Monitoring the Reproductive Success of Naturally Spawning Hatchery- and Natural-Origin Steelhead in a Tributary of the Methow River

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Executive Summary

Hatcheries are one of the main tools used to mitigate for salmon and steelhead losses resulting from the construction and operation of the Columbia River hydropower system, and to recover populations listed under the Endangered Species Act. However, the ability of hatchery programs to achieve salmon recovery goals is uncertain due to genetic effects inherent in hatchery spawning and rearing practices that may result in a decrease in the reproductive success of hatchery fish that spawn in the natural environment. As part of an on-going study to determine the relative reproductive success (RRS) of hatchery- and natural-origin (i.e., wild) summer steelhead *Oncorhynchus mykiss* spawning in the Twisp River, Washington, we evaluated a suite of demographic characteristics of the spawning population of three broods of adult steelhead sampled at the Twisp River weir. We evaluated differences in the run-timing, spawn-timing, age-composition, length-at-age, sex-ratio, and spawning distribution in an effort to determine whether differences existed between hatchery and wild fish that may explain differences in observed RRS, should they occur. We found no significant differences in arrival time at the Twisp River weir between hatchery and wild female steelhead in 2009 (P = 0.96), 2010 (P = 1.00), or 2011 (P = 1.00). Similarly, there were no differences in arrival time between hatchery and wild male steelhead in 2009 (P = 0.17), 2010 (P = 1.00), or 2011 (P = 1.00). We found significant differences in female spawn timing based on fish origin and year (P< 0.01). However, all differences were among years and there were no significant differences between fish of different origin within years (P = 0.44-1.0). There were no significant differences in redd distribution based on fish origin among years (P = 0.13). The proportion of female steelhead within the hatchery and wild populations released upstream of the Twisp River weir was not significantly different for the 2009 (P = 0.751) and 2011 broods (P = 0.325), although 2010 brood hatchery origin fish contained a significantly greater proportion of females (P < 0.001) than did the wild population. No significant differences in the salt-age composition were detected between male and female hatchery and wild steelhead of the same brood year (P = 0.25 – 0.84). Wild fish generally had a greater mean fork length within years compared to hatchery origin fish of the same gender and salt-age, but a statistically significant difference was only detected between 1-salt male fish within the 2009 brood (P < 0.01). We found no significant difference between the fecundity of hatchery and wild fish within brood years (P = 0.06), but fecundity was significantly different between hatchery and wild fish across years (P < 0.001). In general, we found few differences between the hatchery and wild fish released upstream of the Twisp River weir that would influence productivity.

Hatchery steelhead have been released into the Twisp River for the last decade to mitigate losses caused by the construction and operation of the Wells Hydroelectric Project and to boost population demographics in an effort to assist in the recovery of a threatened species. However, hatchery-reared steelhead have exhibited poor reproductive success when compared to naturally-reared con-specifics, putting into question whether recovery efforts that rely on hatchery-reared

fish can succeed. We instituted a relative reproductive success study utilizing hatchery- and naturally-reared summer steelhead in the Twisp River to evaluate reproductive differences using a DNA-based pedigree approach. We analyzed DNA samples collected from 698 adult steelhead from two broods passed over a weir on the Twisp River, and collected 1,453 DNA samples from juvenile *O. mykiss* from one of those broods (2009) using angling, netting, electrofishing, and a rotary screw trap to determine relative reproductive success. Samples were genotyped at 152 single nucleotide polymorphism loci to determine population structure, and to complete parentage assignments. Juvenile samples were assigned a single parent (30.7%), two parents (38.4%), or no parents (30.9%). Of the single-parent assignments, most (66.1%) were assigned the maternal parent. Hatchery- and natural-origin mothers produced similar numbers of offspring (hatchery = 4.6 offspring per female; natural = 5.2 offspring per female). Hatchery males appeared to produce fewer offspring than natural-origin males (hatchery = 3.0 offspring per male; natural = 7.2 offspring per male). Although a slight difference was detected in the reproductive success of hatchery- and naturally-produced fish as measured by juveniles produced, this project is designed to evaluate reproductive success over multiple generations. These preliminary results are similar in some respects to those of other steelhead reproductive success studies, but drawing any major conclusions at this time would be premature.

All data and analyses in this report should be considered preliminary until published in a peer-reviewed scientific journal.

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General Introduction

This project will quantitatively evaluate the relative reproductive success of naturally spawning hatchery and natural origin summer steelhead *Oncorhynchus mykiss* in the Twisp River, Washington. Hatcheries are one of the main tools that have been used to mitigate for salmon losses caused by the construction and operation of the Columbia River hydropower system. In addition to harvest augmentation, hatcheries have recently been used in attempts to protect stocks from extinction and to enhance natural production (supplementation). Surprisingly, little is known about how much the investment in hatcheries benefits or harms natural production. Recent technological advances in genetics have enabled the empirical monitoring of the reproductive success of hatchery and natural summer steelhead using a DNA-based pedigree approach. Specifically, this project will (1) directly measure the relative reproductive success of hatchery and natural-origin summer steelhead in the natural environment, (2) determine the degree to which any differences in reproductive success between hatchery and natural steelhead can be explained by measurable biological characteristics such as run timing, morphology, or behavior, and (3) estimate the relative fitness of hatchery-lineage steelhead after they have experienced an entire generation in the natural environment. The project is intended to last until 2025 in order to evaluate two entire summer steelhead generations.

This project is a collaboration between the Washington Department of Fish and Wildlife (WDFW) and the Public Utility District No.1 of Douglas County (DCPUD). Results and progress are reported jointly. This annual report is a joint-authored report that has been split into two chapters in order to address important topics of the project. This project is an extension of the summer steelhead supplementation programs in the Methow River operated by WDFW and funded by DCPUD.

Description of Project Area

The Twisp River is a major tributary of the Methow River, entering the Methow River at river kilometer (rkm) 64.3. The Methow River is a Columbia River tributary located in north central Washington that enters the Columbia River at rkm 843, approximately 13 km upstream of Wells Dam. The Methow River watershed drains approximately 2,896 km² of which approximately 394 km² is from the Twisp River subbasin (Andonaegui 2000). The Twisp River originates in the Lake Chelan-Sawtooth Wilderness area and flows about 45 kilometers in an easterly direction to its confluence with the Methow River in the Town of Twisp, Washington (Methow Subbasin Plan 2004; Figure 1). Historical river discharge monitored by the United States Geological Survey (USGS gauging station number 12448998 at rkm 2.5) reported a 26-year mean monthly summer low discharge of 1.2 m^3/s and mean monthly spring peak discharge of 26.5 m^3/s.

The Twisp River basin supports self-sustaining populations of spring Chinook salmon *O. tshawytscha*, steelhead, and bull trout *Salvelinus confluentus*. Spring Chinook and bull trout spawning occurs primarily in the mainstem Twisp River during the late summer and fall upstream of rkm 11.4. During this time, a section of the Twisp River adjacent to the Poplar Flats Campground at rkm 35.0 may become dewatered (Andonaegui 2000), affecting the spawning distribution and survival of some species, primarily bull trout (Nelson et al. 2004). Summer steelhead spawning occurs throughout the mainstem Twisp River and within major tributaries such as Little Bridge Creek (rkm 15.0). Stream surveys in 2006-2010 have found that an average of 75.7% of the steelhead redds in the Twisp River and tributaries are located upstream of the Twisp River weir at rkm 11.4 (Snow et al. 2011).

History of Artificial Propagation

When Grand Coulee Dam was constructed in 1939 at rkm 960, an estimated 1,834 km of river was blocked to anadromous salmonids returning to the upper Columbia River (Mullan et al. 1992). In an effort to preserve salmon runs, the Grand Coulee Fish Maintenance Project (GCFMP) was implemented. Under this project, salmon and steelhead returning to the upper Columbia River were trapped at Rock Island Dam (rkm 729) and redistributed into the Wenatchee, Entiat and Methow watersheds, or delivered to the Leavenworth, Entiat, or Winthrop GCFMP hatcheries between 1939 and 1943 for artificial propagation (Fish and Hanavan 1948; Mullan et al. 1992). Although this project was one of the first examples of a mitigation hatchery program in the Columbia River Basin, a coho salmon (*O. kisutch*) hatchery constructed in the Methow River in 1915 also produced steelhead between 1920 and 1931 after coho were extirpated from the Methow River (Whale and Pearson 1984). Artificial production of steelhead in the Methow River basin became institutionalized in 1968 as a mitigation strategy to compensate for losses associated with the construction of Wells Dam at rkm 830. The Wells Fish Hatchery was constructed adjacent to Wells Dam and broodstock were initially collected from fish passage ladders at the dam in 1968. One likely result of the CGFMP was a genetic homogenization of the salmon and steelhead stocks above Rock Island Dam (NOAA 1996). This effect may have been exacerbated by hatchery practices whereby broodstock were collected downstream (i.e., Wells Dam) from multiple populations (Okanogan, Similkameen, Methow, Twisp, Chewuch, Wenatchee, etc.) without maintaining stock separation of the progeny at release. This population mixing may have prevented or delayed the recovery of locally-adapted traits as fish re-colonized tributary habitats after the CGFMP. Genetic integrity has likely been further compromised by inadvertent domestication selection within the hatchery programs and passed on to natural populations through the escapement of adult hatchery fish to spawning grounds.

Progeny of Wells Hatchery stock (Wells-stock) steelhead have been released in all river basins upstream of Rock Island Dam (i.e., Wenatchee, Methow, Okanogan, Entiat), but releases since 1998 have been confined to the Methow and Okanogan rivers, and the Columbia River at the

Wells (rkm 830) and Ringold hatcheries (rkm 567). Although the Wells Hatchery steelhead program has relied on local-origin fish for broodstock, contribution of natural origin fish has been low, reflective of the overall low abundance of natural origin fish in the run-at-large passing Wells Dam. From 1995 to 2010, steelhead releases into the Methow River basin, including Wells-stock fish released into the basin from the Winthrop National Fish Hatchery (WNFH), have averaged about 439,669 fish, with about 24% of those fish released into the Twisp River. Large smolt release numbers and high smolt-to-adult survival rates have routinely resulted in a return of hatchery origin steelhead that greatly exceeded broodstock and spawning ground escapement requirements. Adult steelhead returning to Wells Dam from the 1995 – 2004 broods were primarily hatchery origin fish comprising about 89% of the steelhead returning to Wells Dam from each brood (Snow et al. 2011; Table 1).

Table 1. Summary of hatchery steelhead released into the Methow River basin by Wells Hatchery (WDFW, Chewuch, and Twisp Rivers) or WNFH with adult returns from each brood calculated to Wells Dam. Inc. = incomplete adult return data.

Brood	Methow River		Chewuch River	Twisp River	Methow basin total	Adult returns			% Hatchery
	WDFW	WNFH				H	W	Total	
1995	242,400	141,798	-	-	384,198	3,441	181	3,621	95.0
1996	310,480	104,098	-	-	414,578	2,779	321	3,099	89.6
1997	127,020	-	125,300	126,000	378,320	4,702	710	5,411	86.9
1998	350,431	112,908	116,403	113,583	693,325	14,076	750	14,825	94.9
1999	139,900	105,510	138,300	136,680	520,390	14,691	198	14,889	98.7
2000	116,830	98,834	99,490	109,950	425,104	1,752	993	2,746	63.8
2001	94,020	150,488	85,615	84,475	414,598	11,218	621	11,839	94.8
2002	96,420	119,370	117,495	105,323	438,608	4,577	386	4,964	92.2
2003	80,580	113,603	78,205	117,545	389,933	6,135	420	6,555	93.6
2004	86,041	110,368	82,280	96,405	375,094	4,878	908	5,786	84.3
2005	99,820	102,600	119,500	107,245	429,165	inc.	inc.	inc.	inc.
2006	96,219	122,515	107,545	111,768	438,047	inc.	inc.	inc.	inc.
2007	99,464	116,897	92,670	100,446	409,477	inc.	inc.	inc.	inc.
2008	103,236	102,418	100,373	104,903	410,930	inc.	inc.	inc.	inc.
2009	125,801	100,378	92,760	74,766	393,705	inc.	inc.	inc.	inc.
2010	154,370	107,141	83,861	93,297	438,669	inc.	inc.	inc.	inc.
Mean	145,190	106,808	89,987	105,885	439,669	6,825	549	7,374	89.38

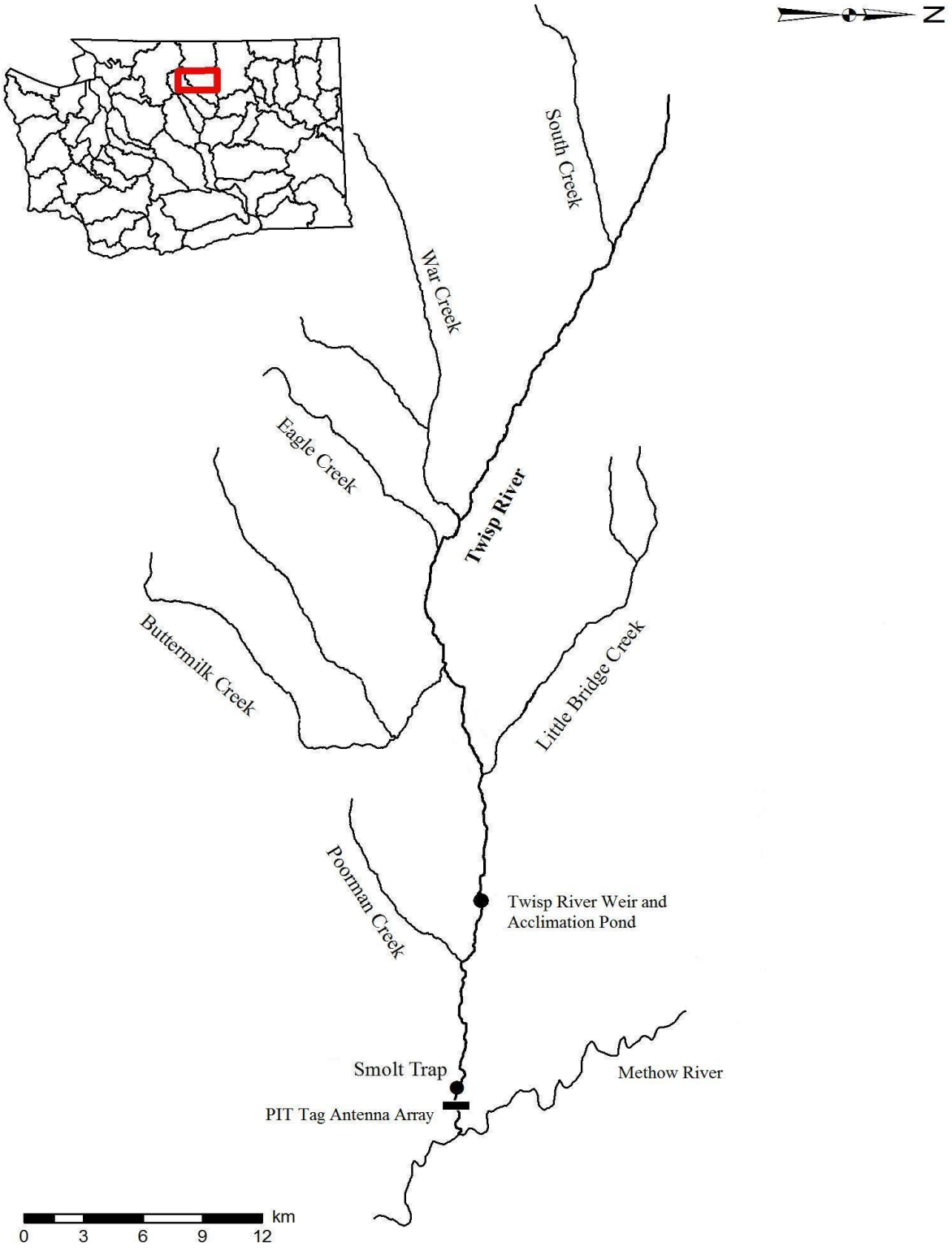

Figure 1. Map of Twisp River basin and primary data collection sites.

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Chapter 1. A comparison of demographic variables that may influence the relative reproductive success of summer steelhead (*Oncorhynchus mykiss*) spawning in the Twisp River, Washington

Abstract

Hatcheries are one of the main tools used to mitigate for salmon and steelhead losses resulting from the construction and operation of the Columbia River hydropower system, and to recover populations listed under the Endangered Species Act. However, the ability of hatchery programs to achieve salmon recovery goals is uncertain due to genetic effects inherent to hatchery spawning and rearing practices that may result in a decrease in the reproductive success of hatchery fish that spawn in the natural environment. As part of an on-going study to determine the relative reproductive success (RRS) of hatchery- and natural-origin (i.e., wild) summer steelhead spawning in the Twisp River, Washington, we evaluated a suite of demographic characteristics of the spawning population of three broods of adult steelhead sampled at the Twisp River weir. We evaluated differences in the run-timing, spawn-timing, age-composition, length-at-age, sex-ratio, and spawning distribution in an effort to determine whether differences existed between hatchery and wild fish that may explain differences in observed RRS, should they occur. We found no significant differences in arrival time at the Twisp River weir between hatchery and wild female steelhead in 2009 (P = 0.96), 2010 (P = 1.00), or 2011 (P = 1.00). Similarly, there were no differences in arrival time between hatchery and wild male steelhead in 2009 (P = 0.17), 2010 (P = 1.00), or 2011 (P = 1.00). We found significant differences in female spawn timing based on fish origin and year (P< 0.01). However, all differences were among years and there were no significant differences between fish of different origin within years (P = 0.44-1.0). There were no significant differences in redd distribution based on fish origin among years (P = 0.13). The proportion of female steelhead within the hatchery and wild populations released upstream of the Twisp River weir was not significantly different for the 2009 (P = 0.751) and 2011 broods (P = 0.325), although 2010 brood hatchery origin fish contained a significantly greater proportion of females (P < 0.001) than did the wild population. No significant differences in the salt-age composition were detected between male and female hatchery and wild steelhead of the same brood year (P = 0.25 – 0.84). Wild fish generally had a greater mean fork length within years compared to hatchery origin fish of the same gender and salt-age but a statistically significant difference was only detected between 1-salt male fish within the 2009 brood (P < 0.01). We found no significant difference between the fecundity of hatchery and wild fish within brood years (P = 0.0576), but fecundity was significantly different between hatchery and wild fish across years (P < 0.001). In general we found few differences between the hatchery and wild fish released upstream of the Twisp River weir that would influence productivity.

