Introgressive Hybridization Between Native and Hatchery-origin Non-native Steelhead (*Oncorhynchus mykiss*)

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Abstract

1. The artificial-propagation and release of individuals from non-local populations is a widespread practice that can threaten the genetic integrity of native, locally adapted populations due to domestication effects from the artificial rearing environments and inter-breeding with the local populations. We examined introgressive hybridization in a threatened population of winter-run steelhead (anadromous *Oncorhynchus mykiss*) in the Willamette Basin, Oregon. The non-local, hatchery, summer-run steelhead is released annually into the basin as mitigation for the impact of numerous dams.
2. Sixteen microsatellite loci were used to detect introgression in natural-origin adult steelhead migrating into the basin prior to spawning during 2013 and 2014. Bayesian clustering analysis (STRUCTURE) was used to identify the level of admixture in the population and assign individuals to clusters.
3. The Bayesian clustering analysis indicated that there are most likely two populations (or clusters) in the study area, a native, coastal, winter-run steelhead and a non-local, summer-run steelhead which was derived from artificial crosses between summer-run coastal and interior redband populations. Introgressive hybridization was detected in 26.4% of the natural-origin adult steelhead. First generation (F_1) hybrids were estimated as 4.9 to 10.1% of the natural-origin adult steelhead. Hybrids backcrossed to the native, coastal, winter-run steelhead were nine times more numerous than backcrosses to the hatchery, summer-run steelhead. Upstream migration timing was significantly different between the native, winter steelhead and the F_1 hybrids.
4. Low numbers of summer steelhead and back-cross summer-run hybrids were identified in the natural-origin population consistent with reduced fitness of hatchery summer steelhead in natural environments. Conservation actions that protect native populations from hatchery fish include altering stocking practices (such as integrated management or sterility), and protecting remaining intact populations by designating genetic preserves and preventing the release of hatchery-origin or hybrid steelhead into these areas.

Keywords: hatchery effects, hybridization, introgression, microsatellite, non-native species, steelhead, *Oncorhynchus mykiss*

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1. Introduction

Introduction of non-native species is a widespread management action implemented to support harvest in forestry, fisheries and wildlife (Laikre et al., 2010). The intentional introductions for the purposes of harvest programs threaten native populations due to the high propagule pressure that is typical in these applications (Lockwood, Cassey, & Blackburn, 2005; Simberloff, 2009). Throughout the western United States, fisheries mitigation programs largely developed in the early and mid-1900's rely heavily on artificial propagation to replace lost fisheries production (*e.g*. U. S. House Document 531, 1949; Naish et al., 2007 and citations therein). Most of these programs pre-date the U.S. Endangered Species Act passed in 1973, and the subsequent listing of numerous salmonid species during the 1990s in the Pacific Northwestern, United States. Fisheries management and mitigation programs are challenged to balance these conflicting mandates, and may use non-local or non-native fish to satisfy harvest goals. Non-local and non-native salmonids cause significant impacts to the native populations, communities and ecosystems (Buoro, Olden, & Cucherousset, 2016). Conflict between harvest or recreation management, and the conservation of native species (*e.g.* Shouse, 2003) can result in hatcheries having undefined or conflicting goals and objectives (Naish et al., 2007). Therefore, the evaluation of interactions between native and propagated populations is important to determine if harvest and conservation programs are in conflict, and identify possible management strategies to reduce or eliminate impacts to native species.

Non-local, domesticated, hatchery salmonids largely impact the native conspecifics through individual-based performance, such as physiological and fitness impacts. (Buoro et al., 2016). Artificially-reared non-native or non-local populations often have reduced fitness in the wild due to domestication effects (Araki et al., 2008; Le Luyer et al., 2017).The demographic effect of continued introductions has been shown to cause rapid extinction of the native species even with reduced fitness of the non-native or non-local population (Huxel, 1999; McGinnity et al., 2003). Reduced fitness of hatchery fish in the wild and loss of production in the native population is identified in several commonly reared salmonid species including: coho salmon (*O. kisutch*, Theriault, Moyer, Jackson, Blouin, & Banks, 2011), steelhead trout (*O. mykiss*, Leider, Hulett, Loch, & Chilcote, 1990; Araki, Cooper, & Blouin, 2007; Berntson, Carmichael, Flesher, Ward, & Moran, 2011), Atlantic salmon (*Salmo salar*, Jonsson, Jonsson, & Hansen, 1991; McGinnity et al., 2003) and Chinook salmon (*O. tshawytscha*, Chilcote, Goodson, & Falcy, 2011). Hatchery and wild crosses also have reduced fitness in the wild with the poorest performance from non-local, hatchery sources (Araki, Berejikian, Ford, & Blouin, 2008).

Hatchery steelhead derived from an artificial mixture of non-local, summer-run subspecies are currently stocked in the Willamette Basin, a tributary to the lower Columbia River, United States, where the native winter-run steelhead population is protected under the Endangered Species Act. The stocking program mitigates for impassable dams that block access for anadromous populations of steelhead and alters the remaining accessible habitats. The hatchery population of summer-run steelhead supports a local recreational fishery due to the timing of return migration (spring to fall) and prolonged freshwater rearing prior to spawning (up to 12 months). The use of highly domesticated hatchery stocks, such as the Skamania stock used in the Willamette Basin (see below), is thought to limit the risk of colonization and hybridization due to low fitness in the wild (Naish et al., 2007). The objective of this study was to identify the extent of genetic interactions between the non-local, hatchery, summer-run steelhead and the native, natural-origin, winter-run steelhead by assessing introgression and run timing of hybrids. This study focuses on the effects of introgression on run timing, the distinguishing phenotype between the summer and winter run steelhead. However, genetic impacts can occur in other phenotypes that were not measured, but can be meaningful to local adaptations and evolutionary potential.

1.1 Steelhead Taxonomy and the Willamette Basin

O. mykiss exhibits diverse life history strategies. Variation is expressed within and among populations and includes multiple return times for adults during spawning migrations, varying periods of freshwater and ocean residency, and plasticity of life history between generations (Behnke, 1992; Busby et al., 1996). Two subspecies of *O. mykiss* are recognized in the Pacific Northwest: a coastal subspecies (*O. m. irideus*, also commonly called rainbow trout) and an interior subspecies (*O. m. gairdneri*, also commonly called redband trout) (Behnke, 1992). Life history expression includes adult rearing in freshwater rivers (resident or fluvial) or ocean (anadromous) in both subspecies. Steelhead, the anadromous form of *O. mykiss*, exhibit different run timing and maturation phenotypes. The winter-run matures in the ocean and migrates upstream immediately prior to spawning. The summer-run matures in freshwater after returning from ocean rearing resulting in a protracted period during freshwater rearing (Prince et al., 2017). Both phenotypes spawn in the late winter/spring months (~January to May) (Van Doornik et al., 2015). The coastal subspecies largely expresses the winter-run maturation phenotype, but may also express an alternate summer-run phenotype in some locations, usually in upper basin habitats. The interior, redband trout only expresses a summer-run, maturation phenotype (Behnke, 1992; Busby et al., 1996).

