Gene Flow between Resident and Anadromous Rainbow Trout in the Yakima Basin: Ecological and Genetic Evidence

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Abstract.—We examined ecological and genetic evidence to determine the potential for gene flow between resident rainbow trout and anadromous steelhead *Oncorhynchus mykiss* in the Yakima River basin. Electrofishing, trapping, radio telemetry, redd surveys, and snorkeling were used to determine the spatial and temporal distribution of spawning *O. mykiss*. Steelhead had a smaller spatial spawning distribution than rainbow trout, but it was entirely within the range of rainbow trout spawning areas. Furthermore, the spawning time of rainbow trout and steelhead was positively related to elevation, and no differences in timing were detected between forms ($P > 0.05$). In addition, we observed many instances of interbreeding between rainbow trout and steelhead. Genetic evidence from starch gel electrophoresis also suggested that rainbow trout and steelhead interbreed. Rainbow trout were genetically indistinguishable from sympatric steelhead collected in the North Fork of the Teanaway River. In addition, estimates of hatchery and wild fish admixtures in naturally produced *O. mykiss* suggested that hatchery rainbow trout had previously spawned with steelhead and that hatchery steelhead had previously spawned with rainbow trout. We speculate that the magnitude of gene flow between rainbow trout and steelhead may vary spatially and temporally, depending in part on the number of anadromous steelhead that spawn within an area or year and on the number of steelhead offspring that rear and mature entirely within freshwater.

Oncorhynchus mykiss may possess the most diverse life-history patterns of any of the Pacific salmonid species. Much of the variation can be attributed to the migrational tendencies that have been observed within the species. One dominant life-history form of *O. mykiss* (steelhead) rears in fresh water, migrates to the ocean, and then returns to spawn in fresh water (Shapovalov and Taft 1954; Withler 1966; Wydoski and Whitney 1979; Behnke 1992; Peven et al. 1994). The other dominant life-history form of *O. mykiss* (rainbow trout) spends its entire life in fresh water (Wydoski and Whitney 1979; Behnke 1992). Within each dominant life-history form there is additional variation in migrational tendencies. For instance, some steelhead will migrate annually between the ocean and freshwater up to five times in order to spawn (Shapovalov and Taft 1954); some steelhead may rear in fresh water for up to 7 years before migrating to the ocean (Peven et al. 1994), and some offspring of steelhead may mature in fresh water without migrating to the ocean (Shapovalov and Taft 1954; Mullan et al. 1992a; Viola and Schuck 1995). Some rainbow trout are extremely sedentary, whereas others migrate considerable distances, particularly as juveniles during the spring and as adults before spawning (Bartrand et al. 1994). It is unclear whether ecological or genetic factors are responsible for the expression of the variation in migrational tendencies within *O. mykiss*, but both factors are probably influential. One mechanism that may influence the expression of intermediate migrational tendencies, or a mixture of such tendencies, is interbreeding between steelhead and rainbow trout (Moring and Buchanan 1978).

There is considerable uncertainty whether resident and anadromous *O. mykiss* interbreed (Busby et al. 1996). Both forms are found together throughout much of their range and spawn primarily during the spring (Rounsefell 1958; Scott and Crossman 1973; Wydoski and Whitney 1979). However, rainbow trout spawn at other times, particularly in spring-fed streams and in streams that have had hatchery stocking (Biette et al. 1981). Although rainbow trout and steelhead generally spawn at similar times and places, little is known about fine-scale spatial and temporal differences (*see* Neave 1944; Shapovalov and Taft 1954). High spatial and temporal overlap in spawning among rainbow trout and steelhead increases the potential for these forms to interbreed.

Genetic comparisons of rainbow trout and steelhead collected within a basin reveal differences in *O. mykiss* that are correlated with geography. In general, rainbow trout and steelhead collected from similar geographic locations are more genetically similar to each other than to rainbow trout and steelhead collected from different geographic locations (Busby et al. 1996). For example, rainbow trout and steelhead collected in the Columbia Basin east of the Cascade mountains are more similar to each other than to rainbow trout and steelhead collected west of the Cascades (Allendorf 1975). At a smaller scale, in the Deschutes River basin, Currens et al. (1990) found that there were significant differences in allozyme frequencies between rainbow trout collected above a barrier falls and *O. mykiss* collected below the falls. These fish from below the falls were probably a combination of rainbow trout and steelhead. Somewhat con-

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trary to the theory that rainbow trout and steelhead share the same gene pool, Campton and Johnston (1985) found that rainbow trout in the upper Yakima Basin were primarily an admixture of hatchery and native rainbow trout despite the stocking of hatchery steelhead into the basin. Furthermore, Neave (1944) found significant differences between average scale counts of rainbow trout and steelhead collected from the same place in the Cowichan River basin, British Columbia. He believed that these differences were genetically based, which suggests the two forms were not interbreeding. We know of no published studies that have compared the genetic structure of wild rainbow trout and steelhead that were collected from populations spawning in sympatry.

