AN ABSTRACT OF THE THESIS OF

Allen F. Evans for the degree of Master of Science in Fisheries Science presented on March 14, 2003. Title: Development and Application of Steelhead (*Oncorhynchus mykiss*) Kelt Identification Techniques.

Abstract approved: Redacted for Privacy

Martin S. Fitzpartrick

The migrations upstream of pre-spawned adult steelhead (*Oncorhynchus mykiss*) and downstream of post-spawned steelhead (referred to as "kelts") can geographically and temporally overlap in the Snake River, leading to management challenges for populations listed under the Endangered Species Act (ESA). This thesis describes the development and application of a method to accurately identify kelts using ultrasound, which is viewed as the first step towards development of effective management practices. In order to develop classification criteria for pre- and post-spawn steelhead, ultrasound images of gonads were taken and plasma levels of testosterone (T), 11-ketotestosterone (11-KT), and 17α,20β-dihydroxyprogesterone (DHP) were determined in adult steelhead before and after spawning. Results indicated that ultrasound images provide quantifiable selection criteria – based on the size, number, location, and/or echogeneity of gonads – for the identification of pre-spawned versus post-spawned adults. Pre-spawn females were easily identifiable by the presence of numerous, well-developed eggs. Conversely, only a few mature eggs remained in the body cavity of female kelts. Males were readily identified by the presence of testicular tissue, but determining their maturation status (i.e., pre- or post-spawned) was more difficult compared to females because some males retained gonad mass after spawning. The average cross-sectional testis area in mature males was 2.86 cm^2 (SD = 0.74) relative to 0.62 cm^2 (SD = 0.24) in post-spawned males, demonstrating a statistically significant difference in testis size before and after spawning. Distributions of testis measurements between the two maturational types did not overlap and results of a discriminant function analysis suggest a classification criteria of 1.25 cm^2 could accurately (*ca.* 99%, based on percentiles) distinguish males as pre- or post-

spawned. Concentrations of T, 11-KT, and DHP were significantly higher in pre-spawn males relative to post-spawned males, confirming an association between gonad size and reproductive hormones. Using classification criteria developed from steelhead of known sex and maturation type, ultrasound imaging of adult steelhead at Lower Granite Dam (LGR) juvenile bypass facility revealed that 94.6% of 3,968 adult steelhead sampled from 1 April to 10 June 2000 were kelts. This included approximately 2,050 wild kelts, or about 17% of the entire protected steelhead run above the dam. The majority of kelts examined were female (77.0%) and most were in good overall morphological condition (69.5%), as indicated by the lack of scars, infections, or other morphological damage. Based on the abundance of kelts at LGR, management initiatives aimed at improving the repeat spawning rate of steelhead may be considered an important part of a comprehensive plan to rebuild ESA-listed steelhead populations throughout the Snake River basin.

Development and Application of Steelhead (*Oncorhynchus mykiss*) Kelt Identification Techniques

by

Allen F. Evans

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented March 14, 2003
Commencement June 2003

Master of Science thesis of Allen F. Evans presented on March 14, 2003.

APPROVED:

Redacted for Privacy

Major Professor, representing Fisheries Science

Redacted for Privacy

Head, Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Allen F. Evans, Author

ACKNOWLEDGMENTS

I sincerely appreciate the assistance of Rebecca Kalamasz, Rex Baxter, and Mike Halter of the U.S. Army Corps of Engineers (USACE). I would also like to thank Dr. Alfred Caudle, University of Georgia, College of Veterinary Medicine, for helping to select the appropriate ultrasound equipment and for providing instruction for its initial use. I would like to thank Gerry Rowan (Confederated Tribes of the Umatilla Indian Reservation), Greg Davis (Oregon Department of Fish and Wildlife) and Joe Blodgett (Yakama Nation) for graciously allowing me to use their hatchery facilities to validate and train with the ultrasound. I would like to thank the Columbia River Inter-Tribal Fish Commission (CRITFC) for their support during my education at Oregon State University. Lastly, the views and interpretation of data presented in this thesis represent those of the author and not necessarily those of the USACE, CRITFC, or Oregon State University.

CONTRIBUTION OF AUTHORS

Ms. Lisbeth Siddens provided the training and logistical support necessary to conduct the radioimmunoassay research associated with Chapter 1. Mr. Roy Beaty was involved with the study design and writing of Chapter 2.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

Figure | Page

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF APPENDIX TABLES

Development and Application of Steelhead (*Oncorhynchus mykiss*) Kelt Identification Techniques

GENERAL INTRODUCTION

Populations of wild steelhead *(Oncorhynchus mykiss)* have dramatically declined from historical levels in both the Columbia and Snake rivers. Steelhead are now listed as endangered in the upper Columbia River and threatened in the Snake River under the Endangered Species Act (NMFS 1997). Causes of the declines are numerous and well known, with passage at mainstem hydroelectric dams being a significant source of mortality during migrations (Raymond 1979, Budy et al. 2002).

Mitigation for dam passage mortalities to salmonids in the Columbia Basin has focused on aiding outmigrating juveniles and upstream-migrating adults of anadromous salmon and steelhead. But unlike Pacific salmon, steelhead are iteroparous (i.e., capable of repeat spawning) and conventional management practices have paid little attention to downstream-migrating post-spawn adult steelhead (referred to as "kelts"). Although overlooked, kelts may be an important genetic resource for rebuilding endangered steelhead populations in the Columbia River Basin.

Repeat spawning steelhead have been documented returning to their natal streams in the Snake and Columbia rivers for decades (Long and Griffin 1937). In the 1950's, scale analysis of Clearwater River (a major tributary of the Snake River) steelhead indicated a repeat spawning rate of approximately 2% - 4% (Whitt 1954). Rates averaging 1.6% have been documented for wild steelhead populations in the Yakima River (Hockersmith et al. 1995) and 3.0% in the Klickitat River (Busby et al. 1996). At the maximum, estimates of repeat spawners in tributaries of the lower Columbia River have exceeded 17.0% (Leider et al. 1986), with individual fish spawning up to four consecutive times.

More kelts from upstream populations would survive to spawn again if they were 1) protected from turbines by increased spill, 2) collected like juveniles and transported around the dams, and/or 3) captured and reconditioned in captivity. If hydroelectric facilities hinder kelt outmigration by prohibiting safe passage, then modifications to mainstem dams that would provide safer and quicker passage of kelts (e.g., increased spill or modifying bypass structures to specifically accommodate adult passage) would increase returns of repeat spawners. Transporting kelts around mainstem dams may also mitigate losses by allowing a larger proportion of the run to reach the ocean and potentially undergo gonad recrudescence. Lastly, reconditioning of iteroparous species has been achieved with arctic charr *Salvelinus alpinus* (Boyer and Toever 1993), Atlantic salmon *Salmo salar* (Johnston et al. 1990, Crim et al. 1992, Moffett et al. 1996) and steelhead (Wingfield 1976) and would also augment iteroparity rates. Reconditoning is the process of culturing post-spawned fish so that they survive, grow, and develop mature gonads. The latter two options for protection of kelts include collecting/capturing kelts, which would require preventing pre-spawners (i.e., mature adults) from being mistaken for kelts and inadvertently diverted away from their spawning migration.

In order to improve survival of outmigrating kelts, it is imperative that methods be developed to accurately differentiate pre-spawn and post-spawn steelhead. In the context used here, the term pre-spawner refers to any fish that is undergoing gonad development or has reached maturity. Conversely, the term kelt refers to any fish that has completed spawning. Kelts moving downstream though the dams are co-mingled with pre-spawners that are coincidentally falling back downstream, and biologists are not sure how to distinguish between the two maturation types. Some pre-spawn steelhead over-winter below mainstem dams (Whitt 1954, Robards and Quinn 2002) and complete their migration to spawning tributaries in March, April, and May when some post-spawners are already moving back downstream as kelts. Fallback is common among pre-spawners at mainstem dams in the Columbia River Basin (Bjornn et al. 2000). Efforts have been made at the dams to enumerate the number of kelts and pre-spawners being removed from each dam's collection system. The approach used has consisted of subjective visual methods that assumed kelts are dark fish in poor morphological condition and that pre-spawners are bright fish in good condition; however, this approach was initiated without any validation. Thus, considerable doubt exists whether unvalidated visual methods – used by the Army Corps of Engineers or by anyone else – will be sufficient to accurately avoid mistaking some pre-spawners as kelts (R. Baxter, USACE Biologist, pers. com.).

Ultrasound imaging offers a rapid and non-invasive alternative method to identify maturation status of adult steelhead removed from juvenile bypass facilities at the dams. Ultrasound imaging has been used to accurately assess both the sex and maturation status of various salmonid species (Martin et al. 1983, Arkush and Petervary et al. 1998). Ultrasound has been used to distinguish immature from mature specimens based on the maximum diameter of the gonad (Blythe et al. 1994, Arkush and Petervary 1998). Fish with large, well-developed gonads are readily identifiable as being mature relative to the small gonads in immature specimens. Despite its ability to identify sex and maturation, an ultrasound approach has yet to be reported for distinguishing pre- and post-spawned steelhead or other iteroparous salmonids. One difficulty with assessing an ultrasound approach with salmonids that are listed as threatened or endangered is the ability to have independent verification of the method. Threatened or endangered steelhead cannot be readily sacrificed following ultrasound imaging in order to determine the stage of gonadal development. Therefore, other non-lethal sampling approaches should be employed to test ultrasound.

Similar to ultrasound imaging, concentrations of reproductive hormones have also repeatedly and successfully been used as indicators of maturation status in salmonid species (Schreck 1972, Scott et al. 1980, Baynes and Scott 1985, Fitzpatrick et al. 1986). As gonads develop during maturation, reproductive hormones follow predictable patterns of changing concentrations within the blood plasma. If steroids associated with reproductive development differ significantly among fish of varying maturational states, and if gonad diameter is a good indication of maturational status, then plasma steroid levels may strongly corroborate ultrasound data. Thus, reproductive hormones could provide an independent assessment of ultrasound imaging of gonads.

The primary goal of this research was to develop effective non-lethal techniques for determining maturation status in adult steelhead removed from juvenile bypass facilities at hydroelectric dams in the Snake River. Comprehensive morphological data were collected and analyzed in conjunction with the ultrasound results to further develop, validate and refine visual identification methods. Improved kelt identification methods will aid in the enumeration and management of kelts from the Snake River, which is a useful first step in improving the survival of kelts and perhaps restoring imperiled steelhead populations from the Columbia River Basin.

Specifically, the primarily objectives of this research were:

1. To develop ultrasound imaging techniques to identify maturational status in adult steelhead and verify the results using concentrations of plasma steroids (Chapter 1).

2. To estimate the abundance of kelts at a Snake River bypass facility using ultrasound imaging techniques (Chapter 2).

3. To assess the accuracy of visual kelt classification traits used by U.S. Army Corps of Engineers personnel (Chapter 2) and to determine which traits might statistically be associated with maturational status in adult steelhead (Appendix).

Ultrasound Imaging as a Potential Identification Tool for Determining Maturation Status in Adult Steelhead (*Oncorhynchus mykiss*) Before and After spawning

Allen F. Evans, Martin S. Fitzpatrick, and Lisbeth K. Siddens

ABSTRACT

The goal of this study was to develop a rapid, non-invasive, and accurate method to distinguish pre-spawn or maturing adult steelhead from post-spawned steelhead (referred to as "kelts"). Ultrasound images of gonads were collected and plasma steroid levels were determined for testosterone (T), 11-ketotestosterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP) from adult steelhead before and after spawning. Results demonstrated that ultrasound images provide quantifiable selection criteria – based on the size, number, location, and/or echogeneity of gonads – for the identification of mature versus post-spawned adults. Mature females were easily identifiable by the presence of numerous, well-developed eggs. Conversely, only a few mature eggs remained in the body cavity of female kelts. Ultrasound investigation of the maximum testis area (cm^2) demonstrated that testes undergo substantial size changes following spawning. Mean maximum testis area in mature specimens was 2.86 cm^2 whereas it was 0.62 cm^2 in post-spawned males. Distributions of testis measurements between the two maturational types did not overlap with one another and results of a discriminant function analysis suggested a classification criteria or threshold of 1.25 cm^2 could accurately distinguish pre-spawned males from post-spawned males. Concentrations of T, 11-KT, and DHP were significantly higher among mature males relative to post-spawned fish, providing an independent assessment of the ultrasound technique. In mature males, median levels of T, 11-KT, and DHP were 49.6 ng/ml, 78.4 ng/ml, and 13.0 ng/ml relative to < 1.2 ng/ml, 6.6 ng/ml, and 1.6 ng/ml for kelts, respective for each steroid. Despite the high degree of corroboration between ultrasound measurements of gonad size and plasma steroid levels, application of ultrasound imaging may result in some classification errors (a few percent), primarily among male steelhead.

INTRODUCTION

Steelhead (*Oncorhynchus mykiss*) differ from most other anadromous Pacific salmonid species by being iteroparous. The upstream migration of maturing or pre-spawn adult steelhead and downstream migration of post-spawned steelhead (referred to as "kelts") can geographically and temporally overlap in the Snake River (Whitt 1954). The majority of adult summer-run steelhead (so-called "freshwater maturing"; Busby et al. 1996) navigate Snake River dams in September and October but do not spawn until the subsequent spring (Robards and Quinn 2002). A sizable portion of the steelhead run over-winters in the Snake River, before traveling to their natal stream for spawning (Whitt 1954). Following natural spawning, surviving individuals must emigrate back to the Pacific Ocean – a distance of over 700 river kilometers – if they are to successfully undergo gonadal recrudescence, which is a process necessary to spawn a second time. Thus between March and May, some pre-spawned fish move to their spawning grounds as kelts leave their spawning grounds enroute to the Pacific Ocean.

Each spring, thousands of adult steelhead – many of which are now listed as endangered (NMFS 1997) – are observed in the juvenile bypass facilities at Snake River dams. Bypass facilities in the Columbia Basin are designed to divert migratory and resident fish around hydroelectric facilities via large screens that partially block turbine intakes (Muir et al. 2001). Although bypass facilities were specifically designed for the non-turbine passage of juvenile salmonids, adult salmonids that "fallback" over the dam (term used to describe fish that initially passed the dam via fishways but subsequently fall back downstream) and downstream migrating adults (e.g., kelts) are also encountered in the collection areas of these juvenile bypass facilities each spring. From 1996 to 2000, a mean number of 5,050 adult steelhead (ranging from 4,182 to 6,504) were counted in the Lower Granite Dam juvenile bypass facility (located 694 river kilometer from the Pacific Ocean) between late March and June of each year (USACE 1996 - 2000). Currently, there is no reliable method to distinguish a pre-spawn adult steelhead that has fallen back over the dam from a downstream migrating post-spawned steelhead (Rex Baxter, USACE Biologist, pers. comm). Because steelhead kelts might be a valuable resource for rebuilding depleted runs on the Snake River and elsewhere, fisheries researchers need access to a rapid, non-invasive and accurate way to distinguish pre-spawn steelhead from post-spawned kelts.

One potential method of identifying maturational status among adult steelhead is ultrasound. Ultrasonic waves – acoustic energy measured in megahertz – produce images of the size, shape, and location of soft tissues (Martin et al. 1983) within biological organisms. Ultrasound technology is an identification method that has been used – albeit not widely – since the early 1980s to determine the sex and maturation status of various freshwater and marine fishes (Martin et al. 1983, Reimers et al. 1987, Shields et al. 1993, Blythe et al. 1994, Arkush and Petervary 1998). When operators are properly trained and know specimen anatomy well, ultrasound has the potential to be a highly accurate non-invasive diagnostic tool in fisheries science. For example, ultrasound studies conducted by Reimers et al. (1987) on adult rainbow trout (*O. mykiss*) were able to distinguish the sex of specimens 5 months prior to spawning with 100% accuracy. Similarly, an ultrasound examination conducted on striped bass (*Morone saxatilis*) was able to identify the sex of fish with 100% accuracy throughout the entire reproductive cycle (Blythe et al. 1994). In addition to sex identification, ultrasound can also be used to distinguish immature from mature specimens based on the maximum diameter of the gonad (Blythe et al. 1994, Arkush and Petervary 1998). Fish with large, well-developed gonads were readily identifiable as being mature relative to the small gonads in immature specimens. Even fish as small as the Pacific herring (*Clupea harengus pallasi*) can be segregated by sex and maturational status with ultrasound (Bonar et al. 1989). Despite its use in both identifying sex and maturation, ultrasound has yet to be reported in the identification of post-spawned maturation status in steelhead or in other iteroparous salmonid species.

A potential obstacle to using ultrasound to identify post-spawned fish relates to the varying size of gonads following spawning. For example, if a male fish retains substantial gonad mass after spawning, then it may be difficult to distinguish a pre-spawn fish from a post-spawned fish. However, studies of gonad development and regression in salmonids provide information to suggest that the physical size of gonads is different among maturation stages. Although the duration of spermiation in salmonids is long-lived (Gjerde 1984, Munkttrick and Moccia 1987), the physical size of testes undergoes wide seasonal variation (Billard 1983). Large variation in testes size has been attributed to the cyclical expansion and contraction of interstitial cells (Billard 1983), the loss of mass via ejaculation (Munkttrick and Moccia 1987), and the subsequent degeneration and loss of dead Leydig cells (Cauty and Loir 1995). Therefore, despite the long duration of spermiation in male steelhead, changes in gonad size (i.e., a decrease) should be detectable with ultrasound imaging.

Along with changes in the gonad size, plasma hormone levels also vary throughout gonad development and regression, and may be used as an indicator of maturation status (Schreck 1972, Scott et al. 1980, Baynes and Scott 1985, Fitzpatrick et al. 1986). For example, the steroid hormones testosterone (T), 11-ketotestosterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP), which are produced in the gonads and circulate within the blood system (Bone et al. 1995), are correlated with sperm formation and production in male rainbow trout (Campbell et al. 1980, Scott et al. 1980, Baynes and Scott 1985, Vizziano et al. 1996). T, 11-KT, and DHP levels increased steadily with the amount of spermatozoa being produced in three different strains of rainbow trout studied by Baynes and Scott (1985). Following spermiation, the steroid levels dramatically declined in all three strains (Baynes and Scott 1985). Vizziano et al. (1996) demonstrated that plasma concentrations of DHP were positively correlated to the volume of milt produced in male testes. In a similar study of steroid concentrations, Fitzpatrick et al. (1986) recorded higher levels of DHP in milt producing coho salmon (*O. kisutch*) relative to non-milt producing males.

Although the complete effects of theses hormones during reproductive development are complex and only partially understood (Vizziano et al. 1996), they are clearly associated with the maturation process. If blood steroid levels of T, 11-KT, and DHP differ significantly among fish of varying maturational states, and if gonad diameter is a good indication of maturational status, then plasma steroid levels may strongly corroborate the ultrasound data. Thus, reproductive hormones could provide an independent assessment of ultrasound imaging of gonads. For example, male steelhead with large testes may also have elevated concentrations of steroids and vice versa. If these steroid concentrations were measured in fish of known maturational status and were found to be positively correlated with gonad size – as determined by ultrasound imaging – then these results would help confirm the accuracy of ultrasound classification.

The primary goal of this study was to develop criteria to identify pre- and post-spawned maturational status for steelhead based on gonad size and determine if plasma steroid levels corroborate the ultrasound data. If quantifiable differences among maturation types emerged, fisheries research would have access to a non-invasive, rapid, and accurate tool to use both in the field and in the laboratory environment. Perhaps the greatest need for such a method is at Snake River hydroelectric facilities. However, the development of rapid, non-invasive maturation identification techniques has broad applications for researchers, natural resource managers, and aquaculturists. Lastly, numerous authors have reported plasma hormone concentrations in non-migratory, captive stocks of *O. mykiss* (Scott et al. 1980, Campbell et al. 1980, Baynes and Scott 1985, Scott and Sumpter 1989, Holloway et al. 1999); however, hormone levels in anadromous forms of *O. mykiss* have not be reported in the literature and are described in the present study.

