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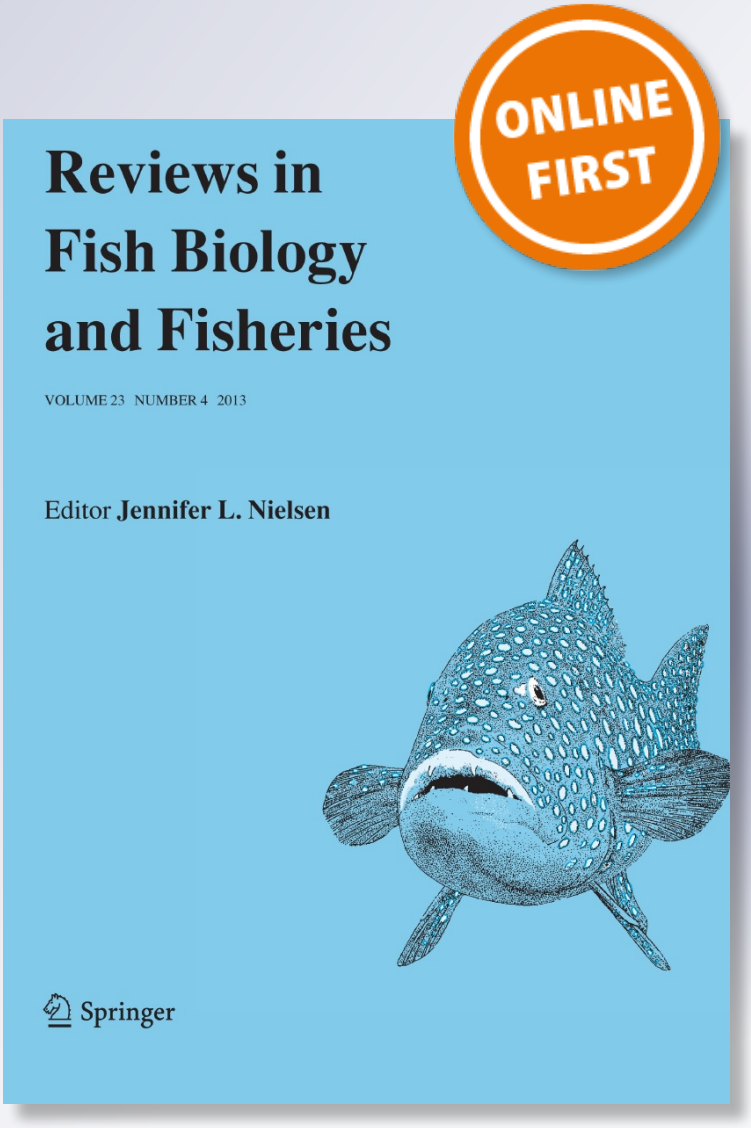

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RESEARCH PAPER

Histological assessment of organs in sexually mature and post-spawning steelhead trout and insights into iteroparity

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Abstract Steelhead trout (*Oncorhynchus mykiss*) are anadromous and iteroparous, but repeat-spawning rates are generally low. Like other anadromous salmonids, steelhead trout fast during freshwater spawning migrations, but little is known about the changes that occur in vital organs and tissues. We hypothesized that fish capable of repeat-spawning would not undergo the same irreversible degeneration and cellular necrosis documented in semelparous salmon. Using Snake River steelhead trout as a model we used histological analysis to assess the cellular architecture in the pyloric stomach, ovary, liver, and spleen in sexually mature and kelt steelhead trout. We observed 38 % of emigrating kelts with food or fecal material in the gastrointestinal tract. Evidence of feeding was more likely in good condition kelts, and feeding was associated with a significant renewal of villi in the pyloric stomach. No vitellogenic oocytes were observed in sections of kelt ovaries, but perinucleolar and early/late stage cortical alveolus oocytes were present suggesting iteroparity was possible. We documented a negative correlation between the quantity of perinucleolar oocytes in ovarian tissues and fork length of kelts suggesting that larger steelhead trout may invest more into a single spawning event. Liver and spleen tissues of both mature and kelt steelhead trout had minimal cellular necroses. Our findings indicate that the physiological processes causing rapid senescence and death in semelparous salmon are not evident in steelhead trout, and recovery begins in fresh water. Future management efforts to increase iteroparity in steelhead trout and Atlantic salmon must consider the physiological processes that influence post-spawning recovery.

Keywords Iteroparity · Histology · Steelhead trout · Fasting

Introduction

Within the Salmonidae family two reproductive life history strategies exist: semelparity and iteroparity. Semelparity is exclusive to the Pacific salmon (*Oncorhynchus* sp.) that spawn once and die, although exceptions to this have occasionally been documented (Tsiger et al. 1994; Unwin et al. 1999). All other salmonids are iteroparous (spawn repeatedly), but the degree of inter-and intra-specific post-spawning survival is highly variable (Crespi and Teo 2002; Quinn and Myers 2004). Steelhead trout *Oncorhynchus*

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mykiss, the anadromous form of rainbow trout, and Atlantic salmon *Salmo salar* are considered iteroparous, but show complex polytypic life history strategies that involve residualism in fresh water (Viola and Schuck 1995; Christie et al. 2011), anadromy (Schaffer and Elson 1975; McDowall 1987; Pascual et al. 2001), and combinations of life history strategies (Docker and Heath 2003; Thrower et al. 2004; Pearse et al. 2009; Null et al. 2013). Iteroparity can increase genetic diversity of stocks and individual lifetime fitness (Seamons and Quinn 2010), as well as provide a safeguard against complete brood year failures (Narum et al. 2008). The energetic and physiological costs of migrations between seawater and fresh water can be high and complicate many of the benefits afforded by iteroparity. Repeat-spawning rates among steelhead trout and Atlantic salmon are generally below 10 % (Busby et al. 1996; Fleming and Reynolds 2004; Quinn and Myers 2004).

Steelhead trout display a range of life history strategies, including stream-maturing (summer/fall run) and ocean-maturing (winter/spring run) forms (Behnke 1992). Stream-maturing steelhead trout return to fresh water early (June–November) and undergo the final stages of gonadal maturation in fresh water, whereas ocean-maturing steelhead trout return to fresh water much closer to the time of spawning (December–April). Low rates of iteroparity in stream-maturing steelhead trout have been commonly attributed to the energy demands of longer migrations and the extended time in fresh water before spawning (Burgner et al. 1992). However, post-spawning mortality in ocean-maturing steelhead trout (Busby et al. 1996) has not been well explained. Considering the differences in migration distance and time spent in fresh water between the two ecotypes, it appears that factors other than simple energetic depletion may contribute to post-spawning survival.

A defining characteristic expressed by both semelparous and iteroparous anadromous salmonids is the prolonged fasting that accompanies freshwater reentry to spawn. Most anadromous salmonids enter a period of voluntary anorexia during spawning migrations and rely on lipids and protein stored in somatic and visceral tissues. It has been theorized that by discontinuing active food consumption in fresh water during spawning, Pacific salmonids and Atlantic salmon can curtail the energy required for digestion that can account for as much as 40 % of basal metabolism in feeding fish (Wang et al. 2006). Through fasting, anadromous salmonids may be able to conserve energy by lowering the energetic demands of their basal metabolism, thus allocating the bulk of their stored energy to support upstream migration, completion of gonadal maturation, the physical exertion of spawning, and in the case of iteroparous species, emigration back to the ocean.

Post-spawn steelhead trout and Atlantic salmon, or kelts (Allan and Ritter 1976), are known to re-initiate feeding activity in fresh water following spawning (Quinn and Myers 2004), but it is presumed that the majority of somatic energy is replaced in the ocean. However, the low proportion of repeat-spawners in steelhead trout and Atlantic salmon populations suggests that many kelts do not survive even when feeding occurs. Larger kelts have been documented to be less likely to repeat-spawn than smaller sized kelts (Dutil 1986; Jonsson et al. 1991; Fleming 1998), presumably because it is more difficult for larger fish to restore energy (Crespi and Teo 2002). In some cases, such as stream-maturing Columbia River steelhead trout, the rates of repeat-spawning are so low that the stocks have often been regarded, as functionally semelparous (Burgner et al. 1992).

Little is known about the effects that prolonged fasting and catabolism have on organ systems in steelhead trout or Atlantic salmon, especially those involved in digestion, energy storage, blood filtration, waste removal, red blood cell production, and vitellogenesis. Studies with other poikilothermic vertebrates (e.g. snakes) have documented a gradual reduction of intestinal epithelium (atrophy) and nutrient transport capacities of the gastrointestinal tract during fasting, but that digestive processes are rapidly restored at the onset of feeding (Secor et al. 2002; Wang et al. 2006). It therefore seems apparent that for steelhead trout or Atlantic salmon kelts to recover, their digestive functions must also be restored. Histological assessments provide information about tissue function at the cellular level and can reveal physiological information that may not be apparent based on external condition and non-lethal measures of nutrition (i.e. blood chemistry, fat-meter). Most histological assessments of spawning anadromous salmonids have focused on semelparous species (Green 1913; Robertson and Wexler 1959). Saunders and Farrell (1988) evaluated coronary tissues in pre- and post-spawn Atlantic salmon, but no histological

assessments of steelhead trout tissues during reproduction and recovery have been reported.