Determination of interbreeding between rainbow trout and steelhead has important management implications. As early as 1944, and most likely before, Neave (1944) suggested that rainbow trout and steelhead should be managed as different species in order to protect both forms. More recently, the National Marine Fisheries Service is considering whether to include rainbow trout as part of a steelhead evolutionarily significant unit (ESU) (Busby et al. 1996; NMFS 1996). If interbreeding between rainbow trout and steelhead occurs frequently, then rainbow trout might be considered part of a steelhead ESU. Furthermore, the abundance of rainbow trout may influence whether a steelhead ESU is worthy of protection by the federal government. Thus, depending on whether rainbow trout and steelhead interbreed and on the abundance of rainbow trout, an ESU may or may not receive federal protection.

The purpose of our study was to determine the potential for gene flow between resident and anadromous forms of *O. mykiss* in the Yakima River basin. We did this by determining the spatial and temporal overlap of spawning, comparing the genetic similarity of rainbow trout and steelhead collected from the same area, and by examining allozyme data for resident x anadromous matings, using hatchery fish as genetic markers.

Study Area

The Yakima Basin drains an area of 15,941 km^2 of south-central Washington, entering the Columbia River near Richland, Washington. Major subbasins of the Yakima River include the Satus, Toppenish, Naches, and upper Yakima (Figure 1). It is estimated that almost 100% of *O. mykiss* spawners in the Satus and Toppenish subbasins are steelhead (Hubble 1992; J. Hubble, Yakama Nation, personal communication), but less than 1% of *O. mykiss* spawners in the upper Yakima Basin are steelhead (WDFW, unpublished data). The proportion of *O. mykiss* that spawn in the Naches subbasin as steelhead is believed to be intermediate between Satus/Toppenish and the upper Yakima River. The lower boundary of the upper Yakima Basin is Roza Dam, which was constructed in 1939. Prior to 1987, fish could not ascend the fish ladder to pass upstream of Roza Dam when the reservoir pool was low. Low pool typically occurred during

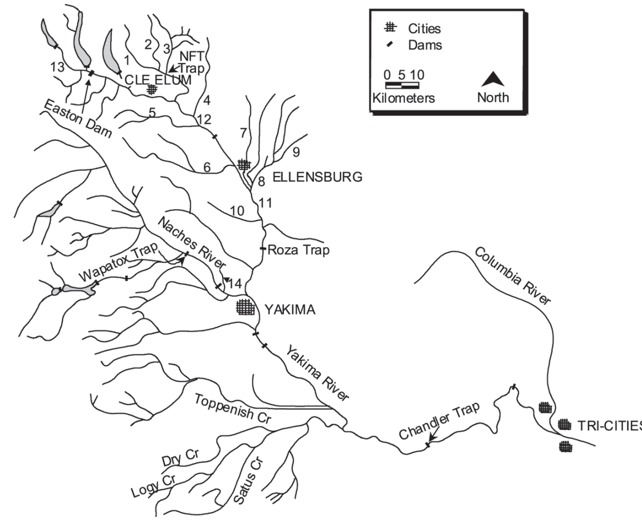

FIGURE 1.—Map of the study area. Numbers refer to study locations in the upper Yakima Basin (1–West Fork of the Teanaway River, 2–Middle Fork of the Teanaway River, 3–North Fork of the Teanway River, 4–Swauk Creek, 5–Taneum Creek, 6–Manastash Creek, 7–Wilson Creek, 8–Cherry Creek, 9–Badger Creek, 10–Umtanum Creek, 11–sections 1-3 of the Yakima River, 12–section 4-6 of the Yakima River, 13–section 7 of the Yakima River, 14–Buckskin Creek).

the winter. During 1987 a modification was made to the fish ladder that allowed fish to pass the dam when the reservoir pool was low. Rainbow trout located upstream of Roza Dam are abundant and provide Washington's best wild stream-trout fishery (Krause 1991; Probasco 1994). The Naches subbasin has several irrigation diversions that span the entire channel width. The Satus subbasin has no dams.

