Identification and Enumeration of Steelhead (*Oncorhynchus mykiss*) Kelts in the Juvenile Collection Systems of Lower Granite and Little Goose dams, 2000.

by

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ABSTRACT

Adult steelhead entering the juvenile collection systems of Lower Granite Dam (LGR) and Little Goose Dam (LGO) were examined with ultrasound to assess maturation status (pre-spawn or kelt), to estimate abundance of kelts, and to investigate the relationship between adult steelhead morphology and maturation. Research conducted at the dams in 2000 was a continuation and expansion of kelt research initiated at Little Goose Dam in 1999.

We used ultrasound images of visceral anatomy to identify kelts and pre-spawners among a 32.4% and 8.1% sample of adult steelhead removed from Lower Granite and Little Goose bypass facilities, respectively. Research was conducted during the peak fallback season of April through June, 2000. Kelts identified by ultrasound at the Lower Granite bypass were marked with Floy tags and released into the tailrace to resume their migration. Floy tags that were subsequently observed at a downstream bypass separators were then used to estimate the total kelt population within the Little Goose reservoir from mark-recapture equations.

The development of visual identification methods for maturation appraisal was another primary objective of the study. Corps personnel were trained by our staff – prior to the onset of the 2000 fish passage season – to visually identify the spawning status of adult steelhead removed from juvenile bypass facilities based on the fish's abdominal appearance. Steelhead with fat, rounded abdomens were visually classified as pre-spawners (i.e., fallbacks), while fish with thin, imployed abdomens were classified as steelhead kelts. After the visual identification by Corps personnel, specimens were examined with ultrasound to determine maturation and an accuracy of visual methods assessed.

Kelt Abundance: Based on ultrasound examinations we calculated that 95.9% (± 1.1, 95% C.I.) and 93.1% (± 5.0, 95% C.I.) of the adult steelhead removed from LGR and LGO, respectively, during the study period were steelhead kelts. We estimated that 3,956 of 4,182 adult steelhead removed from the LGR bypass were kelts and that 1,432 of 1,532 adult steelhead removed from the LGO bypass were kelts. We tagged and released 1,135

kelts into the tailrace of LGR between 4 April and 7 June, 2000. Of these, 104 (9.2%) were recorded reaching the Little Goose bypass, 41 (3.6%) to the Lower Monumental bypass, and 7 (0.6%) to the McNary bypass facility. Based on mark recapture methods, an estimated 7,466 wild steelhead kelts and an estimated 7,657 hatchery kelts were present between Lower Granite and Little Goose dams during the study period. Wild kelts represent between 48% and 79% of the entire wild steelhead run counted passing the LGR fishway from 1 June 1999 to 30 May 2000.

Accuracy of Visual Identification Methods: Visual determination of maturation status was based on the physical size (fat or thin) of the specimen's abdomen. Ultrasound examination at the dams, which was used to assess accuracy of visual appraisals, revealed that visual methods misclassified 62% (81%? and 48%?) of all pre-spawners as kelts but only misclassified 3% (5%? and 2%?) of kelts as pre-spawners. Despite the high misclassification rate among pre-spawner, the majority of fish examined were kelts and overall misclassification was only 7% during the study period. In comparison, visual identification methods employed at Little Goose bypass facility in 1999 – a period when fish condition and coloration were used to visually assess maturation – misclassied at least 32% of the adult steelhead in the separator during research, according to ultrasound examination.

Classification Keys: Consistent with data generated during our 1999 study, statistical analysis (logistic and tree classification models) suggests that the appearance of the steelhead abdomen is the primary visual indicator of maturation status. Steelhead were 25 times more likely to be kelts than pre-spawners if they had an imploded abdomen, according to logistic regression. Conversely, steelhead with fat, rounded abdomens were more likely pre-spawners than kelts. Analysis also suggests that caudal fin wear (ranked by degree of severity) can be used to distinguish pre-spawners from kelts at the dams. We recommend the continued use of visual methods to enumerate kelt passage at Snake R. collector dams. However, we caution that pre-spawner abundance may be underestimated – especially in males – if visual methods alone are used to assess maturation.

The good condition, ready accessibility, and abundance of wild Snake R. kelts would make them excellent candidates for reconditioning studies. Based on the results of this Corps-funded kelt identification and enumeration research project, we encourage the Walla Walla District and regional managers to consider the possible merits of aiding wild Snake River kelts to spawn again via reconditioning and to test the feasibility of kelt transport.

INTRODUCTION

Steelhead differ from most other anadromous Pacific salmonids in that they can be iteroparous. Repeat spawners have been documented returning to their natal streams in the Snake River basin for decades (Long and Griffin 1937, Whitt 1954). However, recent data for upper-basin areas suggest that repeat spawning rates in the Snake River probably average less than 2% under present conditions (Evans and Beaty 2000). Before repeat spawners can contribute to population growth and diversity, they must first successfully emigrate to the ocean following spawning. The term “kelt” has been used to describe this unique post-spawned life history phase within salmonids.

Summer steelhead migrate upstream over Snake River dams in September and October (*US v. Oregon* 1997) and are believed to spawn from early April to May (SSR 1992). However, some steelhead over-winter in the Snake R. and complete their migration to spawning areas in the spring (Whitt 1954). These late, spring pre-spawn migrants can be passing the dams – and falling back over them – when many of the kelts are beginning their downstream migration. Each spring thousands of adult steelhead must be removed from the juvenile bypass facilities at Snake R. dams. For the last decade or so US Army Corps of Engineer employees have attempted to discriminate between maturation types at the bypass facilities based on the fish’s external morphological features; dark fish in poor condition were considered kelts and bright fish in good condition counted as pre-spawners. Unfortunately, it can be difficult to distinguish an outmigrating kelt from a pre-spawn steelhead that has fallen back over the dam using fish condition and coloration as visual identification methods (Evans and Beaty 2000).

One potential alternative method of identifying maturation status among adult steelhead removed from juvenile bypass facilities 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 alternative that has been used since the early 1980s to determine the maturation status of various freshwater and marine fishes (Martin et al. 1983, Reimers et al. 1987, Shields et al. 1993, Blythe et al. 1994, and 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, and 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). Given such relevant ultrasound applications and successful results, we used ultrasound to classify the maturation status of adult steelhead removed from Snake R. bypass facilities.

Project Background

In 1999 the Columbia River Inter-Tribal Fish Commission initiated research to evaluate the use of ultrasound in the identification of steelhead kelts and pre-spawners (Evans and Beaty 2000). A comparison between Corps visual identification methods and ultrasound was also made to assess the accuracy of visual appraisals and to determine if other morphological traits, aside from those currently being used (i.e., condition and coloration), could visually identify kelts. Lastly, ultrasound maturation data collected from specimens in 1999 were used to estimate kelt abundance at the Little Goose bypass facility.

The use of ultrasound as an accurate, rapid and non-invasive method to identify steelhead maturation status was established in 1999 (Evans and Beaty 2000). Ultrasound validation work demonstrated that pre-spawn and freshly post-spawned females are easily distinguished by the presence or absence of an egg mass, although post-spawners frequently retain a few eggs. Despite the clear differences among female steelhead, it proved difficult to identify certain male steelhead that retained substantial testes mass following spawning. In 1999 we concluded that additional ultrasound research was needed to refine the ultrasound technique among male steelhead.

Sampling of adult steelhead at the Little Goose bypass separator in 1999 revealed that 85% (± 4.0, 95 C.I.) of the 2,400 steelhead removed from the separator were kelts. The high proportion of kelts discovered at LGO in 1999 were in contrast to the low (1.4%-26.5%, USACE 1996-98) kelt abundance estimates generated prior to ultrasound research. Ultrasound examinations at Little Goose Dam (LGO) also revealed that Corps visual methods misclassified 27% of the female pre-spawners as kelts and 32%[1] (both sexes) of kelts as pre-spawners. We concluded that the morphological traits used at the dam to visually identify kelts – dark coloration and poor external condition – were very misleading. Conversely, statistical analysis (logistic regression and tree classification) of morphological data collected from specimens in 1999 suggests that the appearance of the fish's abdomen (thin/imploded, intermediate, or fat) was a better indicator of maturation status. Thus, additional research was needed in 2000 to assess the use of abdominal appearance as a new visual indicator of maturation status at the dams.

2000 Research Objectives

In general, research conducted in 2000 is a continuation and expansion of kelt work initiated at the Little Goose juvenile bypass facility in 1999. This study addressed four major objectives in 2000:

[1] True misclassification error rates associated with fish condition and coloration were likely higher than 32% at Little Goose bypass in 1999. For details, please refer to Evans and Beaty (2000).

1. Refine ultrasound technique in the identification of male steelhead.
2. Estimate the proportion of kelts in the Lower Granite and the Little Goose bypass facilities, including an estimate of in-river abundance between these facilities.
3. Train technicians at Walla Walla District collector dams to visually identify kelts and use ultrasound to periodically verify their work.
4. Finalize methods to visually assess maturation based on morphological data.

(1) Further ultrasound refinement is needed regarding the identification of certain male steelhead, which tend to retain testes mass following spawning. Although data generated from 1999 research suggested male kelts and male pre-spawners can be classified based on gonad size, a larger sample of male testes data was needed from steelhead of known maturation to more adequately represent population variability regarding testis size. The development of methods to refine maturation identification techniques among male steelhead would also increase ultrasound enumeration accuracy at the dams by correctly classifying a larger proportion of male specimens.

(2) The high prevalence and overall good condition of kelts encountered at Little Goose bypass in 1999 has raised questions if this pattern of abundance is present at other Snake R. bypass facilities (e.g., at Lower Granite dam). In addition to kelt abundance at the separators, data on kelts outmigrating via spillway, turbine, fishway, and lock was needed to estimate the overall abundance of kelts in the Lower Snake R. system. Such information could help fishery managers monitor the number, condition, and passage timing of kelts in the Lower Snake R. and could ultimately lead to restoration strategies for these unique fish.

