



# **Yakima Steelhead VSP Project:**

## **Resident/Anadromous *O. mykiss* Status and Trend Monitoring**

BPA Project # 2010-030-00

**Report covers work performed under BPA contract #(s) 70353 and 75719**

**Report was completed under BPA contract #(s) 75719**

1/1/2017 - 12/31/2017

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Report Created 09-2017

“This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the authors' and do not necessarily represent the views of BPA.”

## ***Abstract***

The Steelhead Trout *Oncorhynchus mykiss* exhibit some of the most diverse life histories of any Pacific salmonid. Included in the diversity of this species is the variable expression of anadromous and resident life histories. The anadromous form may smolt and migrate to the ocean after one or more years of freshwater residency and return to its natal stream after spending one or more years in the ocean. In contrast, the resident life history form, also known as Rainbow Trout, spends its entire life in freshwater. Our understanding of this species is complicated by the fact that both forms can interbreed and produce offspring of the opposite type. It is unclear how this interaction between life history forms influences the recovery of the anadromous form (Steelhead Trout) as mandated under the Endangered Species Act (ESA). Our project provides information on the Viable Salmonid Population (VSP) metrics for the upper Yakima *O. mykiss* population while generating status and trend monitoring information for the resident and anadromous life history forms. Overall, the *O. mykiss* population in the upper Yakima appears to be gradually increasing and although our recovery targets for the anadromous life history have not yet been achieved, this trend appears unique relative to other regions throughout the Columbia Basin. Upper Yakima tributary streams continue to produce anadromous smolts with the greatest number originating in the mid-elevation tributaries, and fewest from low-elevation tributaries. Preliminary comparisons of productivity indices suggest the Teanaway Basin tributaries maintain high anadromous smolt productivity relative to low elevation tributaries and the main stem Yakima River. This suggests the Teanaway Basin should continue to be the focus of habitat preservation and improvement activities. Finally, we spent a considerable amount of time and effort in attempt to recover from the flooding events that occurred in the winter 2015/2016 that destroyed much of our monitoring infrastructure. Although much of the instream components of our interrogation system were destroyed during these flooding events, this did provide an opportunity to replace much of the instream equipment with improved designs that should improve the long-term performance of our monitoring network.

## ***Introduction***

The Steelhead Trout *Oncorhynchus mykiss* exhibit some of the most diverse life histories of any Pacific salmonid. Included in the diversity of this species is the variable expression of anadromous and resident life histories. The anadromous form may smolt and migrate to the ocean after one, two, three, or more years of residency in freshwater and the return to its natal stream after spending one or more years in the ocean. In contrast, the resident life history form, also known as Rainbow Trout, spends its entire life in freshwater. Our understanding of this species is further complicated by the fact that both forms can interbreed and produce offspring of the opposite type. While Steelhead in the Yakima Basin (Mid-Columbia Distinct Population Segment) are currently listed as threatened under the Endangered Species Act (ESA), the resident form, Rainbow Trout, currently provide one of the best wild trout fisheries in Washington State (Krause 1991; Probasco 1994). Despite the fact that both forms can interbreed when in sympatry, they are managed separately, and the diversity in life history expression complicates effective management of either form (Satterthwaite et al. 2009). The anadromous form is afforded federal protection under the ESA due to depressed abundance and poor adult returns. Management of the resident form is under the jurisdiction of Washington State in the Yakima River and is currently managed as a popular sport fishery. Catch and release fishing regulations for Rainbow Trout have been in effect for the main stem of the Yakima River (upstream from Roza Dam) since 1990 although Rainbow Trout in many tributaries to the Yakima River are open to lawful harvest under Washington State fishing regulations (2 fish over 10 inches in length can be harvested daily). The flexibility in life history expression is thought to provide significant resiliency in unstable environments, although it substantially complicates our ability to manage them and further complicates the recovery of the anadromous form which is mandated under the ESA.

The Yakima Subbasin Plan (Yakima Subbasin Fish and Wildlife Planning Board 2004) identified several key uncertainties and prioritized research needs consistent with Steelhead recovery in the Yakima Basin. In 2009, the Yakima Steelhead Recovery Plan was developed that addressed key uncertainties associated with Steelhead recovery in the Yakima Major Population Group (MPG; Conley et al. 2009). The Yakima Steelhead Recovery Plan was adopted by the National Marine Fisheries Service and was included in the Middle Columbia River Steelhead Distinct Population Segment ESA Recovery Plan (NMFS 2009). One key uncertainty identified for the upper Yakima Steelhead population is the relationship between

resident and anadromous life histories present in the basin. This is particularly important in the upper Yakima River because it supports a robust resident population (Temple et al. 2009) exhibiting some hatchery introgression (Campton and Johnston 1985) and the resident and anadromous forms are known to interbreed (Pearsons et al. 2007; Blankenship et al. 2009). The interplay between the resident and anadromous forms of *O. mykiss* deserves attention because it is poorly understood and there is a strong potential for the resident form to either contribute to, or to limit, the recovery of the anadromous form (Allendorf et al. 2001; Thrower et al. 2004; Kendall et al. 2014). In addition, the interplay between the forms has the potential to confound evaluation of Viable Salmonid Population (VSP) parameters (McElhany et al. 2000) including population level abundance, productivity, spatial structure, and diversity of the anadromous form (Moberg et al. 2005).

Remarkably, very little is known about the interactions between resident and anadromous forms of *O. mykiss* given the wide spatial distribution of the resident form and the generally depressed abundance of the anadromous form in the western United States. Furthermore, there are few locations in Washington State having abundance information generated for sympatric Rainbow Trout and Steelhead Trout (Scott and Gill 2008). In this study, we employ study methods to provide population level status and trend monitoring data for both life history forms in the upper Yakima River.

## ***Methods***

### **General**

The general conceptual design associated with this project is to use large scale Passive Integrated Transponder (PIT) tagging efforts of rearing *O. mykiss* and subsequent detection histories to partition the life histories into their respective anadromous or resident components. Rearing juvenile *O. mykiss* are tagged in their natal tributaries. The proportion of the tags that are detected at downstream locations during the smolt outmigration are assigned to the anadromous life history. The remaining tagged fish that are not detected as migrants are assigned to the resident life history. One complication is that multiple age classes of juveniles are collected and tagged during the tagging period, so the anadromous component from any tagging event in any given year may not be detected for several years post tagging. We address this issue by collecting scale samples from each juvenile fish tagged so we can assign each fish

to the appropriate cohort. In addition, genetic samples are collected from each fish at the time of tagging. Since nearly 100% of the adult Steelhead returning to the Upper Yakima are genetically sampled at Roza Dam, we use genetic parentage assignments to assign each anadromous smolt detected during each smolt migration to its respective parents. In cases that no parents are assigned, smolts are assigned to resident parents by default. The influence of the resident trout population on Steelhead production is of particular interest given the uncertainty surrounding this phenomenon. We describe the information generated from our tagging program in this report in the context of the VSP parameters abundance, productivity, spatial structure, and diversity for the upper Yakima *O. mykiss* population.

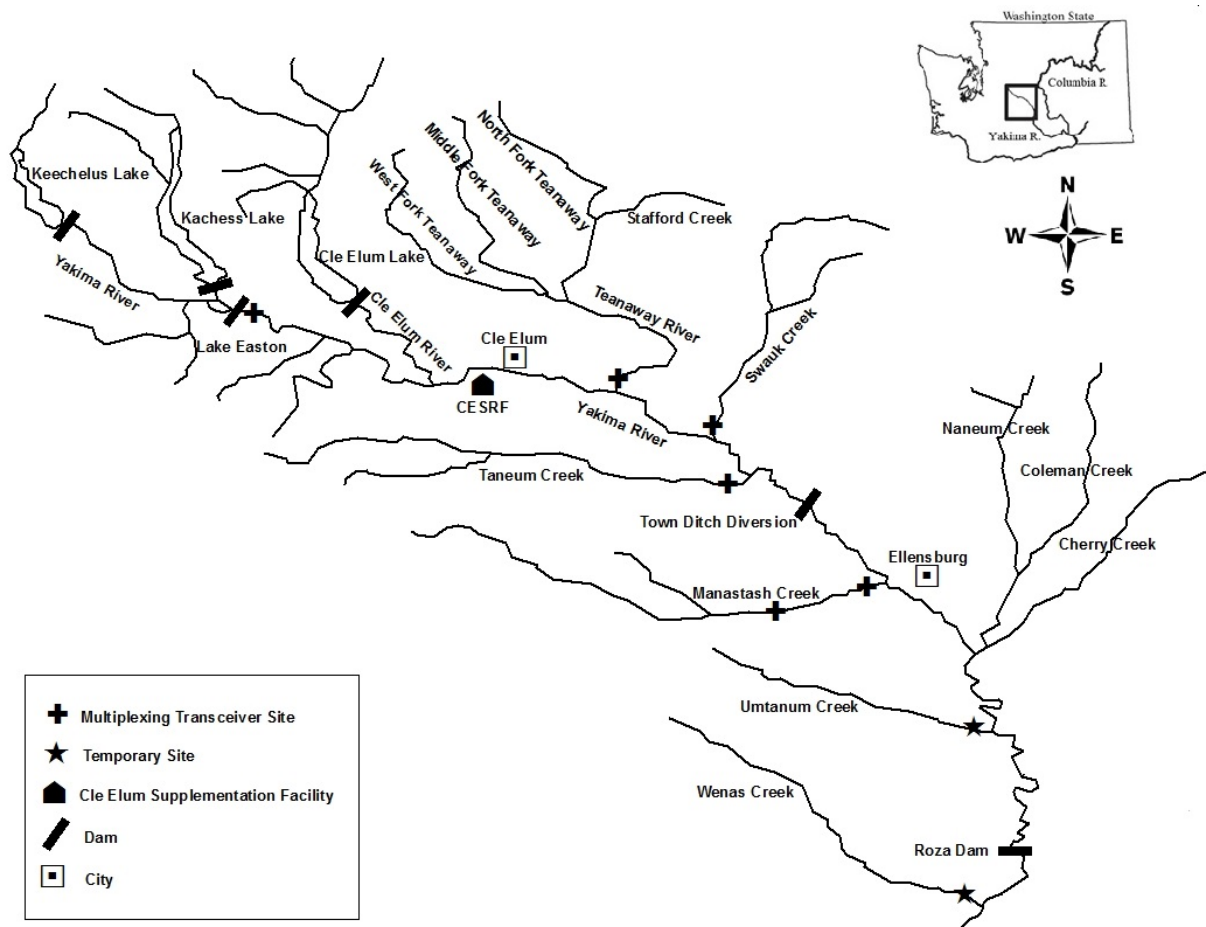


Figure 1. Map of the upper Yakima River basin and fish interrogation sites.

The upper Yakima River contains a unique monitoring infrastructure that complements our study (Figure 1). First, all migratory fish species entering the upper Yakima Basin must pass

through the Roza Adult Monitoring Facility (RAMF) to gain access to the basin. All fish entering the facility enter a fish trap where they can be biologically sampled and returned to the river to continue their upstream migration. The majority of the upper Yakima Steelhead population spawn upstream from Roza Dam in the spring such that fish enumerated in the facility represent ~80%-90% of the run escapement estimate for this population (Figure 1; Frederiksen et al. 2015). The remaining proportion of the upper Yakima Steelhead population spawn in Wenas Creek and Mainstem areas below Roza Dam. Although some fish ascend the dam in the fall (e.g., October) and overwinter in the upper Yakima, the majority of the spawning migration occurs in the spring (e.g., March). The annual run escapement is corrected for pre-spawn mortality to estimate annual spawner escapement. Detailed methods used to generate the total annual spawning escapement for the upper Yakima Steelhead population are presented in Frederiksen et al. (2015).

Juvenile abundance estimates in tributary streams were generated following backpack electrofishing methods as described in [monitoringmethods.org](http://monitoringmethods.org) ([method 118](#)). Juvenile abundance estimates in the main stem Yakima River were generated following drift boat electrofishing methods as detailed in [monitoringmethods.org](http://monitoringmethods.org) ([method 120](#)). Juvenile abundance estimates of rearing *O. mykiss* were partitioned into life history types following published protocols described in [monitoringmethods.org](http://monitoringmethods.org) ([protocol 2165](#)) utilizing recapture information of fish that have been previously PIT tagged ([method 1736](#)). Rearing *O. mykiss* juveniles were assigned to the appropriate age class using scale ageing techniques following methods published in [monitoringmethods.org](http://monitoringmethods.org) ([method 1360](#); [method 1090](#)). Genetic samples collected at the time of tagging (juveniles) or at Roza Dam (adult Steelhead) were combined in a Parentage Analysis to determine the maternal/paternal origins of the Steelhead smolt juvenile migrants. The WDFW Molecular Genetics Laboratory report is included as Appendix 1. Juvenile migrants that did not assign to at least one Steelhead parent were assumed to be the progeny of resident/resident matings by default.

### ***Abundance***

Adult VSP parameters generated during 2017 are described in detail in earlier chapters of this report and are incorporated here as appropriate (primarily the metrics associated with the

upper Yakima population). However, the detailed descriptions of the adult sampling were provided in earlier project annual reports (e.g., Frederiksen et al. 2016; [www.CBFish.org](http://www.CBFish.org)).

Juvenile abundance estimates were collected under the Yakima Klickitat Fisheries Project's Non-Target Taxa of Concern program (1995-063-25). Index monitoring sites were established upper Yakima Basin tributaries as early as 1990. Site selection criteria were described in detail in McMichael et al. (1992). Abundance estimates were generated using efficiency expansions to maintain consistency with historical data collection methods (Temple et al. 2011).

Gaining an understanding of the complex life history traits expressed in this population requires a substantial number of rearing juvenile *O. mykiss* be PIT tagged for subsequent monitoring. Our objective under this contract was to capture, sample, and release 10,000 juveniles in their natal streams throughout the upper Yakima Basin. Our annual target was to capture and tag a minimum of 1000 fish annually in tributaries, including Taneum Creek (TAN), Swauk Creek (SWK), Middle (MFT), and West (WFT) forks of the Teanaway River as well as the main stem Teanaway River (MST), Manastash Creek (MAN), and 4000 fish in the main stem Yakima River (YAK). We also tag 1000 fish annually in the North Fork Teanaway River (NFT) as well as several other smaller tributaries under a separate project (e.g., project 1995-063-25) but their bio-data and tagging histories prove beneficial to this project and thus are included here as appropriate. Finally, we contributed to the juvenile tagging effort in the Naches Basin during 2017 (Figure 2).

Fish are captured as rearing juveniles using backpack mounted electrofishing units using straight DC current. Captured fish are measured, weighed, PIT tagged, and a small genetic sample is collected and stored in ethanol. All sampled fish also have scale samples collected to facilitate age determination allowing for tracking cohorts. Scale samples are collected and placed in the vials containing each individual fish's genetic sample and stored at the Washington Department of Fish and Wildlife's Ellensburg District 8 Field Office until processed. The number and location of juvenile *O. mykiss* PIT tags deployed are presented in Figure 2.

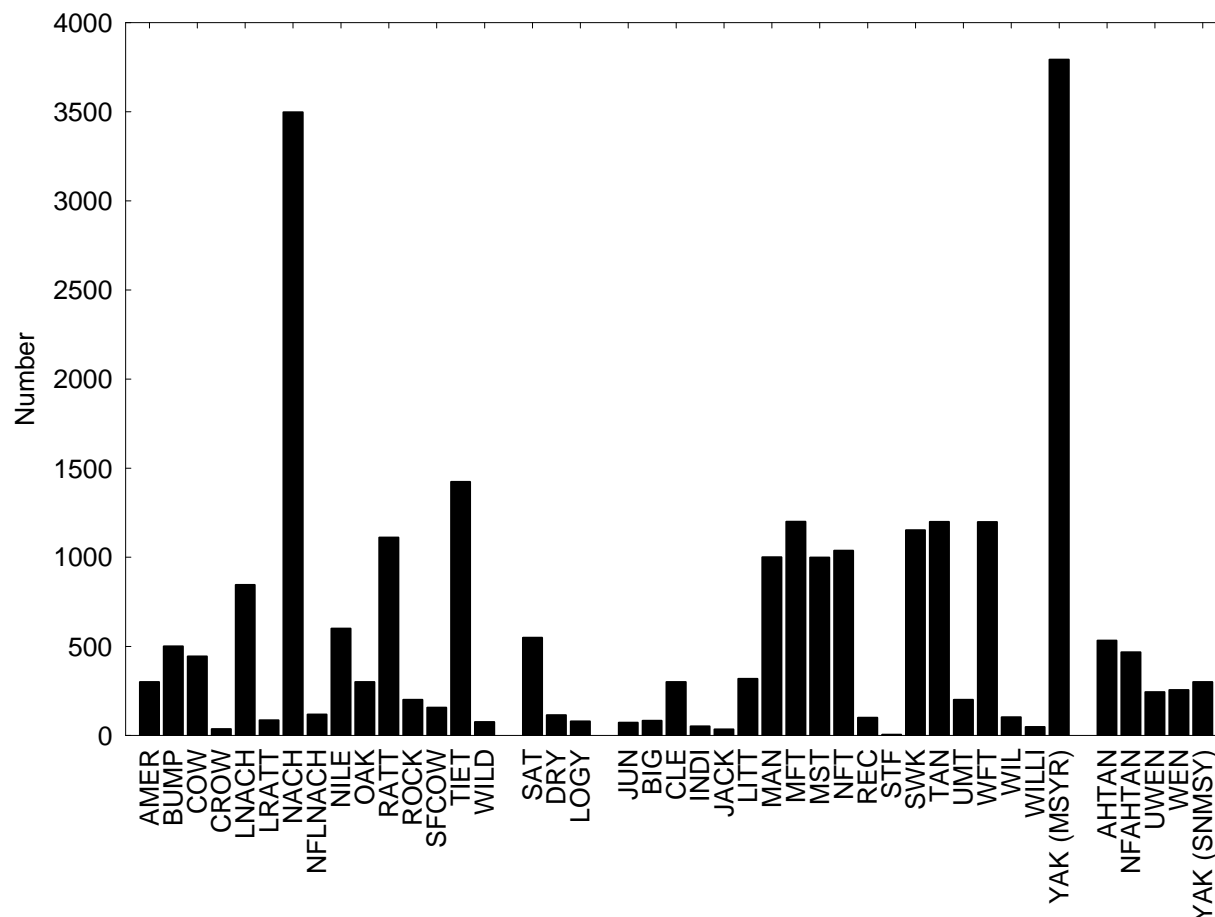


Figure 2. Number of *O. mykiss* PIT tagged in the Yakima Basin including the Naches, Satus, and Upper Yakima sub-basins, and Ahtanum and Wenas creeks in 2017. Stream abbreviations include: the American River (AMER), the Bumping River (BUMP), Cowichee Creek (COW), Crow Creek (CROW), Little Naches River (LNACH), Little Rattlesnake Creek (LRATT), the Naches River (NACH), North Fork Little Naches River (NFLNACH), Nile Creek (NILE), Oak Creek (OAK), Rattlesnake Creek (RATT), Rock Creek (ROCK), South Fork Cowichee Creek (SFCOW), the Tieton River (TIET), Wildcat Creek (WILD), Satus Creek (SAT), Dry Creek (DRY), Logy Creek (LOGY), Jungle Creek (JUN), Big Creek (BIG), the Cle Elum River (CLE), Indian Creek (INDI), Jack Creek (JACK), Little Creek (LITT), Manastash Creek (MAN), Middle Fork Teanaway River (MFT), Mainstem Teanaway River (MST), North Fork Teanaway River (NFT), Reecer Creek (REC), Stafford Creek (STF), Swauk Creek, (SWK), Taneum Creek (TAN), Umtanum Creek (UMT), West Fork Teanaway River (WFT), Wilson Creek (WIL), Williams Creek (WILLI), the main stem Yakima River (YAK(MSYR)), Ahtanum Creek (AHTAN), North Fork Ahtanum Creek (NFAHTAN), upper Wenas Creek (above the lake; UWEN), Wenas Creek (WEN), and the Yakima River (Sunnyside to Naches River confluence; YAK (SNMSY)).

Instream PIT tag interrogation sites were strategically located near the mouth of the major upper Yakima tributary streams (Figure 1) accessible to Steelhead in order to partition



tributary and mainstem Yakima River spawners ([protocol 2165](#)). Previous work indicated that very few Steelhead ascend Easton Dam so interrogation equipment was not installed on tributaries upstream from that point (Karp et al. 2009; Frederiksen et al. 2015). We did install a temporary pass through antenna in the adult ladder to verify our assumption. The U. S. Bureau of Reclamation (USBOR) irrigation reservoirs establish the upper limit for anadromous fish distribution in the upper Yakima Basin because they currently do not contain fish passage facilities, although this is subject to change in future years. Finally, numerous irrigation diversions located throughout the low elevation agriculture lands north of the city of Ellensburg, WA are thought to block anadromous fish passage so we did not install or operate fish monitoring equipment in them (Figure 1). Thus, the major tributaries currently accessible to Steelhead spawners are monitored utilizing instream PIT tag monitoring equipment, and spawners in the main stem Yakima River are estimated by subtraction. This allows us to identify important major and minor spawning areas throughout the basin.

The instream PIT tag interrogation sites were installed to detect fish movement timing and patterns. However, two unusually high water runoff events in November and December 2015 destroyed much of our instream detection equipment in the winter of 2015/2016 (Figure 3). Instream repair efforts were limited to the late summer and early winter periods when instream flow conditions were favorable for repair work. Since most of our instream arrays remain inoperable through this reporting period, we estimated movement, timing, and abundance using the information gained from the 2012-2014 telemetry study until instream arrays can be re-installed.