Non-endemic hatchery rainbow trout and steelhead have been heavily stocked in the Yakima Basin since the early 1940s (Campton and Johnston 1985). Between 1950 and 1980, 3,400,000 rainbow trout and 830,000 juvenile steelhead were stocked into the Yakima Basin from non-endemic hatchery populations (Campton and Johnston 1985). Between 1981 and 1994, about 966,000 rainbow trout and 1,268,500 steelhead were stocked into the Yakima Basin from endemic and non-endemic hatchery sources (Washington Department of Fish and Wildlife stocking records). Most of the hatchery rainbow trout stocked into the Yakima Basin were derived from northern California. Hatchery steelhead smolts stocked into the Yakima Basin were derived mainly from fish native to the Washougal and Klickitat rivers (Skamania strain) (Crawford 1979; Campton and Johnston 1985). These hatchery populations are genetically distinguishable from each other and from *O. mykiss* in the Yakima Basin (Campton and Johnston 1985; Pearsons et al. 1994). Non-endemic hatchery populations contain alleles that are not found in endemic Yakima River populations, and alleles common to hatchery and endemic populations are found in different frequencies (Phelps and Baker 1994). These differences are largely due to the source locations of hatchery strains (e.g., coastal areas). Trout from these areas are known to be genetically different from inland *O. mykiss* (Campton and Johnston 1985).

Methods

Ecology.—Sexual maturity and time of spawning were estimated in seven sections of the mainstem of the Yakima River between Roza Dam and Easton Dam and in 35 study sections in 13 tributaries of the upper Yakima River (Figure 1). The seven mainstem sections were numbered from 1 at the lowest to 7 at the highest elevation. Elevations above sea level at the midpoint of the sections ranged from 390 to 701 m in the mainstem and from 446 to 847 m in the tributaries. Adult steelhead were distinguished from adult rainbow trout by their larger size (> 51 cm FL) and location of capture (e.g., upstream trap). Their identity was often verified from scale pattern analysis. Electrofishing was used to collect rainbow trout for determination of maturity in the upper Yakima River and its tributaries from February through June during 1990 to 1993. Sample sizes were usually 10 to 30 adult-sized rainbow trout per section per survey, for a total of 30 to 90 rainbow trout per tributary per survey. In each tributary, a sample was collected with a back-pack electrofisher in a low, middle, and high elevation stream section. In the mainstem, a driftboat electrofisher was used to collect fish at least once per month from February until June. Fish were anesthetized and checked for spawning condition by gently squeezing the abdomen with thumb and forefinger to see if ova, milt, or resorbing fluids could be extruded. Sexually mature fish were defined to be those that exuded either milt or ova; they were further classified as ripe or spent.

The peak time of rainbow trout spawning was determined by calculating the time at which the greatest percentage of adult rainbow trout were sexually mature. The percentage of sexually mature rainbow trout was calculated as follows. First, the minimum adult size for each tributary stream and mainstem section was estimated to be the fork length of the smallest sexually mature rainbow trout collected during the sampling period (Martin et al. 1994). All others that were equal to, or greater than the minimum length were considered adult size and were defined as "potential" adults. The percentage of sexually mature rainbow trout was calculated by dividing the number of mature rainbow trout in a given sample by the number of "potential" adults.

The peak time of spawning activity was identified using an Open Quasi Cubic Spline method (Manugistics Corporation 1992) and then locating the highest points on the curve. The spline method uses a fifth-order polynomial interpolation to smooth small data sets. We used this smoothing to facilitate location of peaks. Peaks were not interpreted if sample size was less than eight adult-sized fish or if the percentage of sexually mature rainbow trout was less than 16%. The estimated peak time of spawning for each stream section was then compared to elevation at the midpoint of the section to determine if a relationship existed between rainbow trout spawn timing and elevation.

The spawn timing of steelhead was determined using a variety of field methods between 1990 and 1993, such as observations of redds, bankside observations, snorkeling, trapping, electrofishing, and radio telemetry. Panel or picket weir traps were used to trap fish in Wilson, Cherry, Umtanum, Swauk, and Taneum creeks. Spawning times and locations of radio-tagged steelhead were determined from a report by Hockersmith et al. (1995). Redds with fish on or near them were observed by helicopter on April 30, 1991. Bankside and snorkeling observations of steelhead were made throughout the study period. If an adult steelhead was collected by electrofishing or observed in a tributary after March 1, it was assumed to be a spawner at the time of sampling and information on spawning time and location was recorded. Steelhead that spawn in upper Yakima River tributaries frequently enter, spawn, and exit tributaries over a short time (Hockersmith et al. 1995).

Simple regression was used to describe the relationship between spawn timing and elevation for both rainbow trout and steelhead. Multiple regression and *t*-tests were used to compare the slopes and intercepts of regressions for rainbow trout and steelhead.

