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Assess Current and Potential Salmonid Production in Rattlesnake Creek in Association with Restoration Efforts

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Assess Current and Potential Salmonid Production in Rattlesnake Creek Associated with Restoration Efforts

Annual Report

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Executive Summary

This project was designed to document existing habitat conditions and fish populations within the Rattlesnake Creek watershed (White Salmon River subbasin, Washington) before major habitat restoration activities are implemented and prior to the reintroduction of salmon and steelhead above Condit Dam. Returning adult salmon *Oncorhynchus* spp. and steelhead *O. mykiss* have not had access to Rattlesnake Creek since 1913. An assessment of resident trout populations should serve as a good surrogate for evaluation of factors that would limit salmon and steelhead production in the watershed.

Personnel from United States Geological Survey's Columbia River Research Laboratory (USGS-CRRL) attend to three main objectives of the Rattlesnake Creek project. The first is to characterize stream and riparian habitat conditions. This effort includes measures of water quality, water quantity, stream habitat, and riparian conditions. The second objective is to determine the status of fish populations in the Rattlesnake Creek drainage. To accomplish this, we derived estimates of salmonid population abundance, determined fish species composition, assessed distribution and life history attributes, obtained tissue samples for genetic analysis, and assessed fish diseases in the watershed. The third objective was to use the collected habitat and fisheries information to help identify and prioritize areas in need of restoration. As this report covers the third year of at least a five-year study, it is largely restricted to describing our efforts and findings for the first two objectives.

Large woody debris (LWD) was low in frequency in all areas that we surveyed. Water temperatures were above the preferred range for rainbow trout throughout much of

the summer in 2003, as they were in 2001 and 2002, particularly in the section immediately above the confluence with Indian Creek (rkm 0.8). Adequate shading and LWD are likely to continue to be limited in the near future because of lack of large trees, particularly conifers, in the riparian zone.

Although fish habitat was degraded, we found a relatively robust population of rainbow trout in Rattlesnake Creek. All reaches appeared to have some successful reproduction, with age-0 trout collected in every reach. The reach below the lowermost waterfall (rkm 2.4) appears to rear substantially more age-0 trout. The riffles in many sections were nearly dry during summer of 2001, 2002, and 2003, which provided little habitat for older fish. Recaptured PIT-tagged fish showed annual growth, but little or no growth during the summer months, likely attributable to poor flow and temperature conditions.

Several water falls in the watershed are full or partial barriers to upstream migration. The lower waterfalls on Rattlesnake Creek (3.6 m height) appear to be a barrier to resident fish. Lamprey and cutthroat trout were not found above these falls. Only rainbow trout, longnose dace, and shorthead sculpin were found above and below the lower waterfalls. Indian Creek had even fewer species than Rattlesnake Creek, with cutthroat trout dominating the assemblage. The uppermost trout distribution was determined to be a plunge pool at the base of a 2.5 m waterfall at rkm 16.6. If fish were to jump these falls, they would meet a pair of falls, at rkm 17.2, that are each over 22 m in height and are certainly fish barriers.

Results from genetic analysis showed that all of the Indian Creek trout submitted for analysis were hybrids of coastal cutthroat trout and rainbow trout (Graziano and

Nielsen, herein, Appendix C). It also showed that there was little evidence of interbreeding with hatchery fish and that there was a high degree of genetic structure in the White Salmon River and Rattlesnake Creek systems.

Analyses conducted by personnel at the Lower Columbia River Fish Health Center showed heavy infections of diagenic trematodes and suspect cases of BKD in some of the rainbow trout tested. Longnose dace tested positive for BKD and some sculpin were suspected of being infected with BKD, but in general both species appeared healthy. In 2004, we will continue to track the changes in disease presence and severity across time and among reaches.

We conducted extensive PIT-tagging efforts in the Rattlesnake Creek watershed and the mainstem White Salmon River. Over 1,500 PIT tags were inserted in fish in the White Salmon River and Rattlesnake Creek watersheds during 2003, adding to the nearly 1,500 fish that were PIT-tagged in 2001 and 2002. We continued to partner with NOAA fisheries to maintain and upgrade an instream PIT-tag detector system in lower Rattlesnake Creek (rkm 0.3), near its confluence with the White Salmon River. The detector became operational in August 2001. With additional tagging and detection efforts in 2004, we will continue to assess patterns of habitat use and population links between the Rattlesnake Creek watershed and the White Salmon River.

During redd surveys, we observed large rainbow trout on redds. These fish were much larger than those we observed during our population survey work in the summer months. As validated by our PIT-tagging efforts and the PIT-tag detector deployed in lower Rattlesnake Creek, it appears that a population of large rainbow trout that reside in the White Salmon River for most of the year migrate up Rattlesnake Creek for spawning on an annual basis. This documents an important life history linkage for rainbow trout between the mainstem White Salmon River and one of its largest tributaries, Rattlesnake Creek. It demonstrates that these rainbow trout are good surrogates for estimating Rattlesnake Creek's potential productivity for steelhead if and when reintroduced above Condit Dam.

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Introduction

This project was designed to document existing habitat conditions and fish populations within the Rattlesnake Creek watershed (White Salmon River subbasin, Washington) before major habitat restoration activities are implemented in response to the reintroduction of salmon and steelhead above Condit Dam. Returning adult salmon *Oncorhynchus* spp. and steelhead *O. mykiss* have not had access to Rattlesnake Creek since 1914. An assessment of resident trout populations should serve as a good surrogate for evaluation of factors that would limit salmon and steelhead production in the watershed.

Before the construction of Condit Dam in 1913 on the mainstem White Salmon River (at river km 5.1), Rattlesnake Creek (a principal tributary of the White Salmon River at river km 13.8) was likely a productive stream for anadromous salmon, steelhead, and cutthroat trout *O. clarki* (Western Watershed Analysts 1997). With the proposed removal of Condit Dam scheduled for 2008, or at least a retrofit to provide upstream fish passage, Rattlesnake Creek has high potential to support reintroduced or naturally colonizing populations of anadromous salmon and steelhead. This potential is currently limited by existing habitat conditions (Haring 2003).

As noted in previous reports on the Rattlesnake Creek watershed (Stampfli 1994; Western Watershed Analysts 1997; Rawding 2000; Haring 2003), fish habitat has been severely degraded by a number of land-use activities in the watershed. These reports indicated fish habitat conditions in Rattlesnake Creek are compromised by high stream temperatures, low summer flows, lack of woody debris, and lack of riparian vegetation.

Personnel from United States Geological Survey's Columbia River Research Laboratory (USGS-CRRL) attend to three main objectives within the BPA-funded Rattlesnake Creek project. The first is to characterize stream and riparian habitat conditions. This effort includes measures of water quality, water quantity, stream habitat, and riparian conditions. The second objective is to determine the status of fish populations in the Rattlesnake Creek watershed. To accomplish this, we derived estimates of salmonid population abundance, determined fish species composition, assessed distribution and life history attributes, obtained tissue samples for future genetic analysis, and assessed fish diseases in the watershed. The third objective is to use the collected habitat and fisheries information to help identify and prioritize areas in need of restoration.

As this report covers the third year of at least a five-year study, the data collected are partial and the results presented are preliminary. Efforts and results covered by this report include reach-scale habitat surveys (hereafter referred to as "reach surveys"), an intensive large woody debris (LWD) survey, stream temperature, flow, and fish population information that we gathered at key sites within Rattlesnake Creek. This report covers the portion of the work completed under Task 1*a* of Objective 1 (water quantity, stream habitat and riparian conditions) and Tasks 2*a*, 2*b*, and 2*c* of Objective 2 as stated in the Statement of Work submitted in May 2001 by the USGS-CRRL. This report presents our findings from the data we collected through fall 2003.

We used results from habitat surveying, temperature profiling, and flow monitoring to characterize physical habitat conditions and their variation among and within streams of the watershed. Habitat characterization in concert with efforts to assess the fish populations will allow us to assess potential rearing conditions for salmon and steelhead within the watershed. These data should help prioritize sites in need of restoration.

Study Area

The Rattlesnake Creek watershed covers 143 km^2 and supports a third-order stream system with the largest tributary watersheds being the second order systems of Mill and Indian creeks (Figure 1). Rattlesnake Creek enters the White Salmon River at river kilometer (rkm) 13.8, near the town of Husum. Elevations range from 114 m at the mouth of Rattlesnake Creek, which is at the watershed's western boundary, to 927 m at ridge tops near its eastern edge. The watershed's climate is temperate with 75 to 85% of the annual precipitation occurring between October and March. The average annual precipitation at the western downstream end of the watershed is about 127 cm and decreases to about 80 cm in the eastern upstream extension of the watershed (Western Watershed Analysts 1997). Due to the relatively low elevation of the watershed, precipitation in the winter is largely delivered as rain in the lower elevations and as rain or snow in the higher elevations.

There are two sets of waterfalls in Rattlesnake Creek. The lower set of falls, at rkm 2.4, has three individual drops, with the middle one being the largest (about 3.6 m total height, but with a step and 1.5 m deep pocket at 2.1 m). It is most likely a barrier to the resident fish, but may not have been a barrier to salmon and steelhead. Reiser and Peacock (1985) reported a maximum jumping height of 3.3 m for steelhead, and 2.4 m for Chinook salmon. The upper falls, at rkm 17, has two separate drops of about 22 - 25 m each and is certainly a fish barrier.

Indian Creek is a tributary entering at rkm 0.8 of Rattlesnake Creek. There is a culvert approximately 0.1 km from the mouth of Indian Creek and three other culverts at 1.3 km, 1.8 km, and 1.9 km from Indian Creek's confluence that may be resident fish barriers. Mill Creek is a tributary entering at rkm 14 of Rattlesnake Creek.

We divided the drainage into four reaches based on geomorphology and potential fish barriers (Figure 1). The lowermost reach (LRAT) starts at the mouth of Rattlesnake Creek and extends upstream about 2.4 km to the lower set of waterfalls. We had permission to sample 1,100 m at the downstream end of this reach and 440 m at the upstream end of the reach. The next reach (BRAT) is confined by canyon walls and extends from above the lower falls for about 3.1 km to the start of a much less confined section of stream. We had permission to sample all six adjacent sections in this reach totaling 3,140 m. The middle reach (MRAT) is a less constrained alluvial reach that extends 5.3 km between two confined reaches. We had permission to sample on a private landowners section totaling 1,820 m and on Department of Natural Resources (DNR) land totaling 580 m. The uppermost reach (URAT) starts at the base of a canyon and extends about 6.6 km to the base of the upper falls. We had permission to sample the full length of this reach. We had permission to sample two sections of Indian Creek, with the lower section (LIND) being 800-m long and the upper section (MIND) being 880-m long. The two sections were defined by landowner boundaries, but they also constituted parts of two separate reaches defined by their geomorphology.

Methods

Habitat Surveys

To conduct habitat surveys at the reach scale, we walked the stream channel and performed a series of measurements at 20-m intervals. At each 20-m interval, we measured stream width and took a densitometer reading from the stream center. Within each 20-m interval, we measured stream gradient using an Abney level, and we counted boulders (diameter $\geq$ 0.5 m), pools, and pieces of LWD. We classified LWD as conifer or hardwood and tallied pieces into four size classes by length (L) and diameter (D) (L > 5 m with D = 0.3-0.6 m; L > 5 m with D > 0.6 m; L 1-5 m, with D = 0.3-0.6 m; and L 1-5 m with D > 0.6 m). We measured maximum depth in each pool and estimated percent cover for each pool. We also estimated percent spawning area and percent canopy closure within each of these 20-m intervals. Data on pool depth and cover were not analyzed at the time of this report and were not included.

At 100-m intervals, we characterized riparian vegetation within a 10-m x 10-m transect and we assessed channel confinement. Within a transect, we documented dominant species of riparian vegetation. Channel confinement was assessed from estimates of distance to terraces and hill slopes.

Because of the importance of LWD in stream forming processes, and the likelihood that wood placement will be part of restoration efforts in the watershed, a more intensive LWD survey was performed in 2003. Former wood surveys counted wood in the active stream channel by size class and type. However, when reviewing the literature on LWD in streams we found no consensus on the size a piece of wood should be to classify as LWD. Therefore we conducted a wood survey that physically measured each piece larger than 10-cm diameter and 2-m long within the bankfull width.

When conducting this survey we generally followed the methods of Washington State's Timber Fish and Wildlife Monitoring Program Method Manual for the Large Woody Debris Survey (Schuett-Hames et al. 1999). In each reach we measured each qualifying piece's diameter, length, location (thalweg distance from the start of the reach). We categorized the pieces into conifer or deciduous, and categorized the piece(s) as a log, root wad, or jam. To qualify as a log, the roots must have no longer supported the log weight, it must have been dead or completely down, it must have been a minimum of 10-cm diameter for at least 2-m length, and it must have been within the bankfull width. To qualify as a root wad, the piece must have been dead and detached from its original location, the total length must have been less than 2 m, it must have had a 20-cm minimum diameter where the bole meets the root collar, and it must have been within the wetted width. To qualify as a jam there must have been a minimum of 10 pieces touching, with at least one within the bankfull width. We also collected information on the instream wood's influence on the active channel, such as the percent of each piece within the wetted width, bankfull width, and outside bankfull width. We determined if the piece was buried, pinned, stabilized by a root system, or unstable and determined if the piece had any pool forming or sediment storing functions.

Temperature

Personnel from the Underwood Conservation District (UCD) maintained a network of eight thermographs in the Rattlesnake Creek watershed from June 2001 through the present. Sites were chosen to provide thorough coverage of the watershed (Figure 2). All thermograph units deployed and maintained by UCD personnel were Optic StowAway thermograph devices from Onset Computer Corporation (OCC). Prior to deployment, the units were tested for accuracy and adequacy of response time to change in temperature as per instructions from OCC's operating manual.

Thermographs recorded temperature every two hours. Temperature data were downloaded twice a year (spring and fall). To minimize time out of water and missed readings, downloads occurred in the field with use of an OCC optic shuttle. However, the thermographs were removed from the stream in the spring, for up to a week, to be re-calibrated annually. We calculated the daily mean temperature as the mean of the 12 daily readings. We derived the daily minimum and maximum temperatures from the minimum and maximum reading of the 12 daily readings.

Flow

Personnel from CRRL established four flow-monitoring stations in the Rattlesnake Creek subbasin (Figure 3). These stations consisted of a site in the lower Rattlesnake Creek (LRAT), a site in Rattlesnake Creek above the Indian Creek confluence (RAIN), a site in the middle section of Rattlesnake Creek within DNR land (MRAT), and site in the middle section of Indian Creek within DNR land (MIND). These stations were visited about every two weeks during June through October. In addition, occasional attempts were made to measure flow at LRAT throughout the winter of 2002–2003.

An additional air bubbler type automated flow gage was installed in 2003 by the UCD and operated by a subcontractor (Figure 3). With this unit, stage measurements were automatically collected every 15 minutes and a rating curve is established to convert the stage measurements to discharge. This data is collected and reported in a method that is consistent with USGS standards for stream flow measurement.

Stream flow was measured following the protocol of Bain and Stevenson (1999). This protocol entailed anchoring a measuring tape perpendicular to stream flow and recording the distance at the left and right wetted edge. We measured water depth and water velocity with a Marsh-McBirney flow meter at a minimum of 10 (usually about 20) intervals along the measuring tape. Because water depths were always less than 1 m, water velocities were measured at 60% of the depth at each interval. The flow at each interval was computed using the equation:

$$Q_n = d_n \times \left(\frac{b_{n+1} - b_{n-1}}{2} \right) \times v_n$$

Where Q_n = discharge at interval *n*, d_n = depth at interval *n*, b_n = distance along the tape measure from the left wetted edge to point *n*, and v_n = mean velocity of interval *n*. Total flow was calculated by summing the flow of each interval.

Fish

To obtain estimates of salmonid population, density, and biomass, we first conducted intensive habitat surveys of sampling sections, generally following Bisson et al. (1982) for declaring habitat types. Habitat surveys were performed by measuring the length, width, average depth, and maximum depth of each habitat type (e.g., pools, glides, and riffles) from the start to the end of a fish-sampling site. These surveys were usually performed within a few days of fish sampling. For pools, we estimated the percent cover and types of cover (e.g., substrate, undercut bank, instream and overhead wood). In sections of the LRAT, BRAT, MRAT, and LIND reaches, we electrofished a systematic sample of habitat units within strata of habitat types. Habitat units chosen for sampling were blocked off with nets to insure no movement into or out of the unit during sampling. A backpack electrofisher was used to conduct two or more passes under the removal-depletion methodology (Zippin 1956; Bohlin et al. 1982; White et al. 1982). The field guides of Connolly (1996) were used to insure a controlled level of precision in the population estimate (CV < 25% for age-0; CV < 12.5% for age-1 or older trout) was achieved within each sampling unit for each age group considered (two age groups for salmonids age-0 and age-1 or older). These methods were chosen specifically to minimize the number of units sampled by electrofishing and to minimize the number of electrofishing passes conducted. This approach serves to lessen the chance that individual fish will be exposed to potentially harmful effects of electrofishing while insuring a high degree of precision in our estimates.

In addition to the stratified systematic sampling described above, a less intensive method that we termed "index shocking" was used in other sections sampled for fish. The same intensive habitat survey was conducted as described in the population estimate sampling. We then restricted our sampling to pools. One pass was conducted (upstream and back) with no block nets in place. This method allowed us to sample lengths of stream more quickly, while providing information on the fish population within pools and giving us the ability to measure, weigh, insert PIT tags, and recapture previously PIT-tagged fish. In the early spring as soon as the flow was low enough and in the late fall before the flows increased, we electrofished in select reaches without block nets to gain information on fish movement and growth at additional time periods.

Captured fish were anesthetized with the lightest possible dose of MS-222 before handling and were released to their approximate point of capture after handling. The exception to this protocol was when a fish died before or during handling. These mortalities were given to the U.S. Fish and Wildlife Service's Lower Columbia River Fish Health Center (LCRFHC) for disease profiling. All fish captured were measured for fork length to the nearest mm, weighed to the nearest 0.01 g, and inspected for external signs of disease. Scale samples were collected from fish measuring 70 – 100 mm and over 150 mm to estimate age classes. Because of the difficulty identifying rainbow trout from cutthroat trout when the fork length was less than 80 mm, all those collected below this size were simply called "trout". All trout above this size were identified as either rainbow trout or cutthroat trout, and if the fish had hybrid characteristics, it was typically classified as a rainbow trout for our population estimates. In order to track movements, growth, and survival of the trout, we inserted PIT tags (12 mm; 134.2 kHz) in most of the trout that exceeded 80-mm in fork length.

The fish provided to the LCRFHC were given a rigorous inspection for disease. Diseases screened at the LCRFHC by testing or microscopic observations included bacterial (bacterial kidney disease, coldwater disease, columnaris, emphysematous putrefactive disease, furunculosis, enteric redmouth), viral (infectious pancreatic necrosis, infectious hematopoietic necrosis, viral hemorrhagic septicemia), and parasitic agents (whirling disease, *Ceratomyxa*, digenetic trematodes, *Myxobolus kisutchi, Myxidium* *minteri, Hexamita, Gyrodactulus, Scyphidia, Heteropolaria*). During fish collections, all salmonids over 80-mm fork length were visually observed for the presence and severity of black spot (*Neascus).*

Spawning surveys were conducted from 29 March 2002 until no new redds were observed for two consecutive weeks which occurred by 9 May 2002. The LRAT reach was surveyed once a week, with MIND and MRAT surveyed every other week. When redds or spawning fish were seen, we recorded and flagged the location, measured the redd (length, width, depth), estimated dominant and subdominant substrate size, approximated redd age, and recorded the size and species of fish if observed on the redd. To reduce observer error, the same person was involved in all surveys.

Results

Habitat Surveys

Reach surveys were completed on 4.1 km of stream in 2001 and 8.0 km of stream in 2002. In 2003, we completed the URAT reach survey (rkm 14.4 to 17.2) past the uppermost fish distribution to the large waterfalls (Figure 1). We also gained permission to sample and surveyed 1.2 km of additional private property in the BRAT and MRAT reaches. The locations of these reach surveys are shown in Figure 2, and described in Table 1. The average gradient of each reach ranged from 1.3 to 2.7% in Rattlesnake Creek, 2.8% in LIND and 4.7% in MIND (Table 2). The lower 1000 m of Mill Creek had the highest average gradient at 8.1%. In mainstem Rattlesnake Creek, the mean number of pools per 100 m ranged from a low of 2.0 in the URAT reach to a high of 2.8

in the LRAT reach. The tributaries had a higher frequency of pools than the mainstem with 2.7 pools per 100 m in the MIND reach and 3.4 pools per 100 m in both LIND and LMIL reaches. The number of boulders varied from 10 per 100 m at MRAT to 241 per 100 m at LRAT (Table 2). Reach surveys have been completed on all lands that USGS had permission to sample and this dataset should serve as a strong baseline for assessing future habitat change

The amount of coniferous LWD (classified as pieces at least 1 m long and greater than 0.3 m diameter) was low (0.3 pieces per 100 m or fewer in mainstem Rattlesnake Creek, and 0.2 to 0.8 pieces per 100 m in the tributaries; Table 2). Although these data are displayed in 100-m increments, the resolution is in 20 m increments. Hardwood LWD was more abundant at 0.1 to 0.8 pieces per 100 m in mainstem reaches and 0.2 to 1.1 pieces per 100 m in the tributaries. Conifer and hardwood "KEY" pieces (defined as pieces 5 m or longer in length and 0.6 m or larger in diameter) were rare, with 0.3 pieces per 100 m or fewer in all surveyed reaches of Rattlesnake Creek and its tributaries (Figures 4a-4e). Of the mainstem reaches, MRAT reach had the most LWD with 0.9 pieces (conifer and hardwood combined) per 100 m, of which 0.3 per 100 m were KEY pieces. There was mostly hardwood LWD in MIND and LIND, with pieces of coniferous LWD limited to the upstream sections (Figure 4f). Lower Mill Creek (LMIL) had more coniferous LWD per 100 m than any other reach (Table 2), but it had only one KEY piece, which was a conifer (Figure 4g). Figures 4a-g also display the relationship between the gradient, amount of LWD, number of boulders, number of pools, and wetted width.

A more intensive wood survey covering 12.1 km of the Rattlesnake Creek watershed on 10 sections of Rattlesnake Creek and 2 sections of Indian Creek was conducted. While the LWD measured and displayed as part of the reach surveys described above included pieces larger than 0.3 m in diameter and 1 m long, the more intensive LWD survey conducted in 2003 included any piece greater than 0.1 m in diameter and 2 m long. The updated wood survey was collected in high resolution (the data set contains the size, location, stability, and function of each qualifying piece) and is intended to be used to help managers with site specific restoration needs. For display in this report, the information is summarized by sampling section in Table 3 and Figure 5. This survey documented that the existing LWD was of relatively small size (mean diameter for each site ranged from 18-30 cm) and in general does not influence the stream (range of 29% to 55% of the LWD was unstable, and most pieces did not have sediment storing or pool forming functions). There were often long distances with no LWD, 16% to 83% of the 20-m sites had no LWD present. Most of the LWD was deciduous and less than 25 cm in diameter (Figure 5). The LRAT2 site had the most LWD (14 pieces per 100 m), and the URAT2 site had the highest proportion of conifer LWD (73%, Figure 5).

The potential for future natural recruitment of coniferous LWD was poor. The adjacent (0 to 3 m from bankfull width) riparian vegetation on Rattlesnake Creek was dominated by 15 to 40-cm red alder trees (Figures 6a-6g). There were few transects with conifers as the dominant tree type within the adjacent zone in any of the reaches, however conifers tended to dominate in the higher gradient reaches of the tributaries (LMIL and MIND; Figures 6f and 6g).

Hardwoods dominated most outer riparian (3 to 10 m from bankfull width) stands, particularly in the MRAT and URAT reaches, and there were many transects where there were no trees contributing to a canopy layer within the outer riparian zone. Where conifers dominated the canopy in the outer riparian zone, such as in BRAT and MIND, they tended to be small young trees.

Habitat surveys were conducted prior to each fish population and indexing effort to quantify the area of each habitat type for systematic sampling. The LRAT reach had the highest proportion of low gradient riffle (42%) in the mainstem and both LIND and MIND had very high proportions of low gradient riffle (88% and 79% respectively, Table 4). The BRAT3 section had a higher proportion of deep pool (49%), however much of this was due to one pool that was over 130 m long. The information was collected to be used in estimating fish population characteristics by habitat type, but it can also be used to quantify the amount of each habitat type that was available.

Temperature

In the second week of June 2001, UCD placed eight thermographs throughout the Rattlesnake Creek watershed (Table 5, Figure 2). Data from these thermographs were retrieved in the fall of 2001, 2002, and 2003, and the thermographs remained in place to collect temperature information. The thermographs were downloaded again in the spring of 2002 and 2003, and removed from their sites for about one week to calibrate. The analysis in this report covers data collected from June 2001 through September 2003, primarily concentrating on the summer months.

In 2003, the Rattlesnake Creek mainstem site above Indian Creek (RAIN) consistently had the highest daily maximum temperature, and the TOML site consistently had the lowest daily maximum temperature compared with the other mainstem sites (Figure 7). The RAIN also had the highest mean temperature of any of the mainstem sites during July and August of 2001, 2001, and 2003 (Figure 8). The mean water temperature in 2003 was warmer than 2002 and similar to 2001 at all sites (Figure 8). In 2003, the coolest mainstem sites were located in the upper BRAT reach (TOML) and the upper drainage (URAT, Figure 2). There was a period in June when there was a temperature shift at the TOML site (see Figure 2 for location) compared to the other thermograph sites. During that period, it was the coolest site and remained the coolest through mid-September (Figure 7). This site also has the smallest diel range during that period (Figure 9). This was a similar trend as seen in 2001 and 2002, although TOML was the coolest site for only a week in August 2001. There is a large spike in the maximum temperature on July 25, 2003 as seen in Figure 7 and also as a spike in the diel range shown in Figure 9. This sudden change in temperature corresponded to our team's mixing of the water during electrofishing efforts in the pool with the thermograph. It remains to be determined if the difference at this thermograph site is due to pool stratification or spring water influence.

Rattlesnake Creek warmed considerably between rkm 17 downstream to its mouth. The mean water temperature increased at a consistent rate from URAT to MRAT in both July (0.32 °C/ km) and August (0.32 °C/ km), even with the cooling influence of Mill Creek (LMIL; Figure 8). However, in both months but particularly in July of 2003 and August of all years, the monthly mean temperature at the TOML site was much cooler than the upstream site (MRAT). The highest rate of warming was in August from TOML to the RAIN thermograph site, due to the unusually low mean temperature at TOML. During the summer of 2003, a hand held thermometer was used to determine the extent of cooling at the TOML site. The surface water entering and leaving the TOML thermograph pool was notably warmer that the bottom of the pool where the thermograph was and there was no difference between water temperature in the riffles above and below this pool. The bottoms of other large pools in this area were also substantially cooler than the surface water, possibly due to stratification. This illustrates the availability of coldwater refugia in some portions of Rattlesnake Creek. The highest rate of cooling was in August from the RAIN site to the LRAT site, possibly due to the cooling influence of the surface and hyporheic flow from Indian Creek. Further study is needed to quantify the extent of cool water refugia available throughout the creek.

During June through September of 2001, 2002, and 2003, we recorded many daily water temperatures that exceeded 16 °C at all the mainstem Rattlesnake Creek sites and Indian Creek (Table 6). Only Mill Creek did not exceed 16 °C in 2001, 2002, or 2003. This 16 °C limit has been set by the Washington Department of Ecology (Washington Department of Ecology, November 18 1997, Chapter 173-201A, Water Quality Standards for the Surface Waters of the State of Washington) as an indicator of stream health. By comparing either the number of days exceeding 16 °C, 18 °C, and 20 °C, 2003 was a warmer summer than 2001 or 2002. In general 2002 had a higher maximum temperature than 2001 or 2003 (Table 6). However, 2003 had more days with water temperatures above 16 °C than 2001, or 2002 at nearly all the mainstem sites (Table 6). As with 2001 and 2002, the highest temperature recorded (23.8) was at Rattlesnake Creek just above the confluence of Indian Creek (RAIN). This site recorded temperatures higher than 20 °C for 26 days in July, and 18 days in August (Table 7). July was the warmest month in 2003 with the most days above 20 °C among sites. The mainstem location with the lowest maximum temperatures, in the upper canyon below the waterfalls (URAT), still had one day above 20 °C and many days with temperatures above those preferred by salmonids (Table 7).

Most thermograph sites had a diel water temperature range of about 5.5 to 6 °C in July. Most of the other sites matched the annual pattern shown in at the MRAT site with low diel temperature range during the winter and higher fluctuations during the spring and summer (Figure 9). The two sites that are exceptions to this pattern are the thermograph sites in Mill Creek (LMIL) and at TOML. The low annual diel range in Mill Creek is attributed to its well-shaded, high gradient, and north-facing drainage. The pattern at the TOML site is interesting because it mirrors the diel fluctuations of the other sites in the early spring, but in late May through September, the diel range becomes smaller associated with decreasing flows. The increase in the diel range (other than the one day increase due to our presence in the pool, which promoted mixing) corresponds to fall rains, possibly disrupting the thermocline, or reducing the amount of spring influence.

Flow

Five flow measurement sites were sampled in 2003, with three sites on Rattlesnake Creek, one on Indian Creek, and one automated flow gage (Table 8, Figure 3). After June, flow was manually measured every two to three weeks until late October.

In addition, an automated flow meter was installed, which became operational on 9 March 2003. This device collected 15-minute stage height continuously from early March until the present with an outage on 2 May 2003 to 15 May 2003 due to power loss. The stage height was transformed, via a rating curve, to discharge (Figure 10). Flow was measured manually to be 151 cfs at LRAT on 6 January 2003 (Figure 11). We observed higher flows but they could not be measured manually due to personnel safety concerns. The automated flow station measured a maximum flow of 426 cfs on 9 March 2003 and a minimum flow of 0.34 cfs on 31 July 2003. Rattlesnake Creek at this site was consistently at or below 1 cfs from 14 July 2003 to 9 September 2003. The baseline low flow in Rattlesnake Creek was lower than 2001 and similar to 2002 (Figure 12). On three separate occasions there was no flow detected at the MRAT flow site. See Appendix Table A.1 for manual flow measurements at each site in 2003.

During July through October 2003, the upper falls (rkm 17) had no surface flow over the lip of the falls; however, water flowed from the plunge pool at the bottom of the falls throughout the summer. Many of the riffles between pools had no surface flow from July through October.

Fish

A total of 5.3 km of Rattlesnake Creek and 0.8 km of Indian Creek was sampled for fish by electrofishing in 2003 (Table 9, Figure 3). We also hook-and-line sampled on three occasions in the White Salmon River from Husum (rkm 12.7) to about rkm 8.5. This compares with 5.4 km on Rattlesnake Creek, 0.9 km on Indian Creek in 2002 and total of 3.2 km on Rattlesnake Creek and 0.5 km on Indian Creek sampled for fish during summer 2001. A total of 4,411 fish were sampled in 2003. In conjunction with fish sampling for population estimates, 1,501 PIT tags were deployed, including 96 in MIND and 96 in the White Salmon River near the Rattlesnake Creek confluence (Table 9). In 2002 and 2001, 751 PIT tags and 574 Pit tags were deployed, respectively, in the Rattlesnake Creek watershed. Ninety-three PIT-tagged trout were recaptured in 2002 and 282 PIT-tagged fish were recaptured in 2003. Life histories of specific fish will be analyzed as additional data becomes available.