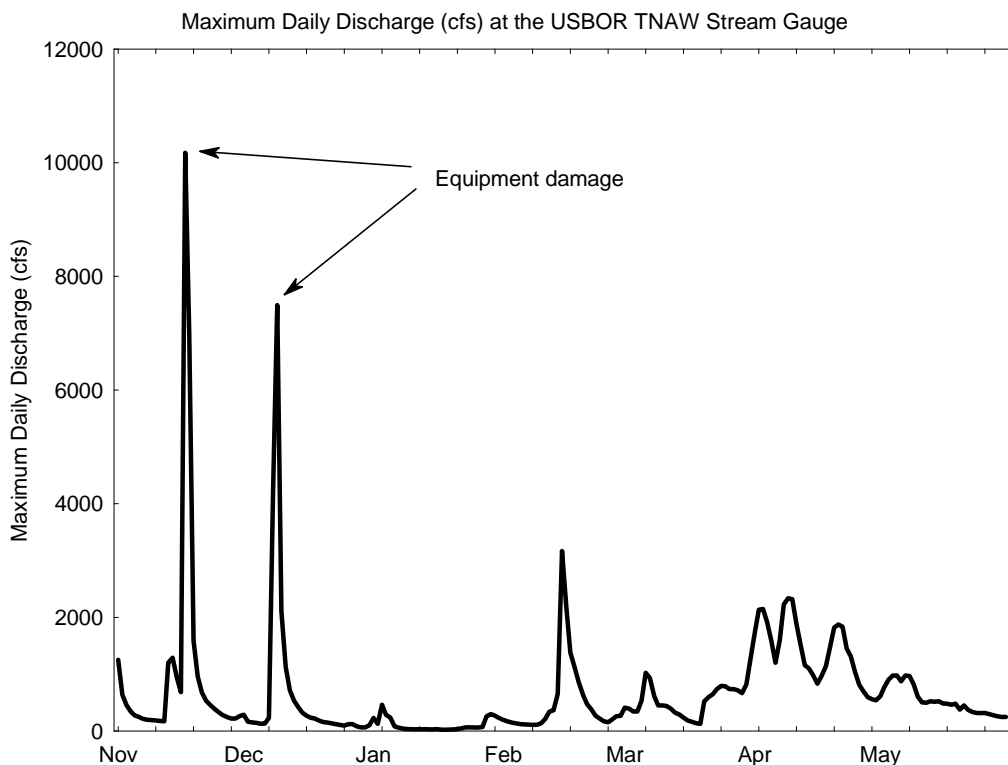


Figure 3. Maximum daily stream discharge (cfs) at the USBOR TNAW stream gauge.

Juvenile *O. mykiss* tagged during their rearing phase in the upper Yakima comprise a combination of anadromous and resident life histories which cannot be distinguished during field sampling prior to the smolt stage. As previously mentioned, we used PIT tag detection histories of *O. mykiss* collected at downstream locations to distinguish migrants from resident juveniles. Juvenile migrants generally display a bimodal emigration from the upper Yakima tributaries with peak emigration in the spring and the fall (Temple et al. 2015). Fish that were detected at downstream locations during the spring smolt migration, either in the Yakima (e.g., Prosser Dam), or at one of the main stem Columbia River interrogation sites were considered to be anadromous smolts. When smolts were identified, their genetic samples were assembled from the collections stored at the WDFW district 8 field office in Ellensburg, WA, and forwarded to the WDFW Molecular Genetics Laboratory for processing. Non-migratory fish samples are simply inventoried and banked for future use. One potential future use may be to process known resident fish samples (exuding gametes upon capture) to compare to the known Steelhead adult spawners to estimate the number of resident trout originating from anadromous parents, although there are no funds currently allocated to do so.

### ***Productivity***

Productivity metrics are generally presented as some measure of recruits produced per adult parent. Recruits can be of any life stage, but typically are adults or smolts. Productivity can be a difficult metric to quantify due to the numerous factors that can influence survival during the freshwater rearing, smolt migration, marine phase, and adult return. In addition, Steelhead are iteroparous so the spawners can have multiple spawning events over multiple years. The juvenile rearing phase can last from one to several years. Thus, a long time series of spawner and recruit data must be collected to ensure spawners and recruits are accounted for in productivity estimates. For example, we predominantly observe smolts that are one or two years of age although it is not uncommon to observe three- and four-year-old smolts. Smolts are aged using scale analysis to assign them to the appropriate brood year parents. This reduces the time series available to generate productivity estimates due to incomplete accounting of the progeny on the ends of the time series as fish from each outmigration are assigned to each brood year of parents.

We conducted small scale PIT tag retention studies to quantify the effect tag loss can have on survival and productivity estimates. Failing to account for tag loss in productivity estimates based on PIT tagged fish can have a profound effect on survival and productivity estimates. We used a dual tagging procedure (Bateman et al. 2009; Dieterman and Hoxmeir 2009; Meyer et al. 2011) conducted in unique tributaries each year 2013-2016 to estimate tag retention rates. We used coded wire tags (CWT; 2013) or Visual Implant Elastomer Tags (VIE; 2014-2016) for the secondary tag type because they are known to have high retention rates (Hale and Gray 1998). Briefly, *O. mykiss* were captured using electrofishing methods during routine tagging surveys during summer low flow conditions, measured (mm) and weighed (g), and marked following standard PIT tagging procedures (Prentice et al. 1990) and either a CWT injected in the dorsal musculature (2013) or a VIE tag injected in the adipose eye tissue (2014-2016). Dual tagged fish served as the tag subjects for the mark group. Recapture sampling was conducted at discreet time intervals following release of tagged fish and ranged from 24h to 365 days. Tag loss was computed as the ratio of the number of recaptured fish possessing only a CWT or a VIE tag without a corresponding PIT tag to the initial group of dual tagged fish released into each tag site.

Generating juvenile migrant abundance is a difficult task. Low juvenile detection probabilities associated with our monitoring infrastructure coupled with low production stemming from depressed population sizes makes generating juvenile migrant estimates difficult. Others have attempted to circumvent these issues by considering recruitment to the returning adult stage. However, as previously discussed, several factors influence survival to the adult stage, so estimating productivity based upon adult returns is also a difficult task. We are attempting to work through these considerations to generate productivity estimates for the upper Yakima population (Frederiksen et al. 2015).

We generated indices of steelhead smolts per adult anadromous steelhead spawner for the major upper Yakima tributaries and for the main stem Yakima River. Smolts were defined as migrants detected leaving the Yakima basin, and spawners were estimated from the 2012-2014 telemetry study. These indices should be considered minimum estimates because they do not account for detection efficiencies at downstream locations. However, these indices can be considered relative measures allowing comparisons of the minimum number of smolts produced per steelhead adult spawner for upper Yakima tributaries and main stem areas.

### ***Spatial Structure***

The spatial distribution of *O. mykiss* in the upper Yakima basin are reported under routine monitoring under the Yakima/Klickitat Fisheries Project (YKFP; 1995-063-25). Utilization (spatial distribution) in tributary streams is monitored via long term 200m long index monitoring sites following electrofishing protocols (Temple and Pearsons 2007). Under the monitoring prescriptions for *O. mykiss* established under the YKFP, tributaries are considered utilized when a minimum of 2 or more individuals occupy any given site. When these minimum utilization criteria are met, the spatial distribution is extrapolated to the stream scale based upon the area that any individual site represents. We began baseline data collection activities in 1990 and have a robust dataset for monitoring trends in spatial distribution. Our monitoring to date suggests *O. mykiss* spatial distribution remains stable in the Upper Yakima and substantial change in utilization has not been detected over the time series of data we have.

Spatial distribution in terms of National Oceanic and Atmospheric Administration's (NOAA) recommendations (e.g., spawner distribution; Crawford and Rumsey 2011) is not calculated for the Upper Yakima because we do not collect spawning information for the large

resident population or for Steelhead adults. This is due to low adult counts (for Steelhead) and the large geographical area encompassing potential spawning locations (i.e., proverbial needle in haystack). The Steelhead spawning distribution for the upper Yakima population is inferred from PIT tag interrogations from our detection arrays at the mouth of each tributary, and in the main stem Yakima River by subtraction. As previously mentioned, equipment malfunctions prevented us from using PIT tag detections to estimate the spawning distribution in 2017, so we used the average proportion of the total run escapement (Roza dam count) apportioned to each tributary as derived from the observations from the previous years.

### ***Diversity***

We report only the status and trend in diversity metrics for naturally produced *O. mykiss* because as previously noted, the upper Yakima is composed predominantly of wild fish, and straying of hatchery origin fish into the Upper Yakima is generally very low. Because of the enormous variability of *O. mykiss* diversity metrics, observed change within these variables may reflect natural variation, rather than change in the diversity metrics. For instance, recent work suggests that *O. mykiss* can spawn during any month of the year in different locales, and that appears to be driven in large part by environmental factors (Bill McMillan, Personal Communication). Thus substantial change in spawn timing may actually reflect the species true plasticity and natural variation for this diversity metric. Detecting small significant changes to highly variable metrics is a difficult task, and generally result in statistical tests with low power (Ham and Pearsons 2000). Other diversity metrics currently monitored include adult spawn timing and distribution of anadromous fish that are radio tagged, age structure of returning anadromous adults, age structure of tributary rearing fish, length at age differences between life histories, and sex ratios of adults sampled at Roza Dam (collected via ultrasound). We also address the long term diversity monitoring strategy (Crawford and Rumsey 2011) by collecting genetic tissue samples on adult Steelhead returning to Roza dam. In addition, genetic samples have been collected and processed intermittently (e.g., prior to this project) for *O. mykiss* in the upper Yakima Basin providing long term genotypic trend monitoring information for the rearing population (e.g., Campton and Johnston 1985).

PIT tagging a large number of juveniles in their natal streams as juveniles has many advantages. For instance, the diversity indices for several variables for the combined resident

and anadromous *O. mykiss* population, as well as each independent life history can be evaluated. Several interesting and important life history characteristics arising from the juvenile tagging studies are described in this report.

## ***Results***

### **General**

Juvenile migrant monitoring within the upper Yakima Basin is somewhat limited by low detection efficiency of instream PIT tag arrays for small fish. For example, in March of 2015, 11,568 PIT tagged spring Chinook salmon were volitionally released from the Jack Creek Acclimation Facility in the North Fork Teanaway (NFT) River. Of those, 1568 were detected on our lower Mainstem Teanaway River instream PIT tag array (13.6%). Knowing that this group of fish passed our North Fork Teanaway River instream PIT tag array, we used the PTagis database to determine that 289 of the fish detected at the Lower Mainstem Teanaway (LMT) site were also detected at the NFT site. The time stamps of the detections at both locations indicated the travel time between the two sites was relatively short on average (4.5hours), although one fish took as long as 64 days to migrate out of the system. The ratio of fish detected vs. those undetected at the NFT site indicated the juvenile detection efficiency following the acclimation release and subsequent downstream migration was approximately 18% illustrating that the juvenile detection efficiencies at this site were quite low. However, the LMT site is being reinstalled with an improved equipment design that we anticipate will significantly improve our detection efficiencies.

To estimate instream PIT tag array juvenile detection efficiencies for Steelhead migrants, we used downstream detections to back calculate detection efficiency of the tributary arrays. Using incidental detections at the Roza Dam, we back calculated the juvenile detection efficiencies for our instream arrays (Table 1). We used the Roza Dam detections due to the proximity to the other instream arrays (Figure 1). Our estimates of detection efficiencies for Steelhead migrants were much improved over those estimated for our Spring Chinook hatchery release. However, we caution that the sample sizes are low for *O. mykiss* (Table 1). Finally, we acknowledge there is still opportunity for improved operations and maintenance to increase the performance of our instream PIT tag arrays for juvenile abundance monitoring although they

have proven useful for generating information on other juvenile monitoring metrics, and for adult monitoring (e.g., migration timing, migration duration, environmental conditions favoring outmigration, species detections, etc).

Table 1. Interrogation site average juvenile *O. mykiss* detection efficiency for fish detected at Roza Dam that were also previously detected the North Fork Teanaway River (NFT), Swauk Creek (SWK), Taneum Creek (TAN), or Lower Mainstem Teanaway (LMT) instream arrays.

Stream	Roza Detections	Array Detections	Efficiency
NFT	8	4	0.50
SWK	8	8	1.0
TAN	7	7	1.0
LMT	20	11	0.55

One of our objectives in monitoring Steelhead status and trends in population abundance is to use our PIT tag infrastructure to determine the spatial distribution and abundance of adult Steelhead spawners in the Upper Yakima population. The radio telemetry study conducted between 2012 and 2014 was used to validate the use of our PIT tag infrastructure to estimate the Steelhead spawning distribution and abundance by tributary. For adult spawner abundance in the upper Yakima, detections of radio tagged adults (that were also PIT tagged) at our PIT tag arrays were compared to the radio-telemetry mobile tracking detections that were conducted 2012-2014 to determine the detection rate of the PIT tagged individuals at our fixed monitoring sites. Fish that were known to have spawned in multiple streams were used to calculate array detection efficiencies for every interrogation site they were known to have passed. The tributary adult spawner abundance estimate was generated for each tributary by expanding the PIT tag detections upstream from each PIT tag array by the detection efficiency estimated at each array (from detections of radio tagged Steelhead; Table 2). The general agreement between the PIT tag array detections and the radio-telemetry verification suggest the fixed site PIT tag arrays can be used to estimate spawner abundance and distribution with reasonable accuracy (Table 2).

Because the majority of our detection infrastructure was not operational during the 2017 spawning migration, we used the average apportionment of the Roza Dam run escapement based upon the radio telemetry study conducted 2012-2014 to partition the run escapement estimate to major tributaries in the upper Yakima (Table 3). Run escapement to the main stem Yakima River (and unmonitored tributaries) was estimated as the difference between the total 2016/2017

Roza adult Steelhead count and the sum of the estimated tributary escapement. The annual run of wild adult Steelhead migrating upstream from Roza Dam was estimated to be 247 during the 2016/2017 spawning migration ([www.YKFP.org](http://www.YKFP.org)).

We used the Taneum Creek and the Manastash Creek instream PIT tag arrays to help validate our run apportionment for 2016/2017 based on the Radio Tag study conducted 2012-2014. Briefly, we estimated the instream PIT tag array detection efficiency for Manastash Creek and for Taneum Creek in 2017 following Connolly (2010). In Manastash Creek, we used the upstream detection array to estimate the downstream detection array efficiency, and the number of unique tags detected at the lower detection array were expanded into the spawning escapement estimate using the calculated detection efficiency. The PIT tag based estimate was compared to the telemetry based estimate as a gauge on the accuracy of using telemetry based apportionment of the Roza Count to index spawner escapement into Manastash Creek (Table 3). The difference between the estimates was 9 fish representing a 22% difference. Using a similar approach in Taneum Creek (following Connolly 2010), we used upstream and downstream antenna detections of unique PIT tags and estimated a total system efficiency of 87.5%. Thus, expanding the total number of unique tags detected, the PIT tag based spawner escapement estimate was 32 fish. This also represented a 9 fish difference between the PIT tag based spawner escapement estimate and the telemetry based apportioning of the Roza Dam count (Table 3). This suggests that using a fixed apportionment of the Roza Dam Steelhead count to estimate tributary and main stem spawner escapement may produce biased estimates so we caution readers and we acknowledge our telemetry based estimate should be regarded as an index and may not be accurate in years that instream PIT array detections are not available.

Table 2. Detections of adult Steelhead that are double tagged (PIT tagged and Radio Tagged) and the adult detection efficiencies estimated during the spring spawning migration in 2014 in each tributary in the Upper Yakima that has an in stream PIT tag detection array.

Stream	Radio tag detections	Radio and Pit tag detections	Detection efficiency	Pit tag Detections (n)	Expanded Estimate	Percent of total run
Swauk Creek	5	5	1	47	47	12.5
Taneum Creek	6	6	1	62	62	16.5
Main stem	14	8	0.57	15	62	7
Teanaway River						
North Fork	6	4	0.67	34	51	13.6



Teanaway Upper Main stem Teanaway River (West and Middle Fork)	8	8	1	60	60	16
Manastash Creek	1	13	1	13	13	3.5
Umtanum Creek	1	1	1	1	1	0.3
Wilson Creek	3	NA	NA	NA	NA	NA

Table 3. Roza Dam expansion factors and spawner escapement indices based upon the apportioning of the Roza dam count using the Radio Telemetry data collected 2012-2014. The PIT tag based spawner escapement estimate generated for Taneum Creek and Manastash Creek in 2017 is presented for comparison. The difference between the index and the estimates is also included for comparison (the relative percent difference is in parenthesis).

Stream	Expansion Factor	Spawner Escapement Index	PIT Based Estimate	Difference
Swauk Creek	0.125	31		
Taneum Creek	0.165	41	32	9 (22%)
Mainstem Teanaway River	0.07	17		
North Fork Teanaway	0.136	34		
Upper Mainstem Teanaway River (West and Middle Fork)	0.16	40		
Manastash Creek	0.035	9	18	9 (-100%)
Umtanum Creek	0.003	1		
Mainstem Yakima and Unsampled Tributaries	Roza – Tributary Escapement	76		
Total Run Escapement		247		

## *Abundance*

Hatchery Steelhead have not been released in the upper Yakima Basin since 1993 and the releases in the early 1990's were relatively small and experimental in nature. Thus, status and trend monitoring under this contract is directed at the upper Yakima River wild population although we do observe a very small number of hatchery strays annually (Figure 4). With the exception of a short winter maintenance period, nearly a complete census of the adult brood year return is collected at Roza Dam during each return year. The geometric mean adult return for the Upper Yakima population as of the most recent status assessment was 246 adults. However, recently, there appears to be an increasing trend in annual wild adult return numbers (Figure 4).

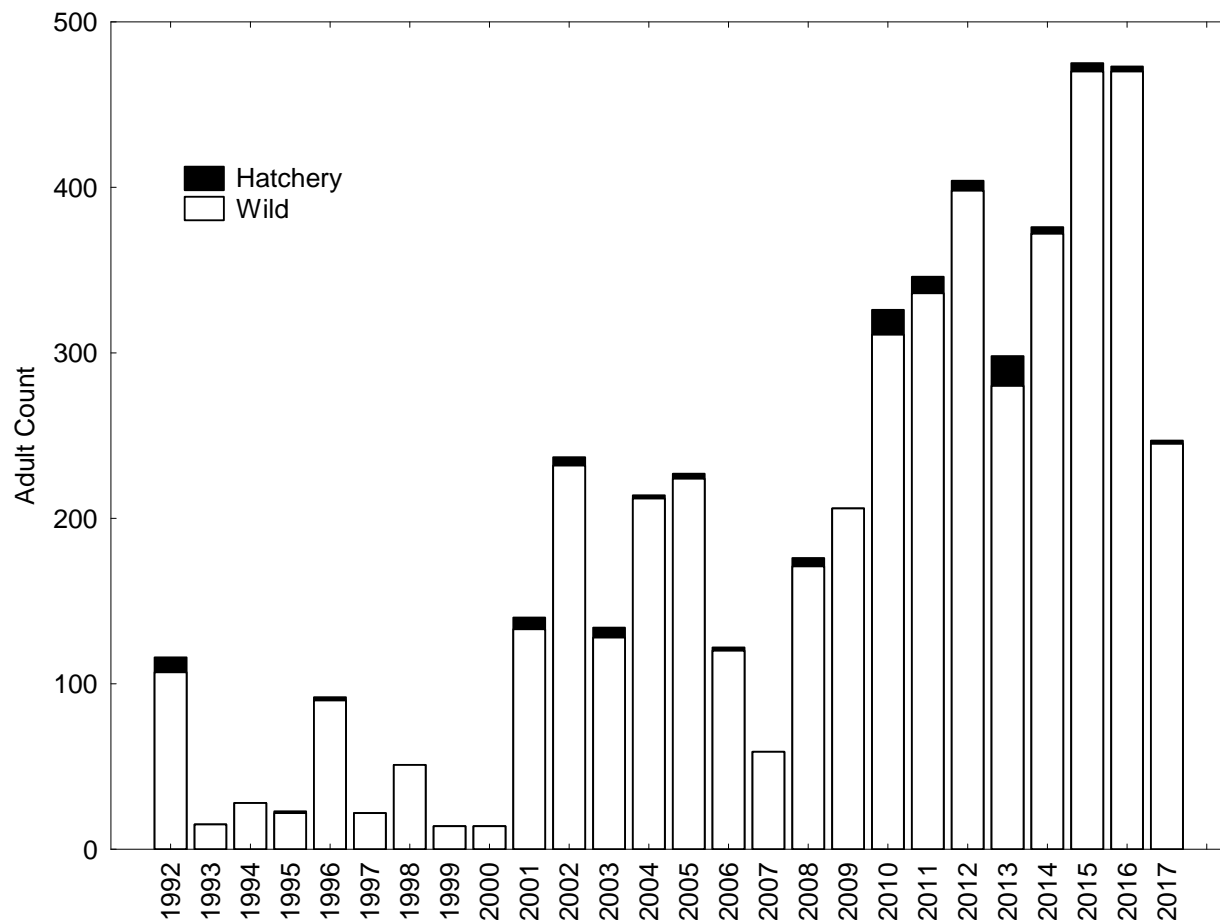


Figure 4. Number of hatchery and wild origin Steelhead adults passing Roza Dam during the annual adult spawning migrations.

It appears the adult Steelhead returns to the Yakima major population group (MPG) are faring well relative to other regions throughout the Columbia Basin (Figure 5). The Prosser Dam

count of wild adult Steelhead (all 4 Yakima populations combined) presented as a proportion of the wild Steelhead count at Bonneville Dam indicates a positive abundance trend since 1995. A similar pattern is observed for the upper Yakima Steelhead population passing upstream from Roza Dam. However, the upper Columbia River region (Priest Rapids Dam count: not differentiated by hatchery or wild origin) and lower Columbia between Bonneville and McNary Dams do not appear to be following the same trajectory. The Snake River region (Ice Harbor Dam count) does indicate an increasing trend but has remained fairly level for the last several years. While the reason for this increase is unknown, it has been the focus of recent discussion. Despite the increasing wild adult trends in the Yakima Basin, there is still significant progress to be made to meet the recovery goals that have been established (Conley et al. 2009; Figure 6).