MATERIALS AND METHODS

A portable Aloka® SSD-500v ultrasound machine, equipped with a 7.5 MHz linear probe, was used to examine the gonads of pre- and post-spawned steelhead. The 7.5 MHz probe or transducer is capable of imaging up to 9 cm of tissue depth with a horizontal plane of 4 cm. The examination consisted of gently placing the ultrasound probe along each fish's abdomen and then moving the probe along the abdominal surface until the gonads were located. For females, the presence or absence of eggs within the body cavity were noted. For males, the maximum cross-sectional area (cm^2) of the single largest testis within the ultrasound's range was measured within the body cavity. Precise measurements of the testis were taken using an elliptical trackball function that is commonly found on ultrasound units. Once the gonads were located and a clear ultrasound image obtained, pictures from each fish were recorded on a standard diskette using a Mavicap® digital adaptor and stored for later viewing.

A preliminary test with ultrasound was conducted at a Columbia Basin steelhead hatchery in March of 1999, i.e., Minthorn Hatchery located on the Umatilla River (465 Rkm from the mouth of the Columbia River). The purpose of the preliminary trial was to become familiar with the ultrasound as a diagnostic tool and to evaluate various examination techniques and protocols. In total, 12 steelhead (6 females and 6 males) were examined with ultrasound before and after artificial stripping of the eggs and milt. Following artificial spawning, autopsies were conducted to correlate anatomical structures (e.g., heart, liver, pyloric caeca, spleen, and gonad) with ultrasound images.

In 2000, researchers focused on males to both confirm the limited number of observations in 1999 and to develop a more comprehensive database. Adult steelhead were again sampled from the Umatilla River with additional samples from Wallowa hatchery on the Wallowa River (293 Rkm from the mouth of the Snake River; a major tributary of the Columbia River) and from Prosser hatchery on the Yakima River (539 Rkm from the mouth of the Columbia River). Steelhead returning to the Wallowa River (WR) and Umatilla River (UR) were examined with ultrasound just prior to artificial stripping on 29 March and 4 April 2000, respectively. All steelhead examined at WR and UR facilities produced milt via a gentle stripping of the abdominal cavity and were thus considered spermiating. Steelhead examined via ultrasound from the Yakima River (YR) were from a sub-sample of naturally post-spawned individuals being held captive for a kelt reconditioning experiment. Unlike the mature specimens examined at WR and UR, post-spawned YR fish did not produce milt upon gentle stripping of the abdomen and were examined during the period spanning 27 March to 18 May 2000. In addition to the naturally post-spawned males examined from the YR, autopsies were conducted on 7 kelts (3 males and 4 females) that died during the reconditioning experiment on 15 June 2000.

Adult steelhead awaiting artificial spawning at UR (n=13) and WR (n=42) hatcheries were killed prior to sampling, while specimens removed from YR (n=34) were transferred via dipnet to a nearby 190-L sampling tank containing fresh river water, where they were anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 parts-per-million and released into the reconditioning tanks following ultrasound examination. Fork-length data was also collected from all specimens. Ultrasound examination time, including the time needed to anesthetize specimens from the YR, was approximately 4 minutes per fish.

For plasma hormone levels, a blood sample was removed from the caudal vein of each male (UR n=12, WR n=15, YR n=13) using a Vacutainer containing heparin to prevent coagulation. Blood samples were kept on ice until the plasma could be separated by centrifugation and stored at -70^0C. All plasma samples were analyzed for testosterone (T), 11-ketotesterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP) by radioimmunoassay using procedures of Fitzpatrick et al. (1986). All plasma samples were assayed in duplicate and intra-assay coefficient of variation was calculated for all duplicates. Coefficients of variation in excess of 10% (n= 3 out of 120) were considered contaminated and removed from the data set. Average extraction efficiency for all three steroid measured was 88% with a minimum assay detection threshold of 1.2 ng/ml, 1.1 ng/ml, and 0.7 ng/ml for T, 11-KT, and DHP, respectively. Intra-assay coefficient of variation was below 3% for all three steroids. Reported steroid levels were corrected for extraction efficiencies.

Ultrasound data on maximum testis cross-sectional area (cm^2) from males were compared between sample populations and maturational types using two sample *t*-tests. Simple linear regression was used to correlate testis area with fork-length. Discriminant function analysis (DFA) was used to develop a testis size classification rule based on the variation of testis area measurements observed between maturational types. DFA develops a classification criterion or rule for observations based on measures of squared distance (Khattree and Naik 2000). The variance-covariance matrices between mature and post-spawned groups were not considered equal (p-value < 0.01, chi-square), and therefore, a quadratic discriminant function analysis was used to compare testis measurements (Khattree and Naik 2000). Percentiles of the standard normal distribution were examined to assess potential error associated with ultrasound classification. Critical values of the standard normal distribution, mean testis values, and standard deviations obtained from the mature and post-spawned samples were used to assess potential error for each group independently.

Distributions of the concentrations of steroid levels were non-normal in mature steelhead due to the presence of outliers and to concentrations from post-spawned individuals often being below the assay detection level. Thus, steroid levels were compared using a distribution free and outlier sensitive test via the Wilcoxon rank-sum procedure (Ramsey and Schafer 1997). Classification criteria for hormone plasma concentrations were again calculated using DFA, in cases where quantifiable levels of hormones were detected (i.e., those within the standard curves). Graphical normality checks (data not shown) suggested the steroid data were not suitable for parametric discriminant analysis (linear or quadratic) and a nonparametric approach (*k*-nearest-neighbor method) was utilized with the natural log of steroid concentrations used for discrimination (Khattree and Naik 2000). A cross-validation method was used in DFA – which reduces model bias by removing each sample from the data set before classification – that allowed estimates of classification error to be generated from the model. In the case of non-parametric DFA, error rates are not dependent on assumptions of normality but are calculated from a density estimation-based approach (Khattree and Naik 2000). Simple linear regression was used to correlate testis area with log transformed steroid concentrations. All statically tests were run using SAS® version 8. Statistical significance was set at $p < 0.05$, although exact p values are reported where available.

RESULTS

Ultrasound Examination

The region just posterior to the pectoral fins provided the most diagnostic images of testes or ovaries. However, other locations along the abdomen (e.g., just posterior to the pelvic fins) also provided useful diagnostic images. In some circumstances, more than one area within the fish's body cavity had to be examined before diagnostic images could be obtained. The imaging of visceral organs (e.g., liver, spleen, pyloric caeca, and gallbladder) just posterior to the transverse septum served as "landmarks" for locating the gonads. Identification of these visceral organs was an important first step in making gonad measurements because these structures are always present, regardless of a fish's sex or reproductive development. Each fish's swim bladder consistently blocked ultrasound images near the posterior end of the gonad, as acoustic waves reverberated off the swim bladder distorting ultrasound imaging. Fortunately, the majority of the gonad resides anterior to the swim bladder and thus was able to be measured.

Results of autopsies from the seven kelts that died during the reconditioning experiment at Prosser hatchery demonstrated that the testes (< 0.20 cm^2) of post-spawned fish were indiscernible from the fish's midgut and surround pyloric caeca due to the gonads' small size and atrophied properties. Autopsies of post-spawned females revealed the presence of new oocytes, often located with a few remnant over-ripe eggs. Similar to post-spawned testes which measured less than 0.20 cm^2, the new pair of female ovaries could not be distinguished via ultrasound imaging because of their small size (oocyte diameter of approximately 1 mm).

Pre-spawn females were easily distinguished from freshly post-spawned females by the presence or absence of a mature egg mass. The ultrasound was sensitive enough and provided such high-resolution images that individual eggs could easily be distinguished within the female body cavity (Figure 1.1). The egg mass in pre-spawn steelhead was so dominant that visceral organs were often obstructed from view by the numerous, well-developed eggs. Conversely, post-spawned females could be characterized by the presence of few remnant eggs within the body cavity, often lodged just underneath the abdominal muscle tissue or anterior to the urogenital papilla (an appendage used to extrude eggs during spawning). Eggs were often uniform in size and with a diameter of approximately 0.30 cm.

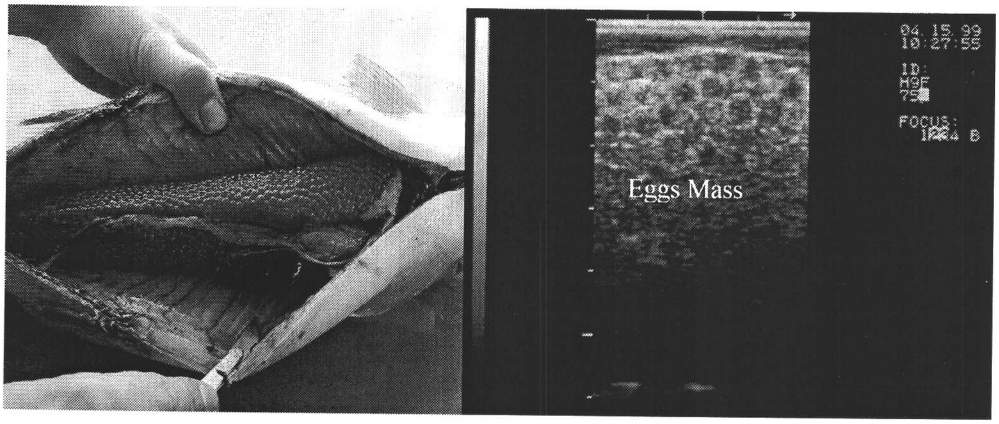

Figure 1.1: A picture of a pre-spawn female steelhead (left) and a cross-sectional ultrasound image (right), showing the tightly packed egg mass of a pre-spawner. Egg membranes are hyperechoic and produce white rings around the darker hypoechoic egg contents. Ultrasound image is made with the specimen inverted.

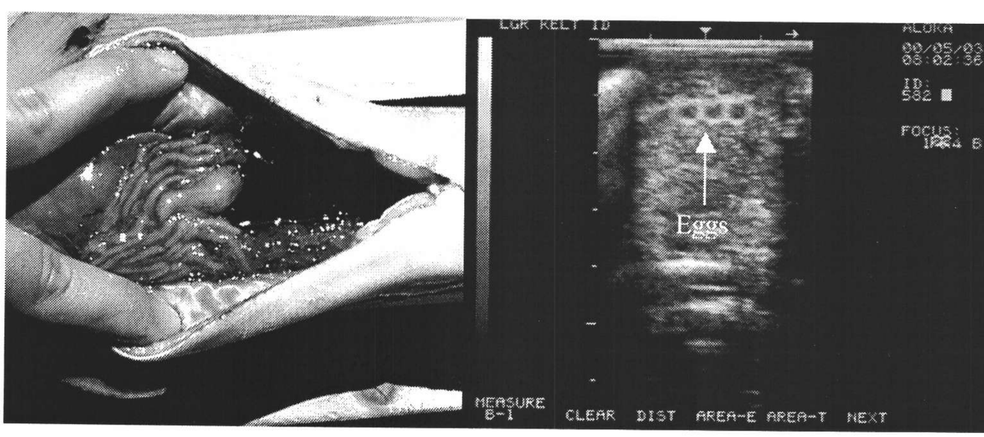

Figure 1.2: A picture of a post-spawned female steelhead (left) and a cross-sectional ultrasound image (right), illustrating three remnant eggs just underneath the specimen's abdominal muscle tissue. Egg membranes are hyperechoic and produce white rings around the darker hypoechoic egg contents. Ultrasound image is made with the specimen inverted.

Unlike female steelhead, some males retain substantial gonad tissue following spawning. An examination of the images from male gonads between the two maturation types revealed two trends: differences in maximum testis area and differences in gonad echogeneity. Echogeneity refers to the acoustic properties of tissues and is depicted on the ultrasound monitor by different black and white color shades. The gonads of pre-spawn steelhead appear more hypoechoic (dark) relative to the hyperechoic (white) gonads of post-spawned fish. Ultrasound images from a mature and post-spawned male help to illustrate the typical testis size difference and echogeneity difference among maturation types encountered during this study (Figure 1.3 and 1.4).

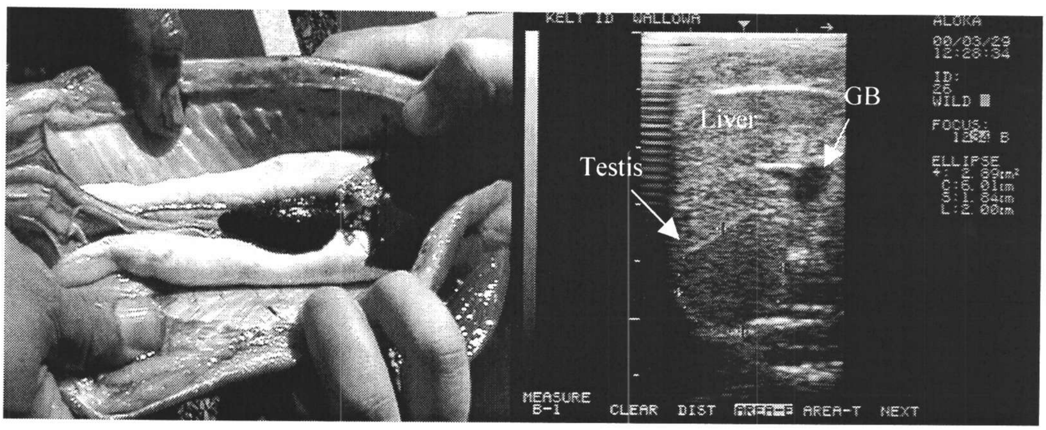

Figure 1.3: A picture of male pre-spawn anatomy (left), testes are the two dominant lobes, and a cross-sectional ultrasound image (right) from a male pre-spawn steelhead. Testis appears as a grayish (hypoechoic), elliptical mass in the center of the image and is highlighted by an arrow. The fish's liver and fluid-filled gallbladder (GB) are also depicted. Testis measurement (2.89 cm^2) is shown along the right margin of the ultrasound image.

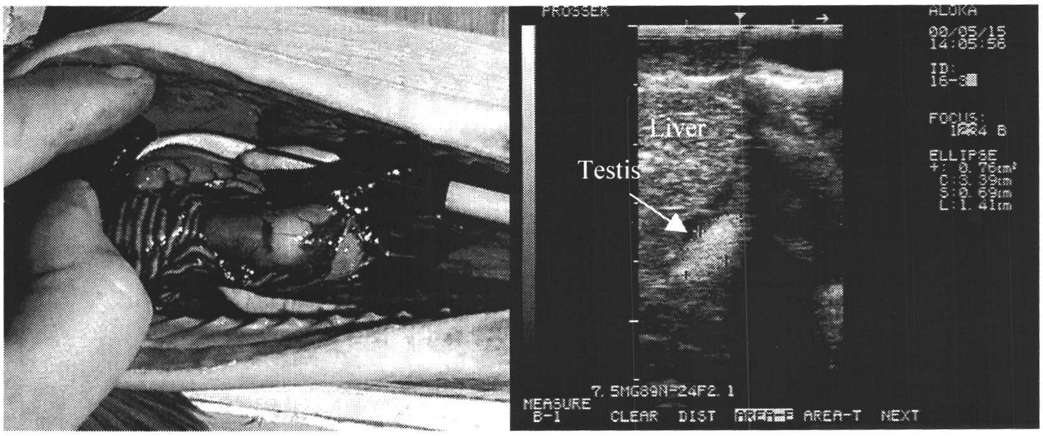

Figure 1.4: A picture of post-spawned male anatomy (left), testis indicated near the tip of a wood-handled probe, and a cross-sectional ultrasound image (right) from a post-spawned male steelhead. Testis appears as a white (hyperechoic), elliptical mass in the center of the image and is highlighted by an arrow. Testis measurement (0.76 cm^2) is shown along the right margin of the ultrasound image.

Investigations of the maximum testis area in 55 pre-spawn and in 34 post-spawned steelhead demonstrated that testes undergo substantial size changes following spawning (Figure 1.5). Testis area measurements were normally distributed and the two groups did not overlap in testis size measurements (Figure 1.5). There was no evidence to suggest testis size among pre-spawn samples (WR versus UR) were significantly different from one another (p-value = 0.25, t-test) but highly significant differences were detected between mean pre-spawn testis area and mean post-spawned (YR) testis area (p-value $<$ 0.001, t-test). Mean testis size (and standard deviation; SD) among post-spawn steelhead was 0.62 cm^2 (SD 0.24) compared to a mean of 2.86 cm^2 (SD 0.73) in mature males. Testis area within the body cavity of mature steelhead was 2.24 cm^2 greater than that of post-spawned steelhead (95% confidence interval: 1.96 to 2.51 cm^2 greater).

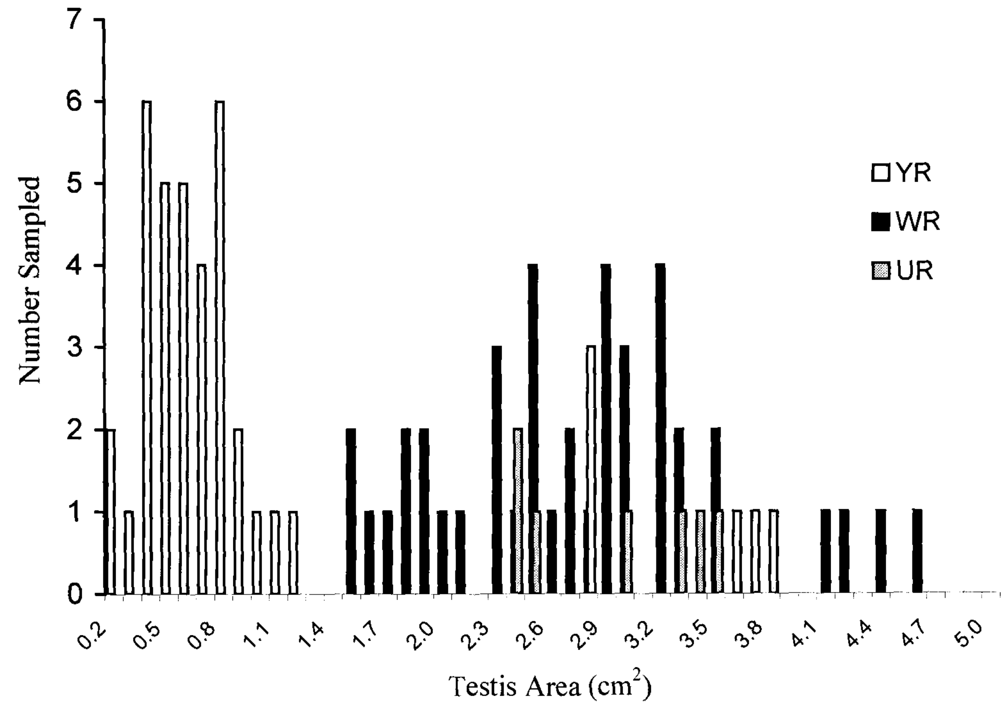

Figure 1.5: Maximum testis area between post-spawned (YR) and mature (WR and UR) steelhead. Measurements of testis size were based on ultrasound examination and represent the largest testis area found along the length of the gonad.

Linear regression analysis of maximum testis area and fork-length provides evidence of a positive association between fish length and ultrasound measurements of gonad size among mature males (p-value =0.01 for the test that the slope differs from zero, with r^2 =0.18). For every 10 cm increase in fork-length there was a 0.63 cm^2 increase in maximum testis area (95% confidence interval; 0.32 to 0.95 cm^2). However, no evidence of an association between fish length and ultrasound measurements of testis area was found for post-spawned steelhead (p-value=0.56 for the test that the slope differs from zero, with r^2= 0.01).