We found six fish species in our sampling areas in 2003 (Table 10): rainbow trout, cutthroat trout, longnose dace, shorthead sculpin *Cottus confusus*, brook lamprey *Lampetra richardsoni*, and brook trout *Salvelinus fontinalis* (collected infrequently in the White Salmon River, and one individual was collected in LRAT on 15 October 2002). Crayfish were present and often abundant in all reaches. All of these fish species were found in the LRAT reach. The fish species present in Indian Creek were limited to cutthroat trout, rainbow trout, and shorthead sculpin. Because brook trout, cutthroat trout, and brook lamprey were not collected above the falls in lower Rattlesnake Creek, they appear to be a barrier to these resident fish.

Pacific giant salamanders *Dicamptodon tenebrosus,* were present, but rare, in Rattlesnake Creek. Two salamanders were observed during sampling efforts from 2001-2003. One was collected in the BRAT reach while electrofishing in 2002 and another was observed in the upper URAT reach (rkm 16.8) during reach surveys in 2003. We have not found Pacific giant salamanders in Mill Creek and Indian Creek, but more intense sampling would be required to verify their absence.

The uppermost fish distribution in Rattlesnake Creek was determined when conducting reach surveys in the upper URAT reach. As we were collecting habitat data in the URAT reach, rainbow trout were readily visible in many pools. The uppermost pool that fish were visible was a plunge pool directly below a 2.5 meter waterfall at rkm 16.6 (Figure 3). Approximately 20 m above this waterfall was an unnamed tributary entering from the southeast. This tributary was in a relatively deep canyon that is easily visible on a map. This tributary was nearly dry and had a 10-m waterfall 60 m from its confluence with Rattlesnake Creek. There were several bedrock pools in the tributary with crayfish but no fish visible. In the mainstem Rattlesnake Creek above the 2.5 m waterfall, there were several bedrock pools, where many crayfish were sighted, but only one Pacific giant salamander. No longnose dace, sculpin, or trout were visible in the remainder of the pools up to and including the large plunge pool below the 27-m waterfall (the lower of two large waterfalls that mark the upper end of our survey).

In Indian Creek, the age-1 or older salmonid population was dominated by cutthroat trout in the MIND reach (93%) and less so in the LIND reach (65%). Several rainbow trout (FL, 87 - 184 mm) that were 80 mm or longer were collected in the MIND and LIND reaches (all trout < 80 mm were identified only as trout), and many of the trout appeared to be hybrids. The MIND reach was above two culverts and above a section of creek that we lacked permission to sample.

To assess the genetic population structure and hybridization of rainbow and cutthroat trout, fin clips of these trout collected from above Husum Falls, from below Husum Falls, from Rattlesnake Creek, and from Indian Creek in 2001 and 2002 were submitted for genetic analysis (see Appendix C; genetics report by Graziano and

Neilsen). This report found that all of the samples submitted from the MIND reach were coastal cutthroat and rainbow trout hybrids (n=13), and 6 of 14 samples from the LRAT reach were hybrids. The genetics results indicated that coastal cutthroat population had not hybridized with westslope cutthroat trout, but had hybridized with rainbow trout. The results also supported the separation of wild trout populations from hatchery rainbow trout populations and a high degree of genetic structure in the system. For more information on the genetics study results, see the attached report by Graziano and Neilsen in Appendix C.

The trout population in Rattlesnake Creek appears to be robust. We conducted a population estimate for trout in the LRAT reach in 2001, 2002, and 2003 (Figure 13). There was an increase in the age-0 trout abundance and biomass in each year. There was a decrease in the age-1 or older rainbow trout population from 2001 to 2002, but the population rebounded slightly in 2003 (Figure 13). Differences between years for cutthroat trout were not as clear, given the small numbers collected in each year. The MRAT reach had population estimates conducted in 2002 and 2003, with an "index" of the population in 2001 (Figure 14). From 2002 to 2003, both the age-0 and age-1 or older trout populations decreased. The index shocking method was used in the LRAT2, BRAT3, BRAT5, MRAT1, URAT, LIND and MIND sections. Population shocking methods were conducted in the LRAT1, BRAT6, and MRAT 2 sections. Figure 15 illustrates the variability in the trout population and biomass among the all the reaches sampled in 2003. There were substantially more age-0 trout in the LRAT reach compared to all other reaches. There was also a notably larger biomass of cutthroat trout per square meter in the MIND reach compared with all sites sampled. Trout populations and age structure varied from year-to-year, and age-0 trout were persistent.

The maximum fork length recorded for an age-0 trout was 90 mm (collected in October 2003 from the BRAT3 section, Table 11). The maximum fork length for an age-0 trout in 2001 was 92 mm from the BRAT reach, and in 2002, it was 95 mm from the MRAT reach. The minimum length of an age-1 fish on Rattlesnake Creek was 80 mm in mid July from the LRAT1 site. The minimum length of an age-1 fish in 2002 was 88 mm from the MRAT2 section in July and in 2001, and it was 78 mm in the LRAT reach in August. The tributaries had smaller fish with a maximum age-0 trout fork length of 46 mm in MIND and 70 in LIND. A 33-mm fish was the largest age-0 sampled in the URAT reach on 10 June 2003 indicating that the age-0 trout had likely recently come out of the gravel. Ages were determined with length-frequency analysis (Figures 16a-e) and by aging scales from those fish near the estimated fork-length limits for each age. Because of the difficulty differentiating between rainbow and cutthroat trout that are smaller than 80 mm, we did not estimate the maximum length of age-0 cutthroat trout in LRAT.

During our fish sampling efforts, we recaptured 282 trout that had been previously PIT tagged (30 in LRAT, 153 in BRAT, 48 in MRAT, 3 in URAT, 2 in LIND and 46 in MIND), which does not include detections of fish on the instream PIT-tag detection system. In 2002 we recaptured 93 PIT-tagged trout. In the LRAT1 section, the change in length of recaptured PIT-tagged fish from initial tagging to each time of recapture showed growth had occurred from year to year, but not during the summer months (Figure 17). We plan to more fully analyze these growth data with additional recaptures and include movement information where available.

Fish were submitted to the LCRFHC for disease assessments from 9 reaches and 10 sampling dates in 2003 (Appendix B). A total of 96 rainbow trout were submitted from Rattlesnake Creek. In general, the trout were in good health with some suspected or confirmed presence of bacterial kidney disease (BKD), *Renibacterium salmoninarum* found in most reaches. *Aeromonas* was found in July in the LRAT reach. As in previous years the trout sampled had the parasite *Nanophyetus* in the hind-gut or gills. Black spot, caused by the parasite *Neascus,* was regularly seen by USGS personnel on salmonids and longnose dace in every reach. This parasite was confirmed in the fish health surveys

Spawning surveys were conducted from 1 April 2003 to 14 May 2003. During the first survey, the water turbidity and high flow made redd detection difficult. As flows subsided and the water cleared, the dark substrate color and lack of algae on the submerged rocks continued to make redd identification difficult. Therefore, only definitive redds and fish seen exhibiting spawning behavior are reported here. New redds or fish with spawning behavior were observed from 8 April 2003 to 6 May 2003. During that time, water temperatures were between 8 °C and 10 °C. During weekly surveys on the LRAT1 section, 19 new redds and 9 fish with spawning behavior were observed. Several trout (300 – 500 mm total length), much larger than those handled during our surveys in the summer, were observed and documented in spawning areas, or on redds in the lower LRAT reach. It is believed that these fish entered Rattlesnake Creek from the White Salmon River for spawning purposes.

Discussion

Large wood and pools were low in frequency throughout the system. Similar to what others have concluded about habitat conditions on Rattlesnake Creek (Western Watershed Analysts 1997; Stampfli 1994; Rawding 2000), these factors indicate degraded fish habitat conditions in Rattlesnake Creek. Our reach surveys showed that the MRAT reach had the highest amount of LWD with 0.7 pieces per 100 m that were at least 0.3-m diameter and 5-m long. The minimum amount of LWD that NMFS (1996) recommend for a stream to be described as "properly functioning" is 1.24 pieces per 100 m (NMFS 1996). However, NMFS defined LWD as pieces with a 0.3-m diameter and 10.4-m length. Therefore, the reach with the most LWD in Rattlesnake Creek had about half of the recommended minimum using our more liberal classification of LWD. In 2003, we re-surveyed all the reaches for LWD and measure the length and width of each piece within the bankfull width. With each LWD piece's length and width, these data can be compared to other studies and prescriptions that use different definitions and classifications.

There were fewer pools in the drainage than the recommended minimum. For a stream of its size, a recommended minimum is 3.5 pools per 100 m (Overton et al. 1997; Platts et al. 1983). The reaches of Rattlesnake Creek that we surveyed ranged from 46% to 80% of this standard. Bisson and Sedell (1984) observed elongated riffles and a reduction in the number of pools in streams where LWD quantities were low. This condition and process appears to fit Rattlesnake Creek.

Our riparian canopy survey showed that most of the Rattlesnake Creek was dominated by small-diameter red alder. There appears to be limited potential for recruitment of LWD large enough to persist, particularly coniferous LWD, in the near future. Likely a result of low levels instream LWD, the creek channel has long low-gradient riffles and few high-quality rearing pools for fish (see Johnson et al. 1985 as referenced in Meehan 1991). The small-diameter deciduous trees do not likely provide adequate shading, as discussed below.

Water temperatures in Rattlesnake Creek are a concern because they were regularly above the preferred range for rainbow trout throughout the summer of 2001, 2002, and 2003, particularly in the section above the confluence with Indian Creek. These high temperatures combined with low base flows could make summer a stressful and potentially lethal time for trout in Rattlesnake Creek. Water from the plateau above the upper waterfall, when flowing, was warm upon entering the fish bearing sections of Rattlesnake Creek. Water in the upper canyon had daily maximum temperatures that were above 16 °C in over half of the days in July and August. These warm temperatures coincided with very low flows (<0.3 cfs at LRAT). The riffles in many sections were nearly dry throughout the summer and did not provide much habitat for adult fish. Many of the larger fish are then concentrated in to the pools. Optimum feeding temperature for rainbow trout is between 13 °C and 16 °C (Cherry et al. 1975; Kaya 1977). As water temperatures increase beyond about 15 °C, metabolic costs escalate rapidly and available food resources support progressively lower densities of juvenile salmonids (Li et al. 1995). At temperatures above 20 °C, rainbow trout can experience high metabolic demands and stress, which can lead to suppressed growth and increased early mortality (Hokanson 1977; Nielsen et al. 1994). At temperatures above 24 °C, high mortalities can occur (Cherry et al. 1975), with the upper incipient lethal temperature reported as 25.6 °C

(Bidgood and Berst 1969; Hokanson 1977). Rattlesnake Creek approached lethal temperatures with the highest temperature recorded of 24.1°C just above the confluence with Indian Creek.

The thermograph site above the confluence with Indian Creek (RAIN) recorded the warmest water temperatures found throughout the summer of 2001, 2002, and 2003. There were many long shallow glides that were exposed to the sun in the BRAT and LRAT reaches, between the TOML and RAIN thermograph sites. However, the temperatures were reduced below the Indian Creek confluence (LRAT), probably due to the surface and hyporheic inflow from Indian Creek. The lack of sufficient canopy shade (ranging by reach from 30% to 67%) likely exacerbates this water temperature problem. There were no 100-m averages, and only a few 20-m sites surveyed in mainstem Rattlesnake Creek that approached 90% shading, the recommended level by Western Watershed Analysts (1997).

The water in Mill Creek (rkm 14) was substantially cooler than Rattlesnake Creek or Indian Creek (rkm 0.8). Mill Creek had some of the highest riparian shade and had low diel temperature variation. Stream temperatures can be affected by characteristics such as ambient air temperature, water velocity, flow, depth, riparian canopy cover, and groundwater inflow. Although Mill Creek and Indian Creek have a similar aspect and similar amounts of shading, Mill Creek has a higher elevation (360 m at mouth) than Indian Creek (128 m at mouth). This may be a primary explanatory factor for the low diel variation and relative coolness of Mill Creek.

Water temperatures at the TOML site were particularly interesting because this site was cooler than the sites either upstream or downstream in 2001, 2002, and 2003.

This may be due to groundwater inflow or pool stratification. This thermograph site was at the downstream end of a 5-km long alluvial reach and just upstream from the more confined BRAT reach. Bounded alluvial valley segments have been associated with increased groundwater inflow (Baxter et al. 1999; Stanford and Ward 1993). In streams with higher than optimal temperatures, salmonids have been shown to use thermal refugia such as coldwater patches created by groundwater seeps, springs, and thermal stratification within stream channels (Nielsen et al. 1994; Ebersole 2001).

The lower waterfalls on Rattlesnake Creek appear to be an upstream passage barrier to resident fish. Lamprey and cutthroat trout were not found above these falls. Only rainbow trout, longnose dace, and shorthead sculpin were found above and below the lower waterfall. Anticipated additional data gathering and analysis of PIT-tag recaptures and the genetic samples may help us assess whether the fish above and below this barrier are distinct populations. The genetic analysis presented in Appendix C lumped the trout from above and below the falls into one group. This was due to an insufficient sample size because of hybridization and sample degradation. Cutthroat trout dominated the assemblage in Indian Creek. The proportion of rainbow trout to cutthroat trout was higher in the LIND reach compared to the MIND reach of Indian Creek, therefore there is a lower potential for introgression of rainbow trout into the cutthroat trout population in the upper reaches of Indian Creek. The results form genetic analysis showed that all of the fish submitted from the MIND reach were hybrids. This result re-emphasizes the difficulty in field identification of rainbow trout and cutthroat trout due to their similar appearance and frequent hybridization (Baumsteiger et al. 2005). The extent of hybridization may vary spatially, with less introgression with rainbow trout higher in the Indian Creek drainage. Further study is needed to assess the viability of a pure coastal cutthroat population in Indian Creek.

We have collected one brook trout but no bull trout in Rattlesnake Creek. The brook trout was collected in the LRAT1 section of Rattlesnake Creek on 15 October 2002. Brook trout are known to inhabit the White Salmon basin, and we believe this fish may have been on a spawning migration (brook trout are fall spawners). No bull trout have been collected in Rattlesnake Creek and water temperature would indicate that it is not suitable for bull trout during the summer months. We have not collected any age-0 brook trout or bull trout throughout three years of intensive sampling, so we do not believe there is a reproducing population of either species of trout in Rattlesnake Creek.

Although fish habitat was degraded, we found a relatively robust population of rainbow trout in Rattlesnake Creek, with several pools containing many age-1 rainbow trout. All reaches seemed to have some successful reproduction, with age-0 trout collected in every reach. In 2001, 2002, and 2003 there was a higher number of age-0 trout in the LRAT reach than other reaches. This may be due to some trout in the White Salmon River using lower Rattlesnake Creek as a spawning tributary.

Over 574 PIT tags in 2001, nearly 751 PIT tags in 2002, and 1,501 PIT tags in 2003 were inserted in fish from the mainstem White Salmon River and the Rattlesnake Creek watershed. Several of those fish were recaptured in 2002 and 2003 and we anticipate substantially more recaptured fish in future years as more PIT tags are deployed throughout the watershed. In 2002 and 2003, the length and weight of the recaptured fish in Rattlesnake Creek showed annual growth, but a lack of growth during the summer months. High metabolic costs due to higher than optimal temperatures may be a factor limiting growth in the summer. We will have opportunities to look at growth, movement, and life history attributes of individual fish when more of these PIT-tagged fish are recaptured in future sampling years. We will continue to monitor the remote PIT-tag reader at the mouth of Rattlesnake Creek, and the PTAGIS database will be queried for any detections.

Results from disease profiling provided by U. S. Fish and Wildlife Service's Lower Columbia River Fish Health Center indicate that longnose dace and shorthead sculpin were relatively healthy. Most trout were healthy, but some individuals had heavy infections of diagenic trematodes and BKD. Black spot infections were common in the longnose dace and trout handled by USGS personnel. There are a variety of chemical, physical, biological, and ecological parameters that influence a fish population's ability to withstand disease (Snieszko, 1974). The elevated parasitic infections of these fish may be due to increased stress during times of high temperature and low flow. Disease can directly influence success of reproduction, performance, susceptibility to predation, and other critical factors required for the survival of a species (Hedrick, 1998). There will be additional disease samples in 2003, and we will track the changes in disease presence and severity over time and among reaches.

The idea that Rattlesnake Creek is an important spawning tributary for the rainbow trout population in the White Salmon River is supported by PIT tagging data and two years of spawning surveys. During spawning surveys, large rainbow trout were observed on redds. These trout were much larger than those we observed during our population survey work in the summer months. These fish are believed to be from the White Salmon River that use Rattlesnake Creek for spawning. The instream PIT-tag detector data includes additional information that supports the use of lower Rattlesnake Creek for spawning by White Salmon River rainbow trout. Additional data such as PIT-tag recaptures and the instream PIT-tag reader will help determine the significance and persistence of what appears to be a potadromous spawning population.

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Table 1. Locations of reach surveys (rapid-reach type) conducted within the Rattlesnake Creek watershed 2001 through 2003. Coordinates were obtained from a hand-held Global Positioning System (GPS) using North American Datum 1927. Sites are listed from upstream to downstream within a watershed.

Watershed Site	Survey year	Start point distance from mouth (km)	Length of survey (km)	Coordinates at start Northing	Coordinates at start Easting	Coordinates at end Northing	Coordinates at end Easting
Rattlesnake Creek							
URAT 2	2003	14.4	2.9	5079978[a]	626878	5081699[a]	628262
URAT 1	2002	10.8	3.6	5078524	624154	5079978[a]	626878
MRAT 2	2001	7.2	0.6	5076347	622077	5076668	622403
MRAT 1	2002	6.0	1.2	5074988[a]	620994	5076351	622064
BRAT 6	2003	4.8	1.2	5074953	620709	5075846	621240
BRAT 5	2002	4.3	0.5	5074092	620731	5074953[a]	620709
BRAT 3 and 4	2001	3.3	1.0	5074176	620038	5074390	620640
BRAT 2	2002	2.9	0.4	5073959	619687	5074176	620038
BRAT 1	2001	2.4	0.5	5073738	619276	5074077	619658
LRAT 2	2002	2.0	0.4	5073589[a]	618900	5073743	619284
LRAT 1	2001	0.2	1.1	5072424	617997	5073141[a]	618415
Mill Creek							
LMIL	2002	0.0	1.0	5079735	626489	5079033	627106
Indian Creek							
MIND	2001	2.2	0.9	5071551	620085	5071699	620025
LIND	2002	0.1	0.8	5072713	618456	5072560[a]	619234

[a] Position obtained with mapping software, all others obtained with hand held GPS device in the field.

Table 2. Reach survey data for streams within the Rattlesnake Creek watershed. Sites are listed from upstream to downstream within a watershed. Bolded numbers are means of results from two or more survey dates.

Watershed Reach	Survey date (mm/yy)	Surveyed length Start	End	Length (m)	Rosgen (1994) channel type	Stream width (m)	Stream gradient (%)	Number per 100m in reach length[a] Pools	Boulders	CLW	HLW	KEY
Rattlesnake Creek												
URAT		**10800**	**17260**	**6460**	**B, F**	**4.2**	**2.7**	**2.0**	**66**	**0.3**	**0.1**	**0.1**
	06/03	14400	17260	2860		4.2	4.2	2.4	91	0.4	0.0	0.1
	07/02	10800	14400	3600		4.3	1.6	1.6	45	0.2	0.3	0.2
MRAT		**5440**	**7940**	**2500**	**C**	**6.4**	**1.4**	**2.4**	**10**	**0.1**	**0.8**	**0.3**
	06/01	7360	7940	580		6.7	1.1	2.2	23	0.2	1.3	0.5
	06/02	6120	7360	1240		6.3	1.6	2.2	3	0.2	0.7	0.3
	06/03	5440	6120	680		6.4	1.4	2.8	9	0.0	1.7	0.0
BRAT		**2400**	**5440**	**3040**	**B, A**	**5.7**	**1.3**	**2.1**	**73**	**0.1**	**0.2**	**0.0**
	06/03	4840	5440	600		6.5	1.3	2.0	27	0.5	0.0	0.2
	08/02	4340	4840	500		5.7	0.8	2.2	163	0.0	0.0	0.0
	8/01-7/02[b]	2400	4340	1940		5.3	1.4	2.6	70	0.1	0.5	0.1
LRAT		**200**	**2400**	**1540**[c]	**B**	**6.1**	**2.7**	**2.8**	**241**	**0.2**	**0.4**	**0.2**
	07/02	1960	2400	440		5.3	4.4	3.6	163	0.2	0	0.2
	07/01	200	1300	1100		6.5	2.0	2.4	257	0.2	0.5	0.2
Mill Creek												
MILL	08/02	0	1000	1000	A	2.2	8.1	3.4	79	0.8	0.2	0.1
Indian Creek												
MIND	07/01	2200	3080	880	B	2.0	4.7	2.7	101	0.2	1.1	0.3
LIND	06/02	100	900	800	B	2.6	2.8	3.4	10	0.4	0.6	0.2

[a] CLW = Conifer large woody debris ≥1 m length and ≥ 0.3 m diameter; HLW = Hardwood large woody debris ≥ 1 m length and ≥ 0.3 m diameter; KEY = "Key pieces" conifer and hardwood large woody debris ≥ 5 m length and ≥ 0.6 m diameter.

[b] A 440 m section of stream not surveyed in 2001, but was surveyed in July 2002 after landowner permission was granted.

[c] A 650 m section of stream has not been surveyed due to lack of landowner permission.

Table 3. The amount, length, diameter, and function of large woody debris (LWD) found in Rattlesnake Creek watershed in 2003. Sites are listed from upstream to downstream within a watershed.

Watershed Site	Reach length (m)	Woody debris (LWD) Number (LWD/100m)	Mean length (m)	Mean diameter (cm)	Percent of 20m sites with no wood	Percent of unstable LWD	Percent of sediment-storing LWD	Percent of pool-forming LWD
Rattlesnake Creek								
URAT2	2860	5	7.08	33.46	48	35	10	2
URAT1	1200	7	6.13	21.44	38	42	6	5
MRAT2	560	7	3.77	30.82	41	35	33	26
MRAT1	1220	9	6.09	25.65	39	44	23	12
BRAT6	1240	7	6.00	22.19	51	37	2	10
BRAT5	480	2	6.35	21.10	83	36	10	0
BRAT3 & 4	980	6	6.00	21.80	29	38	0	0
BRAT2	440	2	4.78	18.78	73	55	0	0
BRAT1	480	4	5.77	21.61	67	50	0	0
LRAT2	420	14	4.61	21.30	19	35	9	2
LRAT1	840	4	6.14	24.73	57	57	6	3
Indian Creek								
MIND	890	10	8.02	24.78	16	45	11	3
LIND	860	11	8.30	24.41	57	29	15	16

Table 4. Location, length, area, and percent of each habitat type from surveyed locations in the Rattlesnake Creek watershed, 2003. Shallow pools were defined as having a depth of ≤90 cm for Rattlesnake Creek and ≤60 cm for tributary streams. Percent habitat was calculated using area. Backwater pools and side-channels were not included in total survey length, but were included for total surface area. Sites are listed in an upstream to downstream pattern.

				Habitat type (%)							
Watershed Site	Start distance from mouth (km)	Total survey length (m)	Total surface area (m^2)	Shallow pool	Deep pool	Back water pool	Glide	High gradient riffle	Low gradient riffle	Side channel	Step
Rattlesnake Creek											
MRAT 2	7.35	627	3,048	28	25	0	11	0	33	2	0[a]
MRAT 1	6.09	812	3,831	44	15	0	7	0	34	0	0[a]
BRAT 6	4.81	717	3,443	24	13	0	23	2	37	0	1
BRAT 5	4.31	532	3,071	37	28	0	1	0	30	3	0
BRAT 3	3.31	828	4,861	21	49	0	5	3	19	1	2
LRAT 2	1.95	420	2,639	46	9	0	9	2	27	5	2
LRAT1	0.20	1,075	6,726	27	14	0	4	13	42	1	0[a]
Rattlesnake Cr. overall		**5,011**	**27,619**	**31**	**23**	**0**	**8**	**4**	**32**	**1**	**1**
Indian Creek											
MIND	2.40	496	1,037	13	0	0	8	0	79	0	0[a]
LIND	0.10	244	627	10	0	0	2	0	88	0	0
Indian Cr. overall		**740**	**1,664**	**12**	**0**	**0**	**6**	**0**	**82**	**0**	**0**

[a] Habitat type present, but consisted of < 0.5% of surveyed habitat area.

Table 5. Locations of thermographs deployed and maintained by Underwood Conservation District within the Rattlesnake Creek watershed. Sites are listed from upstream to downstream within a subbasin. For additional information on thermograph locations, see Figure 2.

Watershed Subwatershed	Code	Coordinates Northing	Coordinates Easting	Elevation (m)	Distance upstream from mouth (km)	Date Start dd/mm/yy	Date End[a] dd/mm/yy
Upper Rattlesnake Creek	URAT	5081213	628410	457	16.9	07/06/01	ongoing
Mill Creek	LMIL	5079549	626619	396	0.2	08/06/01	ongoing
Upper Rattlesnake Creek below canyon	BUPC	5078753	624011	292	11.3	08/06/01	ongoing
Middle Rattlesnake Creek	MRAT	5076576	622218	250	7.7	08/06/01	ongoing
Tomlin property	TOML	5074768	620819	226	5.6	07/06/01	ongoing
Lower Rattlesnake above Indian Creek	RAIN	5072747	618418	131	0.8	07/06/01	ongoing[b]
Indian Creek	LIND	5072689	618451	131	0.0	07/06/01	ongoing
Lower Rattlesnake Creek	LRAT	5072419	617933	122	0.1	07/06/01	ongoing

[a] Thermographs were removed annually in the spring for calibration.
[b] The RAIN thermograph was lost over winter 2003, replaced 19 May 2003.

Table 6. Number of days per year when maximum water temperature exceeded 16, 18, and 20 °C, and yearly maximum water temperature recorded at locations in the Rattlesnake Creek watershed. Thermograph locations are listed from upstream to downstream. Refer to Table 3 and Figure 2 for additional site information.

Site	RKM	Number of days ≥ 16°C			Number of days ≥ 18°C			Number of days ≥ 20°C			Maximum (ºC)		
		2001	2002	2003	2001	2002	2003	2001	2002	2003	2001	2002	2003
Mainstem													
URAT	16.9	47	59	75	15	22	26	0	2	1	19.5	20.3	20.5
BUPC	11.3	81	80	89	48	50	58	16	19	11	21.4	22.4	21.6
MRAT	7.7	87	91	98	51	62	70	11	27	31	21.7	22.3	22.7
TOML	5.6	78	56	14	23	31	3	0	6	0	19.5	21.3	19.2
RAIN	0.8	103	101	110	72	72	83	38	39	51	23.2	24.1	23.8
LRAT	0.1	97	96	109	57	62	67	10	25	25	21.1	23.5	22.1
Tributaries													
LMIL	0.1	0	0	0	0	0	0	0	0	0	15.7	15.4	15.4
LIND	0	85	86	96	41	54	66	9	14	18	20.8	21.8	21.8

Table 7. Number of days per month when maximum water temperature exceeded 16, 18, and 20 °C and the monthly maximum water temperature recorded at locations in the Rattlesnake Creek watershed during 2003. Locations are listed from upstream to downstream. Refer to Table 3 and Figure 2 for additional site information.

Site	RKM	Number of days ≥ 16°C				Number of days ≥ 18°C				Number of days ≥ 20°C				Maximum (°C)			
		Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
Mainstem																	
URAT	16.9	17	30	24	4	0	22	4	0	0	1	0	0	17.9	20.5	18.7	17.7
BUPC	11.3	19	30	31	8	6	26	21	5	0	11	0	0	19.3	21.6	19.6	20.0
MRAT	7.7	21	31	31	14	8	27	31	4	0	21	10	0	19.5	22.7	20.7	19.2
TOML	5.6	0	6	6	1	0	2	1	0	0	0	0	0	15.8	19.2	18.3	16.3
RAIN	0.8	27	31	31	14	15	30	31	7	5	26	18	2	21.6	23.8	22.0	21.0
LRAT	0.1	27	31	31	14	14	30	21	2	5	16	4	0	21.5	22.1	20.3	18.0
Tributaries																	
LMIL	0.1	0	0	0	0	0	0	0	0	0	0	0	0	13.3	15.4	15.2	14.6
LIND	0.0	23	31	31	10	8	27	25	6	0	15	2	1	19.5	21.8	20.5	20.2

Table 8. Locations of flow measurements taken within the Rattlesnake Creek watershed[a]. Coordinates were obtained from a hand-held global positioning system (GPS) using North American Datum 1927. Sites are listed from upstream to downstream within the watershed.

Watershed Site	Coordinates Northing	 Easting	Elevation (ft)	Distance upstream of mouth (km)	Year sampled (Y=Yes, N=No) 2001	 2002	 2003
Rattlesnake Creek							
URAT - upper	5081436	628496	1,500	16.9	Y[b]	N	N
URAT - lower	5078524	624154	900	11.2	Y	Y[c]	Y[d]
MRAT	5076614	622231	820	7.7	Y	Y	Y
LRAT - above Indian Cr.	5072742	618411	430	0.8	Y	Y	Y
LRAT- automated gage	5072699	618186	420	0.6	N	N	Y[e]
LRAT - lower	5072429	617898	400	0.1	Y	Y	Y
Mill Creek							
LMIL - DNR	5079664	626548	1,300	0.1	Y[f]	N	N
Indian Creek							
MIND - middle	5071671	620054	730	2.2	Y	Y	Y
LIND - lower	5072687	618423	430	0.0	Y[b]	N	Y

[a] Flows taken approximately once every two weeks from June through October unless noted otherwise.
[b] Flow measured only on 06/07/01.
[c] Flow measured only on 10/22/02.
[d] Flow measured only on 06/08/01.
[e] Automated flow measurements taken every 15 minutes beginning on 3/09/03.
[f] Flow measured only on 6/10/03.

Table 9. Sites sampled for fish in the Rattlesnake Creek and White Salmon River watershed during summer 2003. Watersheds and streams are listed in an upstream to downstream pattern within the watershed.

Watershed Stream reach or section	Method and length surveyed	Start point distance from mouth (km)	Number of 134.2 kHz PIT tags deployed[a]	Number of fish sampled
Rattlesnake Creek				
URAT	FSNP[b]	11.2	71	96
MRAT2	Population survey, 580 m; FSNP	7.1	188	278
MRAT1	Index survey; 784 m; FSNP	5.9	321	508
BRAT6	Population survey, 717 m; FSNP	5.0	200	292
BRAT5	Index survey, 532 m	4.5	114	214
BRAT3	Index (1 of 2 sections sampled) 829 m	4.0	107	151
BRAT1	FSNP	2.5	70	128
LRAT2	Index survey, 414 m	2.1	96	318
LRAT1	Population survey, 1,100 m; FSNP	0.1	142	1981
Indian Creek				
MIND	Index survey, 536 m; FSNP	2.2	96	282
LIND	Index survey, 244 m; FSNP	0.1	0	32
White Salmon River				
WSR3	FSNP	8.5	96	131
			Total 1,501	**Total 4,411**

[a] Fish tagged were limited to rainbow trout and cutthroat trout with fork length of 80 mm or longer.
[b] FSNP = Fish sampled by electrofishing, not a population survey.