Introduction

Hatcheries are one of the main tools that have been used to mitigate for salmon losses caused by the construction and operation of the Columbia River hydropower system. Historically, the goal of most hatcheries was simply to provide fish for harvest and mitigate for hydro related impacts and habitat loss. As concern for the conservation of wild salmon grew in the late 20th Century, the intent of many hatchery programs changed from providing fish for harvest and mitigation to conserving and rebuilding natural populations. As a result, hatcheries now are a large component of most conservation or recovery programs, particularly for populations in the Interior Columbia River Basin. The use of hatcheries to conserve wild salmon is controversial, due in part to concerns about the genetic impacts of even well intentioned hatchery supplementation on wild populations (Waples and Drake 2004).

Genetic risks associated with hatchery supplementation include the potential for increased inbreeding (Ryman and Laikre 1991; Ryman et al. 1995; Wang and Ryman 2001), outbreeding depression (e.g., Gharrett and Smoker 1991) and domestication selection (Ford 2002). The potential implications of these phenomena is reinforced by a long history of studies showing that hatchery fish often reproduce poorly in the wild when compared to natural origin fish (reviewed by Berejikian and Ford 2004; Araki et al. 2008). Hatchery steelhead have been the most widely studied and have been found to have very low relative reproductive success in several studies (Chilcote et al. 1986; Leider et al. 1990; McLean et al. 2003; Araki et al. 2007a; Araki et al. 2007b; Berntson et al. 2011).

Evaluating the relative reproductive success of Methow River steelhead is particularly important because supplementation is being used as a significant component of the recovery strategy for this population. In addition, Upper Columbia steelhead have a very large ‘gap’ between current productivity and the productivity level needed to meet viability goals (ICTRT 2007). This may be due, at least in part, to the large proportion of hatchery fish in the spawning population. Understanding the relative reproductive success of hatchery steelhead in this population is therefore particularly important for evaluating the recovery potential for this population. We developed a study plan to (1) directly measure the relative reproductive success of hatchery and natural-origin steelhead in the natural environment using a DNA pedigree approach, (2) determine the degree to which any differences in reproductive success between hatchery and natural steelhead can be explained by measurable biological characteristics such as run timing, morphology, spawn timing, or spawning location, and (3) estimate the relative fitness of hatchery-lineage steelhead after they have experienced an entire generation in the natural environment. In this chapter, we focus on the second objective and examine factors that may explain differences in reproductive success between hatchery and naturally produced steelhead spawning in the natural environment.

Materials and Methods

Adult Trapping and Biological Sampling

Adult steelhead were trapped and sampled at a fixed weir in the Twisp River. The weir consists of a series of fixed-panels attached to a concrete sill spanning the river at rkm 11.4. The weir panels are raised and lowered via hydraulic controls located in a protected panel box on the left bank of the river allowing the weir to form a barrier to upstream migration at discharge levels up to about 22.6 m^3/sec. Access to the weir is provided for vehicles and crew through an existing road servicing the weir and the Twisp River spring Chinook (*O. tshawytscha*) acclimation pond. During trapping operations, the weir panels are maintained in a position that prevents upstream migration, but allows the downstream passage of post-spawning fish or other species attempting to migrate downstream. Two aluminum trap boxes constructed from 38 mm bar-stock aluminum pickets are attached to the cement sill on the upstream side of the weir panels. One box is located directly adjacent to the left bank, and the other is located approximately 6.7 m towards the center of the river channel. Each trap box is approximately 3.6 m^3 with a 0.6 m^2 opening at the downstream end fitted with a 0.19 m^2 cod-trigger—a commercial fishing device used to allow one-way passage of target species into crab pots. Each trap box had an aluminum gate at the upstream end that could be raised to allow fish to escape. This gate was kept shut during the entire trapping period on the center-channel box, but a dividing screen was added to the left-bank trap box so that fish could remain trapped within the structure, but the upstream section serves as a volitional release recovery area after sampling. Flow through the trap boxes passes through the cod-trigger and over the sill through a narrow gap in the weir panels. This flow attracts fish attempting to pass the weir and the cod-triggers at the back of each trap box allow fish to enter but prevent emigration. Trap boxes are accessed by a 6.7 m aluminum walkway that spans the shore-side trap box and terminates at the left bank side of the center trap. A removable ladder transferred between each trap box allowed personnel to enter the trap box from the walkway to remove trapped fish.

Trapped fish were netted, placed into a rubber tube with water and transported along the walkway to the sampling area on the left bank. Fish were anesthetized in a 0.48 m^3 container containing approximately 220 L of MS-222 solution at an approximate concentration of 22 mg/L. All salmonids captured at the weir were sampled to determine species, origin, length (fork length or post-orbital to hypural plate [POH]), and were examined for existing marks or tags. Additionally, steelhead and cutthroat trout without existing tags were scale-sampled and tagged with a passive integrated transponder (PIT) tag prior to release. A small (approximately 10 mm^2) piece of caudal fin tissue was collected from steelhead and cutthroat trout released upstream of the weir for DNA analysis. Fish released upstream were allowed to recover briefly in a 210 L recovery tank, then transferred via rubber tube to the volitional release area in the upstream half

of the left bank trap box. Fish to be released downstream were placed in a 1.1 m^3 nylon mesh net pen anchored to the left bank immediately downstream of the weir.

The Twisp River weir was constructed to capture spring Chinook broodstock, but was operated to trap steelhead for the first time in 2009. During the initial year of operation, only wild and known Twisp River hatchery fish were allowed upstream of the weir to spawn. Unknown hatchery fish (i.e., fish not released as juveniles into the Twisp River) were released downstream of the weir to spawn naturally in the lower Twisp or Methow River. Management of returning adult steelhead at the Twisp River weir changed in 2010. All hatchery fish of unknown origin were removed and known Twisp River hatchery steelhead were released upstream of the weir in a similar proportion as wild steelhead. Beginning in 2011, a small number of wild steelhead (13 females and 13 males) were retained for hatchery broodstock, a practice that will continue in the future.

We used arrival date at the weir for steelhead released upstream of the weir to evaluate run timing of hatchery and wild fish. Differences in spawning location and timing between hatchery- and naturally-produced steelhead were evaluated by inserting a PIT tag in the abdominal cavity of female steelhead in the chance that the PIT tag would be deposited with the eggs during spawning. Additionally, because not all returning hatchery fish were adipose fin clipped, we applied 75 mm colored anchor tags, (color based on sex and origin) to fish released upstream of the weir to allow stream surveyors to visually determine the sex and origin of spawning fish. When spawning was near completion, we attempted to scan every completed redd for expelled PIT tags, and used the redd completion date from surveys when a PIT tag was detected to determine spawn timing. Data on spawn timing and location were collected during spawning ground surveys conducted at least weekly by raft or by walking. We recorded the date, redd number, relative location, fish presence, Global Positioning System (GPS) waypoint number, and tag color (if any) on colored bio-degradable flagging and attached it to vegetation on the stream bank adjacent to each redd. At least once during each survey season we scanned completed redds with a PIT tag detector and correlated detected tags with the redd number assigned during stream surveys. Where appropriate, we used fork length and age data from scale samples or hatchery mark information collected at the weir to examine morphological differences between hatchery and naturally produced fish (i.e., length-at-age and age composition). Direct measurement of fecundity was not possible for steelhead released upstream of the weir; therefore, we used fecundity estimates from hatchery and wild fish retained for local broodstocks as a surrogate. These estimates were collected during the incubation phase of hatchery rearing and were calculated as the sum of live and dead eggs at the eyed stage.

Data Analysis

For most comparisons between hatchery and wild fish (except fecundity), only hatchery fish released upstream of the weir were used. Including all fish arriving at the weir would have provided a greater sample size for most analyses in each year. However, our intent was to describe the spawning population contributing to the RRS study, not necessarily to describe the overall Twisp River steelhead population, or the characteristics of individual hatchery stocks. Additional information regarding the entire Twisp River population can be found in Snow et al. (2010, 2011).

Data were tested for normality using Shapiro-Wilk's W-tests and homogeneity of variances using Levene's tests. Data were transformed using standard statistical procedures to achieve normal distributions when necessary. Nonparametric tests were used when normal distributions could not be achieved and variance was unequal between groups. All statistical tests were performed at a significance level of 0.05 (i.e., a 5% chance of erroneously rejecting a null hypothesis). Mean arrival date at the Twisp River weir was compared across years, sexes, and origins using a Kruskal-Wallis rank sum test (KW test) because the assumption of equal variances among groups was not met. Mean spawn timing in the Twisp River was also analyzed across years and origins using a KW test. Redd distribution in the Twisp River based on female origin was analyzed across years using a KW test because normality could not be achieved through log transformations. Fecundity was compared between hatchery and wild steelhead collected for Wells Hatchery broodstock from Wells Dam or the Twisp River weir. We compared the fecundity within each brood by origin using ANCOVA tests with length as a covariate. Length at age was compared within years using factorial ANOVA with age, origin, and fork length as variables. Chi-squared tests were used within brood years by sex and origin, and by salt-age, sex, and origin to test the sex ratio and age composition for independence. Using the age data from scale samples, we removed non-anadromous fish (i.e., wild resident or hatchery residual *O. mykiss*) and repeat-spawning anadromous adult fish from the length at age and age-ratio comparisons because sample sizes within brood years were too small to make meaningful comparisons between hatchery and wild fish of the same gender and age.

Results

Weir Operation

In 2009, the Twisp River weir was operated between 16 March and 27 May when operation ceased because of high river discharge (Figure 1). Trapping resumed on 15 June for spring Chinook salmon when discharge dropped below 30 m^3/s. Of the 376 adult steelhead sampled at the weir in 2009, 343 of those were passed upstream (Table 2). In 2010, the weir was operated between 2 March and 30 June at discharge levels up to 44 m^3/s. A total of 608 adult steelhead

were sampled at the weir in 2010, and 346 were passed upstream. In 2011, the weir was operated between 19 March and 14 May and operation ceased on 15 May when discharge spiked to 69 m^3/s. Trapping resumed on 28 June to target spring Chinook salmon when discharge dropped below 50 m^3/s. A total of 279 adult steelhead were sampled at the weir in 2011 and 236 were passed upstream. All wild adult steelhead were passed upstream of the weir in 2009 and 2010, however, 13 wild females and 13 wild males were retained for broodstock in 2011. Tissue samples for genetics analysis were taken from all wild fish sampled in 2009 (N = 91), 2010 (N = 173), and 2011 (N = 119). Tissue samples were also taken from all hatchery steelhead passed upstream of the weir in 2009 (N = 252), 2010 (N = 173), and 2011 (N = 117). In addition, tissue samples were taken from some hatchery steelhead released downstream of the weir in 2009 (N= 8) and from westslope cutthroat trout (*O. clarkii lewisi*) collected at the weir in 2010 (N = 76) and 2011 (N = 43).

Run Timing

Within each year, adult steelhead migration peaked in April regardless of sex or origin (Figure 1; Table 2). The number of adult steelhead that may have passed the weir after the termination of operation in 2009 and 2011 is unknown. However, run timing appeared roughly similar among years and few fish were sampled after 15 May in 2010 when weir operation was continuous from March through June. Thus, it is likely that the majority of the adult steelhead runs in 2009 and 2011 were sampled despite the termination of weir operations due to high river discharge. Significant differences in run timing were found among years and between sexes and origin ($P < 0.01$); however, differences were inconsistent among years. There were no significant differences in arrival time between hatchery and wild female steelhead (Figure 2) in 2009 (P = 0.96), 2010 (P = 1.00), or 2011 (P = 1.00). Similarly, there were no differences in arrival time between hatchery and wild male steelhead (Figure 3) in 2009 (P = 0.17), 2010 (P = 1.00), or 2011 (P = 1.00). Once in the Twisp River, no significant difference in the migration rate (km/day) between the Twisp River PIT tag antenna array and the Twisp River weir was detected between hatchery and wild fish of the same brood year (ANOVA: P = 0.32; Figure 4).

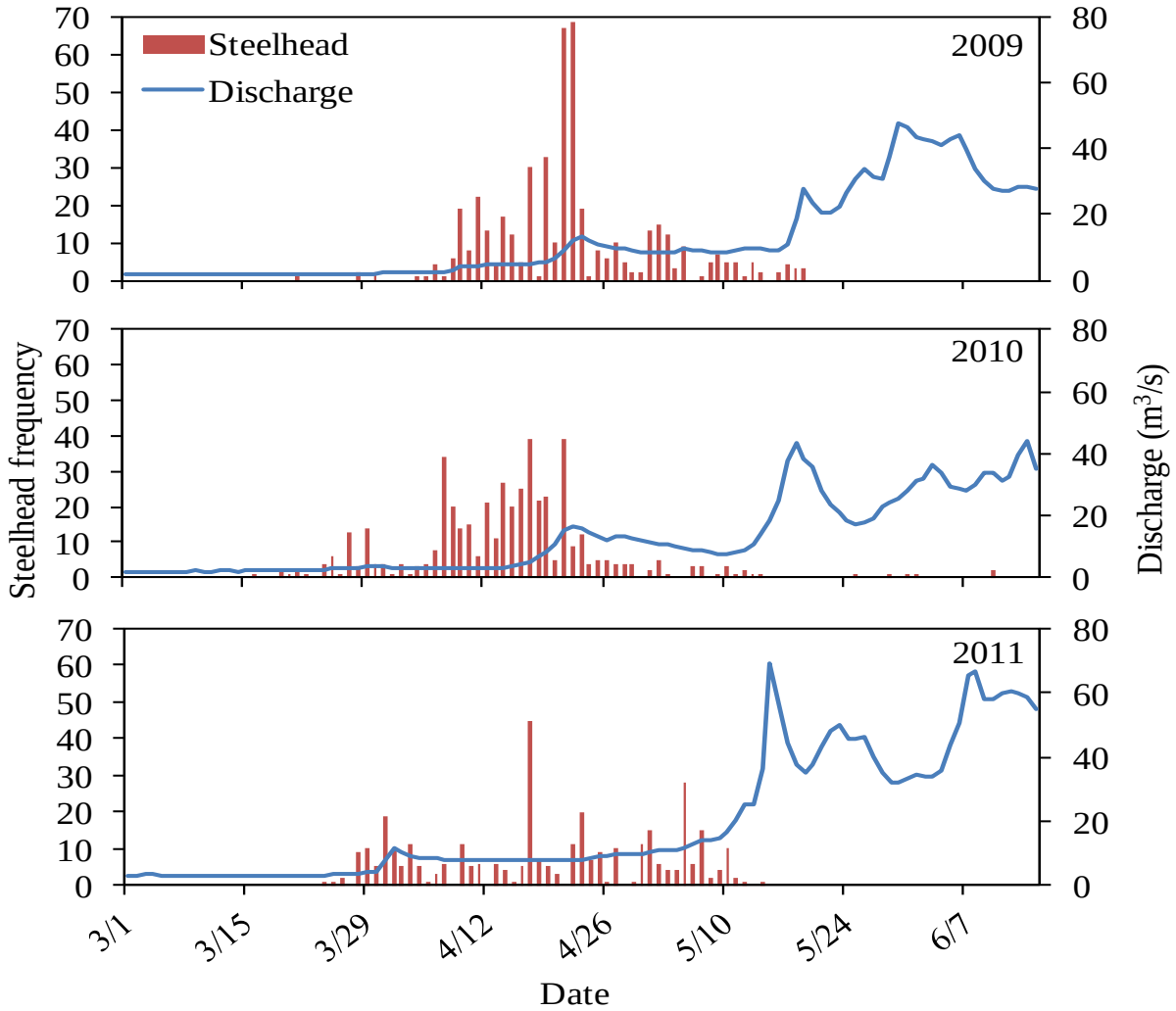

Figure 1: Daily adult steelhead counts at the Twisp River weir and mean daily discharge in the Twisp River from 1 March through 15 June in 2009 (top panel), 2010 (middle panel), and 2011 (bottom panel). Daily discharge derived from USGS gauging station No. 12448998 at rkm 2.5.

Table 2. Summary of steelhead sampled at the Twisp weir from 2009 through 2011.

Year	Origin	Sex	Month				Total	Released upstream
			March	April	May	June		
2009	Wild	F	1	41	7	0	49	49
2009	Wild	M	2	36	4	0	42	42
2009	Hatchery	F	0	108	33	0	141	128
2009	Hatchery	M	2	125	18	0	144	124
2010	Wild	F	8	63	3	1	75	75
2010	Wild	M	20	68	5	0	98	98
2010	Hatchery	F	9	191	13	1	214	113
2010	Hatchery	M	18	198	5	0	221	60
2011	Wild	F	13	53	19	0	85	72
2011	Wild	M	13	35	11	0	59	47
2011	Hatchery	F	8	56	24	0	88	78
2011	Hatchery	M	11	31	5	0	47	39

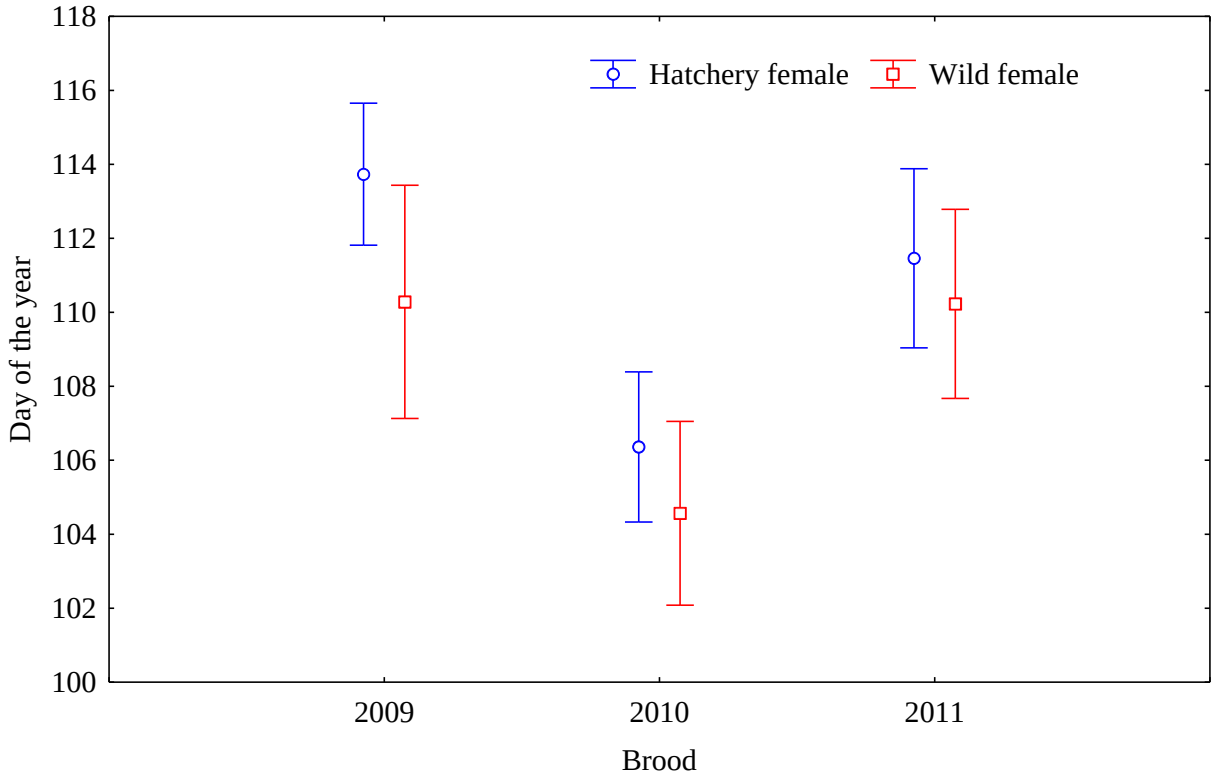

Figure 2. Mean arrival timing of female steelhead at the Twisp River weir by brood. Error bars represent 95% confidence intervals.

