DESCRIPTION OF THE REPRODUCTIVE STRUCTURE, SIZE, GROWTH, AND CONDITION OF HATCHERY-ORIGIN WHITE STURGEON IN THE LOWER COLUMBIA RIVER, BRITISH COLUMBIA, CANADA

by

Paige Anne Camas Maskill

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DEDICATION

This thesis is dedicated to my parents, whose adoration for the outdoors and sense of adventure cultivated a curiosity and passion for conserving our natural world from a very young age. Thank you to both of you for your never-ending love, support, and encouragement. Fish on.

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ABSTRACT

Conservation aquaculture has been successful in preventing extirpation of endangered white sturgeon *Acipenser transmontanus* in the lower Columbia River (LCR), Canada. The goals of this project were to 1) determine if the hatchery-origin (HO) population has reached puberty as many fish are of an age and body size to begin contributing to spawning and 2) assess the size, absolute growth rate (AGR), and relative condition factor (K_n) of the population. The reproductive structure (sex and stage of maturity) of the HO population was assessed using multiple tools (gonadal biopsy analyzed histologically, ultrasound, endoscopy, and plasma sex steroids). The accuracy of each tool was determined by comparing the assigned sex to the true sex determined by histological analysis of a gonadal biopsy. A subobjective was to determine whether gonadal development was homogenous (assessed in fish captured in LCR and Lake Roosevelt, WA, USA). All fish assessed were pre-meiotic males (n=158) or pre-vitellogenic females (n=174) and had yet to reach puberty. Endoscopy and gonadal biopsy analyzed histologically were the most accurate tools with accuracies ≥ 97%. Gonadal development was homogenous in females but 38% of the males exhibited non-homogenous testicular development, with a few precocious cysts that contained germ cells in an advanced stage of development compared to the predominant phase seen throughout the biopsy. The size, AGR, and K_n were compared among sex, age, and river zone. While the mean K_n indicated that the HO white sturgeon were in average condition relative to all HO fish within the LCR, they were lower in condition compared to the full transboundary population (Canada and USA). This reflects a difference in the environmental or physiological conditions of the fish in the LCR and those captured throughout the transboundary reach. Results of this work will be important to determine the reproductive dynamics of the hatchery-origin population over time (e.g., age and size at puberty, sex ratio), and specifically when these fish will begin spawning in the wild. Additionally, this information will help to develop other standardized monitoring programs which can track the reproductive structure of a population over time using tools with known accuracy rates.

CHAPTER ONE

INTRODUCTION TO THESIS

Sturgeon belong to the order Acipenseriformes and are considered to be one of the most endangered groups of fishes worldwide (Billard and Lecointre 2001, Haxton and Cano 2016, Haxton et al. 2016). Sturgeon are prized for their caviar, which created a high demand beginning in the 1800's and led to the overharvest and poaching of numerous species (Billard and Lecointre 2001, Haxton and Cano 2016, Apostle 2017). Anthropogenic impacts including pollution, containments, agricultural run-off, and river regulation have also contributed to sturgeon decline. For example, river regulation has reduced sturgeon habitat, disconnected migration routes, decreased spawning habitat, and altered environmental cues (Beamesderfer et al. 1995, Billard and Lecointre 2001, Haxton and Cano 2016, Apostle 2017). Sturgeon have unique life history characteristics including a long life span, maturation that is not synchronized between sexes and occurs later in life (i.e., greater than 10 years of age), and intermittent spawning once puberty is reached, with males reproducing more frequently than females (Billard and Lecointre 2001, Hildebrand and Parsley 2013, Haxton et al. 2016). These characteristics have evolved over millions of years and allowed for adaptations to change over a much longer time period compared to the recent anthropogenic impacts sturgeon have experienced in less than a single generation. Due to the imperiled status of many species, recovery plans have been developed and management actions have been implemented in an effort to sustain and recover populations (Billard and Lecointre 2001, Haxton and Cano 2016,

Apostle 2017). However, these unique life history characteristics complicate recovery by requiring a significant amount of time before progress of management actions can be assessed (Billard and Lecointre 2001).

White sturgeon *Acipenser transmontanus* are native to western North America and are distributed throughout the Pacific Northwest with populations in the Sacramento, Snake, Fraser, and Columbia River systems (Hildebrand et al. 1999, Hildebrand and Parsley 2013). In the Columbia River, the construction of dams for river regulation and power production resulted in reduced and fragmented populations of white sturgeon. Specifically, the construction of the Grand Coulee Dam (1941) and the Hugh L. Keenleyside Dam (1968) resulted in a subpopulation within the transboundary reach of the Columbia River, which includes the lower Columbia River (LCR) in Canada and Lake Roosevelt in Washington, USA (Hildebrand et al. 1999, Irvine et al. 2007, Hildebrand and Parsley 2013). White sturgeon in the LCR were listed as endangered in 2006 due to recruitment failure, which occurs between the embryonic and juvenile life stages, threatening the persistence of the population (Hildebrand and Parsley 2013, BC Hydro 2013, 2015, 2016, 2017, 2018). In response to recruitment failure, conservation aquaculture was implemented in 2001 in an effort to prevent extirpation and retain the genetic diversity of the existing wild population.

The conservation aquaculture program has been successful in preventing extirpation and has reestablished age classes of white sturgeon which were previously absent from the LCR (BC Hydro 2018). The program has released fish annually into the Canadian and U.S sections of the transboundary reach. The oldest fish in the hatchery-

origin population are now approaching a size and age at which puberty may be initiated as compared to wild populations of white sturgeon (Welch and Beamesderfer 1993, DeVore et al. 1995, Doroshov et al. 1997, Billard and Lecointre 2001, CBWSPF 2013, Haxton et al. 2016). Therefore, as the hatchery-origin population approaches puberty, knowledge of when they may begin to contribute to spawning events with the wild population is critical for management.

Successful management of sturgeons requires knowledge of the reproductive dynamics of individual populations (Jakobsen et al. 2016). Reproductive indices are metrics that can be used to evaluate the reproductive potential of a population and explore future population dynamics (Worm et al. 2009, Lowerre-Barbieri et al. 2011). Reproductive structure, an example of a reproductive indice, is defined as the proportion of the population in each stage of the reproductive cycle (Webb and Doroshov 2011). Describing the reproductive structure of a population can inform demographic models, and tracking changes in the reproductive structure over time can be used to monitor the effects of management actions for threatened or endangered species. Additionally, incorporating information on sex and stage of maturity into monitoring programs is valuable as migratory behavior, habitat use, and sampling gear bias have been found to differ between sexes or among different classes of sex and stage of maturity in some species (McKinley et al. 1998, Richards et al. 2014, and Shaw et al. 2012, 2013). As sturgeons are not sexually dimorphic (Billard and Lecointre 2001, Hildebrand and Parsley 2013, Haxton et al. 2016), numerous tools exist to assign sex and stage of maturity in fishes. These tools include ultrasound (e.g., Moghim et al. 2002, Wildhaber

et al. 2007, Munhofen et al. 2014), endoscopy (e.g., Wildhaber et al. 2005, Bryan 2007, Hurvitz et al. 2007, Munhofen et al. 2014), blood plasma sex steroids (e.g., Webb et al. 2002, Feist et al. 2004, Webb and Doroshov 2011), and a gonadal biopsy analyzed histologically (e.g., Van Eenennaam and Doroshov 1998, Chen et al. 2006, McGuire et al. 2019). Varying levels of accuracy have been found for each tool, but a comparison of these tools has not yet been documented in hatchery-origin white sturgeon (Webb et al. 2018).

Fish size, growth, and condition are other biological metrics that can be easily analyzed and are useful when assessing the reproductive dynamics within a population. Fish of larger sizes are often able to reach puberty at an earlier age compared to smaller individuals within a population (Amano et al. 1997, Huang et al. 1998, Van Eenennaam and Doroshov 1998, Taranger et al. 2010, Ryu et al. 2013, Nater et al. 2018). Additionally, knowledge of growth rates can be of value when assessing reproductive potential in a population, as growth rate slows in fish prior to the onset of puberty when allocation of resources shift from somatic growth to gonadal development (Taranger et al. 2010, Minte-Vera et al. 2016, Nater et al. 2018). Measures of condition in fish are often used to indicate well-being, with the expectation that fish in good condition will likely experience faster growing rates, greater reproductive potential, and higher survival as compared to fish in below-average condition (Pope and Kruse 2007, Neumann et al. 2012). For these reasons, monitoring the size, growth rates, and condition within a population can provide important information in assessing the reproductive status of a population.

Overview of Thesis

The overall goal of this study was to determine if any individuals within the hatchery-origin white sturgeon population in the LCR had reached puberty. The specific objective of Chapter Two was to describe the reproductive structure of the white sturgeon population in the LCR using numerous tools (gonadal biopsy, ultrasound, endoscopy, and plasma sex steroids) available to assign sex to the hatchery-origin population, as well as a small number of the existing wild white sturgeon. The accuracy in using these tools to correctly assign sex was determined using histological analysis of gonadal tissue. The presence of wild fish within the study enabled a comparison of accuracy rates between the prepubertal and post-pubertal populations. Additionally, gonadal homogeneity was assessed to assess whether gonadal development occurred homogeneously across the gonadal lobes of sturgeon in the LCR and Lake Roosevelt in Washington.

In Chapter Three, size, growth, and condition of the hatchery-origin population were described. Conservation aquaculture is expected to be the main source of population growth in LCR white sturgeon, therefore, understanding the condition of the population and whether condition differs by sex, age, and habitat as represented by river zone will be beneficial for long-term management of the population. The specific objectives were to: 1) describe the size, absolute growth rate, and relative condition factor (K_n) of hatchery-origin white sturgeon in the LCR, 2) determine if these metrics differed by sex, 3) determine if the K_n of the hatchery-origin population differed among sexes,

ages, and zone and 4) determine if the K_n of the hatchery-origin fish differed in the LCR as compared to the transboundary reach.

Chapter four summarizes the major findings of the study. Management implications and recommendations for future work were discussed.

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CHAPTER TWO

DESCRIBING THE REPRODUCTIVE STRUCTURE OF THE HATCHERY-ORIGIN POPULATION OF WHITE STURGEON IN THE LOWER COLUMBIA RIVER, BRITISH COLUMBIA, CANADA

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Paige A. C. Maskill

Contributions: Implemented study design, collected and analyzed data, and authored the manuscript.

Co-Author: Molly A. H. Webb

Contributions: Obtained funding, developed study design, discussed the implications of the results, and co-authored the manuscript.

Co-Author: James A. Crossman

Contributions: Obtained funding, developed study design, discussed the implications of the results, and co-authored the manuscript.

Co-Author: Christopher S. Guy

Contributions: Discussed the implications of the results and co-authored the manuscript.

Manuscript Information

Paige A. C. Maskill, James A. Crossman, Christopher S. Guy, and Molly A. H. Webb

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ABSTRACT

Hatchery-origin (HO) white sturgeon *Acipenser transmontanus* in the lower Columbia River, Canada are approaching puberty. Assessing the proportion of HO fish in each stage of the reproductive cycle (i.e., reproductive structure) will enable fisheries managers to determine if the HO population has reached puberty and could contribute to spawning events in the wild. Multiple tools exist to assign sex and stage of maturity in fishes. However, there is a knowledge gap regarding which tool most accurately assigns sex and stage of maturity in prepubertal and post-pubertal sturgeons. Reproductive structure was described in 332 hatchery-origin individuals over two years (2017 and 2018). True sex was determined using histological analysis of gonadal tissue, which is 100% accurate at assigning sex and stage of maturity in fish when germ cells are present in the biopsy. All HO fish were pre-meiotic males (n=158) or pre-vitellogenic females (n=174) and had not reached puberty. The accuracy of each tool was determined by comparing the assigned sex using a specified tool to the true sex which was determined histologically from a gonadal biopsy containing germ cells. Assignment of true sex using histology was 97% and 94% in the HO and wild populations, respectively, as several biopsies did not contain germ cells. Gonadal biopsies that did not contain germ cells were not included in further analyses. The accuracy of tools to assign sex and stage of maturity (gonadal biopsy analyzed histologically, ultrasound, endoscopy, and plasma sex steroids) were compared within HO and between HO (n=332) and wild white sturgeon (n=75). Accuracy in assigning sex to both the HO (98%) and wild (100%) fish was highest using endoscopy (an otoscope). The other tools evaluated were less accurate with 69% accuracy in HO and 93% accuracy in wild fish for plasma sex steroids and 57% accuracy in HO and 70% accuracy in wild fish for ultrasound. Based on these results, endoscopy should be used to assign sex in the white sturgeon population. Long-term monitoring of the reproductive structure of the HO fish is important to determine the reproductive dynamics of this population over time and the effects of management actions.

Introduction

Understanding the reproductive dynamics of a population is essential to the successful management of fishes (Jakobsen et al. 2016), and reproductive indices (e.g., sex ratio, age and size at puberty or first spawning, reproductive structure, fecundity, spawning periodicity) are used to describe the reproductive dynamics of a population. Specifically, the reproductive structure of a population is defined as the proportion of the population in each stage of the reproductive cycle (Webb and Doroshov 2011). The proportion of females and males at each stage of maturity can be used as a metric to evaluate the reproductive potential of a population and explore future population dynamics (Worm et al. 2009, Lowerre-Barbieri et al. 2011). In hatchery-origin populations produced from conservation aquaculture programs, assessing the proportion of the population that is prepubertal and post-pubertal (another way to examine the reproductive structure of a population) allows for the determination of age and size at puberty and if the hatchery-origin population is contributing to spawning events with the wild population.

Describing the reproductive structure of a population can be time consuming, costly, and biological samples may be needed yet difficult to obtain if the species is threatened or endangered (Webb et al. 2018). Many fish species have secondary sexual characteristics allowing for quick and easy assignment of sex and stage of maturity. However, other species, such as the sturgeons and paddlefishes, are not sexually

dimorphic (Billard and Lecointre 2001, Hildebrand and Parsley 2013, Haxton et al. 2016).

Numerous tools (e.g., ultrasound, endoscopy, and plasma sex steroid analysis) exist to assign sex and stage of maturity in fishes (e.g., Moghim et al. 2002, Feist et al. 2004, Munhofen et al. 2014, Du et al. 2017, Webb et al. 2018). However, each tool has different training requirements, a varying level of accuracy, and the accuracy cannot always be equally applied among fish species or, in some cases, populations within a species (Webb et al 2018). Collecting the required information to determine reproductive structure can be challenging but essential to adequately manage and conserve fishes.

White sturgeon *Acipenser transmontanus* in the lower Columbia River, hereafter referred to as LCR, Canada were listed as endangered in 2006 under the Species at Risk Act (SARA). Wild adults in the population continue to spawn; however, age 0 individuals are not surviving to reproduce, and this limited recruitment threatens the persistence of the entire population (Hildebrand and Parsley 2013, BC Hydro 2013, 2015, 2016, 2017, 2018). Without recruitment, Irvine et al. (2007) predicted a rate of decline of 2.7% per year for the LCR white sturgeon population. The LCR white sturgeon is predicted to be functionally extinct by 2044 (BC Hydro 2013, 2015, 2016, 2017, 2018) without management action(s). As a result of these findings, conservation aquaculture was implemented in 2001 in an effort to restore the population.

The conservation aquaculture program has been successful, with current estimates of juvenile abundance being more than 5,500 individuals (BC Hydro 2018) within the LCR. Conservation aquaculture has reestablished juvenile age classes previously absent

from the population, and the oldest year class at the time of this study was 17 years old. In the wild, white sturgeon have been documented to reach puberty at 100-150 cm fork length (FL) and 12-25 years in males and 120-180 cm FL and 15-35 years in females (Welch and Beamesderfer 1993, DeVore et al. 1995, Doroshov et al. 1997, Billard and Lecointre 2001, CBWSPF 2013, Haxton et al. 2016). Thus, some individuals within the LCR white sturgeon hatchery-origin population may be approaching puberty as the median FL is approximately 1.0 m with the distribution exceeding 130.0 cm FL (BC Hydro 2018).

The overall goal of the study was to determine if the hatchery-origin white sturgeon in the LCR, Canada have reached puberty. To accomplish this goal, we described the reproductive structure of the population using numerous tools (gonadal biopsy analyzed histologically, ultrasound, endoscopy, and plasma sex steroids) available to assign sex and stage of maturity. We determined the accuracy in assigning sex using each tool in hatchery-origin white sturgeon and a small number of the existing wild white sturgeon. The wild fish enabled a comparison of accuracy in assigning sex between prepubertal and post-pubertal animals. Sex and stage of maturity was assessed using histological analysis of gonadal biopsies and plasma sex steroids in the wild population. The results from this study will help to further develop a monitoring program which can track changes in the reproductive structure of the hatchery-origin population over time.

Study Area

White sturgeon were sampled throughout a 57-km reach of the LCR, between Hugh L. Keenleyside Dam (HLK; river kilometer (rkm) 0.0) and the United States-Canada border (rkm 57) in British Columbia, Canada (Figure 2.1). Sampling areas were divided into three zones, which were delineated by differences in discharge determined by previously conducted stock assessments. Zone 1 had the lowest discharge (BC Hydro 2015, 2016, 2017) observed in the study reach. It began at the HLK dam and included a small section of the Kootenay River downstream of Brilliant Dam and its confluence with the LCR. Zone 2 was the only section of the study reach which did not contain a confluence and was not in close proximity to a dam. Discharge was highest in Zone 3 (BC Hydro 2015, 2016, 2017), which included the confluence of the Pend d'Oreille River and ended at the United States border at Waneta, Washington. Each zone began at the termination of the previous zone. White sturgeon abundance was highest in Zone 1 (BC Hydro 2013, 2015, 2017, 2018); therefore, in an attempt to equalize the sample sizes of fish captured among zones, the distance (in river kilometers; rkm) was greater in Zones 2 and 3. Zone 1 was 11-rkm(s) and Zones 2 and 3 were each 23-rkm(s).

Hatchery-origin white sturgeon were also collected from the upper Columbia River in Lake Roosevelt, which is located in northeast Washington, USA. Lake Roosevelt is a 209-km reservoir, ranging 1-3 km wide with a maximum depth of 122 m (Polacek et al. 2006). The reservoir is part of the transboundary reach which consists of 243-km of white sturgeon habitat located between Grand Coulee Dam in Washington and

the United States-Canada border (Howell and McLellan 2006, Hildebrand and Parsley 2013).

Methods

Fish Sampling Design

White sturgeon were monitored in 2017-2018, with sampling events in the spring and fall. Sampling took place before and after spawning, which occurred from mid-June to late July (BC Hydro 2016, 2018). The population of LCR white sturgeon has high site fidelity (BC Hydro 2016), therefore, the sampling effort was spatially balanced throughout the fishable areas within the river, and all habitat types were included. Location of sampling sites were randomly assigned with 24 sites in Zone 1 and 48 sites in each of Zones 2 and 3. Probability of catch was assumed to be equal among all sampling zones. Sampling began at the most downstream location in Zone 3 and progressed upstream until all sites were sampled.

During the first sampling season (spring 2017) up to six hatchery-origin fish per year class (2001-2007), and six wild fish were sampled in each zone. Ultrasound or endoscopy were alternately conducted on fish to assign sex and ensure that accuracy for each tool could be determined without bias. This process equated to approximately three hatchery-origin fish sampled with the ultrasound and three hatchery-origin fish sampled with endoscopy per year class within each zone. The same process was repeated on the wild fish, with three wild fish sampled using ultrasound, and three wild fish sampled using endoscopy within each zone.

The sampling design was adjusted prior to the start of fall of 2017 in an effort to decrease the number of minor surgical biopsies conducted due to significant processing time required in the field for the initial sampling design. The adjusted design remained in place for the spring and fall of 2018. As a result, up to four hatchery-origin fish per year class were sampled within each zone, with two sampled using endoscopy and two sampled using ultrasound per year class in each zone. Sampling of the wild population was reduced to five fish sampled in each zone using only the ultrasound to assign sex because endoscopy had been used successfully to assign sex in the wild population of white sturgeon in the LCR in the past. Blood and a gonadal biopsy were collected from all fish. Fish from the 2008-2016 year classes and fish that were captured without a Passive Integrated Transponder (PIT) tag were released without collection of biological samples.

Fish Collection

Baited set lines were used to capture white sturgeon (BC Hydro 2013, 2015, 2017, 2018). One set line was deployed at each sampling location, for a total of 120 set lines (24 in Zone 1 and 48 in both Zones 2 and 3). Set lines were approximately 54 m in length, and 12 circle halibut hooks of four different sizes (14, 16, 18, 20 mm) were attached to the mainline at 6-m intervals. All barbs were removed from hooks prior to sampling to reduce hook related injury. Each set line was baited using pickled squid acquired from Gilmore Fish Smokehouse, Dallesport, WA, USA (BC Hydro 2018).

Captured white sturgeon were lifted into the boat using a stretcher with a wench and davit system. Sturgeon were laid dorsally in the stretcher, and water was pumped over the gills throughout the duration of sampling. All captured fish were scanned for a PIT tag. If a sturgeon without a PIT tag was caught, a PIT tag was implanted subdermally in the tissue layer parallel to the posterior end of the dorsal fin, and the second lateral scute was removed using a sterilized scalpel. All surgical equipment, including the PIT tag and tagging syringe, were immersed in 100% ethanol and rinsed before and after contact with sturgeon. Fork length (± 1 cm) and body weight (± 1 kg) were collected from all fish. All fish were released alive.

Assignment of Sex and Stage of Maturity

Upon retrieval of fish, a blood plasma sample was collected from each individual. Sex was then assigned using either ultrasound or endoscopy. Once sex had been determined, a gonadal biopsy was collected from all fish. The following description of each tool follows the same order as fish were processed in the field.

Plasma Sex Steroids Blood (2 mLs) was collected from the caudal vasculature using a 2-mL syringe, placed in a heparinized vacutainer, and centrifuged at 1,228 x g (relative centrifugal force) for five minutes. Plasma was frozen and stored at -80°C until laboratory analysis. Steroids were extracted from the plasma following the method of Fitzpatrick et al. (1987). Briefly, 100 µL of plasma was extracted twice with 2 mL of diethyl ether. Tubes were vortexed vigorously with ether, and the aqueous phase was removed by snap-freezing in liquid nitrogen. Ether was allowed to evaporate overnight

under a chemical hood. The next day, extracted steroids were resuspended in 1 mL of phosphate-buffered saline with gelatin (PBSG), and 10 or 50 µL was assayed for each steroid. Recovery efficiencies for all steroids were determined by adding tritiated steroids to tubes containing plasma (n=4), which was extracted as described above. Recovery efficiencies were 90-98% for testosterone (T) and 73-93% for estradiol-17β (E2). All steroid assay results were corrected for recovery.

