Genetic Analysis and Photo Documentation of Hybridization between Bull Trout and Brook Trout in the Swan River Basin, Montana

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Abstract

A total of 338 specimens of bull trout, brook trout, and their hybrids were captured in August, 2006, by electrofishing five sections of Goat, Squeezer and Lion Creeks in the Swan River drainage. These *Salvelinus* specimens were identified to species based on standard phenotypic characteristics, photographed in a solarium, and then sampled for later genetic analysis. Results of genetic analysis indicated that field assessment of 338 age 1 and older *Salvelinus*, based on phenotypic characteristics, was over 96% accurate in identifying the two parental species and their hybrids. Only 12 of 338 fish were mis-classified, all of which were either hybrids or brook trout based on genetic results. The use of a solarium (with or without photo documentation) is recommended as a rapid assessment field aid for improving identification of *Salvelinus* species and their hybrids. Throughout the study streams, both in headwater bull trout spawning reaches as well as downstream sections, bull trout and brook trout made up approximately equal proportions of the *Salvelinus* population. Hybrids were more prevalent in lower stream reaches, comprising as much as 21% of one sample, and hybrids made up a disproportionately large share (nearly half) of fish sampled that were over 200 mm. As a result, the biomass of *Salvelinus* in every stream section we sampled was dominated by nonnative brook trout and hybrids. All 34 of the hybrids we sampled were determined to be F1 progeny of matings between bull trout females and brook trout males. Subjective comparison of our findings with those from samples from the same streams collected in the early 1980's and the 1990's show no conclusive evidence that brook trout populations are expanding or that hybrids are becoming more prevalent, though statistical comparisons were not possible. With the emerging threat of an expanding lake trout population in Swan Lake, we are concerned that lake trout will dominate the lacustrine environment in the future and the dominant bull trout life history form in the Swan Lake core area could become less adfluvial and more fluvial or resident in nature. We hypothesize that this could lead to increased competition with brook trout and greater risk of progressive hybridization between bull trout and brook trout in the future. If bull trout stocks become increasingly fragmented and smaller fluvial and resident bull trout begin to dominate, we may witness the breakdown or elimination of the size disparity that currently occurs on the spawning grounds between large adfluvial adult female bull trout and much smaller male brook trout. Based on our interpretation of the sampling results from this study, this size disparity is one of the likely isolating mechanisms that have kept hybridization from progressing to date. We recommend a multi-year experimental electrofishing suppression effort be carried out in one or more of the study streams, which could be highly effective in removing brook trout and hybrids without negatively impacting bull trout. A continuation of the standard monitoring, conducted in conjunction with the experimental suppression, would allow us to assess whether or not the current dominance by brook trout and hybrids of biomass in these streams potentially affects bull trout recruitment and also whether brook trout suppression might be an effective way to boost bull trout recruitment to Swan Lake and reduce the future incidence of hybridization.

Introduction and Study Objectives

The bull trout *Salvelinus confluentus* population native to Swan Lake and the Swan River drainage in northwest Montana has been characterized as one of the healthiest remaining core area populations of this threatened species in the entire Columbia River basin. Bull trout status in Swan Lake is believed to have been enhanced beyond natural conditions since the mid-1900s, first by the presence of an introduced kokanee forage base in Swan Lake and then further by the establishment of *Mysis relicta* introduced by State fishery managers in the late 1970's (MBTSG 1996). Local populations of bull trout spawn in approximately a dozen tributaries to the Swan River (Figure 1). Bull trout spawning redds have been counted in index reaches of select tributaries to the Swan River since 1982. It is estimated in recent years the Swan Lake bull trout core area has supported an annual adfluvial bull trout spawning run of approximately 1,000 adult fish.

The potential for hybridization with non-native brook trout *Salvelinus fontinalis* has existed in the Swan basin ever since brook trout were first introduced and established, early in the 20th century. Hybridization with brook trout represents one of the factors responsible for decline of bull trout throughout their native range (USFWS 2002). However, in the Swan basin, while brook trout X bull trout hybrids have been noted in the past and brook trout remain abundant in some streams, hybridization between the two species has not previously been considered a major or expanding threat to bull trout (MBTSG 1996). One possibility is that behavioral mechanisms may contribute to maintaining reproductive isolation between brook trout and bull trout in the Swan basin, partially as a result of the size disparity between the typically large (18-36 inch) adfluvial bull trout spawners and the much smaller resident brook trout (typically 6-12 inches).

Genetic surveys of bull trout in the Flathead River drainage were conducted in 1992 and 1993, including portions of the Swan system (Kanda 1998). Allozymes at three diagnostic loci were used to assess hybridization in samples collected from South Lost Creek (n = 37), Goat Creek (n = 49), Lion Creek (n = 59) and Elk Creek (n = 65), four of the more productive bull trout spawning tributaries in the Swan. In the 1992-1993 surveys, brook trout hybrids were detected in samples from Elk (1 fish), Goat (1 fish), and Lion Creek (11 fish). Follow-up mitochondrial and nuclear DNA analysis (Kanda 1998) indicated that hybrid fish were mostly

male and primarily F1 progeny of female bull trout mating with male brook trout. Such "sneaker male" spawning behavior by male brook trout has been previously observed in Lion Creek (Kitano et al. 1994). Two post-F1 progeny occurred in the 1992-1993 Swan sample sets; one a Lion Creek fish and the other the only hybrid from Goat Creek. The high prevalence of brook trout hybrids in Lion Creek (19% of the sample), and presence of post-F1 progeny was considered cause for concern (Kanda 1998). Limitations of the 1992-1993 sampling protocol, in that only bull trout or putative bull trout (but not brook trout or putative hybrids) were examined, was noted by Kanda (1998).