(3) Although ultrasound is relatively accurate compared to visual methods, it may not be the best option for identification in the long-term. Ultrasound is capital- and labor-intensive, requiring that the fish be anesthetized prior to an examination. A visual method – one based on easily observed morphological characteristics and that can be accurately applied by many persons – would be preferred to an on-going reliance on

ultrasound. Research conducted at Little Goose in 1999 suggests that fish condition and coloration – traits used prior to ultrasound validation – are poor indicators of maturation status. However, abdominal appearance emerged as a relatively accurate visual indicator. Thus a new visual classification trait (i.e., abdominal appearance) was in need of validation at the dams in 2000.

(4) To ensure that those morphological traits identified as good indicators of maturation during 1999 research are accurate and consistent, another year of data collection is needed. Furthermore, a second year of data might reveal other morphological traits, aside from abdominal appearance, that could be used to classify the spawning status of adult steelhead. Based on these results, a final recommendation will be made regarding the use of morphological traits as indicators of maturation status in adult steelhead encounter at Snake River bypass facilities.

METHODS

Identification of Male Maturation Status Using Ultrasound

In order to develop more comprehensive selection criteria for the identification of male kelts data was collected on the size (cm^2) and echogenity of testes in fish of known maturation status. Steelhead returning to Minthorn Hatchery (Umatilla River) and Wallowa Hatchery (Wallowa River) were used to represent testis size among pre-spawners, while kelts captured from Chandler bypass facility[2] (Yakima River) were used to represent testis size of post-spawned fish. Pre-spawn fish were examined with ultrasound during hatchery spawning operations conducted on 29 March, 2000 at Wallowa Hatchery and on 4 April, 2000 at Minthorn Hatchery. Kelts were examined at Chandler bypass facility during the period spanning 27 March to 18 May, 2000.

A portable Aloka® ultrasound machine, equipment with a 7.5 MHz linear probe, was used to examine the testis diameter of male steelhead. The examination consisted of placing the ultrasound probe along the specimen's abdomen and then moving the probe until the testis was located. The maximum diameter of the single largest testis within the ultrasound's range was measured within the body cavity (the y-axis of the ultrasound monitor denotes cm of penetration). Precise measurements of the testis were taken using an elliptical trackball function that is commonly found on ultrasound units. The trackball function allowed for measurements of testis length (both minor and major axes) and testis circumference.

Ultrasound data on the testis area (cm^2) from males of known maturation (pre-spawn or kelt) were compared using discriminant function analysis (DFA) to develop classification criteria for unknown males encountered at Snake R. bypass facilities. DFA develops a classification criterion or rule for observations based on measures of squared distance (SAS 2000). Once the classification rule was developed from the data set (known male testis area), it was then applied to testis data collected from males encountered on the separator. A cross-validation method was also used in DFA – which reduces model bias by removing each sample from the data set before classification (also referred to as the jack-knife-one procedure) – that allowed us to estimate classification error rates among samples.

Kelt Abundance Estimates

Equipment and methods used to estimate kelt abundance at the bypass facilities were very similar to those used in 1999 at Little Goose Dam (Evans and Beaty 2000). A brief description of sampling procedures at the dams is as follows:

Sampling Methods: Adult steelhead removed from the bypass facility 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 ppm and scanned with the ultrasound machine. We used three portable ultrasounds units, each equipped with 7.5 MHz linear probes, to examine adult steelhead and assess maturation. During the examination, the probe was placed against the specimen's abdomen and then moved anterior or posterior to view the ovaries or testes. Assessment of maturation status among specimens was based on the size, location, and echogenity of the gonads[3]. Following ultrasound examination we recorded our appraisal of maturation status (pre-spawn or kelt) and of sex. Those fish lacking eggs or sperm, thus impossible to sex with ultrasound, were classified as male or female kelts based on sexual dimorphism (e.g., the elongated and partially hooked male jaw).

Examination time varied slightly, averaging approximately 5 min. per fish. Following the examination, fish were allowed to recover in a holding bin within the separator (Little Goose) or just posterior to the separator (Lower Granite). Once normal swimming

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[2] Unlike the Snake River, very few, if any, pre-spawn steelhead are believed to fallback at the Chandler (Yakima River) bypass facility during the spring.
[3] Additional details regarding the methods of ultrasound examination can be found in Evans and Beaty (2000).

behavior was observed, the fish was released via flume into the tailrace of the dam to resume migration.

Sampling Effort: We conducted sampling at the Lower Granite, Little Goose, and Lower Monumental bypass facilities (Figure 1) during the course of the study. However, because adult steelhead numbers are larger at the Lower Granite bypass facility than at any other Snake R. separator we choose to maximum sampling effort at LGR in 2000. We sampled steelhead during ten weekly periods that spanned the peak of the arrival of adults in the juvenile bypass systems: 4 April to 7 June, 2000. A 100% of the adult steelhead arriving on the separator were sampled while the ultrasound machine was in use. Samples were collected during day light hours (6 a.m. to 8 p.m.) and at night (8 p.m. to 6 a.m.), however, randomization was not used to determine what day or time during the week to begin.

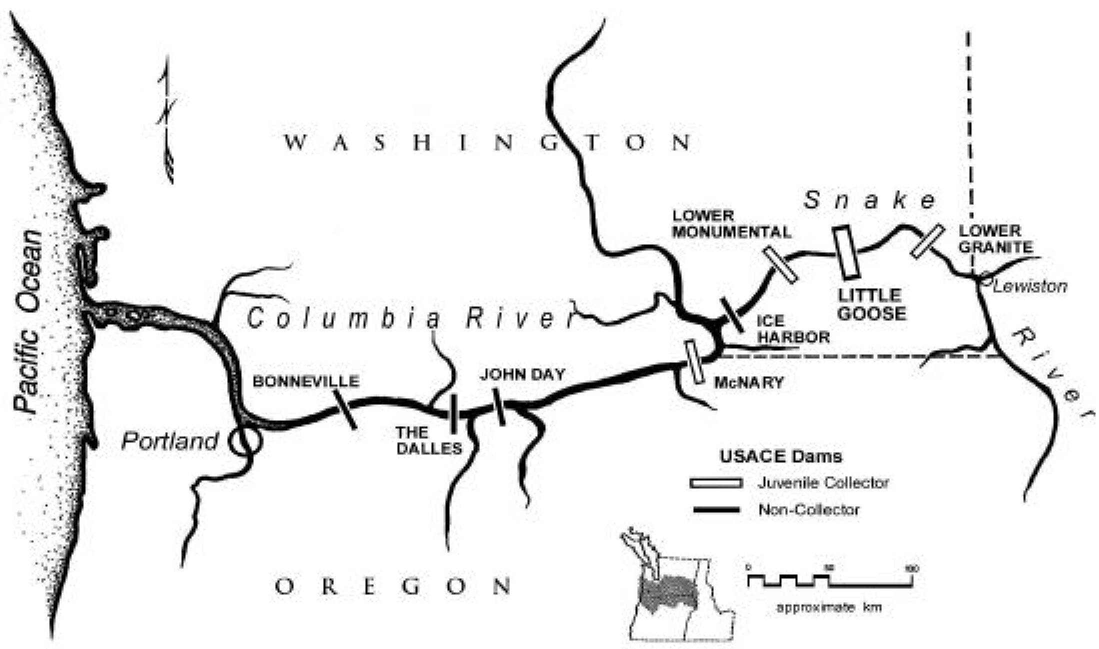

Figure 1. Location of Snake River dams.

The total proportions of kelts in the bypass systems of Lower Granite and Little Goose dams were estimated based on a weighted average of sampling weeks. The proportion of kelts identified by ultrasound during each week of sampling (week = Sunday to Saturday) was multiplied by the total number of steelhead removed from the separator during that week and an estimate determined. We assumed that fish sampled during each weekly period were representative of the fish arriving at the separator during that week. Confidence intervals for each separator estimate (Lower Granite and Little Goose) were then determined based on the relationship between the F-distribution and the binomial distribution for proportions (Zar 1984).

Kelts identified with ultrasound at Lower Granite Dam bypass were implanted with Floy® anchor tags to conduct a mark-recapture experiment. Colored (florescent yellow and orange) and uniquely numbered tags were inserted alongside the fish's dorsal fin using a standard tagging gun. Yellow tags were numbered, while single blank orange tags placed opposite to the numbered tag were used to assess tag loss during migration from Lower Granite to Little Goose bypass. Corps employees at Little Goose, Lower Monumental, and McNary bypass facilities monitored their respective separators for incoming kelts tagged at the Lower Granite bypass. However, only data collected from kelts entering Little Goose bypass were used to assess in-river abundance because tributaries (e.g., Tucannon River) located downstream of Little Goose Dam contribute kelt that can not be accounted for – and potentially tagged – at the Lower Granite separator. Conversely, all kelts that pass Lower Granite Dam must also pass Little Goose Dam.

The total number of tagged kelts recaptured at Little Goose bypass was then used to estimate the total number of kelts in the reservoir by extrapolation. Estimates were calculated using Chapman's modification of the Petersen estimator (Ricker 1975):

$$N_{reservoir} = \frac{(M_{LGR}+1)(C_{LGO}+1)}{R_{LGR@LGO}+1}$$

where:

M_{LGR} = Number of kelts marked at Lower Granite bypass.

C_{LGO} = Total number of adult steelhead kelts collected in the juvenile bypass system at Little Goose.

$R_{LGR@LGO}$ = Number of adult steelhead kelts marked at Lower Granite and recaptured at Little Goose bypass system.