The native coastal steelhead in the upper portion of the Willamette Basin in western Oregon are a distinct population listed as threatened under the Endangered Species Act in 1999 (NMFS, 2006). The native steelhead upstream from Willamette Falls had a unique migration timing that is later than other coastal steelhead populations thought to coincide with seasonal stream flow conditions that allowed passage at Willamette Falls. Willamette Falls is a natural, 12-m high, horseshoe-shaped waterfall located at river km 42 that was altered with navigation locks in 1873 and hydropower facilities in 1889 (OHS, 2018; PGE, 2018). A fish ladder was installed in 1885 to assist passage of salmon (PGE, 2018), prior to this ladder, only the later-migrating native steelhead were able to ascend the falls.

The Willamette River Basin is 29,727 km^2 located in northwest Oregon between the Cascade and the Coast Mountain ranges. The Willamette River is 301 km long and flows from south to north into the Columbia River. Most of the urban population in the state resides in the basin including the cities of Portland, Salem, Corvallis and Eugene (Fig. 1). Hydropower and flood control development in the basin occurred during the 1960s and currently 13 high-head dams block access to 41% of native steelhead habitat (Sheer and Steele, 2006). The accessible, lower elevation areas of the basin are impacted by habitat alteration, urbanization and associated flood control, dam operations and associated altered hydrology, climate trends, and landscape effects (see NMFS, 2016 for more detail).

1.2 Stocking History in the Willamette Basin

Stocking the non-local, summer-run (Skamania stock) steelhead began in 1966. Recently, the hatchery program released an average of 572,411 juvenile steelhead annually between 2012 and 2017 at 6 sites in the study area (ODFW stocking records). Another non-native, hatchery-origin coastal steelhead was artificially-propagated and introduced in the basin upstream from Willamette Falls (commonly called early winter-run), and naturalized populations are thought to migrate into the Willamette Basin during the fall months prior to spawning during the winter and early spring months (~October to February 15) (Van Doornik et al., 2015). Introductions of this hatchery-origin, “early winter” population (non-native coastal) were discontinued in the Basin upstream of Willamette Falls during the 1990s, whereas the introductions of the Skamania summer-run hatchery steelhead are ongoing (Van Doornik et al., 2015).

Juvenile hatchery, summer steelhead are marked by removing the adipose fin and these marked adults are collected for broodstock at hatchery facilities; adults with intact adipose fins are naturally-produced steelhead that can include: offspring of hatchery, summer-run steelhead that spawned in rivers, native winter steelhead, or hybrids. Hatchery-origin, adult, summer-run steelhead out-number the natural-origin steelhead 3:1 during the last 10 years (Fig. 2). Overlap among the migration and spawning timing of these hatchery and native steelhead creates opportunity for spatial and temporal overlap during spawning (Van Doornik et al., 2015).

2 Methods

The collection of steelhead was performed to capture a representative sample of the natural-origin (adipose fin present) steelhead population passing Willamette Falls. Some hatchery-origin, summer-run steelhead were analyzed only to provide a reference for the population clustering, and provide an estimate of classification error from known (adipose fin removed) individuals. Sampling of hatchery, summer steelhead ceased in late June due to high summertime water temperatures and compliance with sampling permits, and did not resume later in the run after summertime temperatures declined. Therefore, the hatchery, summer steelhead sample only captures the first half of the run during 2013.

2.1 Fish Collections

Upstream migrating adult steelhead were captured at the adult fish trap located at the Willamette Falls fish ladder from January 22, 2013 to June 26, 2013 and November 7, 2013 to June 4, 2014. Steelhead were tagged and sampled in approximate proportion to the number passing the fish facility in each year. Sampled individuals represent an estimated 3.4% (2013) and 4.0% (2014) of the natural-origin steelhead (adipose fin present) passing Willamette Falls.

Steelhead were anesthetized with AQUI-S 20E (AquaTactics, Kirkland, WA) and a radio-tag implanted following the methods in Caudill et al. (2014). Total length and weight were measured and fish were checked for marks or tags, sex, and evidence of injuries or disease. Hatchery-origin, summer-run steelhead were identified by the removed adipose fin. Tissue was sampled as a caudal (tail) fin punch and dried on paper. Telemetry records from fixed receiver sites (Fig. 1) and mobile tracking were used to determine upstream extent of migration and infer spawning location using methods described in parallel studies of Chinook salmon (Caudill et al., 2014; Keefer et al., 2017). Additionally, radio-tags provided information on the extent of upstream migration and potential spawning location, as well as the proportion of the individuals passing Willamette Falls that subsequently migrated downstream and out of the study area. All collection, handling and tagging was performed using approved animal care protocols and appropriate scientific collection permits.

2.2 Laboratory Methods

Sixteen microsatellite markers were used to genotype individuals. Thirteen of these markers were standardized across the Columbia River basin and are summarized by Stephenson et al. (2009). Additional primer sets analyzed were *Omy*105, *Omy*2 (Heath, Pollard, & Herbinger, 2001) and *Omy*77 (Morris, Richard, & Wright, 1996). The DNA was isolated from fin clips using Qiagen DNEasy Blood and Tissue^{TM} extraction kits (Qiagen, Inc., Germantown, MD) according to manufacturer's protocols. Sixteen microsatellite loci were amplified by PCR in three multiplex reactions using Qiagen Multiplex PCR Master Mix in 96-well plates on C1000 Touch and S1000 thermal cyclers (Bio-Rad, Hercules, CA). PCR details are provided in the Supporting Information, Section S.1. The PCR products were run on an Applied Biosystems 3130xl Genetic Analyzer. Forward primers were fluorescently labeled (6-FAM from Integrated DNA Technologies, Inc., Coralville, IA; VIC, PET, NED from Applied Biosystems, Inc.). PCR product (1 µl) was added to 10 µl formamide and 0.15 µl of LIZ500 size standard. Peaks were scored using Genemapper software 5 (Applied Biosystems). All samples were amplified two or three times to monitor for genotyping errors. Peaks were also visually checked for conformity to expected profiles. Laboratory error rates were estimated at 1%.