Management actions to enhance iteroparity in steelhead trout and Atlantic salmon have become recognized as important tools for the restoration and conservation of endangered or threatened stocks (Brannon et al. 2004; Gephard and McMenemy 2004). Increasing the number of repeat-spawning individuals within threatened or endangered populations can increase natural production (Hatch et al. 2002), maintain locally adapted traits within a stock (Keefer et al. 2008), and diversify gene flow within the population (Crespi and Teo 2002; Narum et al. 2008). Fish passage around hydro-dams has been improved with modifications to dams, as well as the management of spill (Wertheimer and Evans 2005; Wertheimer 2007). Hatchery reconditioning programs are another management option that can be used to supplement depleted or extirpated stocks of Atlantic salmon (Johnston et al. 1987, 1990, 1992; Gauthier et al. 1989; Eales et al. 1991; Crim et al. 1992) and steelhead trout (Wingfield 1976; Null et al. 2013). In the Connecticut River drainage (CT, MA, NH, and VT), Atlantic salmon kelt reconditioning facilities annually produce between 310,000 and 670,000 eggs for supplementation (Gephard and McMenemy 2004). In the Yakima River, WA, steelhead trout kelt reconditioning has been used to aid in the recovery of imperiled natural stocks by releasing reconditioned individuals back to the river to spawn naturally (Hatch et al. 2013). Yet, despite the success of kelt reconditioning in hatchery settings, we still know little about the physiological and energetic factors that influence post-spawn recovery in the natural environment.

Using Snake River steelhead trout as a model, we assessed the histological architecture of the pyloric stomach, ovary, liver, and spleen tissues to evaluate the effects of prolonged fasting and post-spawn recovery. The primary objectives of our study were to (1) determine if Snake River kelts were attempting to replace energy via feeding during freshwater emigration, (2) use tissue-specific metrics to describe the microscopic changes in the cellular architecture of pre- and post-spawning fish for comparison between phases and across variations in condition, and (3) explore correlations between fish size and the capacity for repeat-spawning based on the functionality of tissues.

Table 1 Number of fish sampled and tissues examined, separated by spawning year, and reproductive stage. All but four sexually mature males and one female in 2010 were sampled at Dworshak National Fish Hatchery. All kelts were sampled at Lower Granite Dam

Phase	Sex	2009	2010	Total
Liver, spleen and pyloric stomach				
Sexually mature	F	16	17	33
	M	0	14	14
Kelt	F	24[a]	29[a]	53
	M	3	10	13
Ovary				
Kelt	F	24	29	53

[a] One sample was missing from analysis of pyloric stomach and liver

Methods

Sample locations and procedures

Snake River steelhead trout are comprised of stream-maturing populations that are categorized into two groups: A-run and B-run. The A-run steelhead trout return to fresh water earlier (July–August), have shorter marine residences (1–2 years), are smaller in size (<70 cm), and generally spawn earlier (March–April) than B-run fish. The B-run steelhead trout return later (August–October), have longer marine residence (2–3 years), are generally larger (>70 cm), and spawn later (April–June). Genetic differentiation between A- and B-run steelhead trout has not yet been fully resolved in the Snake River basin (Nielsen et al. 2009); therefore, the primary means of separating the two groups is by fork length. We identified steelhead trout <70 cm as A-run and steelhead trout ≥70 cm as B-run.

We lethally sampled both A- and B-run steelhead in 2009 and 2010 at two phases of the reproductive cycle, first at or near sexual maturity (mature) and secondly in emigrating kelts (Table 1). Mature steelhead trout were collected at Dworshak National Fish Hatchery (DNFH; 46°30′N, −116°19′W) or intercepted at Nez Perce Tribal Hatchery on the Clearwater River, ID (46°30′N, −116°39′W). Mature steelhead trout were predominantly comprised of large B-run fish (median 82 cm; range 65–90 cm) of known hatchery-origin. Kelts were collected from mixed stocks of emigrating hatchery (adipose fin absent) and natural (adipose fin

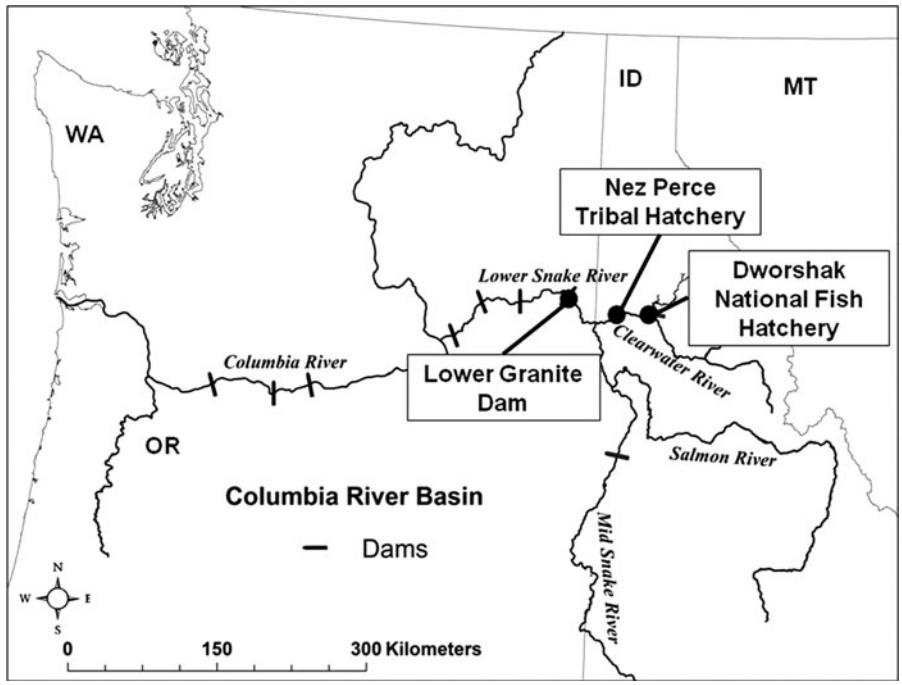

Fig. 1 Map of Columbia/Snake River sub-basin with sampling sites indicated

Table 2 Summary of metrics used to categorize the external condition of steelhead trout kelts at the Lower Granite Dam juvenile bypass facility

Condition	Metrics
Good	No or only minor injuries
	No or only minor fungal infection
	No or only minor fin erosion
	Firm tissue texture
	Active at capture
Fair	Moderate injuries (non-life-threatening)
	Fungal infection 1–10 % of body
	Moderate fin erosion
	Moderate activity at capture
Poor	Severe injuries (life-threatening)
	Fungal infection >10 % of body
	Severe fin erosion
	Flaccid or spongy tissue texture
	Inactive or listless at capture

present) A and B-run Snake River steelhead trout (median 63 cm; range 52–88 cm) at the Lower Granite Dam juvenile bypass facility (46°39′N, −117°26′W), WA (Fig. 1). The date and location of spawning was unknown for all kelts. We attempted to sample equal numbers of female and male steelhead trout at both phases to compare differences in tissue microstructure between sexes, but few male kelts were captured (Table 1).

Steelhead trout were killed with 200 mg/L of MS-222 (Finquel, Argent Laboratories, Redmond, WA, USA) buffered with $NaHCO_3$ or in some cases CO_2 was used at DNFH. Fish were measured for fork length (nearest 0.5 cm). All mature steelhead trout were considered to be in good external condition, whereas the condition of kelts was rated as good, fair, or poor based on visible injuries, fungal infection, fin deterioration, tissue texture, and activity at capture (Table 2).

The gastrointestinal tract of kelts was examined at the time of necropsy for the presence of identifiable food or evidence of digestion via the presence of fecal material. For histological analysis, a section of the pyloric stomach was sagittally bisected at the junction with the pyloric sphincter to provide a cross section of all four layers of the stomach (mucosa, submucosa, muscular coat, and serosa). Sections of the liver, spleen, and ovarian tissue (female only) were also collected for histological analysis. All sampled tissues were transferred to jars containing 10 % buffered neutral formalin (pH 6.8) at a 10:1 ratio of formalin by volume to tissue for fixation. Fixed tissues were shipped to Colorado Histo-Prep (Fort Collins, CO,

Table 3 Summary of histological evaluations by tissue type and magnification in pyloric stomach, liver, spleen, and ovarian tissues. Numerical scores and criteria are presented for each metric

Tissue	Metric (magnification)	Scoring criteria
Pyloric stomach	Submucosa (4×)	1 = Severe detachment from muscularis; 2 = Moderate detachment from muscularis; 3 = Light or no detachment from muscularis
	Villi density (4×)	1 = Low; 2 = moderate; 3 = high
	Villi invagination (10×)	1 = <1/4 distance to stratum compactum; 2 = 1/4 ≤ 1/2 distance to stratum compactum; 3 = 1/2 distance to stratum compactum
	Columnar epithelial cell (40×)	1 = Severe cell necrosis; 2 = moderate cell necrosis; 3 = light to no cell necrosis
	L:W ratio of columnar epithelial cells (40×)	1 = ≤1/2 length to width ratio; 2 = >1/2 length to width ratio;
	Presence of goblet cells (40×)	1 = Absent; 2 = present
Liver	Melanomacrophage aggregates (10×)	1 = Absent; 2 = <5; 3 = >5
	Hepatocyte shrinkage (10×)	1 = Severe shrinkage; 2 = Moderate shrinkage; 3 = Light to no shrinkage
	Hepatocyte spacing (10×)	1 = <10 μm; 2 = >10 μm
	Vacuolization in field of view (40×)	1 = absent; 2 = 1–10 %; 3 = >10 %
	Hepatocyte necrosis (40×)	1 = Severe cell necrosis and nuclear fragmentation/shrinkage; 2 = moderate cell necrosis and nuclear fragmentation/shrinkage; 3 = light to no cell necrosis and nuclear fragmentation/shrinkage
Spleen	Distribution of white pulp	1 = Discrete; 2 = diffuse
	Percentage of red pulp coverage (10×)	1 = 0–39 %; 2 = 40–60 %; 3 = 61–100 %
	Cell production/differentiation (40×)	1 = Absent; 2 = present
Ovary	Quantity of perinucleolar oocytes (4×)	Directly quantified and averaged in 3–4 separate zones
	Quantity of early/late cortical alveolus oocytes (4×)	Directly quantified and averaged in 3–4 separate zones
	Quantity of vitellogenic oocytes (4×)	Directly quantified and averaged in 3–4 separate zones

USA) for processing, where tissues were dehydrated, embedded in paraffin, sectioned at ~4–6 μm, mounted on glass microscope slides, and stained with hematoxylin and eosin (H&E; Luna 1968).