Confidence intervals (±95%) were calculated using Pearson's formula (Ricker 1975), which approximates the confidence intervals for a Poisson distribution for a large number (i.e., >50) of recaptures ($R_{LGR@LGO}$):

$$R_{LGR@LGO} + 1.92 \pm 1.960\sqrt{R_{LGR@LGO} + 1.0}$$

Ancillary data on travel times from kelts tagged at the Lower Granite bypass that were subsequently recovered at the Little Goose, Lower Monumental, and/or McNary bypass were derived from numbered tags recovered at the dams.

Training Corps Personnel

We hope that simple morphological trait(s) – as opposed to the more expensive ultrasound – can be associated with maturation status and ultimately can be used to identify kelts. Toward this goal, Corps personnel were trained by our staff – prior to the onset of the 2000 fish passage season – to visually identify the spawning status (i.e., kelt or pre-spawn) of adult steelhead removed from the Snake R. juvenile bypass facilities based on the fish's abdominal appearance. Those fish with fat abdomens were classified as pre-spawners and fish with thin abdomens as kelts. The Corps' visual appraisal of maturation status was directly compared to an ultrasound appraisal for each fish examined and an accuracy determined. For the purpose of this comparison, we assumed ultrasound correctly classified maturation with 100% accuracy.

Visual Classification Keys

We collected morphometric data from all specimens examined during the study period in an effort to assess the relationship between adult steelhead morphology and maturation status. The appearance of the specimen's abdomen was noted and segregated into four distinct classes[4]: fat, fat-intermediate, thin-intermediate, and thin. We determined that four different abdominal size classes were readily apparent from specimens and that classes could easily be incorporated into statistical tests (e.g., could be pooled in two classes if necessary). In addition to abdominal appearance, data on fish length (cm fork length), condition (good, fair, poor, or dead), coloration (bright, intermediate, or dark), fin wear (ranked by degree of severity), H/W origin (based on fin clips), fungal infections (ranked by degree of severity), and physical anomalies (e.g., head burn) were also collected from specimens prior to ultrasound examination.

Morphological data were analyzed with both logistic regression and tree classification analysis to determine which trait(s) might be statistically associated with maturation status. Logistic regression compares a binary response variable (i.e., maturation status) to a combination of explanatory variables (i.e., morphological traits) and states the odds of any particular steelhead being pre- or post-spawned based on its morphological traits (Ramsey and Schafer 1997). Tree classification analysis can produce a dichotomous key that estimates the probability of correctly classifying maturation status given a combination of morphological traits (S-plus 1998). Both statistical tests can be used to quantify the error rate of any particular set of morphological traits. Logistic analysis was conducted with the aid of the SAS System for Windows v6.12 and tree classification conducted with the aid of S-Plus.

Various modeling procedures (e.g., full model, step-wise procedures, and selection models) 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 maturation status at the bypass facilities. Only those morphological traits that could be readily identified by visual methods were considered for analysis. In total, we determined that five explanatory variable types (abdominal appearance, condition, coloration, caudal fin wear, and fungal infections)

[4] In 1999, we used only three classes of abdomen appearance (fat, intermediate, and thin) but concluded four classes of abdominal size more accurately characterized the fish.

were appropriate for building the best-fitting and most simplistic classification key. To ensure consistency and to reduce any potential variation among multiple observers, only the morphological data collected by A. Evans (project leader in 1999 and 2000) were used for classification analysis purposes.

When evaluating classification methods, whether visual or ultrasound, we place primary emphasis 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), we anticipate that it would not be acceptable to mistakenly divert a pre-spawner out of an ESA-listed population and into a kelt program. Hence, we consider it a *critical error* when a pre-spawner is misclassified (Table 1). We consider it a *non-critical error* when a kelt is misclassified, because the erroneously classified fish would be released into the tailrace unharmed. We are using a provisional accuracy standard of 100% for identifying pre-spawners when evaluating both the ultrasound and the visual methods.

Table 1. Objectives and errors when classifying specimens according to maturation status. The primary objective is to correctly classify (and release) true pre-spawners.

		True Maturation Status	
		Pre-spawner	Kelt
Classification (visual, ultrasound, or other method)	Pre-spawner (release specimen)	**Primary Objective**	**Non-critical Error** (some is acceptable)
	Kelt (retain specimen)	**Critical Error** **(none may be acceptable)**	**Secondary Objective**

RESULTS AND DISCUSSION

Classifying Male Maturation Status with Ultrasound

Investigations of testes area (cm^2) in 55 pre-spawn steelhead (from Minthorn and Wallowa hatcheries) and in 34 kelts (from the Yakima River) demonstrated that testes undergo substantial size changes following spawning (Figure 2). Based on results of a mean comparison test (T-test), there is no evidence to suggest testis area among pre-spawn samples from Minthorn and Wallowa are significantly different (two-sided P= 0.25). However, highly significant differences were detected between mean pre-spawn testis area and mean kelt testis area (two-sided $P < 0.001$).

Simple linear regression analysis between testis area and fish length (cm) provides evidence of a positive association between fish size and testis size among pre-spawners (two-sided P=0.02 with $r^2 = 0.40$, and two-sided P=0.02 with $r^2 = 0.14$; Minthorn and Wallowa, respectively). However, no evidence of a relation between fish size and testis was found for Yakima River kelts (one-sided P=0.56 with $r^2 = 0.01$). Thus, larger pre-spawner individuals had larger testes – although considerable variability existed – but this association may be absent following natural spawning, as indicated by the Yakima River kelts.

Discriminant function analysis (DFA) correctly classified all male steelhead with testis area < 1.30 cm^2 as kelts and all fish with testis area > 1.22 cm^2 as pre-spawners. The overlap suggests that although the populations (kelts and pre-spawners) are significantly different regarding testis size, a "grey area" may exists in which misclassification of maturation status could result. Cross-validation techniques in DFA determined that >95% correct classification of membership could be determined for kelts with testis < 1.0 cm^2 and for pre-spawners with testis area > 1.50 cm^2 . To help reduce error associated with pre-spawners, we selected 1.50 cm^2 as the classification rule for male steelhead encountered at Snake R. bypass facilities (Figure 2). Thus, those male specimens with testis area < 1.50 cm^2 were considered kelts and those specimens with testis area ≥ 1.50 cm^2 were classified as pre-spawners. Such a rule will likely have some error associated with the future classification of kelts because it may misclassify adult males with testis area between 1.0 and 1.5 cm^2.

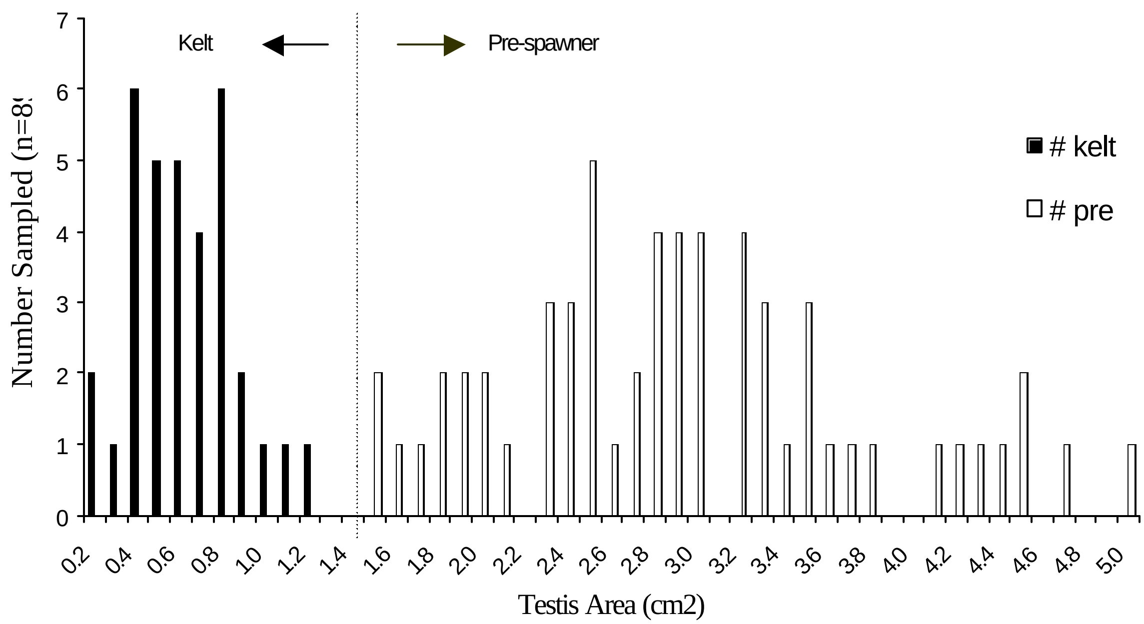

Figure 2: A histogram illustrating the difference in testis size between kelts and pre-spawners of known maturational status. Measurements of testis size are based on ultrasound examination. Horizontal line within the graph depicts the cut-off point between kelts and pre-spawners used at that dams for classifying maturation status.

The ultrasound classification rule or criteria (1.50 cm^2) obtained from the 89 known samples (referred to as the training data set in SAS) was applied to the testis data collected from male steelhead examined at the Little Goose and the Lower Granite bypass systems. Only male steelhead sampled at the dams with precise ultrasound testis measurements (n=273) were analyzed using DFA. Elliptical measurements of testis were not available from 77 males. DFA estimated an overall error rate of 1.5% for all male steelhead examined at the dams with ultrasound during 2000 research. Therefore, ultrasound methods may have misclassified a small fraction (a few percent) of the male steelhead examined at the separators. However, it is important to note that estimated error rates operate under the assumption that the testis data from known samples are representative of male steelhead arriving at Snake R. bypass facilities. The authors have no reason to doubt this assumption and testis data collected from Lower Granite male steelhead were similar to the data collected from Yakima, Wallowa, and Umatilla River steelhead of known maturation.