Steroids (T and E2) were measured by radioimmunoassay (RIA) as described by Fitzpatrick et al. (1987) and modified by Feist et al. (1990). All samples were analyzed in duplicate. Steroid levels were validated by verifying serial dilutions were parallel to standard curves. A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran/L PBSG) was used for all assays to reduce non-specific binding. Quantified plasma sex steroid concentrations below the minimum quantifiable concentration (MQC) of the RIA were assigned the MQC (0.16-0.38 ng/mL for T and 0.10-0.21 ng/mL for E2). Non-detectable plasma sex steroid concentrations (i.e., not quantifiable) were assigned half of the MQC for statistical purposes (0.08-0.19 ng/mL for T and 0.05-0.11 ng/mL for E2; Croghan and Egeghy 2003). The intra- and inter-assay coefficients of variation for all assays were less than 10%.

Ultrasound An ultrasound (Sonosite Edge II with a 6-15 mHz linear transducer) was used to capture images of the gonad in the hatchery-origin and wild populations which enabled the assignment of sex based on differences in echogenicity, echotexture, and uniformity of gonadal tissue. The Small Parts exam type was used with the

optimization set to General, and the scanning depth was set between 4.0 and 6.0 cm. A black sheet was draped over the sonographer to create shade to adequately view the ultrasound. Fish were laid dorsally in the stretcher, and the ultrasound transducer was held 1) posterior to the urogenital pore and perpendicular to the ventral scutes between the anal fin and the second to third ventral scutes and 2) laterally and parallel to the scutes, approximately two to four scutes anterior of the urogenital pore. All ultrasound images were archived.

Fish were assigned as female based on the appearance of fine-grains and an irregular shape of the gonad without a clearly defined tunica or the presence of ovarian follicles (Chebanov and Galich 2009). Fish were assigned as male based on smooth margins of the gonad and a bright hyperechoic tunica (Chebanov and Galich 2009). Sex was assigned blindly by each project member. Field assignment of sex for an individual using ultrasound was based on the majority assignment of the project members (n=3). The accuracy of using ultrasound to assign sex was determined by comparing the sex assignment by ultrasound to true sex determined by gonadal histology.

Endoscopy Endoscopy using an otoscope (Welch Allyn, 3.5 V) was used to macroscopically identify the gonad in the hatchery-origin and wild fish which enabled the assignment of sex based on morphological characteristics of the gonad. The otoscope was inserted into the body cavity through an abdominal incision. The incision site was approximately 1 to 3 cm off the midline between the third and fourth scutes, anterior to the urogenital pore, and approximately 1-to 2-cm long. The otoscope specula was

cleaned and disinfected between each fish with 100% ethanol and rinsed before and after contact with sturgeon.

Fish were assigned as female based on the presence of ovigerous folds, oocytes, or ovarian follicles and assigned as male based on the presence of testicular lobes. Sex was assigned blindly by each project member. Field assignment of sex for an individual was based on the majority assignment of the project members (n=3). The accuracy of using endoscopy to assign sex was determined by comparing the sex assignment by endoscopy to the true sex determined by gonadal histology. Images were captured and archived using a Vividia 2.0 MP handheld USB digital endoscope (Oasis Scientific Inc.).

Gonadal Biopsy Following visual assignment of sex using endoscopy, gonadal tissue (1 cm^3) was collected through the otoscope specula using a Miltex biopsy cup and stored in 10% phosphate buffered formalin. If sex was not assigned using endoscopy, an incision was made as described above, and gonadal tissue was collected through the otoscope specula. The gonadal biopsy was processed histologically to determine the true sex and stage of maturity. Fish were sutured with polydioxanone violet monofilament suture (PDS).

Histology True sex and stage of maturity was determined for white sturgeon using histological analysis of gonadal tissue and assigned according to Webb et al. 2018 (Table 2.1). Accuracy of histological analysis of gonadal tissue is dependent on the collection of germ cells within the biopsy. Gonadal tissue was preserved in 10% phosphate buffered formalin (Webb and Erickson 2007). In the laboratory, gonadal

tissue was processed, embedded in paraffin wax, and sectioned at 5 µm. Tissue from hatchery-origin fish was stained with hematoxylin and eosin, and tissue from wild fish was stained with periodic acid-Schiff (PAS) to assist with identification of post-ovulatory follicles. Slides were examined using a compound scope (Leica DM 2000; 10-100x) (Leica Biosystems Inc., IL, USA). Sex and stage of maturity for all fish in the study was assigned by two readers independently. For fish where sex or stage of maturity differed between readers, the slide was reviewed and if agreement could not be reached it was sent to a third reader. Further, if a slide did not contain germ cells, the slide was sent to the third reader for confirmation, and the sex and stage of maturity was classified as unknown.

Assessment of Gonadal Homogeneity

To our knowledge, the assessment of whether gonadal development (i.e., gametogenesis) occurred homogeneously across the gonadal lobes in sturgeon had not yet been completed. Therefore, we included the assessment of gonadal homogeneity as a sub-objective within this study. To determine the homogeneity of gonadal tissue, we assessed multiple gonadal biopsies collected from hatchery-origin white sturgeon in the LCR as well as Lake Roosevelt, WA. These fish belong to the same conservation aquaculture program, yet some individuals within Lake Roosevelt have begun to initiate puberty (Webb et al. 2016). Assessing the gonads of both prepubertal and fish undergoing puberty enabled us to determine whether development within the gonads of hatchery-origin white sturgeon was homogeneous.

Fish Collection Gonadal tissue was assessed in hatchery-origin white sturgeon from the LCR, Canada (2017) and Lake Roosevelt, Washington, USA, (2016 and 2018) to determine if gametogenesis occurred homogeneously throughout the gonad. Hatchery-origin white sturgeon from Lake Roosevelt were collected during a larger on-going population assessment program conducted by the Confederated Tribes of the Colville Reservation in spring and fall of 2016 and fall of 2018 (Howell and McLellan 2018). Sturgeon were captured using baited setlines at pre-determined, randomly selected locations throughout Lake Roosevelt.

All captured fish for the homogeneity component were euthanized following methods agreed upon with regulatory agencies. Fish were scanned for a PIT tag to determine year class. Fork length (± 1 cm) and body weight (± 1 kg) were collected from all fish.

Homogeneity Biopsy Collection Sample processing differed between years as samples from 2016 had been collected and preserved prior to this study. In 2016, three gonadal tissue biopsies (Miltex biopsy cup) were randomly collected from a 5 x 8-cm section of gonadal tissue (only one lobe sampled) from 24 hatchery-origin male white sturgeon captured from Lake Roosevelt, WA. Each biopsy sample was labelled with the fish ID, stored in 10% phosphate buffered formalin, and processed histologically.

In 2017 and 2018, the gonads were excised and biopsies were collected for histological analysis. An incision was made from the urogenital pore to the pectoral girdle to collect gonadal tissue (n=9 fish in 2017, 5 males and 4 females; n=3 fish in

2018, 3 males and 0 females). A total of twenty biopsies (n=10 per lobe) were collected (anterior to posterior) in the 2017 fish using a Miltex biopsy cup. Due to significant processing time, sample collection was reduced in 2018 with six biopsies (n =3 per lobe) collected (anterior, middle, posterior). Each biopsy was placed and stored in a labelled vial with 10% phosphate buffered formalin and processed histologically.

Gonads were classified as either homogeneous or non-homogenous. Gonads were considered non-homogeneous if biopsies from the same gonad contained different stages of maturity that did not develop progressively. For example, if the testicular cysts contained Stages 2 (spermatogonia) and 4 (predominately spermatocytes and spermatids), the gonadal development was not progressive, but if the cysts contained Stages 2 (spermatogonia) and 3 (spermatogonia and spermatocytes), development was progressive. Gonads that contained portions that were well in advance of the primary stage of maturity (i.e., primarily spermatogonia (Stage 2) with a few cysts containing spermatids (Stage 4) or spermatozoa (Stage 5)) were non-homogeneous, and cysts containing the advanced stage of gametogenesis were considered precocious.

Statistical Analysis

Gonadal biopsy accuracy was determined based on the presence of germ cells within the biopsy. Percent accuracy of collecting a gonadal biopsy containing germ cells was determined as the number of biopsies collected which contained germ cells divided by the total number of biopsies collected.

Accuracy of ultrasound and endoscopy was determined by comparing the assigned sex from each tool to the true sex of the fish determined histologically. Percent accuracy of correctly assigning sex to each fish using ultrasound and endoscopy was determined as the number of fish assigned to a sex (female or male) using each tool divided by the true number of fish (of each sex) determined histologically.

Quadratic discriminant function analysis (DFA) was used to determine the accuracy in assigning sex to the hatchery-origin population of white sturgeon using plasma T and biological data (FL and body weight) and in assigning sex and stage of maturity to the wild population of white sturgeon using plasma T and E2, FL, and body weight. Plasma E2 concentrations were not used in classification of the hatchery-origin population as the steroid was not detected in any hatchery-origin fish. To attain multivariate normality, the steroid data were $\log_{10}$ transformed. Stepwise DFA was conducted using $\log_{10}$ transformed T and E2 (with wild fish only) concentrations, FL, and body weight to determine the best indicators of sex. The accepted significance level for all tests was $\alpha = 0.05$. The error rate associated with assigning sex using the chosen discriminate functions was accomplished through cross validation (see Khattree and Naik 2000). Briefly, one observation was eliminated from the dataset, and a discriminant rule was constructed from the remaining data. The rule was used to classify the observation that was left out. This procedure was repeated for every observation, the numbers of misclassified observations for each group were counted, and the individual and overall error rates were calculated. All DFA analyses were conducted using Statistical Analysis Systems for Windows, release 9.4M6 (SAS Institute, Cary, North Carolina).

Statistical tests were used to determine if differences existed in the size of the fish assessed and blood plasma sex steroid concentrations. All data used for statistical analyses were visually assessed for normality using histograms, side-by-side boxplots, and diagnostic plots which assessed the residuals. Fork length and body weight were normally distributed, and one extreme outlier was identified and removed prior to statistical analyses. One-way ANOVA was used to determine if males and females differed by size (FL and body weight). Plasma T and E2 concentrations collected in 2017 and 2018 were combined, and these data were not normally distributed. To account for this, plasma T was transformed (log_{10} scale). Log_{10} transformation did not normalize the plasma E2 data, therefore non-parametric statistics were used. A paired t-test was used to determine if mean T concentrations differed by sex within the hatchery-origin population. A Kruskal-Wallis test using the package dunn.test (Dinno 2017) was used to determine if E2 concentrations differed by sex within the hatchery-origin population. When assessing the wild population, a one-way ANOVA and a Tukey multiple comparisons test were used to determine if T differed among stages of maturity within each sex. A Kruskal-Wallis test with a Bonferroni correction was conducted using the package dunn.test (Dinno 2017) to assess if E2 differed among stages of maturity within each sex. The single atretic wild female (Stage 8) was not included in the statistical analyses of steroids in the wild population. All data are presented as means with 95% confidence intervals unless otherwise specified. The accepted significance level was $\alpha = 0.05$ unless adjusted by the Bonferroni procedure. All statistical tests, with the exception of the DFA as described above, were completed using R software (R Core Team 2019; version 3.6.1).

Results

Reproductive Structure

Reproductive structure was described in 332 hatchery-origin fish with germ cells. A total of 174 female and 158 male hatchery-origin fish were identified in 2017 and 2018. Three fish were classified as intersex with both female and male germ cells (Figure 2.2), and 12 fish were unknown. All hatchery-origin fish were pre-vitellogenic females (Stages 1 and 2) or pre-meiotic males (Stage 2) and have yet to reach puberty.

Fish Size

Mean size of hatchery-origin males (n =158) was slightly larger compared to the hatchery-origin females (n =174) (Table 2.2). There was no evidence to support a difference in size between female and male hatchery-origin white sturgeon in fork length ($F_{(1, 330)} = 2.02$, $P = 0.157$) and body weight ($F_{(1, 330)} = 1.76$, $P = 0.186$). Within the wild population, there was evidence for a difference in mean size between females (n = 75) and males (n = 25) in fork length ($F_{(1, 74)} = 5.71$, $P = 0.019$) and body weight ($F_{(1, 74)} = 12.94$, $P = < 0.001$), with females being slightly longer and considerably heavier (Table 2.2).

Gonadal Biopsy

In order to use histological analysis of gonadal tissue as a true measure of sex and stage of maturity, gonadal biopsies must contain germ cells. We assessed the accuracy in assigning sex and stage of maturity based on a single gonadal biopsy. Of the 347 gonadal biopsies collected from the hatchery-origin population, only 335 contained germ cells

resulting in a gonadal biopsy accuracy of 97% (94 – 98%; 95% CI). The biopsies without germ cells (n=12) were collected from both females and males based on visual assignment of sex using endoscopy, yet the gonadal biopsies contained adipose tissue and no germ cells. Within the wild population, 80 gonadal biopsies were collected, and 75 of the tissue samples contained germ cells resulting in 94% accuracy (86 – 98%; 95% CI). Similar to the hatchery-origin fish, the biopsies that did not contain germ cells were collected from both females and males (assigned sex using endoscopy) and contained adipose tissue and no germs cells. Fish without germ cells in the gonadal biopsy were not used for further analyses.

Gonadal Homogeneity

Of the 36 hatchery-origin fish assessed, all females (n=4) had homogenous gonads (Table 2.3). Non-homogeneous testicular development was found in 38% (21 – 56%; 95% CI) of males (n=12 out of 32 total, Table 2.3; Figure 2.3). All males from the LCR had homogeneous testicular development (Figure 2.4). Precocious cysts were found in 12 out of the 27 hatchery-origin males collected from Lake Roosevelt, WA. Of the 12 males with gonads that had non-homogeneous testicular development, 85% (78 – 94%; 95 % CI) of the biopsies (n=33 of the 39 biopsies) assessed were found to contain precocious cysts. The precocious cysts were predominately found at or close to the periphery of the gonadal tissue (i.e., outer edges).

Endoscopy

The accuracy in using endoscopy to assign sex was 98% (95 – 100%; 95% CI) in the hatchery-origin population (n=131) and 100% in the wild population (n=7) (Table 2.4). Distinct morphological characteristics between female and male gonads were successfully differentiated using endoscopy. Ovaries with small translucent oocytes and ovigerous folds were apparent in non-reproductive females (Figure 2.5), and larger, vitellogenic ovarian follicles were apparent in reproductive females (Figure 2.6). Testicular tissue appeared smooth, white in color, and often vascularized (Figure 2.7).

Plasma Sex Steroids

Blood plasma sex steroids were analyzed by radioimmunoassay in 331 hatchery-origin and 74 wild white sturgeon with histologically confirmed sex and stage of maturity. Plasma T concentrations were different between hatchery-origin females and males (T = -8.60, df = 329, P = <0.001), with T concentrations higher in males compared to females (Table 2.5). Plasma E2 concentrations were not different between hatchery-origin females and males ($x_2(1) = 0.37$, P = 0.54; Table 2.5).

Plasma T concentrations differed by stage of maturity in wild white sturgeon females ($F_{(2, 45)} = 29.69$, P = <0.001) and males ($F_{(2, 22)} = 22.68$, P = <0.001; Table 2.6). Plasma T was different among pre-meiotic males (Stage 2) compared to meiotic (Stage 4) and mature (Stage 5) wild males (Table 2.6). Within females, T concentrations were different among pre-vitellogenic (Stage 2) females, early to mid-vitellogenic females (Stage 3), and late vitellogenic females (Stage 4; Table 2.6). Evidence of a difference

was found in the plasma E2 concentrations in wild reproductively active females (early to mid-vitellogenic, Stage 3, and late vitellogenic, Stage 4) as compared to pre-vitellogenic females (Stage 2) ($x_2(2) = 27.04$, $P = 0.001$; Table 2.6). All E2 concentrations were non-detectable in wild males.

Plasma T was the best predictor of sex in the hatchery-origin population (Table 2.7). The use of plasma T resulted in the correct assignment of sex in 78% of the females and 60% of the males (Table 2.8). The probability of correctly classifying females and males by chance alone was 50% (two classes of sex; females and males). Overall, 69% of hatchery-origin sturgeon were correctly classified by sex. The cross-validation model showed an error rate of 22% in classifying females and 40% in classifying males using plasma T. All correctly classified hatchery-origin females (n =135) had a distinguishing T concentration of ≤ 0.66 ng/mL, and all correctly classified hatchery-origin males (n=94) had a distinguishing T concentration of ≥ 0.67 ng/mL.

When classifying sex and stage of maturity in the wild population, plasma T, plasma E2, fork length, and body weight resulted in the most accurate prediction of sex and stage of maturity (Table 2.7). The use of these four variables resulted in the correct assignment of sex and stage of maturity in 65% of pre-vitellogenic (Stage 2) females, 100% of early to mid-vitellogenic (Stage 3) females, 100% of late vitellogenic (Stage 4) females, 90% of pre-meiotic (Stage 2) males, 100% of meiotic (Stage 4) males, and 100% of mature (Stage 5) males (Table 2.9). Due to the small sample size (n=1) of atretic females (Stage 8), this stage could not be evaluated in the discriminate function analysis. The probability of correctly classifying females and males by chance alone was

17% (six classes of sex and stage of maturity). Overall, 93% of wild sturgeon were correctly classified by sex and stage of maturity using plasma sex steroids and morphometrics. The cross-validation showed an error rate of 35% in classifying pre-vitellogenic females (Stage 2), 0% in classifying early to mid-vitellogenic females (Stage 3), 0% in classifying late vitellogenic females (Stage 4), 10% in classifying pre-meiotic males (Stage 2), 0% in classifying meiotic males (Stage 4), and 0% in classifying mature males (Stage 5) in classifying sex and stage of maturity using plasma T, plasma E2, fork length, and body weight.

Ultrasound

The overall accuracy in using ultrasound to assign sex was 57% (49 – 65%; 95% CI) in the hatchery-origin population (n=149) and 70% (56 – 81%; 95% CI) in the wild population (n=56). In both populations, accuracy in assigning sex was higher in females compared to males (Table 2.10). No clear morphological or echogenic differences in gonadal tissue were found using the ultrasound, with the exception of reproductively active wild females with vitellogenic ovarian follicles (Stages 3 and 4).

Discussion

Hatchery-origin white sturgeon sampled in this study have yet to reach puberty in the lower Columbia River, Canada. However, the study was successful in assigning sex to hatchery-origin white sturgeon in a variety of age classes within the population using multiple tools. In the wild, white sturgeon have been documented to reach sexual maturity at the ages of 12-25 years in males and 15-35 years in females (Billard and

Lecointre 2001, CBWSPF 2013) and sizes of 100-150 cm FL in males and 120-180 cm FL in females, (Welch and Beamesderfer 1993, DeVore et al. 1995, Billard and Lecointre 2001, CBWSPF 2013, Haxton et al. 2016). The hatchery-origin fish assessed in this study are of similar ages to those that have reached puberty in other reaches of the Columbia River but are of smaller size, with a mean fork length of 101 cm (99–103 cm; 95% CI) in males and 99 cm (97–100 cm; 95% CI) in females. While sample sizes in this study were high (n=332) compared to other work (e.g., Divers et al. 2009, Masoudifard et al. 2011, Matsche et al. 2011, Munhofen et al. 2014), they are small relative to the number of hatchery-origin fish at large in the LCR (5,500; BC Hydro 2018). Further, it should be noted that this study was balanced among age-classes within the hatchery-origin population thus further limiting the sample size of older and larger individuals included in the study. While a balanced sampling design was required given uncertainties in the reproductive structure of the population, we cannot conclude that there are not individuals within the hatchery-origin population which have initiated puberty.

Standardized monitoring programs that track changes in the reproductive structure of hatchery-origin sturgeon have not been developed for many populations. Further, a direct comparison of the tools (i.e., gonadal biopsy analyzed histologically, endoscopy, plasma sex steroids, and ultrasound) that can be used to assign sex in hatchery-origin sturgeon has not yet been completed. The results from this study provide a foundation for the development of monitoring programs to assign sex and track changes in the reproductive structure of the population.

Histological analysis of gonadal tissue is considered one of the most accurate methods to determine sex and stage of maturity in fishes (Blazer 2002) and was used as the true measure of sex and stage of maturity in this study, similar to other sturgeon studies (e.g., Van Eenennaam and Doroshov 1998, Webb et al. 2002, Chen et al. 2006, Webb and Erickson 2007, McGuire et al. 2019). The accuracy results in this study were similar to other studies where histology was used to verify sex and stage of maturity (e.g., Divers et al. 2009, Matsche et al. 2011, Munhofen et al. 2014). Histological analysis of the gonadal tissue was used to assign sex to pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon *Scaphirhynchus platorynchus* with 86% accuracy, and 6% of the tissue collected did not contain germ cells (Divers et al. 2009). Histology was found to be 91% accurate in assigning sex to juvenile Siberian sturgeon *Acipenser baerii* with 9% of the tissue collected containing undifferentiated tissue or being classified as intersex and not included in the analysis (Munhofen et al. 2014). Histology was used to correctly assign sex and developmental stage to prepubertal and post-pubertal Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* and shovelnose sturgeon with 100% accuracy (Matsche et al. 2011). Histological analysis of a gonadal biopsy is an accurate method to determine sex and stage of maturity with 100% accuracy when germ cells are present, yet the success is based on the collection of gonadal tissue that contains germ cells.

To determine if gonadal development occurred homogeneously, we assessed the stage of gametogenesis across the gonadal lobes in individuals. Gonadal development was found to be homogenous in a small number of prepubertal white sturgeon females, however, development was not homogenous in males initiating puberty. Precocious cysts

that contained germ cells in a highly advanced stage of gonadal development compared to the predominant stage of gonadal development within the tissue were found in 12 of the 32 males assessed. All males with precocious cysts were sampled from the Lake Roosevelt hatchery-origin population. Males in this population have reached puberty (Webb et al. 2016), however, despite the presence of precocious cysts the males were classified as prepubertal based on the predominant stage of gonadal development. Precocious cysts with a highly advanced stage of gonadal development have not been described in the literature for any sturgeon or teleost species. At the initiation of puberty in male white sturgeon, it appears that the steroidogenic capability of few testicular cysts is up-regulated as a result of changes in the hypothalamus-pituitary-gonadal axis culminating in maturation of the germ cells (i.e., spermatozoa) within those cysts. It is unclear as to how many cysts are involved and over what time scale precocious cysts may be found prior to the transition of mitosis to meiosis across all testicular cysts. Future histological studies conducted on sturgeons should specify that stage of maturity was based on the predominant stage of gonadal development not the most advanced stage of gonadal development identified. The identification of precocious cysts, while physiologically interesting for identifying early onset of puberty, will not change the assignment of sex or predominant stage of maturity of an individual.

Endoscopy, specifically the use of an otoscope for visual examination of the gonad, is an invasive procedure as it requires insertion of the specula into a small abdominal incision. This tool was found to be the most effective in assigning sex in both hatchery-origin and wild white sturgeon in the LCR with overall accuracy rates of 98%

and 100%, respectively. These results were similar to other studies where endoscopy was used to assign sex in sturgeons (sex confirmed by histological analysis of gonadal tissue; Wildhaber et al. 2005, Bryan 2007, Hurvitz et al. 2007, Munhofen et al. 2014). Male cultured Russian sturgeon *Acipenser gueldenstaedtii* (3.5 years of age) were assessed using endoscopy with 100% accuracy (Hurvitz et al. 2007). Sex was assigned to prepubertal Siberian sturgeon with 97% accuracy using endoscopy (Munhofen et al. 2014). Endoscopy has also been used to assign sex to adult shovelnose sturgeon (> 500 mm total length) with 93% accuracy in males and 92% accuracy in females (Wildhaber et al. 2005). In order to successfully use endoscopy as a tool to assign sex, one must obtain an understanding of the anatomical and morphological features of gonadal tissue in sturgeons. Adipocytes are incorporated directly in gonadal tissue in sturgeons which confounds identification of germ cells (Treanor et al. 2018). Therefore, correct differentiation between adipose tissue and germ cells is important.