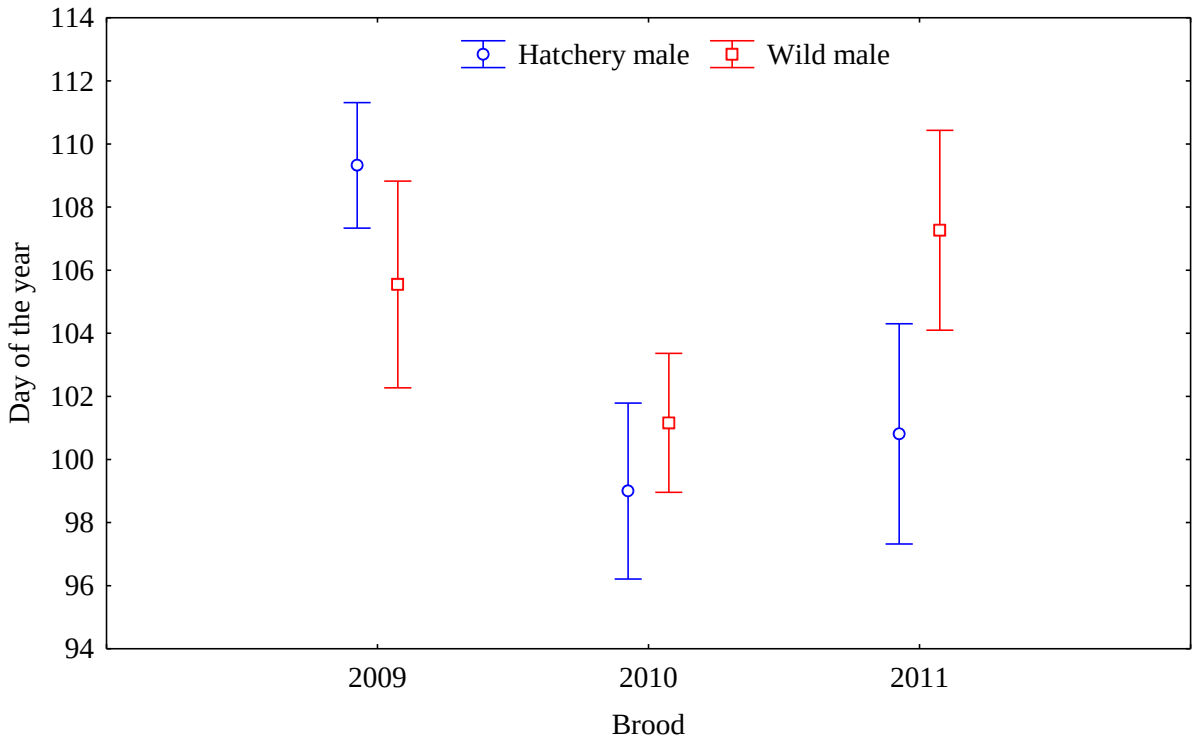

Figure 3. Mean arrival timing of male steelhead at the Twisp River weir by brood. Error bars represent 95% confidence intervals.

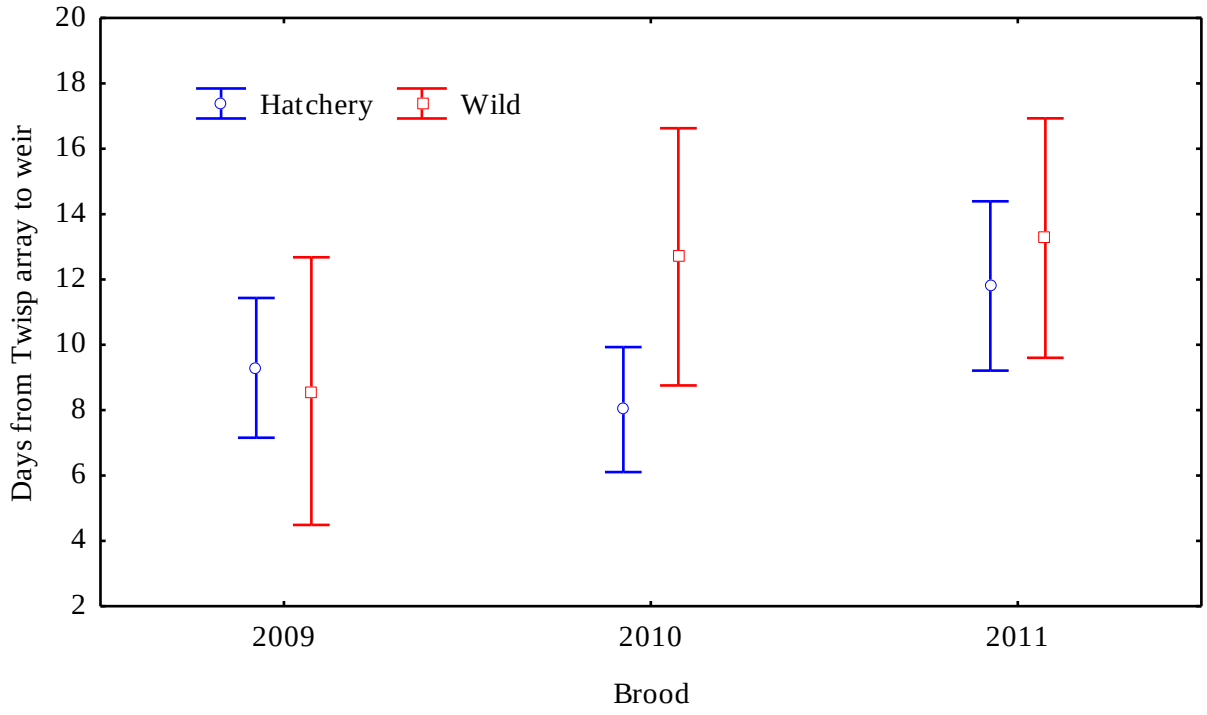

Figure 4. Migration timing of hatchery and wild summer steelhead between the Twisp River PIT tag antenna array (rkm 2) and the Twisp River weir (rkm 11.4). Error bars represent 95% confidence intervals.

Steelhead Spawner Demographics

The proportion of female steelhead within the hatchery and wild populations released upstream of the Twisp River weir was not significantly different for the 2009 (P = 0.751) and 2011 broods (P = 0.325). The 2010 brood of hatchery origin spawners upstream of the weir contained a significantly greater proportion of female fish (P < 0.001) than did the wild population (Figure 5). Because the number of years spent in freshwater differs between hatchery (typically 1-year) and wild fish (typically 2 or more years), we could not compare total age between fish of different origins. Instead, we used the number of winters a fish spent in salt water to compare the age composition within each brood year by sex and origin. No significant differences in the salt-age composition were detected between male and female hatchery and wild steelhead of the same brood year and salt-age (Table 3). Wild fish generally had a greater mean fork length within years compared to hatchery origin fish of the same gender and salt-age (Figure 6), but a statistically significant difference was only detected between 1-salt male fish within the 2009 brood (Mann-Whitney U-test: P < 0.01).

Female steelhead were collected entirely at Wells Dam for the 2009 (N = 150) and 2010 Wells Hatchery broodstocks (N = 156), but the 2011 broodstock (N = 177) included 12 wild females collected at the Twisp River weir. Analysis of covariance of fecundity using POH as a covariate found no significant difference between the fecundity of hatchery and wild fish within brood years (P = 0.06), but fecundity was significantly different between hatchery and wild fish across years (P < 0.001; Figure 7).

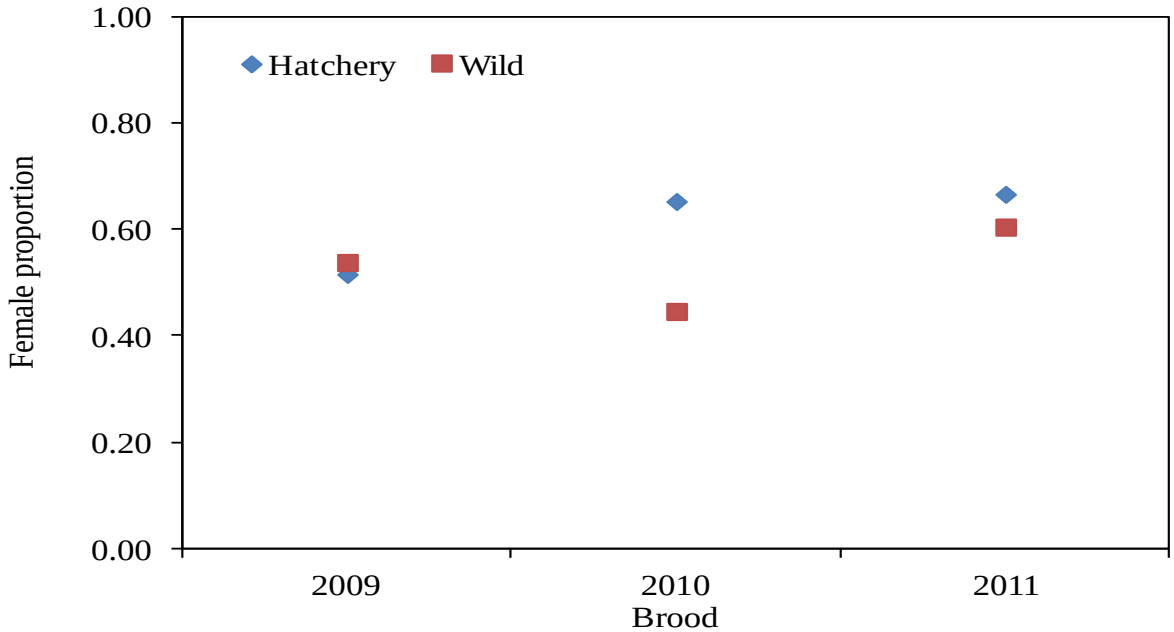

Figure 5. Proportion of female steelhead within the hatchery and wild populations released above the Twisp River weir by brood.

Table 3. Salt-age structure and Chi-squared (χ^2) statistics of steelhead released upstream of the Twisp River weir by age, origin, sex, and brood.

Sex	Year	1-salt H	1-salt W	2-salt H	2-salt W	χ^2	*P*
F	2009	68	26	55	20	0.038	0.84
	2010	61	47	50	27	1.338	0.25
	2011	15	11	63	59	0.315	0.57
M	2009	103	40	12	2	1.219	0.27
	2010	50	76	8	17	0.520	0.47
	2011	22	28	15	17	0.065	0.80

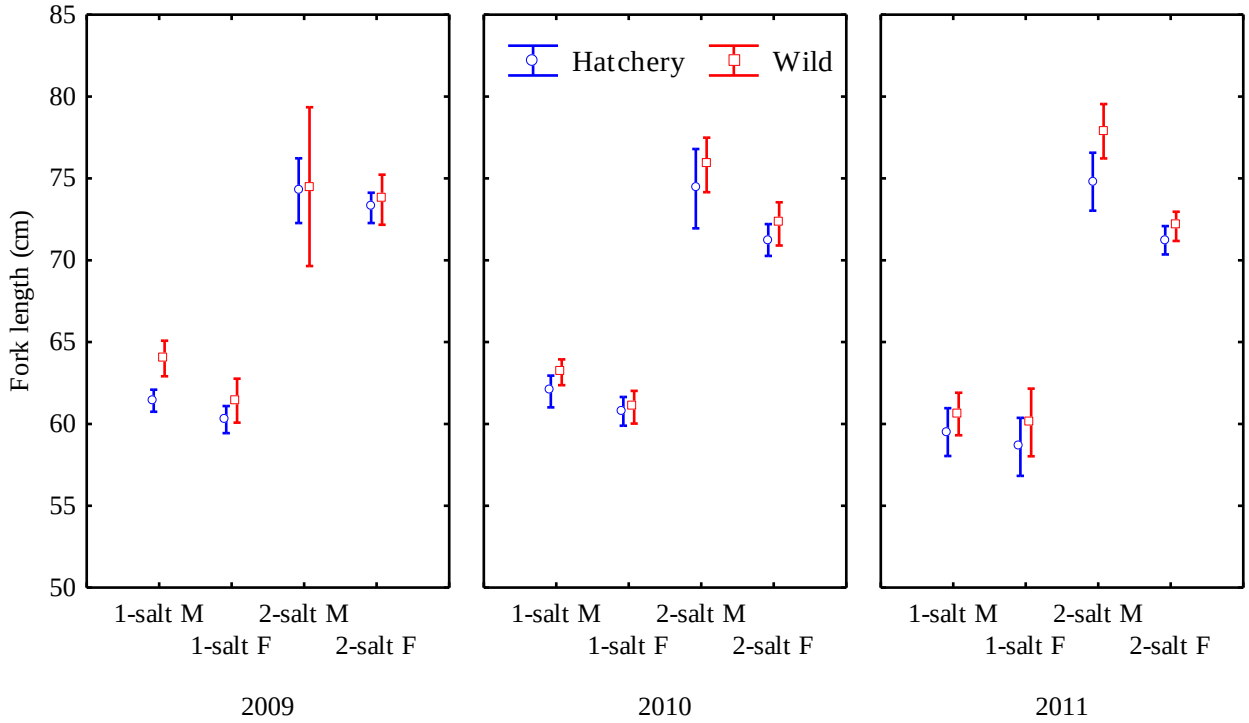

Figure 6. Mean fork length (cm) of steelhead released upstream of the Twisp River weir based on salt-age, brood, and genetic origin. Error bars represent 95% confidence intervals.

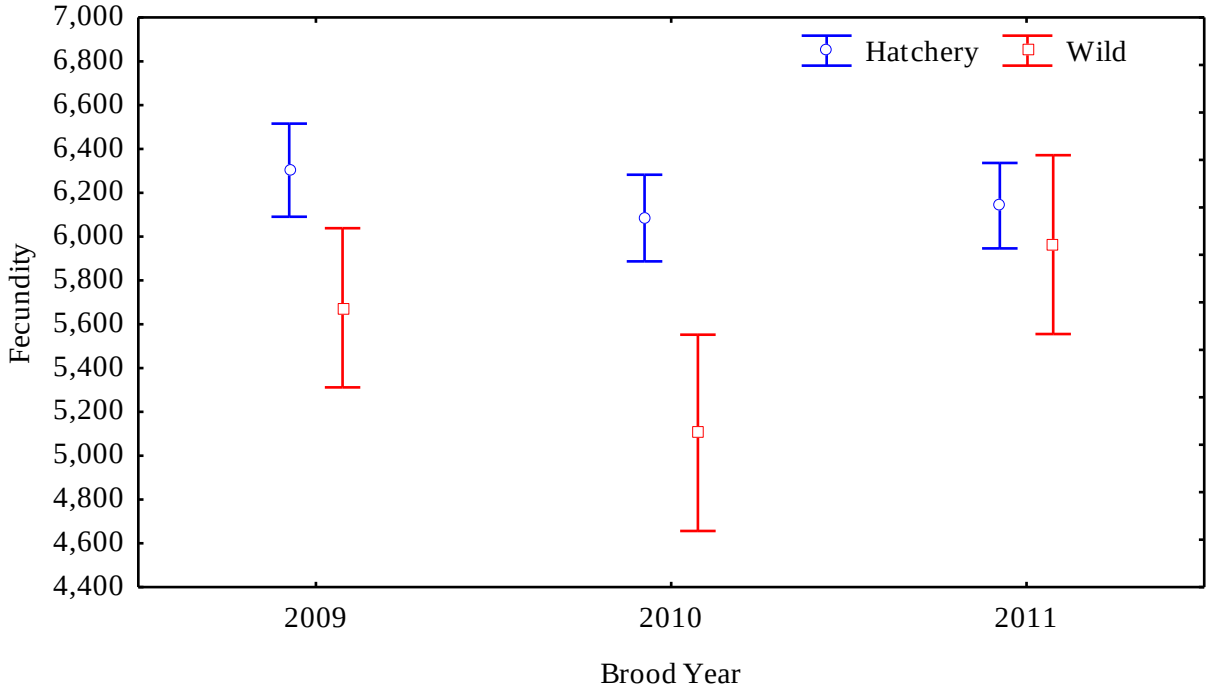

Figure 7. Mean fecundity of hatchery and wild steelhead sampled at the Wells and Methow hatcheries by brood and origin after adjusting for POH as a covariate. Error bars represent 95% confidence intervals.