Along with differences in the size of testis among kelts and pre-spawners, differences regarding the ultrasound echogenity of gonads were also discovered (see Appendix A). The gonads of pre-spawn steelhead appear more hypoachoic (dark) relative to the hyperachoic (white) gonads of kelts. Pictures from a male pre-spawner and a male kelt help to illustrate the typical size difference and echogenity difference among maturation types encountered at the dams (Appendix A; figure 1 and 2). Although differences in echogenity are subjective and not easily quantified with ultrasound because measurements are on the nominal scale, they are useful to assess maturation if the ultrasound operator is well trained.

In conclusion, data collected in 2000 provided distinct, quantifiable testis selection criteria – based on the area and echogenity of testes – for the identification of maturation status among male steelhead encountered at Snake R. dams. In general, we feel research conducted to-date provides ample evidence of the ultrasound's ability to classify maturation types (both in male and female steelhead). In regards to female steelhead, ultrasound validation work conducted at hatcheries in 1999 demonstrated that egg densities were dramatically different between pre-spawn and post-spawned individuals (Evans and Beaty 2000). The ultrasound is sensitive enough and provides such high-resolution images that individual eggs can be distinguished within the female body cavity. The egg mass in pre-spawn steelhead is so dominant that visceral organs (e.g., the liver or spleen) are often obstructed from view due to the numerous, well-developed eggs. Conversely, female kelts can be characterized by the presence of few remnant eggs – typically ranging anywhere from 1 to 20 – within the body cavity, often lodged just anterior to urogenital papilla (an appendage used to discard eggs during spawning). Ultrasound also demonstrated that many adult steelhead completely discard or reabsorb remnant gonads following spawning and although they are more difficult to sex, such individuals are clearly kelts and not pre-spawners.

Kelt Abundance

Lower Granite separator: We examined 1,353 (32.4%) of the 4,182 adult steelhead removed from the separator at Lower Granite Dam bypass during the period of 4 April to 7 June, 2000 (Table 2 and Appendix B). Based on ultrasound examinations we classified 1,297 of the specimens as kelts and the remaining 56 individuals as pre-spawn fallbacks (Table 2). Based on fin clips (adipose, pectoral, and/or ventral), 525 (38.9%) were identified as hatchery kelts, while the remaining 772 (57.1%) kelts were considered naturally produced (i.e., wild) individuals (Table 2).

Samples were collected over the course of 10 weeks (Figure 3), and effort – measured by the percentage of fish sampled each week – fluctuated throughout the study period but never dropped below 13% (Appendix B). The ratio of kelts to pre-spawners changed slightly throughout the study period with kelts becoming more abundant as the season progressed. In April, 91% (391/431) of all fish sampled were kelts, while in May 98% (819/834) were kelts.

Table 2. Maturation status and origin of adult steelhead examined with ultrasound at the Lower Granite bypass, 2000.

Maturation Status	Origin No. *(%)* Hatchery		Wild		**Total**	
Pre-spawn	30	*(2.2)*	26	*(1.9)*	**56**	***(4.1)***
Kelt	525	*(38.8)*	772	*(57.1)*	**1,297**	***(95.9)***
Total	**555**	***(41.0)***	**798**	**(59.0)**	**1,353**	***(100.0)***

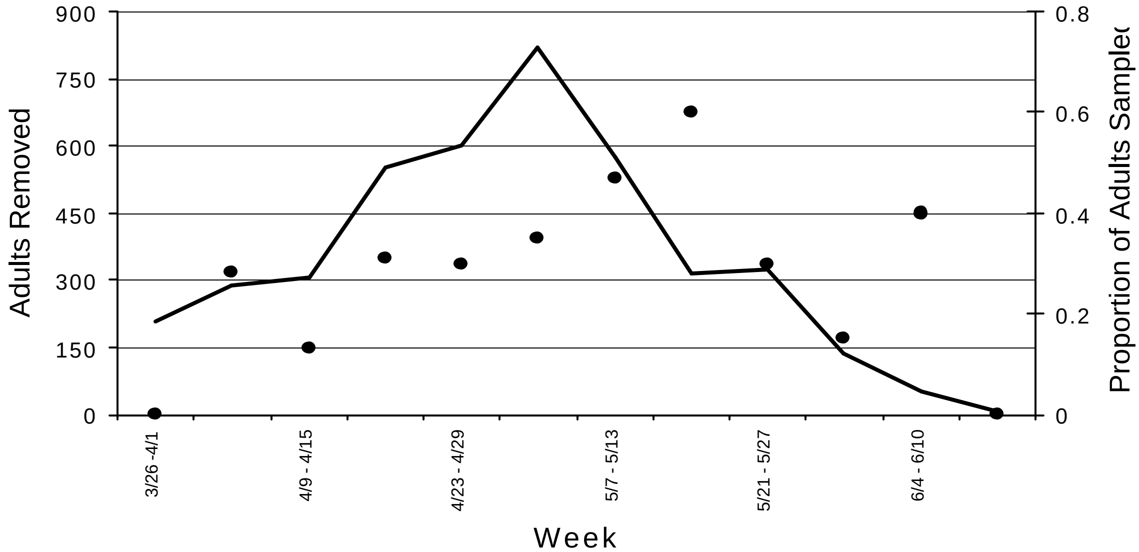

Figure 3: Number (line) of adult steelhead removed from the Lower Granite Dam bypass separator and the proportion (•) sampled with ultrasound, by week, during the study period, 2000.

In total, 4,182 adult steelhead were removed by Corps personnel at Lower Granite bypass separator between March 26 and June 15, 2000. Based on ultrasound examinations and classifications, we calculated that 95.9% (± 1.1%, 95% confidence interval) were steelhead kelts. Based on 10 statistical weeks of sampling, total kelt abundance at the LGR bypass was estimated to be 3,956 fish. This estimate is weighted to account for the higher proportion of pre-spawn fish observed earlier in the season. Assuming Corps employees at LGR accurately identified wild (i.e., non-clipped) fish in the separator when CRITFC staff were not present, 2,152 (54.4%) of the estimated 3,956 kelts were ESA-listed. These fish represent 17.8% (2,152/12,085) of the entire wild steelhead run counted passing LGR fishway from 1 June 1999 to 30 May 2000 (FPC 2000).

Little Goose separator: We examined 116 (8.1%) of the 1,428 "new" or untagged (see *Kelt Abundance in the Little Goose reservoir*) adult steelhead removed from the separator at Little Goose bypass facility during the period of 12 April to 6 June, 2000. Based on ultrasound examinations we classified 108 of the specimens as kelts and the remaining 8 individuals as pre-spawn fallbacks (Table 3). Based on fin clips, 52 (44.8%) were identified as hatchery in origin, while the remaining 64 (55.2%) kelts were considered wild (Table 3). Samples were collected over the course of 9 weeks (Figure 4), and effort was relatively consistent, although substantially lower when compared to Lower Granite, throughout the study period (Appendix B). Similar to the Lower Granite bypass, pre-spawner abundance waned during the study period with kelts becoming more numerous in May and June, although the smaller sample (n) collected from LGO makes population trends regarding abundance more difficult to assess at this facility.

Table 3. Maturation status and origin of adult steelhead examined with ultrasound at Little Goose Dam, 2000.

Maturation Status	Origin No. *(%)* Hatchery		Wild		**Total**	
Pre-spawn	6	*(5.2)*	2	*(1.7)*	**8**	***(6.9)***
Kelt	46	*(39.7)*	62	*(53.4)*	**108**	***(93.1)***
Total	**52**	***(44.8)***	**64**	**(55.2)**	**116**	***(100.0)***

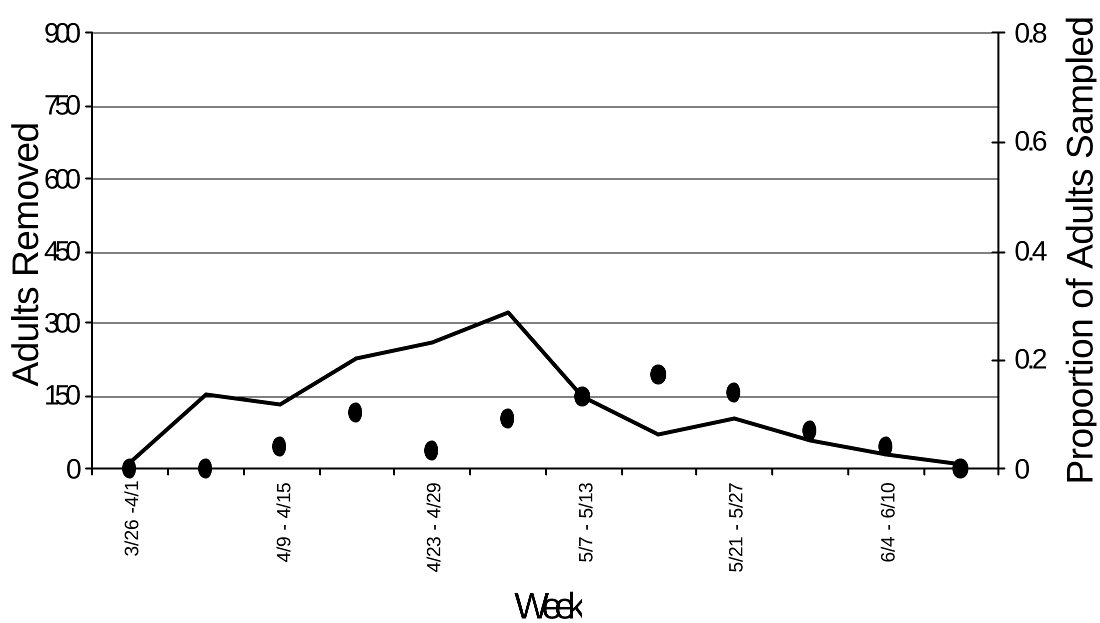

Figure 4: Number (line) of adult steelhead removed from Little Goose bypass separator and the proportion (•) sampled with ultrasound, by week, during the study period, 2000.