The pattern of sex steroid production in sturgeons allows for discrimination of sex and stage of maturity less invasively (Webb and Doroshov 2011). When using blood plasma sex steroids, plasma T was found to be the most reliable predictor of sex within the hatchery-origin population using DFA but with low accuracy (69% overall accuracy; 78% accuracy in females and 60% accuracy in males). Other studies have also found low accuracy in assigning sex to non-reproductive sturgeon since sex steroid concentrations remain low prior to the onset of maturation in both females and males (Webb and Doroshov 2011). There was a high prevalence of prepubertal males with low T concentrations in this study which were misclassified as prepubertal females.

Testosterone concentrations have been found to increase in cultured white sturgeon as early as 18 months of age, and by 21 months of age, all but one male (n=60) had T concentrations of > 2 ng/mL, while female T concentrations remained below 2 ng/mL (Feist et al. 2004). The white sturgeon assessed in Feist et al. (2004) were much younger compared to the hatchery-origin population, but by 18 months of age the cultured white sturgeon had already exceeded the mean body weight of the hatchery-origin fish assessed in this study. When examining individual T concentrations in the hatchery-origin population, the threshold concentration for assigning sex with 100% accuracy in both females and males was 0.67 ng/mL. The use of this concentration to differentiate between sexes should be further examined.

Sex steroids are primarily secreted from the gonads in sturgeons (Schulz et al. 2010, Webb and Doroshov 2011). Therefore, it is possible that fish with smaller body size, and therefore smaller gonads, or fish with higher gonadal fat (often seen in prepubertal sturgeons) may produce less circulating sex steroids. Further work is needed to clearly define the relationship between sex steroid production, body size, and gonadal fat content in sturgeons which may result in changes in the differentiating concentration of plasma T as the fish increase in size and age.

Within the wild white sturgeon population in the LCR, the use of plasma sex steroids and morphometrics (FL and body weight) to assign both sex and stage of maturity had higher accuracy (93% overall accuracy) as compared to the prepubertal fish. However, unlike other studies with sturgeons (e.g., Webb et al 2002, Feist et al. 2004), plasma T was lower in the pre-meiotic males (Stage 2) as compared to the pre-

vitellogenic females (Stage 2). The incorporation of the morphometrics may be driving the increase in accuracy as seen in other sturgeon populations (see Malekzadeh Viayeh et al. 2006). Although the sample size was low, the plasma sex steroid concentrations in the wild adult white sturgeon in the LCR do provide valuable information about steroid concentrations that may be the basis for differentiating among classes of sex and stage of maturity in the post-pubertal population.

Ultrasonography, the least invasive tool, has been the fastest growing technique for the assignment of sex and stage of maturity in sturgeons (see Webb et al. 2018). However, the accuracy of this tool has mixed results. Within this study, ultrasound had the lowest accuracy of all tools tested (overall accuracy rate of 57%; 74% accuracy in females and 39% accuracy in males). Potential explanations for these low accuracy rates may be the limited number of fish that were sampled using the ultrasound in this study (n=149 hatchery-origin and n= 56 wild fish), with an even smaller number of older (i.e., larger) fish assessed. The smaller size of the hatchery-origin fish assessed (mean fork length of 99 cm and mean body weight of 7 kg) indicates that the gonadal tissue within these fish may be less developed, small in size, or both, which could have also effected the accuracy of using the ultrasound to assign sex to the hatchery-origin population. Other studies have also found it difficult to differentiate between less developed gonadal tissue, which lacks distinguishing morphological features (sex confirmed at necropsy; Moghim et al. 2002, sex confirmed using histological analysis of gonadal tissue; Wildhaber et al. 2007, Munhofen et al. 2014). Despite this challenge, some studies have been successful in using ultrasound to assign sex to prepubertal cultured sturgeon

populations (Masoudifard et al. 2011, Munhofen et al. 2014). When using ultrasound to assign sex to assign sex to three-year-old, prepubertal cultured beluga sturgeon *Huso huso* accuracy rates of 97% in females and 98% in males were reported (Masoudifard et al. 2011; sex confirmed by visual inspection of the gonad). Ultrasound was used to assign sex to three- and four-year-old cultured Siberian sturgeon with accuracy rates of 89% and 84%, respectively (Munhofen et al. 2014; sex confirmed using histological analysis of gonadal tissue). Accuracy rates of 91% and 76% were reported by Du et al. (2017) for post-pubertal, non-reproductive (Stage 2) cultured female and male Chinese sturgeon *Acipenser sinensis*, respectively (sex confirmed using histological analysis of gonadal tissue). Examination of the incorrect assignment of sex in the hatchery-origin white sturgeon within a sampling season (i.e., spring 2017), between the seasons (i.e., spring 2017 compared to fall 2017), and between years (i.e., spring 2017 compared to spring 2018) did not reveal a learning curve with increased accuracy over time.

Higher accuracy rates when using ultrasound have been reported when assigning sex to sturgeons with developed gonads due to identifiable features such as decreased adipose tissue and vitellogenic ovarian follicles in female sturgeons (Moghim et al. 2002, Colombo et al. 2004, Chiotti et al. 2016, Du et al. 2017). Within this study, overall accuracy was higher in the post-pubertal wild fish (overall accuracy 70%, 82% accuracy in females and 44% accuracy in males) compared to the hatchery-origin fish and was higher in females compared to males in both populations. Sex was assigned to shovelnose sturgeon with 86% accuracy using ultrasound but when assessing less developed females (Stages 1 and 2) or spent females (Stage 6) accuracy decreased to 75%

and 40%, respectively (Colombo et al. 2004; sex confirmed by visual examination of gonads). Similarly, accuracy rates of 89% and 97% were reported when assessing developed male lake sturgeon *Acipenser fulvescens* by two sonographers and 100% accuracy was reported when assessing reproductive females by both sonographers (Chiotti et al. 2016). Their accuracy decreased when assessing less developed females ("early yellow oocyte" and "late yellow oocyte"; Chiotti et al. 2016) resulting in 33% and 67% accuracy, respectively, by sonographers. Sex was confirmed by visual inspection of the gonad in a subsample of the population assessed (Chiotti et al. 2016). Further training and assessment of more fish, especially larger individuals, is required prior to continued use of the ultrasound to assign sex to the hatchery-origin or wild white sturgeon in the lower Columbia River.

A total of three intersex fish (0.9%) were identified in the hatchery-origin population. Similar to other studies with intersex sturgeon, the fish were predominately males with the presence of oocytes embedded in testicular tissue (Chapman et al. 1996, Van Eenennaam and Doroshov 1998, Colombo et al. 2007, Rzepkowska et al. 2014). Intersex fish are common at low frequencies in both wild and cultured populations of sturgeons (Van Eenennaam and Doroshov 1998, Matsche et al. 2013, Rzepkowska et al. 2014). The low prevalence of intersex fish in this study falls within the normal range found within other sturgeon populations, such as the white sturgeon in the San Francisco Bay (<0.01%; Chapman et al. 1996), Atlantic sturgeon in the Hudson River (1%; Van Eenennaam and Doroshov 1998), and shovelnose sturgeon in the Mississippi River (3%; Carlson et al. 1985 and 2%; Colombo et at. 2007). Prevalence of intersex sturgeon can

be higher in rivers with environmental contaminants, such as the Delaware River, where 12% of shortnose sturgeon *Acipenser brevirostrum* were found to be intersex (Matsche et al. 2013).

Throughout this chapter, we discussed accuracies of several tools for assigning sex and stage of maturity with the emphasis on highlighting the tools with the highest accuracy. However, the level of accuracy required for assigning sex may differ depending on the objective of the monitoring program. For example, programs may be interested in assessing reproductive metrics where some level of error is acceptable, such as estimating the proportion of fish capable of spawning in a given year. In this case, more tools may be useful depending on the level of experience of the sampling crew using the tool(s). Conversely, the approach may differ for programs requiring confirmation on the sex of the individual (i.e., tagging studies). The tools applicable in this scenario may be more limited and require specific experience. We demonstrated lower accuracy for the less invasive tools (i.e., ultrasound and blood plasma sex steroids) evaluated in this study, however these tools have shown higher accuracy in populations which are reproductively active (e.g., Moghim et al. 2002, Wildhaber et al. 2007, Webb et al. 2018, this study). These tools should be reassessed as the hatchery-origin population in the LCR reaches puberty and cycles through gametogenesis.

The hatchery-origin white sturgeon in the LCR Canada assessed in this study had not yet reached puberty as of 2018, however based on the sampling design, we cannot conclude that there are not individuals in the population at large which have initiated puberty. The fish are of similar age, but smaller size compared to other white sturgeon

populations that have reached first maturity (Welch and Beamesderfer 1993, DeVore et al. 1995, Doroshov et al. 1997, Billard and Lecointre 2001, CBWSPF 2013, Haxton et al. 2016). Long-term monitoring will be important to determine the reproductive dynamics of the hatchery-origin population over time (e.g., age and size at puberty, sex ratio), and specifically when these fish will begin contributing to wild spawning events. We suggest initially sampling a suite of age and size classes using endoscopy or histological analysis of gonadal tissue to gain a high-level understanding of the current reproductive structure within a population. Once this information is known, a standardized long-term monitoring program can be developed to track changes in the reproductive structure over time.

Table 2.1. Stages of female and male gonadal development used to stage hatchery-origin and wild white sturgeon in the lower Columbia River, Canada (from Webb et al. 2018).

Developmental Stage		Description
Differentiation	1	Ovarian groove starts to develop into the small and very thin ovigerous "ribbon". Histology reveals clusters of oogonia and potentially a few very small oocytes just beginning the endogenous growth phase.
Pre-vitellogenic	2	Obvious ovigerous folds with small translucent oocytes. Histology reveals perinucleolar oocytes in the endogenous growth phase. The follicular epithelium (granulosa) in the larger oocytes begins mitotic proliferation, and the outer follicular layer (theca) has some vascularization (~ 0.2-0.4 mm).
Early to mid vitellogenic	3	Ovigerous folds contain small white oocytes (0.5-1.0 mm) to larger oocytes that are white, cream, or yellowish in color (1.0-1.5 mm). The distinguishing histological features are the differentiation of the zona radiata and the presence of yolk platelets in the cytoplasm. The granulosa cells increase in thickness and become cuboidal. As ovarian follicle size increases, the density and size of yolk platelets also increases. The nucleus (germinal vesicle) is centrally located.
Late vitellogenic	4	The ovarian follicles darken in color as melanin pigment is deposited under the oolemma. Grey to black ovarian follicles are visible. Ovarian follicle size continues to increase, but maximum size has not been reached (1.5-3.0 mm) The follicular layers (theca, basal lamina, and granulosa) and three layers of the chorion (zona radiata interna, zona radiata externa, and gelatinous coat) are fully differentiated. The nucleus begins to move off-center toward the animal pole.
Post-vitellogenic/Ripe	5	Fully grown ovarian follicles are usually black (but can vary from olive brown, greyish to very light-golden color; >3.0 mm. Cytoarchitectural changes within the oocyte have occurred. The

		nucleus is displaced to the animal pole. The oocyte has a polarized structure with the animal hemisphere containing the bulk of the cytoplasm and small, round yolk platelets and lipid inclusions, while the vegetal hemisphere contains large, oval-shaped yolk platelets and numerous large lipid inclusions.
Oocyte maturation and ovulation	6	Ovarian follicles have undergone the final stages of maturation (i.e., germinal vesicle breakdown) and are ovulated. Eggs are freely flowing from the vent when captured in the wild or from a hormonally induced captive fish.
Post-ovulatory	7	Ovaries contain numerous postovulatory follicles and the next generation of oocytes similar to Stage 2 and sometimes Stage 3. The often reddish appearance of ovarian tissue is a result of vascularization for weeks after spawning.

Developmental Stage		**Description**
Differentiation	1	Testes appear as a thin white thread (≤ 1mm), not discernible by ultrasound. Histological analysis of a testicular biopsy reveals clusters of primary spermatogonia.
Pre-Meiotic	2	Testes appear as a thicker white thread (1-4 mm), not clearly discernible by ultrasound. Histological analysis of testicular tissue reveals spermatogonia undergoing mitosis.
Onset of Meiosis	3	Testes are obvious having a whitish color and turgid texture and ranging in thickness from 0.5 to 2 cm. Each testis "lays" on top of adipose tissue that can add another 1-2 cm to the overall width of the gonad. Testes can be identified by ultrasound and have a distinct white boundary (tunica), while the adipose tissue is dark (hypoechoic). Histological analysis of testicular tissue reveals spermatogonia (≈ 50%) and spermatocytes.

Meiotic	4	The gonad is primarily testicular tissue, with much less adipose tissue as compared to Stage 3. Testes are turgid and white, and testis can be 2-3 cm. Testes can be identified by ultrasound, and areas of each testis start to appear lobular. Histological analysis of testicular tissue reveals the majority of cysts contain spermatocytes and spermatids, with less than 25% of cysts containing spermatogonia.
Mature	5	At the completion of spermatogenesis, the ripe male testis is large (3-8 cm, if sorted in the fall-winter, for spring spermiation) and becomes milky-white, with usually no adipose tissue. Ultrasound reveals a bright white fine-grained homogeneous testis that fills the majority of the screen. Histological analysis of testicular tissue reveals cysts and ducts that contain almost all spermatozoa, but the animal is not actively spermiating.
Spermiation	6	Spermiating males will release thick, white milt to very thin, “cloudy” milt (either at the very start or end of spermiation). The ultrasound sonogram is not as bright white as Stage 5, with no obvious boundaries (tunica). Histological analysis of testicular tissue reveals spermatozoa in all cysts and ducts.
Post-spermiation	7	Histological analysis of testicular tissue reveals residual spermatozoa in regressed testicular cysts, and clear seminal fluid can sometimes be collected from the vent. This fluid when placed into a clear glass beaker reveals white flocculants of residual clumped non-viable spermatozoa. Sonograms of post-spermiation males have not been well documented.

Table 2.2. Mean size (fork length and body weight; 95% confidence intervals (CI) for female and male hatchery-origin and wild white sturgeon in the lower Columbia River, Canada. No statistical difference in size of hatchery-origin fish was found. Different letters denote statistical differences in size between wild female and male white sturgeon.

Origin	Sex	Fork Length (cm; mean, 95% CI)	Body Weight (kg; mean, 95% CI)
Hatchery	Females (n = 174)	99 (97 – 100)	7 (6.0 – 7.0)
	Males (n = 158)	101 (99 – 103)	7 (6.5 – 7.5)
	All (n = 332)	99 (98 - 101)	7 (6.4 - 7.1)
Wild	Females (n = 49)	185 (177 – 192) a	52 (46.5 - 57.1) a
	Males (n = 25)	171 (164 – 177) b	37 (31.5 – 41.6) b
	All (n = 75)	180 (174 – 185)	47 (42.5 – 50.9)

Table 2.3. Proportion of collected gonadal tissue with precocious cysts (95% CI) from 2016, 2017, and 2018 hatchery-origin white sturgeon from Lake Roosevelt, WA (2016, 2018) and the lower Columbia River, Canada (2017). Precocious cysts were only found in male white sturgeon from Lake Roosevelt. Total sample size (n) is provided.

Sex	Precocious cysts present (%)
Females (n=4)	0
Males (n=32)	38 (21 – 56%)
Total (n=36)	33 (19 – 51%)

Table 2.4. Percent accuracy (95% CI) in assigning sex to the hatchery-origin and wild populations of white sturgeon in the lower Columbia River, Canada using endoscopy. Sample sizes (n) are in parentheses.

Origin	Females	Males	Overall
Hatchery	97 (89 – 100%) (n=65)	100 (95 – 100%) (n=66)	98 (95 – 100%) (n=131)
Wild	100 (48 – 100%) (n=5)	100 (16 – 100%) (n=2)	100 (60 – 100%) (n=7)

Table 2.5. Plasma testosterone (T) and estradiol-17β (E2) concentrations (ng/mL) in hatchery-origin white sturgeon in the lower Columbia River, Canada. Data are mean (± SEM), ranges (min. and max.), and sample size (n) in parentheses. Different letters denote statistical differences between females and males within a steroid. Plasma sex steroid concentrations below the minimum quantifiable concentration (MQC) of the radioimmunoassay were assigned the MQC (0.16-0.38 ng/mL for T and 0.10-0.21 ng/mL for E2). Non-detectable plasma sex steroid concentrations were assigned half of the MQC (0.08-0.19 ng/mL for T and 0.05-0.11 ng/mL for E2).

Sex	Stage of Maturity	T (ng/mL)	E2 (ng/mL)
Females	Pre-vitellogenic (n=174)	0.58 ± 0.07 a (0.10 – 9.99)	0.10 ± 0.01 a (0.05 - 0.78)
Males	Pre-meiotic (n=157)	1.51 ± 0.13 b (0.12 - 7.50)	0.10 ± 0.004 a (0.05 - 0.51)

Table 2.6. Plasma testosterone (T) and estradiol-17β (E2) concentrations (ng/mL) in wild white sturgeon in the lower Columbia River, Canada. Data are mean (± SEM), range (min. and max.), and sample size (n) in parentheses. Different letters denote statistical differences among stages of maturity within females or males for T or E2. The single atretic wild female was not included in statistical analyses. Plasma sex steroid concentrations below the minimum quantifiable concentration (MQC) of the radioimmunoassay were assigned the MQC (0.16-0.38 ng/mL for T and 0.10-0.21 ng/mL for E2). Non-detectable plasma sex steroid concentrations were assigned half of the MQC (0.08-0.19 ng/mL for T and 0.05-0.11 ng/mL for E2).

Sex	Stage of Maturity	T (ng/mL)	E2 (ng/mL)
Females	Pre-vitellogenic (n=37)	2.27 ± 0.50 a (0.12 – 12.55)	0.10 ± 0.02 a (0.05 - 0.80)
	Early to mid vitellogenic (n=7)	23.77 ± 12.48 b (3.24 – 97.47)	2.54 ± 0.88 b (0.16 – 5.67)
	Late vitellogenic (n=4)	127.32 ± 25.15 c (77.45 – 195.84)	8.93 ± 1.50 b (7.00 – 13.32)
	Atretic (n=1)	0.37	0.05
Males	Pre-meiotic (n=20)	1.91 ± 0.97 a (0.12 – 19.60)	0.07 ± 0.004 a (0.05 – 0.09)
	Meiotic (n=2)	63.68 ± 7.14 b (56.54 – 70.81)	0.09 ± 0.005 a (0.08 – 0.09)
	Mature (n=3)	79.45 ± 47.08 b (29.15 – 173.53)	0.11 ± 0.03 a (0.080 – 0.16)

Table 2.7. Summary statistics from the stepwise discriminant function analysis predicting sex of hatchery-origin white sturgeon and sex and stage of maturity of wild white sturgeon captured in the lower Columbia River, Canada. The blood plasma indicator used in the analysis was log_{10}-transformed plasma (T) testosterone concentration for determining sex in the hatchery-origin population; and log_{10}-transformed plasma T concentration, log_{10}-transformed plasma estradiol (E2) concentration, fork length, and body weight for determining sex and stage of maturity in the wild population.

Origin	Variable	Partial R^2	F-statistic	Wilks' lambda	Average squared canonical correlation
Hatchery-origin (sex)	T	0.18	73.73	0.82	0.18
Wild fish (sex and stage)	T	0.41	9.15	0.11	0.24
	E2	0.81	57.83	0.19	0.16
	Fork length	0.17	2.60	0.07	0.29
	Body weight	0.20	3.17	0.09	0.27

Table 2.8. Classification summary for determination of sex from the quadratic discriminant function analysis for hatchery-origin white sturgeon in the lower Columbia River, Canada using $\log_{10}$-transformed plasma testosterone (T) concentrations as the predictor. Values in bold are the percentages of fish correctly classified, whereas values not in bold are the percentages of misclassified fish; sample sizes (n) are in parentheses.

	Predicted		
Observed	Female	Male	Total
Hatchery-origin fish			
Female	**78** (135)	22 (39)	100 (174)
Male	40 (63)	**60** (94)	100 (157)

Table 2.9. Classification summary for determination of sex and stage of maturity (see Table 2.1 for descriptions) from the quadratic discriminant function analysis for wild white sturgeon in the lower Columbia River, Canada using log_{10}-transformed plasma testosterone concentrations, log_{10}-transformed plasma estradiol concentrations, fork length, and body weight as the predictors. Values in bold are the percentages of fish correctly classified, whereas values not in bold are the percentages of misclassified fish; sample sizes (n) are in parentheses.

	Predicted						
Observed	Pre-vitellogenic female	Early to mid vitellogenic female	Late vitellogenic female	Pre-meiotic male	Meiotic male	Mature male	Total
Pre-vitellogenic female	**65** (24)	3 (1)	0 (0)	32 (12)	0 (0)	0 (0)	100 (37)
Early to mid vitellogenic female	0 (0)	**100** (7)	0 (0)	0 (0)	0 (0)	0 (0)	100 (7)
Late vitellogenic female	0 (0)	0 (0)	**100** (4)	0 (0)	0 (0)	0 (0)	100 (4)
Pre-meiotic male	10 (2)	0 (0)	0 (0)	**90** (18)	0 (0)	0 (0)	100 (20)

Table 2.9. Continued.

Observed	Predicted						Total
	Pre-vitellogenic female	Early to mid vitellogenic female	Late vitellogenic female	Pre-meiotic male	Meiotic male	Mature male	
Meiotic male	0 (0)	0 (0)	0 (0)	0 (0)	**100** (2)	0 (0)	100 (2)
Mature male	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**100** (3)	100 (3)

Table 2.10. Percent accuracy (95% CI) in assigning sex to the hatchery-origin and wild populations of white sturgeon in the lower Columbia River, Canada using ultrasound. Sample sizes (n) are in parentheses.