Spawn Timing and Distribution

In 2009, steelhead spawning surveys in the Twisp River basin were conducted from 30 March to 12 June. Surveyors found a total of 268 steelhead redds in the Twisp River mainstem, Little Bridge Creek, War Creek, Eagle Creek, and Buttermilk Creek in 2009 (Table 4). In 2010, spawning surveys were conducted from 15 March through 7 June. Surveyors found 329 redds in the Twisp River mainstem, Little Bridge Creek, MSRF ponds, and Buttermilk Creek (Table 4). In 2011, spawning surveys were conducted from 20 March through 13 May. A total of 194 redds were found in the Twisp River mainstem, MSRF ponds, and Eagle Creek (Table 4). The majority of steelhead redds were found upstream of the weir in 2009 (74%), 2010 (64%), and 2011 (54%). No redds were found in Little Bridge Creek in 2011; however, this may be related to the timing of surveys. Surveys in Little Bridge Creek were only conducted from 11 April to 23 April before spring runoff drastically decreased water visibility. A total of 24 adult steelhead were detected by an instream PIT tag array in Little Bridge Creek at approximate rkm 0.2 between 2 April and 13 May suggesting that spawning did occur though no redds were enumerated. Across years, no redds were found in the uppermost reaches of the Twisp River mainstem (T10) and Little Bridge Creek (LBC4), and no redds have been detected in South Creek. There were no significant differences in redd distribution based on fish origin among years ($P = 0.13$; Figure 8).

Spawn timing of hatchery and wild fish was evaluated during spawning ground surveys conducted at least weekly in the Twisp River upstream of the weir using PIT tag detections in completed redds, or by observations of colored anchor tags in spawning fish. We found significant differences in female spawn timing based on fish origin and year ($P < 0.01$; Figure 9). However, all differences were among years and there were no significant differences between fish of different origin within years ($P = 0.44$-1.0; Figure 9).

Table 4. Twisp River mainstem and tributary redd counts by reach and survey year (ns = not surveyed).

Stream reach	Code	Length (km)	2009	2010	2011
Twisp River mainstem					
Road's End C.G. - South Creek Bridge	T10	4.6	0	0	0
South Creek Bridge - Poplar Flats C.G.	T9	3.2	1	3	0
Poplar Flats C.G. - Mystery Bridge	T8	3.2	4	4	0
Mystery Bridge - War Creek Bridge	T7	6.9	24	18	8
War Creek Bridge - Buttermilk Bridge	T6	7.4	60	96	47
Buttermilk Bridge - Little Bridge Creek	T5	5.9	71	52	32
Little Bridge Creek - Twisp Weir	T4	3.8	14	25	13
Twisp Weir - Upper Poorman Bridge	T3	3.5	29	70	48
Up. Poorman Br. - Lower Poorman Br.	T2	5.0	30	35	34
Lower Poorman Bridge - Confluence	T1	2.9	11	13	4
Twisp River mainstem total		46.4	244	316	190
Twisp River tributaries					
Little Br. Cr. (Road's End – Vetch Cr.)	LBC4	1.3	ns	0	0
Little Br. Cr. (Vetch Cr. – 2nd Culvert)	LBC3	3.0	0	1	0
Little Br. Cr. (2nd Culvert – 1st Culvert)	LBC2	2.4	1	3	0
Little Br. Cr. (1st Culvert - Confluence)	LBC1	2.4	17	4	0
MSRF pond outfalls[1]	MSRF 1	0.1	0	1	3
RP-War Creek (log jam barrier - Conf.)	WR1	0.5	2	0	0
RP-Eagle Creek (Rd 4430 - Confluence)	EA1	0.3	2	0	1
RP-Buttermilk Cr. (Fork - Cattle Guard)	BM2	2.1	0	3	0
RP-Buttermilk Cr. (Cattle Guard - Conf.)	BM1	2.0	2	1	0
RP-South Creek (Falls - Confluence)	SO1	0.6	0	0	0
Twisp River tributary total		14.7	24	13	4

[1] Methow Salmon Recovery Foundation pond outfall.

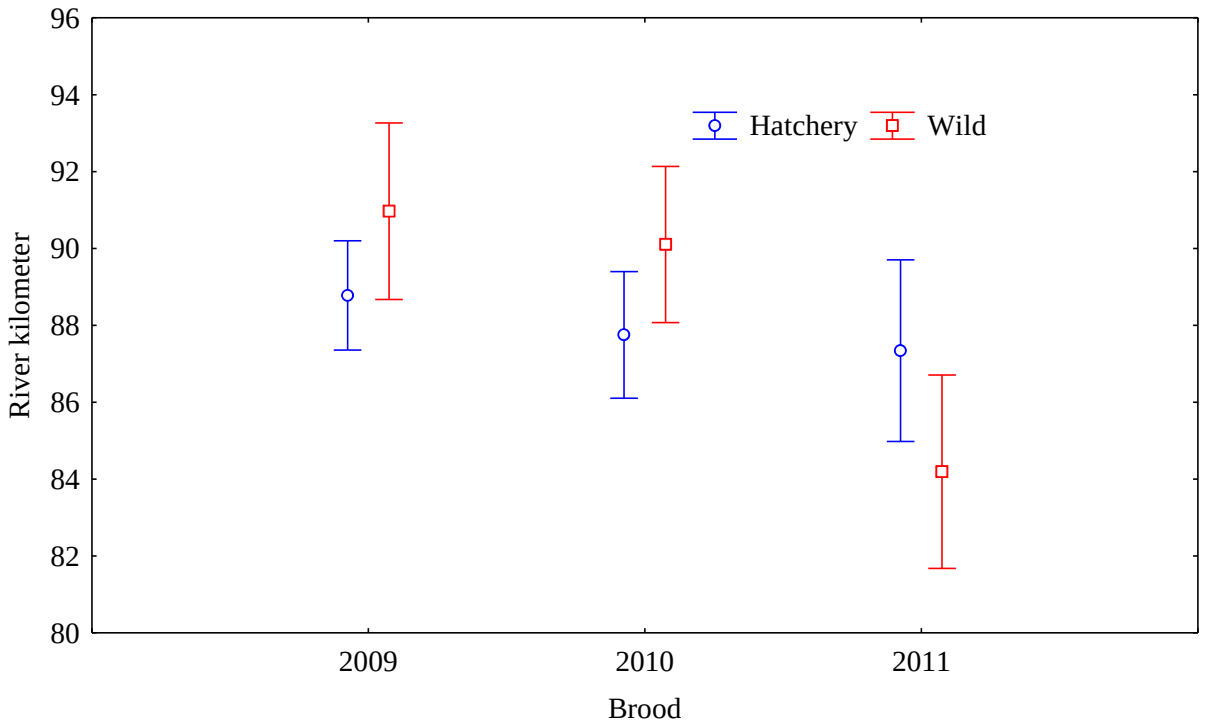

Figure 8. Spawning distribution of hatchery and wild summer steelhead released upstream of the Twisp River weir by brood and kilometer. Error bars represent 95% confidence intervals.

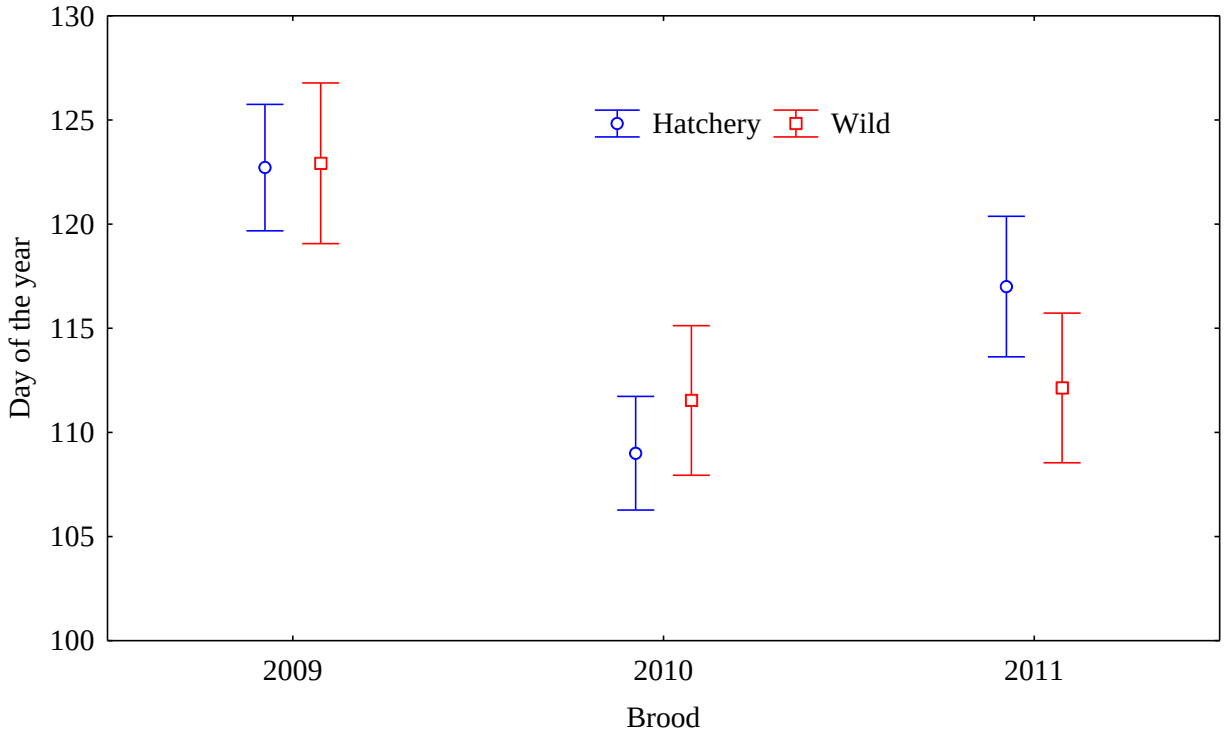

Figure 9. Spawn timing of female steelhead in the Twisp River based on anchor tag and PIT tag recoveries.

Discussion

While it is not possible to sample every potential parent in the natural environment, we attempted to sample every anadromous adult. However, environmental conditions made the weir inoperable at times, potentially allowing steelhead to pass upstream undetected. We estimated the capture efficiency of the weir in 2011 by examining data from the observation of anchor tags used to evaluate spawn timing and location during spawning ground surveys. In that year, a total of 83 steelhead were observed actively spawning, holding on redds, or migrating upstream of the weir, and only three fish did not have visible anchor tags (3.6%). However, based on recaptures of PIT tagged fish at the weir, we estimate that the anchor tag loss rate was 10.5% (i.e., 6 of 57 recaptures had lost anchor tags). This suggests that the capture efficiency of the weir as measured by mark/re-sight of anchor tags was at least 96.4%, but likely higher. A similar analysis was not conducted in prior years because observations were not recorded in a manner that allowed estimation of efficiency (2009) or only female fish received anchor tags (2010), thus confounding efficiency estimates. Despite this, we anticipate that efficiencies between years were similar because operational protocols and environmental conditions were similar.

In general, we found few differences between population demographics of three broods (2009-2011) of hatchery and wild steelhead sampled at the Twisp River weir. Differences in numerical abundance were controlled in 2010 and 2011 by removing excess hatchery origin fish from the spawning population. The resulting populations spawning in the natural environment showed few or inconsistent statistical differences in the spawning location, timing, fecundity, length at age, and age structure. Ultimately, our study will analyze the relation between measured phenotypic traits of the spawning populations of hatchery and wild fish and reproductive success, but such analyses would be premature at this time.

Acknowledgements

We thank the numerous scientific technicians who worked on this project collecting a wide variety of data from adult and juvenile steelhead, without which, this project could not succeed. We also thank the WDFW aging lab for providing scale sample analysis, and employees at the Wells and Methow hatcheries for providing weir operation expertise, staff time, fish transport, and sampling of steelhead fecundity at both facilities. This project was funded by the Bonneville Power Administration and by the Public Utility District No. 1 of Douglas County, Washington. We greatly appreciate their financial and technical support.

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Chapter 2. Pedigree reconstruction and relative reproductive success of hatchery and wild steelhead spawning in the Twisp River, Washington.

Abstract

Hatchery steelhead *Oncorhynchus mykiss* have been released into the Twisp River for the last decade to mitigate for salmon and steelhead losses caused by the construction and operation of the Columbia River hydropower system, and to boost population demographics in an effort to assist in the recovery of a threatened species. However, hatchery-reared steelhead have exhibited poor reproductive success when compared to naturally-reared con-specifics, putting into question whether recovery efforts that rely on hatchery-reared fish can succeed. We instituted a relative reproductive success study utilizing hatchery- and naturally-reared summer steelhead in the Twisp River to evaluate reproductive differences using a DNA-based pedigree approach. We analyzed DNA samples collected from 698 adult steelhead from two broods passed over a weir on the Twisp River, and collected 1,453 DNA samples from juvenile *O. mykiss* from one of those broods (2009) using angling, netting, electrofishing, and a rotary screw trap to determine relative reproductive success. Samples were genotyped at 152 single nucleotide polymorphism loci to determine population structure, and to complete parentage assignments. Juvenile samples were assigned a single parent (30.7%), two parents (38.4%), or no parents (30.9%). Of the single-parent assignments, most (66.1%) were assigned the maternal parent. Hatchery- and natural-origin mothers produced similar numbers of offspring (hatchery = 4.6 offspring per female; natural = 5.2 offspring per female). Hatchery males appeared to produce fewer offspring than natural-origin males (hatchery = 3.0 offspring per male; natural = 7.2 offspring per male). Although a slight difference was detected in the reproductive success of hatchery- and naturally-produced fish as measured by juveniles produced, this project is designed to evaluate reproductive success over multiple generations. These preliminary results are similar in some respects to other steelhead reproductive success studies, but drawing any major conclusions at this time would be premature.

Introduction

Hatcheries are one of the main tools used to mitigate for salmon losses caused by the construction and operation of the Columbia River hydropower system. Despite this, little is known about how much the investment in hatcheries benefits or harms natural production. Within the Habitat Conservation Plan for the Wells Dam Hydroelectric Project (Wells HCP) the Public Utility District No. 1 of Douglas County (DCPUD), Washington agreed to fund a study to investigate the relative reproductive success of hatchery- and naturally-produced (i.e., wild) steelhead (*Oncorhynchus mykiss*) spawning in the Twisp River, Washington. The Washington Department of Fish and Wildlife (WDFW) operates Wells Hatchery, adjacent to Wells Dam, under contract with DCPUD to produce salmon and steelhead for mitigation and harvest

augmentation purposes. Under this agreement, WDFW has released hatchery steelhead in the Methow River basin since about 1969 and in the Twisp River watershed since about 1998.

Prior to 2011, broodstock for Twisp River releases has come from hatchery and wild fish collected from the run at large passing Wells Dam. Starting with the 2011 brood, the Twisp River program changed to an integrated program whereby only naturally produced adults are used as broodstock and the number of naturally spawning hatchery-produced adults are controlled consistent with recommendations made by the Hatchery Scientific Review Group (HSRG 2009). Specifically, only naturally-produced adult steelhead from the Twisp River will be used as broodstock to produce offspring to be released in the Twisp River and the number of hatchery-produced adult steelhead allowed to spawn naturally upstream of the Twisp River weir will be limited.

Our research project will address three key questions related to the efficacy of this hatchery steelhead supplementation program. First, what is the relative reproductive success of hatchery and wild steelhead spawning in the natural environment?; second, if a difference in relative reproductive success is detected, can the difference be explained by differences in measurable biological traits that differ between hatchery and wild fish?; and third, if hatchery steelhead have initially low relative reproductive success, to what degree is this effect reduced in their natural origin progeny? In other words, does the 'hatchery' effect disappear to an appreciable degree after an entire generation in the wild?

The purpose of this report is to summarize the methods and results of genetic analysis of the first two years (2009 and 2010) of adult steelhead sampling at the Twisp River weir and to report on preliminary parentage assignment and reproductive success analysis.

Methods

Sampling Location and Methods

Samples from adult hatchery and wild steelhead (parents) were collected from a weir located in the Twisp River, a tributary to the Methow River in north central Washington State in 2009 and 2010. Adult steelhead collected at the weir were sampled and tagged as necessary to meet study or broodstock objectives as described in Chapter 1. To conduct parent-to-juvenile pedigree analysis, a small fin clip was taken from all steelhead released upstream of the weir for genetic analysis. Fin clips were preserved in 95% ethanol and stored at room temperature.

We collected steelhead parr from the Twisp River basin in 2010 to meet numeric (N = 1,500) tissue sampling and DNA analysis objectives for age-1 progeny of 2009 brood adult steelhead sampled at the Twisp River weir. We used a combination of angling, electrofishing, and netting to collect juvenile steelhead parr. Angling was conducted following equipment rules for

selective fisheries (i.e., unscented artificial flies or lures with a single, barbless hook) defined in annual sport fishing rule pamphlets for Washington State. Electrofishing was conducted using a Halltech HT-2000 pulsed DC battery-powered backpack electrofisher with a telescoping anode pole and stainless steel cable cathode. Electrofisher settings were altered by date and location for voltage (250−350 V) and frequency (40−60 Hz) to maximize capture efficiency and minimize fish injury. Nets of various sizes including 0.14 m^2 hand-held dip nets and 7.4 m^2 seine nets were used on a limited basis to evaluate their feasibility as alternative fish capture methods. Sampling frequency using these techniques varied throughout the sampling period in relation to river flows and staff availability. Sampling was conducted at various locations in the Twisp River mainstem (mouth to rkm 44), and in selected tributaries such as Little Bridge Creek (mouth to rkm 11), and War Creek (mouth to rkm 1; see Figure 1). Sampling locations were initially selected to reflect the spatial distribution of 2009 brood steelhead redds. However, low capture rates in some locations with correspondingly high redd counts led us to sample in downstream reaches to identify rearing locations with a high density of steelhead parr that would assist in meeting sample size targets. Hence, some juvenile sampling was conducted in the Twisp River downstream of the weir.

Regardless of capture method, steelhead parr were held in 19 L plastic buckets filled with river water until the collection event was completed. Captured fish were anesthetized in a solution of tricaine methanesulfonate (i.e., MS-222) at a concentration of 40−60 mg/L, scanned for presence of a PIT tag, measured for fork length to the nearest mm, and weighed to the nearest 0.1 g. A small sample of tissue (approximately 2 mm^2), typically from the distal portion of the dorsal lobe of the caudal fin, was collected from each fish that had not been previously sampled. Tissue samples were placed in individually labeled vials containing 100% ethanol. For age determination and to confirm parental origin (hatchery or wild), scales were collected from steelhead parr from the area between the most posterior ray of the dorsal fin and the anterior ray of the anal fin and about three rows above the lateral line. Tissue and scale samples were sent to the WDFW Molecular Genetics Lab (MGL) and the ageing lab in Olympia, Washington for DNA and age analysis, respectively. All unmarked wild parr were tagged with PIT tags to prevent double sampling of individuals, and to estimate survival to other life history stages (i.e., smolt or adult) or locations (i.e., in-stream or Columbia River hydropower detection facilities). After sampling, location data was collected using a hand-held GPS device. Fish were allowed to fully recover in a bucket of river water then released in a calm part of the river near the sampling location. Tagging data was uploaded to the regional PIT tag database (PTAGIS) maintained by the Pacific States Marine Fish Commission (PSMFC) following standard protocols.