Table 10. Presence and absence of the fish species found in the Rattlesnake Creek watershed by U.S. Geological Survey personnel, 2003. Sites are listed in an upstream to downstream pattern. P = present, A = absent.

Watershed Site	Rainbow trout	Coastal cutthroat trout	Eastern brook trout	Longnose dace	Shorthead sculpin	Brook lamprey
Rattlesnake Creek						
URAT	P	A	A	P	P	A
MRAT	P	A	A	P	P	A
BRAT	P	A	A	P	P	A
LRAT	P	P	P[a]	P	P	P
Mill Creek						
UMIL	P	A	A	A	A	A
LMIL	P	A	A	A	P	A
Indian Creek						
MIND	P	P	A	A	P	A
LIND	P	P	A	A	P	A

[a] One individual found on 15th October 2002.

Table 11. Delimits of age classes of rainbow trout (RBT) and cutthroat trout (CTT) in Rattlesnake Creek and its tributaries during summer 2003. Sites are listed in an upstream to downstream pattern within the watershed. See Figure 3 and Table 6 for information on fish sampling sites. Age classes were estimated by length-frequency analysis and verified by aging scales. FL= fork length (mm).

Watershed Site	Date	Length of stream surveyed (km)	Start point distance from mouth (km)	Species	Max FL age 0	Min FL age 1
Rattlesnake Creek						
URAT	10 Jun	3.60	12.0	RBT	33	94
MRAT 2	7 Oct-9 Oct	0.58	7.2	RBT	52	102
MRAT 1	18 Sep-25 Sep	1.24	6.0	RBT	83	90
BRAT6				RBT	67	94
BRAT 5	29 Sep	0.50	4.4	RBT	85	100
BRAT 3	1 Oct-21 Oct	0.50	3.4	RBT	90	100
LRAT 2	14 Oct-15 Oct	0.44	2.0	RBT	83	92
LRAT 1	14 Jul-21 Jul	1.10	0.2	RBT	68	80
Indian Creek						
MIND	18 Aug	0.88	2.4	CTT	46	79
MIND	18 Aug	0.88	2.4	RBT	46	79
LIND	18 Aug	0.80	0.1	CTT	70	105
LIND	18 Aug	0.80	0.1	RBT	70	110

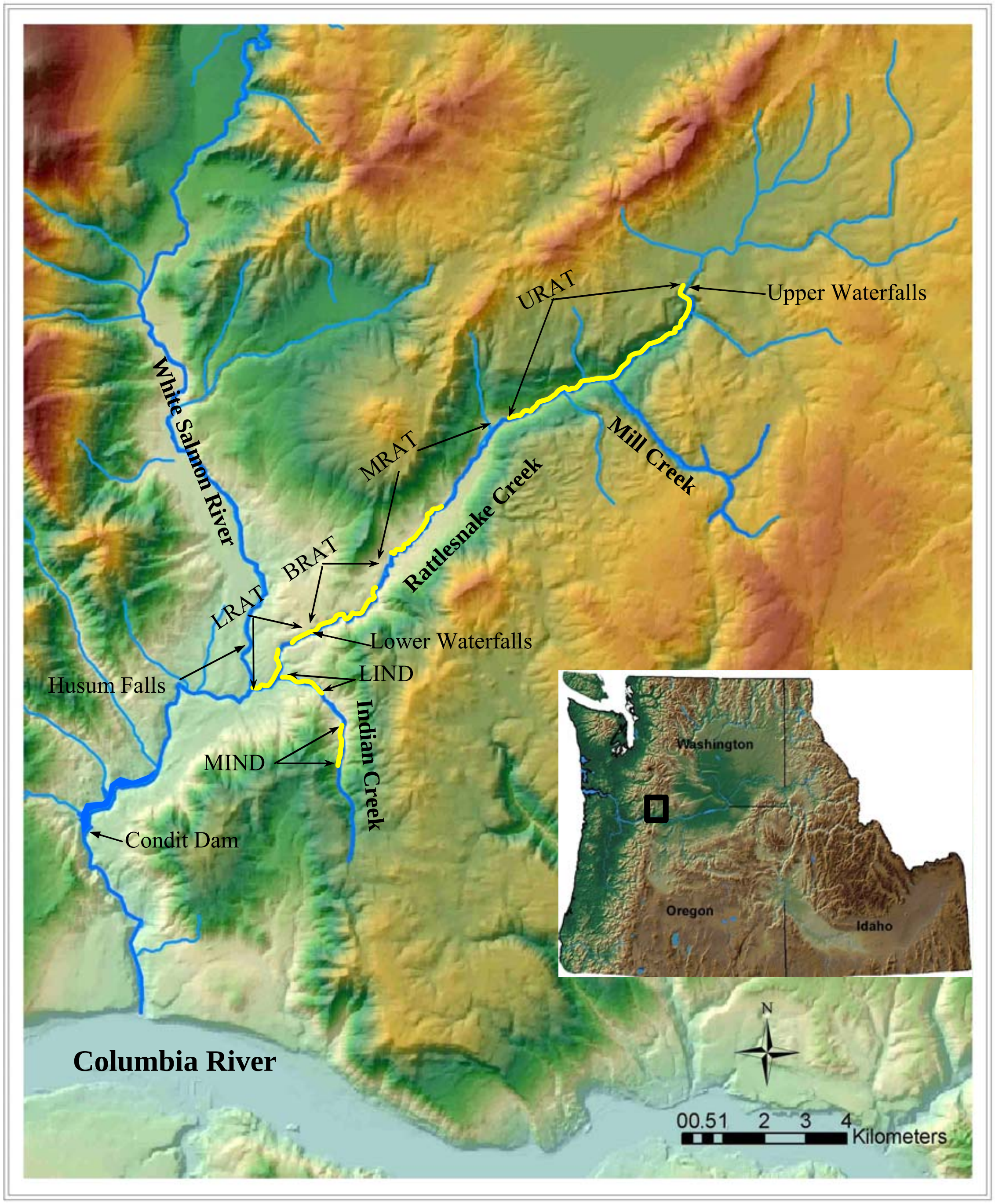


Figure 1. Location of Rattlesnake Creek, WA and study reaches within the Columbia River Gorge. Study reaches are: LRAT = lower Rattlesnake Creek below lower waterfall; BRAT = lower Rattlesnake Creek above lower waterfall; MRAT = middle Rattlesnake Creek; URAT = upper Rattlesnake Creek to upper waterfall. Indian Creek Study reaches are LIND = lower Indian Creek, and MIND = middle Indian Creek.

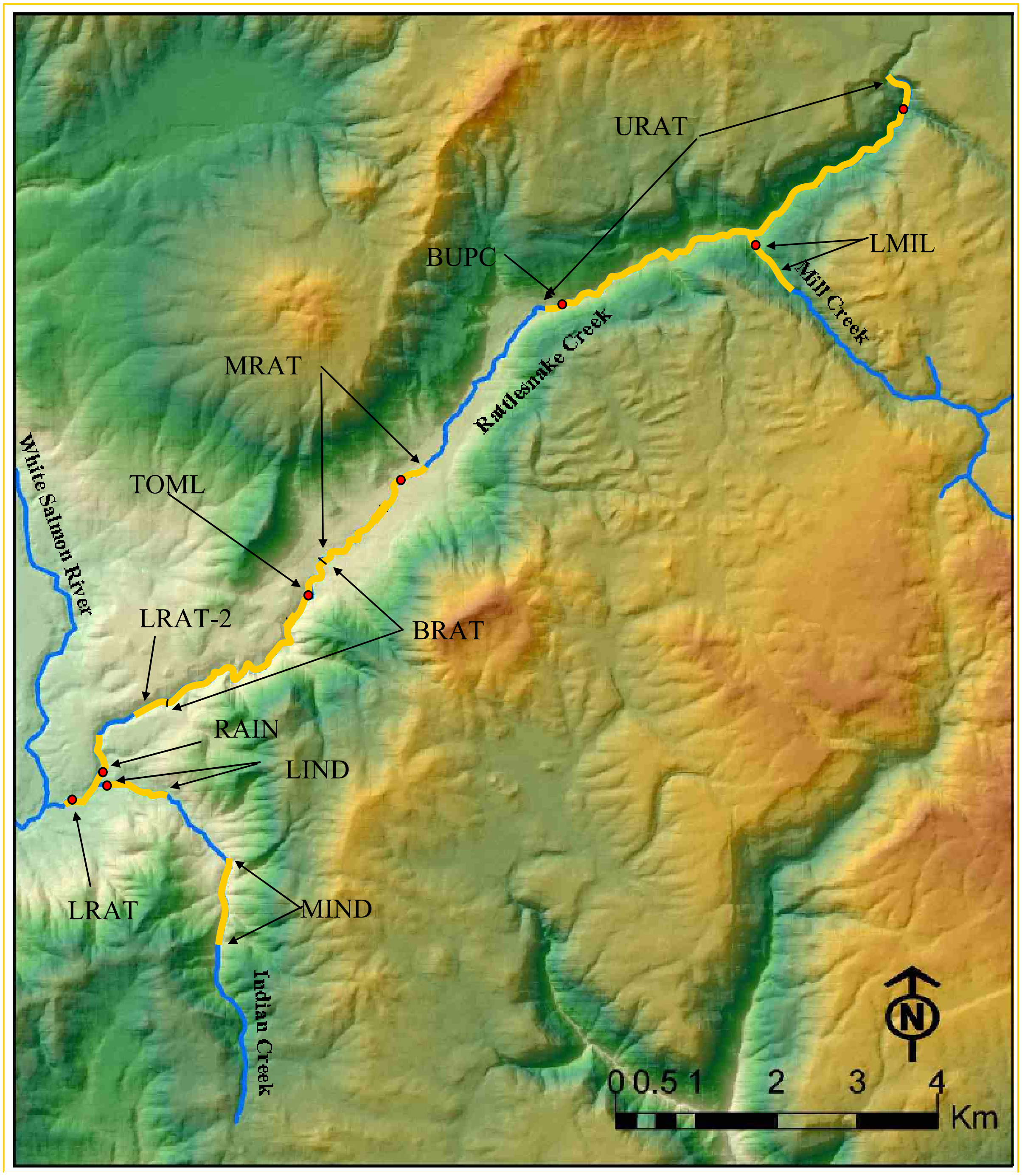


Figure 2. Locations of reach surveys and thermograph sites within the Rattlesnake Creek watershed, 2001-2003. ▬ = Location of reach surveys. ● = Location of thermograph sites. See table 1 for additional information on reach survey sites. Table 5 provides additional information on thermograph sites.

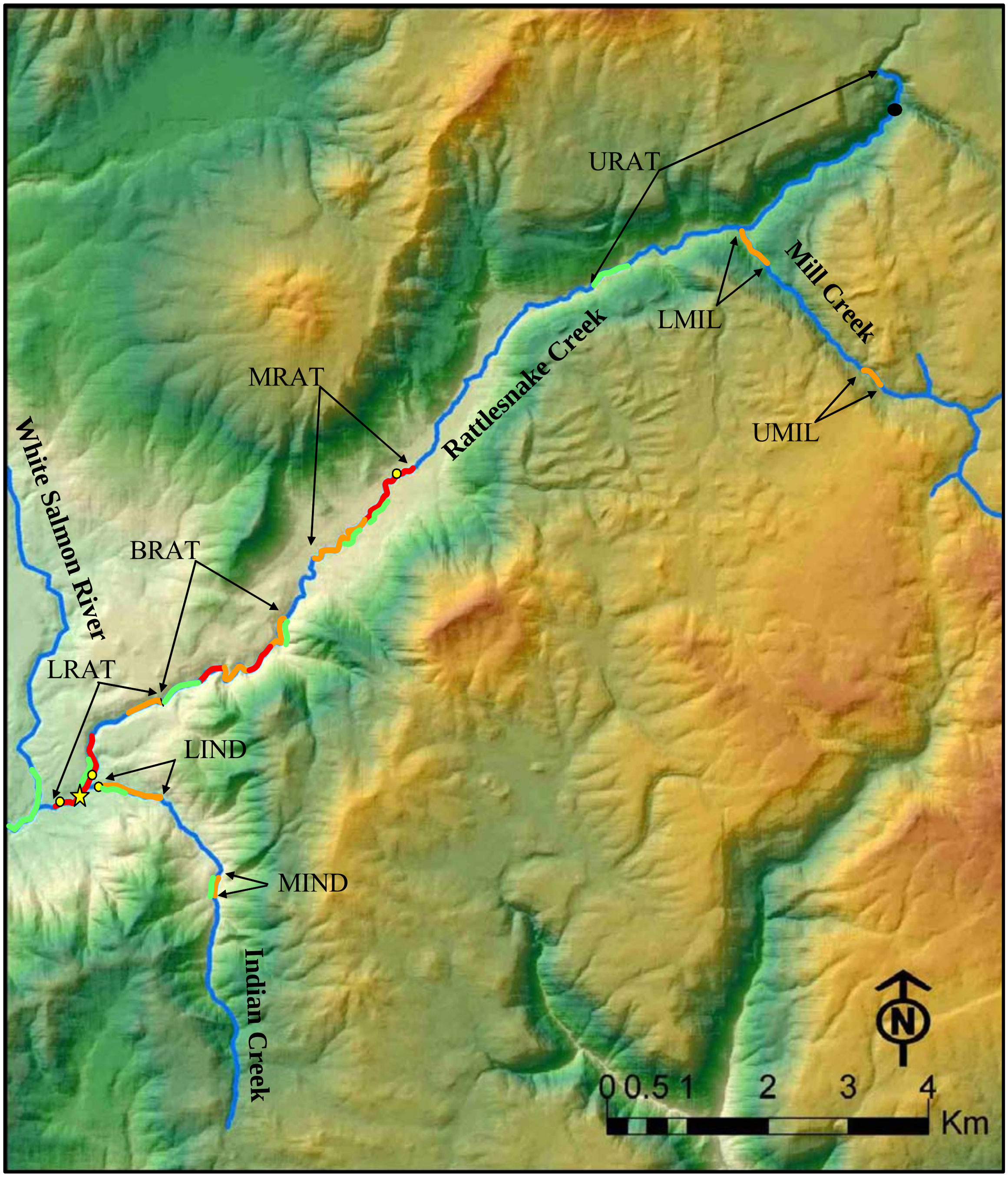


Figure 3. Locations of fish sampling and flow sites within the Rattlesnake Creek watershed, 2003. ○ = Flow measurement locations. ━ = Locations of population surveys (used a systematic sample of habitat units within different habitat types (e.g., pool, glide, riffle) with multiple pass, removal- depletion electrofishing with block nets). ━ = Locations of index shocking (only pool habitats were sampled, one pass was conducted (upstream and back) with no block nets). ━ =Additional fish collections conducted without a population estimate or habitat survey. ● = Uppermost fish distribution in 2003. ☆ = Automated flow gage location.

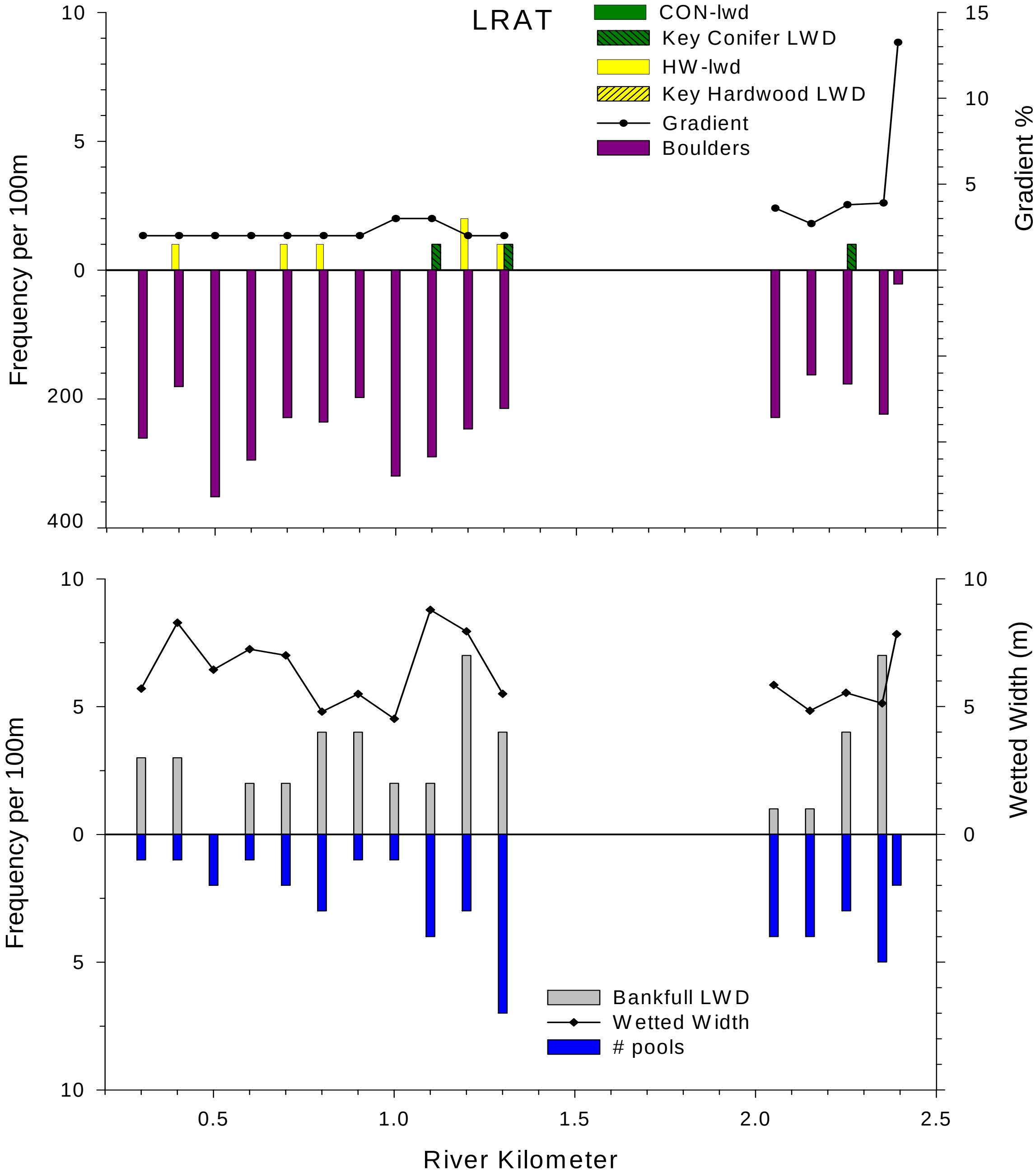


Figure 4a. Reach survey data in 100 m intervals from the LRAT reach of Rattlesnake Creek (rkm 0.2 – 1.3 and rkm 1.9-2.4). Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream. The area between rkm 1.3 and rkm 1.9 was not surveyed.

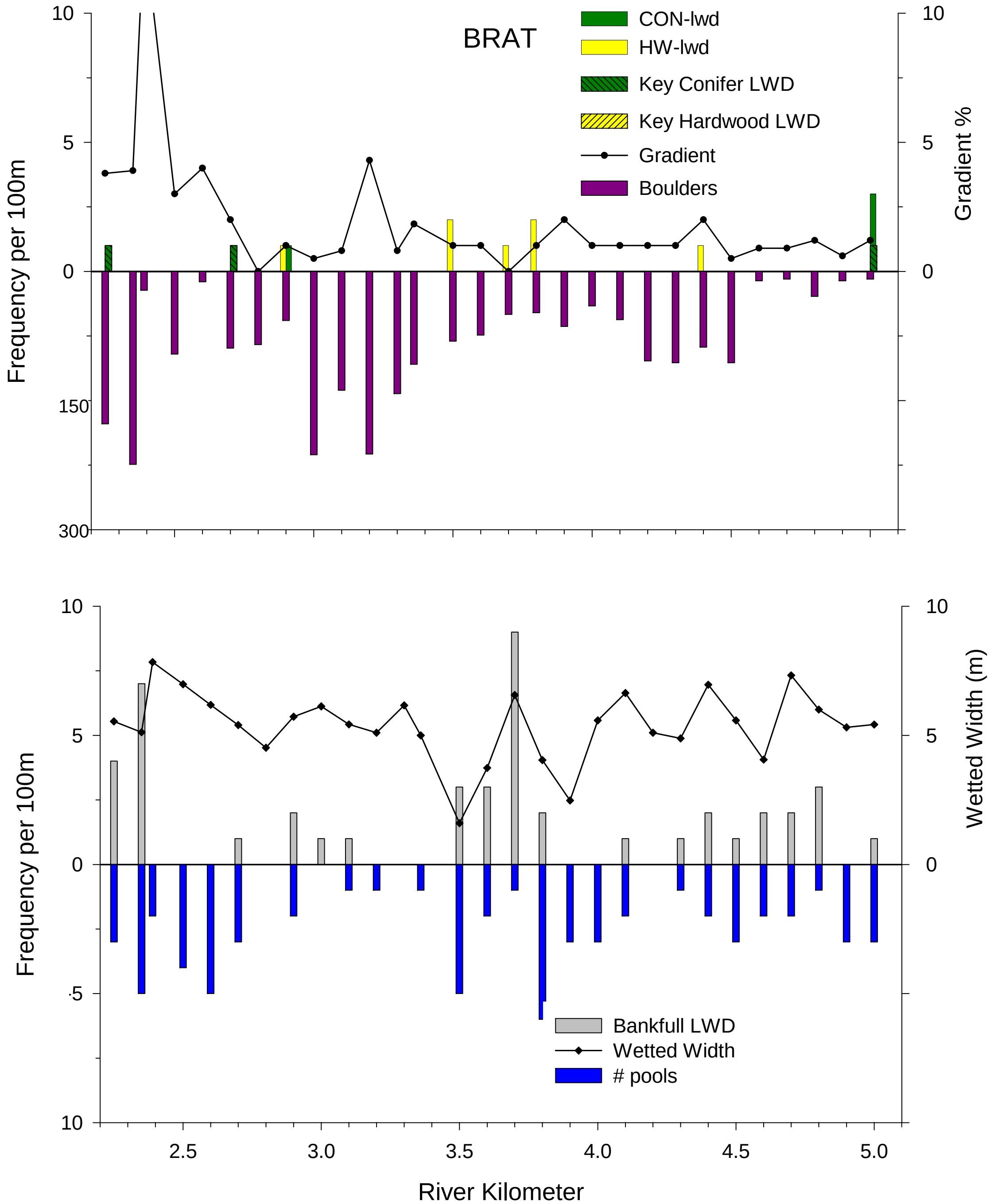


Figure 4b. Reach survey data in 100 m intervals from the BRAT reach of Rattlesnake Creek (rkm 2.4 – 5.5). Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream.

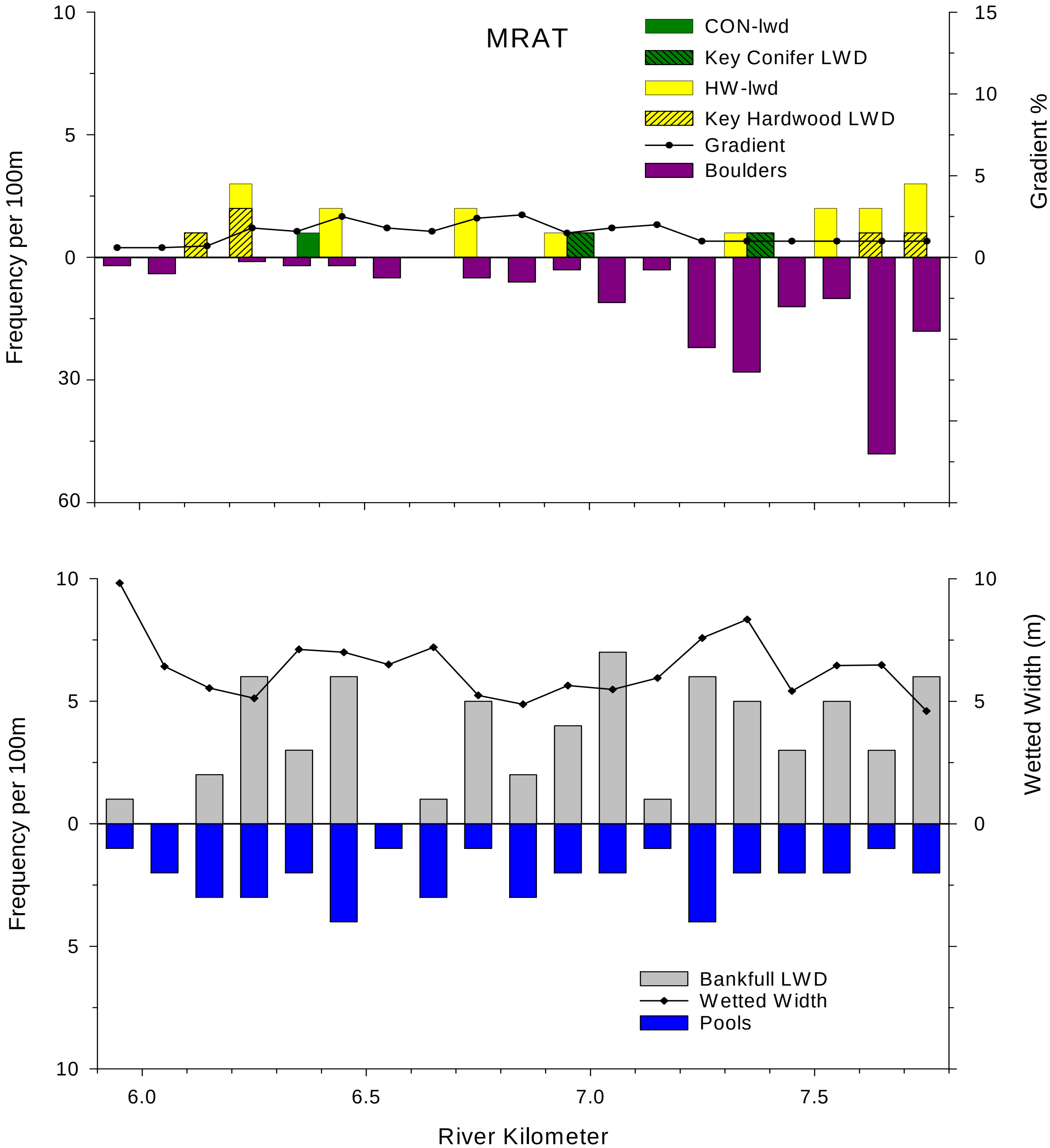


Figure 4c. Reach survey data in 100 m intervals from the MRAT reach of Rattlesnake Creek (rkm 6.0 – 7.8). Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream.

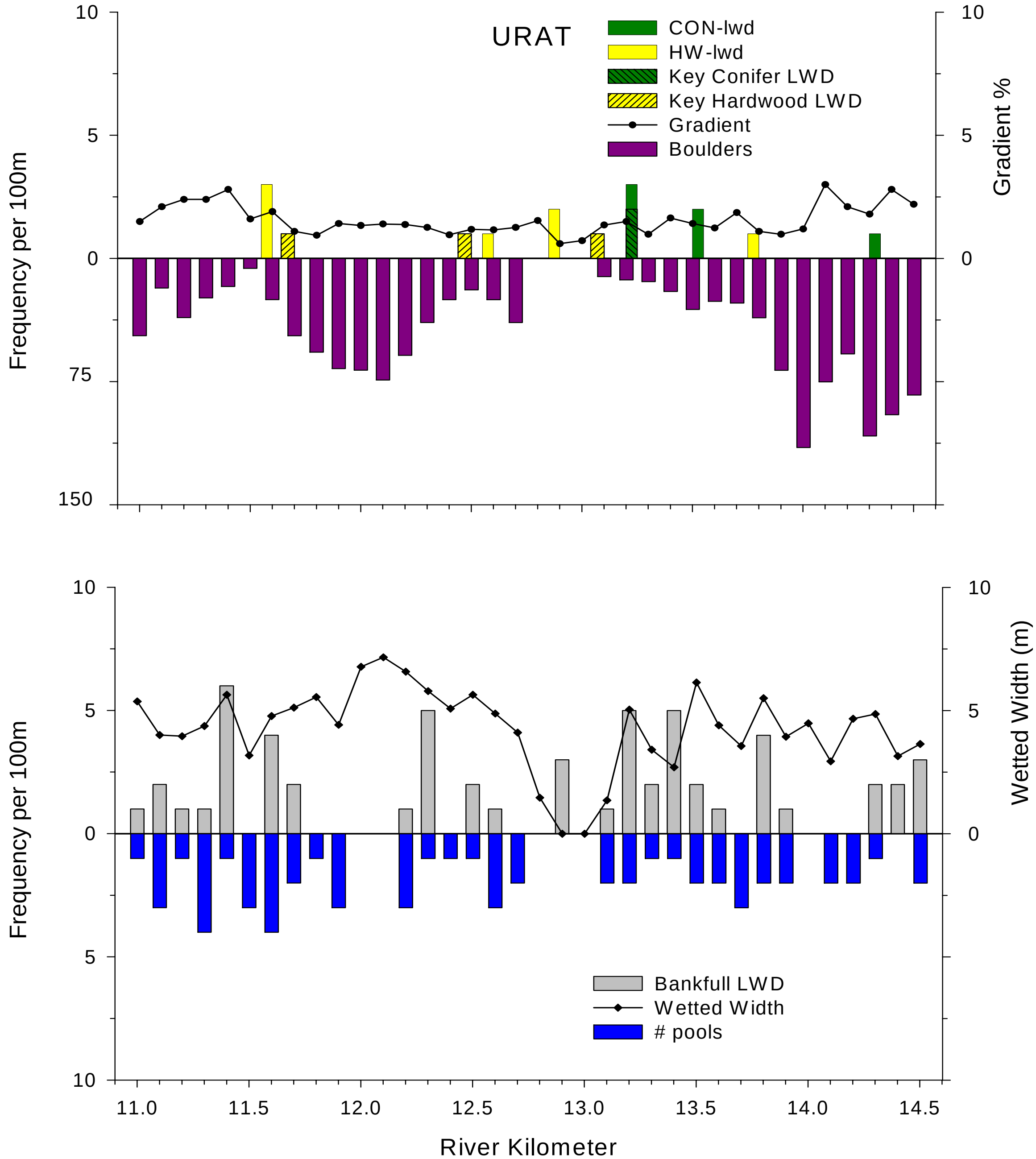


Figure 4d. Reach survey data in 100 m intervals from the URAT reach of Rattlesnake Creek (rkm 10.8 – 14.4). Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream.

