U.S. Fish and Wildlife Service
Columbia River Fish and Wildlife Conservation Office

Tryon Creek Restoration Monitoring

City of Portland FY 2015 Progress Report

Brook P. Silver, J. Michael Hudson, and Timothy A. Whitesel

U.S. Fish and Wildlife Service
Columbia River Fish and Wildlife Conservation Office
Vancouver, WA 98683

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On the cover: *Sampling fish below the Highway 43 culvert pool 2015, photo by Amy Horstman.*

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Abstract

Tryon Creek is a relatively undisturbed urban watershed located in southwest Portland, Oregon. The habitat is well suited for native fish; however, the lower portion of the stream is bisected by a culvert that runs under Oregon State Highway 43. A collaborative project to improve habitat and passage conditions for anadromous fish retrofitted the culvert with a new baffle system, elevated the pool below the culvert, and enhanced floodplain habitat. The U.S. Fish and Wildlife Service is working with the City of Portland to assess the restoration response of multiple species historically present, or believed to be present, in Tryon Creek. Goals in 2015 were to evaluate fish 1) community, 2) relative abundance, and 3) residence time in the confluence habitat, 4) conduct an occupancy survey for larval lamprey 5) and estimate the population abundance of trout species above the Highway 43 culvert. In 2014 and 2015, the fish community below the Highway 43 culvert included 13 species of 2,379 individual fish. Native fish were the most abundant comprising 77% (n = 10) of the species captured and 99.2% (n = 2,359) of the individuals captured. Nearly all juvenile anadromous salmonids (Chinook Salmon, Coho Salmon, and Steelhead Trout) captured below the Highway 43 culvert were of wild origin (99.2%, 971/979) originating from elsewhere in the Willamette River Basin. Juvenile Coho and Chinook Salmon resided in Tryon Creek for 1 - 499 (median = 11) days after tagging. Pacific Lamprey ammocoetes were found both above (likely from previous outplanting in 2013) and below the Highway 43 culvert in 2014 and 2015. The estimated population of Coastal Cutthroat Trout above the Highway 43 Culvert was 156 (95% CI [104, 245]) individuals; density was 0.013 (95% CI [0.008, 0.020]) individuals/ m^2. The majority of PIT-tagged Coastal Cutthroat Trout were not detected leaving Tryon Creek. In general, Coastal Cutthroat Trout populations in the city of Portland appear to be structured such that each watershed entering the Willamette River or Multnomah Channel represented a distinct population. Tryon Creek supports a genetically healthy population of Coastal Cutthroat Trout and appears to represent one distinct population. Coastal Cutthroat Trout from Kelley and Mitchell creeks appear to represent another distinct population. The majority of *O. mykiss* sampled from watersheds in the City of Portland were lower Columbia/Willamette winter steelhead while the remainder were lower Columbia/Willamette summer steelhead. Many of the fish that could be assigned to a specific population with reasonable confidence appeared to be winter steelhead from Eagle Creek National Fish Hatchery.

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Introduction

Tryon Creek (approximately 16.8 km^2) is one of the largest, relatively protected, urban watersheds in Oregon (Callison et al. 2002). Native salmonid species currently found in this stream include Coastal Cutthroat Trout (*Oncorhynchus clarki*), *O. mykiss* (resident Rainbow Trout and anadromous Steelhead Trout), Cutthroat/*O. mykiss* hybrids (hybrids) (Tinus et al. 2003), Coho Salmon (*O. kisutch*), and Chinook Salmon (*O. tshawytscha*) (Hudson et al. 2008). Historically, Pacific Lamprey (*Entosphenus tridentatus*) and Western Brook Lamprey (*Lampetra richardsoni*) may have utilized this stream. However, a culvert that currently runs under Oregon State Highway 43 and the adjacent railroad potentially inhibits, if not prevents, passage of lampreys and salmonids.

A collaborative project was implemented by the Oregon Department of Transportation (ODOT), Oregon Department of Fish and Wildlife (ODFW), Oregon State Parks, National Marine Fisheries Service, Cities of Portland and Lake Oswego, Friends of Tryon Creek, Tryon Creek Watershed Council, National Fish and Wildlife Foundation, and U.S. Fish and Wildlife Service (USFWS) to assess the restoration response of multiple species historically present, or believed to be present, in Tryon Creek. The initial phase of the project (conducted by ODOT in August 2008) retrofitted the existing culvert with a new baffle system to improve fish passage. The effort also provided habitat restoration to the stream, which included raising the level of the pool below the culvert to create a swim-in, rather than jump-in, situation thought to be more beneficial to lamprey and salmonid passage (Silver et al. 2014).

Fish Monitoring

In 2010, the City of Portland completed phase two of the Tryon Creek Confluence Habitat Enhancement Project, which improved floodplain connectivity, removed invasive species, and installed root wads and boulders. The project included stream enhancement of approximately 0.3 km of Tryon Creek from its confluence with the Willamette River to the culvert below Highway 43. In 2012, the U. S. Fish and Wildlife Service continued its partnership with the City of Portland's Bureau of Environmental Services to assess fish abundance, community, and residence time of fish in the Tryon Creek confluence with the following objectives:

1. Estimate relative abundance of fish species present.
    a. Conduct monthly sampling (seine/electrofish) from the Tryon Creek confluence to the Highway 43 culvert for the city of Portland's fiscal years 2013, 2015, 2017, and 2019.
    b. Conduct weekly sampling (seine/electrofish) from the Tryon Creek confluence to the Highway 43 culvert in the spring/summers of 2013, 2015, 2017, and 2019.

   c. Conduct an annual survey in July to electrofish above and below the Highway 43 culvert to detect larval lamprey distribution.
   d. Conduct a two-pass abundance estimate of salmonid species above the Highway 43 culvert in fall 2015 and a single pass in fall 2013 and 2017.
2. Describe fish community throughout the year
3. Document residence time of this habitat by the fish species present during the spring/summer.
   a. Maintain a Passive Integrated Transponder (PIT) array at the mouth of Tryon Creek.

Information collected from this assessment will aid the City of Portland in determining if the project is meeting its goals, evaluating if the site is achieving desired function over time, and improving the design of future projects.

Genetic Assessment

In 2015, the USFWS Columbia River Fish and Wildlife Conservation Office (CRFWCO) and the City of Portland worked with the USFWS Abernathy Fish Technology Center (AFTC) to conduct a genetic characterization of urban salmonids in Portland, Oregon. The investigation consisted of four parts.

Part 1. The genetic health of Coastal Cutthroat Trout from Tryon Creek

Coastal Cutthroat Trout appear to be the most likely self-sustaining, natural population of fish in Tryon Creek, however, their status, or population health is unclear. The goal was to characterize the genetic diversity and several measures of inbreeding in the Tyron Creek population. Estimates of these parameters for Coastal Cutthroat Trout in Tryon Creek were compared to existing information on, presumably, healthy populations of Coastal Cutthroat Trout from non-urbanized streams. Specific objectives included:

1. Provide estimates of genetic diversity and inbreeding for the Coastal Cutthroat Trout population in Tryon Creek
2. Compare these estimates to those obtained for other, presumably healthy, populations in the Columbia River Basin (Smith et al. 2010)
3. Evaluate genetic drift in the Tyron Creek population by measuring divergence among the generations sampled.

Part 2a. Determine whether Coastal Cutthroat Trout in Tryon Creek, upstream and downstream of the Boones Ferry culvert, are from a single spawning population

There is a need to better understand the population structure of Coastal Cutthroat Trout in Tryon Creek. There appears to be a relatively healthy population of Coastal Cutthroat Trout spawning in Tryon Creek, downstream of the culvert under Boones Ferry Road (Smith and Von Bargen, 2015). Coastal Cutthroat Trout can also be found upstream of the culvert. However, the existing culvert is perched, has a trash rack on the upstream end, and has been identified as a

potential barrier to fish passage. Relative to Coastal Cutthroat Trout, it is unclear how much mixing occurs from the lower portion of the creek, through the culvert, to the upper portion of the creek (see Silver et al. 2013). The City of Portland is planning to replace the existing culvert with a bridge that will not be a barrier to fish movement. Once the culvert is removed, Coastal Cutthroat Trout from downstream and upstream of Boones Ferry Road will almost certainly mix.

The objective of this investigation is to determine whether Coastal Cutthroat Trout currently found upstream of Boones Ferry Road are from the same spawning population as those found downstream of Boones Ferry Road. To address the Coastal Cutthroat Trout population structure from a genetic perspective, we:

1. Evaluated genetic diversity in terms of heterozygosity and allelic richness
2. Evaluated inbreeding in terms of linkage disequilibrium, effective population size (N_e), and average pairwise relatedness.
3. Compared results to Coastal Cutthroat Trout collected from Tyron Creek downstream of Boones Ferry Road (but upstream of Highway 43)

Part 2b. Determine the genetic characteristics of Coastal Cutthroat Trout captured residing at the mouth of Tryon Creek (Portland, OR)

Nonnatal tributaries may be used for temporary rearing by juvenile, anadromous salmonids (Scrivener et al. 1994). For anadromous salmonids, juvenile use of nonnatal rearing habitat has been especially well documented in the Lower Columbia River (Roegner et al. 2012). In part, this behavior may be to escape poor conditions in mainstem habitats (Scrivener et al. 1994). Relatively little information exists documenting whether salmonids from resident or fluvial populations (both which may exhibit local or regional movements) also use nonnatal tributaries for rearing. Resident or fluvial Coastal Cutthroat Trout exist within numerous watersheds in the Portland area (Tinus et al. 2003). Some of these trout migrate out of their natal tributary (see Silver et al. 2013) during some point in their life history. Although these fish may eventually return to their natal tributary, there is a need to better understand whether nonnatal tributaries provide rearing habitat for Coastal Cutthroat Trout in the City of Portland.

The objective of this study is to determine the genotypes of Coastal Cutthroat Trout captured at the mouth of Tryon Creek. This information will help managers begin to understand the significance of nonnatal confluence areas as rearing habitat for Coastal Cutthroat Trout. Specific objectives included:

1. Evaluate genetic diversity and divergence between Coastal Cutthroat Trout collected in Tyron Creek, upstream and downstream of upstream of the Highway 43 culvert.

Part 2c. Evaluate the population structure of Coastal Cutthroat Trout from City of Portland (Oregon) watersheds

The conservation value of peripheral populations has been formally recognized (Haak et al. 2010). It is not uncommon for imperiled fish, especially peripheral populations, to inhabit urban, suburban or developed landscapes (see Diefenderfer et al. 2005; NOAA 2010). The

proportion of the landscape that is developed or urbanized continues to increase (see Walsh et al. 2009). Urban ecology is a burgeoning discipline (Adams 2005, Marzluff et al. 2008) and the conservation value of fish populations in urban or suburban areas is becoming increasingly recognized (Miserendino et al. 2008, Brown et al. 2009, Wegner et al. 2010).

The City of Portland manages numerous streams that contain a variety of fish species (Tinus et al. 2003). Coastal Cutthroat Trout are one of those species and can be found in many of these streams. To manage these fish effectively it is critical to understand their population structure. Some Coastal Cutthroat Trout in areas adjacent to Portland (lower Columbia River region) exhibit population structure at the level of the stream or watershed (Johnson et al. 2010). However, it is unclear whether Coastal Cutthroat Trout from City of Portland streams have sufficient mixing to function as one population or have limited mixing and are structured as independent populations.

The objective of this study was to:

1. Characterize the genetic diversity and several measures of inbreeding in groups of Coastal Cutthroat Trout collected from Johnson Creek, Kelley Creek, Mitchell Creek, and Tryon Creek (all within the limits of the City of Portland).
2. Use this information to evaluate whether Coastal Cutthroat Trout from these areas appear to represent independent populations.

Part 3. Determine the species composition of unidentified trout or presumed Hybrid Trout (O. mykiss x Coastal Cutthroat Trout) samples collected from the city of Portland

Steelhead (or Rainbow) Trout (*O. mykiss*) and Coastal Cutthroat Trout coexist in many of the rivers, streams, and creeks within the Portland (Oregon) area. For these closely related species, using morphometric indicators to determine the species of relatively small juveniles (e.g. fry) can be difficult and inaccurate (Baumsteiger et al. 2005). In addition, when *O. mykiss* and Coastal Cutthroat Trout exist sympatrically, they are able to hybridize. Hybridization further obscures the ability to determine species using morphological indicators (Ostberg et al. 2011). Confirming the species identification of a specimen is often a critical component of an investigation and essential for proper management. Thus, there was a need to use genetic tools (Kennedy et al. 2009) to confirm the species (or hybridization) of relatively small *Oncorhynchus* specimens collected from areas where *O. mykiss* and Coastal Cutthroat Trout coexist. To address the question of species and hybrid identification from a genetic perspective, our objectives were to:

1. Determine whether each sampled individual was *O. mykiss*, Coastal Cutthroat Trout, or a hybrid.
2. Incorporate all individuals identified as *O. mykiss* into the investigation of *O. mykiss* origin (Part 4).

Part 4. Determine the origin of Steelhead Trout (O. mykiss) collected from the city of Portland

There is a need to better understand the origin of Steelhead Trout (*O. mykiss*) that use habitats in the City of Portland. Multiple races of Steelhead Trout (summer run and winter run) exist within and use watersheds in the Portland area (Chilcote 1998). Juvenile salmonids in these areas may have originated from local watersheds (the stream from which they were captured) while others may have originated from watersheds outside the Portland area (Teel et al. 2009). In addition, some of these fish may be from listed (threatened or endangered) stocks from outside the area (Good et al. 2007). Thus, to better understand which stocks are using Portland watersheds and protect these fish and these habitats appropriately, managers (in particular the Bureau of Environmental Services from the City of Portland) were seeking to identify the origin of (genetic assignment data for) *O. mykiss* captured from various tributaries. The goal to address the question of *O. mykiss* origin from a genetic perspective included the following objectives:

1. Use the standardized Columbia River Steelhead genetic baseline (Blankenship et al. 2011) and data for *O. mykiss* populations from the Willamette Basin (Williamson et al. 2010, Van Doornik et al. 2015) to assign each *O. mykiss* to run type (i.e., summer or winter).
2. Estimate the probability that each sample originated from a lower Columbia River tributary, a Willamette River tributary, or from an upper basin (e.g. Mid-Columbia River/Lower Snake River, Yakima River, or Upper Columbia River) tributary.

Relationship to the U.S. Fish & Wildlife, Fish & Aquatic Conservation Program's Strategic Plan

Implementation of this project demonstrates application of the Pacific Region's 2009-2013 Strategic Plan. The following National goals (NG) and Regional objectives (RO) have been addressed by this project:

NG1 Open, interactive communication between the Fisheries Program and its partners.

- RO1.1 Develop and maintain relationships with partners throughout the Pacific Region.
- RO1.3 Improve data collection and management and internal and external reporting to reduce redundancy and improve access and usefulness for ourselves and our partners.

NG2 America's streams, lakes, estuaries, and wetlands are functional ecosystems that support self-sustaining communities of fish and other aquatic resources.

- RO2.1 Facilitate management of aquatic habitats on national and regional scales by working with Tribes, States, partners and other stakeholders.
- RO2.2 Develop and expand the use of its expertise to help avoid, minimize or mitigate impacts of habitat alteration on aquatic species and monitor and evaluate completed projects.

RO2.4 Expand opportunities to connect people with nature, engage citizen scientists and volunteers, and temporarily employ youth in the aquatic habitat conservation and monitoring programs and activities we lead or support.

NG3 Self-sustaining populations of native fish and other aquatic resources that maintain species diversity, provide recreational opportunities for the American public, and meet the needs of tribal communities.

RO3.1 Collaborate with Ecological Services (ES) Program, National Oceanographic and Atmospheric Administration Fisheries (NOAA Fisheries) and others, to recover fish and other aquatic resource populations protected under the ESA.

RO3.2 Maintain healthy, diverse, self-sustaining populations of fish and other aquatic resources

RO3.3 Support the research and fish culture needed to prevent listing or to recover native species listed or proposed for listing under ESA.

NG9 Science developed and used by Service employees for aquatic resource restoration and management is state-of-the-art, scientifically sound and legally defensible, and technological advances in fisheries science developed by Service employees are available to partners.

RO9.2 Use state-of-the-art, scientifically sound, legally defensible scientific and technological tools in formulating and executing fishery-related plans and policies.

Study Area

Tryon Creek is a 7.8 km, second order tributary to the Willamette River located in southwest Portland, OR (Figure 1). Its watershed covers 16.8 km^2 in Multnomah and Clackamas counties and its headwaters are located within suburban neighborhoods. The mainstem flows approximately 4 km through privately owned land including culverts at Taylors Ferry Road, SW 18th Place, SW Maplecrest Drive, and a perched pipe culvert at Boones Ferry Road before entering Tryon Creek State Natural Area. Tryon Creek State Natural Area is a 2.59 km^2 area of public land through which the stream flows another 3.5 km. A baffled box culvert bisects the lower portion of Tryon creek at Oregon State Highway 43 and a railroad near the mouth of Tryon Creek. The lowest portion of Tryon Creek flows 0.3 km through public land owned by the City of Lake Oswego and the City of Portland (confluence area) before entering the Willamette River at river kilometer (rkm) 32.

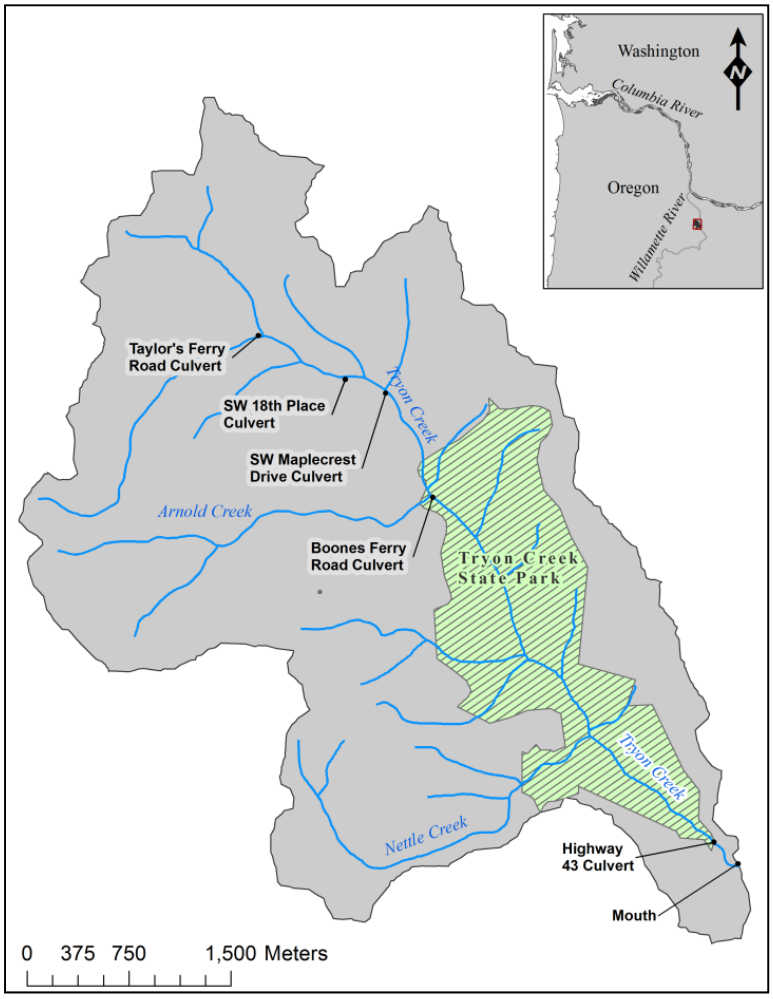

Figure 1. Tryon Creek watershed and major culverts.

The Highway 43 culvert was constructed in the late 1920s. It is approximately 122 m (401 ft.) long with a drop of nearly 6.7 m (22 ft.) from top to bottom, resulting in an average grade of 4.6% (Figure 2). Baffles located within the Highway 43 culvert provide structure, holding water for fish attempting to migrate upstream (Figure 3).

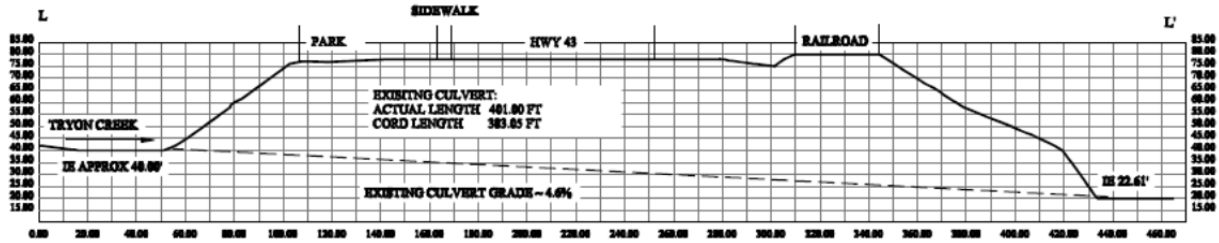

Figure 2. Longitudinal profile of Tryon Creek culvert (Henderson Land Services 2007).

Figure 3. Modified baffles were installed in the Highway 43 culvert to improve fish passage (2008).

Methods

Lamprey Occupancy

Occupancy of larval Pacific and Western Brook Lampreys in Tryon Creek was assessed with an annual electrofishing survey conducted on 8/06/2014 and 7/13/2015. Detailed methods are described in Silver et al. (2013). In brief, the entire reach from the mouth of Tryon Creek to the Highway 43 culvert was sampled. Between the Highway 43 culvert and Boones Ferry Road, six 50 m-long, randomly selected, spatially-balanced reaches were sampled (Figure 4).