We initially sampled sections from the cardiac and pyloric regions of the stomach of each fish, but since preliminary assessment of the cardiac stomach showed similar tissue architecture, we report only results of the pyloric stomach.

Histological analysis

Histological analyses and scoring were accomplished with a compound light microscope (Leitz Laborlux) fitted with a Leica EC3 camera and photographic software (LAS EZ 1.8.0; Leica Microsystems, Cambridge, Ltd). We evaluated the tissue architecture of the pyloric stomach, liver, and spleen with a categorical scoring system at magnifications of 4×, 10×, and 40×, which provided fields of view at 9.6, 1.5, and 0.1 mm^2, respectively (Table 3). Ovarian tissues were scored by direct counts of oocytes in the tissue. All histological scoring was blind.

Pyloric stomach

We evaluated and scored cross sections of the pyloric stomach using six metrics: (1) detachment of the submucosa from the surrounding muscularis and stratum compactum, (2) density of villi lining the stomach lumen, (3) extent of villiar invagination based on the depth or distance from the tip of villi (lamina epithelialis) to the stratum compactum, (4) necrosis of columnar epithelial cells comprising the villi, (5)

length to width ratio of columnar epithelial cells, and (6) presence or absence of goblet cells in the lamina epithelialis as an indication of mucous secretion. All scores for the pyloric stomach were assessed over the entire tissue, regardless of magnification.

Ovary

Ovarian tissues were scored in female kelts. We enumerated the quantity of perinucleolar oocytes (pre-lipid deposition), the quantity of early/late cortical alveolus stage oocytes (post-lipid deposition), and the quantity of vitellogenic oocytes (lipid and yolk deposition) in three to four fields for each ovary section. Only oocytes sectioned through the nucleus or exhibiting clear lipid or yolk deposition were scored and we assumed that all oocytes were randomly distributed within the ovary. To validate this assumption, we compared the frequency of oocyte counts by category (pre-lipid vs. post-lipid vs. vitellogenic) and field. We found 92 % of the ovarian tissues ($N = 51$) showed no significant variations across fields and concluded pre-and post-lipid oocyte distribution was random and could be averaged across all fields by dividing the total sum of pre and post-lipid oocytes by the total number of fields examined.

Liver

Liver tissue was evaluated using five metrics: (1) density of melanomacrophage aggregates dispersed in the parenchyma, (2) shrinkage of hepatocytes within their respective cords, (3) overall separation between heptatocyte cords (sinusoid spaces), (4) proportion of vacuolization within parenchyma, and (5) necrosis of hepatocytes. The density of melanomacrophage aggregates, hepatocyte shrinkage, and sinusoid spacing were scored over the entire liver tissue area at 10×. Scores for the degree of vacuolization and hepatocyte necrosis from each microscopic field were assessed at 40×, averaged and rounded up.

Spleen

Spleen tissue was evaluated using three metrics: (1) distribution of white pulp nodules within the red pulp, (2) proportion of red pulp, and (3) presence of differentiating cells or cell types evidenced by maturing erythrocytes and leukocytes. All scores for the spleen were derived from assessments of the entire tissue, regardless of the magnification used.

Statistical analysis

The frequency of scoring metrics by category within the pyloric stomach, liver, and spleen was compared to explore the differences within and between both mature and kelt phases. Within mature steelhead trout we compared differences between sexes. Within the sample of female kelts we compared metrics by condition for kelts with and without the presence of food at necropsy. Because sample sizes for male kelts were small (poor = 3, fair = 5, and good = 5), we did not make statistical comparisons among males or between male and female kelts. We compared metrics between mature and kelt steelhead trout for females in good condition. Likewise, the relationship between fork length and histology scores was only examined in good condition female mature and kelt steelhead trout. Oocyte counts were compared between good, fair, and poor condition kelts.

The frequencies of histological scores, by category, were compared using exact χ^2 analyses. Monte Carlo simulations (20,000 permutations) were used to account for occasional low frequencies (<5 per cell). Because oocyte counts were determined numerically (not categorically), we compared oocyte counts with fish condition using Wilcoxon rank-sum tests. Spearman correlations were used to examine the relationship between fork length and histological scores. All statistical tests were conducted using SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Evidence of feeding

Of the 65 emigrating kelts examined, 25 had food or fecal material in the gastrointestinal tract. Food items included salmon smolts, other small unidentified fish, fish eggs, and various terrestrial and aquatic invertebrates. Comparatively, the gastrointestinal tract of poor condition female kelts contained significantly lower amounts of food compared to good condition female kelts (58 % of good condition vs. 7 % of poor condition; $P < 0.01$; Table 4).

Table 4 Summary of proportion of kelts with food or evidence of feeding at time of necropsy at Lower Granite Dam, by sex and fish condition. Monte Carlo permutation exact *P* values are provided for comparisons by sex and condition

Sex	Condition	*N*	Food (%)	*P*
Female	Good	24	14 (58)	0.006
	Fair	14	6 (43)	
	Poor	14	1 (7)	
Male	Good	5	2 (40)	
	Fair	5	2 (40)	
	Poor	3	0	
	Total	65	25 (38)	

Pyloric stomach

We found no significant variation between male and female mature steelhead trout in any of the metrics scored in the pyloric stomach. However, scores of submucosa detachment, villi density, villi invagination, and columnar epithelial cell necrosis in female kelts were significantly different by fish condition ($P < 0.02$; Fig. 2). We found 23 of the 38 female kelts in good or fair condition had minor to no detachment of the submucosa from the muscularis of the pyloric stomach, whereas the majority of poor condition kelts (12/14) exhibited severe and moderate detachment of the submucosa. The villi density was highest in good condition female kelts (Figs. 3, 4). The invagination of villi to the stratum compactum was highly variable between good, fair, and poor condition kelts. Invaginations in good condition kelts were all >1/4 the distance to the stratum compactum and 60 % of those evaluated were >1/2 the distance. Fair condition kelts exhibited a wide range of villi invagination, and nearly all poor condition kelts showed villi invagination <1/2 the distance to the stratum compactum (Fig. 2). Regardless of condition, the majority of kelts had minor to no signs of columnar epithelial cell necrosis.

Our comparisons between the stomach tissues of good condition female mature and kelt steelhead trout showed several differences in structural patterns likely related to feeding recovery after spawning. Over 60 % of mature female steelhead trout had low densities of villi. However, over 70 % of female kelts exhibited high villi density ($P < 0.001$; Fig. 5A). We found significant differences in the extent of villi invagination between mature and kelt steelhead trout (exact $P < 0.001$). The villi invagination in mature steelhead

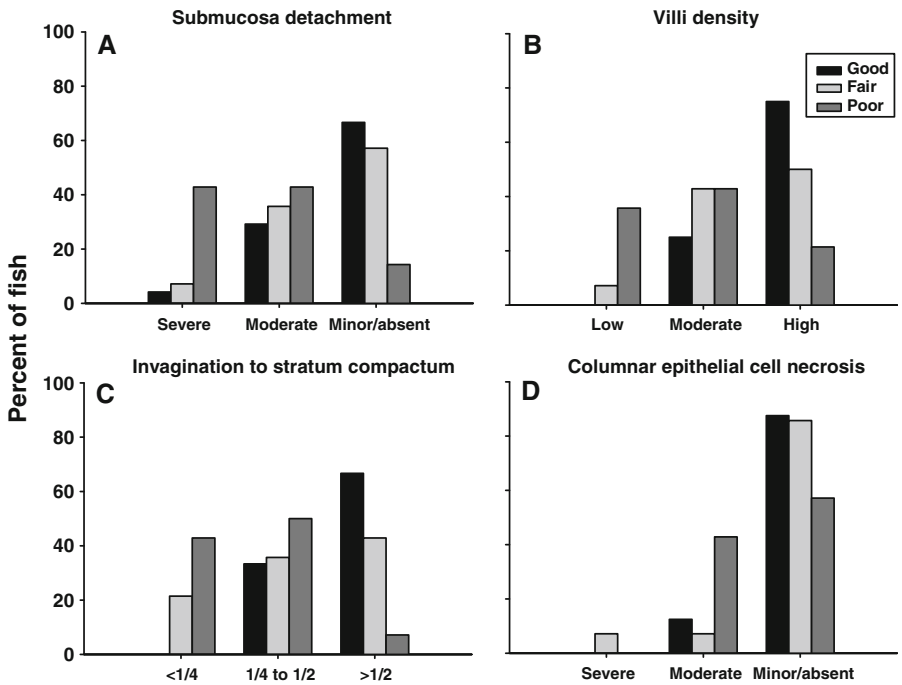

Fig. 2 Significant scoring metrics ($P < 0.02$) for the pyloric stomach among good, fair, and poor condition kelts for scores of **A** submucosa detachment, **B** villi density, **C** villi invagination, and **D** columnar epithelial cell necrosis