2.3 Statistical Analysis

The characterization of the natural-origin run is based on the genetic analysis of 'wild' (adipose fin present) steelhead (n=161 in 2013 and n=206 in 2014). A sample of known hatchery-origin summer steelhead (n=89) were included in the analysis during the 2013 spawning year to provide a genetic reference for this cluster (representing only the first half of this run). Data from the natural-origin run were first tested for allele frequency differences across spawning years for the natural-origin steelhead using GENEPOP version 4.2 (Raymond & Rousset, 1995). All multiple comparisons were adjusted with a Bonferroni correction (Rice, 1989). No significant differences in allele frequency exact tests were identified so data were grouped across years for the remainder of the analyses.

STRUCTURE version 2.3.4 (Prichard, Stephens, & Donnelly, 2000) was used to identify the appropriate K-value for the data set and to identify admixture among these populations in individual samples. STRUCTURE was run using the admixture model without location prior with 10,000 iterations for burn-in followed by 100,000 iterations. Ten independent runs were performed for K ranging from 1 to 8. The optimal number of populations (K) was chosen according to Prichard, Wen, & Falush (2010) and Evanno, Regnaut, & Goudet (2005). A K-means test was performed using GENODIVE (Meirmans, 2013) to confirm the number of clusters identified using STRUCTURE.

Individual admixture values (Q-value) from the STRUCTURE output were averaged from the 3 runs with the lowest log likelihood, which indicates the best fit between the data set and estimated parameters. Hybridized (admixed) individuals were identified from the averaged Q-values at two levels of cluster membership (<0.8 and <0.9) to either parental cluster (native winter or hatchery summer) with hybrids assigning between 0.2 and 0.8 or 0.1 and 0.9, respectively. Individual steelhead were identified based on the Q-values as native, hatchery or hybrid, based on the most supported number of clusters. F_{ST} and allele frequency exact tests were performed on the final population groups using

GENEPOP version 4.2 (Raymond & Rousset, 1995). The proportion of unmarked hatchery, summer steelhead was calculated as the number of hatchery steelhead out of the total number of the natural-origin steelhead. The hybridization rate was estimated as the proportion of the natural-origin first generation (F_1) hybrids in our sample. Due to a wide range of Q-values documented for F_1 hybrids using STRUCTURE (Bohling, Adams, & Waits, 2012), a strict criterion (Q-values from 0.4 to 0.6) and a relaxed criterion (Q-values from 0.3 to 0.7) were evaluated to estimate hybridization in the sample. These Q-values were selected based on STRUCTURE assignments compared to pedigree data that estimated individual F_1 hybrids with Q-values from 23 to 73% (Bohling et al., 2012).

Simulated data were used to identify uncertainty in individual hybrid identifications, potential bias in the population-level estimate of introgression, and to inform error associated with misidentifications related to the different Q-value thresholds (Q=0.8 versus 0.9). Error for the individual assignments was evaluated using HYBRIDLAB version 1.1 (Nielsen, Bach, & Kotlicki, 2006) to simulate F_1 and backcross hybrids from the data set. The input data for the simulated hybrids were derived from 70 individuals from each subspecies collected during 2013 with Q-values > 0.9 for native, winter steelhead and <0.1 for hatchery, summer steelhead, with no missing data. Seventy F_1 hybrids and back-crosses to each parental species were simulated from the parental data. The input parental data and output hybrid data were run in STRUCTURE with K=2 and all settings and procedures were similar to those described above. The assignments of the simulated individuals were compared to the true (known) genotypes.

A principal coordinate analysis on genetic distances was performed using GENALEX version 6.5 (Peakall & Smouse 2006, 2012) for individuals in the identified groups (native winter, hybrid and hatchery summer, Q=0.9). A Chi squared test was used to test for differences from expected counts of native winter, hybrid, and hatchery, summer steelhead (Q=0.9). Differences in the timing of upstream migration based on date of capture between the native winter and hatchery summer steelhead and hybrids were tested using a Kruskal Wallis test in R version 3.4.1 (R Core Team, 2017) followed by a Dunn test for multiple comparisons using the FSA package (Ogle, 2018). Date was converted to the number of days after November 1 when the fish was captured at the collection facility.

3 Results

STRUCTURE output identified the optimal number of clusters as two (Fig. S1), and the K-means analysis supported two clusters in the data based on the maximum pseudo-F value (Meirmans, 2012; 2013). The genetic diversity measures (heterozygosity, allelic richness, etc.) between these two groups are summarized in Table S1. The potential for additional clusters in the data set were explored due to previous research in the vicinity of the study area identifying three clusters in the study area (hatchery summer steelhead, east side and west side tributaries) (Van Doornik et al., 2015). The results considering three populations are included in the Supporting Information (Section S.2). Briefly, the source of a third cluster is undefined, and only a small portion of the natural-origin adult steelhead collected at Willamette Falls (~7% reduced to 5% after removing individuals that subsequently migrated downstream prior to spawning; Fig. S2, Section S.3). Steelhead in this cluster had an earlier passage timing at Willamette Falls than the native (late) winter-run steelhead (Fig. S3), and different F_{ST} and allele frequencies than the summer and winter groups in the data (Table S2). However, PCoA of genetic distances did not indicate clear clustering among the different genotypes (Fig. S4). This additional cluster was not related to adult tributary migration (Fig. S5) or life history. Hereafter, the results are presented in terms of two steelhead populations, a native winter and a hatchery summer.

Hatchery-origin summer-run steelhead clustered together in the STRUCTURE analysis mostly following the hatchery identification with adipose fin removed (Fig. 3). Three hatchery-origin steelhead did not assign to the hatchery steelhead cluster (3.4% misassignment rate) based on these known samples. One of these hatchery-origin steelhead had a Q-value equal to 0.74 which would classify as hybrid based on the Q-value, but did predominantly assign to the correct cluster. One steelhead assigned as a hybrid with equal admixture among the populations, and one steelhead assigned to the native steelhead group with a population assignment of 0.94. Similarly, a small portion of natural-origin steelhead assigned to the summer-run, hatchery group (3.5% at Q=0.9 and 4% at Q=0.8). Individual Q-values were similar across the three independent runs examined (average standard deviation <0.005) with hybrids having higher average standard deviation than the parental populations (Table 1).