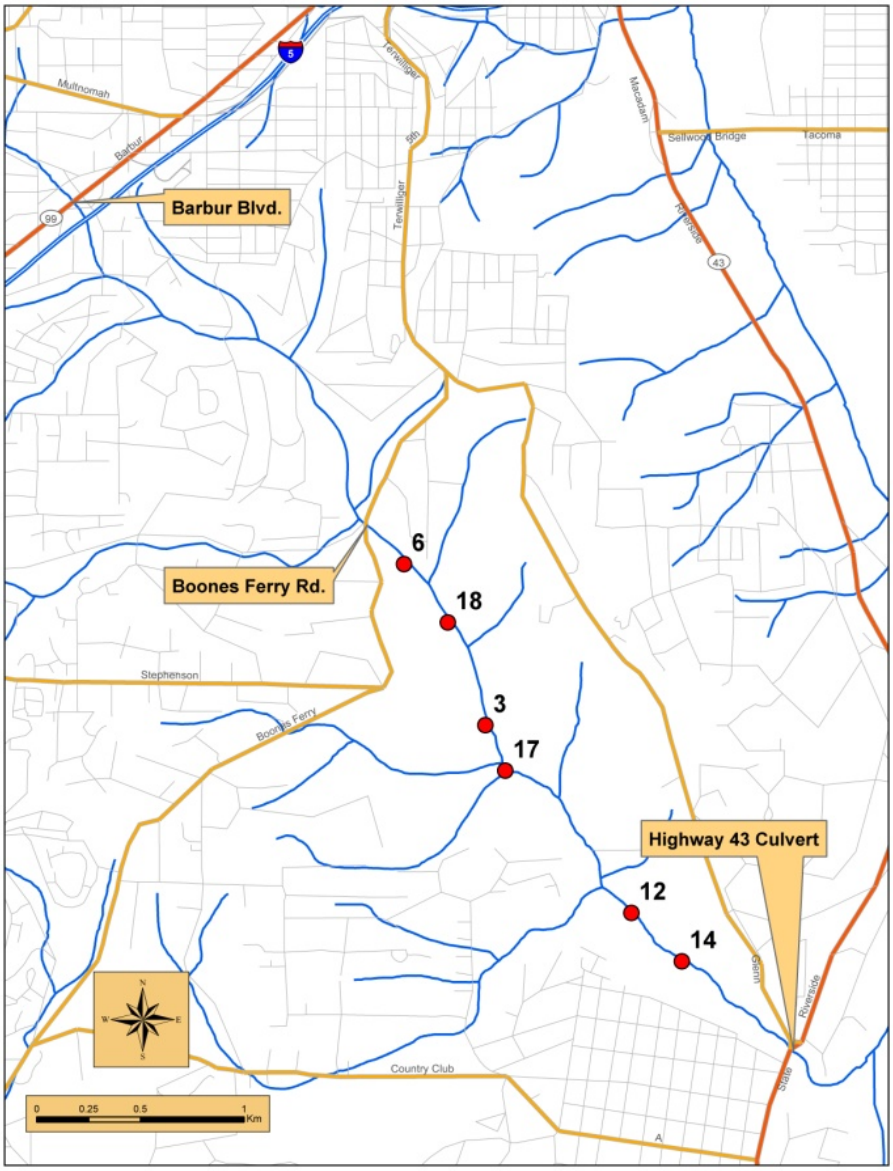

Figure 4. Lamprey survey sites in Tryon Creek between the Highway 43 culvert and Boones Ferry Road.

Below the Highway 43 Culvert - Confluence Sampling

Fish Collection

Sampling in the confluence area for the City of Portland's 2015 fiscal year occurred monthly July 2014 to April 2015; weekly sampling occurred from mid- April to mid-June 2015. Backpack electrofishing occurred in a single pass from the mouth of Tryon Creek to the downstream edge of the Highway 43 culvert pool (Figure 5). Electrofishing was conducted using a Smith-Root model LR-24 shocker in a manner to reduce potential harm to the sampled population. Specifically, any area considered holding habitat for fish species (plunge pools, overhanging banks, eddies, large woody debris, and pocket pools within riffles) were sampled in a "stalk and shock" approach. All fish were collected for identification except sculpin species; because of their high abundance, we estimated the number observed. The LR-24 shocker used pulsed direct current set at a frequency of 25 Hz, 13-15% duty cycle, and voltage 300-375 V. All settings were subject to modification depending on conditions (i.e. water depth, conductivity, flow). Flow was measured by the USGS Discharge Gauge #14211315 located approximately 1.5 km upstream (USGS 2015).

The pool below the Highway 43 culvert was sampled with a seine in two passes. We used an unbagged, 15.2 m long, 1.8 m deep, 0.6 cm mesh seine net with float and lead lines. Two consecutive hauls were pulled by holding one end of the net at the side of the pool, towing the other end around the perimeter of the pool, and pulling the draglines to shore simultaneously. Fish were dip netted out of the seine and transferred into aerated buckets after each haul.

At the completion of each sampling method, all captured fish were anesthetized in a bath containing 60 mg/l MS-222 and 60 mg/l sodium bicarbonate until complete loss of equilibrium was observed (3-4 minutes). Each fish was identified, checked for any external markings, measured (fork length), weighed (grams), and scanned for a PIT tag. If a PIT tag was not found, all non-injured individuals over 70 mm fork length were tagged. After full recovery within an aerated bucket, all fish were released within the reach from which they were captured. Genetic samples collected from salmonids were archived at the USFWS Columbia River Fish and Wildlife Conservation Office.

Relative Abundance

Catch per unit effort (CPUE) was used to determine trends in fish abundance of the confluence habitat. It is an index of relative abundance, which is often related to absolute abundance (Hubert and Fabrizio 2007, Pope et al. 2010). Theoretically, CPUE will increase with an increase in population size or abundance (N), assuming catch (C), effort (E), and catchability (q) remain relatively constant.

$$CPUE = \frac{C}{E} = Nq$$

It is important to note if catchability varies (i.e. by season, number of crewmembers, temperature, time of day, flow, etc.) because it could influence the abundance estimate.

CPUE was calculated differently for electrofishing and seining. For electrofishing, total sample time (in seconds) was recorded at the end of each survey. The seconds of sample time were converted to hours and the total number of fish collected during the electrofishing survey was divided by the number of hours the survey lasted. For each seine, the total number of fish collected was divided by pool volume in m^3 (pool width x pool length x seine max depth). Seine CPUE was averaged each sample event.

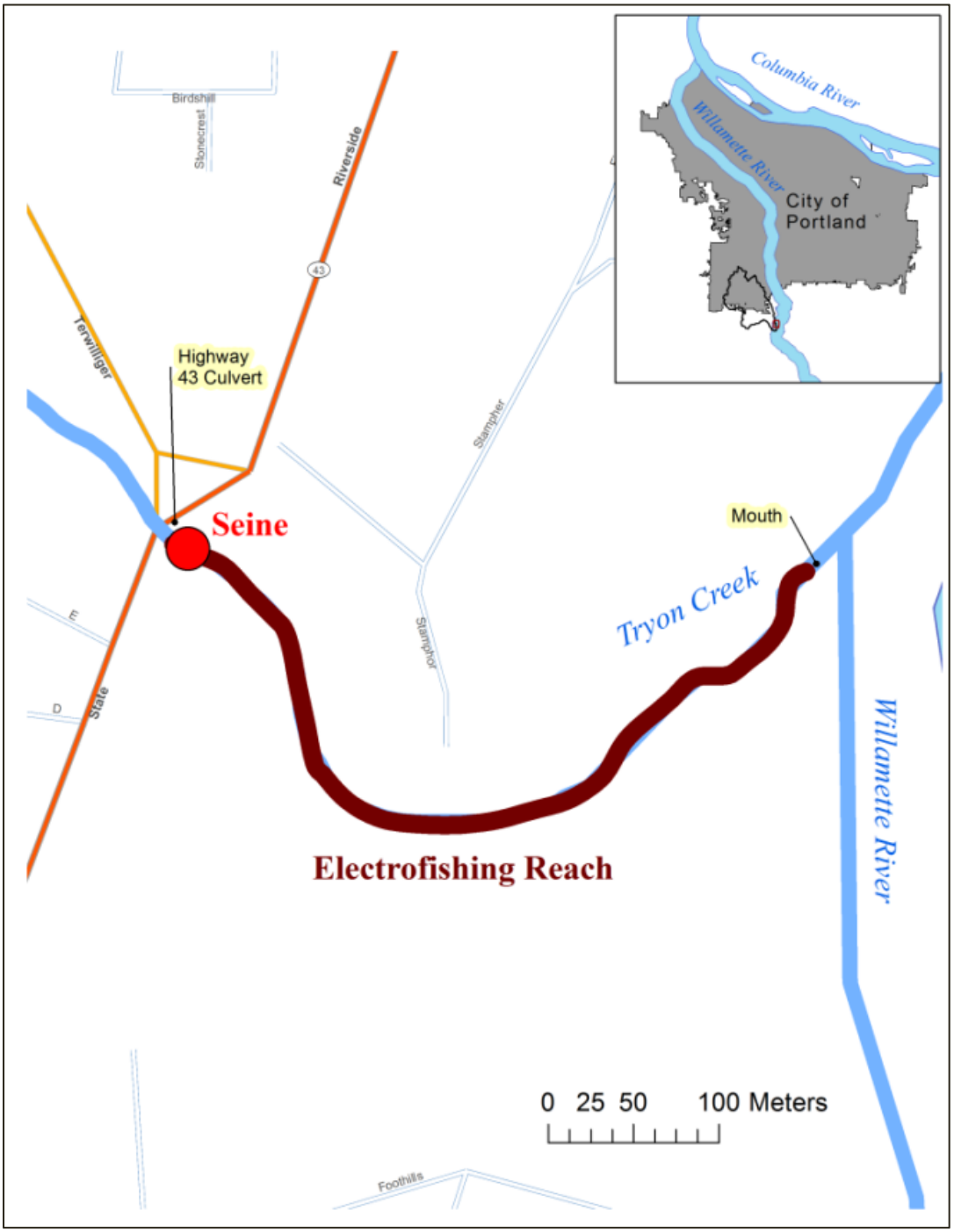

Figure 5. Tryon Creek confluence area monitoring reach

Community

The ratio of native to introduced fish, species richness (Simpson Diversity Index), and relative abundance versus frequency of occurrence (ecological classification) were used to describe fish community.

Native to Introduced Fish Ratio

All fish captured were categorized as "native" or "introduced" according to the Willamette Basin Atlas (Hulse et al. 2002). The proportion of native species to introduced species was calculated for both richness (number of species present) and abundance (number of fish present).

Species Richness

The Simpson (1-D) Diversity Index is an index of species richness, or number of species within a sample area as well as the relative abundance of each species.

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$$

Where *n* is the number of individuals from one particular species and *N* is the total number of individuals found. The index approaches 1.0 when numbers of individuals collected are evenly distributed among the number of species present (evenness of abundance). Biodiversity analysis was calculated each confluence sample event.

Ecological Classification

Species encountered in the confluence were ecologically classified according to relative abundance and percent frequency of occurrence (González-Acosta 1998, González-Acosta et al. 2005). This method of classification is based on Olmstead-Tukey's test (Sokal and Rohlf 1969) and allows an ecological and quantitative classification of the species in each area (González-Acosta et al. 2005). The analysis results in the division of species present into four ecological categories (dominant, common, occasional, and rare) represented by quadrants of a scatter plot that is divided by two axes identifying the mean frequency of occurrence and mean relative abundance for a specific area.

Residence Time

Two PIT tag antennas were installed at the mouth of Tryon Creek in February 2014 (TCM). Efficiency was calculated in 2014 as described in Silver et al. (2015). PIT-tagged fish moving over or through these antennas had the opportunity to be detected and identified. The PIT tag code and time of detection was logged on a Biomark Multiplexing Transceiver (FS 1001M) from which data was downloaded on a monthly basis and uploaded to the Columbia Basin PIT Tag Information System (PTAGIS) online database.

PTAGIS was queried to identify fish detected in Tryon Creek that were tagged and released by other agencies in 2014 and 2015. For fish tagged in Tryon Creek, PTAGIS was used to query detections at all interrogation sites in the Columbia River Basin.

Above the Highway 43 Culvert - Abundance Estimate

We estimated the density (individuals/m^2) of Coastal Cutthroat Trout > 100 mm in Tryon Creek during five years, 2008, 2009, 2011, 2013, 2015, using mark-recapture and single-pass backpack electrofishing. The estimate included the mainstem of Tryon Creek and the lower portion of Arnold Creek between the Boones Ferry Road and the culvert at SW Arnold Street. Twenty sample reaches measured approximately 250 m for a stream segment over 5 km (Figure 6). Data from a habitat survey conducted in 2000 was used to calculate the segment's average wetted width as 4.1 m (Oregon Department of Fish & Wildlife [ODFW] 2012). We did not sample pools created by beaver dams in 2009 and 2015, and ended our survey one reach short in 2015 due to the development of private property. Sample area was estimated each year based on length of habitat sampled. Two to three netters worked with one electrofisher upstream with no blocknets. The LR-24 electrofisher used pulsed direct current set at a frequency of 25 Hz, 13-15% duty cycle, and voltage between 300 and 400 V. All settings were subject to modification depending on conditions (i.e. water depth, conductivity, flow).

In 2008, 2011, and 2015, 2-pass electrofishing was completed. In these three years (i), all cutthroat trout collected in the first pass were marked ($Cap1_i$) and released. During the second pass, we recorded the total number that were captured ($Cap2_i$) and of those, the number of that were marked during the first pass that year ($Recap_i$). Capture probability for each second pass ($2p_i$) was estimated using a binomial distribution:

$$Recap_i \sim Binomial(2p_i, Cap1_i)$$

We estimated abundance in Tyron Creek for those three years ($2Pass.N_i$) using a Poisson model:

$$2Pass.N_i \sim Poisson\ (Expected.2Pass.N_i)$$

with $Expected.2Pass.N_i = Cap2_i/2p_i$. To obtain a larger sample size of capture probability estimates (to estimate abundance for years when mark-recapture was not completed), we used our estimates of $2Pass.N_i$ to estimate capture probability for the first pass in each year ($1p_i$), since it was assumed that abundance would not change between passes, but that capture probability could due to different samplers and environmental conditions (Peterson et al. 2004, Benejam et al. 2012). Thus, for each first pass $1p_i = Cap1_i/2Pass.N_i$.

Expected values for the six capture probability estimates ranged from 0.16 to 0.39 (Table 6). This range was used to estimate abundance in the two years (2009 and 2013) when single pass electrofishing was completed (i.e., to estimate $1Pass.N_i$). To assign an estimate of capture probability (p) when only single pass electrofishing was completed:

$$p \sim Uniform\ (0.16\ to\ 0.39)$$

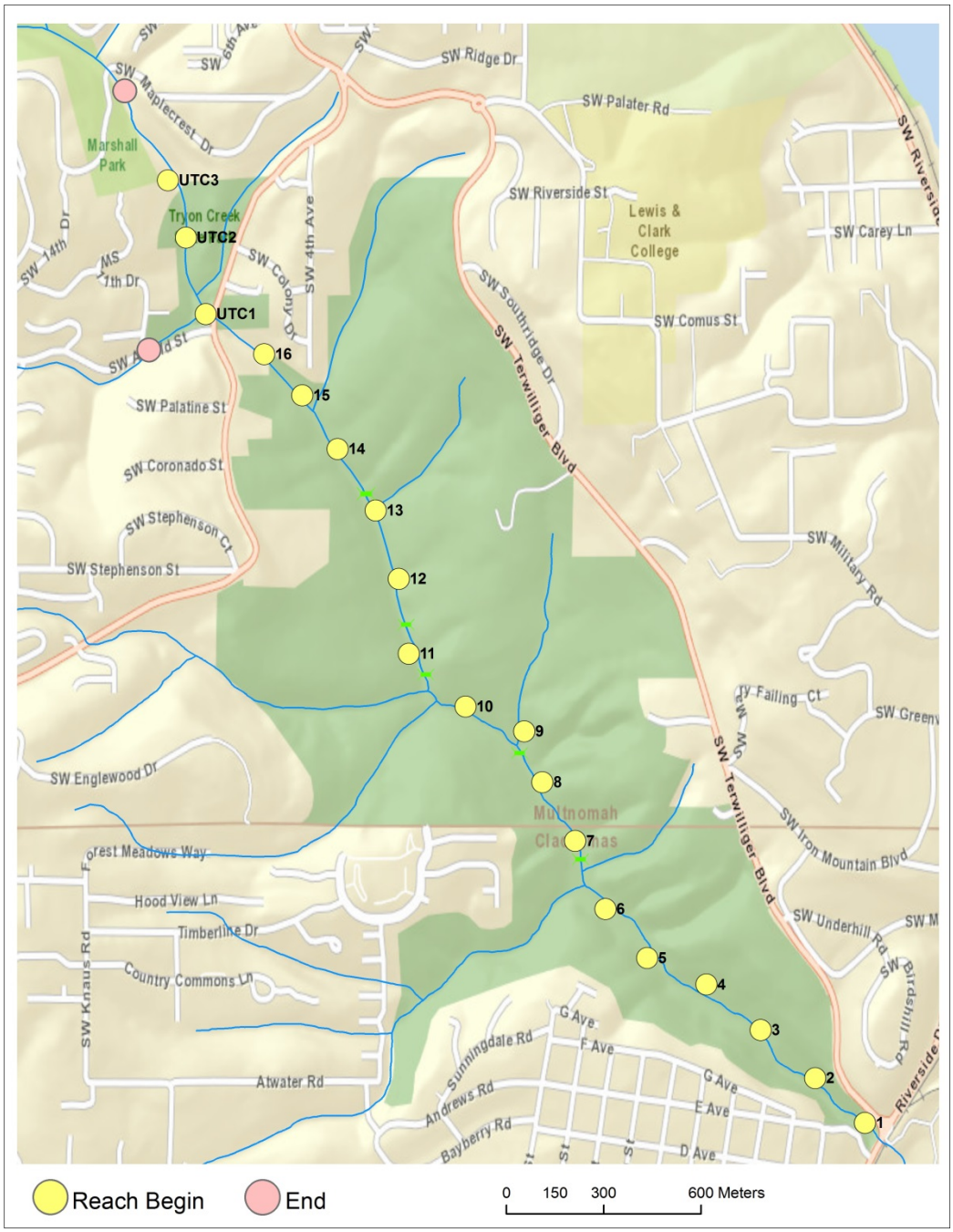

Figure 6. The Tryon Creek study area. Sixteen reaches between the Highway 43 culvert and the Boones Ferry Road crossing and three reaches in Upper Tryon Creek above Boones Ferry Road to Taylors Ferry Road (UTC3 was not sampled in 2015). One reach is located in Arnold Creek above Boones Ferry Road to the SW Arnold Street Culvert.

The expected value for abundance from a single pass was calculated as $Expected.1.Pass.N_i = Cap_i/p$, were Cap_i is the total number caught in the one electrofishing pass completed. Estimates of abundance for single pass electrofishing years ($1Pass.N_i$) were similarly estimated from a Poisson distribution:

$$1Pass.N_i \sim Poisson\ (Expected.1Pass.N_i)$$

Density was calculated for each year by dividing each estimated abundance (i.e., $2Pass.N_i\ or\ 1Pass.N_i$) by the total area that was sampled during that year (Table 6).

All models were evaluated by Bayesian analysis methods using OpenBUGs software (Spiegelhalter et al. 2014). Priors for $2p_i$ and $1p_i$ were from uninformative uniform distributions for capture probabilities (i.e., uniform distributions over the range of 0 to 1). Models were run with two chains for 60,000 iterations with 10,000 removed as burn-in and 50,000 saved to produce posterior distributions. We assessed that convergence had occurred and that parameters reached stable distributions by examining history and density plots (Kéry 2010, Spiegelhalter et al. 2014).

Genetic Assessment

Part 1.

Genetic samples were collected from Coastal Cutthroat Trout in Tryon Creek between 2008 and 2015 for a total sample size of 117 fish. Divergence between the generations sampled in 2008 (n=56) and 2013(n=56) were used to infer divergence over time (i.e., genetic drift). All samples were sent to AFTC where genomic DNA was extracted from each sample. To provide context for the results from the Tryon Creek collections, we incorporated genotype data for coastal cutthroat trout collected from 27 collection sites in other Columbia River Basin tributaries. Genetic divergence between temporal collections at each site was evaluated using contingency tests of allele frequency heterogeneity and pairwise F_{ST} (See Appendix B).

Part 2a.

Abernathy Fish Technology Center analyzed 78 tissue samples taken from Coastal Cutthroat Trout captured in the Tryon Creek watershed, upstream of Boones Ferry Road. Extraction of genomic DNA, microsatellite genotyping of all samples, and removal of *O. mykiss* and hybrids was conducted as described in Appendix B. Genetic diversity was evaluated as expected and observed heterozygosity and allelic richness. Inbreeding was evaluated as linkage disequilibrium, effective population size (N_e) and average pairwise relatedness. Comparisons to Coastal Cutthroat Trout collected from Tyron Creek downstream of Boones Ferry Road were carried out using permutation tests (individuals permuted among collections). Divergence among groups were evaluated using contingency tests for difference in allele frequencies and F_{ST} tests. If these tests were not significant, then collections were pooled, and overall estimates of diversity and inbreeding were provided (See Appendix C).