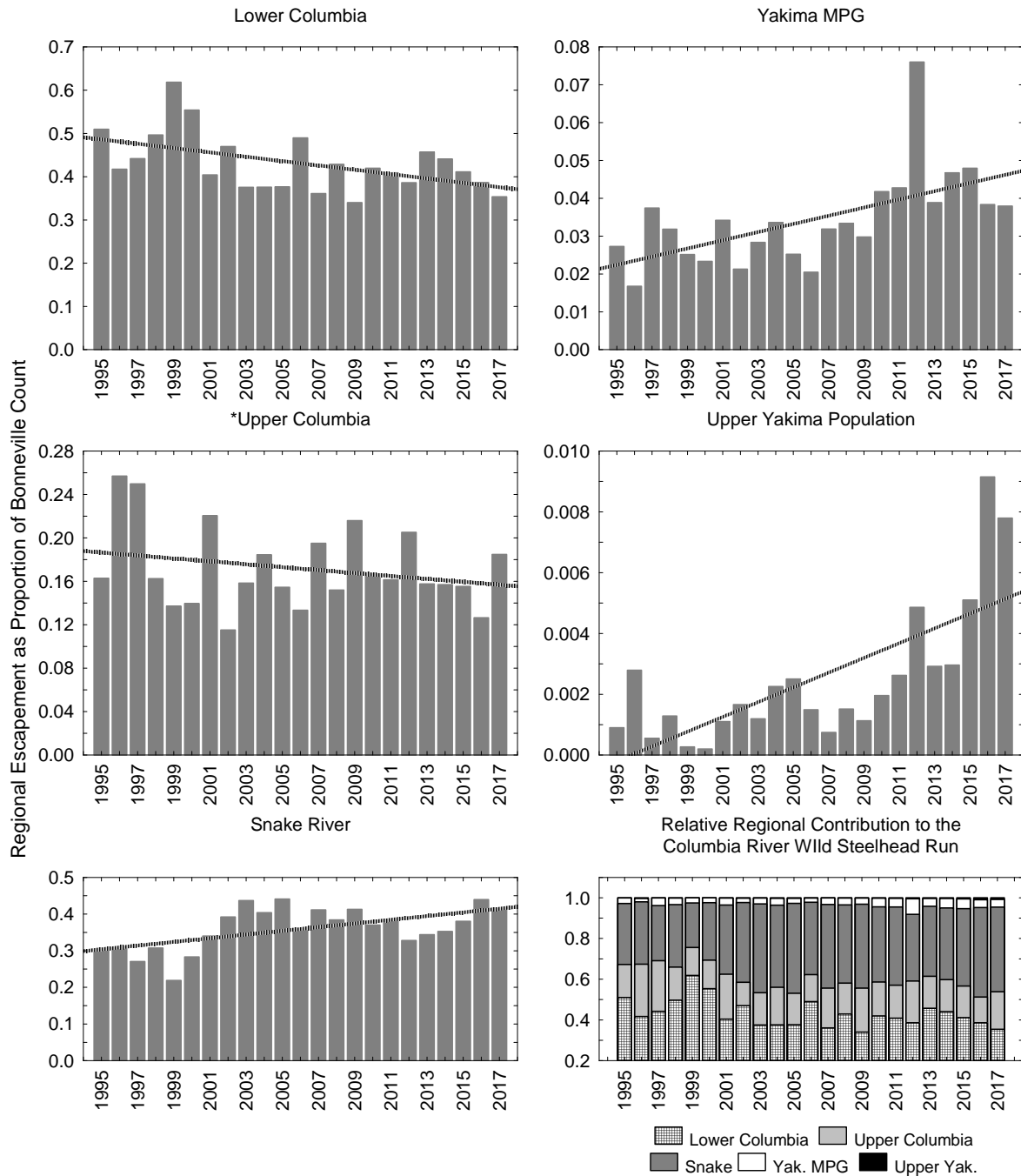


Figure 5. Annual trends in wild Steelhead returns in the various Columbia River Regions as a proportion of the Bonneville Dam Count. The Lower Columbia region depicts difference in the Bonneville and McNary dam counts and therefore does not include populations below Bonneville Dam and should be considered incomplete. The asterisk indicates a complete count, not differentiated by hatchery or wild origin. The dashed lines represent the best fit line.

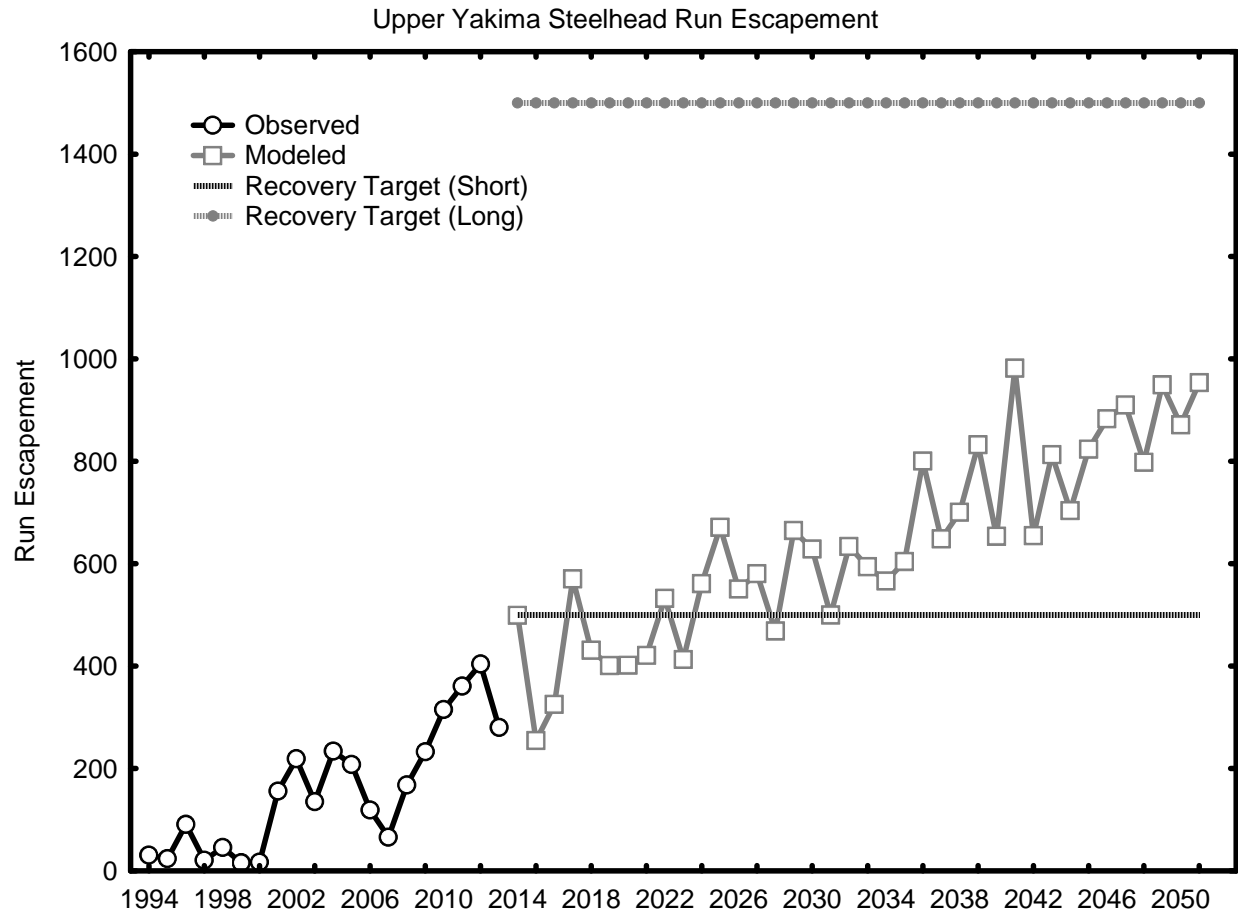


Figure 6. Observed and modeled annual summer Steelhead run escapement into the Upper Yakima. The short term and long term recovery targets are presented as dashed lines.

The population abundance of *O. mykiss* is highly variable from year to year in Yakima River tributary streams (Figure 7). We observed increased abundance in all monitored tributaries in 2017 relative to the previous year. The slope of the best fit trend lines were used to determine if the *O. mykiss* population in each stream is increasing, decreasing, or remaining stable. All of the core long term monitoring tributary streams had abundance trajectories with positive slopes, two of which were significant (North Fork Teanaway  $P = 0.16$ ; Swauk Creek,  $P = 0.01$ ; Taneum Creek  $P = 0.34$ ; Middle Fork Teanaway River  $P = 0.08$ ; West Fork Teanaway River  $P = 0.007$ ; Mainstem Teanaway River  $P = 0.08$ ). The Taneum Creek *O. mykiss* population abundance is also highly variable from year to year although the population appears stable. Migrant production appears loosely correlated with total *O. mykiss* abundance in each stream in some cases (Figure 8).

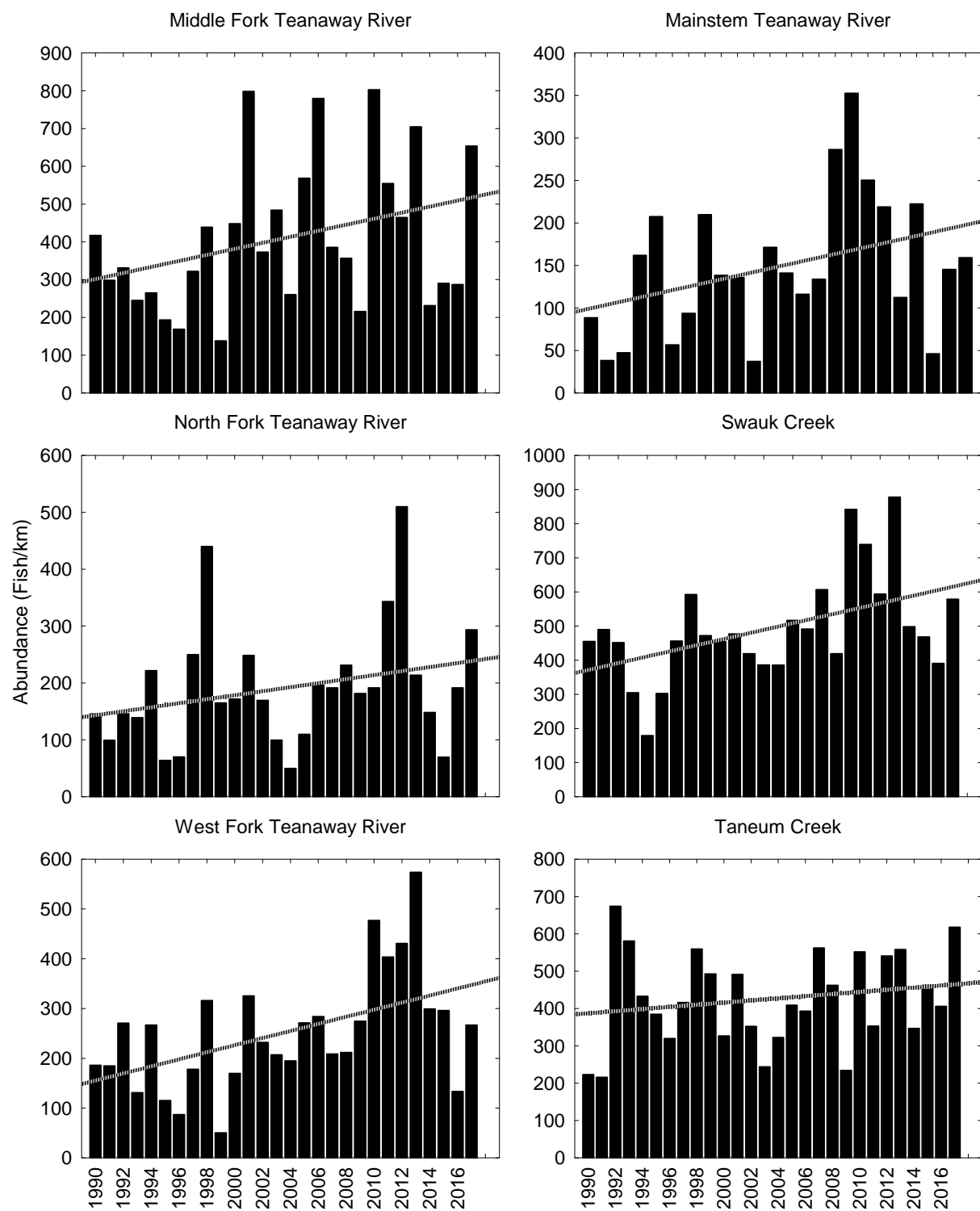


Figure 7. Annual population abundance of *O. mykiss* in core upper Yakima tributary streams. The dashed lines in the individual stream panels represent the best fit trend line.

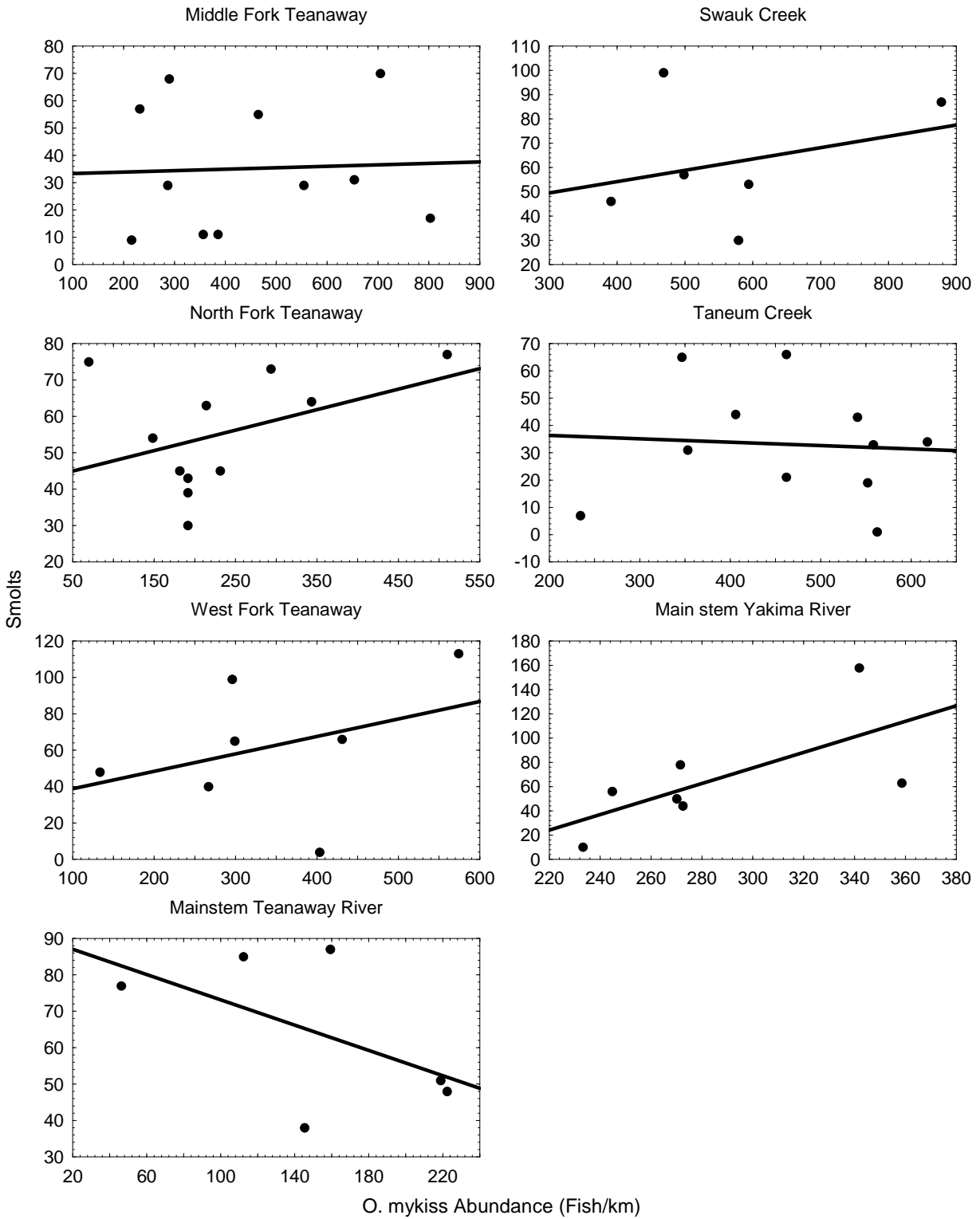


Figure 8. Relationship between total annual *O. mykiss* abundance (Age 1 in the main stem Yakima River) and the number of smolts detected annually in the upper Yakima Basin. Bird mortalities are not yet included as smolts.

### ***Productivity***

A recent description of Yakima Basin Steelhead population productivity is presented in Frederiksen et al. (2015). Additionally, we have made some interesting observations based upon our juvenile tagging data. For instance, we have been able to make relative comparisons of smolt production from upper Yakima tributaries using PIT tag detections. The absolute number of migrants originating in various tributaries that were detected emigrating from the upper Yakima Basin in 2017 are presented in Figure 9, and as a percentage of the tags deployed in Figure 10. Consistently, we observe that the Teanaway Basin produces a larger number of Steelhead trout migrants relative to other upper Yakima Tributaries although the basin consists of 3 major tributaries and a main stem, as well as numerous smaller streams. In contrast, Manastash Creek generally only produces a small number of migrants. Until the fall/winter of 2016, Manastash Creek had irrigation diversions in place that were thought to be complete migration barriers to adult Steelhead Trout. Thus, smolt production in this stream has been attributed to resident trout spawning, which is currently supported by the genetic parentage analysis. The last significant irrigation diversion remaining in Manastash Creek, was removed during 2016 and the entire stream network is now open to anadromous passage. We now have the opportunity to monitor repopulation of an anadromous life history in this system. The absolute number of migrants originating in various tributaries that were detected emigrating from the Naches Basin in 2017 are presented in Figure 11, and as a percentage of the tags deployed in Figure 12.



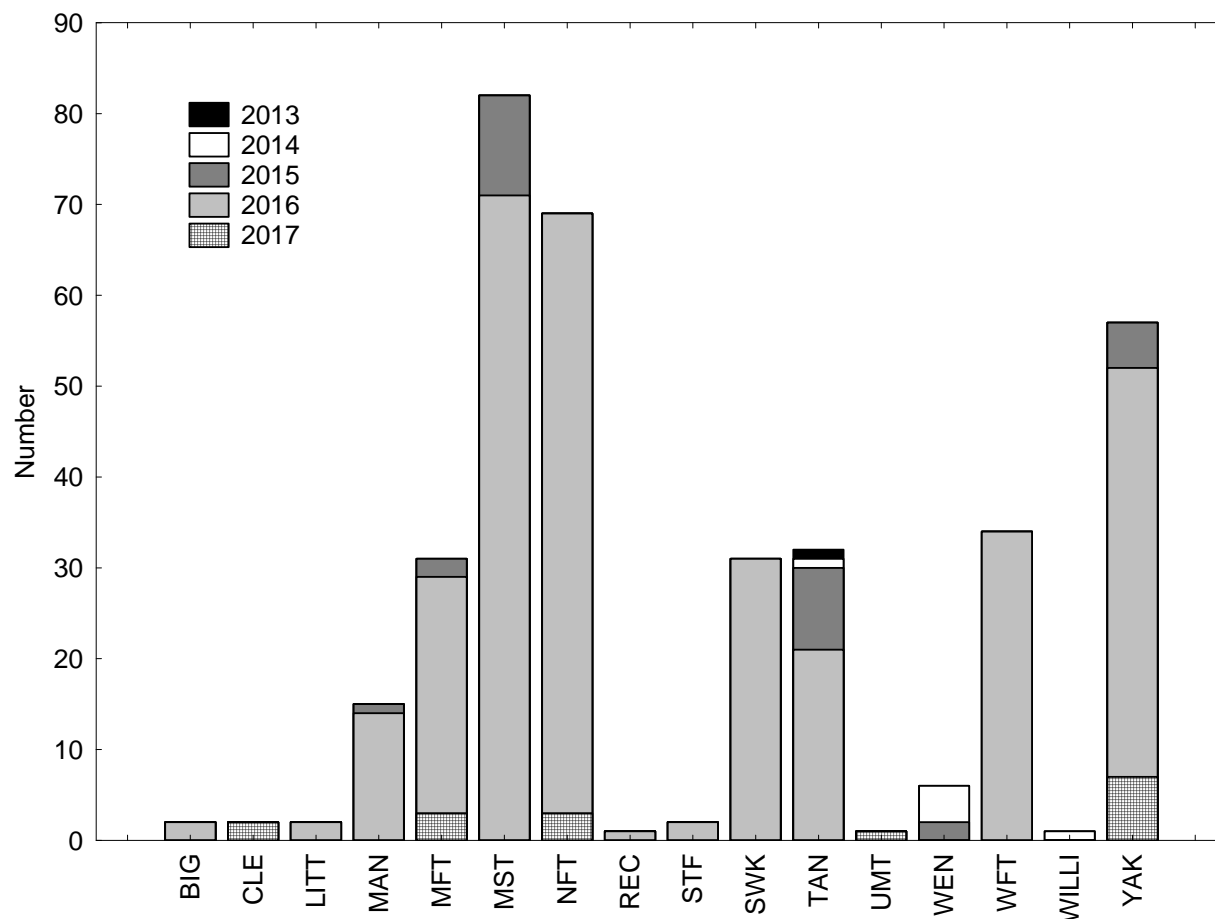


Figure 9. Number of smolts detected during the 2017 spring outmigration and the year they were tagged as juveniles in upper Yakima streams including Big Creek (BIG), the Cle Elum River (CLE), Little Creek (LITT), Manastash Creek (MAN), Middle Fork Teanaway River (MFT), Mainstem Teanaway River (MST), North Fork Teanaway River (NFT), Reecer Creek (REC), Stafford Creek (STF), Swauk Creek (SWK), Taneum Creek (TAN), Umtanum Creek (UMT), Wenas Creek (WEN), West Fork Teanaway River (WFT), Williams Creek (WILLI), and the Yakima River main stem (YAK).