The estimate of introgressive hybridization ranged from 19 to 26.4% of the natural-origin steelhead at Q=0.8 and 0.9, respectively. Hybridization rate, estimated as the number of F_1 hybrids, ranged from 4.9% (Q-values 0.4-0.6) to 10.1% (Q-values 0.3-0.7). The counts of individual native winter and natural-origin summer steelhead and hybrids were not significantly different across the two years (p>0.10). Counts of the natural-origin (hatchery-lineage) summer, native winter and hybrid steelhead were significantly different from the expected counts (X^2 test, p<0.0001), where native, winter steelhead were over-represented and hatchery summer steelhead were under-represented in the natural-origin steelhead (Fig. S6). When we examined the putative back-cross individuals (Q-values 0.7-0.9 and 0.1 to 0.3 using the Q threshold of 0.9 for cluster assignment), there were nine times as many hybrids back-crossed to the native winter steelhead than the non-native hatchery summer steelhead in the sample.

The simulated data showed a wide range of individual Q-values for each genetic assignment (native winter, hatchery summer and hybrids), but the average values across the samples were close to the true genotypes (0.92 for native winter, 0.07 for hatchery summer, 0.49 for F_1 hybrids, 0.75 for F_1 x native winter and 0.22 for F_1 x hatchery summer) (Figs. 4 and S7). Individual misassignments between the native winter or hatchery summer steelhead and hybrid classifications generally ranged from 0 for F_1 hybrids using a Q=0.9 to 59% for back-crosses to hatchery summer steelhead using a Q=0.8 (Fig. 5). The Q-value threshold of 0.9 generally provided lower error rates and the incorrect identifications were nearly equal between the back-cross hybrids identified as parental type and the parental (hatchery or native) groups identified as hybrids providing the most accurate estimate of introgression overall. A Q-value threshold of 0.8 provided an unbalanced proportion of misassignments with fewer parentals misassigned as hybrids, but high proportions of back-cross hybrids misassigned as parentals (>50%). In terms of our F_1 hybrid criteria (relaxed versus strict), the strict criterion only correctly identified 40% of the true F_1 hybrids and 11% of the back cross hybrids, whereas the relaxed criterion identified 73% of the true F_1 hybrids and 31% of the back cross hybrids. Neither the strict nor relaxed criterion identified a true parent as a hybrid.

Pairwise F_{ST} values were similar for the different Q-values, but were slightly greater for the Q-value threshold of 0.9 (Table 2). Hybrids were more similar to the native group (F_{ST} 0.01, 0.02) than to the hatchery summer group (F_{ST} 0.03). Only 9% of the variation of individual genetic distances was explained by the first and second principal coordinate axes; however, the data show a clear gradation of genetic distances among the groups with hybridized individuals intermediate between the native and hatchery groups (Fig. 6).

The date of upstream migration was significantly different among the native winter, hatchery summer and hybrid groups ($p<0.001$); however pairwise tests identified that the F_1 hybrids and native winter steelhead were the only significantly different comparison ($p<0.001$). The median migration date at Willamette Falls was earlier for all hybrid groups than the native winter-run steelhead (Figs. 7 and S8).

4 Discussion

Continued and frequent introduction of non-native or non-local populations results in high propagule pressure that artificially increases the abundance and density of the non-local, hatchery individuals (Lockwood et al., 2005; Simberloff, 2009), and counters the effects of reduced fitness of the hatchery fish in natural environments. In the upper Willamette Basin, the hatchery summer steelhead in the basin out-number the native winter steelhead preceding reproduction (Erdman, Caudill, Naughton, & Jepson, in press). Introgression was detected in 26.4%, and F_1 hybrids were detected in 4.9 to 10.1%, of the natural-origin samples. The simulation results support the estimated rates of introgression. Hatchery summer steelhead were significantly under-represented in the natural-origin steelhead samples indicating a lack of fitness in the natural environment. Individual putative back-cross hybrids to the native winter steelhead were nine times more numerous than the hybrids back-crossed to the hatchery summer steelhead. Hybrid steelhead had earlier median run timing than the native, winter-run steelhead. Although the original (pre-stocking) genetic differentiation between these populations is unknown, introgressive hybridization can alter the genetic integrity of the native population and lead to replacement of the population by the non-native (Huxel, 1999; Ford, 2002). Local adaptations and genetic diversity lost in these processes may not be recoverable in time frames relevant to the management and preservation of natural resources (~100 years) which could reduce the evolutionary potential of the population (Prince et al., 2017; Waples & Lindley, in press).

4.1 Accuracy of Hybridization and Introgression Estimates

A key question is whether admixture is under- or over-estimated at the population-level using the STRUCTURE assignments. Estimation of introgression first depends on the ability to reliably detect the clusters in the analysis which improves with greater genetic differences. Simulated data sets estimate that STRUCTURE is about 97% accurate when F_{ST} is 0.05 (Latch, Dharmarajan, Glaubitz, & Rhodes, 2006), a level of differentiation similar to the populations included in this study. Yet, at the individual-level, hybrids have a wide range of admixture estimated by STRUCTURE, but the mean Q-value of each type of hybrid was close to the expected value when tested with a known pedigree (also shown in Bohling et al., 2012). Further, the user-defined K-value and Q-value thresholds will influence the amount of introgression estimated using these methods (see Supporting Information, Section S.5 for more discussion). In a simulated data set, using the Q-value of 0.8 resulted in 42% of backcrosses being incorrectly assigned to the purebred group while using a Q-value of 0.9 reduced this error to 19% (Vaha & Primmer, 2006), similar to our simulation results. Certainly, at the individual level and with low genetic differentiation (F_{ST}<0.1), there is error in the identification of individuals. Based on the findings in Vaha & Primmer (2006) at an F_{ST} value of 0.06, we expect to correctly identify about 80% of the individuals in this sample using 16 loci. The simulated hybrid data set confirms this 20% error in assignment between parental groups and back-cross hybrids. Overall, these data and other studies suggest high rates of correct assignment for F_1 hybrids and unbiased population-level rates.