Part 2b.

Abernathy Fish Technology Center analyzed 14 tissue samples taken from Coastal Cutthroat Trout captured near the mouth of Tryon Creek. Extraction of genomic DNA, microsatellite genotyping of all samples, and removal of *O. mykiss* and hybrids was conducted as described in Appendix B. Divergence between Coastal Cutthroat Trout collected in Tyron Creek, upstream and downstream of the Highway 43 culvert, was evaluated graphically by plotting the first two axes of a principal component analysis (See Appendix C).

Part 2c.

Abernathy Fish Technology Center analyzed 106 tissue samples taken from Coastal Cutthroat Trout captured in City of Portland (Oregon) watersheds: Johnson Creek (n=31), Kelley Creek (n=45), and Mitchell Creek (n=30). Extraction of genomic DNA, microsatellite genotyping of all samples, and removal of *O. mykiss* and hybrids was conducted as described in Appendix B. Genetic diversity was evaluated as expected and observed heterozygosity and allelic richness. Inbreeding was evaluated as linkage disequilibrium, effective population size (N_e) and average pairwise relatedness. In addition to Coastal Cutthroat Trout collected from Johnson Creek, Kelley Creek, and Mitchell Creek, comparisons from Tyron Creek, Abernathy Creek, and the Chinook River were carried out using permutation tests (individuals permuted among collections). Divergence among groups was evaluated using contingency tests for difference in allele frequencies and F_{ST} tests (See Appendix C).

Part 3.

AFTC analyzed 11 tissue samples from presumed hybrids and 4 tissue samples from unidentified trout. DNA extraction and amplification of twenty microsatellite loci, and genetic species identification was performed on these samples following the methods described in Appendix B.

Part 4.

AFTC analyzed 110 presumed *O. mykiss* tissue samples. DNA extraction and microsatellite genotyping was performed as described in Appendix B. The standardized Columbia River steelhead genetic baseline (Blankenship et al. 2011) plus data for *O. mykiss* populations from the Willamette Basin (Williamson et al. 2010, Van Doornik et al. 2015) were used to assign each individual to run type (i.e., summer or winter). Additionally, the probability that each sample originated from a lower Columbia River tributary, a Willamette River tributary, or from an upper basin (e.g. Mid-Columbia River/Lower Snake River, Yakima River, or Upper Columbia River) tributary was estimated.

Results

Lamprey Occupancy

During the larval lamprey occupancy survey conducted on 8/6/2014, one larval Pacific Lamprey was caught below the Highway 43 culvert, and one larval Pacific Lamprey was caught in reach 18 above the Highway 43 culvert (Figure 5). During the larval lamprey occupancy survey conducted on 7/13/2015, 11 larval Pacific Lamprey were caught below the Highway 43 culvert, and two larval Pacific Lamprey were caught in reach 18 above the Highway 43 culvert.

Below the Highway 43 Culvert - Confluence Sampling

Relative Abundance

We sampled the confluence habitat on nine monthly occasions from 7/2/2014 to 3/4/2015, and thirteen weekly occasions from 4/1/205 to 6/24/2015. The habitat showed seasonal variation of water temperature and flow. In the summer it was warm (mean = 16.7 °C) and flowing slowly (mean = 1cfs); in the winter it was cold (mean 5.1 °C) with higher flows (mean = 6.1 cfs) (Table 1). We cancelled electrofishing 8/27/2014 because of high water temperature (17.5°C) and 6/17/2015 after observing 10 adult salmon at the confluence below the antennas. Between 7/2/2014 and 8/6/2014, a beaver dam was constructed; by 10/8/2014, it was washed out during a fall storm (Figure 7). This beaver dam did not prohibit fish passage, as untagged migratory fish were present in the pool upstream.

Photo Credit Marc Peters 8/6/2014

Figure 7. Beaver dam built in the Tryon Creek confluence habitat. Presence of untagged migratory fish upstream and PIT detections downstream indicate it did not prohibit fish passage.

Electrofishing effort ranged from 427 seconds to 952 seconds, seine effort was the same for all events (pool volume = 225 m^3, two hauls) (Table 1). Mean electrofishing CPUE (± SE) was 298.64 ± 26.70 and mean seine CPUE (± SE) was 0.12 ± 0.02 (Figure 8).

Table 1. Sample conditions and catch per unit effort (CPUE) in the Tryon Creek confluence habitat.

Sample Date	Water Temp (C°)	Flow (cfs)	Sample Method	Seine Effort (pool volume m^3, hauls)	EFish Effort (sec)	Effort (hours)	Fish Captured (n)	EFish CPUE	Seine CPUE
7/2/14	17.0	1.7	EFish	-	796	0.22	118	536.36	-
			Seine	225, 2	-	-	29	-	0.06
8/6/14	16.6	0.7	EFish	-	427	0.12	54	450.00	-
			Seine	225, 2	-	-	43	-	0.09
8/27/14	17.5	0.5	Seine	225, 2	-	-	40	-	0.08
10/8/14	13.9	0.7	EFish	-	800	0.22	119	540.91	-
			Seine	225, 2	-	-	18	-	0.04
11/5/14	13.3	7.5	EFish	-	952	0.26	65	250.00	-
			Seine	225, 2	-	-	23	-	0.05
12/3/14	4.4	4.7	EFish	-	724	0.20	48	240.00	-
			Seine	225, 2	-	-	68	-	0.15
12/31/14	1.7	4.5	EFish	-	597	0.17	14	82.40	-
			Seine	225, 2	-	-	41	-	0.09
2/4/15	8.9	10.0	EFish		681	0.19	42	221.1	-
			Seine	225, 2	-	-	48	-	0.11
3/4/15	4.9	3.9	EFish	-	522	0.15	30	200.0	-
			Seine	225, 2	-	-	14	-	0.03
4/1/15	8.9	5.5	EFish	-	691	0.19	35	184.2	-
			Seine	225, 2	-	-	25	-	0.06
4/8/15	10.4	4.0	EFish	-	932	0.26	58	223.1	-
			Seine	225, 2	-	-	14	-	0.03
4/15/15	6.7	5.7	EFish	-	829	0.23	45	195.7	-
			Seine	225, 2	-	-	33	-	0.07
4/22/15	9.7	3.9	EFish	-	698	0.19	67	352.6	-
			Seine	225, 2	-	-	27	-	0.06
4/28/15	13.4	3.6	EFish	-	665	0.18	50	277.8	-
			Seine	225, 2	-	-	38	-	0.08
5/6/15	11.0	3.1	EFish	-	663	0.18	59	327.8	-
			Seine	225, 2	-	-	66	-	0.15
5/13/15	11.6	3.4	EFish	-	889	0.25	53	212.0	-
			Seine	225, 2	-	-	86	-	0.19
5/20/15	13.4	2.4	EFish	-	532	0.15	49	326.7	-
			Seine	225, 2	-	-	99	-	0.22
5/27/15	12.7	2.0	EFish	-	628	0.17	41	241.2	-
			Seine	225, 2	-	-	90	-	0.20
6/3/15	14.4	3.0	EFish	-	488	0.14	50	357.1	-
			Seine	225, 2	-	-	27	-	0.06
6/10/15	15.2	1.7	EFish	-	593	0.16	67	418.8	-
			Seine	225, 2	-	-	103	-	0.23
6/17/15	14.5	1.4	Seine	225, 2	-	-	175	-	0.39
6/24/15	15.8	1.1	EFish	-	720	0.20	67	335.0	-
			Seine	225, 2	-	-	131	-	0.29

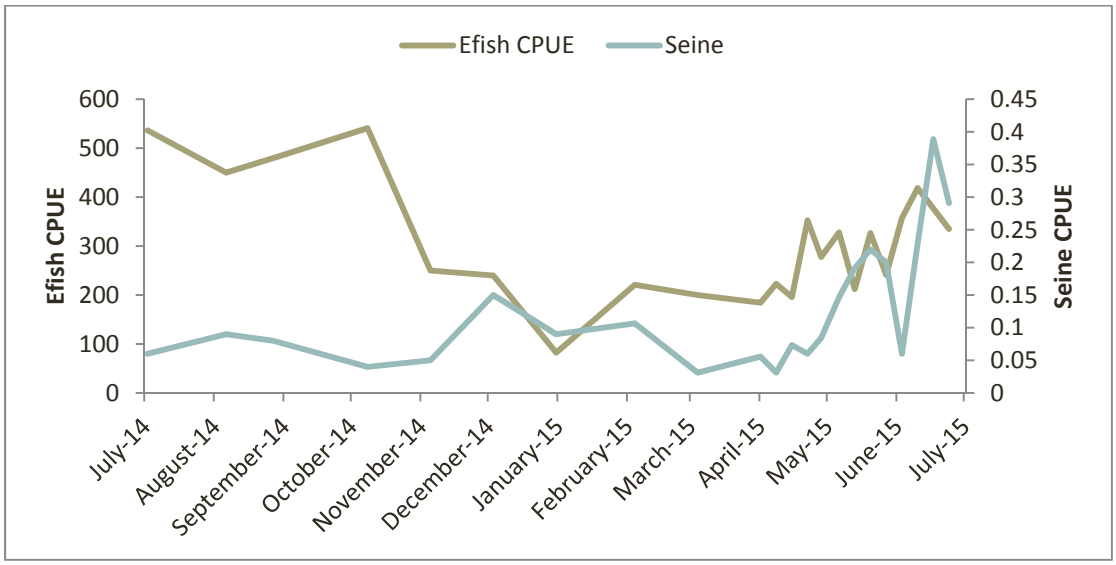

Figure 8. Catch per unit Effort (CPUE) for sample events 7/2014-7/2015 in the Tryon Creek Confluence habitat.

Native salmonids captured included Coastal Cutthroat Trout, *O. mykiss*, Coho Salmon, Chinook Salmon, and Mountain Whitefish (*Prosopium williamsoni*). Additional native species consisted of Peamouth (*Mylocheilus caurinus*), Speckled Dace (*Rhinichthys osculus*), species of sculpin (*Cottus spp.*), and Threespine Stickleback (*Gasterosteus aculeatus*). Three introduced species were captured, Goldfish (*Carassius auratus*), Largemouth Bass (*Micropterus salmoides*), and Smallmouth Bass (*Micropterus dolomieu*) (Figure 9). Genetic samples collected from salmonids were archived at the CRFWCO (n = 502).

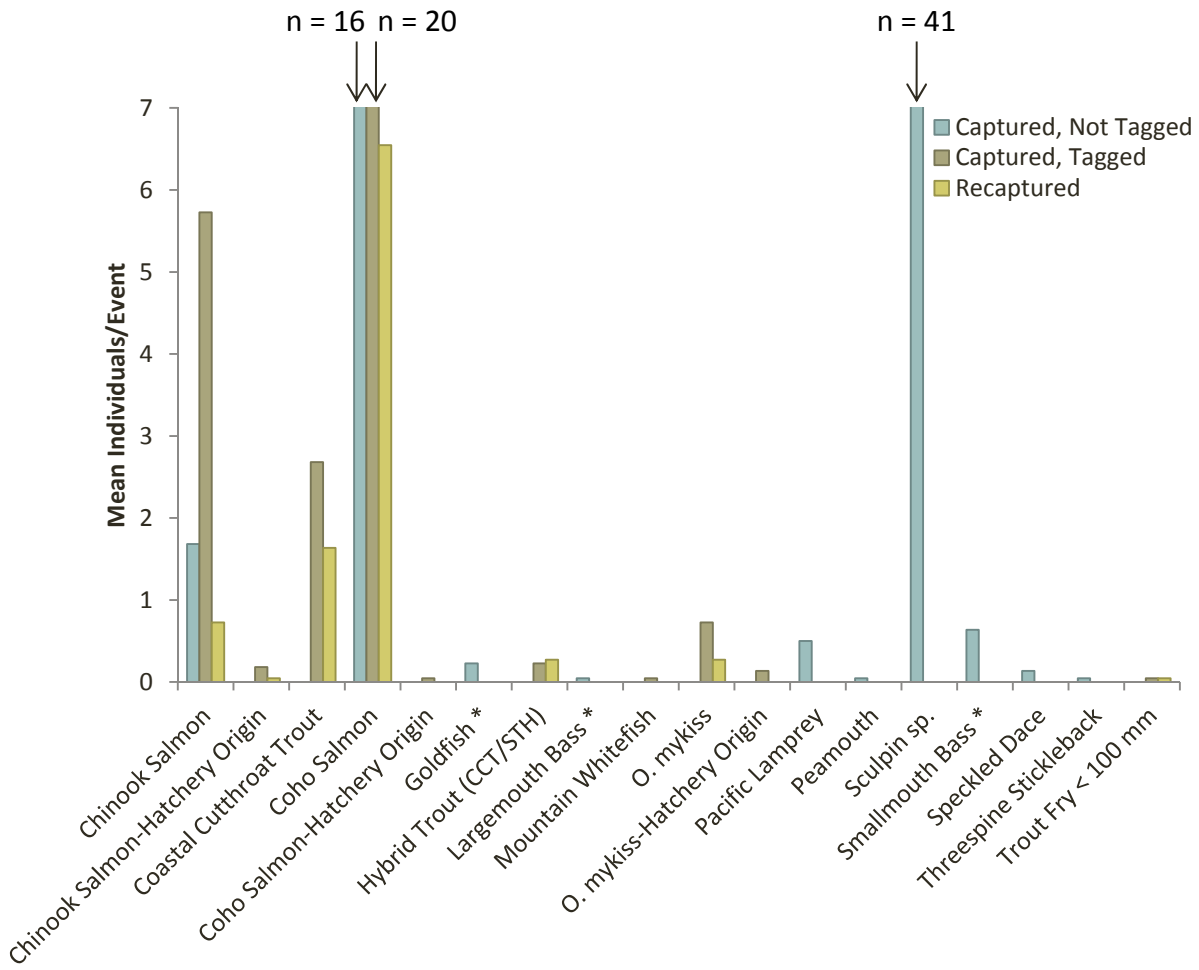

Figure 9. Average number of fish captured per sample event in the Tryon Creek Confluence Habitat 7/2/2014 – 6/24/2015. Captured, not tagged fish may have been recaptured more than once. (Pacific lamprey were captured during a separate larval occupancy survey 8/6/2015 and 7/13/2015). *Introduced species

Recaptures of PIT-tagged fish occurred on all but the first sample event. We recaptured 32% (210/658) of PIT-tagged fish at least once. The recapture rate of juvenile migratory fish, Chinook Salmon and Coho Salmon, was 0.13 and 0.33 respectively. The recapture rate of resident fish, such as Coastal Cutthroat and hybrid trout, was 0.61 and 1, respectively (one hybrid trout tagged in 2013 was recaptured in 2014) (Table 2).

Table 2. Number of PIT-Tagged and recaptured salmonids below the Highway 43 Culvert 7/2/2014 – 6/24/2015

Species	# PIT Tagged	# Recaptured	Recapture Rate (Recaptured/Tagged)	Size Class	Life History
Chinook Salmon	130	17	0.13	Juvenile	Migrant
Coastal Cutthroat Trout	59	36	0.61	Adult/Juvenile	Resident/Migrant
Coho Salmon	443	144	0.33	Juvenile	Migrant
Hybrid Trout	5	6*	1	Adult/Juvenile	Resident
Mountain Whitefish	1	0	0	Juvenile	Migrant
O. mykiss	19	6	0.32	Adult/Juvenile	Migrant/Resident
Trout Fry < 100 mm	1	1	1.00	Juvenile	Resident/Migrant

* Includes one hybrid trout tagged previously in 2013 and recaptured in 2014

Hatchery produced salmonids are marked with an adipose fin clip to facilitate distinction from naturally produced (wild) salmonids. The majority (99.2%, 971/979) of juvenile anadromous salmonids (Chinook, Coho, and *O. mykiss*) captured below the Highway 43 culvert were of wild (naturally produced) origin. Presence of Juvenile Chinook at the mouth of Tryon Creek peaked in winter and juvenile Coho peaked in spring (Figure 10).

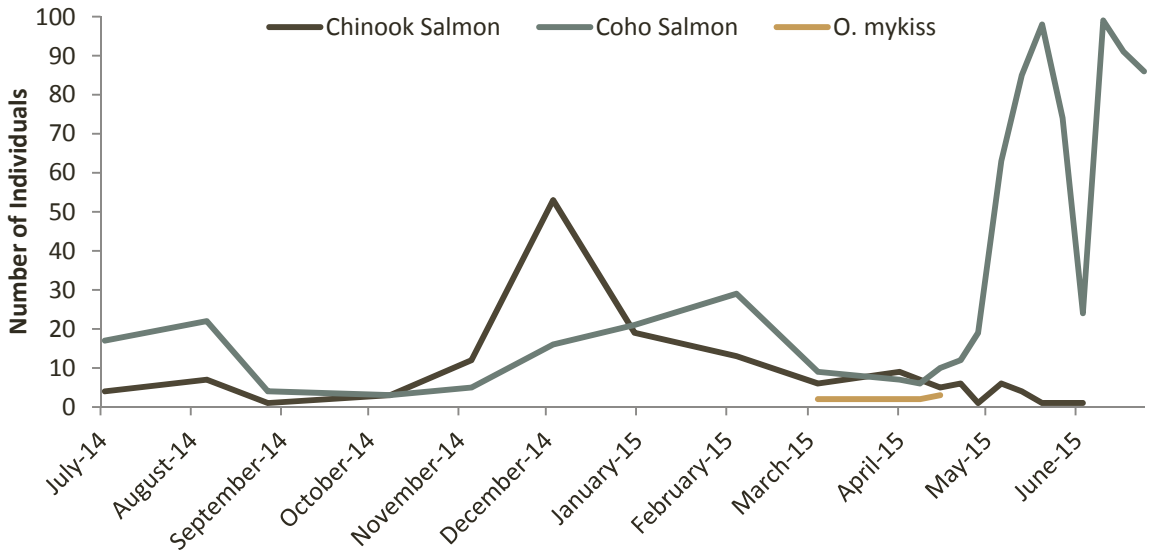

Figure 10. Number of wild migratory salmonids sampled below Highway 43 Culvert 7/2/2014 – 6/24/2015

Community

Native to Introduced Fish Ratio

Sampling below the Highway 43 culvert resulted in the capture of native and introduced fish, wild and hatchery reared salmonids. The fish community below the Highway 43 culvert included 13 species numbering 2,379 total fish (this includes unmarked, recaptured fish) (Table 3, Appendix A). Native fish were the most abundant comprising 77% (10/13) of the species captured and 99.2% (2,359/2,379) of the individuals captured.

Table 3. All species captured in the Tryon Creek Confluence Habitat 2012-2015, (Pacific Lamprey were captured during a separate larval occupancy survey 8/6/2015 and 7/13/2015). Gray shading indicates species captured in prior years.

Family	Genus species	Common Name	Species Abbreviation	Origin (Native/ Introduced) (Hulse 2002)
Catostomidae	*Catostomus sp.*	Sucker	SUK	N
Centrarchidae	*Lepomis macrochirus*	Bluegill	BG	I
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	SMB	I
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	LMB	I
Cottidae	*Cottus sp.*	Sculpin	SCP	N
Cyprinidae	*Carassius auratus*	Goldfish	GF	I
Cyprinidae	*Mylocheilus caurinus*	Peamouth	PEA	N
Cyprinidae	*Rhinichthys cataractae*	Longnose Dace	LND	N
Cyprinidae	*Rhinichthys osculus*	Speckled Dace	SPD	N
Gasterosteidae	*Gasterosteus aculeatus*	Threespine Stickleback	SKB	N
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	BBH	I
Petromyzontidae	*Entosphenus tridentatus*	Pacific Lamprey	PCL	N
Salmonidae	*Oncorhynchus clarki*	Coastal Cutthroat Trout	CCT	N
Salmonidae	*Oncorhynchus clarki/mykiss*	Cutthroat/*O. mykiss* hybrid	HYB	-
Salmonidae	*Oncorhynchus kisutch*	Chinook Salmon	CHN	N
Salmonidae	*Oncorhynchus mykiss*	Steelhead/Rainbow Trout	OMY	N
Salmonidae	*Oncorhynchus tshawytscha*	Coho Salmon	COHO	N
Salmonidae	*Prosopium williamsoni*	Mountain Whitefish	WHF	N

Species Richness

The Simpson Diversity Index was calculated for each sample event. The mean Simpson (1-D) Diversity Index (± SE) was 0.590 ± 0.03 and ranged from 0.254 to 0.786 (Table 4).

Table 4. Simpson (1-D) Diversity Index for each sample event

Sample Event	Season	Simpson (1-D) Diversity Index
7/2/2014	Summer	0.45
8/6/2014	Summer	0.69
8/27/2014	Summer	0.69
10/8/2014	Fall	0.47
11/5/2014	Fall	0.53
12/3/2014	Fall	0.66
12/31/2014	Winter	0.65
2/4/2015	Winter	0.68
3/4/2015	Winter	0.72
4/1/2015	Spring	0.77
4/8/2015	Spring	0.63
4/15/2015	Spring	0.79
4/22/2015	Spring	0.68
4/28/2015	Spring	0.68
5/6/2015	Spring	0.61
5/13/2015	Spring	0.54
5/20/2015	Spring	0.45
5/27/2015	Spring	0.52
6/3/2015	Spring	0.60
6/10/2015	Spring	0.47
6/17/2015	Spring	0.25
6/24/2015	Summer	0.44

Ecological Classification

Ecological classification indicates Chinook Salmon, Coho Salmon, Coastal Cutthroat Trout, and species of sculpin were classified dominant species because their capture was relatively frequent and they were relatively abundant when captured (Figure 11). Common species such as hybrids and *O. mykiss* were captured less frequently and in smaller numbers; species classified rare (Goldfish, Largemouth Bass, Peamouth, Smallmouth Bass, Speckled Dace, Threespine Stickleback, and Mountain Whitefish) were captured infrequently and in small numbers.