In total, 1,532 adult steelhead were removed from the Little Goose bypass separator between April 1 and June 15, 2000. Our total sample (n=116) accounted for 8.1% of all untagged fish (i.e., excludes kelts sampled and marked at LGR that were subsequently observed in the LGO bypass) removed from the separator during the study. We calculated that 93.1% (± 5.0%, 95% confidence interval), of the adult steelhead removed from LGO during the study were steelhead kelts. Based on 9 weeks of sampling, we estimate that 1,432 of 1,532 adult steelhead were kelts. However, the smaller sample at LGO resulted in decreased precision, as evident in the wider confidence intervals for LGO, and the lack of sampling early in the season (week 1 – 3) may have slightly skewed our kelt abundance estimates. Assuming Corps employees at LGO accurately identified wild fish in the separator when CRITFC staff was not present, 628 wild, previously un-sampled kelts were removed from LGO separator during the study period. These fish represent 5.2% (628/12,085) of the entire wild steelhead run counted passing LGR fishway from 1 June 1999 to 30 May 2000 (FPC 2000).

Some error is associated with our kelt abundance estimates for LGR and LGO bypass facilities because of the possible incorrect identification of steelhead kelts by ultrasound, due to the limited samples collected from Little Goose, and because of the possible misidentification of wild fish in the separator. All estimates are generated under the assumption that ultrasound classifies maturation status with 100% accuracy. The error associated with ultrasound applies primarily to male steelhead, which was estimated from discriminant function analysis at 1.5%. However, only 23.9% (350 / 1,459) of all fish examined were male and the author's are confident that ultrasound correctly classified close to 100% of the females. We did not attempt to quantify visual errors associated with the identification of wild versus hatchery fish. However, our appraisal of origin usually concurred with that of Corps personnel working the separator.

Little Goose reservoir: We Floy tagged and released 1,135 (685 wild and 450 hatchery) kelts into the Little Goose reservoir between 4 April and 7 June, 2000. Tagged kelts represent approximately 29% (1,135/3,956) of the entire LGR bypass population (N) available for collection during the study period. Except for those kelts washing into the separator dead (n=2), kelts were tagged regardless of their physical condition. Of the 1,135 kelts tagged, 797 (503 wild and 294 hatchery) were in good condition, 243 (140 wild and 103 hatchery) were in fair condition, and 94 (41 wild and 53 hatchery) were in poor condition upon release into the LGR tailrace.

Of those kelts tagged and recaptured at downstream bypass facilities, 104 (9.2%) were recorded reaching the Little Goose bypass, 41 (3.6%) to the Lower Monumental bypass, and 7 (0.6%) to the McNary bypass facility. According to Chapman's modification of the Peterson estimator, an estimated 7,466 wild kelts ($5{,}810_{\text{lower 95\% C.I.}}$ to $9{,}584_{\text{upper 95\% C.I}}$) were present in the Little Goose reservoir during the study period; where M_{LGRw} = 685, C_{LGOw} = 652, and $R_{LGR@LGOw}$= 59. Furthermore, an estimated 7,657 ($5{,}850_{\text{lower 95\% C.I.}}$ to $10{,}474_{\text{upper 95\% C.I}}$) hatchery kelts were also present in the Little Goose reservoir during the study period; where M_{LGRh} = 450, C_{LGOh} = 780, and $R_{LGR@LGOh}$= 45. Both estimates assume the ratio of wild to hatchery fish observed in the Lower Granite's separator is similar to that of in-river migrants (i.e., separator hatchery-to-wild ratio is representative of those fish passing via spill, turbine, lock, and fishway). The calculation also assumes that reservoir mortality rate is similar for all migrants, regardless of their previous passage route at LGR and that all tagged individuals have an equal probability of recapture. The estimate also assumes that the tagging methods used to mark kelts at LGR did not reduce the survival potential and/or passage behavior. Currently, no data is available to test the validity of these assumptions. Our abundance estimate applies only to the number of kelts present in the Little Goose reservoir and does not predict the eventual fate of outmigrants.

If tagged kelts were to shed their Floy tags between the tailrace of LGR and the LGO bypass facility, total in-river kelt abundance estimates would be exaggerated. Data on tag retention – measured by the presence or absence of a second tag – was recorded for 77 of the 104 kelts that reached LGO bypass[5], the recapture and evaluation point for estimating in-river kelt abundance. Corps personnel were unable to gather tag retention data on 27

[5] Tag retention data from 20 additional specimens were also recorded from fish recaptured at Lower Monumental and McNary bypass facilities. Of these, one fish is suspected to have lost a blank tag during outmigration from LGR to McNary.

kelts because they were not double tagged (n=13) or the information was not recorded (n=14). All of the 77 kelts that were double tagged, released into the LGR tailrace, and recaptured at the LGO bypass maintained both tags during migration. Thus, we assume that all tagged kelts released from LGR separator retained their tags to at least the Little Goose bypass facility.

Based on our calculation of total in-river abundance, wild kelts migrating past LGR represent between 48% and 79% of the entire wild steelhead run counted passing the LGR fishway from 1 June 1999 to 30 May 2000 (FPC 2000). Hatchery migrating kelts represent between 10% and 17% of the hatchery run counted passing the LGR fishway during the same time period (FPC 2000). Overall hatchery kelt proportions (i.e., pre-spawner versus kelt) are presumably lower than wild proportions due to increased harvest of hatchery stocks. Furthermore, many state, federal, and tribal hatcheries kill hatchery steelhead that arrive at their facilities, reducing the proportion of hatchery kelts capable of outmigration following spawning.

Regardless of the lower proportion of hatchery kelts to hatchery pre-spawners, thousands of hatchery steelhead spawned and successfully reached Lower Granite Dam during the spring of 2000. According to our kelt abundance estimates, approximately half of the kelts that reached LGR bypass were of hatchery origin. The biological impact of hatchery fish spawning in the wild is a subject of great debate and controversy in the region. Unfortunately, our kelt data can not provide information regarding where, when, or if hatchery kelts spawned with wild cohorts. However, hatchery steelhead are known to stray and spawn with wild fish in the Snake R. and the shear magnitude of hatchery kelts detected at Lower Granite Dam during the spring months suggests that at least some hatchery fish spawned with wild migrants in 2000.

The biological significance, in terms of repeat spawners, of thousands of kelts present below Lower Granite Dam also can not be determined at this time. The estimate does illustrate the magnitude of kelt passage in the Lower Snake R. but how many of these kelts survived outmigration, recrudesced in the ocean environment, and subsequently migrated back up the Snake R. during the fall of 2000 is currently unknown. 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. Kelt telemetry studies, which are currently being pursued in the Columbia Basin, would greatly augment our understanding of kelt passage, behavior, and outmigration success. Kelt reconditioning studies, which are also currently being conducted in the region, may produce methods to bolster kelt survival and may eventually supplement wild Snake R. stocks through the collection of wild kelts.

We did manage to document the presence of at least one returning repeat spawner during the 2000 research endeavor. On October 1, 2000 a Washington State angler captured a pre-spawn steelhead that was Floy tagged as a kelt at the Lower Granite bypass separator on April 8, 2000. The angler landed the fish via hook and line in the Snake R. about ¼ mile below the mouth of the Grande Ronde River. The angler returned the tag to CRITFC (micro 0070) and noted that the fish was fat and in good overall condition before releasing it back into the river. Upon ultrasound examination at LGR the kelt was a wild, bright, good condition female with a length of 66 cm. This particular fish was never spotted at any downstream dams during its outmigration and presumably passed non-bypass routes to at least McNary Dam.

Some of the incoming adult steelhead to the Lower Granite Dam bypass were not examined or counted during the spring months because they slipped through the separator bars before they could be enumerated. The interstitial spacing between separator bars was just wide enough to allow both narrow steelhead and small steelhead to be collected for barge transportation, similar to the way juveniles are collected at the bypass. The magnitude of this occurrence at LGR during 2000 research is unknown, however, the authors suspect 100's of adult steelhead are accidentally collected for transportation each spring at LGR. Because the separator bars are likely selective (i.e., only divert fish with a narrow girth) we suspect many of the collected adults were kelts and not pre-spawners. If these fish had been allowed to pass LGR – instead of being diverted for juvenile transport – our estimates of overall kelt abundance would be higher.

Travel Times*:* Ancillary data on travel times from kelts tagged at Lower Granite that were subsequently recovered at LGO, LMN, and McNary were derived from numbered tags recovered at the dams. Corps personnel at the downstream bypass facility did not always record the numbers listed on each LGR released tag, thus travel times could not be calculated for all recaptured specimens. Based on tag recoveries with numbers, kelt emigration travel times were calculated for the corresponding bypass facility. Median kelt travel time from the tailrace of the Lower Granite to Little Goose bypass was 85 hrs (n=95, SD 43.56 hrs), from Lower Granite to Lower Monumental bypass was 144 hrs (n=22, SD 56.35), and from Lower Granite to McNary bypass was 201 hrs (n=6, SD 65.3).

Kelt Condition, Coloration, and Sex

Kelt condition and coloration data generated from 2000 is similar to data collected at Little Goose Dam in 1999 (Evans and Beaty, 2000). Many of the kelts examined at Lower Granite and Little Goose bypass during 2000 research were bright and good overall condition (Table 4). Of the 1,297 kelts identified by ultrasound during the study period, 902 (69.5%) were in good overall condition. Pre-spawn fish were also generally in good condition, however, they tended to be darker in coloration than kelts.

