Genetic Composition and Status of Coastal Cutthroat Trout (*Oncorhynchus clarki clarki*) in the San Juan Islands, Washington.

A report for the SeaDoc Society prepared by Wild Fish Conservancy, Long Live the Kings, Kwiaht, and the Washington Dept. of Fish and Wildlife Molecular Genetics Lab

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Summary

Until recently, very little was known about fish use within the watersheds of the San Juan Islands archipelago. These watersheds are small, many flowing only seasonally, and all have been subjected to varying degrees of anthropogenic impacts that affect their ecological integrity and productivity. Nonetheless, studies performed previously by the authors of this report have documented that at least five San Juan County watersheds support self-sustaining populations of coastal cutthroat trout (CCT, *Oncorhynchus clarki clarki*).

This study evaluated the genetic composition and status of CCT within three San Juan County watersheds: Garrison Creek on San Juan Island, and Doe Bay and Cascade Creeks on Orcas Island. Fifty, fifty, and forty-nine trout (respectively) were sampled to compare their genetic relatedness to each other and to other CCT populations sampled within Puget Sound watersheds. Genetic diversity was lower in the San Juan Islands collections than in other CCT collections from Puget Sound, reflecting post-glacial dispersal patterns or specific stream and life history characteristics (anadromy vs. residency) associated with these populations. Genetic analyses also provided estimates of the effective number (N_b) of CCT breeders for each of the three study watersheds, and provide a potential benchmark for documenting changes in genetic diversity in the San Juan Islands populations over time. The values for N_b were estimated in Cascade Creek as 27 (16-48 95% CI), in Doe Bay Creek as 21 (12-39 95% CI), and in Garrison Creek as 20 (12-39 95% CI) – indicating small trout populations persisting in small watersheds.

The genetic analyses also revealed that the small CCT populations in Garrison and Doe Bay Creeks are distinct, native populations that appear to have persisted and evolved at low abundances over time – there is no genetic evidence that they were planted or introduced from other watersheds. Doe Bay CCT had the lowest genetic diversity of any coastal cutthroat trout population in the analyses, suggesting that they are more isolated than the Garrison Creek population and many of the sampled CCT populations in Puget Sound. CCT in Cascade Creek represented two genetic lineages – one clearly descendent from ongoing WDFW hatchery planting (Tokul Creek Hatchery) and another that appeared to be descended from naturalized Tokul Creek Hatchery fish that had moved down from planting sites in Mountain Lake, and/or possibly some remnant of a native population.

We identified five age classes of CCT (the largest trout encountered were likely five years old), documented at least two distinct spotting patterns in Cascade Creek CCT, estimated that in 2014 spawning likely occurred in mid- to late-February, and characterized the length-weight relationships of the three CCT populations finding no significant differences between them.

Given the genetic uniqueness and persistence of these small CCT populations documented in the San Juan Islands, we conclude with a range of recommendations - including additional data that should be collected as well as changes to management that should be undertaken - to protect and preserve these vulnerable salmonid populations.

The project team provides this report to the Washington Department of Fish and Wildlife as a basis for updating the Salmon and Steelhead Inventory (SaSI) for the state.

Background

Coastal cutthroat trout (*O. clarki clarki*) are an endemic fish of Pacific Coastal Ecoregion with a historical distribution from Gore Point, Alaska to Eel River in northern California, overlapping more closely with their ecoregion than any salmonid species (Trotter 2008). They are, however, considered the least-studied group of all the West Coast salmonids (Johnston et al. 1999).

A NOAA Status Review of coastal cutthroat trout from Washington, Oregon, and California concluded that a lack of relevant biological information and basic understanding of their life forms hindered efforts to list CCT under the Endangered Species Act (Johnson et al. 1999). Similar species-at-risk reviews for CCT in British Columbia have been equally challenged by lack of routine monitoring (Costello 2008).

Locally in the San Juan Islands, the situation is similar. The Washington Department of Fish and Wildlife (WDFW) performed a statewide inventory of CCT in 2000; however, the San Juan Islands were not evaluated (WDFW 2000). Washington State resource managers rely on the Salmonid Stock Inventory (SaSI), a standardized, uniform approach to identifying salmonid stocks. These inventories are a first step in the process of identifying stocks and monitoring their status. In 2000, WDFW's SaSI for CCT identified 40 stock complexes, of which only one was rated as healthy. Seven lower Columbia stock complexes were identified as 'depressed,' and

WDFW had insufficient information to assess the status of the remaining 32. Many of these are historically small populations which may be especially vulnerable to negative impacts (Anderson 2008). WDFW did not identify a stock complex that included the San Juan archipelago.

The ecological health of watersheds in the San Juan archipelago faces escalating challenges as the region's climate changes and San Juan County's human population continues to grow. Many of the streams' hydrologic regimes have been altered by poorly designed culverts on rural roads and private driveways, wetland alteration (drainage), pond construction, and increased intensity of stormwater runoff due to increases in effective impervious surface area over the past several decades. Undersized and poorly functioning culverts impede fish passage and interrupt natural stream processes, including the transport of wood, sediment, and water. Riparian vegetation has been lost or compromised by invasive plant species, and water quality has been impacted by loss of riparia, and residential, commercial, and agricultural pollutants delivered via stormwater runoff. In many streams, CCT compete with introduced and exotic fish species for limited spawning, rearing, and foraging resources. The recent documented loss of one population of CCT in the San Juan Islands (Barsh 2010) illustrates the challenges facing them.

Recent studies have documented CCT populations in five of the islands' watersheds: Cascade Creek, Doe Bay Creek, Garrison Creek, West Beach, and Victorian Creek (WFC 2003-2008; Barsh 2010) (Figure 1). Still, little was known about the status of these populations; their origin; their relatedness to each other and to other CCT in Washington; the characteristics of their spawn timing and relative condition; and the anthropogenic impacts that may presently limit their survival and resiliency. The non-profit organizations Long Live the Kings (LLTK), Wild Fish Conservancy (WFC) and Kwiáht, together with the WDFW, have collaborated to begin to answer these questions. We will rely on genetic information to determine whether CCT in each tributary are more similar to each other and/or different from similar populations in Western Washington, and thus, whether CCT sampled in each watershed are unique populations or part of larger stock complexes. Data pertaining to phenotype, behavior (spawn-timing), age structure, and growth (via scales) were also collected to identify potential stock and life history differences among and within CCT populations. Collectively, we hope this information furthers an

understanding of the composition and status of coastal cutthroat trout in the San Juan Island archipelago and ultimately provides a framework for strategic protection and recovery actions.

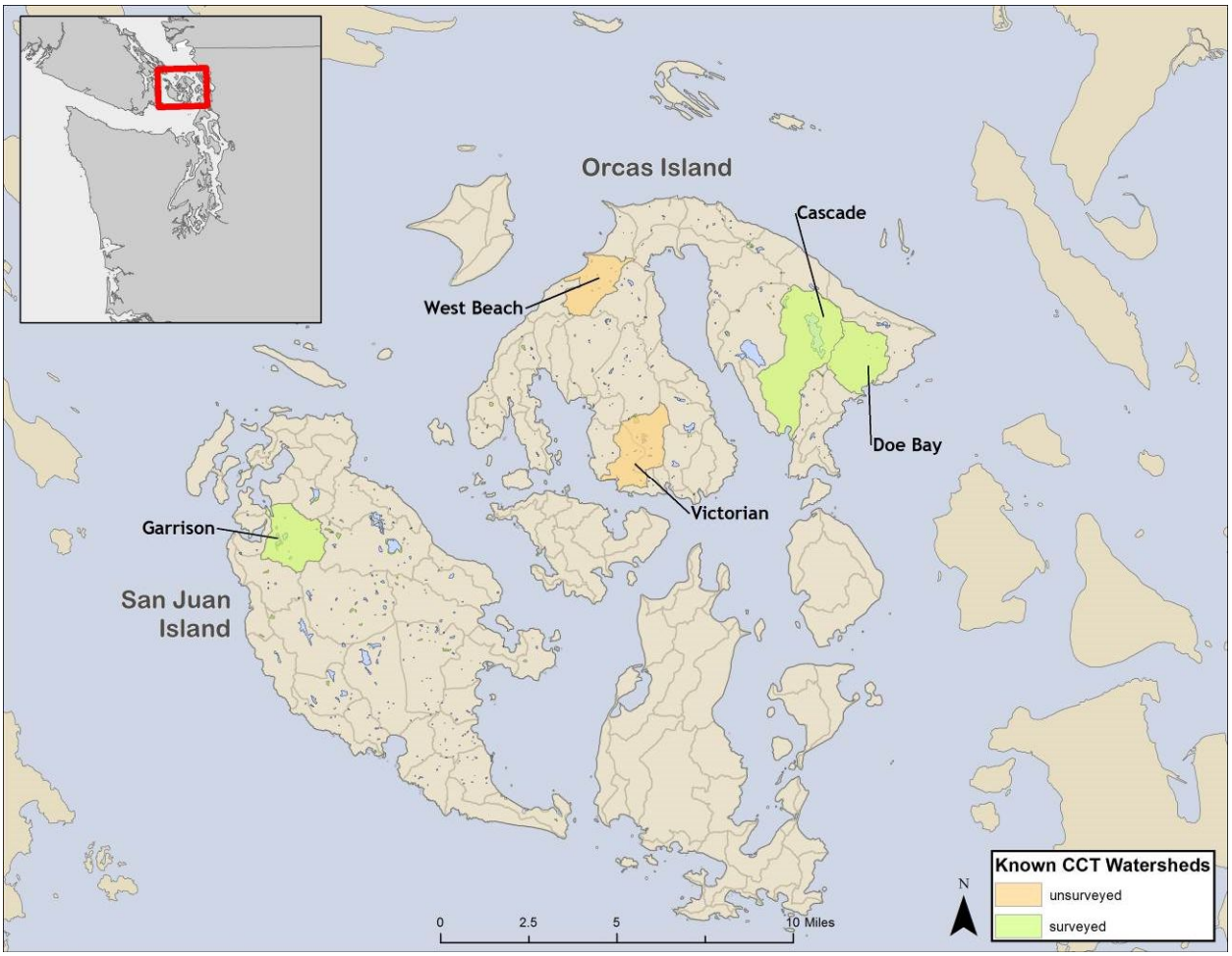


Figure 1. Location of five watersheds with documented coastal cutthroat trout (CCT) presence within the San Juan Islands, WA. The surveyed watersheds that are the focus of this report appear in green: Garrison Creek on San Juan Island, and Doe Bay and Cascade Creeks on Orcas Island.

Methods

We conducted this study within the San Juan Islands, Washington (Figure 1). The San Juan Islands are an archipelago within the Salish Sea, bounded by the Strait of Georgia to the north in British Columbia and the Strait of Juan de Fuca to the south in Puget Sound. They fall within the Pacific Coastal Ecoregion, characterized by high precipitation and a maritime climate with cool, dry summers and warm, wet winters (Naiman & Bilby 2001). The local climate, however, is

strongly influenced by a rainshadow effect from the Olympic Mountains to the south and Vancouver Island to the northwest. As a result, the islands receive less rainfall than neighboring landmasses, and contain some plant species typically found on the drier east side of the Cascade Mountains but not often found west (Atkinson & Sharp 1985). Much of the islands are covered by second- or third-growth forests of Douglas fir (*Pseudotsuga menziesii var. menziesii*), Pacific madrone (*Arbutus menziesii*), red alder (*Alnus rubra*) and bigleaf maple (*Acer macrophyllum*) with the exception of some rare stands of old-growth Douglas fir and (*Thuja plicata*) on Lopez Island and in Moran State Park on Orcas Island.

Bedrock geology dominates the islands, with glacial deposition considered thin compared to other areas in the Puget Sound region (SJC WMC 2000). Catchment water and groundwater recharge come almost exclusively from rainfall, as elevations are too low to provide significant meltwater from snowpack. Microclimates vary dramatically throughout the islands, with annual rainfall accumulations of 48 in. (122 cm) at Mount Constitution on Orcas Island and fewer than 20 in. (51 cm) falling on portions of southern Lopez Island and Cattle Point on San Juan Island (Orr et al., 2002)

Watersheds in the islands are relatively small (< 5 miles^2). With the exception of a few perennial streams, surface flow typically begins between November and January and ceases by June.

Study Areas

Study streams and reaches were selected based on the presence of CCT from previous studies (WFC 2005-7; Barsh 2010; WFC 2010) and with permission from property owners for access. All of these study streams are located within Water Resource Inventory Area 02. These include: Doe Bay Creek, Cascade Creek, Victorian Creek, and West Beach Creek on Orcas Island and Garrison Creek on San Juan Island (Figure 1). All are 3nd Order (Strahler et al. 1957) with the exception of Cascade Creek, which is classified as 2nd Order. The original intent of this study was to describe the cutthroat populations within all five watersheds; however, because of a lack of access (Victorian) and an apparent loss of the cutthroat population since its original discovery (West Beach), we focused this effort on three of the five watersheds: Cascade Creek and Doe Bay Creek on Orcas Island, and Garrison Creek on San Juan Island.

Garrison Creek on San Juan Island flows from atop Cady Mountain (894 ft.) within the San Juan Island National Historical Park's Mitchell Hill unit, downstream through a low-gradient agricultural floodplain to Garrison Bay (Figure 2). It is classified as Stream Number 02-0047 on the Washington States Water Resource catalog for WRIA 2 (Williams 1975). The upper half of the stream course is steep and fish access to the headwaters is restricted by a deteriorating concrete weir as well as other natural barriers (rocks, root wads, chutes). The lower half of the stream is seasonally accessible as a result of topography and failing culverts that are partial fish barriers. A seasonally dry, ditched channel and seasonally flooded and farmed wetland seasonally separate the seaward reach of the stream (reach A in Figure 2) from the perennially-flowing reaches (B and C in Figure 2). Cutthroat trout have consistently been found in these latter reaches, where there are relatively favorable flow and substrate conditions. Connectivity of the presumed spawning and rearing reach with Garrison Bay is consequently seasonal, i.e. when the wetland floods.

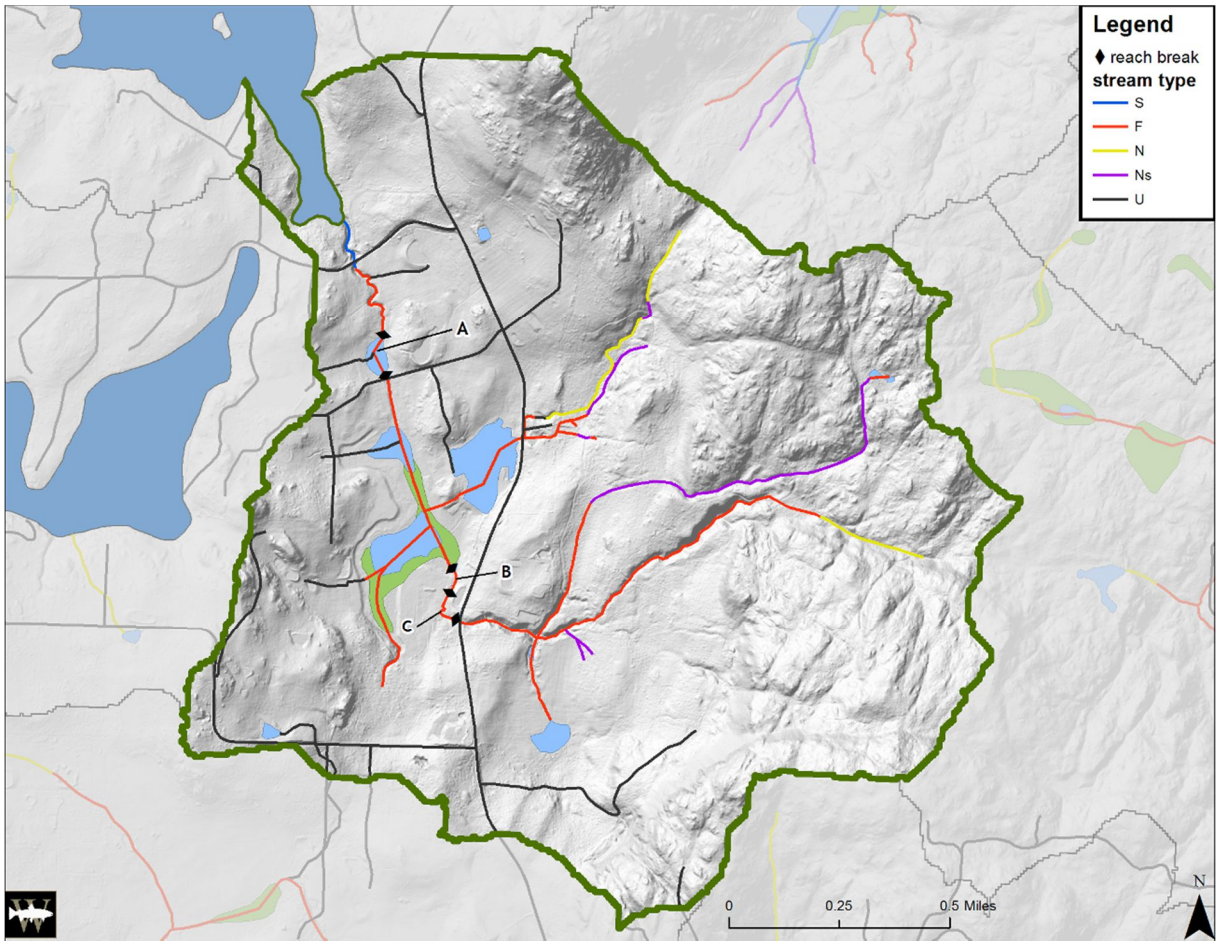


Figure 2. Garrison Creek on San Juan Island, with sample reaches A, B, and C.

Cascade Creek (Stream number 02-0057) flows through Mountain Lake in Moran State Park through densely forested, largely undeveloped land to its terminus at Buck Bay on Orcas Island (Figure 3). Cascade Creek has numerous barriers, both natural and artificial, that impede fish passage. Surface water is diverted and regulated by the Washington Department of Ecology for water users and insured retention for Washington Water Trust. WDFW has stocked CCT in the upper reaches of the watershed in Mountain Lake since the 1930s. Non-native brook trout (*Salvelinus fontanilis*) have also been stocked in Cascade Creek; while they are no longer stocked, the abundance of multiple brook trout age-classes indicates they are reproducing successful there (Figure 4).

The sea-accessible reach of this stream (reach A in Figure 3) extends from the tidal prism beneath a short-span county bridge (built in 2011 to replace a causeway penetrated only by a steel culvert) to a bedrock waterfall approximately 190 m upstream. This reach is a series of riffles, broad shallow pools, and deeper pools where boulders, outcrops, or logjams intrude. A private landowner maintained egg boxes for coho and chum salmon in reach A 10-15 years prior to our study. Reaches B through E exist above anadromy and are isolated by a series of natural and artificial barriers. Reach E is the primary contributor to Mountain Lake. The elevation difference between the outlet of Mountain Lake and the mouth of Cascade Creek (a distance of approximately 3.75 miles) is 900 ft., providing an average gradient of 4.5 percent; however much of the change in elevation occurs at a steep cascade and four bedrock waterfalls (Figure 5).

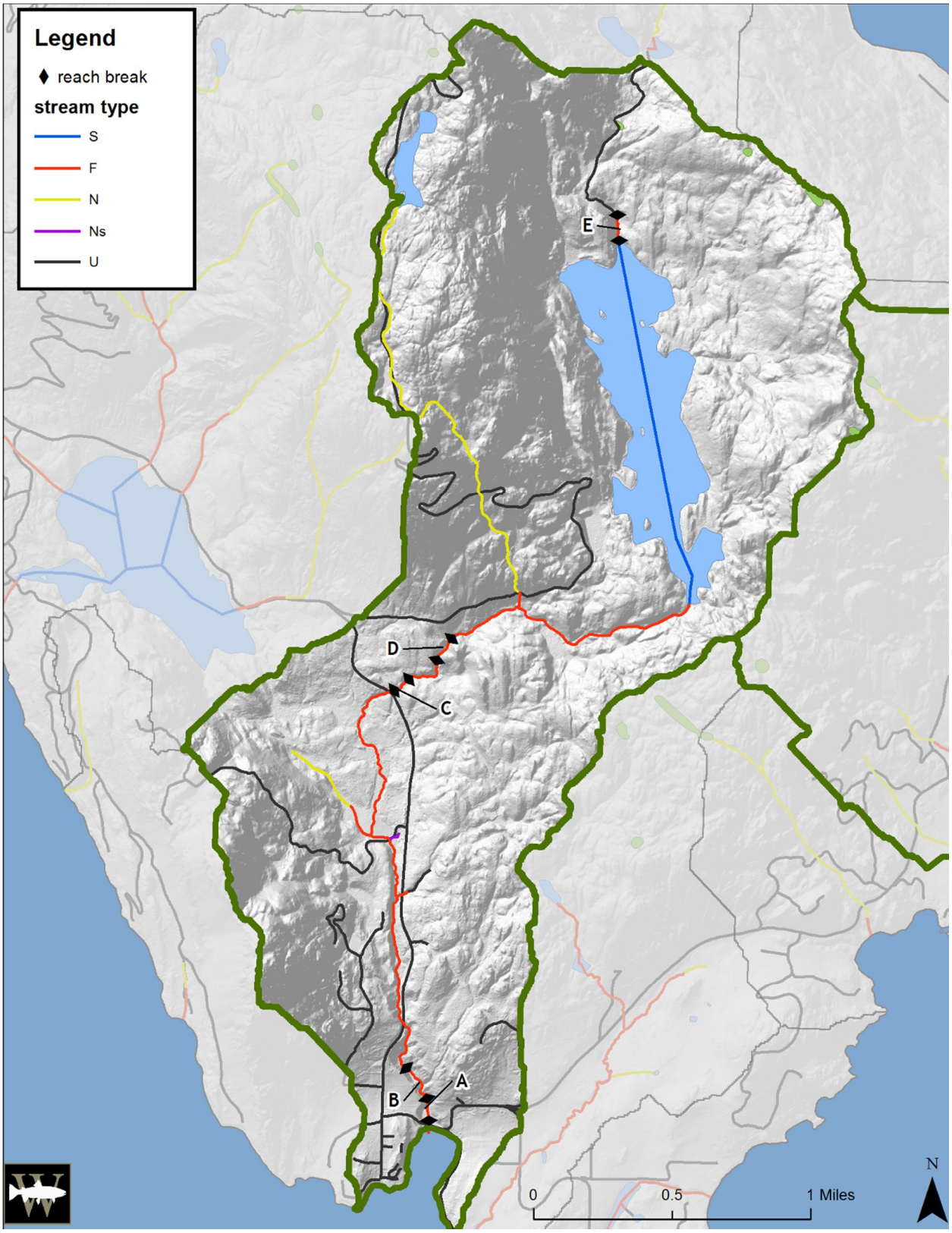


Figure 3. Cascade Creek on Orcas Island, with sample reaches A – E.

Figure 4. Multiple age-classes of brook trout, including young of year, were captured in reaches A and B of Cascade Creek.

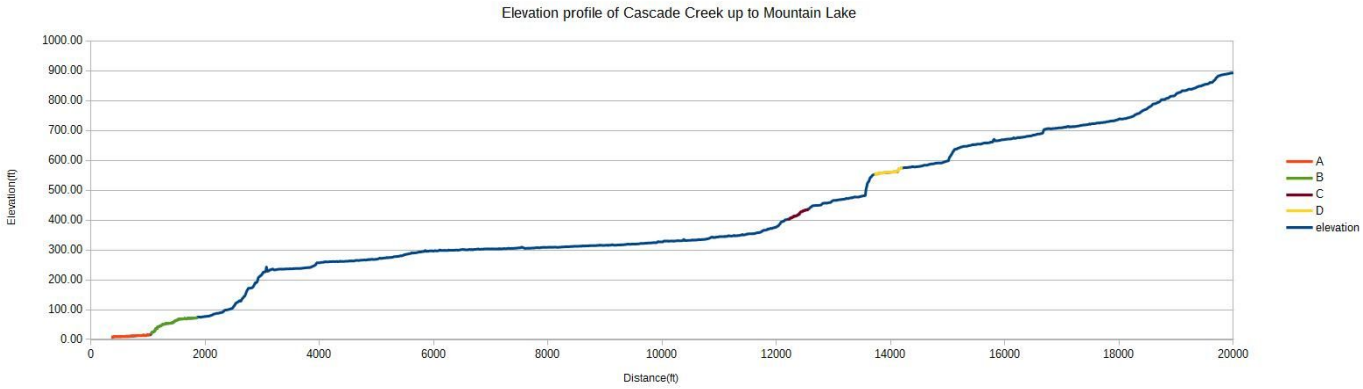


Figure 5. Longitudinal profile of Cascade Creek, from the outlet of Mountain Lake to its mouth at Buck Bay. Survey reaches A-D are shown, reach E is off the profile at the upstream end of Mountain Lake.

Doe Bay Creek originates from Mount Pickett in Moran State Park on Orcas Island and passes through wetlands and ponds, rural neighborhoods, and on towards Doe Bay (Stream Number 02-0055) (Figure 6). Natural bedrock waterfalls at the mouth of Doe Bay bars upstream migration of fish, so all CCT sampled in Doe Bay are presumed to be isolated resident fish. A culvert on Point Lawrence Road (upstream end of reach A in Figure 6) impedes fish passage during the

lower flows in spring through fall. Between the waterfall and the culvert, adult resident cutthroat congregate seasonally. Upstream of this partial barrier, stream conditions vary from riffles and low gradient rapids to shallow pools in bends and under snags.

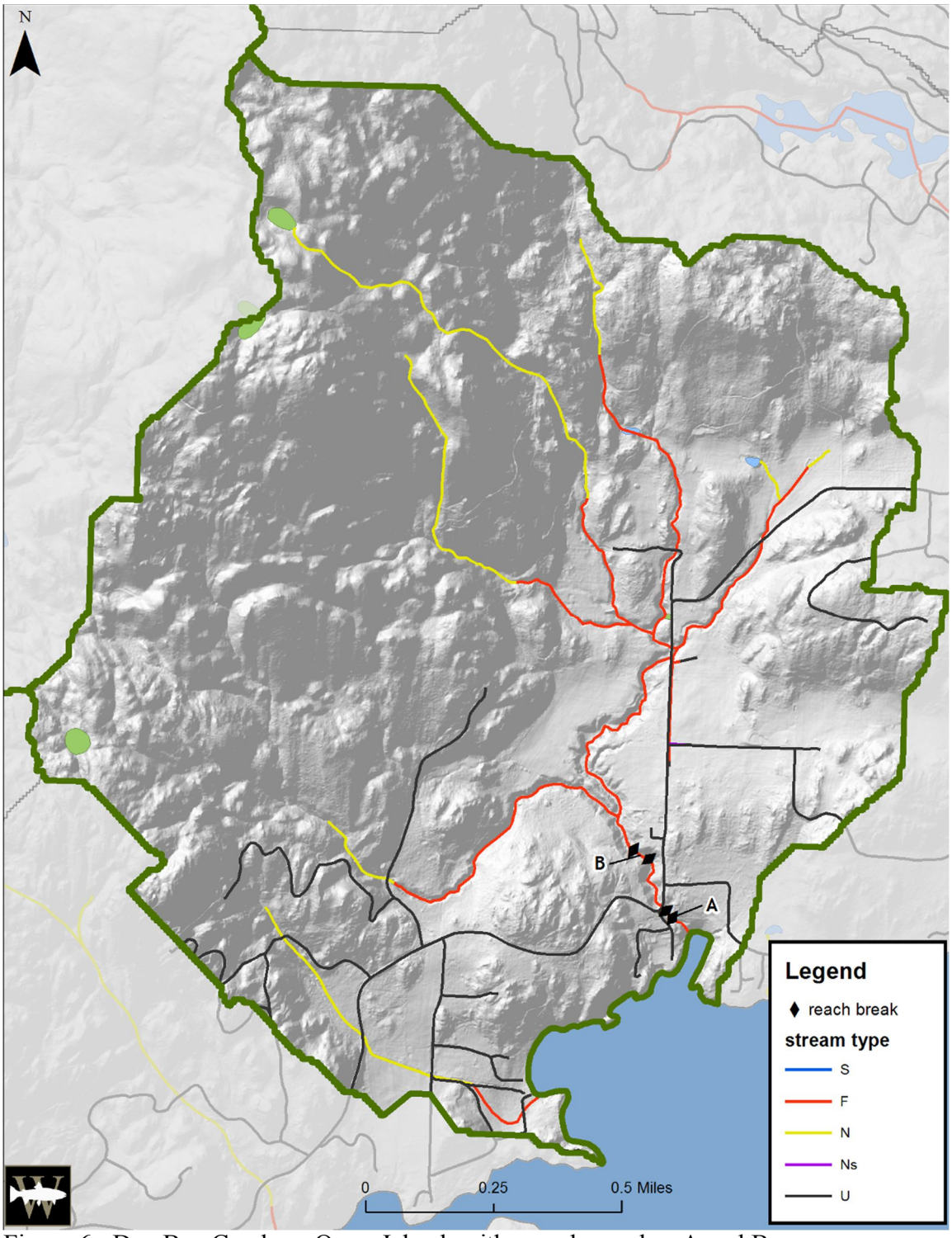


Figure 6. Doe Bay Creek on Orcas Island, with sample reaches A and B.

Sampling effort

Spawner surveys and visual estimates of CCT were conducted weekly from February to May 2014, documenting the presence of redds (fish nests), the first observations of CCT young of the year (YOY), stream conditions (i.e., water temperatures), and any habitat associations (sediment and habitat types). Collection dates for genetic tissues, scales, morphometric and phenotypic samples occurred from June to August 2014, with additional samples collected in Garrison Creek in December 2014 due to sample size limitations.