Hatchery steelhead "smolts" were released into the North Fork Teanaway subbasin annually between the spring of 1991 and 1994 and were examined for sexual maturity and gender. Approximately 150 steelhead from an average release number of 31,155 were examined at the time of release. In addition, hatchery steelhead juveniles and residuals were captured throughout the spring and summer in the North Fork Teanaway subbasin and examined for sexual maturity and gender. These fish were collected by electrofishing and trapping (weirs and screw traps).

Genetics.—Three genetic analyses were performed to determine if gene flow occurred between rainbow trout and steelhead. First, naturally produced sympatric rainbow trout and steelhead juveniles were collected from the North Fork of the Teanaway River and their allele frequencies were compared. Similarities in allele frequencies are an indication that the rainbow trout and steelhead interbreed. Rainbow trout were collected between 1991 and 1993 by electrofishing and angling. Steelhead smolts were collected in a downstream migrant trap during 1991. Second, naturally produced steelhead adults were collected throughout the Yakima Basin and were genetically examined for introgression with hatchery rainbow trout. Third, naturally produced rainbow trout adults were collected throughout the upper Yakima Basin and were genetically examined for introgression with hatchery steelhead.

To avoid mixes of steelhead and rainbow trout in the samples, specific guidelines were used to classify naturally produced rainbow trout and steelhead at the time of collection (see classification above). Only fish that were classified as naturally produced rainbow trout or steelhead were used in the analyses. Fish were classified as rainbow trout if they were < 51 cm, had characteristic coloring and morphology, and if they were sexually mature at the time of collection. Sexual maturity was determined based on the examination of gonads or the exuding of milt or ova. Precociously mature offspring of steelhead parents would be classified as rainbow trout under these criteria. Hatchery-produced rainbow trout were excluded from the samples. Rainbow trout were classified as "hatchery" if they had eroded fins and wavy fin rays. Juvenile fish were classified as steelhead if they had typical smolt characteristics and if they were captured in a downstream migrant trap or by electrofishing in areas without rainbow trout, such as in the Satus or Toppenish subbasins (Hubble 1992; J. Hubble, Yakama Nation, personal communication). Typical smolt charactistics that we used to classify steelhead included silvery coloration, lack of visible parr marks, easy loss of scales, torpedo shape (relatively thin), and dark band on the caudal fin. Hatchery-produced steelhead were excluded from the samples if they had clipped adipose or ventral fins, eroded fins, or wavy fin rays.

Naturally produced steelhead smolts that were used to determine introgression with hatchery rainbow trout were collected between 1989 and 1994. Downstream migrant traps were used to collect steelhead smolts from the Naches subbasin, upper Yakima Basin, and from the Yakima River at Prosser. Steelhead smolts were collected by electrofishing in the Satus and Toppenish subbasins. Naturally produced rainbow trout were collected between 1990 and 1993 in seven mainstem sections of the upper Yakima River and 10 of its tributaries. Rainbow trout were collected by electrofishing, angling, and trapping.

The collected fish were either dissected in the field (most adult specimens) or frozen whole at ultra-low temperatures (–80°C) and transported on dry ice to the Washington Department of Fish and Wildlife's Genetics Laboratory. Muscle, heart, eye, and liver tissues were dissected from each fish and placed into 12 x 75-mm plastic culture tubes. Electrophoresis followed the methods of Aebersold et al. (1987). The electrophoretic protocol, enzymes screened, and alleles observed during this study are described in Phelps et al. (1994). Genetic nomenclature follows the conventions of Shaklee et al. (1990).

A chi-square goodness of fit test was used to compare allozyme data between rainbow trout and steelhead collected in the North Fork of the Teanaway River. Hatchery admixtures were estimated using the program ADMIX (Long 1991). The ADMIX program calculates maximum likelihood estimates of the percentage of the genes that are contributed from hypothetical ancestral parental sources. Stated another way, ADMIX yields admixture proportions that are the best estimates of combinations of hypothetical parental sources. Results can indicate the degree of past breeding events if (1) the putative parental source information is correct, (2) their frequencies are reasonably close to what they would have been at the time the admixture occurred, and (3) there was opportunity for admixture. All of these conditions seem reasonable for our data. Because wild and introduced hatchery fish in the Yakima Basin are so different genetically, intermediate allele frequencies should indicate past breeding events. Three potential parental sources were used in the analysis: wild *O. mykiss*, hatchery rainbow trout, and hatchery steelhead (Table 1). "Wild" is defined as the naturally produced population that would be present in the Yakima Basin in the absence of interbreeding with non-endemic hatchery fish. Potential parental sources for wild *O. mykiss* were determined by examining allele frequencies of fish from the Satus and Teanaway subbasins; these fish were presumed to be representative of wild *O. mykiss*. Allele frequencies for steelhead produced at the Skamania Hatchery were used for the potential parental source of hatchery steelhead. Allele frequencies for rainbow trout produced at the Goldendale Hatchery were used for the potential parental source of hatchery rainbow trout. Some allele frequencies of potential parental sources were adjusted up or down if the "unknown population" was outside of the ranges of potential parental sources. This "adjustment" was necessary in order for the program to run.