Sampling of juvenile steelhead that were considered to be migrating seaward (i.e., smolts) was conducted using a rotary smolt trap in the Twisp River at rkm 2 with the objective of collecting tissue samples for DNA analysis from the progeny of adult steelhead sampled at the Twisp River weir in 2009. We used a rotary smolt trap with a 1.5 m cone diameter operated at the site to estimate annual steelhead and spring Chinook smolt production as part of a comprehensive

monitoring and evaluation plan for hatchery programs funded by DCPUD (Snow et al. 2010, 2011). The Twisp smolt trap was modified after purchase by the addition of a vertical frame structure for raising the trap-cone, a debris deflecting structure mounted in front of the cone, and ancillary 0.37 m^3 fish holding boxes mounted on the downstream end of each pontoon. The Twisp smolt trap typically operated 24h/day with captured fish removed from the trap at least once daily for sampling. Discharge, water velocity, and smolt abundance influenced sampling frequency. During periods of low smolt abundance, fish were removed from the trap each morning. During periods of greater discharge or smolt abundance, the trap was monitored throughout the night to minimize mortality of captured fish and prevent equipment damage. Trapping was occasionally interrupted by unfavorable environmental conditions (e.g., flooding, low flow, ice). Biological sampling of captured fish was similar to that described above for parr. However, natural origin steelhead were visually classified as fry, parr, transitional, or smolt. Fry were defined as newly emerged fish without a visible yolk sac and largely underdeveloped pigmentation, with a fork length < 50 mm. Parr had a fork length ≥ 50 mm and distinct parr marks on their sides. Transitional emigrants had faded parr marks, bright silver coloration, and some scale loss. Juvenile steelhead lacking (or having highly faded) parr marks, bright silver color, and deciduous scales were classified as smolts. We considered fish classified as either a transitional or smolt to be migrating seaward.

Genetic Sample Processing

Samples were genotyped at 152 single nucleotide polymorphism loci (SNPs). Twenty SNP loci were developed to discriminate among trout species; 14 distinguish *O. mykiss* from coastal cutthroat trout (*O. clarkii clarkii*) and westslope cutthroat (*O. clarkii lewisi*), and 6 distinguish steelhead and coastal cutthroat from westslope cutthroat (Table 1). The remaining 132 SNP loci were developed to be used for population structure, parentage assignment, or other population genetic studies of *O. mykiss* (Appendix A). Any fish identified as cutthroat or hybrids were removed from further analysis.

We used Qiagen DNEasy ® kits (Qiagen Inc., Valencia, CA), following the recommended protocol for animal tissues, to extract and isolate DNA from fin tissue. SNP genotypes were obtained through PCR and visualization on Fluidigm EP1 integrated fluidic circuits (chips). Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: samples were pre-amplified by Specific Target Amplification (STA) following Fluidigm's recommended protocol with one modification. The 152 assays were pooled to a concentration of 0.2X and mixed with 2X Qiagen Multiplexing Kit (Qiagen, Inc., Valencia CA), instead of TaqMan PreAmp Master Mix (Applied Biosystems), to a volume of 3.75µl, to which 1.25µl of unquantified sample DNA was added for a total reaction volume of 5µl. Pre-amp PCR was conducted on a MJ Research or Applied Biosystems themal cycler using the following profile:

95°C for 15 min followed by 14 cycles of 95°C for 15 sec and 60°C for 4 minutes. Post-PCR reactions were diluted with 20µl dH_2O to a final volume of 25µl.

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

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

Table 1. List of species identification single nucleotide polymorphic (SNP) loci genotyped in Twisp River steelhead. Primer and probe sequences for unpublished loci available by request.

WDFW name	Locus name	Expected genotype			Reference
		O. mykiss	*O. clarkii clarkii*	*O. clarkii lewisi*	
ASpI001	Ocl_Okerca	T	C	C	(McGlauflin et al. 2010)
ASpI002	Ocl_Oku202	A	C	C	(McGlauflin et al. 2010)
ASpI003	Ocl_Oku211	G	T	T	(McGlauflin et al. 2010)
ASpI004	Ocl_Oku216	C	C	A	(McGlauflin et al. 2010)
ASpI005	Ocl_Oku217	C	C	A	(McGlauflin et al. 2010)
ASpI006	Ocl_SsaHM5	A	A	G	(McGlauflin et al. 2010)
ASpI007	Ocl_u800	T	C	C	(McGlauflin et al. 2010)
ASpI008	Ocl_u801	A	T	T	(McGlauflin et al. 2010)
ASpI009	Ocl_u802	C	C	T	(McGlauflin et al. 2010)
ASpI010	Ocl_u803	C	T	T	(McGlauflin et al. 2010)
ASpI011	Ocl_u804	G	G	C	(McGlauflin et al. 2010)
ASpI012	Omy_B9_228	A	A	C	(Finger et al. 2009)
ASpI013	Omy_CTDL1_243	C	A	A	(Finger et al. 2009)
ASpI014	Omy_F5_136	C	G	G	(Finger et al. 2009)
ASpI016	Omy_myclarp404-111	T	G	G	CRITFC - S. Narum - unpubl.
ASpI017	Omy_myclgh1043-156	C	T	T	CRITFC - S. Narum - unpubl.
ASpI018	Omy_Omyclmk436-96	A	C	C	CRITFC - S. Narum - unpubl.
ASpI019	Omy_RAG11_280	T	A	A	(Sprowles et al. 2006)
ASpI020	Omy_URO_302	T	C	C	(Finger et al. 2009)
ASpI021	Omy_BAC-F5.238	C	G	G	WDFW - S. Young unpubl.

Evaluation of Loci

To check for systematic scoring issues, we performed a two-tailed exact test of Hardy–Weinberg equilibrium (HWE) for each locus in each adult collection using the Markov Chain method implemented in Genepop 4.1 (dememorization number 1000, batches 100, 1000 iterations per batch; (Raymond and Rousset 1995; Rousset 2008)). Significance of probability values were adjusted for multiple tests using false discovery rate (Verhoeven et al. 2005). F_{IS}, a measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of scoring issues, was calculated according to Weir and Cockerham (1984) using Genepop 4.1. Expected heterozygosity was calculated using GENETIX 4.05 software (Belkhir et al. 2001).

Parentage Analysis

Individuals with fewer than 99 SNP loci (>25% missing genotypes) were removed from further analysis. Genetically analyzed juvenile offspring were selected from the entire collections by age to be only those that could have been spawned in brood year 2009, so only adults sampled in brood year 2009 were included as potential parents. Parentage analysis was conducted using the methods employed in the software FRANz (Riester et al. 2009). Initial settings for FRANz included the maximum number of parents (Nmax) = 400, genotyping error rate = 0.01, 5,000,000 burn-in iterations and 30,000,000 more iterations for analysis. Ford and Williamson (2010) showed that bias in parentage assignment can occur when groups of parents (such as hatchery and wild adults) had very different genetically effective population sizes in prior generations. By accepting the maximum likelihood parentage assignment without regard to a statistical confidence threshold, the bias was largely eliminated. Accordingly, we accepted as final parentage assignments those with the largest posterior probability, regardless of the actual values (i.e., without a threshold).

Relative Reproductive Success and Fitness

The intent of this project is to analyze parent-to-progeny productivity at two discreet progeny lifestages; age-1 parr and smolt. For this report however, three juvenile offspring collections representing the entire age-1 parr sample and the age-1 and age-2 smolt samples from the 2009 brood adult spawners were combined for analysis. From parentage assignment, we summarized the absolute number of detected offspring for each adult. From the absolute number of offspring, we calculated the relative fitness by dividing by the average within-sex number of offspring.

Results

Juvenile Sampling

Age-1 parr from the 2009 brood were collected from the Twisp River between 18 June and 12 November, 2010. We captured a total of 1,504 natural origin steelhead parr using angling (N = 1,146), electrofishing (N = 352), and dip netting (N = 6) methods. Of the fish captured, 779 were estimated to be age-1 through scale sample analysis (Table 2). Scale samples from an additional 179 natural origin juvenile fish could not be aged (e.g., regenerated, no samples collected, etc.) and we used fork length (92−169 mm) to estimate ages for this group of fish and concluded that 88 were likely age-1 parr (Figure 2). We included these fish with known age-1 fish and submitted 867 steelhead tissue samples to the WDFW MGL for parentage analysis. We relied on angling techniques when water temperatures were warmer and fish were actively feeding. Most age-1 parr were collected using this method during July and August (Figure 1). When water temperatures and fish activity decreased, we relied primarily on electrofishing to capture fish.

Table 2. Age frequency, length, and weight for wild steelhead parr sampled using angling, electrofishing, and dipnetting in the Twisp River basin in 2010.

Age	*N* (%)	Fork length (mm)		Weight (g)	
		Mean	SD	Mean	SD
0	212 (14.3)	76.0	10.5	5.4	2.4
1	776 (52.4)	138.6	20.8	32.7	13.6
2	201 (13.6)	168.2	30.7	62.0	32.2
3	92 (6.2)	165.5	29.0	58.9	34.6
4	18 (1.2)	196.4	18.6	93.8	30.8
5	2 (0.1)	187.5	34.6	78.7	38.1
Unknown	179 (12.1)	169.6	39.2	61.1	39.9

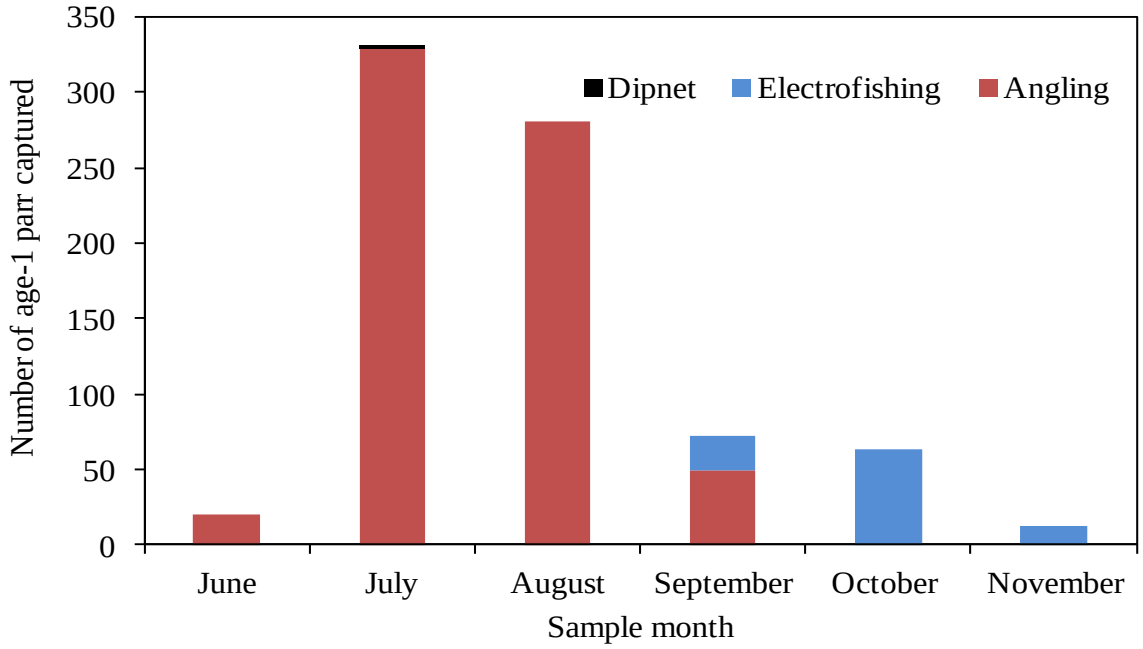

Figure 1. Number of wild age-1 steelhead parr captured by month and method in the Twisp River basin in 2010.

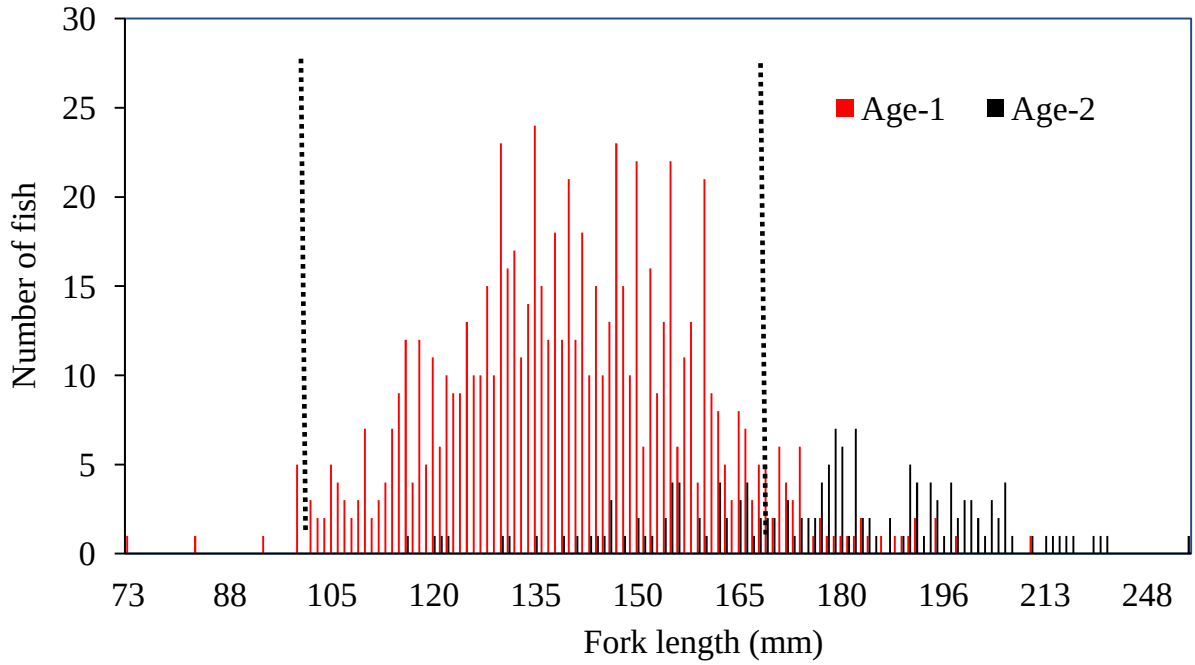

Figure 2. Fork length (mm) of age-1 and age-2 wild steelhead juveniles collected from the Twisp River in 2010 by angling and electrofishing. Dotted lines indicate the fork length range used to estimate age-1 fish when age could not be determined via scale samples.

Sampling of parr- and smolt-stage progeny from the 2009 and 2010 broods occurred during 2010 and 2011 at the WDFW Twisp River smolt trap. In 2010, smolt trapping occurred in the Twisp River between 5 March and 19 November, although the trap was not operated between 17 and 22 May because of high river discharge. A total of 472 wild steelhead parr and 465 wild summer steelhead smolts were sampled in 2010. Capture of smolts was limited to the period between 21 March and 15 July and peak capture occurred on 18 April (N = 99). Most wild steelhead parr were age-1 (84.3%; Table 3) and most wild summer steelhead smolts were age-3 (41.9%; Table 4). Tissue samples from three wild age-1 steelhead smolts were sent to the WDFW MGL for analysis. An additional five smolts for which age could not be determined from scale analysis were estimated to be age 1 fish based on size (fork length: 137−149 mm). Tissue samples from 363 wild age-1 parr and 14 unknown-age parr (fork length: 60−174 mm) sampled in the Twisp River trap were included in the DNA analysis in 2010.

In 2011, rotary smolt trap operation in the Twisp River was initiated on 10 March and continued through 12 May. The trap was not operated between 13 May and 27 July because of high river discharge. A total of 329 wild summer steelhead smolts were sampled between 4 March and 12 May, and peak capture occurred on 1 April (N = 49). Most wild summer steelhead smolts were age-2 (73.9%; Table 7). Tissue samples from a single age-1, 204 age-2, and 13 unknown-age steelhead smolts sampled in the Twisp River trap were submitted to the WDFW MGL for analysis.

Table 3. Age frequency, fork length, and weight for wild steelhead parr collected from the WDFW Twisp River smolt trap in 2010.

Age	*N* (%)	Fork length (mm)		Weight (g)	
		Mean	SD	Mean	SD
0	42 (9.2)	68.2	8.6	3.6	1.3
1	386 (84.3)	87.2	23.5	9.0	11.1
2	11 (2.4)	152.5	41.7	47.1	30.6
3	3 (0.7)	174.3	67.9	72.2	54.7
Unknown	16 (3.5)	98.8	39.5	15.2	17.2

Table 4. Age frequency, fork length, and weight for wild steelhead smolts collected from the WDFW Twisp River smolt trap in 2010 and 2011.

Age	*N* (%)	Fork length (mm)		Weight (g)	
		Mean	SD	Mean	SD
		2010			
1	4 (0.9)	129.3	18.8	22.8	8.1
2	157 (34.3)	157.8	16.2	39.2	11.9
3	192 (41.9)	174.4	18.2	53.8	17.6
4	23 (5.0)	172.0	18.4	51.8	16.5
5	1 (0.2)	167.0	- -	57	- -
Unknown	81 (17.7)	174.1	17.0	51.7	14.8
		2011			
1	1 (0.3)	135.0	- -	45.5	- -
2	224 (73.9)	161.7	15.9	41.6	11.9
3	37 (12.2)	182.4	26.3	60.6	25.2
4	20 (6.6)	182.7	26.8	61.3	23.4
5	1 (0.3)	165.0	- -	50.0	- -
Unknown	20 (6.6)	177.4	23.5	55.8	23.2

Samples received

Tissue samples were collected from 259 hatchery origin (HOR) and 94 natural origin (NOR) adult steelhead in 2009 and from 173 HOR and 173 NOR adult steelhead in 2010 (Table 5). In 2010, natural origin juvenile *O. mykiss* were captured by angling and electrofishing (*N* = 1,501) and from the Twisp River smolt trap (*N* = 517). Of those, 867 captured by angling and electrofishing and 385 captured in the Twisp River smolt trap were identified as age-1 fish (brood year 2009) by scale analysis and sent to the MGL for genetic analysis (Table 5). In 2011, 351 emigrating natural origin juvenile *O. mykiss* were captured at the Twisp River smolt trap. Of

those, 218 fish identified as age-2 (brood year 2009) were sent to the MGL for genetic analysis (Table 5).