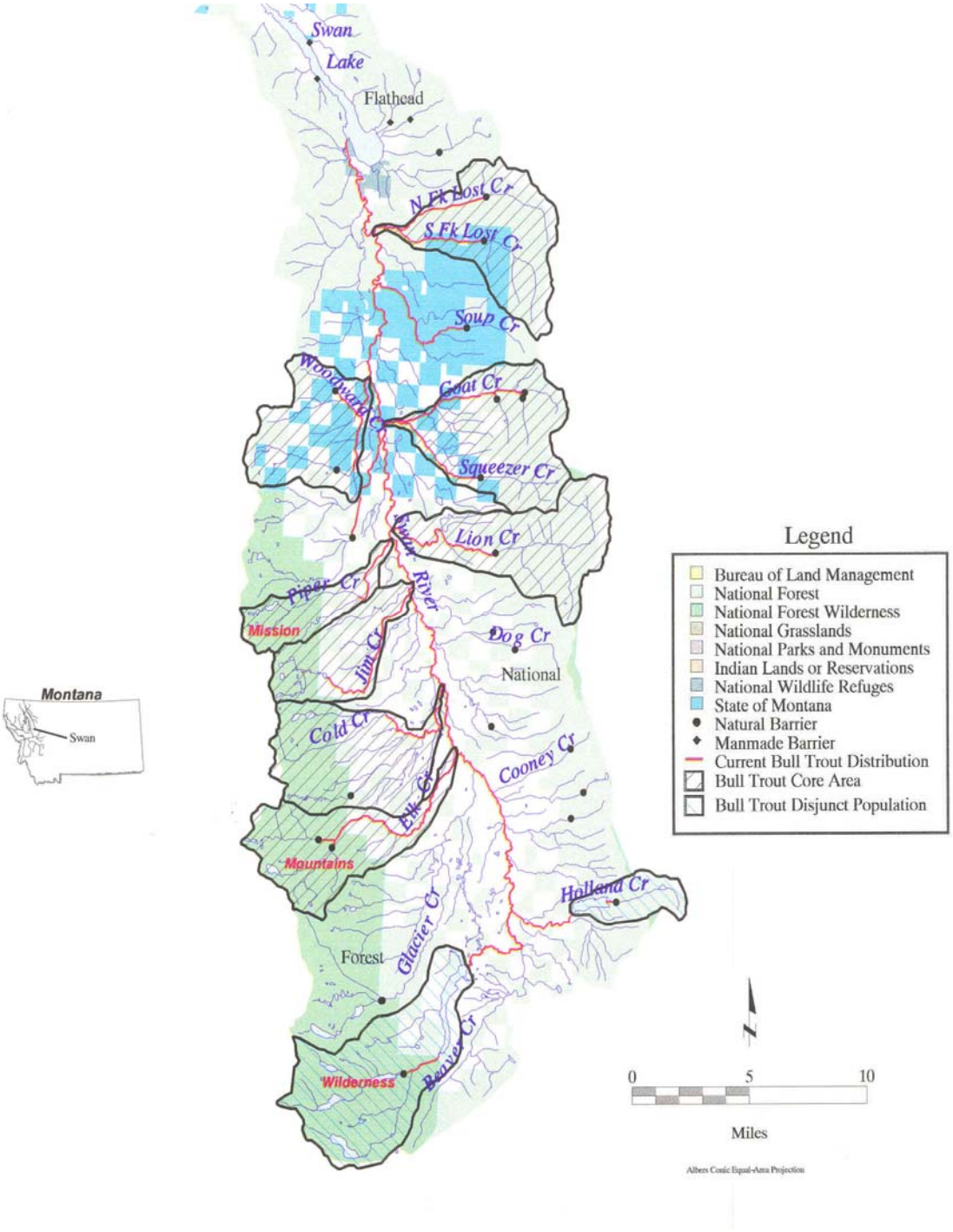
The previous findings regarding brook trout hybridization in the Swan basin led to the current study, designed to address the following objectives:

1.) Assessment of whether the hybridization status in Lion, Goat, and Squeezer Creeks is progressing or relatively stable. Systematic sampling from both upper and lower reaches of the streams, incorporating all *Salvelinus* (including bull trout, brook trout, and their hybrids) will also establish a baseline to be used to accurately assess future changes. We will also determine whether post-F1 hybridization, first detected in 1993, is potentially increasing and whether or not the direction of hybridization (previously all were brook trout males mated with bull trout females) has changed.

2.) Assessment of the *Salvelinus* species balance between bull trout, brook trout, and their hybrids in the stream systems sampled. A quantitative estimate of *Salvelinus* populations in these Swan River tributaries will allow us to determine what portion of the potential bull trout productivity may be comprised of brook trout or hybrids.

3.) Contribution to establishment of a complete genetic baseline, including a set of loci from the Swan River drainage bull trout for comparison to the rangewide database (DeHaan and Ardren 2005).

Figure 1. Bull Trout distribution and local populations in the Swan drainage. From MBTSG 1996.



Study Area

There are about nine primary local populations of bull trout (i.e., spawning and rearing streams) associated with the Swan Lake core area in the Swan River drainage. They are identified in the Montana Bull Trout Status Report (MBTSG 1996) and the Draft Bull Trout Recovery Plan (USFWS 2002) as: Elk, Cold, Jim, Piper, Lion, Goat, Woodward, Soup, and Lost Creeks (Figure 1). For purposes of those determinations, Squeezer Creek, a tributary of Goat Creek, was considered part of the Goat Creek local population.

The current study evaluates the demographic and genetic attributes of the *Salvelinus spp.* complex in two of the major bull trout spawning systems, Goat/Squeezer Creek and Lion Creek. Previous basinwide surveys (MBTSG 1996) had identified Goat/Squeezer and Lion Creeks as two of the three most heavily used bull trout spawning drainages in the Swan River basin (Elk Creek being the other). During the 25 year record of bull trout redd count surveys, from 1982 – 2006, an average of 52 (range 17-91) redds were made by migratory bull trout in Goat Creek (Weaver 2006). Surveys conducted over the same period found an average of 92 redds (range 24-149) in Squeezer Creek and an average of 104 bull trout redds (range 26-190) in Lion Creek.

Background data which quantifies some habitat variables and fish population parameters for these streams is available from an extensive fishery evaluation conducted in response to proposed microhydro development in the Swan Basin in 1982-83 (Leathe et al. 1985). Goat Creek is described (Leathe et al. 1985) as a third order stream with a relatively large drainage area (86.4 km^2). A 3 meter falls, believed to block upstream passage of migratory fish, was noted about 8.5 km upstream of the Swan River confluence (Leathe et al. 1985).

The lower Goat Creek sample site we evaluated was located immediately downstream of the confluence of Squeezer Creek (47° 45.003´ N, 113° 48.959´ W), approximately 1.3 km upstream of the Swan River confluence (Figure 2). This reach was described (Leathe et al. 1985) as low average gradient (0.9%), with a moderate density (8.0 per km) of high quality pools. Streambed was characterized mainly by large gravel (45%) and cobble (30%) with smaller amounts of small gravel (10%), sand (7%), silt (6%), and boulder-bedrock (2%). The stream was rated as containing a moderate amount of channel debris (large wood and logs) and channel stability was rated fair. Bull trout redds in Goat Creek, both historically and currently, are located mostly upstream of the location of this survey reach.

The upper Goat Creek sample site is located approximately 6 km upstream of the Swan River confluence (47° 45.980´ N, 113° 45.736´ W) and 4.7 km upstream of the lower Goat Creek site (Figure 2). This is in the midst of the reach of Goat Creek where most bull trout spawning activity has historically been located. The upper Goat Creek sample site is about midway between two stream reaches where habitat and electrofishing surveys were conducted in 1983 (Leathe et al. 1985). Those habitat surveys indicated that in a section approximately 1.5 km downstream of our sample site the gradient was 1.6% and the stream was characterized by a moderate density (8.5 per km) of high quality pools (8%), with 27% riffle and 65% run habitat. Substrate was composed mainly of large gravel (44%) and cobble (20%) with smaller amounts of small gravel (15%), silt (13%), sand (7%), and boulder-bedrock (1%). In a surveyed reach approximately 1.4 km upstream of our sample site, the gradient was higher (4.6%) and the stream was characterized by a high density (17 per km) of high quality pools (8%), with 15% riffle, 32% run, and 45% pocketwater-cascade habitat. Substrate was composed mainly of boulder-bedrock (35%) and large gravel (31%), with lesser amounts of cobble (27%), small gravel (4%), and silt (3%). The surveyed reaches downstream and upstream of our sample site had moderate to high amounts of large debris and channel stability was rated fair to good (Leathe et al. 1985). We did not directly measure these parameters in our study, but the conditions described in the past survey adequately characterize the range of conditions we observed. Both reaches of Goat Creek were previously considered “critical for migratory bull trout production” (Leathe et al. 1985).