Based on results from a discriminant function analysis, generalized squared distances between maturation types were calculated at -2.8621 and -0.6402 for post-spawned and mature samples, respectively, demonstrating a high degree of separation. Based on result of a quadratic discriminant function analysis, the classification rule or threshold for assigning individual observations was calculated at 1.25 cm^2, which predicted that all males having testis area greater than or equal to 1.25 cm^2 were pre-spawners and males with testis area less than 1.25 cm^2 kelts. An examination of the proportion of a normal distribution that extended beyond a given testis size suggested that testis measurements between the two maturation groups had the potential to overlap and thus, future ultrasound measurement from a sample of unknown spawning types may result in some (a few percent) misclassifications (Table 1.1). The degree of classification confidence will depend on the cost of misclassification. For example, if a testis classification rule of 1.55 cm^2 were used to classify pre-spawners, 0.01 or 1% of a normally distributed sample is expected to be less than 1.55 cm^2 (Table 1.1). Conversely, this same classification rule would result in a misclassification error rate of only 0.0001 for post-spawners (Table 1.1). According to the standard normal distribution, adoption of the discriminant function rule of 1.25 cm^2 would result in an estimated error rate of 0.0041 for post-spawners and 0.0044 for mature specimens, minimizing the cost of error between the two groups (Table 1.1).

Table 1.1: Proportion of pre- and post-spawned male steelhead estimated to lie outside the standard normal distribution based on different testis size classification criteria.

Testis Classification Criteria	Proportion Post-spawned Misclassified	Proportion Mature Misclassified
1.05 cm^2	0.0351	0.0024
1.15 cm^2	0.0136	0.0033
1.25 cm^2	0.0041	0.0044
1.35 cm^2	0.0011	0.0057
1.45 cm^2	0.0002	0.0078
1.55 cm^2	0.0001	0.0104
1.65 cm^2	< 0.0001	0.0133
1.75 cm^2	< 0.0001	0.0170

Plasma Hormone Profiles

Measurements of T, 11-KT, and DHP demonstrated that male steelhead possess significant differences in plasma hormone levels before and after spawning (Figure 1.6). All three steroid hormones measured in post-spawned samples were significantly lower than those from pre-spawn samples (p-value < 0.001, Wilcoxon for T, DHP, and 11-KT comparisons). Plasma concentrations among mature males were highly variable, with T levels ranging between 11.6 to 78.7 ng/ml, 11-KT levels ranging from 25.7 to 172.7 ng/ml, and DHP levels ranging from 2.8 to 54.2 ng/ml (Figure 1.6). Despite the individual differences in plasma hormones found within pre-spawn fish, values were substantially higher than those found in post-spawned male steelhead. Plasma levels in post-spawn fish were often below the minimum detection assay threshold (e.g., T) and only trace amounts of 11-KT and DHP could be detected in fish following spawning (Figure 1.6). Of the three steroids measured, only concentrations of DHP were found to have over-lapping values within the two maturational categories, with one pre-spawn individual having a value typical of those within the post-spawned group (Figure 1.6).

Median testosterone levels among pre-spawn males were 46.2 ng/ml and 50.7 ng/ml for WR and UR samples, respectively, and were not significantly different from one another (p-value = 0.68, Wilcoxon). Testosterone levels from post-spawned fish were below the minimum detection level of the assay and were at least < 1.2 ng/ml. Median levels of DHP for mature fish were calculated at 16.0 and 10.0 ng/L from WR and UR samples, respectively, and differed significantly from one another (p-value=0.02, Wilcoxon). Similar to testosterone levels, DHP levels from the 13 post-spawn fish were often below the assay detection level (*ca.* 0.7 ng/ml) and median level was calculated at 1.6 ng/ml. Median levels of 11-KT for pre-spawn fish from WR and UR (116.0 and 65.5 ng/ml, respectively) were significantly different from each other (p-value=0.01, Wilcoxon). Median concentrations of 11-KT from post-spawned samples were 6.6 ng/ml and all 13 measurements were within the limits of the assay's standard curve (Figure 1.6).

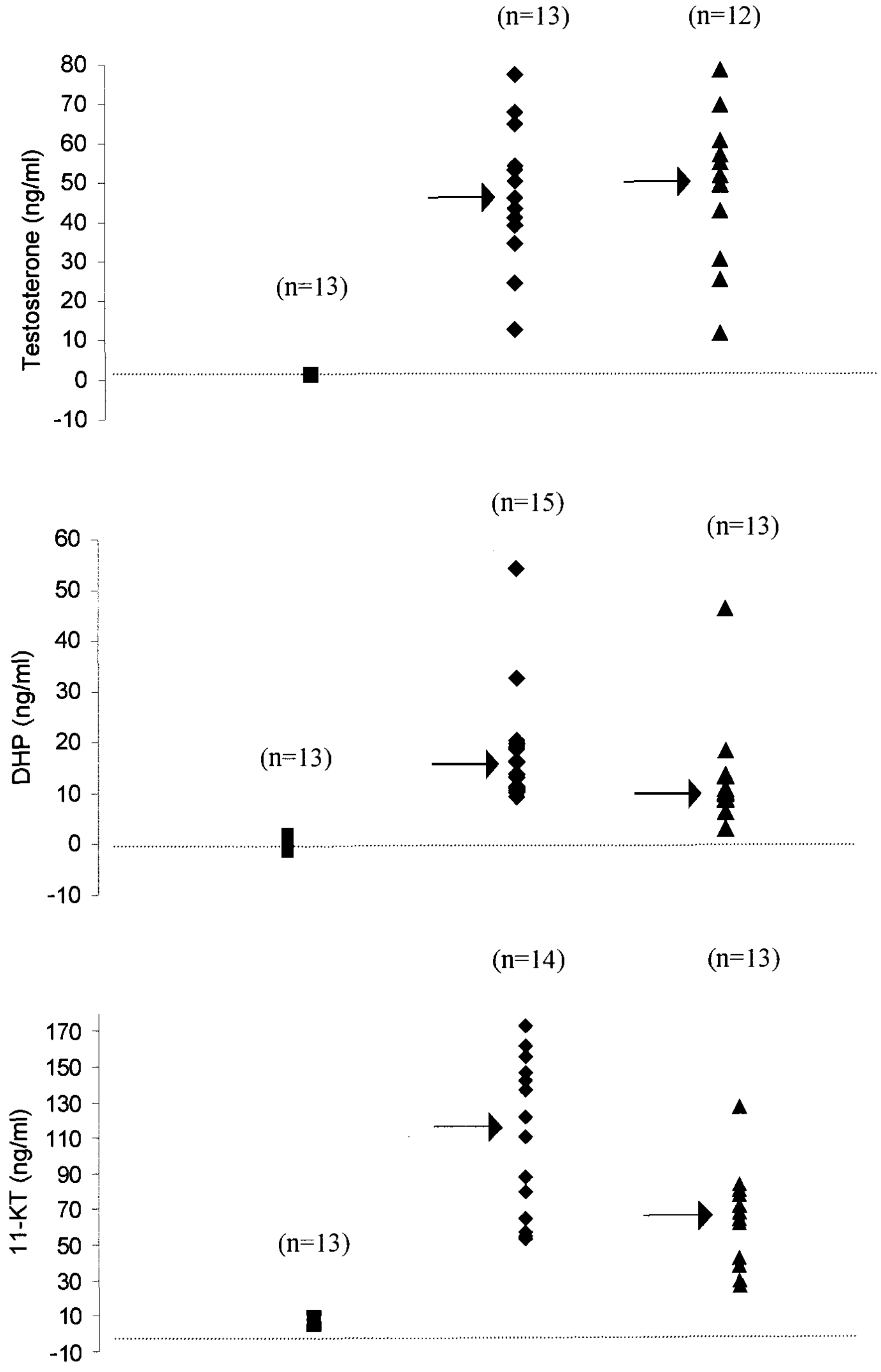

Figure 1.6. Plasma concentrations of testosterone, 17α-hydroxy-20β-dihydroxyprogesterone (DHP), and 11-ketotesterone (11-KT) from Yakima River (YR = ■) post-spawned steelhead and Wallowa River (WR= ♦) and Umatilla River (UR = ▲) mature steelhead. Median values are marked by arrows and the minimum assay detection threshold for each steroid is denoted by a dotted line. Sample sizes (n) are provided.

Nonparametric DFA analysis of the steroid data showed a high degree of separation between steroid levels based on different maturational states. The classification rule or threshold for assigning individual observations was calculated at 15.3 ng/ml and 5.3 ng/ml for 11-KT and DHP, respectively. DFA was not conducted on testosterone levels because all post-spawned fish were below the assay detection threshold (*ca.* 1.2 ng/ml); however, a minimum gap of at 10.3 ng/ml existed between kelt and pre-spawn testosterone concentrations. Cross-validation techniques in DFA resulted in a 100% (35/35) discrimination of 11-KT concentrations between the two maturation types examined, with a minimum gap of 20.7 ng/ml. Cross-validation assignment resulted in 0.02 (1/39) estimated error for DHP samples, with one pre-spawn individual being erroneously classified as a post-spawned fish. With this DHP concentration excluded, the minimum gap between pre-spawner and kelt DHP concentrations was 2.9 ng/ml.

Unlike the positive association between fish length and maximum testis area found among pre-spawners, there was no evidence to suggest that maximum pre-spawn testis area was associated with pre-spawn concentrations of plasma hormones. Regression slopes of testis area versus log values of T, 11-KT, and DHP were not significantly different from zero (p=0.71 and p=0.38 for T, p=0.73 and p=0.25 for 11-KT, p=0.79 and p=0.53 for DHP, respective for WR and UR sample groups). Similarly, post-spawned fish with larger maximum testis area did not have elevated concentrations of 11-KT or DHP (p=0.46 and p=0.96 for 11-KT and DHP, respectively for YR samples) and comparison between post-spawned testis area and T were not possible because all concentrations were below the assay's standard curve.

DISCUSSION

Ultrasound proved to be a rapid and non-invasive way to distinguish between pre-spawn (mature) and post-spawned adult steelhead. Ultrasound images provide quantifiable selection criteria – based on the size, number, location, and/or echogeneity of gonads – for the classification of spawning types among the samples examined. An obvious difference in gonad mass and egg number between mature and post-spawned females was apparent, although post-spawned females frequently retained a few eggs. Ultrasound readily detected the chorionic membrane surrounding the yolk of the eggs as a distinctive ring. It was discovered that male steelhead were readily identifiable by the presence of testicular tissue, but determining their maturation status (i.e., pre-spawn or post-spawn) was more difficult relative to females because some males retained considerable gonad mass after spawning. However, ultrasound measurements of testis size provided overwhelming evidence of differences between mature and naturally post-spawned male testes. The variation in testis measurements among maturation types did not overlap with one another. In addition, differences in gonad echogeneity were detected between mature and post-spawned male steelhead, providing further means of discriminating maturation status. Following spermatogenesis, remnant testicular mass may be absorbed or engulfed by macrophages, which ultimately results in both smaller testes and a denser testicular mass (Billard 1983; Cauty and Loir 1995). Although differences in testis density, as displayed as ultrasound echogeneity, are subjective and not easily quantified with ultrasound because measurements are on the nominal scale, they may be useful for assess maturation if the ultrasound operator is well trained.

In the present study, pre-spawn female ovaries and testes examined via ultrasound were at the final stages of development and dominated the body cavity, maximizing differences between the mature and post-spawned fish. However, the degree of distinction between mature and post-spawned steelhead gonads will depend upon the magnitude of difference in gonad development present at the time of ultrasound appraisals. In the case of female steelhead, this may only be problematic if oocytes and the ovary as a whole are in the very early stages of development (e.g., months prior to spawning). In rainbow trout, the ovaries undergo a pronounced increase in weight 4-5 months prior to spawning as oocytes accumulate exogenous vitellogenin. For example, Sumpter et al. (1984) reported an increase in rainbow trout ovary weight from 9.59 grams to over 200 grams in a 5-month period prior to spawning. Thus, egg and gonad size in maturing females are likely to be large enough to be readily detected via ultrasound months prior to spawning.

If the variation in testis size found among the two maturational types examined in this study are applicable to other summer-run steelhead populations in the region, then individuals with a maximum testis size greater than or equal to 1.25 cm^2 can accurately be classified as mature. Conversely, if the variation in post-spawned testis area found from the YR samples does not persist in other populations or if immature pre-spawn males are encountered during ultrasound exams, misclassification of spawning type could occur. Unlike developing eggs, individual spermatozoa cannot be distinguished within the gonad via 7.5 MHz ultrasound examination and the entire gonad must be at least 0.20 cm^2 to be distinguishable via ultrasound imaging. Billard (1983) demonstrated that brown trout testicular mass was elevated and relatively consistent three months prior to sperimiation. Prior to spermatogenesis, mean testicular mass was only 0.79 grams in immature fish but during spermatogenesis mean testicular mass was 17.01 grams, with 95% of the testicular mass generated during the first four weeks of development. In the two months following spawning, mean testicular mass dropped by 85% of their mature weight. In rainbow trout, a 10-fold increase in the gonadosomatic (GSI) index has been documented over the course of two months, with peak GSI values occurring 1-2 months prior to spawning (Scott and Sumpter 1989). Once testicular mass and size had peaked, it remained fairly constant until the conclusion of spawning, at which time a dramatic decrease was noted (Scott and Sumpter 1989). Based on data presented here and those of other researchers, differences in gonad size between pre-spawned and post-spawned males should remain elevated for long enough time periods to accurately determine maturational status using the ultrasound approach.

To minimize the potential for erroneous classification using ultrasound measurements, researchers should have knowledge of the reproductive cycle of the species under examination, should have an indication of the potential for mature and post-spawned individuals to have overlapping migrations, and should have sufficient preliminary ultrasound studies to describe the variation in gonad size present in the population of interest. Furthermore, the cost of misclassification should also be considered, depending on the particular study goals and objectives. For example, there may be circumstances in which it is not permissible to misclassify a mature individual as a post-spawned fish. If previous data exist on gonad size variation, then conservative classification criteria can be adopted to minimize the probability of misclassifying a specific maturational category. Various modeling exercises (e.g., discriminant function analysis) can be utilized to both describe the variation in gonad size and to estimate classification error rates.

The use of ultrasound images to identify pre- and post-spawned males in this study was corroborated by blood plasma steroid data. Milt producing specimens not only had significantly larger maximum testis area but also had significantly elevated levels of T, 11-KT, and DHP relative to post-spawned fish. Concentrations of T, 11-KT, and DHP observed in the present study among mature male steelhead are similar to those reported by other researchers. During the spawning period (i.e., in mature fish), Campbell et al. (1980) and Scott et al. (1980) reported mean testosterone levels of approximately 60 and 70 ng/ml, respectively, in resident forms of male rainbow trout (*O. mykiss*). Mean levels of 11-KT were measured at 98 ng/ml by Campbell et al. (1980) and ranged from 80 - 100 ng/ml in study of male rainbow trout conducted by Baynes and Scott (1985). Baynes and Scott (1985) also observed that mean concentrations of DHP ranged between 20 to 30 ng/ml in mature male rainbow trout. In the present study, all post-spawned males had concentrations of testosterone below < 1.2 ng/ml and only 8 out 13 fish had measurable concentrations of DHP (ranging from 1.1 to 3.2 ng/ml) in post-spawned males. Post-spawn males did have measurable concentrations of 11-KT (ranging from 5.3 to 9.2 ng/ml), but the values were significantly lower than that found in mature fish. In a study linking gonad regression with changes in hormone concentrations in rainbow trout, Baynes and Scott (1985) observed similar values of T, 11-KT and DHP in post-spawned males as those reported in the present study. In the three months following sperimiation, concentrations of these hormones dramatically declined and were approximately 10, 4, and 3 ng/ml, respectively for each hormone, in non-milt producing males (Baynes and Scott 1985).

Profiles of steroids in resident forms of rainbow trout have linked peak levels of T with spermatogenesis (Billard 1978, Scott et al. 1980), while peak levels of 11-KT correspond with spermiation (Campbell et al. 1980, Baynes and Scott 1985). Numerous studies have demonstrated that T peaks prior to 11-KT and both steroids continue to decline throughout the spawning period. Similar cyclical patterns in T and 11-KT have also been reported in male coho salmon (*O. kisutch*) by Fitzpatrick et al. (1986), in arctic charr (*Salvelinus alpinus*) by Elofosson et al. (2000), and in Atlantic salmon (*Salmo salar*) by Hunt et al. (1981). DHP has been positively correlated with milt volume, spermatocrit, and the total number of spermatozoa produced in the testes and is suspected to control the ionic composition of seminal fluids in male rainbow trout (Byanes and Scott 1985, and Vizziano 1996). Thus, mature anadromous forms of *O.mykiss* examined here had levels of steroids similar to resident, captive stocks, suggesting that similar cyclical changes in hormone concentration were taking place. However, plasma steroid concentrations were taken at a single sampling time and thus do not represent changes in hormone profiles throughout gonad regression.

Although both ultrasound measurements of gonads and concentrations of steroids could be used to discriminate between the mature and post-spawned male steelhead examined here, the ultrasound method has advantages relative to radioimmunoassy (RIA) measurements of steroid concentrations. Unlike the RIA technique, ultrasound is rapid and a truly non-invasive technique that can be conducted both in the field and in a laboratory environment. Furthermore, the training, labor and costs associated with using ultrasound are minimal, at least when compared to the cost of radioimmunassay laboratory equipment and the expertise and time needed to conduct the assays. An ultrasound operator must simply be familiar with the equipment, the specimen's reproductive cycle and general visceral anatomy in order to interpret diagnostic images. Once anesthetized, individual fish can be examined with ultrasound in less than 30 seconds and images can be stored on a computer for future viewing.

Although the primary objective of this study was to develop ultrasound classification criteria to distinguish pre-spawn from post-spawned steelhead encountered at mainstem hydroelectric facilities in the Snake River basin (see Chapter 2), ultrasound has a much broader potential for fisheries research. In aquaculture of salmonids, ultrasound can be used to distinguish maturing brood fish from immature individuals, allowing mature fish to ripen in isolation and immature fish to continue somatic growth and reproductive development via feeding. Since ultrasound can identify sex in captive salmonids up to five months prior to spawning (Martin et al. 1983, Arkush and Petervary 1998), a single ultrasound examination can substantially reduce handling of brood stock and may ultimately reduce stress and disease transmission associated with multiple handlings. Ultrasound may also provide an alternative method to biopsy checks in white sturgeon (*Acipenser transmontanus*). Assessment of reproductive development in white sturgeon is typically performed via surgical biopsies, allowing a visual inspection of the gametes (Webb et al. 2002). Webb et al. (2002) recently used profiles of reproductive hormones to develop a less invasive method of determining both the sex and reproductive development of white sturgeon, with classification success ranging from 96% in mature males to 98% in maturing females. Ultrasound may provide a more rapid and non-invasive way to diagnose maturing sturgeon, while achieving similar levels of accuracy relative to reproductive hormone concentrations.

REFERENCES

Arkush, K.D., and N.A. Petervary. 1998. The use of ultrasonography to predict reproductive development in salmonid fish. International Association for Aquatic Animal Medicine. 29: 19-20.

Baynes, S.M., and A.P. Scott. 1985. Seasonal variations in parameters of milt production and in Plasma concentration of sex steroids of male rainbow trout (*Salmo gairdneri*), General and Comparative Endocrinology. 75: 150-160.

Billard, R. 1983. A quantitative analysis of spermatogensis in the trout, Salmo trutta fario. Cell and Tissue Research. 230: 495-502.

Blythe, B., L.A. Helfrich, W.E. Beal, B. Boswarth, and G.S. Libey. 1994. Determination of sex and maturational status of striped bass (*Morone saxatilis*) using ultrasonic imaging. Aquaculture 125:175-184.

Bonar, S.A., G.L. Thomas, and G.B. Pauley. 1989. Use of ultrasonic images for rapid nonlethal determination of sex and maturity of Pacific herring. North American Journal of Fisheries Management. 9:364-366.

Bone, Q., N.B. Marshall, and J.H.S. Blaxter. 1995. Biology of Fishes second edition. Chapman and Hall.

Busby, P.J., T.C. Wainwright, E.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz, and I.V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. NOAA Technical Memorandum. Available from National Marine Fisheries Service. Seattle, WA.

Campbell, C.M., A. Fostier, B. Jalabert, and B. Truscott. 1980. Identification and quantification of steroids in the serum of rainbow trout during spermiation and oocyte maturation. Journal of Endocrinology. 85: 371-378.

