Movement of resident rainbow trout (*Oncorhynchus mykiss*) transplanted below barriers to anadromy in Freshwater Creek, California.

By

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ABSTRACT

Movement of resident rainbow trout (*Oncorhynchus mykiss*) transplanted below barriers to anadromy in Freshwater Creek, California.

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I conducted an experiment to determine if resident rainbow trout (*Oncorhynchus mykiss*) isolated upstream of a barrier to anadromy in Freshwater Creek, California would exhibit migratory behavior after individuals were relocated to a downstream reach with access to the Pacific Ocean. Between 2005 and 2006 a total sample of 131 age 1+ trout upstream of a 5-m-high waterfall were captured and individually marked with 23-mm passive integrated transponder (PIT) tags. Genetic analysis determined that above-barrier individuals have extensively hybridized with coastal cutthroat trout (*Oncorhynchus clarki clarki*). At each sampling event, half of the tagged individuals (n=22 and n=43 for trout in 2005 and 2006 respectively) were transplanted approximately 10 km from tidewater. Analysis of otolith microchemistry indicated that above-barrier trout were derived from residential parental lineages. An equal number were released at the point of capture above the barrier. Tagged individuals in above- and below- barrier reaches were subsequently relocated and/or recaptured to track their movement. The majority of transplanted trout displayed little downstream movement from the transplant location. Forty percent of transplanted individuals remained within 500 m of the release location in all sightings. The percentage of transplanted trout that moved into tidally influenced water (6%, or 4 of 65 individuals) did not appear to be different from the percentage of prior downstream resident (4%, or 9 of 210 individuals) that were captured and tagged in the vicinity of the transplant release location and later captured in tidally influenced water. Five, tagged individuals from above the barrier-were detected in below barrier reaches. Four of the transplanted individuals were last detected within the tidally influenced reach of the lower river. Downstream migrant traps captured seven tagged individuals, of which, two were determined to have smolted (one of which was not transplanted below the waterfall) and one was a pre-smolt. The similarity in movements observed in the transplant group and the below-barrier population, the smoltification of transplanted individuals, and the leakage of above-barrier fish downstream suggests the potential for resident trout to exhibit migratory behavior and contribute to breeding populations of steelhead.

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INTRODUCTION

The rainbow trout, *Oncorhynchus mykiss*, is a polytypic species characterized by populations of resident, adfluvial, and fluvial rainbow trout as well as anadromous steelhead (Neave 1944; Shapovalov and Taft 1954; Behnke 1992). In some instances, both resident rainbow trout and anadromous steelhead forms can co-occur within the same waterbody. This phenomenon is referred to as partial migration (Jonsson and Jonsson 1993). The underlying basis of this migratory polymorphism is poorly understood. Migratory polymorphism in rainbow trout may be derived from phenotypic plasticity within one gene pool or from fixed differences between sympatric but reproductively isolated populations. Some studies have shown high levels of gene flow between resident and anadromous life history types (Docker and Heath 2003; Seamons et al. 2004; Olsen et al. 2006; Araki et al. 2007) and each life history type can be derived from the progeny of the other (Zimmerman and Reeves 2000; Thrower et al. 2004). There have been only a few documented cases of genetic differentiation between life history forms in the same basin (e.g. Narum et al. 2004). Environmental control of life history expression is suggested in the adoption of anadromy by populations of rainbow trout (Zaugg and Wagner 1973; Pereira and Adelman 1985).

Whether resident rainbow trout and anadromous steelhead represent a randomly mating gene pool or exhibit reproductive isolation has significant implications for the management of steelhead populations, which have undergone a precipitous decline in recent years. Steelhead are listed under the US Endangered Species Act (ESA) as threatened in the northern California ESU (evolutionary significant unit) and elsewhere (U.S. Office of the Federal Register 1997, 2000). If resident and anadromous populations within a basin share a common gene pool, resident fish could be managed to help buffer extinction risks to anadromous populations. Resident fish above barriers could provide a “reserve” gene pool in times of unfavorable ocean conditions and could colonize newly available habitat (e.g. through dam removal) with anadromous progeny. Currently, resident populations of rainbow trout are not considered to be part of recognized steelhead ESUs. Busby et al. (1996) acknowledged that resident populations that inhabit areas upstream from numerous smaller barriers in California might contain genetic resources similar to those of anadromous fish in the ESU, but concluded that little information is available on these fish or the role they might play in conserving natural populations of steelhead.

The objectives of this study were to determine if trout isolated above waterfalls in a northern California stream would exhibit migratory behavior when individuals are relocated to downstream reaches with access to the Pacific Ocean and to compare movement and growth between above- and below-barrier populations of fish. I also sought to estimate the extent of genetic differentiation between above- and below-barrier populations of fish, and to determine the anadromous versus resident parentage of above-barrier individuals.

METHODS

Study site.-This study was conducted in Freshwater Creek, a 4th order coastal stream in northern California (Figure 1). Freshwater Creek was selected for study for several reasons. It offers a barrier to upstream fish passage in the form of a waterfall on its upper mainstem, with a small population of resident rainbow trout above the barrier. The origin of this above-barrier population is not known and there are no known records of stocking. A permanent weir operated by the Humboldt Fish Action Council allows for steelhead escapement to be tracked. Finally, the California Department of Fish Game (CDFG) conducted a full life-cycle salmonid monitoring program within the Freshwater Creek basin and major tributaries during the course of my study. This monitoring effort included the use of downstream migrant traps, electrofishing and snorkel surveys, as well as mobile and stationary antennae for detecting PIT-tagged fish.

Freshwater Creek drains a catchment of 9227 hectares, and empties into Humboldt Bay via the diked estuarine channels of Freshwater and Eureka Slough. Elevations in the watershed range from 823 meters at the headwaters to sea level at the mouth. The mainstem and five major tributaries provide approximately 30 km of habitat for anadromous salmonids (Ricker 2006). The mainstem of Freshwater Creek is approximately 23 km long, of which approximately 16 km supports anadromous salmonids. In the mainstem, upstream salmonid migration is prevented by a barrier waterfall located at 40º 44’ 18.27”N, 124 º 00’ 04.47”W. The waterfall measures 5 m from the waterfall crest to the downstream pool surface at summer base flow, and does not present step pools to allow for fish passage. Several smaller waterfalls lie upstream of this barrier. The land upstream of the waterfalls is undeveloped with access limited to secondary logging roads. Five main tributaries, including McCready Gulch, Little Freshwater, Cloney

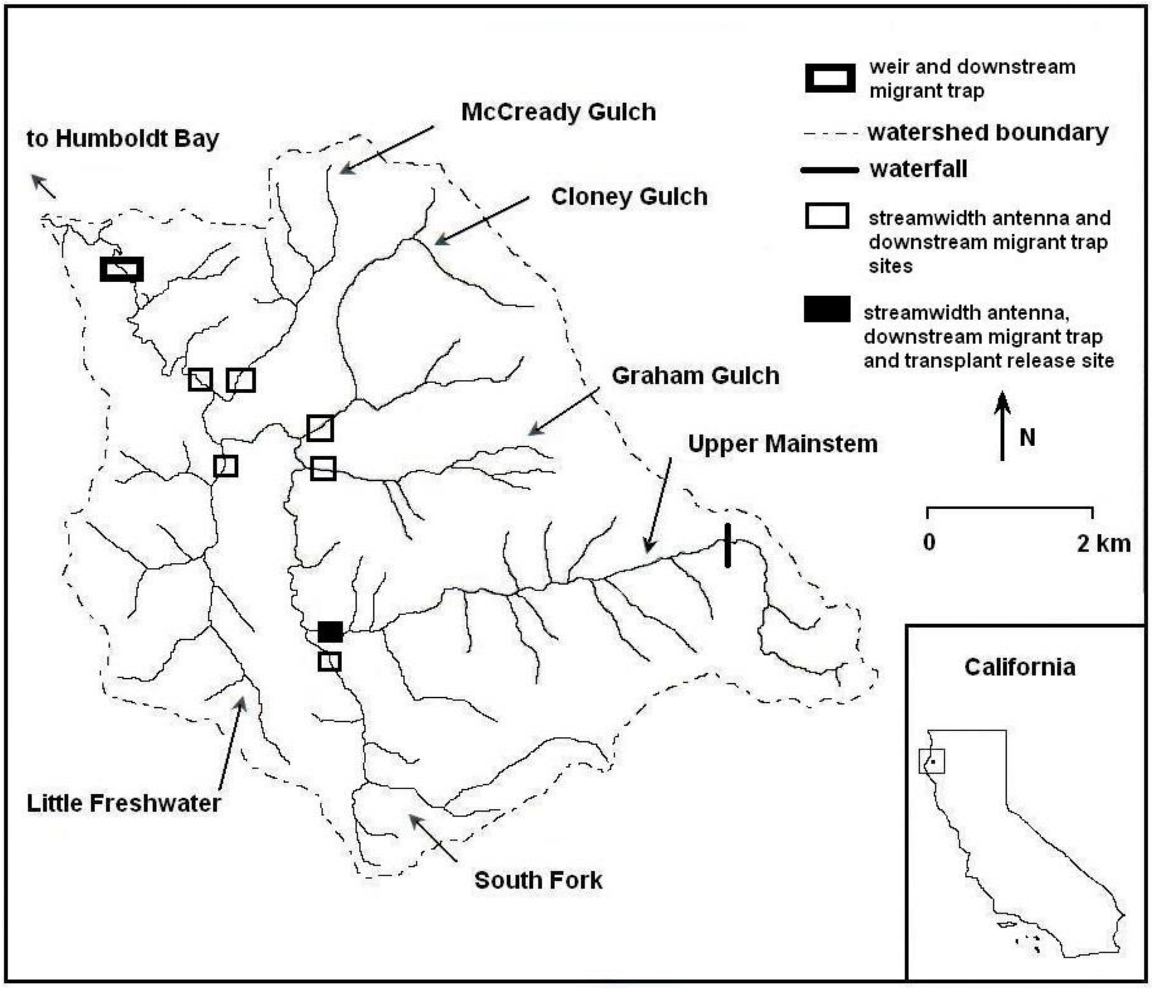

Figure 1. Location of Freshwater Creek (latitude 40°44', longitude 124°02') in northern California (inset) and a watershed map showing the locations of fixed antennae, downstream migrant traps, weir, and anadromous barrier waterfall.

Gulch, Graham Gulch, and South Fork Freshwater each provide an additional 2 to 4 km of habitat for anadromous salmonids (Figure 1).

The release site utilized for transplanted individuals during this study was located within the mainstem in a 100-m reach immediately upstream of the South Fork confluence (Figure 1). The South Fork of Freshwater Creek is the furthest upstream major confluence in the watershed. From this location fish were upstream from all fixed antenna and downstream migrant trap locations, and had an unobstructed pathway to Humboldt Bay and coastal waters. The stream width antenna that was furthest downstream (Howard Heights Road, Figure 1) was 8.5 km below the transplant release reach and within tidally influenced water (Humboldt Bay Watershed Advisory Council 2005).