Systematic CCT spawner surveys, to our knowledge, had never been fully initiated in the San Juan Islands prior to this study. The presence of redds, the dates of first emergence of YOY in each stream, and stream temperatures were compared with published estimates for "degree days" or thermal units (Losee et al., *in press*; Merriman 1935) to estimate spawn timing for CCT in each stream.

Fish were enumerated by visual counts and underwater videography with a GoPro© video camera mounted on a meter-length pole within a watertight housing. Surveyors started at the lower end of each reach, walking upstream while recording footage and visually searching for trout. Once trout were observed, surveyors recorded the number, estimate age class, the location, and any stream variables such as flow and substrate types. Underwater videography (GoPro ©) was used to confirm and/ or strengthen visual estimates of trout.

During the tissue sampling surveys, fish were captured with a triple-pass electrofishing of block-netted sections and single pass (absent block nets) electrofishing using a Smith Root backpack electrofisher from June through August 2014, and mid-December 2014 (Garrison only). Fish were anesthetized with tricaine methanesulfonate (MS-222) and weighed on a digital scale to the nearest 0.1 gram. Fork lengths were measured to the nearest 1.0 millimeter using a measuring board. For CCT greater than 50mm, surveyors used surgical scissors to remove a 1-2mm^2 fin clip from the dorsal lobe of the caudal fin, preserved in ethanol, and delivered to the WDFW Molecular Genetics Lab in Olympia, WA. See Small et al. (Appendix 1) for methodology. Scales (n = 89) were collected from a subset of trout from each watershed and analyzed for age estimation from scale annuli. Trout brought to hand for this study were photographed in

photariums, and images were compared to characterize any morphological or phenotypic (e.g., proportions, spotting patterns, etc.) differences or similarities. Once trout recovered from their anesthesia, they were returned to their original location.

A goal of this study was to establish effective population size estimates from genetic samples, with a secondary goal of collecting reliable abundance estimates from field observations. Genetic samples did permit estimates of the number of effective breeders (Nb) and genetically effective population sizes (Ne) to be made. Reliable abundance estimates, were not possible, since many of the assumptions for depletion were not consistently met (Meyer & High 2011). Block nets were not consistently used (the population could not be assumed to be closed) and the pace of electrofishing surveys was variable (fishing effort was not constant).

Age structures were estimated and compared across streams from length-frequency distributions and scale analyses to assess similarities and/or differences across streams.

Length-Weight analyses

The relationship between weight and length of individual trout samples from the three populations was examined using Bayesian linear regression to determine whether length-weight relationship differed significantly between populations. Weight was measured in grams and fork length was measured in millimeters. Linear regressions were conducted by regressing natural log-transformed weight (Ln(W) on natural log-transformed fork length (Ln(L), assuming normal regression (process) errors in natural log space. Broad uninformative uniform prior distributions were placed on the intercepts, slopes and errors and the posterior distribution of the three parameters of the normal likelihood was sampled using a Fortran based Metropolis-within-Gibbs sampling routine. To assure ample coverage of the posterior probability space for parameter estimation a total of 500,000 samples were retained using a thinning interval of 50.

Before conducting any regression analyses, the length-weight data were first examined for outliers by examining the Fulton Condition Factors (K) and removing all outliers before conducting regressions on the remaining data points. To identify outliers K was calculated for each of the 164 length-weight data points using the formula

$K = W*100,000/L^3$,

where W is weight in grams and L is fork length in millimeters. A value of K of 1.0 indicates that a fish's weight is directly proportional to the cube of its length. Fulton's K provides an indication of how well fish weight scales with the cube of fish length. Salmonid weight should approximate the cube of length given the assumption that salmon girth is approximately cylindrical and hence salmon girth should scale with the cube of length. Average values of K for resident salmonids typically range between 0.9 and 1.1 (Wild Fish Conservancy, unpublished data). We assumed that on average cutthroat trout weight should scale close to the cube of fork length across the range of lengths in our samples (40 to 233 mm), with a population mean value near 1.0 and individual variation around the mean. To identify outliers (weights that were unreasonably low or high at a given length), the sample mean, sample standard deviation (sd), and central 99-percentile of the expected distribution of K within each of the populations was calculated as values lying within plus or minus 2.57 times the sample standard deviation. Any value of K lying outside the central 99 percentile of the expected distribution calculated for the population was considered to be an outlier. After first calculating the mean, standard deviation, and central 99 percentile of K for each population, data points identified as outliers were removed and the mean, sd, and central 99 percentile recalculated and the remaining data examined for outliers. This process was repeated separately for each population until no outliers remained.

This resulted in removing 4 data points from Cascade Creek, 3 from Doe Bay Creek, and 5 from Garrison Creek. The final length-weight data consisted of 60 samples from Cascade, 47 from Doe Bay, and 45 from Garrison (total n = 152).

Results

Sampling effort

A total of 167 coastal cutthroat trout were brought to hand during the sampling effort. Of 167 individuals, three were recaptures (2 in Cascade Creek, 1 in Garrison Creek). Trout were not captured from an electrofishing effort at West Beach Creek on August 20, 2014; consequently, West Beach Creek was not further sampled.

Fin clips were collected from 149 CCT, and scale samples were collected from 115 CCT (Table 1; Appendix 2).

Table 1. Site names, sampling dates, reach length, and sampling results.

			Avg. Reach	Coastal Cutthroat Trout		
Site		2014 Dates	Length (m)	Captured	Fin clipped	Scales samp.
Cascade	A	6/9, 7/4, 7/28, 8/4	190	12	10	10
Cascade	B	4-Aug	240	14	14	14
Cascade	C	5-Aug	100	19	10	8
Cascade	D	5-Aug	140	11	5	4
Cascade	E	5-Aug	140	10	10	0
Doe Bay	A	2-Jul	70	22	22	20
Doe Bay	B	2-Jul	90	28	28	18
Garrison	A	22-Aug	125	1	1	0
Garrison	B	7/1, 8/22,	100	19	18	18
Garrison	C	7/1, 8/22, 12/16	170	31	31	23
				167	149	115

The largest live trout (fork length, 233 mm: mass, 125.5 g) was caught on July 2, 2014 in Doe Bay Creek. The longest trout was 290 mm, a decomposing carcass found on August 22, 2014 in Garrison Creek. The cause of mortality was not determined. The smallest CCT, captured in Doe Bay creek, was 37mm.

Additional fish species captured in Cascade Creek included: Pacific staghorn sculpin (*Leptocottus armatus,* n= 9, length range 28-93 mm), other sculpins (including reticulated *Cottus perplexus,* n= 100+, length range 27-117 mm), brook trout (*Salvelinus fontinalis*; n= 29, length range 35– 215 mm, weight range 0.5– 82.5 g), adipose-intact juvenile coho salmon (*O. kisutch*; n= 35, length range 62- 90 mm, weight range 2.6- 10.6 g), and one adipose-clipped juvenile Chinook salmon (*O. tshawytscha,* length = 66 mm, weight= 3.8 g). Pumpkinseed (*Lepomis gibbosus,* n= 6, length range 40-89 mm) and three-spined stickleback (*Gasterosteus aculeatus,* n= 9, length range 23-70 mm.) were captured at West Beach Creek. No additional species were captured in Doe Bay and Garrison Creek.

Abundance

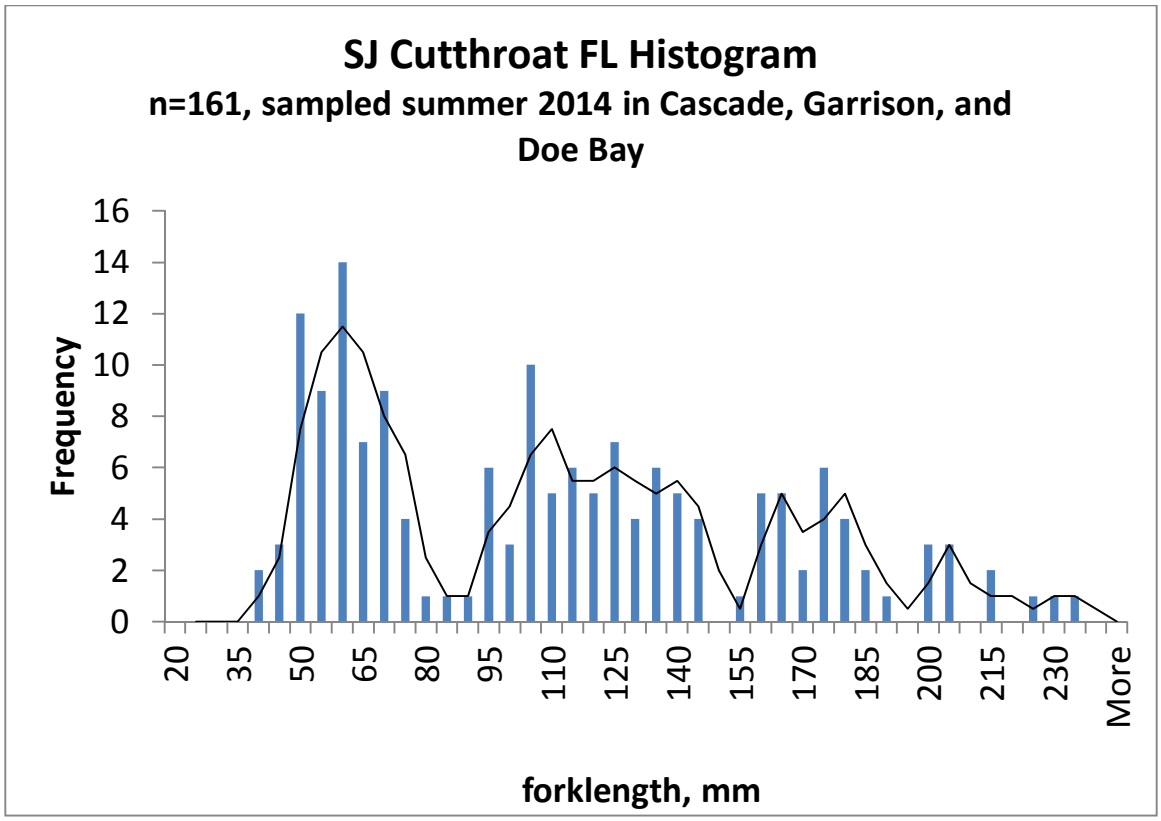
The team originally planned to block net and do multiple-pass or removal population estimates, but that approach was abandoned after the first day of sampling in order to ensure our ability to obtain at least fifty CCT fin clips from each study watershed. Instead, we used genetic analyses to estimate the effective number of CCT breeders that would have given rise to the sampled genetic diversity for each of the three study watersheds using the genetics data. These were estimated in Cascade as 27 (16-48 95% CI), in Doe as 21 (12-39 95% CI), and in Garrison as 20 (12-39 95% CI) (Appendix 1).

Age structure

A total of 166 coastal cutthroat trout fork lengths were collected during the course of the project. Additionally, approximately 2 to 8 scales were sampled from each of 115 CCT, but scale analyses were confounded due to regeneration, resorption, and illegibility of annuli. Of the 115 trout samples, scales were readable from 89.

Length-frequency and scale histograms represent at least five age classes of CCT, as observed by the five modes for both histograms (Figure 7). Five Garrison samples collected in December 2014 were excluded from the length histograms. In general, the size of age 0 (or young of the year), age 1, age 2, age 3, and older (age 4+) live trout constitute 39%, 39%, 16%, 4%, and 2%, respectively, of the trout captured in the San Juan Islands in the summer of 2014. Length estimates include: 0 to <90 mm. (young of year), 90 to <155 mm. (age 1), 155 to <195mm. (age 2), 195 to <220 mm. (age 3), and 220+ mm. (age 4+).

CCT sampled in Doe Bay trout were significantly smaller than those sampled in Cascade Creek and Garrison Creek (ANOVA, $F_{2, 166} = 6.07$, $P= 0.03$, Tukey < 0.05), with the distribution of trout captured in Doe Bay skewed towards the young of the year (YOY) age class. YOY constituted 62% of the captures in Doe Bay Creek, as compared to 38% Cascade Creek, and 13% in Garrison Creek (Figure 8).



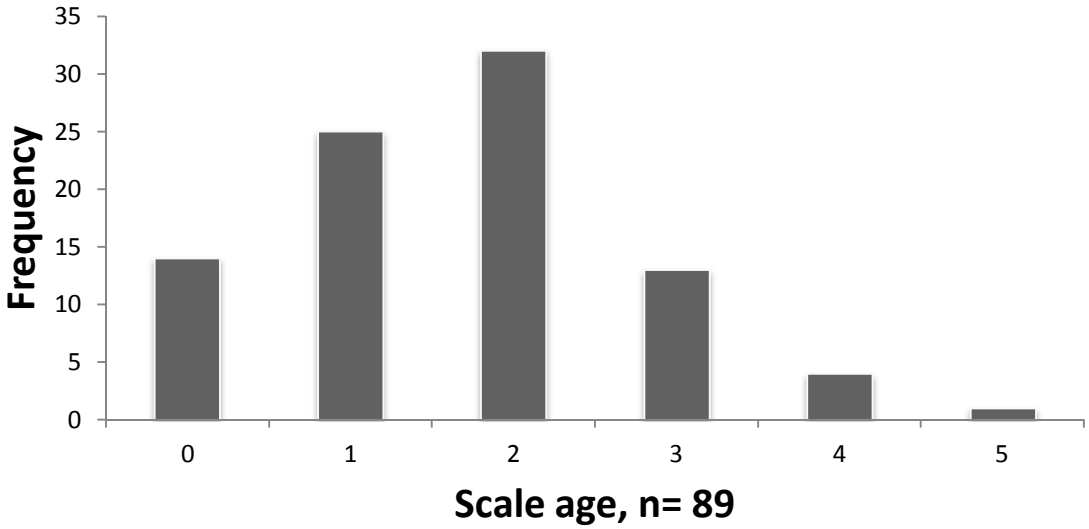


Figure 7. Length frequency (n = 161) and scale frequency distributions (n = 89) for CCT captured in the Garrison, Doe Bay, and Cascade creeks in summer 2014 (five December samples from Garrison excluded). Five distinct age classes exist.

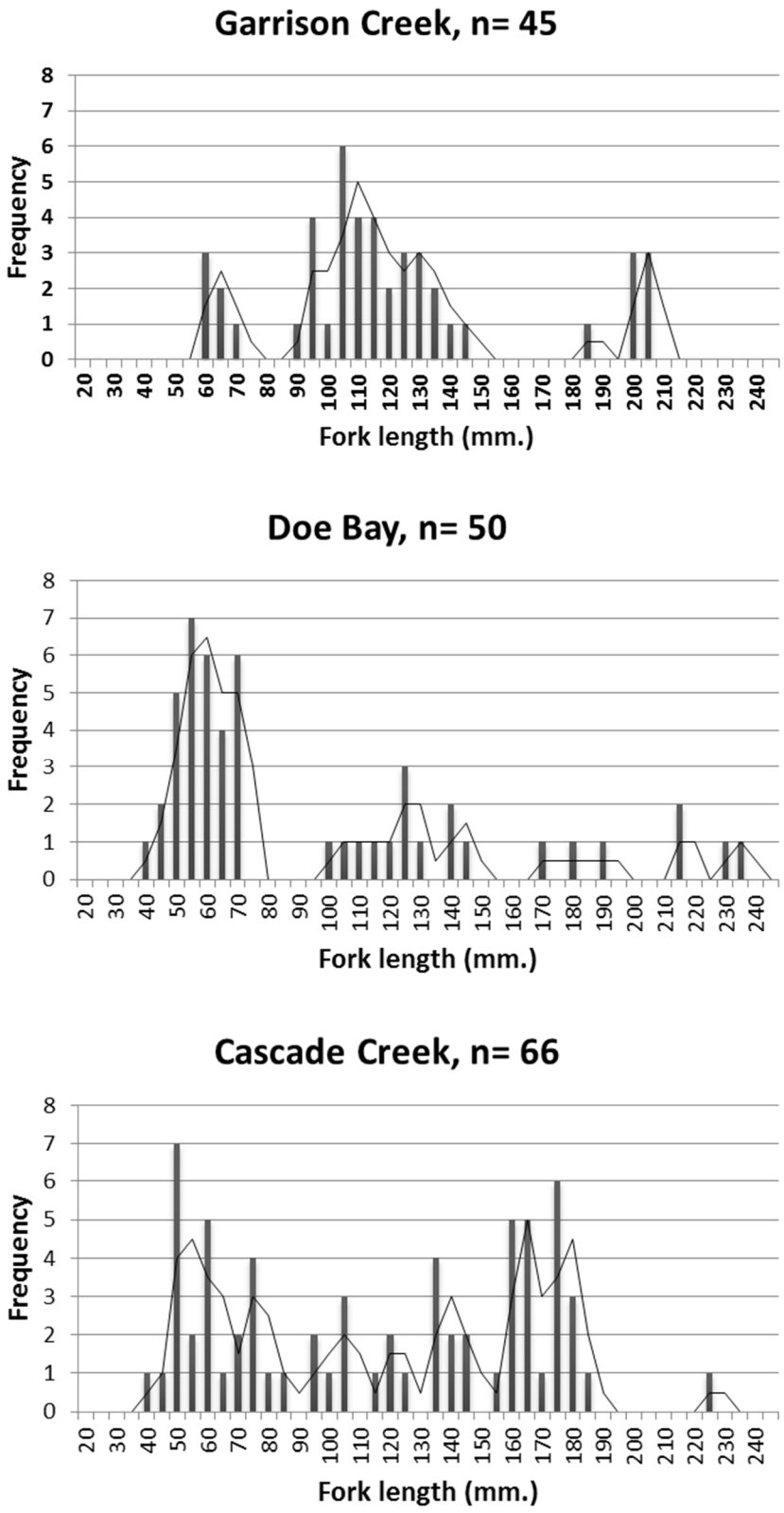


Figure 8. Summer 2014 CCT fork length histograms for each of the three study basins.

Length-Weight Analyses

A total of 164 samples with both length and weight measurements were available. These included 64 samples from Cascade Creek and 50 samples each from Doe Bay and Garrison Creek. Outliers likely representing errors in measurement were identified in all three populations: 4 from Cascade Creek, 3 from Doe Bay Creek, and 5 from Garrison Creek. After removing all 12 outliers, the final length-weight data consisted of 60 samples from Cascade, 47 from Doe Bay, and 45 from Garrison (total n = 152).

Regressions of Ln(W) on Ln(L) were conducted on the remaining 152 data points using the Bayesian program described in Methods. The first regression analysis consisted of estimating separate regression parameters (intercept A, slope B, and regression error S) for each of the three populations. This was done within a single program which enabled derived parameters for the difference in the values of each of the three parameters between each pair of populations to be calculated simultaneously with the primary regression parameters of each population. That is, in addition to calculating the posterior distribution of A, B, and S for each population 1 through 3, A1-A2, A1-A3, A2-A3 were calculated (where A1 is the intercept for Cascade Creek, A2 the intercept for Doe Bay Creek, and A3 the intercept for Garrison Creek) and similarly for slopes B and errors S. The distributions of the derived parameters were examined to determine whether there was evidence that the distribution of the primary parameters differed significantly between populations. A complementary examination of the posterior distributions of parameters between pairs of populations was also conducted, and is illustrated for the slopes and intercepts in Appendix Figures A1 – A6.

The results indicated that all three populations had similar, though not identical, weight-length relationships, and regression errors for all three indicated reasonably precise regressions. In a Bayesian analysis using broad uninformative prior distributions, the posterior mode of each parameter is the single most probable value of the parameter and will be equal to the conventional maximum likelihood estimate (MLE) of the parameter. The posterior modes of the regression errors of the three populations ranged from 0.077 to 0.116. The conventional frequentist R-square statistic is approximately equal to $1-S^2$, where S is the estimate of the regression error, so R-square corresponding to the posterior modes of each of the three

regressions are all greater than 0.98. Thus the distribution of the process errors indicated that it would be reasonable to assume a common regression error for all three populations.

Similarly the posterior distributions of the regression slopes were very close to one another with similar shape. Posterior modes ranged from 2.927 to 2.948. On the basis of the similarity of slopes and regression errors among the three populations, a tradition ANCOVA regression was run on all the data for all three populations assuming a common regression slope and error but individual intercepts to determine whether there were differences between populations in the heights of the regression lines. This resulted in normally distributed posterior distributions (mode equal to the mean) for the regression error and slope with modes of 0.09, and 2.936, respectively, and normal distributions for each of the three intercepts with nearly identical means and standard deviations. The means were -11.19, -11.14, and -11.14, respectively.

On the basis of these results it was concluded that the length-weight relationships of all three populations are fundamentally the same. Therefore, a final linear regression was conducted on the full data set (n = 152) to estimate the posterior distributions of common intercept, slope, and regression error. The posterior modes (single most probable values) for the three parameters from this regression are: Intercept = -11.137, Slope = 2.931 and Error = 0.093. This yields the following regression equations:

$$Ln(W) = -11.137 + 2.931*Ln(L). \quad (1a)$$

Equation [1a] gives the mean of W in natural log space, which is equal to the median in the original lognormally-distributed space, which is smaller than the lognormal mean. When back-transforming from logarithmic space the regression error variance should strictly be accounted for, though in this case it is very small (as evidenced by the high approximate R-square). Accounting for the regression error, this yields the following equation:

$$W = \exp[-11.137+(0.093^2/2)+2.931*Ln(L)]$$

$$= \exp[-11.1327 + 2.931*Ln(L)], \quad (1b)$$

where Ln is the natural logarithm, W is weight in grams, and L is fork length in millimeters.

After carrying out the exponentiation in [1b], the equation for the predicted weight in the original length and weight space is

W = 0.000014627*L^2.931. (1c)

The predicted weights are graphed together with the length-weight data for all three populations in Figure 9.

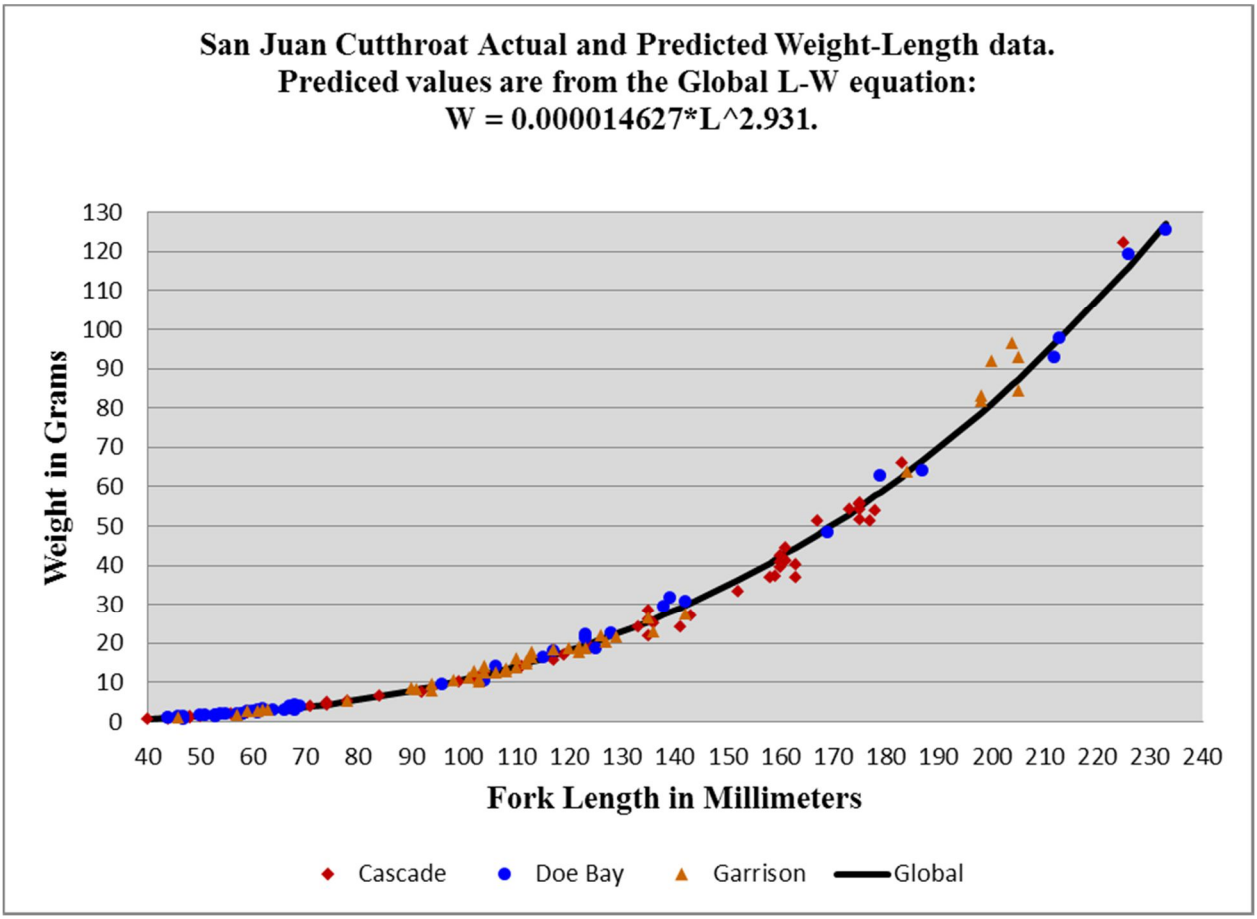


Figure 9. Actual (points) and predicted (line) weight-length data from pooled samples collected in Garrison, Doe Bay, and Cascade Creeks.

Spawn Timing

A single group of possible CCT tests or redds was observed at Cascade Creek on February 10, 2014 at 48 37.418 N, 122 49.921 W. The width and length of the entire redds area was 4 m by 6 m, or 24 m^2. No other definitive CCT redds were observed during the spawning surveys.

The first CCT young of year were sighted in 2014 on April 15, April 22, and April 29 in Garrison Creek, Cascade Creek, and Doe Bay Creek, respectively. Spawner surveys were conducted weekly from February 10 to May 15, 2014, with emergence of YOY assumed to have occurred within a week of the date they were first observed.

Stream temperatures (grab samples at time of surveys, n= 165) ranged from 3 to 15 C. from February to May 2014 for Garrison Creek, Cascade Creek, and Doe Bay Creek, respectively. The mean stream temperature recorded was 9.4 (SE 0.1) C during this time period. The lowest water temperature (3 C) was recorded on February 27 in Cascade Creek, and the highest (15 C) on April 25 in Garrison Creek.

Spawn timing was back-calculated using published degree days of fertilization to hatching (Merriman, 1935) and hatching to swim up (Trotter, personal comm.) estimates for CCT. If the average degree days from spawn to swim up is roughly 570 degree days (Trotter, personal comm.), it would take 63 days for trout to emerge based on the average 9 C (mean= 9.1 (0.2 SE). Back-calculating spawn timing, based on the first YOY observed in each stream, would put an estimated spawn time (for each of the streams) at:

- ~ February 11, 2014 for Garrison Creek
- ~ February 18, 2014 for Cascade Creek
- ~ February 25, 2014 for Doe Bay Creek

CCT spawning is estimated to have occurred mid- to late February for all study streams in the San Juan Islands during 2014. This corroborates well with the redd observed at Cascade Creek on February 10, 2014. This is, however, nearly a month earlier (late February vs. late March) than observed in Garrison Creek from a previous study (WFC 2010). Redds were noted in late March to early April with correspondingly cooler stream temperatures in Garrison Creek in 2008

than observed in 2014 (this study). For example, a redd was observed in Garrison Creek on March 11, 2008 with a water temperature of 7.1 C as compared to 10 C on March 14, 2014, roughly, a three-degree difference between studies. Higher stream temperatures would certainly account for shorter times for emergence, but it is interesting to note that spawning times were estimated at a far earlier time that previously noted, at least for Garrison Creek.

Phenotypic Observations

145 CCT were photographed in 2014. No consistent patterns in differences in coloration, spotting and general morphology of trout were observed from the study streams; with one exception: CCT from Cascade Creek appeared to exhibit two distinct spotting patterns. Six adult CCT from Cascade Creek had noticeably larger spotting then the rest; the six represented fish captured from several subreaches and fish that demonstrated both Tokul (hatchery) and Cascade (naturalized) genetic lineages. Representative CCT photos are presented in Appendix 4. Detailed morphometric measurements of the CCT might demonstrate significant phenotypic differences between the CCT in each of the three study watersheds, but such analyses are beyond the scope of this project.

Genetics

The WDFW Molecular Lab in Olympia, WA conducted genetic analyses of fin clips from Cascade Creek (n= 49), Doe Bay Creek (n= 50), and Garrison Creek (n= 50). Trout samples were genotyped at seven microsatellite loci, and were also genotyped at 96 single nucleotide polymorphism loci (SNPs).

The San Juan Islands collections had the highest number of fixed loci among coastal cutthroat trout collections in comparisons to other coastal cutthroat trout collections from the WDFW coastal cutthroat trout baseline. Genetic diversity, measured as allelic richness and heterozygosity, was lower in the San Juan Islands collections than in other coastal cutthroat trout collections from Puget Sound and the WA coast. The genetic analyses revealed that the small CCT populations in Garrison and Doe Bay Creeks are distinct native populations that appear to have persisted and evolved at low abundances over time. Doe Bay CCT had the lowest genetic

diversity of any coastal cutthroat trout population in the analyses, suggesting that they are more isolated than Garrison. CCT in Cascade Creek represented two genetic lineages – one clearly descendent from WDFW hatchery planting (Tokul) and another that appeared to be descended from naturalized Tokul Creek Hatchery fish that had moved down from planting sites in Mountain Lake, and/or possibly some remnant of a native population. Detailed information is provided in Appendix 1.