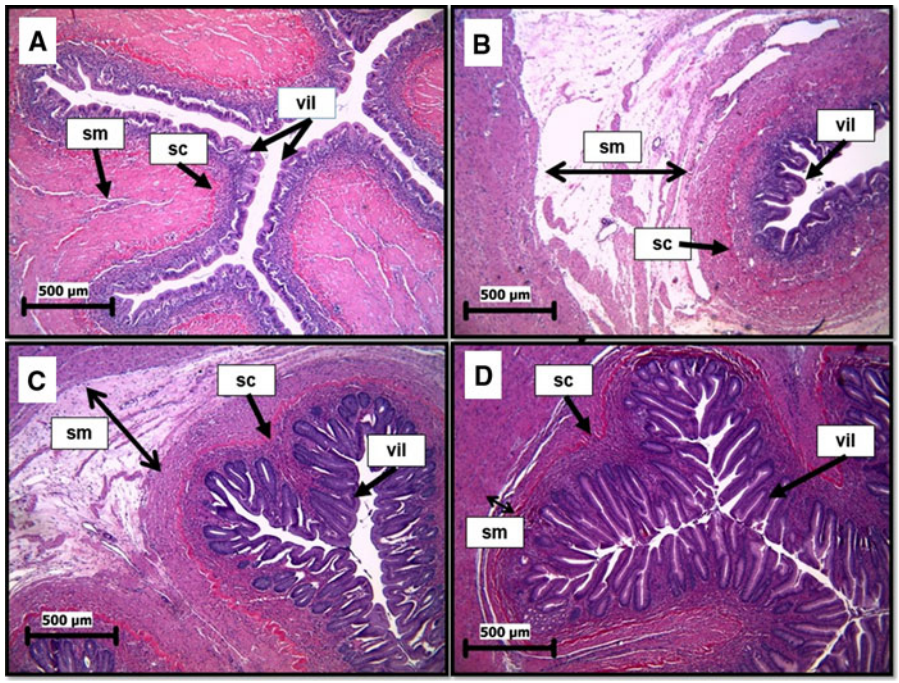

Fig. 3 Photomicrograph (×4) of pyloric stomach architecture in **A** mature steelhead trout (minor to no submucosa detachment, low villi density, <1/4 invagination), **B** poor condition kelt (severe submucosa detachment, low villi density, <1/4 invagination), **C** fair condition kelt (moderate submucosa detachment, moderate villi density, and <1/2 invagination), and **D** good condition kelt (minor to no submucosa detachment, high villi density, >1/2 invagination). *sm* submucosa, *sc* stratum compactum, *vil* villi

trout was very shallow, with nearly 80 % of all villi measured <1/4 the distance to the stratum compactum (Fig. 5B). In kelts, villi invagination was more pronounced and all measures were >1/4 the distance to the stratum compactum. No significant variations in submucosa detachment, columnar epithelial cell necrosis, length to width ratio of columnar epithelial cells, or presence of goblet cells were found between mature or kelt steelhead trout.

We found a negative correlation between submucosa detachment and fork length in mature steelhead trout ($r = -0.465$; $P < 0.007$; $N = 33$). Over 85 % of mature steelhead trout displayed no or only minor submucosa detachment, 15 % displayed moderate detachment, and none exhibited severe detachment. We found no relationship between fork length and submucosa detachment in kelts (Table 5).

Ovary

No vitellogenic oocytes were observed in any of the ovarian tissues from kelts. However, perinucleolar and early/late stage cortical alveolus stage oocytes were observed indicating that Snake River steelhead trout are capable of repeat-spawning. Oocyte atresia was infrequent. We found no variations in the frequency of perinucleolar and early/late stage cortical alveolus oocytes attributed to fish condition (Fig. 6). However, we detected a negative correlation between fork length and perinucleolar oocytes ($r = -0.606$; $P < 0.002$; $N = 24$). We found no correlation between fork length and counts of early/late stage cortical alveolus oocytes (Table 5).

Liver

We detected a significantly higher proportion of vacuolization in liver parenchyma of mature steelhead trout males compared to that observed in mature females ($P = 0.043$; Fig. 7). Mature female steelhead trout had significantly lower proportions of cellular necrosis in hepatocytes than proportions observed in males ($P < 0.001$). We found no significant differences in the density of melanomacrophage aggregates, the amount of hepatocyte shrinkage, or sinusoid

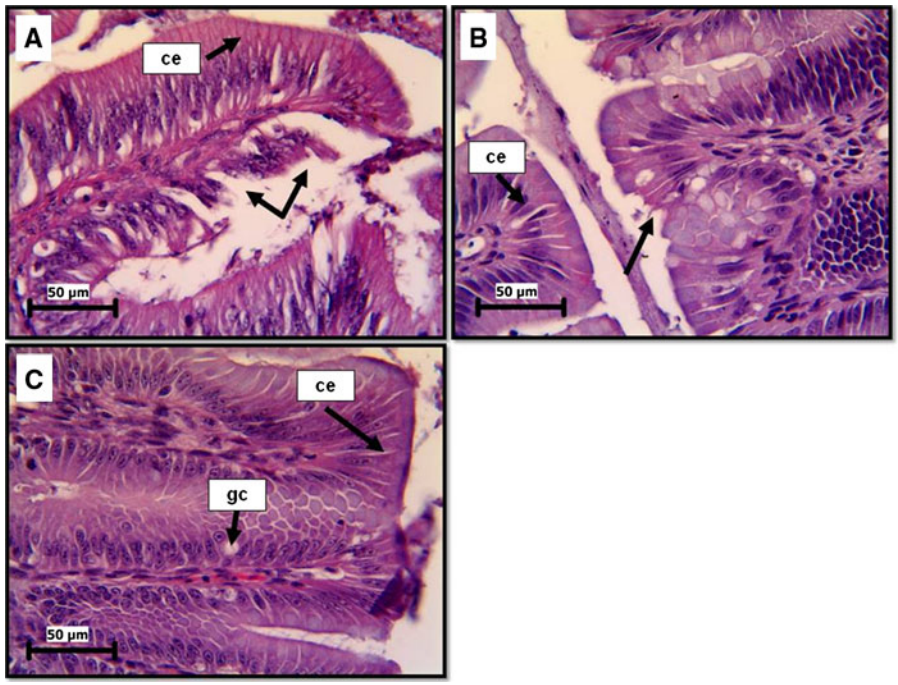

Fig. 4 Photomicrograph (×40) of columnar epithelial cell necrosis in the pyloric stomach villi of **A** poor condition kelt (severe to moderate cellular necrosis indicated by *arrow*), **B** fair condition kelt (moderate to minor cellular necrosis indicated by *arrow*), and **C** good condition kelt (minor to no cellular necrosis). *ce* columnar epithelialis, *gc* goblet cell

spacing between male and female mature steelhead trout.

In comparisons of liver tissues between good condition female mature and kelt steelhead trout we found the proportion of mature females with minor to no hepatocyte cellular necrosis was significantly higher over that of kelts ($P = 0.006$; Figs. 8, 9). Good condition female kelts exhibited moderate hepatocyte shrinkage within hepatocyte cords ($P = 0.002$), in contrast with samples from mature fish that had little to none. Variation in the degree of vacuolization in livers between mature and kelt steelhead trout were noted ($P = 0.05$). We observed approximately 60 % of livers from mature steelhead trout with vacuolization scores between 1 and 10 %, and 20 % with scores >10 %, while nearly 50 % of kelts had a complete absence of vacuolization. Small melanomacrophage aggregates clustered near bile ducts and veins were noted in liver tissues of kelts and mature steelhead trout. Sinusoid spacing was consistent between phases and rarely exceeded 10 µm between hepatocyte cords.

In kelts, we observed hepatocyte necrosis varied among good, fair, and poor condition females. Over 60 % of good condition ($N = 24$) and poor condition ($N = 14$) kelts had minor to no hepatocyte necrosis, whereas approximately 80 % of fair condition kelts ($N = 13$) showed moderate necrosis (Fig. 9). In samples with necrosis, we noted disintegration or loss of the cellular membrane and shrinkage or fragmentation of the nucleus within hepatocytes. Hypertrophy of hepatocytes was occasionally observed and some exhibited vacuolization within the cytoplasm (Fig. 9). We found no significant variation in the proportion of melanomacrophage aggregates, shrinkage of hepatocytes, spacing of sinusoids, or vacuolization among good, fair, or poor female kelts.