Table 5. Collection and genotyping results of adult and juvenile steelhead and cutthroat from the Twisp River by code, year, and method.

Code	Year	Species/stage	Collection method	# Received	# Genotyped
09DA, DC	2009	Hatchery steelhead adult	Twisp weir	259	259
09CZ, DB	2009	Wild steelhead adult	Twisp weir	94	94
10DR	2010	Wild steelhead adult	Twisp weir	173	173
10DQ	2010	Hatchery steelhead adult	Twisp weir	173	172
10DS	2010	Wild cutthroat adult	Twisp weir	76	52
10DW	2010	Wild age-1 *O. mykiss*	Twisp smolt trap	385	380
10DX	2010	Wild age-1 *O. mykiss*	Angling/electro	867	855
11CM	2011	Wild age-2 *O. mykiss*	Twisp smolt trap	218	218

Evaluation of loci

One locus was monomorphic in all collected Twisp River *O. mykiss* (A*Omy*123, Appendix B). Several loci were monomorphic in one or more, but not all of the 4 collections (A = 1, Appendix B). No loci deviated from HWE after correction for multiple tests and no loci were estimated to be linked. Eleven pairs of adults had matching genotypes, five of which were identified as individuals that fell back downstream over the weir, and were recaptured and resampled. One pair of matching genotypes was identified from the 10DR collection (10DR0105 and 10DR0153). Scale-based estimated ages for these samples were different by one year (age-3 and age-4), so it is possible that these samples came from different fish. However, both were identified as male rainbow trout and had identical length measurements, so it is likely that these two samples were from the same fish sampled one week apart. Two matches (four individuals) were sampled within the same year, but the biological data did not unambiguously indicate that they represented a recapture of the same individual. A fourth match included a sample from 2009 and one from 2010. Both samples were identified as hatchery fish with a PIT tag, but the biological data were too incomplete to be certain that it was a repeat spawner. The remaining two matches had sequential individual IDs, indicating that the same individual was sampled twice in the field or processed twice in the laboratory. Recapture samples were dropped from further analysis as were both samples from fish with sequential IDs. The remaining four pairs of matching individuals were included in the parentage assignment dataset.

Parentage assignment and relative reproductive success

One parent was assigned to 439 (30.7%) progeny, two parents were assigned to 550 (38.4%) juvenile progeny, and no parents could be assigned to 442 (30.9%) progeny. Of those offspring we assigned a single parent, 66.1% were assigned a mother only and 33.9% were assigned a father only. Among all adults, the average number of offspring per mother (4.8) and per father (4.1) were similar. Distributions of reproductive success were skewed toward smaller numbers of offspring (i.e., we detected few offspring from most families and a few families were detected at high frequency; Figure 3). Hatchery and natural origin mothers produced similar numbers of offspring (HOR mean = 4.6 offspring per female, NOR mean = 5.2 offspring per female). Hatchery males appeared to produce fewer offspring than natural origin males (HOR mean = 3.0 offspring per male, NOR mean = 7.2 offspring per male). One-salt natural origin mothers produced approximately 1.6 times the number of offspring of 1-salt hatchery origin mothers, but the average number of offspring of 2-salt hatchery and natural origin mothers was roughly equal (Table 6). Overall patterns of male reproductive success were driven mainly by the reproductive success of 1-salt males, the most common age group of male Twisp River steelhead in the 2009 brood (Table 6). Two-salt hatchery origin males had slightly higher reproductive success than natural origin males, but sample sizes were very small.

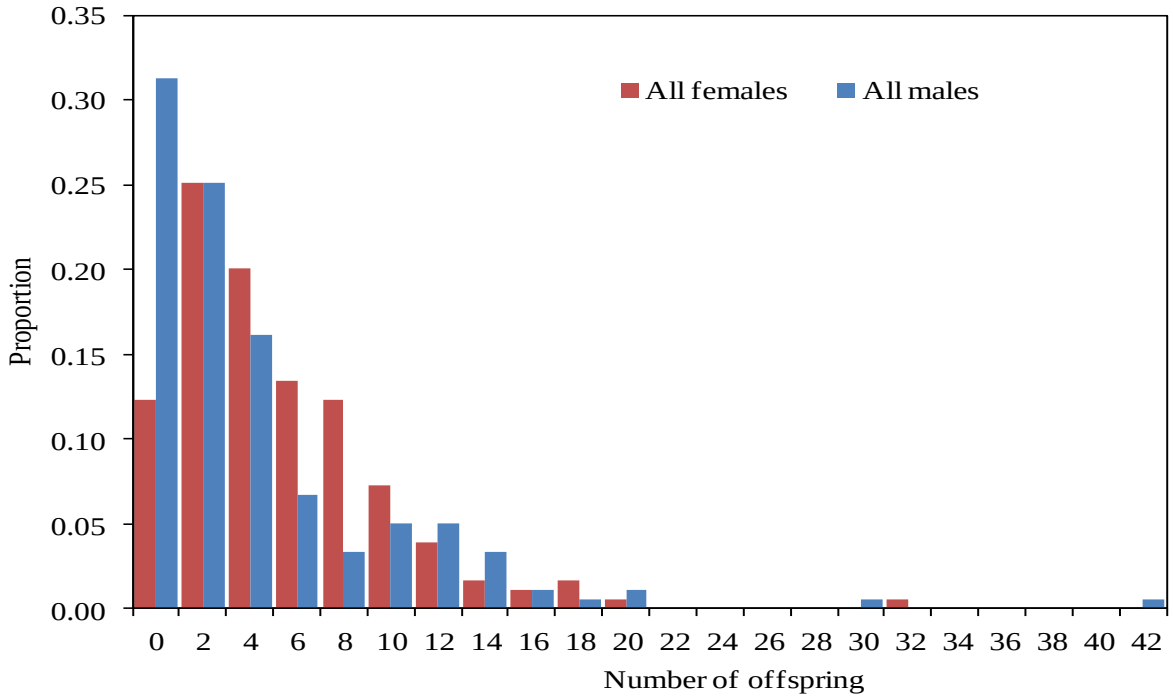

Figure 3. The distribution of reproductive success for Twisp River adult steelhead breeding in 2009.

Table 6. Mean reproductive success by saltwater age and origin of 1-salt and 2-salt 2009 brood steelhead. Sample sizes for all groups are included within parentheses.

Collection code	Female			Male		
	Saltwater age		No age data	Saltwater age		No age data
	1	2		1	2	
09CZ - wild	4.44 (25)	6.17 (23)	- -	7.39 (41)	6.00 (2)	- -
09DA - hatchery	2.74 (69)	6.45 (55)	12.67 (3)	2.38 (112)	7.50 (14)	9.00 (2)

Discussion

Determining the RRS of steelhead spawning in the natural environment is made problematic by a number of factors, many of which are difficult to account for in experimental designs. Of primary concern is the genetic contribution from fish not represented in the tissue samples necessary to successfully make two parent assignments (i.e., un-sampled anadromous adults, resident *O. mykiss*, and residual hatchery fish). For steelhead in particular this problem is an important one to consider because of the varied life history and extended freshwater rearing period of wild juveniles and the presence of hatchery origin residuals, both of which can and do contribute to spawning populations (e.g., Seamons et al. 2004, Christie et al. 2011). Within the two broods of adult fish sampled at the weir, only seven wild-resident and ten hatchery-resident *O. mykiss* were captured moving upstream to spawn, all of which were males. Trapping results suggest that the weir should be capable of capturing *O. mykiss* with a fork length as small as 350 mm, although few fish that size were observed. However, stream surveyors routinely reported seeing small (e.g., < 200 mm) fish in redds or in the immediate vicinity of spawning females while attempting to identify anchor tag color of spawning fish. These resident fish are likely contributing genetic material to our study population and decreasing the number of juvenile fish we are able to assign to two parents. Further, many of our juvenile samples (517 out of 867 captured via angling and electrofishing) were collected in areas downstream of the Twisp River weir. Redd surveys indicated that about 30% of the 2009 brood spawning occurred in areas downstream of the weir where no adults were sampled. Hence, we would expect our juvenile collection from this area to have a low parental assignment rate. Despite this, the number of progeny we were able to assign to two parents was only slightly lower than that reported in a similar study (40%, Berntson et al. 2011). Of those offspring, 66.1% were assigned to a mother only indicating that most unassigned fish were males, similar to that observed by Christie et al. (2011). We intend to collect a greater proportion of the annual juvenile samples from areas upstream of the Twisp River weir, and as our study progresses, juvenile samples from wild steelhead parr can be included as parent samples for future broods to increase assignment rates and help identify missing parents.

Acknowledgements

We thank the numerous scientific technicians who worked on this project collecting a wide variety of data from adult and juvenile steelhead, without which, this project could not succeed. This project was funded by the Bonneville Power Administration and by the Public Utility District No. 1 of Douglas County, Washington. We greatly appreciate their financial and technical support. We acknowledge and thank Cherril Bowman, Sonia Peterson, Edith Martinez, and Sarah Bell for their work in the laboratory generating the genetic data.

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Appendix A. List of general use, diploid single nucleotide polymorphic (SNP) loci genotyped in Twisp River steelhead. Primer and probe sequences for unpublished loci available by request.

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy005	Omy_aspAT-123	T	C	(Campbell et al. 2009)
AOmy014	Omy_e1-147	G	T	(Sprowles et al. 2006)
AOmy015	Omy_gdh-271	C	T	(Campbell et al. 2009)
AOmy016	Omy_GH1P1_2	C	T	(Aguilar and Garza 2008)
AOmy021	Omy_LDHB-2_e5	T	C	(Aguilar and Garza 2008)
AOmy023	Omy_MYC_2	T	C	(Aguilar and Garza 2008)
AOmy027	Omy_nkef-241	C	A	(Campbell et al. 2009)
AOmy028	Omy_nramp-146	G	A	(Campbell et al. 2009)
AOmy047	Omy_u07-79-166	G	T	WDFW - S. Young unpubl.
AOmy051	Omy_121713-115	T	A	(Abadía-Cardoso et al. 2011)
AOmy056	Omy_128693-455	T	C	(Abadía-Cardoso et al. 2011)
AOmy059	Omy_187760-385	A	T	(Abadía-Cardoso et al. 2011)
AOmy061	Omy_96222-125	T	C	(Abadía-Cardoso et al. 2011)
AOmy062	Omy_97077-73	T	A	(Abadía-Cardoso et al. 2011)
AOmy063	Omy_97660-230	C	G	(Abadía-Cardoso et al. 2011)
AOmy065	Omy_97954-618	C	T	(Abadía-Cardoso et al. 2011)
AOmy067	Omy_aromat-280	A	T	WSU - J. DeKoning unpubl.
AOmy068	Omy_arp-630	G	A	(Campbell et al. 2009)
AOmy071	Omy_cd59-206	C	T	WSU - J. DeKoning unpubl.
AOmy073	Omy_colla1-525	C	T	WSU - J. DeKoning unpubl.
AOmy079	Omy_g12-82	T	C	WSU - J. DeKoning unpubl.
AOmy081	Omy_gh-475	C	T	(Campbell et al. 2009)
AOmy082	Omy_gsdf-291	T	C	WSU - J. DeKoning unpubl.
AOmy089	Omy_hsp90BA-193	C	T	(Campbell and Narum 2009)
AOmy094	Omy_inos-97	C	A	WSU - J. DeKoning unpubl.
AOmy095	Omy_mapK3-103	A	T	CRITFC - N. Campbell unpubl.
AOmy096	Omy_mcsf-268	T	C	WSU - J. DeKoning unpubl.
AOmy100	Omy_nach-200	A	T	WSU - J. DeKoning unpubl.
AOmy107	Omy_Ots249-227	C	T	(Campbell et al. 2009)
AOmy108	Omy_oxct-85	A	T	WSU - J. DeKoning unpubl.
AOmy110	Omy_star-206	A	G	WSU - J. DeKoning unpubl.
AOmy111	Omy_stat3-273	G	Deletion	WSU - J. DeKoning unpubl.
AOmy113	Omy_tlr3-377	C	T	WSU - J. DeKoning unpubl.
AOmy117	Omy_u09-52-284	T	G	WDFW - S. Young unpubl.
AOmy118	Omy_u09-53-469	T	C	WDFW - S. Young unpubl.
AOmy120	Omy_u09-54.311	C	T	WDFW - S. Young unpubl.
AOmy123	Omy_u09-55-233	A	G	WDFW - S. Young unpubl.
AOmy125	Omy_u09-56-119	T	C	WDFW - S. Young unpubl.
AOmy129	Omy_BAMBI4.238	T	C	WDFW - S. Young unpubl.

Appendix A, continued.

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy132	Omy_G3PD_2.246	C	T	WDFW - S. Young unpubl.
AOmy134	Omy_Il-1b-028	T	C	WDFW - S. Young unpubl.
AOmy137	Omy_u09-61.043	A	T	WDFW - S. Young unpubl.
AOmy151	Omy_p53-262	T	A	CRITFC - N. Campbell unpubl.
AOmy173	BH2VHSVip10	C	T	Pascal & Hansen unpubl.
AOmy174	OMS00003	T	G	(Sánchez et al. 2009)
AOmy176	OMS00013	A	G	(Sánchez et al. 2009)
AOmy177	OMS00018	T	G	(Sánchez et al. 2009)
AOmy179	OMS00041	G	C	(Sánchez et al. 2009)
AOmy181	OMS00052	T	G	(Sánchez et al. 2009)
AOmy182	OMS00053	T	C	(Sánchez et al. 2009)
AOmy183	OMS00056	T	C	(Sánchez et al. 2009)
AOmy184	OMS00057	T	G	(Sánchez et al. 2009)
AOmy185	OMS00061	T	C	(Sánchez et al. 2009)
AOmy186	OMS00062	T	C	(Sánchez et al. 2009)
AOmy187	OMS00064	T	G	(Sánchez et al. 2009)
AOmy189	OMS00071	A	G	(Sánchez et al. 2009)
AOmy190	OMS00072	A	G	(Sánchez et al. 2009)
AOmy191	OMS00078	T	C	(Sánchez et al. 2009)
AOmy192	OMS00087	A	G	(Sánchez et al. 2009)
AOmy193	OMS00089	A	G	(Sánchez et al. 2009)
AOmy194	OMS00090	T	C	(Sánchez et al. 2009)
AOmy195	OMS00092	A	C	(Sánchez et al. 2009)
AOmy196	OMS00094	T	G	(Sánchez et al. 2009)
AOmy197	OMS00103	A	T	(Sánchez et al. 2009)
AOmy198	OMS00105	T	G	(Sánchez et al. 2009)
AOmy199	OMS00112	A	T	(Sánchez et al. 2009)
AOmy200	OMS00116	T	A	(Sánchez et al. 2009)
AOmy201	OMS00118	T	G	(Sánchez et al. 2009)
AOmy202	OMS00119	A	T	(Sánchez et al. 2009)
AOmy203	OMS00120	A	G	(Sánchez et al. 2009)
AOmy204	OMS00121	T	C	(Sánchez et al. 2009)
AOmy205	OMS00127	T	G	(Sánchez et al. 2009)
AOmy206	OMS00128	T	G	(Sánchez et al. 2009)
AOmy207	OMS00132	A	T	(Sánchez et al. 2009)
AOmy208	OMS00133	A	G	(Sánchez et al. 2009)
AOmy209	OMS00134	A	G	(Sánchez et al. 2009)
AOmy210	OMS00153	T	G	(Sánchez et al. 2009)
AOmy211	OMS00154	A	T	(Sánchez et al. 2009)

Appendix A, continued.

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy216	OMS00176	T	G	(Sánchez et al. 2009)
AOmy218	OMS00180	T	G	(Sánchez et al. 2009)
AOmy220	Omy_1004	A	T	(Hansen et al. 2011)
AOmy221	Omy_101554-306	T	C	(Abadía-Cardoso et al. 2011)
AOmy222	Omy_101832-195	A	C	(Abadía-Cardoso et al. 2011)
AOmy223	Omy_101993-189	A	T	(Abadía-Cardoso et al. 2011)
AOmy225	Omy_102505-102	A	G	(Abadía-Cardoso et al. 2011)
AOmy226	Omy_102867-443	T	G	(Abadía-Cardoso et al. 2011)
AOmy227	Omy_103705-558	T	C	(Abadía-Cardoso et al. 2011)
AOmy228	Omy_104519-624	T	C	(Abadía-Cardoso et al. 2011)
AOmy229	Omy_104569-114	A	C	(Abadía-Cardoso et al. 2011)
AOmy230	Omy_105075-162	T	G	(Abadía-Cardoso et al. 2011)
AOmy231	Omy_105385-406	T	C	(Abadía-Cardoso et al. 2011)
AOmy232	Omy_105714-265	C	T	(Abadía-Cardoso et al. 2011)
AOmy233	Omy_107031-704	C	T	(Abadía-Cardoso et al. 2011)
AOmy234	Omy_107285-69	C	G	(Abadía-Cardoso et al. 2011)
AOmy235	Omy_107336-170	C	G	(Abadía-Cardoso et al. 2011)
AOmy238	Omy_108007-193	A	G	(Abadía-Cardoso et al. 2011)
AOmy239	Omy_109243-222	A	C	(Abadía-Cardoso et al. 2011)
AOmy240	Omy_109525-403	A	G	(Abadía-Cardoso et al. 2011)
AOmy241	Omy_110064-419	T	G	(Abadía-Cardoso et al. 2011)
AOmy242	Omy_110078-294	A	G	(Abadía-Cardoso et al. 2011)
AOmy243	Omy_110362-585	G	A	(Abadía-Cardoso et al. 2011)
AOmy244	Omy_110689-148	A	C	(Abadía-Cardoso et al. 2011)
AOmy245	Omy_111005-159	C	T	(Abadía-Cardoso et al. 2011)
AOmy246	Omy_111084-526	A	C	(Abadía-Cardoso et al. 2011)
AOmy247	Omy_111383-51	C	T	(Abadía-Cardoso et al. 2011)
AOmy248	Omy_111666-301	T	A	(Abadía-Cardoso et al. 2011)
AOmy249	Omy_112301-202	T	G	(Abadía-Cardoso et al. 2011)
AOmy250	Omy_112820-82	G	A	(Abadía-Cardoso et al. 2011)
AOmy252	Omy_114976-223	T	G	(Abadía-Cardoso et al. 2011)
AOmy253	Omy_116733-349	C	T	(Abadía-Cardoso et al. 2011)
AOmy254	Omy_116938-264	A	G	(Abadía-Cardoso et al. 2011)
AOmy255	Omy_117259-96	T	C	(Abadía-Cardoso et al. 2011)
AOmy256	Omy_117286-374	A	T	(Abadía-Cardoso et al. 2011)
AOmy257	Omy_117370-400	A	G	(Abadía-Cardoso et al. 2011)
AOmy258	Omy_117540-259	T	G	(Abadía-Cardoso et al. 2011)
AOmy260	Omy_117815-81	C	T	(Abadía-Cardoso et al. 2011)
AOmy261	Omy_118175-396	T	A	(Abadía-Cardoso et al. 2011)

Appendix A, continued.