Discussion

A lack of data has contributed to the widely-held misconception that there are no native stocks of salmonids left in the San Juan Islands' watersheds. A Salmon and Steelhead Habitat Limiting Factors Report (Kerwin 2002) noted the presence of coastal cutthroat trout in one stream and numerous lakes but concluded that "there are no known naturally sustaining populations of anadromous or resident salmonids in the freshwater habitats of WRIA 2." And again, in a 2004 *Handbook for Salmon Recovery in San Juan Island*, "This stream (Garrison Creek) is reported to have had a population of sea-run cutthroat trout, but the presence of these fish is unverified" (SJC WRMC 2004). Several studies performed in the San Juan Islands in the past decade have greatly expanded our understanding of the watersheds, the fish that inhabit them, and potential factors limiting their overall production (WFC 2003-8; Barsh 2010; WFC 2010). The present collaborative study is the first to provide baseline stock information on CCT populations in the San Juan Islands that is directly applicable for Washington State resource managers in the process of identifying stocks and monitoring their statuses.

This study verifies that there are distinct, native populations of CCT in the San Juan Islands in Doe Bay Creek and Garrison Creek, and possibly in Cascade Creek as well. CCT genetics in Cascade Creek appear to be strongly associated with Tokul Creek hatchery trout but maintain some genetic diversity unique from the hatchery genetics. Hatchery CCT have been stocked in Mountain Lake, the headwaters of Cascade Creek, since 1934, and at least since 1982, were identified as Lake Whatcom brood stock from the Tokul hatchery. It is unclear to what extent the current populations of CCT in Cascade Creek retain some genetic diversity from remnant, native CCT populations that pre-date stocking. Certainly there are numerous natural and artificial barriers in Cascade Creek that limit fish migration, and there are different environs

(lake, stream, and nearshore) that may influence isolation among different life history forms (e.g., fluvial, lacustrine, resident, and sea-run; Trotter 2008). Regardless, the genetics data demonstrate that hatchery CCT planted in Mountain Lake are accessing the entire watershed, from above the lake down to saltwater (Reaches A – E).

The observation of CCT redds in Cascade Creek and the subsequent sightings of YOY in Doe Bay Creek and Garrison Creek demonstrate that CCT populations in the San Juan Islands are reproducing naturally and should be classified as "wild" for SaSi production type. Juvenile and adult CCT were consistently observed on spawning surveys in the spring, during capture efforts throughout the summer, and at least for Garrison Creek, upon one winter survey in the same year (2014).

This study estimates the spawn timing for San Juan Island CCT populations from mid- to late February with a corresponding mid- to late April emergence. This observed spawn timing corresponds well with historical estimates of January to March, with peak timing in February, for other CCT populations in Washington (Johnston 1999). It is also in agreement with the early spawn timing (i.e. mid-February) for anadromous CCT populations in South Puget Sound, WA (Losee et al., *in press*), though researchers found wide variability (February to April) across study years. Estimated incubation time for the San Juan Islands' CCT is, however, nearly a month shorter than observed CCT populations in the lower South Fork of the Snoqualmie River, WA (Thompson et al. 2011) and in Garrison Creek in an earlier study (WFC 2010). Spawn timing and emergence of CCT populations may be explained by ambient stream temperatures, access to available spawning sites, severity of stream flows, interspecific competition with other stream species, and may ultimately be a selective adaptation for coastal cutthroat trout in an unpredictable environment (McMillan et al. 2014).

The multiple age classes observed of cutthroat trout captured in Garrison Creek, Doe Bay Creek, and Cascade Creek from length-frequency distributions and scale analyses also provide support that CCT populations are rearing and reproducing naturally in the San Juan Islands. CCT populations in Washington show great variation in sexual maturation, with resident forms typically maturing at 2 to 3 years and sea-run populations at closer to 4 years (Johnson et al.

1999). With the exception of Garrison Creek, all the observed age classes (YOY, 2, 3, and 4+) were represented in the SJIs' CCT populations sampled in this study. One decomposing 290 mm trout was found in Garrison Creek, possibly suggesting a potential upper size limit for trout in this stream. Smaller-sized bodies at maturation may confer a selective advantage on trout in small streams (Johnson et al. 1999).

All three of the sampled CCT populations appear to have small effective population sizes as demonstrated from genetic analyses and small relative abundances observed in these study streams. Genetic drift is thus a strong potential factor influencing genetic structure among these populations and in relation to other CCT populations in Puget Sound. Habitat likely imposes limitations on population sizes of coastal cutthroat trout inhabiting streams on the San Juan Islands (Barsh 2010). Natural falls at Doe Bay Creek and Cascade Creek certainly reduce upstream migration for CCT populations, though it is important to note that resident CCT are known to produce anadromous offspring. The climatic and geomorphological characteristics of the San Juan Islands, e.g. drier summers and relatively small catchment basin areas, likely impose hydrological limitations on these CCT populations that persist as small population sizes. Other factors (e.g., food availability, availability of spawning substrate, intra- and interspecific competition, genetic introgression with hatchery stocks) may also influence the effective population size of CCT populations within these watersheds (Rosenfeld et al. 2000) and should not be discounted.

Genetic diversity, measured as allelic richness and heterozygosity, was lower in cutthroat trout populations from the San Juan Islands than from elsewhere in Puget Sound and the WA coast. Without accounting for stream size or population size, there was a north-to-south cline in genetic diversity in Puget Sound, suggesting that latitude explained 53% of the genetic variation and that genetic diversity increased towards south Puget Sound and on the coast. This north-south pattern has been observed previously, with one exception, the Strait of Juan de Fuca populations tended to be more closely related to Hood Canal and southern Puget Sound populations than they were to either northern Puget Sound or to Olympic Peninsula CCT populations, possibly suggesting a recolonization pattern following the retreat of glaciers (Johnston 1999). Wenberg et al. (1998) were unable to find a correlation between geographic distances and genetic distances, arguing

that postglacial population structure of coastal cutthroat trout has been determined largely by individual stream-processes rather than dispersal from a single refugium along the contemporary WA coastlines. The authors, however, conceded that CCT populations may reflect different patterns of postglacial recolonization. Certainly, dispersal and specific stream characteristics (physical and environmental) may have collectively influenced the genetic structuring of coastal cutthroat trout populations in the San Juan Islands.

Doe Bay Creek had the lowest genetic diversity of any coastal cutthroat trout population in the analyses, suggesting that these CCT are more isolated and/or have been isolated for longer than the Garrison and Cascade Creek populations. The long branch lengths observed in the neighbor-joining dendrogram and remote clustering in the principal components analyses often signals high genetic drift from the Garrison Creek, the Puget Sound, and WA coast CCT collections. Cutthroat trout populations are restricted by multiple, natural bedrock waterfalls at the mouth of Doe Bay Creek, and at least in the summer months, by a perched road culvert underneath Point Lawrence Road. Barsh (2010) suggested that this population of CCT may represent an unusual, post-glacial relic isolated by isostatic rebound of Orcas Island relative to sea level more than 4,000 years ago. Regardless of the mechanism, the long persistence of this small population above a natural barrier is noteworthy, and worthy of special conservation consideration.

Management implications and habitat protection/restoration opportunities

Responsible management in data-poor situations requires use of the precautionary principle. In the case of the San Juan CCT, what few data exist document small, isolated populations of CCT that are subject to considerable threat from habitat loss and fragmentation. The extensive logging and diversion of water for agriculture in the early to late 1900's have left little if any intact riparian corridors along much of Garrison Creek. Riparian buffers are important to CCT populations because they regulate stream temperatures, provide large woody debris inputs that create and maintain instream habitats, provide organic inputs and terrestrial insects that are important for their food webs, etc. Livestock grazing in Garrison Creek has degraded stream banks and reduced water quality with nutrient loading. Fragmentation of habitat from culverts and other artificial barriers in Garrison Creek have also reduced the amount of available habitat for CCT populations. Introduced fish species, such as bass and rainbow trout, compete with and

potentially prey on CCT in the large seasonal wetland of Garrison Creek, which receives winter overflow from stocked ponds. Similar threats to habitat, though less immediate, exist in Cascade Creek and Doe Bay Creek. Both streams have numerous artificial barriers that restrict movement for CCT populations. Stream flows in Cascade Creek are often not enough in spring to fall months to satisfy all water users (RH2 Engineering 2015), and may exacerbate given climate warming scenarios. Invasive, eastern brook trout and hatchery-origin cutthroat trout compete for space and resources with existing wild CCT populations in Cascade Creek. All three study streams in the San Juan Islands maintain small, effective population sizes of CCT that may be far more susceptible to stochastic events and/or threats from habitat loss than larger populations conceivably would.

Caution is also recommended in managing these small, isolated populations. Headwater CCT populations are known to persist at very small spatial scales (Rosenfeld et al. 2002). Inland cutthroat trout populations can persist in isolation and at very small population sizes (~50) if quality habitat is available (Peterson et al. 2014; Peacock et al. 2012). Persistence of cutthroat trout populations is often believed to be the result of the amount of quality habitat available, the connectivity of these habitats, and not necessarily the time since isolation (Hilderbrand & Kershner 2004; Whiteley et al. 2010). Barriers to movement can lead to reduced coastal cutthroat trout genetic diversity as a result of genetic drift (not natural selection), which ultimately may compromise the long-term persistence of these populations (Wofford et al. 2005). Vincenzi et al. (2009), in their aptly titled paper, *The management of small, isolated salmonid populations: do we have to fix it if it aint broken?*, argue that there are, as of yet, not enough examples of small, isolated salmonid populations that were extirpated due to loss of genetic diversity and inbreeding depression. Rather, they contend, many small, isolated salmonid populations with low genetic variability prove to be viable and well adapted to their environment if given enough quality habitat. Removing artificial barriers in order to reconnect available habitat for CCT populations should be a management objective, but it seems prudent to recognize that small, isolated CCT populations like those observed in Doe Bay Creek and Garrison Creek may have evolved to local ecological conditions, conferring some adaptation in a changing environment.

Effective implementation of existing state and county regulations designed to protect environmentally-sensitive areas is needed to ensure that the San Juan Islands' CCT populations persist into the future. Protecting instream and riparian habitat from damage or destruction is critical, as is protecting the hydrology of the San Juan watersheds. Protection can and should be incentivized and pursued at a watershed scale with the full participation of neighboring landowners, rather than left solely to parcel-by-parcel permit approvals. Formal recognition by the county of CCT as a species of local economic, cultural and ecological value would help raise public awareness and engender a sense of stewardship for these small but persistent fish populations.

Stream flow recommendations have never been developed for San Juan County streams, as many streams fall below the threshold for regulation with the Department of Ecology, and the San Juan County Water Resource Management Committee for WRIA 2 largely assumed there were no self-sustaining salmonids within the county (SJC WRMC, 2004). It appears that surface water diversions for ponds continue to be approved in San Juan County without apparent regard for impacts on stream flows; such diversions reduce the quality and quantity of habitat available to trout during the summer, when they can least afford it.

The practice of stocking of Lake Whatcom/Tokul hatchery cutthroat trout in Mountain Lake should be reconsidered, as the genetic data demonstrate that hatchery fish are distributing throughout the watershed and reproducing with wild (naturally reproducing) populations of CCT in Cascade Creek. Johnson et al. (1999) suggested the potential for genetic interactions between Lake Whatcom/Tokul hatchery CCT and Puget Sound CCT stocks as both spawn at the same time, but concluded that there were no studies to demonstrate the extent of genetic exchange between CCT populations in their natural environments. If Cascade Creek had a native stock of CCT in the past, which is highly likely given the basin size and short distance to its marine outlet (and considering its physical attributes compared to Doe Bay and Garrison), any native stock that may have existed is currently subject to hatchery genetic introgression and subsequent impacts to fitness and reproductive success associated with the annual influx of maladapted Tokul genes. Furthermore, WDFW has unwritten policy stating the agency will not plant hatchery trout in lakes where fish have egress to streams containing wild fish (Larry Phillips, pers. com,); it is now clear that the ongoing planting of Mountain Lake contradicts this policy. Because of this,

WDFW should strongly consider terminating the releases of hatchery cutthroat trout to prevent further ecological and genetic interactions with stream resident and potentially anadromous CCT populations. If this practice of stocking is not stopped, at minimum, WDFW should find a way to prevent hatchery/wild fish interactions (e.g., through exclusion devises at the outfall of the Lake). Alternative measures such as exclusion devices must include monitoring and adaptive management to ensure effectiveness. (Note: A cost-benefit analysis may indicate that the costs, both fiscal and ecological, of stocking CCT in Mountain Lake may prove unwarranted. Most recreational fishing in Moran State Park occurs in Cascade Lake, which is hydrologically independent from the Mountain Lake / Cascade Creek watershed.).

Interspecific competition and direct predation among nonnative fish species, such as eastern brook trout, have been known to severely reduce coastal and inland cutthroat trout populations (Dunham et al. 2002). Eastern brook trout spawn earlier (fall vs. spring spawners), rear earlier, and reach larger sizes with greater fecundity than similar-aged cutthroat trout in the same stream. Larger-bodied brook trout have a selective advantage for food and habitat, often forcing cutthroat trout into less optimal and largely inferior rearing and spawning grounds. Many coastal cutthroat trout populations are sympatric with other species (e.g. coho salmon, reticulated sculpin) in streams like Cascade Creek, and the severity of nonnative impacts on native species is little understood. Brook trout eradication programs in the interior west have largely been unsuccessful as the removals of target species can often harm the species they are meant to protect (Meyer et al. 2006). Recent studies have suggested that brook trout controls may be more feasible and effective under climate warming scenarios, as brook trout are more sensitive to warmer temperatures and higher fall flows than coastal cutthroat trout in western streams (Wenger et al. 2011).

Numerous opportunities exist for future habitat restoration and protection in San Juan County streams. Recommended measures for habitat restoration have been proposed for all of the CCT streams in this study, but with the exception of West Beach Creek, have as of yet not been implemented (WFC 2010; Barsh 2010). San Juan County's Land Bank recently acquired a section of Cascade Creek with Salmon Recovery Funds, protecting this lower reach in perpetuity from future development. Acquisitions of functionally intact stream reaches are often the best way to ensure an ongoing, functional ecosystem, as it is often easier and cheaper to protect

habitat than it is to restore it (Beechie et al. 2008).

Culvert and artificial barrier removals are also high- priority restoration projects: the reconnection of isolated, off-channel habitats or blocked tributaries is likely to last for many decades, and has a high likelihood of success (Beechie et al. 2008). A systematic inventory and assessment of anthropogenic fish barriers in the San Juan Islands is the first step in restoring natural connectivity within watersheds and is directly applicable to WDFW's Fish Passage and Diversion Screening Inventory (FPDSI) database. Anthropogenic fish passage barrier removals should be done without sacrificing pools created by long-term blockage, and must include an understanding of the benefits to all species. Removing the culvert under Point Lawrence Road may be beneficial for CCT in Doe Bay Creek so long as this restoration action maintains, replaces, or enhances the pool that currently exists below the culvert.

Other restoration possibilities exist in areas that are more degraded, like Garrison Creek, and may be more challenging; but the initial investment, study design, and support of property owners have largely been developed by an early feasibility study (WFC 2010). Culverts under the county road, driveways, and livestock crossings should be replaced to fully restore fish passage within the reaches where our study has observed adult and juvenile cutthroat trout in Garrison.

There are also opportunities to reconnect ponds and divert water back into streams that were lost from artificial impoundments. This may be the only way to ensure adequate stream flows and reduce the amount of water lost to evaporation in the drier months of the year. Several historical irrigation and recreational ponds in the Garrison watershed could be re-connected to the stream, for example, taking care to screen out rainbows and bass stocked in some of these ponds in the past.

Many islanders also expressed an interest in reintroducing CCT in streams that historically had them. This is certainly a possibility, but among other genetic and ecological considerations such decisions must be made by balancing the reality of translocating a fish population and the risk of removing individuals from streams with already low population sizes.

Data Gaps / Next steps / Limitations

Improved estimates of CCT abundance in the three study watersheds are recommended to improve population status and trend monitoring in the coming years. There are several well-established field methods available to provide rigorous estimates of salmonid abundance, either using electrofishing (removal, mark-recapture, or mark-resight techniques; see Bateman et al. 2005) and/or underwater video.

Relationships between effective population sizes and the required habitat needs of CCT populations will need to be quantified, as these relationships are not well understood (Whiteley et al. 2010). Data will be needed over multiple years to fully understand the status and characteristics of CCT populations in the San Juan Islands. This study and previous CCT studies in the San Juan Islands examined these populations in a single year and season without adequately understanding their needs in different seasons (winter), in subsequent years, and as new pressures (i.e. climate changes) may present themselves. Limitation of our data set include: understanding the movement of CCT within reaches (including anadromy vs. residency) and how this movement is related to available habitat sizes and types; identifying whether reproductive isolation may or may not occur in the Cascade Creek CCT populations (are there native stocks in the lower reach as compared to the upper reaches where fish were stocked?); and further describing phenotypic differences among the three study populations.

The next steps in data collection include characterizing the CCT populations in Victorian Creek and West Beach Creek. Are there still CCT populations in these streams or in other San Juan Islands streams? A more rigorous effort must be put forth to determine the abundance and genetic characteristics of trout in these study streams for status and trend monitoring. Additional habitat typing and further stream monitoring with the installation of stream gauges and temperature loggers, would help to determine what habitat and stream variables are important requirements for San Juan Islands CCT populations, and how any changes in these variables may limit these populations. Public outreach and awareness is also critically necessary to conserve these rare and endemic fish in the San Juan Islands.

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Appendix 1. [see appended report].

Genetic Composition and Status of Coastal Cutthroat Trout in the San Juan Islands

Maureen P. Small[1], Nick Gayeski[2], Jamie Glasgow[2], and Vanessa Smilansky[1]

Final report, March 2016

[1]Molecular Genetics Lab, Washington Department of Fish and Wildlife, 1111 Washington St. SE, Olympia, WA 98501

[2]Wild Fish Conservancy

Appendix 2. Raw Coastal Cutthroat Trout Field Data from Cascade, Doe Bay, and Garrison Creeks in San Juan County.

Reach ID	2014 Date	Length	Weight	(141J-) Tissue Collected?	Scale Collected?	Photos
Cascade A	9-Jun	160	42.5	1	Y	0512-0513
Cascade A	9-Jun	135	28.3	2	Y	0514-0515
Cascade A	4-Jul	78	5.3	85	Y	6020
Cascade A	4-Jul	163	40.1	86	Y	Y
Cascade A	4-Jul	141	24.2	87	Y	6038
Cascade A	28-Jul	163	36.8	89	Y	Y
Cascade A	28-Jul	177	51.2	90	Y	
Cascade A	28-Jul	175	51.5	91	Y	
Cascade A	4-Aug	152	33.4	92	Y	1020606-0611
Cascade B	4-Aug	183	66.1	93	Y	1020621-0623
Cascade B	4-Aug	167	51.4	94	Y	1020624-0626
Cascade B	4-Aug	175	55.4	95	Y	1020627-0628
Cascade B	4-Aug	161	41.3	96	Y	1020630-0632
Cascade B	4-Aug	161	44.3	97	Y	1020633-0635
Cascade B	4-Aug	173	54.3	98	Y	1020636-0638
Cascade B	4-Aug	135	26.1	99	Y	1020640
Cascade B	4-Aug	225	122.3	100	Y	1020641-0643
Cascade B	4-Aug	159	37.4	101	Y	1020644-0646
Cascade B	4-Aug	161	41.3	102	Y	1020647-0649
Cascade B	4-Aug	178	53.9	103	Y	1020650-0652
Cascade B	4-Aug	160	40.8	104	Y	1020653-0655
Cascade B	4-Aug	160	39.6	105	Y	1020656-0658
Cascade B	4-Aug	135	21.8	106	Y	1020659-0661
Cascade A	4-Aug	175	54.3	107	Y	1020662-0664
Cascade A	4-Aug	175	55.8	N	N	
Cascade A	4-Aug	177	N/A	N	N	
Cascade C	5-Aug	124	18.9	118	Y	726-728
Cascade C	5-Aug	103	11.5	119	Y	729-731
Cascade C	5-Aug	71	3.8	120	N	732-734
Cascade C	5-Aug	94	8.4	121	Y	735-737
Cascade C	5-Aug	102	11	122	Y	738-740
Cascade C	5-Aug	46	1.1	N	N	
Cascade C	5-Aug	40	0.8	N	N	
Cascade C	5-Aug	50	1.4	N	N	
Cascade C	5-Aug	50	1.6	N	N	
Cascade C	5-Aug	59	2.3	N	N	
Cascade C	5-Aug	59	2.3	N	N	
Cascade C	5-Aug	51	1.5	N	N	
Cascade C	5-Aug	104	12.4	N	N	
Cascade C	5-Aug	143	27	123	Y	741-743
Cascade C	5-Aug	99	10.1	124	Y	744-746
Cascade C	5-Aug	111	14.1	125	Y	747-749

Cascade C	5-Aug	136	25.1	126	Y	750-752
Cascade C	5-Aug	60	3.2	127	N	753-755
Cascade C	5-Aug	139	N/A	N	N	
Cascade D	5-Aug	175	55.7	128	Y	756-758
Cascade D	5-Aug	119	17	129	Y	759-761
Cascade D	5-Aug	158	36.8	130	Y	762-765
Cascade D	5-Aug	67	3.3	131	N	766-768
Cascade D	5-Aug	117	15.6	132	Y	769-771
Cascade D	5-Aug	133	24.2	N	N	
Cascade D	5-Aug	68	3.8	N	N	
Cascade D	5-Aug	84	6.7	N	N	
Cascade D	5-Aug	61	2.4	N	N	
Cascade D	5-Aug	74	5	N	N	
Cascade D	5-Aug	74	4.1	N	N	
Cascade E	5-Aug	72	5.2	108	N	1020665-0668
Cascade E	5-Aug	57	1.6	109	N	1020669-0671
Cascade E	5-Aug	56	1.9	110	N	1020672-0674
Cascade E	5-Aug	92	7.6	111	N	1020675-0677
Cascade E	5-Aug	53	1.5	112	N	0708-0710
Cascade E	5-Aug	48	1.2	113	N	0711-0713
Cascade E	5-Aug	48	1.1	114	N	0714-0716
Cascade E	5-Aug	44	0.8	115	N	0717-0719
Cascade E	5-Aug	47	0.8	116	N	0720-0722
Cascade E	5-Aug	46	1	117	N	0723-0725
Doe Bay A	2-Jul	68	3	35	Y	5932
Doe Bay A	2-Jul	53	2.9	36	Y	5933
Doe Bay A	2-Jul	47	0.8	37	N	5934
Doe Bay A	2-Jul	47	1	38	N	5935
Doe Bay A	2-Jul	59	2.5	39	Y	5936
Doe Bay A	2-Jul	64	2.8	40	Y	5937
Doe Bay A	2-Jul	53	1.5	41	Y	5938
Doe Bay A	2-Jul	226	119.3	42	Y	5939
Doe Bay A	2-Jul	233	125.5	43	Y	5942
Doe Bay A	2-Jul	212	92.9	44	Y	5943
Doe Bay A	2-Jul	213	97.6	45	Y	5944
Doe Bay A	2-Jul	187	64.2	46	Y	5945
Doe Bay A	2-Jul	179	62.8	47	Y	5946
Doe Bay A	2-Jul	169	48.4	48	Y	5948
Doe Bay A	2-Jul	138	29.4	49	Y	5949
Doe Bay A	2-Jul	139	31.6	50	Y	5950
Doe Bay A	2-Jul	117	17.9	51	Y	5951
Doe Bay A	2-Jul	62	3.3	52	Y	5954
Doe Bay A	2-Jul	66	3	53	Y	5955
Doe Bay A	2-Jul	68	4.1	54	Y	5956
Doe Bay A	2-Jul	58	2	55	Y	5957
Doe Bay A	2-Jul	56	2.7	56	Y	5960
Doe Bay B	2-Jul	125	18.6	57	Y	5961
Doe Bay B	2-Jul	123	21.3	58	Y	5963

Doe Bay B	2-Jul	123	22.2	59	Y	5963
Doe Bay B	2-Jul	104	10.6	60	Y	5964
Doe Bay B	2-Jul	142	30.6	61	Y	5965-5966
Doe Bay B	2-Jul	96	9.6	62	Y	5968
Doe Bay B	2-Jul	106	14.1	63	Y	5969
Doe Bay B	2-Jul	53	1.3	64	Y	5970
Doe Bay B	2-Jul	128	22.6	65	Y	5971
Doe Bay B	2-Jul	61	2.8	66	Y	5872
Doe Bay B	2-Jul	44	1.1	67	Y	5973
Doe Bay B	2-Jul	46	1.3	68	N	5974
Doe Bay B	2-Jul	58	1.9	69	N	5976
Doe Bay B	2-Jul	69	3.8	70	Y	5977
Doe Bay B	2-Jul	60	2.5	71	Y	5978
Doe Bay B	2-Jul	54	1.9	72	N	5980
Doe Bay B	2-Jul	55	2.1	73	N	5981
Doe Bay B	2-Jul	115	16.4	74	Y	5982-5983
Doe Bay B	2-Jul	67	3.8	75	Y	5984
Doe Bay B	2-Jul	37	1	76	N	5985
Doe Bay B	2-Jul	47	1.3	77	N	5986
Doe Bay B	2-Jul	57	1.9	78	Y	5988
Doe Bay B	2-Jul	68	3.8	79	Y	5989
Doe Bay B	2-Jul	53	1.6	80	N	5990
Doe Bay B	2-Jul	51	1.7	81	N	5991
Doe Bay B	2-Jul	50	1.5	82	N	5997
Doe Bay B	2-Jul	61	2.3	83	Y	5998-5999
Doe Bay B	2-Jul	44	0.9	84	N	59?
Garrison B	1-Jul	112	14.7	3	Y	1
Garrison B	1-Jul	200	91.9	4	Y	2
Garrison B	1-Jul	205	92.8	5	Y	3
Garrison B	1-Jul	135	26.6	6	Y	4
Garrison B	1-Jul	120	18.7	7	Y	5
Garrison B	1-Jul	198	83	8	Y	6
Garrison B	1-Jul	57	1.4	9	Y	7
Garrison B	1-Jul	113	16.3	10	Y	8
Garrison B	1-Jul	108	12.6	11	Y	9
Garrison B	1-Jul	103	10.8	12	Y	10
Garrison B	1-Jul	101	11.2	13	Y	11
Garrison C	1-Jul	117	18.3	14	Y	12
Garrison C	1-Jul	110	13.6	15	Y	13
Garrison C	1-Jul	91	8.3	16	Y	14
Garrison C	1-Jul	94	3.7	17	Y	15
Garrison C	1-Jul	112	15.3	18	Y	16
Garrison C	1-Jul	133	16.8	19	Y	17
Garrison C	1-Jul	94	7.9	20	Y	18
Garrison C	1-Jul	57	1.8	21	Y	19
Garrison C	1-Jul	102	12.6	22	Y	20
Garrison C	1-Jul	108	13.5	23	Y	21
Garrison C	1-Jul	127	20.1	24	Y	22

Garrison C	1-Jul	184	63.7	25	Y	23
Garrison C	1-Jul	123	18.6	26	Y	24
Garrison C	1-Jul	104	12.3	27	Y	25
Garrison C	1-Jul	113	17.7	28	Y	26
Garrison C	1-Jul	106	12.3	29	Y	27
Garrison C	1-Jul	129	21.5	30	Y	28
Garrison C	1-Jul	90	8.5	31	Y	29
Garrison C	1-Jul	94	9.4	32	Y	30
Garrison C	1-Jul	98	10.4	33	Y	31
Garrison C	1-Jul	69	4.7	34	Y	32
Garrison B	22-Aug	205	84.3	141	Y	779
Garrison B	22-Aug	104	14.1	N	N	780
Garrison B	22-Aug	142	27.5	143	Y	781
Garrison B	22-Aug	136	22.8	144	Y	782
Garrison B	22-Aug	122	17.7	145	Y	783
Garrison B	22-Aug	204	96.4	146	Y	784-787
Garrison B	22-Aug	103	10.1	147	Y	788-789
Garrison B	22-Aug	198	81.6	148	Y	790-793
Garrison C	22-Aug	63	3	149	N	793
Garrison C	22-Aug	61	2.7	150	N	794-795
Garrison C	22-Aug	59	2.5	151	N	796-797
Garrison C	22-Aug	122	19	152	Y	798-799
Garrison C	22-Aug	126	22	153	Y	800-802
Garrison A	22-Aug	290	N/A	135	N	
Garrison C	16-Dec	78	5.2	154	N	0064 - 0066
Garrison C	16-Dec	110	15.9	155	N	67-69
Garrison C	16-Dec	60	2.9	156	N	70-72
Garrison C	16-Dec	46	1.1	157	N	73-75
Garrison C	16-Dec	62	2.9	158	N	76-78

Appendix 3. Comparison of posterior distributions of intercepts and slopes of Cascade, Doe Bay, and Garrison Creeks to the posterior distributions of the global regression on the combined length-weight data from all three populations.