Gametic disequilibrium analysis was used to determine whether rainbow trout collections contained a mixture of distinct gene pools or recent mixtures of gene pools (Nei and Li 1973; Waples and Smouse 1990; Phelps et al. 1994). In our samples, we had the ability to find significant gametic disequilibrium due to the presence of "pure" hatchery and "pure" wild rainbow trout, recent interbreeding of hatchery

TABLE 1.—Allele frequencies of potential parental sources used in admixture analyses. Sources for wild *O. mykiss* were from Satus and Teanaway subbasins, hatchery rainbow trout from Goldendale Hatchery, and hatchery steelhead from Skamania Hatchery.

Locus and Allele	Allele Frequency of Parental Source		
	Wild *O. mykiss*	Hatchery Rainbow	Hatchery Steelhead
*ADA-1*100*	1.00	0.25	1.00
*sAH*100*	0.80	1.00	0.97
*ALAT*100*	0.96	1.00	0.94
*CK-A1*100*	1.00	0.94	1.00
*GAPDH-3*100*	0.96	1.00	1.00
*bGLUA*100*	1.00	0.67	1.00
*mIDHP-2*100*	1.00	0.63	0.99
*sIDHP-2*72*	0.30	0.05	0.32
*LDH-B2*100*	0.50	1.00	0.82
*LDH-C*100*	1.00	0.90	1.00
*sMDH-B2*83*	0.00	0.55	0.01
*MPI*100*	0.90	1.00	1.00
*mSOD*100*	0.90	1.00	1.00
*sSOD-1*152*	0.05	0.36	0.24
*sSOD-1*38*	0.05	0.00	0.00
*TPI-3*100*	0.96	1.00	0.99
*ADH*100*	1.00	1.00	0.96
*GPI-A*100*	1.00	1.00	0.94
*G3PDH-1*100*	1.00	0.99	0.84
*sMEP-1*100*	1.00	1.00	0.94
*PEPA*100*	1.00	1.00	0.98
*PEPD-1*100*	1.00	1.00	0.91

and wild rainbow trout, or mixtures of rainbow trout cohorts that were genetically dissimilar (our samples were pooled among years). Failure to detect significant disequilibrium in collections would suggest that either parental gene pools have introgressed and represent one gene pool, or sample sizes were insufficient (Nei and Li 1973). We did not examine the steelhead collections because some of these were from traps that collected multiple stocks of steelhead. Otherwise, gametic disequilibrium analysis was performed on the same data that were used in the ADMIX analysis.

Results

Ecology

The spatial and temporal overlap of rainbow trout and steelhead spawning was very high. Except for one steelhead that spawned in a high elevation tributary that we did not electrofish, sexually mature rainbow trout were collected in all areas where steelhead spawned. However, rainbow trout spawned over a much larger geographic area than did steelhead. Rainbow trout spawned throughout all sampled reaches of the upper Yakima River basin. Rainbow trout spawned in tributaries and mainstem areas, and sexually mature individuals were collected at elevations between 375 and 1,061 m. Steelhead also spawned in tributaries and mainstem areas but spawned at elevations between 375 and 896 m.

Rainbow trout and steelhead spawned at similar times during the spring. Spawning generally began in February and continued through June. The earliest date that sexually mature rainbow trout were collected was February 1, and the latest date for spring spawners was June 28. Some sexually mature fish were also collected during fall sampling in some locations but not during the summer (Pearsons et al. 1996). Our analysis was restricted to spring spawning. Steelhead spawning occurred between February 28 and July 2.