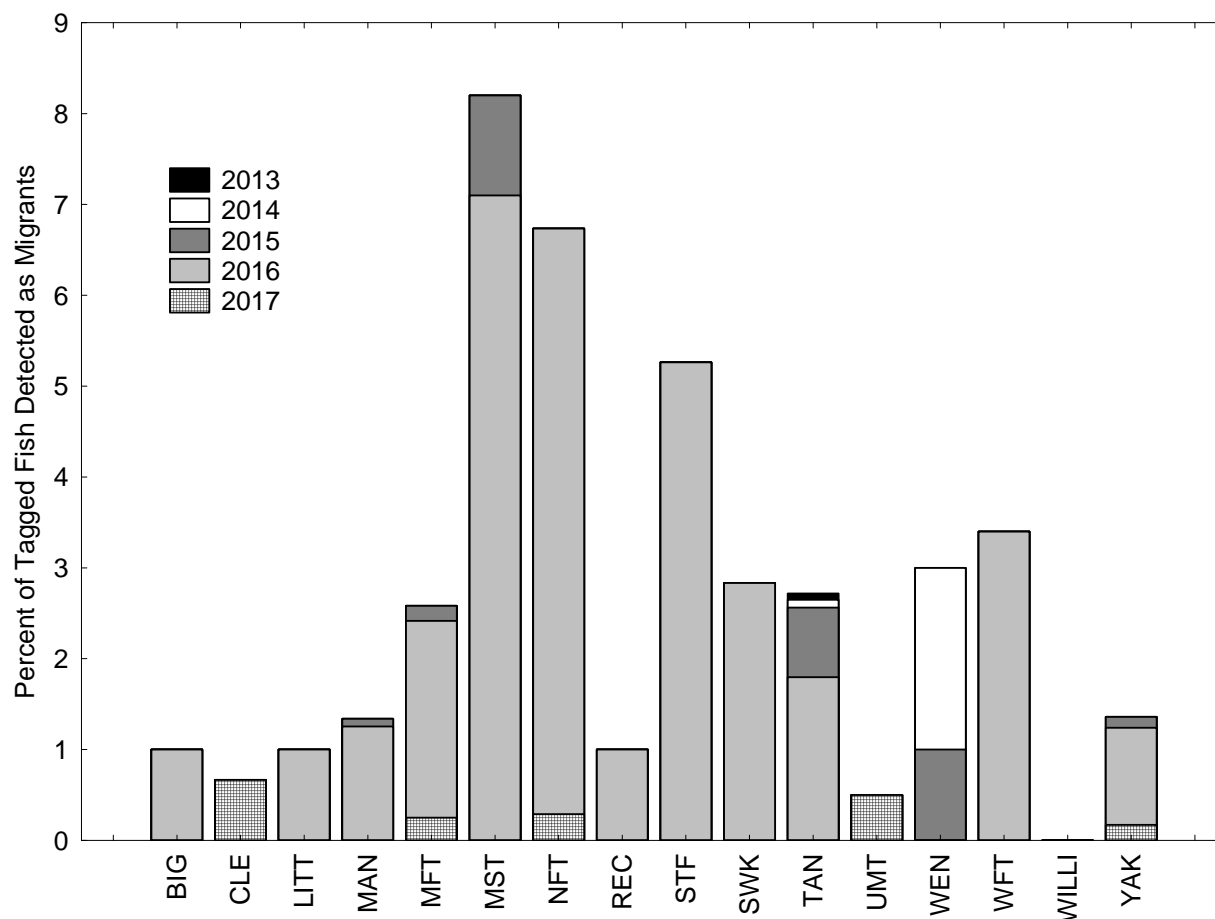


Figure 10. Percent of *O. mykiss* tagged in select streams that were detected as migrants during the 2017 outmigration. Stream abbreviations include: Big Creek (BIG), the Cle Elum River (CLE), Little Creek (LITT), Manastash Creek (MAN), Middle Fork Teanaway River (MFT), Mainstem Teanaway River (MST), North Fork Teanaway River (NFT), Reecer Creek (REC), Stafford Creek (STF), Swauk Creek (SWK), Taneum Creek (TAN), Umtanum Creek (UMT), West Fork Teanaway River (WFT), Williams Creek (WILLI), and the Yakima River (YAK) by year when they were tagged.

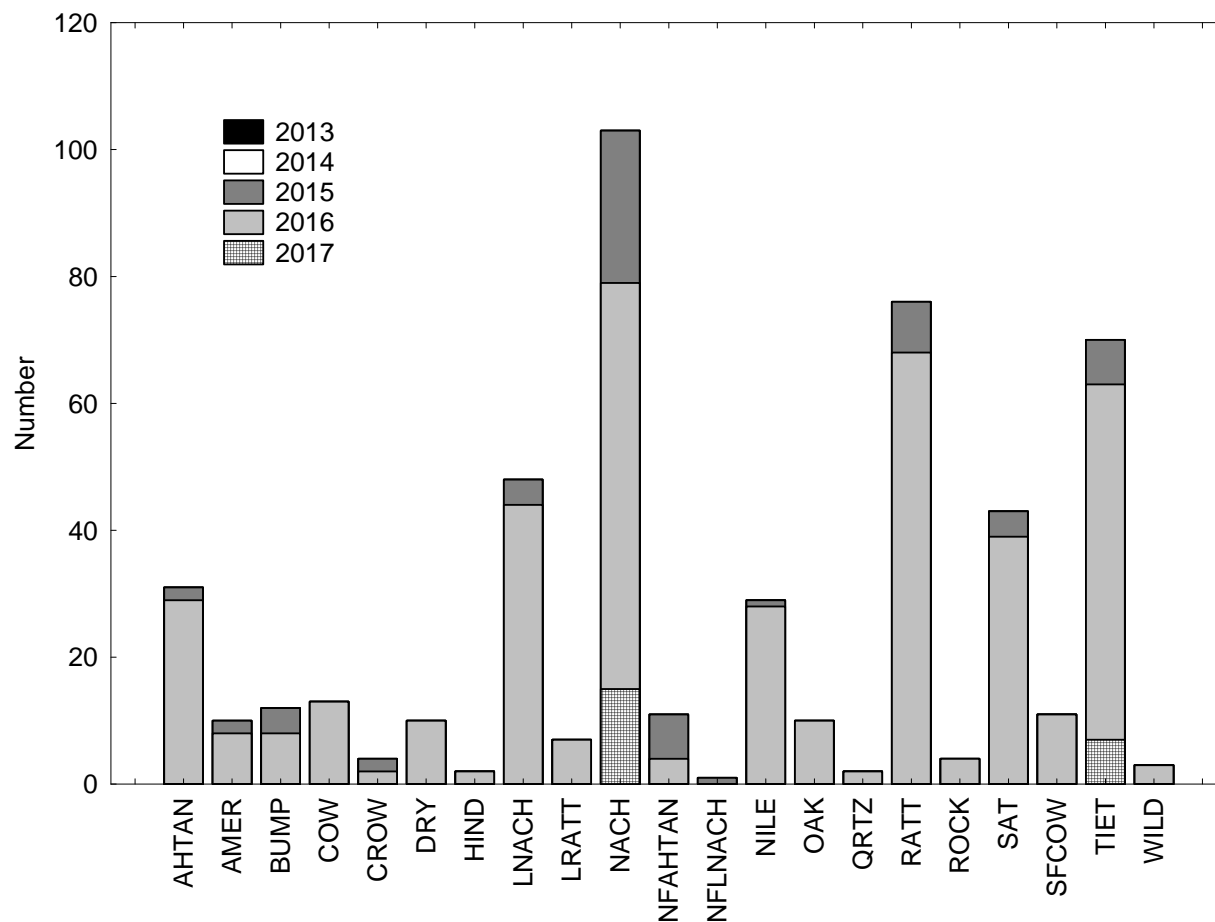


Figure 11. Number of smolts detected during the 2017 spring outmigration and the year they were tagged as juveniles in streams in the Naches Basin including Ahtanum Creek (AHTAN), the American River (AMER), Bumping River (BUMP), Cowichee Creek (COW), Crow Creek (CROW), Dry Creek (DRY), Hindoo Creek (HIND), Little Naches River (LNACH), Little Rattlesnake Creek (LRATT), Naches River (NACH), North Fork Ahtanum (NFAHTAN), North Fork Little Naches River (NFLNACH), Nile Creek (NILE), Oak Creek (OAK), Quartz Creek (QRTZ), Rattle Snake Creek (RATT), Rock Creek (ROCK), Satus Creek (SAT), South Fork Cowichee Creek (SFCOW), Tieton River (TIET), and Wildcat Creek (WILD).

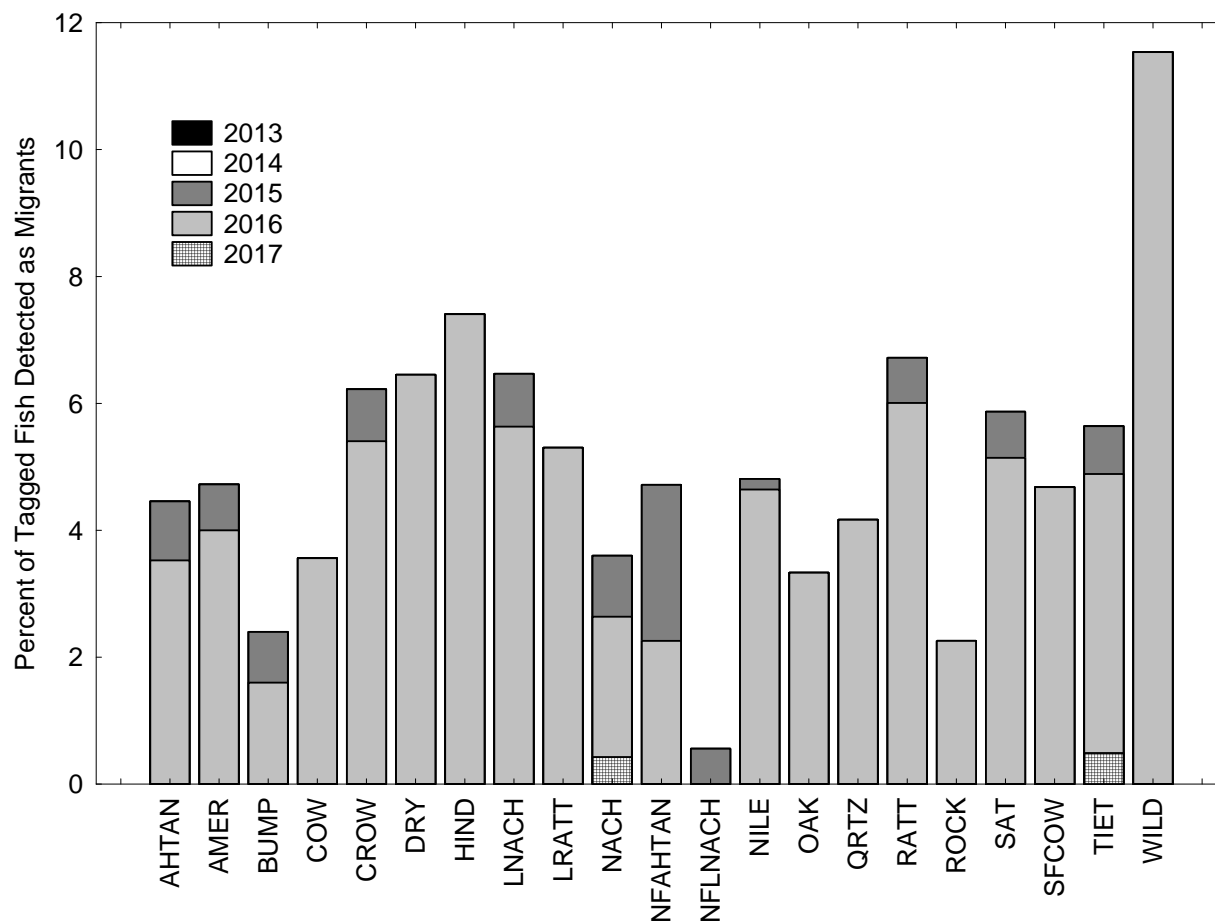


Figure 12. Percentage of *O. mykiss* detected during the 2017 outmigration that were tagged in streams in the Naches Basin including Ahtanum Creek (AHTAN), the American River (AMER), Bumping River (BUMP), Cowichee Creek (COW), Crow Creek (CROW), Dry Creek (DRY), Hindoo Creek (HIND), Little Naches River (LNACH), Little Rattlesnake Creek (LRATT), Naches River (NACH), North Fork Ahtanum (NFAHTAN), North Fork Little Naches River (NFLNACH), Nile Creek (NILE), Oak Creek (OAK), Quartz Creek (QRTZ), Rattle Snake Creek (RATT), Rock Creek (ROCK), Satus Creek (SAT), South Fork Cowichee Creek (SFCOW), Tieton River (TIET), and Wildcat Creek (WILD) by year when they were tagged.

Tag retention studies conducted in Manastash Creek (2013), Cowichee Creek (2014), Rattlesnake Creek (2015), and Wenas Creek (2016) indicate tag retention of stream dwelling *O. mykiss* was generally high. Pit tag retention was typically over 90% for time intervals between 48 h and 90 days. Tag retention dropped to 84% following a 1-year time period between marking and release (Table 3).

Table 3. Pit tag retention rates (%) for *O. mykiss* dual tagged (tag group) over various time intervals in several Yakima Basin tributaries.

Time interval	Tag group	Recaptured	PIT retained	Retention rate (%)
<b>Manastash Creek 2013</b>				
48 hours	275	155	152	98.06
1 week				
2 weeks	275	242	233	96.28
1 month				
3 months	558	340	325	95.59
1 year	558	73	61	83.56
<b>Cowiche Creek 2014</b>				
48 hours	98	34	32	94.12
1 week	98	28	27	96.43
2 weeks	98	31	29	93.55
1 month				
3 months	98	30	29	96.67
1 year				
<b>Rattlesnake Creek 2015</b>				
48 hours	158	106	104	98.11
1 week				
2 weeks	158	75	75	100.00
1 month				
3 months	158	40	40	100.00
1 year				
<b>Wenas Creek 2016</b>				
48 hours	115	21	20	95.24
1 week				
2 weeks	115	23	22	95.65
1 month				
3 months	115	11	10	90.91
1 year				

Accounting for tag retention rates in tagging studies is critical when making comparative estimates of population parameters based upon tagged fish. In general, high PIT tag retention rates for migrating anadromous juveniles have been reported in the literature. Our tag retention study based upon dual tagging procedures indicated that tag retention rates of tagged *O. mykiss*

were generally high in our tributaries. Recent studies of resident fish in Idaho suggested spawning females can shed their tags during the act of spawning (Meyer et al. 2011). Thus tag retention of resident and anadromous *O. mykiss* may not be equivalent after the migration (smolts) and adult life stages (resident trout). The information generated from these studies will be necessary to incorporate when generating comparisons of resident and anadromous abundance, survival, and productivity estimates over long time intervals (e.g., 3 months or greater). We will also need to account for tag induced mortality rates in our tagging studies. However, long term tag induced mortality is very difficult to measure in the natural stream setting. We initiated a small scale tag mortality study in conjunction with a re-conditioned Kelt breeding study that is being conducted in the semi-natural spawning channel at the Cle Elum Supplementation and Research Facility during the spring spawning period. In 2017, 10 resident Rainbow Trout were stocked into the artificial spawning channel in early March. Eight of them survived until the spawning period and were accounted for until mid-May representing a minimum survival estimate of 80% for 75 days (Jeff Stephenson, Personal Communication).

We caution readers that developing true productivity estimates for Steelhead trout takes a substantial amount of time. Crawford and Rumsey (2011) recommend a minimum of 12 brood years be collected to provide productivity estimates. This is due to the complex time requirements necessary to observe all possible combinations of freshwater residency and ocean migration over the lifespan of the adults. Our project began in 2010 and we implemented juvenile tagging efforts in the upper Yakima Basin in earnest in 2011. We are now beginning to accumulate an adequate time series such that we can track entire cohorts back to their respective broodyear, and hence generate minimum estimates of recruits per spawner. Although rare, some migrants that are six years old have been detected and thus, we have complete cohort tracking for two brood years (BY2010 and 2011). However, the majority of the migrants are of the one- and two-year-old age class and we do commonly observe three- and some four-year-old migrants and with this consideration we have near complete accounting for an additional two broodyears (BY2012 and 2013; Table 4), and partial accounting for brood years 2014 and 2015 (Table 4).

Table 4. Adult spawning brood year (BY) versus the respective age of recruits for each migration year. The light gray shaded area indicates the current juvenile recruitment time series data collected for each brood year over the duration of this project and the dark grey box represents complete or nearly complete brood years of migrant data collected through 2017.

Migrants							
BY	Age0*	Age1	Age2	Age3	Age4	Age5	Age6
2008	2008	2009	2010	2011	2012	2013	2014
2009	2009	2010	2011	2012	2013	2014	2015
2010	2010	2011	2012	2013	2014	2015	2016
2011	2011	2012	2013	2014	2015	2016	2017
2012	2012	2013	2014	2015	2016	2017	2018
2013	2013	2014	2015	2016	2017	2018	2019
2014	2014	2015	2016	2017	2018	2019	2020
2015	2015	2016	2017	2018	2019	2020	2021
2016	2016	2017	2018	2019	2020	2021	2022
2017	2017	2018	2019	2020	2021	2022	2023

\*We generally do not observe age0 migrants

The ratio of juvenile recruits produce per spawning adult must be greater than 1 for any population to persist (Ricker 1975). It appears that the anadromous recruitment per spawner ratio (R/S) for the upper Yakima population currently exceeds 1 because the trend in the Roza Dam count is steadily increasing (refer to Figure 5). Comparisons of R/S between tributary and main stem Yakima River areas show a general trend of increased productivity with increasing distance (Rkm) from the Columbia River (Figure 13). Relative comparisons of R/S also indicate that the Teanaway Basin exhibits a greater number of R/S than lower elevation tributaries or the main stem Yakima River (Figure 13).

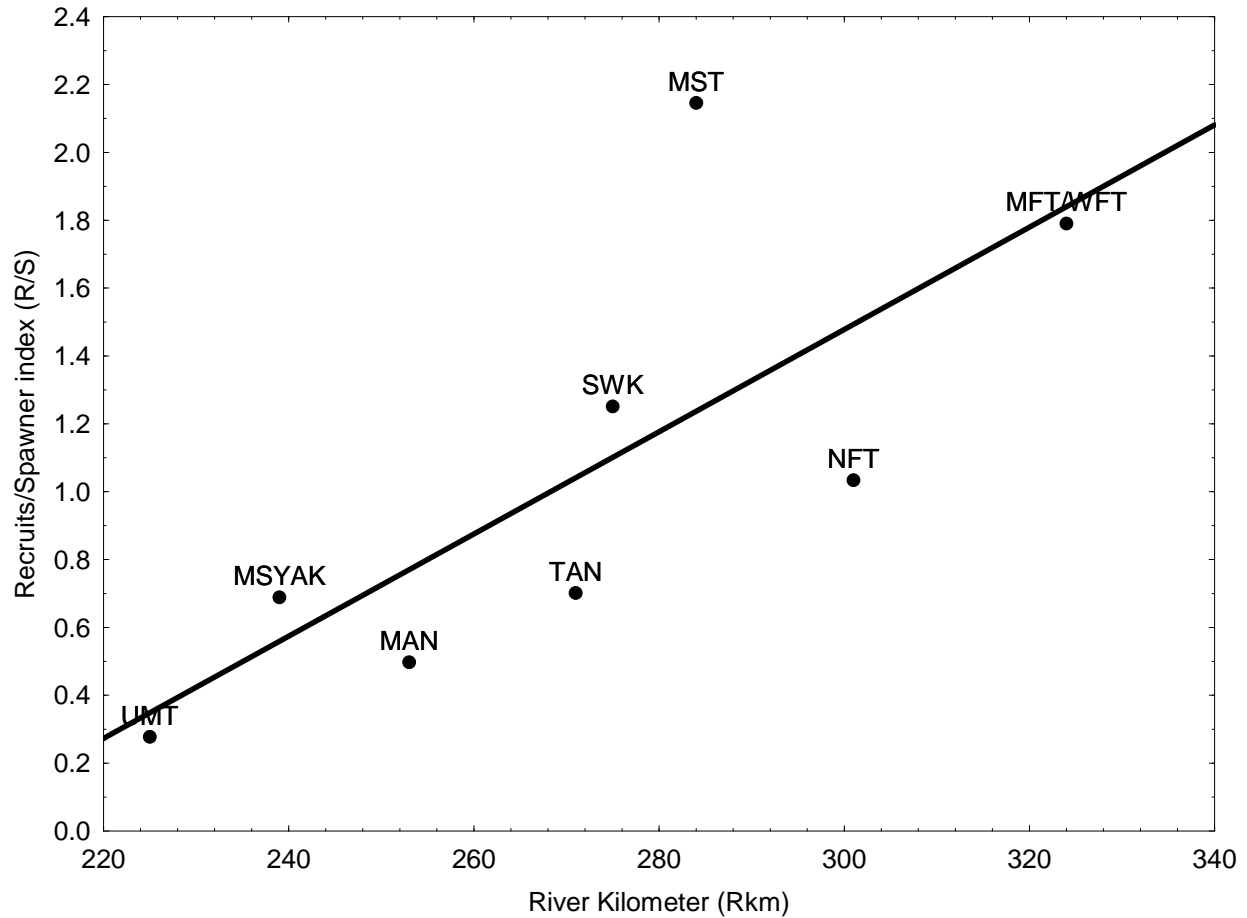


Figure 13. Average (2011-2014 broodyears) indices of steelhead recruits (smolts) per spawner (R/S) for Umtanum Creek (UMT), Manastash Creek (MAN), Taneum Creek (TAN), Swauk Creek (SWK), North Fork Teanaway River (NFT), Mainstem Teanaway River (MST), the combined West and Middle Fork Teanaway Rivers (MFT/WFT), and the main stem Yakima River (MSYAK), per river kilometer upstream from the Columbia River confluence. R/S should be considered a minimum index (unexpanded estimates) and as a relative measure.

### *Spatial Structure*

In 2014, we standardized our description of Steelhead rearing distribution by stratifying each tributary into 200m sampling sections throughout its entire length and the main stem Yakima River into 500 m sections (Figure 14). The tagging location of each fish tagged is known to the nearest 200m in tributaries, and 500m in main stem river sections. We constructed simple frequency plots of Steelhead smolt rearing origin in the main stem upper Yakima River and from each tributary by river kilometer for the upper Yakima Basin (Figure 15 and Figure 16



respectively) as well as for the Naches Basin and Naches Basin tributaries (Figure 17 and Figure 18 respectively). There has been much interest in Wenas Creek recently because some *O. mykiss* tagged upstream from Wenas Dam have been detected migrating out of the Yakima River as Steelhead smolts. Wenas dam is currently a migration barrier to anadromous Steelhead so anadromous smolt production upstream from the dam is the result of resident trout matings. The distribution of anadromous smolts originating from Wenas Creek in 2017 are presented in Figure 19.