The climate of the region is characterized as marine west coast with an annual average precipitation of 170–200 cm, most of which falls as rain between November and March. During the study, summer discharge in Freshwater Creek averaged 0.357 $m^3 \cdot s^{-1}$, while winter flows averaged 2.55 $m^3 \cdot s^{-1}$. Winter flows in Freshwater Creek can be highly variable based upon the occurrence of storms. Based on 30 years of data from an adjacent watershed (Bigelow 2003), a storm event with a 50 year recurrence interval occurred during the first winter of study (December 28, 2005, producing a discharge of 15.9 $m^3 \cdot s^{-1}$). The peak flow during the second winter of study had a 10 year recurrence interval. Mainstem flows averaged 1.25 $m^3 \cdot s^{-1}$ in winter during this study. Temperatures in Freshwater Creek ranged between a minimum of 2.9ºC in the winter and a maximum of 19.2ºC during the summer months, with an annual average of 11.0ºC.

Three quarters of the Freshwater Creek watershed is managed for industrial timber production of second growth (~70 year old) redwood (*Sequoia sempervirens*) and Douglas-fir

(*Pseudotsuga menziesii*). The fish assemblage of Freshwater Creek includes Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), coastal rainbow trout/steelhead (*Oncorhynchus mykiss irideus*), coastal cutthtroat trout (*Oncorhynchus clarki clarki*), prickly sculpin (*Cottus asper*), coast-range sculpin (*Cottus aleuticus*), Pacific lamprey (*Entosphenus tridentatus*), brook lamprey (*Lampetra pacifica*) and three spine stickleback (*Gasterosteus aculeatus*).

Experimental Design.-Resident rainbow trout isolated above a waterfall were relocated to a downstream reach that provided access to the Pacific Ocean. Captured fish above the barrier were individually marked using 23 mm passive integrated transponder tags (PIT tags) at each of 3 sampling events. During each sampling event, approximately half of the sample of tagged individuals were randomly selected and released at the location of their capture (total n=66). Half of the sample (total n=65) of captured individuals was transplanted to a downstream reach approximately 5 km downstream of the barrier. Fish in both above- and below- barrier reaches were subsequently recaptured and/or relocated to compare movement and growth patterns.

This experiment was supplemented with genetic analyses to identify pure strain and hybrid (*O. mykiss iredeus* and *O. clarki clarki*) individuals in the above-barrier population. The identification of pure strain *O. mykiss* was to be used in a comparison with pure strain below-barrier *O. mykiss*. Otolith microchemistry analysis was conducted on above-barrier individuals to determine the maternal lineage of the fish.

Fish Capture, Marking and Transplantation.-All procedures on vertebrates were approved by the Humboldt State University Institutional Animal Care and Use Committee (protocol NO. 05106F19-A). Above-barrier fish were sampled using multiple-pass electrofishing within naturally segregated habitat units (i.e plunge pools). A total sample of 131 individuals greater than or equal to 100 mm in fork length were captured during three sampling events conducted during October 2005, July 2006, and October 2006. Fish were collected from the top of the barrier waterfall to approximately 3.5 km upstream. These fish were anesthetized with a solution of tricaine methanesulfonate (MS-222), and a 23 mm PIT tag was implanted into the body cavity of each individual. Coding of tag numbers was coordinated with CDFG to ensure that duplicate numbers were not assigned. Tagged fish were weighed to the nearest .01g, and measured to the nearest millimeter. Scale samples and caudal fin clips were collected from all fish greater than or equal to 100 mm in fork length. Fin clips were air dried and stored in a freezer for a maximum of one year before being analyzed. Tagged fish were allowed to recover in an aerated five gallon bucket for approximately 10 minutes, or until recovery was complete. Individuals less than 100mm in fork length were enumerated and assigned to age categories of 0+ (less than 60mm fork length) or 1+ (greater than 60mm fork length). Age class was estimated using length-frequency data and histograms.

Transplanted individuals were transported in aerated five gallon buckets on the day of capture to the release point in the mainstem, below the barrier. In fall 2005, 22 trout were released below the barrier and 20 were released above the barrier. In the sampling events of 2006, 43 trout were released below- and 44 were released above- the barrier.

Relocation and Recapture of Tagged Individuals.-Several different methods were used to relocate and/or recapture transplanted and non-transplanted individuals. Tagged fish transplanted below the barrier waterfall were detected while operating downstream migrant traps, during juvenile electrofishing and night-dive snorkel surveys, with stationary stream width antennae systems, and with the use of a mobile PIT tag interrogation system. Tagged fish above the barrier waterfall were recaptured by electrofishing efforts conducted in the fall of 2006 and 2007.

Pairs of stream width antennae were established in seven fixed locations in Freshwater Creek, including upper mainstem, South Fork, Graham Gulch, Cloney Gulch, McCready Gulch, Little Freshwater and the lower mainstem (Figure 1). The lower mainstem site is 13 km upstream from the mouth of Freshwater Slough, and occasionally experiences tidal influence (Humboldt Bay Watershed Advisory Council 2005). An antenna consisted of a single loop of braided copper electrical wire formed into a rectangle, with the bottom of the rectangle buried in the substrate and the top of the rectangle positioned above the surface of the stream. Size of the antennae varied with stream width. Two antennae were located at each site to allow direction of tagged fish to be determined and to evaluate capture efficiency. Detection data were recorded onto a battery-powered data logger circuit board from Oregon RFID ™, and records were uploaded weekly to a PDA (Palm Pilot M130™). The antennae were operated continuously by the California Department of Fish and Game during this study, but were sometimes interrupted during storm events. Based on tests with tagged fish, the antenna detection rate was close to 100% when in operation. Stream width antennae were located downstream from all of the downstream migrant traps, as close to the confluence with the mainstem as possible. Tagged fish were also located during a watershed survey (16 km of anadromous mainstem habitat) conducted by California Department of Fish and Game personnel from May-June and again in October of each year using a battery powered, mobile, radio frequency identification (RFID) scanner. The same hardware used for the stationary antennae was fit onto a backpack frame and enclosed within a waterproof housing. The antenna, a 61 cm diameter loop, was attached to a PVC wand approximately two meters long, and had a detection range of approximately one meter. Data collected from this survey included PIT tag number, date, time of detection, location (± 1 meter), habitat type and whether each scanned individual appeared to be alive or dead, based on movement. Upon detection, date, time and PIT tag number were electronically recorded on a PDA (Palm Pilot M130™). An audible "beep" alerted surveyors to the presence of a PIT tag detection, at which point, location, time, habitat type and status (alive or dead) was manually recorded. Manually and electronically collected data were synthesized by time of detection at the completion of each day's survey.

The Anadromous Fish Research and Monitoring Program of the California Department of Fish and Game operated seven downstream migrant traps immediately upstream from stream width antennae sites from March through June of each year (Figure 1). Pipe traps were constructed in each of the five major tributaries, as well as, on the upper mainstem. In the lower mainstem a floating inclined plane trap was deployed. Additionally, a permanent weir located 4 km downstream of the Howard Heights site was retrofitted to operate as another pipe trap for collection of migrating individuals. At each downstream migrant trap captured fish were scanned with handheld RFID readers for the presence of a PIT tag, weighed (± 0.1g), measured (± 1 mm fork length) and scale samples were collected for age and growth analysis. Fish were visually examined and classified as smolt, parr, or resident on the basis of body morphology and skin reflectance (Beeman et al. 1995; Haner et al. 1995). Parr and resident individuals were distinguished primarily on body shape, size and coloration patterns. Parr were individuals showing intermediate morphological characteristics between fry and smolt or mature resident trout, accompanied by distinguished parr marks. Fish were released downstream from each trap after measurements were completed.

Summer juvenile abundance surveys and bi-annual night dives conducted each year by the California Department of Fish and Game provided another opportunity for recapture of tagged fish. Abundance surveys were conducted during this study from August-October using a modified Hankin and Reeves (1988) protocol, as described by Brakensiek and Hankin (2007). This protocol randomly selects habitat units that are snorkeled while a subset of these same units are electrofished, to depletion (multiple pass), as a calibration measure to the snorkel counts. Fish captured during the electrofishing process were scanned for the presence of a PIT tag with a handheld RFID tag unit, weighed (± 0.1g) and measured (± 1 mm in fork length). Location of capture was also recorded. Bi-annual night dive snorkel surveys were conducted in pool habitats on the Upper Mainstem and South Fork of Freshwater Creek from June 10 to July 10 in 2005, from June 10 to July 1 in 2006, and from October 1 to November 1 in 2006. During night dives individuals were captured using dip nets and flashlights. Location of captured individuals was recorded, and fish were weighed (± 0.1g) and measured (± 1 mm in fork length).

Previously tagged individuals above the barrier were recaptured during sampling events conducted from October 2 to October 11 2006, and from June 25 to July 2 2007. Multiple-pass electrofishing was utilized for recapture efforts within the same naturally segregated habitat units that were originally sampled. Captured individuals were scanned for the presence of a PIT tag, weighed (± 0.1g), and measured (± 1 mm in fork length).

Otolith Microchemistry.-Five untagged above-barrier individuals (greater than 100mm fork length) were sacrificed for otolith extraction and analysis. Sagittal otoliths were collected and prepared for analysis following the methods described in Wells et al. (2003). Strontium (Sr) occurs at greater concentrations in seawater than freshwater, and it replaces calcium (Ca) in the calcium carbonate matrix of a fish otolith at levels relative to its environmental concentration (Kalish 1990). Based on an assumption that the Sr/Ca ratio of the otolith primordium reflects the Sr/Ca ratio of the environment in which yolk precursors develop, this ratio can be used to establish whether the maternal origin is anadromous (yolk precursors developing in seawater) or resident (yolk precursors developing in freshwater) (e.g., Rieman et al. 1994; Zimmerman and Reeves 2000). A higher Sr/Ca ratio in the primordial growth region relative to the freshwater growth region suggests an anadromous maternal parent, while the lack of a difference in Sr/Ca ratios between growth regions suggests a resident maternal origin. Ratios were measured at 10 points each within the primordial and freshwater growth regions at the USGS Alaska Science Center. I compared Sr/Ca ratios using paired one-tailed paired *t*-tests with a significance level of $\alpha = 0.05$.

Genetic Analysis.-Fin clips from 18 above-barrier trout were analyzed, using restriction fragment length polymorphism (RFLP) technologies, and insertion/deletion loci to determine if and to what degree hybridization between rainbow trout and cutthroat trout had occurred in the above barrier population. A total of 11 nuclear loci and 1 mitochondrial loci were examined for variation. Insertion/deletion loci were analyzed following the methods described by Ostberg and Rodriguez (2004), whereas RFLP analysis followed the methods described by Baumsteiger et al. (2005).