Origin	Females	Males	Overall
Hatchery	74 (63 – 83%) (n=77)	39 (28 – 51%) (n=72)	57 (49 – 65%) (n=149)
Wild	82 (65 – 92%) (n=38)	44 (22 – 69%) (n=18)	70 (56 – 81%) (n=56)

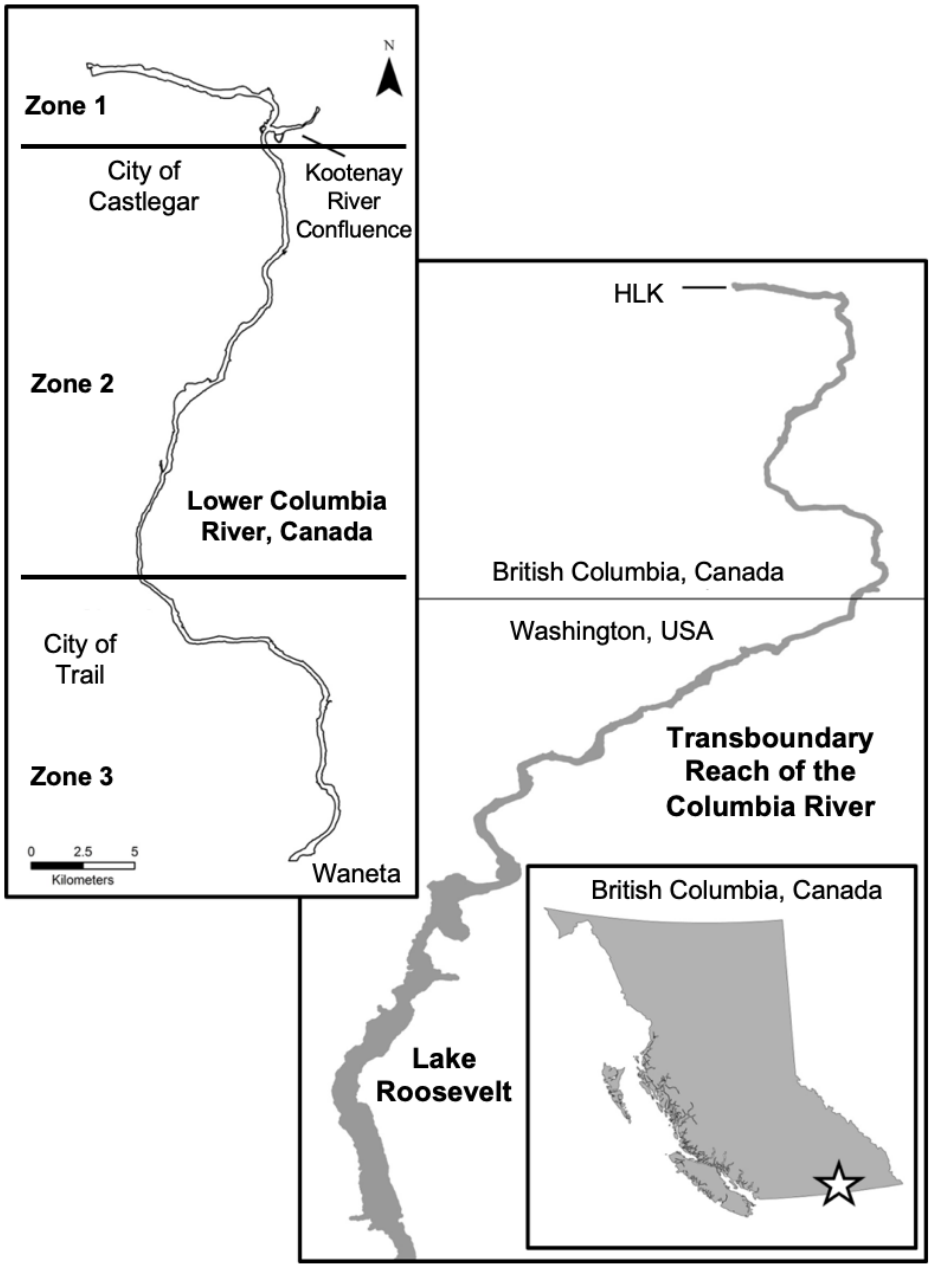

Figure 2.1. Map depicting the study site that was 57-km of the Columbia River between Hugh L. Keenleyside Dam (HLK) and Waneta, WA on the lower Columbia River (LCR), Canada. The black horizontal lines represent the different zones within the LCR. Zone 1 was 11-rkm and Zones 2 and 3 were 23-rkm each. The transboundary reach of the Columbia River, which includes the LCR, Canada and Lake Roosevelt in Washington, is shown on the right side of the figure. Figure modified from BC Hydro (2016).

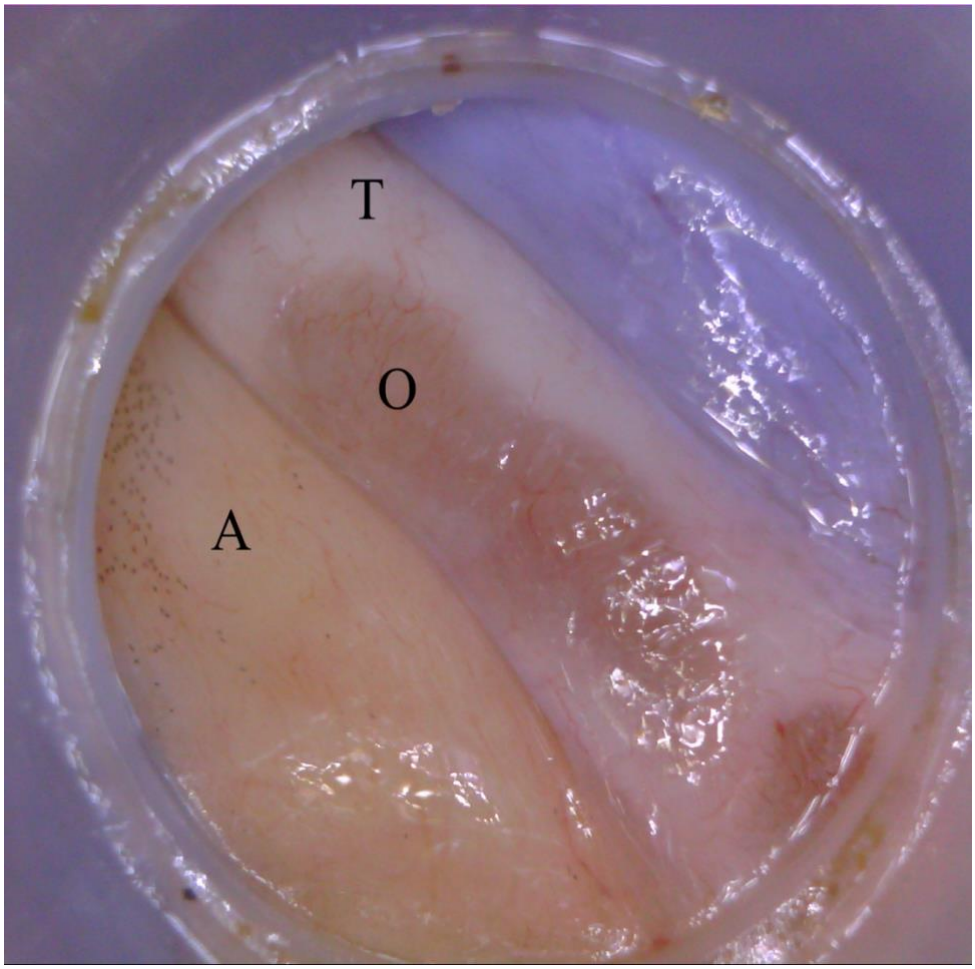

Figure 2.2. Endoscopic image of primary oocytes (O), testicular tissue (T), and adipose tissue (A) in an intersex white sturgeon in the lower Columbia River, Canada.

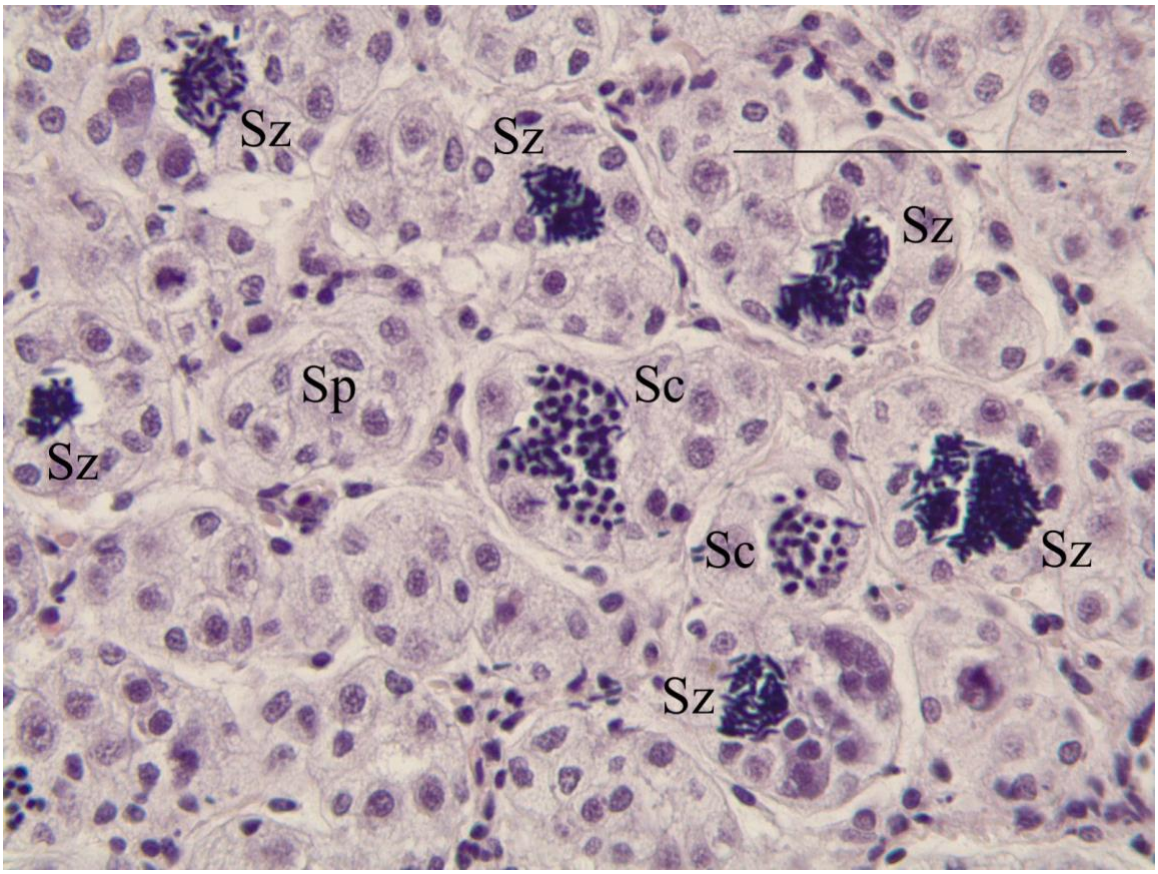

Figure 2.3. Microscopic image (40x; H&E stain) of non-homogeneous testicular development with precocious cysts in a Stage 2 prepubertal hatchery-origin male white sturgeon in Lake Roosevelt, Washington. The majority of testicular cysts contain spermatogonia (Sp; Stage 2). Spermatocytes (Sc; Stage 3) are the next stage of development following natural progression of the gonad. Precocious cysts containing spermatozoa (Sz; Stage 5) are present. Scale bar represents 100 μm.

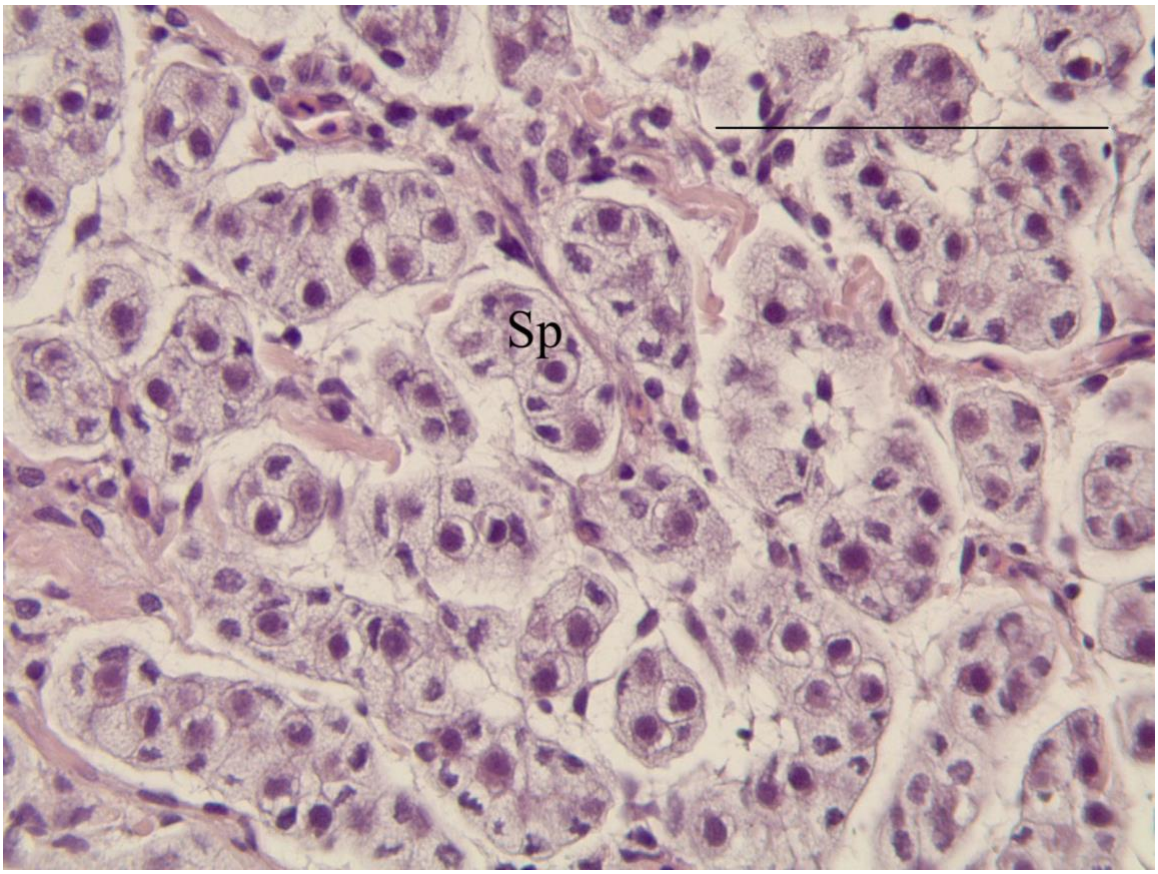

Figure 2.4. Microscopic image (40x; H&E stain) of homogeneous testicular development in a Stage 2 prepubertal hatchery-origin male white sturgeon in the lower Columbia River, Canada. The testicular cysts contain spermatogonia (Sp; Stage 2). Scale bar represents 100 μm.

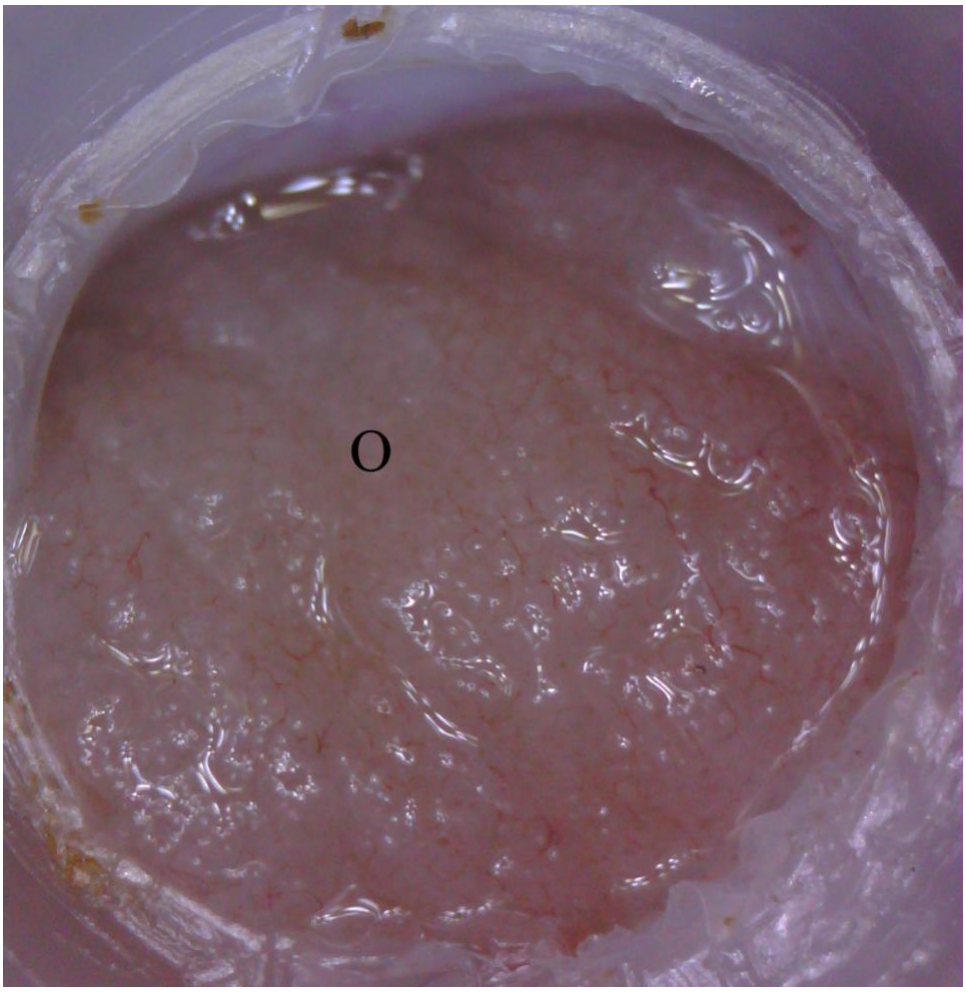

Figure 2.5. Endoscopic image of primary oocytes (O) and ovigerous folds in a non-reproductive female white sturgeon in the lower Columbia River, Canada.

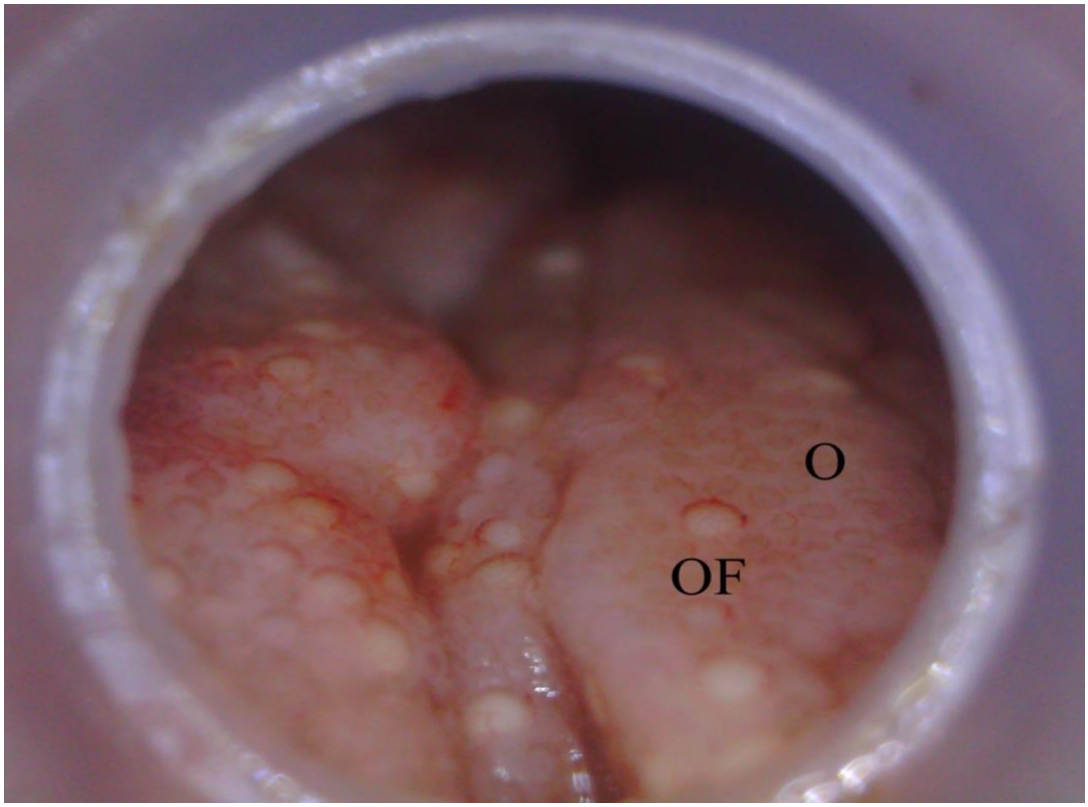

Figure 2.6. Endoscopic image of primary oocytes (O) and early vitellogenic ovarian follicles (OF) in a reproductive female white sturgeon in the lower Columbia River, Canada.

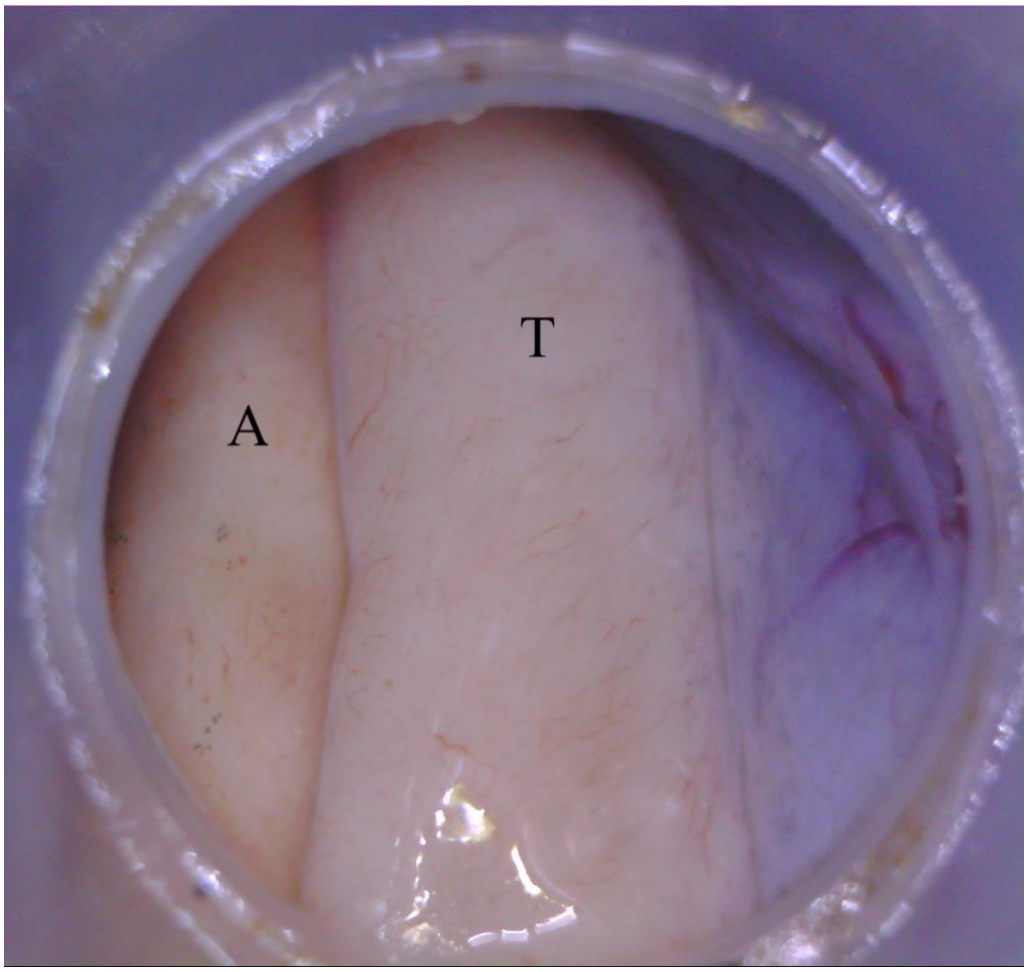

Figure 2.7. Endoscopic image of testicular tissue (T) and adipose tissue (A) in a male white sturgeon in the lower Columbia River, Canada.