Table 4. Condition and coloration by classification (pre-spawner or kelt) of steelhead examined with ultrasound at the Lower Granite bypass (LGR) and Little Goose bypass (LGO) facilities between 4 April and 7 June, 2000.

No. LGR	**Pre-spawner**				**Kelt**			
Condition	Bright	Inter-mediate	Dark	**Total**	Bright	Inter-mediate	Dark	**Total**
Good	9	13	8	**30**	531	324	47	**902**
Fair	0	8	11	**19**	33	158	94	**285**
Poor	0	2	4	**6**	2	48	58	**108**
Dead	0	1	0	**1**	1	0	1	**2**
Total	**9**	**24**	**23**	**56**	**567**	**530**	**200**	**1,297**
No. LGO								
Good	1	4	0	**5**	53	16	4	**73**
Fair	0	1	0	**1**	1	15	5	**21**
Poor	0	0	2	**2**	2	4	8	**14**
Dead	0	0	0	**0**	0	0	0	**0**
Total	**1**	**5**	**2**	**8**	**56**	**35**	**17**	**108**

In addition to the typically bright coloration and good condition of kelts, the majority of fish examined were female (Table 5). Conversely, the numbers of male and female pre-spawners were nearly equal throughout the study period. Female kelts also composed the vast majority of the sample during the early part of the study, while male kelt proportions increased as the season progressed. For example, during the month of April 91% (565/623) of all kelts were female while only 72% (601/834) were female in May. The abundance and early arrival of female kelts relative to male kelts suggest that more female steelhead survive the act of spawning and that females are the first to leave the spawning grounds come spring.

Table 5. Sex and maturation status of adult steelhead examined at the Lower Granite and the Little Goose bypass facilities between 4 April and 7 June, 2000.

Maturation Status	**Sex**		**Ratio (? to?)**
	Male	Female	
Kelt	321	1,084	1 : 3.4
Pre-spawn	29	35	1 : 1.2

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, Jonsson et al. 1991, Fleming 1998, and Niemel et al. 2000). Niemel et al. (2000) research on Atlantic salmon (*S. salar)* kelts from the Scandinavian River Teno, indicated that female kelts were more abundant than male kelts (861**?** to 516**?**) following spawning and that this influence was carried over to 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 ratio of post-spawned males to females may be a result of increased 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). Our 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.

Accuracy of Corps Visual Classifications

Visual classification of steelhead maturation status was compared directly to ultrasound appraisal and different degrees of visual identification error determined based on two primary standards; *critical error* and *non-critical error.* Critical error refers to the misclassification of pre-spawners as kelts, while non-critical error refers to the misclassification of kelts as pre-spawners (see Table 1 in *Methods*).

Lower Granite separator: In total, 821 (60.6%) of the 1,353 adult steelhead sampled from the LGR bypass were identified by both visual and ultrasound techniques during the study period. Visual methods correctly identified 98% (560/574) of all female kelts and 95% (193/203) of all male kelts (Table 6). The non-critical error (i.e., the erroneous classification of kelts) at the LGR separator was estimated to be only 3.1% (24/777), with male kelts composing the bulk of misclassifications. The accuracy of visual methods regarding pre-spawn fish, however, was substantially lower relative to kelts. Visual methods correctly identified only 46% of female pre-spawners and only 20% of male pre-spawners (Table 6). Based on these rates, critical-error associated with visual methods was assessed to be 65.9% during the study period. Of the male pre-spawners identified by ultrasound technique at LGR, 80.0% (16/20) of them were misclassified via visual appraisal. Overall, however, misclassification (both kelts and pre-spawners) was only 6.5% during the study period because the bulk of fish examined were kelts and not pre-spawners (Table 6).

Table 6. Percent visual classification agreement with ultrasound results of maturation status at the Lower Granite separator. Weeks are numbered from the beginning of bypass operations, with 26th March to 1 April = week 1.

Sampling Week		Ultrasound Classification					
Week	Sampling	Pre-spawner		Kelt			
No.	Dates	?	?	?	?	Both Classes (n)	
2	4/2 - 8	50%	33%	94%	50%	80%	(56/70)
3	4/9 - 15	50%	100%	96%	100%	94%	(31/33)
4	4/16 - 22	100%	33%	98%	80%	92%	(102/111)
5	4/23 - 29	50%	0%	97%	100%	94%	(88/94)
6	4/30 - 5/6	0%	0%	97%	91%	91%	(136/149)
7	5/7 - 13	-	-	99%	98%	99%	(152/154)
8	5/14 - 20	-	0%	97%	100%	98%	(121/124)
9	5/21 - 27	0%	0%	100%	95%	95%	(54/57)
10	5/28 - 6/3	-	0%	100%	100%	92%	(11/12)
11	6/4 - 6/10	-	-	100%	100%	100%	(17/17)
Overall Total (n):		46% (11/24)	20% (4/20)	98% (560/574)	95% (193/203)	94% (768/821)	

Little Goose separator*:* In total, 109 (93.9%) of 116 adult steelhead sampled from the LGR bypass facility were identified by both visual and ultrasound techniques during the study period. Visual methods correctly identified 100% (84/84) of the female kelts and 94.1% (16/17) of the male kelts (Table 7). Non-critical error at the LGR separator was only 0.9% (1/101) because only one male kelt was misclassified as a pre-spawner during the study. Similar to Lower Granite, the accuracy of visual methods regarding pre-spawn fish was substantially lower, although not as dramatic, at Little Goose separator in 2000. Visual methods correctly identified 71.4% of the female pre-spawners and 0% of male pre-spawners (Table 7). However, only one male pre-spawner was sampled at LGO during the study period, making the comparison weak. Furthermore, overall sample size (both male and female) was smaller at LGO compared to LGR data. Critical error rates were assessed to be 37.5% at Little Goose during the study period, while overall misclassification (both kelts and pre-spawners) was only 3.7% during the study period

Table 7. Percent visual classification agreement with ultrasound results of maturation status at the Little Goose separator. Weeks are numbered from the beginning of bypass operations, with 26th March to 1 April = week 1.

Sampling Week		Ultrasound Classification				
Week No.	Sampling Dates	Pre-spawner ?	Pre-spawner ?	Kelt ?	Kelt ?	Both Classes (n)
3	4/9 - 15	100%	-	100%	-	100% (4/4)
4	4/16 - 22	50%	-	100%	-	95% (21/22)
5	4/23 - 29	-.	-	100%	100%	100% (7/7)
6	4/30 - 5/6	66%	-	100%	100%	96% (25/26)
7	5/7 - 13	100%	-	100%	100%	100% (18/18)
8	5/14 - 20	-	-	100%	100%	100% (12/12)
9	5/21 - 27	-	-	100%	86%	93% (14/15)
10	5/28 - 6/3	-	0%	100%	100%	75% (3/4)
11	6/4 - 6/10	-	-	100%	-.	100% (1/1)
Overall Total (n):		71% (5/7)	0% (0/1)	100% (84/84)	94% (16/17)	96% (105/109)

Lower Monumental separator: Only five (0.6%) of the 779 adult steelhead removed from the Lower Monumental separator were examined with ultrasound during the study period. Of these, only three were classified with both ultrasound and visual methods. All three were identified as female kelts and were also correctly classified with visual methods by Corps personnel.

Male pre-spawn steelhead were particularly difficult to identify with visual methods, with only 19.0% (4/21) being correctly identified at both LGR and LGO combined. Difficulty regarding male identification is likely related to the fact that abdominal differences between male pre- and post-spawned fish are less dramatic than the difference among female steelhead (i.e., it’s easier to notice a fat female because the egg mass is larger than the male testes). Conversely, the severely imploded abdomens of female kelts relative to the fat abdomens of pre-spawners made visual classification methods for female kelts highly accurate (97.8%; 644 / 658) at both the LGR and LGO separators.

The overall inability of visual methods to identify pre-spawners may be a result of several factors. First, the reduced abdominal difference between male kelts and male pre-spawner may make abdominal appearances difficult to assess. Secondly, the specific location of visual exams may influence the accuracy of appraisal. At the Lower Granite bypass facility adult steelhead were rarely dip-netted off the separator and technicians had to identify maturation without handling or removing specimens. It was also difficult to view the fish's ventral surface at LGR because it was often "wedged" between separator bars, obscuring view. Lastly, observer bias may have influenced the accuracy of pre-spawn visual methods during 2000 research. Corp Biological Technicians quickly learned that kelts composed the majority of adult steelhead washing into the separator and if distinct morphological maturation traits were not apparent or a good view of the fish was not attainable, the fish was often classified as a kelt and not a pre-spawner. In this context, a classification of kelt was often the "default" option for technicians monitoring the separators and may have contributed to the overall misidentification of pre-spawners.

Visual Classification Keys

An ongoing objective of this research endeavor is to determine if adult steelhead possess morphological traits indicative of their maturation status. Toward this goal, we continued to collect and analyze morphological data from adult steelhead examined at the separators in the hope of further developing and refining current visual keys[6]. Results of ultrasound appraisals were used to determine the "true" maturation status of all specimens. Thus, ultrasound results are the basis against which the classification keys are constructed and evaluated. We recognize, however, that the ultrasound may have misidentified the maturation of some individuals – although we suspect a very low proportion – and could contribute to misleading classification results and interpretations.

[6] An analysis of 1999 ultrasound data from Little Goose Dam determined that abdominal appearance was the best-suited visual trait for the classification of maturation status at the dams.