Nuclear and mitochondrial markers were chosen that were fixed for alternate alleles in coastal cutthroat and steelhead, based on Baumsteiger et al. (2005). The nuclear genes used for RFLP analysis were growth hormone intron D (GH2D), gonadotropin II beta subunit (GTH II β*), gonadotropin releasing hormone (GnRH*), heat shock cognate 71 (hsc 71*), ikaros (IK*), insulin-like growth factor (IGF-2*) and recombination activation gene (RAG 3'*). Differentiation at the p53 locus used by Baumsteiger et al. (2005) proved to be unreliable in differentiating known controls (pure strain *O. mykiss iredeus* and *O. clarki clarki* obtained from the Humboldt State University Fish Hatchery) , and was therefore not used. The mitochondrial gene NADH dehydrogenase-1 (ND-1) was used to differentiate steelhead and coastal cutthroat trout mitochondrial DNA haplotypes. Mitochondrial DNA is maternally inherited and can therefore be used to determine the maternal lineage of hybrid individuals. In addition to the eight markers used by Baumsteiger et al (2005), I also used the markers Occ-42 (*O. clarki clarki*-42), OM-47 (*O. mykiss*-47), Occ-35 and Occ-38 (Ostberg and Rodriguez 2004), with both forward and reverse primers. These loci represent insertion/deletion locations and do not require cutting with restriction enzymes before genotyping. Analyses were conducted at the Fisheries Genetics Laboratory at Humboldt State University. Electrophoresis was performed on each loci/marker combination to determine genotype. The number of cutthroat alleles was then plotted in a histogram to show levels of hybridization.

Growth Analysis.-Length-weight regressions were compared-between rainbow trout populations in the above- and below-barrier populations by covariance analysis to determine whether above- and below-barrier fish differed in growth. Because many of the trout scales lacked a detectable first-year annulus, I was unable to compare growth through analysis of fish length or mass at age. Individuals judged in the field to be pre-smolts or smolts were excluded from the datasets. Transplanted trout recaptured in subsequent years were also excluded from comparison on the basis of potentially unequal growth opportunities to those of the established resident population.

Movement Analysis.-Movement was graphically examined to determine direction and distances traveled by both transplanted trout and prior occupants of the below-barrier population. Distance was measured as the distance from the transplant release location (for transplanted trout) or the site of tagging (for below-barrier trout) to the location of last detection. Migration behaviors were examined between the transplant group and below-barrier trout. Below-barrier trout that were included in the comparison consisted of tagged trout greater than 100mm in fork length captured within a 3300m reach (100m downstream and 3200m upstream of transplant site) of river that encompassed the transplant location. Below-barrier individuals of greater than 100mm fork length were chosen for comparison due to their indeterminate life history and proximity of size to the transplant group. Distance traveled was determined by a combination of stationary and mobile antennae reads, and downstream migrant trap captures. Distance traveled was expressed as the distance from the transplant or tagging location to the last point of detection or capture.

RESULTS

Above-barrier population description.-All fish captured above the barrier waterfall were field identified as being rainbow trout. No other field-identified species of fish were encountered during surveys conducted above the barrier. Of 364 individuals captured above the barrier 32 were determined to be young-of -the year (Figure 2). The above-barrier population consisted primarily of age 1+ resident fish occupying high gradient, segmented, pool habitats within a confined channel (i.e. plunge pools). No more than 3 individuals were captured in any habitat unit. The mode of the capture dataset was 1 individual captured per habitat unit with various units producing zero individuals. Many of the habitat units which did not produce fish were occupied by large (>200mm) Pacific Giant Salamanders (*Dicamptodon tenebrosus*). Transplanted individuals varied in size at capture from 100mm-226mm in fork length. The randomly chosen transplant group mimicked the natural above-barrier population in size frequency.

The age and size structure of the above-barrier population differed greatly from that of the below-barrier population into which they were transplanted (Figure 3). The below-barrier population dataset was compiled from surveys conducted in the upper mainstem reach nearest to the transplant location. The above-barrier population was dominated by 1+ and older resident trout, while the below-barrier assemblage consisted primarily of young-of-the-year anadromous progeny of steelhead, cutthroat trout, and coho salmon.

Otolith microchemistry showed that Sr/Ca ratios did not significantly differ between the two growth regions suggesting that all individuals were derived from a freshwater (resident) maternal parentage (Table 1). Genetic analysis of 16 above-barrier rainbow trout indicated that

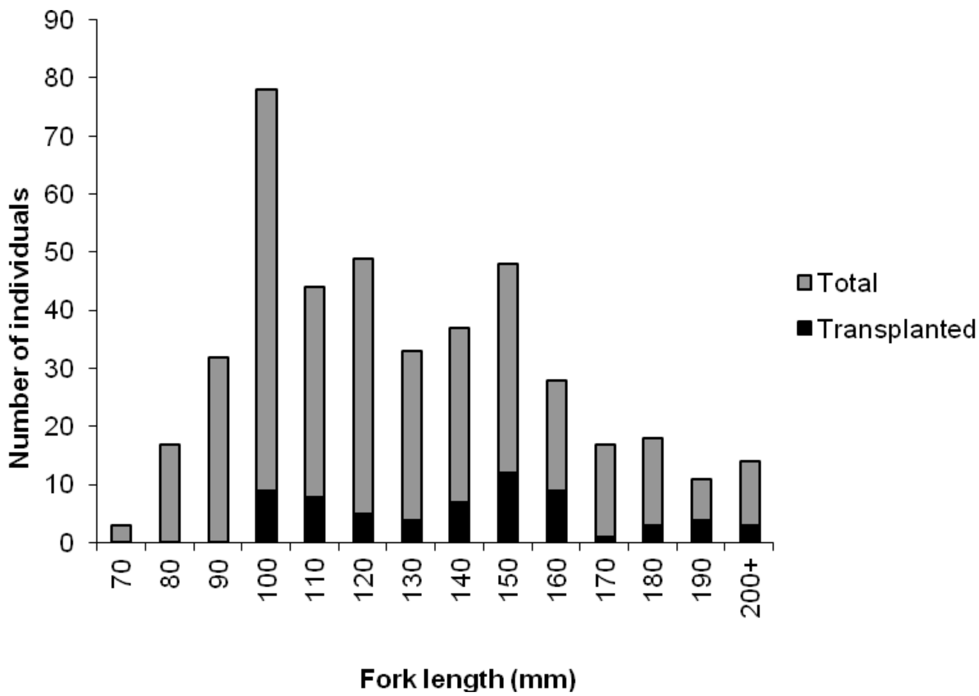

Figure 2. Size frequency relationships for the above-barrier population of rainbow trout in Freshwater Creek, CA, and for the sample of above-barrier individuals (n = 65) that were transplanted below the waterfall. Fish were captured above the waterfall during four sampling events over the period of study from October 2005 to July 2007.

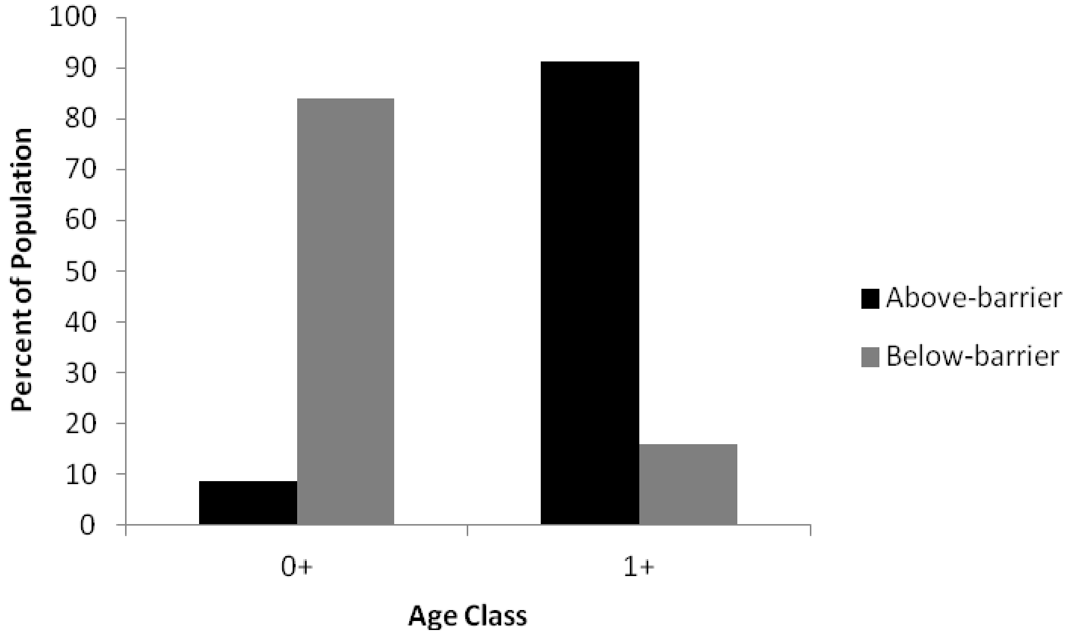

Figure 3. Age class structure of trout above the barrier waterfall (n = 364) and below the waterfall (n = 1274) in Freshwater Creek, CA. Age class was estimated from length-frequency distributions. Age 1+ individuals were greater than 60 mm in fork length with individuals less than 60 mm being categorized as 0+.

Table 1. Strontium/Calcium (Sr/Ca) ratios in the primordial and freshwater growth regions of otoliths (n=5) collected from above-barrier trout in Freshwater Creek, CA. Means and standard deviations of five otoliths examined at 10 points in each growth region. One-tailed paired *t*-tests were used to test the hypothesis that Sr/Ca is greater in the primordial than in the freshwater region (df=4). $P > 0.17$ suggests resident rather than anadromous maternal parentage.

Otolith	SR/CA Primordial Region Mean(standard deviation)	SR/CA Freshwater Region Mean(standard deviation)	*P*-value
1	$.0013(6.85x10^{-5})$	$.0012(8.78x10^{-5})$	.443
2	$.0011(1.1x10^{-4})$	$.00096(8.20x10^{-5})$	.496
3	$.0011(7.77x10^{-5})$	$.0011(8.82x10^{-5})$	.419
4	$.0011(8.71x10^{-5})$	$.0012(1.30x10^{-4})$	.486
5	$.0013(8.88x10^{-5})$	$.0011(3.43x10^{-5})$	.499

all individuals showed some degree of introgression with coastal cutthroat trout. The mitochondrial ND-1 marker showed all of the genotyped individuals to be the progeny of rainbow trout females. The above-barrier population was composed entirely of backcrossed hybrids and no pure rainbow trout, coastal cutthroat trout, or putative F1 hybrids. All hybrids were backcrossed towards rainbow trout containing one to seven cutthroat trout alleles (Figure 4). Previous genotyping studies in the below-barrier population have shown the majority (84.6%) of hybrids to backcross towards coastal cutthroat trout (Voight et al. 2008).

Movement.-Movement of transplanted fish varied considerably among individuals (Figure 5). More than two-thirds of the trout that were transplanted below the barrier to anadromy were located or recaptured during the two-year study (n = 44 of 65 transplanted fish). Stationary antennae and mobile PIT tag units provided the majority of recapture points and were approximately equally effective in detecting tagged fish with 27 and 30 individuals detected respectively (Appendices A, B, and C). Downstream migrant traps captured seven tagged trout during the two-year study period (Appendix D). Tagged trout were not detected in summer juvenile abundance surveys or during fall and summer night dive surveys.