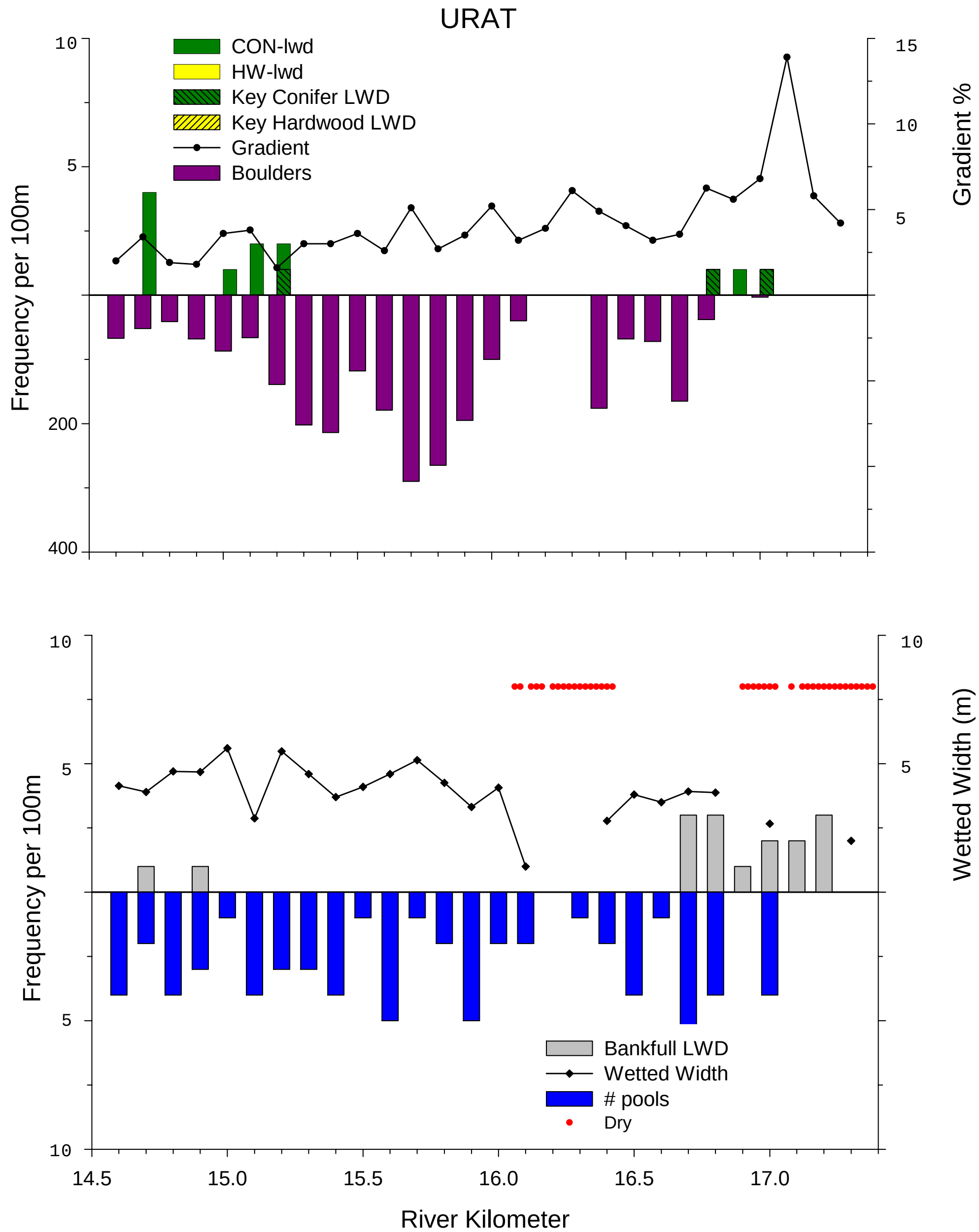


Figure 4e. Reach survey data in 100 m intervals from the URAT reach of Rattlesnake Creek (rkm 14.4 – 17.2). Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream.

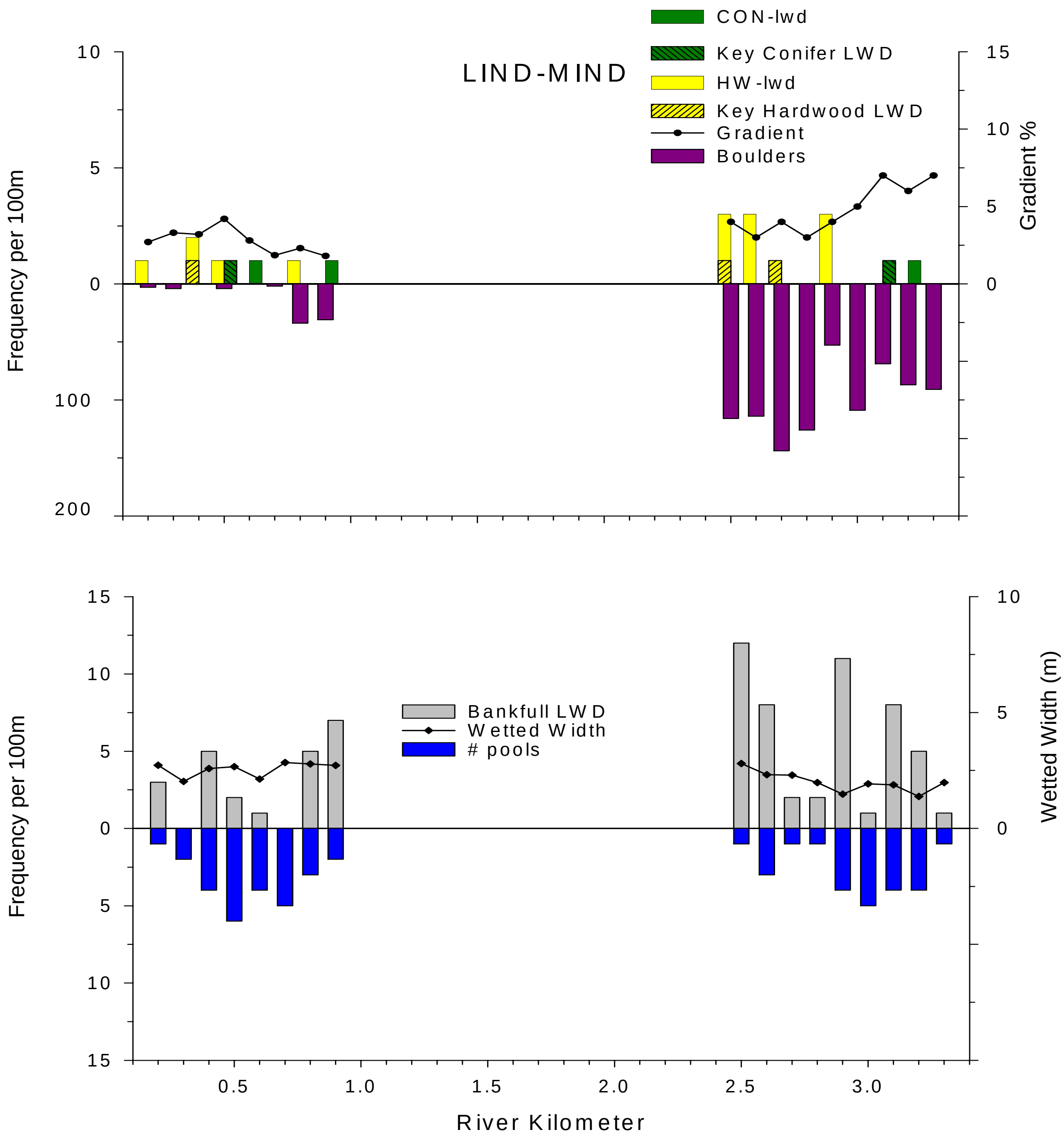


Figure 4f. Reach survey data in 100 m intervals from Indian Creek (LIND; rkm 0.1-0.9 and MIND; rkm 2.4-3.3). Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream. The area between rkm 0.9 and rkm 2.4 was not surveyed.

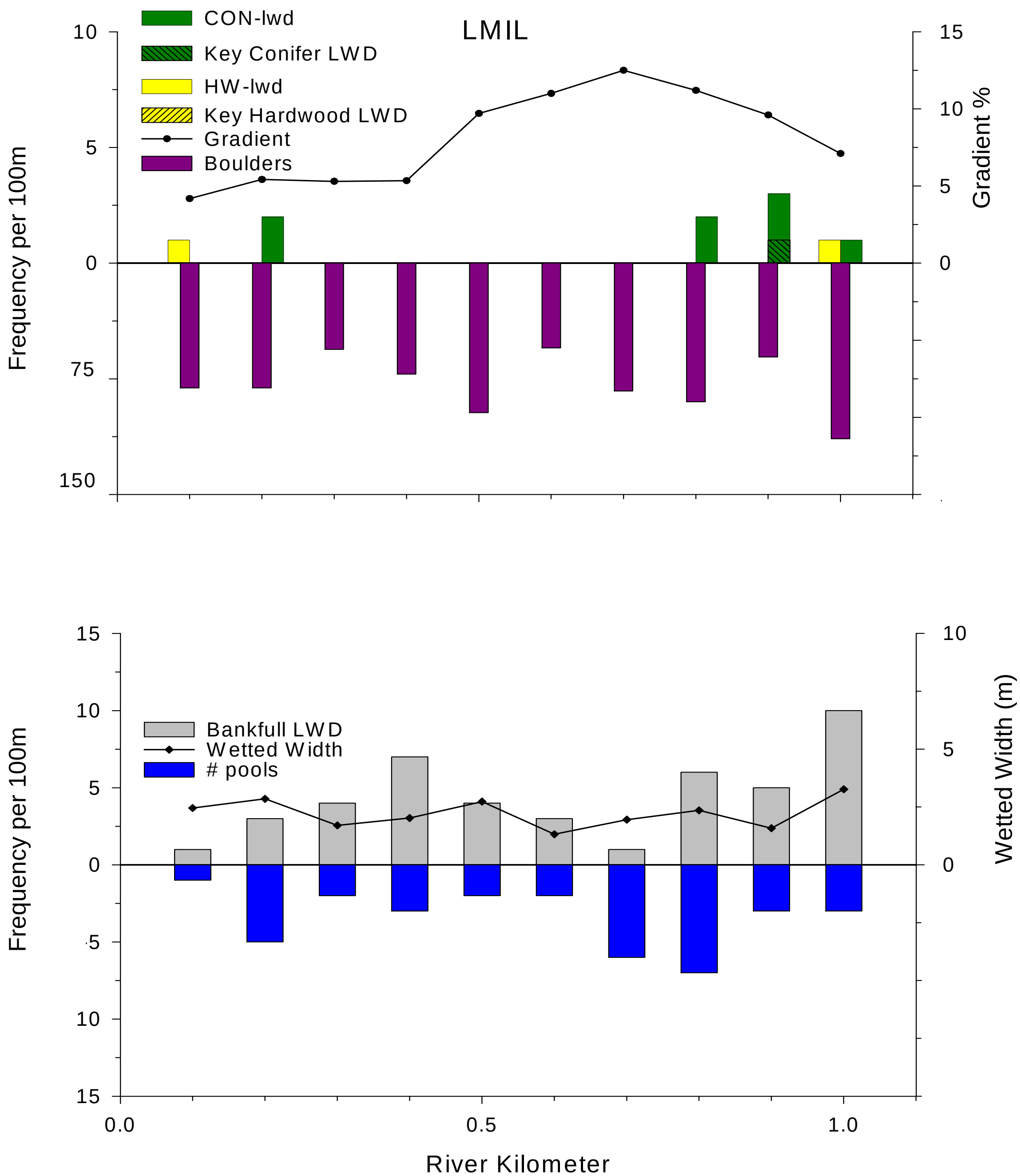


Figure 4g. Reach survey data in 100 m intervals from lower Mill Creek (rkm 0.0 - 1.0) a tributary of Rattlesnake Creek. Top graph shows the total number per 100 m of coniferous and deciduous large woody debris (LWD; >1 m long and >30 cm diameter) key LWD pieces (>5 m long and >60 cm diameter), boulders, and the average gradient. Bottom graph shows the total number of bankfull LWD, pools, and the average wetted width of the stream.

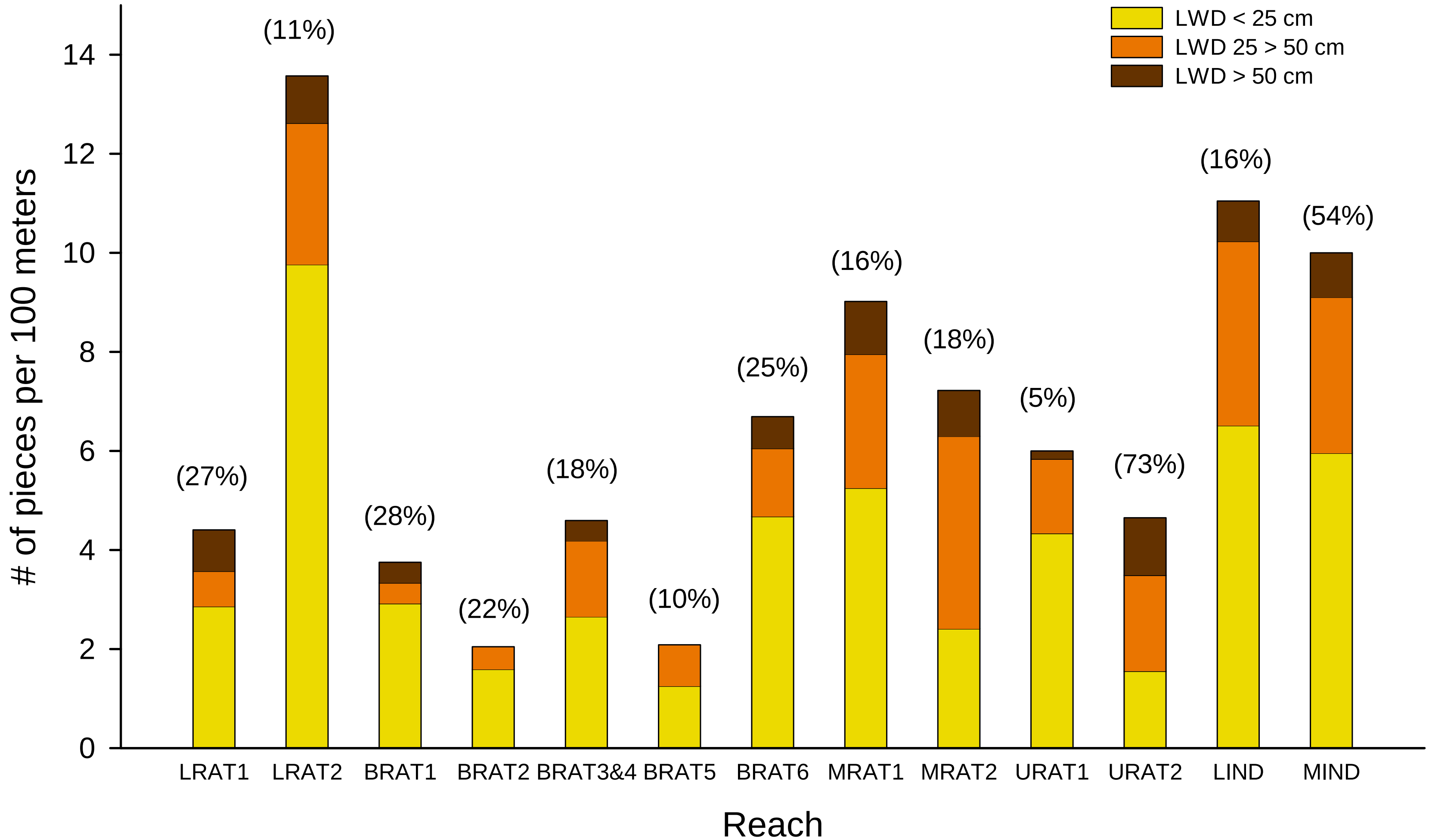


Figure 5. The mean number of pieces of large woody debris (LWD) per 100 m by site and size range for the Rattlesnake Creek watershed. The percent of coniferous pieces are displayed in parentheses above each site. Stream codes are: LRAT1 = section 1 of lower Rattlesnake Cr.(rkm 0.2-1.3), LRAT2 = Rattlesnake Cr. below 1st waterfall (rkm 2.0-2.4), BRAT1 = Rattlesnake Cr. above falls (rkm 2.4-2.9), BRAT2 = Rattlesnake Cr. 500 m above falls (rkm 2.9-3.3), BRAT3 and 4 = Rattlesnake Cr. 1000 m above falls (rkm 3.3-3.8), BRAT5 = Rattlesnake Cr. 2000 m above falls (rkm 4.3-4.8), BRAT6 = Rattlesnake Cr. rkm 4.8-6.1, MRAT1 = Rattlesnake Cr. rkm 5.6-7.2, MRAT2 = Rattlesnake Cr. rkm 7.2-7.8, LIND = Indian Cr. 100 m above mouth, MIND = Indian Cr. rkm 2.4-3.3. Additional information on stream code locations are provided on Figure 3 and Table 1.

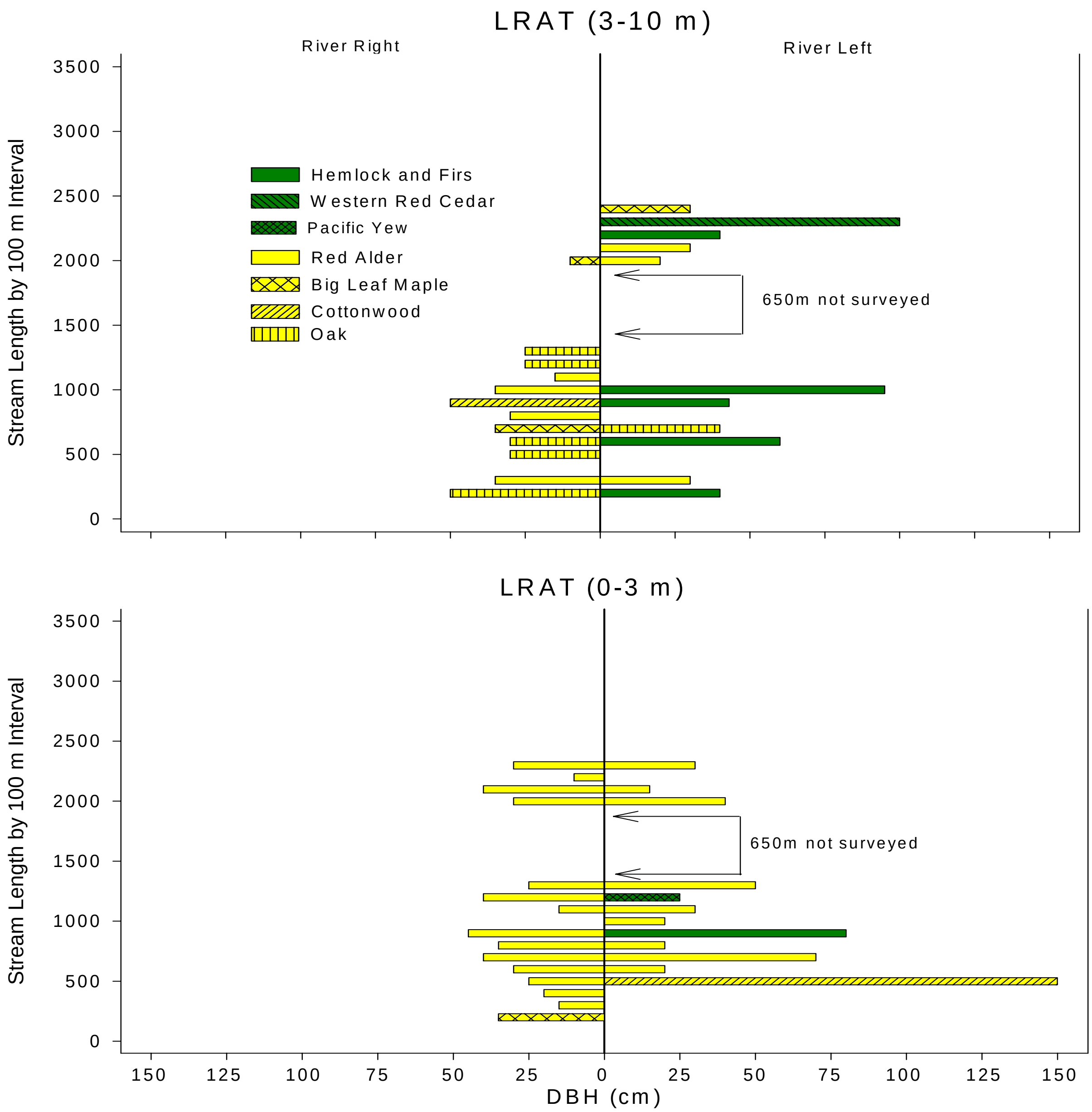


Figure 6a. Characterization of outer (3-10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in lower Rattlesnake Creek (LRAT; rkm 0.2-2.4). The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

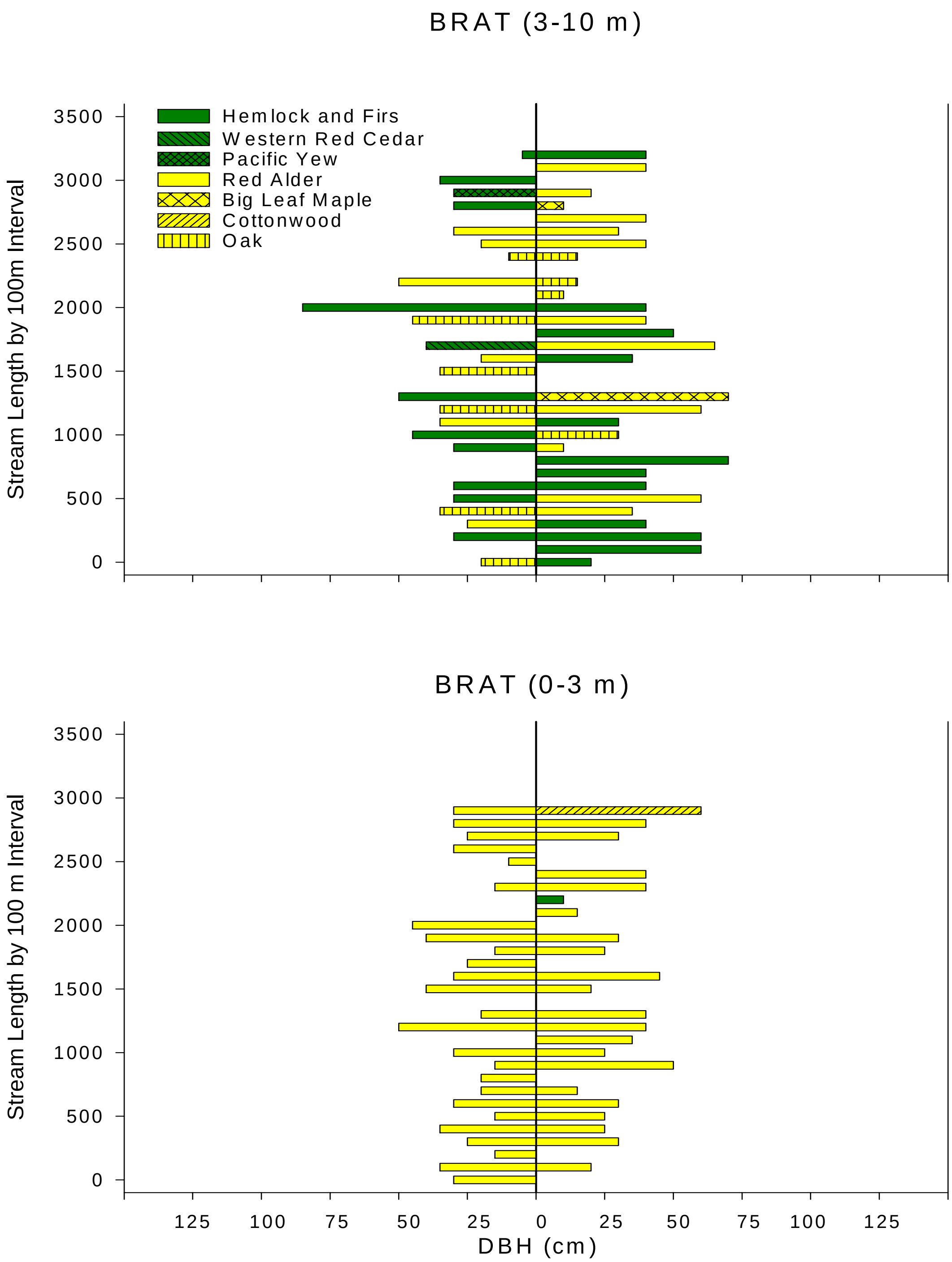


Figure 6b. Characterization of outer (3-10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in the BRAT reach of Rattlesnake Creek (rkm 2.4-5.5). The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

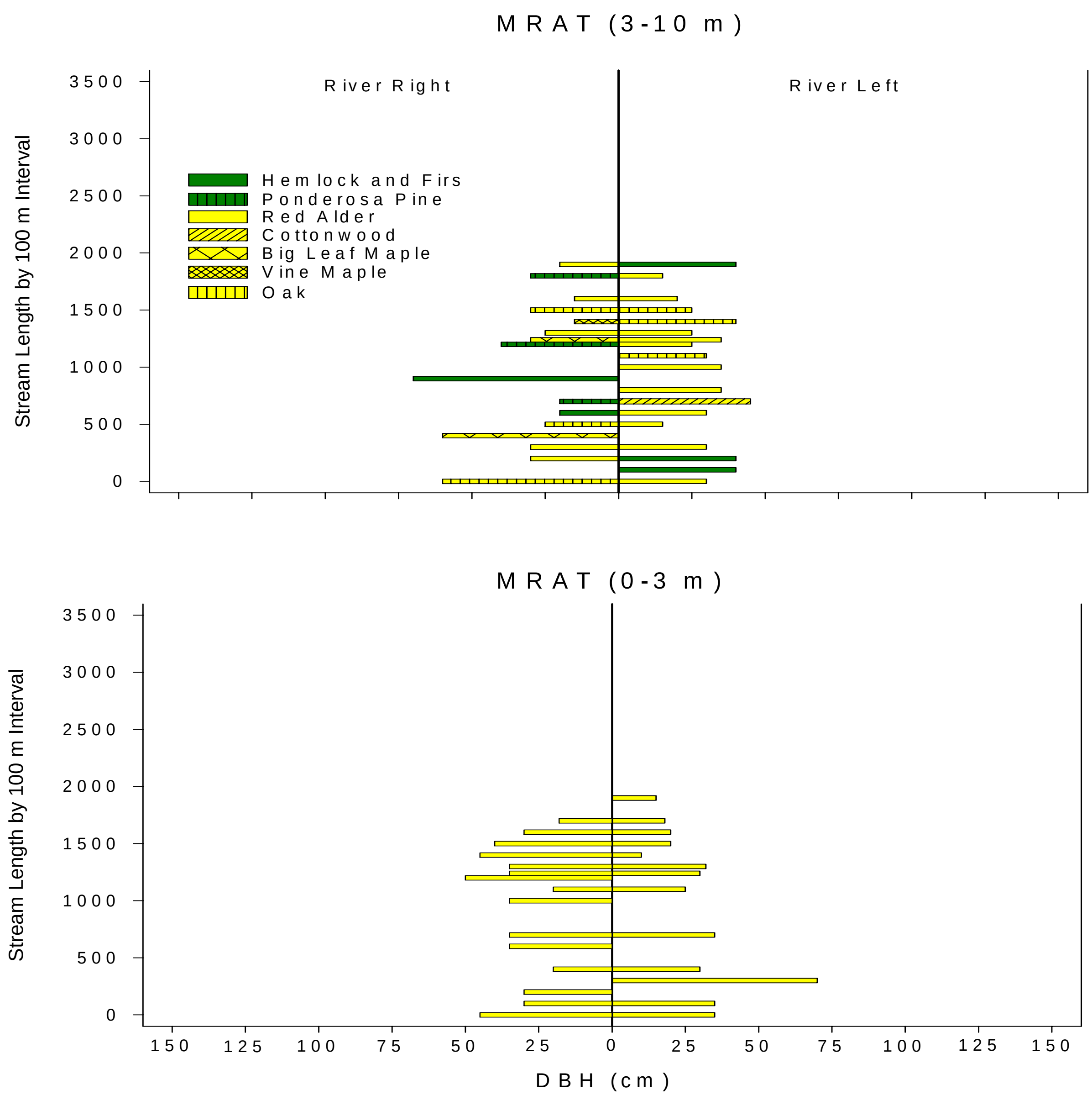


Figure 6c. Characterization of outer (3-10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in middle Rattlesnake Creek (MRAT; rkm 6.0-7.8). The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

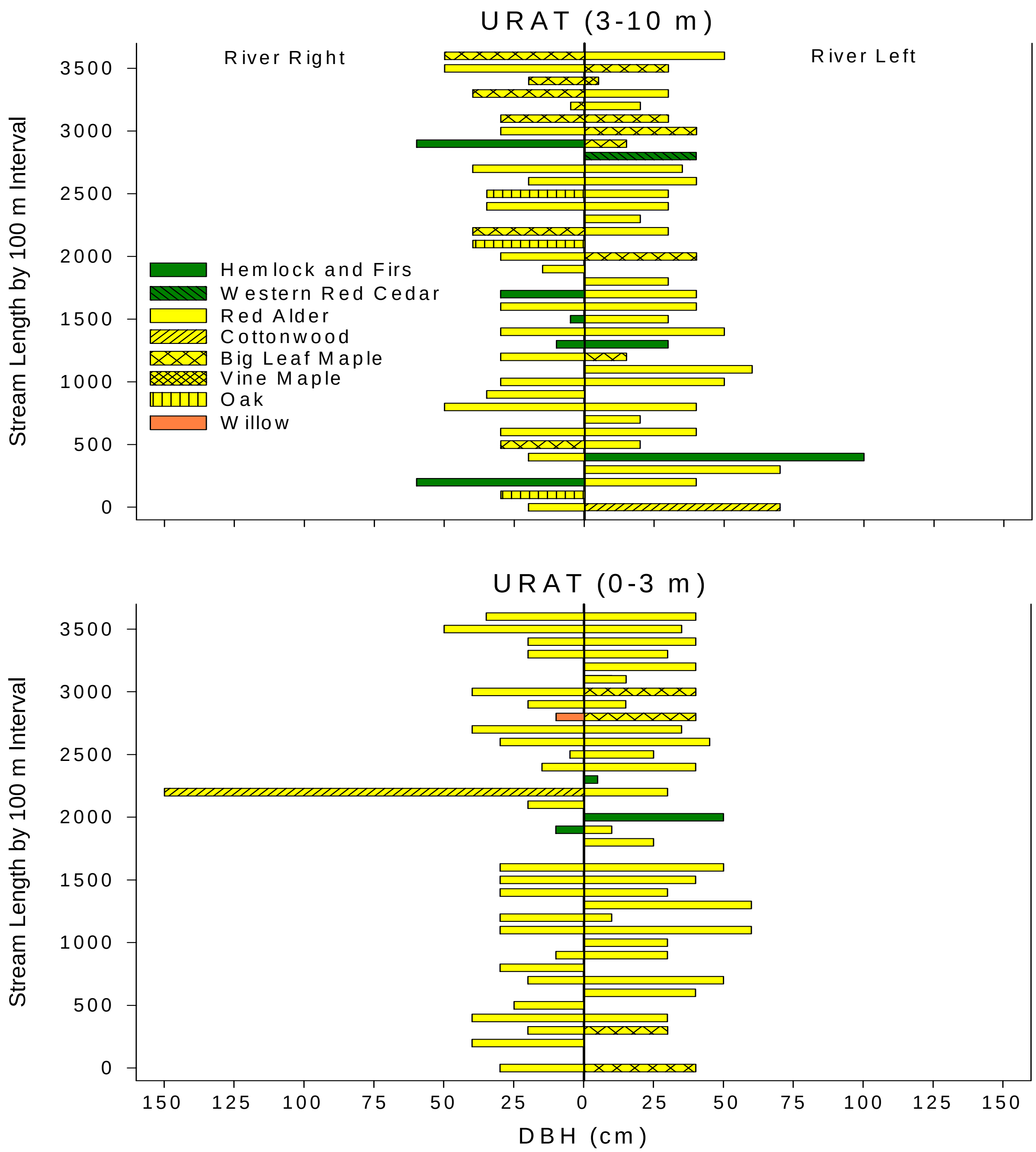


Figure 6d. Characterization of outer (3–10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in upper Rattlesnake Creek (URAT; rkm 10.8-14.4). The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