Squeezer Creek is described as a third order stream with a medium sized drainage area (35.4 km²) and a low (2.5%) average channel gradient (Leathe et al. 1985). The reach of Squeezer Creek we sampled is about 2.0 km upstream of the confluence with Goat Creek (47° 44.593´ N, 113° 47.680´ W), some 3.3 km upstream of the Swan River confluence (Figure 2). This location is near the lower end of the high density bull trout spawning reach in Squeezer Creek, with most redds historically located in the several km reach immediately upstream. This reach was surveyed by Leathe et al. (1985) and described as containing a moderate density (9.0 per km) of high quality pools, with habitat comprised of about 5% pool, 28% riffle and 67% run habitat. Streambed was characterized mainly by small gravel (37%) and large gravel (34%), with smaller amounts of sand (17%), silt (9%), and cobble (3%). The stream was rated as containing a high amount of channel debris (large wood and logs) and channel stability was rated

fair (Leathe et al. 1985). Fish passage is precluded beyond about 7 km in Squeezer Creek by a series of falls and cascades. Squeezer Creek was also considered "critical for migratory bull trout production" (Leathe et al. 1985).

Lion Creek is described (Leathe et al. 1985) as a third order stream with a relatively large drainage area (81.6 km²) and low average channel gradient (2.5%). Our lower and upper Lion Creek study reaches are located approximately 1.0 km (47° 40.927´ N, 113° 48.516´ W) and 7.6 km (47° 40.373´ N, 113° 45.038´ W) upstream of the confluence of the Swan River, respectively (Figure 2). The lower Lion sample site is downstream of several km of high density bull trout spawning habitat and the upper Lion sample site is near the upper end of the spawning reach, but still within heavily used habitat. Leathe et al. (1985) documented steep cascades and waterfalls up to 6m in height in a reach of Lion Creek 10.4–10.9 km upstream from the Swan River. A certain passage barrier to migratory bull trout occurs at an 8.0 m high falls on Lion Creek, located in a bedrock canyon 11.1 km upstream of the Swan River confluence (Leathe et al. 1985).

Habitat surveys overlapping our upper Lion Creek survey site were conducted in 1982 (Leathe et al. 1985). The surveys indicated the entire lower 10 km of Lion Creek is very low gradient (0.9%) and in 1982 contained a low density (2.5 per km) of high quality pools (Leathe et al. 1985). The channel was comprised of about 3% pool, 10% riffle, 52% run, and 35% pocketwater-cascade habitat. Streambed was characterized mainly by large gravel (37%) and cobble (32%), with lesser amounts of boulder-bedrock (13%), small gravel (8%), silt (8%), and sand (2%). The stream was rated as containing a moderate amount of channel debris (large wood and logs) and channel stability was considered fair. As with the other streams we studied, the lower 11.1 km of Lion Creek were considered "critical for migratory bull trout production" (Leathe et al. 1985).

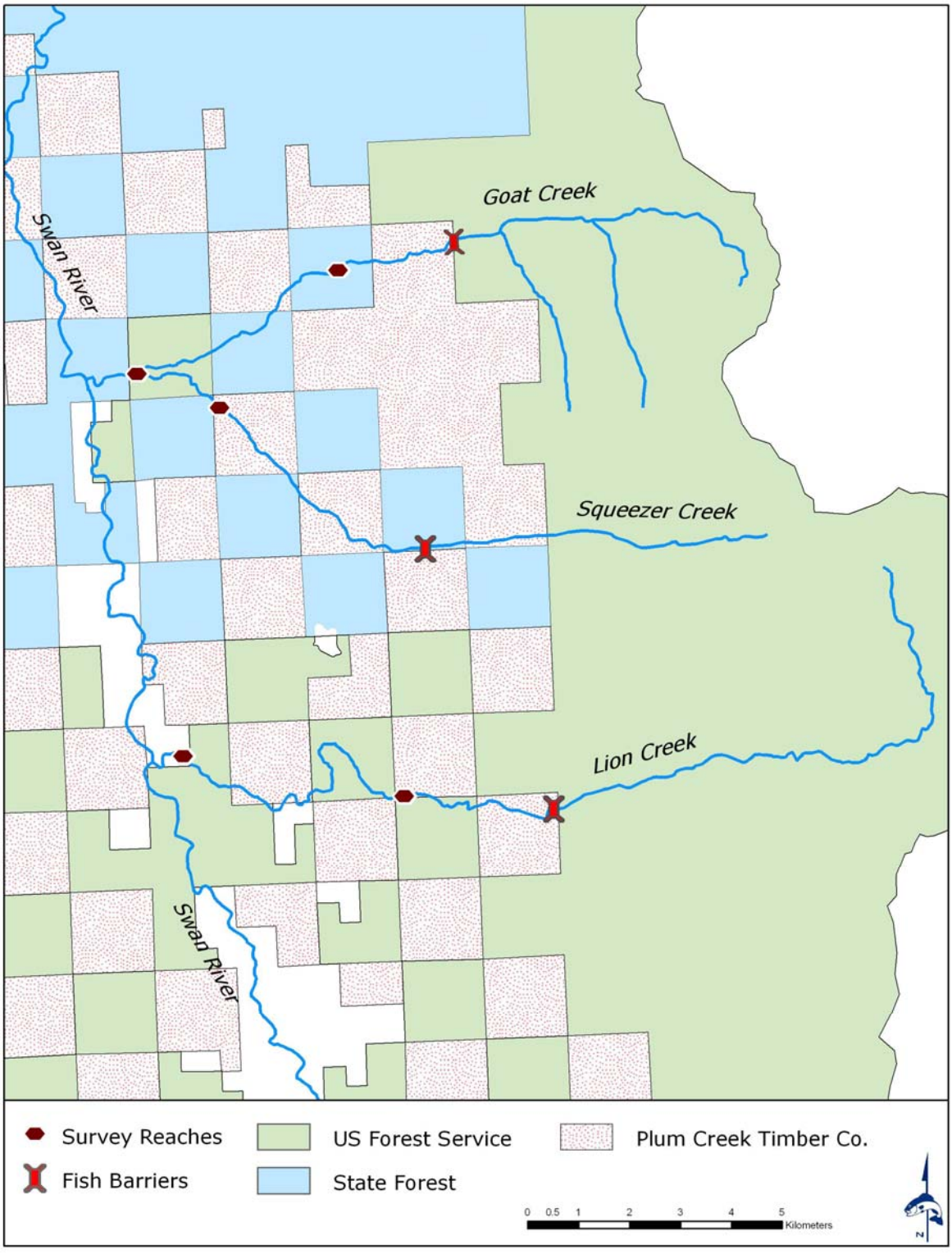


Figure 2. Map of study sites showing Swan River and upstream fish passage barriers.