WDFW Name	Locus Name	Allele 1	Allele 2	Reference
AOmy262	Omy_118205-116	A	G	(Abadía-Cardoso et al. 2011)
AOmy263	Omy_118654-91	A	G	(Abadía-Cardoso et al. 2011)
AOmy265	Omy_120255-332	A	T	(Abadía-Cardoso et al. 2011)
AOmy266	Omy_128996-481	T	G	(Abadía-Cardoso et al. 2011)
AOmy267	Omy_129870-756	C	T	(Abadía-Cardoso et al. 2011)
AOmy268	Omy_131460-646	C	T	(Abadía-Cardoso et al. 2011)
AOmy269	Omy_98683-165	A	C	(Abadía-Cardoso et al. 2011)
AOmy270	Omy_cyp17-153	C	T	WSU - J. DeKoning unpubl.
AOmy271	Omy_ftzf1-217	A	T	WSU - J. DeKoning unpubl.
AOmy272	Omy_GHSR-121	T	C	CRITFC - N. Campbell unpubl.
AOmy273	Omy_metA-161	T	G	CRITFC - N. Campbell unpubl.
AOmy274	Omy_UBA3b	A	T	(Hansen et al. 2011)

Appendix B. Genetic parameters for all Twisp River steelhead collections at all SNP loci.