Spleen

We found significant differences in the proportion of red pulp in male versus female mature steelhead trout ($P = 0.001$; Figs. 10, 11). Proportionally, 70 % of male steelhead trout exhibited red pulp ratios greater than 61 %. In female kelts, differences in the proportions of cellular differentiation in the spleen were associated with external fish condition. We observed

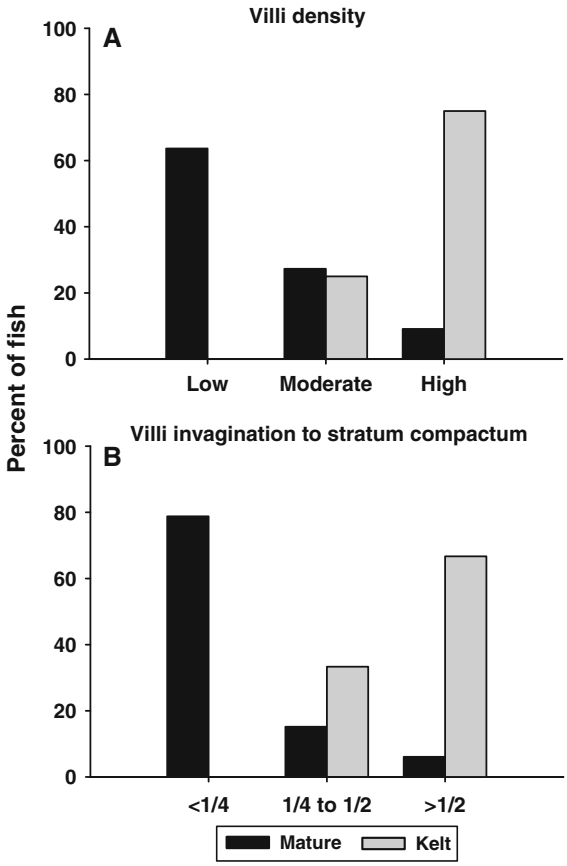

Fig. 5 Significant scoring metrics ($P < 0.001$) for pyloric stomach histology between good condition female sexually mature and kelt steelhead trout: **A** villi density and **B** villi invagination

some evidence of red blood cell differentiation as well as leukocyte production or infiltration in 75 % of kelt spleens. Only fair and poor condition kelt spleens were observed without cell production or monocyte activity (Fig. 11). No other metrics were associated with fish condition. Regardless of reproductive phase, we observed that spleens with higher proportions of white pulp had greater amounts of cell differentiation in the white pulp. In spleens with elevated proportions of red pulp, mature erythrocytes were observed as the dominant cell type.

We found a statistically significant relationship between fish length and the proportion of red pulp in kelts ($r = 0.435$; $P = 0.034$; $N = 24$). Although this trend suggested that larger kelts had higher proportions of red pulp in spleens, these relationships were primarily driven by two large kelts (73 and 75 cm) with red pulp ratios >61 % (Table 5).

Discussion

The energetic, physiological, and tissue deterioration of semelparous Pacific salmon during spawning has been documented extensively (Green 1916; Robertson and Wexler 1960; Robertson et al. 1961; Hane and Robertson 1959; Brett 1995; McPhee and Quinn 1998; Barry et al. 2010; Morbey et al. 2005), but has not been well described in anadromous iteroparous salmonids (Belding 1934). Intuitively, it would seem that any fish capable of repeat-spawning would not undergo the same irreversible cellular degeneration as occurs in semelparous salmon. We found little evidence of extensive cellular necrosis in the pyloric stomach, ovary, liver or spleen in mature or kelt steelhead trout suggesting that the physiological processes causing rapid senescence and death in semelparous salmon are likely not the same in steelhead trout. Furthermore, the observation of active feeding in emigrating kelts indicates that these fish are attempting to replace energy as they emigrate from fresh water.

Gastrointestinal response

Steelhead trout are not believed to feed actively during freshwater migrations prior to spawning, but occasional freshwater feeding has been documented in mature Pacific (Garner et al. 2009) and Atlantic salmon (Johansen 2001). Reductions or loss of villi and microvilli in the gastrointestinal tract are common in fasting or starving poikilothermic organisms (i.e. fish, snakes; Ehrich et al. 1976; Secor et al. 2002; Krogdahl and Bakke-McKellep 2005; Wang et al. 2006). Considering that the majority of mature steelhead trout at DNFH were held on site for 2–3 months and not provided any opportunities to feed prior to sampling, we are confident these fish were in a fasting state. The pyloric stomach of mature steelhead trout had low densities of villi and little to no villi invagination. These observations were similar to the histological assessments of post-spawn sockeye *O. nerka* and Chinook *O. tshawytscha* salmon (Robertson and Wexler 1960). The reduction of villi in fasting fish has also been documented in non-salmonid species. Zeng et al. (2012) showed that the morphology and function of the gastrointestinal tract and liver in food deprived juvenile catfish (*Siluris meridionalis*) were reduced as a physiological response to endure unfavorable environmental conditions. However, it is important to note that cellular

Table 5 Summary of spearman correlations between fork length (cm) and histology scores for good condition female mature and kelt steelhead trout

Tissue	Metric	Mature $N = 33$		Kelt $N = 24$	
		r	P value	r	P value
Pyloric stomach	Submucosa detachment	−0.465	0.006	−0.053	0.807
	Density of villi	0.088	0.628	−0.098	0.650
	Invagination of villi	0.123	0.496	0.102	0.634
	Columnar epithelial cell necrosis	−0.061	0.738	−0.055	0.800
	L:W ratio of columnar epithelial cells	−0.250	0.160	All scores the same	
	Presence of goblet cells	−0.252	0.157	−0.009	0.966
Liver	Melanomacrophage aggregates	−0.071	0.693	0.017[a]	0.935[a]
	Hepatocyte shrinkage	−0.034	0.853	0.046[a]	0.826[a]
	Hepatocyte spacing	0.252	0.157	0.296[a]	0.151[a]
	Vacuolization	−0.063	0.726	0.332[a]	0.105[a]
	Hepatocyte necrosis	−0.252	0.157	0.234[a]	0.261[a]
Spleen	Proportion of red pulp	0.129[b]	0.482[b]	0.435	0.034
	Distribution of white pulp	0.129	0.473	0.146	0.495
	Presence of cell production/destruction	−0.299	0.092	All scores the same	
Ovary	Pre-lipid oocytes	Not tested		-0.606	0.002
	Post-lipid oocytes	Not tested		-0.214	0.315

Correlation coefficients and P values are provided for comparisons by fork length and histology scores. Sample sizes are indicated with the following exceptions

[a] $N = 25$

[b] $N = 32$

necrosis of the columnar epithelial cells rarely occurred in mature steelhead trout suggesting that an irreversible degeneration was not occurring.

From an evolutionary perspective, the volitional anorexia in anadromous semelparous and some iteroparous salmonids is likely the result of returning to a less productive environment (fresh water) at a generally much larger size (McDowall 1987; Gross 1987; Fleming 1998). Wang et al. (2006) hypothesized that reductions in organ size and enzymatic activity, specifically in the gastrointestinal tract, could provide considerable energy savings to fasting or starving organisms. This hypothesis is especially relevant to fasting anadromous salmonids preparing to spawn, which primarily rely on somatic energy stores for migration and spawning. Bioenergetically, it is conceivable that anadromous salmon returning to spawn would save energy by fasting because they would not actively pursue food with little energetic gain, lower their basal metabolic costs by not operating the gastrointestinal tract, and also forgo the costs of specific dynamic action (loss of energy during digestion) by using stored energy. Therefore we agree with the Wang et al. (2006) hypothesis and further hypothesize that the gastrointestinal tract of iteroparous salmonids enters a cellular stasis or hibernation during reproduction, which is supported by the observed renewal of villi and invagination found in kelts.

It is unclear if kelt steelhead trout must first ingest food to begin gastrointestinal recovery. Secor et al. (2002) found that following prolonged periods of fasting in pythons that intestinal mass and microvilli density rapidly increased following the ingestion of specific amino acids and peptides. The columnar epithelial cells and goblet cells comprising the villi are important in the secretion of enzymes, mucous, as well as the uptake of nutrients in the gastrointestinal tract (Mader 2001). Presumably, the observed increases in villi density and invagination provide more surface area for the digestion and absorption of food. Significant variations in villi density and invagination did occur among good, fair, and poor kelts, which is possibly due to the finding that good condition kelts were more likely to feed than fair or poor condition kelts. However, comparisons between feeding and

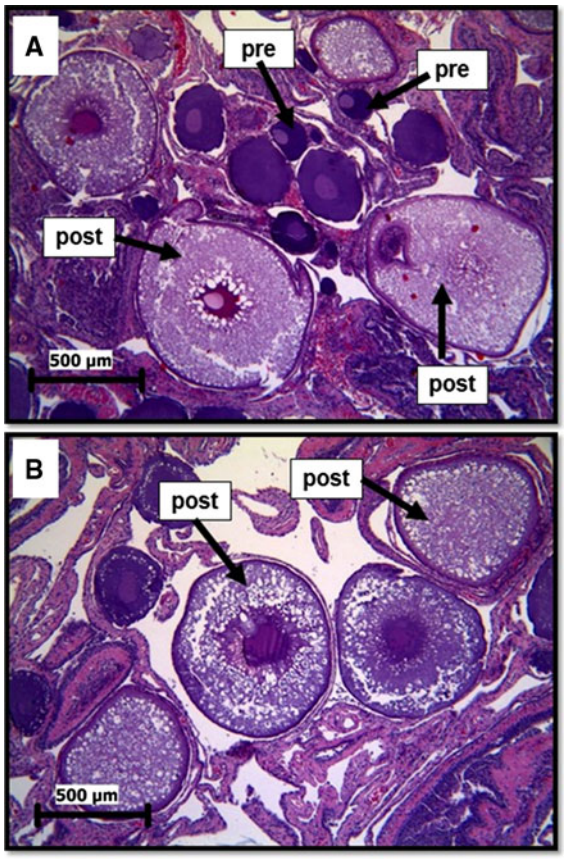

Fig. 6 Photomicrograph (×4) of kelt ovaries **A** kelt ovary with high quantity of perinucleolar (pre-lipid) and some early and late stage cortical alveolus (lipid deposition present) oocytes and **B** ovary from a large kelt (>70 cm) with only early and late stage cortical alveolus oocytes. *pre* pre-lipid oocyte, *post* post-lipid oocyte