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CHAPTER THREE

LENGTH, WEIGHT, ABSOLUTE GROWTH RATE, AND RELATIVE CONDITION OF PREPUBERTAL HATCHERY-ORIGIN WHITE STURGEON IN THE LOWER COLUMBIA RIVER, BRITISH COLUMBIA, CANADA

Introduction

As the number of imperiled fish species continues to increase, conservation aquaculture is often implemented to sustain or recover threatened and endangered populations of fishes worldwide (e.g., Clark et al. 1989, Tyus and Saunders 2001, Ireland 2002, USFWS 2007, Worm 2009, BC Hydro 2018). Crucial to the success of these programs are monitoring efforts providing biological knowledge about hatchery populations after release into the wild. Collection of biological data can inform population dynamics, vital rates, and reproductive potential which are needed for adaptively managing conservation aquaculture efforts over the length of time required to evaluate success for sturgeons (Ireland 2002, USFWS 2007, Worm 2009, Lowerre-Barbieri et al. 2011, Shuman et al. 2011, BC Hydro 2018). Assessing population dynamics, such as fish growth and condition, will provide valuable information for recovery programs interested in assessing the effects of management actions on threatened or endangered populations.

Assessing fish growth over time can provide insight into the utilization of resources, population dynamics, and effect of management actions (Isely and Grabowski 2007). Multiple factors drive growth in fishes, including but not limited to temperature,

habitat, age, food quality and quantity, and competition (LeBreton et al. 2004, Moyle and Cech 2004). Information regarding growth of fishes can be used to assess environmental factors (i.e., habitat effects) and changes in growth can aid in understanding population structure (Maceina 1992, Blackwell et al. 2000, Moyle and Cech 2004, Neumann et al. 2012). Habitat suitability plays a role in the growth of fishes, and conversely, size, growth, and condition of a population provides information which can be used to assess fish habitat (Hayes et al. 1996, Tupper and Boutilier 1997, Amara et al. 2007). Abiotic and biotic factors that drive growth can be highly variable resulting in variable growth rates within fish populations and making identification of the primary driver(s) of growth for a species challenging.

Assessment of biological metrics such as size, growth, and condition provide insight into the reproductive dynamics of a population. Size (i.e., length and weight) and age are highly correlated in fishes at puberty, with larger fish often reaching puberty at an earlier age compared to smaller individuals within a population (Amano et al. 1997, Huang et al. 1998, Van Eenennaam and Doroshov 1998, Taranger et al. 2010, Ryu et al. 2013, Nater et al. 2018). Knowledge of growth rates are valuable when assessing reproductive potential in a population because growth rates shift in fishes as they approach puberty (e.g., allocation of resources shift from somatic growth to gonadal development; Taranger et al. 2010, Minte-Vera et al. 2016, Nater et al. 2018). Measures of condition in fish are often used to indicate well-being, with the expectation that fish in good condition will likely experience faster growing rates, greater reproductive potential, and higher survival as compared to fish in below-average condition (Pope and Kruse

2007, Neumann et al. 2012). For these reasons, monitoring the size, growth rates, and condition within a population can provide important information in assessing the reproductive status of a population.

White sturgeon *Acipenser transmontanus* in the lower Columbia River, hereafter referred to as LCR, Canada were listed as endangered in 2006 under the Species at Risk Act (SARA). Wild adults in the population continue to spawn; however, age 0 individuals are not surviving to reproduce, and this limited recruitment threatens the persistence of the entire population (Hildebrand and Parsley 2013, BC Hydro 2013, 2015, 2016, 2017, 2018). As a result of recruitment failure, conservation aquaculture was implemented in 2001 in an effort to recover and maintain the population. The conservation aquaculture program has been successful and has reestablished juvenile age classes previously absent from the population. There is some information available regarding growth within the hatchery-origin population however, growth rates for individuals with known sex is limited.

The original goal of this study was to assess the biological and environmental factors that could contribute to the size and age at puberty within the hatchery-origin population of white sturgeon in the LCR. However, no individuals sampled within this study from the hatchery-origin population were found to have reached puberty as of 2018 (see Chapter 2). Therefore, the specific objectives of this study were amended to 1) describe the size, absolute growth rate (AGR), and relative condition factor (K_n) of hatchery-origin white sturgeon in the LCR, 2) determine if these metrics differed by sex, 3) determine if the K_n of the hatchery-origin population differed among sexes, ages, and

zone, and 4) determine if the K_n of the hatchery-origin fish differed in the LCR as compared to the transboundary reach. Since conservation aquaculture is expected to be the main source of population abundance in the LCR white sturgeon, understanding the K_n of the population and how it differs by sex, age, and habitat will be beneficial for long-term monitoring to understand the well-being of the population, as well as individual age classes within the population, and fish in different zones throughout the study reach.

Study Area

White sturgeon were sampled throughout the transboundary reach of the Columbia River, which includes fish in the LCR in Canada and the upper Columbia River in Lake Roosevelt, Washington, USA. The primary focus of this study was the LCR, where fish were captured throughout a 57-km reach between Hugh L. Keenleyside Dam (HLK; river kilometer (rkm) 0.0) and the United States-Canada border (rkm 57) in British Columbia, Canada (Figure 2.1). Sampling areas were divided into three zones, which were delineated by differences in discharge determined by previously conducted stock assessments. Zone 1 had the lowest discharge (BC Hydro 2015, 2016, 2017) observed in the study reach. It began at the HLK dam and included a small section of the Kootenay River downstream of Brilliant Dam and its confluence with the LCR. Zone 2 was the only section of the study reach which did not contain a confluence and was not in close proximity to a dam. Discharge was highest in Zone 3 (BC Hydro 2015, 2016, 2017), which included the confluence of the Pend d'Oreille River and ended at the United

States border at Waneta, Washington. Each zone began at the termination of the previous zone. White sturgeon abundance was highest in Zone 1 (BC Hydro 2013, 2015, 2017, 2018); therefore, in an attempt to equalize the sample sizes of fish captured among zones, the distance (in river kilometers; rkm) was greater in Zones 2 and 3. Zone 1 was 11-rkm(s) and Zones 2 and 3 were each 23-rkm(s).

We compared results of fish collected in our study to fish collected in the transboundary reach which includes Lake Roosevelt, located in northeast Washington. Hatchery-origin fish in the LCR and Lake Roosevelt originated from the same conservation aquaculture program and are therefore similar in age.

Methods

Fish Sampling Design

White sturgeon were monitored in 2017-2018, with sampling events in the spring and fall. Sampling took place before and after spawning, which occurred from mid-June to late July (BC Hydro 2016, 2018). The population of LCR white sturgeon has high site fidelity (BC Hydro 2016), therefore, the sampling effort was spatially balanced throughout the fishable areas within the river, and all habitat types were included. Location of sampling sites were randomly assigned with 24 sites in Zone 1 and 48 sites in each of Zones 2 and 3. Probability of catch was assumed to be equal among all sampling zones. Sampling began at the most downstream location in Zone 3 and progressed upstream until all sites were sampled. During the first sampling season (spring 2017), up

to six hatchery-origin fish per year class (2001-2007) were sampled in each zone. The numbers of sampled fish were reduced in the fall of 2017 in an effort to decrease the number of minor surgical biopsies conducted. For the duration of sampling, four hatchery-origin fish per year class within each zone were sampled.

Fish Collection

Baited set lines were used to capture white sturgeon (BC Hydro 2013, 2015, 2017, 2018). One set line was deployed at each sampling location for a total of 120 set lines (24 in Zone 1 and 48 in both Zones 2 and 3). Set lines were approximately 54 m in length, and 12 circle halibut hooks of four different sizes (14, 16, 18, 20 mm) were attached to the mainline at six m intervals. Catch per unit effort (CPUE) was calculated as the total number of fish captured per set line hour and was calculated among zones each sampling season. All barbs were removed from hooks prior to sampling to reduce hook related injury. Each set line was baited using pickled squid acquired from Gilmore Fish Smokehouse, Dallesport, WA, USA (BC Hydro 2018).

Captured white sturgeon were lifted onto the boat in a stretcher using a wench and davit system. Sturgeon were laid dorsally in the stretcher, and water was pumped over their gills throughout the duration of sampling. All captured fish were scanned for a Passive Integrated Transponder (PIT) tag and assessed for removed scutes to determine age class. If a sturgeon without a PIT tag was caught, a PIT tag was implanted subdermally in the tissue layer parallel to the posterior end of the dorsal fin, and the second lateral scute was removed using a sterilized scalpel. All surgical equipment,

including the PIT tag and tagging syringe were immersed in 100% ethanol and rinsed before and after contact with sturgeon.

Biological Sampling

Fork length (± 1 cm) and body weight (± 1 kg) were collected from hatchery-origin white sturgeon from the 2001-2007 year classes. Only fish that had retained their original PIT tag at release from the hatchery were used to assess AGR (see Absolute Growth Rate section below).

A gonadal biopsy was collected from all fish using an otoscope that was inserted into the body cavity through an abdominal incision. The incision site was approximately 1 to 3 cm off the midline between the third and fourth scutes, anterior to the urogenital pore, and approximately 1 to 2 cm long. Gonadal tissue (1 cm^3) was collected through the otoscope specula using a Miltex biopsy cup and stored in 10% phosphate buffered formalin (Webb and Erickson 2007). The gonadal biopsy was processed histologically to determine the true sex and stage of maturity. Fish were sutured with polydioxanone violet monofilament suture (PDS) and released back into the water. The otoscope specula was cleaned and disinfected between each fish with 100% ethanol and rinsed before and after contact with sturgeon.

Histology

True sex and stage of maturity was determined for white sturgeon using histological analysis of gonadal tissue and assigned by two readers according to Webb et al. 2018 (Table 2.1). Gonadal tissue was processed, embedded in paraffin wax, and

sectioned at 5 µm. Tissue from hatchery-origin fish was stained with hematoxylin and eosin. Slides were examined using a compound scope (Leica DM 2000; 10-100x) (Leica Biosystems Inc., IL, USA). If gonadal tissue did not contain germ cells, the fish was removed from the analyses.

Physical Parameters

During the 2017-2018 study period, water temperature and discharge were collected from several locations throughout the study reach. Thermographs were used to record water temperatures (Vemco Minilogs, ± 0.1°C) every hour at six locations (rkm 0.1, 10.5, 13.4, 26.0, 37.5, and 56.0; BC Hydro 2018). Discharge (cubic meters per second; cms) was collected by BC Hydro at four locations (rkm 0.1, 10.5, 29.0, and 57.0; BC Hydro 2018). Data for discharge were recorded at one-minute intervals and averaged per hour.

Absolute Growth Rate

Absolute growth rate (AGR) was assessed for the hatchery-origin population (2017-2018 data) using data from recaptured individuals. Absolute growth rate was calculated for fork length (cm) and body weight (kg) as:

$$AGR = \frac{s_2 - s_1}{number\ of\ years}$$

where s_1 is size at release from the hatchery, s_2 is size at recapture, and number of years refers to the number of years between release from the hatchery and recapture (Jones 2002, Shuman et al. 2011) – AGR was an annual rate.

Weight-Length Relationship

Weight-length data from this study (2017-2018) were combined with a larger dataset collected in 2013-2018 (fork length and body weight; no sex and stage of maturity data) from hatchery-origin white sturgeon throughout the transboundary reach of the Columbia River. The data was used to develop two weight-length relationships, one for individuals captured in the LCR and one for individuals captured throughout the transboundary reach. Fish captured in 2013-2016 were sampled during an on-going population assessment program and capture and handling were standardized with the protocol described in this study. The combined dataset included data from 8,065 fish captured within the transboundary reach and 2,738 fish captured within the LCR.

The weight-length relationship for the LCR white sturgeon population was determined using linear regression of $\log_{10}$-transformed weight-length data (2013-2018 data). The weight-length relationship was defined using the following equation:

$$log_{10}(W) = log_{10}(a) + b * log_{10}(L)$$

where a is the intercept value, b is the slope of the line, W is the weight (body weight), and L is the length (fork length) of the fish (Le Cren 1951, Neumann et al. 2012).

Relative Condition Factor

The established weight-length relationship was used to calculate the relative condition factor (K_n; LeCren 1951) of the hatchery-origin population (2017-2018 data) as follows:

$$Kn = \left(\frac{W}{W'}\right)$$

where *W* is an observed body weight and *W'* is a predicted mean length-specific weight based on a defined region or population (Le Cren 1951, Blackwell et al. 2000, Neumann et al. 2012). The calculation of *W'* was determined using:

$$W' = 10^{(a+b*log_{10}(L)}$$

where *a* is the intercept value, *b* is the slope of the weight-length equation defined above, *W'* is the weight (body weight; kg), and *L* is the length (fork length; cm) of the fish.

Statistical Analysis

All 2017-2018 data used for statistical analyses were assessed for outliers, defined as residuals greater or less than three standard deviations of the mean - three outliers were identified and removed prior to statistical analyses. All residuals were visually assessed for normality using histograms, side-by-side boxplots, and diagnostic plots. Fork length, body weight, AGR (in fork length and body weight), and K_n were normally distributed. All recaptured fish (n=29) were removed prior to statistical analyses (i.e., the first capture data was used). One-way ANOVA was used to assess how size and AGR differed by sex. Linear regression was used to assess fork length and body weight-at-age of the hatchery-origin population as a function of sex. Three-way additive ANOVA with Type II sum of squares using package car (Fox and Weisburg 2011) was used to assess K_n by sex, zone, and age. Mean K_n did not differ between females and males ($F_{(1, 296)}$ = 0.00,1 P = 0.97);

therefore, sex was excluded from further analyses. Post hoc pairwise comparison tests (Tukey HSD) were preformed to further determine differences among age classes and zones using the package multcomp (Hothorn et al. 2008). The single 15-year old fish in Zone 3 was removed from the mean comparison test. One-way ANOVA was used to compare K_n among age classes in each zone. All data are presented as means and 95% confidence intervals unless otherwise specified. The accepted significance level was $\alpha = 0.05$ unless corrected for using the Bonferroni procedure. All statistical tests were conducted using R software (R Core Team 2019; version 3.6.1) and figures were produced using package ggplot2 (Wickham 2016).

Results

Physical Parameters

The thermal gradient within the LCR was assessed during 2017 and 2018. Mean daily water temperatures (^oC) measured within the LCR were variable but followed a seasonal trend with warmest temperatures in July – September and coldest temperatures in January – March during 2017 and 2018 (Figure 3.1). On average over the two-year period, Zone 1 had the coldest water temperatures, and the warmest temperatures were in Zone 3 (Table 3.1).

Water discharge was compared throughout the LCR in 2017 and 2018. Mean daily discharge varied in each zone (Figure 3.2), with the lowest discharge in Zone 1 and highest discharge in Zone 3 (Table 3.2).

Fish Capture

Over the two-year study, 1,337 white sturgeon were captured during sampling in the LCR, Canada, with 586 fish captured in 2017 and 751 fish captured in 2018. Of those fish, 1,118 were hatchery-origin, 210 were wild, and the origin could not be confirmed for 9 fish. A total of 307 hatchery-origin white sturgeon met the age and zone criteria to be included in this study. Additional sampling days (n=4) were conducted in Zone 3 in the spring of 2017 due to low sample sizes among age classes. Therefore, the total setline effort was higher in 2017 (4,852 hours) compared to 2018 (4,737 hours; Table 3.3).

Assignment of Sex and Stage of Maturity

Sex and stage of maturity was determined histologically in 307 hatchery-origin fish. A total of 162 female and 145 male hatchery-origin fish were identified in 2017 and 2018. All hatchery-origin fish were pre-vitellogenic females (Stages 1 and 2) with the presence of primary oocytes or pre-meiotic males (Stage 2) with spermatogonia and have not reached puberty.

Fish Size

Mean fork length of the hatchery-origin fish assessed in this study was 100 cm (99 – 101 cm; 95% CI; n = 307), and mean body weight was 7 kg (6.6 – 7.4 kg; 95% CI; n = 307). Fork length and body weight were normally distributed (Figures 3.3 and 3.4). Mean size was similar between female and male hatchery-origin white sturgeon (Table 3.4) in fork length ($F_{(1, 305)} = 1.93$, $P = 0.17$) and body weight ($F_{(1, 305)} = 1.29$, $P = 0.27$).

Absolute Growth Rate

Mean annual AGR in fork length for the hatchery-origin fish assessed in this study was 6.1 cm/year (6.0 – 6.2 cm; n=307), and mean annual AGR in body weight was 0.5 kg/year (0.50 - 0.55 kg; n=307). Absolute growth rate in fork length was normally distributed (Figure 3.5), whereas distributions of AGR in body weight were positively skewed with few individuals accumulating more than 0.5 kg in growth per year (Figure 3.6). Mean annual AGR was similar between female and male hatchery-origin white sturgeon (Table 3.5) in fork length (fork length $F_{(1, 305)} = 2.88$, $P = 0.09$) and body weight ($F_{(1, 305)} = 1.86$, $P = 0.17$).

Linear Regression of Size at Age

Size-at-age in fork length and body weight was compared between female and male white sturgeon. The size-at-age relationships for both sexes had considerable variability. The slopes for fork length-at-age were positive and differed from zero for females ($F_{(1, 160)} = 27.78$, $P = < 0.001$) and males ($F_{(1, 143)} = 12.64$, $P = < 0.001$), and adjusted R^2 values varied from 0.07 to 0.14 (Table 3.6, Figure 3.7). Similarly, the slopes for body weight-at-age were positive and differed from zero for females ($F_{(1, 160)} = 13.43$, $P = < 0.001$) and males ($F_{(1, 143)} = 13.38$, $P = < 0.001$). Adjusted R^2 values varied from 0.07 to 0.08 (Table 3.6, Figure 3.8).

Weight-Length Relationship

Log_{10}-transformed weight and length data were correlated to determine the relationship between weight and length for the hatchery-origin white sturgeon in the LCR

(n=2,738) and the transboundary reach (n=8,065). The resulting weight-length relationship for the hatchery-origin white sturgeon in the LCR was:

$$\log_{10}(W) = -5.46 + 3.13 * \log_{10}(L)$$

The relationship between $\log_{10}$-transformed weight and length had a positive slope and differed from zero ($R^2 = 0.95$, $P = < 0.001$). The hatchery-origin fish assessed provide an accurate representation of the white sturgeon population in the LCR based on the overlap of individuals captured in this study compared to those previously captured throughout the LCR (Figure 3.9). The weight-length relationship for the hatchery-origin white sturgeon in the transboundary reach was:

$$\log_{10}(W) = -5.37 + 3.12 * \log_{10}(L)$$

The relationship between $\log_{10}$-transformed weight and length had a positive slope and differed from zero ($R^2 = 0.95$, $P = < 0.001$). Majority of the hatchery-origin fish assessed in this study were plotted below the regression line, indicating a difference in K_n in the fish assessed in this study as compared to white sturgeon captured throughout the transboundary reach (Figure 3.10).

Relative Condition Factor

Mean K_n was normally distributed within the hatchery-origin fish assessed in this study (Figure 3.11) with a mean K_n of 1.03 (1.02 – 1.04; 95% CI; n=307). Mean K_n was similar between female and male hatchery-origin white sturgeon in the LCR ($F_{(1, 296)} = 0.003$ $P = 0.96$), but differed among age classes ($F_{(7, 297)} = 3.79$, $P = < 0.001$; Table 3.7) and zones ($F_{(2, 297)} = 7.85$, $P = < 0.001$; Table 3.8). For example, 17-year old fish had the

highest mean K_n (Table 3.7), and fish captured in Zone 3 had the highest mean K_n (Table 3.8). Mean K_n was similar among age classes in each zone (Figure 3.12); Zone 1 ($F_{(7, 144)} = 2.04$, $P = 0.05$), Zone 2 ($F_{(7, 90)} = 1.02$, $P = 0.42$), and Zone 3 ($F_{(6, 90)} = 1.91$, $P = 0.10$). When comparing mean K_n of the fish assessed this study to the entire transboundary reach the mean was lower, 0.87 (0.86 - 0.88; 95% CI; n=307; Figure 3.13).

Discussion

Relative condition of the fish assessed in this study (mean $K_n = 1.03$) indicated that the hatchery-origin white sturgeon population (n=307) were in average condition relative to all hatchery-origin fish within the LCR. While there were no obvious trends among age-classes, individuals sampled in downstream habitats had higher mean K_n within the study area. This could be attributed to differences in density of white sturgeon in the downstream zones (Zones 2 and 3) shown by the lower CPUE. Alternatively, when comparing K_n of the hatchery-origin population sampled in this study to other hatchery-origin white sturgeon sampled throughout the transboundary reach the mean K_n decreased to slightly below average (mean $K_n = 0.87$). This reflects a difference in the environmental or physiological conditions of the fish assessed in this study and those captured throughout the transboundary reach. Continued monitoring of changes in the K_n of white sturgeon in the LCR and transboundary reach may help to identify the different environmental or physiological changes these fish are experiencing.

Age and size at puberty has not yet been described in hatchery-origin white sturgeon. Determining this reproductive index is valuable for monitoring population

dynamics (Jakobsen et al. 2016). In the wild, white sturgeon have been documented to reach puberty at the ages of 12-25 years in males and 15-35 years in females (Billard and Lecointre 2001, CBWSPF 2013) and sizes of 100-150 cm FL in males and 120-180 cm FL in females (Welch and Beamesderfer 1993, DeVore et al. 1995, Billard and Lecointre 2001, CBWSPF 2013, Haxton et al. 2016). Hatchery-origin fish in this study varied in age from 10-17 years old with a mean FL of 101 cm (99–103 cm; 95% CI) in males and 99 cm (97–101 cm; 95% CI) in females. These fish are of similar ages to those that have reached puberty in other reaches of the Columbia River but are of smaller size. Considerable variation was found in the size and growth rates of the hatchery-origin population. This level of variability is common among sturgeons (Welch and Beamesderfer 1993, Ireland 2002, CBWSPF 2013, Hildebrand and Parsley 2013), yet it makes distinguishing factors which may be contributing to growth challenging.