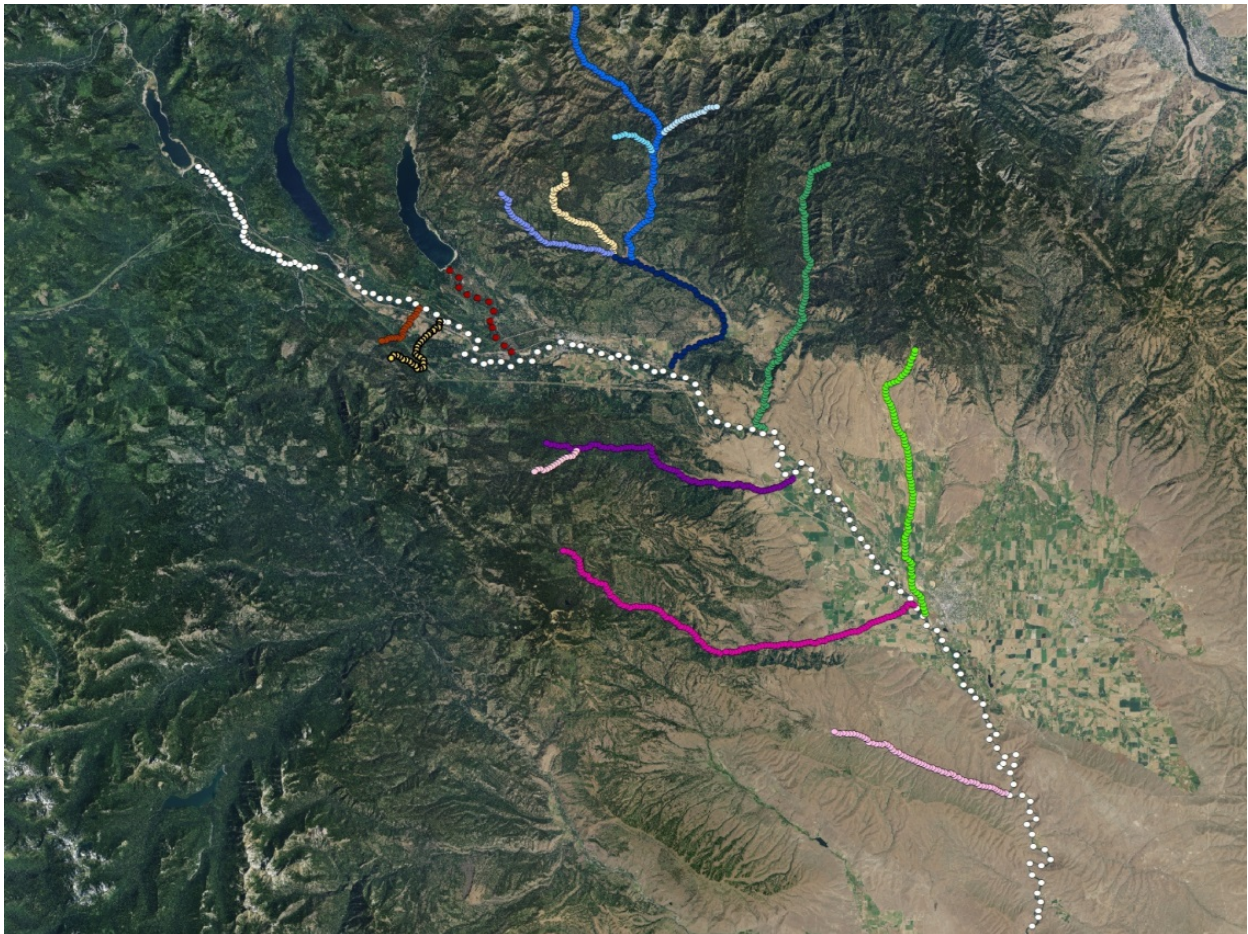


Figure 14. PIT tag collection sites in each tributary stream of the upper Yakima Basin. Collection site names are labeled sequentially moving up the stream channel. Each dot represents 200 m in tributary streams, and 300 m or 500 m in main stem stream sections.

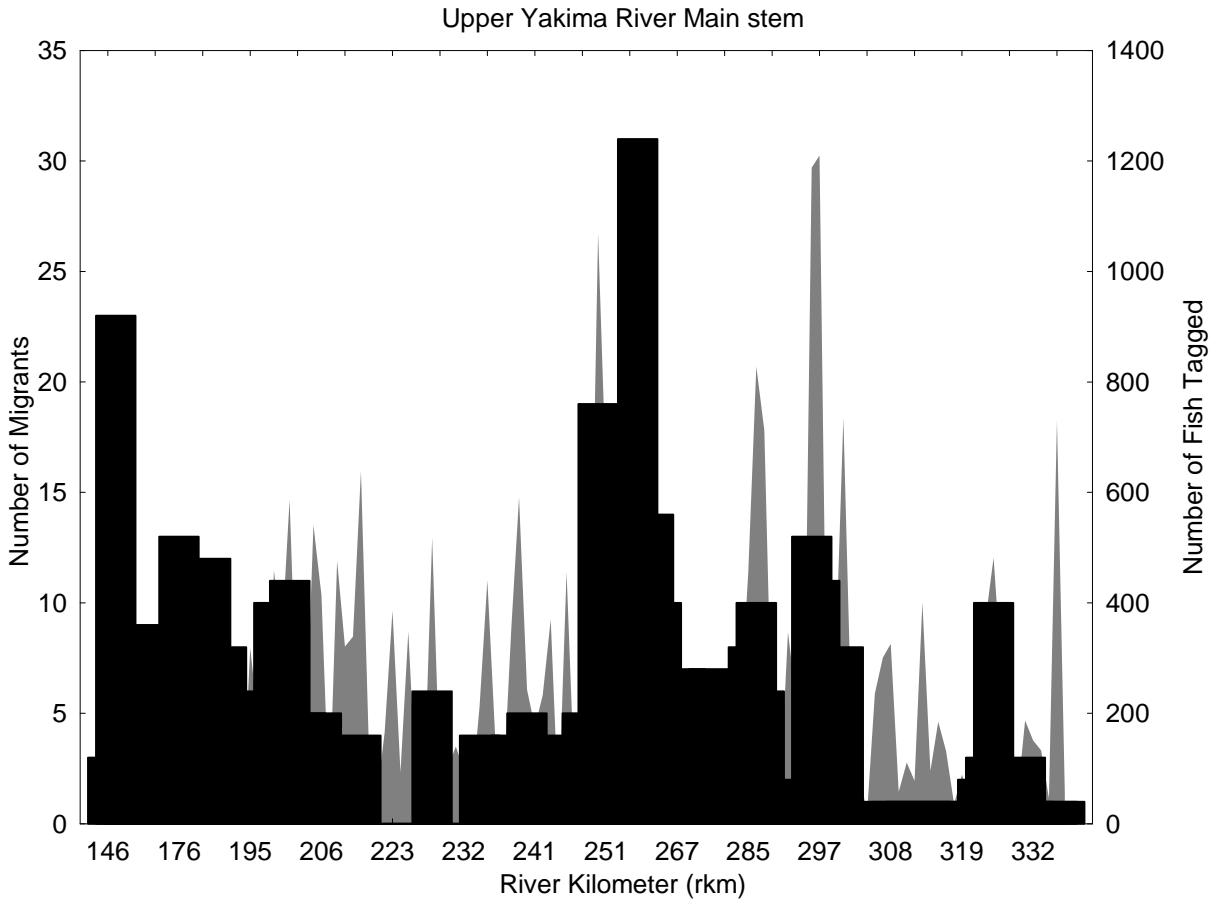


Figure 15. Number and origin (river kilometer; rkm) of Steelhead smolts (black bars; Left Y axis) detected during the spring smolt migration (all years combined; 2011-2017) that were tagged in the main stem Yakima River. The grey area (Right Y axis) represents total number of *O. mykiss* tagged per rkm between 2011 and 2017.

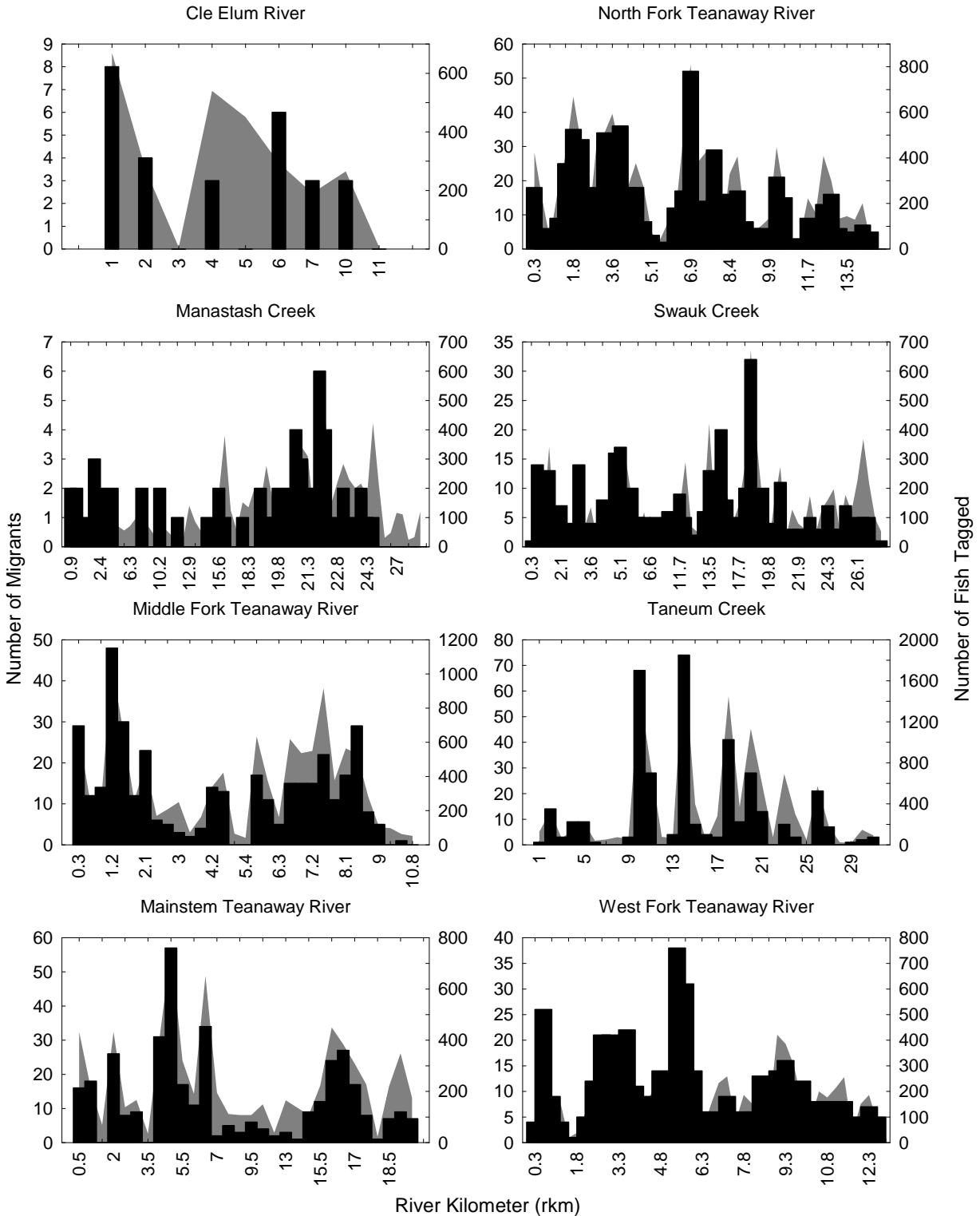


Figure 16. Number and origin (river kilometer; rkm) of Steelhead smolts (black bars; left Y axis) detected during the spring smolt migration (all years combined; 2011-2017) that were tagged in upper Yakima tributary streams. Grey area (right Y axis) represents total number of *O. mykiss* tagged per rkm between 2011 and 2017.

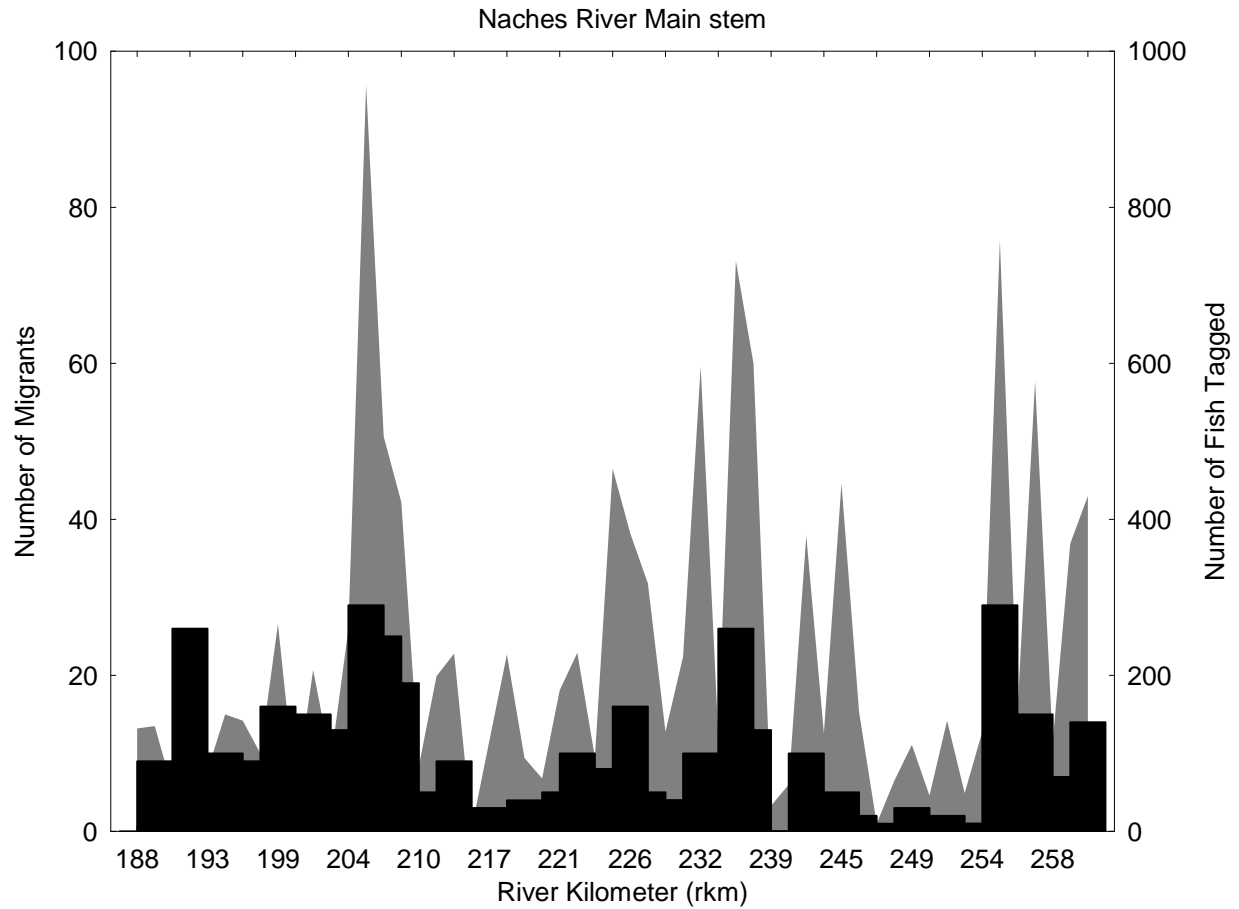


Figure 17. Number and origin (river kilometer; rkm) of Steelhead smolts (black bars; left Y axis) detected during the spring smolt migration (all years combined; 2011-2017) that were tagged in the Naches River. Grey area (right Y axis) represents total number of *O. mykiss* tagged per rkm between 2011 and 2017.

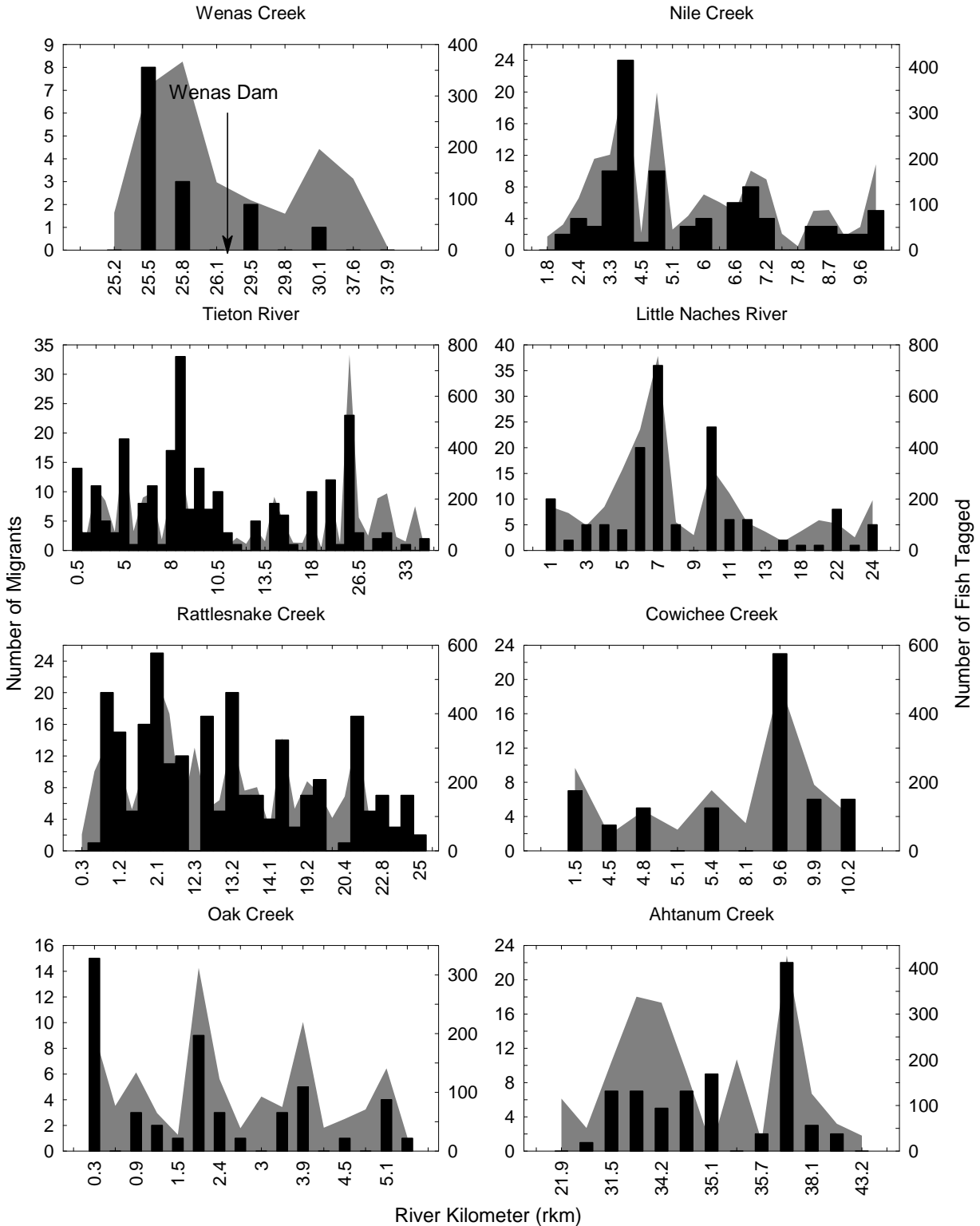


Figure 18. Number and origin (river kilometer; rkm) of Steelhead smolts (black bars; left Y axis) detected during spring smolt migration (all years combined; 2011-2017) that were tagged in Naches Basin tributaries. Grey area (right Y axis) represents total number of *O. mykiss* tagged per rkm between 2011 and 2017.



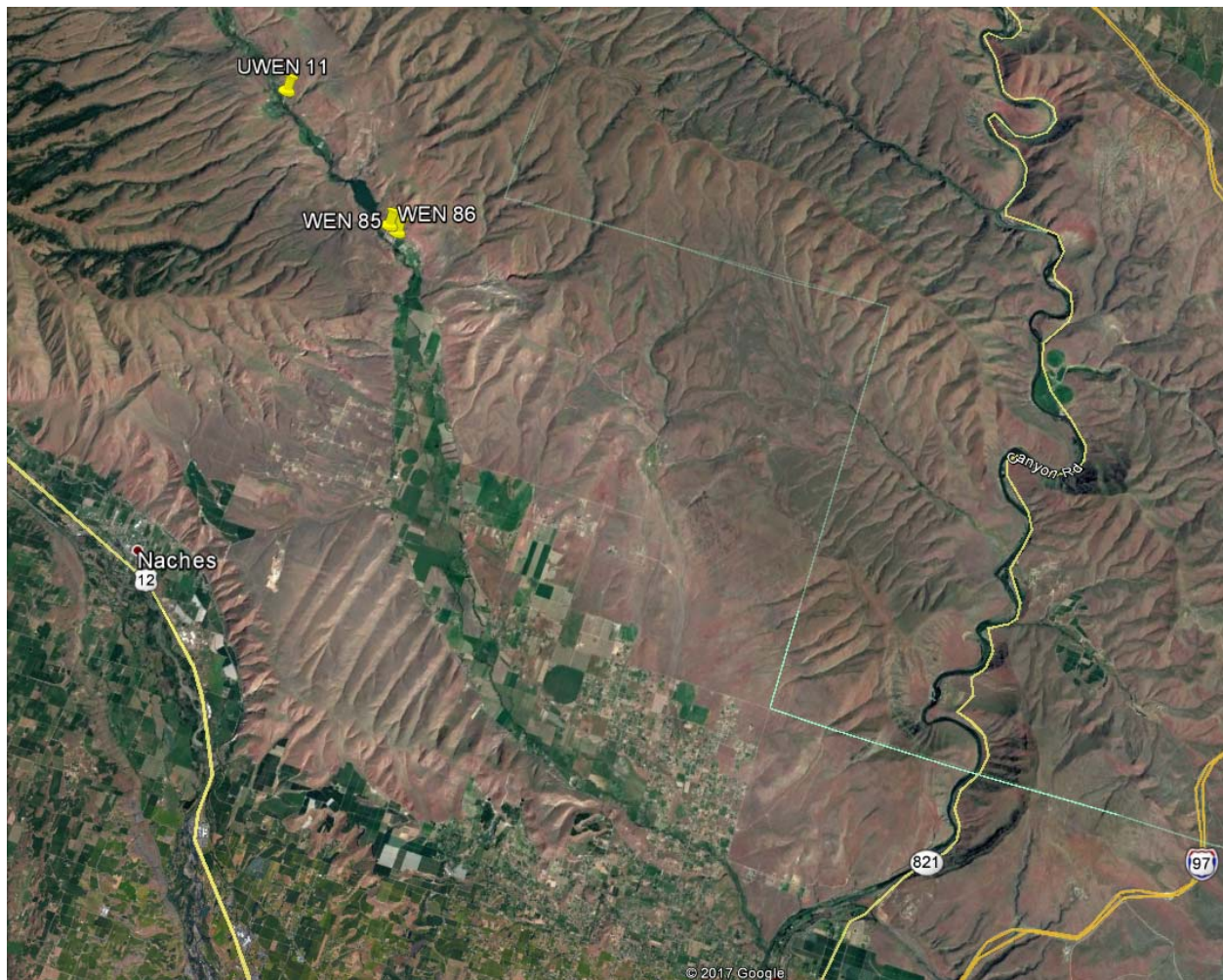


Figure 19. Origin of Steelhead smolts detected during the 2017 smolt outmigration that were tagged in Wenas Creek collection sites including UWEN11 upstream from the reservoir, and WEN85 and WEN85 downstream from the reservoir.