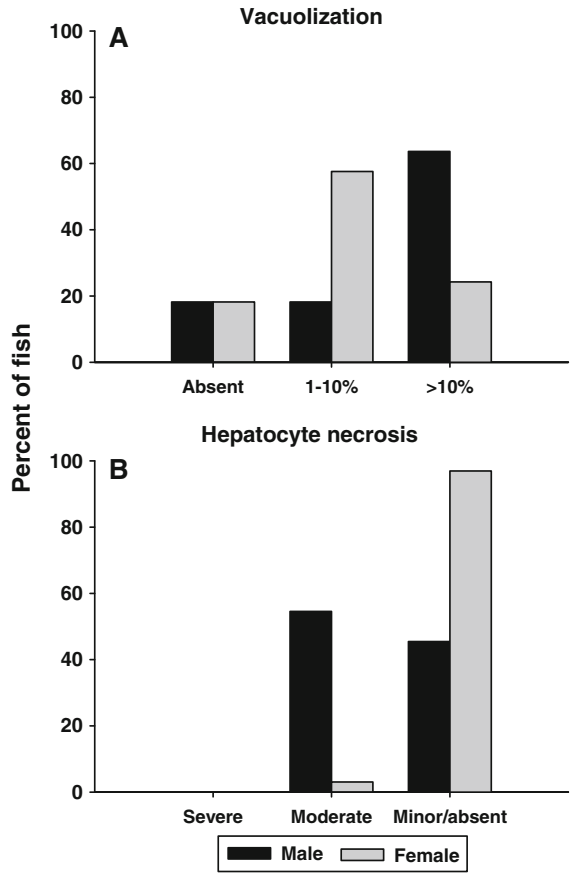

Fig. 7 Significant scoring metrics ($P < 0.05$) for the liver between male and female pre-spawn steelhead trout: **A** vacuolization and **B** hepatocyte necrosis

non-feeding female kelts in good condition displayed no significant variations in any of the pyloric stomach scoring metrics.

In reconditioning experiments with Atlantic salmon kelts, Johnston et al. (1987) found that many kelts began feeding soon after spawning, but that many had difficulties in swallowing feed and only after swallowing food several times did the rejection of feed become less prevalent. Gastrointestinal regeneration may not occur at the same rate in kelts or may be more difficult for some over others. It is possible that kelts observed in our study with empty gastrointestinal tracts had simply evacuated food prior to sampling. Carnivorous species like steelhead trout have short gastrointestinal tracts and the residence period of food in the gut is relatively short (Arrington et al. 2002). Additionally, acute stress can alter the cellular composition and reduce the permeability of the gastrointestinal tract (Olsen et al. 2005, 2008).

In addition to its role in digestion, the gastrointestinal tract is also important to osmoregulation in emigrating kelts. As in smolts, the emigration of kelts from a hypo- to hyper-tonic environment requires a change in the osmoregulation of ions from the gills to the gastrointestinal tract. The process of the re-acclimation to seawater has been shown to be greatly accelerated in kelts. Talbot et al. (1992) found that Atlantic salmon kelts were able to re-adapt to seawater within 48 h, thus indicating that the gut and all organs responsible for the salt and water balance had recovered some or all function 4–6 weeks after spawning. A rapid acclimation and gastrointestinal recovery is likely beneficial to kelts on two levels. Not only does it reduce the stress and time required to re-acclimate in the estuary allowing for quicker access to

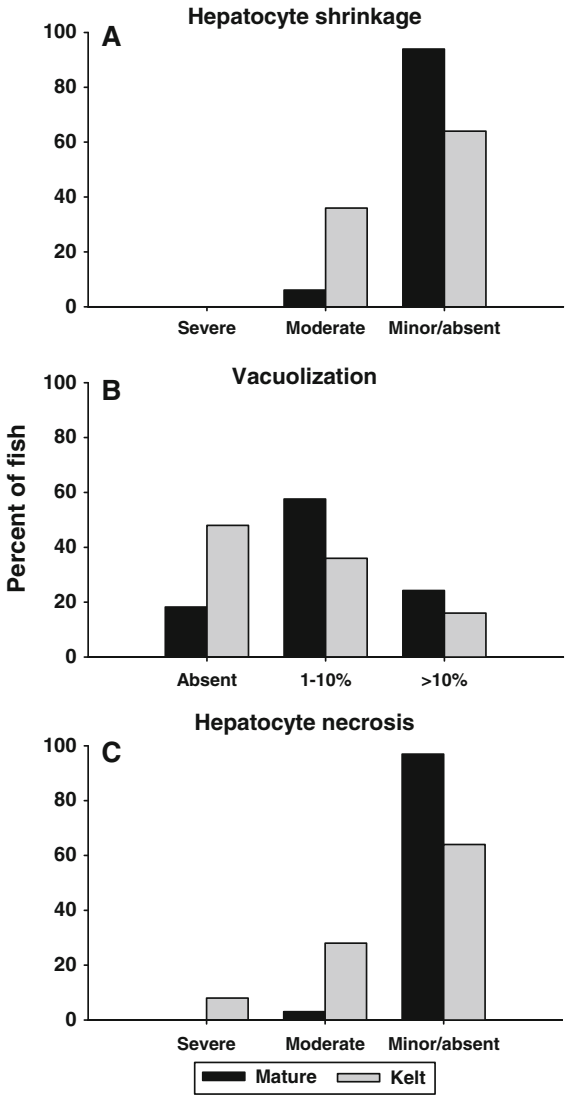

Fig. 8 Significant scoring metrics (all $P \leq 0.05$) for liver histology between good condition female sexually mature and kelt steelhead trout: **A** hepatocyte shrinkage, **B** vacuolization, and **C** hepatocyte necrosis

high energy food in the ocean, but also aids in the recovery from many freshwater-specific external infections such as fungus (*Saprolegnia* sp.) and parasitic copepods (*Salmincola* sp.).

Liver and spleen responses

Our assessments of mature and kelt steelhead trout livers showed little evidence of severe liver necrosis or loss of function at the cellular level. Of the two phases examined, tissues in kelts had the most hepatocyte shrinkage and necrosis, although samples from <10 % of kelts showed severe hepatocyte necrosis. Robertson and Wexler (1960) reported pronounced hepatocyte necrosis that included decreases in cytoplasm and occasionally a complete loss of nuclear material in spawning semelparous salmon.

Compared to white muscle tissue, the liver is not a primary energy storage tissue in anadromous salmonids (Brett 1995), but it does serve as a readily accessible energy source during fasting or starvation. In semelparous pink salmon *O. gorbuscha*, Phleger (1971) found that the liver lost the ability to synthesize triglycerides following freshwater re-entry and spawning. Mommsen et al. (1980) examined the enzymatic activity of Fraser River sockeye salmon *O. nerka* livers during spawning migrations and found a gradual decrease in metabolic enzymes and protein content as fish progressed toward spawning. In salmonids, carbohydrates comprise only a very small component of white muscle tissue composition and are not a significant source of energy (Brett 1995); however, the liver does store glycogen as a small source of energy for immediate use when needed. French et al. (1983) found that the liver of post-spawn sockeye salmon increased its capacity to synthesize glucose following complete exhaustion of somatic lipids, perhaps providing a final energy source for nest guarding. Thus, it appears that a complete loss of liver function is rare even among spawning semelparous species and may explain the overall lack of hepatocyte necrosis found in Snake River steelhead trout livers.

The effects of starvation and prolonged fasting on the cellular architecture of the liver have been documented for a variety of fish species (Hochachka 1961; Vijayan and Moon 1992; Hur et al. 2006; Rios et al. 2007; Kullgren et al. 2010). Among the noticeable histological changes in the livers of starving fish are losses of glycogen or lipid vacuolization (Wolf and Wolfe 2005; Hur et al. 2006). In our study, the only significant variation in liver vacuolization was observed between male and female pre-spawn steelhead trout, where males had a higher proportion of vacuolization. French et al. (1983) found that liver glycogen reserves in Fraser River sockeye were highest in females just before spawning, but did not examine males. As livers are important to vitellogenin synthesis, it is possible that glycogen reserves in females were used to complete oogenesis before spawning. This difference between sexes may also partially explain the variations in hepatocyte necrosis

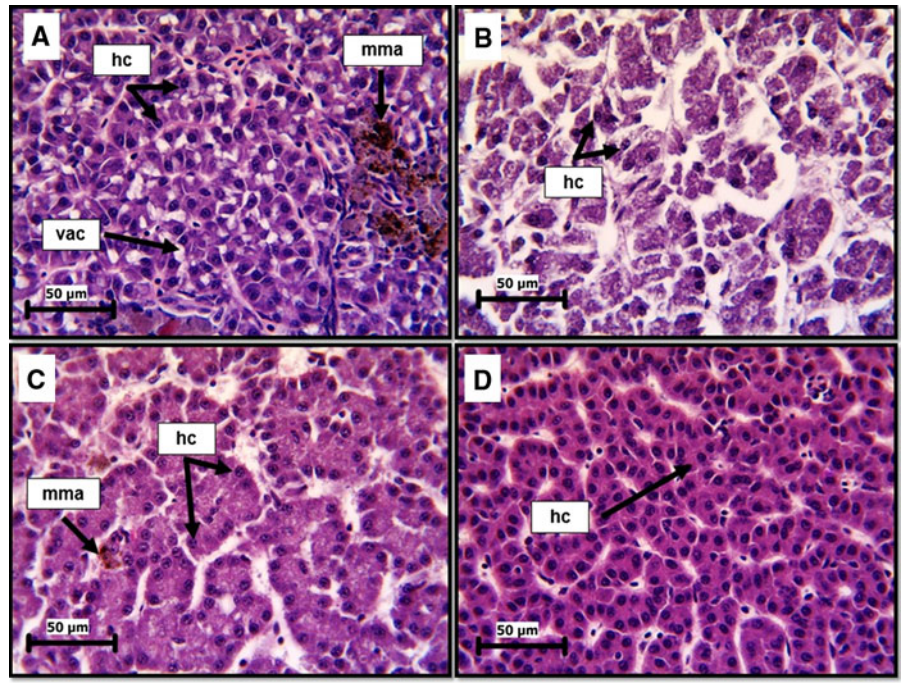

Fig. 9 Photomicrograph (×40) of liver cellular architecture in **A** mature steelhead trout (high vacuolization, minor to no hepatocyte shrinkage, minor to no hepatocyte necrosis), **B** poor condition kelt (no vacuolization, severe hepatocyte shrinkage, severe hepatocyte necrosis), **C** fair condition kelt (no vacuolization, minor to no hepatocyte necrosis), and **D** good condition kelt (vacuolization present, minor to no hepatocyte shrinkage, minor to no hepatocyte necrosis). *hc* hepatocytes, *vac* vacuolization, *mma* melanomacrophage aggregates