Methods

Field Methods

We intensively sampled two reaches of upper and lower Lion Creek, two reaches of upper and lower Goat Creek, and a reach of Squeezer Creek (a tributary to Goat Creek) by backpack electrofishing during August, 2006 (Figure 3). Two-pass electrofishing depletion estimates were conducted in each 100 meter stream section, employing two backpack shockers working in tandem in downstream fashion, with two or three dipnetters and an additional crew person carrying a bucket to shuttle fish to holding pens. Standard MFWP protocol (Weaver et al. 2006) was followed, requiring placement of a nylon block net (6.35 mm mesh) at the lower boundary of the electrofishing section prior to the first pass, to reduce escapement or immigration of fish into the section during the procedure (Figure 4). Equal effort was applied to each pass and each section was "rested" for approximately 90 minutes between passes. Probability of capture exceeded 0.6 on the first pass for each section, satisfying quality control concerns. Stream width (wetted surface) was measured every 10 meters along the section and the average stream width multiplied by the section length to calculate surface area, in order to facilitate density comparisons. Sections were considered representative of the reaches in which they occurred (Figure 5).

All fish captured during electrofishing were measured, weighed and field identified, using standard MFWP procedures and based on standard phenotypic characters (Figure 6). At least one, but typically two or more experienced MFWP fisheries personnel were involved in sorting and classifying the fish to species, based on their appearance. Fish were phenotypically classified as bull trout if they had unmarked dorsal fins, relatively distinct spots on the dorsal surface rather than vermiculations, spots on the flanks that were yellow or cream colored with no halos, and if they generally conformed to other standard bull trout traits including a slender and elongated body shape (Figure 7). Fish were classified as brook trout if the dorsal fin was marked by black, the dorsal surface had vermiculations rather than spots, spots on the flanks were red and/or blue and typically with halos, and the fish generally conformed to the standard brook trout appearance including a more robust body shape (Figure 8). Fish exhibiting characteristics intermediate between the two prototypes were considered hybrids, especially if they were intermediate in several of the above-described categories (Figure 9, 10, and 11).

For each stream section, a random sample of *Salvelinus* was collected with each electrofishing pass, without any species-specific sorting. The entire collection from each electrofishing pass was incorporated into our further photo documentation and genetic sampling, until sample sizes sufficient to meet study objectives were reached. Thus, in some cases, fish from the first pass were completely sampled (i.e., identified, measured, photographed, genetic samples) and fish from the second pass were processed only for population estimate purposes (i.e., identified and measured). In other cases, the total catch from both electrofishing passes was insufficient to meet our study sample size objectives, so additional electrofishing was conducted immediately downstream of the study reach until a larger random sample of additional *Salvelinus* specimens were obtained for complete processing.

The total randomly sampled group of 338 fish of the genus *Salvelinus*, ranging from 75 to 320 mm total length, were placed broadside in a solarium and digitally photographed (Figure 12). Photographs, correlated to genetic sample numbers, were kept in proper order by occasionally interspersing a whiteboard with written identification numbers into the photo sequence. A plastic measuring device, taped to the bottom of the outside of the solarium, was also used to verify approximate sizes of fish in the photos (Figures 7 and 8). Two solariums were used, one measuring 12 inches long by 8 inches high by 1.5 inches deep and the other 6 inches long by 4 inches high by 1 inch deep, with choice depending on the size of the fish. Specimens were centered as much as possible in the photos. Generally, fish placed in solariums were relatively calm, following electrofishing, and sedation was not required. Occasionally, a clear plastic probe (barely visible in the water) was used to move the fish or hold them in position. An Olympus Stylus 410 digital camera mounted on a tripod was used for the photos, set on either macro or super macro mode, depending on fish size.

Fin clips for genetic analysis were collected from all photographed *Salvelinus* between 75 and 320 mm total length (previously determined from length frequency data to be Age 1 and older). As described, sampling efforts did not target specific numbers of hybrids, pure bull trout or pure brook trout, but rather approximated a random collection of the fish present from each sample location. A subset of smaller fish (i.e., young of the year) were also finclipped for genetic evaluation, but were outside our study design and these results are not included in the data set for purposes of this analysis.

Laboratory Methods

DNA was extracted from all fin clips in a *Chelex 100* (Sigma Chemical Co.) resin solution as described by Miller and Kapuscinski (1996). All individuals were then genotyped at 12 microsatellite loci: *Sco102, Sco105, Sco109* (Washington Department of Fish and Wildlife, *unpublished*), *Sco200, Sco202, Sco212, Sco215, Sco216, Sco220* (DeHaan and Ardren 2005), *Smm22* (Crane et al. 2004), *Omm1128* (Rexroad et al. 2001) and *Sfo18* (Angers et al. 1995). The major advantage of using microsatellite loci to distinguish bull trout, brook trout, and individuals of hybrid ancestry, is that microsatellites are expressed codominantly, thus allowing homozygotes and heterozygotes at a single locus to be distinguished. This in turn allows complete population genetic analyses of hybridization through multiple generations (see Campton 1987) and further examination of any fish that may represent a hybrid swarm.

Polymerase chain reactions (PCR) were conducted in 15 µl volumes containing 1X polymerase buffer (10 mM Tris-HCL, 50 mM KCL, 1% Triton X-100), 1.5 or 2mM $MgCl_2$, 0.2mM of each dNTP, 0.5µM of each primer and 0.5 units of Taq DNA polymerase (Promega Corporation). Amplified products were pooled for electrophoresis on an *ABI 3100* Genetic Analyzer (Applied Biosystems, Inc.). Automated electrophoresis was carried out using the G5 filter set following the manufacturer's protocols. *Genescan* and *Genotyper* software (Applied Biosystems, Inc., Foster City California) were used to identify alleles at each locus and to determine the multi-locus genotype of each fish at those loci.

In addition to microsatellite DNA markers, we also amplified a region of the mitochondrial genome (specifically the cytochrome B gene) for all fish identified as hybrids to determine the direction of hybridization (M. Campbell, Idaho Dept. of Fish and Game, *personal communication*). PCR reactions for mtDNA analysis were conducted in 15µl volumes and contained 1X PCR buffer (10 mM Tris-HCL, 50 mM KCL, 1% Triton X-100), 0.5mM dNTPs, 0.5µM of each primer, 2.5 mM $MgCl_2$, and 0.2 units of *Taq* DNA polymerase (Promega Co.). PCR conditions were as follows: 38 cycles of 94°C for 45 seconds, 54°C for 40 seconds and 70°C for 2 minutes 30 seconds, followed by a final extension at 72°C for 7 minutes. PCR products were then digested overnight with the restriction enzyme *RSA-I* (New England Biolabs) according to the manufactures' instructions. Following restriction digests, samples were run on 2% agarose gels stained with ethidium bromide at 95V for 45 minutes. Banding patterns

produced by each sample were then compared to samples of known bull trout and brook trout to determine the mtDNA lineage for each hybrid fish.