Morphological data from 881 adult steelhead (50 pre-spawners and 761 kelts) were analyzed using both logistic regression and tree classification analyses. Of the 881 fish examined, 191 were male and the remaining 620 specimens were female. We determined that only five morphological traits were suitable for classification analysis (see *Methods* for details): abdominal appearance, fin wear, condition, coloration and fungus. A pictorial of the five morphological traits used for analysis, along with examples of the different categories within traits, can be viewed in Appendix C.

Logistic regression*:* Of the five morphological traits examined in the full model, logistic regression identified abdominal appearance as the primary visual indicator of maturation status for adult steelhead encountered at the dams. Abdominal appearance was significantly related to maturation status at the $P<0.001$ probability level (two-sided p-value). According to logistic regression results, individuals with fat, rounded abdomen were 24.98 times (12.98 to 57.63 times, 95% C.I.) more likely to be pre-spawners than to be kelts. Conversely, the odds of a specimen with a thin or imploded abdomen being a pre-spawner were only 0.04. Logistic regression also provided some evidence that caudal fin wear was significantly related to maturation status (two-sided $P=0.05$). Further examination of the data set by fish sex, revealed that caudal fin wear was only related to maturation status in females steelhead (two-sided $P=0.01$), while no evidence of a relation existed for male specimens (two-sided $P=0.17$). The odds of a female pre-spawner having noticeable fin wear (> 5% erosion) were 0.11 (0.03 to 0.33 times, 95% C.I.), providing evidence that kelts and not pre-spawners were likely to have eroded caudal fins.

No other morphological characteristic was significantly associated with pre-spawners or kelts in the logistic regression analysis, regardless of the modeling approach (full model, step-wise model, or other reduced models) attempted. Other variables and their two-sided P-values in the full model were; fungus infections ($P= 0.30$), condition ($P= 0.23$), and coloration ($P= 0.11$).

Tree classification: Tree classification analysis produced results similar to those of logistic regression. Like logistic regression, tree classification selected abdominal appearance as the most conclusive trait – based on classification accuracy –associated with maturation status. In fact, abdominal appearance was selected as the primarily indicator of maturation regardless of the combination or number of variables tested with the different models (i.e., all tree classification runs identified abdominal appearance as the primary node in which to classify spawning type). If all five morphological traits are considered simultaneously in the full model, abdominal appearance alone correctly classifies 90.5% (736/812) of the total sample, making it the most simplistic and accurate visual indicator.

Similar to results obtained from 1999 data, tree classification analysis was very successful at winnowing out the spawning status of fish possessing thin abdomens. This is because of the 396 steelhead classified with thin abdomens, ultrasound identified all 396 as kelts. Perhaps more importantly, the critical error associated with the presence of a thin abdomen was non-existent because no true pre-spawners (as determined by ultrasound) were misclassified as a kelts due to the presence of a thin abdomen. Fish possessing thin-intermediate abdomens were also easily classified by tree analysis, with 97.5% (314/322) identified via ultrasound as kelts. However, critical error could result from this visual indicator because some true male pre-spawners had thin-intermediate abdomens and could be erroneously classified as kelts. Overall, adult steelhead with imploded abdomens (thin and thin-intermediate combined) were predominately kelts and the probability of committing a critical error was estimated at 0.01, according to tree classification analysis.

Tree analysis was also successful at winnowing out maturation among those fish with fat abdomens. Of the 18 fish classified as having fat abdomens during the study, ultrasound considered all to be pre-spawners. Thus, the presence of a fat abdomen is an excellent visual indicator of the pre-spawner type. Despite this finding, abdominal appearance alone was unable to adequately distinguish maturation among those individuals having a fat-intermediate abdominal appearance, especially among male steelhead. For example, of the 34 male specimens with fat-intermediate abdomens, ultrasound determined that 14 were pre-spawners and the remaining 20 kelts. Unfortunately, tree analysis was unable to incorporate other morphological traits (e.g., fungus, fin wear, and coloration) to adequately decipher maturation status among fat-intermediate males. However, the use of additional morphological traits did help resolve the classification accuracy of female steelhead with fat-intermediate abdomens. Female steelhead with both fat-intermediate abdomens and substantial caudal fin wear were 83.9% more likely to be kelts than pre-spawners, while the probability of being a female pre-spawner with just a fat-intermediate abdomen was only 58.3%. According to tree classification analysis, if abdominal appearance alone were used to assess maturation at the dams, an estimated misclassification error rate of 0.105 could be expected for male steelhead and an error rate of 0.048 for female steelhead. Again, the ambiguity of using abdominal appearance to visually assess maturation is primarily associated with male steelhead that possesses fat-intermediate abdomens. Fortunately, the presence of fat-intermediate abdomen among males was rare, observed in only 4.2% (34/812) of the total sample.

In conclusion, both the tree classification analysis and the logistic regression determined that maturation status could most consistently, most easily (based on time necessary to classify), and most accurately (based on error rates) be determined by the fish's abdominal appearance. However, analysis pertains specifically to those five traits examined and does not exclude other variables – traits not considered during our study – from being possible indicators of maturation. Despite this statistical caveat, the authors feel confident that abdominal appearance is best suited in the identification of maturation status among adult steelhead removed from Snake R. bypass facilities during the spring. Lastly, results generated from 2000 data corroborate with results obtained in 1999 at Little Goose bypass, strengthening the overall validity of conclusions with a second year of data.

Final recommendations*:* We recommend that Corps personnel monitoring the Snake R. bypass facilities continue to use abdominal appearance to classify maturation among adult steelhead. Those fish observed with a fat abdomen should be considered pre-spawner fallbacks, while fish with a thin/imploded abdomen (they often appear emaciated) should be considered steelhead kelts. The use of this simple visual method should adequately distinguish the bulk of adult steelhead entrained in the juvenile bypass facilities each spring. We also recommend that caudal fin wear be used in steelhead that also have fat-intermediate abdomens. The presence of substantial fin wear, used in conjunction with females possessing fat-intermediate abdomens, may increase the classification accuracy and reduce the probability of committing non-critical error. Unfortunately, of those additional traits considered for analysis (e.g., fungus, coloration, condition, and fin wear) none were able to adequately distinguish maturation for male specimens observed with fat-intermediate abdomens. Despite this finding, we recommend all male steelhead with fat or fat-intermediate abdomens be classified as pre-spawners, acknowledging that classification will occasional result in non-critical error.

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. Given that the incorrect identification – from either ultrasound or visual methods – of kelts or pre-spawners currently results in the same treatment of individuals (i.e., released into the tailrace), attempts to reduce error in identification methods will result in a more accurate kelt monitoring program but may not significantly influence fish survival. Thus, we recommend the continued use of visual methods for kelt enumeration purposes at the dams. However, we recommend visual methods be periodically validated with ultrasound whenever feasible to quantify error rates, especially those associated with male pre-spawners.

If kelt collection or kelt reconditioning were the primary research or management objective, the authors recommend the use of ultrasound over visual methods at the Snake R. dams. 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 a 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 were very successful in facilitating repeat spawning, whereas a higher non-critical error rate may be acceptable if the best available kelt program were not very successful. In regards to the ultrasound technique, we believe critical error associated with the 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.

Other Morphological Traits

During the examinations we also recorded data on morphological traits that were not expected to be directly related to maturation status, but that might be of general interest (Table 8). "Head burn" was equally common in both pre-spawners and kelts, at least relative to their abundance. In most circumstances, the head burn did not appear to be "fresh," because the damaged tissue was worn and faded. Fungal infections were often associated with head burn areas. The high prevalence of freshwater parasites was common in both kelts (87%) and pre-spawners (61%) and was also observed during 1999 research at Little Goose Dam. In extreme cases the parasites covered much of the gill lamella and could potentially inhibit respiration. Most of the ectoparasites were copepods (class Copepoda) located on the fish's gills and/or fins. Leeches (class Hirudinea) were the second most common parasite. The presence of scarred tissue on the dorsal side, primarily along the dorsal fin, of adult steelhead was also apparent in some individuals examined during 2000 research. In many cases these injuries seemed fresh and were still bleeding upon examination. None of the 257 adult steelhead examined at LGO in 1999 had evidence of this condition.

Table 8. Incidence of three pathological conditions among adult steelhead sampled at LGO and LGR bypass facility during the 2000 study.

Pathological Condition	Incidence (%) in: Pre-spawners	Kelts
Head Burn	3 (4.7)	38 (2.7)
Dorsal Scars	0 (-)	8 (0.1)
Ectoparasites	39 (60.9)	1,140 (81.1)

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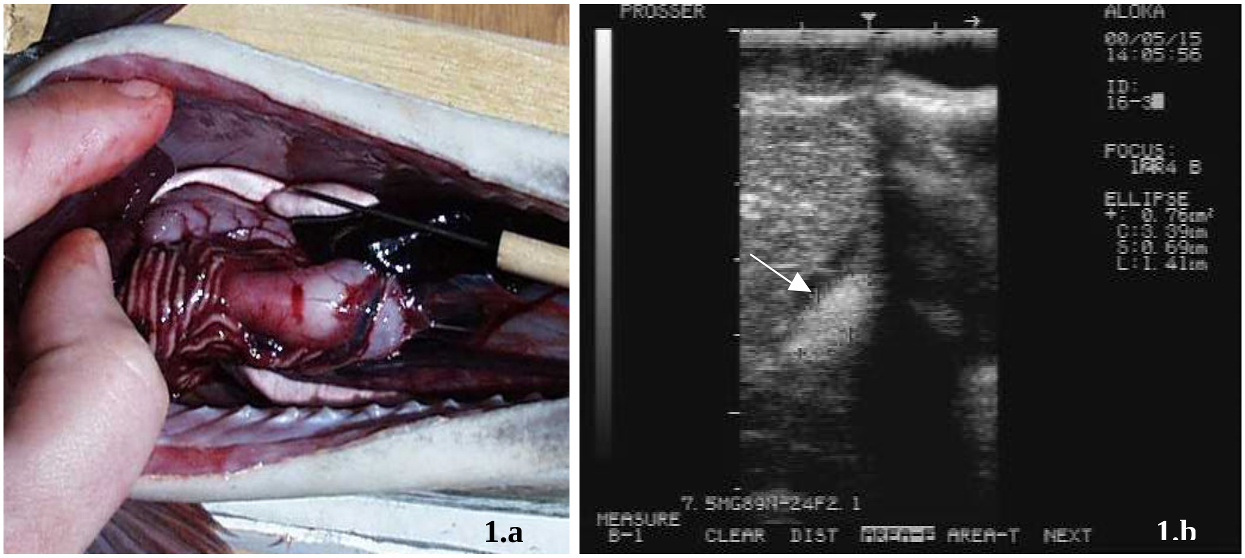

Figure 1. A picture of kelt anatomy (1.a), testis indicated near the tip of a wood-handled probe, and a cross-sectional ultrasound image (1.b) from a male steelhead kelt examined on the Yakima River. Testis appears as a white (hyperachoic), elliptical mass in the center of the image. Testis measurement (0.76 cm^2) is shown along the right margin of the ultrasound image.