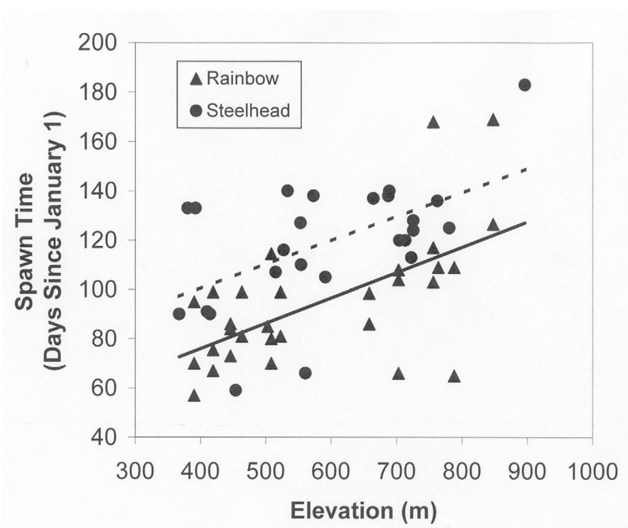

FIGURE 2.—Peak of spawning time for rainbow trout and spawning time of steelhead relative to elevation in the upper Yakima River basin. Regression lines are also presented (top line–steelhead, bottom line–rainbow trout).

Spawn timing of both rainbow trout and steelhead was positively related to elevation (Figure 2):

$$\text{PSTRBT} = 0.103 \cdot (\text{E}) + 34.8 \ (n = 30, r^2 = 0.37, P < 0.05)$$

$$\text{STSTH} = 0.098 \cdot (\text{E}) + 61.1 \ (n = 28, r^2 = 0.30, P < 0.05).$$

Where PSTRBT is the peak of spawn timing of rainbow trout measured in days since January 1, STSTH is the time of steelhead spawning in days since January 1, and E is the elevation measured in meters. There was no statistical difference in the intercepts ($t = 0.142$, $P = 0.89$) or slopes ($t = 0.978$, $P = 0.33$) of regressions between the peak of rainbow trout spawning and steelhead spawning.

We probably underestimated the duration of time that sexually mature rainbow trout were present because sexually mature fish were often collected during the first and last sampling period. However, sexually mature rainbow trout were rarely collected during other sampling activities in the summer, although some sexually mature rainbow trout have been collected in Badger, Wilson, and Cherry creeks and the Middle Fork of the Teanaway River during the fall.

We have observed many instances of suspected breeding between rainbow trout and steelhead. In 1992 a ripe female steelhead was trapped while migrating into Umtanum Creek and later was recaptured "spent" while migrating back downstream. This occurred near the peak of rainbow trout spawning activity in the creek. No other steelhead were observed to have entered the creek through the trap that year. In 1990, a spent female steelhead was collected adjacent to her redd in association with several ripe male rainbow trout. No other steelhead were collected in Umtanum Creek during 1990 despite intensive sampling. In 1995, one female steelhead was observed with mature rainbow trout on a redd in Buckskin Creek (tributary to the lower Naches River). Again, no male steelhead were captured in an adult migrant trap located below the redd. Lastly, many precocious hatchery steelhead have been observed in the North Fork Teanaway Basin. Up to 4.0% of hatchery steelhead released were sexually mature males. Less than 0.1% of precocious hatchery steelhead encountered during all sampling activities were females. Some precocious hatchery steelhead have been collected after they have spawned and others have been observed spawning with rainbow trout in the North Fork Teanaway subbasin.

Genetics

Genetic data supported the hypothesis that rainbow trout and steelhead were interbreeding where they were found in sympatry. Rainbow trout were genetically indistinguishable from steelhead collected in the North Fork of the Teanaway River ($X^2 = 54.99$, df = 43, $P = 0.104$). Except for the Satus and Toppenish subbasins, steelhead were estimated to be an admixture of hatchery rainbow trout, hatchery steelhead, and wild steelhead (Table 2). This result suggests that some steelhead have spawned with hatchery rainbow trout sometime

TABLE 2.—Admixture analysis of parental source (Long 1991) for Yakima Basin steelhead stocks. Smolts were collected by electrofishing or trapping. One standard error of the point estimate is shown in parentheses.

			Potential parental sources (%)		
			Hatchery		Wild
Location	Years	*n*	Rainbow	Steelhead	Yakima River
Satus Creek subbasin					
Dry Creek	1989, 1991	153	2 (1)	0	98 (1)
Logy Creek	1990–1991	186	1 (0.3)	0	99 (0.3)
Satus Creek	1990–1991, 1994	263	2 (1)	0 (1)	98 (1)
Toppenish Creek subbasin					
Toppenish Creek	1990, 1994	172	0 (0.5)	0	100 (0.5)
Naches River subbasin					
Wapatox trap	1989–1991	366	6 (1)	4 (2)	90 (3)
Upper Yakima Basin					
Roza trap	1989–1990	175	16 (2)	6 (3)	78 (3)
N.F. Teanaway trap	1991	25	9 (4)	11 (9)	80 (10)
Yakima River (composite)					
Chandler trap	1989, 1994	373	11 (2)	3 (2)	86 (2)
Yakima Hatchery	1991	40	5 (2)	44 (10)	52 (10)

in the past. Conversely, with the exception of Wilson and Cherry creeks and the mainstem Yakima River above the Ellensburg Town Diversion, rainbow trout were estimated to be an admixture of hatchery rainbow trout, hatchery steelhead, and wild rainbow trout (Table 3). Again, this result further indicates that some rainbow trout and steelhead spawned together.