Statistical Analyses

Statistical methods for calculating two-pass population estimates follow MFWP protocol (Weaver et al. 2006) according to the following formula (Seber and LeCren 1967):

$$\hat{N} = \frac{C_1^{\,2}}{C_1 - C_2}$$

Where $\hat{N}$ = the estimated population size prior to the time of the first pass

C_1 = the number of Age I and older fish captured during the first pass (by species)

C_2 = the number of Age I and older fish captured during second pass (by species)

Variance of the estimate:

$$V(\hat{N}) = \frac{(C_1)^2 (C_2)^2 (C_1 + C_2)}{(C_1 - C_2)^4}$$

For purposes of standard comparison, all sections were converted to an areal basis and results reported as number of fish / 100 m².

We used the program GENETIX (Belkhir et al. 2004) to perform a factorial correspondence analysis (FCA) for all samples genotyped. This analysis provides an unbiased graphical representation of the data with individuals whose genotypes are more similar to one another grouping together. We used the program NEWHYBRIDS (Anderson & Thompson 2002) to classify each individual as either pure bull trout, pure brook trout, first generation hybrid (F1), second generation hybrid (F2), hybrid backcross with bull trout or hybrid backcross with brook trout. NEWHYBIRDS uses Bayesian statistical methods to calculate the probability that an individual fish assigns to each of the six different classes (Anderson & Thompson 2002). Based on these classifications, we determined the relationship between morphological (phenotypic) identifications of each individual fish and their genetic identification.

Figure 3. MFWP electrofishing crew sampling upper section of Goat Creek.

Figure 4. Block net installed at lower end of electrofishing section, lower Lion Creek (Hwy. 83) bridge.

Figure 5. Typical fish habitat in lower reach of Lion Creek.

Figure 6. MFWP biologists processing fish collected by electrofishing.

Figure 7. Typical bull trout (103 mm), from Goat Creek.

Figure 8. Typical brook trout (168 mm), from Lion Creek.

Figure 9. Typical F1 hybrid (bull trout x brook trout) (245 mm), from Lion Creek.

Figure 10. Typical dorsal fin markings and spotting pattern of F1 hybrid (bull trout x brook trout) from Lion Creek.

Figure 11. Brook trout, hybrid, and bull trout (left to right) from Lion Creek.

Figure 12. Two coauthors collecting genetic samples and taking solarium photos.

Results

In total, there were 338 *Salvelinus* specimens over 75 mm in length (age 1 and older) that were randomly collected at five sampling locations and incorporated into this study. Each underwent a complete series of field identification (Table 1), photo documentation, and genetic analysis (Table 2). Some additional fish captured and utilized only for population estimates (no photographs or genetics) are not included in these results.

Of the 338 fish intensively studied, a total of 150 were identified in the field as bull trout (44%), 156 were identified in the field as brook trout (46%), and 32 fish were classified as hybrids (9%) based on phenotypic characteristics. Fish ranged in length from 75-320 mm. In all cases, sample sizes from each stream approximated the original study design, with 61-80 fish from each sample site and roughly equal numbers of Age 1+ bull trout and brook trout captured at each location. Putative hybrids were also captured at all sites, with highest numbers observed in the lower reaches of Goat Creek and Lion Creek.

Table 1. *Salvelinus* samples incorporated in the Swan hybridization study. Identifications are based on phenotypic characters (field identification).

Site	Age 1+ Bull #	Length Range (mm)	Age 1+ Brook #	Length Range (mm)	Age 1+ Hybrids (mm)	Length Range (mm)
Squeezer Creek	30	89-158	29	101-294	4	189-249
Upper Goat Cr.	35	88-144	40	86-320	5	114-315
Lower Goat Cr.	25	91-189	25	85-230	11	109-133
Upper Lion Cr.	31	97-168	38	95-209	3	82-219
Lower Lion Cr.	29	112-226	24	75-233	9	91-245
TOTAL	**150**	88-226	**156**	75-320	**32**	82-315

Results of the genetic analysis (NEWHYBRIDS program) showed that the field ID agreed with the genetic ID over 96% of the time (12 of 338 individuals, or 3.6% were mis-classified). Genetic results identified the "true" composition of our sample of 338 fish as 146 bull trout (43%), 158 brook trout (47%) and 34 hybrids (10%), from the 5 sample sites within the Swan River Basin (Table 2). Of the 12 loci that we analyzed, one locus, *Sco105*, did not amplify well in many of the samples; therefore we excluded this locus from the statistical analyses. The FCA plot (Figure 13) showed three distinct groupings of fish which corresponded well to individuals classified as bull trout, brook trout and hybrids in the field. Individuals classified in the field as hybrids appeared to be intermediate to the parental species on the FCA plot.

Table 2. *Salvelinus* samples incorporated in the Swan hybridization study. Identifications are based on genotypes (lab identification).

Site	Age 1+ Bull #	Length Range (mm)	Age 1+ Brook #	Length Range (mm)	Age 1+ Hybrids (mm)	Length Range (mm)
Squeezer Creek	29	89-169	27	101-212	7	116-294
Upper Goat Cr.	35	88-151	41	86-320	4	136-315
Lower Goat Cr.	23	91-192	25	85-230	13	97-141
Upper Lion Cr.	31	97-168	39	82-209	2	133-219
Lower Lion Cr.	28	112-226	26	75-233	8	147-245
TOTAL	**146**	88-226	**158**	75-320	**34**	97-315