The relationship between size and the onset of puberty in fishes is strongly supported (e.g., Le Gac et al. 1993, Tupper and Boutilier 1997, Huang et al. 1998, Okuzawa 2002, Taranger et al. 2010, Ryu et al. 2013, Nater et al. 2018), and larger fish are often able to reach puberty at an earlier age compared to smaller individuals (Amano et al. 1997, Huang et al. 1998, Van Eenennaam and Doroshov 1998, Taranger et al. 2010, Ryu et al. 2013, Nater et al. 2018). Particularly, body weight has been correlated with the onset of puberty in fishes (Doroshov et al. 1997, Huang et al. 1998, Okuzawa 2002, Ryu et al. 2013). Hatchery-origin white sturgeon from the same conservation aquaculture program have also been released into Lake Roosevelt, WA, USA, downstream from the Canadian population (BC Hydro 2018). The Lake Roosevelt hatchery-origin population

is slightly larger in body size compared to the Canadian population with a mean FL of 102 cm and a mean body weight of 9 kg (n=252; Howell and McLellan 2018) in the Lake Roosevelt fish and a mean FL of 100 cm (99-101 cm; 95% CI) and mean body weight of 7 kg (6.6-7.4 kg; 95% CI) in the Canadian population (n=307). This slight difference in size between the populations may not seem significant, however some males in the Lake Roosevelt population have reached puberty (Webb et al. 2016). The hatchery-origin fish in the LCR and Lake Roosevelt are of similar ages but different sizes. This difference in size, particularly in body weight (2 kg), could be a contributing factor to why fish in Lake Roosevelt have reached puberty, whereas fish in the LCR remain prepubertal (see Chapter 2). Further work should be conducted to compare the body weight of males in Lake Roosevelt to the LCR to define a potential threshold at which puberty is initiated.

Fish have indeterminate growth (e.g., Jobling 1995, Jones et al. 2002, Minte-Vera et al. 2016), but the rate of growth changes during different life stages (Jones 2002, Minte-Vera et al. 2016, Nater et al. 2018). Rapid growth is usually experienced in early life before tapering off and slowing over time (Maceina 1992, Taranger et al. 2010). This period of slow, continued growth is highly associated with the onset of puberty, where allocation of resources shift from somatic growth to reproductive output (Taranger et al. 2010, Minte-Vera et al. 2016, Nater et al. 2018). There is a poor correlation between fork length and age in the hatchery-origin population (female R^2= 0.14, and male R^2= 0.07). However, the trajectory of the regression line in males was lower compared to females (see Figure 3.7). This trend may suggest a shift from somatic growth to testicular development prior to the onset of puberty. The trajectory of the regression line in

females was steeper compared to the males suggesting that females may be continuing to invest in somatic growth and are likely going to require more time before the onset of puberty as compared to males in this population. The relationship between body weight and age was similar in both female and male fish within the hatchery-origin population, although once again the R_2 values were low (female R_2= 0.07, and male R_2= 0.08). Due to the poor correlation between size-at-age in the hatchery-origin population caution should be taken when interpreting the results of these relationships. Future work should continue to assess the size-at-age of the hatchery-origin population which may provide another metric for recovery programs interested in monitoring the shifts in growth rate that may be associated with the onset of puberty within the population.

Understanding the variation in the growth in fish requires many years of data and the ability to recapture individuals on several occasions. Our study was limited to collecting data over two years, whereas most monitoring programs tend collect data over a longer period of time resulting in descriptions or trends that are more representative of the entire population. Ireland et al. (2002) described a mean growth rate of 6.4 cm/yr FL in hatchery-origin white sturgeon in the Kootenai River, Idaho, USA, which is similar to the mean growth rate described in the hatchery-origin white sturgeon assessed in this study. Annual growth rates of 5-10 cm/yr FL (predicted from von Bertalanffy growth curves or actual growth) were described in wild white sturgeon in the Columbia River, USA (Jones et al. 2001, CBWSPF 2013). Hatchery-origin white sturgeon in the LCR experienced mean annual growth rates of 6 cm/year FL, which is similar to the lower end of the range experienced by wild white sturgeon in the USA. Numerous factors may be

contributing to the differences in annual growth, including but not limited to age, food source availability, water temperature, and habitat (Jobling 1995, Moyle and Cech 2004, Barth and Anderson et al. 2015, Boltana et al. 2017, Porter and Schramm 2018). Future monitoring of annual growth rates of the hatchery-origin white sturgeon will provide valuable information about potential changes in environmental conditions.

Because sturgeon are not sexually dimorphic, the parameters used within population dynamic models do not often include sex-specific information. For example, condition is often analyzed within a population rather than compared between females and males within a population (e.g., Ireland et al. 2002, Amara et al. 2007, Shuman et al. 2011, Barth and Anderson 2015). Within this study, we had the opportunity to examine differences in size, AGR, and K_n between females and males. No meaningful differences were found in these metrics between the sexes. This indicates that determination of sex, which may be costly and time consuming, does not provide additional value for understanding population dynamics in prepubertal fish.

Continuing long term monitoring of the hatchery-origin white sturgeon in the LCR will allow for the assessment of biological and environmental factors that could contribute to the size and age at puberty. Because fish had not reached puberty in this study, size, AGR, and K_n were described. In 2017 and 2018, the mean K_n of the hatchery-origin population indicated that the fish were in average condition, and mean AGR was similar to other white sturgeon populations in the Columbia River. High variability was found in the size of the fish (FL and body weight), similar to other sturgeons (Welch and Beamesderfer 1993, Ireland et al. 2002, Shuman et al. 2011,

Hildebrand and Parsley 2013, Barth and Anderson 2015, Porter and Schramm 2018). Increased biological knowledge of hatchery-origin populations is important for conservation aquaculture programs.

Table 3.1. Mean annual temperature (°C; 95% confidence intervals (CI)) for Zones 1-3 in the lower Columbia River, Canada during 2017 and 2018. Differences in sample sizes are due to lost or damaged temperature loggers.

Year	Zone	n	Temperature (°C; mean, 95% CI)
2017	1	730	9.0 (8.6 – 9.04)
	2	730	9.1 (8.7 – 9.5)
	3	564	10.7 (10.2 – 11.2)
2018	1	612	8.9 (8.5 – 9.3)
	2	730	9.4 (9.0 – 9.8)
	3	366	9.6 (9.2 – 10.0)

Table 3.2. Mean annual discharge (cms; 95% confidence intervals (CI)) for Zones 1-3 in the lower Columbia River, Canada. Two of the four discharge readers were located in Zone 1, hence the larger sample size.

Year	Zone	n	Discharge (cms; mean, 95% CI)
2017	1	732	1075 (1032 - 1119)
	2	366	2191 (2101 - 2281)
	3	366	3089 (2949 – 3230)
2018	1	732	1039 (996 – 1081)
	2	365	2677 (2576 – 2777)
	3	366	3803 (3618 – 3988)

Table 3.3. White sturgeon catch per unit effort (CPUE) for sampling among zones by sampling season in the lower Columbia River during 2017 and 2018. Catch per unit effort was calculated as the total number of fish captured per set line hour.

Season	Zone	Fish captured	Effort (hrs)	CPUE
Spring 2017	1	133	926	0.14
	2	27	520	0.05
	3	30	1039	0.03
Fall 2017	1	237	977	0.24
	2	111	807	0.14
	3	48	583	0.08
Spring 2018	1	253	936	0.27
	2	59	813	0.07
	3	16	610	0.03
Fall 2018	1	235	952	0.25
	2	141	797	0.18
	3	47	629	0.07

Table 3.4. Mean size (fork length and body weight; 95% confidence intervals (CI)) for the hatchery-origin population of white sturgeon in the lower Columbia River, Canada. No statistical differences in size of hatchery-origin fish were found.

	Mean fork length (cm; mean, 95% CI)	Mean body weight (kg; mean, 95% CI)
Females (n=162)	99 (97 - 101)	7 (6.5 – 7.5)
Males (n=145)	101 (99 - 103)	7 (6.5 – 7.5)
Total (n=307)	100 (99 - 101)	7 (6.6 – 7.4)

Table 3.5. Mean absolute growth rate (AGR; fork length and body weight; 95% confidence intervals (CI)) for the hatchery-origin population of white sturgeon in the lower Columbia River, Canada. No statistical difference in AGR of hatchery-origin fish was found.

	Mean AGR fork length (cm/yr; mean, 95% CI)	Mean AGR body weight (kg/yr; mean, 95% CI)
Females (n=163)	6.0 (5.8 - 6.1)	0.5 (0.48 - 0.52)
Males (n=145)	6.2 (6.0 - 6.4)	0.5 (0.47 - 0.53)
Total (n=308)	6.1 (6.0 – 6.2)	0.5 (0.50 - 0.55)

Table 3.6. The regression models for fork length (FL) at age and body weight (WT) at age within the hatchery-origin population (n=307) in the lower Columbia River, Canada.

Dependent Variables	Regression Equation	*P*	R_2
FL (cm)	Female FL = 63.9 + 2.5 *(Age)	< 0.001	0.14
	Male FL = 75.1 + 1.9 *(Age)	< 0.001	0.07
WT (kg)	Female WT = 0.2 + 0.5 *(Age)	< 0.001	0.07
	Male WT = 0.8 + 0.5*(Age)	< 0.001	0.08

Table 3.7. Mean relative condition factor (K_n; mean, 95% confidence intervals (CI)) of the hatchery-origin population of white sturgeon among age classes in the lower Columbia River, Canada. Different letters denote statistical differences in K_n.

Age (years)	Mean K_n (mean, 95% CI)
10 (n=14)	0.99 (0.95 – 1.03) a
11 (n=36)	1.02 (0.99 – 1.05) ab
12 (n=44)	1.05 (1.03 – 1.07) ab
13 (n=48)	0.99 (0.97 – 1.01) a
14 (n=36)	1.01 (0.99 – 1.03) ab
15 (n=50)	1.01 (0.99 – 1.03) ab
16 (n=59)	1.05 (0.79 – 1.03) a
17 (n=20)	1.06 (1.03 – 1.09) b

Table 3.8. Mean relative condition factor (K_n; mean, 95% confidence intervals (CI)) of the hatchery-origin population of white sturgeon among zones in the lower Columbia River, Canada. Different letters denote statistical differences in K_n.

Zone	Mean K_n (mean, 95% CI)
1 (n=152)	1.01 (0.99 – 1.02) a
2 (n=98)	1.04 (1.02 – 1.06) b
3 (n=57)	1.05 (1.03 – 1.07) b

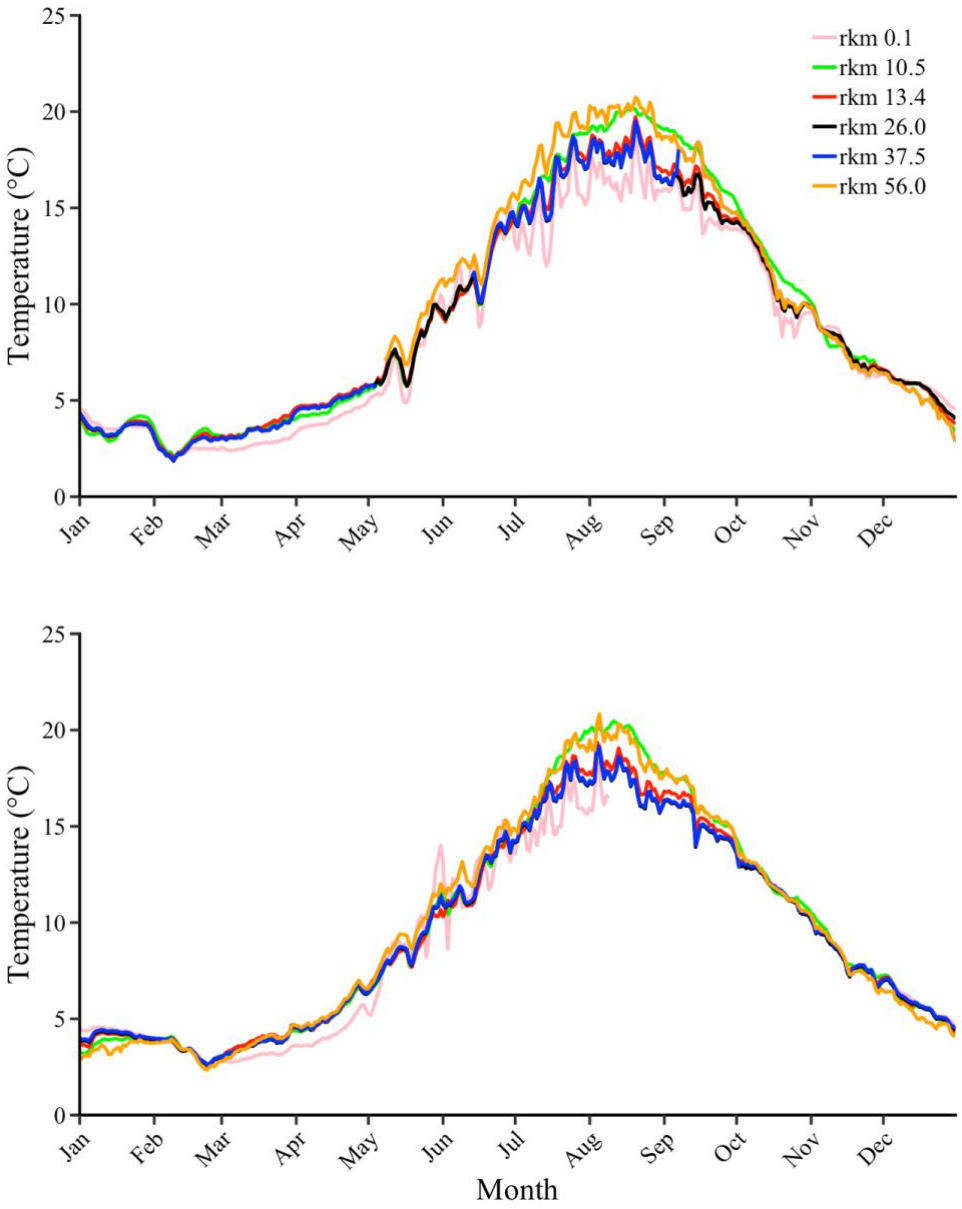

Figure 3.1. Mean daily water temperature (oC) of the lower Columbia River, Canada in 2017 (top) and 2018 (bottom). Missing data is the result of lost temperature logger(s) at rkm 26.0 from January to May in 2017 and rkm 0.1 from September to December 2018. Zone 1 consists of rkm 0.1 - 10.5, Zone 2 consists of rkm 13.4 - 26.0, and Zone 3 consists of rkm 37.5 - 56.0.

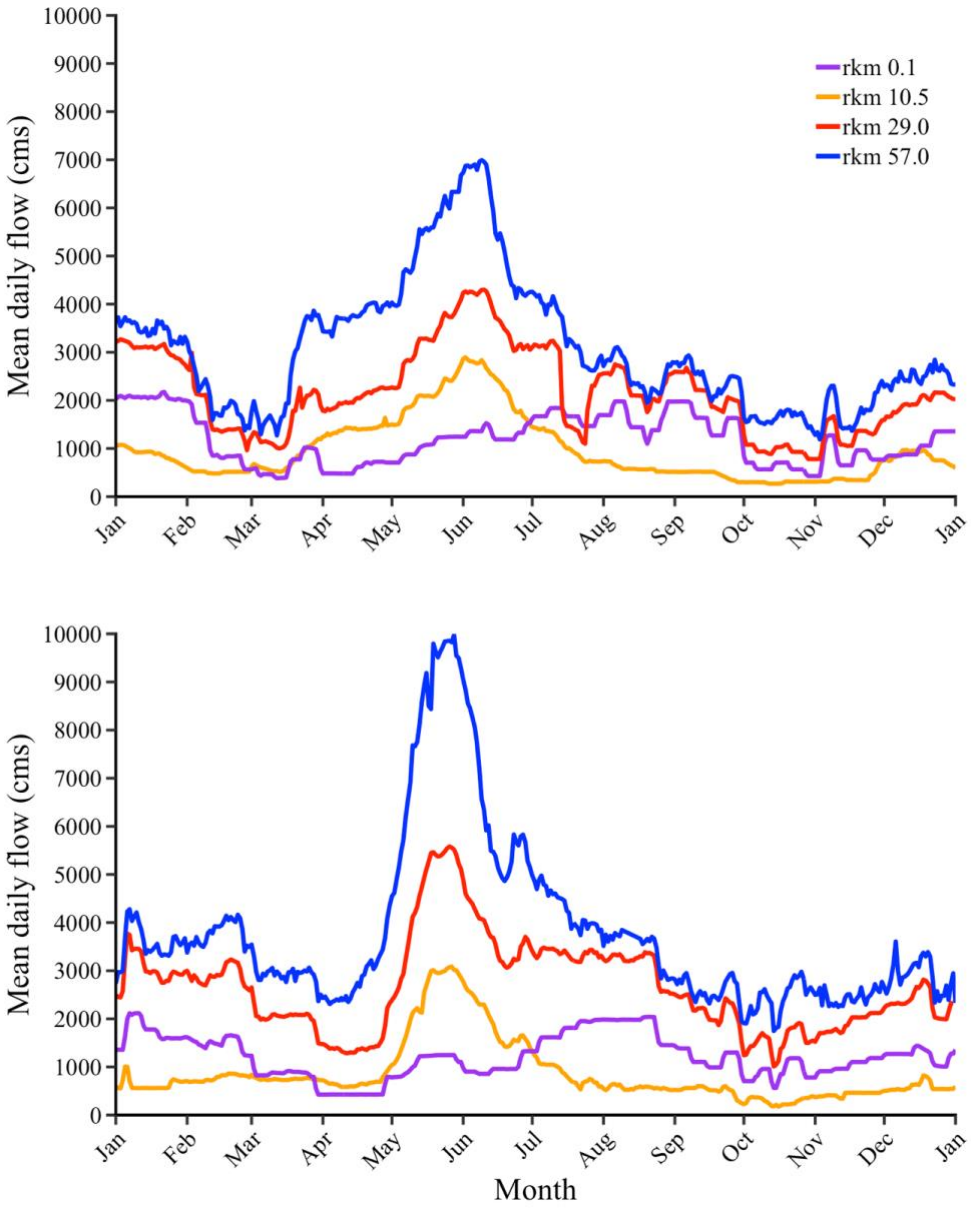

Figure 3.2. Mean daily discharge (cubic meters per second, cms) of the lower Columbia River, Canada in 2017 (top) and 2018 (bottom). Zone 1 consists of rkm 0.1 – 10.5, Zone 2 consists of rkm 29.0, and Zone 3 consists of rkm 57.0.

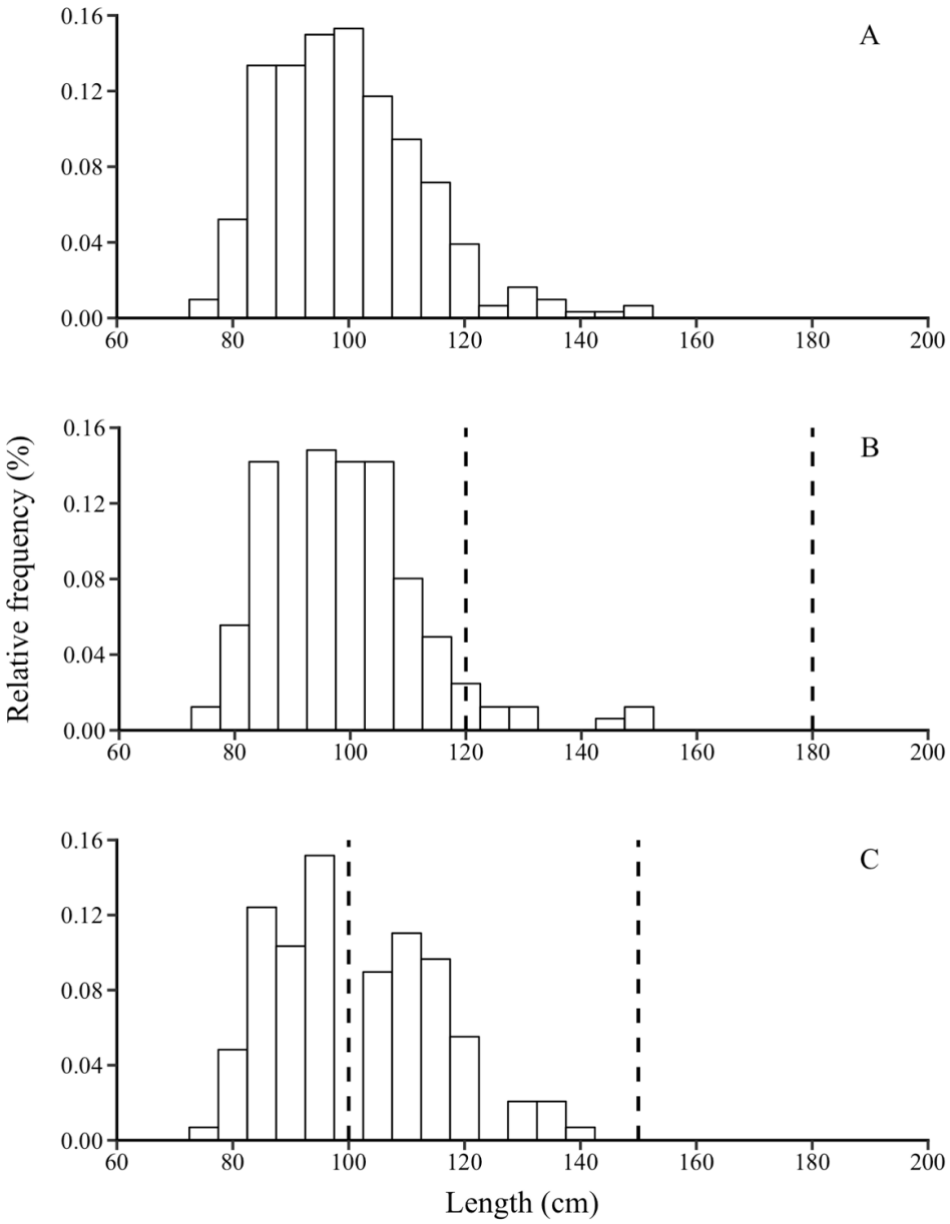

Figure 3.3. Relative frequency (%) distributions of fork length (5 cm bins) for hatchery-origin white sturgeon in the lower Columbia River, Canada. Plot A represents all hatchery-origin fish (n=307), plot B represents females only (n=162), and plot C represents males only (n=145). The vertical lines represent the reported fork length range for reproductively active white sturgeon females (120 - 180 cm) and males (100 - 150 cm).