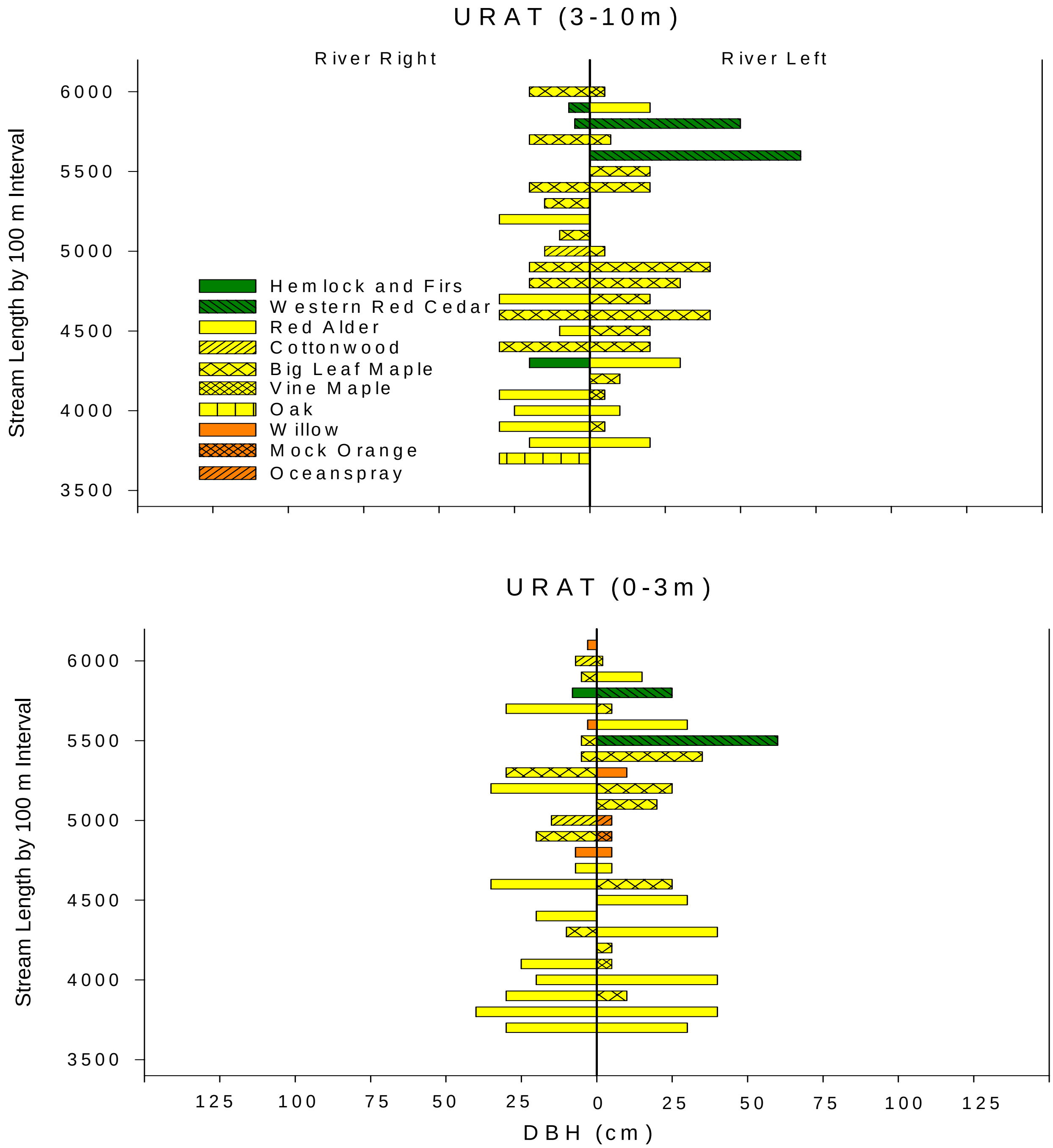


Figure 6e. Characterization of outer (3–10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in upper Rattlesnake Creek (URAT; rkm 14.4-17.2). The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

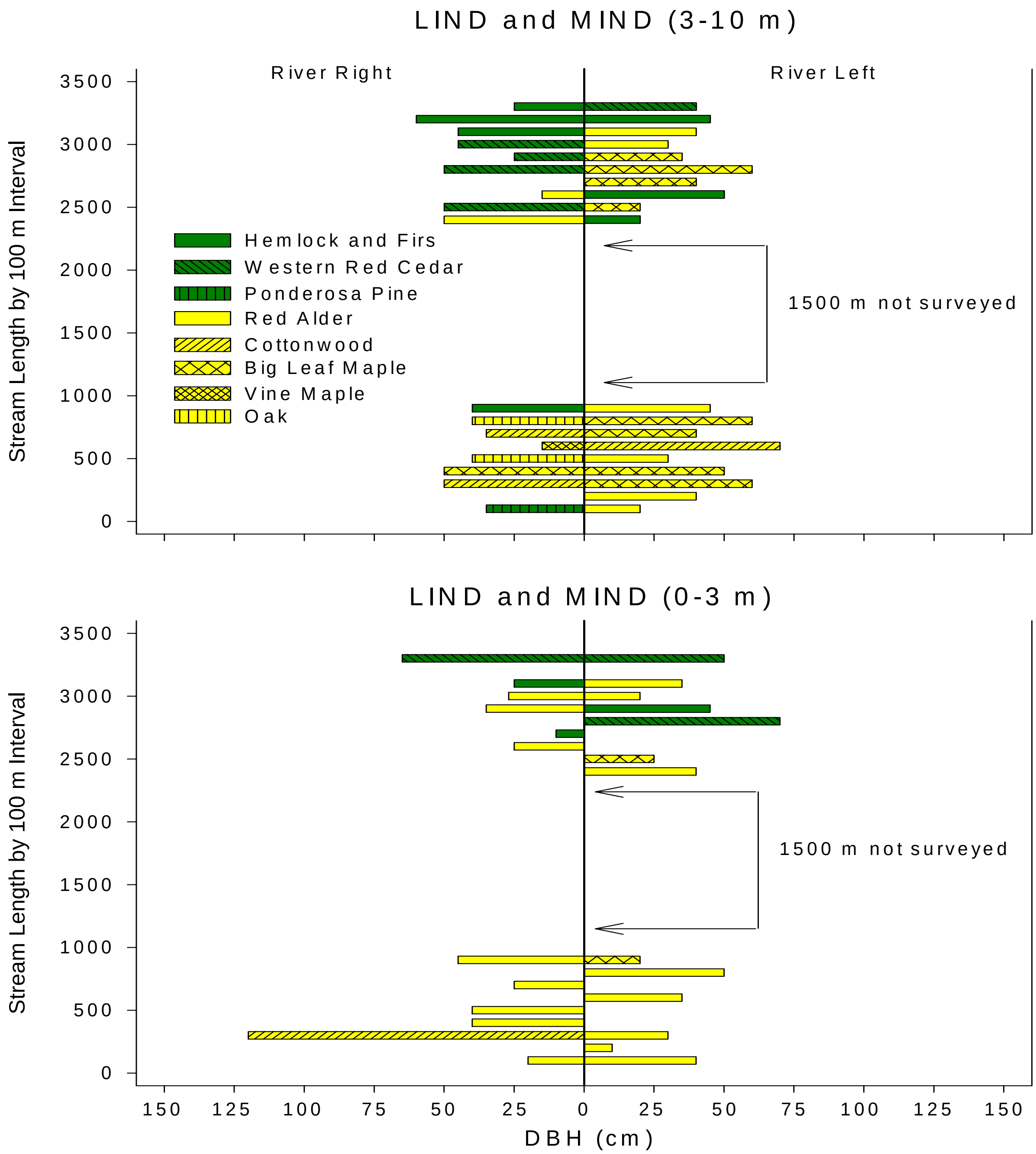


Figure 6f. Characterization of outer (3–10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in upper Rattlesnake Creek (LMIL; rkm 0-1.0). The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

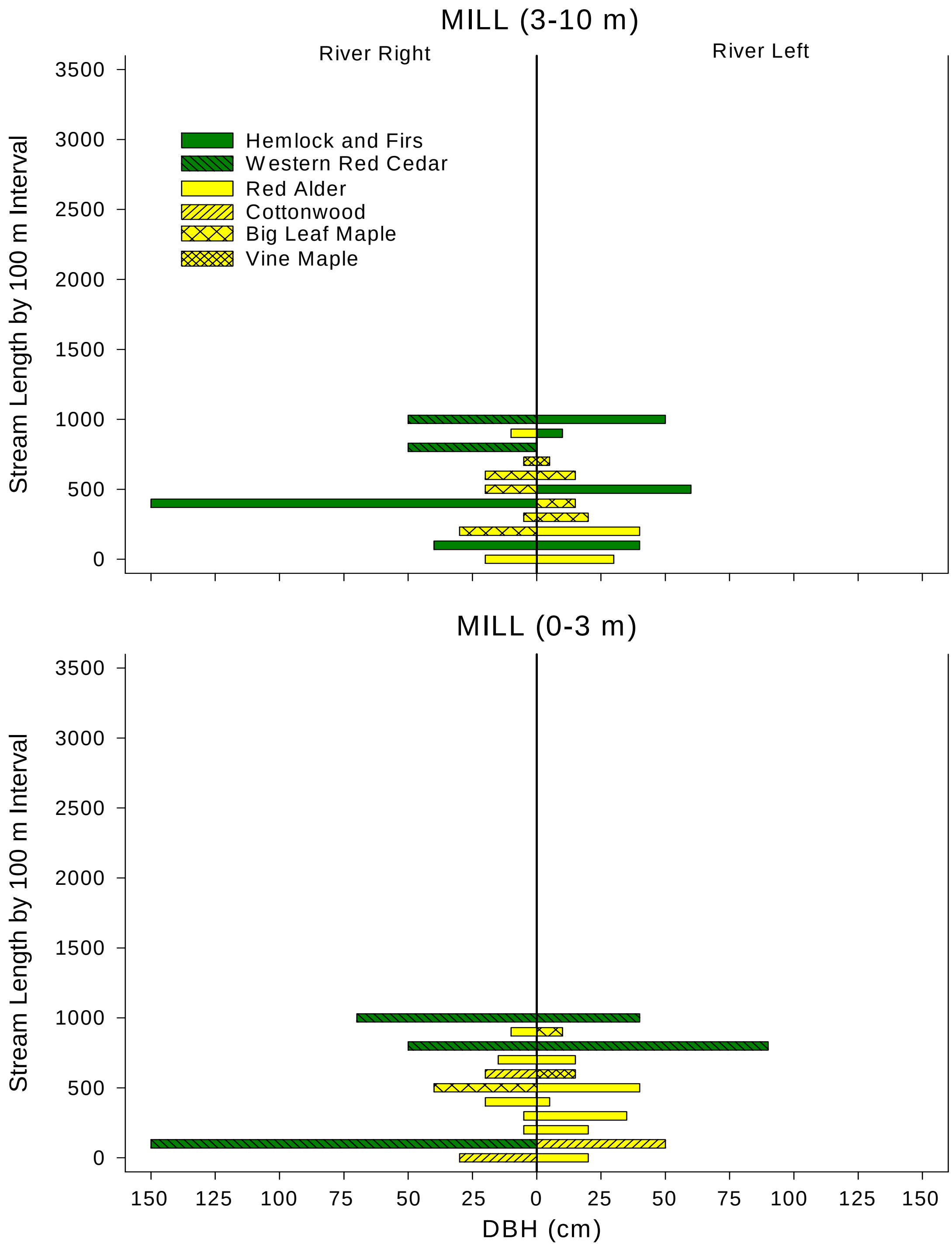


Figure 6g. Characterization of outer (3-10 m from bankfull) and adjacent (0-3 m from bankfull) riparian vegetation in lower Mill Creek (LMIL; rkm 0-1.0), a tributary to Rattlesnake Creek. The diameter at breast height (DBH) of the dominant tree type within a 10-m section at each 100-m transect is shown. Blanks indicate the lack of canopy-height trees (approx. >3 m tall) within the 10-m section.

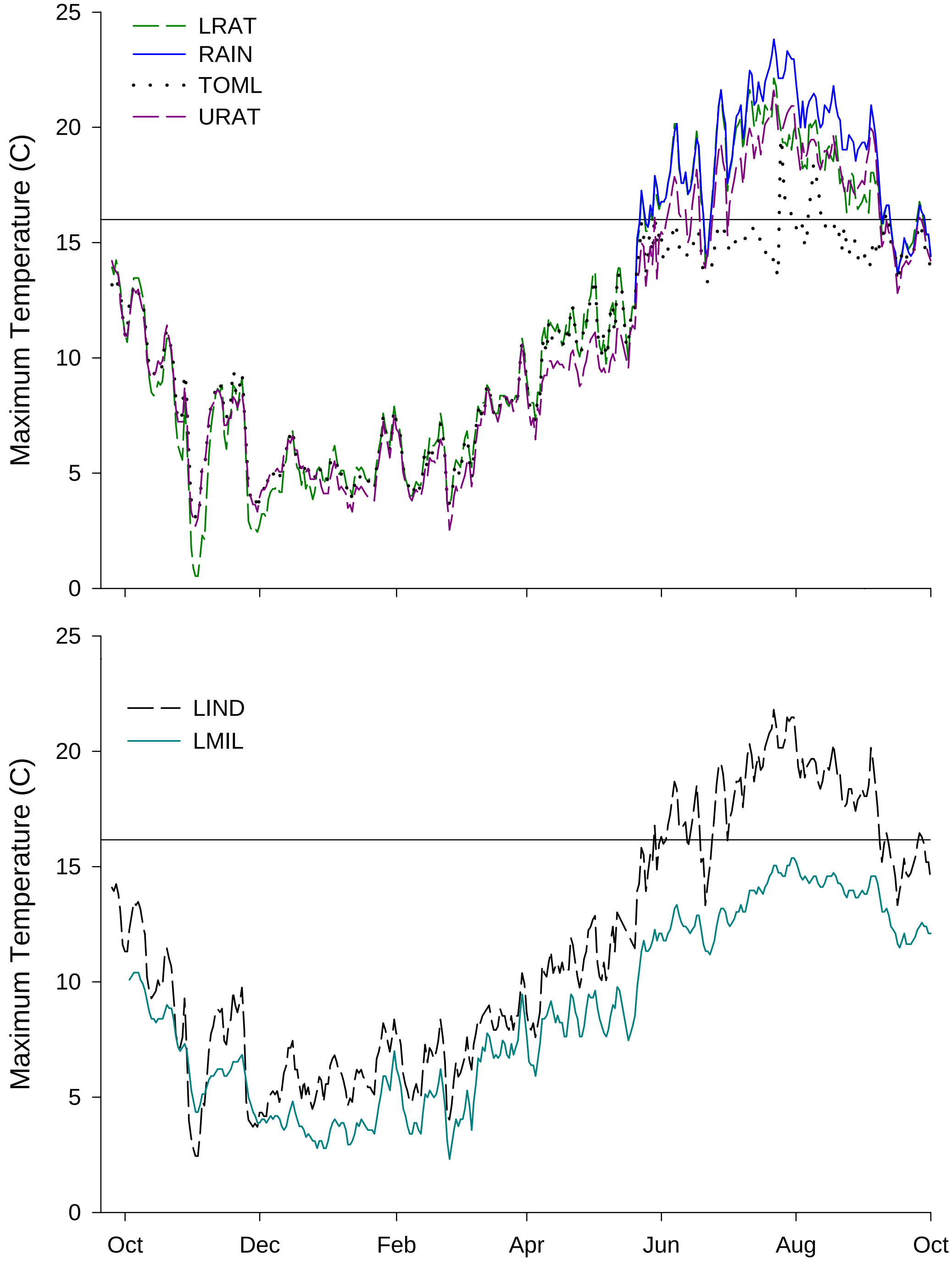


Figure 7. Daily maximum temperatures at six sites in Rattlesnake Creek from September 24, 2002 to October 1, 2003. Thermograph sites are mapped on Figure 2, and coordinates and elevation are provided in Table 5. The line at 16° C marks the maximum surface water temperature standard set by the Washington Department of Ecology (Chapter 173-201A, Nov. 18, 1997, Water Quality Standards for the Surface Waters of the State of Washington). Data from RAIN thermograph missing up to 5/20/2003.

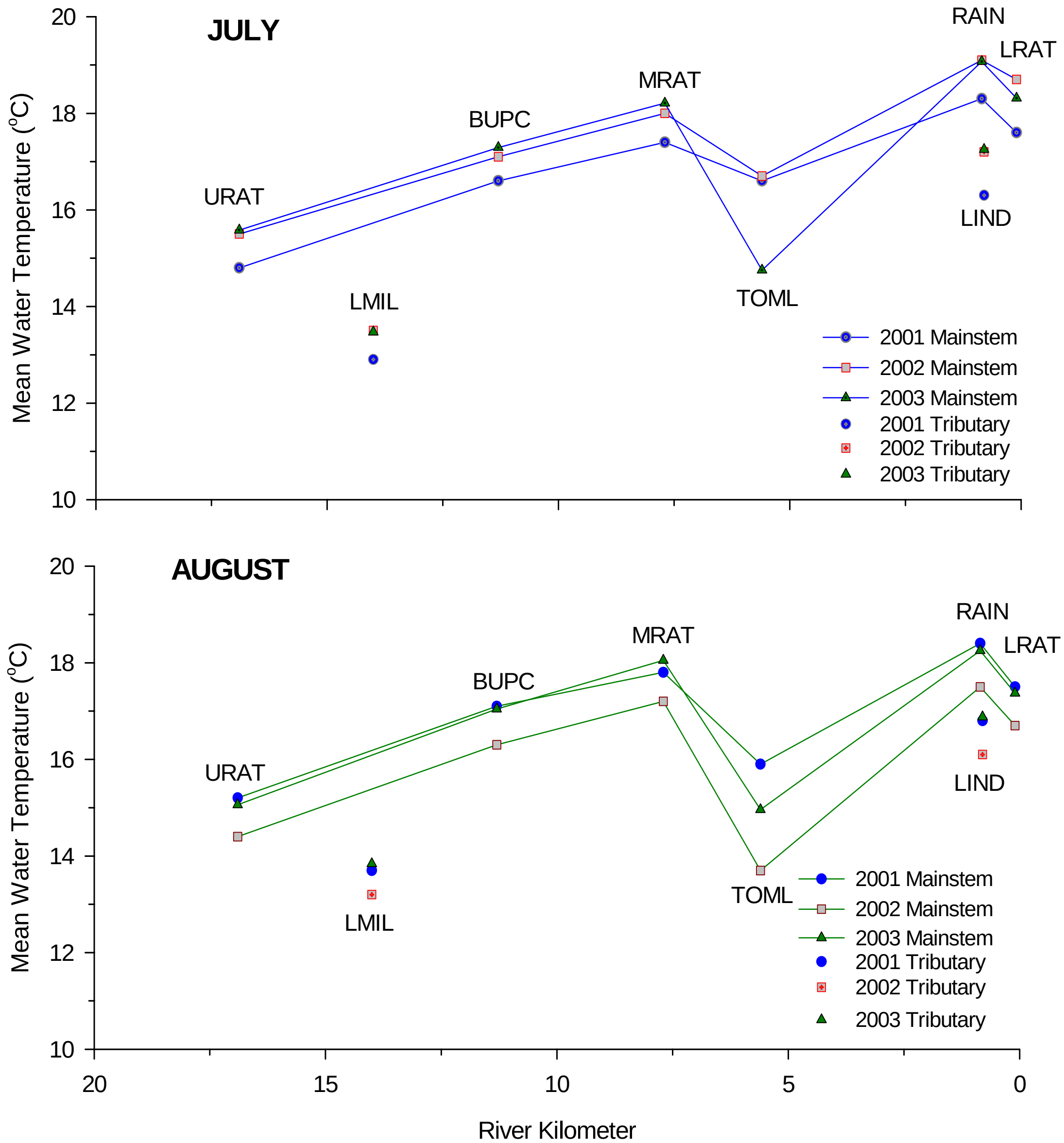


Figure 8. Mean water temperature during July and August 2001 through 2003 in the mainstem Rattlesnake Creek and its tributaries. Sites, from left to right, are shown from upstream to downstream. River kilometer zero is the mouth of Rattlesnake Creek. Refer to Figure 2 for a map of thermograph locations, and refer to Table 5 for additional information on thermograph coordinates, elevations and start and end dates.

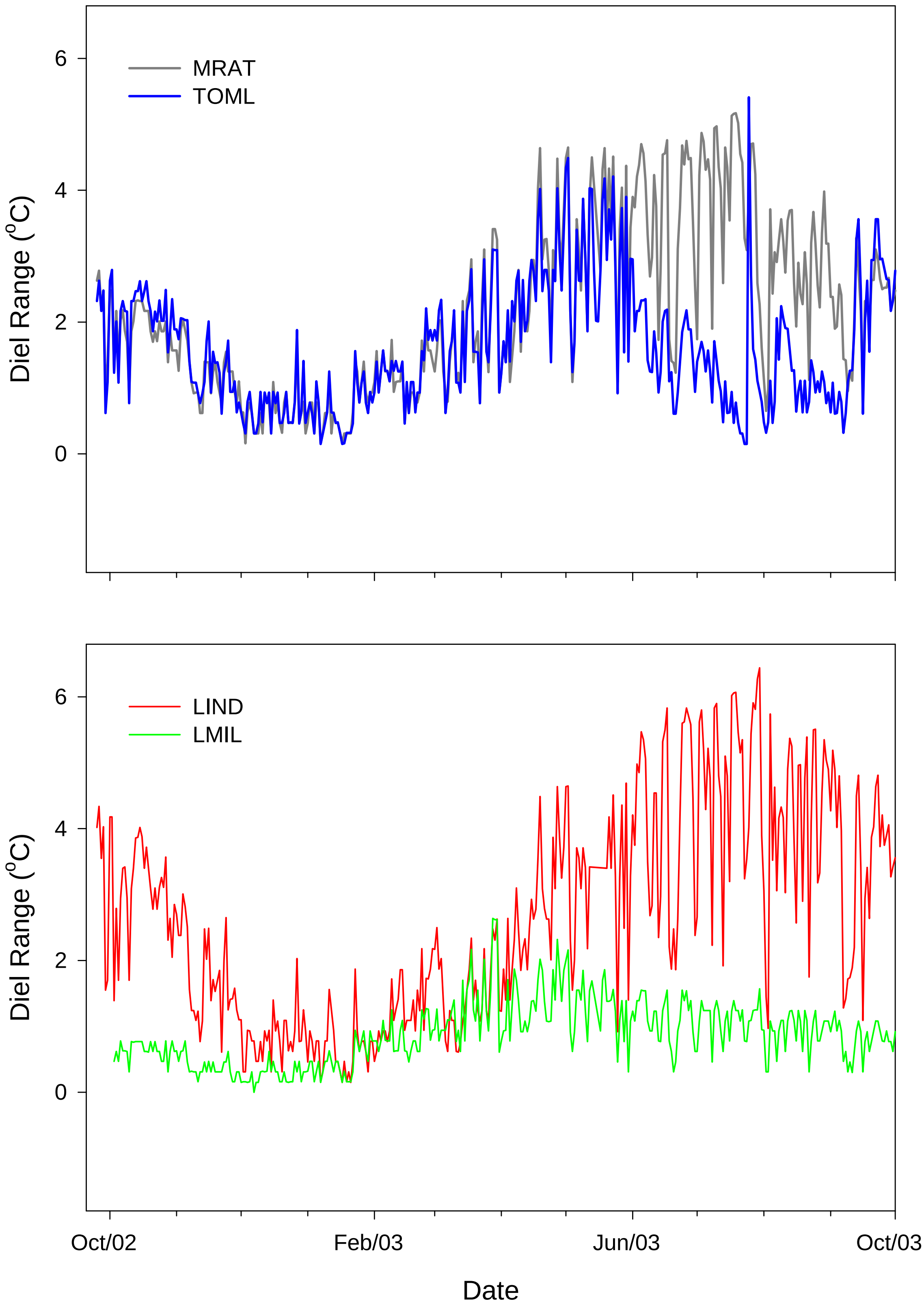


Figure 9. Diel fluctuation of water temperatures at select sites in Rattlesnake (MRAT, TOML), Indian (LIND), and Mill (LMIL) creeks. See Figure 2 for a map of thermograph locations. For additional information on thermograph coordinates, elevations, and start and end dates, see Table 5. Spike in TOML is July 25, 2003 when we were electrofishing for population estimates.

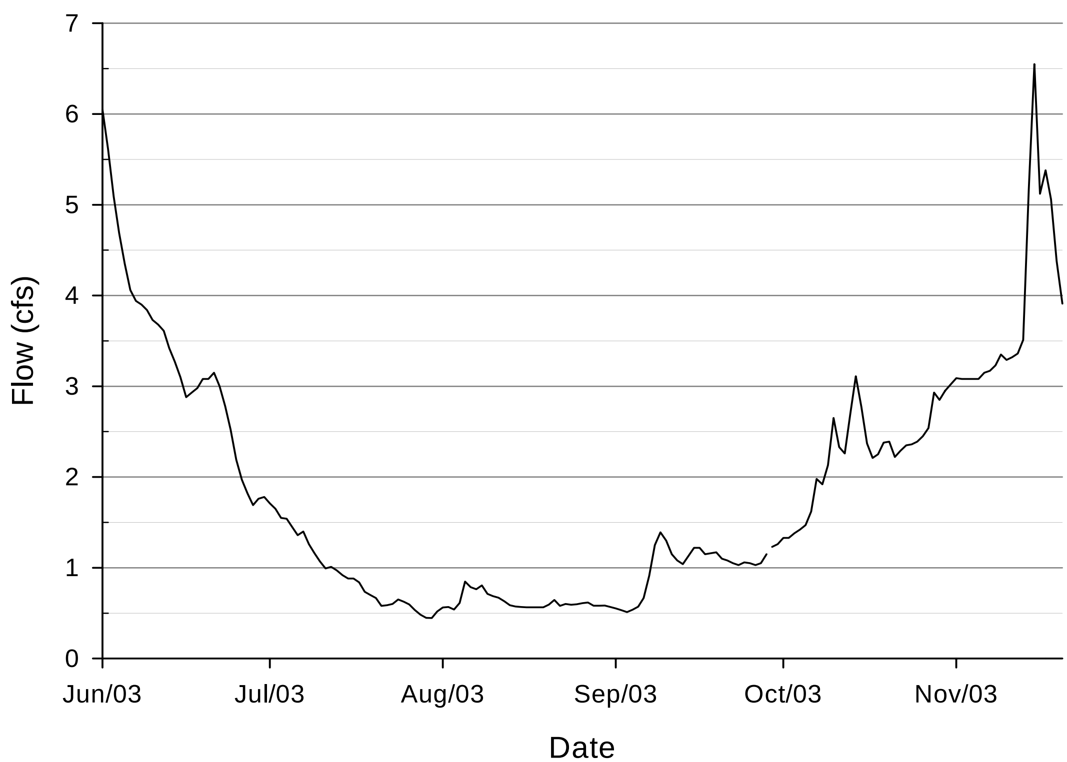


Figure 10. Mean daily flow measured in 15 minute intervals at the LRAT automated gaging station (rkm 0.2) from March 03- March 04 (top graph) and June – November, 2003 (bottom graph). Note log scale on top graph. For information on flow measurement locations, see Table 8 and Figure 3.

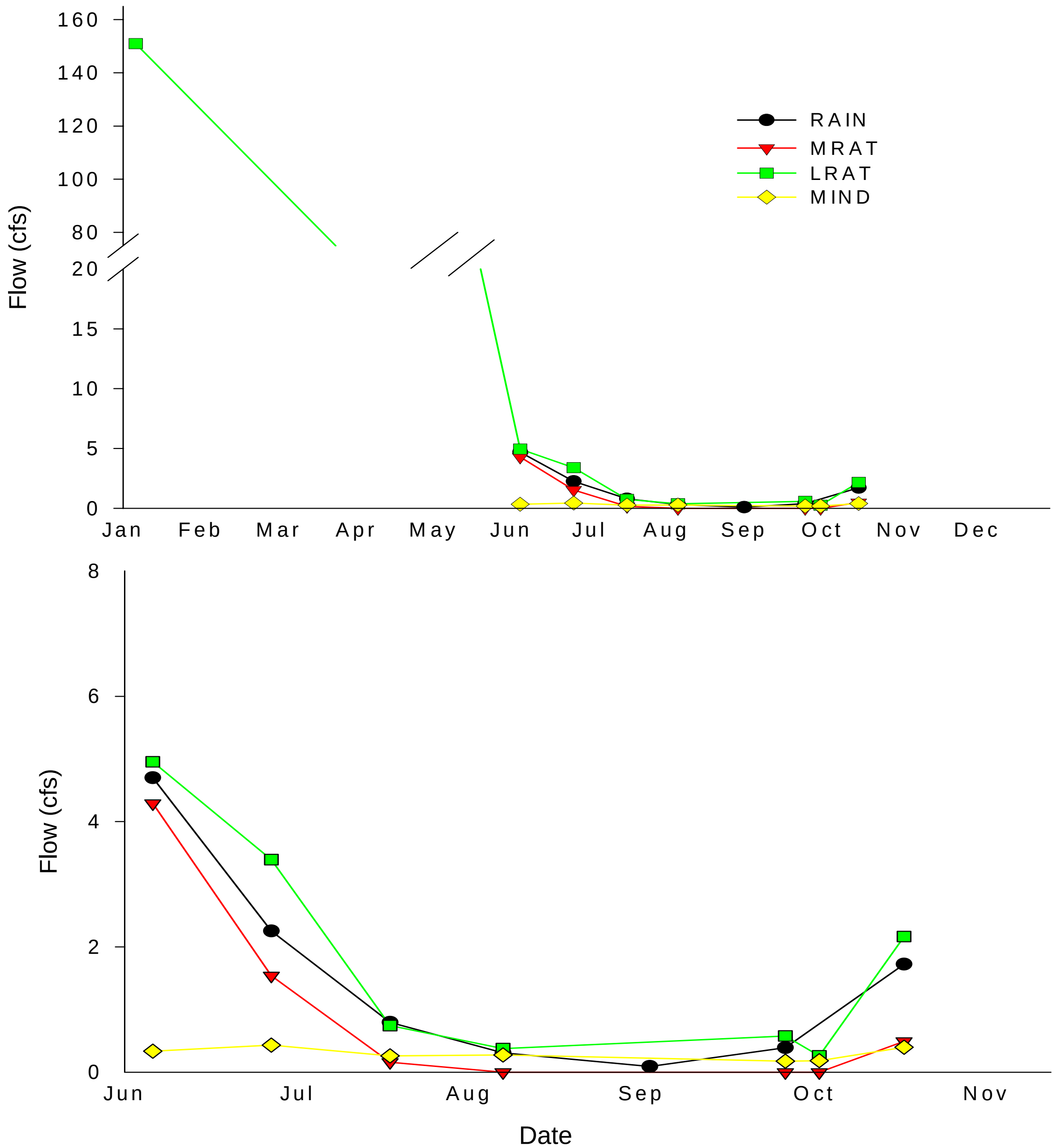


Figure 11. Flow measured at three sites on Rattlesnake Creek (LRAT, rkm 0.2; RAIN, rkm 0.8; MRAT, rkm 7.7) and one site on Indian Creek (MIND, rkm 2.2). The top graph shows flow measurements collected from January - December 2003. The bottom graph shows low flows from June – November 2003. For information on flow measurement locations, see Table 8 and Figure 3. See Appendix Table 1 for flow measurements and dates.