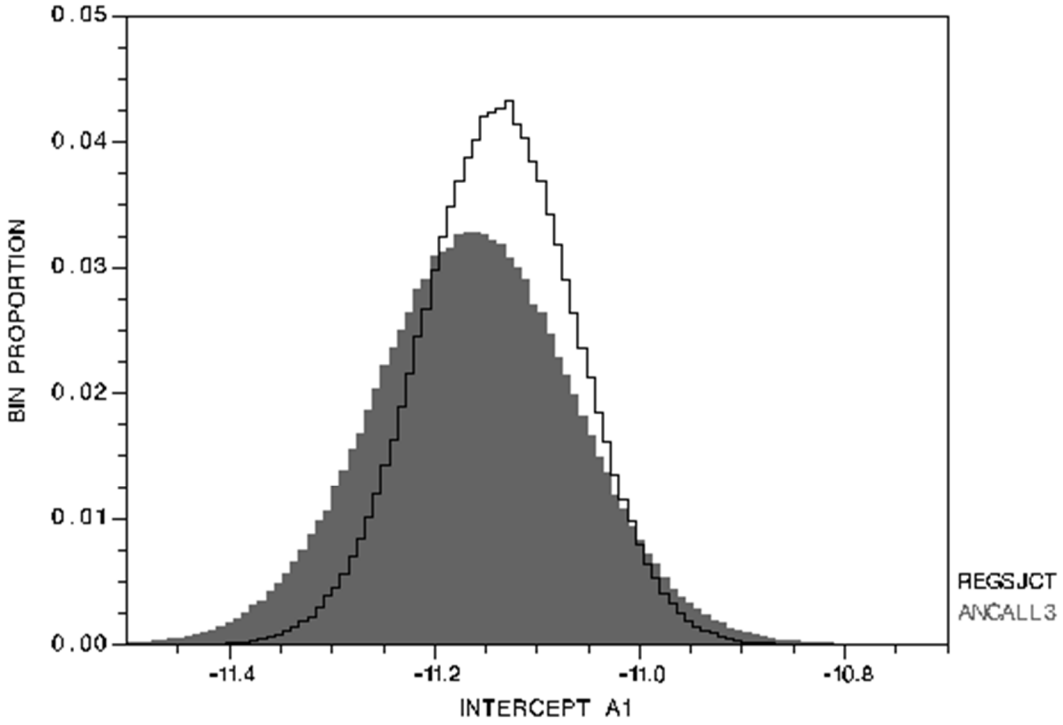


Figure A1. Posterior distribution of the Intercept from the global regression (black line, Regsjct) overlain on the posterior distribution from the regression on Cascade Creek length-weight data (solid grey. Ancall).

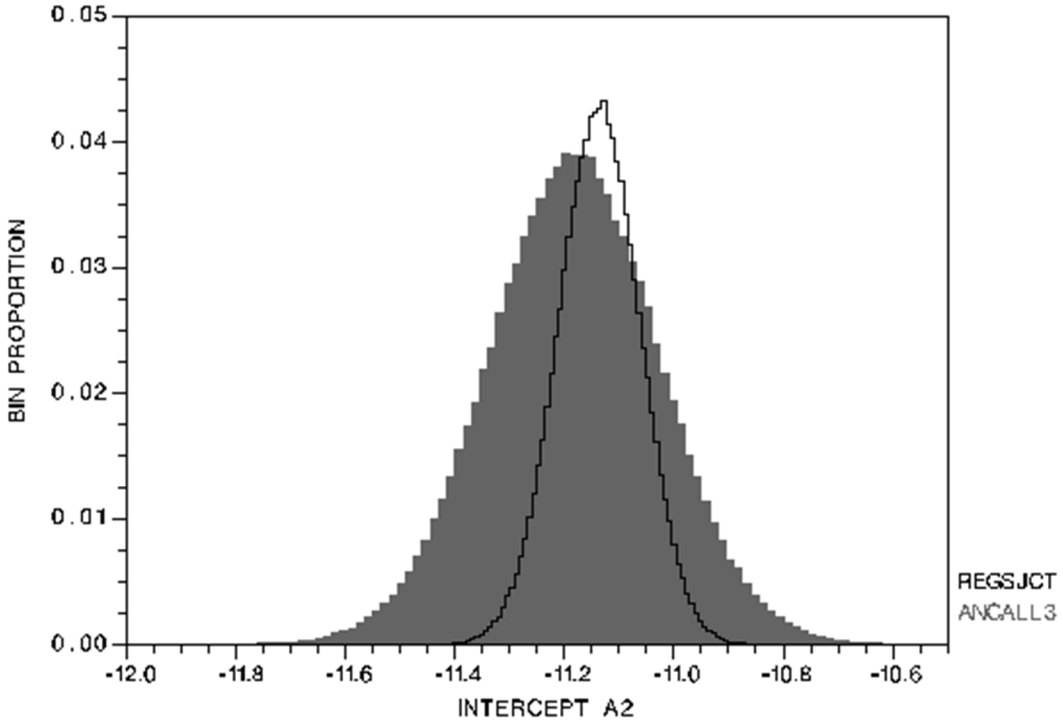


Figure A2. Posterior distribution of the Intercept from the global regression (black line, Regsjct) overlain on the posterior distribution from the regression on doe Bay Creek length-weight data (solid grey. Ancall).

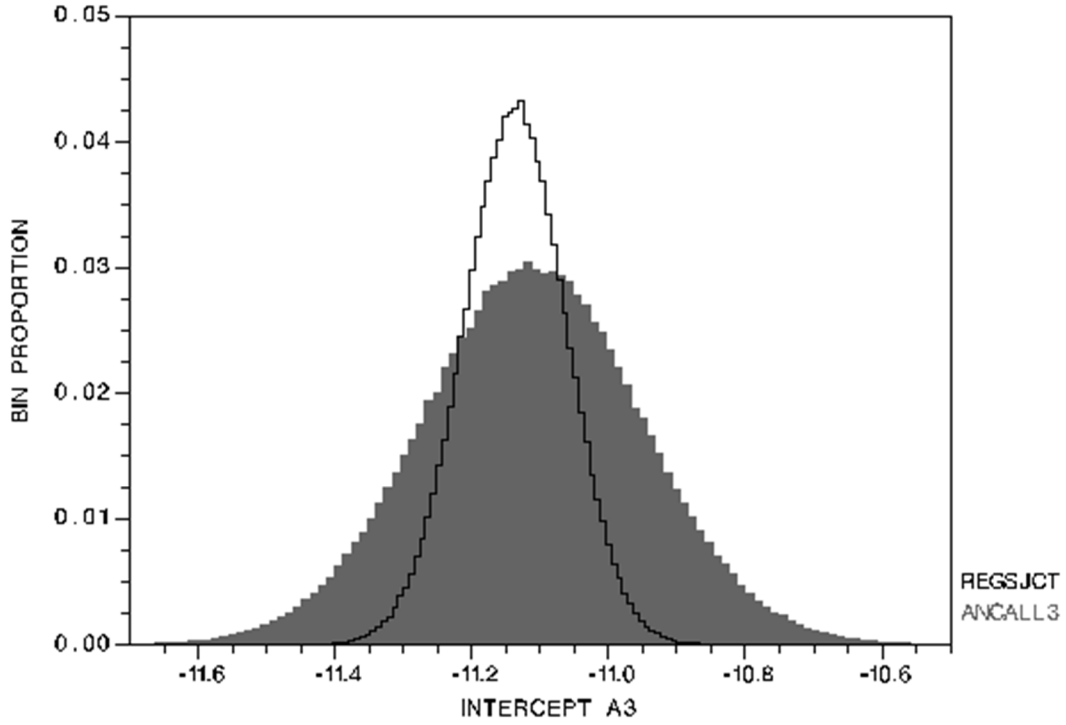


Figure A3. Posterior distribution of the Intercept from the global regression (black line, Regsjct) overlain on the posterior distribution from the regression on Garrison Creek length-weight data (solid grey. Ancall).

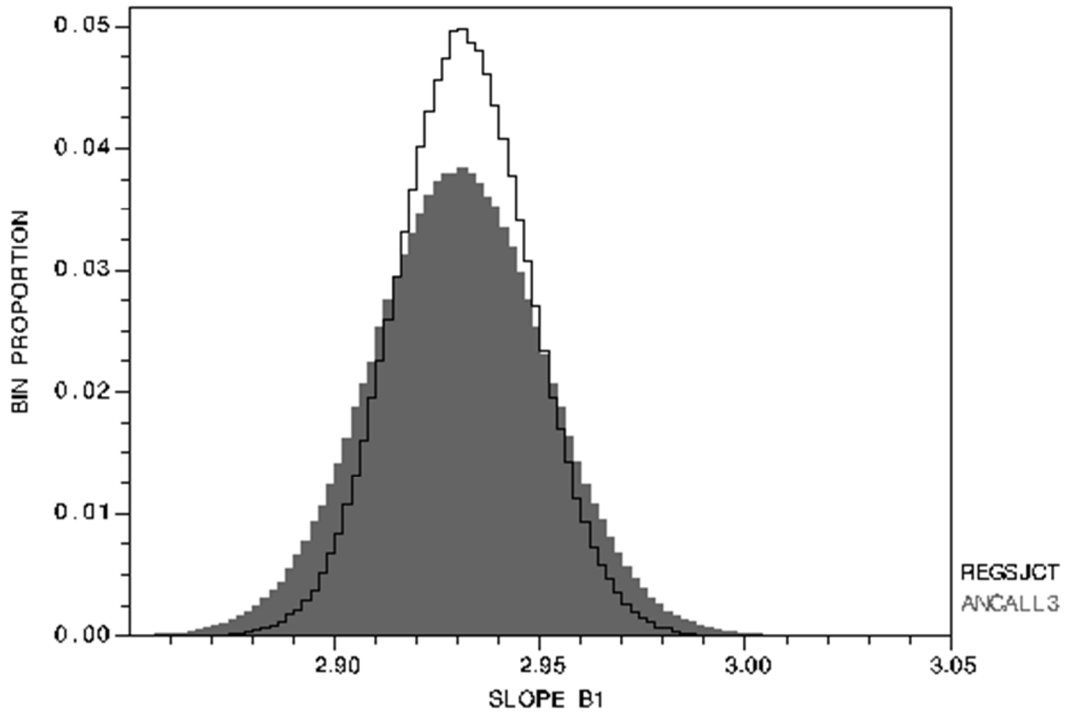


Figure A4. Posterior distribution of the Slope from the global regression (black line, Regsjct) overlain on the posterior distribution from the regression on Cascade Creek length-weight data (solid grey. Ancall).

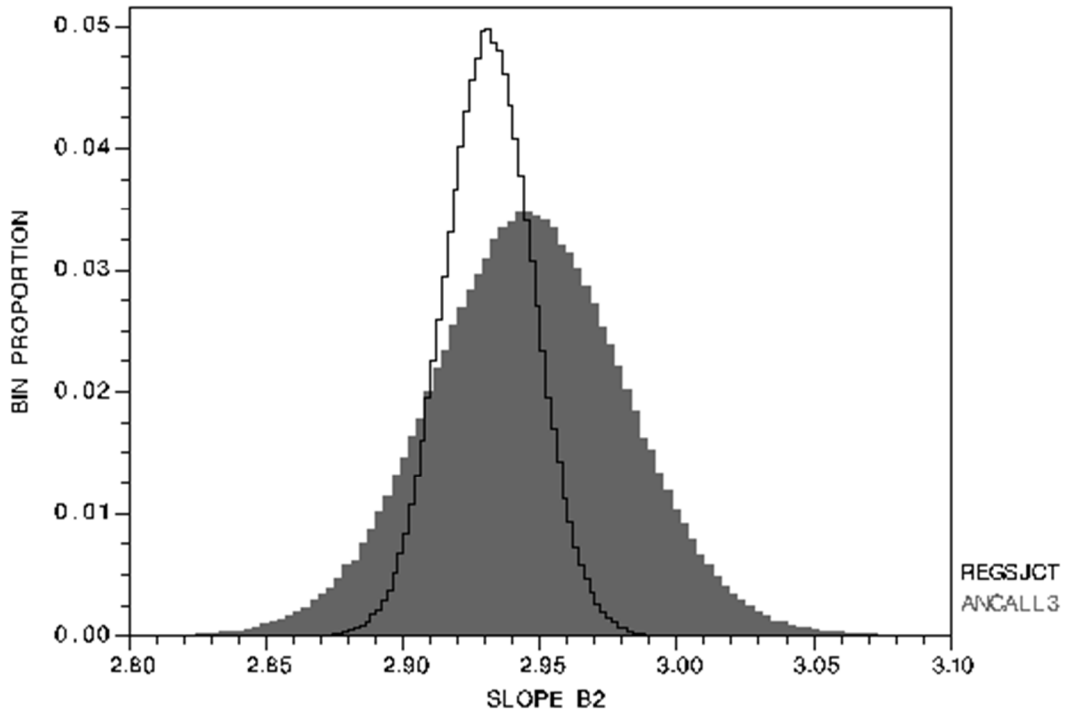


Figure A5. Posterior distribution of the Slope from the global regression (black line, Regsjct) overlain on the posterior distribution from the regression on Doe Bay Creek length-weight data (solid grey. Ancall).

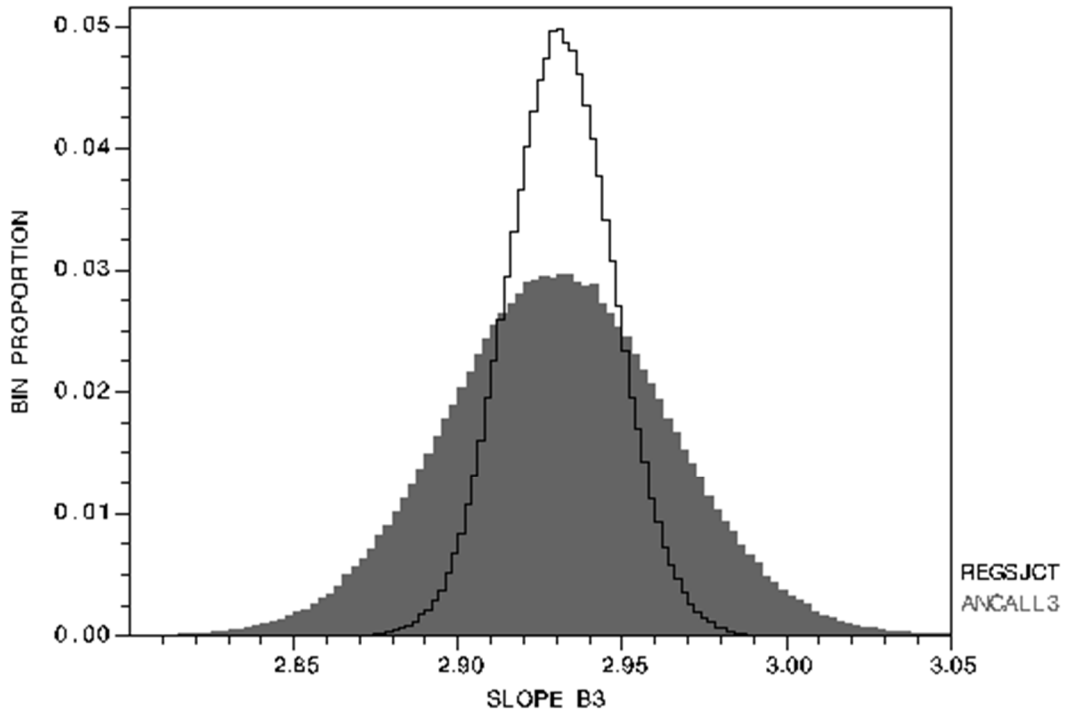


Figure A6. Posterior distribution of the Intercept from the global regression (black line, Regsjct) overlain on the posterior distribution from the regression on Garrison Creek length-weight data (solid grey. Ancall).

Appendix 4. Representative Coastal Cutthroat Trout Photographs from Garrision, Doe Bay, and Cascade Creeks, San Juan County, WA. All photographs were taken during 2014 sampling effort.

CASCADE CR. FINE-SPOTTED VS. COARSE-SPOTTED

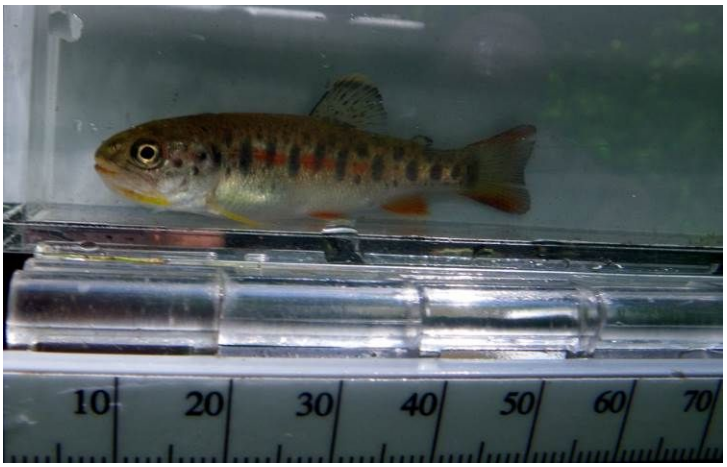
Cascade adult, fine-spotted (0757)

Cascade adult, large-spotted (0637).

JUVENILE CCT COMPARISON, TYPICAL

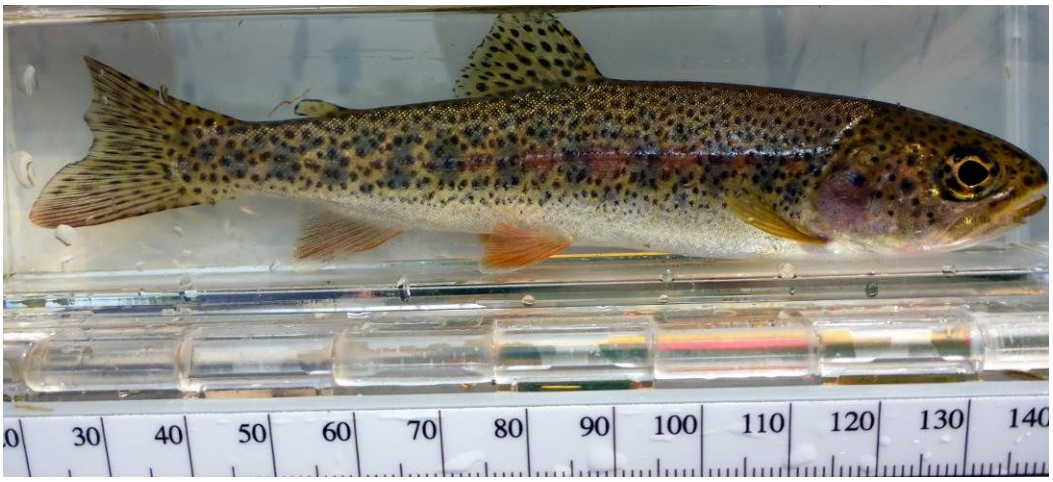
Garrison Juvenile (0076)

Cascade Juvenile (0674)

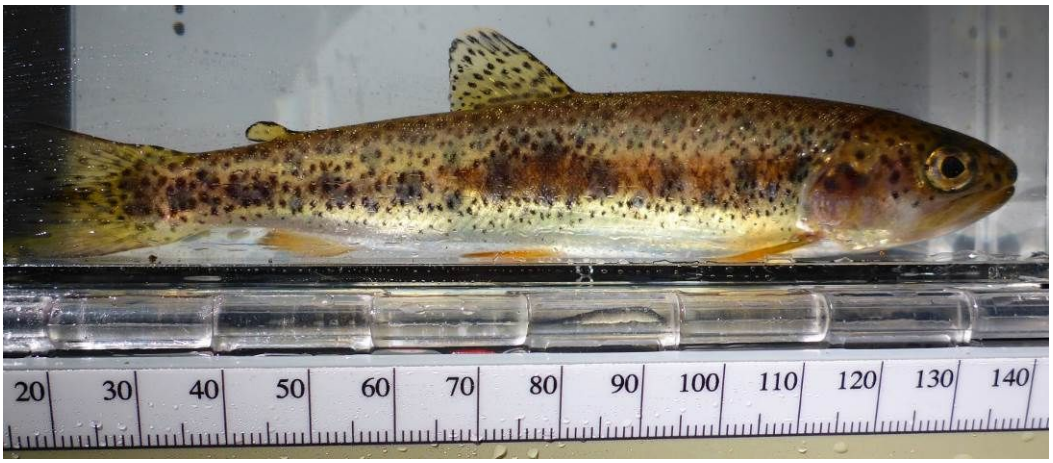


Doe Bay Juvenile (5978)

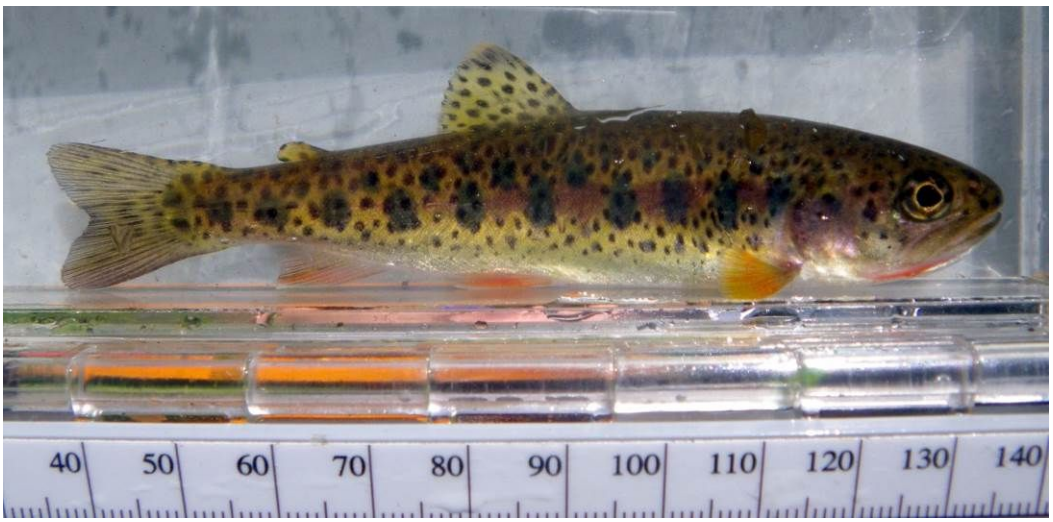
ADULT CCT COMPARISON, TYPICAL



Garrison adult (0782)



Cascade adult (0742)



Doe Bay adult (5983)

Appendix 1

Genetic Composition and Status of Coastal Cutthroat Trout in the San Juan Islands

Maureen P. Small[1], Nick Gayeski[2], Jamie Glasgow[2], and Vanessa Smilansky[1]

Final report, March 2016

[1]Molecular Genetics Lab, Washington Department of Fish and Wildlife, 1111 Washington St. SE, Olympia, WA 98501

[2]Wild Fish Conservancy

Prepared for Wild Fish Conservancy and Long Live the Kings

Overview: San Juan Islands residents, biologists, and local conservation groups are concerned about the status of resident and sea-run coastal cutthroat trout (*Oncorhynchus clarki*) that inhabit the San Juan Islands. Coastal cutthroat trout have historically been caught in recreational fisheries in the San Juan Islands, and long-standing residents of San Juan Islands recall a time when the fish appeared to be more abundant than they are now. However, little is known about the current status of coastal cutthroat trout in the San Juan archipelago. To understand the conservation needs of these coastal cutthroat trout, we need a starting point. This project seeks to describe the composition and status of the San Juan Island spawning aggregate to provide a basis for determining appropriate recovery efforts, establish priorities, and assess recovery actions.

Washington State resource managers rely on the Salmonid Stock Inventory (SaSI), a standardized, uniform approach to identifying salmonid stocks, including coastal cutthroat trout, and monitoring their status. The Washington Department of Fish and Wildlife (WDFW) performed an inventory of coastal cutthroat trout in 2000; however, the San Juan Islands were not evaluated (WDFW 2000). Initial steps toward identifying cutthroat trout stocks in the San Juan Islands occurred recently. The Wild Fish

Conservancy (WFC) noted the presence of cutthroat trout in five streams—Cascade, Doe Bay, Garrison, Victorian, and West Beach (Table 1) — during their Puget Sound Water Type Assessment (2005-2007). Local salmon recovery nonprofit, Long Live the Kings (LLTK), developed a collaborative effort with WFC, Kwiáht (nonprofit center for historical ecology of the Salish Sea on San Juan Island), and WDFW to analyze the composition and status of San Juan coastal cutthroat trout. Eventually, genotypic, phenotypic, and behavioral (spawn-timing) characteristics of cutthroat trout from each stream will be evaluated in an attempt to determine whether distinct stocks exist within or among the five watersheds and whether the San Juan coastal cutthroat trout spawning aggregate constitutes a distinct stock complex (composition). Assessment will rely primarily on genetic information; however, phenotype, spawn-timing, age structure, and growth (via scales) data will also be collected. The status of San Juan Island coastal cutthroat trout will be evaluated based upon the current level of abundance and distribution of fish in each stream, for each stock identified through the genetic analysis. Because initial collection efforts were successful in three streams: Cascade, Doe Bay and, Garrison, this report documents the genetic composition and status of coastal cutthroat trout collected in these three streams.

Methods: Project partners collected coastal cutthroat trout samples from Cascade Creek (n=49), Doe Bay Creek (n=50), and Garrison Creek (n=50) (Table 2, Table 3, Figure 1, Appendix 1) from June through Decemberin 2014. Based on analyses of scale annuli, cutthroat sampled ranged in age from age 0 fish to age 5. Survey crews brought fish to hand for sampling using backpack electrofishing surveys throughout representative stream reaches where permission was granted by landowners. In addition to coastal cutthroat trout, the survey teams also captured brook trout, sculpin, juvenile chinook, and juvenile coho salmon within Cascade Creek.

WDFW's hatchery trout plantings in the San Juan Islands are summarized in Appendix 2. Over 270,000 hatchery coastal cutthroat trout have been released in Mountain Lake -- the headwaters of Cascade Creek -- since 1934, averaging almost 19,000 fry plants annually since 2012.

Sample processing: Genomic DNA was extracted from tissue samples using Clone-tech® extraction kits. Trout samples were genotyped at seven microsatellite loci (Table 4). Microsatellite alleles were PCR-amplified using fluorescently labeled primers. PCRs were conducted in 96 well plates in 10 µl volumes employing 1 µl template with final concentrations of 1.5 mM $MgCl_2$, 200µM of each dNTP, and 1X Promega PCR buffer. The following microsatellite loci were used at the following concentrations (concentration in µM after locus name): One-108 [0.075], Ots-103 [0.037], Omy-77 [0.075], Ots-1 [0.08], Ots-3M [0.05], Ogo-3 [0.07], and Omm-1138 [0.08]). After initial two minute denature at 94°, there were 3 cycles consisting of 94° denaturing for 30 seconds, 60° annealing for 30 seconds, at 72° extension for 60 seconds. These were followed by 30 cycles with the same parameters but the annealing temperature was dropped to 50° and then there was a final 10-minute extension at 72°. Samples were run on an ABI 3730 automated DNA Analyzer and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GeneMapper software (Applied Biosystems).

Samples were also genotyped at 96 single nucleotide polymorphism loci (SNPs, see Table 4 for list) through PCR and visualized on Fluidigm EP1 integrated fluidic circuits (chips). Nineteen of the SNP loci were developed to discriminate among trout species and 77 of the SNP loci have been used to identify population structure and other genetic attributes of coastal cutthroat trout. To enhance SNP locus DNA in preparation for PCR, specific target amplification (STA) reactions were conducted using 96-well plates in 5 ul volumes with 1.25 ul of DNA template and pooled TaqMan® assays concentrated at 1X. Samples were run for 15 minutes at 95.0°C, followed by 14 cycles of 15 second denaturing at 95.0°C and 4 minute annealing at 60.0°C. Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: assay loading mixture contains 1X Assay Loading Reagent (Fluidigm), 2.5X ROX Reference Dye (Invetrogen) and 10X custom TaqMan Assay (Applied Biosystems); sample loading mixture contains 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 0.05X AmpliTaq Gold DNA

polymerase (Applied Biosystems), 1X GT sampling loading reagent (Fluidigm) and 6.5 µL STA. Four µL assay loading mix and 5 µL sample loading mix were pipetted onto the chip and loaded by the IFC loader (Fluidigm). PCR was conducted on a Fluidigm thermal cycler using a two-step profile. Initial mix thermal profile was 70°C for 30min, 25°C for 5 min, 52.3° for 10 sec, 50.1°C for 1 min 50sec, 98°C for 5 sec, 96°C for 9 min 55 sec, 96°C for 15 sec, 58.6°C for 8 sec, and 60.1°C for 43 sec. Amplification thermal profile was 40 cycles of 58.6°C for 10 sec, 96°C for 5 sec, 58.6°C for 8 sec and 60.1°C for 43 sec with a final hold at 20°C. The TaqMan assays were visualized on the Fluidigm EP1 machine using the BioMark data collection software and analyzed using Fluidigm SNP genotyping analysis software. All data were scored by two researchers.

Data analysis: San Juan Islands coastal cutthroat trout genotypic data were compared to each other and to data from the WDFW coastal cutthroat trout genetic baseline to provide greater perspective on San Juan Island coastal cutthroat trout. To examine genetic diversity of populations and confirm that collections were random samples representing randomly mating populations, we used FSTAT (Goudet 1995) to calculate basic population genetic statistics. Genetic diversity measures included allelic richness (average number of alleles per locus corrected for unequal sample sizes to a minimum of 7 individuals with full genotypes) and heterozygosity (gene diversity or expected heterozygosity, also corrected for unequal sample sizes, averaged over all loci). Conformance to Hardy-Weinberg equilibrium (HWE) expectations was tested at each locus and over all loci in each collection (as expressed by F_{IS} values) to confirm that samples met assumptions for statistical analyses. Deviations from HWE at single loci can signal lab processing problems such as null alleles (mutation at the primer site that causes PCR failure). Deviations from HWE over all loci can signal sampling problems such as samples with family groups or including members from more than one population. We used GenePop (Rousset 2008) to calculate linkage disequilibrium among all locus pairs in each population. Although loci were screened previously for physical linkage – loci located close together on the same chromosome such that they are inherited together – physically unlinked loci can generate linkage signals when there are related individuals in a

sample such as parents and offspring or siblings, and also in small populations subject to genetic drift. Familial linkage signals would be detected in samples that include family members (e.g. a sample of juveniles that included siblings from a single family) because the parental allele combinations are represented in multiple individuals. Small populations subject to drift would have lower genetic diversity and linkage in the absence of family groups. Thus, linkage signals could be a sign of non-random sampling or family members in the sample or that the sample was a random sample from a small population. Samples were examined for family structure using the software COLONY (Wang 2004). The program uses maximum likelihood to identify full- and half-sibling relationships and parent-offspring relationships. Population size was examined by comparing genetic diversity measures, which would be lower in smaller populations, and calculating effective population sizes (discussed below).