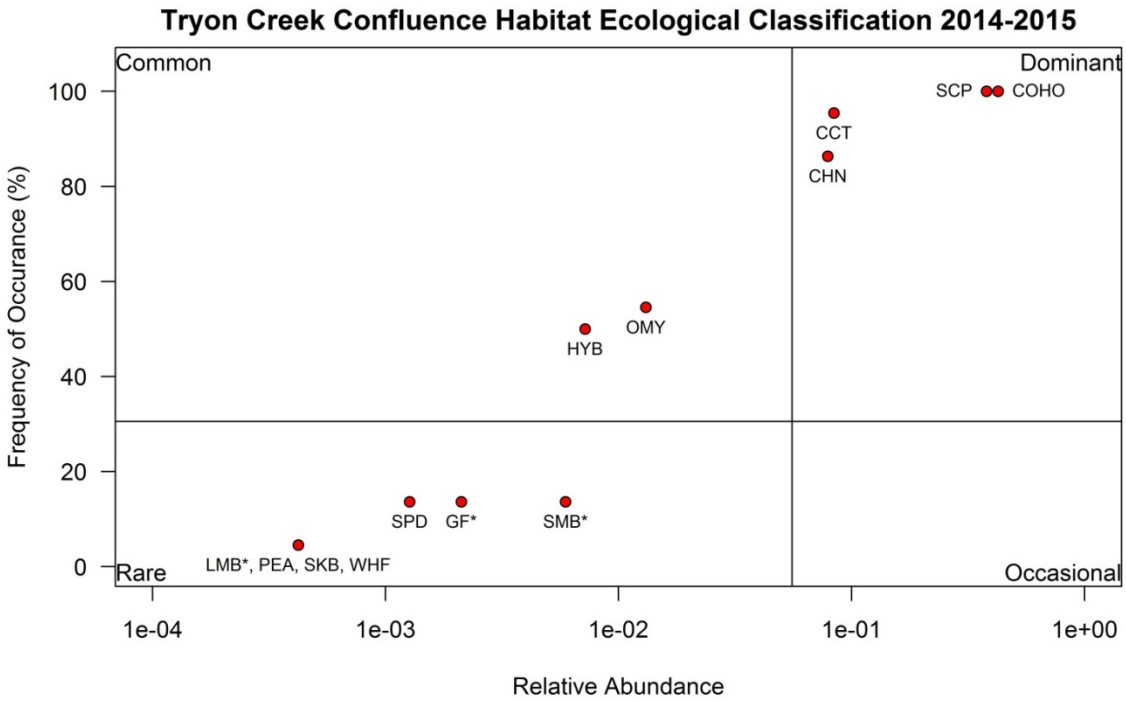

Figure 11. Ecological Classification of species captured in 2014 and 2015. Chinook Salmon (CHN), Coho Salmon (COHO), Coastal Cutthroat Trout (CCT), Cutthroat/*O. mykiss* hybrid (HYB), Goldfish (GF), Largemouth Bass (LMB), Mountain Whitefish (WHF), *O. mykiss* (OMY), Peamouth, (PEA), Sculpin species (SCP), Smallmouth Bass (SMB), Speckled Dace (SPD), Threespine Stickleback (SKB). *Introduced species

Residence Time

The Tryon Creek Mouth (TCM) antenna site monitored for PIT tags from 2/14/2014 to 12/31/2015. On 12/7/2015, a high water event (600 cfs) washed out all but one antenna. The site had 280,378 detections of 404 unique PIT tags (Table 5). Of these unique detections, 78% (314/404) were PIT tags implanted in juvenile migratory salmonids (Chinook and Coho). The majority (383/404) of all PIT tags detected were from fish tagged in Tryon Creek below the Highway 43 Culvert. The peak of unique detections per month occurred between December 2014 and June 2015 (Figure 12). Resident fish such as Coastal Cutthroat and hybrids were detected throughout the year.

Table 5. All detections at the TCM antenna site 2014 - 2015. ( ) = Hatchery origin fish released in the North Santiam River

Species	Total PIT Tag Detections	Unique PIT Tag Detections	Year Tag Used 2007	2008	2009	2010	2012	2013	2014	2015
Chinook Salmon	820	99					1	7 (1)	51 (12)	25 (2)
Coastal Cutthroat Trout	224,214	54			2	2	2	13	23	12
Coho Salmon	54,751	215					1	4 (1)	47 (1)	161
Hybrid Trout	422	13		1			1	6	2	3
O. mykiss	171	23	1					5	2	11 (4)
Total	280,378	404	1	1	2	2	5	37	138	218

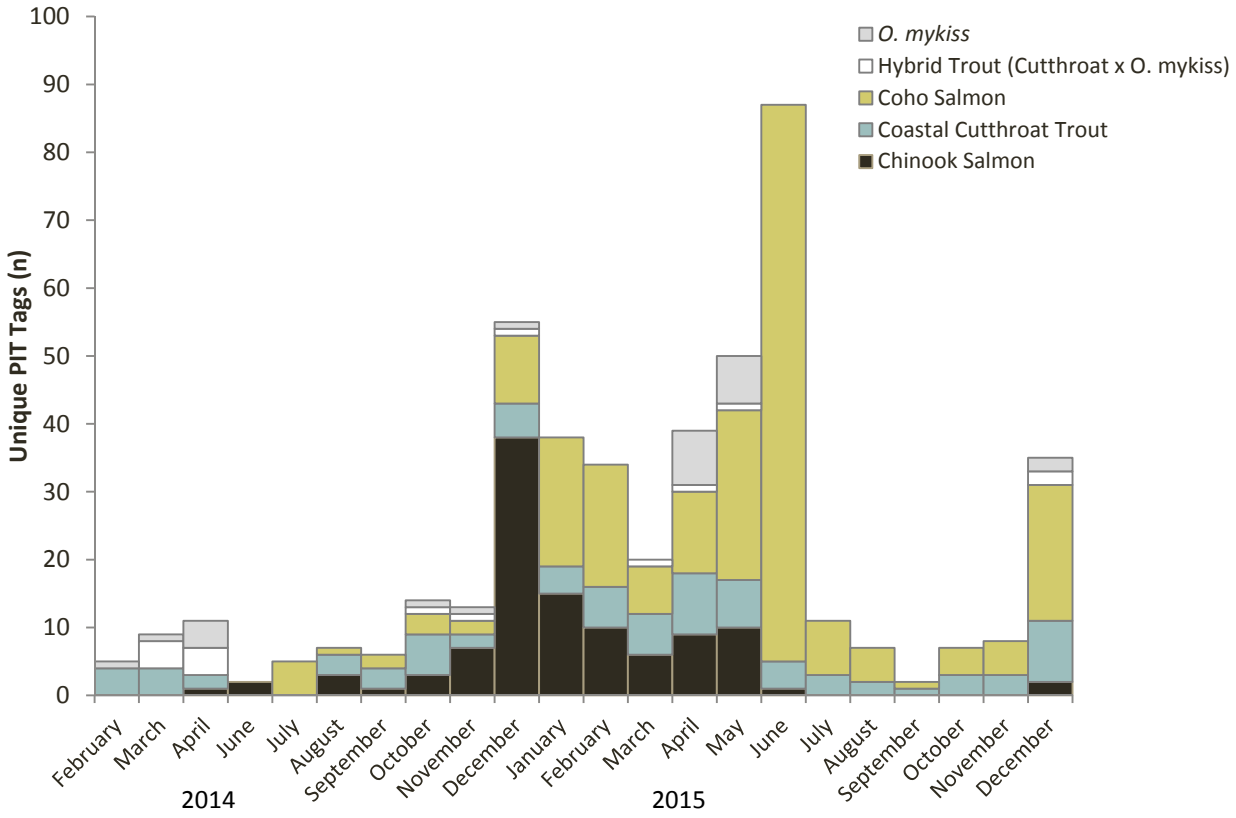

Figure 12. Number of unique detections by month and species.

The TCM antenna site detected salmonids tagged in Tryon Creek between 2014 and 2015. Juvenile anadromous salmonids (Chinook and Coho) (n = 289) were detected emigrating 1 - 499 (median = 11) days after their initial capture. Salmonids exhibiting both resident and anadromous behaviors (Coastal Cutthroat, hybrids, and *O. mykiss*) (n = 55) were detected by the TCM antenna 1 - 534 days (median = 45) after tagging.

Movement

In 2014 and 2015, the TCM antenna site detected thirteen salmonids originating from outside the Tryon Creek watershed. We detected eleven hatchery Chinook released by the Marion Forks Hatchery in the North Santiam River between 2013 and 2014. In 2015, we detected two *O. mykiss* released by the Marion Forks Hatchery in the South Santiam River (Figure 13).

The National Marine Fisheries Service deploys the experimental trawl detector (TWX) in the Columbia River estuary. Two salmonids tagged in Tryon Creek between 2014 and 2015 were detected by the TWX. One Coastal Cutthroat Trout (181 mm) was tagged 4/22/2015, detected at TCM 5/6/2015, and detected six days later at TWX on 5/12/2015. One *O. mykiss* (161 mm) tagged 4/28/2015 was last detected at TCM 5/2/2015 before its detection three days later at TWX on 5/5/2015.

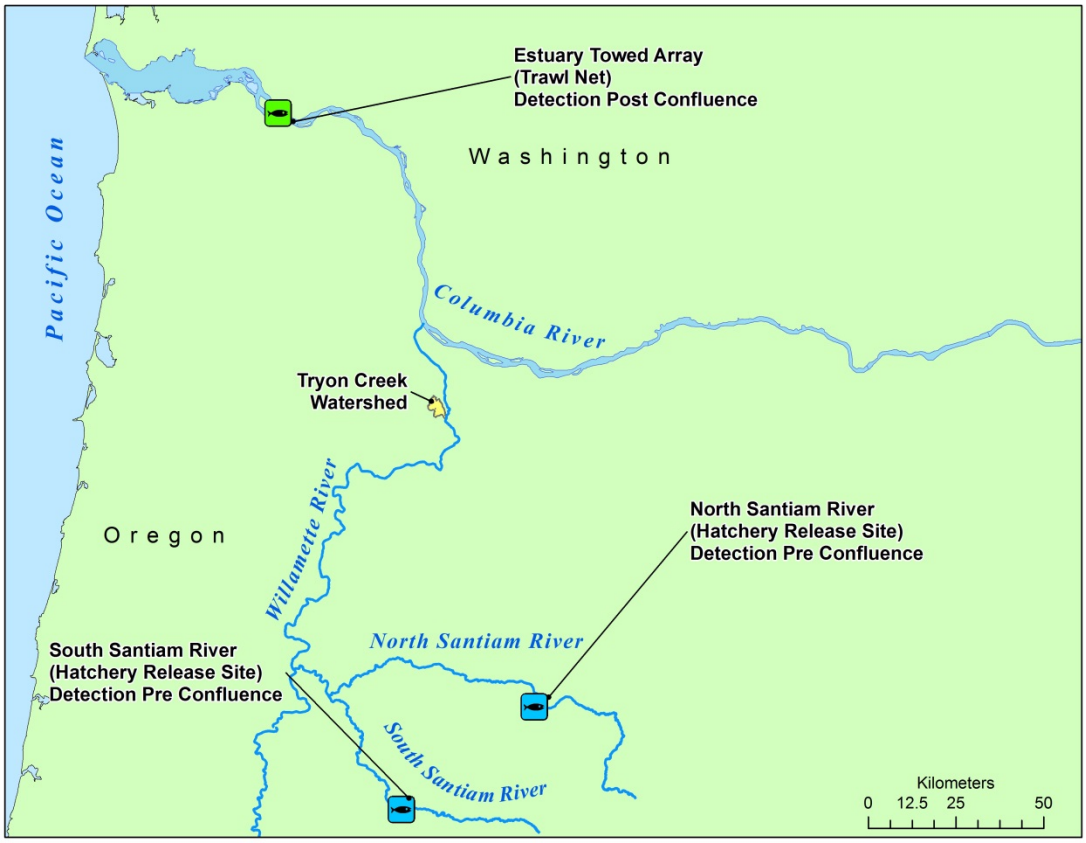

Figure 13. The Tryon Creek Mouth antenna (TCM) detected Chinook Salmon and *O. mykiss* released in the North and South Santiam River. The Estuary Towed array (TWX) detected *O. mykiss* and Coastal Cutthroat Trout released in Tryon Creek.

Above the Highway 43 Culvert - Abundance Estimate

The first electrofishing pass (9/21 - 9/23) above the Highway 43 culvert resulted in 61 Coastal Cutthroat Trout, the second electrofishing pass (10/5 - 10/8) resulted in one *O. mykiss* and 43 Coastal Cutthroat Trout, 17 of which were recaptured from pass one. The estimated abundance of Coastal Cutthroat Trout > 100 mm above the Highway 43 Culvert and below UTC03 was 156 (95% CI [104, 245]) individuals. The estimated area sampled in 2015 was 12,293 m^2. The estimated density of Coastal Cutthroat Trout > 100 mm was 0.013 (95% CI [0.008, 0.020]) individuals/ m^2 (Table 6). A length/frequency histogram of Tryon Creek trout shows a range of sizes from 100 mm to 274 mm (Figure 14).

Table 6. Abundance and density estimates for salmonids in Tryon Creek, by year. Area= the area sampled by backpack electrofishing, $1p_i$ = capture probability in the first pass, $2p_i$ = capture probability in the second pass.

Year	Area (m^2)	$1p_i$	$2p_i$	Abundance	Density
2008	21,181	0.28 (0.21 – 0.36)	0.32 (0.25 – 0.40)	475 (370 – 625)	0.022 (0.017 – 0.030)
2009	19,932			270 (184 – 450)	0.014 (0.009 – 0.023)
2011	21,181	0.20 (0.14 – 0.29)	0.16 (0.11 – 0.23)	702 (494 – 1,050)	0.033 (0.023 – 0.050)
2013	21,181			571 (398 – 950)	0.027 (0.019 – 0.045)
2015	12,293	0.39 (0.25 – 0.59)	0.28 (0.18 – 0.40)	156 (104 – 245)	0.013 (0.008 – 0.020)

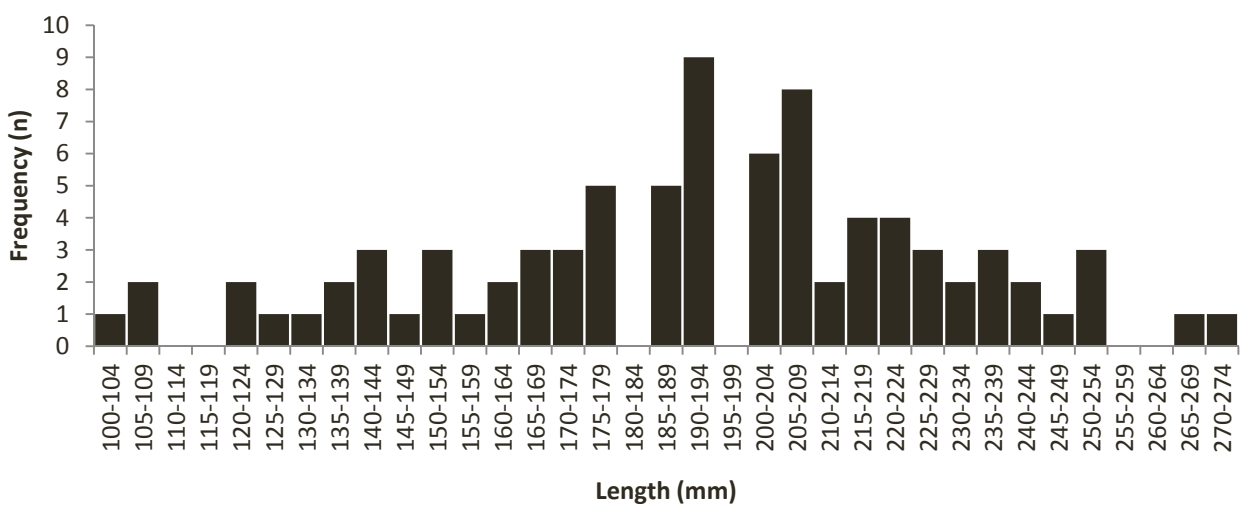

Figure 14. Length-frequency histogram of Coastal Cutthroat Trout captured in Tryon Creek, Passes 1 and 2, September – October 2015.

Genetic Assessment

Part 1.

Of 117 fish analyzed with the species ID markers, 113 were identified as Coastal Cutthroat Trout and four were identified as Coastal Cutthroat Trout x *O. mykiss* hybrids (none were identified as pure *O. mykiss*). Of 113 Coastal Cutthroat Trout, three individuals failed to amplify at the majority of population structure markers and were excluded from subsequent analyses. The remaining 110 fish successfully amplified at 8-12 loci (overall genotyping success rate 97%)

Linkage disequilibrium was observed in four pairs of loci in the 2008 Tryon Creek collection and two pairs of loci in the 2013 collection. When the two collections were pooled and the analysis rerun, linkage disequilibrium was observed in five pairs of loci. The estimate of F_{ST} between the 2008 and 2013 collections from Tryon Creek was essentially 0.000 (See Appendix B).

Part 2a.

The Coastal Cutthroat Trout collected from Tyron Creek upstream and downstream of Boones Ferry Road had deviations from Hardy-Weinberg Proportions at a single locus each. Fish collected below or above Boones Ferry Road had 11 and nine private alleles, respectively. The pairwise comparison produced low F_{ST}-values (<0.02) with 95% confidence intervals that overlapped zero, suggestive of fish from a single breeding population. The N_e estimate for the Tryon Creek population resulted in N_e=77.2 with 95% CI=65-92.5. Individuals upstream and downstream of Boones Ferry Road were more related than would be expected by chance (p<0.002) (See Appendix C).

Part 2b.

Pairwise exact tests of allele frequencies were not significant between upstream and downstream of the Highway 43 culvert (p=0.503). Pairwise estimates of F_{ST} produced low F_{ST}-values (<0.02) with 95% confidence intervals that overlapped zero (See Appendix C).

Part 2c.

Three of the collection sites (Kelley Creek, Mitchell Creek, Tryon Creek mouth) showed no substantial deviations from HWP for any of the 12 loci (p>0.057). The five collection sites produced similar levels of H_o (range 0.74-0.80). All but three pairwise exact tests of allele frequencies produced p-values less than 0.01, suggestive of population differentiation. Overall the entire system displayed a signal of individuals within watersheds being more related than expected (p<0.002) (See Appendix C).

Part 3.

The four tissue samples collected from unidentified trout were identified as Coastal Cutthroat Trout (n = 3), and hybrid trout (n = 1), there were zero *O. mykiss.* The 11 tissue

samples from presumed hybrids were identified as Coastal Cutthroat Trout (n = 6), hybrid trout (n = 3), and *O. mykiss* (n = 2). The two *O. mykiss* were included in our assessment for part 4. Accuracy of visually identifying hybrids was 27.3% which corroborates the suggestion from the literature (Matala et al. 2009) that genetic confirmation is necessary to be accurate.

Part 4.

Samples from 110 presumed *O. mykiss* were evaluated for population assessment. Of those, 81 (73.6%) were confirmed as *O. mykiss,* 6 (14.5%) were confirmed as *O. clarki,* 7 (6.4%) were confirmed as hybrids, and 6 (5.5%) failed to amplify. Two confirmed *O. mykiss* were added to the analysis from part 3 for a total 83 *O. mykiss* (Tryon Creek 15, Kelley Creek 3, Crystal Springs 5, Johnson Creek 60) available for population analysis.

All *O. mykiss* from Tryon Creek, Kelley Creek and Crystal Springs assigned to the reporting group = Willamette winter steelhead. From Johnson Creek, 54 (90%) assigned to the reporting group = Willamette River winter steelhead; 6 (10%) assigned to the reporting group = Willamette River summer steelhead. For *O. mykiss* that assigned to a population at a probability of at least 0.90 (n=17) or 0.80 (n=25), it was most common for them to assign to Eagle Creek Hatchery (Tables 7 and 8). The confidence in population assignments was highly variable. For example, for eight samples collected from fish in Johnson Creek, Germany Creek had the highest probability of assignment. While Germany Creek was the most likely population of assignment, the probability varied from < 0.40 to > 0.90 (Figure 15).