4.2 Potential Factors Mediating Introgression from Hatchery Steelhead

Hatchery, summer steelhead have been stocked in the study area for about 10 generations, and two explanations are possible for the observed hybridization pattern in the natural-origin population. First, the genetic differentiation in the native winter and hatchery summer populations could be a result of genetic mixing that is reducing genetic differentiation over time and moving the populations toward a hybrid swarm. Briefly, the sample represents a “snapshot” during a temporal process. Under this scenario, introgression is expanding toward an inevitable hybrid swarm, and delaying a management action will result in the extinction of the native genotype. An alternative hypothesis suggests that the level of introgression could be mediated by loss in fitness of hatchery summer steelhead and hybrids – thereby resulting in a stable level of introgression (Chilcote et al., 2011). Epifanio & Philipp (2001) show that introgression will asymptote at about 20% admixture in 10 generations when fitness of the hybrids is low. This scenario requires very low relative fitness of the hybrids at 0.15. In their model, even a slight increase of relative fitness to 0.20 resulted in complete introgressive hybridization within 11 generations. If introgression is stabilized at these levels, then it is possible that discontinuing the release of artificially propagated summer-run steelhead which have very low fitness in the natural environment could result in the decline of the summer-run steelhead in the population, and the potential negative effects of introgression may be limited. Although assortative mating could be a mechanism that maintains genetic differentiation providing another alternative hypothesis, Epifanio & Philipp (2001) show that strong pre-mating isolation alone is insufficient to prevent a complete hybrid swarm if fitness of the hybrids is at least 0.20 of the fitness of the native species or population. Even if considering a conservative estimate of F_1 hybridization, the spatial and temporal overlap between the native winter and hatchery summer steelhead is enough to cause introgression. Additionally, the timing of spawning between the native winter and the hatchery summer steelhead overlaps (Van Doornik et al., 2015) minimizing the potential for segregation in the natural environment.

Few natural-origin, summer steelhead were detected in the population, and lower numbers of putative hybrids back-crossed to the hatchery summer steelhead despite high abundances of hatchery summer steelhead in the population during reproduction. Jones et al. (2015) found similar proportions of hatchery, hybrid and wild steelhead in a stream in Southwest Washington (1% natural-origin, hatchery-lineage steelhead and 29% hybrids), and conclude that the earlier spawning hatchery steelhead were emerging during unfavorable conditions; however, the later emerging hatchery steelhead were more likely to encounter better environmental conditions for early rearing resulting in selection for this later migration timing that is more similar to the native population. The timing of adult migration is a heritable trait in salmonids and has substantial fitness consequences if not synchronized with local environmental conditions (Flagg, Waknitz, Maynard, Milner, & Mahnken, 1995; Quinn, Peterson, Gallucci, Hershberger, & Brannon, 2002; McLean, Bentzen, & Quinn, 2005; Jones et al., 2015; Hess, Zendt, Matala, & Narum, 2016).

Recent research indicates that modifications at the GREB1L gene are associated with premature migration, and that premature migration (expressed in summer steelhead) is not masked in the heterozygote (Prince et al., 2017). Yet, upstream migration after maturation is not a conduit for the premature migration allele, and data patterns indicate selection against the intermediate phenotypes (Prince et al., 2017). This interaction between run timing, maturation and spawning timing is likely a complex expression of multiple gene complexes (Waples & Lindley, in press, and citations therein). The earlier run timing expressed via the summer introgression in this data set suggests that the later migrating hatchery summer steelhead (August to October) may have a greater likelihood to hybridize with the native winter steelhead which spawn after the summer steelhead. An alternate explanation for the observed influence of the later migrating hatchery summer steelhead in the hybridized individuals could be less opportunity for removal from the population due to reduced fishing pressure or other mortality during freshwater maturation.

4.3 Conservation and Management

Options for harvest-focused hatchery programs are to maintain programs with complete segregation between the native, wild and hatchery populations, or to fully integrate the two populations with the hatchery programs providing surplus production to meet harvest goals. Unfortunately, the efficacy of these approaches has not been tested over the long term (Naish et al., 2007). Complete segregation relies on the ability to collect all artificially propagated adults prior to spawning. Various other management strategies to support segregation have been attempted to address threats from hatchery fish, such as introducing sterile hatchery fish (Tiwary, Kurbagaran, & Ray, 2004) where the threat of introgression or inter-breeding is high (*e. g.*, Cotter et al., 2000), and the designation of genetic preserves (also called gene banks) to protect and promote the recovery of native populations (*e.g.* WDFW, 2015). Integration is also commonly implemented, but the rapid epigenetic effects creates challenges to limit the transmission of genetic impacts to wild populations (Le Luyer et al., 2017).

Reduced fitness and misidentification are a concern when stocking hatchery fish without complete segregation. Hybrids and natural-origin, hatchery individuals will be counted as native species during abundance estimates and population demographic analyses. Currently, managers in the study area are transplanting wild and presumably native winter steelhead returning to the basin into inaccessible spawning areas upstream from dams, thereby introducing the hatchery-origin summer steelhead genes into these areas. Although the majority (~70%) of the natural-origin returning adults to the upper Willamette Basin are identified as native winter steelhead, the estimate of encounter rates based on the observed proportions of F_1 hybrids in the data set is a concern. This likely underestimates the effect of the hatchery summer steelhead due to the low expected fitness. For example, at a hybridization rate of 5% and a relative fitness of 0.10, the hybrid encounter rates not expressed in the observed data could be as high as 50%. If relative fitness is increased to 0.40, then hybrid encounter rates would be about 12%.

Future research should examine the fitness consequences to the hybridized individuals and identify the effects of the mitigation stocking program on the population demographic. Fitness can be acquired from individual-based lifetime tagging studies and genotyping over time; however spawning behavior, hybridization attempts and redd disturbance may be difficult to estimate due to high stream flows, low water visibility and large habitats where steelhead occur in the study area. Importantly, long term studies are needed to best identify strategies to prevent impacts to the native, wild populations. Information on the migration and spawning behavior of the later migrating (passing Willamette Falls between August 1 and October 31) hatchery summer run steelhead is lacking, and information on whether these individuals are more likely to contribute to hybridization could assist in identifying potential management actions to reduce effects in the basin. Additionally, fisheries management in the local basin has included the practice of “re-cycling” hatchery, summer steelhead to maximize angler opportunity (*i.e.* trapping, transporting and re-releasing adult hatchery steelhead downstream). However, while this program boosts angler harvest by an estimated 15%, it also likely increases the reproductive interactions between hatchery and wild steelhead by leaving many mature, hatchery steelhead in-river (Erdman et al., in press).

Areas, like the Columbia River Basin, where existing harvest and conservation policies are conflicting, demonstrate that clear guidance on priorities and updated policies that ensure and encourage the conservation of native species and ecosystem function are needed to assist in the complex processes implementing fishery programs under these laws. Yet, defining common policies has proven challenging when management actions span multiple agencies with different underlying objectives. Even within agencies, different departments may have their own competing objectives and complex infra-structure. Areas currently developing new hydropower projects should strive to more fully balance the conservation of native species and the replacement (or mitigation) for lost subsistence or commercial fisheries, and create programs and policies that can evolve with scientific advances and socio-political concerns. Areas where artificial propagation is planned or ongoing should implement genetic monitoring practices to ensure the preservation and evolutionary potential of native genotypes (Schwartz, Luikart & Waples, 2007; Bohling 2016), and promote adaptive management based on scientific findings and best-available science.