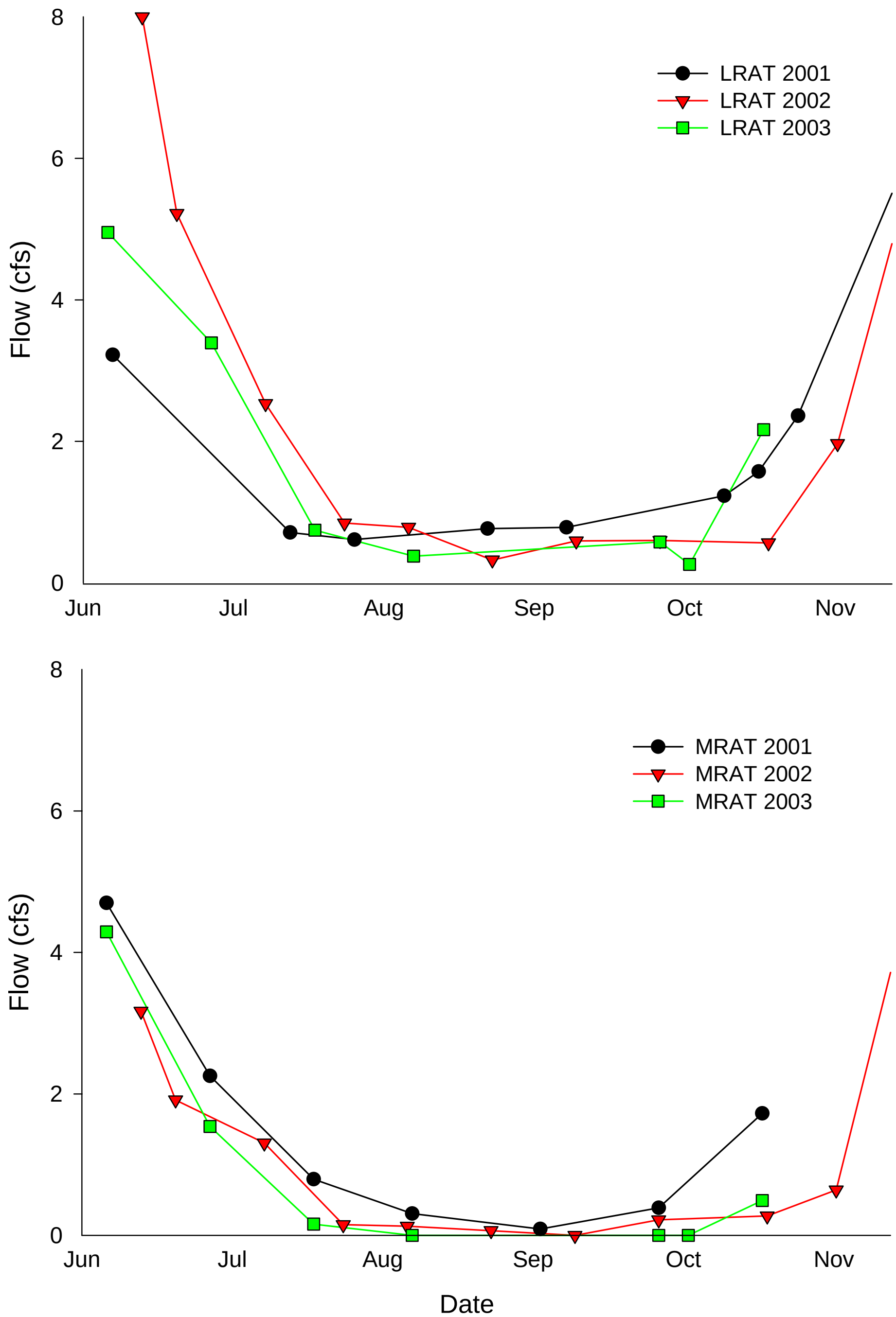


Figure 12. Flow for LRAT (rkm 0.2) and MRAT (rkm 7.7) from June – November, 2001-2003. For information on flow measurement locations, see Table 8 and Figure 3.

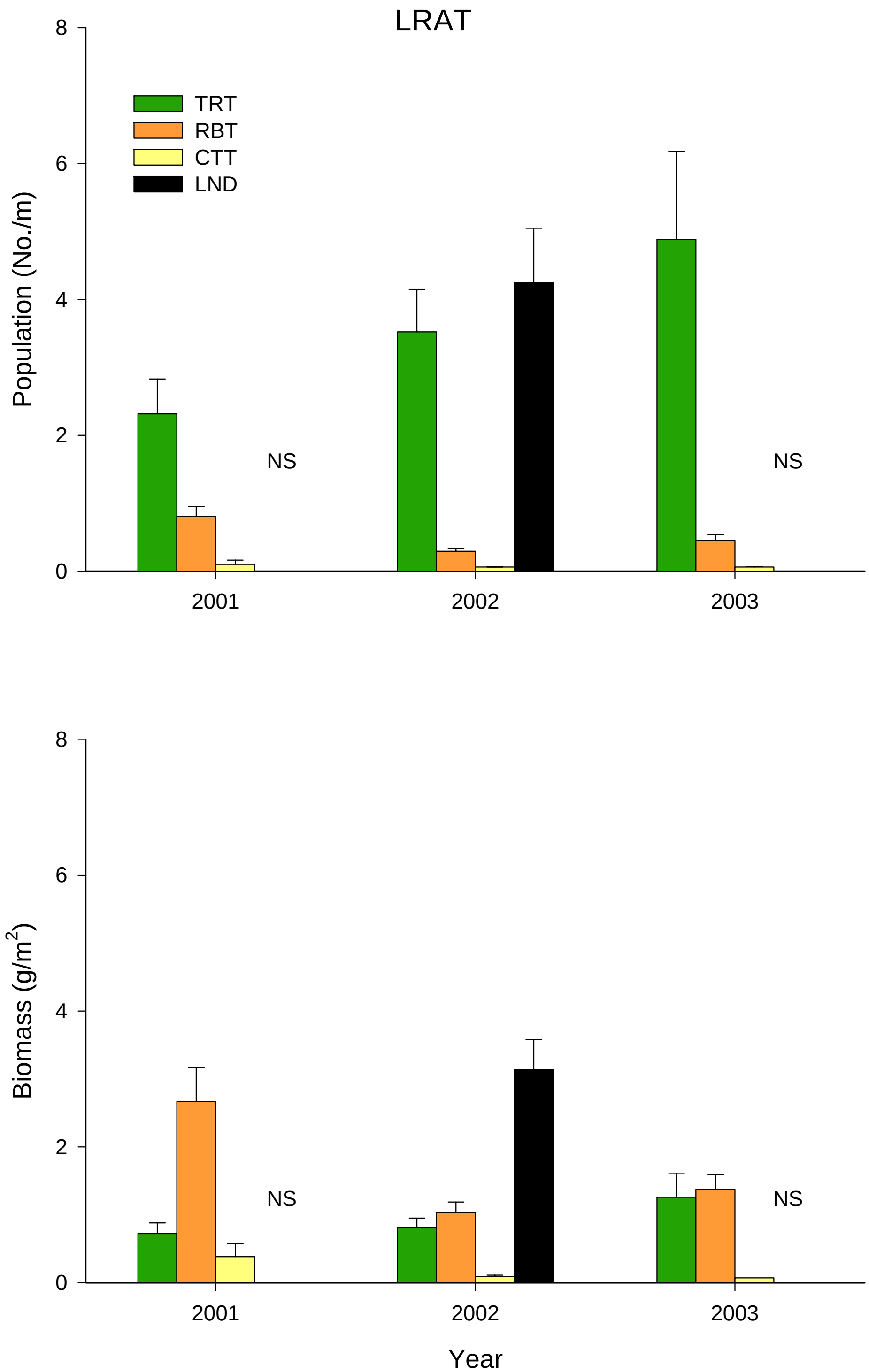


Figure 13. Comparison of fish population and biomass estimates in lower Rattlesnake creek (LRAT; rkm 0.2-1.3). Salmonids <80 mm long were lumped as trout (TRT). Rainbow trout (RBT) and cutthroat trout (CTT) were collected in 2001, 2002, and 2003. Longnose dace (LND) were not sampled (NS) in 2001 or 2003, but were in 2002. Error bars represent 2 SE, which is approximately a 95 % confidence interval.

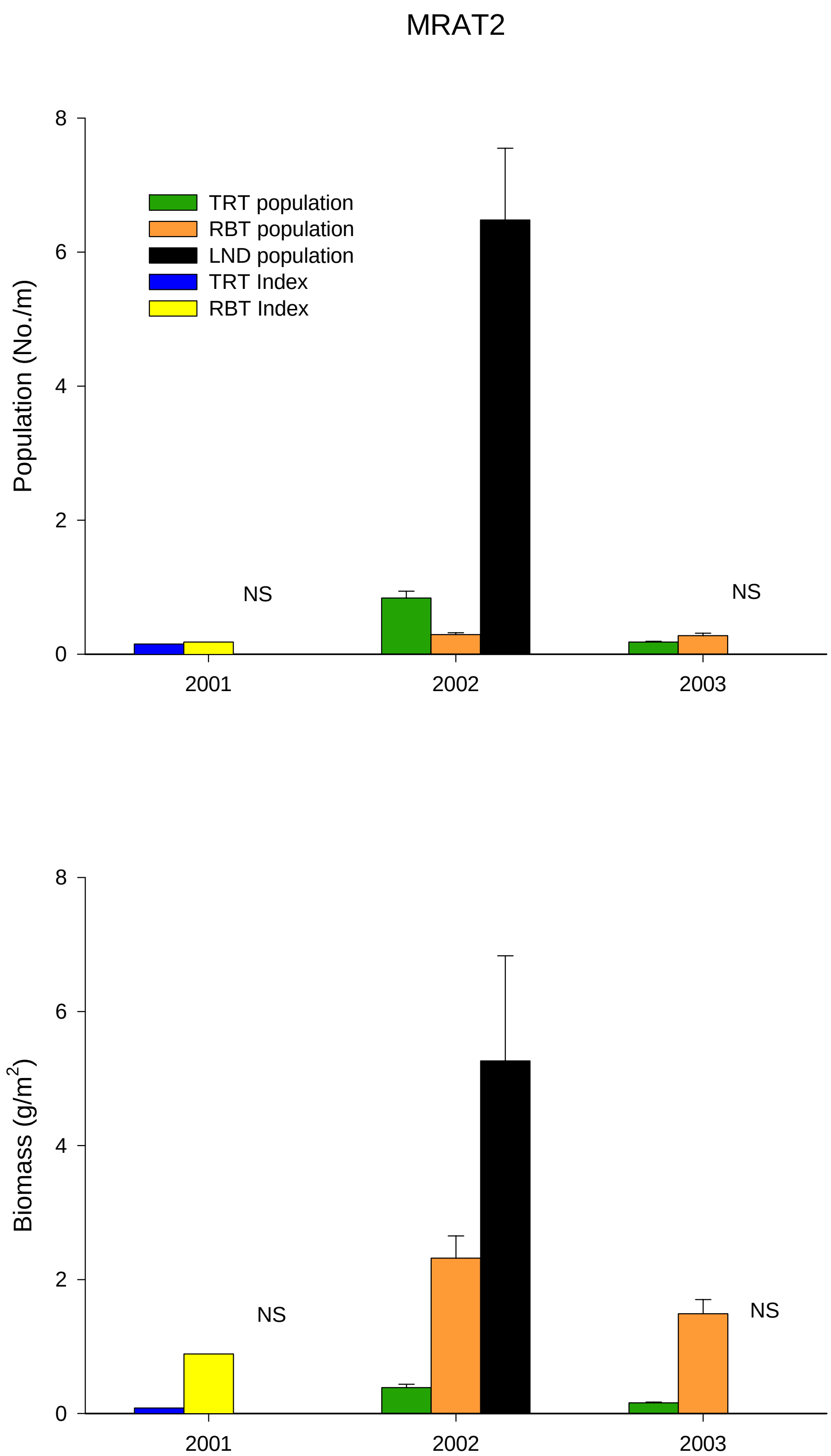


Figure 14. Comparison of fish population and biomass estimates in middle Rattlesnake creek (MRAT2; rkm 7.35-7.91). Salmonids <80 mm long were lumped as trout (TRT). Rainbow trout (RBT) were collected in 2001, 2002, and 2003. Longnose dace (LND) were not sampled (NS) in 2001 or 2003, but were in 2002. Error bars represent 2 SE, which is approximately a 95 % confidence interval.

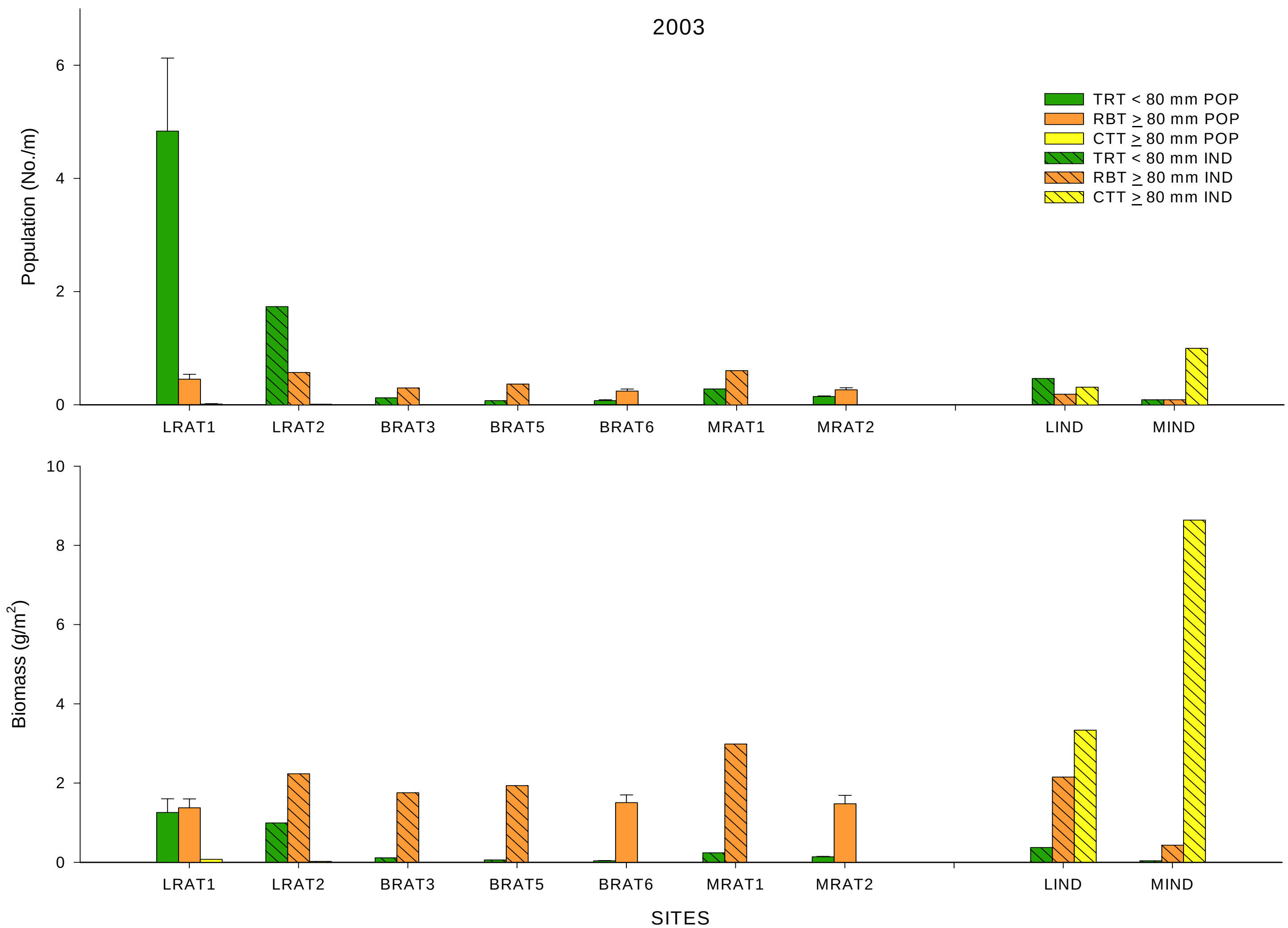


Figure 15. Population (POP) and Index (IND) electrofishing population and biomass estimates (with 1 SE bars for population estimates) of rainbow trout (RBT), and cutthroat trout (CTT) found in Rattlesnake Creek watershed, 2003. Stream codes are: LRAT1 = section 1 of lower Rattlesnake Cr.(rkm 0.2-1.3), LRAT2 = Rattlesnake Cr. below 1st waterfall (rkm 2.0-2.4), BRAT3 = Rattlesnake Cr. 1000 m above falls (rkm 3.3-3.8), BRAT5 = Rattlesnake Cr. 2000 m above falls (rkm 4.3-4.8), BRAT6 = Rattlesnake Cr. rkm 4.8-6.1, MRAT1 = Rattlesnake Cr. rkm 5.6-7.2, MRAT2 = Rattlesnake Cr. rkm 7.2-7.8, LIND = Indian Cr. 100 m above mouth, MIND = Indian Cr. rkm 2.4-3.3. Additional information on stream code locations are provided on Figure 3 and Table 9.

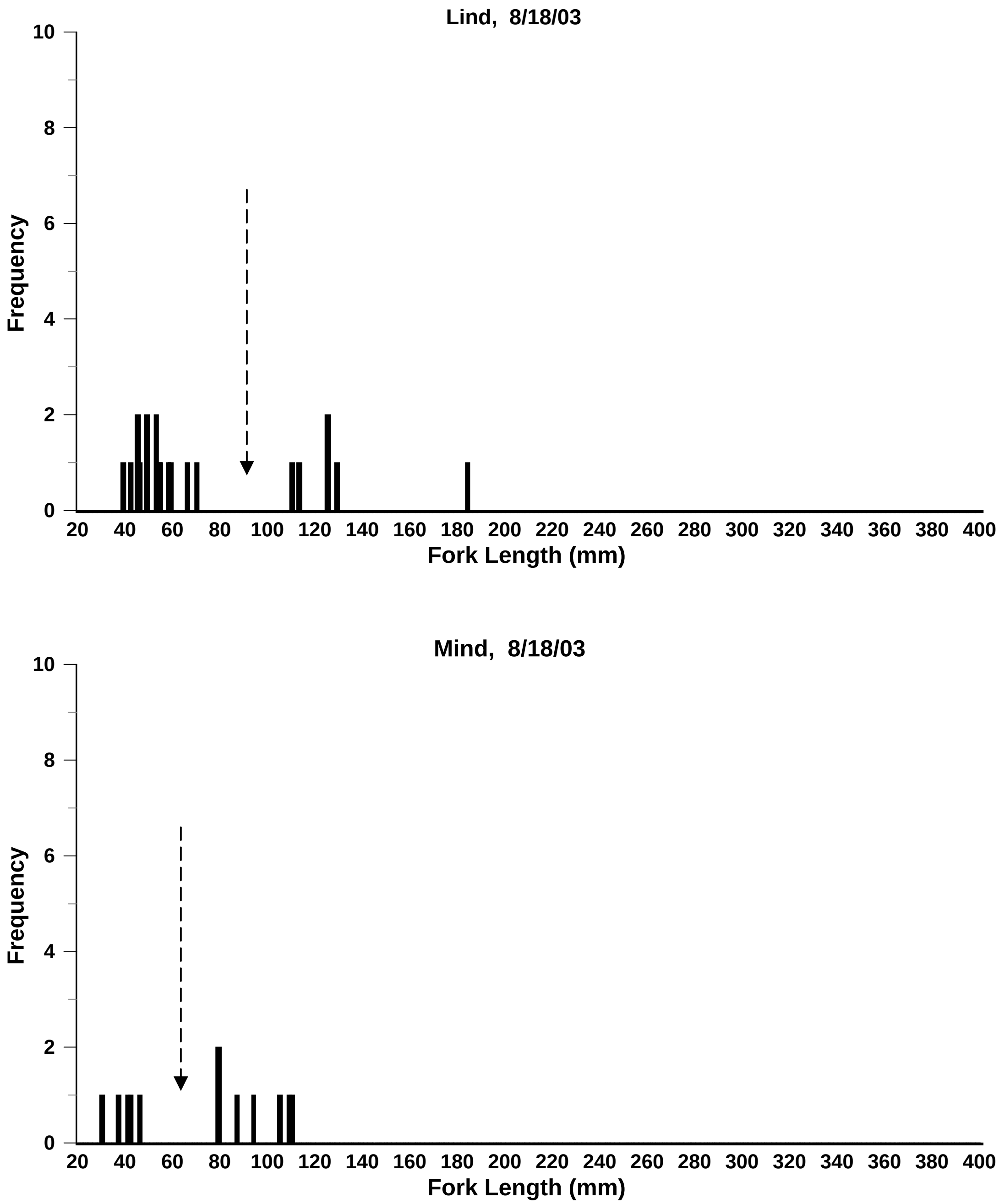
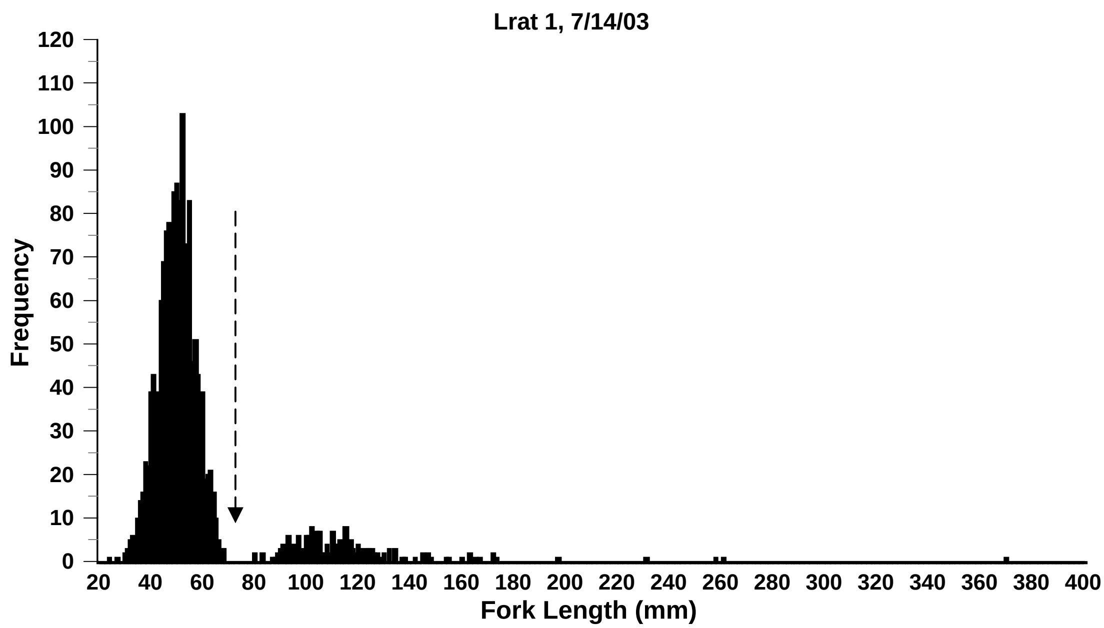


Figure 16a. Length frequency in 1-mm increments of rainbow trout sampled in LIND (rkm 0.0-0.9) and MIND (rkm 2.40-3.28) reaches of Indian Creek of the Rattlesnake watershed in 2003. The arrow indicates the break between age-0 and age-1 or older fish.



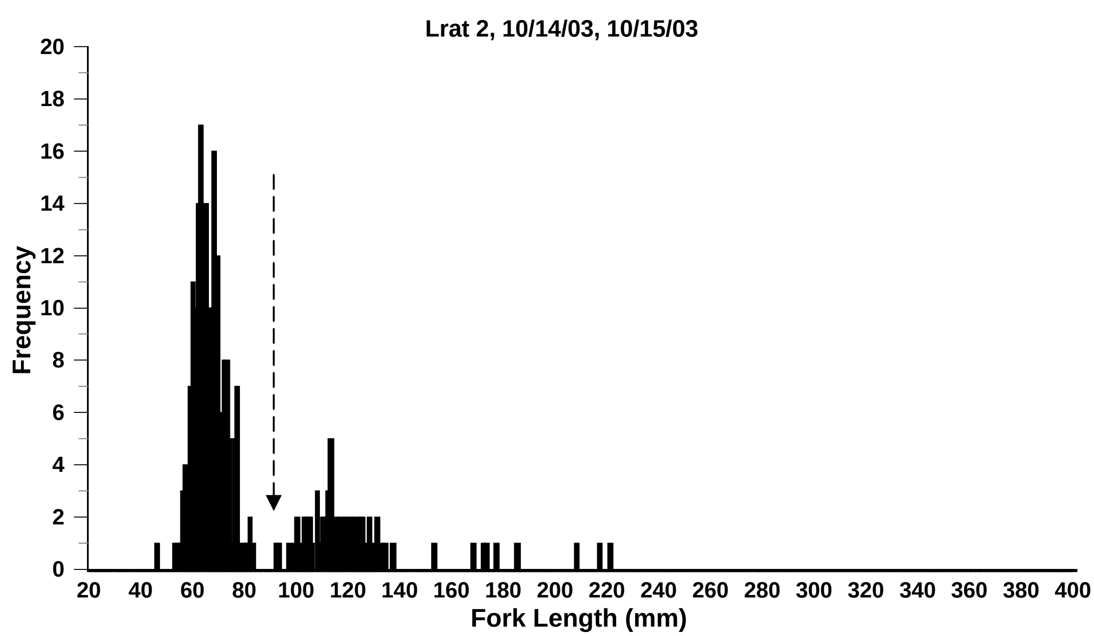
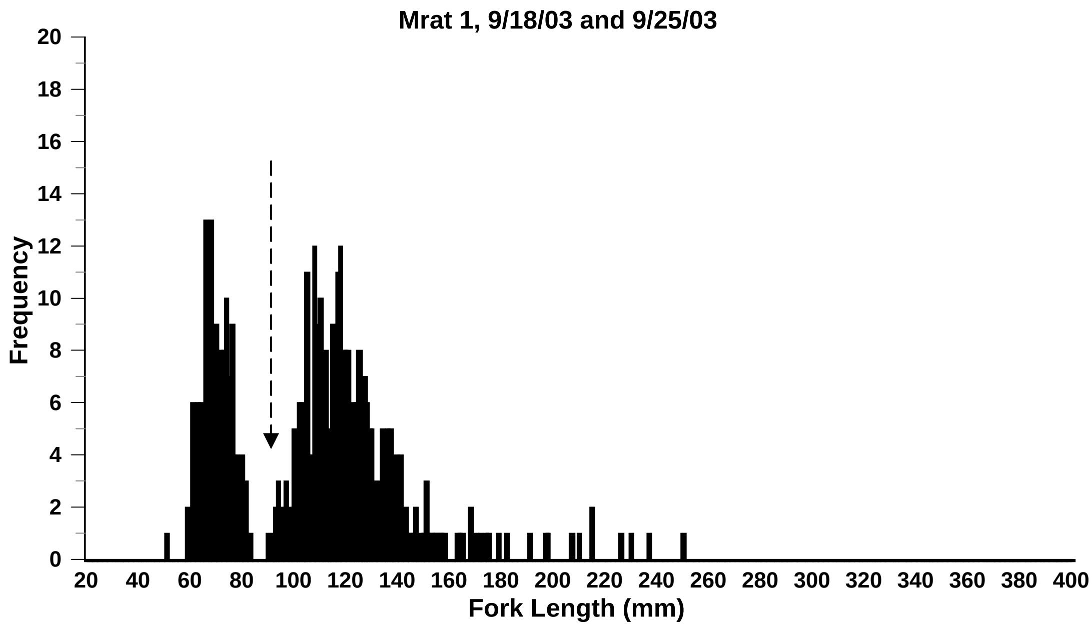


Figure 16b. Length frequency in 1-mm increments of rainbow trout sampled in section 1 (rkm 0.2-1.3) and section 2 (rkm 1.9-2.4) of the LRAT reach of Rattlesnake Creek in 2003. The arrow indicates the break between age-0 and age-1 or older fish.