The estimated percentage of hatchery admixture among *O. mykiss* varied with location and life-history type. The percentage of hatchery rainbow trout alleles detected in rainbow trout samples was negatively related to elevation (Figure 3). However, the percentage of hatchery steelhead alleles detected in rainbow trout was unrelated to elevation. The highest percentage of hatchery rainbow trout admixture in steelhead samples in subbasins of the Yakima Basin was found in the upper Yakima Basin (16%), followed by the Naches (6%), Satus (2%), and Toppenish (0%) subbasins (Table 2). Rainbow trout had higher percentages of hatchery admixtures than did steelhead (Tables 2 and 3).

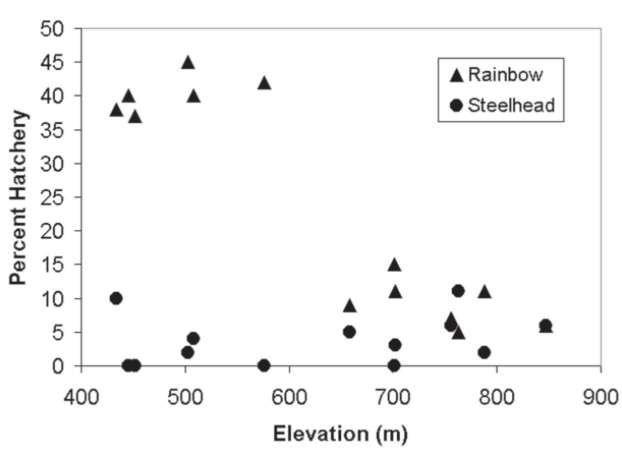

FIGURE 3.—Percentage of non-endemic hatchery rainbow trout and steelhead alleles found in naturally produced rainbow trout collected in the upper Yakima River basin.

Gametic disequilibrium analyses generally indicated that gene pools of parental sources were mixed through interbreeding. In other words, most of the rainbow trout collections (8 of 12) were not separate populations of non-endemic hatchery and wild fish but rather mixed populations of hatchery and wild fish. Those collections that were in disequilibrium were Wilson Creek, Cherry Creek, Manastash Creek, and the Middle Fork of the Teanaway River ($P < 0.05$). These collections were probably not in equilibrium because they contained samples with recent hatchery admixtures or were from pooled samples. Wilson Creek is the last stream location in the upper Yakima Basin that is still stocked with non-endemic rainbow trout, and some of these trout probably disperse into nearby Cherry Creek.

Discussion

Ecological and genetic evidence indicates that rainbow trout and steelhead in the Yakima Basin interbreed when in sympatry. Interbreeding between the two life-history forms may occur in a variety of ways. For example, the following

TABLE 3.—Admixture analysis of parental source (Long 1991) for upper Yakima Basin rainbow trout. Mature (ripe or spent) adults were collected by electrofishing. One standard error of the point estimate is shown in parentheses.

			Potential parental sources (%)		
			Hatchery		Wild
Location	Years	*n*	Rainbow	Steelhead	Yakima River
Tributaries					
Wilson[a]	1990–1993	74	40 (3)	0	60 (3)
Cherry[a]	1990–1991	12	37 (5)	0	63 (5)
Badger	1991–1992	45	45 (7)	2 (5)	53 (8)
Umtanum	1990–1993	102	40 (7)	4 (6)	56 (8)
Taneum	1990–1992	59	11 (4)	2 (4)	87 (5)
Swauk	1990–1992	64	11 (2)	3 (3)	86 (3)
Manastash	1991–1992	69	9 (3)	5 (4)	86 (5)
M.F. Teanaway	1991–1993	86	5 (2)	11 (6)	84 (6)
W.F. Teanaway	1991–1993	72	7 (2)	6 (4)	87 (4)
N.F. Teanaway	1991–1993	55	6 (3)	6 (6)	88 (7)
Mainstem					
Sections 1–3	1990–1992	108	38 (4)	10 (6)	52 (6)
Sections 4–6[a]	1990–1992	52	42 (4)	0	58 (4)
Section 7	1991–1992	14	15 (3)	0	85 (3)