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Table 1. Average Q-value for individual assignments and standard deviation for hatchery and native steelhead and hybrids in the upper Willamette River Basin. Results for the two tested Q-values (0.9 and 0.8) are presented.

	Q=0.90		Q=0.80	
	Avg	SD	Avg	SD
Native, winter	0.96	0.00098	0.95	0.00132
Hatchery, summer	0.03	0.00069	0.04	0.00076
Hybrid	0.65	0.00375	0.56	0.00423

Table 2. F_{ST} and allele frequency tests among clusters identified by STRUCTURE at K=2. F_{ST} values using a Q-value 0.8 are shown above the diagonal and Q-value 0.9 is shown below the diagonal. All pairwise comparisons had significantly different allele frequencies.

	Native, winter	Hybrid	Hatchery, summer
Native, winter		0.015	0.057
Hybrid	0.013		0.022
Hatchery, summer	0.065	0.028	

Figure Legends

Figure 1. Map of study area and locations of the downstream-most impassable dams (gray rectangles) and stationary radio-telemetry receiver sites (blue circles) in the study area. Fish were captured and tagged at Willamette Falls fish ladder.

Figure 2. Counts of hatchery summer and native winter steelhead at Willamette Falls by year. Identification is based on adipose fin presence/absence. Data from Oregon Department of Fish and Wildlife (ODFW 2017).

Figure 3. STRUCTURE plot K=2 for adult steelhead in the upper Willamette Basin. Individuals are represented by one bar in the plot and samples are ordered by date captured during the upstream migration (x-axis). The clusters group with native winter (green) and hatchery summer (red) steelhead. Hybridized individuals show contributions from both winter and summer steelhead corresponding to the Q-value shown on the y-axis.

Figure 4. STRUCTURE output for individual simulated genotypes (y-axis) versus true genotype (x-axis). The simulated hybrids are shown on the x-axis according to the proportion of native winter steelhead (0.5 for F_1 hybrids, 0.25 for F_1 x summer and 0.75 for F_1 x winter).

Figure 5. Percentage of individuals misassigned in each genotype category using a minimum Q-value of 0.9 and 0.8 for cluster assignment. Misassignment rate is the proportion of true (simulated) individuals assigned as: known winter or summer as hybrid; known F_1 or back cross hybrids as winter or summer.

Figure 6. Principal coordinate analysis (PCoA) of individual genetic distances for steelhead in the upper Willamette Basin (Q=0.9) for native winter (blue diamonds), hatchery summer (black triangles), and hybrids (orange squares) identified by STRUCTURE output.

Figure 7. Migration timing (range and median) of natural-origin, steelhead by genotype (native, winter steelhead, F_1 and back cross hybrids), and hatchery-origin, summer steelhead at Willamette Falls. The native winter and hybrid run timing is based on the genotyped sample collected for this study. The hatchery summer steelhead run timing is based on the run counts at Willamette Falls for the adult returns for the 2014 spawning year (return date 1 November 2013 to 31 October 2014) (ODFW, 2017).

Figure 1

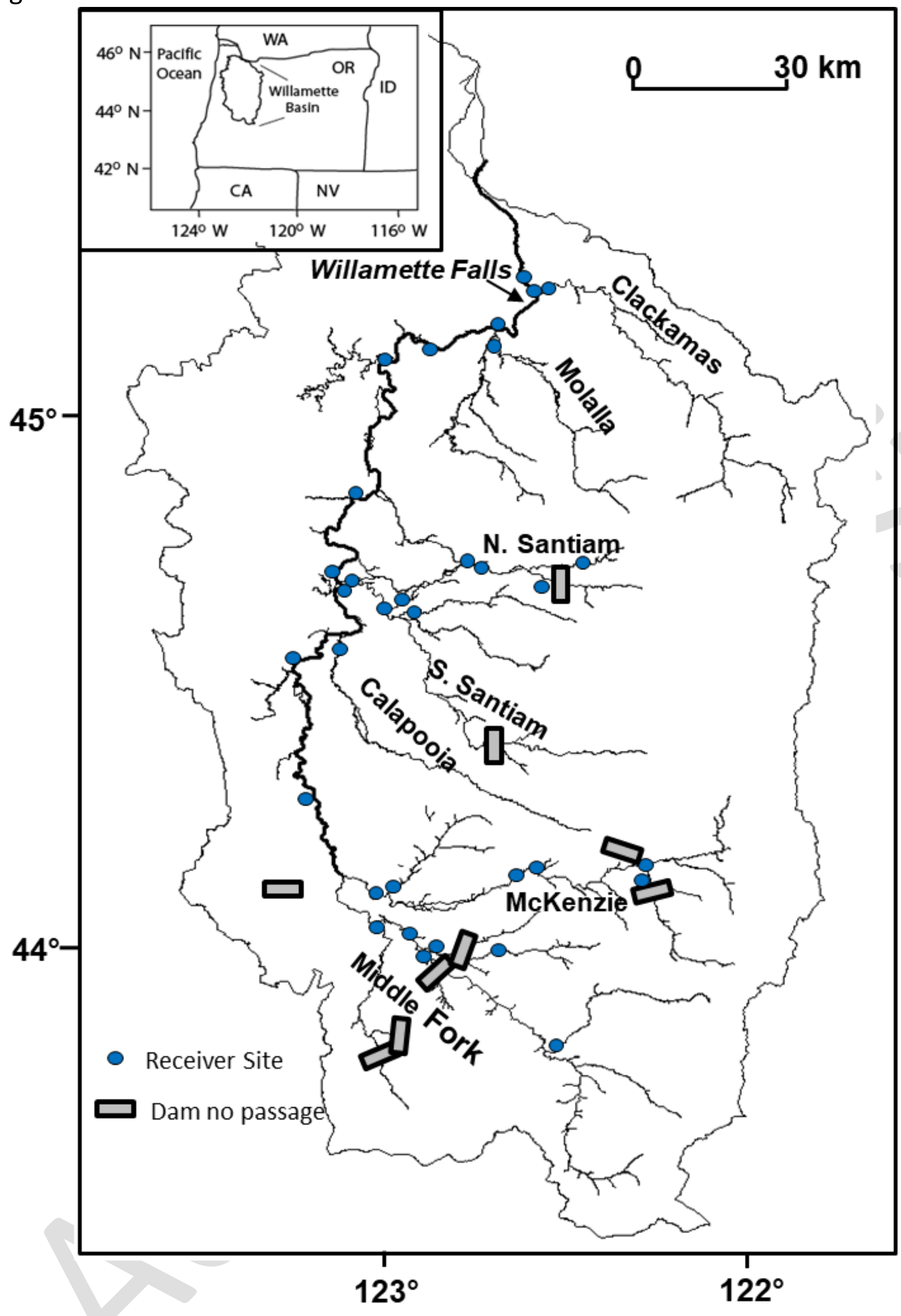
46° N
44° N
42° N
124° W
120° W
116° W
Pacific Ocean
WA
OR
ID
CA
NV
Willamette Basin
0
30 km
Willamette Falls
Clackamas
Molalla
N. Santiam
S. Santiam
Calapooia
McKenzie
Middle Fork
45°
44°
123°
122°
● Receiver Site
▭ Dam no passage