The percentage of transplanted trout (6%, or 4 of 65 transplants) that moved into tidally influenced water did not appear to be different from the percentage of prior downstream residents (4%, or 9 of 210 individuals greater than 100mm in fork length) that were captured and tagged in the vicinity of the transplant release location and later captured in tidally influenced water. The majority of the detected individuals (n = 26) remained within 500 m of the release location in all sightings. Upstream movement was observed in four of the transplants, with two individuals last detected within 300 m of the waterfall, approximately 4.5 km from the release

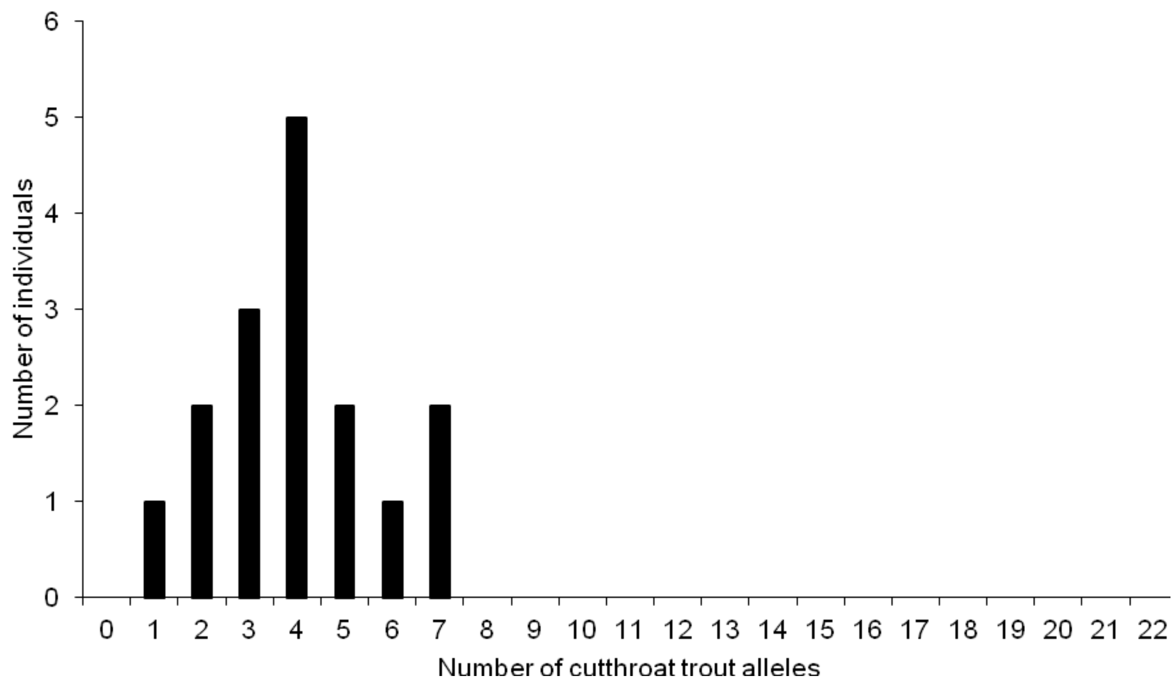

Figure 4. Allelic frequency of 18 above-barrier individuals in Freshwater Creek, CA at 11 differentiating loci, with zero alleles representing pure strain rainbow trout, 22 representing a pure strain coastal cutthroat trout, and 11 alleles representing a putative F1 hybrid.

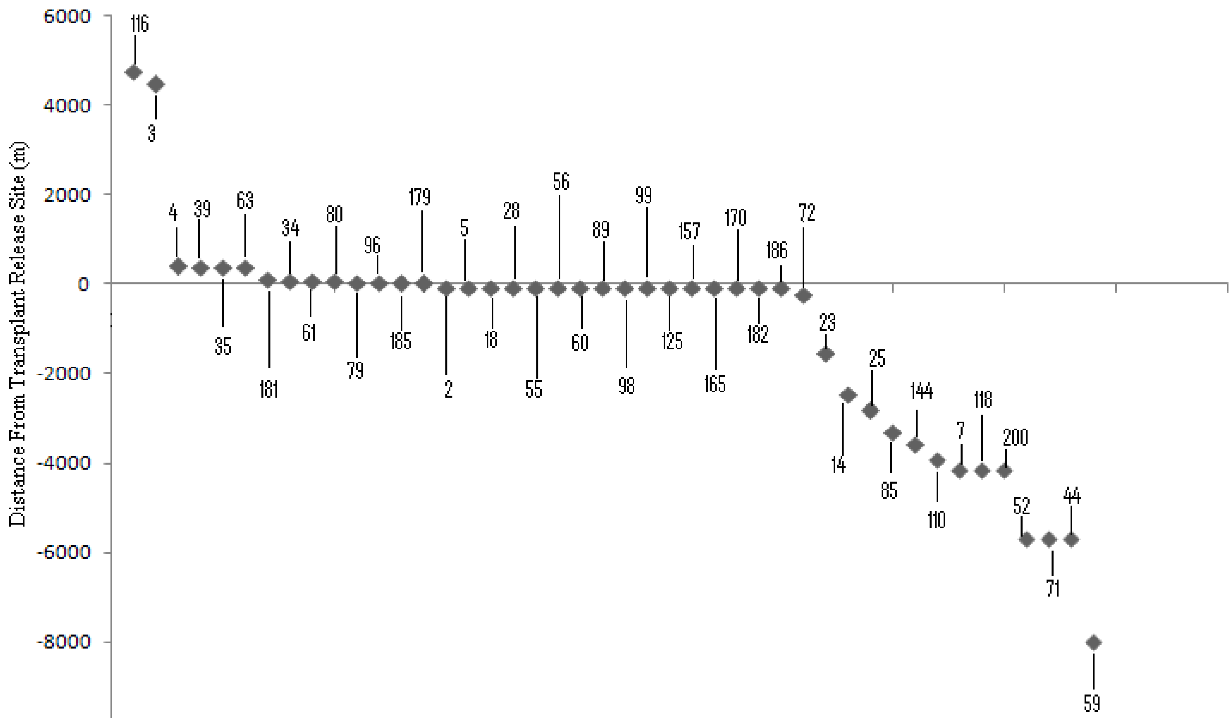

Figure 5. Distance moved (in meters) by transplanted rainbow trout in Freshwater Creek, CA from release point below the waterfall to last known live location. Negative numbers represent downstream movement while positive numbers represent upstream movement from the transplant release point. Each point represents one transplanted individual identified by the final digit(s) of its PIT tag number (Appendix A). Individuals that passed 5860 m downstream from the release point were last located in tidal water.

location. Individuals transplanted below the barrier waterfall were never detected above the waterfall. Eight individuals were found to have traveled in a downstream direction while remaining in freshwater. Of these eight individuals, only one entered and remained in a tributary while the remaining seven inhabited the mainstem. In a five-day period, a single individual (tag number 71) traveled over six-km downstream, entering three separate tributaries before returning to the mainstem, whereupon it entered tidally influenced water and returned within five hours to the non-tidal mainstem. Tag number 71 was never detected after these movements and presumably remained in the lower reaches of the system. Four of the transplanted individuals traveled downstream from the release location and were last detected in tidally influenced waters. All individuals last detected in tidal water moved there within one year of their release below the waterfall. Three of these four individuals were last detected in tidal waters during the spring while the fourth individual was last detected in tidal water immediately preceding a storm event in December of 2006. Capture of transplanted trout in downstream migrant traps allowed assessment of smolting status. Of the five transplanted trout that were captured, one was determined to have smolted (Figure 6), and a second was determined to be a pre-smolt.

The size of individuals at the time of transplantation did not appear to affect migratory behavior. The average length of transplanted individuals that assumed seaward migration was 143 mm in fork length (n=4, range 125mm-185mm) whereas the average size of individuals who remained within 5km of the transplant location was 146mm in fork length (n=38, range of 104mm-226mm).

Five tags that were released above the barrier were found below the barrier in Freshwater Creek. Two of these individuals were recaptured in the downstream migrant trap on the lower mainstem, where it was determined that one had smolted, and one had not. Two tags from

Figure 6. Top: Photo of above-barrier trout at time of initial capture above the waterfall on Freshwater Creek, CA; Bottom: Photo of a transplanted trout (# 44, Appendix D) recaptured in a downstream migrant trap 183 days following its release. At release, the fish measured 185 mm in fork length and weighed 82 g. At recapture, the fish increased 7 mm in fork length, and lost 12 g in weight.

individuals from the above-barrier population were discovered near the base of the waterfall, presumably shed tags or washed over mortalities. All tags from the above-barrier release group that were found below the barrier were from individuals captured within 1000m upstream of the waterfall.

Growth.-. Growth potential/body morphology did not appear to differ between populations of above- and below-barrier populations for each year of study (2006 and 2007). Length-weight regressions of above- and below- barrier fish did not differ in 2006 ($p=.514$, Figure 7) or in 2007 ($p=.355$, Figure 8).

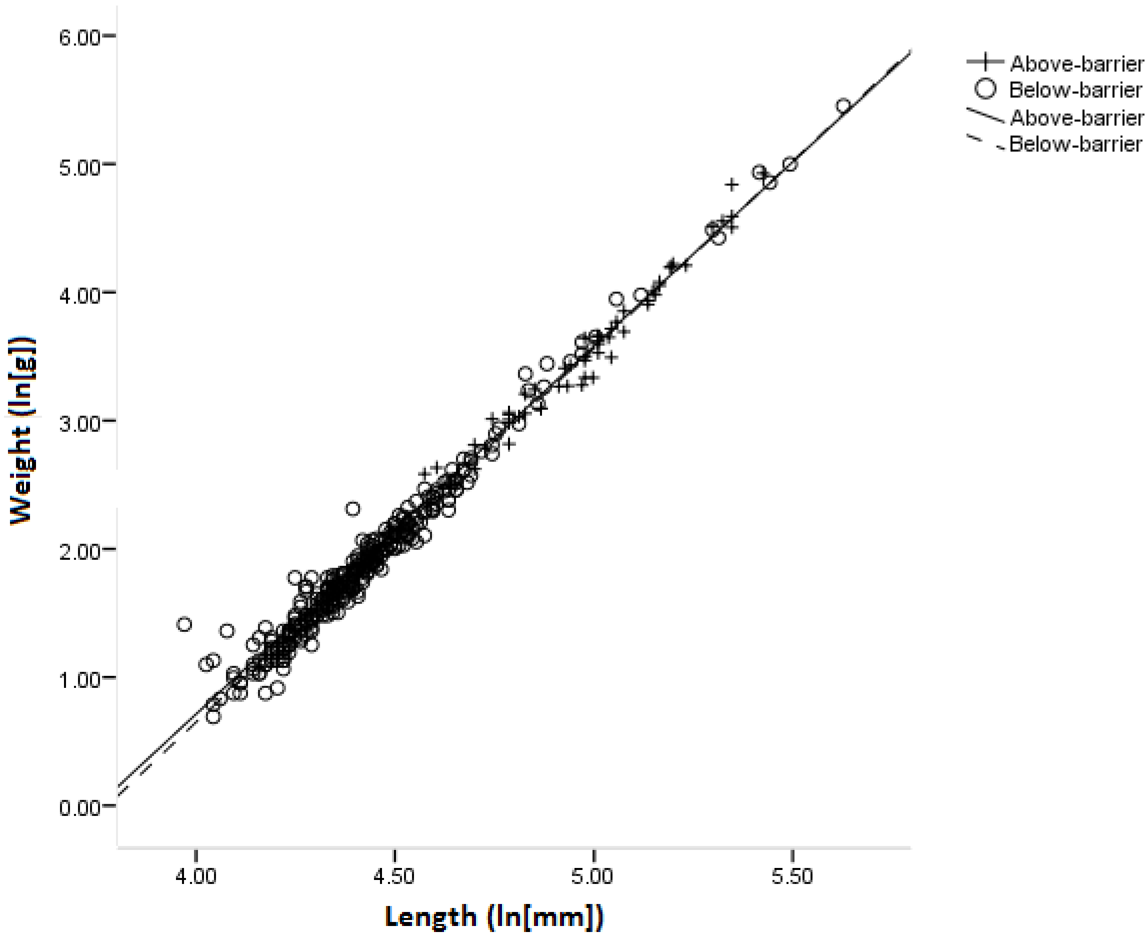

Figure 7. Natural logarithms of length-weight relationships of above- and below-barrier rainbow trout in Freshwater Creek, CA in 2006.