Table 7. *O. mykiss* that assigned to a population at a probability of at least 0.90 (based on the populations and fish included to construct the baseline, > 0.90 probability that the fish was most closely related to an assignment population)

Assignment population	**Stream of Collection**			
	Tryon Creek	**Kelley Creek**	**Crystal Springs**	**Johnson Creek**
Big Creek, Columbia River (w)	0	0	0	2 (12%)
Clackamas River (w)	0	0	0	1 (6%)
Clackamas River, North Fork Dam (w)	0	0	0	1 (6%)
Eagle Creek Hatchery (w)	2 (100%)	0	0	7 (41%)
Germany Creek (w)	0	0	0	1 (6%)
Lewis River, NF (w)	0	1 (50%)	0	1 (6%)
Mollala River, NF (w)	0	0	0	2 (12%)
Sandy River (w)	0	0	0	1 (6%)
Santiam River, NF (w)	0	0	0	1 (6%)
Washougal River (w)	0	1 (50%)	0	0 (0%)
TOTAL FISH	2	2	0	17

w = Winter Steelhead s = Summer steelhead

Table 8. *O. mykiss* that assigned to a population at a probability of at least 0.80 (based on the populations and fish included to construct the baseline, > 0.80 probability that the fish was most closely related to an assignment population)

Assignment population	Stream of Collection			
	Tryon Creek	Kelley Creek	Crystal Springs	Johnson Creek
Big Creek, Columbia River (w)	0	0	0	2 (8%)
Clackamas River (w)	0	0	0	1 (4%)
Clackamas River, North Fork Dam (w)	0	0	0	2 (8%)
Cowlitz Hatchery (w)	0	0	0	2 (8%)
Eagle Creek (w)	0	0	0	1 (4%)
Eagle Creek Hatchery (w)	2 (100%)	0	0	8 (32%)
Elochoman River (w)	0	0	0	1 (4%)
Germany Creek(w)	0	0	0	1 (4%)
Lewis River, NF (w)	0	1 (50%)	0	1 (4%)
Mollala River, NF (w)	0	0	0	2 (8%)
Sandy River (w)	0	0	0	1 (4%)
Santiam River, NF (w)	0	0	0	1 (4%)
Santiam River, NF (s)	0	0	0	1 (4%)
Skamania Hatchery (s)	0	0	0	1 (4%)
Washougal River (w)	0	1 (50%)	0	0 (0%)
TOTAL FISH	2	2	0	25

w = Winter Steelhead s = Summer steelhead

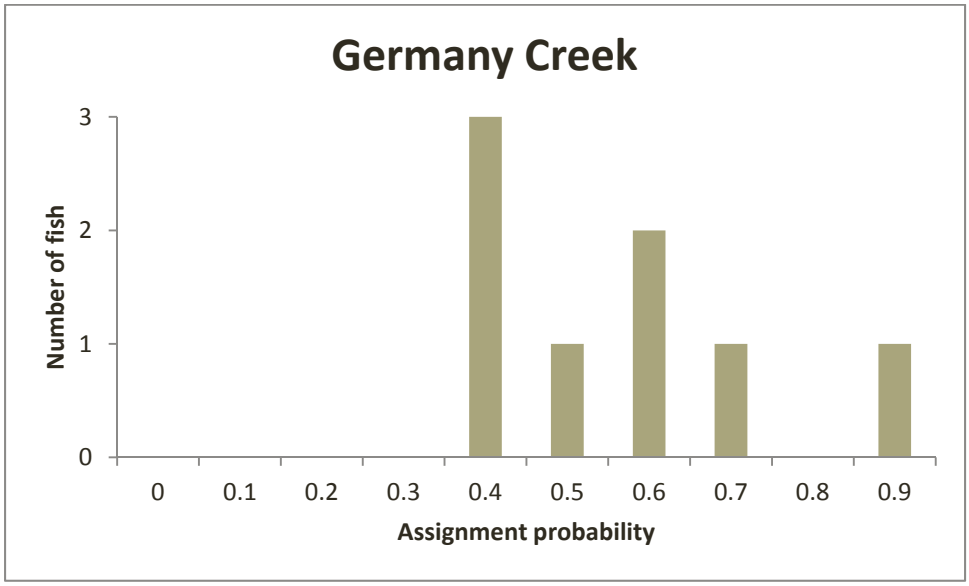

Figure 15. The distribution of assignment probabilities for mykiss collected in Johnson Creek whose highest assignment probability was to Germany Creek (n= 8).

Findings

Restoration actions in the Tryon Creek confluence appear to support native fish, predominantly salmonids during their various life history stages. Native fish are the most abundant species captured, species richness is consistent, and resident fish are present throughout the year. In winter and spring, juvenile, out-of-basin fish rear in the pool below the Highway 43 culvert. Above the Highway 43 culvert, a relatively stable resident coastal cutthroat population persists and appears to include a migratory component. Additional years of sampling will allow a comprehensive inventory of fish species and contribute to our understanding of fish use Tryon Creek over time.

Below the Highway 43 culvert, we have identified 12 native species and five introduced species in Tryon Creek to date (2012-2015); the Willamette Basin contains 31 native species and 29 introduced species (Hulse et al. 2002). According to Hulse et al. (2002), all species in the confluence habitat are common in the Willamette River, except coho, which are rare. Our ecological classification in the confluence area identified native species (Chinook, Coho, sculpin species, Coastal Cutthroat, *O. mykiss*, and their hybrids) as dominant or common. Species classified rare were both native (Mountain Whitefish, Peamouth, Speckled Dace, and Threespine Stickleback) and introduced (Goldfish, Largemouth Bass, and Smallmouth Bass). The number of species present and their relative abundance was similar for each sample event.

Both juvenile and adult Chinook and Coho salmon utilize the Tryon Creek confluence as part of their migration. Because there is no evidence of anadromous salmonids spawning upstream of the Highway 43 culvert, we assume all migratory salmonids originate from other locations in the Willamette River basin. The off channel habitat created by the confluence enhancement project may be important for these juvenile migratory fish in winter months. Flooding in the Willamette creates strong currents and juveniles need access to floodplains that contain slower moving water (Schroeder et al. 2014). The pool below the Highway 43 culvert appears to provide such refuge for weeks and months at a time. This off channel habitat is especially important in the lower Willamette River where there has been development along the riverbanks and loss of channels due to urbanization.

Juvenile Chinook and Coho salmon were the majority of fish detected by our PIT tag antennas. These fish were tagged and released below the Highway 43 culvert in the winter and spring months. It is important to note that shed PIT tags move downstream during high flow events. These shed PIT tags may account for false positive detections when detected by our antennas. Additionally, our PIT antennas detected Chinook Salmon tagged and released in the North Santiam River by ODFW as they were migrating downstream. Resident fish such as Coastal Cutthroat Trout and hybrids were detected throughout the year, which may indicate the presence of a migratory component of the population. However, the majority of tagged Coastal Cutthroat Trout have not been detected leaving the system, suggesting the resident form may be the most common. Detections of fish tagged in 2014 and 2015 will continue for several more years as fish mature and complete their migration.

Upstream of the Highway 43 culvert, larval Pacific Lamprey were present near a release site that was part of a 2013 larval Pacific Lamprey outplanting effort (Silver et al. 2014). Although all of the outplanted larvae received a visual implant elastomer (VIE) tag, no VIE tags were observed on any of the larval lamprey we captured in 2014 or 2015. Despite this, based on the size and location of the larvae as well as the challenges adults would encounter to migrate upstream through the Highway 43 culvert, it is most likely that they were larvae from the 2013 outplanting.

An apparently stable population of Coastal Cutthroat Trout persists above the Highway 43 culvert. In 2015, the population density was relatively low, similar to 2009. Additional species observed above the culvert during fall electrofishing efforts included numerous sculpin and crawfish, and one hybrid trout, Coho Salmon, and Fathead Minnow. Pacific Lamprey ammocoetes were found both above (from previous outplanting in 2013) and below the Highway 43 culvert in 2014 and 2015.

Tryon Creek Tryon supports a genetically healthy population of Coastal Cutthroat Trout. It appears to contain a single population intermediate in genetic diversity compared to other healthy populations in the Columbia River Basin. There was no evidence of genetic drift. Cutthroat Trout from Tryon Creek possessed less diversity than coastal populations but more than interior montane populations (Smith et al. 2015).

Coastal Cutthroat Trout from above and below Boones Ferry Road appear to be part of the same genetic population. Although the culvert under Boones Ferry Road inhibits upstream passage for Coastal Cutthroat Trout, they do have at least a limited ability to pass upstream and can pass downstream. The result of fish mixing between the two areas is enough to make them indistinguishable from a genetic perspective.

The majority of Coastal Cutthroat Trout found in the mouth of Tryon Creek, below the Highway 43 Culvert, are genetically indistinguishable from Coastal Cutthroat Trout above the Highway 43 Culvert. This may suggest that the majority of Coastal Cutthroat Trout at the mouth came from upper Tryon Creek, and that the mouth of Tryon Creek is not a significant rearing area for non-natal (out-of-watershed) Coastal Cutthroat Trout. Alternatively, the Coastal Cutthroat Trout in the mouth of Tryon Creek may have originated from other watersheds whose Coastal Cutthroat Trout have similar genetic characteristics to Coastal Cutthroat Trout in upper Tryon Creek.

In general, Coastal Cutthroat Trout populations from neighboring watersheds in Portland (Oregon) (Kelley Creek, Mitchell Creek, Crystal Springs, and Tryon Creek) appear to represent distinct populations. Fish within watersheds were more closely related than between watersheds. Estimates of genetic diversity were moderate and there were not tremendous departures from neutral expectations that often accompany small populations. Coastal Cutthroat Trout from Tryon Creek appear to represent one distinct population. Coastal Cutthroat Trout from Kelley and Mitchell creeks appear to mix and, collectively, represent another distinct population. Results for Coastal Cutthroat Trout from Johnson Creek were somewhat more equivocal, but were also suggestive of a distinct population. These results are expected for resident fish

dwelling in small streams with few breeders. In general, this evidence supports the notion that Coastal Cutthroat Trout populations in the city of Portland may be structured such that each watershed (at a minimum) entering the Willamette River or Multnomah Channel represents a distinct population.

The majority of *O. mykiss* sampled from watersheds in the City of Portland were lower Columbia/Willamette winter steelhead while the remainder were lower Columbia/Willamette summer steelhead. Most of these fish did not assign very confidently to any specific population. However, of the fish that could be assigned to a population with reasonable confidence, the majority appeared to be winter steelhead from Eagle Creek National Fish Hatchery. It is important to note that these assignments are influenced by which populations and which fish from those populations were used to develop the baseline for assignment. Not every population that these fish could have originated from has been characterized and included in the baseline. Thus, while this assignment information may be suggestive of certain patterns and help guide additional research, specific questions about populations or origin should be formulated and studied directly.

FY 2016 Tasks

- Maintain a Passive Integrated Transponder (PIT) array at the mouth of Tryon Creek
- Conduct monthly sampling (seine/electrofish) from the Tryon Creek confluence to the Highway 43 culvert 7/2016-12/2016
- Conduct an annual survey in July to electrofish above and below the Highway 43 culvert to detect larval lamprey distribution

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Appendix A: Fish Capture

Sample Date	Sample Method	Reach	Chinook Salmon	Coastal Cutthroat	Coho Salmon	Crawfish	Fathead Minnow	Goldfish	Mountain whitefish	*O. mykiss* x cutthroat	Pacific lamprey	*O. mykiss*	Sculpin sp.	Smallmouth bass	Speckled dace	Threespine stickleback	Trout Fry <100mm
07/02/2014	Seine & Electrofish	BELOWHWY43	4	18	17							2	106				1
08/06/2014	Electrofish	18									1						
08/06/2014	Seine & Electrofish	BELOWHWY43	7	20	24					1	1		45				
08/27/2014	Seine	BELOWHWY43	1	14	14					1			10				
10/08/2014	Seine & Electrofish	BELOWHWY43	4	12	4					3		2	98	12	1		
11/05/2014	Seine & Electrofish	BELOWHWY43	12	7	6					1		2	59	1	1		
12/03/2014	Seine & Electrofish	BELOWHWY43	54	7	16					1		4	33				
12/31/2014	Seine & Electrofish	BELOWHWY43	22		21								11				
02/04/2015	Seine & Electrofish	BELOWHWY43	14	4	31				1	1			38	1			
03/04/2015	Seine & Electrofish	BELOWHWY43	6	3	10					1		4	20		1		
04/01/2015	Seine & Electrofish	BELOWHWY43	10	6	12			1		1		9	23			1	
04/08/2015	Seine & Electrofish	BELOWHWY43	8	7	12							7	41				
04/15/2015	Seine & Electrofish	BELOWHWY43	10	12	22					3		13	24				
04/22/2015	Seine & Electrofish	BELOWHWY43	8	12	22					3		4	47				
04/28/2015	Seine & Electrofish	BELOWHWY43	3	14	28			3		1		4	37				
05/06/2015	Seine & Electrofish	BELOWHWY43	10	12	69								34				
05/13/2015	Seine & Electrofish	BELOWHWY43	6	9	87							4	35				
05/20/2015	Seine & Electrofish	BELOWHWY43	1	9	105			1				4	30				
05/27/2015	Seine & Electrofish	BELOWHWY43	1	8	81								41				
06/03/2015	Seine & Electrofish	BELOWHWY43	1	8	28								40				
06/10/2015	Seine & Electrofish	BELOWHWY43		6	112								52				
06/17/2015	Seine	BELOWHWY43		6	150								19				
06/24/2015	Seine & Electrofish	BELOWHWY43		6	135								55				
07/13/2015	Seine & Electrofish	18									2						
07/13/2015	Seine & Electrofish	BELOWHWY43									10						
09/21/2015	Electrofish	TC01		2		9							180				
09/21/2015	Electrofish	TC02		4		11							189				
09/21/2015	Electrofish	TC03		1		8							201				
09/21/2015	Electrofish	TC04		3		7							75				
09/21/2015	Electrofish	TC05		3		4							280				
09/21/2015	Electrofish	TC06		2		12							293				
09/22/2015	Electrofish	TC07		7		7							187				
09/22/2015	Electrofish	TC08		3		7							160				
09/22/2015	Electrofish	TC09		2		4							145				

Sample Date	Sample Method	Reach	Chinook Salmon	Coastal Cutthroat	Coho Salmon	Crawfish	Fathead Minnow	Goldfish	Mountain whitefish	*O. mykiss* x cutthroat	Pacific lamprey	*O. mykiss*	Sculpin sp.	Smallmouth bass	Speckled dace	Threespine stickleback	Trout Fry <100mm
09/22/2015	Electrofish	TC10		4		4							225				
09/22/2015	Electrofish	TC11		8		8							176				1
09/22/2015	Electrofish	TC12		3		7							186				
09/23/2015	Electrofish	AC01				6							170				9
09/23/2015	Electrofish	TC13		7		15							296				
09/23/2015	Electrofish	TC14		5		8	1						98				
09/23/2015	Electrofish	TC15		5		3							159				3
09/23/2015	Electrofish	TC16				2							153				5
09/23/2015	Electrofish	UTC01		6		4							200				1
09/23/2015	Electrofish	UTC02		3		8							89				
10/05/2015	Electrofish	TC01		4		2						1	65				
10/05/2015	Electrofish	TC02		3	1	4							160				
10/05/2015	Electrofish	TC03				4							55				
10/05/2015	Electrofish	TC04		1		2							65				
10/05/2015	Electrofish	TC05		3		4							173				
10/05/2015	Electrofish	TC06		2		4							123				
10/06/2015	Electrofish	TC07		4		5							84				
10/06/2015	Electrofish	TC08				4							90				
10/06/2015	Electrofish	TC09		1		3							115				
10/06/2015	Electrofish	TC10		1		2							23				
10/06/2015	Electrofish	TC11		3		6							54				1
10/06/2015	Electrofish	TC12		6		7							73				2
10/06/2015	Electrofish	TC13		1		7							119				
10/07/2015	Electrofish	TC14		7		9							82				2
10/07/2015	Electrofish	TC15				6							78				
10/08/2015	Electrofish	AC01				7							89				8
10/08/2015	Electrofish	TC16				8							50				6
10/08/2015	Electrofish	UTC01		5		11							77				6
10/08/2015	Electrofish	UTC02		2		10							75				4

Appendix B: Population Structure of Coastal Cutthroat Trout from Tryon Creek, OR

U.S. Fish and Wildlife Service

Population structure of coastal cutthroat trout from Tryon Creek, OR

FY2015 Final Report

January 2016

By Christian T. Smith and Jennifer Von Bargen

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Photo Credit: T. Whitesel

Population structure of coastal cutthroat trout from Tryon Creek, OR

Report for work done in FY2015

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Introduction

Tryon Creek is one of the largest, relatively protected, urban watersheds in Oregon. The stream is located in southwest Portland and its headwaters are located within urbanized neighborhoods. The stream flows approximately 5 km through privately owned land before entering Tryon Creek State Natural Area, a 259 hectare area of public land, through which the stream flows another 5 km. The lower most portion of Tryon Creek flows through public land owned by the City of Lake Oswego and the City of Portland. Since the 1920's, this portion of the stream has been bisected by a culvert that runs under Oregon Highway 43 and a railroad near the mouth of Tryon Creek. A number of native fish species are associated with Tryon Creek. Species that currently can be found and appear to spawn in Tryon Creek include coastal cutthroat trout (*Oncorhynchus clarki*) and rainbow trout (*O. mykiss*; Tinus et al. 2003). The status, or population health, of coastal cutthroat trout in Tryon Creek is unknown.

The US Fish and Wildlife Service (USFWS) Columbia River Fisheries Program Office (CRFPO) has conducted monitoring over several years to investigate whether Tryon Creek supports a healthy population of coastal cutthroat trout. The objective of that work has been to characterize the health of the coastal cutthroat population in Tryon Creek by examining 1) morphological characteristics, 2) disease prevalence, 3) population abundance and density, 4) migratory behavior and 5) genetic health (i.e., genetic diversity and inbreeding).

This report describes the results of a genetic analysis of samples of coastal cutthroat trout collected from Tryon Creek. Our goal was to compare genetic diversity, effective population size, and genetic drift in Tryon Creek to values observed in other (non-urban) populations of coastal cutthroat trout from the Lower Columbia River Basin.

Methods and materials

Coastal cutthroat trout were captured in Tryon Creek by CRFPO staff in 2008 (n=56) and 2013 (n=56) (Appendix 1). In addition, fish were captured in lower Tryon Creek by City of Portland staff in 2013 (n=5) (Appendix 2). The total sample size for this work was thus 117 fish. Each fish was measured, and a genetic sample was preserved in 95% non-denatured ethanol. Given

that 5 years elapsed between collection events and information from growth rates determined from recaptured fish, it was assumed that the fish captured in 2013 represented a different cohort (brood year) than those captured in 2008. Based on this assumption, we used divergence between the collections to infer divergence over time (i.e., genetic drift). Genetic samples were sent to USFWS Abernathy Fish Technology Center (AFTC) for processing.

Genomic DNA was extracted from each sample using DNeasy 96-Tissue Kits (QIAGEN), and stored at -20˚C. All samples were genotyped at 17 microsatellite loci (Table 1). Three of these loci were developed as species / hybrid identification markers (Ostberg and Rodriguez 2004) and are thought to be largely fixed within species (i.e., CCT v. RBT). These are referred to below as the "species ID loci". The remaining 14 loci were chosen based on polymorphism in coastal cutthroat trout and were known at the outset of this study to be variable in both of the species of interest (Stephenson et al. 2009; Johnson et al. 2010). These are thus referred to below as the "population structure loci". Polymerase chain reaction (PCR) was conducted in 10µL volumes containing 1X Multiplex PCR Mastermix (QIAGEN), 0.2µL Primer Mix (Table 1), and 2.0µL template DNA. Temperature profiles included a polymerase activation step of 15 minutes at 95˚C; 29 cycles of 30 seconds at 95°C; 1.5 minutes at annealing temperature (Table 1); and one minute at 72˚C; followed by a 20 minute extension step at 60˚C. Following PCR, products were size-fractionated using an *ABI 3130xl* Genetic Analyzer (Applied Biosystems). GENEMAPPER v5.0 software (Applied Biosystems) was used to determine the multi-locus genotype of each fish.

To provide context for the results from our Tryon Creek collections, we incorporated genotype data for coastal cutthroat trout collected from 27 collection sites in other Columbia River Basin tributaries (Smith et al. 2010; Table 3). A comparison of alleles for a subset of samples (n=10), which were genotyped previously and re-genotyped with the samples for this study, revealed that two loci (Och29 and Omm1231) produced inconsistent genotypes. We expect this was due to different running conditions (e.g., Seeb et al. 2007). In any case, these two loci were excluded from the population genetic analysis to allow comparison of data from Tryon to those from other systems.

Individuals exhibiting *O. mykiss* alleles at the species identification loci (Table 1) were excluded from the population analyses described below.

Genotypes for the 12 variable loci were examined in each collection for evidence of disequilibrium. We tested for genotypic ratios that departed from Hardy-Weinberg Equilibrium (HWE) using Fisher's exact tests in GENEPOP (Raymond and Rousset 1997). The log likelihood ratio statistic (G test) was also used to test for Genotypic Disequilibrium (GD) between each pair of loci in each collection. Critical values were adjusted for the number of simultaneous tests (i.e.: for HWE α=0.05/12 loci per collection; for GD α=0.05/66 pairwise comparisons per collection).

Genetic divergence between temporal collections at each site was evaluated using two methods. Contingency tests of allele frequency heterogeneity (Raymond and Rousset 1995) were performed using GENEPOP (Rousset 2008). Pairwise F_{ST}s (Weir and Cockerham 1984) were estimated using the program GENETIX (Belkhir et al. 2004). Statistical significance of each pairwise F_{ST} was evaluated by comparing observed values to a null distribution based on 10^4 replicates in which individuals were permuted among sites. To provide context for the measures of temporal divergence in Tryon Creek, we repeated these analyses on temporal replicate samples from four reference populations (Upper Dog River, Lower Dog River, Abernathy Creek, and Chinook River). A visual evaluation of divergence between temporal samples from Tryon Creek was performed by plotting the first two axes of a principal component analysis conducted in R (R Development Core Team 2014).