Figure 2

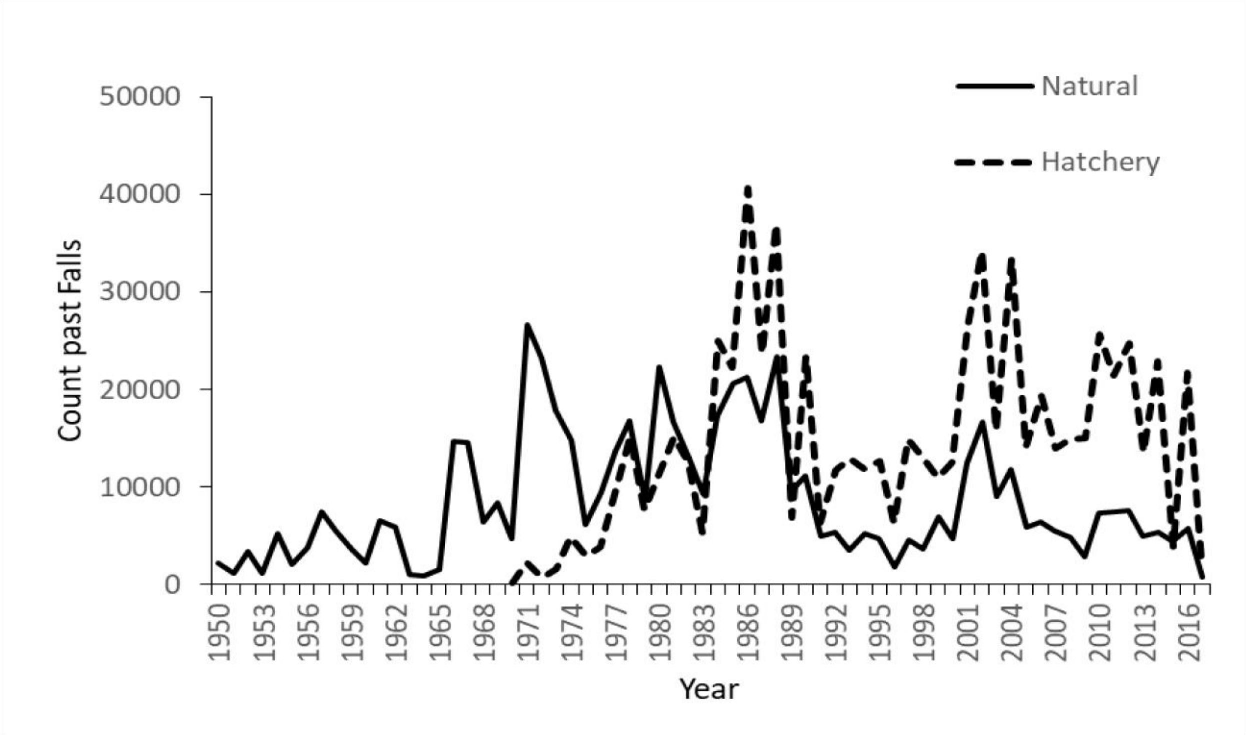

Figure 3

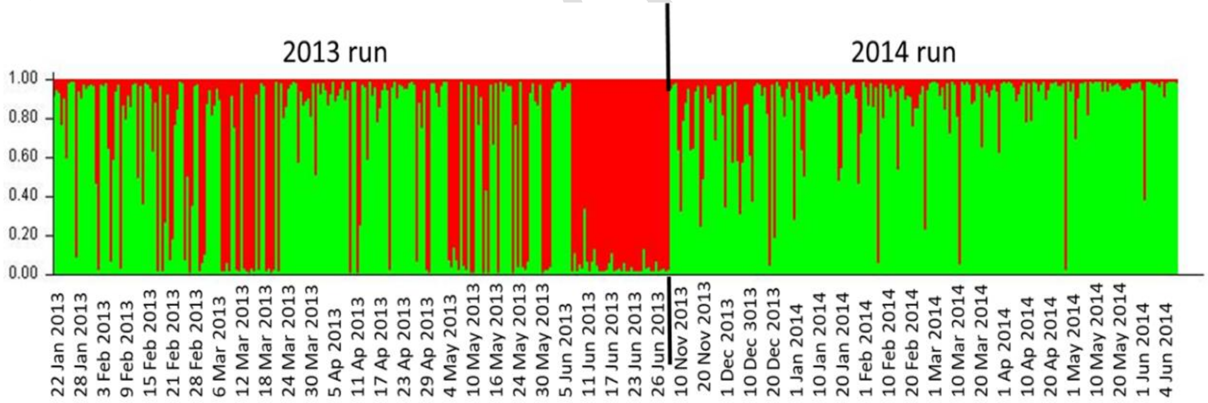

Figure 4