### ***Diversity***

Pit tagging a large number of juvenile *O. mykiss* in their natal streams provided several interesting and important results related to life history diversity. First, it appears the bulk of the juvenile Steelhead smolts, and perhaps pre-smolts, emigrate from their natal streams during the spring (Figure 20). We also observed a fall migration of tagged juvenile *O. mykiss* out of the upper Yakima tributary streams (Figure 20). We speculated that the fall migration may be driven by dropping stream temperatures and increased fall discharge. While there was no clear relationship between these variables, there may be an inverse relationship between average monthly stream temperature and monthly emigration from the Teanaway Basin (Figure 21).

While the juvenile emigration from the tributary streams did occur primarily in the spring and fall period, fish did move past our interrogation site during most months of the calendar year. These observations are based upon the 2015 emigration due to incomplete detections for our instream arrays following the flood events that destroyed our instream equipment. Repairs are currently underway.

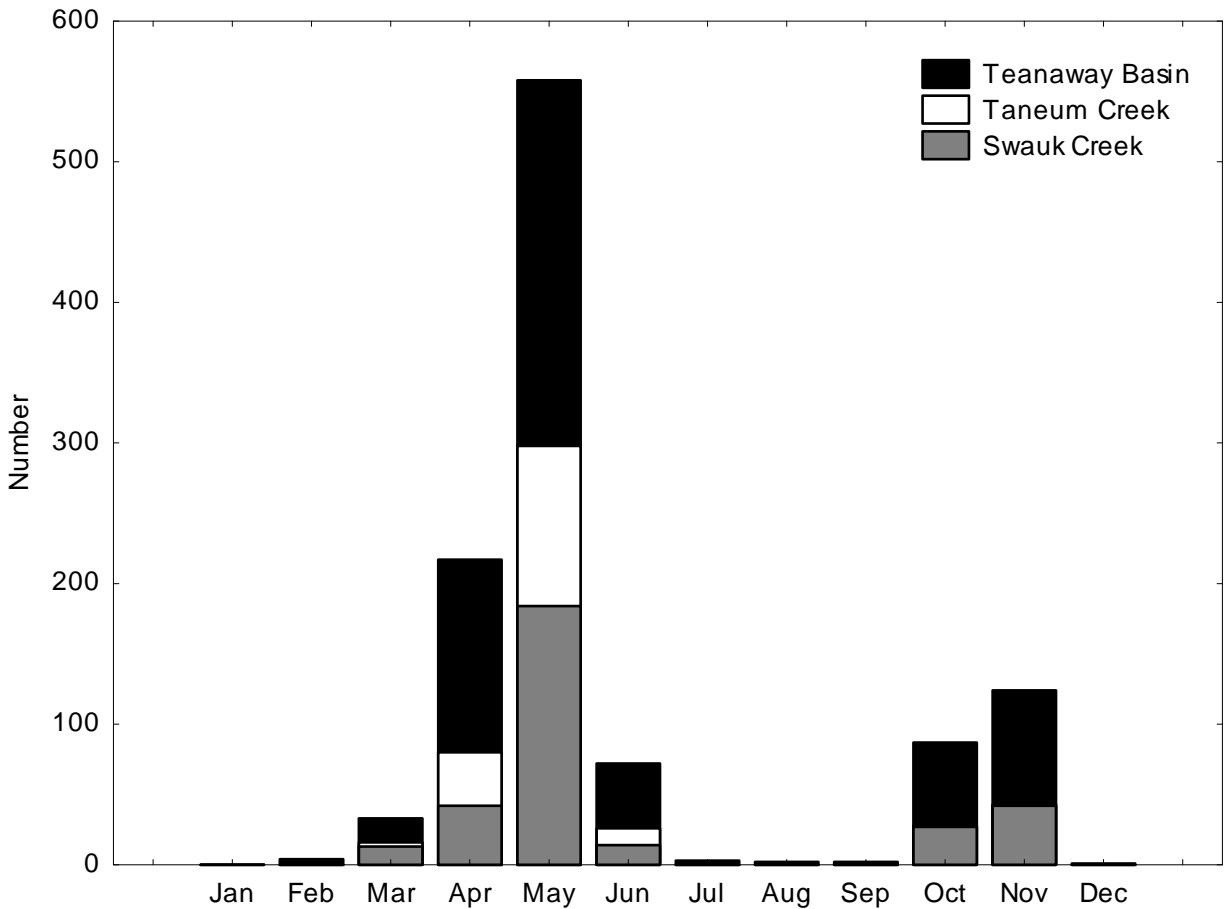


Figure 20. Number of fish migrating from select upper Yakima tributaries by month during 2015.

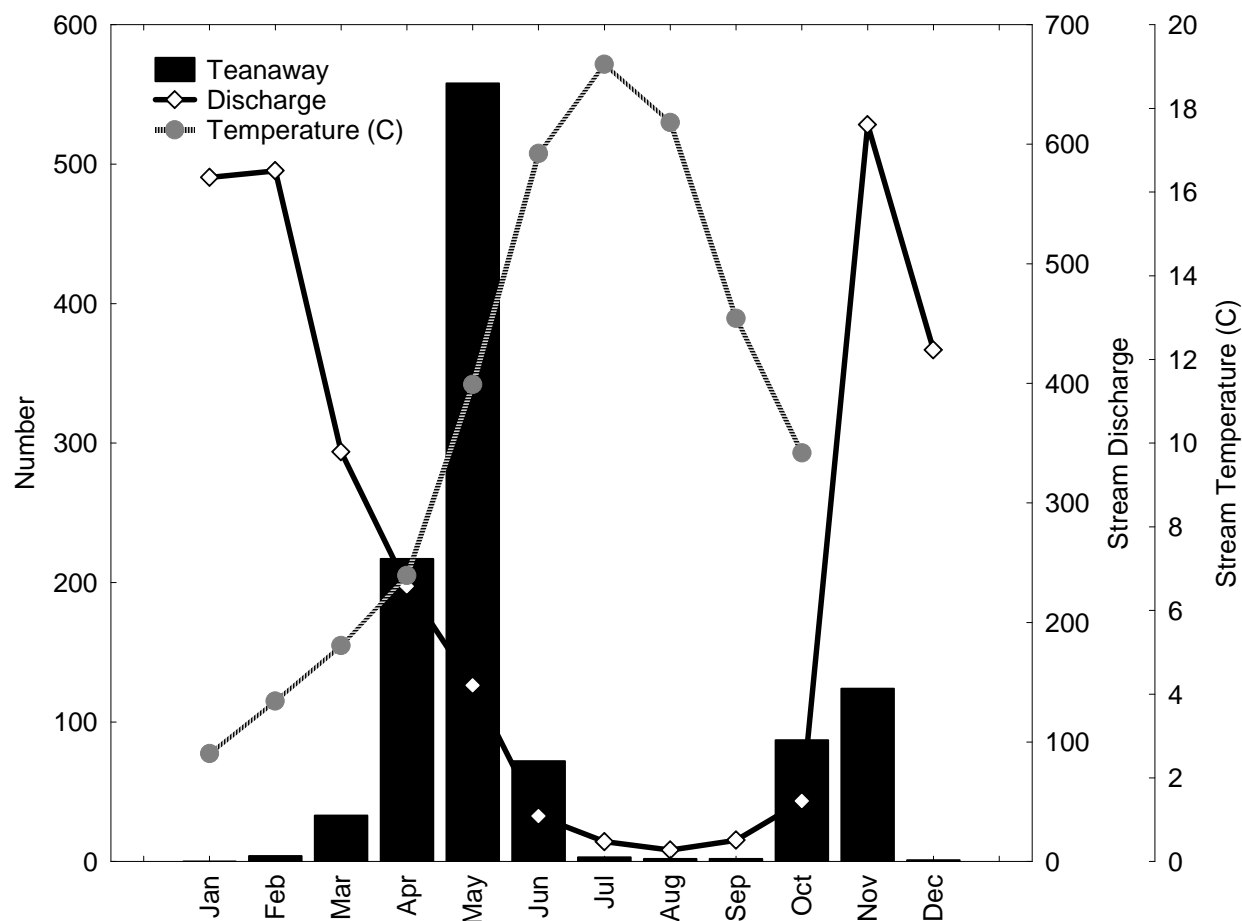


Figure 21. Number of juvenile emigrants detected each month at the mouth of the Teaway River relative to average monthly stream discharge (cfs; right axis) and stream temperature (C; Second right axis). Water temperature was monitored until Oct. 15, 2015 when the monitoring equipment failed.

We were interested to know if the length vs. weight relationship of anadromous juveniles at the time of tagging were any different than that of the resident or rearing *O. mykiss* population. An analysis of co-variance (ANCOVA) of the  $\log_{10}$  transformed length vs. weight relationship indicates that there is a slight, but significant, difference in the length/weight relationship between life history forms ( $P < 0.001$ ). Anadromous juveniles generally weigh less at a given length than their resident counterparts (Figure 22) although the variation around these average relationships would make it difficult to distinguish between life histories for individual fish.



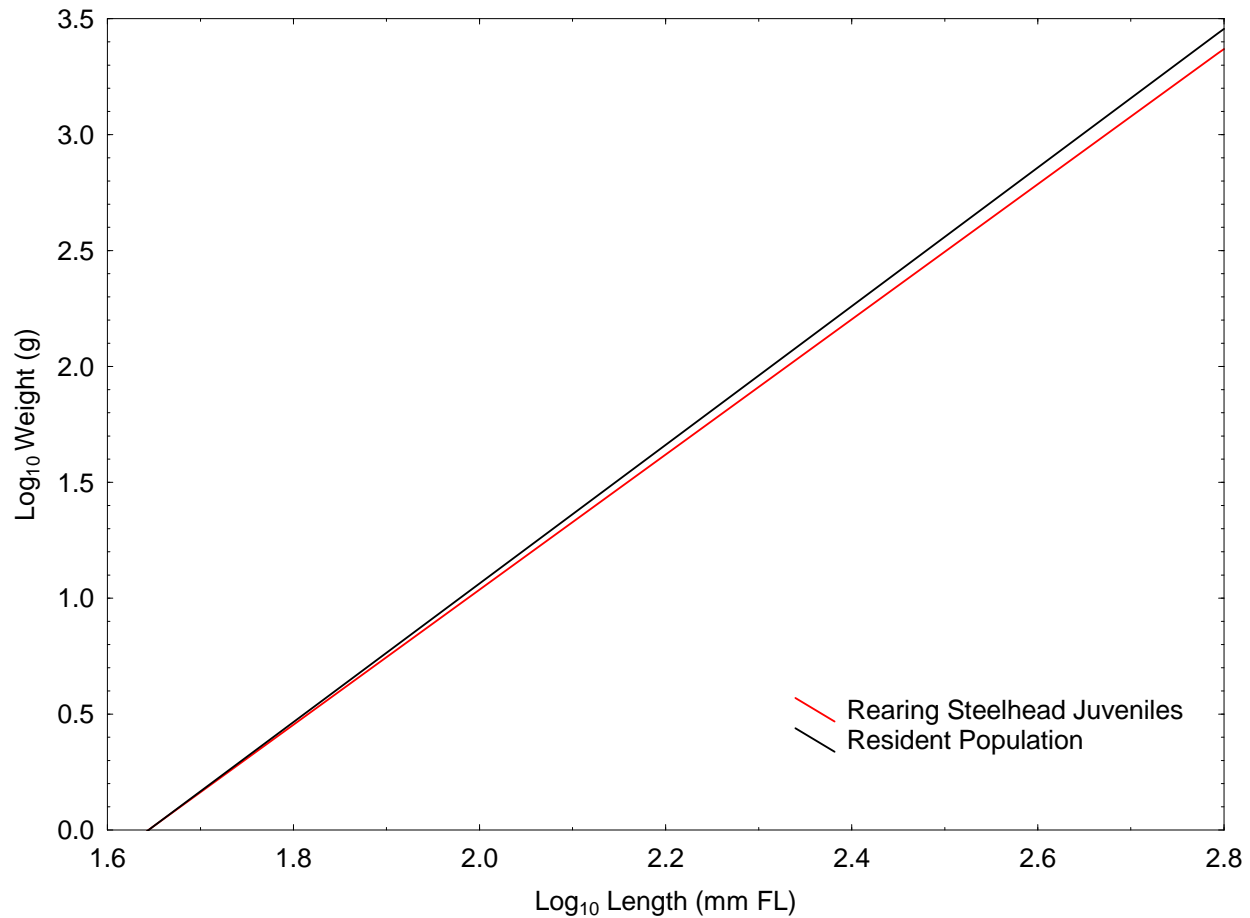


Figure 22.  $\text{Log}_{10}$  transformed length weight relationship for resident *O. mykiss* and rearing Steelhead juveniles. The Steelhead were tagged as juveniles and detected as returning adults in subsequent years. The resident population was defined as tagged individuals that were not detected as migrants in subsequent years.

Back calculations of the length at age for resident Rainbow Trout and anadromous Steelhead Trout smolts for the Middle Fork Teanaway River, North Fork Teanaway River, Swauk Creek, and the West Fork Teanaway River indicated there may be slight differences in the growth trajectories of the two life histories during the freshwater rearing phase (Figure 23). Low sample sized limited our comparisons to length at age 0 and age 1. There was no significant difference between resident and anadromous back calculated length at age 0 ( $P = 0.33$ ) but there was for age 1 fish ( $P = 0.05$ ) with smolts being slightly longer in body length at age 1 than their resident counterparts (Figure 23).

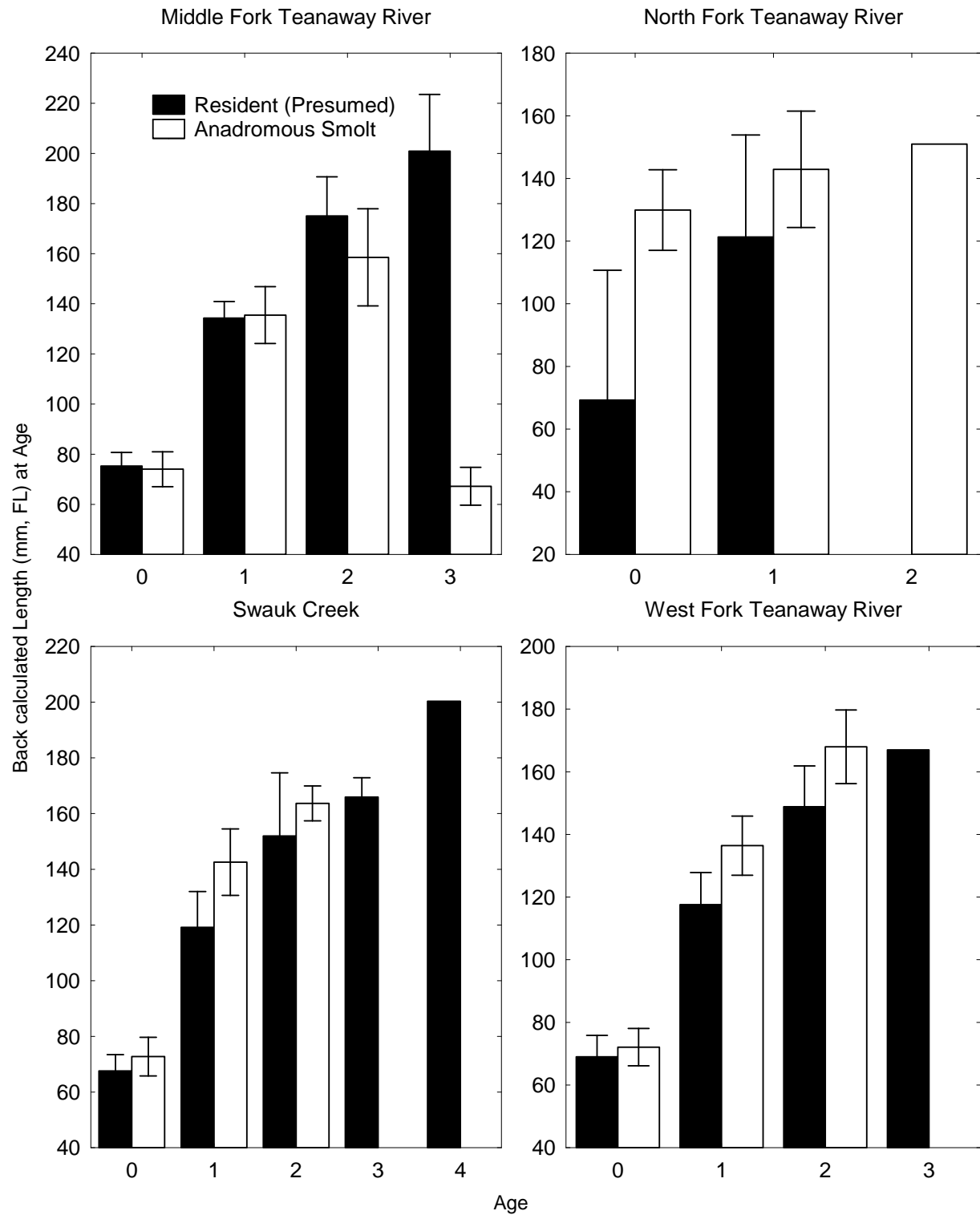


Figure 23. Back calculated length at age (mm; fork length, FL) from scale samples collected from resident Rainbow Trout based on PIT tag recapture histories, and known smolts from four upper Yakima Tributary streams.

As our project progresses, we are beginning to observe increased number of Steelhead adults returning to the Yakima Basin that were tagged as juveniles in their natal streams several years prior. In 2017, we detected 9 adult Steelhead at Bonneville Dam that were tagged as juveniles in their natal streams in the upper Yakima Basin during the freshwater rearing phase. We also detected 25 adults that originated from the Naches Basin (including 1 from Ahtanum Creek). This information is used to track diversity metrics for the Naches population and the Upper Yakima population for resident and anadromous life histories. We have a smaller dataset collected in the Naches Basin as we began expanding our tagging study to include the Naches in 2011 vs. 2006 for the upper Yakima. In addition, we deployed a smaller number of tags each year in the Naches in comparison with the upper Yakima basin. However, we expect that we will see increased information in the coming years as additional adult fish from the Naches population begin returning. Until that time, the comparisons of adult diversity metrics of fish tagged as juveniles are based upon small numbers of fish.

It appears that adult Steelhead returning to the Naches and Upper Yakima populations have similar run timing (entry into the Columbia River). Steelhead trout that were tagged as rearing juveniles in tributaries in both the Upper Yakima population and the Naches population were detected as returning adults at Bonneville Dam at approximately the same Julian Date (Figure 24) during the spawning migration. An Analysis of Variance indicated that there was no significant difference in the detection date at Bonneville dam for fish tagged in tributary streams in both basins (ANOVA;  $F_{15,140}=0.78$ ;  $P = 0.69$ ).