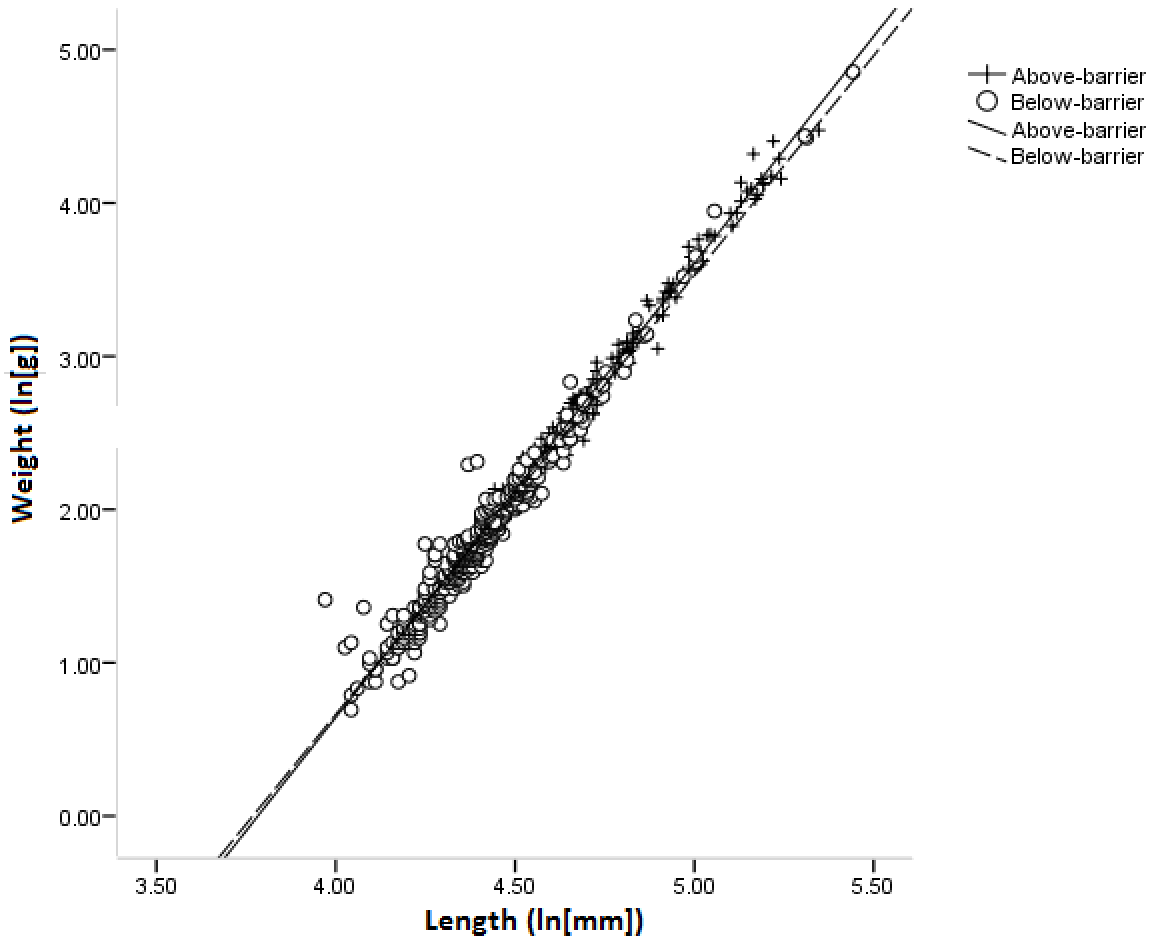

Figure 8. Natural logarithms of length-weight relationships of above- and below-barrier rainbow trout in Freshwater Creek, CA in 2007.

DISCUSSION

Movement and behaviors displayed by transplanted fish in this study may have been biased by the act of transplantation, as the below-barrier habitat may have been saturated with fish. Above-barrier residents enjoyed lower than equilibrium density with the removal of transplants. Prior to transplanting fish, density in the below-barrier population was much higher than the density of the above-barrier population. A reciprocal transplant of below-barrier trout to above-barrier habitat would have completed the study design and strengthened the results, but permission for a reciprocal transplant was not obtained. Although the downstream salmonid assemblage includes three other salmonid species that may compete for the same resources, similarity of trout length-weight relationships between above- and below-barrier reaches suggests a similarity in the availability and utilization of resources of above- and below-barrier reaches was similar.

This study provided no direct evidence that any of the transplanted individuals successfully migrated to the Pacific Ocean. The weir/downstream migrant trap was located within the tidal region where a salt wedge occasionally reaches the weir fence. Downstream of the weir the riverine habitat transitions to an estuarine slough. Due to low ocean survival rates (Quinn 2005) and the relatively small sample size of this study it is not anticipated that any returning fish will be recaptured at the weir as adult steelhead. Therefore, confirmation of successful survivorship to escapement of those individuals last encountered in tidewater is not likely. The highly variable movements and behaviors displayed by transplanted individuals speak to the plasticity and adaptability of rainbow trout to changing environments and conditions.

Morphological changes associated with smolting were directly observed in two individuals over the two years this study was conducted. One of the individuals was from the transplant group while the other was released above the barrier waterfall. The lengths of the two smolting individuals in this study (192 and 203mm in fork length) were larger than the median fork length range (81mm-146mm) for smolting steelhead reported by Ricker (2006). This demonstration of smolting and apparent migratory behavior demonstrates the potential for previously resident trout to adapt an anadromous life history.

The detection of above-barrier individuals volitionally migrating below the barrier demonstrates the potential for gene flow from an above-barrier population into downstream reaches. This finding contradicts that of Deiner et al. (2007) who found through genetic analysis that trout populations above and below barriers in the same tributary were not significantly interbreeding. Girman and Garza (2006) did find that resident and anadromous forms from the same river were genetically more similar to each than their respective forms in neighboring basins. Further support of life history interbreeding is provided by Heath et al. (2008), who found sympatric populations of resident and anadromous *O. mykiss* were not genetically differentiated in four of five study drainages. This suggests some level of interbreeding between life history morphologies does take place. However, Pearse et al. (2009) suggest that washed over above-barrier trout form a below-barrier subpopulation and remain reproductively isolated from the now sympatric anadromous population. Seamons et al. (2004) and McMillan et al. (2007) documented mature resident males (“sneakers”) mating with adult female steelhead providing gene flow between life histories. Planned genetic comparisons of the above and below barrier populations were abandoned when pure strain rainbow trout were not identified.

All 18 genotyped individuals in this study showed some level of introgressive hybridization between rainbow trout and coastal cutthroat trout. Through the use of maximum likelihood and maximum parsimony analyses, Crespi and Fulton (2004) determined it is likely that these two species are allopatric sister taxa that came into secondary contact. Among the factors potentially contributing to the threatened status of steelhead in northern California is hybridization with coastal cutthroat trout. Currently, these two species are sympatric in most coastal streams from northern California to Alaska and recent studies have documented numerous cases of hybridization (Campton and Utter 1985; Young et al. 2001; Baker et al. 2002; Docker and Heath 2003; Ostberg et al. 2004). However, the exact extent of hybridization between these two species is not known. Hybridization is of concern because it can result in the loss of unique gene pools and threaten long-term persistence of a species. Recent studies have documented deleterious effects of hybridization on the genetic integrity of inland subspecies of cutthroat (*Oncorhynchus clarki lewisi*) and rainbow trout (Allendorf et al. 2001; Hitt et al. 2003; Rubridge and Taylor 2004). Hybrid individuals display different morphological characteristics which affect the length weight relationships used to determine growth potential. A difference in growth potential between above- and below-barrier populations was not detected in this study. As yet, effect of this hybridization on life history expression and migratory behavior in salmonid fishes has not been studied.

Genotyped individuals from this study were shown to backcross towards *O. mykiss*. This finding is in direct contradiction to the findings of Voight et al. (2008) who found genotyped individuals in the below-barrier population to backcross towards *O. clarki*. It is likely that the above-barrier population structure is indicative of a hybrid swarm, as defined by Harrison (1993), consisting of an initial population with a higher proportion of rainbow trout individuals.

The downstream waterfall created an isolated population with a one way barrier to gene flow in which no new individuals could enter the breading population. With no known stockings or plantings of new individuals the populations interbred in a genetic ratio reflective of the founding population (i.e. higher initial proportion of *O.mykiss*).

Rainbow trout in California exhibit numerous life histories, varying from anadromous to resident as well as “half pounders” (Shapovolov and Taft 1954). Shapovolov and Taft (1954) also described a life history in which individuals reached sexual maturity in freshwater prior to smolting. Whether individuals in this study reached sexual maturity prior to the act of transplantation is unknown. It appears that some of the variability in life history can be attributed to phenotypic plasticity and some of it may be heritable. Smolting individuals were derived through the crossing of two lake bound resident trout after 70 years of isolation from anadromous water (Thrower et al. 2004). This study showed that the genetic potential for smolting may lie dormant or be expressed under a variety of environmental conditions and at various life stages. Zimmerman and Reeves (2000) found anadromous fish whose maternal parent was resident and resident fish whose maternal parent was anadromous in a British Columbia drainage, showing that parental lineage does not necessarily determine the life history traits of their progeny. Furthermore, McPhee et al. (2007) found, through analysis of microsatellite variation, there to be no evidence of genetic divergence in resident and anadromous populations of *O. mykiss* in Russia’s Sopochnaya River. In Argentina, a population of anadromous steelhead was established from an initial stocking of resident rainbow trout (Pascual et al. 2001); however, it is unknown whether the original stock was derived from an anadromous or resident lineage (Behnke 2002). This study adds to the body of knowledge supporting the plasticity of life history expression in rainbow trout.

The similarity in movements observed in the transplant group and the below-barrier population, the smoltification of transplanted individuals, and the leakage of above-barrier fish downstream suggests the potential for resident trout to exhibit migratory behavior and contribute to breeding populations of steelhead. These findings have important conservation and management implications. Currently, under the U.S. Endangered Species Act resident populations of rainbow trout are considered separate from anadromous steelhead and are therefore not provided the same level of protection (National Marine Fisheries Service 2005). Based on our results these decisions appear to be misguided in the case of steelhead / rainbow trout in Freshwater Creek.

REFERENCES

Allendorf, F.W., R.F. Leary, N.P. Hitt, K.L. Knudsen, L.L. Lundquist, and P. Spruell. 2004. Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? Conservation Biology. 18:1203-1213.