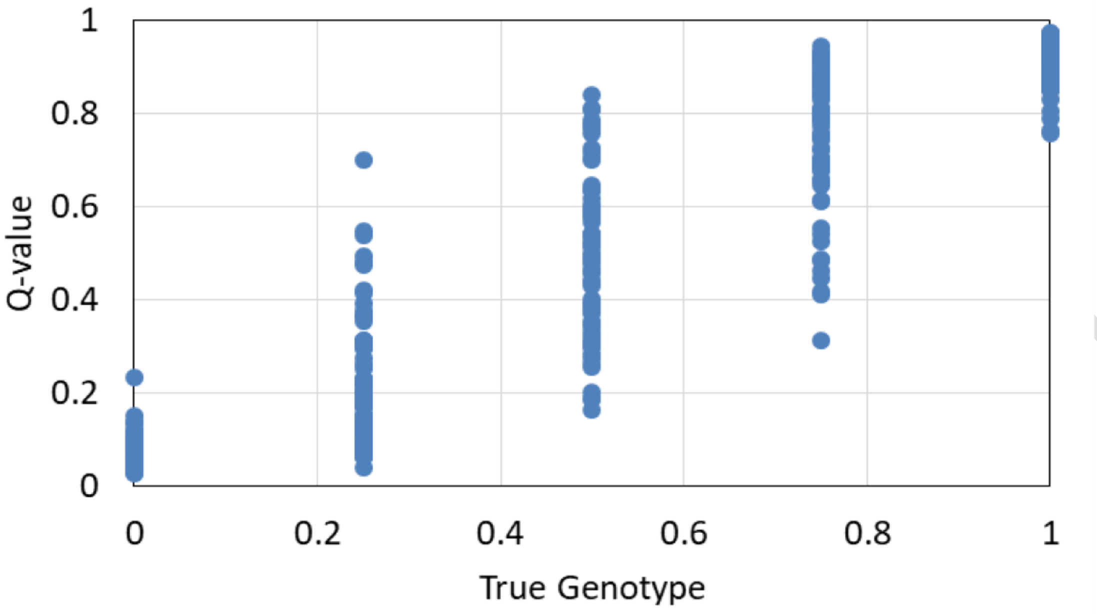

Figure 5

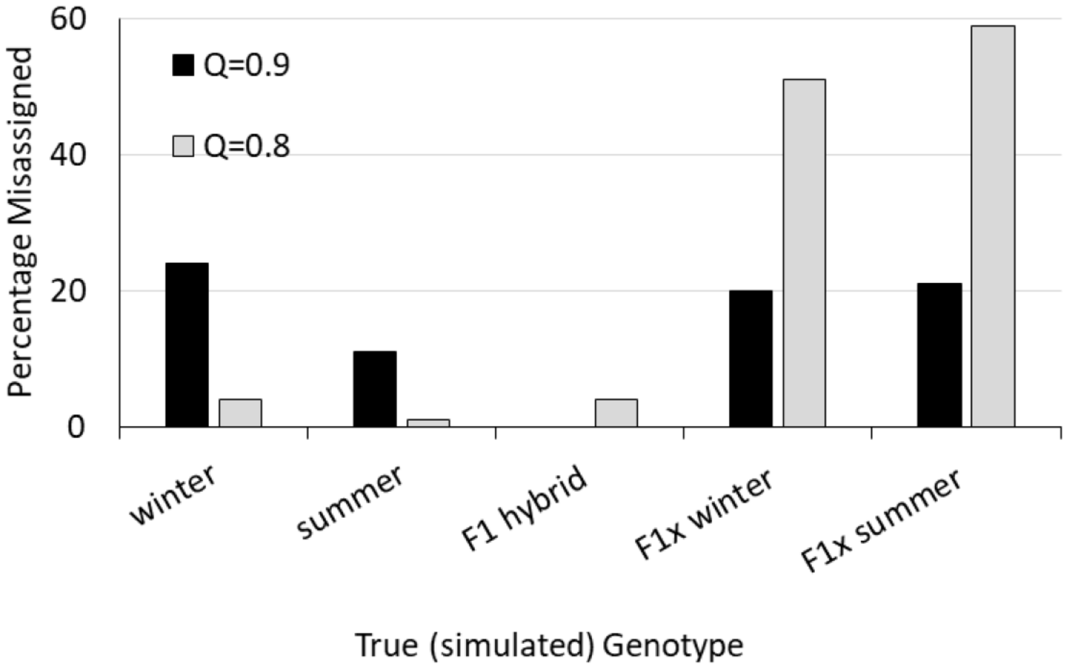

Figure 6

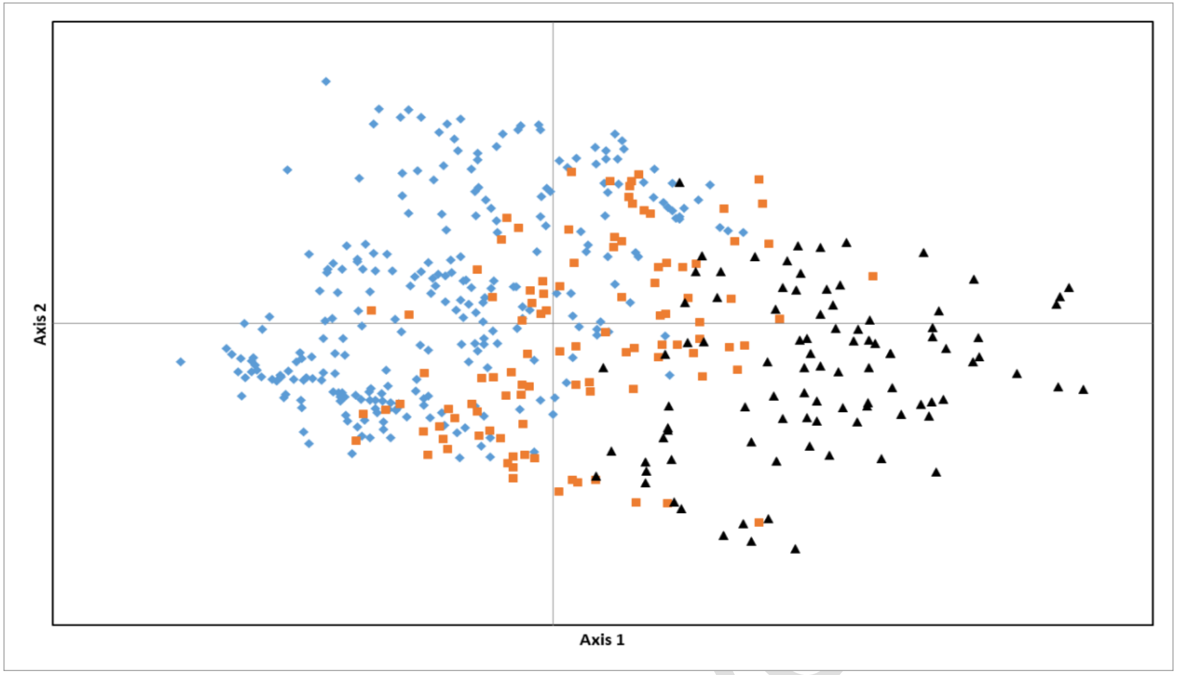

Figure 7