Cauty, C., and M. Loir. 1995. The interstitial cells of the trout testis (*Oncorhynchus mykiss*): ultrastructural characterization and changes throughout the reproductive cycle. Tissue and Cell. 27 (4) : 383-395.

Elofosson, U.O.E., I. Mayer, B. Damsgard, and S.Winberg. 2000. Intermale competition in sexually mature Arctic charr: Effects on brain monoamines, endocrine stress response, sex hormone levels, and behavior. General and Comparative Endocrinology. 118: 450-460.

Fitzpatrick, M.S., G.V. Kraak, and C.B. Schreck. 1986. Profile of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch,* during final maturation. General and Comparative Endocrinology. 62: 437-451.

Gjerde, B. 1984. Variation in semen production of farmed Atlantic salmon and rainbow trout. Aquaculture. 40:109-114.

Holloway, A.C., M.A. Sheridan, G.V.D. Kraak, and J.F. Leatherland. 1999. Correlations of plasma growth hormones and thyroid hormones in rainbow trout during sexual recrudescence. Comparative Biochemistry and Physiology. 123: 251-260.

Hunt, S.M.V., T.H Simpson, and R.S.Wright. 1981. Season changes in the levels of 11-oxotestosterone and testosterone in the serum of male salmon, Salmo salar L., and their relationship to growth and maturation cycle. Journal of Fish Biology. 20: 105-119.

Khattree, R.,and D.N. Naik. 2000. Multivariate data reduction and discrimination with SAS software. SAS Institute Inc. Cary, NC.

Martin, R.W., J.Myers, S.A. Sower, D.J. Phillips, and C. McAuley. 1983. Ultrasonic imaging, a potential tool for sex determination of live fish. North American Journal of Fisheries Management. 3:258-264.

Muir, W.D., S.G. Smith, J.G. Williams, and B.P. Sandford. 2001. Survival of Juvenile salmonids passing through bypass systems, turbines, and spillways with and without flow deflectors at Snake River dams. North American Journal of Fisheries Management. 21:125-146.

Munkittrick, K.R., and R.D. Moccia. 1987. Seasonal changes in the quality of rainbow trout (Salmo gairdneri) semen: Effects of a delay in stripping on spermatocrit, motility volume and seminal plasma constituents. Aquaculture. 64: 147-156

NMFS (National Marine Fisheries Service). 1997. Endangered and threatened species: Listing of several evolutionary significant units (ESUs) of west coast steelhead. Final rule. Federal Register 62 (159):43937-43940.

Ramsey, F.L., and D.W. Schafer. 1997. The Statistical Sleuth. Duxbury Press: An Alexander Kugushev publication. Belmont, CA.

Reimers E., P. Landmark, T. Sorsdal, E. Bohmer, and T. Solum. 1987. Determination of salmonids' sex, maturation and size: an ultrasound and photocell approach. Aquaculture Magazine. November/December pp. 41-44.

Robards, M.D., and T.P.Quinn. 2002. The migratory timing of adult summer-run steelhead in the Columbia River over six decades of environmental change. Transactions of the American Fisheries Society. 131: 523-536.

Schreck, C.B. 1972. Evaluation of diel variation in androgen levels of rainbow trout, *Salmo gairdneri.* Copeia 4: 865-868.

Scott, A.P., V.J. Bye, and S.M. Baynes. 1980. Seasonal variation in plasma concentrations of 11-ketotestoterone and testosterone in male rainbow trout, *Salmo gairdnerii Richardson*. Journal of Fish Biology 17: 495-505.

Scott, A.P. and J.P. Sumpter. 1989. Seasonal variations in testicular germ cell storage and in plasma concentrations of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old. General and Comparative Endocrinology. 73: 46-58.

Shields R.J., J Davenport, C. Young and P.L. Smith. 1993. Oocyte maturation and ovulation in the Atlantic halibut, *Hippoglossus hippoglossus* (L.), examined using ultrasonography. Aquaculture and Fisheries Management. 24: 181-186.

Sumpter J.P., A.P. Scott, S.M. Baynes, and P.R. Witthames. 1984. Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri Richardson*). Aquaculture. 43: 235-242.

USACE (US Army Corps of Engineers). 1996-00. Juvenile Fish Transportation Program. Annual reports for 1996-2000. U.S. Army Corps of Engineers, Walla Walla District, Walla Walla, WA.

Vizziano, D., F.L. Gac, and A. Fostier. 1996. Effect of 17β-estradiol, testosterone, and 11-ketotestosterone on 17, 20β-diydroxy-4-pregnen-3-one production in the rainbow trout testis. General and Comparative Endocrinology. 104: 179 -188.

Webb, M.A., E.P.Foster, C.B. Schreck, and M.S. Fitzpatrick. 2002. Potential classification of sex and stage of gonadal maturity of wild white sturgeon using blood plasma indicators. Transactions of the American Fisheries Society. 131: 132-142.

Whitt, C.R. 1954. The Age, Growth, and Migration of Steelhead Trout in the Clearwater River, Idaho. MS thesis, University of Idaho, Moscow, ID.

Identification and Enumeration of Steelhead (*Oncorhynchus mykiss*) Kelts in the Bypass Collection Facility at Lower Granite Dam

Allen F. Evans, Roy E. Beaty and Martin S. Fitzpatrick

ABSTRACT

Listing of steelhead (*Oncorhynchus mykiss*) populations under the Endangered Species Act (ESA) has raised questions about whether post-spawned adult steelhead (referred to as "kelts") can be used to bolster iteroparity rates in the Snake River. Biologists monitoring Snake River hydroelectric dams have attempted to distinguish pre-spawned (mature) steelhead from kelts based on external appearance: dark and/or poor condition fish are classified as kelts and good and/or bright condition fish pre-spawn fish. Visual methods to determine maturational status in steelhead are subjective and the persons using them have little confidence of their unproven accuracy. This study used ultrasound images of visceral anatomy to identify kelts and pre-spawn fish among adult steelhead removed from the Lower Granite Dam (LGR) juvenile bypass facility. Steelhead were sampled during ten weekly periods between April and June 2000 that spanned the peak of the arrival of adults in the juvenile bypass system. Based on ultrasound examinations, 94.6% of the 3,968 adults examined at the LGR facility during the study period were identified by ultrasound as kelts. Of these post-spawned adults, 2,050 were wild, ESA-listed steelhead. These 2,050 wild kelts encountered at the dam constituted 17% of the protected population counted migrating upstream at the LGR fishway from 1 June 1999 to 31 May 2000, which is the time period used to calculate yearly run counts. The majority (69.5%) of kelts sampled were in good morphological condition – as determined by their lack of physical damage – and the kelt run was predominantly (77.0%) female. An examination of reproductive hormones in a sub-sample of male steelhead suggested that kelts can also be readily detected via the low or non-detectable levels of testosterone, 11-ketotesterone, and 17α-hydroxy-20β-dihydroxyprogesterone. However, plasma steroid concentrations in pre-spawn fish varied sufficiently to result in more classification errors relative to the ultrasound method. Relative to ultrasound appraisals, visual methods grossly under-estimated kelt abundance with only 18% of the ultrasound sampled adults being classified as post-spawned.

INTRODUCTION

Populations of wild steelhead *(Oncorhynchus mykiss)* have declined in both the Columbia and Snake rivers; those in the upper Columbia River are endangered and those in the Snake River are threatened under the Endangered Species Act (NMFS 1997). Causes of the declines are numerous and well known (Budy et al. 2002), and regional plans uniformly recognize the need to protect and enhance weak upriver steelhead populations while maintaining their genetic integrity (NPPC 1996). However, efforts to enhance wild populations are limited and protection from hydroelectric facility passage mortalities has focused on aiding juvenile migrants and upstream-migrating adults of all anadromous salmonid species.

Steelhead differ from most other anadromous Pacific salmonids in that they can be iteroparous. Over a century ago, residents of Idaho recognized that Snake River steelhead, unlike salmon, did not die after spawning, but appeared to travel back to the ocean (US Fish Commission 1895). Historically, the number that survived to spawn again is not known; however, between 2 and 4% of the steelhead returning to Idaho's Clearwater River in the 1950s (a period when two downstream dams, Lewiston and Bonneville Dams, blocked migration) were estimated to be repeat spawners (Whitt 1954). Rates averaging 1.6% and 3% have been documented for wild steelhead populations in the Yakima River (above 4 mainstem dams; Hockersmith et al. 1995) and Klickitat River (above 2 main stem dams; Busby et al. 1996), respectively. In contrast, estimates of repeat spawners in tributaries of the lower Columbia River have exceeded 17%, with some fish returning to spawn four consecutive times (Leider et al. 1986).

Most summer-run steelhead migrate upstream over Snake River dams from September through October and are believed to spawn from March to May (Busby et al. 1996). Many steelhead over-winter in the mainstem Snake River and complete their migration to tributary spawning areas in the spring (Whitt 1954, Robarts and Quinn 2002). These late, spring pre-spawn or maturing migrants can be passing the dams – and falling back over them – when many of the post-spawned steelhead are beginning their downstream migration. Thus, the upstream migration of pre-spawn adult steelhead and downstream migration of kelts can geographically and temporally overlap in the Snake

River. Until recently, kelts were generally considered moribund animals and methods to enhance their survival were not explored. However, steelhead kelts may be an important but overlooked source of genetic material for rebuilding depleted populations of summer-run steelhead. Because wild kelts could be a valuable resource for rebuilding depleted steelhead populations in the region, the National Marine Fisheries Service has requested that research be conducted to enumerate kelt passage and to reduce dam passage mortality of kelts as a component of species recovery plan in the Columbia River Basin (NMFS 2000).

Distinguishing a pre-spawn steelhead from a downstream migrating kelt is not simple. Only those that are entrained in the juvenile bypass systems at mainstem Columbia and Snake River dams can be observed on a regular basis, and there has been no quantifiable way to classify maturation status based on external characteristics. However, biologists working at Snake River dams attempted to make the distinction based on the coloration and condition of each fish. Generally, dark and/or poor condition fish were counted as kelts; bright and/or good condition fish were counted as pre-spawners. Biologists have a limited amount of time to view adult steelhead at the dams and because the fish are partially submerged and actively moving in the water during visual examination, the accuracy of visual appraisals are in question (Rex Baxter, USACE Biologist, pers. comm.). Using these visual methods, it was estimated between 1.4% and 26.5% of the approximately 5,000 adult steelhead annually removed from the juvenile separator at Little Goose Dam and Lower Granite Dam on the Snake River were kelts and that pre-spawners compose a very large majority (USACE 1996-00).

To develop a more quantifiable kelt and pre-spawner identification technique, research was previously conducted to determine maturation status in adult steelhead via ultrasound imaging (see Chapter 1). Ultrasound has been used to diagnose both the sex and maturation status of various freshwater and marine fishes by producing acoustic images of the size, shape and location of gonads (Martin et al. 1983, Reimers et al. 1987, Shields et al. 1993, Blythe et al. 1994, Arkush and Petervary 1998). Ultrasound research demonstrated that pre-spawn and freshly post-spawned females were easily distinguishable by the presence or absence of an egg mass. For males, an investigation of testis size (characterized by the cross-sectional area) demonstrated that gonads undergo substantial size changes following spawning, with testis of mature males 2.24 cm^2 greater than that of kelts (Chapter 1). An examination of distribution of testis sizes from pre- and post-spawned steelhead suggests that the testis size gap between kelts and pre-spawners was large enough to classify each maturation type with little estimated error (a few percent).

In addition to the ultrasound method, plasma hormones can be used as indicators of reproductive development in fish (Campbell 1980, Baynes and Scott 1985, Fitzpatrick et al. 1986). A comparison between the ultrasound method and concentrations of testosterone, 11-ketotestosterone, and 17α-hydroxy-20β-dihydroxyprogesterone demonstrated that both approaches could accurately assess maturation status in male steelhead (Chapter 1). However, mature fish sampled during this ultrasound and reproductive hormone validation study were spermiating at the time of sampling and questions remained as to whether steroids can be used to accurately identify steelhead in the field which might be of vastly different maturational phases. Thus, further investigations of reproductive hormones in male steelhead were needed to assess the adequacy of the technique.

After the successful development of ultrasound identification techniques (Chapter 1), the following studies were undertaken to examine the abundance and run-timing of kelts and pre-spawners at Snake River dams, sex ratios, production origin (hatchery versus wild) and other basic biological information. Furthermore, the visual method previously employed at Snake River dams to determine maturation status (i.e., fish condition and coloration) were evaluated relative to the ultrasound approach. Lastly, reproductive hormones from a sample of male steelhead of mixed pre-spawn maturational phases were measured to examine the adequacy of using steroids to determining maturation status at the dams.

MATERIALS AND METHODS

Location

Research was conducted at the Lower Granite Dam's (LGR) juvenile bypass collector located on the Snake River approximately 696 river kilometers (Rkm) from the mouth of the Columbia River (Figure 2.1). More adult steelhead are counted during the spring months at the LGR bypass than at any other Snake or Columbia River bypass facility. Furthermore, LGR bypass facility is the first mainstem collection point in which downstream-migrating kelts can be sampled on the Snake River. Three major downstream fish passage routes exist at LGR: the spillway, the bypass facility, and the turbine units. Bypassed fish are collected in the dam's forebay via large submersible screens that partially block turbine intakes and divert fish into the bypass facilities "separator" (a term used to describe a series of horizontal bars that separate fish based on size). The primary purpose of the bypass facility is to collect juvenile salmonids for barge transportation. However, adult salmonids and other resident fish species are routinely diverted into the LGR juvenile bypass. U.S. Army Corps personnel monitored the LGR separator 24 hours per day, seven days a week throughout the study period and counted the total number of adult steelhead encountered.

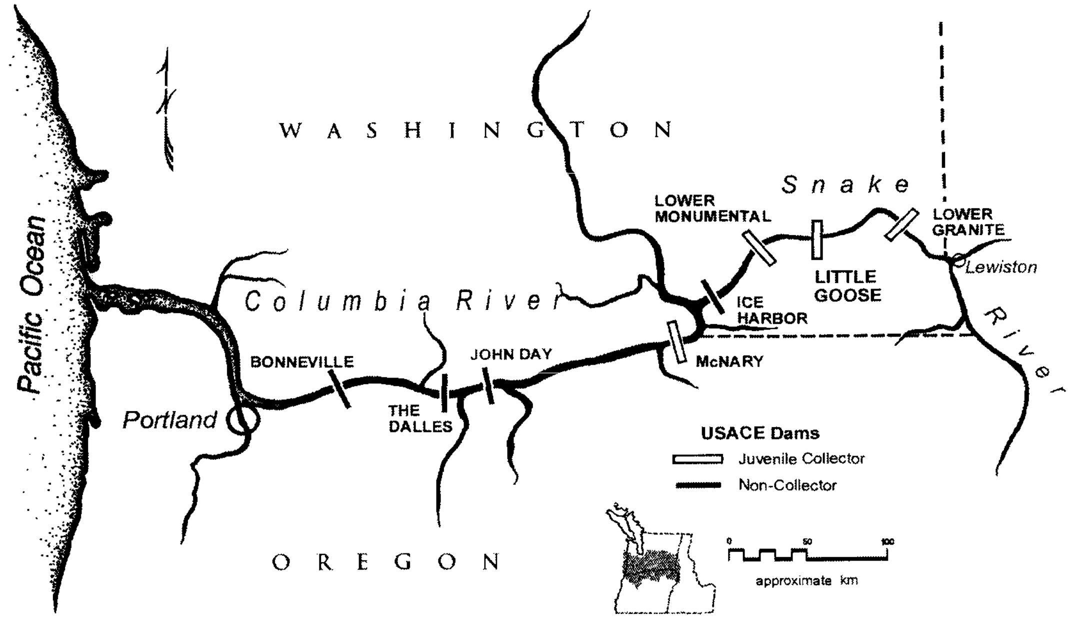

Figure 2.1: Location of Snake and Columbia River dams.

Sampling Procedures

Adult steelhead were removed from the LGR separator and transferred via dipnet to a nearby 190-L sampling tank containing fresh river water, where they were anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 parts-per-million. A portable Aloka® SSD-500v ultrasound machine, equipped with 7.5 MHz linear probe, was used to examine the gonads of adult steelhead. During the examination, the probe was gently placed against the specimen's abdomen and then moved anterior or posterior to view the ovaries or testes. Assessment of maturational status among specimens was based on presence/absence of gonads or the size gonads (Chapter 1). Because of the protected status of steelhead in the Snake River, fish could not be sacrificed during the study to confirm ultrasound appraisals of maturational status via gonad histology or other lethal techniques. Assessment of spawning status was based on ultrasound classification data collected from known pre- and post-spawned individuals (hereafter referred to as the "known" sample) at three steelhead aquaculture facilities, located both above and below

LGR, during the months of March, April and May, 2000 (Chapter 1). Ultrasound classification at LGR operated under the assumption that steelhead sampled from LGR were at similar stages of gonad development relative to the known samples.

For female specimens, spawning type (pre- or post-spawned) was determined based on the presence or absence of egg masses running the length of the gonads. For male specimens, the maximum cross-sectional area of the single largest testis within the ultrasound's range was measured using a trackball function commonly found on ultrasound units. All male steelhead with maximum testis area greater than or equal to 1.25 cm^2 were classified as pre-spawners and those with testis size less than 1.25 cm^2 as kelts. Based on ultrasound data obtained from known samples, the classification criteria of 1.25 cm^2 has an estimated error rate of 0.0041 for kelts and 0.0044 for pre-spawners (Chapter 1). Kelts lacking detectable gonads, making them impossible to sex with ultrasound were classified as male or female based on sexual dimorphism (e.g., the elongated and partially hooked male jaw) using methods described by Groot and Margolis (1991), slighted modified for steelhead.

Concurrent with the ultrasound examinations, data on fish length (cm fork length), morphological condition (rated by degree of external damage as "good", "fair", or "poor"), coloration (rated by skin coloration as "bright", "intermediate", or "dark"), and rearing type ("hatchery" or "wild") were collected (see Appendix for a description of the traits used to classify fish condition and coloration). Rearing type was based on the presence (wild) or absence (hatchery) of an adipose fin, a marking technique used to identify hatchery steelhead in the Columbia and Snake rivers. Total examination time at the LGR separator averaged approximately 4 minutes per fish. Following the examination, fish were allowed to recover in a holding bin at the downstream end of the separator from which they could escape once normal swimming behavior resumed.

Concentrations of testosterone (T), 11-ketotesterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP) were determined by radioimmunoassay using procedures of Fitzpatrick et al. (1986). Blood samples were removed from the caudal vein of male specimens (n= 34) using a Vacutainer containing heparin to prevent coagulation. Blood samples were kept on ice until the plasma could be separated by centrifugation and stored at -70^0C. All plasma samples were assayed in duplicate and intra-assay coefficient of variation was calculated for all duplicates. Coefficients of variation in excess of 10%

(n= 4 out of 102) were considered contaminated and removed from the data set prior to analysis. Average extraction efficiency for all three steroids measured was 88% with a minimum assay detection threshold of 1.2 ng/ml, 1.1 ng/ml, and 0.7 ng/ml for T, 11-KT, and DHP, respectively. Intra-assay coefficient of variation was below 3% for all three steroids. Reported steroid levels were corrected for extraction efficiencies.

Determination of spawning status from steroid levels was based on plasma hormone criteria obtained from the same known sample used to generate male ultrasound classification criteria (Chapter 1). Individuals with concentrations of T, 11-KT, and DHP greater than or equal to 11.5 ng/ml, 15.3 ng/ml, or 5.3 ng/ml, respectively, were considered pre-spawners and all others classified as kelts. The classification rule for 11-KT and DHP were based on results of a discriminant function analysis, while the testosterone classification criteria of 11.5 ng/ml represents the lowest level encountered within the known pre-spawner group (Chapter 1).