Araki, H., William R. Ardren, Erik Olsen, Becky Cooper, and Michael S. Blouin. 2007. Reproductive success of captive-bred steelhead trout in the wild: Evaluation of three hatchery programs in the Hood river. Conservation Biology. 21: 181-910.

Baker, J., P. Bentzen, and P. Moran. 2002. Molecular markers distinguish coastal cutthroat trout from coastal rainbow trout/steelhead and their hybrids. Transactions of the American Fisheries Society. 131:404-417.

Baumsteiger, J., Hankin, D., Loudenslager, E.J. 2005. Genetic analyses of juvenile steelhead, coastal cutthroat trout, and their hybrids differ substantially from field identifications. Transactions of the American Fisheries Society. 134: 829-840.

Beeman, J. W., D. W. Rondorf, M.E. Tilson, and D. A. Venditti. 1995. A non-lethal measure of smolt status of juvenile steelhead based on body morphology. Transactions of the American Fisheries Society. 124: 764-769.

Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society Monograph 6. Bethesda, Maryland.

Behnke, R.J. 2002. Comment: First Documented Case of Anadromy in a Population of introduced Rainbow Trout in Patagonia, Argentina. Transactions of the American Fisheries Society. 131: 582-585

Bigelow, P.E. 2003. Scour, fill, and salmon spawning in a northern California coastal stream. M.S. Thesis, Humboldt State University, Arcata, California.

Brakensiek, K.E. and D.G. Hankin. 2007. Estimating overwinter survival of juvenile Coho salmon in a northern California stream: Accounting for effects of passive integrated transponder tagging mortality and size-dependent survival. Transactions of the American Fisheries Society. 136: 1423-1427.

Busby, P.J., T.C. Wainwright, G.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz, and I.V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon and California. NOAA Technical Memorandum NMFS-NWFSC-27.

Campton, D.E. and F.M. Utter. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. Canadian Journal of Fisheries and Aquatic Sciences 42:110-119.

Crespi, B.J., and M.J. Fulton. 2004. Molecular systematics of salmonidae: combined nuclear data yields a robust phylogeny. Molecular Phylogenetics and Evolution 31:658-679.

Deiner, K., J.C. Garza, R. Coey, and D.J. Girman. 2007. Population structure and genetic diversity of trout (Oncorhynchus mykiss) above and below natural and man-made barriers in the Russian River, California. Conservation Genetics 8:437–454.

Docker, A. Dale and D.D. Heath. 2003. Erosion of interspecific reproductive barriers resulting from hatchery supplementation of rainbow trout sympatric with cutthroat trout. Molecular Ecology. 12:3515-3521.

Girman, D. and J. C. Garza. 2006. Population structure and ancestry of O. mykiss populations in south-central California based on genetic analysis of microsatellite data. Final Report. Prepared for California Department of Fish and Game Project No. P0350021 and Pacific States Marine Fisheries Contract No. AWIP-S-1.

Haner, P. V., J. C. Faler, R. M. Schrock, D. W. Rondorf,and A. G. Maule. 1995. Skin reflectance as a nonlethal measure of smoltification for juvenile salmonids. North American Journal of Fisheries Management. 15:814–822.

Hankin, D. G., and G. H. Reeves. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. Canadian Journal of Fisheries and Aquatic Sciences. 45:834–844.

Harrison R.G. 1993. Hybrids and Hybrid Zones: Historical Perceptive. Oxford University Press, New York.

Heath, D. D., C.M. Bettles, S. Jamieson, I. Stasiak, and M.F. Docker. 2008. Genetic differentiation among sympatric migratory and resident life history forms of rainbow trout in British Columbia. Transactions of the American Fisheries Society. 137: 1268-1277.

Hitt, N.P., C.A. Frissell, C.C. Muhlfeld, and F.W. Allendorf. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi* and nonnative rainbow trout, *Oncorhynchus mykiss*. Canadian Journal of Fisheries and Aquatic Sciences. 60:1440-1451.

Humboldt Bay Watershed Advisory Committee. 2005. Humboldt Bay Watershed and Salmon and Steelhead Conservation Plan. Prepared for California Department of Fish and Game and the California Coastal Conservancy by the Humboldt Bay Watershed Advisory Committee and the Natural Resources Services Division of Redwood Community Action Agency. 232 pp.

Jonsson, B. and N. Jonsson. 1993. Partial migration: niche shift versus sexual maturation in fishes. Reviews in Fish Biology and Fisheries 3: 348-365.

Kalish, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fisheries Bulletin U.S. 88: 657–666.

McMillan, J.R., S.L. Katz, and G.R. Pess. 2007. Observational evidence of spatial and temporal structure in a sympatric Anadromous (winter steelhead) and resident rainbow trout mating system on the Olympic Peninsula, Washington. Transactions of the American Fisheries Society. 136: 736–748.

McPhee, M.V. F. Utter, J.A. Stanford, K.V. Kuzishchin, K.A. Savvaitova, D.S. Pavlov and F.W. Allendorf. 2007. Population structure and partial anadromy in *Oncorhynchus mykiss* from Kamchatka: relevance for conservation strategies around the Pacific Rim. Ecology of Freshwater Fish. 16: 539-547.

Narum, S.R., C. Contor, A. Talbot, M.S. Powell. 2004. Genetic divergence of sympatric resident and anadromous *Oncorhynchus mykiss* in the Walla Walla River, U.S.A. Journal of Fish Biology. 65: 471-488.

National Marine Fisheries Service. 2005. Endangered and threatened species: request for comments on alternative approach to delineating 10 evolutionarily significant units of west coast Oncorhynchus mykiss. Federal Register 70: 67130–67134.

Neave, F. 1944. Racial characteristics and migratory habits in *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada. 6: 245-251.

Olsen J.B., Wuttig, K., Fleming, D., Kretschmer, E.J., and Wenburg, J.K. 2006. Evidence of partial anadromy and resident form dispersal bias on a fine scale in populations of *Oncorhynchus mykiss*. Conservation Genetics. 7: 53-67.

Ostberg, C.O., and R.J. Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. Molecular Ecology Notes 4:26-29.

Ostberg, C.O., S.L. Slatton and R.J. Rodriguez. 2004. Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*) and interspecific hybrids. Molecular Ecology. 13:2773-2778.

Pascual, M., P. Bentzen, C.R. Rossi, G. Mackay, M.T. Kinnison, and R. Walker. 2001. First documented case of anadromy in a population of introduced rainbow trout in Patagonia, Argentina. Transactions of American Fisheries Society. 130:53-67.

Pearse, D.E., S.A. Hayes, M.H. Bond, C.V. Hanson, E.C. Anderson, R.B. MacFarlane, and J.C. Garza. 2009. Over the Falls? Rapid Evolution of Ecotypic Differentiation in Steelhead/Rainbow Trout (*Oncorhynchus mykiss*). Journal of Heredity. 100(5):515-525.

Pereira, D.L., and Adelman, I.R. 1985. Interactions of temperature, size, and photoperiod on growth and smoltification of Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture. 46: 185-192.

Quinn, T.P. 2005. The behavior and ecology of Pacific salmon and trout. The University of Washington Press, Seattle. 378 p.

Ricker, S. 2006. Results of Juvenile Salmonid Downstream Migrant Trapping Conducted on Freshwater Creek, 2005. Scientific report prepared in partial fulfillment of California Adaptive Watershed Improvement. Anadromous Fisheries Resource Assessment and Monitoring Program. California Department of Fish and Game. Northern California – North Coast Region.

Rieman, B.E., Myers, D.L, and Nielsen, R.L. 1994. Use of otolith microchemistry to discriminate Oncorhynchus nerka of resident and anadromous origin. Canadian Journal of Fisheries and Aquatic Science. 51: 68–77.

Rubridge, E.M. and E.B. Taylor. 2004. Hybrid zone structure and the potentialrole of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). Molecular Ecology. 13:3735-3749.

Seamons, T.R., Bentzen, P. and Quinn, T.P. 2004. The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. Environmental Biology of Fish. 69: 333-344.

Shapovalov, L., and A.C. Taft. 1954. The life histories of the steelhead rainbow trout (*salmo gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Wadell Creek, California, and recommendations regarding their management. California Department of Fish and Game Fisheries Bulletin 98: 375 pages.

Thrower, F.P., Hard, J.J., and Joyce, J.E. 2004. Genetic architecture of growth and early life-history transitions in anadromous and derived freshwater populations of steelhead. Journal of Fish Biology. 65: 286-307.

U.S. Office of the Federal Register. 1997. Endangered and threatened species: listing of several evolutionarily significant units (ESUs) of west coast steelhead. Federal Register 62: 159 (18 August 1997): 43937-43954.

U.S. Office of the Federal Register. 2000. Endangered and threatened species: threatened status for one steelhead evolutionarily significant unit (ESU) in California. Federal Register 65: 110 (16 September 1999): 50393-50415.

Voight, H.N., D.G. Hankin, and E.J. Loudenslager. 2008. Errors in visual identifications of juvenile steelhead, coastal cutthroat trout, and their hybrids. Pages 92-93 *in* P.J. Connolly, T.H. Williams, and R.E. Gresswell, editors. The 2005 coastal cutthroat trout symposium: status, management, biology, and conservation. Oregon Chapter, American Fisheries Society, Portland.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, C.M. Jones. 2003. Relationships between water, otolith, scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society. 132(3): pp. 409-424.

Young, W.P., C.O. Ostberg, P. Keim, G.H. Thorgaard. 2001. Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*). Molecular Ecology 10:921-930.

Zaugg, B.S., and Wagner, H.H. 1973 Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comparative Biochemistry and Physiology, Part B. 45: 955-965.

Zimmerman, C.E., and Reeves, G.H. 2000. Population structure of sympatric anadromous and non-anadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences. 57: 2152-2162.

APPENDIX A: Identity, transplant status (yes or no), and subsequent detection of tagged above-barrier rainbow trout in below-barrier stationary or portable PIT tag interrogation sytems or capture in downstream migrant traps during the 2 years of study in Freshwater Creek, CA. PIT # gives the final digit(s) of the PIT tag code. Tagged trout that were not transplanted were released at their site of capture in the above-barrier reach. For each detection type, data are coded as 0 when an individual was not detected and 1 when the individual was detected one or more times during the study.