Genetic diversity was estimated as expected heterozygosity (i.e., gene diversity; eqn 8.1 in Nei 1987) and allelic richness (AR; number of alleles observed, corrected for unequal sample sizes) using the program HPRARE (Kalinowski 2005). We estimated the inbreeding effective sizes (N_e) of the populations represented by our collections using the linkage disequilibrium method (Waples 2006) in the program NeEstimator v2.0 (Do et al. 2014). Estimates of diversity and effective size were based on all temporal replicates sampled at each site.

Results

Of 117 fish analyzed with the species ID markers, 113 were identified as coastal cutthroat trout and 4 were identified as coastal cutthroat trout x rainbow trout (*O. mykiss)* hybrids (none were identified as rainbow trout). Of the individuals identified as hybrids, three exhibited a single

rainbow trout allele, and one exhibited two rainbow trout alleles, suggesting that these were back-crossed rather than F1 individuals. Of 113 coastal cutthroat trout, 3 individuals failed to amplify at the majority of population structure markers (one failed at 7 loci, one failed at 9 loci, and one failed at 10 loci), and were excluded from subsequent analyses. The remaining 110 fish were successfully amplified at 8-12 loci (overall genotyping success rate 97%).

We observed one departure from Hardy Weinberg proportions in each Tryon Creek collection. In both cases, the locus was *Ssa408*. When we pooled the two collections and re-ran the analysis, *Ssa408* was still out of equilibrium due to a homozygote excess but no other departures were detected. We observed linkage disequilibrium in 4 pairs of loci in the 2008 Tryon Creek collection and 2 pairs of loci in the 2013 collection. When we pooled the two collections and re-ran the analysis, we observed linkage disequilibrium in 5 pairs of loci.

Estimates of F_{ST} between temporal replicates were significant ($\alpha = 0.05$) for collections from two reference sites (Lower Dog River and Chinook River), non-significant for collections from the other two (Upper Dog River and Abernathy Creek), and non-significant for the collections from Tryon Creek (Table 2). Our estimate of F_{ST} between the two collections from Tryon Creek was essentially 0.000. Allele frequency heterogeneity tests yielded significant ($\alpha = 0.05$) results for pairs of collections from all sites except Tryon Creek. Similarly, the principal component analysis did not indicate any difference between the two collections from Tryon Creek (Figure 1).

We observed the greatest genetic diversity in the two Lower Columbia River populations (Abernathy Creek and Chinook River) and the lowest diversity in a few of the more isolated populations in the Mt Hood National Forest (e.g., Clear Branch, Tony Creek, and Upper Dog River; Table 3). Diversity in Tryon Creek, as indicated by both AR and H_e, was comparable to that in the most diverse populations in the Mt. Hood National Forest. Both measures of genetic diversity (H_e and AR) indicated that Tryon Creek exhibited comparable levels to those observed in the Mt. Hood National Forest populations.

Our estimate of effective population size for Tryon Creek was significantly lower ($N_e = 62$; 95% C.I. = 53-72) than estimates for the two Lower Columbia River sites (Abernathy Creek $N_e = 326$; 95% C.I. = 219 – 605; and Chinook River $N_e = 127$; 95% C.I. = 100 – 169), as indicated by non-

overlapping confidence intervals. Approximately half (13/22) of the reference collections from Mt Hood National Forest yielded N_e point estimates higher than Tryon Creek, but only two were significant (lower Dog River Ne = 119; 95% C.I. = 100-146; Powerdale Dam Ne=158; 95% C.I. = 98-367). Lower confidence intervals for Mt Hood National Forest reference collections were all lower than those for Lower Columbia River reference collections.

Conclusions

Our results suggest that the two temporal collections taken in 2008 and 2013 from Tryon Creek were representative of a single population. We did not detect excessive Hardy-Weinberg or linkage disequilibrium in either of these collections alone, and linkage only increased slightly when the two were pooled. We were also not able to detect allele frequency differences between the two temporal collections. Estimates of pairwise F_{ST} and results of the heterogeneity test indicated that divergence among the two collections from Tryon was less than that observed between pairs of collections from the reference sites. This suggests that genetic drift has not been stronger in the Tryon Creek population than in these other populations.

Greater genetic diversity in the heart of a species' range than near the fringe of its distribution is typical, and the two Lower Columbia River reference sites are known to have anadromous components (Johnson et al. 2010), so it was not surprising to observe greater genetic diversity in these sites than in the Columbia River Gorge (Mt. Hood National Forest sites). We do not know whether Willamette River populations were historically similar to one of these groups or contained some intermediate level of diversity. In any case, diversity (H_e, AR and N_e) in Tryon Creek appears comparable to that in populations in the Mt. Hood National Forest.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Figure 1. Principal component analysis of genotype data for coastal cutthroat trout collected in Tryon Creek in 2008 (blue) and 2013 (red).

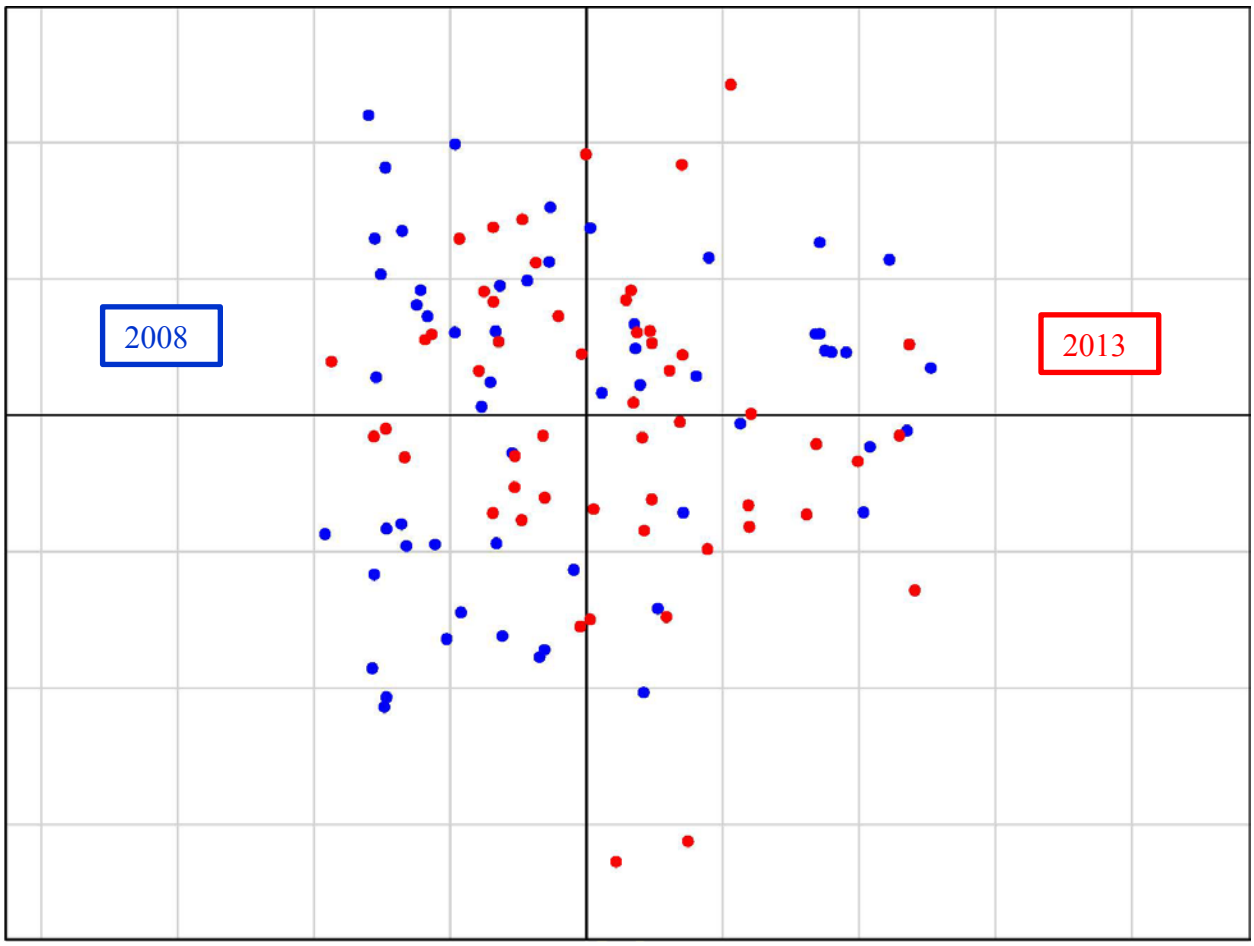

Table 1. Amplification details for seventeen markers in six multiplex reactions. Three of the loci were used for species identification (Species ID) and fourteen were used to evaluate population structure (Pop structure) in coastal cutthroat trout. PCR primer concentration (Primer [ ] in nM), and annealing temperature (T_A in ˚C) are listed for each locus. Sizes of alleles (in nucleotides) used to identify individuals as rainbow trout (RBT) or coastal cutthroat trout (CCT) are listed for each species identification locus.

Marker	Multiplex	Purpose	Primer [ ]	T_A	RBT alleles	CCT alleles
Occ35	1	Species ID	20	60	203	235
Occ36	1	Species ID	7	60	268	297
Occ42	1	Species ID	13	60	170	188, 192
Och29	2	Pop structure	60	57		
Oki10	2	Pop structure	27	57		
Omm1050	2	Pop structure	33	57		
Ocl34H04	3	Pop structure	27	57		
Ocl35D01	3	Pop structure	40	57		
Ssa408	3	Pop structure	40	57		
Och15	4	Pop structure	33	57		
Och16	4	Pop structure	20	57		
Ocl30H10	4	Pop structure	53	57		
Och20	5	Pop structure	20	60		
Och24	5	Pop structure	40	60		
Omm1231	6	Pop structure	13	59		
Omy1001	6	Pop structure	33	59		
Ssa407	6	Pop structure	27	59		

Table 2. Divergence among pairs of collections sampled at Tryon Creek and the four reference sites for which temporal replicates were available. Reference data were taken from Smith et al. (2010).

Site	Years sampled	F_{ST}	F_{ST} p value	Heterogeneity p value
Tryon Creek	2008, 2013	-0.001	0.658	0.095
Dog River upper	2002, 2008	0.006	0.080	0.001
Dog River lower	2002, 2008	0.019	0.000	0.000
Abernathy Creek	2002, 2003	0.002	0.208	0.030
Chinook River	2002, 2003	0.009	0.001	0.005

Table 3. Genetic diversity observed in coastal cutthroat trout captured in Tryon Creek, as well as from reference sites connected to the Columbia River above (Mt. Hood National Forest) and below (Lower Columbia River) Tryon Creek. Expected heterozygosity (H_e), allelic richness (AR; based on a sample size of 20 alleles), and effective population size (N_e, with 95% CI) are listed for each site. Note that the estimates below were based on pooling all temporal replicate samples available for each site. Reference data were taken from Smith et al. (2010).

Site	H_e	AR	N_e
Tryon Creek	0.760	6.6	62 (53-72)
Reference sites in Mt. Hood National Forest			
Fivemile Creek	0.784	6.3	99 (69 - 166)
Fifteenmile Creek	0.824	7.1	108 (61 - 385)
Bear Creek lower	0.818	7.1	75 (59 - 99)
Bear Creek middle	0.782	6.4	50 (39 - 67)
Oak Grove Fork	0.700	5.8	74 (39 - 309)
Timothy Lake	0.845	7.9	48 (34 - 80)
Clear Branch	0.549	3.1	29 (16 - 60)
Cold Spring	0.750	5.8	64 (37 - 162)
Crow Creek	0.674	4.3	593 (57 - inf.)
Dog River upper	0.547	3.1	53 (33 - 96)
Dog River lower	0.832	7.5	119 (100 - 146)
Powerdale Dam	0.852	8.5	158 (98 - 367)
Indian Creek lower	0.786	6.6	52 (38 - 80)
Indian Creek upper	0.789	6.1	82 (37 - inf.)
Mosier Creek	0.757	5.4	273 (37 - inf.)
Neal Creek middle	0.734	6.0	90 (56 - 193)
Neal Creek upper	0.737	5.6	27 (23 - 32)
SF Mill Creek	0.659	4.4	93 (66 - 148)
Tony Creek middle	0.474	3.2	29 (16 - 66)
Tony Creek lower	0.536	3.3	34 (17 - 112)
Viento Creek upper	0.706	5.1	78 (40 - 427)
Viento Creek lower	0.693	5.0	50 (28 - 138)
Reference sites in Lower Columbia River			
Abernathy Creek	0.844	8.1	326 (219 - 605)
Chinook River	0.812	7.4	127 (100 - 169)

Sample / data disposition:

Raw (genotype) data generated in the course of the work described here has been archived in the AFTC Progeny Database. Samples will be archived at AFTC, or returned to the CRFPO upon request.

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Appendix 1. Capture dates and lengths of coastal cutthroat trout sampled by CRFPO staff in Tryon Creek, OR. Data are presented for 56 fish captured in 2008 (left column) and 56 fish captured in 2013 (right column).

Genetic_ID	Sample_Date	Length (mm)	Genetic_ID	Sample_Date	Length (mm)
2008-1011	10/07/2008	121	2013-0272	09/11/2013	115
2008-0935	10/06/2008	122	2013-0280	09/11/2013	116
2008-0980	10/07/2008	122	2013-0281	09/11/2013	121
2008-0904	10/06/2008	124	2013-0255	09/11/2013	123
2008-1003	10/07/2008	125	2013-0263	09/11/2013	129
2008-1037	10/08/2008	125	2013-0204	09/09/2013	131
2008-1046	10/08/2008	125	2013-0254	09/11/2013	133
2008-0943	10/06/2008	126	2013-0276	09/11/2013	133
2008-0965	10/07/2008	126	2013-0269	09/11/2013	134
2008-0990	10/07/2008	128	2013-0292	09/12/2013	135
2008-1009	10/07/2008	128	2013-0245	09/09/2013	141
2008-1039	10/08/2008	128	2013-0265	09/11/2013	142
2008-1002	10/07/2008	130	2013-0288	09/12/2013	145
2008-1040	10/08/2008	130	2013-0202	09/09/2013	146
2008-0909	10/06/2008	131	2013-0222	09/09/2013	146
2008-0900	10/06/2008	132	2013-0266	09/11/2013	147
2008-1079	10/08/2008	132	2013-0297	09/13/2013	150
2008-0991	10/07/2008	133	2013-0293	09/12/2013	151
2008-1055	10/08/2008	133	2013-0289	09/12/2013	154
2008-0938	10/06/2008	136	2013-0223	09/09/2013	155
2008-0917	10/06/2008	137	2013-0295	09/13/2013	157
2008-0939	10/06/2008	137	2013-0259	09/11/2013	158
2008-0912	10/06/2008	138	2013-0296	09/13/2013	158
2008-0910	10/06/2008	139	2013-0300	09/13/2013	159
2008-0925	10/06/2008	139	2013-0299	09/13/2013	159
2008-1049	10/08/2008	139	2013-0253	09/11/2013	162
2008-1005	10/07/2008	140	2013-0267	09/11/2013	165
2008-1035	10/08/2008	140	2013-0287	09/12/2013	165
2008-1081	10/08/2008	140	2013-0298	09/13/2013	165
2008-1047	10/08/2008	141	2013-0224	09/09/2013	168
2008-1058	10/08/2008	141	2013-0203	09/09/2013	169
2008-1001	10/07/2008	142	2013-0286	09/11/2013	170
2008-1078	10/08/2008	142	2013-0261	09/11/2013	171

Genetic_ID	Sample_Date	Length (mm)	Genetic_ID	Sample_Date	Length (mm)
2008-1077	10/08/2008	143	2013-0221	09/09/2013	173
2008-1080	10/08/2008	143	2013-0246	09/09/2013	173
2008-0896	10/06/2008	144	2013-0264	09/11/2013	173
2008-0915	10/06/2008	144	2013-0250	09/09/2013	176
2008-1031	10/08/2008	144	2013-0270	09/11/2013	176
2008-1042	10/08/2008	144	2013-0290	09/12/2013	185
2008-1070	10/08/2008	144	2013-0279	09/11/2013	186
2008-1048	10/08/2008	145	2013-0240	09/09/2013	192
2008-0936	10/06/2008	148	2013-0291	09/12/2013	192
2008-1052	10/08/2008	148	2013-0205	09/09/2013	193
2008-0913	10/06/2008	149	2013-0278	09/11/2013	200
2008-0986	10/07/2008	149	2013-0262	09/11/2013	203
2008-1073	10/08/2008	149	2013-0274	09/11/2013	204
2008-0911	10/06/2008	150	2013-0243	09/09/2013	205
2008-0963	10/07/2008	150	2013-0249	09/09/2013	206
2008-0985	10/07/2008	150	2013-0284	09/11/2013	207
2008-0994	10/07/2008	150	2013-0271	09/11/2013	208
2008-1057	10/08/2008	150	2013-0252	09/09/2013	213
2008-0997	10/07/2008	151	2013-0206	09/09/2013	215
2008-1075	10/8/2008	151	2013-0251	09/09/2013	215
2008-1053	10/8/2008	157	2013-0256	09/11/2013	216
2008-1076	10/8/2008	157	2013-0258	09/11/2013	218
2008-1063	10/8/2008	168	2013-0277	09/11/2013	220

Appendix 2. Capture dates of coastal cutthroat trout sampled by City of Portland staff in Tryon Creek, OR. Data are presented for 5 fish captured in 2013.

Genetic_ID	Sample_Date
3058-053	03/22/2013
3058-054	03/28/2013
3058-055	04/18/2013
3058-056	05/08/2013
3058-057	05/31/2013

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Appendix C: Population structure of coastal cutthroat trout inhabiting urban watersheds in Portland, OR

U.S. Fish and Wildlife Service

Population structure of coastal cutthroat trout inhabiting urban water-sheds in Portland, OR

Final Report

November 2015 *By Justin Bohling and Christian Smith*

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Population structure of coastal cutthroat trout inhabiting urban watersheds in Portland, OR

Report for work done in FY2015/2016

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Introduction

Urban watersheds are often heavily manipulated by human activity but can also be home to many native aquatic species. Numerous studies have documented the negative impacts of urbanization on aquatic organisms (Paul & Meyer 2001; Walsh *et al.* 2005; Stranko, Hilderbrand & Palmer 2011), which creates challenges for maintaining aquatic biodiversity in urban areas. From a genetic perspective urban development can destroy habitat and reduce abundance, which may reduce genetic diversity and facilitate inbreeding, and reduce connectivity, creating fragmented populations (Delaney, Riley & Fisher 2010; Noël & Lapointe 2010). Thus, genetic monitoring can serve as a tool to assess the health of populations located in urban watersheds and provide insights into the biology of aquatic organisms located in heavily urban areas.

Within the greater Portland metropolitan area there are a number of watersheds that are part of the Willamette and Columbia River basins. Several drain primarily urban areas that are a mixture of heavily developed commercial zones, residential areas, and urban green spaces. Two such watersheds are Tryon Creek and Johnson Creek. Tryon Creek is located in the southwest of the city of Portland and runs about 7.81 km to the Willamette. Much of mainstem of the creek runs through a state park, although the near the mouth the stream is bisected by a culvert supporting a highway and railroad. Johnson Creek enters the Willamette about 1.5 km downstream of Tryon's mouth and drains portions of the cities of Gresham, Milwaukie, and Portland. About 40 km in length, Johnson Creek passes through a heavily urban area but has been the recent target of stream restoration projects. Both streams historically supported populations of anadromous and resident salmonids. Several of these species are still present, albeit in lesser abundance, including the cutthroat trout (*Oncorhynchus clarki*). Since cutthroats are year-long residents of these watersheds, they could be heavily impacted by urbanization. The

US Fish and Wildlife Service (USFWS) Columbia River Fisheries Program Office (CRFPO) is interested in evaluating the health of these resident populations. One objective is to assess the genetic attributes of cutthroats in Tryon Creek and neighboring basins. In an initial analysis, our lab analyzed genetic samples of individuals collected from Tryon and compared them to other cutthroat populations in the Columbia River basin (Smith *et al.* 2015). Levels of genetic diversity and effective population size were lower than coastal populations but higher than montane populations near Mt. Hood. In this report we expanded our analysis to include samples taken from different locations within Tryon Creek and Johnson Creek to assess patterns of genetic structure and diversity.

Methods

CRFPO field personnel sampled cutthroat trout from 2008-2015 in the Johnson and Tryon Creek watersheds within the Portland metropolitan area. Three sections of Johnson Creek (Kelley Creek, Mitchell Creek, and the mainstem Johnson) were sampled via electroshock fishing. Within Tryon Creek three sections were surveyed: one at the mouth near the Willamette River (Tryon creek mouth), one further upstream above the Boones Ferry Rd. culvert (Boones Ferry), and one along the main stem of Tryon between the two culverts (Tryon Creek upstream). Upon capture, each fish was measured for biological data and a genetic sample was removed and placed in 95% non-denatured ethanol. Genetic samples were transported to the USFWS Abernathy Fish Technology Center (AFTC) where DNA was extracted using DNeasy 96-tissue Kits (Qiagen) and then stored at -20ºC. We genotyped all samples at the same panel of 14 microsatellite loci as Smith *et al.* (2015) and divided them into several multiplexes that were amplified under the same set of polymerase chain reaction (PCR) conditions (Table 1). Each PCR was 10 µL in volume with 1X Multiplex PCR Mastermix (Qiagen), 0.2 µL of primer mix,

and 2.0 µL of template DNA. PCR temperature profiles consisted of a activation phase of 95ºC for 15 minutes followed by 29 cycles of 30 seconds at 95ºC, 1.5 minutes of annealing temperature, and one minute at 72ºC with a final 20 minute extension cycle at 60ºC. These PCR products were segregated according to size using an ABI 3130xl genetic Analyzer (Applied Biosystems) and we reviewed the electropherograms using GeneMapper v5.0 software. Smith *et al.* (2015) reported difficulties in scoring genotypes at two loci (*Och29* and *Omm1231*). They excluded those data from their analyses and to maintain consistency we also excluded those two loci from our analysis.