Sampling Effort

Steelhead were sampled during ten week-long periods (week = Sunday to Saturday) that spanned the peak of the arrival of adults in the juvenile bypass system: 1 April to 10 June 2000. All of the adult steelhead arriving on the separator were sampled while the ultrasound machine was in use. Samples were collected during daylight hours and at night (as determined by sunrise and sunset hours for Moscow, Idaho); however, randomization was not used to determine which days of the week or hours of the day to sample. To help ensure blood samples were taken throughout the ten-week sampling period, blood was removed and assayed from at least one male during each sample week, with a minimum of four males from sample weeks 1 to 5, which is the period when pre-spawn fallbacks were most likely to occur.

Analysis

The overall abundance of kelts at the LGR juvenile bypass facility was estimated by multiplying the proportion of kelts identified by ultrasound during each week of sampling by the total number of steelhead removed from the separator during that week and adding the weekly estimates. Variance and confidence bounds were calculated using a stratified estimate (Schaeffer 1989; equation provided), which helped account for variability in kelt and pre-spawner abundance among weekly samples.

Estimated variance of the weekly or stratified kelt proportion:

$$(v_i) = \frac{1}{N^2}\sum_{i}^{L} N_i^2 \left(\frac{N_i - n_i}{N_i}\right)\left(\frac{p_i q_i}{n_i - 1}\right)$$

Confidence error bound:

$$p_{st} \pm 2\sqrt{v(p_{st})}$$

Fisher's exact tests were used to assess if the proportions of kelt and pre-spawners differed significantly with changing light conditions. Chi-square tests were used to assess if kelt and pre-spawner counts were significantly different regarding sex under the assumption of a male to female ratio of 1:1. All statistical tests were run using SAS® version 8. Data on fish condition and coloration were compared to ultrasound appraisals of maturational status to assess the adequacy of prior visual classification criteria. Morphological data on fish condition and coloration were directly compared to an ultrasound appraisal for each fish examined and an accuracy determined. For the purpose of this comparison, it was assumed that ultrasound classified maturational status with 100% accuracy. Maturation appraisals from the ultrasound and plasma steroid techniques were directly compared to one another based on the number of individual agreements. Again, ultrasound appraisals of maturational status were the basis on which steroid classification accuracy was evaluated.

RESULTS

In total, 1,353 (34.1%) of the 3,968 adult steelhead encountered at the Lower Granite Dam bypass separator during the period of 1 April to 10 June 2000 were examined with ultrasound. Of these, 330 (24.4%) were considered male and the remaining 1,023 female. Of the fish examined with ultrasound, 38.8% (128/330) of the males and 42.0% (430/1,023) of the females had to be visually classified by sex because gonads could not be detected within the body cavity via ultrasound imaging. Thus, many kelts completely discard, reabsorb or have remnant gonads too small for ultrasound detection following spawning.

Of the fish with identifiable gonads, pre-spawn and post-spawned females were easily distinguishable by egg number and the general length of the egg mass. Ultrasound provided such high-resolution images that individual eggs could be distinguished within the body cavity (*see* Chapter 1 *to view ultrasound images*). The egg mass in pre-spawners was continuous; running just posterior of the transverse septum to anterior of the urogenital papilla (an appendage used to extrude eggs during spawning). Individual egg size and the shape of the gonads varied among pre-spawners. Larger egg masses, resulting from larger individual eggs, filled the body cavity so that the top portion of the gonad was adjacent to the abdominal muscle tissue and visceral organs (e.g., liver and digestive track) were obscured due to the well-developed egg masses. Less developed pre-spawners could be characterized by two distinctive egg-filled lobes separated by the liver and underlying digestive track. In contrast, female kelts often had a few remnant, mature or over-ripe eggs – typically 10 to 50 – within the body cavity. For kelts, a few eggs were often lodged just anterior to urogenital papilla or spread throughout the body cavity.

Unlike females, males were much harder to classify according to maturation type, because many kelts retained testicular mass. Of the 330 male steelhead sampled at LGR, precise ultrasound measurements were available from 202 (61.2%). In the remaining 128 male specimens, testes were too small or too atrophied to be distinguishable from surrounding tissue via ultrasound imaging, but the fish were clearly post-spawned based on the absence of any measurable gonad. Based on the pre-determined classification rule of testicular cross-sectional area greater than or less than 1.25 cm^2, 169 males with identifiable gonads were classified as post-spawned and the remaining 33 individuals as pre-spawned (Figure 2.2). Application of the classification rule to partition maturation groups resulted in a mean and standard deviation (SD) testis area of 0.64 cm^2 (SD 0.24) and 2.27 cm^2 (SD 0.67) for kelts and pre-spawners, respectively. For comparison, the means of testis size from male steelhead of known spawning status were 0.62 cm^2 (SD 0.24) and 2.86 cm^2 (SD 0.73) from 34 kelts and 55 mature males, respectively (Chapter 1).

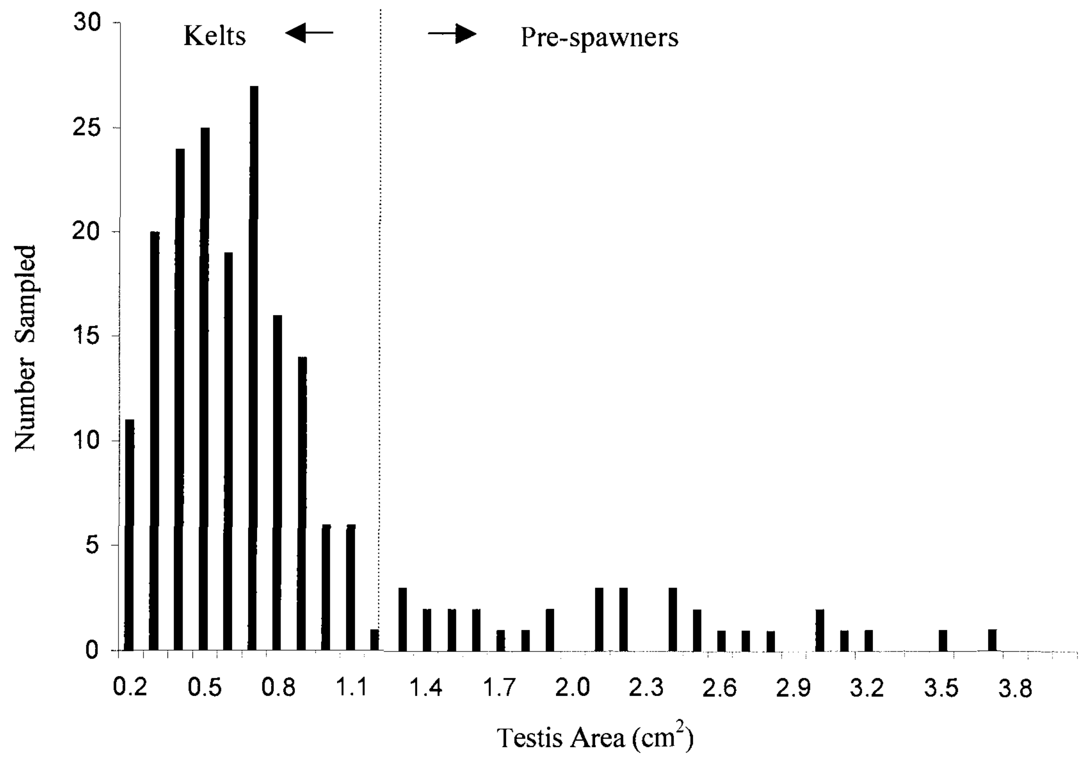

Figure 2.2: Ultrasound measurements of the maximum testis area from steelhead sampled at Lower Granite Dam's juvenile bypass facility. Vertical line (dotted) is the dividing point used to classify maturation types.

Kelt Abundance

Adult steelhead were collected over the course of 10 weeks and effort – measured by the percentage of fish sampled each week – fluctuated throughout the study period but never dropped below 13% (Figure 2.3). Weekly sampling effort averaged 4.4 days per week with a mean of 8.5 hours per day. In general, sampling effort intensified in concert with increasing adult abundance in the separator but was more intense during the latter half of the sampling period. Adult steelhead numbers were elevated the week prior to ultrasound examinations, suggesting the early portion of the run was not sampled. Numbers of adult fish in the separator peaked during the last two weeks of April and the first two weeks of May.

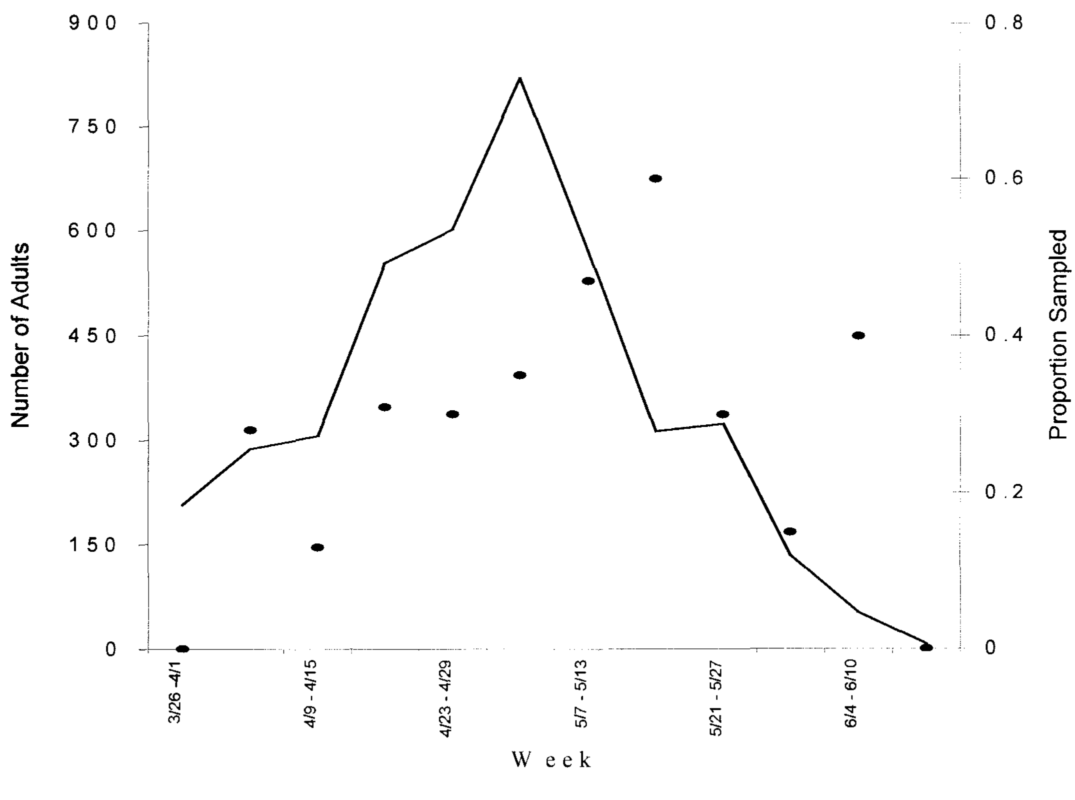

Figure 2.3: Number (line) of adult steelhead encountered at the Lower Granite Dam bypass separator and the proportion (•) sampled with ultrasound, by week, during the study period. In total, 3,968 adult steelhead were encountered during a 10-week sampling period.

Ultrasound examination of the 1,352 adult steelhead indicated that 1,292 (95.5%) of the fish were kelts and the remaining 61 individuals pre-spawn fallbacks (Table 2.1).

Based on adipose fin clips (marks used to identify fish originating from a hatchery), 521 (38.5%) were identified as hatchery kelts, while the remaining 771 (57.0%) kelts were considered naturally produced (i.e. wild) individuals (Table 2.1). For pre-spawners, 34 (2.5%) were hatchery and 27 (2.0%) wild (Table 1.1). The proportions of kelts and pre-spawners changed slightly throughout the study period, with the kelt proportion increasing from 89% (429/471) in April to 97.9% (823/841) in May and 97.6% (40/41) in June.

Table 2.1: Maturational status and origin of adult steelhead examined with ultrasound at the Lower Granite bypass.

Maturational status	Origin No. *(%)* Hatchery		Wild		Total	
Pre-spawn	34	*(2.5)*	27	*(2.0)*	61	*(4.5)*
Kelt	521	*(38.5)*	771	*(57.0)*	1,292	*(95.5)*
Total	555	*(41.0)*	798	(59.0)	1,353	*(100.0)*

Based on 10 weeks of ultrasound sampling, total kelt abundance at the LGR bypass was estimated to be 3,752 fish or 94.6% of the total bypass population. Upper and lower confidence intervals suggest the percentage lies between 95.8% and 93.3%. The total estimate is weighted to account for seasonal differences in relative abundance. Assuming U.S. Army Corps biologist at LGR accurately identified wild (i.e., non-adipose clipped) fish in the separator during non-sampling periods, 2,050 of the 3,752 kelts were ESA-listed steelhead. The total number of wild summer-run steelhead counted passing upstream through the Lower Granite Dam fishway from 1 June 1999 to 31 May 2000 – the period used to determine yearly run counts – was 12,075 pre-spawners (FPC 2002). Thus, an estimated 17.0% (2,050/12,075) of the entire ESA-listed steelhead run was observed as kelts in the LGR bypass system during a ten-week period. In regards to hatchery fish, an estimated 2.8% (1,702/61,736) of the run was observed as kelts during the same interval.

Limited nighttime sampling suggested that no diurnal pattern existed between kelt and pre-spawner proportions when samples were compared on a daily basis. Within the ten-week sampling period, daytime and nighttime samples were collected on weeks 3, 4, 6, and 7. In total, direct comparisons were available from 112 adult steelhead sampled at night and 254 adult steelhead sampled during daylight hours. At night 97.3% (109/112) of the adults were kelts compared to 96.5% (245/254) kelt abundance during day light hours. In all cases examined where both pre-spawners and kelts were observed on a daily basis, no statistical difference between proportions was detected (p-value > 0.50, Fisher's exact). If the data are pooled by sample date and week, no significant difference was detected (p-value = 0.7073, Fisher's exact) between day and night relative abundance of kelts.

Differences regarding fish origin (hatchery versus wild) and sex (male versus female) were observed throughout the study period. Among kelts, the proportion of wild kelts increased from 0.48 to 0.95 during the season and dominated the proportion of hatchery kelts during the later weeks (Figure 2.4). Hatchery kelt abundance peaked one week prior to that of wild kelts (Figure 2.4). The vast majority of kelts were female (Figure 2.5). In total, 995 kelts were classified as female and the remaining 297 male. With the exception of the last two weeks of the sampling period, females significantly outnumbered males (p-value < 0.001, chi-square). However, male kelt abundance steadily increased as the season progressed (Figure 2.5).

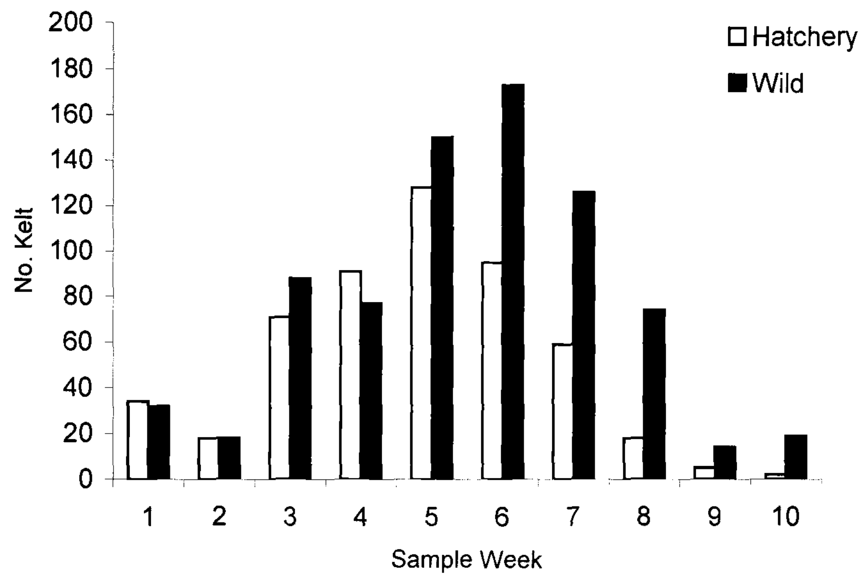

Figure 2.4: Number of hatchery and wild steelhead kelts observed over the course of a ten-week (Sunday to Saturday) sampling period at the Lower Granite bypass facility.

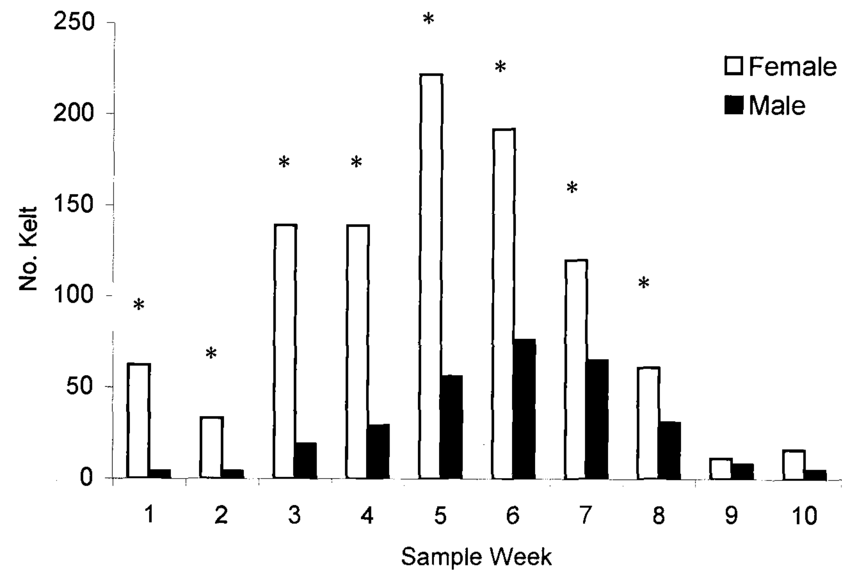

Figure 2.5: Number of female and male steelhead kelts observed over the course of a ten-week (Sunday to Saturday) sampling period at the Lower Granite bypass facility. Asterisk denotes significant difference at the 0.05 level.

Kelts were more silvery in appearance ("brighter") and in better condition than pre-spawners, an observation contrary to prior assumptions. Of the 1,292 kelts identified by ultrasound during the study period, 898 (69.5%) were in good overall condition and 566 (43.8%) bright in coloration (Table 2.2). For pre-spawners, 34 (55.7%) were in good condition and only 10 (16.4%) bright in coloration (Table 2.2).

Table 2.2: Condition and coloration by classification (pre-spawner or kelt) of steelhead examined with ultrasound at the Lower Granite bypass facilities.

No. LGR	Pre-spawner				Kelt			
Condition	Bright	Inter-mediate	Dark	Total	Bright	Inter-mediate	Dark	Total
Good	10	15	9	34	530	322	46	898
Fair	0	8	12	20	33	158	93	284
Poor	0	2	4	6	2	48	58	108
Dead	0	1	0	1	1	0	1	2
Total	10	26	25	61	566	528	198	1,353

Relative to ultrasound appraisals of maturational status, visual methods grossly under-estimate kelt abundance at LGR during the study period. Based on the morphological traits of fish condition and coloration – as utilized by Corp personnel in years past –18.0% (244/1,353) of the LGR sampled adult steelhead were classified as kelts and the remaining 82% (1,109/1,253) pre-spawners (Table 2.3). Of the two morphological traits, fish coloration was the more misleading, as kelts tended to be bright in coloration and pre-spawners tended to be darker. The appendix has detailed analysis of the morphological data observed in kelts and pre-spawners sampled from LGR in 2000.

Table 2.3: Comparison of maturation appraisals for both visual and ultrasound based methods at Lower Granite bypass facility. Visual methods are based on fish condition and coloration while ultrasound imaging is based on the presence/absence of gonads or the size of gonads in adult steelhead.