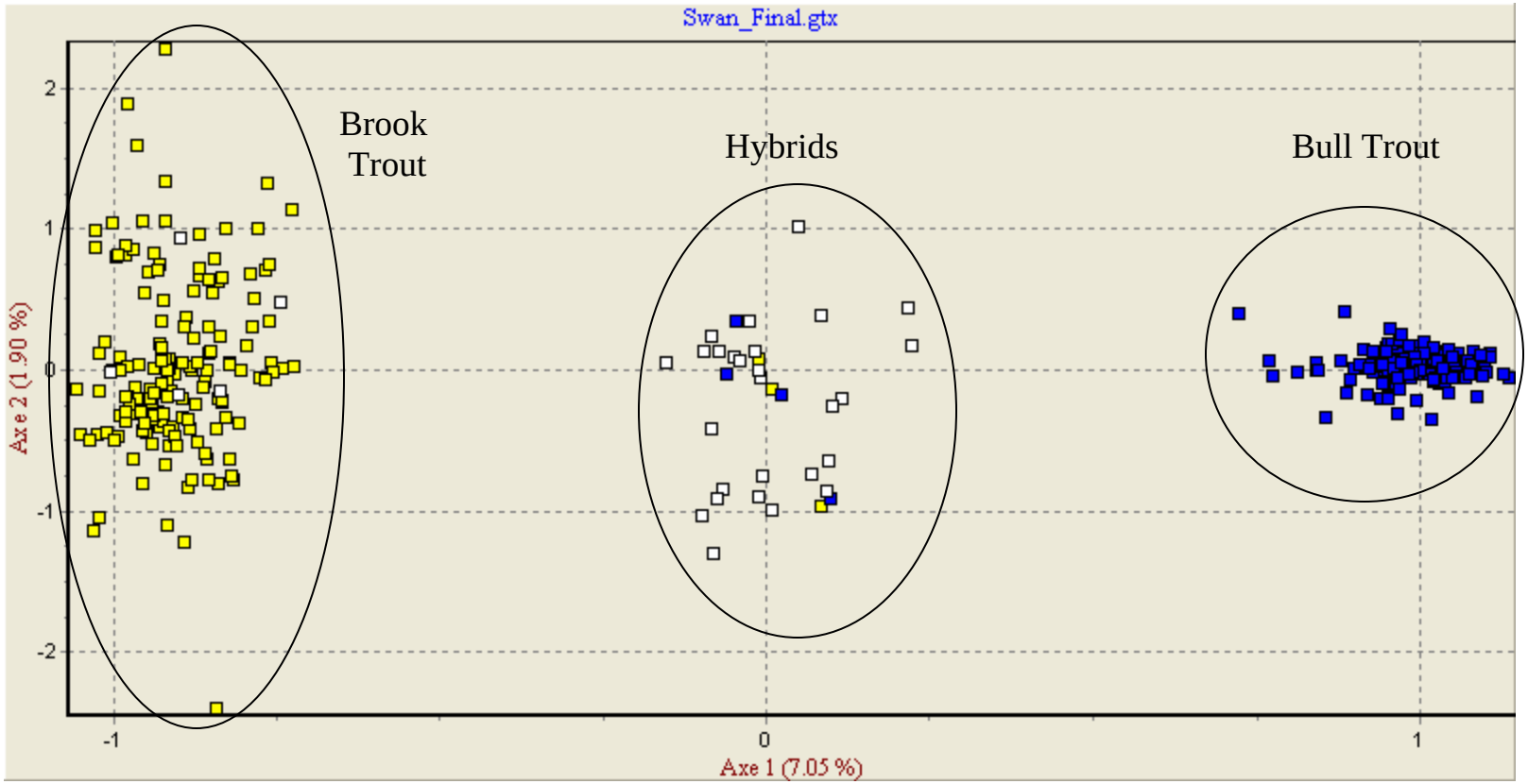


Figure 13. Multidimensional scaling analysis (FCA) based on 11 microsatellite loci for 338 *Salvelinus* samples from the Swan River Basin, MT. The blue points represent individuals classified in the field as bull trout, the yellow points represent individuals classified in the field as brook trout and the white points represent individuals classified as hybrids.

Most (7) of the 12 fish mis-classified, based on comparison between the "true" genetic identification and the observed phenotypic characteristics, were determined genetically to be hybrid fish that were incorrectly identified in the field as one of the parental species (4 were identified as bull trout and 3 as brook trout). In 5 other cases, brook trout were misidentified in the field as hybrids. There was not a single case amongst 338 samples where a pure bull trout was mis-classified as either a brook trout or a hybrid.

Of the 34 fish genetically identified as hybrids, all of them were identified as first generation (F1) with a probability assigned of 0.84 or greater (NEWHYBRIDS program), with the exception of a single individual (#117-070) collected in upper Goat Creek (Figure 14). The ability to recall and re-examine the photo of this specimen, which clearly shows phenotypic characteristics of a hybrid, shows the further value of the solarium series. This individual was assigned to multiple hybrid classes with a relatively low probability (NEWHYBRIDS program). The anomalous result was reevaluated by the genetics lab and the coauthors concluded the fish was an F1 hybrid that produced inconsistent results because one or more alleles did not amplify well at certain loci.

Figure 14. Hybrid *Salvelinus* specimen from Upper Goat Creek (136 mm).

The mtDNA analysis showed that all 34 individuals genetically identified as hybrids had bull trout mtDNA. This indicates that all of the hybrids in this study were the result of bull trout females mating with brook trout males.

Montana Fish, Wildlife and Parks (Tom Weaver, personal communication 2007) calculated fish population estimates for age 1 and older (75 mm and longer) bull trout (Table 3) and brook trout (Table 4) for each of our sample sites. The numbers of hybrids were low enough that separate estimates were not valid and so the hybrids are not included in these results.

The size distribution of the two parental species and the hybrid fish were markedly different. In Lion Creek, most bull trout were between 95-145 mm long with a small group of fish 160-180 mm (Figure 15). Brook trout were more broadly distributed, from 75-235 mm in length. In Goat and Squeezer Creeks, size ranges of bull trout and brook trout were distributed similarly to Lion Creek. Bull trout averaged 122.0 mm in length in Goat/Squeezer and 125.9 mm in length in Lion Creek. Brook trout averaged 150.9 mm in Goat/Squeezer and 133.3 mm in Lion Creek.

A relatively high proportion of the larger fish in both systems were hybrids. Half of the fish over 200 mm in length from the combined stream sample (13/26) were brook trout, roughly proportional to their occurrence in the combined sample. But only one fish over 200 mm was a bull trout and the remaining 12 were hybrids. The hybrids averaged 164.9 mm in length in Goat/Squeezer and 191.9 mm in length in Lion Creek, larger than either parental species. The streams are closed to angling, so the age and size structure probably reflects a largely unexploited population.

Photo documentation of the 338 sampled specimens was organized and individual photos were filed in sequence, with labels corresponding to genetic samples. Photo quality was generally high (see e.g., Figures 7, 8, 9, and 14) with only about 5 of the photos considered insufficient in quality to adequately demonstrate phenotypic characteristics that would allow trained observers to accurately assess whether the individual was a bull trout, brook trout, or hybrid.

Table 3. Estimated bull trout numbers, density, and biomass in five 100 meter stream reaches of Squeezer, Goat, and Lion Creek in the Swan River drainage, based on two-pass electrofishing.

Stream Section (100 m)	**Number of bull trout (95% CI)**	**Density of bull trout (# / 100m^2)**	**Biomass of bull trout (g / m^2)**
Squeezer Creek	21 (21-30)	2.73	0.42
Upper Goat Creek	33 (33-37)	3.29	0.55
Lower Goat Creek	27 (27-34)	2.18	0.46
Upper Lion Creek	41 (41-45)	2.58	0.38
Lower Lion Creek	29 (29-40)	2.48	0.62
Range	**21 - 41**	**2.18 - 3.29**	**0.38 – 0.62**

Table 4. Estimated brook trout numbers, density, and biomass in five 100 meter stream segments of Squeezer, Goat, and Lion Creek in the Swan River drainage, based on two-pass electrofishing.