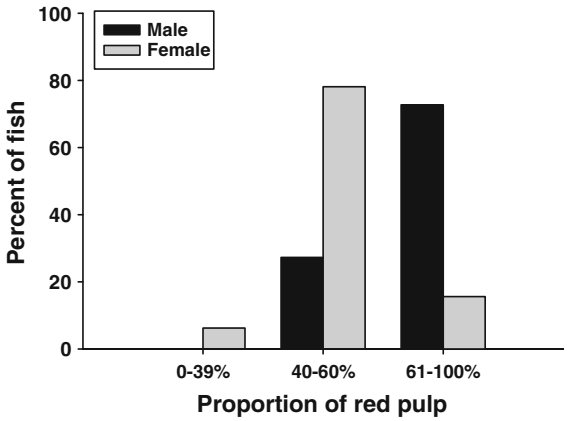

Fig. 10 Proportion of *red pulp* coverage in spleens of male and female pre-spawn steelhead trout. Male steelhead trout had significantly more *red pulp* ($P < 0.05$) than females

between males and females. Wolf and Wolfe (2005) note that it is not uncommon to observe hepatocyte hypertrophy in the livers of sexually maturing females due to estrogen induced vitellogenin production. Therefore, it is possible that the variations in vacuolization and hepatocyte necrosis between mature male and female steelhead trout reflected these differences in energy allocation for oogenesis.

Regardless of condition, sex, or phase all steelhead trout livers had >5 melanomacrophage aggregates. Agius and Roberts (2003) noted the melanomacrophage aggregates are generally prolific in the kidney, spleens, and livers in starving fish. Passantino et al. (2013) reported that melanomacrophage aggregates acted as effective biomarkers for determining the health status of Atlantic bluefin tuna *Thunnus thynnus* (Walbuam), where high densities were indicative of disease, physiological stress, nutritional deficiencies, or exposure to pollutants. We did not observe large numbers of aggregates, but perhaps a more refined method of scoring aggregates would have resolved potential variation among factors of condition, sex, and mature and kelt phases.

We found little evidence of cellular necrosis or loss of spleen function in either mature or kelt steelhead trout. Only fair and poor condition kelts showed a complete loss of cellular activity in the spleen suggesting other potential pathological problems. Robertson and Wexler (1960) detected a reduction in the quantity or complete disappearance of lymphoid cells and increase in fibrous tissue in the spleen of spawning semelparous Chinook and sockeye salmon, but found only 1 of 16 spleens with cellular necrosis.

We found a higher proportion of red pulp in male mature steelhead trout over females. Rebok et al. (2011) found that white pulp increased in the spleens of female Ohrid trout *Salmo letnica* during spawning,

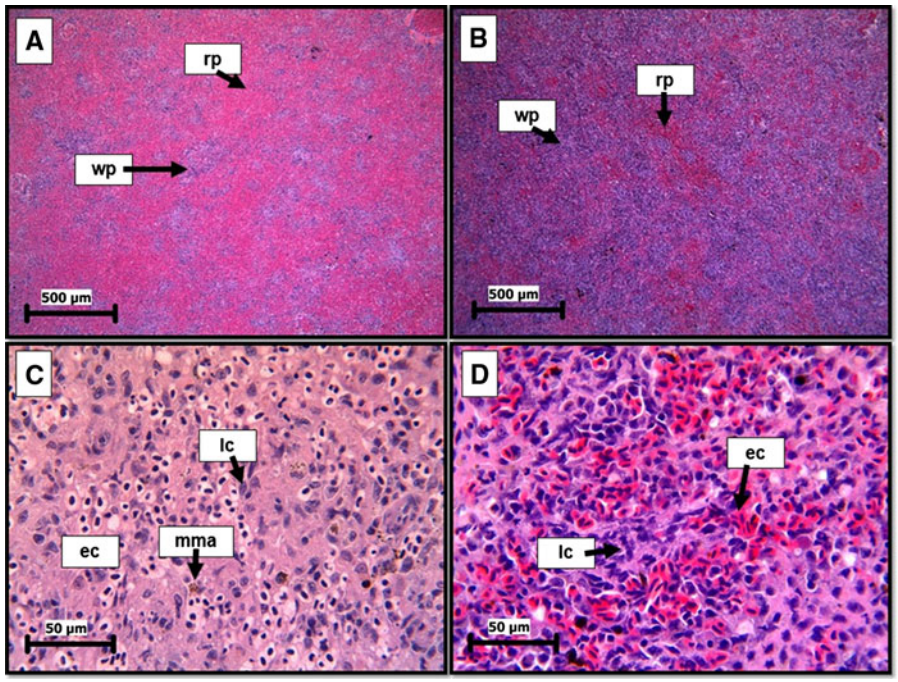

Fig. 11 Photomicrograph of spleen cellular architecture in **A** mature male steelhead trout (>61 % *red pulp*) at ×4 **B** mature female steelhead trout (40–60 % *red pulp*) at ×4, **C** poor condition kelt (no cell differentiation, severe necrosis) at ×40, and **D** good condition kelt (erythrocyte and lymphocyte differentiation) at ×40. *wp* white pulp, *rp* red pulp, *ec* erythrocytes, *lc* lymphocytes, *mma* melanomacrophage aggregates

but did not examine males. Rebok et al. (2011) postulated that gonadal maturation likely reduced the proportion of circulating lymphocytes thereby potentially increasing the white pulp in the spleen of females. Variations in red pulp can be caused by the use of erythrocytes for exercise and activity. Strenuous exercise influences the proportion of erythrocytes stored in the spleens of *O. mykiss*, and other teleosts (Kita and Itazawa 1989; Franklin et al. 1993). It is possible that the spleens of mature male steelhead trout in our study contained higher proportions of erythrocytes in red pulp in preparation for competition on the spawning grounds.

We found no variations in the arrangement of white pulp nodes by sex, condition, or phase in our study, but red and white pulp nodes in teleosts are reported to be more diffuse than in mammals (Mumford et al. 2007). Although not scored, melanomacrophage aggregates were present in all steelhead trout spleens. The number of melanomacrophage aggregates in the spleen generally increases with prolonged fasting and starvation (Agius and Roberts 1981; Mizuno et al. 2002), similar to changes observed in the liver and kidney. Future evaluations of the spleen in response to prolonged fasting during spawning may be warranted.

Repeat-spawning potential and fish size

We observed perinucleolar (pre-lipid) and early/late stages of cortical alveolus oocytes (post-lipid) in Snake River kelts, suggesting that these populations are capable of repeat-spawning. Like their freshwater resident conspecifics, steelhead trout exhibit group synchronous ovarian development characterized by the development of one primary group of oocytes at a time, which is accompanied by numerous "resting" previtellogenic oocyte stages (Blazer 2002). This type of ovarian development pattern has also been defined as determinant fecundity, where the recruitment of oocytes in relation to secondary growth is arrested prior to spawning (Rideout and Tomkiewicz 2011). Interestingly, Robertson and Wexler (1960) found small nucleated oocytes present in the ovaries in spawning sockeye salmon, but not in the ovaries of Chinook salmon. Exceptions to repeat-spawning have been found for some salmonids that were previously

considered to follow strict semelparous life histories. Tsiger et al. (1994) found that male masu salmon *O. masou* maturing and spawning in fresh water could later adopt anadromous life histories and return to spawn again. Unwin et al. (1999) found that precocious male Chinook salmon could be spawned and undergo gonadal recrudescence to spawn again under experimental conditions. We are presently not aware of any experiments that have been able to achieve repeat-spawning in semelparous female salmonids.

All oocytes observed in Snake River steelhead trout ovaries were in previtellogenic stages indicating that vitellogenesis was not occurring. This finding is not surprising considering that these fish had recently ovulated the primary group of oocytes during spawning (<1–2 months earlier). Oogenesis is an energetically expensive process for all fish species (Rideout and Tomkiewicz 2011), thus immediate gonadal recrudescence would not be expected to occur in a post-spawning fish with depleted somatic energy. Johnston et al. (1987) found oogenesis and vitellogenesis proceeded in Atlantic salmon kelts only after restoration of body energy reserves. In our studies, the exact date and location of spawning was unknown for kelts, but it is unlikely that any of these fish had adequate time to restore their total body energy to re-initiate oogenesis and vitellogenesis, even with the re-initiation of feeding.