We estimated the effective number of annual breeders (N_b) and the per-generation genetic effective population sizes (N_e) for collections using the linkage disequilibrium method implemented in LDNe (Waples and Do 2008). The N_b is an estimate of the number of breeders that produced a particular cohort and the N_e is a theoretical estimate of the effective number of breeders in a generation. The N_e is the number of breeders in an ideal, randomly mating population that would have the same amount of genetic diversity and experience the same amount of genetic drift as the population under study, regardless of the population census size (N_c). Because reproductive success and sex ratios are unequal in natural populations, which reduce genetic diversity, N_e is usually smaller than N_c (Ruzzante et al. 2016). In iteroparous species, the N_b is multiplied by a correction factor to calculate the N_e (Waples et al. 2014, Ruzzante et al. 2016). The N_e and amount of linkage disequilibrium in samples obtained over a single generation allows us to estimate how genetic drift might be impacting populations. Because collections were mixed-ages from an iteroparous species, calculated N_b values were between annual and per-generation values: the calculated N_b was multiplied by ~two to roughly estimate the effective population size N_e (Waples 2006, Waples et al. 2014). However, the relationship between N_b, N_e and N_c is complicated and variable within species (Waples et al. 2014, Ruzzante et a. 2016) and our samples were

inadequate to fully address this relationship in coastal cutthroat trout. We include the analysis to allow tentative comparisons to other coastal cutthroat trout populations in Puget Sound and to provide benchmarks for documenting changes in genetic diversity in the San Juan Islands populations.

Pairwise genetic comparisons

To explore spatial genetic relationships between the sampled populations and other populations in the WDFW genetic baseline, we calculated pairwise F_{ST} values among tributary collections with FSTAT. Pairwise F_{ST} is an estimate of genetic variation among collections (higher genetic variation indicates higher genetic distinction and lower gene flow or longer time since sharing common ancestors). Pairwise F_{ST} values were tested for whether they were significantly different from zero with a permutation test (100 permutations).

Neighbor-joining Dendrogram

As another means to visualize genetic relationships among coastal cutthroat trout populations, we plotted Nei's genetic distances among collections in a neighbor-joining tree using programs within the PHYLIP software package (Felsenstein 2004). We assessed the repeatability of the groupings on the tree with 10,000 bootstrap replications.

Principle Coordinates Analysis

We conducted a principle coordinates analysis of the pairwise F_{ST} matrix using the software GenAlEx (Peakall and Smouse 2006), as a non-hierarchical means to view genetic relationships among collections. The analysis finds axes that explain the maximum amount of genetic variation in the data set and plots the collections along the axes.

Factorial Correspondence Analysis

To view genetic diversity and relationships on an individual level, we conducted a factorial correspondence analysis using the program GENETIX (Belkhir et al. 2001). This analysis finds axes that explain the maximum amount of genetic variation in the data set and plots individual samples along the axes.

STRUCTURE analysis

To examine individual fish from Cascade for hatchery influence, we conducted a pairwise STRUCTURE analysis (Pritchard et al. 2000) in comparison to the 2014 Tokul Creek hatchery collection. STRUCTURE divides the data set into genetic clusters that minimize Hardy-Weinberg and linkage disequilibrium. Individuals that have membership in the same genetic group cluster in the same genetic cluster and individuals that are hybrid may have membership in more than one genetic cluster. We ran the analysis using default options (admixture model and correlated allele frequencies) with 50,000 burn-in runs to move the analysis away from starting conditions, and 200,000 iterations in 5 runs with the number of clusters set at 1, 2, and 3. Because family structure can be identified by the analysis as population structure, family members were restricted to two per family in final STRUCTURE analyses.

Results:

Genotyping was mostly successful for the two marker types. Samples with missing data were rerun to try and complete genotypes. The following samples were excluded from analyses due to missing 50% or more genotypic data: 14QW0011, 14QW0012, 14QW0033, 14QZ0002, 14QZ0005, 14QZ0010, 14QZ0020, 14QZ0022, 14QZ0023, and 14QZ0027. Most samples were genetically unique with the exception of two pairs of samples collected in Garrison that had identical genotypes: 14QZ0003 and 14QZ0038; 14QZ0006 and 14QZ0040. Matching genotypes can arise when population size is small and samples include related individuals such as parent-offspring or full siblings. Three of the Species ID SNPs have proven useful for identifying cutthroat-rainbow hybrids (WDFW unpublished data) and

genotypes at these loci indicated that all samples were pure coastal cutthroat trout. Fourteen SNP loci were fixed (had a single allele) in all collections (ASpID002, ASpID014, ASpID018, ASpID037, ASpID038, ASpID044, ASpID046, ASpID048, ASpID052, ASpID053, ASpID055, AOcl034, AOcl043, and AOcl054) and were excluded from analyses because they provided no information. Six additional loci were excluded because they failed to amplify in one or more collections (ASpID027, ASpID056, AOmy180, AOmy279, AOcl0002, and AOcl021), leaving a total of 83 loci (7 microsatellites and 76 SNPs) in the final genotypes that were analyzed. Allele frequencies for all loci with variation are presented in Appendix 3.The conditional formatting in Appendix 3 highlights some similarities and differences in allele frequencies between Doe and Garrison and between Cascade and Tokul Hatchery.

The San Juan Islands collections had the highest number of fixed loci among coastal cutthroat trout collections (Table 5) in comparisons to other coastal cutthroat trout collections from the WDFW coastal cutthroat trout baseline. Genetic diversity, measured as allelic richness and heterozygosity, was lower in the San Juan Islands collections than in other coastal cutthroat trout collections from Puget Sound and the WA coast. This pattern was consistent between marker types and diversity measures. However, collections compared were a mix of resident (San Juan Islands, Tokul Hatchery, Snoqualmie) and anadromous coastal cutthroat trout (Cedar, Goodman, Grays Harbor, Nooksack, Kennedy, McLane, and Skookum), so comparisons should be treated cautiously. Without accounting for stream or population size, there was a north to south cline in genetic diversity in Puget Sound, suggesting that latitude explained 53% of the genetic variation (Figure 2) and that genetic diversity increased in the anadromous collections towards south Puget Sound and on the coast. Without correcting for multiple tests, the San Juan Islands collections departed from HWE expectations with excess homozygosity (Cascade and Garrison) and excess heterozygosity (Doe), suggesting that samples departed from random expectations. The San Juan Islands collections also had a tendency towards linkage disequilibrium with higher number of locus pairs in disequilibrium than expected by chance at the $p < 0.05$ level.

Because of a few matching genotypes, departures from HWE, and higher than expected linkage, we examined the San Juan Islands collections for family structure. In the Cascade collection COLONY estimated a single full sibling family of eight, one of three, and four sets of two full siblings (Figure 3, see Appendix 3 for identities of fish within large families). In the Doe collection, COLONY estimated a single full sibling family of five, one of four, two sets of three full siblings, and nine sets of two full siblings. In the Garrison collection, COLONY estimated a single full sibling family of nine, two sets of three full siblings, and five sets of two full siblings. Because the samples were mixed ages and genetic statistics indicated that population sizes were small, some of these relationships could have been parents or grandparents and offspring or slightly more removed relationships such as aunts, uncles, nieces, nephews, and cousins. The program also generated an estimate of the number of breeders giving rise to the samples using a pairwise sibship method (Wang 2004) and these were estimated in Cascade as 27 (16-48 95%CI), in Doe as 21 (12-39 95%CI), and in Garrison as 20 (12-39 95%CI). Although there has been no formal analysis of the relationship between the number of breeders calculated with pairwise sibship method and the effective population size in a mixed aged sample from an iteroparous species, similar to the linkage disequilibrium method (see below) the estimated number of breeders would be less than the effective population size.

The LDNe calculated using the linkage disequilibrium method supported small effective population sizes for the San Juan Islands populations. The N_b value for Garrison was much lower than the value from the pairwise sibship method (5.8 versus 20) because of the high amount of linkage in the Garrison sample (14%). As mentioned in the methods section, values should be multiplied by a correction factor (Waples et al. 2014) to estimate N_e. The estimated N_e is an imprecise measure which varies according to the method employed but is useful for comparative purposes and as a benchmark for assessing management strategies. In Westslope cutthroat trout the N_e can be very small for resident populations, but resident populations can persist with low census sizes (~50 fish (Whiteley et al. 2013)) if there is sufficient good quality habitat to support them (Peterson et al. 2013). In Peterson et al. (2013) small Westslope cutthroat

trout populations persisted at least 100 years in 0.2 km of good habitat. It is possible that resident coastal cutthroat trout follow similar requirements for habitat and persistence (Rosenfeld et al. 2002). Sea-run cutthroat trout, such as populations from South Puget Sound, have greater feeding opportunities and supported larger populations with higher genetic diversity.

The pairwise F_{ST} values (Table 6) showed that there were significant genetic differences among the San Juan Islands coastal cutthroat trout collections and between the San Juan Islands coastal cutthroat trout and the populations from the WDFW coastal cutthroat trout baseline (these are primarily sea-run cutthroat trout). The closest relationship was between Cascade and the Tokul Creek Hatchery collections (0.0125 and 0.0082 in comparisons to 14Tokul and 01Tokul, respectively) – the values were an order of magnitude lower than other comparisons, suggesting a close relationship between these populations (Tokul Creek Hatchery uses resident Lake Whatcom coastal cutthroat trout broodstock (Crawford 1979)). Because of an interest in examining the relationship between Cascade Creek fish and Tokul Creek Hatchery fish, pairwise F_{ST} values were calculated after removing from the Cascade Creek collection all but one member of the single large family. The pairwise F_{ST} values between Cascade and the Tokul Creek Hatchery collections were slightly smaller (0.0089 and 0.0055, see Table 6), demonstrating that some of the genetic differentiation between Cascade and Tokul Creek Hatchery arose from the large family.

The neighbor-joining tree (Figure 4) showed a similarly close relationship between Cascade and Tokul Creek hatchery collections: they grouped on the same branch with 100% bootstrap support. If the single large family was removed from the Cascade collection, the Cascade Creek collection moved slightly down the branch from the hatchery collections but still grouped with the hatchery collections with 100% bootstrap support (not shown). The Puget Sound and Coastal sea-run cutthroat trout collections occupied the center of the tree and Garrison and Doe resident coastal cutthroat trout collections occupied a supported branch on the opposite side of the tree. The pairwise F_{ST} values showed that Garrison and Doe

were significantly differentiated and the tree showed that they shared genetic similarity with each other. But Doe was more differentiated, as indicated by the long branch length, often a signal of high genetic drift. The principle coordinates analysis showed the same pattern where Cascade clustered with Tokul Creek hatchery collections, the Puget Sound and Coast collections clustered together, with Garrison closer to this cluster than Doe, which was off in its own genetic space (Figure 5). The first axis explained 33% of the genetic variation and the second axis explained 25% of the genetic variation.

The factorial correspondence analysis (FCA) was another line of evidence showing genetic relationships among collections at the individual and population level (Figure 6 upper and lower plots). The FCA showed a close relationship between Cascade and Tokul Creek Hatchery fish and that the Garrison fish were closer to the central cluster of Puget Sound and coast fish. As in the neighbor-joining tree and PCoA, the Doe fish were in their own genetic space. This structure was the same when only the San Juan Islands fish and Tokul Creek Hatchery fish were included in the analysis (lower plot in Figure 6). However, with only Cascade and Tokul Creek Hatchery fish in the analysis, some of the Cascade fish cluster with the Tokul Creek Hatchery fish and others plotted slightly away from the hatchery cluster.

Because analyses had indicated a close genetic relationship between Cascade and Tokul Creek Hatchery fish, we considered two hypotheses: 1) that the Cascade cutthroat trout were a population of naturalized hatchery fish and 2) that the Cascade cutthroat trout were a population of naturalized hatchery fish with a remnant component of a native population. We used a STRUCTURE analysis to examine these hypotheses (Figure 7). When the STRUCTURE analysis included all the collections (not shown), the fish from Cascade Creek clustered with the Tokul Creek Hatchery fish, as expected from the low genetic variance between the collections indicated by pairwise F_{ST} values, and STRUCTURE was unable to separate Cascade fish from the hatchery fish. When we ran STRUCTURE in a pairwise test with only fish from Cascade Creek (limiting family size to two individuals) and Tokul Creek Hatchery collections, the analysis indicated that there were two genetic clusters. Some fish from Cascade Creek occupied the

cluster shared by the majority of the Tokul Creek Hatchery fish and other Cascade fish occupied a second genetic cluster (see Appendix 4 for individual ancestry values) shared by a minority of the hatchery fish. This diversity among the Cascade fish was similar to the results from the FCA where some Cascade fish clustered tightly with the hatchery fish and others plotted away from the hatchery fish cluster. Further, in the neighbor-joining tree, although the Cascade collection was strongly associated with the Tokul Creek Hatchery fish, the Cascade collection inserted along the hatchery branch, close to but not at the terminus, reflecting the small genetic difference between Cascade Creek and the Tokul Creek Hatchery collections quantified in the pairwise F_{ST} values. Because hatchery fish have been planted in Cascade and Mountain lakes at the headwaters of Cascade Creek since the 1930's and we have no samples of cutthroat trout from Cascade Creek prior to hatchery planting, it is impossible to determine whether this small difference between Cascade Creek trout and the Tokul Creek Hatchery trout reflects a remnant of a native population.

We also examined allele distributions for evidence supporting or refuting hypotheses for the status of the Cascade Creek population. One piece of evidence that might suggest a native population would be microsatellite alleles that are found in Cascade and not in the hatchery. Allele frequencies are similar at most loci (Appendix 3), but there were two microsatellite alleles that were found in Cascade and not in Tokul Creek Hatchery: one allele was found only in two individuals in Cascade Creek (Omm1138*153) and another was found in one individual in Cascade Creek but was common in other populations besides the hatchery (One108*175). The allele found only in Cascade Creek was in step with other alleles and could have been a mutation within the breeding population in the creek or could have been an allele found in Tokul Creek Hatchery in the past that was introduced into the creek but lost in the hatchery through genetic drift.

We considered various possibilities for the genetic diversity in Cascade Creek: the creek may have had a native cutthroat trout population prior to hatchery planting and hatchery fish mixed with native fish, there

may have been variation in the Tokul Creek hatchery broodstock over time and hatchery fish falling down from Cascade and Mountain lakes colonized the creek. We explored the possibility of a native gene pool by removing the large family (which can distort genetic relationships) and recalculating genetic distances. But the remaining Cascade gene pool remained closely related to the hatchery gene pool. Further, in STRUCTURE tests, both the 2001 and 2014 Tokul Creek Hatchery collections had individuals that clustered in the "Cascade" gene pool (fewer in the 2014 collection). If the remaining gene pool in Cascade Creek was a remnant of a native gene pool there should be no individuals in Tokul Creek Hatchery with ancestry in this pool. One possibility is that the remaining gene pool in Cascade Creek reflects changes in the hatchery broodstock that had naturalized in Cascade Creek, rather than representing native Cascade Creek genetic diversity. Hatchery planting in Mountain Lake has averaged almost 19,000 fish per planting year over the past 3 years and analyses described below suggest that hatchery fish drop down into the creek. Another possibility is that the breeding population in Cascade Creek is so small that the gene pool diverged from the Tokul Creek Hatchery gene pool through genetic drift.

The STRUCTURE analysis showed that the contemporary Tokul Creek Hatchery ancestry was non-uniformly distributed throughout Cascade Creek (Figure 7 and Table 7). If fish collected in Cascade Creek that clustered with the contemporary Tokul Creek Hatchery gene pool had been born in the hatchery then these were hatchery fish planted in Mountain Lake that had moved down into Cascade Creek and were found in the lowest and highest reaches. However, because hatchery fish are unmarked, the fish with primarily contemporary "Tokul Creek Hatchery" ancestry could be either escaped hatchery fish or members of the breeding population in Cascade Creek. Ancestry values in Appendix 4 show that over half (26/40) of the assigned fish had greater than 80% "Cascade" ancestry. However, this "Cascade" ancestry is also found in the Tokul Creek Hatchery collections (Figure 7) and was represented more in the 2001 collection. The data support the hypothesis that fish collected in Cascade Creek represent a breeding population that descended from naturalized hatchery fish and that the breeding population

differs from the Tokul Creek Hatchery broodstock because of genetic drift (N_e for Cascade = 21, suggesting a very small breeding population). Genetic drift is likely a force in the hatchery as well because the 2001 Tokul Creek collection differed significantly from the 2014 collection. However, we are unable to rule out the possibility that there is some remnant component of a native gene pool in Cascade Creek because there were two microsatellite alleles that were found in the Cascade Creek gene pool that were absent from the hatchery gene pool.

Summary:

Coastal cutthroat trout in creeks on the San Juan Islands have different evolutionary histories: coastal cutthroat trout sampled in Cascade Creek appear to be descended from naturalized Tokul Creek Hatchery fish that had moved down from planting sites in lakes and possibly some remnant of a native population. Coastal cutthroat trout from Doe and Garrison Creeks are distinct native populations. Doe had the lowest genetic diversity of any coastal cutthroat trout population in the analyses, suggesting that they are more isolated than Garrison. All three San Juan Islands populations have small effective population sizes and genetic drift is thus a strong factor influencing genetic structure among these populations and in relation to other coastal cutthroat trout populations in Puget Sound. Habitat likely imposes limitations on population sizes of coastal cutthroat trout inhabiting streams on the San Juan Islands (Barsh 2010). Other environmental factors influence population size and potential for gene flow with other populations (Wenberg et al. 1998).

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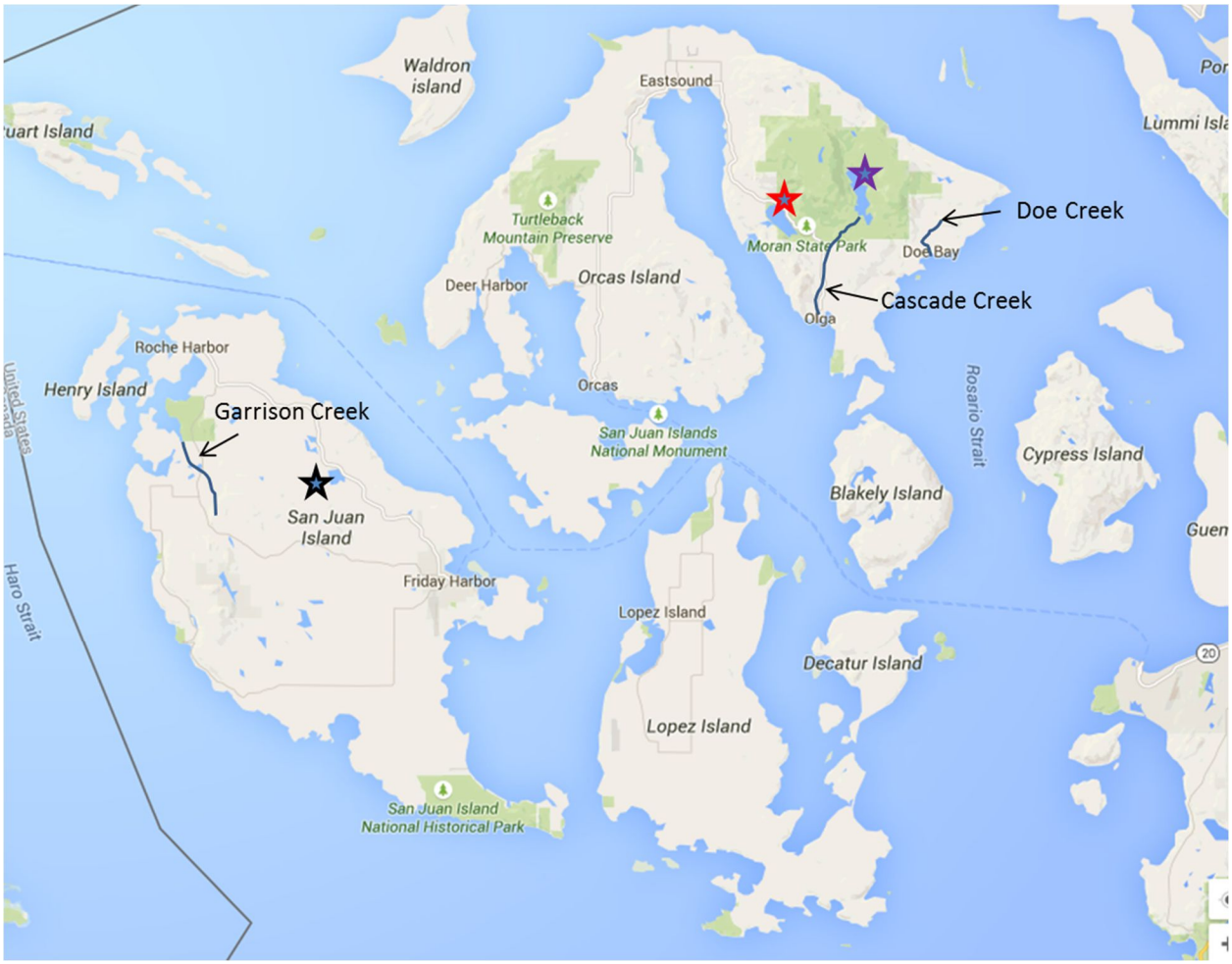

Figure 1. Approximate location of San Juan Islands coastal cutthroat trout collection sites. Stars indicate lakes where hatchery coastal cutthroat trout were planted: black – Egg Lake, red – Cascade Lake, and purple – Mountain Lake. Hatchery planting data detailed in Appendix 2.

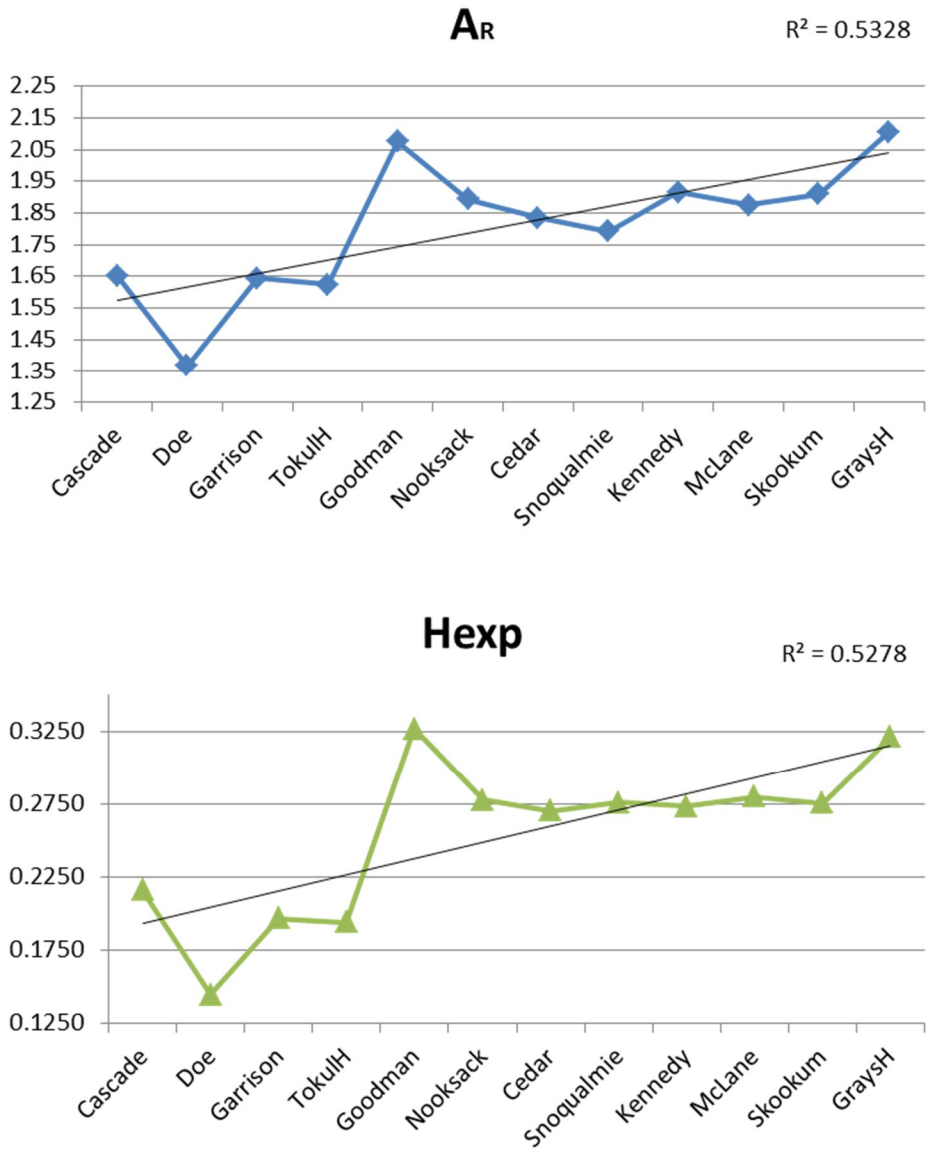

Figure 2. Graph of allelic richness and expected heterozygosity (full genotypes) versus north to south in Puget Sound – Grays Harbor is on the coast.

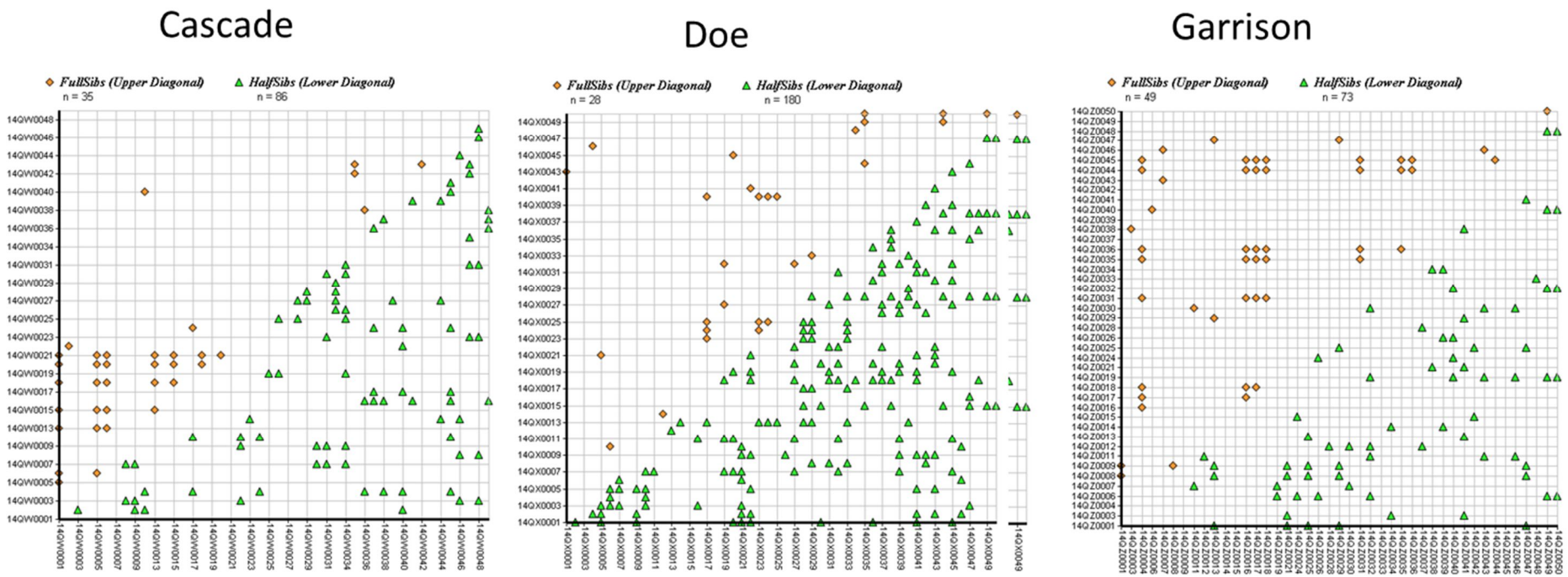

Figure 3. Plot of pairwise sibling relationships in the three San Juan Islands coastal cutthroat trout collections. For Cascade and Doe the sample sizes were too small to list all the samples on the axes. Full sibling relationships are indicated by a yellow diamond on the intersection of two samples and half siblin g relationships are indicated by a green triangle on the intersection of two samples. Families are visualized as diamonds along a single row or column. For instance, in Cascade the first individual is estimated to be full siblings with seven other samples.