Not all samples amplified at the full suite of 12 microsatellite loci. Of the 314 individuals that were analyzed, only 198 amplified at all 12. We set a cut-off value of nine loci to ensure that we maintained adequate power to detect population structure while minimizing the number of samples excluded from the analysis. This allowed inclusion of 15 individuals genotyped at 11 loci, 18 at ten, and seven at nine. The resulting dataset had fewer individuals and several sample years were excluded, but every tributary was still represented by at least 14 individuals (Table 2). One individual fish contained multiple new alleles at several loci that have not been observed in other cutthroat trout populations. We suspect this individual may have been a hybrid or misidentified, so it was excluded from the analyses.

Since genetic samples were collected from the same creek sections across multiple years, we wanted to address the potential for temporal variation in allele frequencies to influence the interpretation of genetic structure. We divided each collection site based on years samples were collected (Table 2). Some of the resulting sample sizes were small (n=3), so we were limited in the analyses we could conduct. For each site with multiple years we calculated pairwise F_{ST} in Arlequin v.3.5 (Excoffier & Lischer 2010) with 1000 permutations to assess significance. Also,

we performed an Analysis of Molecular Variance (AMOVA, Excoffier *et al.* 1992) with individuals grouped by collection site and year sampled. The goal was to determine the amount of genetic variance that was attributed to differences in temporal samples. The AMOVA was performed in Arlequin with 20000 permutations.

Our results suggested temporal variation was minimal (see Results), so we combined samples across years for each collection site: Johnson Creek, Kelley Creek, Mitchell Creek, Tryon Creek mouth, Tryon Creek upstream, and Boones Ferry. First we examined deviations from neutral Hardy-Weinberg Proportions (HWP) using permutation tests based on 1000 permutations implemented by the package *pegas* (Paradis 2010) in R v3.2 (R Core Development Team). For each collection site we calculated estimates of observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A_r), and a population-specific inbreeding coefficient (F_{IS}). These calculations were performed using the R package *diveRsity* (Keenan *et al.* 2013). Within this package A_r is standardized based on 1000 re-samples of the smallest sample size with replacement and bias-corrected 95% confidence intervals are estimated for F_{IS}. We counted the number of private alleles per collection site using GenAlEx 6.5 (Peakall & Smouse 2012).

We performed Fisher's exact test of independent allele frequencies as a measure of population differentiation. These pairwise tests were based on multi-locus genotypes with 1000 Monte Carlo replicates. For further estimation of differentiation, we estimated pairwise F_{ST} (Weir & Cockerham 1984) along with bias-corrected 95% CIs between all five collection sites. Both the exact tests and estimates of F_{ST} were conducted with *diveRsity*.

Natural patterns of genetic structure do not necessarily correspond to obvious *a priori* classifications of individuals and populations. To discern such underlying structure, we

implemented several analyses. Mitchell and Kelley Creeks are tributaries of Johnson Creek, which is itself a tributary of the Willamette River. The two primary tributaries enter from opposite sides of the Willamette (Tryon from the west, Johnson from the east), meaning any fish moving from one watershed to the other would have to cross the width of the Willamette. To compare genetic structure between and within these primary watersheds we used an AMOVA with collection sites grouped by the two main watersheds. Again, though, this test requires populations to be grouped based collection sites. For assessing genetic variation among individual fish instead of *a priori* populations, we conducted a discriminant analysis of principal components (DAPC) in the R package *adegenet* (Jombart, Devillard & Balloux 2010). This multivariate procedure provides a representation of genetic variation along a two-dimensional plane based upon principal components generated from allele frequencies. We performed a cross-validation with 10% of the individuals and 100 replicates to determine the optimal number of principal components to retain for the dataset.

Our final technique to evaluate genetic structure was to evaluate the number of distinct genetic clusters in the dataset using the Bayesian approach implemented in STRUCTURE (Pritchard, Stephens & Donnelly 2000; Falush, Stephens & Pritchard 2003). This program simultaneously estimates the number of genetic clusters in the data and assigns the probability of individual genotypes originating from those clusters. We used the admixture model with correlated allele frequencies and 500,000 MCMC repetitions following a 100,000 step burn-in. *K* varied from 1-15 with 10 replicates of each value. To determine the optimal value of *K*, we used the method of Evanno *et al.* (2005) as estimated with the R package *CorrSeive* (Campana *et al.* 2011). Once the optimal *K*-value was identified, we used CLUMPAK to estimate a composite ancestry value (*q*) for individual fish across the 10 replicates.

Given the potential for habitat degradation and loss of connectivity, urban fish populations may be isolated in small reaches that restrict population size and limit gene flow. Estimating effective population size (N_e) can provides insight into these processes and serves as a proxy for evaluating population trends. We estimated N_e using the linkage disequilibrium method (Waples & Do 2010) implemented in NeEstimator v2 (Do *et al.* 2014) for each collection site. In the model we assumed random mating and set a minor allele frequency threshold of 0.02.

Low N_e and restricted gene flow can produce populations composed of highly related, potentially inbred individuals. We assessed the degree of relatedness within collection sites by calculating relatedness values (r) between individuals. We utilized the triadic likelihood estimator (trioml) of Wang (2007) implemented in the R package *related* (Pew *et al.* 2015). The trioml metric ranges from zero to one with higher values indicating higher probability of shared ancestry. *Related* also performs a procedure to compare the relatedness scores within populations to the other populations to evaluate whether individuals are more or less related then expected by chance. This is an iterative procedure that randomly shuffles individuals among populations and recalculates relatedness scores. In our analysis we included all five collection sites and used 500 iterations.

Results

When individuals from each primary tributary (Johnson and Tryon creeks) were combined, the global dataset deviated from neutral HWP ($p<0.001$). Three of the collection sites (Kelley Creek, Mitchell Creek, Tryon Creek mouth) showed no substantial deviations from HWP for any of the 12 loci ($p>0.057$). The Johnson Creek population showed deviations at five loci, while the Boones Ferry and Tryon Creek upstream had deviations at a single locus each. Because

we conducted 72 independent tests (i.e. population-locus combinations) of HWP, we determined whether the number of deviations we observed (n=7) was greater than expected due to random error caused by performing multiple tests. Under neutral HWP and α=0.05, we would expect four tests to produce Type I errors. With a binomial distribution, the probability of observing sevent deviations (as we did) fell within the 95% confidence interval for the expected number of Type I errors with our number of tests (n=72) and our significance value (α=0.05). Thus, the deviations we observed could be due to random Type I error. Since the deviations were restricted to three collection sites and did not show any patterns among loci, we believe these deviations reflect population-specific processes. We chose not to exclude these populations from our analysis, for further investigation revealed patterns of genetic structure that may be responsible for these deviations (see below).

The five collection sites produced similar levels of H_o (range 0.74-0.80). H_e was more variable with Johnson Creek producing a higher value (H_e=0.87) than the other five (range 0.74-0.80). There was a similar pattern with allelic richness, with Johnson Creek possessing an average of two more alleles per locus than the next highest site (Figure 1). The 95% CIs for allelic richness from Johnson Creek did not overlap with errors bars for Kelley and Mitchell Creeks; otherwise all CIs overlapped. Aside from Johnson Creek, the other five sites produced small values of F_{IS} with 95% confidence intervals that overlapped zero (Figure 1). In contrast, Johnson Creek displayed a strong heterozygote deficiency (F_{IS}=0.13). Johnson Creek had the largest number of private alleles (33), followed by Tryon Creek upstream (11) and Boones Ferry (9). Kelley Creek had six, Mitchell had two, and Tyron mouth had one each.

All but three pairwise exact tests of allele frequencies produced p-values less than 0.01, suggestive of population differentiation. The three that were not significant were the comparison

of Kelley and Mitchell Creeks (p=0.289), Boones Ferry and Tryon mouth (p=0.167), and the two Tryon Creek sites (p=0.58). This same pattern was observed for the pairwise estimates of F_{ST}; both the Kelley-Mitchell and three Tryon Creek comparisons produced low F_{ST}-values (<0.02) with 95% confidence intervals that overlapped zero (Figure 2). Estimates from other comparisons ranged from 0.03 to greater than 0.1 with lower bounds greater than zero.

Results from the global AMOVA indicate that a greater percentage of the genetic variation is partitioned among the primary tributaries compared to individual collection sites within them (Table 3). Most of the variation (~88%) was distributed within individuals, with the next highest percentage (~7.5%) among the two tributaries. The smallest percentage (~1.4%) was among collection sites within those two tributaries.

For the DAPC, the cross-validation procedures revealed that the highest mean percentage of correct assignments occurred with 40 retained PCs (~42.7%). The resulting DAPC showed two primary groups separated along the primary axis: one consisting of the three Tryon Creek populations and another composed of Kelley and Mitchell Creeks (Figure 3). Along this axis Johnson Creek was intermediate between the two main clusters. The secondary axis split some Johnson Creek fish from the global population, creating an indistinct cluster.

The mean likelihood scores and the ΔK statistic produced by the STRUCTURE analysis clearly indicated that most likely number of clusters in our dataset was two (Figure 4). Not only did higher values of K have less support with those two metrics, individual membership probabilities were highly unstable. The primary division at K=2 fell along the primary tributaries with one cluster composed of the three Johnson Creek sites and the second with the two Tryon Creek sites (Figure 5). All fish in Mitchell and Kelley Creeks were assigned with high probability to the 'Johnson Creek' cluster. Two individuals from Johnson Creek had high

probability of assignment (q>0.99) to the 'Tryon Creek' cluster. One fish from Tryon Creek mouth had high assignment (q=0.95) to the 'Johnson Creek' cluster. Three individuals from the upstream portion of Tryon Creek and three from above the Boones Ferry culvert had high probability of assignment (q>0.8) to Johnson Creek.

Estimates of N_e were variable across sites and their confidence intervals were generally wide and overlapped in many cases. By far the highest estimated N_e was produced by Mitchell Creek with a point estimate of 313.4 (95% CI=42.6-Inf.). The next highest was Johnson Creek with an estimate of 152.4 (CI=48.4-Inf.). Boones Ferry (45.1, CI=36.9-56.3), Tryon Creek upstream (70.8, CI=59.7-85.2), Tryon Creek mouth (30.2, CI=21.6-46.8), and Kelley Creek (21.6, CI=15.5-32.6) produced lower estimates. Given the genetic similarity of Kelley and Mitchell Creeks and the two Tryon Creek sites, we performed additional estimates of N_e with those combined populations (i.e. Kelley and Mitchell combined and Tryon Creek combined). The combined Mitchell/Kelley population produced N_e=34.3 with 95% CI=25.1-50.6. Together, the combined Tryon Creek population resulted in N_e=77.2 with 95% CI=65-92.5.

The highest levels of relatedness were observed in Kelley Creek (mean r=0.19, sd=0.151), Mitchell Creek (mean r=0.206, sd=0.136), and Boones Ferry (mean r=0.109, sd=0.136)(Figure 6). None of the other sites produced a mean r>0.1. Similarly, Kelley and Mitchell Creek had the highest percentage of pairwise values (3.68% and 4.16%, respectively) greater than 0.5. For the other populations values of r>0.5 were less than 2% of all values. Based on the iterative reshuffling procedure implemented in *related*, individuals in Kelley, Mitchell, Boones Ferry, and Tryon Creek upstream were more related than would be expected by chance (p<0.002, Figure 7). Johnson and Tryon Creek mouth were no more or less related than expected

(p<0.448). Overall the entire system displayed a signal of individuals within populations being more related than expected (p<0.002).

Conclusions

Our results provide insights into patterns of genetic structure and gene flow among cutthroat trout populations in an urban watershed. Previous analysis of Tryon Creek cutthroat trout revealed that these fish were intermediate in genetic diversity compared to other populations in the Columbia River Basin, possessing less diversity than coastal populations but more than interior montane populations (Smith *et al.* 2015). Across the six collection sites, aside from Johnson Creek, there were fairly consistent levels of heterozygosity and allelic richness. No one site appeared to be lacking in genetic diversity. The mean and distribution of relatedness values were elevated in both Mitchell and Kelley Creeks, suggesting the presence of moderate number of related family groups. Such observations are not unexpected for trout in small streams given that related individuals often occupy the same stretch of river. Considering the high levels of diversity and lack of deviations from neutral conditions, the Kelley and Mitchell Creek populations are likely not highly inbred.

Analyses of genetic differentiation provided clear evidence that the primary division was between the two main watersheds. Within those watersheds, though, there was little partitioning of genetic variation. Mitchell and Kelley Creeks, which are close in proximity, are also close genetically. The three collection sites from Tryon Creek appear to form a single genetic unit. Although such observations could be a legacy of shared ancestry, given the short generation time of trout and decades of human alteration the genetic similarity of collection sites within the primary tributaries is suggestive of contemporary gene flow and homogenization. Aside from Johnson Creek, the remaining four sites displayed little evidence of departures from neutral

expectations. These four sites produced F_{IS}-values that did not deviate from expectations of random mating. Interestingly, there is evidence of bidirectional dispersal between the two main genetic units, especially in the Tryon Creek upstream and Boones Ferry sites. The STRUCTURE analysis identified several apparent migrants and the DAPC further corroborated these findings. Although migrants composed a small number of individuals, they suggest that the Willamette River may not act as a major barrier to dispersal. Unexpected was the detection of more migrants in the upstream portion of Tryon Creek than the area around the mouth. Perhaps the upstream portions of the tributary, much of which is surrounded by protected natural areas, contain preferred habitat for cutthroat trout compared to the entrance to the Willamette. Further research is needed to explain this pattern, especially since the upstream portions of Johnson Creek (Kelley and Mitchell Creeks) did not contain any migrants. We also acknowledge that the migrants detected via STRUCTURE may not have originated from our collection sites *per se* but perhaps outside populations.

Despite evidence of gene flow and movement, estimates of N_e were low. This is typical of many stream-dwelling fishes in which there can be high variance in reproductive success between individuals and generations. Also, the size of these streams likely restricts population size, further limiting the available gene pool. Moreover, if the sites we examined represent multiple cohorts, then our estimates may have been further biased downward. Still, the estimates of N_e were comparable to cutthroat trout populations located in Mt. Hood National Forest (Smith et al. 2015). The high estimate of N_e for the Mitchell Creek site should be interpreted with caution. The method used to estimate N_e measures increases in linkage disequilibrium (LD) relative to an infinitely large population, and our small sample size for Mitchell Creek (n=15 fish) resulted in limited power to detect LD. The observed estimate of 313 was higher than all

but one reference population analyzed by Smith et al. (2015) and defies reasonable expectations for a stream of this size. This population displayed very low departures from expected levels of LD, which likely inflated N_e. Much more reasonable and informative is the combined Kelley/Mitchell Creek estimate, for the high genetic similarity between these populations warrants a joint estimate and is closer to biological expectations.

Several of our results indicated that the Johnson Creek samples were distinct. Although they clustered with Kelley and Mitchell Creeks in the STRUCTURE analysis, the second axis of the DAPC suggested that these fish formed their own indistinct cluster compared to the other four sites. Plus, Johnson Creek was differentiated from the Kelley and Mitchell sites according to the exact tests and pairwise F_{ST}. Despite a low sample size, it had the highest levels of genetic diversity and private alleles. In contrast it also had the highest level of site-specific F_{IS}, indicating a deficiency of heterozygotes. The unique genetic character of Johnson Creek compared to the other sites could be the product of gene flow, which would explain the elevated genetic diversity and positive F_{IS} via the Wahlund effect. Presence of numerous rare alleles resulting from gene flow could also explain the high N_e observed for this site. In the N_e calculations, using a minor allele frequency threshold of 0.05 produced a point estimate of 29.2, much lower than estimates produced when more rare alleles remained in the dataset. One possible explanation is that the samples we analyzed from Johnson Creek represent a larger population or meta-population. Another possibility is that they represent a population which is more closely integrated with *O. mykiss*. Additional sampling, especially from upper portions of the Johnson Creek watershed, could resolve these patterns.

Overall, cutthroat trout populations from these two neighboring watersheds display patterns of genetic structure similar to populations in more ‘natural’ watersheds. Sites within

watersheds were more closely related than between watersheds. Estimates of genetic diversity were moderate and there were not tremendous departures from neutral expectations that often accompany small populations. N_e-estimates were typically low and individuals within populations, especially Kelley and Mitchell Creeks, were more highly related than expected by chance. Again, though, this is not unexpected for resident fish dwelling in small streams with only a few breeders.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service.

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Table 1: Details of amplification conditions for 14 microsatellite markers used in this study.

This includes PCR primer concentrations (Concentration) and annealing temperature (T_A).

Locus	Multiplex	Concentration (nM)	T_A (ºC)
Och29	1	60	57
Oki10	1	27	57
Omm1050	1	33	57
Ocl34H04	2	27	57
Ocl35D01	2	40	57
Ssa408	2	40	57
Och15	3	33	57
Och16	3	20	57
Ocl30H10	3	53	57
Och20	4	20	60
Och24	4	40	60
Omm1231	5	13	59
Omy1001	5	33	59
Ssa407	5	27	59

Table 2: Sample size, observed heterozygosity (H_O), and expected heterozygosity (H_E) per population.

Population	Sample size	H_O	H_E	Private alleles
Johnson Creek	14	0.76	0.87	33
Kelley Creek	20	0.8	0.8	6
Mitchell Creek	16	0.76	0.76	2
Tryon Creek mouth	17	0.79	0.76	1
Tryon Creek upstream	108	0.75	0.75	11
Boones Ferry	63	0.73	0.74	9

Table 3: Partitioning of genetic variation by an AMOVA with samples grouped by population and then by main tributary (i.e. Johnson or Tryon Creek). "Groups" are the two main tributaries and "populations" refer to the six populations that were sampled.

Source of variation	Sum of squares	Variance components	Percentage variation	*F*-statistics
Among groups	67.27	0.411	8.08	F_{CT}=0.08
Among populations within groups	29.65	0.04	0.79	F_{SC}=0.009
Among individuals within populations	1079.11	0.14	2.73	F_{IS}=0.03
Within individuals	1044.5	4.50	88.40	F_{IT}=0.12
Total	2219.53	5.09		

Figure 1: Allelic richness (A_r) and population-specific F_{IS} for each tributary. Each point estimate is bounded by 95% confidence intervals.

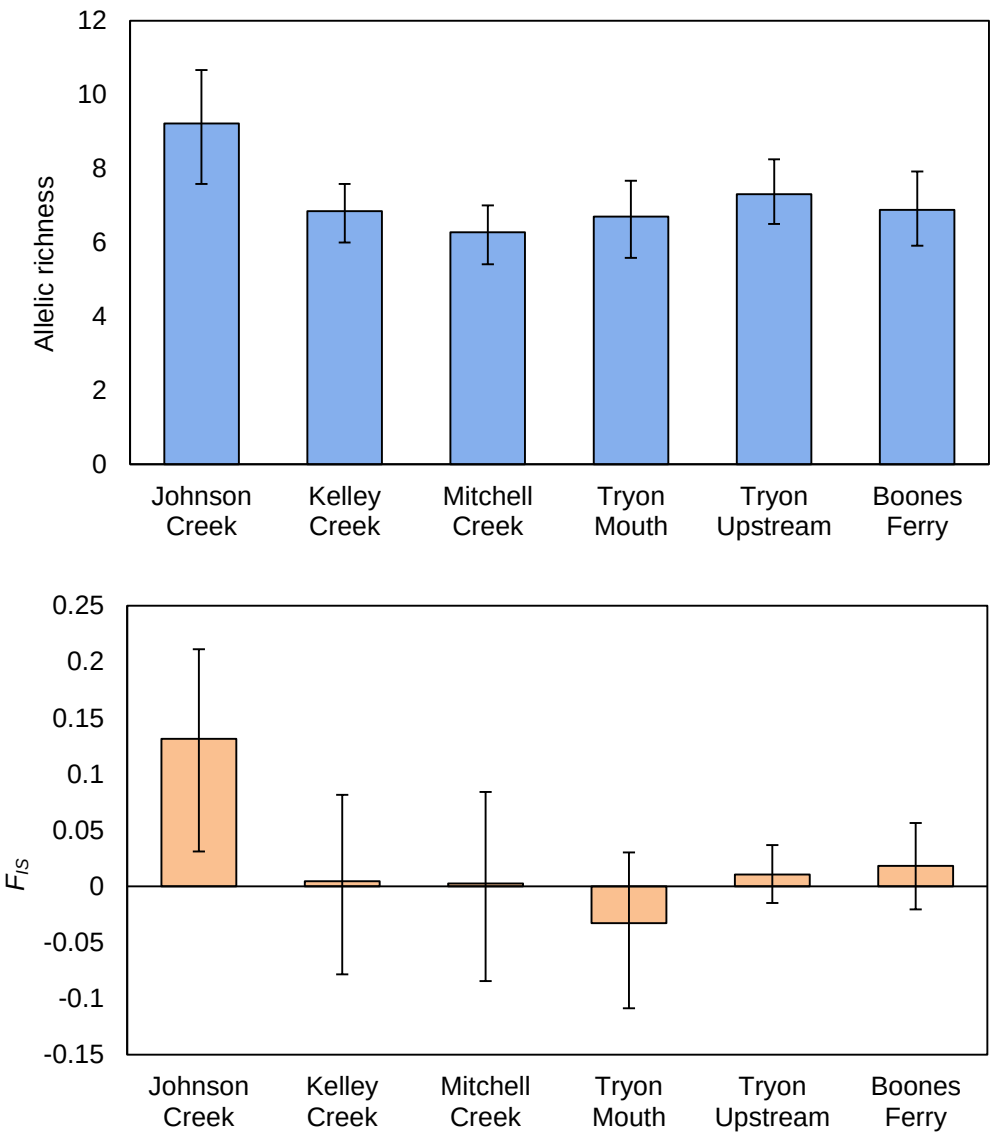


Figure 2: Estimates of pairwise F_{ST} between tributaries and their associated 95% confidence intervals. JC=Johnson Creek, KC=Kelley Creek, MC=Mitchell Creek, TM=Tryon Creek mouth, TU= Tryon Creek upstream, BF=Boones Ferry.