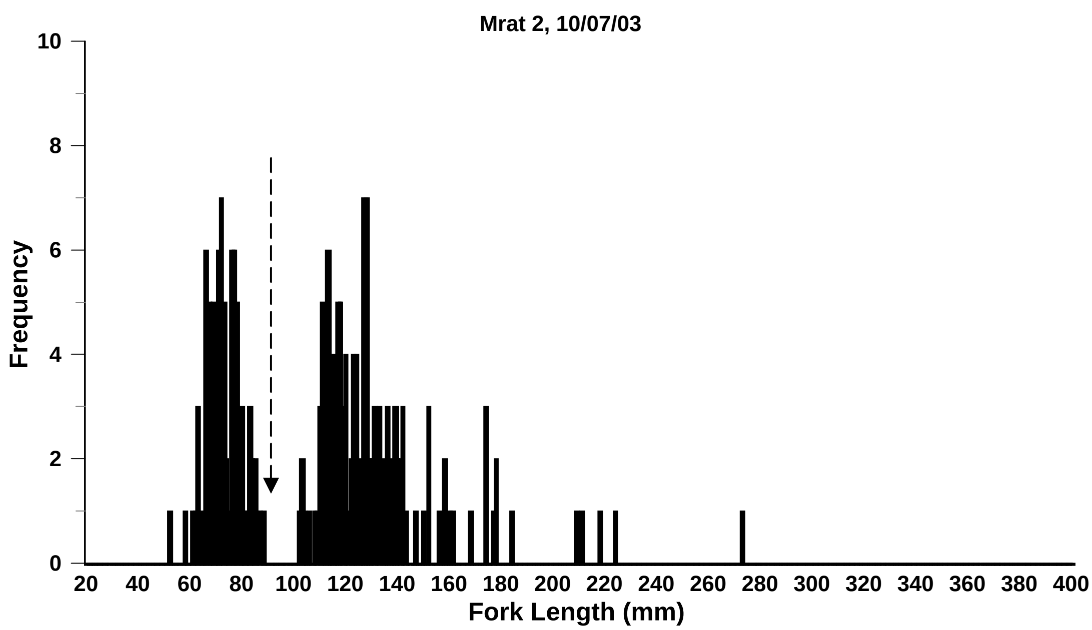
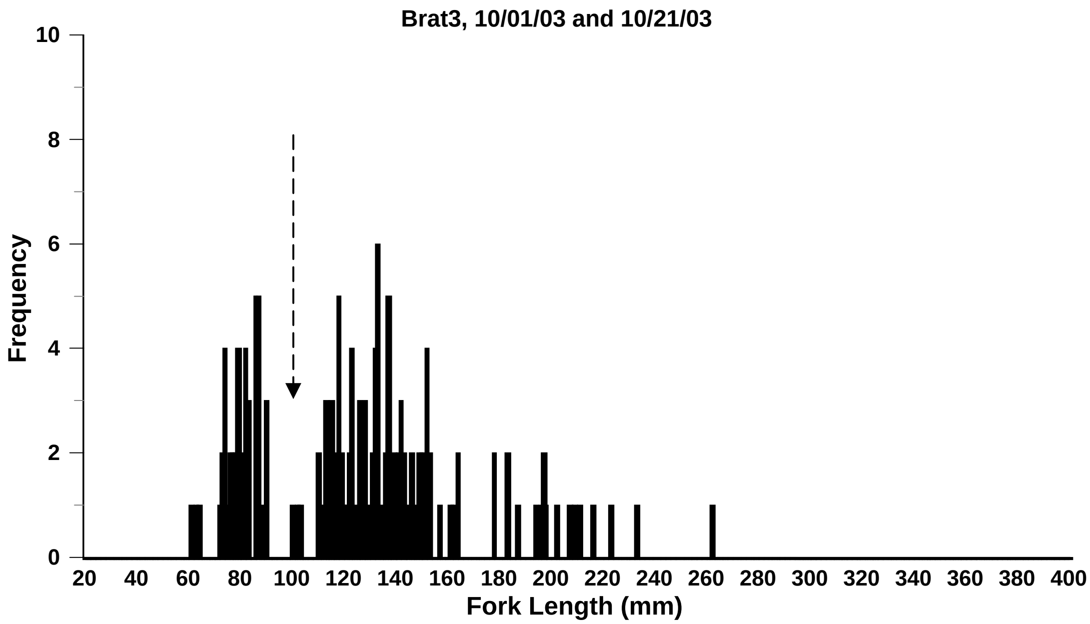


Figure 16c. Length frequency in 1-mm increments of rainbow trout sampled in section 1 (rkm 6.0-7.2) and section 2 (rkm 7.2-7.8) of the MRAT reach of Rattlesnake Creek in 2003. The arrow indicates the break between age-0 and age-1 or older fish.



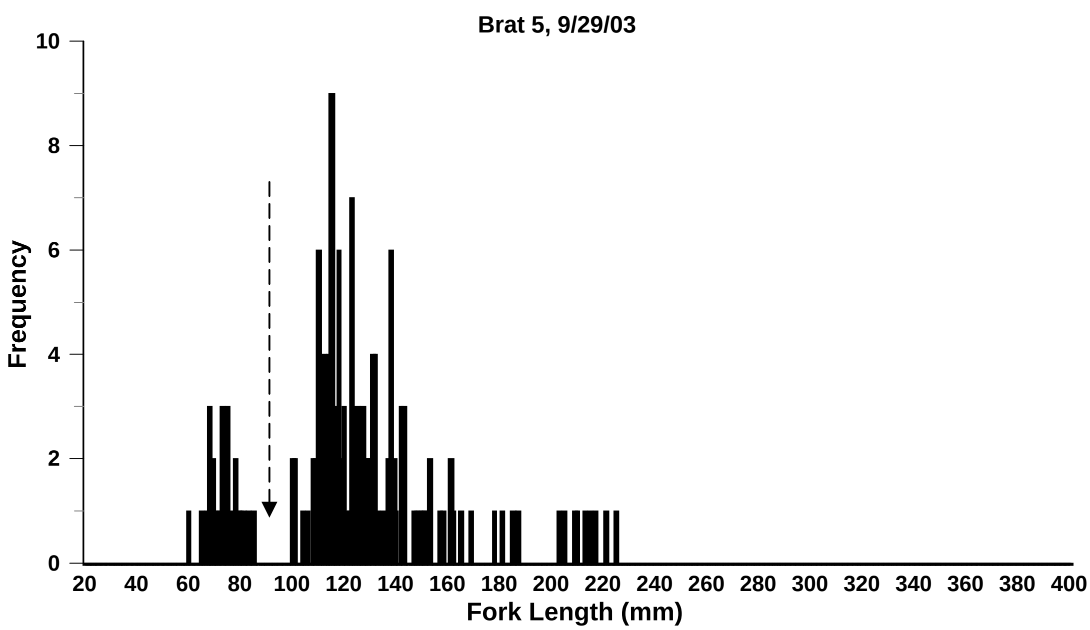


Figure 16d. Length frequency in 1-mm increments of rainbow trout sampled in section 3 (rkm 3.3-3.8) and section 5 (rkm 4.3-4.8) of the BRAT reach of Rattlesnake Creek in 2003. The arrow indicates the break between age-0 and age-1 or older fish.

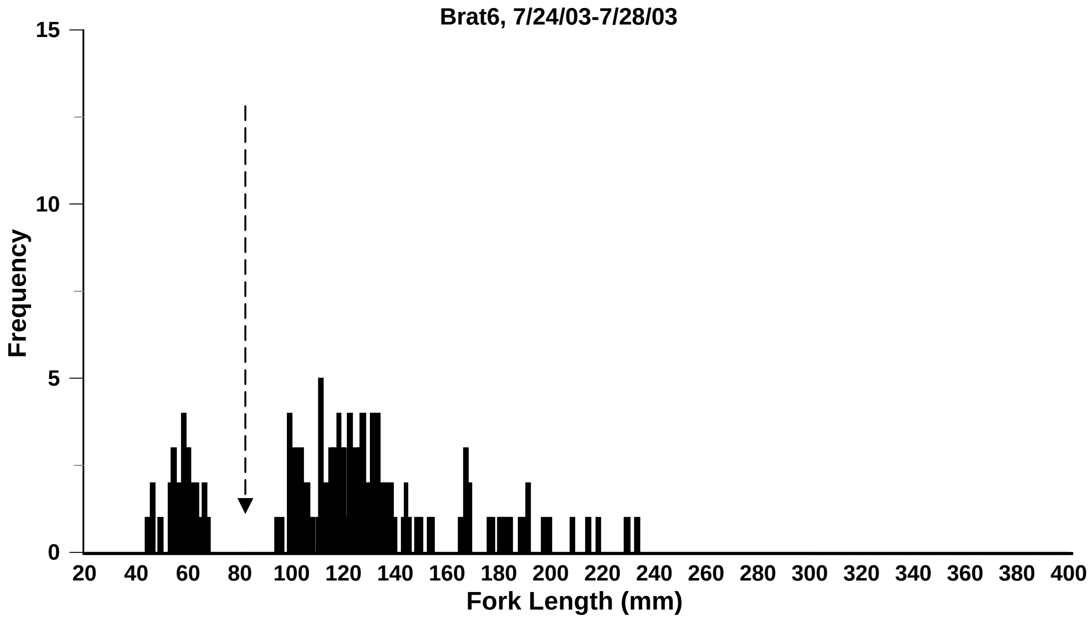


Figure 16e. Length frequency in 1-mm increments of rainbow trout sampled in section 6 (rkm 4.81-5.51) of the BRAT reach of Rattlesnake Creek in 2003. The arrow indicates the break between age-0 and age-1 or older fish

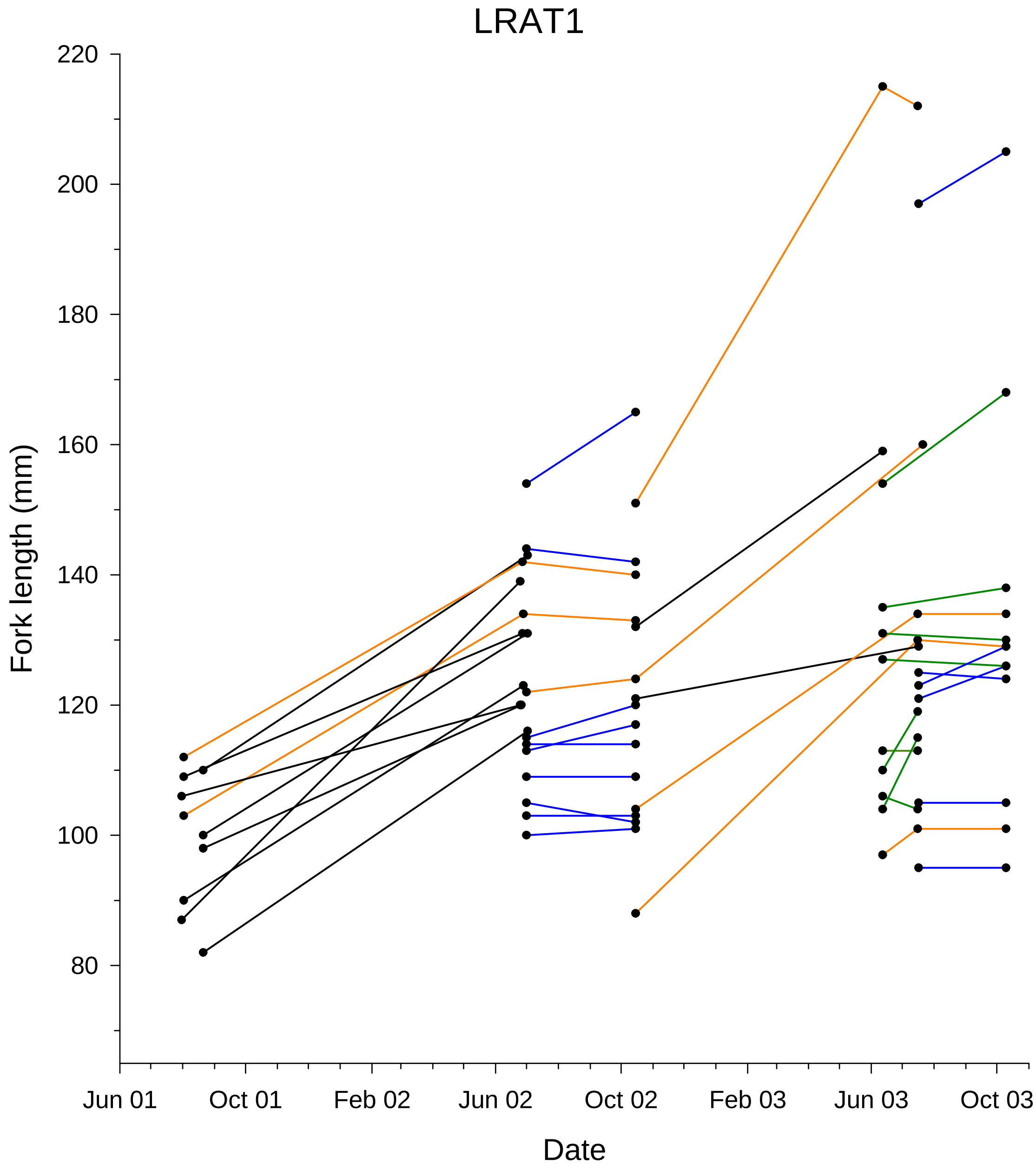


Figure 17. Change in the fork length of PIT-tagged rainbow trout over time within lower Rattlesnake Creek (LRAT1; rkm 0.2-1.3). Lines between the fork length at initial tagging and at each time of recapture connect individual fish.

Appendixes

Appendix A

Appendix B

Appendix C

Appendix A

Appendix Table A.1. Actual flow measures for dates and sites listed below. Sites are listed in an upstream to downstream order. See Table 8 and Figure 3 for additional information on flow site locations.

Watershed				**Date**					
Site	01/06/03	06/06/03	06/27/03	07/18/03	08/07/03	09/02/03	09/26/03	10/02/03	10/17/03
Rattlesnake Creek									
URAT		2.836							
MRAT		4.289	1.539	0.160	0.000		0.000	0.000	0.492
RAIN		4.699	2.254	0.794	0.307	0.091	0.389		1.723
LRAT	150.955	4.954	3.393	0.744	0.377		0.577	0.259	2.164
Indian Creek									
MIND				0.154	0.078		0.030		0.061
LIND		0.334	0.430	0.259	0.274		0.176	0.183	0.398

Appendix B

Results from the U. S. Fish and Wildlife Service's Lower Columbia River Fish Health Center disease profiling for rainbow trout collected in Rattlesnake Creek during 2003.

U.S. Fish & Wildlife Service
LOWER COLUMBIA RIVER FISH HEALTH CENTER
61552 S.R. 14
Underwood, WA 98651
Phone: 509-493-3156
Fax: 509-493-2748

FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-099 **Number of fish:** 4 **Date Sampled:** 06-11-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	4	negative	Negative by EPC and CHSE-214 cells
IHNV	4	negative	Negative by EPC and CHSE-214 cells
VHS	4	negative	Negative by EPC and CHSE-214 cells
AS	4	negative	Negative by BHIA medium
YR	4	negative	Negative by BHIA medium
RS	4	not detected	ELISA
BCD	4	negative	Negative by TYES medium
CD	4	negative	Negative by TYES medium
ESC	4	negative	Negative by BHIA medium
WD	4	negative	Pepsin/Trypsin Digest
CS	4	negative	microscopic examination
Comments			*Neascus* on the skin (heavy). *Nanophyetus* on the gills of two fish (heavy).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri),* **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-103 **Number of fish:** 1 **Date Sampled:** 06-12-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	1	negative	Negative by EPC and CHSE-214 cells
IHNV	1	negative	Negative by EPC and CHSE-214 cells
VHS	1	negative	Negative by EPC and CHSE-214 cells
AS	1	negative	Negative by BHIA medium
YR	1	negative	Negative by BHIA medium
RS	1	**Suspect**	Detected by ELISA, not confirmed by PCR 0/1
BCD	1	negative	TYES medium
CD	1	negative	Negative by TYES medium
ESC	1	negative	Negative by BHIA medium
WD	1	negative	Pepsin/Trypsin Digest
CS	1	negative	microscopic examination
Comments			*Nanophyetus* in the hind-gut (low).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri)*, **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri)*, **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-114 **Number of fish:** 7 **Date Sampled:** 07-14-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	7	negative	Negative by EPC and CHSE-214 cells
IHNV	7	negative	Negative by EPC and CHSE-214 cells
VHS	7	negative	Negative by EPC and CHSE-214 cells
AS	7	negative	Negative by BHIA medium
YR	7	negative	Negative by BHIA medium
RS	7	**Positive**	1/4 detected by ELISA, confirmed by PCR 1/1
BCD	7	negative	TYES medium
CD	7	negative	Negative by TYES medium
ESC	7	negative	Negative by BHIA medium
WD	7	negative	Pepsin/Trypsin Digest
CS	7	negative	microscopic examination
Comments			*Neascus* on the skin(heavy). *Nanophyetus* in the hind-gut (low).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri),* **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connelly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-116 **Number of fish:** 44 **Date Sampled:** 07-15-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	44	negative	Negative by EPC and CHSE-214 cells
IHNV	44	negative	Negative by EPC and CHSE-214 cells
VHS	44	negative	Negative by EPC and CHSE-214 cells
AS	44	negative	Negative by BHIA medium
YR	44	negative	Negative by BHIA medium
RS	30	**Suspect**	1/3 pools detected by ELISA, not confirmed by PCR 0/1
BCD	44	negative	TYES medium
CD	44	negative	Negative by TYES medium
ESC	44	negative	Negative by BHIA medium
WD	44	negative	Pepsin/Trypsin Digest
CS	-	not tested	microscopic examination
Comments			*Aeromonas* bacterial growth on BHIA medium. ELISA pooled into 10 fish pools.

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri),* **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connelly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-117 **Number of fish:** 10 **Date Sampled:** 07-16-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	10	negative	Negative by EPC and CHSE-214 cells
IHNV	10	negative	Negative by EPC and CHSE-214 cells
VHS	10	negative	Negative by EPC and CHSE-214 cells
AS	10	negative	Negative by BHIA medium
YR	10	negative	Negative by BHIA medium
RS	10	not detected	ELISA
BCD	10	negative	TYES medium
CD	10	negative	Negative by TYES medium
ESC	10	negative	Negative by BHIA medium
WD	10	negative	Pepsin/Trypsin Digest
CS	-	not tested	microscopic examination
Comments			

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri)*, **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-127 **Number of fish:** 3 **Date Sampled:** 07-25-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	3	negative	Negative by EPC and CHSE-214 cells
IHNV	3	negative	Negative by EPC and CHSE-214 cells
VHS	3	negative	Negative by EPC and CHSE-214 cells
AS	3	negative	Negative by BHIA medium
YR	3	negative	Negative by BHIA medium
RS	3	**Positive**	Detected by ELISA, confirmed by PCR
BCD	3	negative	TYES medium
CD	3	negative	Negative by TYES medium
ESC	3	negative	Negative by BHIA medium
WD	3	negative	Pepsin/Trypsin Digest
CS	3	negative	microscopic examination
Comments			*Nanophyetus* in the gills and skin (moderate). *Nanophyetus* also found in the kidney (high).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri)*, **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri),* **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Indian Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Cutthroat trout **Age:** Juvenile **CHN:** W03-144 **Number of fish:** 6 **Date Sampled:** 08-19-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	6	negative	Negative by EPC and CHSE-214 cells
IHNV	6	negative	Negative by EPC and CHSE-214 cells
VHS	6	negative	Negative by EPC and CHSE-214 cells
AS	6	negative	Negative by BHIA medium
YR	6	negative	Negative by BHIA medium
RS	6	not detected	ELISA
BCD	6	negative	TYES medium
CD	6	negative	TYES medium
ESC	6	negative	Negative by BHIA medium
WD	6	negative	Pepsin/Trypsin Digest
CS	3	negative	microscopic examination
Comments			Large female spawned out.

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri),* **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH SOURCE	FISH EXAMINED
Location: Indian Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Cutthroat trout **Age:** Juvenile **CHN:** W03-145 **Number of fish:** 3 **Date Sampled:** 08-19-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	3	negative	Negative by EPC and CHSE-214 cells
IHNV	3	negative	Negative by EPC and CHSE-214 cells
VHS	3	negative	Negative by EPC and CHSE-214 cells
AS	3	negative	Negative by BHIA medium
YR	3	negative	Negative by BHIA medium
RS	3	not detected	ELISA
BCD	3	negative	TYES medium
CD	3	negative	TYES medium
ESC	3	negative	Negative by BHIA medium
WD	3	negative	Pepsin/Trypsin Digest
CS	2	negative	microscopic examination
Comments			Collected in the middle section. *Nanophyetus* in the gills (low).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri)*, **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri)*, **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-183 **Number of fish:** 5 **Date Sampled:** 09-18-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	5	negative	Negative by EPC and CHSE-214 cells
IHNV	5	negative	Negative by EPC and CHSE-214 cells
VHS	5	negative	Negative by EPC and CHSE-214 cells
AS	5	negative	Negative by BHIA medium
YR	5	negative	Negative by BHIA medium
RS	5	**Positive**	3/5 detected by ELISA, confirmed by PCR 2/3
BCD	5	negative	TYES medium
CD	5	negative	Negative by TYES medium
ESC	5	negative	Negative by BHIA medium
WD	5	negative	Pepsin/Trypsin Digest
CS	5	negative	microscopic examination
Comments			*Nanophyetus* in the gills (low).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri),* **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W03-187 **Number of fish:** 4 **Date Sampled:** 09-26-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	4	negative	Negative by EPC and CHSE-214 cells
IHNV	4	negative	Negative by EPC and CHSE-214 cells
VHS	4	negative	Negative by EPC and CHSE-214 cells
AS	4	negative	Negative by BHIA medium
YR	4	negative	Negative by BHIA medium
RS	4	**Suspect**	1 /2 detected by ELISA, not confirmed by PCR 0/1
BCD	4	negative	TYES medium
CD	4	negative	Negative by TYES medium
ESC	4	negative	Negative by BHIA medium
WD	4	negative	Pepsin/Trypsin Digest
CS	4	negative	microscopic examination
Comments			*Nanophyetus* in the kidney (high). *Neascus* on the skin (high).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri)*, **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri)*, **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

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FISH HEALTH REPORT 2003

FISH SOURCE	FISH EXAMINED
Location: Rattlesnake Creek (MDNR) **County:** Klickitat **Contact Person:** Pat Connolly **Affiliation:** USGS CRRL **Phone:** (509) 538-2299 ext 269	**Species:** Rainbow trout **Age:** Juvenile **CHN:** W04-005 **Number of fish:** 9 **Date Sampled:** 10-07-03

DISEASE AGENT [1]	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	9	negative	Negative by EPC and CHSE-214 cells
IHNV	9	negative	Negative by EPC and CHSE-214 cells
VHS	9	negative	Negative by EPC and CHSE-214 cells
AS	9	negative	Negative by BHIA medium
YR	9	negative	Negative by BHIA medium
RS	9	**Positive**	2/4 detected by ELISA, confirmed by PCR 2/2
BCD	9	negative	Negative by TYES medium
CD	9	negative	Negative by TYES medium
ESC	9	negative	Negative by BHIA medium
WD	9	negative	Pepsin/Trypsin Digest
CS	9	negative	microscopic examination
Comments			*Neascus* on the skin (heavy). 3 fish with short stocky look (small body with big heads). Digenetic trematodes in the hind-gut(low).

[1] **IPNV** Infectious Pancreatic Necrosis Virus, **IHNV** Infectious Hematopoietic Necrosis Virus, **VHS** Viral Hemorrhagic Septicemia Virus, **AS** Furunculosis (*Aeromonas salmonicida)* , **YR** Enteric Redmouth (*Yersinia ruckeri),* **RS** BKD (*Renibacterium salmoninarum*), **BCD** Coldwater Disease (*Flexibacter psychrophilum)*, **CD** Columnaris (*Flexibacter columnaris)*, **ESC** Emphysematous Putrefactive Disease *(Edwardsiella ictaluri)*, **WD** Whirling Disease (*Myxobolus cerebralis*), **CS** Salmonid Ceratomyxosis (*Ceratomyxa shasta).*

Appendix C

Genetic Population Structure of Rainbow Trout from the White Salmon River Subbasin, Including Inference on Hybridization with Coastal Cutthroat Trout

By

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Draft Technical Report submitted to Paul Powers, US Forest Service, Gifford Pinchot National Forest, Wind River Work Center

ABSTRACT

The U.S. Forest Service (USFS) collected samples for genetic analyses from three wild rainbow trout (*Onchorynchus mykiss)* populations in the White Salmon River subbasin (Husum Falls-below, Husum Falls-above, and Rattlesnake Creek) and three hatcheries (Goldendale, Spokane and Eells Springs). DNA was amplified and analyzed for 340 fish samples. Twenty-two samples were removed from our genetic structure analyses because microsatellite and RFLP analyses indicated hybridization with coastal cutthroat trout (*O. clarki clarki*). Hybrids were found in Rattlesnake Creek (N = 6) and Middle Indian Creek (N = 13). One sample was removed because microsatellite analysis and USFS field observations identified it as a brook trout (*Salmo fontinalis*). Genetic variation found at ten microsatellite loci was used to describe the population structure for the six *O. mykiss* populations in this study. Average heterozygosity (Hz) for the 10 loci across all populations was Hz = 0.653. Pairwise genetic distance based on *Fst* analyses between all possible pairs of populations ranged from *Fst* = 0.032 (Husum Falls, below and Rattlesnake Creek) to *Fst* = 0.264 (Rattlesnake Creek and Eells Springs Hatchery). All pairwise *Fst* comparisons were significant ($p \leq 0.05$) suggesting a high degree of genetic structure in this system. Neighbor-joining (NJ) analysis of genetic distance supported the separation of wild populations from hatchery populations (99% support based on 2000 bootstrap replicates). AMOVA analysis showed that 15.13% of the molecular variance could be attributed to differences among populations. Garza and Williamson's (2001) *M* across all populations was *M* = 0.623, below the published threshold ($M \leq 0.680$), supporting recent population reductions for *O. mykiss* in the White Salmon River subbasin. Average estimated effective population size (*Ne* based on SMM) across all populations in this study was *Ne* = 5134. Additional sampling using appropriate collection strategies for genetic analyses is recommended to gain greater insight into the fine-scale genetic structure of *O. mykiss* throughout the White Salmon River subbasin.

INTRODUCTION

The tributaries of the White Salmon River subbasin were former natal grounds to anadromous steelhead (*Onchorynchus mykiss*), coastal cutthroat trout (*O. clarki clarki*), Chinook (*O. tschawytscha*) and coho salmon (*O. kisutch*) (Western Watershed Analysts 1997). Upon construction of Condit dam (1913), approximately 40 miles of anadromous habitat was blocked above Northwestern Lake (Washington Conservation Commission 1999). Husum Falls (a natural 20-foot falls) is suspected to also act as a barrier to some anadromous fish (Rawding 2000). Resident rainbow trout (*O. mykiss*) are present throughout the White Salmon River system.

The impact of hatchery *O. mykiss* on wild stocks in streams and reservoirs throughout North America over the last 150 years has been the subject of many studies (see reviews in Reisenbicher and McIntyre 1977, Waples and Do 1994, Campton 1995 and Nielsen 1999). To address the status and current genetic relationships among *O. mykiss* populations isolated by Condit dam, population genetic structure was analyzed in trout collected from the White Salmon River above and below Husum Falls, and Rattlesnake Creek. Putative hatchery introgression onto wild *O. mykiss* populations was considered using three reference hatchery populations in our analysis (Goldendale, Eells Springs and Spokane hatcheries).

Coastal cutthroat trout and steelhead are thought to have coevolved in coastal ecosystems and are considered reproductively isolated under most circumstances (Leary et al. 1987; Mullan et al. 1992; Nielsen 1999; McCusker et al. 2000). However, hybridization has been well documented in areas where these two species co-occur, primarily in inland freshwater habitats where rainbow trout have been introduced (Allendorf and Leary 1988; Henderson et al. 2000; Rubidge et al. 2001; Hitt et al. 2003). In the laboratory, coastal cutthroat/steelhead hybrids did not exhibit decreased fitness (Hawkins 1997). Persistent bi-directional natural hybridization has also been documented in some coastal areas where these species occur sympatrically including documentation of backcross or later generation (Fn) hybrids (Hawkins and Foote 1998; Young et al. 2001; Ian Williams, USGS Alaska Science Center, unpublished data). Molecular genetic data have been used to depict interspecific hybridization in many trout species (Campton 1987; Leary et al. 1995; Wenburg et al. 1996; Baker et al. 2002; Ostberg and Rodriguez 2002). The conservation value of hybrid fishes and introgressed populations, especially in salmonids, presents a major controversy for natural resource managers (Allendorf et al. 2001; Peacock and Kirchoff 2004).

This study represents a minimal understanding of the genetic structure of *O. mykiss* in the White Salmon subbasin. The number of populations and population sample sizes were small. Therefore, additional genetic analyses may modify the results presented in this study. We highly recommend a more extensive sampling protocol ($N \geq 50$) including all of the major tributaries documented to support rainbow trout (Buck, Little Buck, Mill, Spring, Rattlesnake and Indian creeks) to properly assess population structure of *O. mykiss* throughout the White Salmon subbasin.

METHODS

Sample Collections and DNA Extraction

The U.S. Forest Service collected fin tissues from 340 fish (putatively *O. mykiss*) in the White Salmon River subbasin during 2001-2003. DNA was extracted two times from each sample, independently, using Puregene® DNA isolation tissue kits (Gentra Systems, Minneapolis, MN, U.S.A.).

Microsatellite Amplification Protocols

Microsatellite loci taken from the published literature were selected for analysis based on documented variability in *O. mykiss*, ease of amplification in polymerase chain reaction (PCR), and allele scoring rigor. G. K. Sage (Alaska Science Center, Conservation Genetics Laboratory) developed multiplex systems using 13 loci grouped together for amplification of steelhead allelic size structure. G. K. Sage redesigned several primers in order to establish the three multiplex protocols used in this study, one containing 5 loci and two containing 4 loci each (see Table 3). *Oneμ10-(F)*, *Ogo4-(F)*, *Ogo4-(R)* and *Ogo3-(R)* were redesigned as follows: *Oneμ10-(F)* was renamed *Oneμ10.2-(F)* (5'-TGTTGGCACCATTGTAACAG-3'); *Ogo4-(F)* was renamed *Ogo4.2-(F)* (5'-CAGAATGAGTAACGAACG C-3'); *Ogo4-(R)* was renamed *Ogo4.2-(R)* (5'-GAGGATAGAAGA GTTTGGC-3'); and *Ogo3-(R)* was renamed *Ogo3.2-(R)* (5'-CACAATGGAAGACCAT-3'). *Ogo1a*, *Ogo4.2*, *Oneμ10.2* and *Ots3* forward primers were modified by the addition of M13R tails, and *Oneμ8* and *Oneμ11* forward primers were modified by the addition of M13F tails. All M13 tails were added to the primers at the 5' end. These tails allowed allele fragment visualization by annealing to labeled complementary M13 tails added to the PCR mix. The remaining loci were visualized by adding directly labeled forward primers. Allele sizes (from adapted primers) were standardized to single locus products by running known standards for allelic size for each locus on all multiplex gels.

In general, PCR reactions were conducted in 10µl volumes using approximately 50ng of genomic DNA, 0.1-0.2 U of DNA polymerase (Perkin Elmer), 10mM Tris-HCl (pH 8.3), 1.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100, and 200µM each dNTP. To visualize loci with directly labeled primers, the total of forward (F) and reverse (R) primers per locus per reaction equaled 4 pmoles, with the F primer concentration being a combination of labeled and unlabeled primer. Tailed F and R primer concentrations for the multiplex systems were as follows: *Oneµ10* (10 pmoles), *Ogo1a*, *Ogo4*, *Oneµ11*, *Ots3* (5 pmoles) and *Oneµ8* (1 pmole).

The following amounts of labeled primers were added in each of the three multiplex systems. Multiplex A had between 0.06-0.20 pmoles per reaction (*Omy325*, 0.06; *Ots1*, 0.20; *Oneµ14*, 0.40; *Ots4*, 0.06). Multiplex B was between 0.1-1.5 pmoles (*Omy77*, 0.2; M13F, 0.3; M13R, 1.5), and Multiplex C had between 0.1-1.5 pmoles (M13F, 1.5; M13R, 1.5; *Omy27*, 0.1; *Omy207*, 0.2). Gel electrophoresis and visualization of microsatellite alleles was performed using LI-COR Model 4200 and IR2 automated fluorescent DNA Sequencers and sizing was performed using V3.00 Gene ImagIR (LI-COR, Lincoln, NE, USA). Microsatellite allele sizes (including the amplified primer) were determined in relation to the M13 ladder or to the GeneScan-350 internal size standard (P-E Biosystems, Foster City, CA, USA), and *O. mykiss* DNA samples of known size that were included on each gel. Approximately 10% of all samples were re-amplified independently and run on a second gel to verify allelic size.

Microsatellite data for *Omy207* was removed from statistical analysis because of a problem with low allele fallout and/or null alleles. *Ots3* and *Ots4* were removed from the analysis because of PCR amplification problems.