Code	Metric	AOmy005	AOmy014	AOmy015	AOmy016	AOmy021	AOmy023	AOmy027
09CZ	n (N=92)	92	92	92	91	90	91	91
09CZ	H_e	0.419	0.185	0.131	0.022	0.321	0.053	0.498
09CZ	F_{IS}	0.05	-0.11	-0.07	-0.01	-0.04	-0.02	-0.10
09CZ	*P*	0.802	0.589	1.000	1.000	1.000	1.000	0.404
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.30	0.10	0.07	0.01	0.20	0.03	0.47
09DA	n (N=265)	265	264	265	265	260	265	263
09DA	H_e	0.450	0.130	0.127	0.066	0.373	0.033	0.493
09DA	F_{IS}	0.07	0.10	-0.07	0.20	0.00	-0.02	0.03
09DA	*P*	0.284	0.118	0.623	0.030	1.000	1.000	0.716
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.34	0.07	0.07	0.03	0.25	0.02	0.56
10DQ	n (N=170)	169	168	170	168	166	0.04	0.517
10DQ	H_e	0.385	0.148	0.111	0.035	0.315	0.006	0.482
10DQ	F_{IS}	-0.04	0.00	-0.06	-0.02	0.07	a	0.11
10DQ	*P*	0.683	1.000	1.000	1.000	0.460	a	0.202
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.26	0.08	0.06	0.02	0.20	0.003	0.59
10DR	n (N=176)	176	176	176	175	170	175	176
10DR	H_e	0.414	0.127	0.097	0.050	0.283	0.077	0.499
10DR	F_{IS}	-0.03	0.11	-0.05	0.20	0.13	-0.04	0.07
10DR	*P*	0.854	0.178	1.000	0.104	0.100	1.000	0.363
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.29	0.07	0.05	0.03	0.17	0.04	0.52
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy028	AOmy047	AOmy051	AOmy056	AOmy059	AOmy061	AOmy062
09CZ	n (N=92)	92	92	92	92	92	90	92
09CZ	H_e	0.032	0.194	0.280	0.000	0.000	0.075	0.053
09CZ	F_{IS}	-0.01	0.00	0.04	a	a	0.56	-0.02
09CZ	*P*	1.000	1.000	0.714	a	a	0.003	1.000
09CZ	A	2	2	2	1	1	2	2
09CZ	MAF	0.02	0.11	0.17	0.000	0.000	0.04	0.03
09DA	n (N=265)	265	264	264	265	265	251	265
09DA	H_e	0.011	0.175	0.184	0.000	0.004	0.012	0.062
09DA	F_{IS}	0.00	-0.11	0.22	a	a	0.00	-0.03
09DA	*P*	1.000	0.146	0.002	a	a	1.000	1.000
09DA	A	2	2	2	1	2	2	2
09DA	MAF	0.01	0.10	0.10	0.000	0.002	0.01	0.03
10DQ	n (N=170)	0.0028	0.1761	0.1886	0.0028	0.0057	0.0271	0.0455
10DQ	H_e	0.006	0.208	0.245	0.000	0.000	0.025	0.017
10DQ	F_{IS}	a	-0.07	0.13	a	a	-0.01	-0.01
10DQ	*P*	a	0.473	0.110	a	a	1.000	1.000
10DQ	A	2	2	2	1	1	2	2
10DQ	MAF	0.003	0.12	0.14	0.000	0.000	0.01	0.01
10DR	n (N=176)	176	176	175	176	176	166	176
10DR	H_e	0.006	0.290	0.306	0.006	0.011	0.053	0.087
10DR	F_{IS}	a	-0.09	0.40	a	0.00	0.43	-0.04
10DR	*P*	a	0.302	0.000	a	1.000	0.003	1.000
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.003	0.18	0.19	0.003	0.01	0.03	0.05
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy063	AOmy065	AOmy067	AOmy068	AOmy071	AOmy073	AOmy079
09CZ	n (N=92)	88	92	90	90	90	92	91
09CZ	H_e	0.430	0.219	0.358	0.473	0.364	0.370	0.495
09CZ	F_{IS}	-0.08	0.16	0.07	-0.05	-0.06	0.03	0.12
09CZ	*P*	0.617	0.137	0.555	0.665	0.771	0.781	0.293
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.31	0.13	0.23	0.38	0.24	0.24	0.45
09DA	n (N=265)	262	265	265	265	264	265	265
09DA	H_e	0.412	0.120	0.346	0.489	0.283	0.414	0.494
09DA	F_{IS}	0.08	0.06	0.02	0.06	0.01	0.06	0.09
09DA	*P*	0.227	0.288	0.727	0.364	0.827	0.373	0.130
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.29	0.06	0.22	0.43	0.17	0.29	0.44
10DQ	n (N=170)	0.3353	0.1023	0.2857	0.4261	0.267	0.2816	0.5
10DQ	H_e	0.451	0.132	0.344	0.497	0.391	0.403	0.497
10DQ	F_{IS}	-0.07	0.11	0.03	0.06	0.00	-0.12	0.20
10DQ	*P*	0.403	0.190	0.821	0.539	1.000	0.128	0.012
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.34	0.07	0.22	0.46	0.27	0.28	0.46
10DR	n (N=176)	173	176	175	176	176	174	175
10DR	H_e	0.446	0.184	0.408	0.489	0.391	0.405	0.500
10DR	F_{IS}	-0.03	0.07	0.05	-0.09	0.04	-0.13	-0.04
10DR	*P*	0.734	0.390	0.575	0.291	0.563	0.093	0.648
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.34	0.10	0.29	0.43	0.27	0.28	0.50
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy081	AOmy082	AOmy089	AOmy094	AOmy095	AOmy096	AOmy100
09CZ	n (N=92)	92	91	91	88	91	92	92
09CZ	H_e	0.185	0.000	0.053	0.156	0.187	0.083	0.063
09CZ	F_{IS}	0.01	a	-0.02	0.06	0.24	-0.04	-0.03
09CZ	*P*	1.000	a	1.000	0.476	0.049	1.000	1.000
09CZ	A	2	1	2	2	2	2	2
09CZ	MAF	0.10	0.000	0.03	0.09	0.10	0.04	0.03
09DA	n (N=265)	265	265	265	260	265	263	265
09DA	H_e	0.107	0.030	0.120	0.070	0.062	0.084	0.033
09DA	F_{IS}	0.08	-0.01	-0.07	0.07	-0.03	0.23	-0.02
09DA	*P*	0.200	1.000	0.609	0.288	1.000	0.009	1.000
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.06	0.02	0.06	0.04	0.03	0.04	0.02
10DQ	n (N=170)	168	169	170	170	164	167	169
10DQ	H_e	0.228	0.052	0.090	0.131	0.130	0.080	0.029
10DQ	F_{IS}	0.01	-0.02	0.08	0.11	-0.07	0.11	-0.01
10DQ	*P*	1.000	1.000	0.313	0.191	1.000	0.248	1.000
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.13	0.03	0.05	0.07	0.07	0.04	0.01
10DR	n (N=176)	173	176	176	175	175	175	176
10DR	H_e	0.191	0.023	0.082	0.133	0.108	0.034	0.044
10DR	F_{IS}	-0.06	-0.01	0.10	-0.07	0.05	0.32	-0.02
10DR	*P*	0.697	1.000	0.269	1.000	0.434	0.043	1.000
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.11	0.01	0.04	0.07	0.06	0.02	0.02
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy107	AOmy108	AOmy110	AOmy111	AOmy113	AOmy117	AOmy118
09CZ	n (N=92)	91	91	92	90	92	90	92
09CZ	H_e	0.430	0.187	0.202	0.364	0.211	0.054	0.466
09CZ	F_{IS}	0.11	-0.11	-0.02	0.06	-0.03	-0.02	0.17
09CZ	*P*	0.336	0.590	1.000	0.565	1.000	1.000	0.121
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.31	0.10	0.11	0.24	0.12	0.03	0.37
09DA	n (N=265)	265	265	264	264	265	263	265
09DA	H_e	0.454	0.212	0.254	0.334	0.204	0.090	0.458
09DA	F_{IS}	-0.04	-0.10	0.12	-0.04	0.06	-0.05	-0.07
09DA	*P*	0.591	0.144	0.050	0.584	0.358	1.000	0.296
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.35	0.12	0.15	0.21	0.12	0.05	0.35
10DQ	n (N=170)	167	169	169	169	169	169	162
10DQ	H_e	0.422	0.200	0.200	0.409	0.181	0.101	0.407
10DQ	F_{IS}	0.00	0.05	0.11	-0.11	-0.04	-0.05	0.00
10DQ	*P*	1.000	0.442	0.236	0.193	1.000	1.000	1.000
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.30	0.11	0.11	0.29	0.10	0.05	0.28
10DR	n (N=176)	172	176	175	175	175	176	176
10DR	H_e	0.441	0.146	0.198	0.376	0.233	0.066	0.434
10DR	F_{IS}	0.04	-0.08	-0.01	0.03	0.04	-0.03	-0.04
10DR	*P*	0.606	0.607	1.000	0.688	0.520	1.000	0.598
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.33	0.08	0.11	0.25	0.13	0.03	0.32
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy120	AOmy123	AOmy125	AOmy129	AOmy132	AOmy134	AOmy137
09CZ	n (N=92)	90	92	89	76	76	92	92
09CZ	H_e	0.461	0.000	0.273	0.000	0.000	0.266	0.112
09CZ	F_{IS}	-0.08	a	0.14	a	a	0.06	-0.06
09CZ	*P*	0.504	a	0.232	a	a	0.696	1.000
09CZ	A	2	1	2	1	1	2	2
09CZ	MAF	0.36	0.000	0.16	0.000	0.000	0.16	0.06
09DA	n (N=265)	265	265	264	201	201	265	265
09DA	H_e	0.422	0.000	0.246	0.000	0.000	0.277	0.103
09DA	F_{IS}	-0.02	a	-0.04	a	a	-0.01	-0.06
09DA	*P*	0.886	a	0.621	a	a	1.000	1.000
09DA	A	2	1	2	1	1	2	2
09DA	MAF	0.30	0.000	0.14	0.000	0.000	0.17	0.05
10DQ	n (N=170)	168	169	168	169	168	169	169
10DQ	H_e	0.411	0.000	0.258	0.006	0.029	0.325	0.096
10DQ	F_{IS}	0.15	a	-0.13	a	0.39	-0.04	-0.05
10DQ	*P*	0.061	a	0.127	a	0.028	0.811	1.000
10DQ	A	2	1	2	2	2	2	2
10DQ	MAF	0.29	0.000	0.15	0.003	0.01	0.20	0.05
10DR	n (N=176)	176	175	175	146	175	176	176
10DR	H_e	0.421	0.000	0.157	0.007	0.006	0.287	0.112
10DR	F_{IS}	0.06	a	-0.02	a	a	0.03	-0.06
10DR	*P*	0.471	a	1.000	a	a	0.793	1.000
10DR	A	2	1	2	2	2	2	2
10DR	MAF	0.30	0.000	0.09	0.003	0.00	0.17	0.06
	A_{TOT}	2	1	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy151	AOmy173	AOmy174	AOmy176	AOmy177	AOmy179	AOmy181
09CZ	n (N=92)	92	91	92	92	92	91	92
09CZ	H_e	0.176	0.426	0.131	0.176	0.235	0.022	0.328
09CZ	F_{IS}	-0.10	0.08	-0.07	0.02	-0.15	-0.01	0.08
09CZ	*P*	0.598	0.472	1.000	1.000	0.205	1.000	0.522
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.10	0.31	0.07	0.10	0.14	0.01	0.21
09DA	n (N=265)	264	265	264	260	265	259	265
09DA	H_e	0.175	0.433	0.168	0.112	0.097	0.019	0.356
09DA	F_{IS}	0.07	-0.13	0.12	-0.06	-0.05	-0.01	0.00
09DA	*P*	0.283	0.036	0.058	0.607	1.000	1.000	1.000
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.10	0.32	0.09	0.06	0.05	0.01	0.23
10DQ	n (N=170)	166	170	170	166	170	168	169
10DQ	H_e	0.225	0.468	0.225	0.092	0.111	0.052	0.311
10DQ	F_{IS}	0.07	-0.12	0.01	0.08	0.05	-0.02	0.03
10DQ	*P*	0.484	0.142	1.000	0.313	0.451	1.000	0.630
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.13	0.37	0.13	0.05	0.06	0.03	0.19
10DR	n (N=176)	176	175	175	175	175	176	176
10DR	H_e	0.244	0.418	0.175	0.216	0.133	0.066	0.348
10DR	F_{IS}	-0.07	0.26	0.29	0.07	-0.07	-0.03	0.01
10DR	*P*	0.533	0.002	0.002	0.306	1.000	1.000	1.000
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.14	0.30	0.10	0.12	0.07	0.03	0.22
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy182	AOmy183	AOmy184	AOmy185	AOmy186	AOmy187	AOmy189
09CZ	n (N=92)	92	92	91	91	92	89	92
09CZ	H_e	0.483	0.315	0.449	0.104	0.346	0.494	0.499
09CZ	F_{IS}	0.04	0.04	0.22	-0.05	0.03	-0.11	-0.02
09CZ	*P*	0.827	0.744	0.034	1.000	0.763	0.386	1.000
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.41	0.20	0.34	0.05	0.22	0.44	0.47
09DA	n (N=265)	264	265	265	264	264	260	264
09DA	H_e	0.492	0.262	0.472	0.187	0.369	0.495	0.488
09DA	F_{IS}	0.06	0.02	0.03	0.09	0.01	-0.15	-0.14
09DA	*P*	0.321	0.812	0.700	0.180	1.000	0.018	0.033
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.44	0.15	0.38	0.10	0.24	0.45	0.42
10DQ	n (N=170)	170	170	170	170	170	170	170
10DQ	H_e	0.490	0.298	0.462	0.180	0.344	0.474	0.475
10DQ	F_{IS}	0.02	0.02	0.00	0.02	-0.11	-0.03	0.11
10DQ	*P*	0.874	0.798	1.000	0.670	0.185	0.746	0.154
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.43	0.18	0.36	0.10	0.22	0.39	0.39
10DR	n (N=176)	176	175	174	176	175	175	174
10DR	H_e	0.500	0.288	0.412	0.184	0.310	0.484	0.481
10DR	F_{IS}	0.05	-0.09	0.15	0.01	-0.09	-0.06	-0.10
10DR	*P*	0.547	0.300	0.063	0.696	0.325	0.539	0.214
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.51	0.17	0.29	0.10	0.19	0.41	0.40
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy190	AOmy191	AOmy192	AOmy193	AOmy194	AOmy195	AOmy196
09CZ	n (N=92)	92	92	91	92	91	92	91
09CZ	H_e	0.463	0.457	0.245	0.401	0.495	0.250	0.104
09CZ	F_{IS}	-0.05	0.08	0.20	-0.11	-0.06	0.01	0.16
09CZ	*P*	0.657	0.494	0.077	0.435	0.661	1.000	0.232
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.36	0.35	0.14	0.28	0.45	0.15	0.05
09DA	n (N=265)	265	265	262	264	263	265	265
09DA	H_e	0.451	0.454	0.220	0.439	0.497	0.215	0.069
09DA	F_{IS}	-0.04	-0.02	0.14	0.05	-0.06	-0.10	-0.04
09DA	*P*	0.596	0.795	0.046	0.409	0.324	0.141	1.000
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.34	0.35	0.13	0.33	0.46	0.12	0.04
10DQ	n (N=170)	170	170	170	170	170	170	170
10DQ	H_e	0.457	0.392	0.131	0.413	0.498	0.317	0.131
10DQ	F_{IS}	0.13	0.18	0.20	0.05	0.01	-0.09	-0.07
10DQ	*P*	0.132	0.033	0.036	0.577	1.000	0.331	1.000
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.35	0.27	0.07	0.29	0.47	0.20	0.07
10DR	n (N=176)	175	176	175	175	176	176	174
10DR	H_e	0.486	0.483	0.265	0.408	0.495	0.275	0.128
10DR	F_{IS}	0.20	0.04	0.08	-0.12	-0.06	0.09	-0.07
10DR	*P*	0.011	0.642	0.389	0.144	0.446	0.270	1.000
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.42	0.41	0.16	0.29	0.45	0.16	0.07
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy197	AOmy198	AOmy199	AOmy200	AOmy201	AOmy202	AOmy203
09CZ	n (N=92)	90	89	87	92	90	91	92
09CZ	H_e	0.011	0.500	0.270	0.063	0.437	0.229	0.294
09CZ	F_{IS}	a	0.02	0.07	-0.03	0.04	0.05	0.23
09CZ	*P*	a	1.000	0.455	1.000	0.805	0.649	0.036
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.006	0.49	0.16	0.03	0.32	0.13	0.18
09DA	n (N=265)	262	263	264	265	262	265	167
09DA	H_e	0.015	0.498	0.273	0.008	0.412	0.146	0.279
09DA	F_{IS}	-0.01	0.13	-0.03	0.00	-0.02	-0.03	0.14
09DA	*P*	1.000	0.038	0.820	1.000	0.881	1.000	0.088
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.01	0.53	0.16	0.00	0.29	0.08	0.17
10DQ	n (N=170)	170	169	170	170	163	169	169
10DQ	H_e	0.029	0.500	0.230	0.052	0.423	0.116	0.345
10DQ	F_{IS}	-0.01	0.12	-0.10	-0.02	0.12	-0.06	0.09
10DQ	*P*	1.000	0.164	0.314	1.000	0.132	1.000	0.267
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.01	0.49	0.13	0.03	0.30	0.06	0.22
10DR	n (N=176)	176	172	173	176	168	174	174
10DR	H_e	0.017	0.494	0.251	0.060	0.434	0.199	0.262
10DR	F_{IS}	-0.01	-0.02	-0.17	-0.03	-0.05	-0.07	0.17
10DR	*P*	1.000	0.880	0.026	1.000	0.591	0.698	0.037
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.01	0.44	0.15	0.03	0.32	0.11	0.16
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy204	AOmy205	AOmy206	AOmy207	AOmy208	AOmy209	AOmy210
09CZ	n (N=92)	91	91	91	90	92	92	92
09CZ	H_e	0.462	0.000	0.094	0.499	0.122	0.022	0.053
09CZ	F_{IS}	0.10	a	-0.05	0.05	0.11	-0.01	-0.02
09CZ	*P*	0.365	a	1.000	0.676	0.318	1.000	1.000
09CZ	A	2	1	2	2	2	2	2
09CZ	MAF	0.36	0.000	0.05	0.48	0.07	0.01	0.03
09DA	n (N=265)	265	264	264	265	263	265	264
09DA	H_e	0.494	0.000	0.069	0.497	0.073	0.008	0.111
09DA	F_{IS}	0.03	a	0.07	-0.04	0.07	0.00	-0.06
09DA	*P*	0.616	a	0.282	0.534	0.314	1.000	1.000
09DA	A	2	1	2	2	2	2	2
09DA	MAF	0.44	0.000	0.04	0.54	0.04	0.00	0.06
10DQ	n (N=170)	170	170	170	170	170	170	170
10DQ	H_e	0.482	0.006	0.063	0.499	0.106	0.029	0.095
10DQ	F_{IS}	-0.07	a	0.16	-0.08	-0.06	-0.01	0.20
10DQ	*P*	0.425	a	0.152	0.348	1.000	1.000	0.055
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.41	0.003	0.03	0.52	0.06	0.01	0.05
10DR	n (N=176)	175	175	176	174	176	176	175
10DR	H_e	0.486	0.006	0.033	0.499	0.132	0.023	0.056
10DR	F_{IS}	-0.13	a	-0.01	0.12	0.01	-0.01	-0.03
10DR	*P*	0.121	a	1.000	0.127	0.600	1.000	1.000
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.42	0.003	0.02	0.53	0.07	0.01	0.03
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy211	AOmy212	AOmy213	AOmy215	AOmy216	AOmy218	AOmy220
09CZ	n (N=92)	92	91	92	91	22	90	92
09CZ	H_e	0.375	0.453	0.064	0.480	0.127	0.424	0.235
09CZ	F_{IS}	-0.10	-0.09	-0.03	-0.07	-0.05	-0.12	0.22
09CZ	*P*	0.411	0.482	1.000	0.525	1.000	0.323	0.057
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.25	0.35	0.03	0.40	0.07	0.31	0.14
09DA	n (N=265)	265	263	264	264	263	265	264
09DA	H_e	0.358	0.419	0.033	0.494	0.048	0.438	0.168
09DA	F_{IS}	0.01	0.01	-0.02	-0.04	-0.02	-0.01	0.08
09DA	*P*	0.863	0.881	1.000	0.545	1.000	0.889	0.252
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.23	0.30	0.02	0.45	0.02	0.32	0.09
10DQ	n (N=170)	170	168	170	170	170	164	170
10DQ	H_e	0.397	0.459	0.035	0.493	0.131	0.421	0.156
10DQ	F_{IS}	-0.05	-0.09	-0.02	-0.09	-0.07	0.18	-0.01
10DQ	*P*	0.566	0.320	1.000	0.271	1.000	0.028	1.000
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.27	0.36	0.02	0.44	0.07	0.30	0.09
10DR	n (N=176)	176	174	176	175	156	176	176
10DR	H_e	0.345	0.450	0.044	0.495	0.114	0.404	0.175
10DR	F_{IS}	0.08	-0.11	-0.02	0.03	0.05	-0.02	0.03
10DR	*P*	0.279	0.177	1.000	0.768	0.448	0.852	0.665
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.22	0.34	0.02	0.45	0.06	0.28	0.10
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy221	AOmy222	AOmy223	AOmy225	AOmy226	AOmy227	AOmy228
09CZ	n (N=92)	91	89	88	90	90	92	92
09CZ	H_e	0.160	0.480	0.386	0.482	0.134	0.168	0.415
09CZ	F_{IS}	-0.09	0.19	0.12	0.11	-0.07	-0.10	-0.10
09CZ	*P*	1.000	0.123	0.271	0.387	1.000	1.000	0.447
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.09	0.40	0.26	0.41	0.07	0.09	0.29
09DA	n (N=265)	265	265	265	262	265	265	265
09DA	H_e	0.162	0.486	0.356	0.455	0.212	0.218	0.420
09DA	F_{IS}	0.04	0.05	-0.03	-0.08	-0.03	-0.04	-0.12
09DA	*P*	0.440	0.450	0.734	0.234	0.778	0.779	0.056
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.09	0.42	0.23	0.35	0.12	0.12	0.30
10DQ	n (N=170)	169	170	170	170	170	169	170
10DQ	H_e	0.096	0.474	0.418	0.483	0.189	0.171	0.444
10DQ	F_{IS}	-0.05	0.07	0.03	0.19	0.07	0.24	0.01
10DQ	*P*	1.000	0.419	0.709	0.017	0.402	0.006	1.000
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.05	0.39	0.30	0.41	0.11	0.09	0.33
10DR	n (N=176)	175	175	175	175	176	175	175
10DR	H_e	0.108	0.422	0.353	0.490	0.146	0.207	0.443
10DR	F_{IS}	0.05	0.19	-0.03	0.05	-0.01	-0.07	-0.05
10DR	*P*	0.438	0.022	0.830	0.647	1.000	0.473	0.508
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.06	0.30	0.23	0.43	0.08	0.12	0.33
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy229	AOmy230	AOmy231	AOmy232	AOmy233	AOmy234	AOmy235
09CZ	n (N=92)	91	90	91	90	92	91	92
09CZ	H_e	0.053	0.223	0.488	0.499	0.235	0.229	0.340
09CZ	F_{IS}	-0.02	-0.14	-0.19	-0.04	0.31	-0.05	-0.08
09CZ	*P*	1.000	0.351	0.083	0.833	0.010	1.000	0.549
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.03	0.13	0.42	0.48	0.14	0.13	0.22
09DA	n (N=265)	263	265	265	265	264	265	265
09DA	H_e	0.019	0.229	0.500	0.496	0.295	0.251	0.240
09DA	F_{IS}	-0.01	-0.02	0.04	-0.02	0.01	-0.02	0.15
09DA	*P*	1.000	1.000	0.618	0.800	0.833	1.000	0.019
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.01	0.13	0.49	0.45	0.18	0.15	0.14
10DQ	n (N=170)	170	170	170	170	169	170	170
10DQ	H_e	0.017	0.185	0.497	0.492	0.332	0.230	0.194
10DQ	F_{IS}	-0.01	-0.05	-0.15	0.05	0.16	0.06	-0.12
10DQ	*P*	1.000	1.000	0.064	0.651	0.037	0.499	0.227
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.01	0.10	0.46	0.56	0.21	0.13	0.11
10DR	n (N=176)	174	175	176	176	176	175	176
10DR	H_e	0.017	0.189	0.497	0.495	0.287	0.202	0.227
10DR	F_{IS}	-0.01	-0.05	0.02	-0.12	-0.01	0.10	0.10
10DR	*P*	1.000	0.696	0.880	0.129	1.000	0.245	0.184
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.01	0.11	0.46	0.55	0.17	0.11	0.13
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy238	AOmy239	AOmy240	AOmy241	AOmy242	AOmy243	AOmy244
09CZ	n (N=92)	91	92	91	92	91	92	91
09CZ	H_e	0.498	0.243	0.413	0.440	0.253	0.063	0.260
09CZ	F_{IS}	0.08	-0.07	-0.03	0.21	0.09	-0.03	-0.01
09CZ	*P*	0.528	0.687	0.806	0.059	0.406	1.000	1.000
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.47	0.14	0.29	0.33	0.15	0.03	0.15
09DA	n (N=265)	264	265	259	265	264	265	265
09DA	H_e	0.448	0.329	0.362	0.437	0.184	0.011	0.274
09DA	F_{IS}	0.10	0.04	0.07	0.08	-0.07	0.00	0.11
09DA	*P*	0.127	0.582	0.232	0.207	0.495	1.000	0.115
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.34	0.21	0.24	0.32	0.10	0.01	0.16
10DQ	n (N=170)	170	170	170	170	164	170	170
10DQ	H_e	0.487	0.298	0.337	0.422	0.303	0.023	0.330
10DQ	F_{IS}	0.00	0.17	0.11	0.01	0.10	-0.01	0.02
10DQ	*P*	1.000	0.037	0.174	0.858	0.294	1.000	0.818
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.42	0.18	0.21	0.30	0.19	0.01	0.21
10DR	n (N=176)	174	176	173	176	175	174	176
10DR	H_e	0.487	0.279	0.382	0.446	0.133	0.056	0.275
10DR	F_{IS}	0.06	0.05	-0.01	0.01	-0.07	-0.03	0.05
10DR	*P*	0.427	0.592	1.000	1.000	1.000	1.000	0.583
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.42	0.17	0.26	0.34	0.07	0.03	0.16
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy245	AOmy246	AOmy247	AOmy248	AOmy249	AOmy250	AOmy252
09CZ	n (N=92)	92	91	87	92	92	92	91
09CZ	H_e	0.150	0.361	0.500	0.073	0.053	0.063	0.417
09CZ	F_{IS}	-0.08	0.12	0.13	-0.03	-0.02	0.32	0.16
09CZ	*P*	1.000	0.259	0.282	1.000	1.000	0.081	0.134
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.08	0.24	0.49	0.04	0.03	0.03	0.30
09DA	n (N=265)	265	264	261	263	265	264	265
09DA	H_e	0.097	0.390	0.500	0.073	0.037	0.062	0.492
09DA	F_{IS}	-0.05	0.13	-0.12	0.17	0.19	-0.03	0.04
09DA	*P*	1.000	0.058	0.064	0.047	0.084	1.000	0.515
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.05	0.27	0.51	0.04	0.02	0.03	0.44
10DQ	n (N=170)	163	170	168	169	170	170	169
10DQ	H_e	0.082	0.366	0.500	0.052	0.040	0.029	0.449
10DQ	F_{IS}	-0.04	0.10	0.05	-0.02	0.27	-0.01	0.25
10DQ	*P*	1.000	0.207	0.547	1.000	0.061	1.000	0.002
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.04	0.24	0.51	0.03	0.02	0.01	0.34
10DR	n (N=176)	175	176	168	174	176	176	176
10DR	H_e	0.166	0.357	0.499	0.103	0.066	0.044	0.478
10DR	F_{IS}	0.18	0.05	0.04	0.06	-0.03	-0.02	0.23
10DR	*P*	0.040	0.528	0.639	0.406	1.000	1.000	0.003
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.09	0.23	0.52	0.05	0.03	0.02	0.39
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy253	AOmy254	AOmy255	AOmy256	AOmy257	AOmy258	AOmy260
09CZ	n (N=92)	92	92	90	91	91	91	92
09CZ	H_e	0.460	0.053	0.043	0.123	0.478	0.237	0.450
09CZ	F_{IS}	-0.08	-0.02	-0.02	-0.07	-0.14	-0.06	0.02
09CZ	*P*	0.501	1.000	1.000	1.000	0.189	1.000	1.000
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.36	0.03	0.02	0.07	0.40	0.14	0.34
09DA	n (N=265)	264	263	265	264	264	265	264
09DA	H_e	0.447	0.026	0.022	0.127	0.498	0.221	0.388
09DA	F_{IS}	0.04	-0.01	-0.01	-0.07	-0.05	-0.04	0.09
09DA	*P*	0.576	1.000	1.000	0.614	0.479	0.779	0.146
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.34	0.01	0.01	0.07	0.47	0.13	0.26
10DQ	n (N=170)	170	170	170	170	170	170	170
10DQ	H_e	0.442	0.012	0.006	0.126	0.500	0.161	0.405
10DQ	F_{IS}	-0.04	0.00	a	-0.07	0.00	-0.09	-0.13
10DQ	*P*	0.731	1.000	a	1.000	1.000	0.368	0.126
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.33	0.01	0.003	0.07	0.51	0.09	0.28
10DR	n (N=176)	175	176	173	175	176	175	173
10DR	H_e	0.398	0.033	0.017	0.185	0.500	0.284	0.359
10DR	F_{IS}	-0.03	-0.01	-0.01	-0.11	-0.08	-0.12	0.05
10DR	*P*	0.852	1.000	1.000	0.220	0.366	0.111	0.532
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.27	0.02	0.01	0.10	0.49	0.17	0.23
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy261	AOmy262	AOmy263	AOmy265	AOmy266	AOmy267	AOmy268
09CZ	n (N=92)	92	89	92	91	91	92	91
09CZ	H_e	0.022	0.182	0.370	0.074	0.213	0.258	0.053
09CZ	F_{IS}	-0.01	0.14	0.03	-0.03	0.28	-0.17	-0.02
09CZ	*P*	1.000	0.212	0.783	1.000	0.023	0.119	1.000
09CZ	A	2	2	2	2	2	2	2
09CZ	MAF	0.01	0.10	0.24	0.04	0.12	0.15	0.03
09DA	n (N=265)	264	263	264	264	263	265	265
09DA	H_e	0.015	0.172	0.293	0.023	0.150	0.329	0.033
09DA	F_{IS}	-0.01	0.03	0.07	-0.01	-0.09	0.13	-0.02
09DA	*P*	1.000	0.713	0.285	1.000	0.234	0.037	1.000
09DA	A	2	2	2	2	2	2	2
09DA	MAF	0.01	0.10	0.18	0.01	0.08	0.21	0.02
10DQ	n (N=170)	170	170	170	170	170	170	170
10DQ	H_e	0.006	0.298	0.298	0.035	0.146	0.294	0.063
10DQ	F_{IS}	a	-0.02	-0.10	-0.02	0.32	0.10	-0.03
10DQ	*P*	a	1.000	0.300	1.000	0.001	0.196	1.000
10DQ	A	2	2	2	2	2	2	2
10DQ	MAF	0.003	0.18	0.18	0.02	0.08	0.18	0.03
10DR	n (N=176)	176	175	176	175	176	176	175
10DR	H_e	0.033	0.257	0.375	0.103	0.165	0.305	0.039
10DR	F_{IS}	-0.01	-0.09	0.12	-0.05	0.25	-0.04	-0.02
10DR	*P*	1.000	0.371	0.108	1.000	0.008	0.803	1.000
10DR	A	2	2	2	2	2	2	2
10DR	MAF	0.02	0.15	0.25	0.05	0.09	0.19	0.02
	A_{TOT}	2	2	2	2	2	2	2

Appendix B, continued.

Code	Metric	AOmy269	AOmy270	AOmy271	AOmy272	AOmy273	AOmy274
09CZ	n (N=92)	29	91	88	88	91	91
09CZ	H_e	0.285	0.000	0.011	0.252	0.317	0.330
09CZ	F_{IS}	-0.19	a	a	0.19	-0.03	-0.06
09CZ	*P*	0.559	a	a	0.086	1.000	0.755
09CZ	A	2	1	2	2	2	2
09CZ	MAF	0.17	0.000	0.006	0.15	0.20	0.21
09DA	n (N=265)	265	261	264	264	264	265
09DA	H_e	0.136	0.000	0.011	0.187	0.343	0.306
09DA	F_{IS}	-0.08	a	0.00	-0.03	-0.17	-0.03
09DA	*P*	0.378	a	1.000	0.751	0.006	0.691
09DA	A	2	1	2	2	2	2
09DA	MAF	0.07	0.000	0.006	0.10	0.22	0.19
10DQ	n (N=170)	170	170	170	169	169	170
10DQ	H_e	0.106	0.000	0.000	0.209	0.373	0.221
10DQ	F_{IS}	0.06	a	a	0.04	-0.01	0.02
10DQ	*P*	0.411	a	a	0.705	1.000	0.732
10DQ	A	2	1	1	2	2	2
10DQ	MAF	0.06	0.000	0.000	0.12	0.25	0.13
10DR	n (N=176)	158	175	176	175	175	175
10DR	H_e	0.073	0.006	0.006	0.171	0.302	0.207
10DR	F_{IS}	-0.04	a	a	0.10	-0.04	0.09
10DR	*P*	1.000	a	a	0.178	0.803	0.263
10DR	A	2	2	2	2	2	2
10DR	MAF	0.04	0.003	0.003	0.09	0.19	0.12
	A_{TOT}	2	2	2	2	2	2