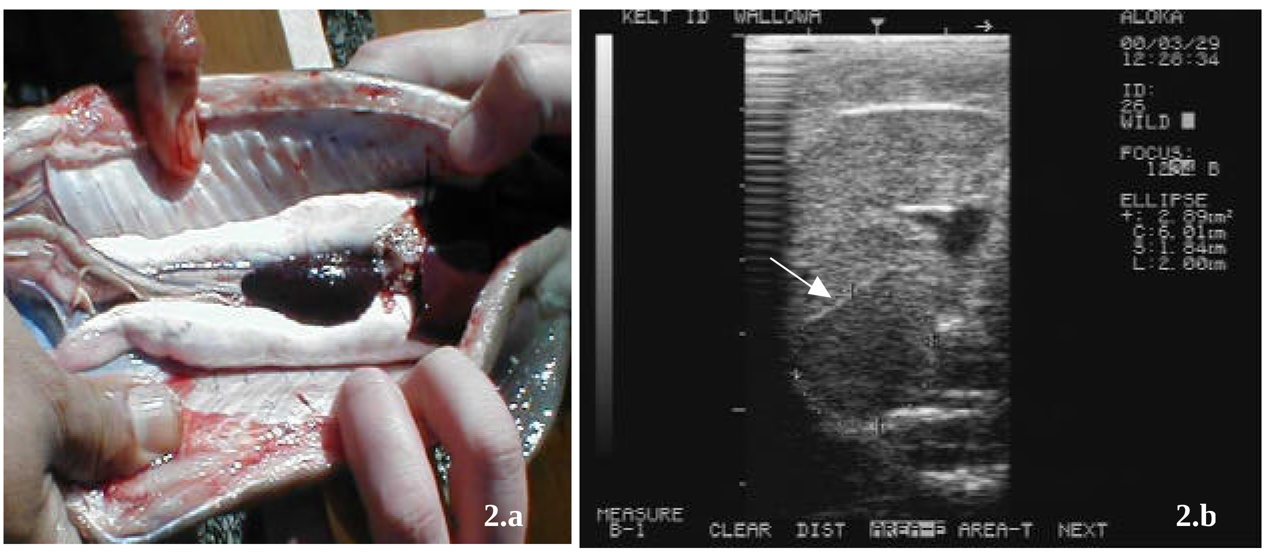

Figure 2. A picture of pre-spawn anatomy (2.a), testes are the two dominant lobes, and a cross-sectional ultrasound image (2.b) from a male pre-spawn steelhead examined at Wallowa hatchery. Testis appears as a grayish (hypoachoic), elliptical mass in the center of the image. Testis measurement (2.89 cm^2) is shown along the right margin of the ultrasound image.

Table 1: Number of adult steelhead removed, sampled, and classified by ultrasound for each day of sampling conducted at the Lower Granite Dam bypass separator, 2000. Weekly totals represent all the adult steelhead removed during that week (Saturday to Sunday) by Corp personnel. Estimated kelt abundance is calculated for each week of sampling, producing an overall weighted estimate.

Date	No. Adults Removed	No. (%) Sampled	No. Kelts	No. Pre	% Est. Weekly Kelt Abundance
Week 1 total	**207**	**0**	**0**	**0**	**N.A.**
4/4/00	37	12	8	4	
4/6/00	31	22	18	4	
4/7/00	56	34	30	4	
4/8/00	70	13	11	2	
Week 2 total	**288**	**81 (28%)**	**67**	**14**	**82.7%**
4/9/00	22	17	15	2	
4/11/00	44	23	22	1	
Week 3 total	**306**	**40 (13%)**	**37**	**3**	**92.5%**
4/18/00	62	30	29	1	
4/20/00	100	35	32	3	
4/21/00	91	50	46	4	
4/22/00	92	55	51	4	
Week 4 total	**554**	**170 (31%)**	**158**	**12**	**92.9%**
4/23/00	91	21	19	2	
4/24/00	88	35	33	2	
4/25/00	73	41	40	1	
4/26/00	104	18	16	2	
4/27/00	61	27	25	2	
4/28/00	82	38	36	2	
Week 5 total	**602**	**180 (30%)**	**169**	**11**	**93.8%**
5/1/00	88	73	68	5	
5/2/00	127	32	32	0	
5/3/00	138	43	43	0	
5/4/00	143	73	71	2	
5/5/00	124	42	41	1	
5/6/00	108	25	24	1	
Week 6 total	**821**	**288 (35%)**	**279**	**9**	**96.9%**
5/7/00	104	48	48	0	
5/8/00	107	28	27	1	
5/9/00	62	64	64	0	
5/10/00	88	85	85	0	
5/11/00	104	24	24	0	
5/13/00	48	21	21	0	
Week 7 total	**572**	**270 (47%)**	**269**	**1**	**99.6%**
5/14/00	39	25	25	0	
5/15/00	47	31	31	0	
5/16/00	49	29	29	0	
5/17/00	42	21	21	0	
5/18/00	49	17	17	0	
5/19/00	33	37	36	1	
5/20/00	54	27	26	1	
Week 8 total	**313**	**187 (60%)**	**185**	**2**	**98.9%**
5/21/00	43	19	18	1	
5/22/00	46	30	30	0	
5/23/00	68	19	19	0	
5/24/00	55	16	16	0	

5/26/00	39	12	10	2	
Week 9 total	**324**	**96 (30%)**	**93**	**3**	**96.8%**
5/30/00	15	8	8	0	
6/1/00	11	2	1	1	
6/2/00	14	7	7	0	
6/3/00	13	3	3	0	
Week 10 total	**135**	**20 (15%)**	**19**	**1**	**95%**
6/4/00	4	4	4	0	
6/5/00	5	7	7	0	
6/6/00	8	5	5	0	
6/7/00	7	5	5	0	
Week 11 total	**53**	**21 (39%)**	**21**	**0**	**100%**
Week 12 total	**7**	**0**	**0**	**0**	**N.A.**
Total Removed and Sampled	**4182**	**1353 (32%)**	**1297**	**56**	**95.9%[a]**

[a] Total kelt abundance is calculated based on a weighted average from each weekly sample.

Table 2: Number of adult steelhead removed, sampled, and classified by ultrasound for each day of sampling conducted at the Little Goose bypass separator, 2000. Weekly totals represent all the adult steelhead removed during that week (Saturday to Sunday) by Corp personnel. Estimated kelt abundance is calculated for each week of sampling.

Date	No. Adults Removed	No. Sampled	No. (%) Kelts	No. (%) Pre	% Est. Kelt Abundance
Week 1 total	**11**	**0**	**0**	**0**	**N.A.**
Week 2 total	**152**	**0**	**0**	**0**	**N.A.**

4/12/00	13	4	3	1	
Week 3 total	**135**	**4 (3%)**	**3**	**1**	**75%**
4/19/00	43	24	22	2	
Week 4 total	**229**	**24 (10%)**	**22**	**2**	**91.6%**
4/25/00	36	3	3	0	
4/27/00	33	4	4	0	
Week 5 total	**260**	**7 (3%)**	**7**	**0**	**100%**
5/2/00	32	12	12	0	
5/3/00	56	17	14	3	
Week 6 total	**323**	**29 (9%)**	**26**	**3**	**89.6%**
5/9/00	22	9	8	1	
5/11/00	29	11	11	0	
Week 7 total	**149**	**20 (13%)**	**19**	**1**	**95%**
5/16/00	11	2	2	0	
5/17/00	11	5	5	0	
5/18/00	5	5	5	0	
Week 8 total	**71**	**12 (17%)**	**12**	**0**	**100%**
5/23/00	21	11	11	0	
5/24/00	18	4	4	0	
Week 9 total	**105**	**15 (14%)**	**15**	**0**	**100%**
6/1/00	6	4	3	1	
Week 10 total	**59**	**4 (7%)**	**3**	**1**	**75%**
6/6/00	7	1	1	0	
Week 11 total	**28**	**1 (4%)**	**1**	**0**	**100%**
Week 12 total	**10**	**0**	**0**	**0**	**N.A.**
Total Removed[a] and Sampled	**1532**	**116 (8%)**	**108**	**8**	**93.1%[b]**

[a] 104 of the Total Removed were known kelts tagged at Lower Granite and recaptured at Little Goose. Tagged kelts were not "re-sampled" at Little Goose during ultrasound examinations.

[b] Estimate accounts for the 104 recaptured known kelts.