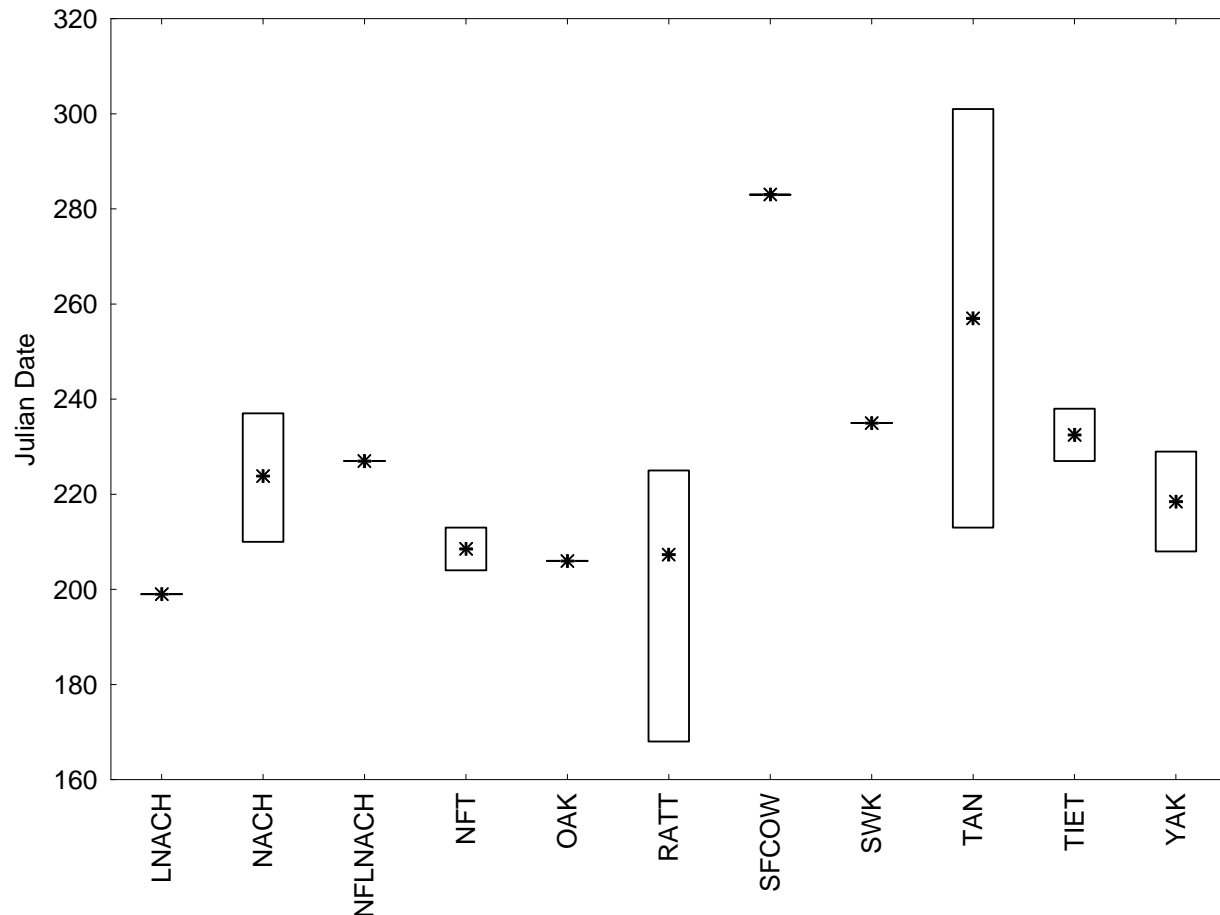


Figure 24. Average and the range (Min and Max) of dates (Julian Day) of the first detection of returning Steelhead adults at Bonneville Dam in 2017 for fish PIT tagged in their natal streams as juveniles. Stream abbreviations include the Little Naches River (LNACH), Naches River (NACH), North Fork Little Naches River (NFLNACH), Oak Creek (OAK), Rattlesnake Creek (RATT), South Fork Cowichee Creek (SFCOW), Swauk Creek (SWK), Taneum Creek (TAN), Tieton River (TIET), and the main stem Yakima River (YAK).

The wide spread detections of PIT tagged upper Yakima Steelhead throughout the Columbia Basin suggests that it is not uncommon for these fish to wander during their adult migration. Similar to previous years, we observed Yakima Steelhead making extensive use of the entire Columbia River Basin during the 2017 adult spawning migration (Figure 25). Several Yakima Steelhead were detected at the Deschutes River mouth, and in the Snake River Basin. Fish were also detected in the upper Columbia Basin passing upstream from Priest Rapids Dam. Several of these fish were detected in the juvenile fishways at mainstem Columbia River Dams as well, presumably in an attempt to move downstream through the hydro-system as they

migrated throughout the basin or as post spawned kelts. In contrast, recapture information collected on rearing juveniles (combined life histories) indicated very little movement prior to the smolt stage.

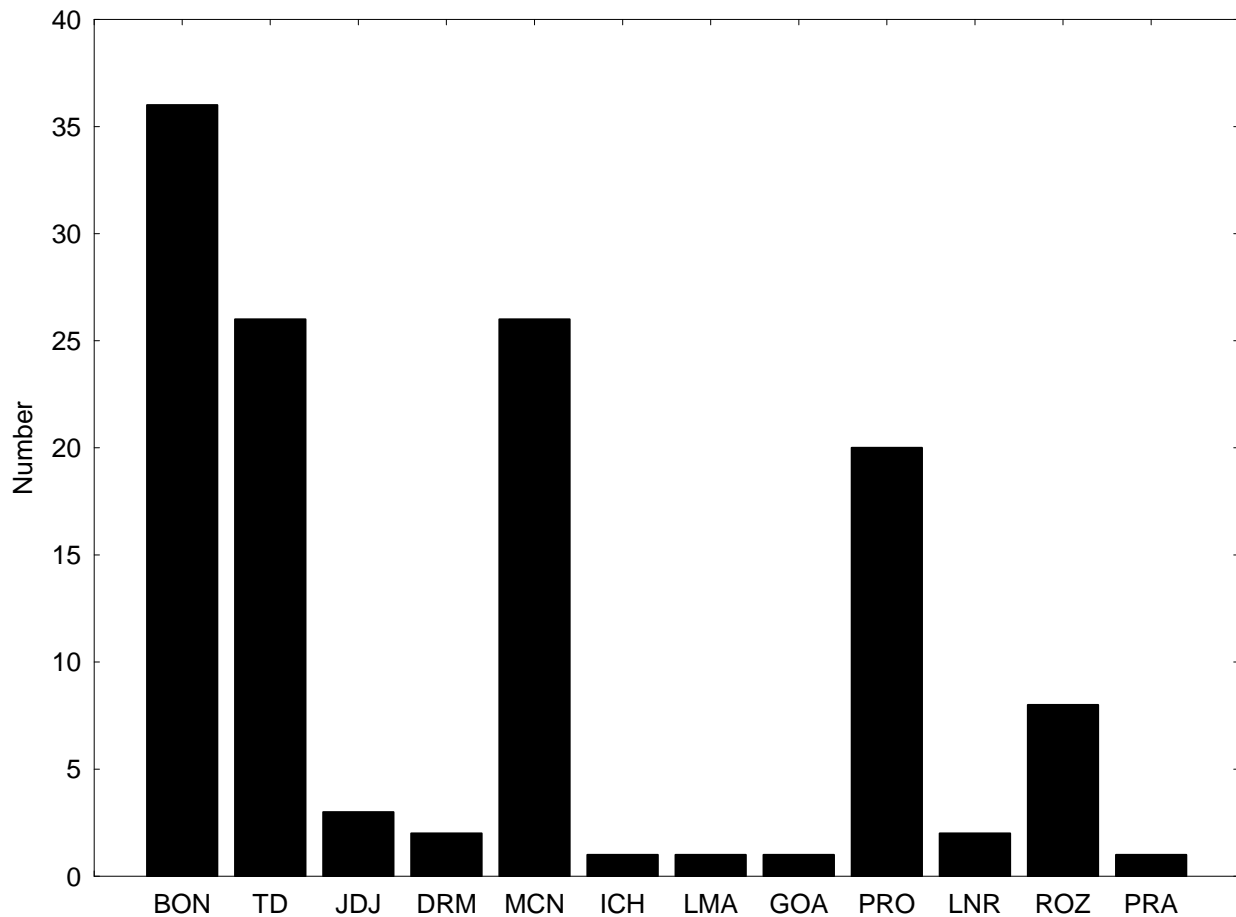


Figure 25. Number of Yakima Steelhead adults detected at instream PIT tag arrays at Bonneville Dam (BON), The Dalles Dam (TD), John Day Dam (JDJ), the Deschutes River mouth (DRM), McNary Dam (MCN), Ice Harbor Dam (ICH), Lower Monumental Dam (LMA), Little Goose Dam (GOA), Prosser Dam (PRO), Naches River (LNR), Roza Dam (ROZ), and Priest Rapids Dam (PRA) in 2017.

Adult summer Steelhead generally migrate into the Teanaway Basin between mid-February and late May. In spring of 2015, detections from the upstream North Fork, and upper Mainstem arrays were used to back calculate the passage timing of adults that were not detected on the lower array by using the average migration speed of fish that were detected at both an upstream and downstream interrogation site. The date that adults were detected or estimated to

have passed the Lower Teanaway array in 2015 were overlayed on a line plot of average daily discharge measured at the USBOR Teanaway Forks gauging station (Figure 26). Adults entered the Teanaway during the months of February, March, April, and May when they were presumed to have spawned. The adult detection efficiency was improved in 2015 relative to earlier years yet 3 adults passed the lower Mainstem Teanaway instream PIT tag array without being detected.

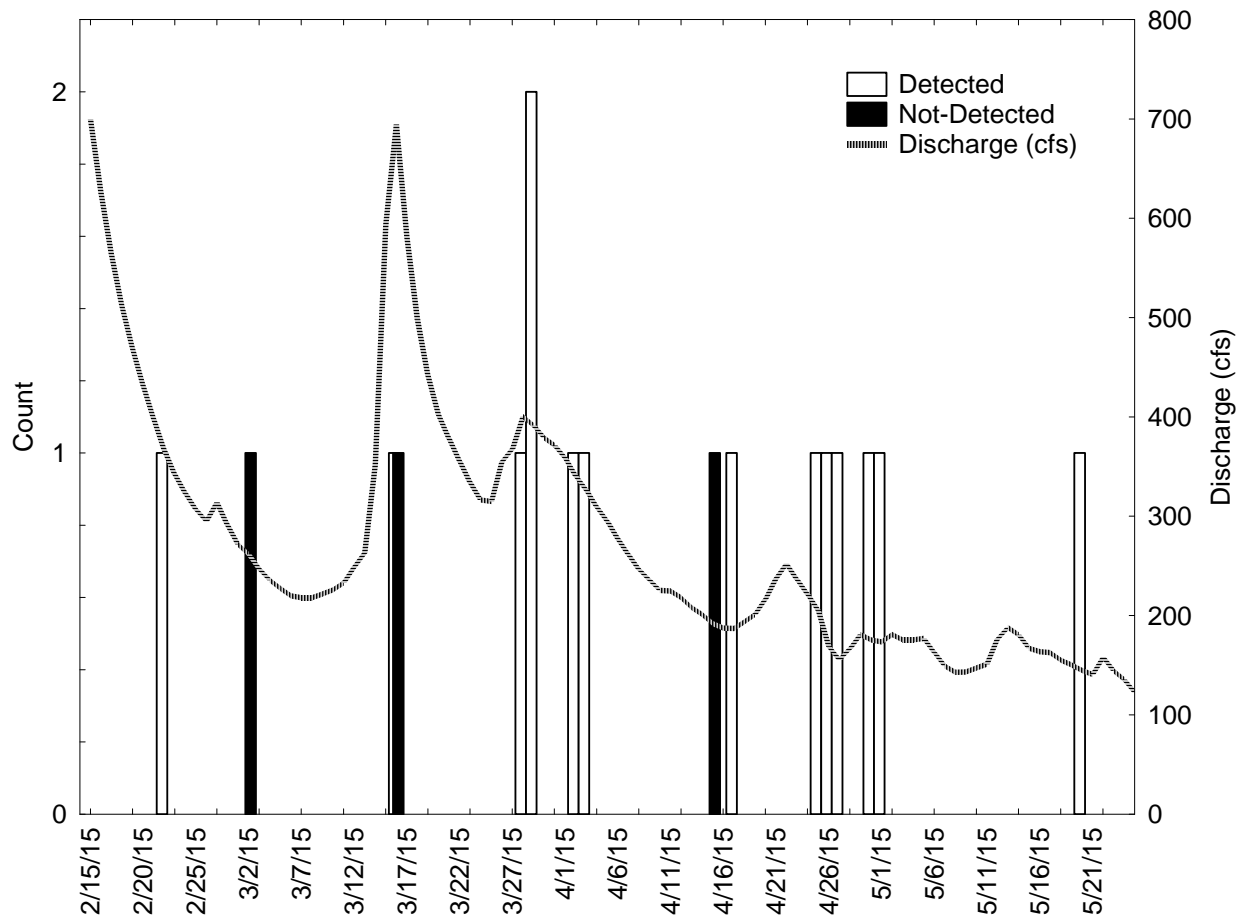


Figure 26. Mean daily stream discharge (cfs: dashed line) in the Teanaway River during the 2015 summer Steelhead spring spawning migration and the number of Steelhead detected (white) and not-detected (black) at the Lower Teanaway River instream PIT tag detection array (LMT).

## Discussion/Conclusion

One of the primary objectives of this work is to collect population level status and trend data for the upper Yakima *O. mykiss* population (sympatric life histories). These data collection efforts are ongoing. One of the secondary benefits is that the data are collected in a manner to answer critical uncertainties associated with the interactions of life history types in this sympatric

population. Little is known about how the interactions between resident and anadromous forms of *O. mykiss* affects the recovery objectives mandated for the anadromous form. Bettering our understanding of these interactions will fill these data gaps, and help facilitate our recovery efforts.

Our monitoring yielded several new and exciting results this contract period, particularly with respect to diversity and spatial structure metrics. This information will be useful for monitoring trends in the diversity and spatial structure metrics in future years that will support NOAA fisheries and the Columbia River BiOp and provide critical information improving the long term management of the sympatric life histories. Many of the variables monitored are currently being used to inform life cycle modeling efforts, and can be used in high level documents for the populations in the MPG (e.g., Steelhead at risk report; Status assessments). Steelhead are notably the most complex species in the Pacific Salmonid group and recent research conducted under this project, and elsewhere, are beginning to improve our understanding of the complexities of this species which will in turn, support their best management.

Another useful product generated during this contract period includes the geo-referenced plots of smolt production from each tributary stream. One strategy for recovering anadromous fish resources in the Yakima Basin is to repair fish habitat. Plots of *O. mykiss* smolt production per river kilometer in each tributary display stream reaches that are important for the natural production of anadromous Steelhead Trout juveniles. While we have identified the stream reaches that are producing Steelhead smolts in the upper Yakima, we will work to improve the evaluation by attempting to identify causative factors. By identifying links between specific habitats and Steelhead smolt production, we will be able to provide recommendations for habitat protection or specific habitat improvement actions that will benefit anadromous Steelhead Trout rearing so habitat managers can prioritize actions aimed to benefit Steelhead production in the freshwater rearing environment.

### ***Adaptive Management & Lessons Learned***

The instream PIT tag arrays provide a wealth of information pertaining to abundance, productivity, spatial structure, and diversity metrics: migration timing, run size, production, movement and movement/environmental relationships for example. However, the instream

arrays have proven difficult to keep operational during large environmental events. In the late fall of 2015 (November and December), two large unanticipated runoff events occurred rendering several of our instream arrays inoperable. Repair of these sites, and the installation of several large sites including the main stem Naches River, and Sunnyside Dam instream equipment coupled with a short work window arising from unusually hard winter in 2016/2017, has delayed complete repair of many sites during this contract period. In these instances, we apply the 2012-2014 radio telemetry information to model the metrics for the adult abundance, and productivity until the instream equipment is operational. We have engaged in testing different antenna material that may be more suitable and resilient to high stream discharge/flow events. In addition, we have redistributed much of the detection equipment in a strategy to increase the security, performance, and resiliency of our detection equipment for the future.

In 2017 we acquired three field tablet computers from the Washington Department of Fish and Wildlife. We are developing an electronic field data collection protocol using PTagis based P4 software to streamline the data collection and quality control of our field data. This should translate to a shorter data handling period so field data can be made accessible to PTagis with minimum delay. We anticipate adopting electronic data collection beginning in 2018.

The Teanaway Basin continues to produce a large proportion of the steelhead smolts originating from the upper Yakima Basin. The Teanaway also harbors a large number of steelhead spawners as evidenced from the radio telemetry data. The productivity information suggests that this basin is an important stronghold for Steelhead production for the upper Yakima population despite its long history of habitat degradation. As such, we recommend continuing to pursue protective measures for fish and fish habitat in this basin, particularly when considering the potential adverse effects of climate change.

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## ***Appendices***

Appendix 1. WDFW Molecular Genetics Laboratory assessment.

## Methods

### *Sampling Location and Methods*

Samples from adult wild steelhead (parents) were collected as they passed through Roza Dam in years 2009 through 2017 representing fish spawning in the spring each year of 2010 through 2017. Adult steelhead handled at the dam were sampled for sex, length, weight, origin, and a small fin clip was taken for genetic analysis. Fin clips were preserved in 100% ethanol and stored at room temperature.

Steelhead juveniles were collected and sampled from throughout the Yakima River and its tributaries upstream of Roza Dam via electrofishing. Captured fish scanned for presence of a PIT tag, measured for fork length and body weight. Untagged fish were given a PIT tag and a small sample of tissue was collected from each fish. Tissue samples were placed in individually labeled vials containing 100% ethanol. Scales were taken from a subset of individuals for age determination. After sampling, fish were released alive back into the river from where they were taken. Fish subsequently detected by PIT tag detectors downstream of Roza Dam were identified as migrants. Tissue from migrant juveniles was forwarded to the WDFW Molecular Genetics Laboratory for genetic processing and analysis.

### *Genetic Sample Processing*

All samples were genotyped at the WDFW panel of *O. mykiss* single nucleotide polymorphic loci (SNPs) that is used for analysis statewide. The suite of 192 SNP markers included 189 SNP loci developed to be used for population structure, parentage assignment, or other population genetic studies of *O. mykiss* (Table 1) and three SNP loci developed to distinguish cutthroat trout (*O. clarki*) from steelhead and rainbow trout (Table 2). Any fish genetically identified as cutthroat or *mykiss/clarki* hybrids was removed from further analysis.

To extract and isolate DNA from fin tissue from samples processed prior to 2018, Qiagen DNEasy ® kits (Qiagen Inc., Valencia, CA) were used, following the recommended protocol for animal tissues. SNP genotypes were obtained through PCR and visualization on Fluidigm EP1 integrated fluidic circuits (chips). Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: Samples were pre-amplified by Specific Target Amplification (STA) following Fluidigm's recommended protocol with one modification. The 192 assays were pooled to a concentration of 0.2X and mixed with 2X Qiagen Multiplexing Kit (Qiagen, Inc., Valencia CA), instead of TaqMan PreAmp Master Mix (Applied Biosystems), to a volume of 3.75µl, to which 1.25µl of unquantified sample DNA was added for a total reaction volume of 5µl. Pre-amp PCR was conducted on a MJ Research or Applied Biosystems thermal cycler using the following profile: 95°C for 15 min followed by 14 cycles of 95°C for 15 sec and 60°C for 4 minutes. Post-PCR reactions were diluted with 20µl dH<sub>2</sub>O to a final volume of 25µl.

Specific SNP locus PCRs were conducted on the Fluidigm chips. Assay loading mixture contained 1X Assay Loading Reagent (Fluidigm), 2.5X ROX Reference Dye (Invetrogen) and 10X custom TaqMan Assay (Applied Biosystems); sample loading mixture contains 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 0.05X AmpliTaq Gold DNA polymerase (Applied Biosystems), 1X GT sampling loading reagent (Fluidigm) and 2.1 µL template DNA. Four µL assay loading mix and 5 µL sample loading mix were pipetted onto the chip and loaded by the IFC loader (Fluidigm). PCR was conducted on a Fluidigm thermal cycler using a two-step profile. Initial mix thermal profile was 70°C for 30min, 25°C for 5 min, 52.3° for 10 sec, 50.1°C for 1 min 50sec, 98°C for 5 sec, 96°C for 9 min 55 sec, 96°C for 15 sec, 58.6°C for 8 sec, and 60.1°C for 43 sec. Amplification thermal profile was 40 cycles of 58.6°C for 10 sec, 96°C for 5 sec, 58.6°C for 8 sec and 60.1°C for 43 sec with a final hold at 20°C.

The SNP assays were visualized on the Fluidigm EP1 machine using the BioMark data collection software and analyzed using Fluidigm SNP genotyping analysis software. To ensure all SNP markers were being scored accurately and consistently, all data were scored by two researchers and scores of each researcher were compared. Disputed scores were called missing data (i.e., no genotype).

All samples were analyzed for matching genotypes. Any individuals with matching genotypes were interrogated to elucidate possible explanations for having matching genotypes. In some cases (see results), pairs or one member of a pair of samples with matching genotypes were removed from further analysis.

Samples processed in 2018 were processed using different methods. In 2018, we used a cost effective method based on custom amplicon sequencing called Genotyping in Thousands (GTseq, Campbell et al. 2015) to amplify 269 SNP loci, which included almost all of the SNP loci included in the previously used panel. The SW269 SNP panel included 265 SNP loci developed for population structure analysis, parentage assignment, or other population genetic studies of *O. mykiss*, three SNPs that distinguish cutthroat trout from steelhead and rainbow trout, and one sex-linked locus that allowed genetic determination of sex.

To extract and isolate genomic DNA from tissue, 30uL of 10% Chelex (Sigma Aldrich, C7901) and 5uL of Proteinase K solution (Qiagen, 1018332) were added to fin tissue and incubated overnight at 55°C. To start the library preparation, an ExoSAP cleanup was performed on 10uL of extracted DNA. 1.3uL of Exonuclease I (New England BioLabs, M0293L), 0.3 uL of SAP (New England BioLabs, M0371L), 0.15uL of Exonuclease 1 Buffer (New England BioLabs, B0293S), and 1.25uL of nuclease free water were added to the extracted DNA for a combined volume of 13uL. Thermal cycling was conducted in 96-well PCR plates for all reactions and had

the following conditions for the ExoSAP reaction: 37°C-60 min, 80°C-20 min, 4°C-hold.

Following the ExoSAP reaction, amplification of the multiplexed pool of targeted loci was performed. The multiplex PCR cocktail reaction was 2uL of cleaned DNA extract, 3.5uL of Qiagen Multiplex PCR Plus mix (Qiagen, 10672201), and 1.5uL pooled primer mix (IDT, Tables 3 and 4, final volume = 7uL; final primer concentrations at each locus = 54nM). Thermal cycling conditions were as follows: 95°C-15 min; 5 cycles [95°C – 30 s, 5% ramp down to 57°C – 30 s, 72°C – 2 min]; 10 cycles [95°C – 30 s, 65°C – 30 s, 72°C – 30 s]; 4°C hold. Following the multiplex PCR, the amplified samples were diluted 20-fold. 3uL of diluted multiplex PCR product was then used in the barcoding PCR. The barcoding PCR is used to add indexes that identify each sample by well and by plate. For the barcoding PCR, 1uL of 10uM well-specific i5 tagging primer (IDT) and 1uL of 10uM plate-specific i7 tagging primer were added to the 3uL of amplified sample. 5uL of Qiagen Multiplex PCR Plus mix (Qiagen, 10672201) was then added for a final reaction volume of 10uL. Thermal cycling conditions were: 95°C – 15 min; 10 cycles [98°C – 10 s, 65°C – 30 s, 72°C – 30 s]; 72°C – 5 min; 4°C hold. Following the barcode PCR, each plate of samples (library) was normalized using the SequelPrep™ Normalization Plate Kit (Applied Biosystems, A1051001) according to the manufacturer's instructions. Upon completion of normalization, 10uL of each sample per 96-well plates was pooled into a 1.5mL tube constituting a library.