Maturational status	Identification Method	
	Visual No. (%)	Ultrasound No. (%)
Kelt	244 (18.0)	1,292 (95.5)
Pre-spawn	1,109 (82.0)	61 (4.5)

Steroid levels

Direct comparisons between ultrasound measurements of testis area and concentrations of steroids were available from 34 specimens or 10% (34/330) of the males sampled throughout the study period. Based on ultrasound appraisals, 19 were classified as kelts and the remaining 15 as pre-spawn fallbacks. Mean testis area was 0.62 cm^2 (SD 0.27) and 2.39 cm^2 (SD 0.38) for kelts and pre-spawner, respectively. Steroid concentrations provide a clear distinction between ultrasound-derived measurements of testis area and the lack of reproductive hormones in kelts. All fish determined to be kelts via ultrasound examination had concentrations of testosterone (T), 11-ketotesterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP) within the range anticipated for the post-spawned maturational phase (Figure 2.6). Median levels of 11-KT and DHP were 8.6 ng/ml (ranging from 6.4 to 12.5) and 2.1 ng/ml (ranging from 1.2 to 3.2), respectively.

Concentrations of testosterone were below the assay minimum detection threshold in all but one kelt.

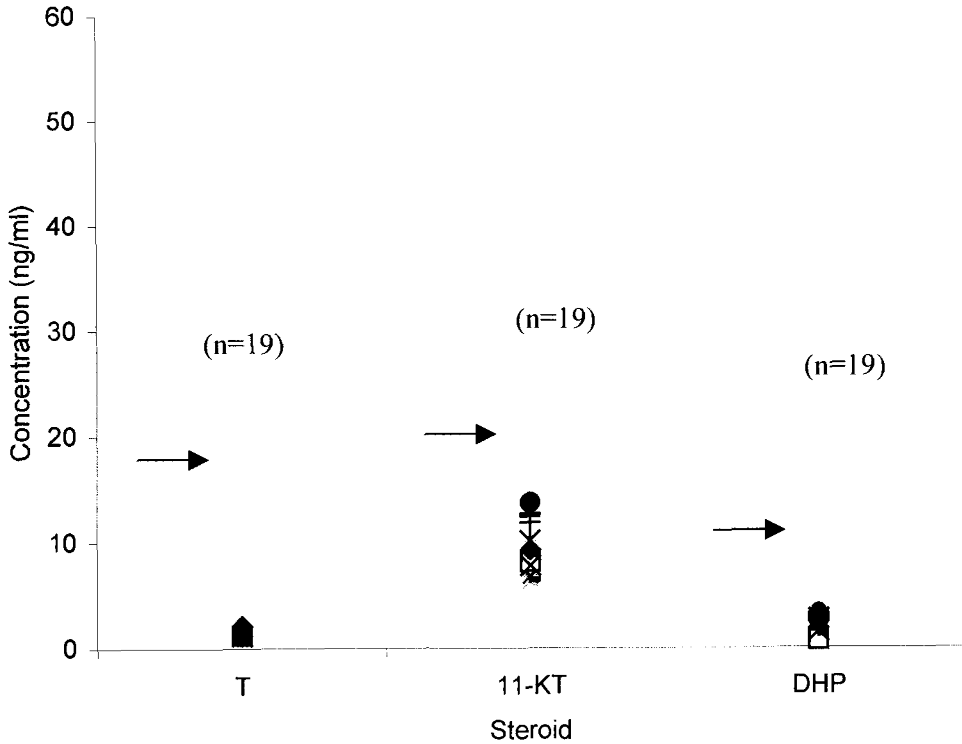

Figure 2.6: Plasma concentrations of testosterone (T), 17α-hydroxy-20β-dihydroxyprogesterone (DHP), and 11-ketotesterone (11-KT) from adult male steelhead determined to be kelts via ultrasound imaging. All symbols, which correspond to steroid levels for individual fish, below arrows had steroid concentrations within the range previously described for kelts. Sample sizes (n) are provided.

Steroid concentration provided a less clear distinction between ultrasound-determined pre-spawners and kelts. Agreement between ultrasound and steroid appraisals for pre-spawners were highly variable, with corroborations of only 20% (3/15) for T, 46% (6/13) for 11-KT, and 62% (8/13) for DHP (Figure 2.7). Median steroid levels for pre-spawners were 18.0 ng/ml (ranging from 1.7 to 35.0), 13.5 ng/ml (ranging from 7.9 to 53.5), and 5.9 ng/ml (ranging from 1.2 to 34.1) for T, 11-KT, and DHP, respectively. In total, 33% (5/15) of the ultrasound determined pre-spawners had steroid levels more typical of the post-spawned maturational phase than that of a pre-spawner. A temporal pattern between elevated concentrations of steroids and pre-spawners was also observed, with the bulk of pre-spawners sampled in April with at least one pre-spawn level of steroid (82% or 9/11) relative to those sampled in May (25% or 1/4). Limited pre-spawn samples during May, however, prevent a meaningful statistical comparison based on sample date.

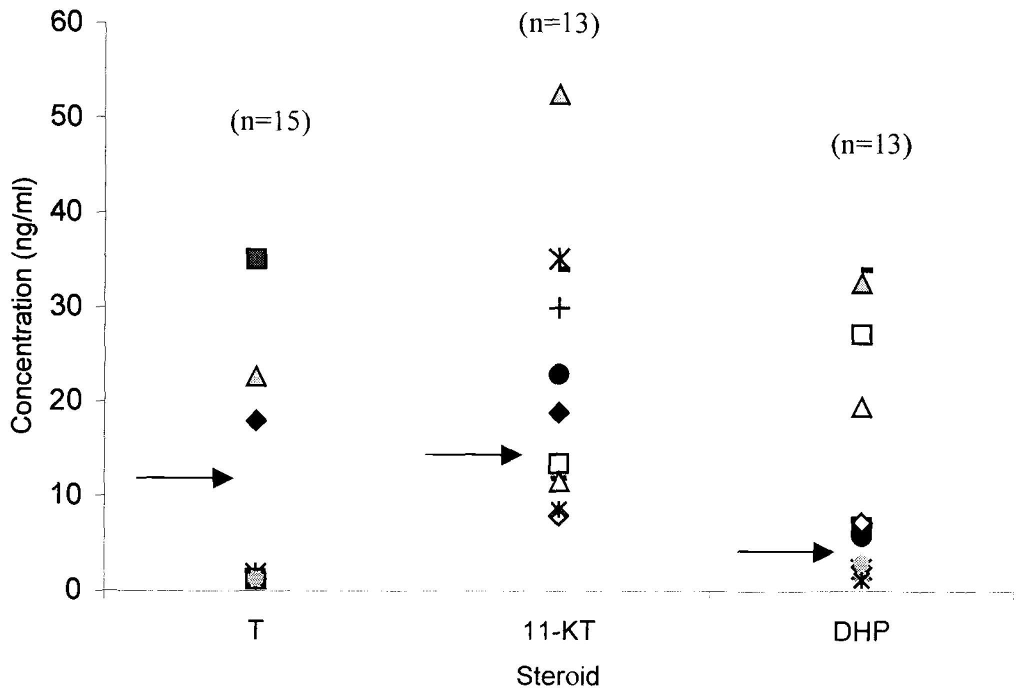

Figure 2.7: Plasma concentrations of testosterone (T), 17α-hydroxy-20β-dihydroxyprogesterone (DHP), and 11-ketotesterone (11-KT) from adult male steelhead determined to be pre-spawned via ultrasound imaging. All symbols, which correspond to steroid levels for individual fish, below arrows had steroid concentrations within the range previously described for kelts while symbols above the solid line had concentration with the range anticipated for pre-spawners. Sample size (n) are provided.

DISCUSSION

Ultrasound

Ultrasound proved to be a non-invasive, rapid and accurate method of classifying kelts and pre-spawners at the Lower Granite Dam (LGR) juvenile bypass facility. Females were easily classified as pre- or post-spawned based on egg number and the general length of the egg mass. Ultrasound images of female steelhead obtained from Lower Granite bypass were comparable to those previously described (Chapter 1), with the exception that pre-spawn gonads were more variable in regards to individual egg size and gonad size relative to the consistently large egg masses observed in mature or ripe females. Based on the high-resolution ultrasound images obtained and the clear dichotomy between pre- and post-spawned egg numbers, there is no reason to believe ultrasound appraisal of maturational status at LGR was less than 100% accurate for females with noticeable gametes.

Determination of spawning status was more difficult in male specimens because 61% of the males sampled retained noticeable testicular mass in the anterior portion of the gonad. As maturing male testes increase in size during gonad development and post-spawned male testes decrease in size following spawning, there is potential for overlap in testis area between the two maturation types. The classification accuracy of the ultrasound method used at LGR operates under the assumption that the pre-determined testis size classification rule of 1.25 cm^2 was representative of male steelhead sampled at the bypass separator. The authors have no reason to doubt this assumption was substantially violated and testis data collected from LGR male steelhead were similar – in regards to average values and variation about the mean – to the data collected from steelhead of known spawning types (Chapter 1). Males were relatively uncommon compared to females at the dam (24.4% of the sampled fish were male) and potential ultrasound error is most likely to occur in males with testis measurements near the 1.25 cm^2 threshold. In total, only 5.5% (18/330) of the sampled males or 1.3% (18/1,353) of the entire sample had testis sizes between 1.00 cm^2 and 1.50 cm^2. Classification confidence beyond this range was estimated to > 99.9% for kelts and > 99.8% for pre-spawners (Chapter 1). Thus, evidence suggests the overall accuracy of the ultrasound method at LGR was high, although some males near the classification threshold were probably misclassified.

Visual and Steroid Identification Methods

Results presented here suggest that the visual methods previously employed to classify maturational status at Snake River bypass facilities were not adequate. The errors produced by present visual methods were not a complete surprise. Although based on very reasonable assumptions (e.g., kelts *must* be dark and in poor condition), the methods had not been validated and the persons using them generally had little confidence in their unproven accuracy. Ironically, it was the kelts that tended to be bright in coloration and not the pre-spawners. Coloration may be indicative of spawning type but in fashion contrary to the logic used previously at the dams. Similar to smolts, kelts must undergo changes – both physiological and morphological – in preparation for the ocean environment. One of these changes is in coloration, whereby fish develop a more silvery appearance to become more cryptic in the ocean environment (Groot and Margolis 1991). It is possible that other morphological traits – aside from fish condition and coloration – can be used to accurately distinguish pre-spawned steelhead from kelts (Appendix). However, unless other morphological traits can accurately be used to distinguish kelts from pre-spawners, visual methods should be replaced with the more quantifiable and accurate ultrasound method.

Contrary to visual methods, which under-estimated kelt abundance, steroid concentrations tended to over-estimate kelt abundance, with 33% of pre-spawn males having steroids within the range previously described for kelts. Kelts could readily be detected by the low plasma levels of testosterone (T), 11-ketotesterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP), but some ultrasound-identified pre-spawners also had low steroid concentrations. Because individual differences in metabolic rates and individual differences in gonad development are linked to variation in plasma hormone levels (Baynes and Scott 1985, Vizziano et al. 1996), it is not surprising that T, 11-KT, and DHP levels did not concur 100% of the time with ultrasound appraisals. If male pre-spawners sample from LGR were in vastly different maturation phases, it would help explain the lack of corroboration between the two approaches. It is possible that some of the male pre-spawners, especially those sampled during the latter half of the season, were reproductive "duds" (fish that are reproductively mature but, for whatever reason, unable to spawn). Although duds would still possess a full complement of sperm, reproductive hormones associated with gonadal development might be lower than expected. During the first four weeks (April 7 to April 26, 2000), 82% (9/11) of the pre-spawners had concentrations of hormones that would classify these fish as pre-spawners. Conversely, during the latter half of the sampling period (from May 5 to May 21, 2000), only 25% (1/4) of the ultrasound determined pre-spawners had elevated concentrations of steroids. Given that the majority of Snake River steelhead spawn from March to May (Busby et al. 1996), the lack of reproductive hormones in males sampled during May provides some evidence that either gonad recrudescence had completely ceased and/or these individuals were reproductive duds.

Kelt Abundance

The abundance and early arrival of female kelts relative to male kelts observed at LGR suggests that more female steelhead survive the act of spawning and that females are the first to leave the spawning grounds in the spring. A trend toward higher post-spawn female survival, relative to males, is consistent with data from other iteroparous populations (Withler 1966, Leider et al. 1986, Fleming 1998, and Niemel et al. 2000). Niemel et al. (2000) found that female Atlantic salmon (*S. salar)* kelts from the Scandinavian River Teno were more abundant than male kelts (861 females to 516 males) following spawning and that this was reflected in a female-dominated repeat spawner population the following year. A study investigating steelhead populations along the Pacific Coast concluded that females composed 81.5% of all repeat spawners that were examined in eight different coastal rivers (Withler 1966). The lower number of post-spawned males to females may be a result of intensive male-male competition on the spawning grounds (Fleming 1998, Niemel et al. 2000), resulting in higher male post-spawned mortality rates (Leider et al. 1986). The observation that the number of male kelts increased as the season progressed is also supported in literature. Shapovalov and Taft (1954) noted that male steelhead were often the last to leave the spawning grounds.

Hatchery kelt abundance at the LGR bypass facility peaked one week prior to that of wild kelts, and wild kelts substantially outnumbered hatchery kelts during the last few weeks of the sampling period. Temporal differences in the proportion of hatchery kelts versus wild kelts may be a reflection of hatchery practices. Many steelhead and salmon hatcheries in the Columbia River Basin select the first returning individuals as broodstock, which in turn may result in selection for earlier migration and spawning of hatchery fish relative to wild cohorts (Leider et al. 1986, Mackey et al. 2001). Early selection has been imposed to increase egg and juvenile rearing times at the hatcheries and to broaden the gap between hatchery and wild spawners to minimize interbreeding (Mackey et al. 2001).

Steelhead that return to state, federal or tribal fish hatcheries in the Snake River Basin are typically used as broodstock, harvested by anglers, or killed to prevent hatchery fish from spawning with wild cohorts (Busby et al. 1996). Although wild kelts outnumbered hatchery kelts in our sample, thousands of hatchery steelhead spawned and successfully reached Lower Granite Dam during the spring of 2000. Relative to wild kelts, hatchery kelts composed 45% of all post-spawned steelhead sampled during the study period. Given that management practices at hatcheries attempt to limit interbreeding opportunities, the large number of post-spawned hatchery fish present at LGR is surprising. The biological impact of hatchery fish spawning in the wild is a subject of great debate and controversy in the Pacific Northwest (Busack and Currens 1995, Campton 1995). Recent research suggests that hatchery steelhead are less fit relative to wild spawners (Leider et al. 1990) and hatchery steelhead are known to stray and spawn with wild fish in the Snake River and elsewhere (Leider et al. 1990, Busby et al. 1996, Mackey et al. 2001). Unfortunately, kelt data collected from LGR can not provide information regarding where, when, or if hatchery kelts spawned with wild cohorts, although the abundance of hatchery kelts suggests that interbreeding was possible.

Many of the ESA-listed steelhead that spawned in the Snake River in 2000 were collected as kelts in the juvenile bypass system at Lower Granite Dam, with an estimated 17% of the protected run encountered during just ten weeks of sampling. Assuming a pre-spawn male to female ratio of 1:1 in the Snake River and the finding that kelts were predominately female, the potential contribution of kelts toward future steelhead runs – in terms of egg production – is much greater than 17%. In addition, it is likely wild kelts observed in the LGR bypass facility represent only a fraction of the total number of kelts present in-river. Only those adults entrained by the LGR bypass system were examined with ultrasound and the bypass estimate does not account for the number of kelts that utilized the dam's spillway or turbine units to emigrate during the spring of 2000. If the juvenile bypass systems collects about the same proportion of kelts passing LGR as it does smolts (estimated between 38% and 63% for smolts; FPC 2002), then about twice as many wild kelts passed LGR via all routes as the 2,050 fish counted in the bypass system alone. Whether kelts have a similar passage behavior to that of juveniles is yet to be reported; however, the total number of ESA-listed kelts present in the Lower Snake River was certainly higher than 17% or 2,050 kelts.

Despite the thousands of ESA-listed kelts that attempt emigration in Snake River, there are currently no fishery management initiatives to protect kelts or to enhance iteroparity in Columbia Basin steelhead stocks. Kelt abundance at LGR does illustrate the magnitude of kelt passage in the Lower Snake River, but the number of kelts that survived outmigration, underwent recrudescence in the ocean environment, and subsequently migrated back up the Snake R. during the fall of 2000 is not known. Clearly, the iteroparous life history trait that makes the steelhead so unique has not been eliminated in the Snake R., as evident from the thousands of good condition kelts attempting outmigration. However, the fate of these kelts and their eventual contribution to subsequent spawning runs needs investigation. Furthermore, research to assess the passage behavior and overall system survival of kelts during emigration to the Pacific Ocean is needed. Kelt tagging studies (e.g., radio telemetry and passive integrated transponder tagging) would greatly augment our understanding of kelt passage behavior, passage efficiency, and outmigration success to the Pacific ocean. In addition, kelt reconditioning studies may also produce methods to bolster kelt survival and may eventually supplement wild Snake R. stocks through the collection of wild kelts at mainstem hydroelectric facilities. Reconditioning is the process of culturing post-spawned fish in a captive environment until they are able to grow and develop mature gonads for a second spawning. Successfully reconditioning of iteroparous species has been achieved with arctic char *Salvelinus alpinus* (Boyer and Toever 1993), Atlantic salmon *Salmo salar* (Johnston et al. 1990, Crim et al. 1992, Moffett et al. 1996) and steelhead (Wingfield 1976).

Ultimately, fishery managers must be able to evaluate which approaches (e.g., kelt reconditioning) and operational modifications (e.g., increased spill regimes or bypass design features) can most cost-effectively increase iteroparity. Although addressing these needs presents a challenge, such information may help address a central question to salmon restoration in the Columbia Basin: how to rebuild natural populations expeditiously in ways that preserve their genetic and life history diversity.

REFERENCES

Arkush, K.D., and N.A. Petervary. 1998. The use of ultrasonography to predict reproductive development in salmonid fish. International Association for Aquatic Animal Medicine. 29: 19-20.

Baynes, S.M., and A.P. Scott. 1985. Seasonal variations in parameters of milt production and in Plasma concentration of sex steroids of male rainbow trout (*Salmo gairdneri*). General and Comparative. Endocrinology. 75: 150-160.

Blythe, B., L.A. Helfrich, W.E. Beal, B. Boswarth, and G.S. Libey. 1994. Determination of sex and maturational status of striped bass (*Morone saxatilis*) using ultrasonic imaging. Aquaculture 125:175-184.

Boyer, J.N., and W.Van Toever. 1993. Reconditioning of Arctic charr (*Salvelinus alpinus*) after spawning. Aquaculture 110:279-284.

Budy, P., G.P. Thiede, N. Bouwes, C.E. Petrosky, and H. Schaller. 2002. Evidence linking delayed mortality of Snake River salmon to their earlier hydrosystem experience. North American Journal of Fisheries Management. 22:35-51.

Busack, C.A. and K.P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium. 15: 71-80.

Busby, P.J., T.C. Wainwright, E.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz, and I.V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. NOAA Technical Memorandum. Available from National Marine Fisheries Service. Seattle, WA.

Campbell, C.M., A. Fostier, B. Jalabert, and B. Truscott. 1980. Identification and quantification of steroids in the serum of rainbow trout during spermiation and oocyte maturation. Journal of Endocrinology. 85: 371-378.

Campton, D.E. 1995. Genetic effects of hatchery fish on wild populations of pacific salmon and steelhead: what do we really know. American Fisheries Society Symposium. 15: 337-353.

Crim, L.W., C.E. Wilson, Y.P. So, D.R. Idler, and C.E. Johnston. 1992. Feeding, reconditioning, and rematuration responses of captive Atlantic salmon (*Salmo salar*) kelt. Canadian Journal of Fisheries and Aquatic Sciences. 49:1835-1842.

Fitzpatrick, M.S., G.V. Kraak, and C.B. Schreck. 1986. Profile of plasma sex steroids and gonadotropin in coho salmon, Oncorhynchus kisutch, during final maturation. General and Comparative Endocrinology. 62: 437-451.