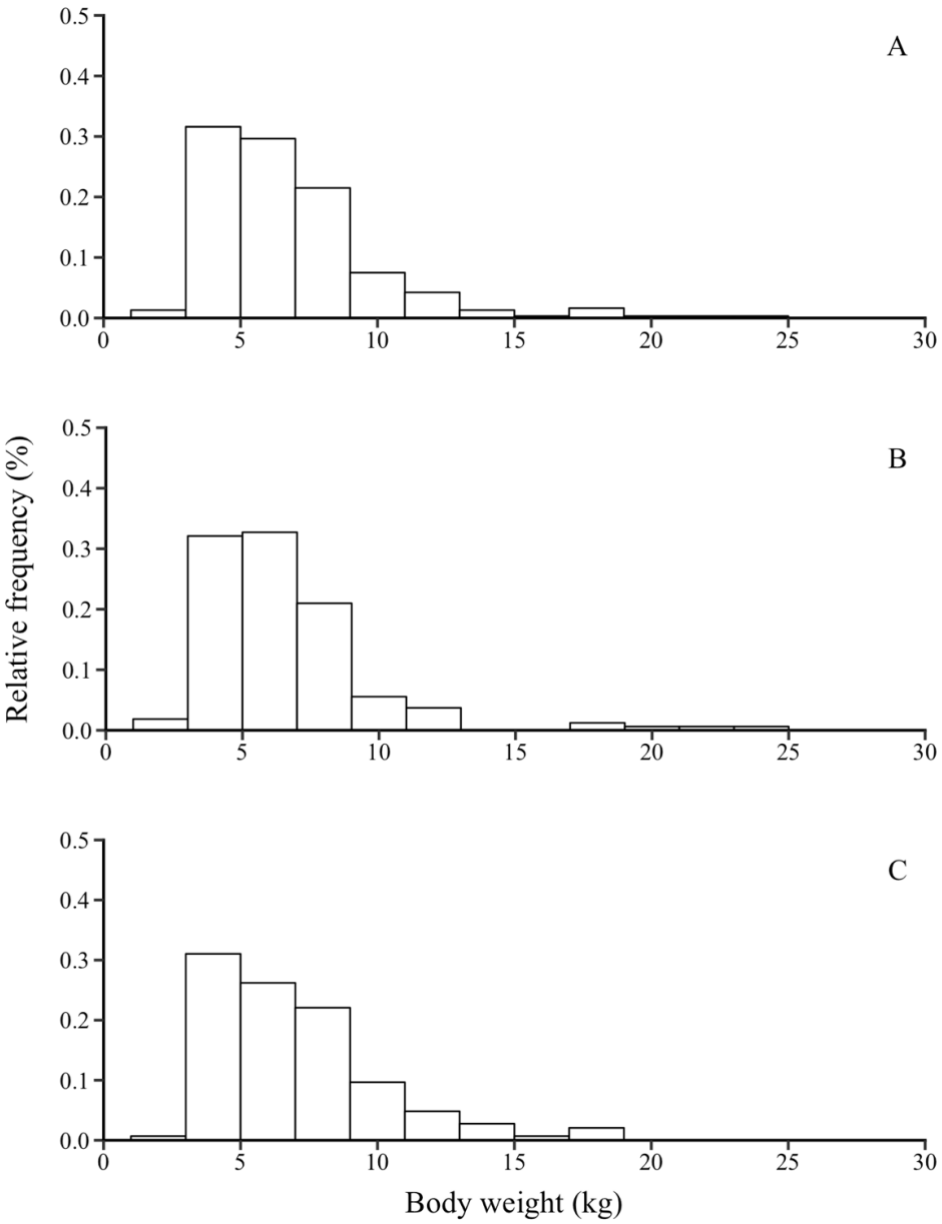

Figure 3.4. Relative frequency (%) distributions of body weight (3 kg bins) for hatchery-origin white sturgeon in the lower Columbia River, Canada. Plot A represents all hatchery-origin fish (n=307), plot B represents only females (n=162), and plot C represents only males (n=145).

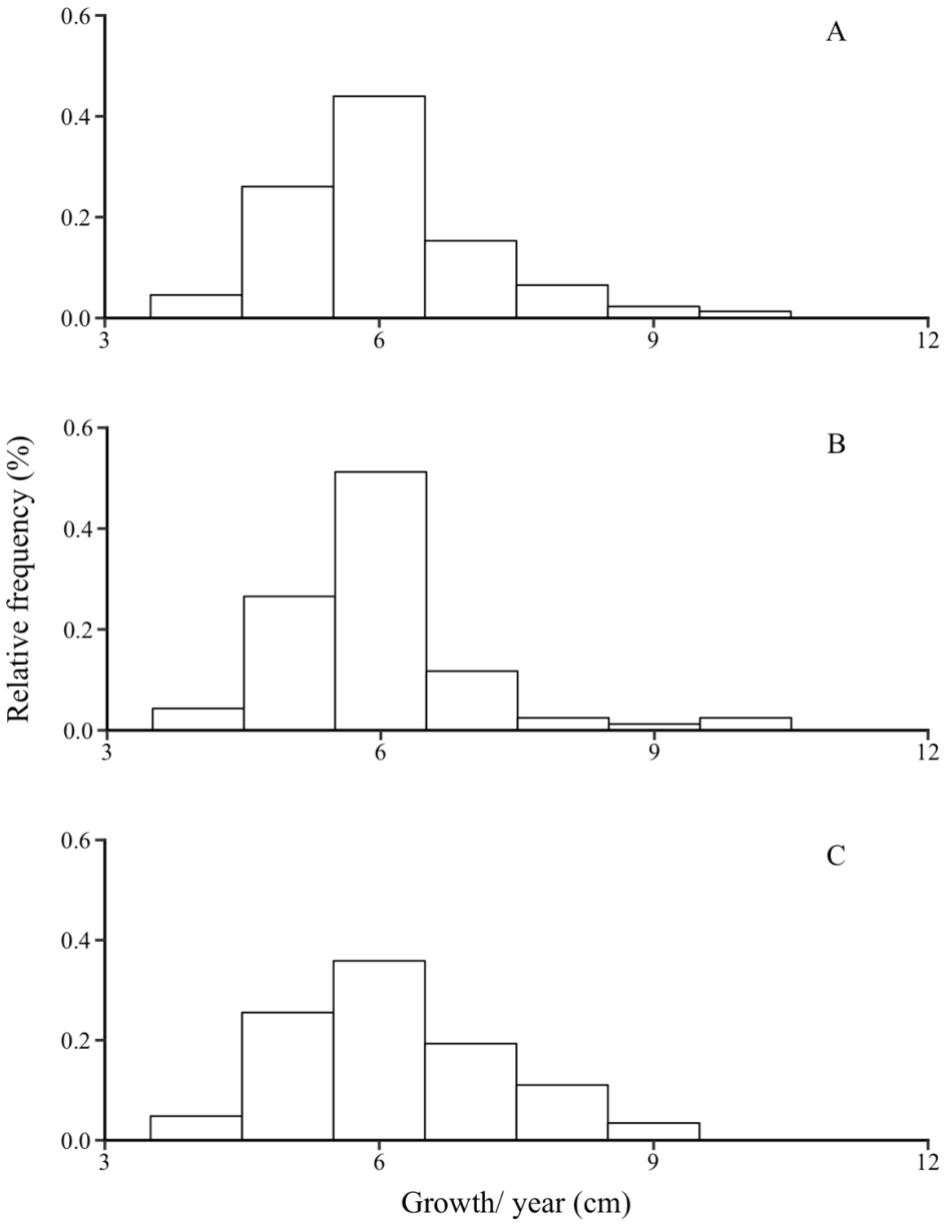

Figure 3.5. Relative frequency (%) distributions of absolute growth rate in fork length (1 cm bins) for hatchery-origin white sturgeon in the lower Columbia River, Canada. Plot A represents all hatchery-origin fish (n=307), plot B represents only females (n=162), and plot C represents only males (n=145).

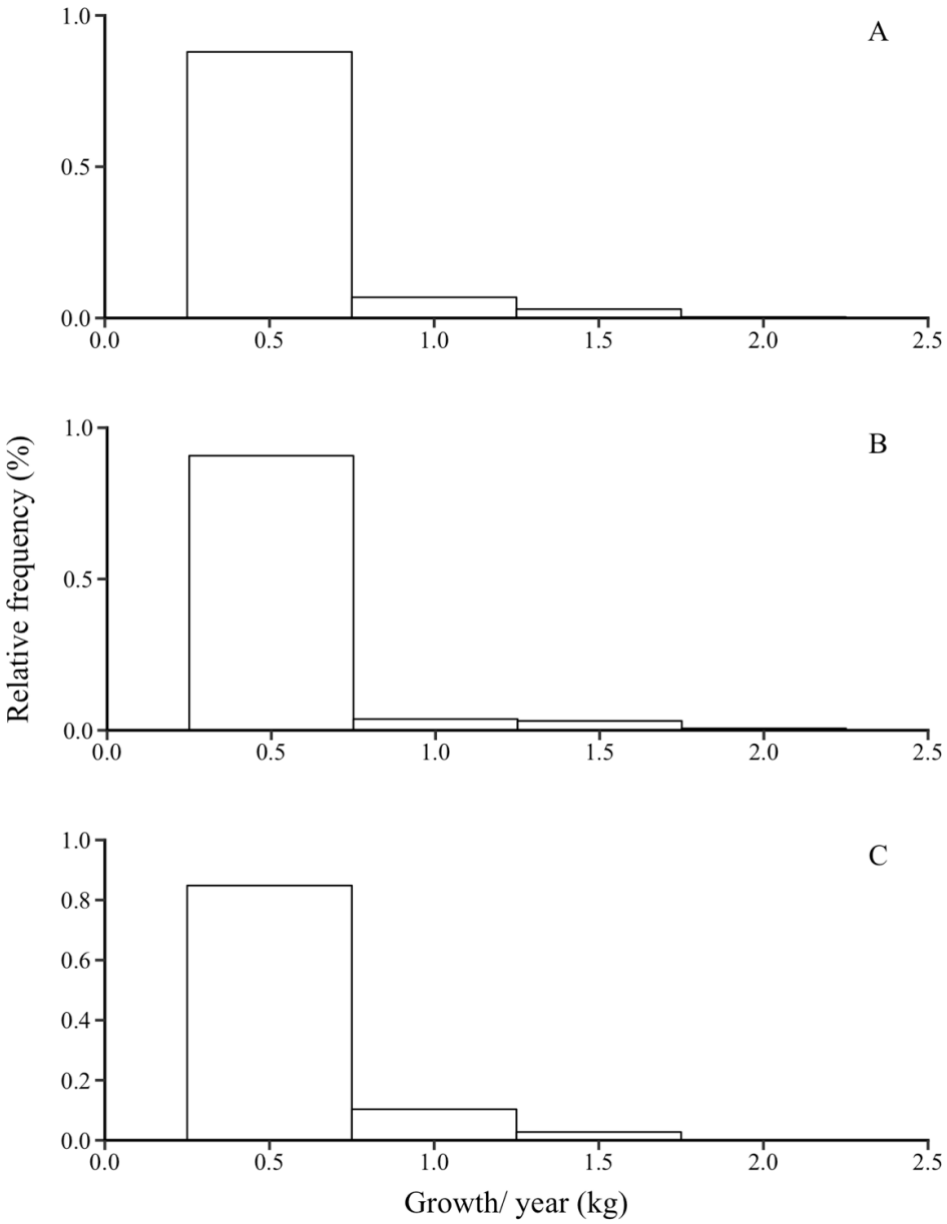

Figure 3.6. Relative frequency (%) distributions of absolute growth rate in body weight (0.5 kg bins) for hatchery-origin white sturgeon in the lower Columbia River, Canada. Plot A represents all hatchery-origin fish (n=307), plot B represents only females (n=162), and plot C represents only males (n=145).

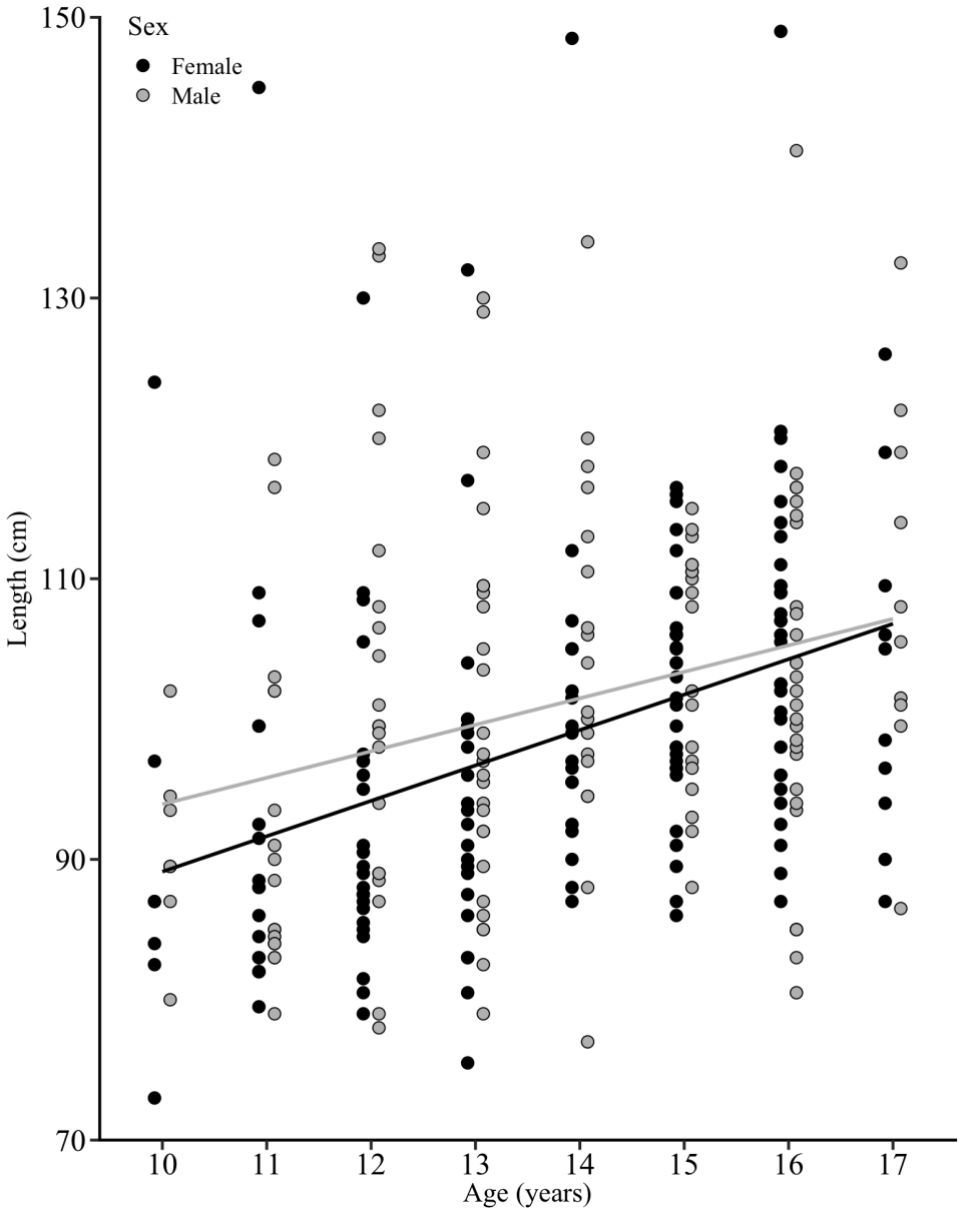

Figure 3.7. Length (FL; cm) at age of females and males within the hatchery-origin population of white sturgeon in the lower Columbia River, Canada. Adjusted R_2 values for regressions were 0.14 for females and 0.07 for males.

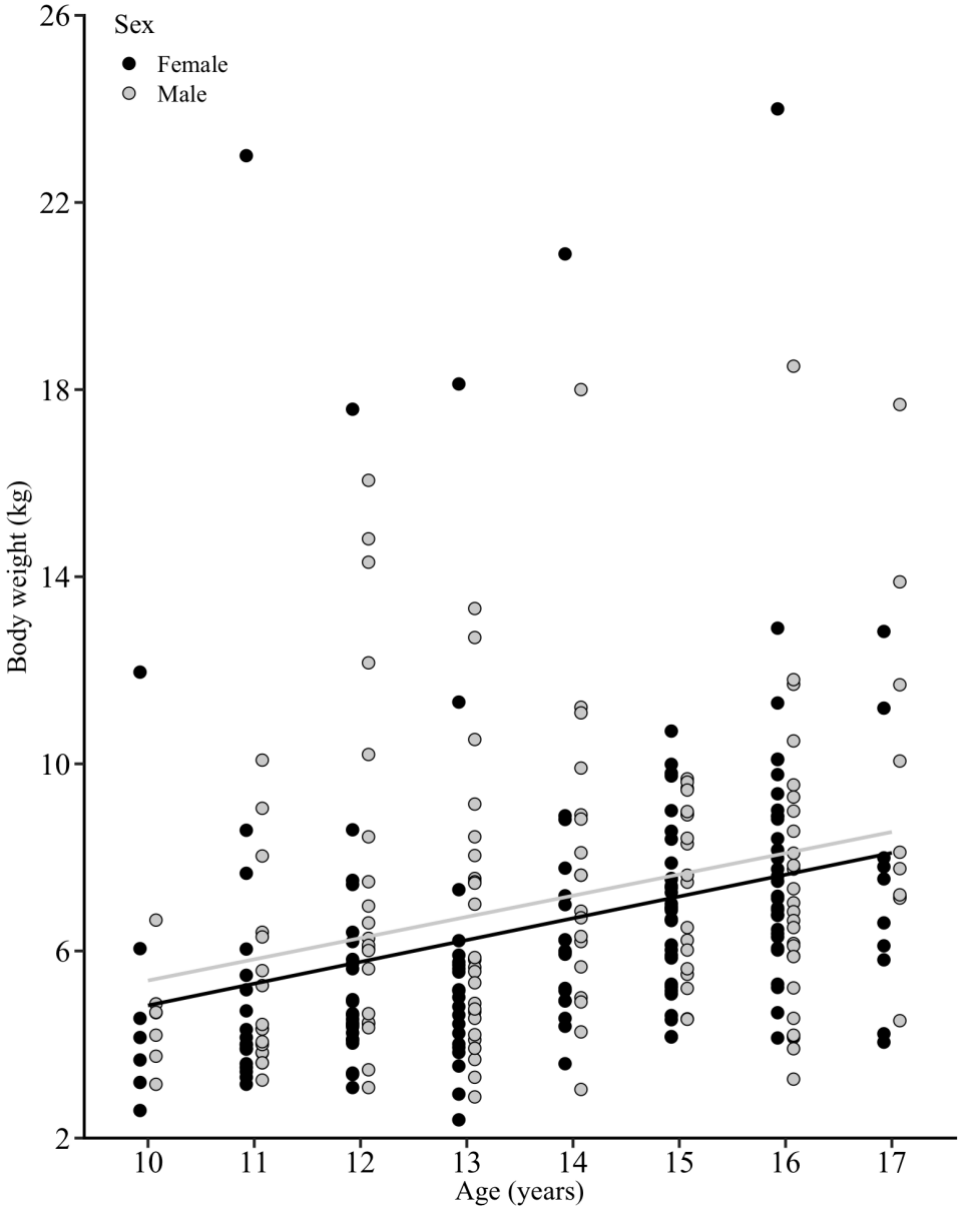

Figure 3.8. Body weight (kg) at age of females and males within the hatchery-origin population of white sturgeon in the lower Columbia River, Canada. Adjusted R_2 values for regressions were 0.07 for females and 0.08 for males.

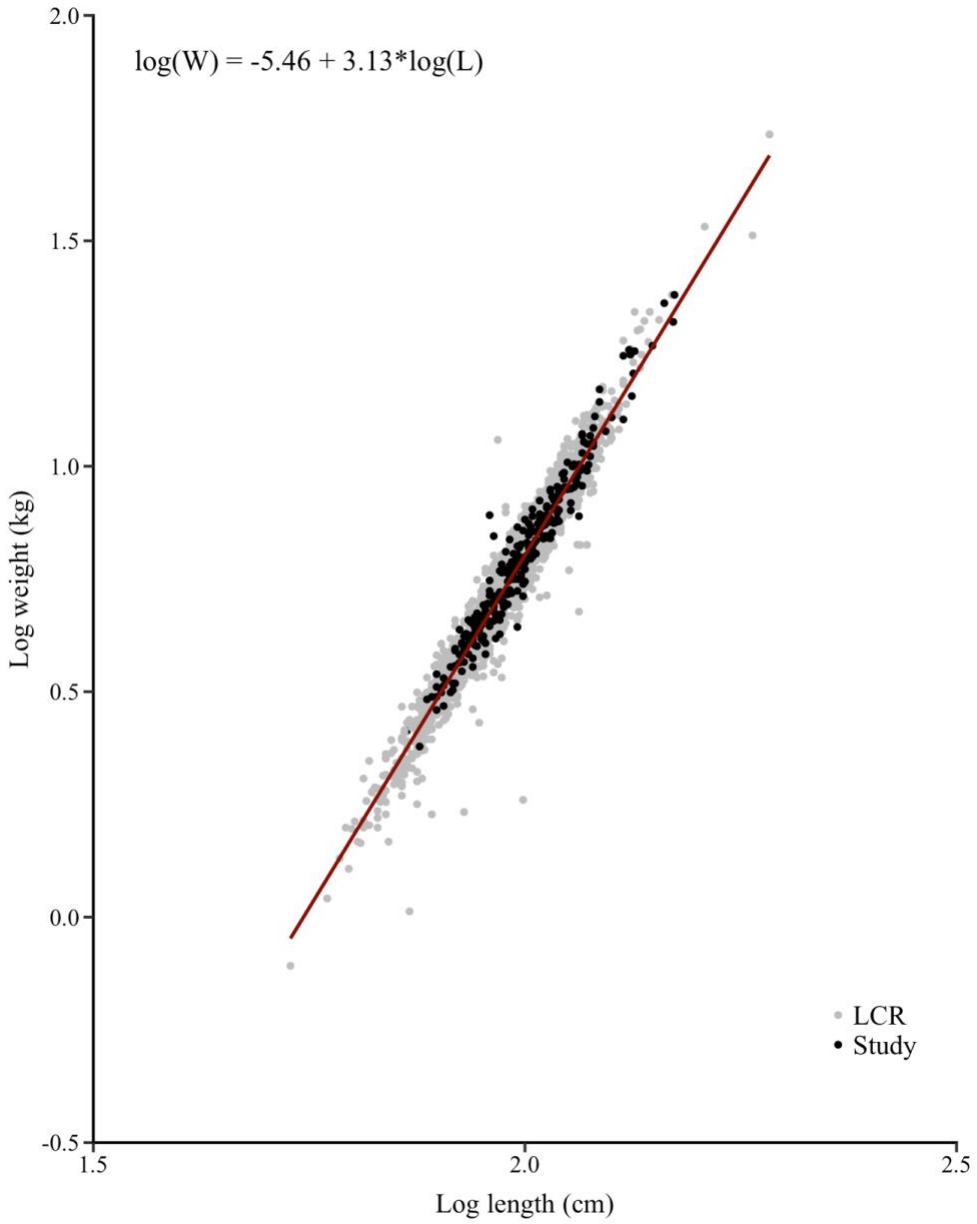

Figure 3.9. Log-transformed length (FL; cm) and log-transformed body weight (kg) of hatchery-origin white sturgeon (n=2,738) in the lower Columbia River, Canada. Study, shown in black, refers to the fish captured in this study (n=307), and LCR, shown in grey, refers to all fish captured in the lower Columbia River, Canada (n=2,738). This regression was used to determine the coefficients for the weight length relationship (displayed) which was used to calculate the relative condition factor for the hatchery-origin population in this study (n=307).