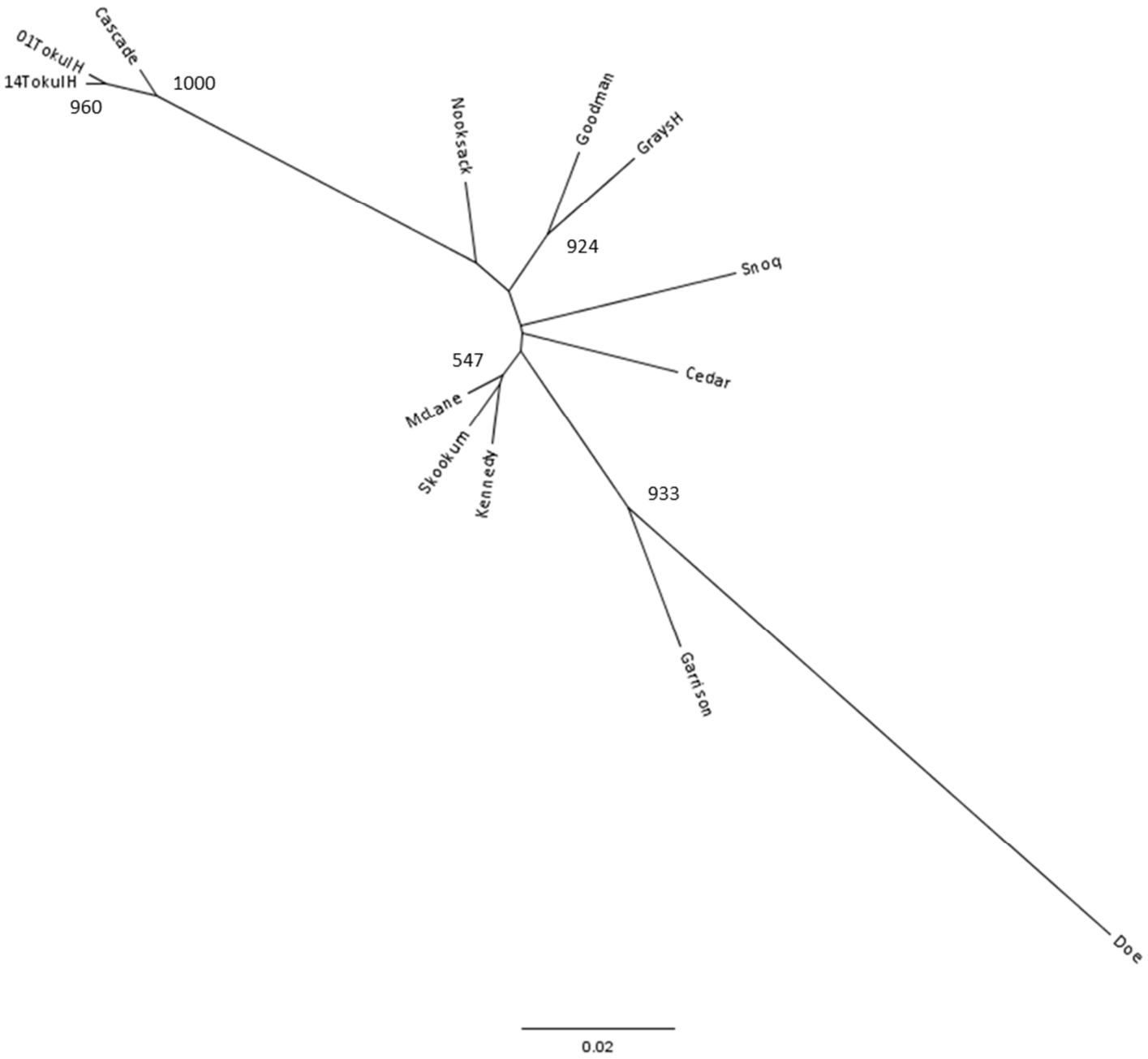

Figure 4. Neighbor-joining tree of Nei's genetic distances among coastal cutthroat trout collections. Numbers at the nodes are the bootstrap values indicating the number of trees (out of 1000) in which the collections beyond the node clustered together.

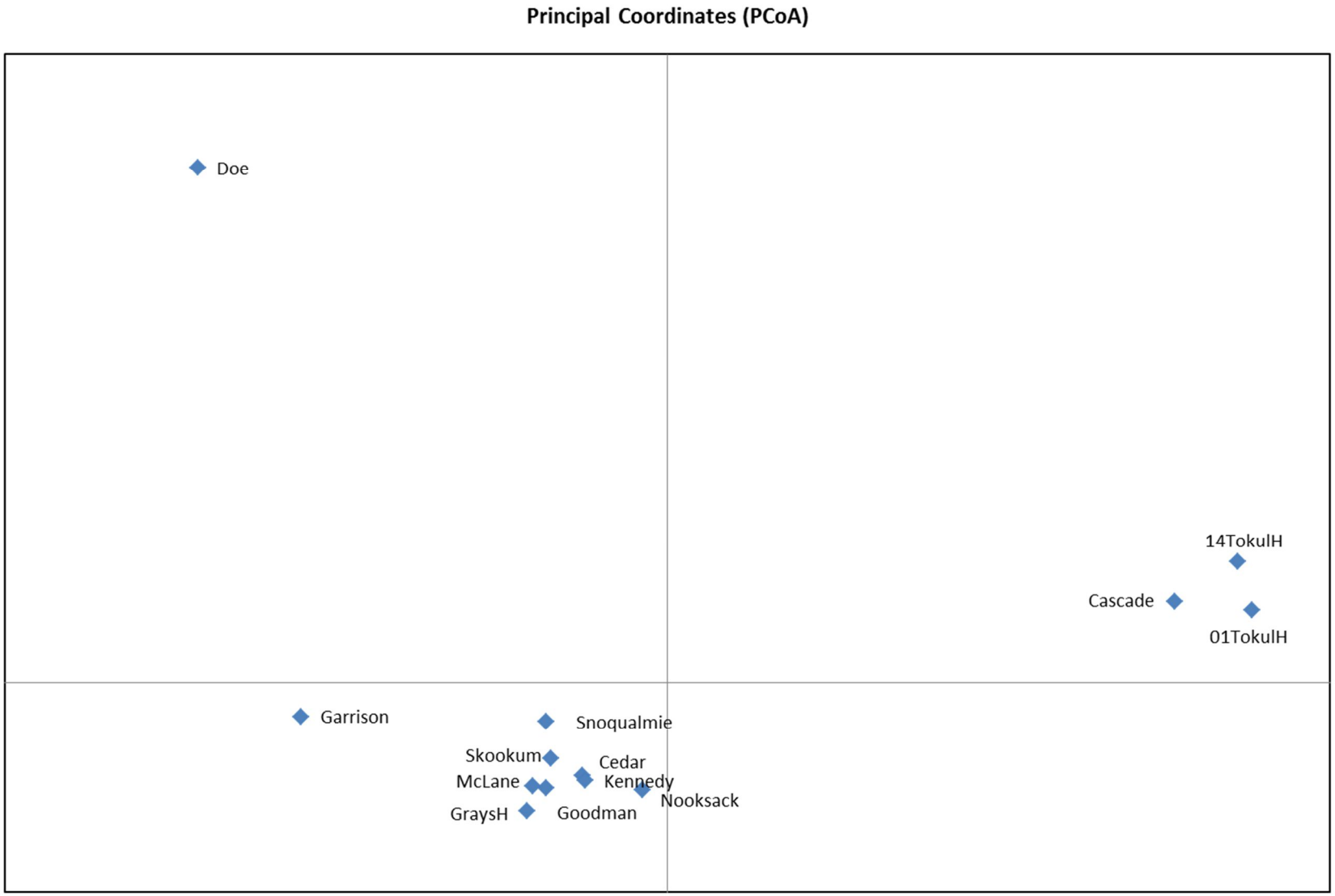

Figure 5. Principal coordinates plot of pairwise F_{ST} values from GenAlEx.

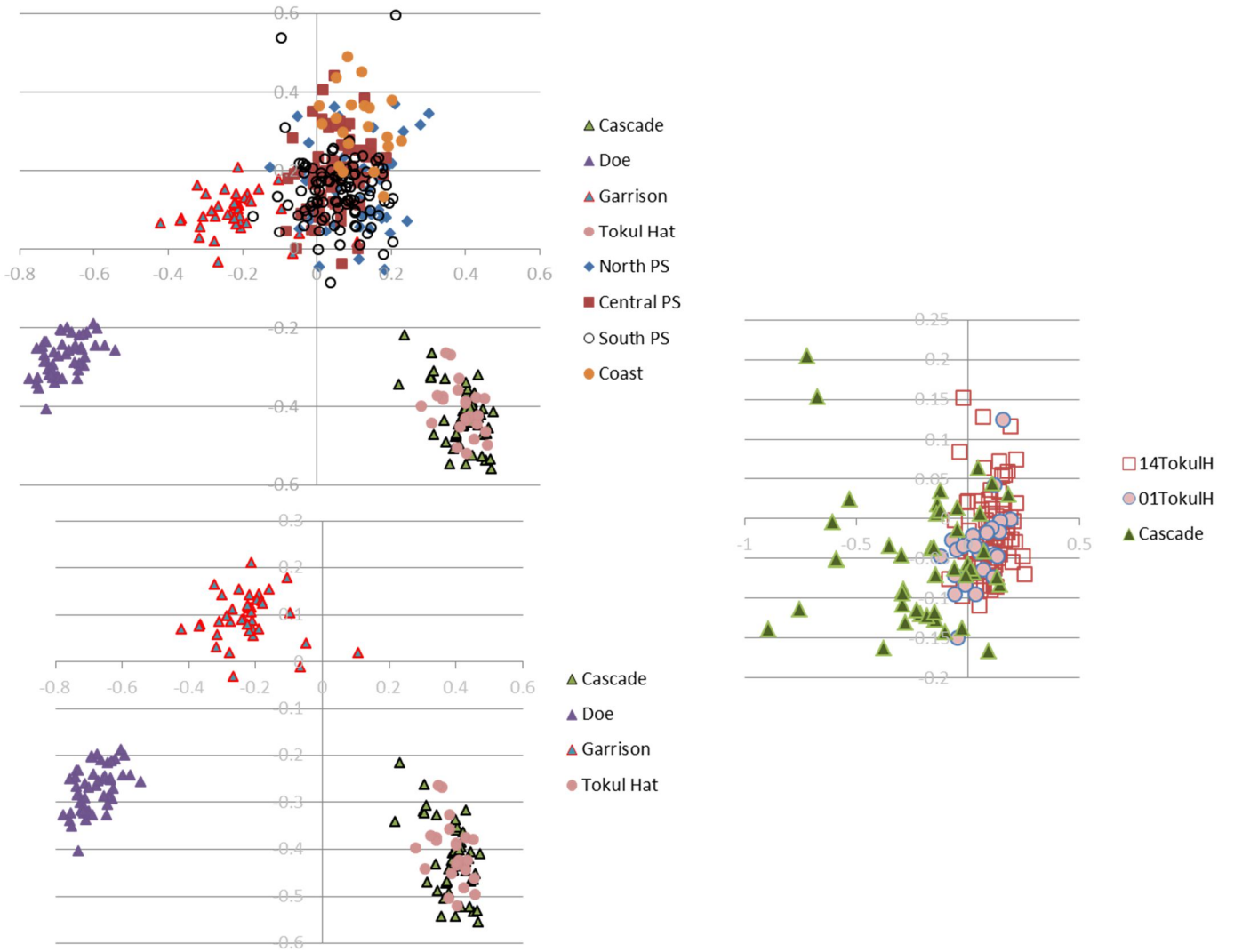

Figure 6. Factorial correspondence analysis plot of genetic relationships among individual samples. Lower left plot shows relationships just among San Juan Islands collections and Tokul Hatchery. Right plot shows relationships among Cascade and Tokul hatchery collections. Individual positions changed slightly because genetic variation was recalculated based only on samples included in the analysis.

Figure 7. STRUCTURE analysis of Coastal cutthroat trout from Tokul Creek Hatchery and Cascade Creek and K = 2, averaged over five runs, using 80 loci (3 additional loci were removed for this comparison because there was no variation). Cascade Creek samples were re-organized by reach, which is indicated by the reach letter before the individual ID. Up to two individuals from a single full-sibling family were included in analyses. Individual ancestry values for Cascade fish are listed in Appendix 4.

Table 1. Description of streams hosting coastal cutthroat trout in San Juan Islands (Barsh 2010). Streams with asterisks by name and island locations were sampled for this project.

Stream	*Fish groups*	*Cutthroat observed*	*Seaward barriers*
*Cascade on Orcas Island	Buck Bay	Age 0+, 1+, 2+	Undersized culvert
	Olga Tank	Age 0+, 1+, 2+ & redds	Waterfall
	Kahboo Hill	Age 0+, 1+ & redds	Waterfalls
*Doe Bay on Orcas Island	DB Resort	Age 2+	Waterfalls
*Garrison on San Juan Island	Yacht Haven	Age 0+, 2+	
	Roadside Inn	Age 0+, 1+ & redds	Channel-less wetland
	Troutbeck	Age 1+, redds	Partly collapsed culvert
	Mitchell Hill	Age 1+	Concrete weir
Victorian	Bay Head	Age 2+	
West Beach	WB Road	Age 0+, 1+	Stagnant online pond
	Deer Ravine	Age 0+, 1+	Perched decaying culvert

Table 2. List of coastal cutthroat trout samples analyzed in this study: samples at top were collected for the San Juan Islands project and other samples are in the WDFW coastal cutthroat trout genetic baseline.

Region	San Juan study	Code	N
NorthPS	Cascade	14QW	49
NorthPS	Doe	14QX	50
NorthPS	Garrison	14QZ	50
	WDFW baseline		
NorthPS	01TokulH	01NZ	24
NorthPS	14TokulH	14MK	90
CentralPS	Cedar	05BB	20
CentralPS	Snoqualmie	09IJ	42
NorthPS	Goodman	00CU	21
Coast	GraysH	11OI	21
NorthPS	Nooksack	95VF	22
SouthPS	Kennedy	14JG	32
SouthPS	McLane	14JG	34
SouthPS	Skookum	14JG	35

Table 3. Location details and dates for sampling coastal cutthroat trout on San Juan Islands.

Location	description	date in 2014	N
Cascade A	Anadromous reach, bridge to first falls	6/9, 7/4, 7/28, 8/4	10
Cascade B	From first falls upstream for several hundred feet	8/4	14
Cascade C	From Olga Rd. (diversion dam) upstream for ~200 feet	8/5	10
Cascade D	From ~200 ft DS Cascade Falls to Cascade Falls.	8/5	5
Cascade E	Upstream from Mtn Lake: footbridge US for ~500 ft.	8/5	10
Doe Bay A	Reach DS culvert	7/2	22
Doe Bay B	Reach US culvert	7/2	28
Garrison A	Alpaca Ponds	8/22, 12/16	1
Garrison B	The Clearing (State's Inn) DS driveway culvert	7/1, 8/22	18
Garrison C	The Clearing (State's Inn) US driveway culvert	7/1, 8/22, 12/16	31

Table 4. List of microsatellite (msat) and single nucleotide polymorphism (SNP) loci genotyped in the study. The SNP loci had a WDFW nickname assigned (see Appendix3 for allele frequencies for all loci with more than one allele). The amount of genetic variation among all samples was assessed with F_{ST} values: p-values indicate F_{ST} values that were significantly different from zero. Fixed loci under F_{ST} had the same allele in all populations and no variation.

Type	WDFW name	Locus ID	F_{ST}	p-value	Type	WDFW name	Locus ID	F_{ST}	p-value
msat		Ogo-3	0.03472	0	SNP	AOcl050	Ocl_120751c	0.15282	0
msat		Omm-1138	0.29703	0	SNP	AOcl051	Ocl_123048c	0.23088	0
msat		Omy-77	0.2736	0	SNP	AOcl052	Ocl_123205c	0.19459	0
msat		One-108	0.20065	0	SNP	AOcl053	Ocl_124454c	0.15126	0
msat		Ots-1	0.23103	0	SNP	AOcl055	Ocl_128302c	0.15696	0
msat		Ots-103	0.24584	0	SNP	AOcl056	Ocl_128757c	0.10198	0
msat		Ots-3M	0.19616	0	SNP	AOcl057	Ocl_128923c	0.12827	0
SNP	AOcl001	Ocl_gdh-33	0.27720	0	SNP	AOcl058	Ocl_128996c	0.37211	0
SNP	AOcl003	Ocl_94903c	0.20099	0	SNP	AOcl059	Ocl_129144c	0.37114	0
SNP	AOcl004	Ocl_95769c	0.12775	0	SNP	AOcl060	Ocl_129170c	0.12713	0
SNP	AOcl005	Ocl_96127c	0.20516	0	SNP	AOcl061	Ocl_130524c	0.23723	0
SNP	AOcl006	Ocl_96500c	0.08405	0	SNP	AOcl062	Ocl_131460c	0.23494	0
SNP	AOcl007	Ocl_97077c	0.07401	0	SNP	AOcl063	Ocl_131785c	0.20794	0
SNP	AOcl008	Ocl_97865c	0.02854	0	SNP	AOcl064	Ocl_131802c	0.01247	0.09677
SNP	AOcl009	Ocl_98188c	0.21680	0	SNP	AOcl065	Ocl_impa1ya	0.21801	0
SNP	AOcl010	Ocl_98409c	0.12646	0	SNP	ASpI029	Ocl_impa1-189	0.26859	0
SNP	AOcl011	Ocl_101704c	0.23279	0	SNP	ASpI030	Ocl_ca050-39	0.59351	0
SNP	AOcl012	Ocl_102420c	0.07722	0	SNP	ASpI032	Ocl_gh1-633	0.07494	0
SNP	AOcl013	Ocl_102510c	0.09134	0	SNP	ASpI033	Ocl_MK3p-145	0.30259	0
SNP	AOcl014	Ocl_103122c	0.25292	0	SNP	ASpI040	Ocl_cin-90	0.22953	0
SNP	AOcl015	Ocl_104216c	0.00026	0.42229	SNP	ASpI042	Ocl_hbad-264	0.12863	0
SNP	AOcl016	Ocl_105385c	0.44677	0	SNP	AOmy004	Omy_ALDOA_1	0.13323	0
SNP	AOcl017	Ocl_105407c	0.17860	0	SNP	AOmy048	Omy_113490-159	0.00826	0.47605
SNP	AOcl018	Ocl_105768c	0.36389	0	SNP	AOmy049	Omy_114315-438	0.02202	0.00293
SNP	AOcl019	Ocl_105897c	0.14185	0	SNP	AOmy063	Omy_97660-230	0.00556	0.21701
SNP	AOcl020	Ocl_106172c	0.26297	0	SNP	AOmy064	Omy_97865-196	0.53434	0
SNP	AOcl022	Ocl_106747c	0.07939	0	SNP	AOmy210	OMS00153	0.11757	0
SNP	AOcl023	Ocl_107074c	0.20481	0	SNP	AOmy252	Omy_114976-223	-0.00446	0.62757
SNP	AOcl024	Ocl_107607c	0.08146	0	SNP	AOmy258	Omy_117540-259	0.15056	0
SNP	AOcl025	Ocl_108007c	0.02067	0.02542	SNP	AOmy330	Omy_109894-185	0.01547	0.06452
SNP	AOcl026	Ocl_109243c	0.07997	0	SNP	AOmy342	Omy_GH1-prom1-1	0.03103	0.19746
SNP	AOcl027	Ocl_109894c	0.05936	0.00684	SNP	AOcl002	Ocl_myo1b-16	NA	
SNP	AOcl028	Ocl_110064c	0.07541	0	SNP	AOcl034	Ocl_113109c	fixed	
SNP	AOcl029	Ocl_110495c	0.10151	0	SNP	AOcl043	Ocl_117144c	fixed	
SNP	AOcl030	Ocl_111084c	0.21951	0	SNP	AOcl054	Ocl_125998c	fixed	
SNP	AOcl031	Ocl_111312c	0.13037	0	SNP	ASpI002	Ocl_Oku202	fixed	
SNP	AOcl032	Ocl_111383c	0.21440	0	SNP	ASpI014	Omy_F5_136	fixed	
SNP	AOcl033	Ocl_112419c	0.18995	0	SNP	ASpI018	Omy_Omyclmk436-96	fixed	
SNP	AOcl035	Ocl_113128c	0.14924	0	SNP	ASpI037	Ocl_fKbp2-62	fixed	
SNP	AOcl036	Ocl_113600c	0.10177	0	SNP	ASpI038	Ocl_mx1-129	fixed	
SNP	AOcl037	Ocl_114315c	0.39846	0	SNP	ASpI044	Ocl_gshpx-104	fixed	
SNP	AOcl038	Ocl_114336c	0.13826	0	SNP	ASpI046	Ocl_mk3pro-69	fixed	
SNP	AOcl039	Ocl_114448c	0.16499	0	SNP	ASpI048	Ocl_hsc71p-71	fixed	
SNP	AOcl040	Ocl_115987c	0.22630	0	SNP	ASpI053	Ocl_bcAKala-259	fixed	
SNP	AOcl041	Ocl_116865c	0.11083	0	SNP	ASpI055	Ocl_msra-168	fixed	
SNP	AOcl042	Ocl_116938c	0.04486	0.00098	SNP	AOmy180	OMS00048	NA	
SNP	AOcl044	Ocl_117259c	0.34412	0	SNP	AOmy279	OMS00015	NA	
SNP	AOcl045	Ocl_117370c	0.59759	0	SNP	ASpI052	Ocl_aldB-79	fixed	
SNP	AOcl046	Ocl_117432c	0.12500	0	SNP	ASpI027	Ocl_arp-117	NA	
SNP	AOcl047	Ocl_117540c	0.29201	0	SNP	ASpI056	Ocl_metB-106	NA	
SNP	AOcl048	Ocl_118654c	0.18776	0	SNP	AOcl021	Ocl_106419c	NA	

Table 5. Table of genetic statistics for coastal cutthroat trout including the average number of alleles (Avg alleles) for microsatellite loci, SNP loci and for both loci combined, the number of loci fixed for a single allele, allelic richness (A_R, the average number of alleles corrected to a collection size of 7 individuals), heterozygosity (H_{EXP}, the average expected heterozygosity over microsatellite loci, SNP loci and both loci, corrected to a collection size of 7 individuals), the HWE value over all loci (F_{IS}) and p values for departures from expected values in a positive (pos) or negative (neg) direction as well as the number of loci departing from equilibrium in both directions, the number of pairwise tests for linkage disequilibrium (total tests – values differ because number of fixed loci differed), the number of tests significant at $p < 0.05$ and at $p < 0.0001$, and the effective population size (LDNe) calculated using a linkage disequilibrium method and the 95% confidence limits.

Region Name		NPS Cascade	NPS Doe	NPS Garrison	NPS 14TokulH	NPS 01Tokul	NPS Goodman	NPS Nooksack	CPS Cedar	CPS Snoqualmie	SPS Kennedy	SPS McLane	SPS Skookum	Coast GraysH
Avg alleles	msats	3.71	1.86	3.71	4.50	4.00	8.71	6.00	5.14	5.71	6.86	6.57	7.29	8.71
	SNPs	1.68	1.43	1.70	1.58	1.61	1.83	1.80	1.71	1.74	1.79	1.78	1.86	1.84
	both	1.85	1.46	1.87	1.83	1.81	2.41	2.16	2.00	2.07	2.21	2.18	2.31	2.42
	fixed	27	47	25	39	31	14	16	23	20	17	18	12	13
A_R	msats	2.81	1.71	2.81	2.93	2.86	5.53	4.03	3.91	3.65	4.54	4.11	4.37	5.51
	SNPs	1.54	1.34	1.54	1.45	1.51	1.76	1.70	1.64	1.62	1.67	1.67	1.68	1.79
	both	1.65	1.37	1.64	1.56	1.62	2.08	1.89	1.84	1.79	1.91	1.87	1.91	2.11
H_{EXP}	msats	0.4194	0.2509	0.3839	0.4036	0.4036	0.6064	0.4947	0.4779	0.5420	0.5363	0.4919	0.5060	0.5707
	SNPs	0.1973	0.1350	0.1796	0.1624	0.1749	0.3012	0.2579	0.2515	0.2519	0.2492	0.2607	0.2546	0.2979
	both	0.2158	0.1447	0.1966	0.1825	0.1939	0.3269	0.2778	0.2703	0.2761	0.2731	0.2799	0.2755	0.3209
HWE	F_{IS} overall	0.072	-0.134	0.089	-0.013	0.014	0.038	-0.085	0.055	0.059	0.041	-0.004	0.030	0.030
	p value pos	0.0006	1	0.0001	0.7862	0.3176	0.081	0.9986	0.0308	0.0019	0.0297	0.5626	0.0766	0.1341
	nloci pos F_{IS}	7	1	8	3	0	6	1	2	6	5	2	7	3
	p value neg	0.9995	0.0000	0.9999	0.2142	0.6834	0.9191	0.0014	0.9693	0.9982	0.9705	0.4379	0.9236	0.866
	nloci neg F_{IS}	2	7	0	4	0	1	1	1	0	0	3	3	1
linkage	total tests	1537	665	1627	1590	1324	2313	2193	1757	1941	2072	2480	2139	2331
	N linked 0.05	135	59	229	97	43	76	112	63	81	78	94	80	66
	% tests	8.78%	8.87%	14.07%	6.10%	3.25%	3.29%	5.11%	3.59%	4.17%	3.76%	3.79%	3.74%	2.83%
	N linked 0.0001	6	2	8	1	1	0	0	1	3	0	0	0	0
	LDNe	21	21.8	5.8	108.0	59.4	89.5	13.7	13.4	235.2	56.8	68.4	83.7	196.7
	low	17.6	15.4	4.1	81.7	35.2	52.9	11.3	10.8	114.7	42.6	47.8	59.4	77.2
	high	25.2	31.9	7.1	152.0	150.2	248.7	16.9	17	5608.9	81.8	112.4	134.5	Infinite

Table 6. Table of pairwise F_{ST} values (upper matrix) and their associated p values (lower matrix). The F_{ST} values are color coded from low (green) to high (red). Lower table shows pairwise F_{ST} values for Cascade compared to 01Tokul and 14Tokul with the large family removed from Cascade.

	14TokulH	01TokulH	Cascade(all)	Doe	Garrison	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum
14TokulH		0.0030	0.0125	0.1659	0.1230	0.0727	0.0825	0.0801	0.0826	0.0652	0.0676	0.0750	0.0704
01TokulH	0.00		0.0082	0.1693	0.1216	0.0715	0.0821	0.0738	0.0794	0.0616	0.0678	0.0733	0.0719
Cascade(all)	0.00	0.00		0.1614	0.1106	0.0713	0.0798	0.0683	0.0743	0.0608	0.0647	0.0707	0.0664
Doe	0.00	0.00	0.00		0.1061	0.1204	0.1184	0.1247	0.1320	0.1312	0.1206	0.1160	0.1112
Garrison	0.00	0.00	0.00	0.00		0.0641	0.0738	0.0544	0.0588	0.0663	0.0588	0.0515	0.0546
Cedar	0.00	0.00	0.00	0.00	0.00		0.0364	0.0394	0.0352	0.0282	0.0267	0.0248	0.0246
Snoqualmie	0.00	0.00	0.00	0.00	0.00	0.00		0.0412	0.0427	0.0407	0.0365	0.0360	0.0354
Goodman	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.0159	0.0227	0.0295	0.0252	0.0323
GraysH	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.0234	0.0281	0.0273	0.0319
Nooksack	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.0284	0.0243	0.0314
Kennedy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.0087	0.0094
McLane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01		0.0112
Skookum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

	01TokulH	Cascade
14TokulH	0.0030	0.0089
01TokulH		0.0055

Table 7. Cascade Creek Ancestry values within Appendix 4 show 26 of the 40 assigned fish had Cascade ancestry >0.8, representing possibly an older Tokul Creek Hatchery broodstock that had naturalized in Cascade Creek. Analyzing Cascade Creek sample ancestry by reach location (reaches identified in Table 3), there is a 44% Tokul Creek Hatchery signature in Reach E (the upstream most reach which feeds Mountain Lake, where the hatchery fish are planted), with lower contemporary Tokul Creek Hatchery signature with distance downstream from the Lake, to the highest contemporary Tokul Creek Hatchery signature in reach A, the anadromous zone at the mouth of Cascade Creek.

Reach	# >0.8 Cascade	# total	% Assign Cascade	% Assign Tokul
A	3	9	0.33	0.67
B	6	7	0.86	0.14
C	10	10	1.00	0.00
D	4	5	0.80	0.20
E	5	9	0.56	0.44
	26	40		

Appendix 1. Sample collection details for individual samples – number under "tissue collected" column is the WFC ID collection number. Red cells were a large single full-sib family in Cascade. Yellow cells were a single full-sib family in Doe. Green cells were a large single full-sib family in Garrison. In some cases full-sib relationships could also be parent-offspring because samples included multiple age classes and coastal cutthroat trout are iteroparous.