Recent studies by Null et al. (2013) on acoustically tagged kelt steelhead trout in the Sacramento River, CA documented that 10 % of kelts residualized in fresh water. Although residualized kelts grew less than those that returned to the ocean, their survival was higher. Steelhead trout re-building energy stores and undergoing gonadal recrudescence can remain in the ocean a year or more before return migration (Burgner et al. 1992). Variations in spawning periodicity, known as skip-spawning, are common and can occur in mature fish due to low somatic energy, poor physical health, or poor environmental conditions (Rideout et al. 2005; Rideout and Tomkiewicz 2011). In anadromous species, migrating long distances, skip spawning is common. We found no female kelts in our study that had retained ripe eggs, and some follicular atresia was observed. The absence of vitellogenisis in Snake River kelt ovaries in our studies suggests that the pre-vitellogenic oocytes were still in a resting stage.

With the extended migration distance to return to the ocean, it is likely that most Snake River steelhead trout would exhibit skip-spawning to re-build energy stores. Jonsson et al. (1991) found smaller Atlantic salmon kelts (<60 cm) were more likely to exhibit consecutive spawning, whereas larger kelts were more likely to exhibit skip-spawning (>90 cm), and smaller kelts had higher survival. The residualized and anadromous kelt steelhead trout tagged by Null et al. (2013) were observed as consecutive spawners, and were <60 cm. Fork length was found to be negatively correlated to the number of pre-lipid oocytes. Our finding is of particular interest in the Snake River basin considering that large B-run steelhead have been reported to represent only a small proportion of emigrating kelts at the Lower Granite Dam juvenile bypass facility (Narum et al. 2008).

During our sampling of kelts in 2009 and 2010, we observed low proportions of large kelts (>70 cm) in the juvenile bypass system at the Lower Granite Dam (unpublished data). In 2010, we determined that 92.0 % of kelts were <70 cm indicating that most emigrating individuals were A-runs. Geographically, A-run steelhead trout spawn throughout most of the Columbia and Snake River basin, whereas B-run steelhead trout primarily originate in the Clearwater, Middle Fork Salmon, and South Fork of the Salmon Rivers, ID (Campbell et al. 2012). Using multilocus microsatellite genotypes, Narum et al. (2008) reported that only 7.5 and 9.4 % of kelt steelhead trout sampled at the Lower Granite Dam juvenile bypass facility in 2002 were assigned to the Clearwater and Salmon River's even though these populations represented the largest populations within the Snake River subbasin. There is evidence supporting that larger body size decreases the likelihood of repeat-spawning in anadromous iteroparous salmons due to increased energetic investments in size to increase fecundity, egg size, and competitive advantages on the spawning grounds (Fleming 1998; Crespi and Teo 2002; Fleming and Reynolds 2004). Keefer et al. (2008) hypothesized that high post-spawn mortality of large kelts on or near spawning grounds possibly reflected the selection of a life history strategy more in-line with semelparity. In this study, the lower number of perinucleolar oocytes observed in B-run kelts lends some support to the Keefer et al. (2008) hypothesis; however, there is little evidence indicating that B-run steelhead trout die soon after spawning.

Jones (2013) acoustically tagged 30 B-run kelts in two upper tributaries of the Clearwater River, ID and

detected 29 that successfully emigrated to the Lower Granite Dam forebay, providing evidence that large B-run kelts do not die on or near the spawning grounds, as would occur in a semelparous Pacific salmon. One alternative explanation for the lack of large B-run kelts in the Lower Granite Dam juvenile bypass facility may be related to preferential passage routes through the dam. Tagging by Wertheimer and Evans (2005) and Colotelo et al. (2013) in the Snake River reported that steelhead trout kelts primarily passed dams using the spillways or spillway weirs. It is possible B-run steelhead avoid the juvenile bypass portals due to their larger size and prefer to pass dams via spillway routes. Further research evaluating how kelt size affects downstream dam passage is warranted.

Saunders and Farrell (1988) examined coronary arteriosclerosis in pre- and post-spawning Atlantic salmon and found that fish size and growth rate influenced the prevalence and severity of coronary lesions in arterial tissues. Farrell (2002) reported that increased fish size was directly correlated with the severity of coronary arteriosclerosis in salmonids, regardless of origin (natural vs. hatchery) or maturity status. However, unlike in mammals the pathological consequences of severe coronary arteriosclerosis in salmonids is not well understood. It has been demonstrated that the main coronary artery of rainbow trout can be ablated without impairing cardiac function (Gamper et al. 1994); therefore, it seems unlikely that severe coronary arteriosclerosis is the primary cause of mortality in large spawning salmonids. However, Farrell (2002) commented that coronary lesions could certainly impair blood circulation and regulation in salmonids and affect spawning success. The severity of coronary arteriosclerosis, especially in large kelts, may be a factor affecting post-spawning recovery of steelhead trout and Atlantic salmon.

Another plausible explanation for low post-spawn survival rates in B-run kelts is energetic exhaustion. Most (>60 %) emigrating Snake River kelts were reported to die before reaching the Pacific Ocean (Wertheimer and Evans 2005; Colotelo et al. 2013). Narum et al. (2008) commented that larger kelts generally require more energy for recovery over smaller kelts. To determine if fish size affected external condition, we examined the length distributions of good, fair, and poor condition kelts (Fig. 12), and found no evidence that B-run kelts were in poorer condition than A-run kelts. Penney and Moffitt (in press) found somatic lipids were exhausted in all Snake River steelhead kelts, regardless of size. It is currently unknown how much energy is required for post-spawn recovery in Snake River A- or B-run steelhead trout; however we speculate that it is highly variable among individuals.

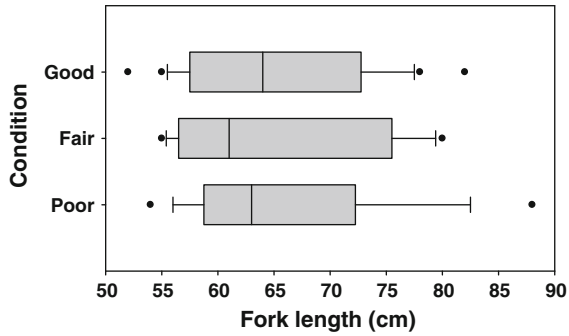

Fig. 12 Fork length (cm) of steelhead kelts by external condition. No significant differences ($P > 0.05$) were detected between condition and fork length

Management implications

Iteroparity provides a unique conservation tool for increasing or maintaining threatened and endangered populations of iteroparous salmonids. Live or air-spawning of steelhead trout (Null et al. 2013) and Atlantic salmon (Gephard and McMenemy 2004) is commonly practiced in hatcheries that supplement systems with low return rates. Kelt reconditioning programs have demonstrated that human intervention can bypass many of the energetic constraints that limit post-spawning survival in the natural environment (Gauthier et al. 1989; Hatch et al. 2013), as well as expedite spawning periodicity (Johnston et al. 1990, 1992). Our results provide further insight into the internal factors that influence post-spawn recovery in anadromous iteroparous salmonids and can be helpful to programs attempting to increase iteroparity.

Steelhead trout are considered to be the closest "ecological parallel" to Atlantic salmon (Fleming and Reynolds 2004); therefore we speculate the changes in cellular architecture during reproduction are similar between the two species. One key finding of this study not listed in the original objectives was the relationship between external condition and histological assessments. External evaluations are commonly used to grade the physiological condition of kelts and have been reported to influence the capacity for iteroparity.

Keefer et al. (2008) reported that repeat-spawning rates in good condition steelhead kelts were an order of magnitude higher than kelts in poor condition. Hatch et al. (2013) noted that kelts in good external condition were more likely to survive reconditioning than kelts in poor condition. We found that histological assessments further validated external evaluations of fish condition. For example, severe submucosa detachment in the pyloric stomach was most common in poor condition kelts, whereas the majority of good condition kelts exhibited no or only minor detachment of the submucosa. Our validation that external condition is largely reflective of internal condition has merit in the selection or treatment of kelts for reconditioning.

A comparison not examined in this study was the potential variation in tissue histology between hatchery and natural origin steelhead trout. It is possible that differences in migration and spawning behavior between hatchery and natural-origin could affect energy use and post-spawning condition. However, for the purposes of this study we assumed that all mature steelhead trout would be in a fasting state and that all kelts intercepted at the Lower Granite Dam juvenile bypass facility had spawned naturally, regardless of smolt origin. Keefer et al. (2008) reported that 52 % of kelts collected from the Lower Granite Dam juvenile bypass facility from 2002 to 2004 were of natural-origin and that most repeat-spawners were of also of natural-origin (0.95 vs. 0.40 %). In our sampling of kelts in 2010, we could not unequivocally separate natural from hatchery-origin smolts. We documented that 69 % of kelts had adipose fins, but numerous kelts with adipose fins were observed with healed, eroded dorsal fins potentially indicating hatchery-origin (Winfree et al. 1998). Future research examining variations in the behavior and energy use of hatchery and natural-origin steelhead should be pursued.

Summary

This study was the first to examine in detail the histological architecture of organs of steelhead trout at maturity and as emigrating kelts. Although many changes were detected between these two stages, the majority of steelhead trout showed little cellular atrophy or necrosis that would be indicative of loss of cellular function. The evidence of feeding and presence of oocytes in kelts, as well as changes in the epithelial structures of the pyloric stomach between maturity and kelt emigration, all provide strong indications of the potential for iteroparity in these stocks.

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