Fleming, I.A. 1998. Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. Canadian Journal of Fisheries and Aquatic Sciences. 55(Supplement 1): 59-76.

FPC (Fish Passage Center). 2002. Fish Passage Center 2001 Annual Report. Available through the internet at *http://www.fpc.org/reports*

Groot, C. and L. Margolis. 1991. Pacific Salmon Life Histories. UBC Press. Vancouver, B.C.

Hockersmith, E. J. Vella, L. Stuehrenberg, R.N. Iwamoto, and G. Swan. 1995. Yakima River radio-telemetry study: Steelhead, 1989-93. Report to US Dept. Energy, Bonneville Power Administration, for Proj. No. 89-089, Contract No. DE-AI79-89BP00276, by Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA.

Holloway, A.C., M.A. Sheridan, G.V.D. Kraak, and J.F. Leatherland. 1999. Correlations of plasma growth hormones and thyroid hormones in rainbow trout during sexual recrudescence. Comparative Biochemistry and Physiology. 123: 251-260.

Johnston, C.E., S.R. Farmer, R.W. Gray, and M. Hambrook. 1990. Reconditioning and reproductive responses of Atlantic salmon kelts (*Salmo salar*) to photoperiod and temperature manipulation. Canadian Journal of Fisheries and Aquatic Sciences. 47: 701-710.

Leider, S.A., M.W. Chilcote, and J.J. Loch. 1986. Comparative life history characteristics of hatchery and wild steelhead trout (*Salmo gairdneri*) of summer and winter races in the Kalama River, Washington. Canadian Journal of Fisheries and Aquatic Sciences. 43: 1398-1409

Leider, S.A., P.L. Hulett, J.J. Loch, and M.W. Chilocote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture. 88: 239-252.

Mackey, G., J.E. McLean, and T.P. Quinn. 2001. Comparison of run timing, spatial distribution, and length of wild and newly established hatchery populations of steelhead in Forks Creek, Washington. North American Journal of Fisheries Management. 21:717-724.

Martin, R.W., J.Myers, S.A. Sower, D.J. Phillips, and C. McAuley. 1983. Ultrasonic imaging, a potential tool for sex determination of live fish. North American Journal of Fisheries Management. 3:258-264.

Moffett, I.J.J., G.J.A. Kennedy, and W.W. Crozier. 1996. Freshwater reconditioning and ranching of Atlantic salmon, *Salmo salar* L., kelts: growth and reproductive performance. Fisheries Management and Ecology. 3:35-44.

Niemela, E., T.S. Makinen, K. Moen, E. Hassinen, J. Erkinaro, M. Lansman, and M. Julkunen. 2000. Age, sex ratio and timing of the catch of kelts and ascending Atlantic salmon in the subarctic River Teno. Journal of Fish Biology. 56: 974-985.

NMFS (National Marine Fisheries Service). 1997. Endangered and threatened species: Listing of several evolutionary significant units (ESUs) of west coast steelhead. Final rule. Federal Register 62 (159):43937-43940.

NMFS (National Marine Fisheries Service). 2000. Biological Opinion on the operation of the Federal Columbia River Power System. National Marine Fisheries Service Northwest Region. Seattle, WA.

NPPC (Northwest Power Planning Council). 1996. Return to the River. Northwest Power Planning Council. Portland, OR. Available on the internet at *http://www.nwcouncil.org/library*

Reimers E., P. Landmark, T. Sorsdal, E. Bohmer, and T. Solum. 1987. Determination of salmonids' sex, maturation and size: an ultrasound and photocell approach. Aquaculture Magazine. November/December: 41-44.

Robards, M.D., and T.P.Quinn. 2002. The migratory timing of adult summer-run steelhead in the Columbia River over six decades of environmental change. Transactions of the American Fisheries Society. 131: 523-536.

Schaeffer, R.L., L.O. Mendenhall, and L. Ott. 1989. Elementary Survey Sampling. Fourth Edition. PWS-KENT Publishing Company. Boston, MA 02116.

Scott, A.P., V.J. Bye, and S.M. Baynes. 1980. Seasonal variation in plasma concentrations of 11-ketotestoterone and testosterone in male rainbow trout, *Salmo gairdnerii Richardson.* Journal of Fish Biology 17: 495-505.

Shapalov, L. and A.C. Taft. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri* gairdneri) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. California Department of Fish and Game. Bulletin 98.

Shields R.J., J Davenport, C. Young and P.L. Smith. 1993. Oocyte maturation and ovulation in the Atlantic halibut, *Hippoglossus hippoglossus* (L.), examined using ultrasonography. Aquaculture and Fisheries Management. 24: 181-186.

USACE (US Army Corps of Engineers). 1996-00. Juvenile Fish Transportation Program Annual Reports 1996 - 2000. U.S. Army Corps of Engineers, Walla Walla District, Walla Walla, WA.

US Fish Commission. 1895. A preliminary report upon salmon investigation in Idaho. Steamer Alabtross. Bulletin of the US Fish Commission. Government Printing Office. Washington D.C.

Vizziano, D., F.L. Gac, and A. Fostier. 1996. Effect of 17β-estradiol, testosterone, and 11-ketotestosterone on 17, 20β-diydroxy-4-pregnen-3-one production in the rainbow trout testis. General and Comparative Endocrinology. 104: 179 -188.

Whitt, C.R. 1954. The Age, Growth, and Migration of Steelhead Trout in the Clearwater River, Idaho. MS thesis, University of Idaho, Moscow, ID.

Wingfield, B. 1976. Holding summer steelhead adults over to spawn second year. Northwest Fish Culture Conference. 27: 63-64

Withler I. L. 1966. Variability in life history characteristics of steelhead trout (Salmo Gairdneri) along the Pacific Coast of North America. Journal of the Fisheries Research Board of Canada. 23: 365-393.

GENERAL CONCLUSION

Ultrasound imaging provides a way to determine maturational status in adult summer-run steelhead. The accuracy of ultrasound will depend on both the sex and maturational status of individual specimens at the time of ultrasound examination. Mature adult steelhead examined here were at the final stages of reproductive development, which maximized the differences between the mature and post-spawned fish. The large, well developed egg masses of pre-spawn steelhead – in concert with the highly visible chorion of the egg membrane – results in a potentially error free identification tool for females with noticeable gametes. In cases where eggs (or milt) are absent, other techniques must be used to identify sex (e.g., sexual dimorphism) in post-spawned fish. Results indicate that some male steelhead retain testicular mass in the anterior region of the gonad following spawning. Although the testicular tissue is readily identifiable via ultrasound imaging, a more quantifiable approach is needed to determine maturational status in males via cross-sectional measurements of testis area. Testis measurements from males of known spawning types indicate that significant differences exist between mature and post-spawn males. Testis area measurements within each maturation type did not overlap with one another. Due to large differences in testis size between pre- and post-spawned steelhead, classification criteria can be used to predict maturational status with little estimated error.

The accuracy of determining maturational status in steelhead will depend on the distribution of gonad measurements obtained and to some degree the cost of error the researcher is willing to incur. For example, if the primary goal is to accurately identify kelts, adoption of a right-shifted classification rule will reduce error associated with kelts but simultaneously increase error associated with the classification of pre-spawners. The classification rule for determining maturational status in male steelhead developed here also operates under the assumption that known testis sizes are representative of males encountered at Lower Granite Dam's (LGR) juvenile bypass facility. The sampling of known males during the same general time period and at locations adjacent to the dam increases the likelihood of obtaining a representative model population. Regardless, it is difficult to assess the accuracy of ultrasound appraisals for LGR sampled males because there was no confirmatory measurement and it certainly possible that maturational status was erroneously determined in some individuals.

Concentrations of reproductive hormones have been used as indicators of reproductive development in numerous salmonid species and the belief was that steroid concentrations could be used to verify ultrasound data. As anticipated, known mature males had significantly higher concentrations of testosterone (T), 11-ketotesterone (11-KT), and 17α-hydroxy-20β-dihydroxyprogesterone (DHP) relative to post-spawn males. Comparisons of gonad size and reproductive hormones demonstrated that quantifiable differences could be detected with changing maturational phases in male steelhead. Thus, an association between gonad size and reproductive physiology was confirmed. Ultrasound-classified kelts at LGR had concentrations of T, 11-KT, and DHP within the range anticipated for the post-spawned maturational phase, based on concentrations obtained from known kelts. However, steroid levels in LGR pre-spawners varied sufficiently that only 67% had at least one steroid concentration within the range anticipated for a maturing fish. Of the three steroid examined, DHP was the mostly commonly elevated hormone among pre-spawners. Conversely, T and 11-KT levels were no longer elevated in many of the ultrasound determined pre-spawners, suggesting spermatogenesis – an early phase of gonad develop – had ceased. These observations suggest that the lower-than-expected levels of hormones that are involved in gonad development indicate that the majority of male pre-spawners from LGR had either completed gonad development or were reproductive "duds". Regardless, the variable and cyclical patterns of reproductive hormones during gonad development limited the corroboration between steroids and ultrasound appraisals of maturational status.

Using the ultrasound method and the classification criteria developed from fish of known maturational statuses, it was revealed that 94.6% of the adult steelhead encountered at LGR during the spring of 2000 were kelts and not pre-spawn fallbacks. Although pre-spawners were encountered at the dam during the sampling period, the majority (85.2%) were observed during the first five weeks of the ten-week sampling period. The abundance of kelts in the LGR bypass facility followed a classic bell-shaped curve with peak numbers observed during the last two weeks of April and first two week of May. In total, an estimated 2,050 wild kelts, equivalent to *ca.* 17% of the 1999 wild run above Lower Granite Dam, passed through the juvenile collections system at LGR. Kelts were considered to be in good condition and the kelt run was predominately female. Assuming that kelts not only use the juvenile bypass to emigrate, kelts in bypass facilities represent only a portion of the total number of kelts emigrating in the Snake River each spring.

Based on the morphological traits of fish condition and coloration, it was previously believed that majority (> 73%) of adult steelhead present in the Lower Snake river during the spring were pre-spawners, i.e. fish that had temporarily over-wintered in the Snake River before completing their migration to spawning areas. Although based on reasonable assumptions (e.g., kelts *must* be dark and in poor condition), visual methods had not been validated and the persons using them generally had little confidence in their unproven accuracy. Relative to ultrasound appraisals, visual methods grossly under-estimated kelt abundance with only 18% of the ultrasound sampled adults being classified as post-spawned. Fish coloration was the most misleading of the two visual traits, with kelts tending to be brighter in coloration than pre-spawners. Ironically, visual methods assumed that kelts would be dark in coloration relative to pre-spawners. Unless other morphological traits can accurately be used to distinguish kelts from pre-spawners (see Appendix A), visual methods should be replaced with the more quantifiable and accurate ultrasound method.

Development of a kelt identification tool (i.e., ultrasound) is just the first step in broadening our understanding of kelts. Despite the large numbers and good condition of kelts observed in the juvenile bypass facility at Lower Granite Dam, data relating to the outmigration success and passage behavior of kelts are needed. After passing Lower Granite Dam, kelts must navigate seven additional dams and travel approximately 700 river kilometers to the Columbia River estuary. Information regarding the downstream survival and passage behavior of kelts will be important in making future decisions about operations, facilities, and options to improve kelt survival. This information will probably be needed soon to decide whether and how to protect the thousands of steelhead kelts that attempt to migrate downstream through the Federal Columbia River Power System each spring. If kept alive, kelts could contribute to rebuilding wild populations in the Snake River.

BIBLIOGRAPHY

Allison, P.D. 1999. Logistic Regression Using the SAS System: Theory and Application. SAS Institue Inc. Cary, NC.

Arkush, K.D., and N.A. Petervary. 1998. The use of ultrasonography to predict reproductive development in salmonid fish. International Association for Aquatic Animal Medicine. 29: 19-20.

Baynes, S.M., and A.P. Scott. 1985. Seasonal variations in parameters of milt production and in Plasma concentration of sex steroids of male rainbow trout (*Salmo gairdneri*). General and Comparative Endocrinology. 75: 150-160.

Billard, R. 1983. A quantitative analysis of spermatogensis in the trout, Salmo trutta fario. Cell and Tissue Research. 230: 495-502.

Bjornn, T.C., M.L. Keefer, C.A.Peery, K.R. Tolotti, M.A.Jepson, and R.R. Ringe. 2000. Adult chinook and sockeye salmon, and steelhead fallback rates at John Day Dam. Available from the Biological Resources Division Idaho Cooperative Fish and Wildlife Research Unit. University of Idaho. Moscow, ID.

Blythe, B., L.A. Helfrich, W.E. Beal, B. Boswarth, and G.S. Libey. 1994. Determination of sex and maturational status of striped bass (*Morone saxatilis*) using ultrasonic imaging. Aquaculture 125:175-184.

Bonar, S.A., G.L. Thomas, and G.B. Pauley. 1989. Use of ultrasonic images for rapid nonlethal determination of sex and maturity of Pacific herring. North American Journal of Fisheries Management. 9:364-366.

Bone, Q., N.B. Marshall, and J.H.S. Blaxter. 1995. Biology of Fishes second edition. Chapman and Hall.

Boyer, J.N., and W.Van Toever. 1993. Reconditioning of Arctic char (*Salvelinus alpinus*) after spawning. Aquaculture 110:279-284.

Budy, P., G.P. Thiede, N. Bouwes, C.E. Petrosky, and H. Schaller. 2002. Evidence linking delayed mortality of Snake River salmon to their earlier hydrosystem experience. North American Journal of Fisheries Management. 22:35-51.

Busack, C.A. and K.P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium. 15: 71-80.

Busby, P.J., T.C. Wainwright, E.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz, and I.V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. NOAA Technical Memorandum. Available from National Marine Fisheries Service. Seattle, WA.

Campbell, C.M., A. Fostier, B. Jalabert, and B. Truscott. 1980. Identification and quantification of steroids in the serum of rainbow trout during spermiation and oocyte maturation. Journal of Endocrinology. 85: 371-378.

Campton, D.E. 1995. Genetic effects of hatchery fish on wild populations of pacific salmon and steelhead: what do we really know. American Fisheries Society Symposium. 15: 337-353.

Cauty, C., and M. Loir. 1995. The interstitial cells of the trout testis (*Oncorhynchus mykiss*): ultrastructural characterization and changes throughout the reproductive cycle. Tissue and Cell. 27 (4) : 383-395.

Crim, L.W., C.E. Wilson, Y.P. So, D.R. Idler, and C.E. Johnston. 1992. Feeding, reconditioning, and rematuration responses of captive Atlantic salmon (*Salmo salar*) kelt. Canadian Journal of Fisheries and Aquatic Sciences. 49:1835-1842.

Elofosson, U.O.E., I. Mayer, B. Damsgard, and S.Winberg. 2000. Intermale competition in sexually mature Arctic charr: Effects on brain monoamines, endocrine stress response, sex hormone levels, and behavior. General and Comparative Endocrinology. 118: 450-460.

Fitzpatrick, M.S., G.V. Kraak, and C.B. Schreck. 1986. Profile of plasma sex steroids and gonadotropin in coho salmon, Oncorhynchus kisutch, during final maturation. General and Comparative Endocrinology. 62: 437-451.

Fleming, I.A. 1998. Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. Canadian Journal of Fisheries and Aquatic Sciences. 55(Supplement 1): 59-76.

FPC (Fish Passage Center). 2002. Fish Passage Center 2001 Annual Report. Available through the internet at *http://www.fpc.org/reports.*

Gjerde, B. 1984. Variation in semen production of farmed Atlantic salmon and rainbow trout. Aquaculture. 40:109-114.

Groot, C. and L. Margolis. 1991. Pacific Salmon Life Histories. UBC Press. Vancouver, B.C.

Hockersmith, E. J. Vella, L. Stuehrenberg, R.N. Iwamoto, and G. Swan. 1995. Yakima River radio-telemetry study: Steelhead, 1989-93. Report to US Dept. Energy, Bonneville Power Administration, for Proj. No. 89-089, Contract No. DE-AI79-89BP00276, by Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA.

Holloway, A.C., M.A. Sheridan, G.V.D. Kraak, and J.F. Leatherland. 1999. Correlations of plasma growth hormones and thyroid hormones in rainbow trout during sexual recrudescence. Comparative Biochemistry and Physiology. 123: 251-260.

Hunt, S.M.V., T.H Simpson, and R.S.Wright. 1981. Season changes in the levels of 11-oxotestosterone and testosterone in the serum of male salmon, Salmo salar L., and their relationship to growth and maturation cycle. Journal of Fish Biology. 20: 105-119.

Johnston, C.E., S.R. Farmer, R.W. Gray, and M. Hambrook. 1990. Reconditioning and reproductive responses of Atlantic salmon kelts (*Salmo salar*) to photoperiod and temperature manipulation. Canadian Journal of Fisheries and Aquatic Sciences 47:701-710.

Khattree, R.,and D.N. Naik. 2000. Multivariate data reduction and discrimination with SAS software. SAS Institute Inc. Cary, NC.

Leider, S.A., M.W. Chilcote, and J.J. Loch. 1986. Comparative life history characteristics of hatchery and wild steelhead trout (*Salmo gairdneri*) of summer and winter races in the Kalama River, Washington. Canadian Journal of Fisheries and Aquatic. Sciences. 43: 1398-1409.

Leider, S.A., P.L. Hulett, J.J. Loch, and M.W. Chilocote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture. 88: 239-252.

Long, J.B., and L.E. Griffin. 1937. Spawning and migration habits of Columbia river steelhead trout as determined by scale studies. Copeia. 62: 27-32.

Mackey, G., J.E. McLean, and T.P. Quinn. 2001. Comparison of run timing, spatial distribution, and length of wild and newly established hatchery populations of steelhead in Forks Creek, Washington. North American Journal of Fisheries Management. 21:717-724.

Martin, R.W., J.Myers, S.A. Sower, D.J. Phillips, and C. McAuley. 1983. Ultrasonic imaging, a potential tool for sex determination of live fish. North American Journal of Fisheries Management. 3:258-264.

Moffett, I.J.J., G.J.A. Kennedy, and W.W. Crozier. 1996. Freshwater reconditioning and ranching of Atlantic salmon, *Salmo salar* L., kelts: growth and reproductive performance. Fisheries Management and Ecology. 3:35-44.

Muir, W.D., S.G. Smith, J.G. Williams, and B.P. Sandford. 2001. Survival of Juvenile salmonids passing through bypass systems, turbines, and spillways with and without flow deflectors at Snake River dams. North American Journal of Fisheries Management. 21:125-146.

Munkittrick, K.R., and R.D. Moccia. 1987. Seasonal changes in the quality of rainbow trout (Salmo gairdneri) semen: Effects of a delay in stripping on spermatocrit, motility volume and seminal plasma constituents. Aquaculture. 64: 147-156.

Niemela, E., T.S. Makinen, K. Moen, E. Hassinen, J. Erkinaro, M. Lansman, and M. Julkunen. 2000. Age, sex ratio and timing of the catch of kelts and ascending Atlantic salmon in the subarctic River Teno. Journal of Fish Biology. 56: 974-985.

NMFS (National Marine Fisheries Service). 1997. Endangered and threatened species: Listing of several evolutionary significant units (ESUs) of west coast steelhead. Final rule. Federal Register 62 (159):43937-43940.

NMFS (National Marine Fisheries Service). 2000. Biological Opinion on the operation of the Federal Columbia River Power System. National Marine Fisheries Service Northwest Region. Seattle, WA.

NPPC (Northwest Power Planning Council). 1996. Return to the River. Available from the Northwest Power Planning Council. Portland, OR 1996.

Ramsey, F.L., and D.W. Schafer. 1997. The Statistical Sleuth. Duxbury Press: An Alexander Kugushev publication. Belmont, CA.

Raymond, H.L. 1979. Effects of dams and impounds on migrations of juvenile Chinook salmon and steelhead from the Snake River, 1966 – 1975. North American Journal of Fisheries Management. 8: 1-24.

Reimers E., P. Landmark, T. Sorsdal, E. Bohmer, and T. Solum. 1987. Determination of salmonids' sex, maturation and size: an ultrasound and photocell approach. Aquaculture Magazine. November/December: 41-44.