PIT #	Transplanted (Y/N)	Stationary antennae	Mobile PIT tag reader	Downstream migrant trap
1	N	0	0	0
2	Y	1	0	0
3	Y	0	0	0
4	Y	0	1	0
5	Y	1	0	0
6	Y	0	0	1
7	Y	1	0	0
8	N	0	0	0
9	Y	0	0	0
10	N	0	0	0
11	N	0	0	0
12	Y	0	0	0
13	Y	0	0	0
14	Y	1	1	0
15	N	0	0	0
16	N	0	0	0
17	Y	0	0	0
18	Y	1	0	0
19	N	0	0	0
20	N	0	0	0
21	N	0	0	0
22	N	0	0	0
23	Y	0	1	1
24	N	0	0	0
25	Y	1	0	0
26	Y	0	0	0
27	N	0	0	0
28	Y	1	1	0
29	N	0	0	0
30	N	0	0	0
31	Y	0	0	0
32	N	0	0	0
34	Y	0	1	0
35	Y	0	1	0
37	N	0	0	0

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PIT #	Transplanted (Y/N)	Stationary antennae	Mobile PIT tag reader	Downstream migrant trap
38	N	0	0	1
39	Y	0	1	0
40	N	0	0	0
41	N	0	0	0
42	N	0	0	0
43	N	0	0	0
44	Y	0	0	1
45	N	0	0	0
46	Y	0	0	1
47	Y	0	0	0
48	N	0	0	0
49	N	0	0	0
50	N	0	0	0
51	N	0	0	0
52	Y	1	1	0
53	Y	0	0	0
55	Y	1	0	0
56	Y	1	0	0
57	Y	0	0	0
58	N	0	0	0
59	N	0	0	1
60	Y	1	1	0
61	Y	1	1	0
62	N	0	0	0
63	Y	0	1	0
64	Y	0	0	0
65	N	0	0	0
66	N	0	0	0
67	Y	0	0	0
68	N	0	0	0
69	Y	0	0	0
70	N	0	0	0
71	Y	1	1	0
72	Y	1	1	0
73	N	0	0	0
74	N	0	0	0

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PIT #	Transplanted (Y/N)	Stationary antennae	Mobile PIT tag reader	Downstream migrant trap
75	Y	0	0	0
76	N	0	0	0
77	N	0	0	0
78	N	0	0	0
79	Y	0	1	0
80	Y	0	1	0
81	Y	0	0	0
82	N	0	0	0
83	Y	0	0	0
84	N	0	0	0
85	Y	0	1	0
86	N	0	0	0
87	N	0	0	0
88	Y	0	0	0
89	Y	1	0	0
90	N	0	0	0
91	N	0	0	0
92	N	0	0	0
93	N	0	0	0
94	N	0	0	0
95	N	0	0	0
96	Y	0	1	0
97	Y	0	0	0
98	Y	1	1	0
99	Y	1	0	0
100	Y	0	0	0
107	N	0	0	0
110	Y	0	1	0
114	N	0	0	0
116	Y	0	0	0
117	N	0	0	0
118	Y	1	1	0
119	N	0	0	0
120	N	0	0	0
121	N	0	0	0
123	N	0	0	0

APPENDIX A: Identity, transplant status (yes or no), and subsequent detection of tagged above-barrier rainbow trout in below-barrier stationary or portable PIT tag interrogation sytems or capture in downstream migrant traps during the 2 years of study in Freshwater Creek, CA. PIT # gives the final digit(s) of the PIT tag code. Tagged trout that were not transplanted were released at their site of capture in the above-barrier reach. For each detection type, data are coded as 0 when an individual was not detected and 1 when the individual was detected one or more times during the study (continued).

PIT #	Transplanted (Y/N)	Stationary antennae	Mobile PIT tag reader	Downstream migrant trap
125	Y	1	0	0
130	N	0	0	0
135	N	0	0	0
139	N	0	0	0
144	Y	0	1	0
145	N	0	0	0
157	Y	1	1	0
158	Y	0	0	0
164	N	0	0	0
165	Y	1	0	0
167	N	0	0	0
170	Y	1	1	0
171	N	0	0	0
175	Y	0	0	0
177	Y	0	0	0
179	Y	0	1	0
181	Y	1	1	0
182	Y	1	0	1
185	Y	1	1	0
186	Y	1	0	0
187	N	0	0	0
188	N	0	0	0
197	N	0	0	0
200	Y	1	1	0

APPENDIX B: Detection of tagged rainbow trout downstream of the waterfall in Freshwater Creek, California by pairs of stationary PIT tag antennae. Date, time, direction of movement, and location of tagged, transplanted trout detected at pairs of stationary antennae. PIT # refers to the final digit(s) of the PIT tag code for each individual.

PIT #	Date	Time	Direction of movement	Location
2	12/1/2005	11:35:39	Upstream	Upper Mainstem
5	11/15/2006	7:20:53	Downstream	South Fork confluence
	11/15/2006	16:52:23	Downstream	South Fork confluence
	11/15/2006	6:12:19	Upstream	Upper Mainstem
7	11/20/2006	23:49:37	Downstream	Upper Mainstem
	11/20/2006	19:11:34	Upstream	Upper Mainstem
	11/20/2006	23:49:25	Upstream	Upper Mainstem
	11/21/2006	8:34:27	Downstream	Upper Mainstem
	2/11/2007	11:56:33	Downstream	Little Freshwater confluence
	2/11/2007	11:56:34	Downstream	Little Freshwater confluence
14	11/3/2005	21:25:33	Downstream	Upper Mainstem
18	7/21/2006	3:25:11	Downstream	Upper Mainstem
	7/21/2006	3:25:14	Downstream	Upper Mainstem
	7/21/2006	3:22:41	Upstream	Upper Mainstem
	7/21/2006	3:23:40	Upstream	Upper Mainstem
25	12/18/2005	21:15:09	Upstream	Cloney Gulch confluence
	12/18/2005	21:50:37	Upstream	Cloney Gulch confluence
28	7/6/2006	6:13:51	Downstream	Upper Mainstem
	7/6/2006	21:38:26	Downstream	Upper Mainstem
	7/6/2006	22:07:31	Downstream	Upper Mainstem
	7/6/2006	6:20:40	Upstream	Upper Mainstem
	7/6/2006	21:37:59	Upstream	Upper Mainstem
	7/6/2006	22:09:44	Upstream	Upper Mainstem
	7/11/2006	23:34:21	Downstream	Upper Mainstem
	7/11/2006	23:34:36	Downstream	Upper Mainstem
	7/11/2006	22:20:57	Upstream	Upper Mainstem
	7/11/2006	23:38:39	Upstream	Upper Mainstem
	7/16/2006	0:13:33	Downstream	Upper Mainstem
	7/16/2006	0:10:53	Upstream	Upper Mainstem
52	7/7/2006	0:18:28	Downstream	Upper Mainstem
	7/7/2006	0:16:55	Upstream	Upper Mainstem
	7/7/2006	0:16:56	Upstream	Upper Mainstem
	11/14/2006	6:48:23	Downstream	Howard Heights
	11/14/2006	6:48:11	Upstream	Howard Heights
55	7/10/2006	5:51:42	Upstream	Upper Mainstem
	7/10/2006	5:51:43	Upstream	Upper Mainstem
	7/13/2006	11:06:19	Downstream	Upper Mainstem
	7/13/2006	11:12:25	Upstream	Upper Mainstem
	7/22/2006	22:35:05	Upstream	Upper Mainstem
	7/23/2006	3:27:40	Downstream	Upper Mainstem
	7/23/2006	3:27:41	Downstream	Upper Mainstem
56	11/15/2005	6:52:31	Downstream	Upper Mainstem
	11/15/2005	6:47:43	Upstream	Upper Mainstem
	11/26/2005	17:38:43	Downstream	Upper Mainstem

APPENDIX B: Detection of tagged rainbow trout downstream of the waterfall in Freshwater Creek, California by pairs of stationary PIT tag antennae. Date, time, direction of movement, and location of tagged, transplanted trout detected at pairs of stationary antennae. PIT # refers to the final digit(s) of the PIT tag code for each individual (continued).

PIT #	Date	Time	Direction of movement	Location
	11/26/2005	17:40:35	Upstream	Upper Mainstem
	11/30/2005	7:12:56	Downstream	Upper Mainstem
	11/30/2005	6:50:46	Upstream	Upper Mainstem
	11/30/2005	7:28:00	Upstream	Upper Mainstem
	11/30/2005	7:28:08	Upstream	Upper Mainstem
60	11/4/2005	7:19:43	Downstream	Upper Mainstem
61	11/15/2006	6:56:38	Downstream	Upper Mainstem
	11/15/2006	6:56:59	Downstream	Upper Mainstem
	11/15/2006	6:44:32	Upstream	Upper Mainstem
71	7/8/2006	0:20:37	Upstream	Upper Mainstem
	7/8/2006	1:29:45	Upstream	Upper Mainstem
	11/17/2006	9:55:31	Downstream	Howard Heights
	11/17/2006	9:55:04	Upstream	Howard Heights
	11/20/2006	11:46:53	Downstream	Howard Heights
	11/20/2006	20:18:35	Downstream	Howard Heights
	11/20/2006	11:47:50	Upstream	Howard Heights
	11/20/2006	20:18:22	Upstream	Howard Heights
72	7/7/2006	2:39:47	Downstream	Upper Mainstem
	7/7/2006	2:39:48	Downstream	Upper Mainstem
	7/7/2006	2:38:54	Upstream	Upper Mainstem
	7/7/2006	2:38:55	Upstream	Upper Mainstem
89	1/23/2006	7:14:05	Downstream	Upper Mainstem
	1/23/2006	7:14:06	Downstream	Upper Mainstem
	1/23/2006	7:10:30	Upstream	Upper Mainstem
	1/23/2006	7:10:31	Upstream	Upper Mainstem
98	11/14/2006	13:13:50	Downstream	Upper Mainstem
	11/14/2006	14:03:47	Downstream	Upper Mainstem
	11/14/2006	13:13:43	Upstream	Upper Mainstem
	11/21/2006	7:42:59	Downstream	Upper Mainstem
	11/21/2006	8:19:11	Downstream	South Fork confluence
	11/21/2006	7:42:40	Upstream	Upper Mainstem
	11/21/2006	8:33:11	Upstream	South Fork confluence
	11/21/2006	19:06:28	Upstream	South Fork confluence
	12/15/2006	7:10:25	Downstream	South Fork confluence
	12/15/2006	7:11:22	Downstream	South Fork confluence
	12/15/2006	7:22:22	Downstream	South Fork confluence
	12/15/2006	13:02:34	Downstream	South Fork confluence
	12/15/2006	17:09:07	Downstream	South Fork confluence
	12/15/2006	23:18:17	Downstream	South Fork confluence
	12/15/2006	23:36:24	Downstream	South Fork confluence
	12/15/2006	7:20:11	Upstream	South Fork confluence
	12/15/2006	13:02:58	Upstream	South Fork confluence
	12/15/2006	17:08:45	Upstream	South Fork confluence
	12/15/2006	23:19:18	Upstream	South Fork confluence

APPENDIX B: Detection of tagged rainbow trout downstream of the waterfall in Freshwater Creek, California by pairs of stationary PIT tag antennae. Date, time, direction of movement, and location of tagged, transplanted trout detected at pairs of stationary antennae. PIT # refers to the final digit(s) of the PIT tag code for each individual (continued).