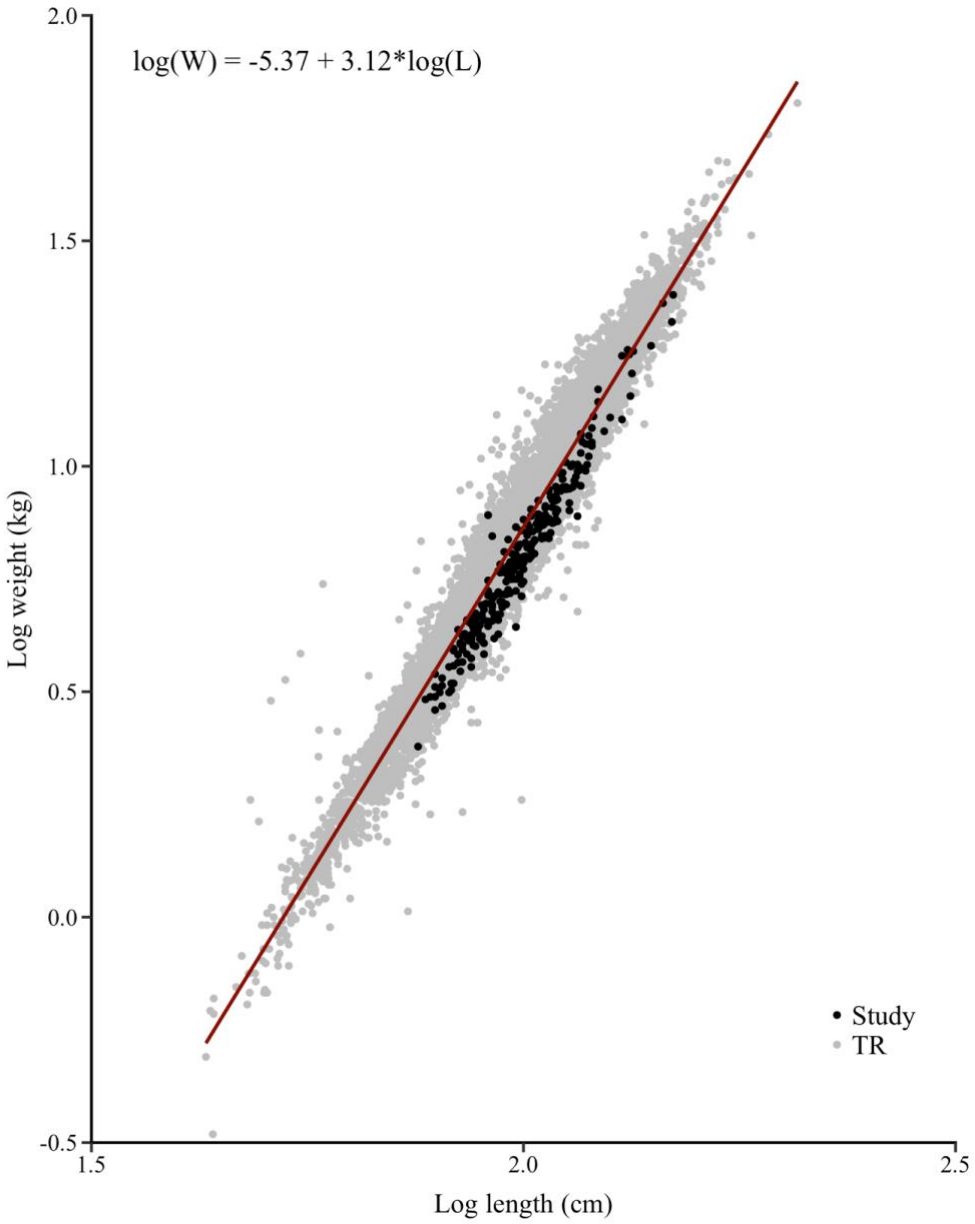

Figure 3.10. Log-transformed length (FL; cm) and log-transformed body weight (kg) of hatchery-origin white sturgeon in the transboundary reach of the Columbia River. Study, shown in black, refers to the fish captured in this study (n=307) and TR, shown in grey, refers to all fish captured (n=8,065) in the transboundary reach. This regression was used to determine the coefficients for the weight-length relationship (displayed) for white sturgeon within the transboundary reach.

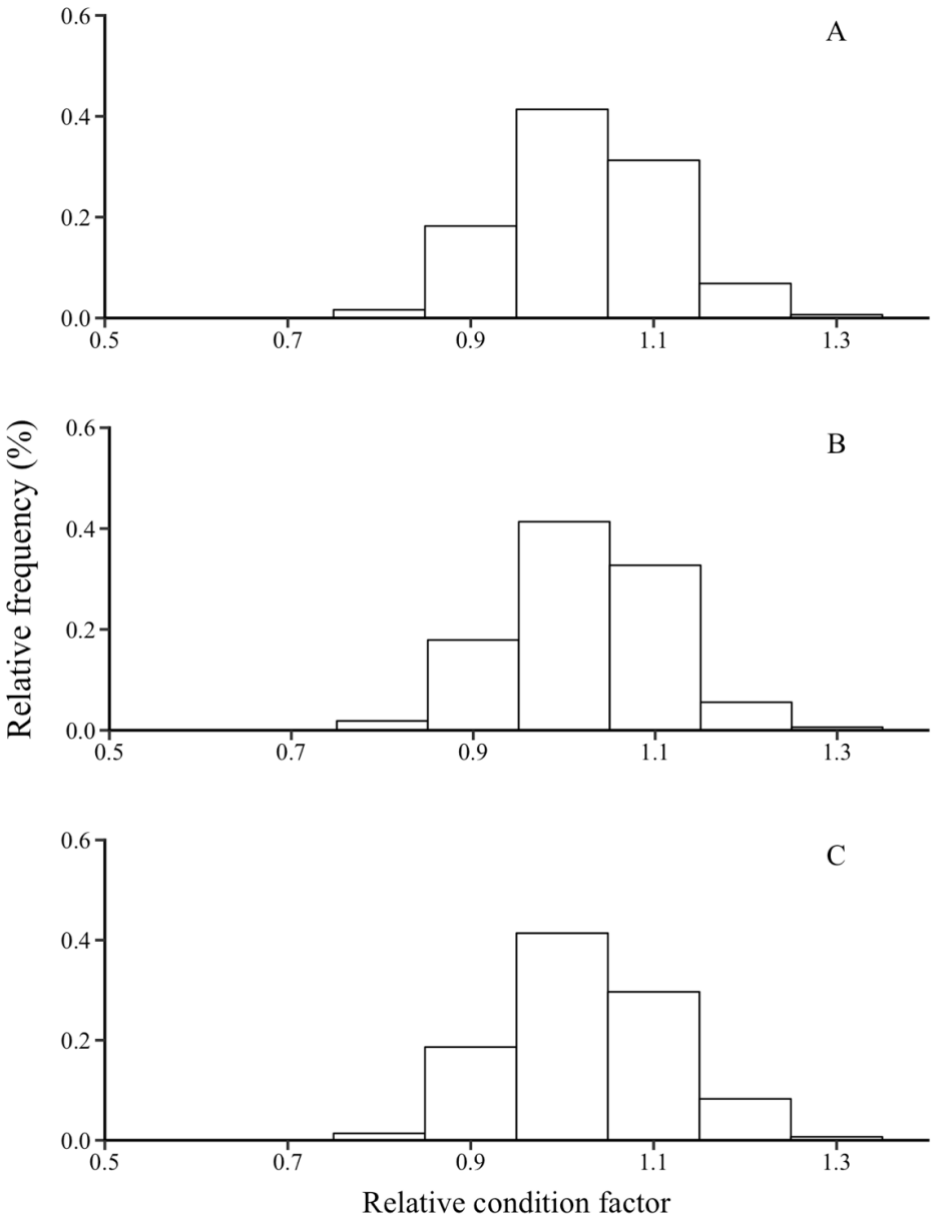

Figure 3.11. Relative frequency (%) distributions of relative condition factor (K_n) (0.1 bins) for hatchery-origin white sturgeon in the lower Columbia River, Canada. Plot A represents all hatchery-origin fish (n=307), plot B represents only females (n=162), and plot C represents only males (n=145).

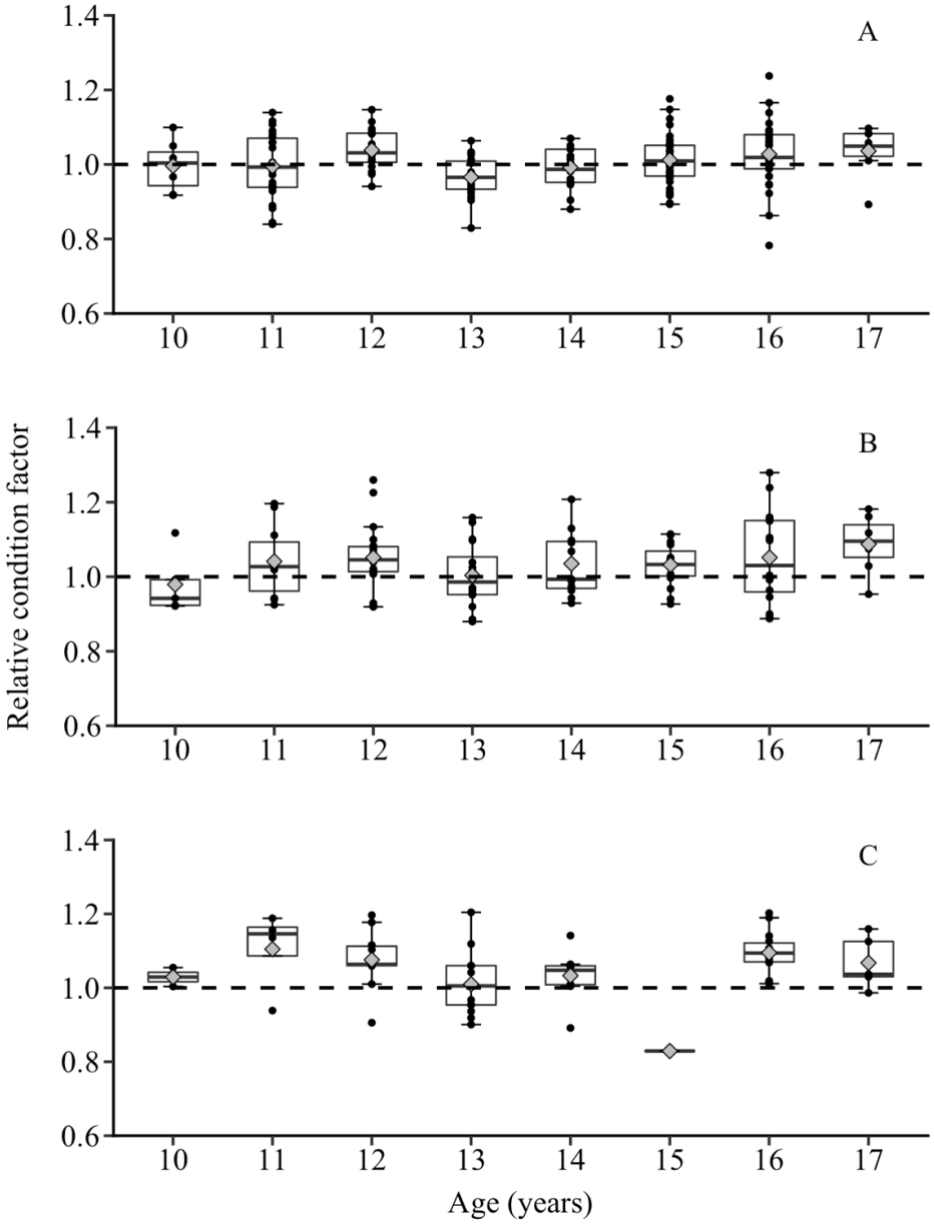

Figure 3.12. Relative condition factor at age of hatchery-origin white sturgeon among zones in the lower Columbia River (LCR), Canada based on the weight-length relationship from the LCR. Plot A represents fish in Zone 1 (n=152), plot B represents fish in Zone 2 (n=98), and plot C represents fish in Zone 3 (n=57). The median is represented within each box by a horizontal line, and the mean is represented by the diamond. Minimum and maximum values are represented by whiskers. Individual points represent raw data. The dashed horizontal line represents the standard relative condition factor (1.0). Relative condition factor was similar among age classes in all zones 1 and 2. The single 15-year old fish captured in Zone 3 was not included in the mean comparison test for Zone 3.

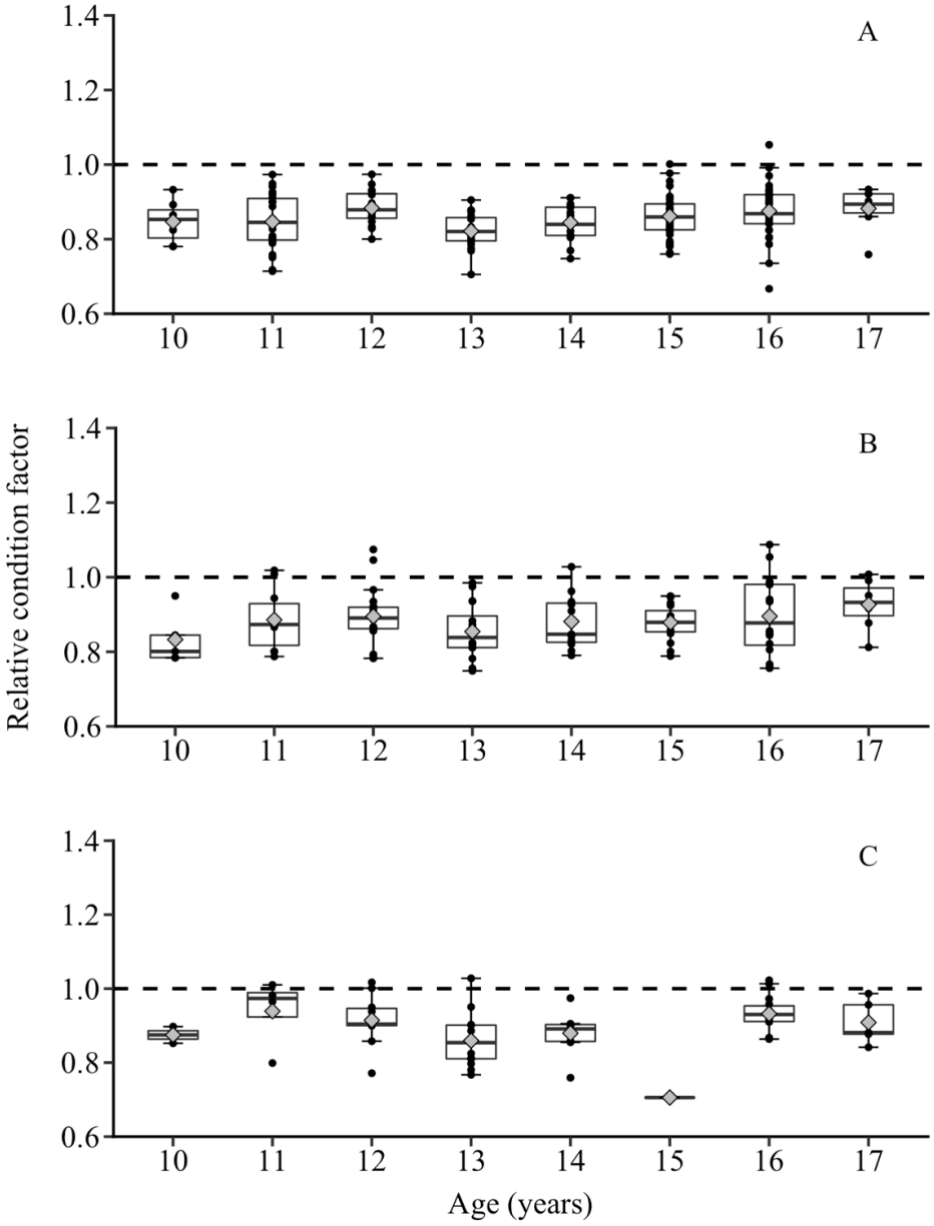

Figure 3.13. Relative condition factor at age of hatchery-origin white sturgeon among zones in the lower Columbia River, Canada based on the weight-length relationship from the transboundary reach. Plot A represents fish in Zone 1 (n=152), plot B represents fish in Zone 2 (n=98), and plot C represents fish in Zone 3 (n=57). The median is represented within each box by a horizontal line, and the mean is represented by the diamond. Minimum and maximum values are represented by whiskers. Individual points represent raw data. The dashed horizontal line represents the standard relative condition factor (1.0). Relative condition factor was similar among age classes in all zones 1 and 2. The single 15-year old fish captured in Zone 3 was not included in the mean comparison test for Zone 3.

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CHAPTER FOUR

CONCLUSIONS

The overall goal of this study was to determine if any individuals within the hatchery-origin white sturgeon population in the lower Columbia River (LCR), Canada had reached puberty. The specific objective of Chapter Two was to describe the reproductive structure of the white sturgeon population in the LCR using numerous tools (gonadal biopsy analyzed histologically, ultrasound, endoscopy, and plasma sex steroids) available to assign sex to the hatchery-origin population, as well as a small number of the existing wild white sturgeon. The specific objectives of Chapter Three were: 1) to describe the size, absolute growth rate (AGR), and relative condition factor (K_n) of hatchery-origin white sturgeon in the LCR, 2) determine if these metrics differed by sex, 3) determine if the K_n of the hatchery-origin population differed among sexes, ages, and zone, and 4) determine if the K_n of the hatchery-origin fish differed in the LCR as compared to the transboundary reach.

As of 2018, individuals sampled from the hatchery-origin white sturgeon population in the LCR have sexually differentiated but had not yet reached puberty. All hatchery-origin fish were pre-vitellogenic females (Stages 1 and 2) or pre-meiotic males (Stage 2). Tools were evaluated to determine their accuracy in assigning sex to a prepubertal population. Endoscopy and histological analysis of gonadal tissue collected by minor biopsy were the most accurate in assigning sex in the hatchery-origin population with 98% and 97% accuracy, respectively. Both tools require a small

abdominal incision. The less-invasive tools evaluated, blood plasma sex steroids and ultrasound, had lower accuracy in the hatchery-origin population with 69% and 57% accuracy, respectively. As the hatchery-origin population reaches puberty and continues to cycle through gametogenesis, the accuracy of less-invasive tools should be reevaluated, as previous research has shown higher accuracy in using these tools to assign sex in sturgeon populations which are reproductively active (e.g., Moghim et al. 2002, Wildhaber et al. 2007, Webb et al. 2018, see Chapter Two). The accuracies of several tools for assigning sex and stage of maturity to white sturgeon were discussed in Chapter two, however, the level of accuracy required may differ depending on the objectives of other monitoring programs. Long-term monitoring will be important to determine the reproductive dynamics of the hatchery-origin population over time (e.g., age and size at puberty, sex ratio), and specifically when these fish will begin contributing to wild spawning events.

Non-homogeneous testicular development was identified in 38% (21 – 56%; 95% CI; n=12) of male hatchery-origin white sturgeon in Lake Roosevelt, USA (the same population of hatchery-origin fish in Canada). It was recently documented that a proportion of males in the Lake Roosevelt hatchery-origin population have initiated puberty (Webb et al. 2016). The predominant stage of gonadal development in the testicular tissue of these males was pre-meiotic (Stage 2; spermatogonia), however, precocious cysts, which have not yet been described in sturgeon or teleost species, containing spermatozoa (Stage 5) were found in 85% of the total number of biopsies collected from these males. Therefore, we recommend that future histological studies

conducted on sturgeons specify whether the predominant stage of gonadal development or the most advanced stage of gonadal development was identified. Determining the prevalence of precocious cysts and over what time scale precocious cysts may be found prior to the transition into meiotic development across all testicular cysts would directly inform knowledge on reproductive development and improve accuracy when assigning sex and stage of maturity.

In Chapter Three, the fork length, body weight, and absolute growth rate (AGR) was described in hatchery-origin white sturgeon in the LCR, and relative condition factor (K_n) was described in hatchery-origin white sturgeon in the LCR and the transboundary reach of the Columbia River. High variability was found in the size of the hatchery-origin population (fork length (FL) and body weight), similar to other sturgeons (i.e., Welch and Beamesderfer 1995, Ireland et al. 2002, Shuman et al. 2011, Barth and Anderson 2015, Porter and Schramm 2018). The mean AGR of the population was 6 cm/year in FL and was similar to other populations of white sturgeon located in the Columbia River, USA. Assessing fork length-at-age revealed that the trajectory of the regression line in males was lower compared to females within the population which could indicate that the older males were beginning to allocate more resources into gonadal development rather than only somatic growth. This same shift was not observed in the females. However, due to the poor correlation between fork length and body weight-at-age in the hatchery-origin population caution should be taken when interpreting the results of these relationships. When comparing the hatchery-origin fish to other white sturgeon in the LCR, the mean K_n was 1.03 which represents average condition.

However, when comparing the hatchery-origin fish to other white sturgeon in the transboundary reach, the mean K_n was 0.87 which is slightly below average condition. The sex of the fish did not contribute to meaningful differences in size, AGR, or K_n. This finding suggests that determination of sex, which can be time consuming and costly in non-sexually dimorphic species, did not provide additional value for understanding population dynamics in prepubertal fish. Fish with the highest mean K_n were found in Zones 2 and 3. Fish in good condition are more likely to experience faster growth and greater reproductive potential as compared to individuals with below-average condition (Pope and Kruse 2007, Neumann et al. 2012). Therefore, fish in Zones 2 and 3 may be among the first individuals to begin initiating puberty.

Future work should include continued monitoring of the hatchery-origin population in the LCR to track changes in their reproductive structure, size, AGR, and K_n, which could provide insight into the reproductive dynamics within the population. Given the correlation between large size and the onset of puberty in fishes (Amano et al. 1997, Huang et al. 1998, Van Eenennaam and Doroshov 1998, Taranger et al. 2010, Ryu et al. 2013, Nater et al. 2018), larger fish in the hatchery-origin population are likely to be among the first individuals to reach puberty. We suggest that monitoring programs interested in tracking the reproductive structure within a population begin by sampling a suite of age and size classes using endoscopy or histological analysis of gonadal tissue to gain a high-level understanding of the current reproductive structure. Once this information is known, a standardized long-term monitoring program can be developed to track changes in the reproductive structure over time potentially using other tools if their

accuracy is acceptable given the goals of the program. Continued assessment of growth rates within the population can also provide value in assessing the reproductive potential in a population since growth rates shift in fishes as they approach puberty (Taranger et al. 2010, Minte-Vera et al. 2016, Nater et al. 2018). Additionally, further work should be conducted to compare the body weight of hatchery-origin males in Lake Roosevelt to the LCR to define a potential threshold at which puberty is initiated, which has not yet been documented in hatchery-origin sturgeons. Once individuals begin to initiate puberty, assessment of the biological and environmental factors that could contribute to the size and age at puberty can be conducted.

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