Stream Section (100 m)	**Number of brook trout (95% CI)**	**Density of brook trout (# / 100m^2)**	**Biomass of brook trout (g / m^2)**
Squeezer Creek	17 (17-20)	2.24	0.84
Upper Goat Creek	22 (22-24)	2.16	0.81
Lower Goat Creek	17 (17-20)	1.36	0.38
Upper Lion Creek	51 (51-56)	3.19	0.91
Lower Lion Creek	17 (17-24)	1.46	0.31
Range	**17 - 51**	**1.36 – 3.19**	**0.31 – 0.91**

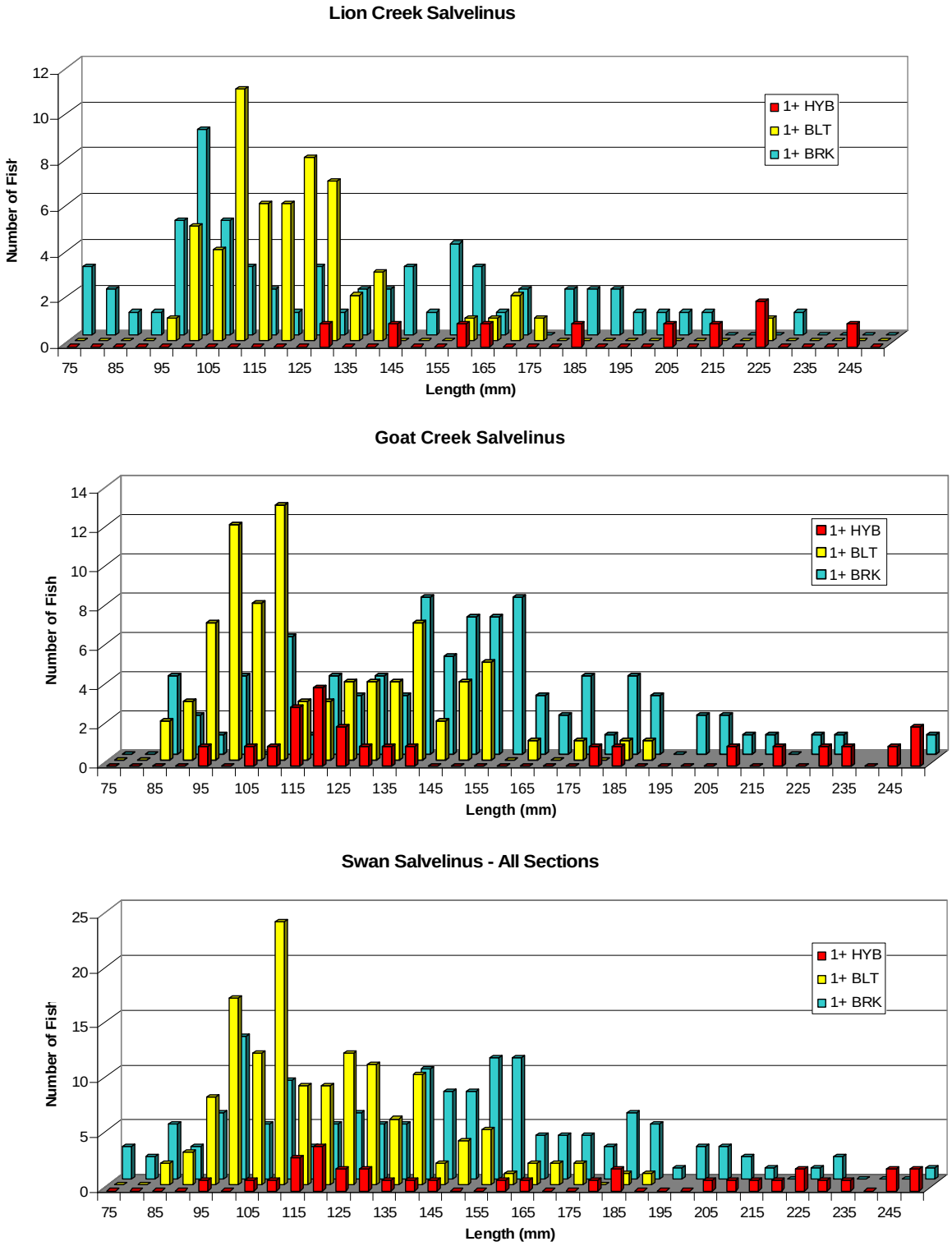


Figure 15. Length frequency of Salvelinus specimens over 75 mm captured from Lion Creek (top) Goat/Squeezer (middle) and combined (bottom). All species identifications were based on genetic results.

Discussion

Results of the genetic analysis indicated that standard field assessment of age 1 and older *Salvelinus*, based on phenotypic characteristics, was generally very accurate in identifying the two parental species and their hybrids. Only 12 of 338 specimens (3.6%) were mis-classified by field identification, and all 12 field mis-classifications involved errors related to hybrid status (either hybrids were misclassified as true bull or brook trout, or brook trout were mistakenly classified as hybrids).

Management implications of these results are very important. Had we planned to use field identification to sort and systematically remove brook trout and hybrids from the five studied stream sections, we would have removed 156 putative "brook trout" and 32 putative "hybrids", retaining only 150 putative "bull trout". Following genetic verification, we conclude that such an action would have resulted in removing 100% of the brook trout (158/158, even though some were misclassified as hybrids) and 88% of the hybrids (30 of 34, even though a few were misclassified as bull trout or brook trout) from the sample. Only four hybrid fish that were misidentified as bull trout would have been left in the stream.

The population estimates conducted in conjunction with this study provided quantitative assessments of distribution of the two *Salvelinus* species. In the three uppermost stream sections (Squeezer, upper Goat, and upper Lion Creeks), closest to the known bull trout spawning areas, bull trout density ranged from 2.58 – 3.29 fish per 100m² and biomass was estimated at 0.38 – 0.55 grams per square meter. In lower Goat and lower Lion Creeks, several km downstream of known spawning locations, densities of bull trout were slightly lower, ranging from 2.18 – 2.48 fish per 100m². However, biomass of bull trout at the lower sites was slightly higher than upstream and ranged from 0.46 – 0.62 grams per square meter, due to a higher proportion of larger (and logically, older) juvenile bull trout.

In the three uppermost sample sites (Squeezer, upper Goat, and upper Lion Creeks), closest to the bull trout spawning areas, brook trout density ranged from 2.24 - 3.19 fish per 100m² and biomass was estimated at 0.81 – 0.91 grams per square meter. In lower Goat and lower Lion Creeks, brook trout densities were considerably lower, ranging from 1.36 – 1.46 fish per 100m². Brook trout biomass was also lower, ranging from 0.31 – 0.38 grams per square meter.

It is the biomass estimates that are, perhaps, most revealing. In the upper stream sections (Squeezer, upper Goat, and upper Lion) brook trout biomass was 1.5 – 2.4 times higher than bull trout biomass, indicating that 60-70% of the stream biomass was occupied by nonnative brook trout. This does not consider the additional numbers of hybrids, which were not estimated, but which would have skewed the biomass even further toward nonnatives. In lower Goat and lower Lion Creeks the biomass of the two parental species was more nearly equal, but if hybrids were factored in, nonnatives would also dominate the biomass of *Salvelinus* in these reaches.

In general, estimates of 21-41 juvenile bull trout and 17-52 juvenile brook trout per 100 meter of stream section were consistent with results (Leathe et al.1985) found in similar locations on the same streams over 20 years ago. Previous researchers (Leathe et al. 1985) found 16-33 juvenile bull trout and 11-58 juvenile brook trout in those same stream sections. Leathe et al. (1985) found considerably higher densities of brook trout in Squeezer Creek (58 per 100m vs. 17 per 100m in our survey) but also considerably lower numbers of brook trout in Lion Creek (13 per 100m vs. 51 per 100m in our survey), indicating that perhaps the species balance is a dynamic equilibrium that changes depending on local habitat conditions or other factors.

We did not observe a consistent spatial pattern in the distribution of the two *Salvelinus* parental species in our study. We did, however, observe increasing numbers and larger sizes of hybrids in the lowermost reaches of the two stream systems. These results may have been related to habitat preferences of the hybrids. An additional factor is that these streams are closed to angling, potentially eliminating human exploitation as a mortality factor on larger fish.