[a] did not use *PEPD-1*100* for the estimate

crosses may occur: female steelhead x male rainbow trout, male steelhead x female rainbow trout, residual female steelhead (offspring of steelhead x steelhead mating) x male rainbow trout, residual male steelhead (offspring of steelhead x steelhead mating) x female rainbow trout, female steelhead x anadromous male rainbow trout (offspring of rainbow x rainbow trout mating) and male steelhead x anadromous female rainbow trout (offspring of rainbow x rainbow trout mating). All of the interbreedings that we observed that involved an anadromous adult were between female steelhead and male rainbow trout. At Waddell Creek, California, Shapovalov and Taft (1954) observed that female steelhead were very often accompanied by male rainbow trout during spawning. Only rarely were resident females observed with steelhead during spawning. Furthermore, as in other salmonids, male rainbow trout may successfully spawn with female steelhead, even in the presence of male steelhead, by sneak spawning (Shapovalov and Taft 1954; Hutchings and Myers 1985; Foote and Larkin 1988; Wood and Foote 1996). The sex ratio of anadromous steelhead may also be skewed toward females when large proportions of male offspring residualize (Thorpe 1987; Peven et al. 1994). A high proportion of anadromous females would increase the potential for female steelhead to spawn with male rainbow trout, particularly if anadromous male steelhead were scarce. This has been shown to occur in other salmonids (Jonsson 1985; Myers and Hutchings 1987).

Precocious male steelhead that do not migrate to the ocean may also spawn with female rainbow trout. We know of no studies that have definitively documented residualized precocious steelhead in natural populations below barriers. However, Mullan et al. (1992a) provide evidence to suggest that "steelhead" at high elevations are thermally fated to a resident life history. Humans have created self-sustaining populations of residualized steelhead in locations above impassable barriers. It is well documented that hatchery-reared populations of steelhead produce numerous precocious male steelhead (Tipping et al. 1995; Viola and Schuck 1995) that can spawn with female steelhead (Viola and Schuck 1995). Spawning between precocious males and anadromous females has also been documented in other salmonids (Myers and Hutchings 1987; Mullan et al. 1992b).

The amount of gene flow in the upper Yakima Basin may be artificially high due to low escapement of steelhead and a high number of rainbow trout. For example, three instances of gene flow between rainbow trout and steelhead occurred when only one female steelhead had ascended a stream that contained many mature rainbow trout. If the steelhead was to spawn in that stream it had to spawn with a rainbow trout or with another species. In addition, steelhead in the upper Yakima Basin collected at Roza Dam had the highest estimated percentage of hatchery rainbow trout ancestry, which further supports the contention that interbreeding may be artificially high in the upper Yakima Basin. Other explanations for the relatively high percentage ancestry of hatchery rainbow trout in steelhead in the upper Yakima Basin are also possible. For example, the number of hatchery rainbow trout stocked, or their survival and reproductive success, may have been higher in the upper Yakima Basin than in other areas of the basin. Unfortunately, we do not have the data that could be used to eliminate either of the explanations. Roza Dam was a probable contributor to the reduction in steelhead abundance, and to the ecological release of rainbow trout in the absence of strong anadromous fish runs

(Campton and Johnston 1985). The high proportion of spawning steelhead found in the Satus and Toppenish subbasins might be more representative of a natural population in the Yakima Basin.

We speculate that the magnitude of gene flow between rainbow trout and steelhead may vary spatially and temporally. If steelhead are in low enough numbers that they have difficulty finding steelhead mates, then there is a higher probability that they will spawn with rainbow trout if rainbow trout are present in spawning condition. Thus, in times or locations that experience low steelhead spawning escapements and that have sympatric rainbow trout populations, the extent of interbreeding could be relatively high. However, in times or locations with high steelhead spawning escapements and/or low numbers of sympatric rainbow trout, interbreeding could be relatively low. Interbreeding may be high when conditions that promote maturation of steelhead in fresh water are good. This might occur when growing conditions in fresh water are poor (Mullan et al. 1992a) or good (Thorpe 1987).

Results from our work suggest that endemic rainbow trout in the Yakima Basin should be included within a steelhead ESU because the two forms are not reproductively isolated when in sympatry. In fact, rainbow trout may be a good source of natural genes if steelhead are extirpated or if wild steelhead are excessively admixed with hatchery steelhead. Indeed, rainbow trout located in high elevation areas of the upper Yakima Basin could have a more natural complement of genes than steelhead spawning in the upper Yakima Basin. However, breeding between rainbow trout and steelhead poses genetic risks to steelhead. Interbreeding between hatchery admixed rainbow trout and steelhead may decrease the long-term fitness of steelhead due to loss of adaptation. In the upper Yakima Basin, this may occur at relatively low elevations, where hatchery admixtures of rainbow trout are high.

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