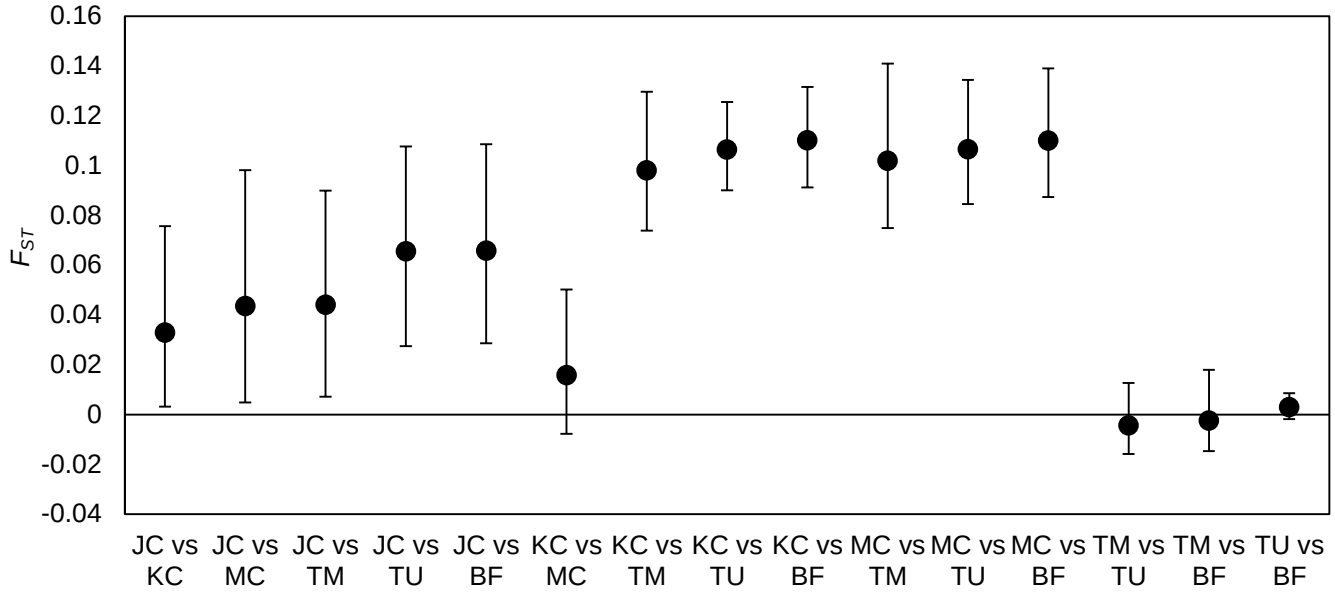


Figure 3: Scatterplot plot of the results from the discriminant Analysis of principal Components (DAPC). Each individual fish is represented by an individual point and points are colored based on tributary of sample. The insets refer to the number of principal components (*n*=40) and number of eigenvalues (*n*=2) that were included.

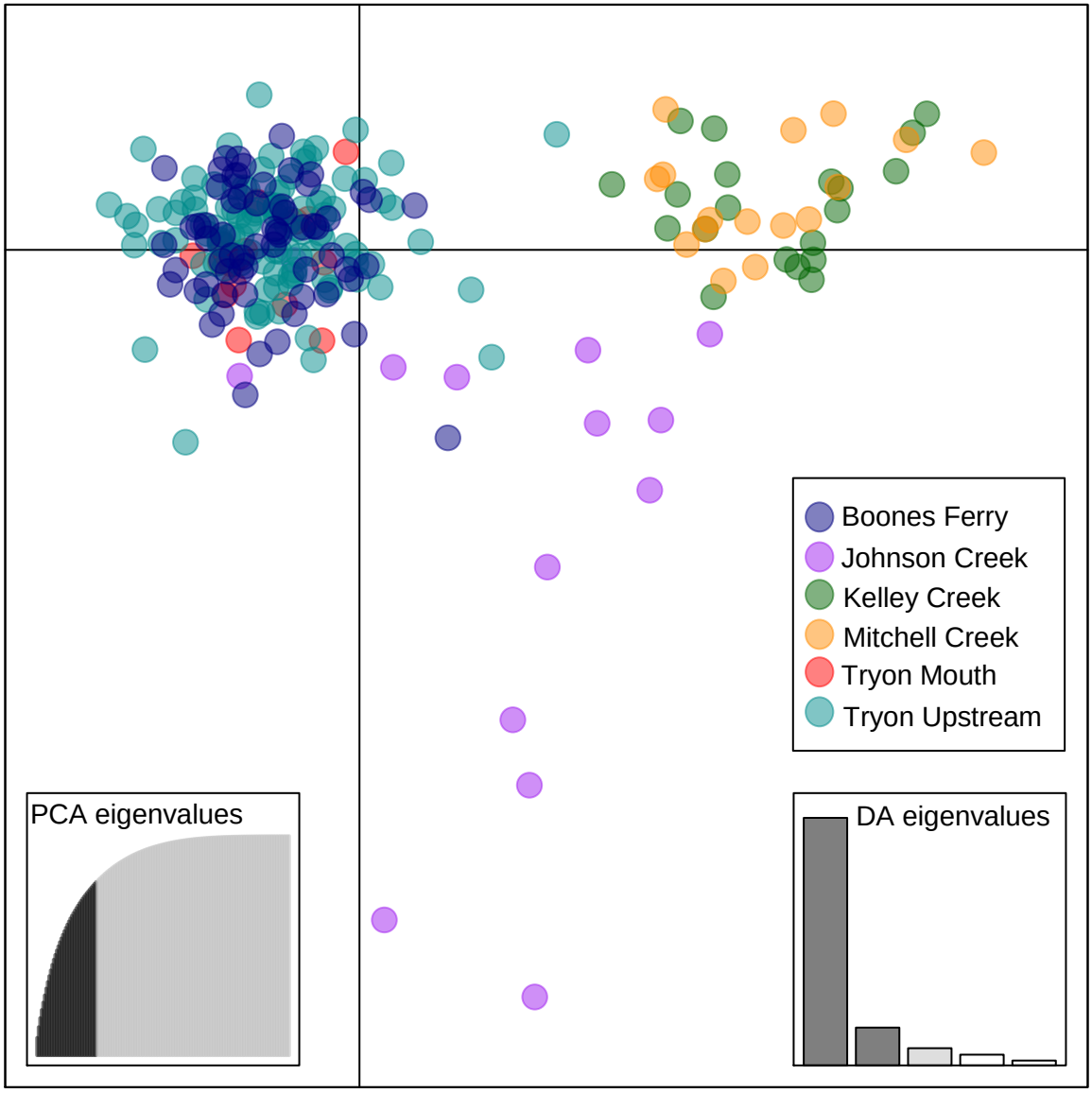


Figure 4: Estimates of mean likelihood (black line, circles) produced by STRUCTUE and Δ*K* (gray dashed line, squares) for values of *K* from 1-15.

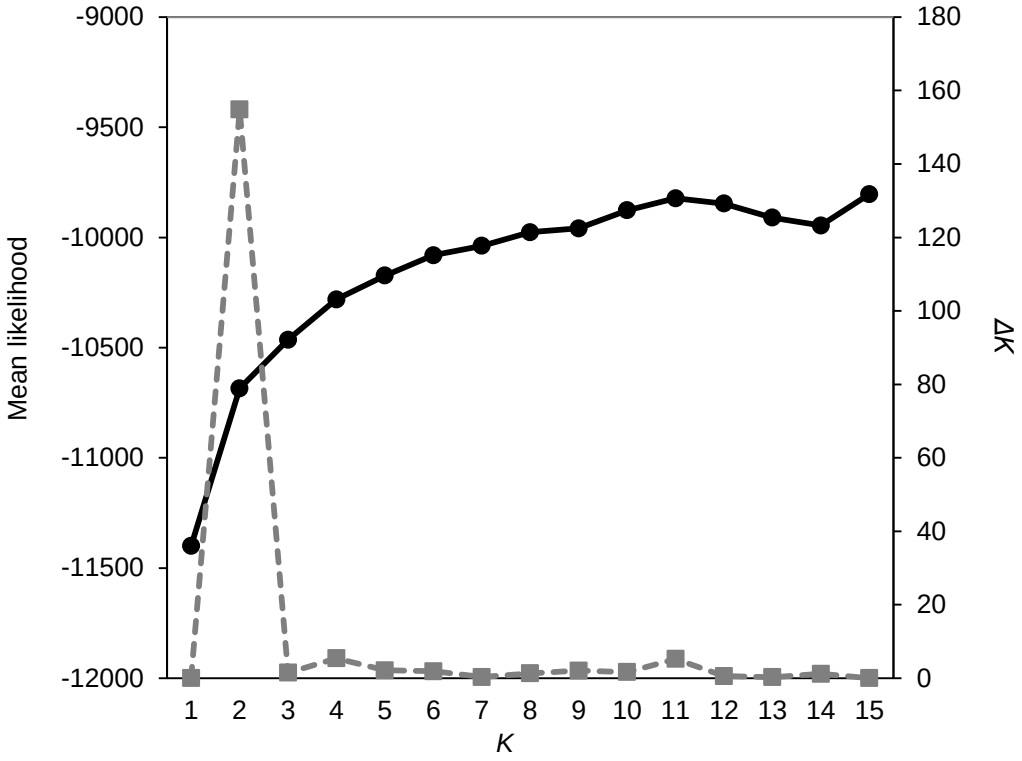


Figure 5: Barplot of ancestry values for individual fish as estimated by STRUCTURE at *K*=2. Each fish represents an individual vertical bar indicating the proportion of its ancestry assigned to each cluster. Thick black lines group fish by tributary. JC=Johnson Creek, KC=Kelley Creek, MC=Mitchell Creek, TM=Tryon Creek mouth, TU= Tryon Creek upstream, BF=Boones Ferry.

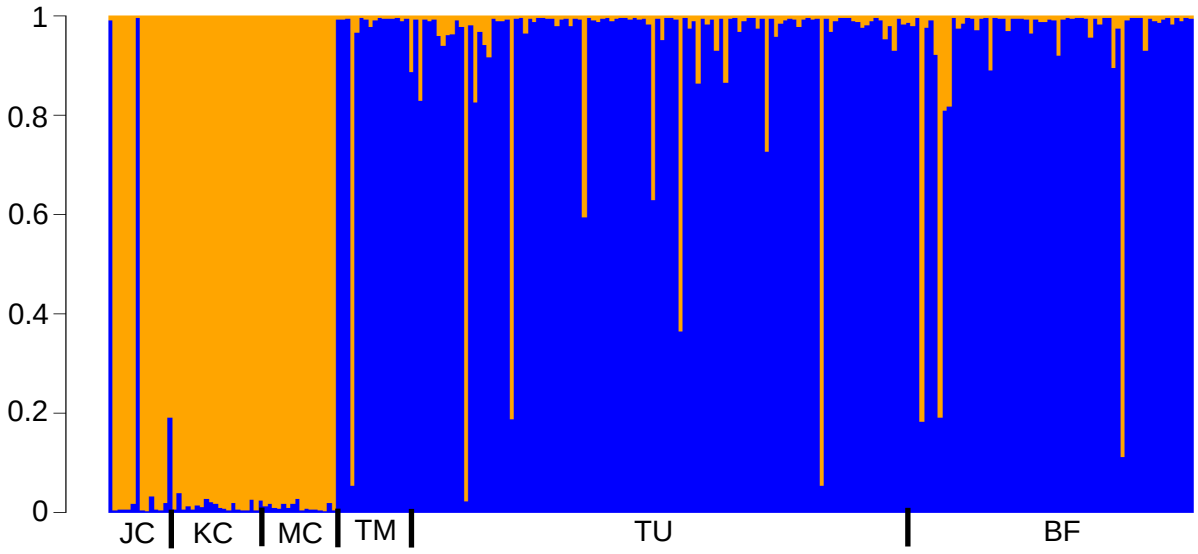


Figure 6: Violin plots displaying the distribution of pairwise relatedness values between individuals within each population. Wider plot widths indicate a greater number of values that correspond to values on the y-axis. Within each distribution is a box and whiskers plot with the median represented by a white point. Relatedness values are based on the triadic maximum-likelihood method of Wang (2007).

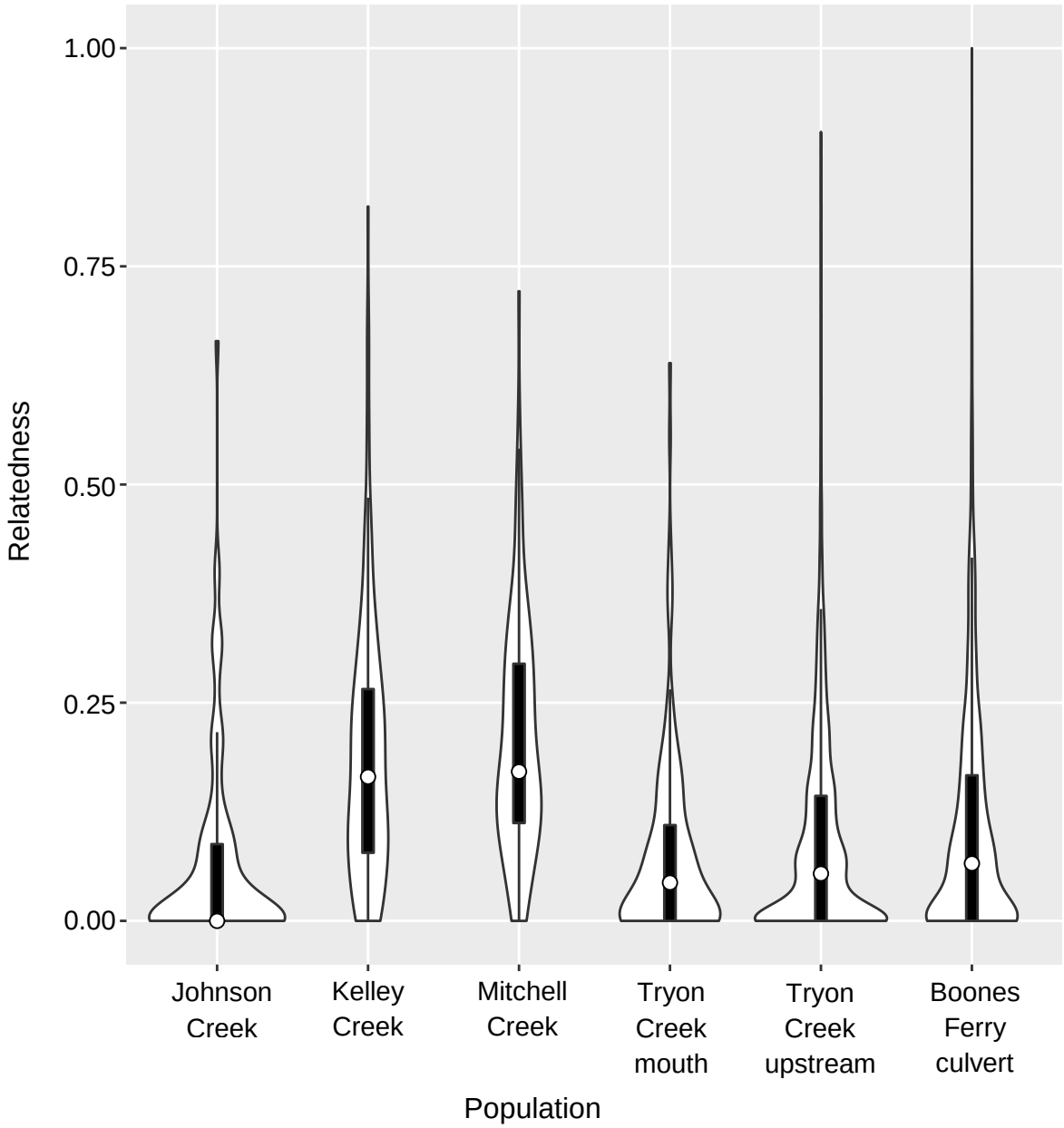
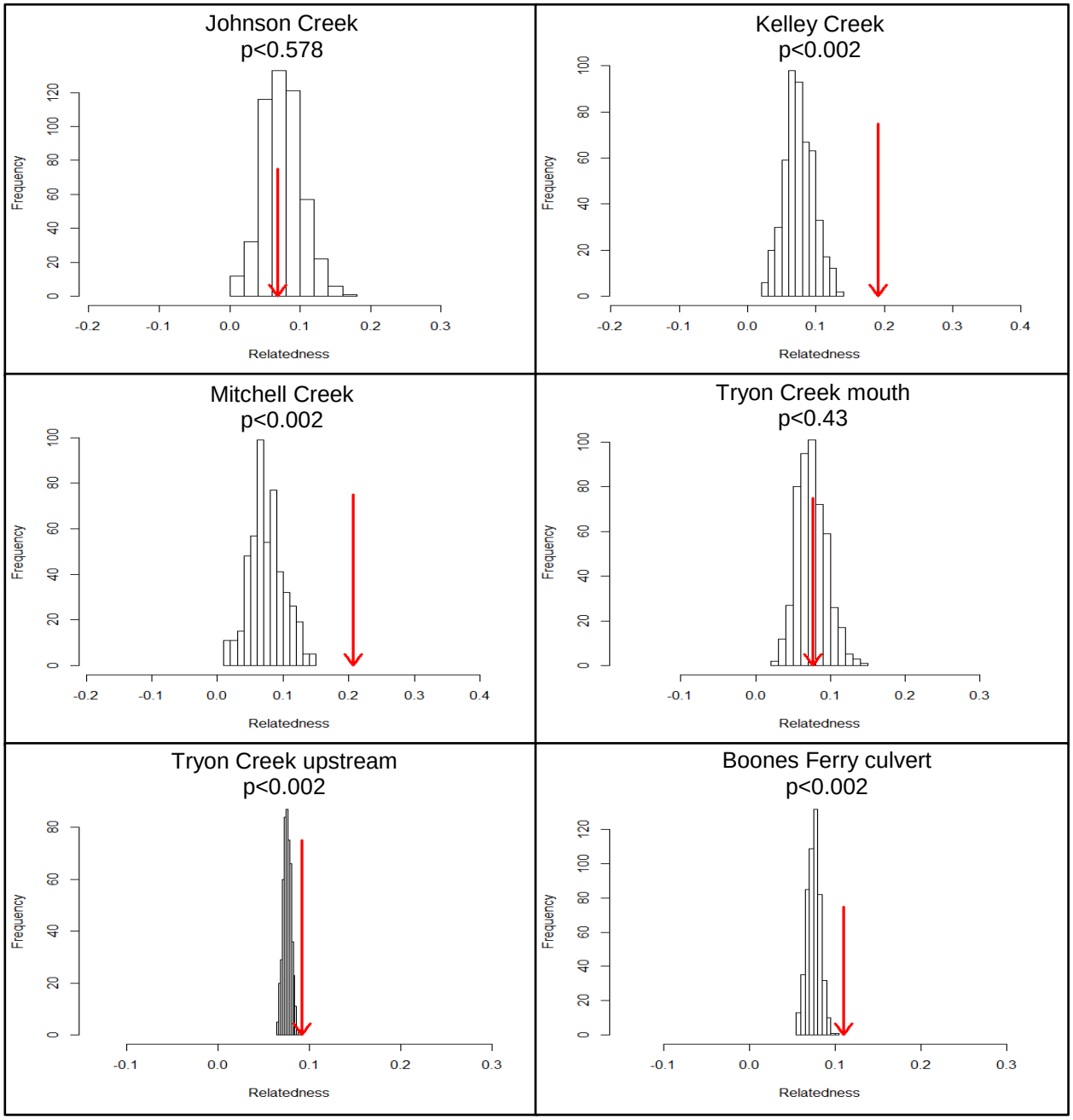


Figure 7: Frequency distribution of simulated relatedness values generated for each collection site and the overall sample of fish. Frequency is on the *y*-axis and relatedness value on the *x*-axis. The distributions were generated with 500 iterations that randomly shuffled individuals between populations and recalculated the mean relatedness value. The red arrow indicates the actual observed mean relatedness value produced by the collection site and the *p*-value refers to whether the simulations deviated from the observed value.



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