PIT #	Date	Time	Direction of movement	Location
	12/15/2006	23:35:35	Upstream	South Fork confluence
	12/16/2006	6:57:56	Downstream	South Fork confluence
	12/16/2006	16:40:44	Downstream	South Fork confluence
	12/16/2006	7:02:32	Upstream	South Fork confluence
	12/16/2006	8:18:01	Upstream	South Fork confluence
	12/17/2006	7:16:21	Downstream	South Fork confluence
	12/17/2006	7:17:05	Upstream	South Fork confluence
	12/17/2006	16:58:22	Upstream	South Fork confluence
	12/18/2006	5:25:56	Downstream	South Fork confluence
	12/18/2006	5:26:44	Upstream	South Fork confluence
	12/18/2006	5:37:35	Upstream	South Fork confluence
	12/19/2006	7:21:29	Downstream	South Fork confluence
	12/19/2006	16:59:13	Downstream	South Fork confluence
	12/19/2006	19:07:40	Downstream	Upper Mainstem
	12/19/2006	21:42:11	Downstream	Upper Mainstem
	12/19/2006	7:21:42	Upstream	South Fork confluence
	12/19/2006	16:59:10	Upstream	South Fork confluence
	12/19/2006	19:13:11	Upstream	Upper Mainstem
	12/19/2006	21:41:43	Upstream	Upper Mainstem
	12/20/2006	5:27:24	Downstream	South Fork confluence
	12/20/2006	5:27:31	Downstream	South Fork confluence
	12/20/2006	17:15:52	Downstream	South Fork confluence
	12/20/2006	19:03:25	Downstream	South Fork confluence
	12/20/2006	5:28:15	Upstream	South Fork confluence
	12/20/2006	17:15:46	Upstream	South Fork confluence
	12/20/2006	19:04:16	Upstream	South Fork confluence
	12/21/2006	6:52:50	Downstream	South Fork confluence
	12/21/2006	9:36:50	Downstream	South Fork confluence
	12/21/2006	9:44:17	Downstream	South Fork confluence
	12/21/2006	13:15:04	Downstream	Upper Mainstem
	12/21/2006	6:50:37	Upstream	South Fork confluence
	12/21/2006	9:37:06	Upstream	South Fork confluence
	12/21/2006	9:44:10	Upstream	South Fork confluence
	12/21/2006	13:19:33	Upstream	Upper Mainstem
	12/28/2006	11:33:20	Downstream	South Fork confluence
	12/28/2006	13:27:04	Downstream	South Fork confluence
	12/28/2006	14:16:00	Downstream	South Fork confluence
	12/28/2006	15:33:48	Downstream	South Fork confluence
	12/28/2006	15:34:02	Downstream	South Fork confluence
	12/28/2006	16:23:04	Downstream	South Fork confluence
	12/28/2006	23:05:52	Downstream	South Fork confluence
	12/28/2006	23:55:10	Downstream	South Fork confluence
	12/28/2006	11:34:00	Upstream	South Fork confluence
	12/28/2006	13:26:48	Upstream	South Fork confluence

APPENDIX B: Detection of tagged rainbow trout downstream of the waterfall in Freshwater Creek, California by pairs of stationary PIT tag antennae. Date, time, direction of movement, and location of tagged, transplanted trout detected at pairs of stationary antennae. PIT # refers to the final digit(s) of the PIT tag code for each individual (continued).

PIT #	Date	Time	Direction of movement	Location
	12/28/2006	14:16:54	Upstream	South Fork confluence
	12/28/2006	15:15:06	Upstream	South Fork confluence
	12/28/2006	15:39:59	Upstream	South Fork confluence
	12/28/2006	16:22:45	Upstream	South Fork confluence
	12/28/2006	23:07:59	Upstream	South Fork confluence
	12/29/2006	6:49:03	Downstream	Upper Mainstem
	12/29/2006	6:52:17	Downstream	Upper Mainstem
	2/23/2007	11:05:14	Downstream	South Fork confluence
	2/23/2007	12:07:16	Downstream	South Fork confluence
	2/23/2007	13:05:43	Downstream	South Fork confluence
	2/23/2007	13:37:51	Downstream	South Fork confluence
	2/23/2007	13:37:52	Downstream	South Fork confluence
	2/23/2007	11:55:31	Upstream	South Fork confluence
	2/23/2007	12:06:56	Upstream	South Fork confluence
	2/23/2007	13:10:25	Upstream	South Fork confluence
	2/23/2007	13:35:49	Upstream	South Fork confluence
99	12/3/2005	13:24:54	Downstream	Upper Mainstem
	12/3/2005	13:42:32	Upstream	Upper Mainstem
	7/9/2006	21:44:56	Downstream	Upper Mainstem
	7/9/2006	21:45:00	Downstream	Upper Mainstem
	7/9/2006	21:43:30	Upstream	Upper Mainstem
	7/9/2006	21:43:40	Upstream	Upper Mainstem
118	11/18/2006	4:30:56	Upstream	Upper Mainstem
	12/23/2006	11:25:57	Downstream	Little Freshwater confluence
	12/23/2006	14:32:07	Downstream	Howard Heights
	12/23/2006	9:44:02	Upstream	Cloney Gulch confluence
	12/23/2006	11:26:05	Upstream	Little Freshwater confluence
	12/23/2006	13:04:22	Upstream	Howard Heights
	12/23/2006	14:32:1	Upstream	Howard Heights
	12/28/2006	9:25:55	Downstream	Little Freshwater confluence
	12/28/2006	9:26:3	Downstream	Little Freshwater confluence
	12/28/2006	9:46:58	Downstream	Little Freshwater confluence
	12/28/2006	9:46:59	Downstream	Little Freshwater confluence
	12/28/2006	9:26:11	Upstream	Little Freshwater confluence
	12/28/2006	9:46:54	Upstream	Little Freshwater confluence
125	7/10/2006	3:19:19	Upstream	Upper Mainstem
	7/10/2006	3:20:20	Upstream	Upper Mainstem
157	7/18/2006	4:29:00	Upstream	Upper Mainstem
	7/18/2006	4:37:36	Upstream	Upper Mainstem
165	7/11/2006	1:56:46	Upstream	Upper Mainstem
	7/11/2006	1:56:53	Upstream	Upper Mainstem
170	11/20/2006	3:51:19	Upstream	Upper Mainstem
	11/20/2006	3:51:31	Upstream	Upper Mainstem
	12/18/2006	8:10:17	Downstream	South Fork confluence

APPENDIX B: Detection of tagged rainbow trout downstream of the waterfall in Freshwater Creek, California by pairs of stationary PIT tag antennae. Date, time, direction of movement, and location of tagged, transplanted trout detected at pairs of stationary antennae. PIT # refers to the final digit(s) of the PIT tag code for each individual (continued).

PIT #	Date	Time	Direction of movement	Location
	12/18/2006	8:10:46	Upstream	South Fork confluence
	12/18/2006	18:23:33	Upstream	South Fork confluence
	12/19/2006	16:59:27	Downstream	South Fork confluence
	12/25/2006	0:44:18	Downstream	Upper Mainstem
	12/27/2006	10:23:34	Downstream	South Fork confluence
	12/27/2006	10:26:22	Upstream	South Fork confluence
	2/21/2007	11:28:45	Downstream	South Fork confluence
	2/21/2007	11:29:37	Downstream	South Fork confluence
181	11/16/2006	17:01:37	Downstream	Upper Mainstem
	11/16/2006	16:59:31	Upstream	Upper Mainstem
	11/17/2006	12:29:05	Downstream	Upper Mainstem
	11/17/2006	12:29:34	Upstream	Upper Mainstem
182	4/2/2007	13:22:58	Upstream	South Fork confluence
	4/2/2007	13:23:35	Upstream	South Fork confluence
185	7/10/2006	6:21:21	Upstream	Upper Mainstem
	7/19/2006	13:04:27	Downstream	Upper Mainstem
	7/19/2006	13:04:27	Upstream	Upper Mainstem
	7/19/2006	13:04:33	Upstream	Upper Mainstem
	7/24/2006	12:44:41	Upstream	Upper Mainstem
	4/9/2007	5:23:24	Upstream	Upper Mainstem
186	7/12/2006	3:27:41	Downstream	Upper Mainstem
	7/12/2006	4:39:54	Downstream	Upper Mainstem
	7/12/2006	3:24:38	Upstream	Upper Mainstem
	7/12/2006	3:24:47	Upstream	Upper Mainstem
	7/12/2006	4:44:25	Upstream	Upper Mainstem
	7/15/2006	4:29:39	Downstream	Upper Mainstem
	7/15/2006	9:41:00	Downstream	Upper Mainstem
	7/16/2006	20:45:54	Upstream	Upper Mainstem
	7/16/2006	20:45:56	Upstream	Upper Mainstem
200	11/16/2006	6:58:20	Downstream	Upper Mainstem
	3/1/2007	18:4:59	Upstream	Howard Heights
	3/2/2007	9:28:09	Upstream	Howard Heights
	3/7/2007	8:00:20	Downstream	Little Freshwater confluence
	3/7/2007	8:00:21	Downstream	Little Freshwater confluence
	3/7/2007	8:04:24	Downstream	Little Freshwater confluence
	3/7/2007	8:00:32	Upstream	Little Freshwater confluence
	3/7/2007	8:00:40	Upstream	Little Freshwater confluence

APPENDIX C: Detection of tagged rainbow trout downstream of the waterfall in Freshwater Creek, California from surveys conducted using mobile PIT-tag interrogation systems. PIT # refers to the final digit(s) of the PIT tag code for each individual. PIT tag numbers with an asterisk represent individuals that were released above the barrier waterfall at their points of capture. Also given is the date of detection, distance moved from the transplant location (negative distance represents downstream movement), and whether the individual appeared to be alive or dead (coded Y for yes and N for no). Duplicate records for the same individual on the same day and at the same approximate location (± 5m) were removed from the dataset.

PIT #	DATE	Distance from Transplant	Dead (Y/N)
3	10/11/2006	4468	N
4	5/22/2007	386	N
14	6/13/2007	-4841	Y
23	6/14/2007	-3601	N
28	10/10/2006	-357	Y
34	10/11/2006	65	N
35	10/11/2006	374	N
39	10/11/2006	378	N
	5/22/2007	380	N
52	10/10/2006	-305	N
60	10/9/2006	-2262	Y
61	10/11/2006	68	N
63	10/11/2006	367	N
71	10/11/2006	66	Y
72	10/10/2006	-249	N
79	6/20/2006	19	Y
	10/11/2006	18	Y
80	10/11/2006	-51	N
85	10/9/2006	-2838	Y
87*	5/30/2007	4844	N
96	6/20/2006	18	Y
	10/11/2006	18	Y
98	10/11/2006	168	N
107*	6/19/2006	3317	N
110	6/12/2007	-3938	Y
116	10/11/2006	4752	N
117*	5/30/2007	4752	N
118	10/11/2006	379	N
144	6/19/2006	-3317	N
157	10/10/2006	-359	Y
170	10/11/2006	192	N
179	10/11/2006	1	N
181	10/11/2006	100	N
185	10/11/2006	6	N
200	10/11/2006	160	N

APPENDIX D: Capture of tagged rainbow trout in downstream migrant traps below the barrier waterfall in Freshwater Creek, California. PIT # refers to the final digit(s) of the PIT tag code used to identify each individual. Also given are the basin in which the trap was located, date of capture, length, weight and field identification of migratory stage at time of capture. PIT tag numbers with an asterisk represent individuals that were released above the barrier waterfall at their points of capture.

PIT #	Trap Location	Date	Length (mm)	Weight (g)	Migratory Stage
6	Upper Mainstem	4/20/2006	172		Non-smolting
23	Upper Mainstem	5/7/2007	157	51.8	Non-smolting
38*	Upper Mainstem	5/3/2007	126	25.4	Non-smolting
44	Upper Mainstem	4/29/2006	192	70.7	Smolt
46	Upper Mainstem	4/8/2006	121		Pre-smolt
59*	Lower Mainstem	5/4/2007	203	83.5	Smolt
182	Upper Mainstem	4/1/2007	132	18	Unknown