	Tissue					Scale	
WDFW Code	**Collected?**	**Reach ID**	**Date**	**Length**	**Weight**	**Collected?**	**Photos**
14QW0001	001	Cascade A	9-Jun	160	42.5	Y	0512-0513
14QW0002	002	Cascade A	9-Jun	135	28.3	Y	0514-0515
14QW0003	085	Cascade A	4-Jul	78	5.3	Y	6020
14QW0004	086	Cascade A	4-Jul	163	40.1	Y	Y
14QW0005	087	Cascade A	4-Jul	141	24.2	Y	6038
14QW0006	089	Cascade A	28-Jul	163	36.8	Y	Y
14QW0007	090	Cascade A	28-Jul	177	51.2	Y	
14QW0008	091	Cascade A	28-Jul	175	51.5	Y	
14QW0009	092	Cascade A	4-Aug	152	33.4	Y	1020606-0611
14QW0010	093	Cascade B	4-Aug	183	66.1	Y	1020621-0623
14QW0011	094	Cascade B	4-Aug	167	51.4	Y	1020624-0626
14QW0012	095	Cascade B	4-Aug	175	55.4	Y	1020627-0628
14QW0013	096	Cascade B	4-Aug	161	41.3	Y	1020630-0632
14QW0014	097	Cascade B	4-Aug	161	44.3	Y	1020633-0635
14QW0015	098	Cascade B	4-Aug	173	54.3	Y	1020636-0638
14QW0016	099	Cascade B	4-Aug	135	26.1	Y	1020640
14QW0017	100	Cascade B	4-Aug	225	122.3	Y	1020641-0643
14QW0018	101	Cascade B	4-Aug	159	37.4	Y	1020644-0646
14QW0019	102	Cascade B	4-Aug	161	41.3	Y	1020647-0649
14QW0020	103	Cascade B	4-Aug	178	53.9	Y	1020650-0652
14QW0021	104	Cascade B	4-Aug	160	40.8	Y	1020653-0655
14QW0022	105	Cascade B	4-Aug	160	39.6	Y	1020656-0658
14QW0023	106	Cascade B	4-Aug	135	21.8	Y	1020659-0661
14QW0024	107	Cascade A	4-Aug	175	54.3	Y	1020662-0664
14QW0025	108	Cascade E	5-Aug	72	5.2	N	1020665-0668
14QW0026	109	Cascade E	5-Aug	57	1.6	N	1020669-0671
14QW0027	110	Cascade E	5-Aug	56	1.9	N	1020672-0674
14QW0028	111	Cascade E	5-Aug	92	7.6	N	1020675-0677
14QW0029	112	Cascade E	5-Aug	53	1.5	N	0708-0710
14QW0030	113	Cascade E	5-Aug	48	1.2	N	0711-0713
14QW0031	114	Cascade E	5-Aug	48	1.1	N	0714-0716
14QW0032	115	Cascade E	5-Aug	44	0.8	N	0717-0719
14QW0033	116	Cascade E	5-Aug	47	0.8	N	0720-0722
14QW0034	117	Cascade E	5-Aug	46	1	N	0723-0725
14QW0035	118	Cascade C	5-Aug	124	18.9	Y	726-728
14QW0036	119	Cascade C	5-Aug	103	11.5	Y	729-731
14QW0037	120	Cascade C	5-Aug	71	3.8	N	732-734
14QW0038	121	Cascade C	5-Aug	94	8.4	Y	735-737
14QW0039	122	Cascade C	5-Aug	102	11	Y	738-740
14QW0040	123	Cascade C	5-Aug	143	27	Y	741-743
14QW0041	124	Cascade C	5-Aug	99	10.1	Y	744-746
14QW0042	125	Cascade C	5-Aug	111	14.1	Y	747-749
14QW0043	126	Cascade C	5-Aug	136	25.1	Y	750-752
14QW0044	127	Cascade C	5-Aug	60	3.2	N	753-755
14QW0045	128	Cascade D	5-Aug	175	55.7	Y	756-758

	Tissue					Scale	
WDFW Code	**Collected?**	**Reach ID**	**Date**	**Length**	**Weight**	**Collected?**	**Photos**
14QW0046	129	Cascade D	5-Aug	119	17	Y	759-761
14QW0047	130	Cascade D	5-Aug	158	36.8	Y	762-765
14QW0048	131	Cascade D	5-Aug	67	3.3	N	766-768
14QW0049	132	Cascade D	5-Aug	117	15.6	Y	769-771
14QX0001	035	Doe Bay A	2-Jul	68	3	Y	5932
14QX0002	036	Doe Bay A	2-Jul	53	2.9	Y	5933
14QX0003	037	Doe Bay A	2-Jul	47	0.8	N	5934
14QX0004	038	Doe Bay A	2-Jul	47	1	N	5935
14QX0005	039	Doe Bay A	2-Jul	59	2.5	Y	5936
14QX0006	040	Doe Bay A	2-Jul	64	2.8	Y	5937
14QX0007	041	Doe Bay A	2-Jul	53	1.5	Y	5938
14QX0008	042	Doe Bay A	2-Jul	226	119.3	Y	5939
14QX0009	043	Doe Bay A	2-Jul	233	125.5	Y	5942
14QX0010	044	Doe Bay A	2-Jul	212	92.9	Y	5943
14QX0011	045	Doe Bay A	2-Jul	213	97.6	Y	5944
14QX0012	046	Doe Bay A	2-Jul	187	64.2	Y	5945
14QX0013	047	Doe Bay A	2-Jul	179	62.8	Y	5946
14QX0014	048	Doe Bay A	2-Jul	169	48.4	Y	5948
14QX0015	049	Doe Bay A	2-Jul	138	29.4	Y	5949
14QX0016	050	Doe Bay A	2-Jul	139	31.6	Y	5950
14QX0017	051	Doe Bay A	2-Jul	117	17.9	Y	5951
14QX0018	052	Doe Bay A	2-Jul	62	3.3	Y	5954
14QX0019	053	Doe Bay A	2-Jul	66	3	Y	5955
14QX0020	054	Doe Bay A	2-Jul	68	4.1	Y	5956
14QX0021	055	Doe Bay A	2-Jul	58	2	Y	5957
14QX0022	056	Doe Bay A	2-Jul	56	2.7	Y	5960
14QX0023	057	Doe Bay B	2-Jul	125	18.6	Y	5961
14QX0024	058	Doe Bay B	2-Jul	123	21.3	Y	5963
14QX0025	059	Doe Bay B	2-Jul	123	22.2	Y	5963
14QX0026	060	Doe Bay B	2-Jul	104	10.6	Y	5964
14QX0027	061	Doe Bay B	2-Jul	142	30.6	Y	5965-5966
14QX0028	062	Doe Bay B	2-Jul	96	9.6	Y	5968
14QX0029	063	Doe Bay B	2-Jul	106	14.1	Y	5969
14QX0030	064	Doe Bay B	2-Jul	53	1.3	Y	5970
14QX0031	065	Doe Bay B	2-Jul	128	22.6	Y	5971
14QX0032	066	Doe Bay B	2-Jul	61	2.8	Y	5872
14QX0033	067	Doe Bay B	2-Jul	44	1.1	Y	5973
14QX0034	068	Doe Bay B	2-Jul	46	1.3	N	5974
14QX0035	069	Doe Bay B	2-Jul	58	1.9	N	5976
14QX0036	070	Doe Bay B	2-Jul	69	3.8	Y	5977
14QX0037	071	Doe Bay B	2-Jul	60	2.5	Y	5978
14QX0038	072	Doe Bay B	2-Jul	54	1.9	N	5980
14QX0039	073	Doe Bay B	2-Jul	55	2.1	N	5981
14QX0040	074	Doe Bay B	2-Jul	115	16.4	Y	5982-5983
14QX0041	075	Doe Bay B	2-Jul	67	3.8	Y	5984
14QX0042	076	Doe Bay B	2-Jul	37	1	N	5985
14QX0043	077	Doe Bay B	2-Jul	47	1.3	N	5986
14QX0044	078	Doe Bay B	2-Jul	57	1.9	Y	5988
14QX0045	079	Doe Bay B	2-Jul	68	3.8	Y	5989
14QX0046	080	Doe Bay B	2-Jul	53	1.6	N	5990
14QX0047	081	Doe Bay B	2-Jul	51	1.7	N	5991
14QX0048	082	Doe Bay B	2-Jul	50	1.5	N	5997
14QX0049	083	Doe Bay B	2-Jul	61	2.3	Y	5998-5999
14QX0050	084	Doe Bay B	2-Jul	44	0.9	N	59?
14QZ0001	003	Garrison B	1-Jul	112	14.7	Y	1

	Tissue					Scale	
WDFW Code	**Collected?**	**Reach ID**	**Date**	**Length**	**Weight**	**Collected?**	**Photos**
14QZ0002	004	Garrison B	1-Jul	200	91.9	Y	2
14QZ0003	005	Garrison B	1-Jul	205	92.8	Y	3
14QZ0004	006	Garrison B	1-Jul	135	26.6	Y	4
14QZ0005	007	Garrison B	1-Jul	120	18.7	Y	5
14QZ0006	008	Garrison B	1-Jul	198	83	Y	6
14QZ0007	009	Garrison B	1-Jul	57	1.4	Y	7
14QZ0008	010	Garrison B	1-Jul	113	16.3	Y	8
14QZ0009	011	Garrison B	1-Jul	108	12.6	Y	9
14QZ0010	012	Garrison B	1-Jul	103	10.8	Y	10
14QZ0011	013	Garrison B	1-Jul	101	11.2	Y	11
14QZ0012	014	Garrison C	1-Jul	117	18.3	Y	12
14QZ0013	015	Garrison C	1-Jul	110	13.6	Y	13
14QZ0014	016	Garrison C	1-Jul	91	8.3	Y	14
14QZ0015	017	Garrison C	1-Jul	94	3.7	Y	15
14QZ0016	018	Garrison C	1-Jul	112	15.3	Y	16
14QZ0017	019	Garrison C	1-Jul	133	16.8	Y	17
14QZ0018	020	Garrison C	1-Jul	94	7.9	Y	18
14QZ0019	021	Garrison C	1-Jul	57	1.8	Y	19
14QZ0020	022	Garrison C	1-Jul	102	12.6	Y	20
14QZ0021	023	Garrison C	1-Jul	108	13.5	Y	21
14QZ0022	024	Garrison C	1-Jul	127	20.1	Y	22
14QZ0023	025	Garrison C	1-Jul	184	63.7	Y	23
14QZ0024	026	Garrison C	1-Jul	123	18.6	Y	24
14QZ0025	027	Garrison C	1-Jul	104	12.3	Y	25
14QZ0026	028	Garrison C	1-Jul	113	17.7	Y	26
14QZ0027	029	Garrison C	1-Jul	106	12.3	Y	27
14QZ0028	030	Garrison C	1-Jul	129	21.5	Y	28
14QZ0029	031	Garrison C	1-Jul	90	8.5	Y	29
14QZ0030	032	Garrison C	1-Jul	94	9.4	Y	30
14QZ0031	033	Garrison C	1-Jul	98	10.4	Y	31
14QZ0032	034	Garrison C	1-Jul	69	4.7	Y	32
14QZ0033	135	Garrison A	22-Aug	290	N/A	N	
14QZ0034	141	Garrison B	22-Aug	205	84.3	Y	779
14QZ0035	143	Garrison B	22-Aug	142	27.5	Y	781
14QZ0036	144	Garrison B	22-Aug	136	22.8	Y	782
14QZ0037	145	Garrison B	22-Aug	122	17.7	Y	783
14QZ0038	146	Garrison B	22-Aug	204	96.4	Y	784-787
14QZ0039	147	Garrison B	22-Aug	103	10.1	Y	788-789
14QZ0040	148	Garrison B	22-Aug	198	81.6	Y	790-793
14QZ0041	149	Garrison C	22-Aug	63	3	N	793
14QZ0042	150	Garrison C	22-Aug	61	2.7	N	794-795
14QZ0043	151	Garrison C	22-Aug	59	2.5	N	796-797
14QZ0044	152	Garrison C	22-Aug	122	19	Y	798-799
14QZ0045	153	Garrison C	22-Aug	126	22	Y	800-802
14QZ0046	154	Garrison C	16-Dec	78	5.2	N	1030064 - 0066
14QZ0047	155	Garrison C	16-Dec	110	15.9	N	67-69
14QZ0048	156	Garrison C	16-Dec	60	2.9	N	70-72
14QZ0049	157	Garrison C	16-Dec	46	1.1	N	73-75
14QZ0050	158	Garrison C	16-Dec	62	2.9	N	76-78

Appendix 2. Coastal cutthroat trout hatchery plantings on San Juan Islands. Cascade Lake is adjacent to but separate from Cascade Creek. Mountain Lake is in the Cascade Creek headwaters. Egg Lake is on San Juan Island, but is independent from Garrison Cr. Tokul Creek Hatchery cutthroat broodstock was developed from Lake Whatcom resident cutthroat trout in 1947 and is the broodstock planted in these lakes after 1950 when the first eggs were obtained from the broodstock (Crawford 1979). While the Mountain Lake broodstock program is maintained at Tokul Creek Hatchery, the cutthroat for Mountain Lake are reared at WDFW's Kendall Creek Hatchery on the Nooksack River (Justin Spinelli, WDFW, pers. comm.).

Cascade Lake				**Mountain Lake**	
year	cutthroat			year	cutthroat
1934	18,500			1934	21,000
1951	40,800			1969	29,150
1952	3,208			1978	15,180
1953	123,730			1979	20,125
1954	151,201			1980	15,444
1955	125,625			1981	15,052
1963	7,000			1983	10,000
1980	10,098			2005	15,000
1981	15,052			2006	15,000
1982	14,994			2007	10,000
1983	10,000			2008	20,000
1984	10,664			2009	10,000
1985	10,062			2010	7,575
1987	7,641			2011	10,000
1988	7,905			2012	18,900
1994	10,152			2013	18,900
1995	20,625			2014	18,900
1997	10,198			**Total**	**270,226**
1998	10,010				
1999	40,000				
2000	30,000				
2001	25,000			**Egg Lake**	
2002	27,000			year	cutthroat
2003	20,500			1969	10,500
2004	25,000			1971	5,820
2005	30,000			**Total**	**16,320**
2006	65,000				
2007	30,000				
2009	30,000				
2010	20,000				
2011	30,000				
Total	**979,965**				

Appendix 3. Allele frequencies for microsatellites and all variable SNPs for coastal cutthroat trout populations compared in this study. Allele frequencies are shaded as follows: yellow 0.75 to 1, red 0.5 to 0.75, green 0.1 to 0.5. Dark green cells highlight two alleles that were found in the San Juan Islands Cascade population and not in the TokulCr hatchery collections.

Locus	Allele#	Size	Doe	Garrison	Cascade	14TokulH	01TokulH	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
Ogo-3	1	189	1	1	1	1	1	1	0.975	1	1	0.925	1	1	1	
Ogo-3	2	200	0	0	0	0	0	0	0	0	0	0.075	0	0	0	Nooksack
Ogo-3	3	232	0	0	0	0	0	0	0.025	0	0	0	0	0	0	Snoq
Ogo-3	#	samples:	49	41	46	81	24	19	40	21	20	20	31	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
Omm-1138	1	151	0	0	0	0	0	0	0	0	0	0.025	0	0	0	Nooksack
Omm-1138	2	153	0	0	0.0217	0	0	0	0	0	0	0	0	0	0	Cascade
Omm-1138	3	155	0	0	0	0	0	0.0263	0.0125	0	0	0	0	0	0	
Omm-1138	4	157	0	0	0	0	0	0	0	0	0.025	0	0	0	0	GraysH
Omm-1138	5	159	0	0	0	0	0	0	0.175	0	0	0	0	0	0	Snoq
Omm-1138	6	161	0.3776	0	0.5761	0.5556	0.5625	0.0526	0	0.0476	0	0.075	0.0161	0	0.0147	
Omm-1138	7	165	0.6224	0.8625	0.4022	0.4198	0.4375	0.9211	0.8125	0.881	0.95	0.9	0.9194	0.9844	0.9559	
Omm-1138	8	167	0	0.1375	0	0.0247	0	0	0	0.0714	0.025	0	0.0645	0.0156	0.0294	
Omm-1138	#	samples:	49	40	46	81	24	19	40	21	20	20	31	32	34	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
Omy-77	1	103	0	0	0	0	0	0	0.0119	0	0	0	0	0	0	Snoq
Omy-77	2	113	0.16	0.697	0	0	0	0	0	0.2857	0	0.05	0	0	0	
Omy-77	3	115	0	0	0	0	0	0.3846	0.1905	0	0	0	0	0	0	
Omy-77	4	117	0	0	0	0	0	0	0	0.0476	0.025	0	0.0161	0.0625	0.0588	
Omy-77	5	121	0	0	0	0.0192	0	0	0	0	0	0	0	0	0	14TokulH
Omy-77	6	123	0	0	0	0.0321	0.0455	0.1154	0	0	0.05	0	0.2903	0.3125	0.2353	
Omy-77	7	125	0	0.0455	0.2222	0.0705	0.0227	0	0	0.0238	0.075	0.025	0	0	0.0294	
Omy-77	8	127	0.84	0.2121	0	0	0	0.0769	0	0.0238	0	0	0.0645	0.1094	0.2206	
Omy-77	9	129	0	0	0	0.0064	0	0	0	0	0	0	0.1129	0	0.0294	
Omy-77	10	131	0	0	0	0	0	0	0	0	0	0	0.0161	0.0625	0	
Omy-77	11	133	0	0	0	0.0513	0.0227	0.1538	0.0476	0	0	0.225	0.1613	0.0781	0.1912	
Omy-77	12	135	0	0	0	0	0	0	0.0476	0	0.05	0.05	0	0.0156	0	
Omy-77	13	137	0	0	0.5111	0.3462	0.5682	0.1154	0	0.0952	0.1	0.4	0.0484	0.1094	0.0588	
Omy-77	14	139	0	0	0.2333	0.4038	0.2045	0.0769	0.5	0.0476	0.175	0.2	0.0968	0.0469	0.0882	
Omy-77	15	141	0	0.0455	0.0111	0.0128	0.0227	0	0	0.0476	0.025	0	0	0.0938	0	
Omy-77	16	143	0	0	0	0	0	0.0769	0.1429	0.0714	0.225	0	0.1774	0.0938	0.0294	
Omy-77	17	145	0	0	0	0	0	0	0	0.2381	0.075	0.05	0	0.0156	0.0441	
Omy-77	18	148	0	0	0.0222	0.0385	0.1136	0	0.0595	0	0.05	0	0	0	0	
Omy-77	19	150	0	0	0	0.0192	0	0	0	0.0714	0.05	0	0	0	0	
Omy-77	20	154	0	0	0	0	0	0	0	0	0.1	0	0	0	0	GraysH
Omy-77	21	158	0	0	0	0	0	0	0	0	0	0	0.0161	0	0	Kennedy
Omy-77	22	160	0	0	0	0	0	0	0	0.0238	0	0	0	0	0	Goodman
Omy-77	23	162	0	0	0	0	0	0	0	0.0238	0	0	0	0	0	Goodman
Omy-77	24	164	0	0	0	0	0	0	0	0	0	0	0	0	0.0147	Skookum
Omy-77	#	samples:	50	33	45	78	22	13	42	21	20	20	31	32	34	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
One-108	1	141	0	0	0	0	0	0	0	0	0.15	0	0	0	0.0156	
One-108	2	154	0	0.3125	0	0	0	0.2353	0.0385	0.0476	0.05	0.025	0.0806	0	0.2031	
One-108	3	158	1	0.3875	0.0795	0.0065	0.0455	0.4706	0.4103	0.119	0.275	0.175	0.2903	0.5312	0.2344	
One-108	4	162	0	0	0.1705	0.2727	0.2955	0.1176	0.4615	0.4048	0.15	0.475	0.1613	0.3125	0.25	
One-108	5	166	0	0	0.1591	0.0649	0.1136	0.0294	0	0.2381	0.05	0.05	0.0484	0.0625	0.0781	
One-108	6	171	0	0	0	0	0	0.0588	0.0128	0.0476	0.075	0.1	0	0.0156	0.0156	
One-108	7	175	0	0.25	0.0114	0	0	0	0.0128	0.0476	0.15	0.1	0.0161	0.0156	0.0781	
One-108	8	179	0	0	0.0341	0.0519	0.0227	0	0	0.0714	0.1	0.05	0.2742	0.0469	0.0781	
One-108	9	183	0	0.0125	0	0	0	0	0	0	0	0	0.0806	0.0156	0.0469	

One-108	10	187	0	0.0375	0	0	0	0	0	0	0	0	0	0	Garrison
One-108	11	203	0	0	0	0	0	0	0.0128	0	0	0	0	0	Snoq
One-108	12	208	0	0	0.4659	0.2727	0.3409	0.0882	0	0	0	0	0.0484	0	0
One-108	13	211	0	0	0.0795	0.3312	0.1818	0	0	0	0	0	0	0	0
One-108	14	216	0	0	0	0	0	0	0	0.0238	0	0	0	0	0	Goodman
One-108	15	244	0	0	0	0	0	0	0.0128	0	0	0	0	0	0	Snoq
One-108	16	248	0	0	0	0	0	0	0.0128	0	0	0	0	0	0	Snoq
One-108	17	251	0	0	0	0	0	0	0	0	0	0.025	0	0	0	Nooksack
One-108	18	268	0	0	0	0	0	0	0.0256	0	0	0	0	0	0	Snoq
One-108	#	samples:	50	40	44	77	22	17	39	21	20	20	31	32	32	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
Ots-1	1	172	0	0	0	0	0	0	0	0	0	0.025	0	0	0	Nooksack
Ots-1	2	236	0	0	0	0	0	0	0	0.0476	0	0	0	0	0	Goodman
Ots-1	3	243	0	0	0	0	0	0.0278	0	0	0	0	0.0161	0	0.0441	
Ots-1	4	247	0	0	0	0	0	0	0	0	0	0	0	0.0156	0	McLane
Ots-1	5	258	0	0	0	0	0	0	0	0.0238	0	0	0	0	0	Goodman
Ots-1	6	260	0	0	0	0	0	0	0	0.119	0	0	0	0	0	Goodman
Ots-1	7	262	0	0	0	0.0125	0	0	0	0	0	0	0	0	0	14TokulH
Ots-1	8	264	0	0	0	0	0	0	0.1316	0	0.05	0	0	0	0.0147	
Ots-1	9	266	0	0	0	0	0	0.3889	0.0789	0	0	0	0	0	0	
Ots-1	10	268	0	0.0641	0.8478	0.8	0.9167	0.1667	0.2632	0	0.125	0.275	0.0323	0.0469	0.0588	
Ots-1	11	270	0	0	0	0	0	0	0	0	0.025	0.05	0.0323	0.0469	0.0294	
Ots-1	12	272	0	0	0	0	0	0.0278	0	0.0238	0	0.025	0	0.0156	0	
Ots-1	13	274	0	0	0	0	0	0.0278	0	0	0	0	0	0	0.0147	
Ots-1	14	276	0	0	0.1196	0.0813	0.0625	0	0.2368	0	0	0.025	0.2097	0.1406	0.1029	
Ots-1	15	278	0	0.0769	0	0	0	0.0278	0.0658	0	0.275	0.125	0.2258	0.3438	0.0882	
Ots-1	16	280	0.39	0.2692	0	0	0	0	0	0.119	0.025	0.1	0.0161	0.0156	0.0294	
Ots-1	17	282	0	0.2821	0	0	0	0.3333	0	0	0.175	0.25	0.1613	0.1094	0.1176	
Ots-1	18	285	0.01	0	0	0	0	0	0	0.0476	0	0	0.0323	0.0625	0	
Ots-1	19	287	0	0.0385	0	0	0	0	0.0789	0.0714	0.1	0.025	0.0645	0.1406	0.2647	
Ots-1	20	289	0	0	0	0	0	0	0.0921	0.0714	0.05	0	0.0968	0	0.0588	
Ots-1	21	291	0	0	0	0	0	0	0	0.0714	0.075	0.025	0	0.0469	0.1176	
Ots-1	22	293	0	0	0	0.0063	0	0	0	0.0476	0	0	0	0	0.0147	
Ots-1	23	295	0	0	0.0326	0.0938	0.0208	0	0.0526	0.0476	0	0	0.1129	0	0.0441	
Ots-1	24	297	0	0.0513	0	0	0	0	0	0	0	0	0	0	0	Garrison
Ots-1	25	299	0.18	0.1923	0	0	0	0	0	0	0.05	0.025	0	0.0156	0	
Ots-1	26	301	0.42	0	0	0	0	0	0	0.0476	0	0.05	0	0	0	
Ots-1	27	303	0	0	0	0.0063	0	0	0	0.0952	0	0	0	0	0	
Ots-1	28	306	0	0	0	0	0	0	0	0	0.025	0	0	0	0	GraysH
Ots-1	29	308	0	0.0256	0	0	0	0	0	0.0476	0.025	0	0	0	0	
Ots-1	30	314	0	0	0	0	0	0	0	0.0238	0	0	0	0	0	Goodman
Ots-1	31	318	0	0	0	0	0	0	0	0.0476	0	0	0	0	0	Goodman
Ots-1	32	322	0	0	0	0	0	0	0	0.0476	0	0	0	0	0	Goodman
Ots-1	#	samples:	50	39	46	80	24	18	38	21	20	20	31	32	34	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
Ots-103	1	54	0	0	0	0	0	0	0.3902	0	0	0	0	0	0	Snoq
Ots-103	2	63	0	0	0	0	0	0	0	0.1667	0	0	0.1	0.1406	0.0152	
Ots-103	3	66	1	1	1	1	0.9583	0.9737	0.5976	0.6667	0.825	1	0.9	0.8594	0.9697	
Ots-103	4	70	0	0	0	0	0	0	0	0.1667	0.125	0	0	0	0.0152	
Ots-103	5	74	0	0	0	0	0	0	0	0	0.05	0	0	0	0	GraysH
Ots-103	6	86	0	0	0	0	0	0.0263	0	0	0	0	0	0	0	Cedar
Ots-103	7	88	0	0	0	0	0.0417	0	0.0122	0	0	0	0	0	0	
Ots-103	#	samples:	50	41	45	78	24	19	41	21	20	20	30	32	33	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
Ots-3M	1	134	0	0.0769	0.1444	0.1899	0.1364	0.1667	0	0.0714	0.075	0.225	0.2097	0.1875	0.2206	
Ots-3M	2	143	0	0	0	0	0.0227	0	0	0	0	0.025	0	0	0	
Ots-3M	3	147	0	0	0	0	0	0	0	0	0	0	0.1452	0	0	Kennedy
Ots-3M	4	156	0	0	0	0	0	0	0	0	0.025	0	0	0	0	GraysH

Ots-3M	5	158	0	0	0	0	0	0	0	0	0.05	0	0	0	0.0294	
Ots-3M	6	160	0	0	0	0	0	0	0	0.0238	0	0	0	0	0	Goodman
Ots-3M	7	162	0	0	0	0	0	0	0	0	0.1	0	0	0	0	GraysH
Ots-3M	8	164	0	0	0	0	0	0	0	0	0.1	0	0	0	0	GraysH
Ots-3M	9	166	0	0.1026	0	0	0	0.25	0	0.0238	0	0	0	0	0	
Ots-3M	10	168	0	0	0.2	0	0.0455	0	0	0.0238	0	0	0.0484	0	0	
Ots-3M	11	170	0	0	0.0111	0.038	0	0.0417	0.0366	0.0952	0.1	0.075	0.0806	0.2031	0.2206	
Ots-3M	12	172	0	0	0.1222	0.0633	0.0909	0	0	0.119	0	0	0.0323	0	0	
Ots-3M	13	174	0	0	0	0.0063	0	0.0417	0	0.119	0.025	0.025	0	0.0156	0	
Ots-3M	14	176	0	0	0	0	0	0	0.0976	0.119	0	0	0	0	0	
Ots-3M	15	178	0	0.0769	0	0	0	0.2083	0.3415	0	0	0.025	0.129	0.2188	0.25	
Ots-3M	16	180	0.76	0.7179	0	0	0.0227	0.0417	0.2927	0.1429	0	0.075	0.0806	0.0469	0.1471	
Ots-3M	17	182	0	0	0	0	0	0.0833	0.0366	0.0476	0	0.125	0.0806	0.0156	0	
Ots-3M	18	184	0	0	0	0	0	0	0.0122	0.0238	0.075	0.25	0	0	0	
Ots-3M	19	186	0.24	0.0256	0	0	0	0.0417	0.061	0.0476	0.05	0.175	0	0	0	
Ots-3M	20	188	0	0	0	0	0	0	0.122	0	0.05	0	0	0	0	
Ots-3M	21	190	0	0	0	0	0	0	0	0	0.025	0	0.0323	0.0781	0.0147	
Ots-3M	22	192	0	0	0	0	0	0	0	0	0.075	0	0	0	0	GraysH
Ots-3M	23	194	0	0	0	0	0	0	0	0.0476	0.025	0	0	0	0	
Ots-3M	24	197	0	0	0	0.0063	0	0.0833	0	0.0238	0	0	0	0.0469	0	
Ots-3M	25	199	0	0	0.3111	0.3924	0.3636	0	0	0	0	0	0	0	0	
Ots-3M	26	201	0	0	0	0	0	0.0417	0	0	0.025	0	0	0	0	
Ots-3M	27	203	0	0	0	0.019	0	0	0	0	0.1	0	0	0	0	
Ots-3M	28	205	0	0	0.2111	0.2848	0.3182	0	0	0.0238	0	0	0	0	0.0147	
Ots-3M	29	207	0	0	0	0	0	0	0	0.0238	0.05	0	0	0.0625	0	
Ots-3M	30	209	0	0	0	0	0	0	0	0	0	0	0.0161	0	0.0441	
Ots-3M	31	210	0	0	0	0	0	0	0	0.0238	0	0	0.0806	0.0156	0	
Ots-3M	32	213	0	0	0	0	0	0	0	0	0.025	0	0.0323	0.1094	0.0441	
Ots-3M	33	215	0	0	0	0	0	0	0	0	0.025	0	0	0	0	GraysH
Ots-3M	34	217	0	0	0	0	0	0	0	0	0	0	0	0	0.0147	Skookum
Ots-3M	35	218	0	0	0	0	0	0	0	0	0	0	0.0323	0	0	Kennedy
Ots-3M	#	samples:	50	39	45	79	22	12	41	21	20	20	31	32	34	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl001	1	2	0.88	0.3125	0.0326	0.0185	0	0.525	0.1548	0.4286	0.275	0.4762	0.5938	0.4219	0.4571	
AOcl001	2	4	0.12	0.6875	0.9674	0.9815	1	0.475	0.8452	0.5714	0.725	0.5238	0.4062	0.5781	0.5429	
AOcl001	#	samples:	50	40	46	81	23	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl002	1	3	0	0.907	0.3902	0.3395	0.2917	0.4	0.5139	~~~~~~	~~~~~~	~~~~~~	0.7812	0.85	0.6429	
AOcl002	2	5	1	0.093	0.6098	0.6605	0.7083	0.6	0.4861	~~~~~~	~~~~~~	~~~~~~	0.2188	0.15	0.3571	
AOcl002	#	samples:	46	43	41	81	24	20	36	~~~~~~	~~~~~~	~~~~~~	32	10	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl003	1	2	0.01	0.1628	0.3913	0.2778	0.1875	0.15	0.631	0.7143	0.725	0.4762	0.5312	0.4375	0.4	
AOcl003	2	4	0.99	0.8372	0.6087	0.7222	0.8125	0.85	0.369	0.2857	0.275	0.5238	0.4688	0.5625	0.6	
AOcl003	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl004	1	2	0.42	0.3214	0.9024	0.7	0.8	0.8333	0.55	0.4062	0.5	0.5588	0.6875	0.4643	0.4412	
AOcl004	2	5	0.58	0.6786	0.0976	0.3	0.2	0.1667	0.45	0.5938	0.5	0.4412	0.3125	0.5357	0.5588	
AOcl004	#	samples:	50	42	41	80	20	15	30	16	14	17	32	28	34	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl005	1	3	0.5761	0.7791	0.2738	0.1235	0.2708	0.525	0.7976	0.6429	0.8421	0.775	0.7812	0.7344	0.9429	
AOcl005	2	4	0.4239	0.2209	0.7262	0.8765	0.7292	0.475	0.2024	0.3571	0.1579	0.225	0.2188	0.2656	0.0571	
AOcl005	#	samples:	46	43	42	81	24	20	42	21	19	20	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl006	1	2	0.54	0.5976	0.8261	0.8519	0.8542	0.5	0.4762	0.619	0.6053	0.9737	0.7031	0.5968	0.5714	
AOcl006	2	4	0.46	0.4024	0.1739	0.1481	0.1458	0.5	0.5238	0.381	0.3947	0.0263	0.2969	0.4032	0.4286	