Genetic Analyses

Genetic data from 317 fish samples were analyzed using a variety of software from different statistical packages including ARLEQUIN version 1.1 (Schneider et al. 2000), BOTTLENECK (Piry et al. 1999), WHICH LOCI (Banks and Eichert 2000), NEIGHBOR application of PHYLIP (Felsenstein 1993), and GENEPOP version 3.3 (Raymond and Rousset 1997). It should be noted that 22 samples were removed from the statistical analysis because hybridization was detected and one sample (O1CK 18) was removed because it was identified as a brook trout (*Salmo fontinalis*). Putative hybrid fish (cutthroat X rainbow) were identified using variation in microsatellite allelic structure that fell outside of expected values for *O. mykiss* samples and in relationship to allelic variation typically found in cutthroat trout (Ian Williams, USGS, unpublished data).

Tests of Hardy-Weinburg equilibrium (HWE) were performed to look at the performance of different loci among these *O. mykiss* populations to gain insight into population structure. Heterozygosity and simulated Fisher's exact tests using randomization for HWE were performed using GENEPOP and ARLEQUIN. ARLEQUIN's *Fst* pairwise comparisons were used to test for differences in allele frequencies between and among populations. Statistical significance levels for allelic frequency comparisons were set using sequential Bonferonni tests (Rice 1989). Partitioning of microsatellite allelic variation based on analysis of molecular variance (AMOVA) was performed using ARLEQUIN.

Detection of recent reductions in population size using microsatellite data was performed on the White Salmon River subbasin populations using Garza and Williamson's *M* (2001). Effective population size (*Ne*) estimates were made under the assumption of mutation-drift equilibrium using the Single-Step Mutation Model (SSM) and the Infinite Allele Model (IAM) with a mutation rate of $2.05E^{-4}$ (Garza and Williamson 2001 based on methods from Lehmann et al. 1998 and Rooney et al. 1999).

Genetic distance values reflecting the proportion of shared alleles between individuals and groups of individuals can be used to graphically depict genetic relationships and population structure. A Cavalli-Sforza and Edward's chord genetic distance (1967) matrix based on allele frequency data was generated using Treemaker version 1.0 (Cornuet et al. 1999). An unrooted Neighbor-Joining tree (NJ) was generated using the NEIGHBOR application of PHYLIP and visualized with TreeView version 1.6.6 (Page 1996). To assess the reproducibility of branching patterns on the consensus tree, bootstrapping over loci (n = 2000; Felsenstein 1985) was performed using NJBPOP

(Cornuet et al. 1999). The program WHICHLOCI was used to rank the microsatellite loci used in this study based on their relative allelic differential derived from White Salmon River *O. mykiss* populations (Banks and Eichert 2000).

Restriction Fragment Length Polymorphism Analysis

Select samples identified as putative hybrids through microsatellite analysis were evaluated for restriction fragment length polymorphisms (RFLP) at two sites within the mitochondrial genome: NADH dehydrogenase 5/6 (ND 5/6) and cytochrome b. RFLP's (ND 5/6 region cut with *Dde I* and cytochrome b cut with *Dpn II)* were used to test the species status between *O. mykiss* (steelhead/rainbow trout) and *O. clarki* (cutthroat trout) in this study. Amplification and visualization followed procedures from Scribner *et al.* (1998), with minor modification.

Mitochondrial DNA Sequence Analysis

Total genomic DNA from selected samples (including all putative hybrids) was extracted from fin clips using Chelex-100 resin (BioRad) following the methods of Nielsen et al. (1994). Conserved primers (S-Phe and P2) were used to amplify a segment of the mtDNA control region (D-loop). These primers are known to amplify the highly variable segment of the right-domain of the mtDNA control region in salmonids. See Nielsen et al. (1994) for primer sequences, amplification and sequencing protocols, and sequence of the entire region amplified by these primers in several Pacific salmonids. *O. mykiss* (rainbow trout haplotype, MYS-1), *O. clarki clarki* (cutthroat) and *O. clarki lewisi* (Westslope cutthroat trout) were included as reference samples for alignment and evaluation of maternal contribution to putative hybrids.

RESULTS

Microsatellite Loci, HWE and Genetic Population Structure

Allelic diversity at 10 microsatellite loci was determined for three *O. mykiss* populations in the White Salmon River subbasin: Husum Falls-below, Husum Falls-

Figure 1. Map of the White Salmon River subbasin study area. Arrow indicates the approximate location of Husum Falls.

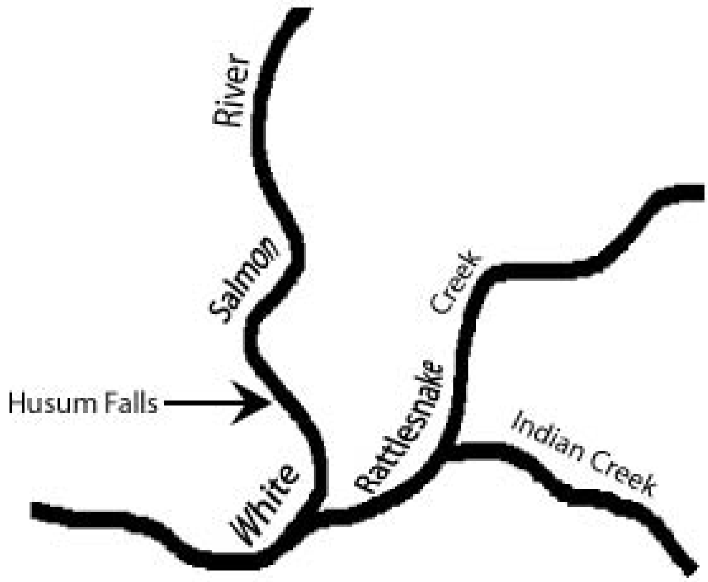

Table 1. White Salmon River project populations, total number of non-hybrid samples (N) and sampling year for populations evaluated in the genetic structure analysis. The Rattlesnake Creek population contains samples from lower Rattlesnake (N=8), middle Rattlesnake (N=17) and the Bramhill property (N=75). Goldendale hatchery contains a compilation of samples collected in 2001 and 2002. All samples were collected by the US Forest Service.

Population	N	Sampling Year
Husum Falls - below	33	2001
Husum Falls - above	45	2001
Rattlesnake Creek	100	2001
Goldendale hatchery	47	2001 / 2002
Eells Springs hatchery	48	2002
Spokane hatchery	44	2003
TOTAL	317	

above, Rattlesnake Creek (Figure 1); and three hatchery rainbow trout populations: Goldendale Hatchery, Eells Springs Hatchery and Spokane Hatchery (Table 1). Six other populations were not included in the analysis because proposed samples were never received in the laboratory or fin clip tissues were improperly stored and the DNA was degraded (Table 2). Year-to-year analysis could not be performed for Middle Indian Creek for two reasons: lack of adequate sample size (N = 13) for the 2001 sampling year and the 2002 sampling year tissues were never received. It should also be noted that Middle Indian Creek, 2001 (N=13), was excluded from the population structure analysis presented in this report because all 13 samples indicated hybridization in the microsatellite analysis. Another year-to-year analysis between two populations occurring above Husum Falls (Husum Falls-above, 2001 and White Salmon-BZ to Husum, 2002) was not performed because DNA from the White Salmon-BZ to Husum samples was not recoverable (i.e. degraded tissues).

Table 2. White Salmon River project populations not included in our analysis. Rationale for removing these populations from the analysis is listed below.

Population	Population Code	N	Sampling Year	Rationale for not including in analysis
Lower Rattlesnake Creek	LRAT 1	51	2002	degraded DNA*
White Salmon: BZ to Husum	WS-C	34	2002	degraded DNA*
Lower Mill Creek, WA	LMIW	51	2002	degraded DNA*
Middle Mill Creek, WA	MMIW	50	2002	never received samples
Lower Indian Creek	LIND	49	2002	never received samples
Middle Indian Creek	MIND 02	50	2002	never received samples
TOTAL		285		

* Fin clips were not stored in 100% ethanol

Amplification protocols for multiplex systems used to assess microsatellite loci are described in Table 3. Microsatellite loci were ranked according to their accuracy for correct population assignment using the program WHICHLOCI (Banks and Eichert 2000). After iteration over 100 datasets, WHICHLOCI indicated that all 10 loci were required for 94.9% reassignment accuracy (Table 4). Individual contributions (% relative score) to this analysis of allelic frequency differences were distributed evenly among microsatellite loci, excluding Oneμ11, which posted a relative score of 3.8%. Omy325 was the top ranked locus with a 13.8% relative score.

Table 3. Multiplex systems comprised of 13 microsatellite loci were used to amplify DNA from White Salmon River samples. Amplification products were analyzed on the LI-COR automatic sequencer. Additional primer modifications made to enhance these multiplexes are given in the text. The "700" and "800" columns represent different dyes used on the LI-COR platform. Ots3 and Ots4 were removed from the analysis due to amplification problems and Omy207 was removed due to potential low allele fallout.

Multiplex	Anneal temp(oC)/ # cycles	30 min. extension	Loci 700	Loci 800
A	52/40	NO	Omy325 Ots1	Ots4 Oneμ14
B	52/40	YES	Omy 77 Oneμ8	Ogo1a Ogo4 Ots3
C	52/40	YES	Omy207 Oneμ10	Omy27 Oneμ11

Table 4. Microsatellite loci rank using the allele frequency differential method from WHICHLOCI (Banks and Eichert 2000).

Rank	Locus	Score	% Relative Score
1	Omy325	9.366	13.846
2	Omy77	8.287	12.251
3	Ogo4	8.198	12.121
4	Oneμ8	8.077	11.941
5	Ots1	7.867	11.631
6	Oneμ14	6.893	10.191
7	Oneμ10	6.215	9.189
8	Omy27	5.336	7.888
9	Ogo1a	4.841	7.157
10	Oneμ11	2.561	3.787

Allelic size ranges, number of alleles and mean heterozygosity (Hz) for each locus is given in Table 5. Average number of alleles per locus was 10.9 and mean Hz across all loci and populations was 0.653. Omy325 recorded the highest number of alleles (26), the largest allelic size range (87 – 149) and the greatest mean Hz across all populations (0.782). Only three alleles occurred at Oneμ11 and this locus had the lowest mean Hz (0.439). Table 6 indicates the unbiased (Nei 1987) and observed Hz for each population across all loci. Husum Falls-below recorded the highest unbiased and observed Hz, 0.7693 and 0.6996, respectively.

Table 5. List of microsatellite loci used in this study. Mean heterozygosity (Hz) is given per locus across all populations statistically analyzed in the White Salmon River.

Locus	Source	Number of Alleles	Allelic Size Range (bp)	Mean Hz
Ogo1a	Olsen et al. 1998	6	123 - 168	0.670
Ogo4	Olsen et al. 1998	11	118 - 140	0.642
Omy27	Heath et al. 2001	7	99 - 113	0.603
Omy77	Morris et al. 1996	16	97 - 141	0.766
Omy325	O'Connell et al. 1997	26	87 - 149	0.782
Oneμ8	Scribner et al. 1996	12	152 - 188	0.600
Oneμ10	Scribner et al. 1996	6	121 - 131	0.708
Oneμ11	Scribner et al. 1996	3	145 - 149	0.439
Oneμ14	Scribner et al. 1996	6	147 - 161	0.663
Ots1	Banks et al. 1999	16	155 - 241	0.654
	average # of alleles/locus =	10.9	mean Hz across all loci =	0.653

Table 6. Unbiased and observed heterozygosity (Hz) for populations used in the genetic structure analysis. Unbiased Hz is based on Nei's unbiased genetic diversity (1987).

Population	Unbiased Hz	Observed Hz
Husum Falls - below	0.7693	0.6996
Husum Falls - above	0.6726	0.6086
Rattlesnake Creek	0.7178	0.6583
Goldendale hatchery	0.6827	0.6141
Eells Springs hatchery	0.5071	0.4944
Spokane hatchery	0.5661	0.5258
mean =	0.653	0.600

ARLEQUIN's Hardy-Weinburg equilibrium (HWE) expectations for all populations and loci are reported in Table 7. All populations were within HWE for Oneµ11. Husum Falls-below was within HWE for 9 out of 10 loci. The two remaining wild populations, Husum Falls-above and Rattlesnake Creek, each fell out of HWE for 5 out of 10 loci. Eells Springs and Spokane hatcheries were within HWE for 8 out of 10 loci and Goldendale hatchery was within HWE for 6 out of 10 loci (Table 7).

ARLEQUIN's population pairwise comparison (*Fst*) found significant differences in allelic frequencies among all populations analyzed (Table 8). *Fst* values ranged from 0.032 to 0.264 and reflect the most similar and the most diverged population pairs, respectively. Wild populations below Husum Falls (Husum Falls-below and Rattlesnake Creek) were most similar with regards to allele frequencies (*Fst* = 0.032). Eells Springs hatchery was highly divergent from all other populations in the study, however was most diverged from the Rattlesnake Creek population (*Fst* = 0.264).

The largest estimation of effective population size (*Ne*) under the assumptions of the single mutation model (SMM) was recorded for Husum Falls-below, *Ne* = 9629 (Table 9). The mean effective population size under SMM for all populations was *Ne* = 5134. Garza and Williamson's (2001) *M* demonstrates a recent reduction in population size, i.e. population bottleneck, when $M \leq 0.680$. Based on this test, all populations were predicted to have undergone recent reductions in population size (Table 9).

ARELQUIN's analysis of molecular variance (AMOVA) was used to determine the partitioning of genetic variance among groups of populations, among populations within groups and within populations. When all populations were considered as one group, 15.13% of the genetic variance was attributed to differences among populations, 84.87% to individuals within populations. When wild and hatchery populations were considered as separate groups, the molecular variance was proportioned as follows: 5.87% among groups; 11.2% among populations within groups; 82.93% within populations. AMOVA analysis of wild populations above (Husum Falls-above) and below Husum Falls (Husum Falls-below and Rattlesnake Creek) divided 3.74% of the genetic variability among groups and 3.2% within groups (93.06% within populations).

Table 7. Hardy-Weinburg equilibrium (HWE) results for 10 loci indicating populations within HWE "-" and out of HWE "+" based on exact tests performed by ARLEQUIN 1.1.

POPULATION	N	LOCUS Ogo1a	Ogo4	Omy27	Omy77	Omy325	Oneμ8	Oneμ10	Oneμ11	Oneμ14	Ots1	Loci within HWE
Husum Falls, below	33	-	-	-	-	-	-	-	-	+	-	9
Husum Falls, above	45	-	+	-	-	+	-	+	-	+	+	5
Rattlesnake Creek	100	+	-	+	-	-	+	-	-	+	+	5
Goldendale Hatchery	47	+	-	-	-	-	+	-	-	+	+	6
Eells Springs Hatchery	48	-	-	-	-	-	-	+	-	+	-	8
Spokane Hatchery	44	-	-	-	+	-	-	-	-	+	-	8
Total within HWE		4	5	5	5	5	4	4	6	0	3	

Table 8. Pairwise *Fst* comparisons between White Salmon River populations. Pairwise *Fst* values are given below the diagonal and the matrix of significant *Fst* P values ($p < 0.05$) is given above the diagonal. The "+" symbol represents a significant pairwise difference.

POPULATION	1	2	3	4	5	6
1. Husum Falls, below		+	+	+	+	+
2. Husum Falls, above	0.052		+	+	+	+
3. Rattlesnake Creek	0.032	0.076		+	+	+
4. Goldendale hatchery	0.045	0.129	0.113		+	+
5. Eells Springs hatchery	0.211	0.240	0.264	0.193		+
6. Spokane hatchery	0.100	0.171	0.170	0.112	0.249	

Genetic Distance Analysis

A consensus Neighbor-Joining (NJ) tree based on Cavalli-Sforza and Edwards chord distance (1967) for the set of samples analyzed in this report is presented in Figure 2. Branch bootstrap values (% iteration over 2000 replicate trees) are given for all branches in this unrooted tree. NJ analyses supported clustering of the hatchery populations (99% bootstraps). Trout sampled at Husum Falls (below) clustered with the hatchery populations with 92% bootstrap support. Seventy-three percent bootstraps supported the branch clustering Spokane and Eells Springs hatcheries.

Table 9. Estimations of recent reductions in population size (Garza and Williamson's *M*) and effective population size (*Ne*) based on the Infinite Allele (IAM) and Single Mutation (SMM) models.

Population	Garza & Williamson's *M*	*Ne* IAM	*Ne* SMM
Husum Falls, below	0.639	3778	9629
Husum Falls, above	0.646	2413	4800
Rattlesnake Creek	0.585	3042	6835
Goldendale Hatchery	0.635	2533	5164
Eells Springs Hatchery	0.636	1228	1846
Spokane Hatchery	0.598	1547	2528
mean	0.623	2424	5134

Hybrid Samples, RFLP analysis and mtDNA Sequencing

Twenty-two samples, representing four populations indicated hybridization (Table 10). Lack of compliance to allele size ranges expected for *O. mykiss* (rainbow trout), and demonstrated *O. clarki clarki* (coastal cutthroat) alleles in the microsatellite analyses were used as criteria to designate hybrids. Five microsatellite loci were used to infer cutthroat-rainbow trout introgression in this study (Table 10). The entire Middle Indian Creek population (N=13) exhibited mainly cutthroat microsatellite alleles, as did the six hybrid samples from Rattlesnake Creek. Two samples from Husum Falls-below indicated hybridization, one of which recorded cutthroat signatures for all alleles across five microsatellite loci used to detect hybridization. One allele at Omy325 was of putative cutthroat origin for a Goldendale hatchery (2001) sample.

Hybrid samples from Middle Indian and Rattlesnake creeks were analyzed for restriction fragment length polymorphisms (RFLP) used as species designators between rainbow and cutthroat trout. ND 5/6 cut with *Dde I* (RFLP 1, Table 10) was used on all Middle Indian and Rattlesnake creek samples and select samples from these populations were further analyzed at cytochrome b cut with *Dpn II* (RFLP 2, Table 10). The majority of RFLP signatures were of *O. mykiss* origin.

The D-loop of the mitochondrial DNA (mtDNA) control region was chosen for sequencing. Eight samples were sequenced and aligned against *O. mykiss* (haplotype MYS-1), *O. clarki clarki* (coastal cutthroat) and *O. clarki lewisi* (Westslope cutthroat) as reference samples (Table 11). Four samples (MIND 06, MIND 09, RAT 14, RAT 15) aligned perfectly with *O. mykiss* haplotype MYS-1. Four other samples (MIND 04, MIND 11, RAT 16 and RAT 18) aligned perfectly with *O. clarki clarki* mtDNA. No sample shared alignment with the Westslope cutthroat reference sample.

Figure 2. Unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards (1967) genetic chord distance for *O. mykiss* populations in the White Salmon River subbasin. Bootstrap values (% confidence after 2000 replicates) are given for major branches.

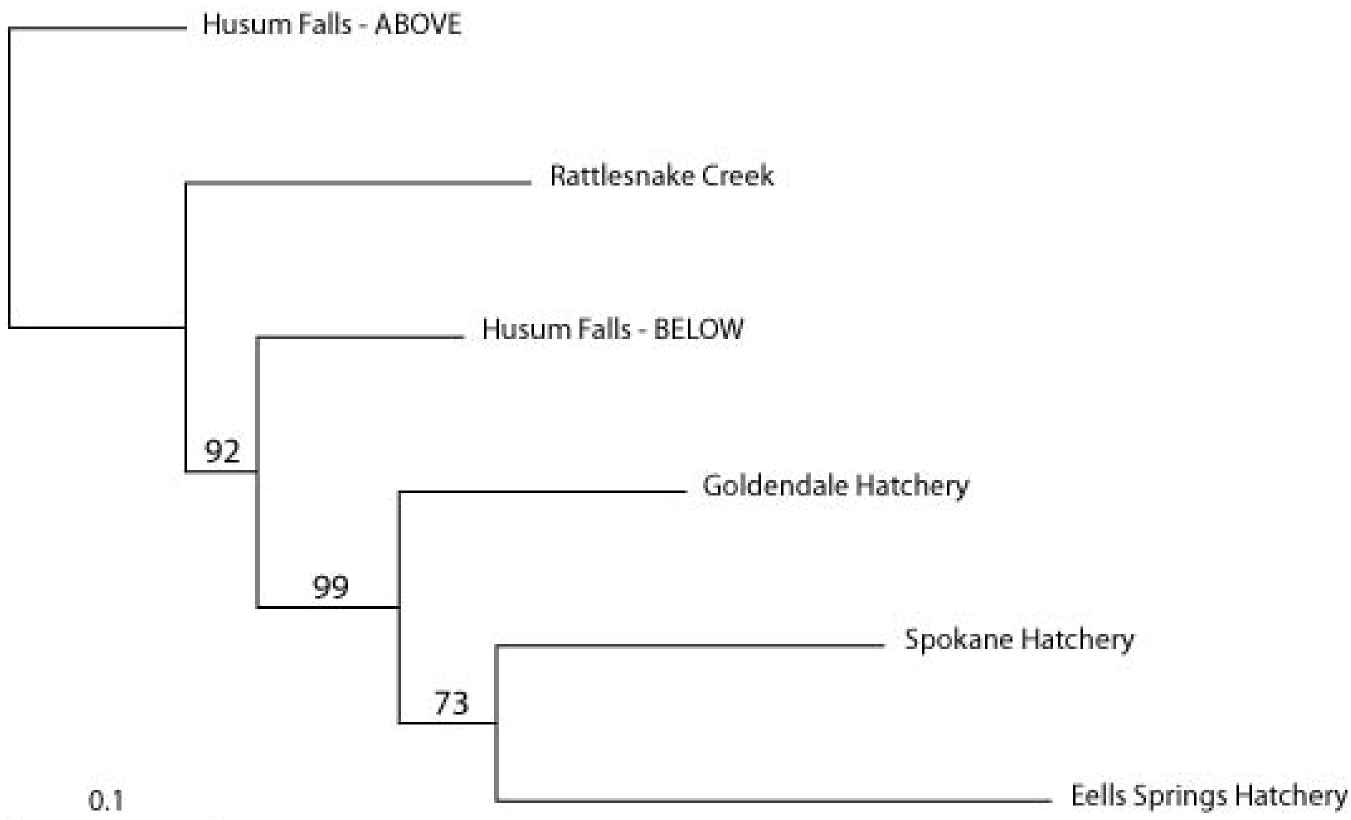

Table 10. List of samples from the White Salmon River subbasin indicating hybridization. *Onchorynchus mykiss* (rainbow trout – "RBT") and *O. clarki* (cutthroat trout – "CTT") phenotypes were based on physical characteristics determined in the field (US Forest Service). Results from mtDNA and RFLP loci are given using species-specific diagnostic indicators ("RBT" for *O. mykiss*, "CTT" for *O. clarki*). The symbol "-" indicates that no analysis was done. For microsatellite diploid data, allele pairs at each locus are given as "R" and/or "C" separated by a slash ("/"), representing *O. mykiss* and *O. clarki* alleles, respectively. The notation "na" indicates that no amplification occurred for that sample.

			Loci Indicating Hybridization							
POPULATION	Number of Hybrids	Phenotype	mtDNA sequence	RFLP 1*	RFLP 2**	Ogo 4	Omy 325	Oneμ8	Oneμ14	Ots 1
Goldendale hatchery	1									
GOL 69		RBT	-	-	-	R/R	R/C	R/R	R/R	R/R
Husum Falls, below	2									
O1CK 50		RBT	-	-	-	R/C	R/C	R/R	R/R	R/C
O1CK 54		RBT	-	-	-	C/C	C/C	C/C	C/C	C/C
Middle Indian Creek	13									
MIND 01		CTT	-	RBT	-	C/C	R/C	C/C	C/C	R/C
MIND 02		CTT	-	RBT	RBT	C/C	C/C	C/C	C/C	C/C
MIND 03		CTT	-	CTT	CTT	R/C	C/C	C/C	C/C	C/C
MIND 04		CTT	CTT	CTT	CTT	C/C	C/C	C/C	C/C	C/C
MIND 05		CTT	-	CTT	-	C/C	C/C	C/C	C/C	C/C
MIND 06		CTT	RBT	RBT	RBT	C/C	C/C	C/C	C/C	C/C
MIND 07		CTT	-	CTT	-	C/C	R/C	C/C	C/C	C/C
MIND 08		CTT	-	RBT	-	R/C	R/C	C/C	C/C	R/C
MIND 09		RBT	RBT	RBT	RBT	R/C	R/C	R/C	R/C	R/C
MIND 10		CTT	-	RBT	-	C/C	R/C	C/C	C/C	R/C
MIND 11		CTT	CTT	CTT	-	C/C	C/C	C/C	na	C/C
MIND 12		CTT	-	RBT	RBT	C/C	R/C	C/C	C/C	C/C
MIND 13		CTT	-	RBT	-	R/C	C/C	C/C	C/C	C/C
Rattlesnake Creek (lower)	6					C/C	C/C	C/C	C/C	C/C
RAT 13		CTT	-	RBT	-	R/C	R/C	R/C	C/C	R/C
RAT 14		CTT	RBT	RBT	RBT	C/C	R/C	C/C	C/C	R/C
RAT 15		CTT	RBT	RBT	RBT	C/C	C/C	C/C	C/C	C/C
RAT 16		CTT	CTT	CTT	-	C/C	R/C	C/C	C/C	C/C
RAT 17		CTT	-	RBT	RBT	C/C	R/C	C/C	C/C	R/C
RAT 18		CTT	CTT	CTT	CTT	R/C	C/C	C/C	C/C	C/C

* RFLP signature 1 resulted from *Dde I* digestion of the ND 5/6 region

** RFLP signature 2 resulted from *Dpn II* digestion of cytochrome b

Table 11. Mitochondrial DNA (mtDNA) control region variable sites and nucleotide changes found in White Salmon River hybrid samples. *Onchorynchus mykiss* haplotype 1 (rainbow trout – "MYS-1"), *O. clarki clarki* (coastal cutthroat – "CCT") and *O. clarki lewisi* (Westslope cutthroat – "WSCT") were used as reference samples to create the alignment. Base pair (bp) numbers reflect *O. mykiss* sequence data published by Digby *et al.* (1992). The "-" indicates a gap exists in the sequence alignment.

		base pair number																							
mtDNA Reference	Sample	964	967	979	980	986	1006a*	1015a*	1015b*	1032	1039	1040	1058	1085	1086	1089	1093	1100	1103	1115	1119	1123	1127	1143	1147	1149
MYS-1		C	A	T	T	A	-	-	-	G	A	A	A	A	T	A	T	C	A	G	T	C	G	G	G	C
	MIND 06	C	A	T	T	A	-	-	-	G	A	A	A	A	T	A	T	C	A	G	T	C	G	G	G	C
	MIND 09[#]	C	A	T	T	A	-	-	-	G	A	A	A	A	T	A	T	C	A	G	T	C	G	G	G	C
	RAT 14	C	A	T	T	A	-	-	-	G	A	A	A	A	T	A	T	C	A	G	T	C	G	G	G	C
	RAT 15	C	A	T	T	A	-	-	-	G	A	A	A	A	T	A	T	C	A	G	T	C	G	G	G	C
CCT		A	G	T	-	G	G	T	A	A	G	G	T	G	C	C	C	G	G	A	A	T	A	A	A	T
	MIND 04	A	G	T	-	G	G	T	A	A	G	G	T	G	C	C	C	G	G	A	A	T	A	A	A	T
	MIND 11	A	G	T	-	G	G	T	A	A	G	G	T	G	C	C	C	G	G	A	A	T	A	A	A	T
	RAT 16	A	G	T	-	G	G	T	A	A	G	G	T	G	C	C	C	G	G	A	A	T	A	A	A	T
	RAT 18	A	G	T	-	G	G	T	A	A	G	G	T	G	C	C	C	G	G	A	A	T	A	A	A	T
WSCT		A	G	-	-	G	G	T	A	A	G	G	T	G	C	C	C	G	A	A	A	C	A	A	G	T

* base pairs 1006a, 1015a and 1015b represent gaps in the MYS-1 reference sequence relative to the CCT reference sequence.

[#] MIND 09 was the only sample sequenced that was identified phenotypically as a rainbow trout (*O. mykiss*) by the US Forest Service.

DISCUSSION

This study focused on three wild and three hatchery populations and only touches the surface of our understanding the genetic structure of *O. mykiss* in the White Salmon River system. Given the extent of this study, we highly recommend a sampling approach prioritizing increased sample sizes that represent populations from all major tributaries of the White Salmon River - Buck, Little Buck, Spring, Mill, Indian and Rattlesnake creeks, as suggested by Rawding (2000).

Pairwise *Fst* comparisons indicated that the two wild populations below Husum Falls were more similar to Goldendale hatchery than Eells Springs or Spokane hatcheries (see *Fst* comparisons in Table 8). Eells Springs Hatchery exhibited more rare alleles and was distinctly diverged with respect to allelic pairwise comparisons (all *Fst* values ≥ 0.193; Table 8). The unrelated genetic nature of Eells Springs Hatchery relative to all other populations examined may have skewed estimates of effective population size for this population (*Ne* = 1846; Table 9). Excluding Eells Springs Hatchery, all other estimates of *Ne* were relatively high (mean *Ne* = 5134; Table 9). Estimates of effective population size (*Ne*) incorporate relative parameters related to demographic information. In small populations, *Ne* is important because it is inversely related to the rate of loss of genetic diversity. Estimates of *Ne*, however, are based on several assumptions (identity-by-descent, random mating, temporal stability in finite populations) that are generally difficult to support for *O. mykiss* and can often overestimate population size (Heath et al. 2002, Palm et al. 2003; Ardren and Kapuscinski 2003). The relationship between effective population size (i.e. the estimated number of individuals contributing genes to the next generation) and actual demographic population size is important in understanding the effects of artificial husbandry on the genetic composition of hatchery stocks (Waples and Do 1994).

The NJ tree supported the branch containing all hatchery populations with 99% bootstraps. Ninety-two per cent of the NJ trees clustered Husum Falls (below) with the hatchery populations with a low pairwise Fst = 0.045 (Goldendale Hatchery). Trout below Husum Falls represent a valuable recreational fishery that is stocked with hatchery *O. mykiss*, therefore hatchery fish many have influenced the genetic signature of the trout sampled below Husum Falls. Rattlesnake Creek, which is also below Husum Falls, carried more diverse alleles in pairwise comparison with the Goldendale Hatchery (Fst = 0.113).

The genetic composition of the Middle Indian Creek population (N = 13) warrants further research in this tributary and its mainstem, Rattlesnake Creek (see hybrids in Table 10 and 11). The presence of coastal cutthroat-rainbow trout hybrids indicates the existence of coastal cutthroat (*O. clarki clarki*) populations in the White Salmon River system above Condit dam. These habitats above Condit dam have been identified as primary targets for restoration prior to dam removal (2006) to aid in the reestablishment of anadromy to these former natal grounds (CBFWA 1995). Sequence analysis of mtDNA did not indicate any trends in the directionality of hybridization (i.e. was the hybridization driven by female *O. mykiss* breeding with male *O. clarki clarki* or vice versa). To address this issue, sequence analysis of a greater number of hybrids is required. Hybridization between *O. mykiss* and Westslope cutthroat trout (*O. clarki lewisi*) does not appear to be an issue based on the hybrid samples analyzed in this report.

Relationships presented in this report will most likely change with increased sample size and coverage of a broader geographic area within this basin. These data should be considered preliminary management tools working toward a more rigorous analysis of the *O. mykiss* populations in the White Salmon River. We recommend a multi-species sampling approach to address the presence of hybrids and to identify baseline population(s) and genetic structure for *O. mykiss* and *O. clarki clarki* within this system.

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