In tributaries of the Malheur River drainage DeHaan et al. (2005) observed a *Salvelinus* species gradient, with streams dominated by bull trout in the upper drainage, brook trout in the lower reaches, and a greater proportion of hybrids in between. Differences in spatial patterns of hybridization between the Malheur and Swan systems may be the result of both habitat factors and life history. In the Swan, the stream reaches available to bull trout are relatively short (approximately 8.5 km in Goat Creek, 7 km in Squeezer Creek, and 11 km in Lion Creek), due to upstream passage barriers. Bull trout use is confined to relatively low gradient bounded alluvial valley bottom reaches (Watson and Hillman 1998) that are heavily groundwater-influenced, with consistently cold thermal regimes (Baxter and Hauer 2000). Also, nearly all the bull trout using the Swan streams are the progeny of large migratory adfluvial adults. In the Malheur system, bull trout occupy the headwaters of longer tributary systems which tend to have pronounced

thermal gradients. Habitat has been highly modified in some areas, and both resident and migratory fish are present (DeHaan et al. 2005). Another difference between the two systems is that in the Malheur brook trout are stocked into a headwater lake.

Differences between the Swan and Malheur habitats may also account for observed differences between patterns of hybridization. In the Swan, hybridization appears to be unidirectional, with all 34 hybrids we evaluated being F1 progeny of matings between male brook trout and female bull trout. In the Malheur, hybridization was found to be proceeding in both directions (female brook trout also crossing with male bull trout), and a high proportion of the fish in some streams were post-F1 hybrids.

Our tentative conclusions are that hybridization of bull trout does not appear to be progressively influencing the streams that we evaluated in the Swan, but rather appears to be a somewhat stable condition that has persisted over at least the past few decades. However, we emphasize that the current study provides the first real quantitative baseline for examining that issue into the future, because previous studies examined only the putative bull trout portion of the *Salvelinus* population and did not assess fish that appeared to be brook trout, but were in fact hybrids.

Management Implications

A recent, major threat to bull trout in the Swan Lake core area is the establishment of a reproducing population of lake trout in Swan Lake (SVBTWG 2006). If lake trout seriously erode the population base of adfluvial bull trout in the lake, as is currently an issue of concern, then the bull trout population in the Swan Lake and River core area could experience a shift toward remnant fluvial and resident populations of bull trout. If this occurs, the historically existing large size disparity between bull trout spawners and brook trout spawners would be reduced or could potentially disappear. Ultimately, this size disparity between spawners may be the primary mechanism that favors behavioral resistance to random hybridization in the Swan system (see e.g., Kitano et al. 1994). Thus, a totally unanticipated consequence of lake trout establishment in Swan Lake could be that bull trout could be subjected to a new and compounding threat, that of progressive post-F1 hybridization with similarly-sized brook trout, more similar to what has occurred in the Malheur system.

Digital photo documentation proved to be a very useful way of archiving representative specimens for reexamination of phenotypic characteristics following genetic evaluation; providing sort of a visual virtual recall. Specimens in a solarium are much easier to accurately assess, as fins are erect and colors are true. We highly recommend the solarium technique as a field tool for improving species identification calls. If field crews wish to rapidly and accurately identify *Salvelinus* and their hybrids, a quick visual examination in the solarium of any questionable specimens should improve the accuracy of identification. We intend to use the sequence of solarium photos, in conjunction with the genetic identifications, to further systematically examine phenotypic characteristics such as markings on dorsal, pelvic, and anal fins; coloration and shape of spotting, vermiculations, and halos; and body and head shapes and dimensions. These results could be used to assess whether more systematic keys to *Salvelinus* hybridization may offer promise for improving accuracy of field assessments in the future, especially for observers with less experience than those in our study.

Further investigation of hybridization and brook trout competition within the Swan system is warranted. The fact that the biomass of *Salvelinus* in some of the best bull trout spawning and rearing streams in the Swan is dominated by brook trout and hybrids is cause for concern. However, this does not appear to be a recent development and begs questions about the role that interspecific competition may play in affecting bull trout carrying capacity and recruitment in these streams. Across the range of bull trout, the likelihood for bull trout to be replaced by brook trout appears to depend on a complex set of variables, including temperature, elevation, and perhaps life history form; results are known to be highly variable and stream dependent (Rieman et al. 2006, McMahon et al. 2007).

In Sun Creek, Oregon, brook trout were successfully eradicated from 8 km of stream with a resident bull trout population, over a ten year period, and concurrently the population of bull trout increased almost 300% (Renner 2005). The author concluded that brook trout may have limited recruitment of bull trout in that situation (Renner 2005). McMahon et al. (2007) reported on a series of laboratory investigations, which demonstrated brook trout grew significantly faster than bull trout at water temperatures between 8° and 20° C, with growth differences increasing linearly with increased temperature. Bull trout feeding and aggression were significantly reduced when they were held in sympatry with brook trout at 8° and 16° C (Gunckel et al. 2002, McMahon et al. 2007).

Given the extremely high accuracy cooperators in this study were able to demonstrate in their ability to recognize brook trout and hybrids in the Swan, we believe it would be valuable to conduct an experimental series of electrofishing removals of brook trout and hybrids from one or more of the three study streams over a period of years, with follow-up assessment to determine whether or not bull trout numbers will expand to fill the potential habitat void created by removal of nonnative *Salvelinus*. Lion Creek may be the best choice for such a study, as bull trout densities have historically been lower in this stream than the others (Weaver 2006). Also, based on our comparison to the data of Leathe et al. (1985), it is possible expanded colonization of the upper reaches of Lion Creek by brook trout may be a more recent phenomenon.

While there is currently no evidence that juvenile recruitment is limiting the adult bull trout population in Swan Lake, we do have concerns for the immediate future in the face of lake trout expansion. Since the study streams are already closed year-round to angling, electrofishing removal from these systems would not be encumbered by the usual public concern over loss of sportfishing opportunities. The density of juvenile bull trout in the Swan study streams we surveyed in August, 2006, ranged from 2.18-3.29 fish per 100 m^2, which appears to be within normal range but on the low end compared to past survey results of standard electrofishing reaches on Goat, Squeezer, and Lion Creeks (Weaver 2006). It is unknown whether the fluctuation of bull trout numbers in the past may have been correlated or synchronized with variations in brook trout densities, since routinely estimates have been conducted only for bull trout. In bull trout spawning streams in the North Fork and Middle Fork Flathead River, where brook trout generally do not occur, densities of age 1 and older juvenile bull trout in the better quality spawning and rearing streams were frequently in the range of 3.0-7.0 fish per 100m^2 (Weaver et al. 2006), prior to 1990's declines in that population.

Experimental suppression of brook trout and hybrids in one of the Swan tributaries might provide some indication whether congeneric brook trout and their hybrids are currently reducing the carrying capacity for juvenile bull trout in Swan basin spawning and rearing streams. It would also be interesting to determine whether suppression of brook trout would eventually result in reduced opportunity for hybridization and eventual declines in the presence of hybrids in important bull trout spawning and rearing streams.

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