AOcl006	#	samples:	50	41	46	81	24	20	42	21	19	19	32	31	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl007	1	2	0.26	0.369	0.6304	0.6049	0.5625	0.35	0.3214	0.2619	0.25	0.2381	0.625	0.5156	0.3143	
AOcl007	2	4	0.74	0.631	0.3696	0.3951	0.4375	0.65	0.6786	0.7381	0.75	0.7619	0.375	0.4844	0.6857	
AOcl007	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl008	1	2	0.47	0.2381	0.5	0.2778	0.2917	0.3	0.3659	0.4524	0.4	0.0952	0.3906	0.2812	0.4286	
AOcl008	2	4	0.53	0.7619	0.5	0.7222	0.7083	0.7	0.6341	0.5476	0.6	0.9048	0.6094	0.7188	0.5714	
AOcl008	#	samples:	50	42	45	81	24	20	41	21	20	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl009	1	2	0.4457	0.3488	0	0	0	0	0.1125	0.1842	0.1667	0	0	0.1613	0.0143	
AOcl009	2	4	0.5543	0.6512	1	1	1	1	0.8875	0.8158	0.8333	1	1	0.8387	0.9857	
AOcl009	#	samples:	46	43	39	79	24	20	40	19	15	18	32	31	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl010	1	2	0	0.0714	0.0122	0.0123	0	0.025	0.0119	0.1875	0.4286	0.1875	0.0938	0.0517	0.0429	
AOcl010	2	3	1	0.9286	0.9878	0.9877	1	0.975	0.9881	0.8125	0.5714	0.8125	0.9062	0.9483	0.9571	
AOcl010	#	samples:	46	42	41	81	24	20	42	16	14	16	32	29	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl011	1	3	1	0.9767	0.4556	0.3642	0.3958	0.9	0.939	0.7143	0.8684	0.825	0.8594	0.7031	0.5714	
AOcl011	2	5	0	0.0233	0.5444	0.6358	0.6042	0.1	0.061	0.2857	0.1316	0.175	0.1406	0.2969	0.4286	
AOcl011	#	samples:	50	43	45	81	24	20	41	21	19	20	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl012	1	4	1	1	1	1	1	1	1	0.9737	0.8824	1	1	1	1	
AOcl012	2	5	0	0	0	0	0	0	0	0.0263	0.1176	0	0	0	0	
AOcl012	#	samples:	48	42	42	81	24	20	42	19	17	19	32	31	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl013	1	3	1	1	1	1	1	1	1	0.8571	0.8421	0.9762	0.9062	1	0.8571	
AOcl013	2	5	0	0	0	0	0	0	0	0.1429	0.1579	0.0238	0.0938	0	0.1429	
AOcl013	#	samples:	50	42	46	81	24	20	42	21	19	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl014	1	3	1	0.593	1	0.9259	1	1	1	1	1	1	0.9062	0.9839	0.9429	
AOcl014	2	5	0	0.407	0	0.0741	0	0	0	0	0	0	0.0938	0.0161	0.0571	
AOcl014	#	samples:	50	43	46	81	19	12	11	17	16	19	32	31	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl015	1	2	0	0	0	0	0	0	0	0	0	0	0.0156	0	0	Kennedy
AOcl015	2	4	1	1	1	1	1	1	1	1	1	1	0.9844	1	1	
AOcl015	#	samples:	50	43	46	81	24	20	42	17	14	18	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl016	1	3	0.08	0.8372	0.5217	0.6605	0.5625	0.875	1	0.7381	0.875	0.7619	0.9062	0.875	0.9286	
AOcl016	2	4	0.92	0.1628	0.4783	0.3395	0.4375	0.125	0	0.2619	0.125	0.2381	0.0938	0.125	0.0714	
AOcl016	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl017	1	3	0	0.1395	0.2159	0.1914	0.2083	0.175	0.1548	0.5	0.725	0.6053	0.2188	0.25	0.1	
AOcl017	2	4	1	0.8605	0.7841	0.8086	0.7917	0.825	0.8452	0.5	0.275	0.3947	0.7812	0.75	0.9	
AOcl017	#	samples:	48	43	44	81	24	20	42	21	20	19	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl018	1	3	0.75	0.8953	0.25	0.0556	0	0.275	0.0119	0.381	0.2368	0.2857	0.2031	0.4844	0.2	
AOcl018	2	5	0.25	0.1047	0.75	0.9444	1	0.725	0.9881	0.619	0.7632	0.7143	0.7969	0.5156	0.8	

AOcl018	#	samples:	50	43	46	81	24	20	42	21	19	21	32	32	35

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl019	1	3	0	0	0	0	0	0.125	0.1905	0.2647	0.0333	0.1667	0.0156	0	0.0429	
AOcl019	2	5	1	1	1	1	1	0.875	0.8095	0.7353	0.9667	0.8333	0.9844	1	0.9571	
AOcl019	#	samples:	50	42	46	81	24	20	42	17	15	18	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl020	1	2	0.59	0.0349	0.0109	0.1111	0.0625	0.25	0.2143	0.3095	0.15	0.2143	0.5781	0.5	0.5143	
AOcl020	2	5	0.41	0.9651	0.9891	0.8889	0.9375	0.75	0.7857	0.6905	0.85	0.7857	0.4219	0.5	0.4857	
AOcl020	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl022	1	4	1	0.9767	0.9783	1	1	0.925	0.7857	0.75	0.85	0.925	0.8281	0.8906	0.9143	
AOcl022	2	5	0	0.0233	0.0217	0	0	0.075	0.2143	0.25	0.15	0.075	0.1719	0.1094	0.0857	
AOcl022	#	samples:	50	43	46	81	24	20	42	20	20	20	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl023	1	2	0.98	0.9651	0.5109	0.716	0.4792	0.875	0.6786	0.4762	0.725	0.8571	0.9062	0.9219	0.9	
AOcl023	2	3	0.02	0.0349	0.4891	0.284	0.5208	0.125	0.3214	0.5238	0.275	0.1429	0.0938	0.0781	0.1	
AOcl023	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl024	1	3	0.99	0.75	0.7717	0.9938	0.9167	0.575	0.7738	0.5952	0.75	0.6667	0.75	0.5781	0.7	
AOcl024	2	5	0.01	0.25	0.2283	0.0062	0.0833	0.425	0.2262	0.4048	0.25	0.3333	0.25	0.4219	0.3	
AOcl024	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl025	1	3	1	0.9535	1	1	1	1	1	0.95	1	1	1	1	0.9857	
AOcl025	2	4	0	0.0465	0	0	0	0	0	0.05	0	0	0	0	0.0143	
AOcl025	#	samples:	50	43	46	81	24	20	42	20	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl026	1	2	0	0	0	0	0	0.025	0.0119	0.0476	0.175	0.0952	0	0	0	
AOcl026	2	3	1	1	1	1	1	0.975	0.9881	0.9524	0.825	0.9048	1	1	1	
AOcl026	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl027	1	2	0.97	0.9419	0.9457	0.9815	0.9792	1	0.8095	0.9737	0.9375	1	0.9688	0.9844	0.9714	
AOcl027	2	4	0.03	0.0581	0.0543	0.0185	0.0208	0	0.1905	0.0263	0.0625	0	0.0312	0.0156	0.0286	
AOcl027	#	samples:	50	43	46	81	24	20	42	19	16	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl028	1	3	0	0	0.1744	0.2469	0.1458	0	0	0.1471	0.0714	0.1111	0.0625	0.0167	0.0286	
AOcl028	2	5	1	1	0.8256	0.7531	0.8542	1	1	0.8529	0.9286	0.8889	0.9375	0.9833	0.9714	
AOcl028	#	samples:	46	43	43	81	24	20	42	17	14	18	32	30	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl029	1	2	0.57	0.6512	0.8913	0.9012	0.875	0.675	0.881	0.5833	0.7333	0.3824	0.6562	0.4833	0.6143	
AOcl029	2	4	0.43	0.3488	0.1087	0.0988	0.125	0.325	0.119	0.4167	0.2667	0.6176	0.3438	0.5167	0.3857	
AOcl029	#	samples:	50	43	46	81	24	20	42	18	15	17	32	30	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl030	1	2	0	0	0.2283	0.5309	0.4167	0	0	0.05	0.0556	0.0263	0	0	0.0571	
AOcl030	2	4	1	1	0.7717	0.4691	0.5833	1	1	0.95	0.9444	0.9737	1	1	0.9429	
AOcl030	#	samples:	50	42	46	81	24	20	42	20	18	19	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl031	1	2	1	0.6395	0.7826	0.5556	0.4583	0.6	0.4762	0.7857	0.5789	0.7619	0.5781	0.625	0.8286	
AOcl031	2	5	0	0.3605	0.2174	0.4444	0.5417	0.4	0.5238	0.2143	0.4211	0.2381	0.4219	0.375	0.1714	

AOcl031	#	samples:	50	43	46	81	24	20	42	21	19	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl032	1	2	0.6	0.9302	0.9674	0.8642	0.9792	0.3	0.8095	1	0.8611	0.7	0.875	0.6875	0.5286	
AOcl032	2	4	0.4	0.0698	0.0326	0.1358	0.0208	0.7	0.1905	0	0.1389	0.3	0.125	0.3125	0.4714	
AOcl032	#	samples:	50	43	46	81	24	20	42	21	18	20	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl033	1	2	0.49	0.6905	1	0.9877	1	0.35	0.6585	0.8571	0.7368	0.675	0.875	0.8871	0.8	
AOcl033	2	3	0.51	0.3095	0	0.0123	0	0.65	0.3415	0.1429	0.2632	0.325	0.125	0.1129	0.2	
AOcl033	#	samples:	50	42	46	81	24	20	41	21	19	20	32	31	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl034	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	
AOcl034	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl035	1	3	1	1	1	1	1	1	1	0.9211	0.7632	0.9474	1	1	1	
AOcl035	2	5	0	0	0	0	0	0	0	0.0789	0.2368	0.0526	0	0	0	
AOcl035	#	samples:	50	43	46	81	24	20	42	19	19	19	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl036	1	2	0	0	0	0	0	0.125	0	0.1667	0.275	0	0.0781	0.1562	0.0571	
AOcl036	2	4	1	1	1	1	1	0.875	1	0.8333	0.725	1	0.9219	0.8438	0.9429	
AOcl036	#	samples:	50	43	46	81	24	20	42	21	20	20	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl037	1	3	1	0.1163	0.8587	0.8457	0.8333	0.3	0.4881	0.3	0.3947	0.3421	0.25	0.2344	0.2714	
AOcl037	2	4	0	0.8837	0.1413	0.1543	0.1667	0.7	0.5119	0.7	0.6053	0.6579	0.75	0.7656	0.7286	
AOcl037	#	samples:	50	43	46	81	24	20	42	20	19	19	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl038	1	4	0.42	0.7442	0.0652	0.0926	0.1458	0.25	0.369	0.5	0.5526	0.25	0.3125	0.3438	0.4429	
AOcl038	2	5	0.58	0.2558	0.9348	0.9074	0.8542	0.75	0.631	0.5	0.4474	0.75	0.6875	0.6562	0.5571	
AOcl038	#	samples:	50	43	46	81	24	20	42	21	19	20	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl039	1	3	0	0.3571	0.5	0.2593	0.125	0.45	0.5714	0.5476	0.575	0.3571	0.4688	0.4375	0.6143	
AOcl039	2	5	1	0.6429	0.5	0.7407	0.875	0.55	0.4286	0.4524	0.425	0.6429	0.5312	0.5625	0.3857	
AOcl039	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl040	1	4	1	0.8333	0.7609	0.7407	0.6667	0.725	0.9762	0.7381	0.65	0.9286	0.4219	0.625	0.5	
AOcl040	2	5	0	0.1667	0.2391	0.2593	0.3333	0.275	0.0238	0.2619	0.35	0.0714	0.5781	0.375	0.5	
AOcl040	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl041	1	2	0	0.0595	0	0	0	0.025	0.2619	0.1111	0.1111	0.2105	0.0156	0.1207	0.1714	
AOcl041	2	5	1	0.9405	1	1	1	0.975	0.7381	0.8889	0.8889	0.7895	0.9844	0.8793	0.8286	
AOcl041	#	samples:	50	42	46	81	24	20	42	18	18	19	32	29	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl042	1	3	0	0	0.0761	0.037	0.0833	0	0.0595	0.0952	0.15	0.15	0.0781	0.125	0.1857	
AOcl042	2	5	1	1	0.9239	0.963	0.9167	1	0.9405	0.9048	0.85	0.85	0.9219	0.875	0.8143	
AOcl042	#	samples:	50	43	46	81	24	20	42	21	20	20	32	32	35	
Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl043	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	
AOcl043	#	samples:	50	43	46	81	24	20	42	16	14	18	32	30	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl044	1	2	0.5556	1	0.9756	1	0.9792	0.975	1	1	1	1	0.9844	1	0.9857	
AOcl044	2	4	0.4444	0	0.0244	0	0.0208	0.025	0	0	0	0	0.0156	0	0.0143	
AOcl044	#	samples:	45	43	41	81	24	20	42	18	15	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl045	1	4	0.163	1	1	1	1	1	1	0.9474	0.8438	0.9722	0.9219	0.9375	0.8857	
AOcl045	2	5	0.837	0	0	0	0	0	0	0.0526	0.1562	0.0278	0.0781	0.0625	0.1143	
AOcl045	#	samples:	46	43	43	81	24	20	42	19	16	18	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl046	1	2	0	0	0.2174	0.2099	0.1875	0	0.1905	0.2105	0	0.1579	0	0	0.0143	
AOcl046	2	4	1	1	0.7826	0.7901	0.8125	1	0.8095	0.7895	1	0.8421	1	1	0.9857	
AOcl046	#	samples:	50	42	46	81	24	20	42	19	16	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl047	1	2	0.93	0.4762	0	0	0	0.2632	0.4286	0.5	0.5556	0.3158	0.2969	0.371	0.2571	
AOcl047	2	4	0.07	0.5238	1	1	1	0.7368	0.5714	0.5	0.4444	0.6842	0.7031	0.629	0.7429	
AOcl047	#	samples:	50	42	46	81	24	19	42	19	18	19	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl048	1	3	1	1	1	0.9691	1	1	1	0.75	0.9286	1	1	1	1	
AOcl048	2	5	0	0	0	0.0309	0	0	0	0.25	0.0714	0	0	0	0	
AOcl048	#	samples:	46	43	39	81	24	20	42	16	14	18	32	30	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl049	1	3	1	1	1	1	1	1	1	0.7	0.7778	0.9737	0.9688	1	1	
AOcl049	2	5	0	0	0	0	0	0	0	0.3	0.2222	0.0263	0.0312	0	0	
AOcl049	#	samples:	50	43	46	81	24	20	42	20	18	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl050	1	2	0.25	0.7375	0.3333	0.3827	0.3261	0.375	0.0357	0.2778	0.5	0.4643	0.4844	0.371	0.4857	
AOcl050	2	4	0.75	0.2625	0.6667	0.6173	0.6739	0.625	0.9643	0.7222	0.5	0.5357	0.5156	0.629	0.5143	
AOcl050	#	samples:	46	40	42	81	23	20	42	18	18	14	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl051	1	3	0	0.25	0.7375	0.7284	0.7708	0.3	0.2949	0.5312	0.7083	0.625	0.4062	0.2778	0.2714	
AOcl051	2	5	1	0.75	0.2625	0.2716	0.2292	0.7	0.7051	0.4688	0.2917	0.375	0.5938	0.7222	0.7286	
AOcl051	#	samples:	46	42	40	81	24	20	39	16	12	16	32	27	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl052	1	3	0	0.2143	0.087	0.1605	0.1458	0.625	0.4048	0.3571	0.5	0.5476	0.5156	0.4219	0.3286	
AOcl052	2	5	1	0.7857	0.913	0.8395	0.8542	0.375	0.5952	0.6429	0.5	0.4524	0.4844	0.5781	0.6714	
AOcl052	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl053	1	2	0.99	0.4405	0.6957	0.9383	0.9167	0.65	0.4881	0.4286	0.425	0.5952	0.7812	0.7969	0.7	
AOcl053	2	4	0.01	0.5595	0.3043	0.0617	0.0833	0.35	0.5119	0.5714	0.575	0.4048	0.2188	0.2031	0.3	
AOcl053	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl054	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	
AOcl054	#	samples:	50	41	46	81	24	20	42	18	14	18	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl055	1	2	0	0.1375	0.3415	0.5325	0.4583	0.65	0.0769	0.4118	0.625	0.1471	0.2258	0.2333	0.3286	
AOcl055	2	3	1	0.8625	0.6585	0.4675	0.5417	0.35	0.9231	0.5882	0.375	0.8529	0.7742	0.7667	0.6714	
AOcl055	#	samples:	46	40	41	77	24	20	39	17	16	17	31	30	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl056	1	3	0.7128	0.869	0.9878	1	1	0.85	0.9286	0.5938	0.8846	0.8056	0.9375	0.9167	0.9714	

AOcl056	2	5	0.2872	0.131	0.0122	0	0	0.15	0.0714	0.4062	0.1154	0.1944	0.0625	0.0833	0.0286
AOcl056	#	samples:	47	42	41	81	24	20	42	16	13	18	32	30	35

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl057	1	2	0.28	0.8721	0.7609	0.4568	0.5833	0.85	0.5238	0.6429	0.55	0.6667	0.3906	0.5312	0.4857	
AOcl057	2	3	0.72	0.1279	0.2391	0.5432	0.4167	0.15	0.4762	0.3571	0.45	0.3333	0.6094	0.4688	0.5143	
AOcl057	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl058	1	2	1	1	0.0444	0.0679	0.0833	0.55	0.5595	0.6389	0.4167	0.2632	0.5469	0.5938	0.5143	
AOcl058	2	3	0	0	0.9556	0.9321	0.9167	0.45	0.4405	0.3611	0.5833	0.7368	0.4531	0.4062	0.4857	
AOcl058	#	samples:	46	43	45	81	24	20	42	18	18	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl059	1	3	0.99	0.1512	0.087	0.2654	0.1875	0.65	0.7024	0.325	0.25	0.35	0.375	0.1094	0.4	
AOcl059	2	4	0.01	0.8488	0.913	0.7346	0.8125	0.35	0.2976	0.675	0.75	0.65	0.625	0.8906	0.6	
AOcl059	#	samples:	50	43	46	81	24	20	42	20	20	20	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl060	1	3	0.26	0.186	0.163	0.1938	0.2708	0.25	0.0976	0.6111	0.625	0.5714	0.0833	0.2353	0.0147	
AOcl060	2	5	0.74	0.814	0.837	0.8063	0.7292	0.75	0.9024	0.3889	0.375	0.4286	0.9167	0.7647	0.9853	
AOcl060	#	samples:	50	43	46	80	24	18	41	9	8	7	30	17	34	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl061	1	3	1	0.9881	0.9111	0.9938	0.9792	0.75	0.4643	0.6667	0.8056	0.7895	0.8438	0.8125	0.8714	
AOcl061	2	5	0	0.0119	0.0889	0.0062	0.0208	0.25	0.5357	0.3333	0.1944	0.2105	0.1562	0.1875	0.1286	
AOcl061	#	samples:	50	42	45	81	24	20	42	18	18	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl062	1	3	0.18	0.0233	0.7065	0.8704	0.9167	0.35	0.375	0.5789	0.0833	0.4474	0.5968	0.5	0.4286	
AOcl062	2	4	0.82	0.9767	0.2935	0.1296	0.0833	0.65	0.625	0.4211	0.9167	0.5526	0.4032	0.5	0.5714	
AOcl062	#	samples:	50	43	46	81	24	20	40	19	18	19	31	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl063	1	3	1	0.9419	0.9565	0.8333	0.8542	0.55	0.5714	0.7619	0.525	0.7619	0.6094	0.6094	0.7429	
AOcl063	2	5	0	0.0581	0.0435	0.1667	0.1458	0.45	0.4286	0.2381	0.475	0.2381	0.3906	0.3906	0.2571	
AOcl063	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl064	1	2	1	1	1	1	1	1	1	1	1	1	1	0.9844	0.9714	
AOcl064	2	5	0	0	0	0	0	0	0	0	0	0	0	0.0156	0.0286	
AOcl064	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOcl065	1	2	0.35	0.0244	0.8261	0.8827	0.9167	0.525	0.4762	0.3333	0.325	0.6053	0.6719	0.5625	0.6429	
AOcl065	2	5	0.65	0.9756	0.1739	0.1173	0.0833	0.475	0.5238	0.6667	0.675	0.3947	0.3281	0.4375	0.3571	
AOcl065	#	samples:	50	41	46	81	24	20	42	21	20	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy004	1	2	0	0.0244	0.0978	0.216	0.1458	0.225	0.6071	0.1579	0.1316	0.2368	0.1562	0.371	0.1143	
AOmy004	2	3	1	0.9756	0.9022	0.784	0.8542	0.775	0.3929	0.8421	0.8684	0.7632	0.8438	0.629	0.8857	
AOmy004	#	samples:	50	41	46	81	24	20	42	19	19	19	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy048	1	3	0	0.8537	1	1	1	0.875	0.4881	1	0.8667	0.8611	0.9688	0.9483	0.8714	
AOmy048	2	5	1	0.1463	0	0	0	0.125	0.5119	0	0.1333	0.1389	0.0312	0.0517	0.1286	
AOmy048	#	samples:	48	41	45	81	24	20	42	17	15	18	32	29	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy049	1	4	0	0.1977	0.0978	0.0185	0.0208	0	0	0.0952	0.0526	0.1579	0	0.0156	0.0857	
AOmy049	2	5	1	0.8023	0.9022	0.9815	0.9792	1	1	0.9048	0.9474	0.8421	1	0.9844	0.9143	

AOmy049	#	samples:	50	43	46	81	24	20	42	21	19	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy063	1	3	0.01	0.2619	0.9239	1	0.9375	0.675	0.4762	0.525	0.4474	0.5789	0.5156	0.6	0.7429	
AOmy063	2	4	0.99	0.7381	0.0761	0	0.0625	0.325	0.5238	0.475	0.5526	0.4211	0.4844	0.4	0.2571	
AOmy063	#	samples:	50	42	46	81	24	20	42	20	19	19	32	30	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy064	1	2	1	0.9524	0.5326	0.3086	0.3333	0.325	0.5714	0.7381	0.75	0.275	0.7188	0.6875	0.8	
AOmy064	2	4	0	0.0476	0.4674	0.6914	0.6667	0.675	0.4286	0.2619	0.25	0.725	0.2812	0.3125	0.2	
AOmy064	#	samples:	50	42	46	81	24	20	42	21	20	20	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy210	1	4	0.45	0.9881	0.7841	0.8704	0.9375	0.875	0.7073	0.9762	0.8158	0.8947	0.7188	0.8281	0.6857	
AOmy210	2	5	0.55	0.0119	0.2159	0.1296	0.0625	0.125	0.2927	0.0238	0.1842	0.1053	0.2812	0.1719	0.3143	
AOmy210	#	samples:	50	42	44	81	24	20	41	21	19	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy252	1	4	0.0102	0	0.3784	0.2778	0.0625	0.075	0.0238	0.1111	0.2188	0.0278	0.175	0.05	0.1304	
AOmy252	2	5	0.9898	1	0.6216	0.7222	0.9375	0.925	0.9762	0.8889	0.7812	0.9722	0.825	0.95	0.8696	
AOmy252	#	samples:	49	37	37	72	24	20	42	18	16	18	20	30	23	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy258	1	4	0.7805	0.7955	0.7037	0.7279	0.8333	0.8947	0.7561	0.881	0.7	0.875	0.881	0.875	0.7609	
AOmy258	2	5	0.2195	0.2045	0.2963	0.2721	0.1667	0.1053	0.2439	0.119	0.3	0.125	0.119	0.125	0.2391	
AOmy258	#	samples:	41	22	27	68	24	19	41	21	20	20	21	28	23	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy330	1	3	0	0	0	0	0	0.025	0	0	0.0882	0.0263	0	0.0156	0.0143	
AOmy330	2	5	1	1	1	1	1	0.975	1	1	0.9118	0.9737	1	0.9844	0.9857	
AOmy330	#	samples:	46	41	39	81	24	20	41	19	17	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
AOmy342	1	2	1	1	1	1	1	1	1	1	1	0.9762	1	1	1	
AOmy342	2	5	0	0	0	0	0	0	0	0	0	0.0238	0	0	0	Nooksack
AOmy342	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpl029	1	4	1	1	1	1	1	1	0.5238	1	1	1	1	1	1	
ASpl029	2	5	0	0	0	0	0	0	0.4762	0	0	0	0	0	0	Snoq
ASpl029	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpl030	1	3	0	0.0595	0	0	0	0.15	0.0119	0	0	0	0.125	0.2097	0.1	
ASpl030	2	5	1	0.9405	1	1	1	0.85	0.9881	1	1	1	0.875	0.7903	0.9	
ASpl030	#	samples:	50	42	46	81	24	20	42	20	19	19	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpl032	1	3	1	0.9881	1	1	1	1	1	1	1	1	1	1	1	
ASpl032	2	5	0	0.0119	0	0	0	0	0	0	0	0	0	0	0	Garrison
ASpl032	#	samples:	50	42	46	81	24	18	41	21	19	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpl033	1	2	0	0.0349	0.413	0.2037	0.1875	0.3	0.0366	0	0.125	0.0238	0.1406	0.1562	0.0143	
ASpl033	2	3	1	0.9651	0.587	0.7963	0.8125	0.7	0.9634	1	0.875	0.9762	0.8594	0.8438	0.9857	
ASpl033	#	samples:	50	43	46	81	24	20	41	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpl037	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpl037	#	samples:	50	42	46	81	24	20	42	19	15	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI038	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI038	#	samples:	46	42	41	81	24	20	42	17	13	18	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI040	1	2	0	0	0	0	0	0	0.0238	0	0	0	0	0	0	Snoq
ASpI040	2	4	1	1	1	1	1	1	0.9762	1	1	1	1	1	1	
ASpI040	#	samples:	50	42	46	81	24	20	42	21	20	21	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI042	1	4	0	0	0	0	0	0	0	0.05	0	0	0	0	0	Goodman
ASpI042	2	5	1	1	1	1	1	1	1	0.95	1	1	1	1	1	
ASpI042	#	samples:	50	42	46	81	24	20	42	20	18	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI044	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI044	#	samples:	50	43	46	81	24	20	42	19	16	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI046	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI046	#	samples:	50	42	46	81	24	20	41	20	19	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI048	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI048	#	samples:	50	40	46	80	24	20	40	19	18	19	32	31	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI052	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI052	#	samples:	49	43	46	81	24	20	42	19	17	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI053	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI053	#	samples:	50	42	46	81	24	20	42	19	15	19	32	32	35	

Locus	Allele#	Size	Doe	Garrison	Cascade	14Tokul	01Tokul	Cedar	Snoq	Goodman	GraysH	Nooksack	Kennedy	McLane	Skookum	Private?
ASpI055	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	
ASpI055	#	samples:	50	43	46	81	24	20	42	21	20	21	32	32	35	

Appendix 4. Individual ancestry values and average over all Cascade individuals from STRUCTURE analysis of Tokul Hatchery versus Cascade Creek cutthroat trout at K =2, averaged over five runs. Values are plotted in Figure 7. Individuals 1 and 5 were the remaining individuals in the large full-sibling family. Green cells were 0.1 to 0.6 ancestry and pink cells were >0.6 ancestry.

Reach	avg "Tokul"	avg "Cascade"
A	0.486	0.514
B	0.205	0.795
C	0.055	0.945
D	0.116	0.884
E	0.385	0.615
A_14QW0001*	0.025	0.975
A_14QW0002	0.770	0.230
A_14QW0003	0.832	0.168
A_14QW0004	0.018	0.982
A_14QW0005*	0.014	0.986
A_14QW0007	0.921	0.079
A_14QW0008	0.773	0.227
A_14QW0009	0.977	0.023
A_14QW0024	0.039	0.961
B_14QW0010	0.029	0.971
B_14QW0014	0.057	0.943
B_14QW0016	0.014	0.986
B_14QW0017	0.065	0.935
B_14QW0019	0.188	0.812
B_14QW0022	0.879	0.121
B_14QW0023	0.202	0.798
C_14QW0035	0.046	0.954
C_14QW0036	0.043	0.957
C_14QW0037	0.033	0.967
C_14QW0038	0.019	0.981
C_14QW0039	0.147	0.853
C_14QW0040	0.080	0.920
C_14QW0041	0.038	0.962
C_14QW0042	0.032	0.968
C_14QW0043	0.042	0.958
C_14QW0044	0.071	0.929
D_14QW0045	0.021	0.979
D_14QW0046	0.103	0.897
D_14QW0047	0.029	0.971
D_14QW0048	0.351	0.649
D_14QW0049	0.074	0.926
E_14QW0025	0.155	0.845
E_14QW0026	0.045	0.955
E_14QW0027	0.273	0.727
E_14QW0028	0.482	0.518
E_14QW0029	0.045	0.955
E_14QW0030	0.869	0.131
E_14QW0031	0.832	0.168
E_14QW0032	0.075	0.925
E_14QW0034	0.689	0.311