Robards, M.D., and T.P.Quinn. 2002. The migratory timing of adult summer-run steelhead in the Columbia River over six decades of environmental change. Transactions of the American Fisheries Society. 131: 523-536.

Scheaffer, R.L., L.O. Mendenhall, and L. Ott. 1989. Elementary Survey Sampling. Fourth Edition. PWS-KENT Publishing Company. Boston, MA 02116.

Schreck, C.B. 1972. Evaluation of diel variation in androgen levels of rainbow trout, *Salmo gairdneri.* Copeia 4: 865-868.

Scott, A.P., V.J. Bye, and S.M. Baynes. 1980. Seasonal variation in plasma concentrations of 11-ketotestoterone and testosterone in male rainbow trout, *Salmo gairdnerii Richardson.* Journal of Fish Biology 17: 495-505.

Scott, A.P. and J.P. Sumpter. 1989. Seasonal variations in testicular germ cell storage and in plasma concentrations of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old. General and Comparative Endocrinology. 73: 46-58.

Shapalov, L. and A.C. Taft. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri* gairdneri) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. California Department of Fish and Game. Bulletin 98.

Shields R.J., J Davenport, C. Young and P.L. Smith. 1993. Oocyte maturation and ovulation in the Atlantic halibut, *Hippoglossus hippoglossus* (L.), examined using ultrasonography. Aquaculture and Fisheries Management. 24: 181-186.

Sumpter J.P., A.P. Scott, S.M. Baynes, and P.R. Witthames. 1984. Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri Richardson*). Aquaculture. 43: 235-242.

USACE (US Army Corps of Engineers). 1996-00. Juvenile Fish Transportation Program. Annual reports for 1996-2000. U.S. Army Corps of Engineers, Walla Walla District, Walla Walla, WA.

US Fish Commission. 1895. A preliminary report upon salmon investigation in Idaho. Steamer Alabtross. Bulletin of the US Fish Commission. Government Printing Office. Washington D.C.

Vizziano, D., F.L. Gac, and A. Fostier. 1996. Effect of 17β-estradiol, testosterone, and 11-ketotestosterone on 17, 20β-diydroxy-4-pregnen-3-one production in the rainbow trout testis. General Comparative Endocrinology. 104: 179 -188.

Webb, M.A., E.P.Foster, C.B. Schreck, and M.S. Fitzpatrick. 2002. Potential classification of sex and stage of gonadal maturity of wild white sturgeon using blood plasma indicators. Transactions of the American Fisheries Socity. 131: 132-142.

Whitt, C.R. 1954. The Age, Growth, and Migration of Steelhead Trout in the Clearwater River, Idaho. MS thesis, University of Idaho, Moscow, ID.

Wingfield, B. 1976. Holding summer steelhead adults over to spawn second year. Northwest Fish Culture Conference. 27: 63-64

Withler I. L. 1966. Variability in life history characteristics of steelhead trout (Salmo Gairdneri) along the Pacific Coast of North America. Journal of the Fisheries Research Board of Canada. 23: 365-393.

APPENDIX

APPENDIX: Visual Identification Techniques

The morphological traits of fish condition and coloration grossly under-estimated kelt abundance at the Lower Granite Dam (LGR) bypass facility during the spring of 2000. However, it is possible that other morphological trait (s) – aside from or in addition to condition and coloration – can be used to accurately classify spawning status. Although ultrasound proved to be an accurate and non-invasive way to classify maturational status, the cost and expertise necessary to use ultrasound may limit its widespread application to Columbia Basin hydroelectric facilities. Therefore, one objective established for this study was to try to develop a better visual method for identifying maturation types at the dams.

Morphological data collected from 227 adult steelhead examined with ultrasound at Little Goose juvenile bypass facility in 1999 indicated that abdominal appearance was significantly associated with maturational status, based on results of a logistic regression analysis (data not provided). In anticipation of this trait being a key variable in the visual identification of maturation types, U.S. Army Corps staff at LGR were trained by the author – prior to the onset of the 2000 fish passage season – how to visually determine maturation types based on abdominal appearance: those fish with fat, rounded abdomens were classified as pre-spawners and those fish with slim, imploded abdomens classified as kelts. The Corps' visual appraisal of maturational status was directly compared to an ultrasound appraisal for each fish examined and an accuracy determined. For the purpose of this comparison, it was assumed that ultrasound correctly classified maturation with 100% accuracy. Due to time constraints, Corps biological technicians monitoring the separator only had time to examine abdominal appearance and other morphological traits of possible relevance were not examined by Corps staff in 2000.

To continue to refine visual models/keys and perhaps identify additional morphological traits associated with maturational status, morphometric data were collected from anesthetized adult steelhead prior to ultrasound examination at Lower Granite Dam's (LGR) juvenile bypass during the spring of 2000, during the period spanning 1 April to 10 June 2000. Only those morphological traits that could be readily identified by Corps staff at the bypass facilities were considered for analysis, although a more thorough examination was feasible on anesthetized fish compared to fish examined by Corps staff in the bypass separator.

In total, five explanatory variable types were appropriate for building a classification model (Table 1): abdominal appearance (ab), condition (cond), coloration (col), caudal fin wear (cw), and anal fin wear (aw). In addition to being readily identifiable to persons monitoring the separator, these explanatory variables were chosen because the researchers expected them to be different for kelt and pre-spawners based on their biological relevance to spawning. For example, fin wear results from a female digging redds for egg deposition and it was logical to assume female kelts would have eroded fins. Male steelhead may also incur fin wear during defense of redds, especially those located in shallow water. Differences in abdominal size may result from the loss of eggs or milt during spawning, making pre-spawn fish appear fatter relative to kelts. As a result of the long spawning migration that summer steelhead must undergo and the subsequent building and defending of redds, kelts may also be in worse morphological condition relative to pre-spawners. Lastly, coloration may also be indicative of spawning type but in fashion contrary to the logic used previously at the dams. Similar to smolts, kelts must undergo changes – both physiological and morphological – in preparation for the ocean environment. One of these changes is in coloration, whereby fish develop a more silvery appearance to become more cryptic in the ocean environment. Thus, it is possible that kelts are brighter in coloration than pre-spawners and not vice versa, as previously employed at the dams.

Table 1: Description of the five morphological traits used in the analysis of maturational status (pre- or post-spawned) via logistic regression. Each morphological trait was ranked or coded for inclusion in the model.

Morphological Category		Coded for Analysis	Description
Coloration	Bright	0	Overall silvery appearance on both sides of the fish with a white abdominal surface.
	Intermediate	1	Mixture of silver and dark-grey blotches along the sides of the fish.
	Dark	2	Dark-grey blotches dominate the sides, dorsal, and ventral surface.
Condition	Good	0	No noticeable external damage (e.g., scars, lesions, fungal infections, etc..).
	Fair	1	Minor scars, lesions, and/or fungal infections are present.
	Poor	2	Substantial lesions, fungus, scars, or other body damaged that suggests fish is moribund.
Caudal Fin wear	None	0	< 5 % of the fin eroded
	Minor	1	Between 5 and 25% erosion
	Major	2	> 25% erosion
Anal Fin wear	None	0	< 5 % of the fin eroded
	Minor	1	Between 5 and 25% erosion
	Major	2	> 25% erosion
Abdominal Appearance	Slim	0	Imploded, concave appearance when view from the side. Dorsal surface is wider in girth than abdominal surface. Bony ridge is apparent along the fish's dorsal surface. Abdominal surface also forms a ridge that runs from the pectoral to pelvic fins.
	Slim-medium	1	Abdominal surface is $\leq$ in girth relative to the dorsal surface.
	Fat-medium	2	Rounded abdomen, although girth appears uniform between pectoral and pelvic fins. Abdominal surface is $\geq$ in girth relative to the dorsal surface.
	Fat	3	Rounded abdomen, with area posterior to the pectoral fins larger in girth than the area just anterior to the pelvic fins. Abdominal surface is $>$ in girth relative to the dorsal surface.

Morphological data collected from LGR sampled adult steelhead were analyzed with logistic regression to determine which trait(s) might be statistically associated with maturational status. Morphological data were collected from each specimen before the ultrasound examination (i.e., the observer did not know the maturational status during sampling) and results are consistent with a prospective study design. Results of ultrasound appraisals were used to determine the "true" maturational status of all specimens. Thus, ultrasound results were the basis against which the classification models were constructed and evaluated. However, the author recognizes that the ultrasound method may have misidentified the maturational status of some individuals – primarily males – which could contribute to misleading morphological classification results and interpretations.

Various modeling procedures were attempted until the most parsimonious model was determined from the data set. In the context used here, parsimony refers to the fewest number of morphological traits that can be used to accurately assess maturational status at the bypass facilities. Because biologists have a limited amount of time in which to view adult steelhead at the dams, criteria for the model stressed accuracy (i.e., high probability of correctly classifying spawning status), speed, and parsimony (fewest number of steps or traits used preferred). Logistic regression compares a binary response variable (i.e., maturational status) to a combination of explanatory variables (i.e., morphological traits) and states the probability of any particular steelhead being pre- or post-spawned based on its morphological traits (Allison 1999). Each explanatory variable was tested separately to determine inclusion or exclusion based on significance, using criteria from Ramsey and Schafer (1997). Akaike information criterion (AIC) model ranking was used to determine which Logistic Regression model(s) most adequately assessed maturational status. AIC ranking is a relative measure of model adequacy (e.g., used to compare multiple models from the same data set) and can be used to assess model fit while imposing penalties as the number of explanatory variables increase in the model (Khattree and Naik 2000). The AIC ranking with the lowest value is typically preferred (Allison 1999). Logistic analysis was conducted with the aid of the SAS System for Windows v 8.

When evaluating visual classification methods, primary emphasis was placed on the proportion of pre-spawners that would be misclassified. Looking beyond the present study objectives to the time when kelts may be diverted into a program to improve their survival (e.g., transportation downstream or reconditioning *via* feeding in captivity), it would not be acceptable to mistakenly divert a pre-spawner out of an ESA-listed population and into a kelt program. Hence, it was considered a *critical error* when a pre-spawner was misclassified and a *non-critical error* when a kelt was misclassified, because the erroneously classified fish would be released into the tailrace unharmed.

Accuracy of Visual Classifications

In total, 434 steelhead were identified by both visual and ultrasound methods at the LGR bypass facility during the spring of 2000. Based on ultrasound examinations, 401 were kelts and the remaining 33 pre-spawners. Visual methods – those utilizing abdominal appearance – correctly identified 96.3% of all female kelts and 95.0% of males kelts (Table 2). The non-critical error (i.e., the erroneous classifications of kelts) at the LGR separator was estimated to be 4% (Table 2). Overall, 92.2% of the 434 adult steelhead examined via visual methods were in agreement with ultrasound appraisals, primarily because the majority of adults sampled were kelts. The accuracy of visual methods regarding pre-spawn fish was substantially lower relative to kelts. Visual methods correctly identified only 45.5% of pre-spawners, resulting in a critical error rate of 55.5% (Table 2). Male pre-spawn steelhead were particular difficult to identify based on abdominal size, with only 30.8% (4/13) being correctly identified with visual methods.

Table 2: Percent visual classification agreement with ultrasound results of maturational status at the Lower Granite separator.

Sex	Ultrasound Classification		Both Classes (n)
	Pre-spawner (n)	Kelt (n)	
Male	30.8% (4/13)	95.0% (96/101)	87.7% (100/114)
Female	55.0% (11/20)	96.3% (289/300)	93.8% (300/320)
Total	45.5% (15/33)	96.0% (385/401)	92.2% (400/434)

The overall inability of visual methods to identify pre-spawners may be a result of several factors. First, difficulty regarding male identification is likely related to the finding that abdominal differences between male pre- and post-spawned fish are less dramatic than the difference among female steelhead. Conversely, the severely imploded abdomens of kelts relative to fat, rounded abdomens of many pre-spawners – especially females – made visual classification methods for kelts highly accurate, with 96% agreement between ultrasound and visual methods. Secondly, the specific location of visual exams may influence the accuracy of appraisals. At LGR, adult steelhead are not handled and must be examined while semi-submerged in water, making it difficult to view the fish's ventral surface. Lastly, observer bias may have influenced the accuracy of pre-spawn visual methods at LGR. Corps Biological Technicians quickly learned that kelts composed the majority of adult steelhead washing into the facility and if distinct morphological traits were not apparent or a good view of the fish was not attainable, a fish was often classified as a kelt and not a pre-spawner. In this context, a classification of kelt was often the "default" options for technicians and ultimately may have contributed to the overall misidentification of pre-spawners.

Morphological Data

In total, morphological data were available from 739 of 1,353 adult steelhead sampled with ultrasound at LGR during the spring of 2000. Of the 739 fish with morphological data recorded, ultrasound examination determined that 44 were pre-spawners and 695 were kelts. As anticipated, kelts tended to be brighter in coloration than pre-spawner and kelts tended to have more fin erosion relative to pre-spawners. In total, 50.8% (353/695) of the kelts were bright in coloration, 33.1% (230/695) intermediate, and only 16.1% (112/695) characterized as dark in coloration. For pre-spawners, only 18.2% (8/44) were bright, 47.7% (21/44) intermediate, and 34.1% (15/44) characterized as being dark. In total, 74.8% (520/695) of kelts had caudal and anal fin wear covering at least 5% of the surface area. Conversely, only 29.5 % (13/44) of pre-spawners had noticeable evidence of fin wear. Of those fish determined to be pre-spawners via ultrasound examination, 14 were characterized with fat abdomens (14 female and 0 males), 23 with fat-medium abdomens (9 female and 14 males), seven with slim-medium abdomens (1 female and 6 males), and zero with slim abdomens. In comparison, no kelts were observed with fat abdomens, 51 with fat-medium abdomens (31 female and 20 males), 300 with slim-medium abdomens (210 female and 90 male), and 344 with slim abdomens (295 female and 49 male).

In general, all models that utilized abdominal appearance best described maturation types among steelhead examined in this study (Table 3), which is consistent with data collected from Little Goose Dam in 1999. All model combinations constructed without inclusion of the abdominal appearance variable yielded AIC values greater than 196 and probabilities of correct classification less than 80.0% (Table 3; combinations of non-abdominal explanatory variables not shown). Of the five variables initially tested, condition was not significantly related to maturational status (p-value = 0.90, chi-square) and was removed from further consideration. No tests could initially be run on abdominal size (coded 0-3), because no variation existed within two of four classes: those with slim abdomens (coded 0) and those with fat abdomens (coded 3). A 100% of the steelhead with slim abdomens were kelts and 100% of those with fat abdomens were pre-spawners. In total, fat and thin abdominal appearance alone correctly classified 48.4% (358/739) of all adult steelhead examined during the study as kelts (n=344) or pre-spawners (n=14). Since no variation exists (i.e., no error to measure) within these two categories, logistic regression cannot be used. However, since other morphological traits may also be good indicators of maturation, analysis was continued with the 381 individuals with abdomens coded 1 (slim-medium) and 2 (fat-medium) along with the other three remaining traits of interest (caudal fin-wear, anal fin-wear, and coloration).

Even after exclusion of fat and slim fish, abdominal appearance is still significantly related to maturational status (p-value < 0.001, 1 df, chi-square), with the probability of a slim-medium fish being a kelt estimated at 97.7% (95.3%$_{\text{lower C.I.}}$ to 98.9%$_{\text{upper C.I}}$). After accounting for the effects of abdominal appearance, coloration was significantly related to maturational status (p-value = 0.03, df 2, chi-square), with the probability of being a kelt with slim-medium abdomen and bright coloration estimated at 99.5% (97.6%$_{\text{lower C.I.}}$ to 99.9%$_{\text{upper C.I}}$). Conversely, a kelt with a fat-medium abdomen and dark coloration was estimated at only 56.9% (39.9%$_{\text{lower C.I.}}$ to 72.3%$_{\text{upper C.I}}$). The model with the lowest AIC contained the explanatory variables of abdominal appearance, caudal fin-wear, and coloration (Table 3).

Table 3: Comparison of various logistic regression models using Akaike information criterion (AIC) ranking. Four different explanatory variables were used to construct models; abdominal appearance (ab), caudal fin-wear (cw), anal fin-wear (aw), and coloration (col.).

Model	Log Value	AIC
U (maturation) = $B_0 + B_{1ab} + B_{2cw} + B_{3col}$	-71.29	154.88
U (maturation) = $B_0 + B_{1ab} + B_{2aw} + B_{3col}$	-71.54	155.07
U (maturation) = $B_0 + B_{1ab} + B_{2col}$	-73.62	155.23
U (maturation) = $B_0 + B_{1ab} + B_{2aw} + B_{3cw} + B_{4col}$	-70.24	156.49
U (maturation) = $B_0 + B_{1ab} + B_{2cw}$	-75.96	159.92
U (maturation) = $B_0 + B_{1ab}$	-79.25	162.49
U (maturation) = $B_0 + B_{1ab} + B_{2aw} + B_{3cw}$	-75.29	162.58
U (maturation) = $B_0 + B_{1ab} + B_{2aw}$	-77.91	163.82
U (maturation) = $B_0 + B_{1\text{(any combination of cw, aw, or col.)}}$	Variable	> 196

Very little additional variation was explained by using all three explanatory traits (abdominal appearance, caudal fin wear, and coloration) relative to using abdominal appearance alone and only a slight increase in classification confidence can be achieved. In addition, time constraints at the dams make it impractical to collected data on three traits from each individual specimen without having to anesthetize fish, at which time an ultrasound examination could easily be conducted. Given that each of the models were within 8 AIC units apart from the "best" model, the most parsimonious model to assess maturation types was determined to be one that utilizes only abdomen appearance.

Summary

Vast improvements in visual identification methods were achieved using abdominal appearance relative to the morphological traits of fish condition and coloration, traits previously used at the dams. Overall, visual methods using abdominal appearance correctly classified 92.2% of the adult examined. Fish condition was not statistically associated with maturational status and although fish coloration was, it was being applied incorrectly at the dams in years past (e.g., kelts tended to be bright in coloration, not pre-spawners). Eroded fins, caudal fins, and anal fins were also statistically associated with maturational status, but like coloration, they provided little overall increase in estimated precision relative to abdominal appearance alone. Based on pre-determined criteria for model parsimony, abdominal appearance alone is best suited and the most practical morphological trait identified among those examined here.

Despite an estimated classification accuracy of 92.2%, visual methods based on abdominal appearance tested at LGR in 2000 still misclassified the bulk of pre-spawners. The finding that male pre-spawers were predominantly observed with fat-medium or slim-medium abdomens likely contributes to misclassification accuracy. It should be noted that the visual methods tested at LGR apply only to abdominal appearance and critical error may have been reduced if both fin wear and/or coloration were used. However, since visual error was primarily associated with pre-spawners and not kelts, utilization of fin wear and/or coloration would likely have had little effect.

For enumeration purposes, critical or non-critical error is less important than if kelts were removed for reconditioning or other management purposes to bolster iteroparity in Snake R. steelhead. However, if kelt collection or kelt reconditioning were the primary research or management objective, ultrasound is clearly the preferred method. Contrary to using identification methods for enumeration purposes, the use of identification techniques for kelt restoration measures may impact future populations. This is because any true pre-spawners that are erroneously classified as kelts would be removed from the spawning population. Furthermore, a trade-off exists between maintaining a low critical-error and low non-critical error. This trade-off will depend to some degree on the magnitude of the benefit afforded by whatever kelt program(s) might be in operation in the future. For example, a lower non-critical error standard may be applied if a kelt program was very successful in facilitating repeat spawning, whereas a higher non-critical error rate may be acceptable if the best available kelt program was not very successful. In regard to the ultrasound technique, issues of critical and non-critical error are also applicable. However, evidence suggests critical error associated with the ultrasound identification of female kelts is very minimal, if not non-existent. The development of a specific testis size rule or criteria from specimens of known maturation during this research endeavor significantly reduces critical error but does not completely remove the risk of erroneously classifying some males with ultrasound.