A purification step was then performed on each library with Agencourt AMPure® XP magnetic beads (Agencourt, A63881) according to the manufacturer's instructions for size selection with a 2:1 and 1.43:1 ratio of library to beads. The purified libraries were then eluted with 15uL of TE pH 8.0. In order to complete the final process of library preparation, each library was quantified and normalized. The libraries were quantified using a Qubit 3 Fluorometer (Invitrogen) and

Qubit™ DNA HS Assay Kit reagents (Invitrogen, Q32854) according to the manufacturer's instructions. Following the quantification, the concentration of each library was calculated using the molecular weight specific to the multiplex pool used. Then each library was normalized to 4nM and pooled with other libraries that were sequenced on the same sequencing run. Pooled libraries were then sequenced at a 2.5pM loading concentration on an Illumina NextSeq 500 instrument of a single-end read flow cell using 111 cycles with dual-index reads of six cycles each.

To genotype the samples a bioinformatics pipeline was used (available online at <https://github.com/GTseq/GTseq-Pipeline>; (Campbell et al. 2015)). Essentially, there are a series of custom PERL scripts that ultimately create individual fastq files and genotype files for every individual that can be compiled for further analysis. Allele calling (nucleotide identification) is performed by counting amplicon-specific sequences for each allele, and allele ratios are used to determine the genotypes.

#### *Evaluation of Loci*

To evaluate genetic qualities of loci, we quantified several genetic parameters of the collections of adult samples collected at Roza Dam grouped by spawning year. To check for systematic scoring issues, we performed a two-tailed exact test of Hardy–Weinberg equilibrium (HWE) for each locus in each collection using the Markov Chain method implemented in Genepop 4.2 (dememorization number 1000, batches 100, 1000 iterations per batch; (Raymond and Rousset 1995; Rousset 2008)). Significance of probability values was adjusted for multiple tests using false discovery rate (Verhoeven et al. 2005).  $F_{IS}$ , a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of scoring issues, was calculated according to Weir and Cockerham (1984) using



Genepop 4.2. Expected heterozygosity was calculated using GDA software (Lewis and Zaykin 2001).

## **Results and Discussion**

### *Juvenile and Adult Sampling*

Over 3,000 adult steelhead sampled at Roza Dam from 2007 through 2016 were SNP genotyped. Of those, 2,580 spawned in years 2010 through 2016 and had sufficient genetic data for parentage analysis. Of the many thousands of juvenile steelhead sampled and PIT tagged in the upper Yakima River basin, 1,821 were determined to be expressing a migrant life history, were spawned in years 2010 through 2016, and had sufficient genetic data for parentage analysis. An additional 91 non-migrant upper Yakima juveniles genotyped for baseline purposes were also included in parentage analysis, as were 26 migrant juveniles sampled in the mainstem Yakima River upstream of the mouth of the Naches, but downstream of Roza Dam.

### *Evaluation of Loci*

Based on poor amplification and scoring performance in previous projects, three loci were eliminated prior to evaluation of loci, *AOmy056*, *AOmy179*, and *AOmy289*. With the remaining 177 loci, adult collections from each spawning year showed unusually high levels of statistically significant deviations from Hardy-Weinberg Equilibrium and linkage disequilibrium suggesting systematic scoring issues of major effect or natural processes that lead to deviations from HWE and LD. Adult collections were submitted to sibship analysis using COLONY to identify related individuals, which could cause HWE and LD problems if found in large proportions. Many related individuals were found in each brood year. Removal of a subset of related individuals from brood year 2010 adults slightly improved (i.e., reduced) levels of deviations from HWE or LD. Four loci (*AOmy067*, *AOmy105*, *AOmy192*, and *AOmy266*) deviated from HWE in five of

six brood years. Visual inspection of statistics for juvenile collections from the upper Yakima River at the same loci revealed that these loci also displayed comparably large deviations from HWE expectations that were not statistically significant at the  $\alpha = 0.05$  level. Differences in statistical significance thus appear likely due to sample size differences (i.e., the Roza Dam adult collections are much larger than juvenile collections). These loci do not show other evidence of scoring issues and do not show high levels of HWE or LD issues in other Washington *O. mykiss* collections. Further investigation is needed regarding these loci, but the inclusion of them in parentage assignment analysis should not affect the accuracy or precision of parent assignments.

#### *Matching genotypes and resampling adults*

Eighty-four pairs of adults from spawn years 2010 to 2016 had matching genotypes. Of those, 36 pairs were the same fish sampled in two different spawn years, i.e., repeat spawners, verified by recaptured PIT tag numbers. Another 19 pairs appeared to be repeat spawners based on the spawn years in which they were sampled, but were not verified by PIT tag information. Finally, 29 pairs were fish sampled twice in the same spawn year, which could be fish that dropped back downstream over Roza Dam, re-ascended, and were sampled a second time. All but two of these pairs were identified in individuals from spawn year 2014, 2015, and 2016.

#### **Acknowledgements**

We acknowledge and thank Cherril Bowman, Sarah Bell, Garrett Gee, and Mitch Kissler for their work in the laboratory generating the genetic data and thank Todd Kassler for project and contract administration.

**Table 1. List of general use, diploid single nucleotide polymorphic (SNP) loci genotyped in Yakima River steelhead**

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy005	Omy_aspAT-123	T	C	(Campbell et al. 2009)
AOmy010	Omy_CRB2677.106	G	T	(Sprowles et al. 2006)
AOmy014	Omy_e1-147	G	T	(Sprowles et al. 2006)
AOmy015	Omy_gdh-271	C	T	(Campbell et al. 2009)
AOmy016	Omy_GH1P1_2	C	T	(Aguilar and Garza 2008)
AOmy021	Omy_LDHB-2_e5	T	C	(Aguilar and Garza 2008)
AOmy023	Omy_MYC_2	T	C	(Aguilar and Garza 2008)
AOmy026	Omy_myoD.178	A	C	(Campbell et al. 2009)
AOmy027	Omy_nkef-241	C	A	(Campbell et al. 2009)
AOmy028	Omy_nramp-146	G	A	(Campbell et al. 2009)
AOmy029	Omy_Ogo4.212	T	C	(Campbell et al. 2009)
AOmy042	Omy_BAC-F5.284	C	T	(Limborg et al. 2012)
AOmy047	Omy_u07-79-166	G	T	(Limborg et al. 2012)
AOmy048	Omy_113490-159	C	T	(Abadía-Cardoso et al. 2011)
AOmy049	Omy_114315-438	T	G	(Abadía-Cardoso et al. 2011)
AOmy051	Omy_121713-115	T	A	(Abadía-Cardoso et al. 2011)
AOmy056	Omy_128693-455	T	C	(Abadía-Cardoso et al. 2011)
AOmy058	Omy_130524-160	C	G	(Abadía-Cardoso et al. 2011)
AOmy059	Omy_187760-385	A	T	(Abadía-Cardoso et al. 2011)
AOmy061	Omy_96222-125	T	C	(Abadía-Cardoso et al. 2011)
AOmy062	Omy_97077-73	T	A	(Abadía-Cardoso et al. 2011)
AOmy065	Omy_97954-618	C	T	(Abadía-Cardoso et al. 2011)
AOmy067	Omy_aromat-280	T	C	WSU - J. DeKoning unpubl.
AOmy068	Omy_arp-630	G	A	(Campbell et al. 2009)
AOmy072	Omy_cd59b-112	C	T	WSU - J. DeKoning unpubl.
AOmy073	Omy_colla1-525	C	T	WSU - J. DeKoning unpubl.
AOmy074	Omy_cox2-335	T	G	WSU - J. DeKoning unpubl.
AOmy078	Omy_g1-103	T	C	(Stephens et al. 2009)
AOmy079	Omy_g12-82	T	C	WSU - J. DeKoning unpubl.
AOmy081	Omy_gh-475	C	T	(Campbell et al. 2009)
AOmy082	Omy_gsdf-291	T	C	WSU - J. DeKoning unpubl.
AOmy084	Omy_hsc715-80	C	A	WDFW - S. Young unpubl.
AOmy087	Omy_hsp47-86	T	A	WDFW - S. Young unpubl.
AOmy088	Omy_hsp70aPro-329	A	G	(Campbell and Narum 2009)
AOmy089	Omy_hsp90BA-193	C	T	(Campbell and Narum 2009)
AOmy091	Omy_IL17-185	G	A	WSU - J. DeKoning unpubl.
AOmy092	Omy_IL1b-163	T	G	WSU - J. DeKoning unpubl.
AOmy094	Omy_inos-97	C	A	WSU - J. DeKoning unpubl.
AOmy095	Omy_mapK3-103	A	T	CRITFC - N. Campbell

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
				unpubl.
AOmy096	Omy_mcsf-268	T	C	WSU - J. DeKoning unpubl.
AOmy100	Omy_nach-200	A	T	WSU - J. DeKoning unpubl.
AOmy105	Omy_OmyP9-180	C	G	(Sprowles et al. 2006)
AOmy107	Omy_Ots249-227	C	T	(Campbell et al. 2009)
AOmy108	Omy_oxct-85	A	T	WSU - J. DeKoning unpubl.
AOmy110	Omy_star-206	A	G	WSU - J. DeKoning unpubl.
AOmy111	Omy_stat3-273	G	Deletion	WSU - J. DeKoning unpubl.
AOmy113	Omy_tlr3-377	C	T	WSU - J. DeKoning unpubl.
AOmy114	Omy_tlr5-205	T	A	WSU - J. DeKoning unpubl.
AOmy117	Omy_u09-52-284	T	G	(Limborg et al. 2012)
AOmy118	Omy_u09-53-469	T	C	(Limborg et al. 2012)
AOmy120	Omy_u09-54.311	C	T	WDFW - S. Young unpubl.
AOmy123	Omy_u09-55-233	A	G	(Limborg et al. 2012)
AOmy125	Omy_u09-56-119	T	C	(Limborg et al. 2012)
AOmy129	Omy_BAMBI4.238	T	C	WDFW - S. Young unpubl.
AOmy132	Omy_G3PD_2.246	C	T	WDFW - S. Young unpubl.
AOmy134	Omy_Il-1b-028	T	C	WDFW - S. Young unpubl.
AOmy137	Omy_u09-61.043	A	T	WDFW - S. Young unpubl.
AOmy144	Omy_UT16_2.173	C	T	WDFW - S. Young unpubl.
AOmy147	Omy_U11_2b.154	T	C	WDFW - S. Young unpubl.
AOmy149	Omy_gluR-79	C	T	CRITFC - unpubl.
AOmy152	Omy_SECC22b-88	T	C	CRITFC - unpubl.
AOmy173	BH2VHSVip10	C	T	Pascal & Hansen unpubl.
AOmy174	OMS00003	T	G	(Sánchez et al. 2009)
AOmy176	OMS00013	A	G	(Sánchez et al. 2009)
AOmy177	OMS00018	T	G	(Sánchez et al. 2009)
AOmy179	OMS00041	G	C	(Sánchez et al. 2009)
AOmy180	OMS00048	T	C	(Sánchez et al. 2009)
AOmy181	OMS00052	T	G	(Sánchez et al. 2009)
AOmy182	OMS00053	T	C	(Sánchez et al. 2009)
AOmy183	OMS00056	T	C	(Sánchez et al. 2009)
AOmy184	OMS00057	T	G	(Sánchez et al. 2009)
AOmy185	OMS00061	T	C	(Sánchez et al. 2009)
AOmy186	OMS00062	T	C	(Sánchez et al. 2009)
AOmy187	OMS00064	T	G	(Sánchez et al. 2009)
AOmy189	OMS00071	A	G	(Sánchez et al. 2009)
AOmy190	OMS00072	A	G	(Sánchez et al. 2009)
AOmy191	OMS00078	T	C	(Sánchez et al. 2009)
AOmy192	OMS00087	A	G	(Sánchez et al. 2009)
AOmy193	OMS00089	A	G	(Sánchez et al. 2009)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy194	OMS00090	T	C	(Sánchez et al. 2009)
AOmy195	OMS00092	A	C	(Sánchez et al. 2009)
AOmy197	OMS00103	A	T	(Sánchez et al. 2009)
AOmy198	OMS00105	T	G	(Sánchez et al. 2009)
AOmy199	OMS00112	A	T	(Sánchez et al. 2009)
AOmy200	OMS00116	T	A	(Sánchez et al. 2009)
AOmy201	OMS00118	T	G	(Sánchez et al. 2009)
AOmy202	OMS00119	A	T	(Sánchez et al. 2009)
AOmy203	OMS00120	A	G	(Sánchez et al. 2009)
AOmy204	OMS00121	T	C	(Sánchez et al. 2009)
AOmy205	OMS00127	T	G	(Sánchez et al. 2009)
AOmy206	OMS00128	T	G	(Sánchez et al. 2009)
AOmy207	OMS00132	A	T	(Sánchez et al. 2009)
AOmy208	OMS00133	A	G	(Sánchez et al. 2009)
AOmy209	OMS00134	A	G	(Sánchez et al. 2009)
AOmy210	OMS00153	T	G	(Sánchez et al. 2009)
AOmy211	OMS00154	A	T	(Sánchez et al. 2009)
AOmy212	OMS00156	A	T	(Sánchez et al. 2009)
AOmy213	OMS00164	T	G	(Sánchez et al. 2009)
AOmy214	OMS00169	A	G	(Sánchez et al. 2009)
AOmy215	OMS00175	T	C	(Sánchez et al. 2009)
AOmy216	OMS00176	T	G	(Sánchez et al. 2009)
AOmy218	OMS00180	T	G	(Sánchez et al. 2009)
AOmy220	Omy_1004	A	T	(Hansen et al. 2011)
AOmy221	Omy_101554-306	T	C	(Abadía-Cardoso et al. 2011)
AOmy222	Omy_101832-195	A	C	(Abadía-Cardoso et al. 2011)
AOmy223	Omy_101993-189	A	T	(Abadía-Cardoso et al. 2011)
AOmy225	Omy_102505-102	A	G	(Abadía-Cardoso et al. 2011)
AOmy226	Omy_102867-443	T	G	(Abadía-Cardoso et al. 2011)
AOmy227	Omy_103705-558	T	C	(Abadía-Cardoso et al. 2011)
AOmy228	Omy_104519-624	T	C	(Abadía-Cardoso et al. 2011)
AOmy229	Omy_104569-114	A	C	(Abadía-Cardoso et al. 2011)
AOmy230	Omy_105075-162	T	G	(Abadía-Cardoso et al. 2011)
AOmy231	Omy_105385-406	T	C	(Abadía-Cardoso et al. 2011)
AOmy232	Omy_105714-265	C	T	(Abadía-Cardoso et al. 2011)
AOmy233	Omy_107031-704	C	T	(Abadía-Cardoso et al. 2011)
AOmy234	Omy_107285-69	C	G	(Abadía-Cardoso et al. 2011)
AOmy235	Omy_107336-170	C	G	(Abadía-Cardoso et al. 2011)
AOmy237	Omy_107806-34	C	T	(Abadía-Cardoso et al. 2011)
AOmy238	Omy_108007-193	A	G	(Abadía-Cardoso et al. 2011)
AOmy239	Omy_109243-222	A	C	(Abadía-Cardoso et al. 2011)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy240	Omy_109525-403	A	G	(Abadía-Cardoso et al. 2011)
AOmy241	Omy_110064-419	T	G	(Abadía-Cardoso et al. 2011)
AOmy242	Omy_110078-294	A	G	(Abadía-Cardoso et al. 2011)
AOmy243	Omy_110362-585	G	A	(Abadía-Cardoso et al. 2011)
AOmy244	Omy_110689-148	A	C	(Abadía-Cardoso et al. 2011)
AOmy246	Omy_111084-526	A	C	(Abadía-Cardoso et al. 2011)
AOmy247	Omy_111383-51	C	T	(Abadía-Cardoso et al. 2011)
AOmy248	Omy_111666-301	T	A	(Abadía-Cardoso et al. 2011)
AOmy249	Omy_112301-202	T	G	(Abadía-Cardoso et al. 2011)
AOmy250	Omy_112820-82	G	A	(Abadía-Cardoso et al. 2011)
AOmy252	Omy_114976-223	T	G	(Abadía-Cardoso et al. 2011)
AOmy253	Omy_116733-349	C	T	(Abadía-Cardoso et al. 2011)
AOmy254	Omy_116938-264	A	G	(Abadía-Cardoso et al. 2011)
AOmy255	Omy_117259-96	T	C	(Abadía-Cardoso et al. 2011)
AOmy256	Omy_117286-374	A	T	(Abadía-Cardoso et al. 2011)
AOmy257	Omy_117370-400	A	G	(Abadía-Cardoso et al. 2011)
AOmy258	Omy_117540-259	T	G	(Abadía-Cardoso et al. 2011)
AOmy260	Omy_117815-81	C	T	(Abadía-Cardoso et al. 2011)
AOmy261	Omy_118175-396	T	A	(Abadía-Cardoso et al. 2011)
AOmy262	Omy_118205-116	A	G	(Abadía-Cardoso et al. 2011)
AOmy263	Omy_118654-91	A	G	(Abadía-Cardoso et al. 2011)
AOmy265	Omy_120255-332	A	T	(Abadía-Cardoso et al. 2011)
AOmy266	Omy_128996-481	T	G	(Abadía-Cardoso et al. 2011)
AOmy267	Omy_129870-756	C	T	(Abadía-Cardoso et al. 2011)
AOmy268	Omy_131460-646	C	T	(Abadía-Cardoso et al. 2011)
AOmy269	Omy_98683-165	A	C	(Abadía-Cardoso et al. 2011)
AOmy270	Omy_cyp17-153	C	T	WSU - J. DeKoning unpubl.
AOmy271	Omy_ftzfl-217	A	T	WSU - J. DeKoning unpubl.
AOmy272	Omy_GHSR-121	T	C	CRITFC - unpubl.
AOmy273	Omy_metA-161	T	G	CRITFC - unpubl.
AOmy274	Omy_UBA3b	A	T	(Hansen et al. 2011)
AOmy275	M09AAC.055	C	T	WDFW - S. Young unpubl.
AOmy276	M09AAE-082	T	G	WDFW - S. Young unpubl.
AOmy277	OMGH1PROM1-SNP1	A	T	(Abadía-Cardoso et al. 2011)
AOmy279	OMS00015	A	T	(Sánchez et al. 2009)
AOmy280	OMS00024	T	G	(Sánchez et al. 2009)
AOmy283	OMS00070	T	C	(Sánchez et al. 2009)
AOmy284	OMS00074	T	G	(Sánchez et al. 2009)
AOmy285	OMS00096	T	G	(Sánchez et al. 2009)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy286	OMS00111	T	C	(Sánchez et al. 2009)
AOmy288	OMS00149	T	G	(Sánchez et al. 2009)
AOmy289	OMS00173	T	C	(Sánchez et al. 2009)
AOmy290	Omy_105105-448	C	T	(Abadía-Cardoso et al. 2011)
AOmy291	Omy_110201-359	T	G	(Abadía-Cardoso et al. 2011)
AOmy292	Omy_128923-433	T	C	(Abadía-Cardoso et al. 2011)
AOmy293	Omy_anp-17	C	A	CRITFC - N. Campbell unpubl.
AOmy294	Omy_bcAKala-380rd	G	A	CRITFC - N. Campbell unpubl.
AOmy295	Omy_cin-172	C	T	CRITFC - N. Campbell unpubl.
AOmy296	Omy_ndk-152	A	G	CRITFC - N. Campbell unpubl.
AOmy297	Omy_nips-299	T	Deletion	CRITFC - N. Campbell unpubl.
AOmy298	Omy_ntl-27	G	A	CRITFC - N. Campbell unpubl.
AOmy299	Omy_rbm4b-203	Deletion	T	CRITFC - N. Campbell unpubl.
AOmy300	Omy_sys1-188	C	A	CRITFC - N. Campbell unpubl.
AOmy301	Omy_txnip-343	T	C	CRITFC - N. Campbell unpubl.
AOmy302	Omy_vamp5-303	A	Deletion	CRITFC - N. Campbell unpubl.
AOmy303	Omy_vatf-406	T	C	CRITFC - N. Campbell unpubl.
AOmy305	OMS00077	C	G	(Sánchez et al. 2009)
AOmy306	OMS00101	A	G	(Sánchez et al. 2009)
AOmy311	Omy_G3PD_2-371	C	A	CRITFC - N. Campbell unpubl.
AOmy320	Omy_redd1-410	C	T	CRITFC - N. Campbell unpubl.
AOmy322	Omy_srp09-37	C	T	CRITFC - N. Campbell unpubl.
AOmy324	Omy1011	C	A	(Hansen et al. 2011)
AOmy326	OMS00068	A	G	(Sánchez et al. 2009)
AOmy327	OMS00079	T	C	(Sánchez et al. 2009)
AOmy328	OMS00106	T	G	(Sánchez et al. 2009)
AOmy329	OMS00179	A	C	(Sánchez et al. 2009)
AOmy331	Omy_114587-480	T	G	(Abadía-Cardoso et al. 2011)

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy335	OMS00017	A	G	(Sánchez et al. 2009)
AOmy341	Omy_metB-138	T	A	CRITFC - unpubl.

**Primer and probe sequences for unpublished loci available by request.**



**Table 2. List of species identification single nucleotide polymorphic (SNP) loci genotyped in Yakima River steelhead.**

WDFW Name	Locus Name	Expected genotype			Reference
		<i>O. mykiss</i>	<i>O. clarkii clarkii</i>	<i>O. clarkii lewisi</i>	
ASpI001	Ocl_Okerca	T	C	C	(McGlaufflin et al. 2010)
ASpI014	Omy_F5_136	C	G	G	(Finger et al. 2009)
ASpI018	Omy_Omyclmk436-96	A	C	C	CRITFC - S. Narum - unpubl.

**Primer and probe sequences for unpublished loci available by request.**

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