Microsatellite Analyses of Alameda Creek Rainbow/Steelhead Trout

by

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Report submitted to Applied Marine Sciences, Inc.
October 7, 1999

Abstract

Microsatellite genetic diversity found in Alameda Creek rainbow trout support a close genetic relationship with coastal trout found in Lagunitas Creek, Marin County, California. No significant genotypic or allelic frequencies associations could be drawn among Alameda Creek trout and fish collected from the four primary rainbow trout hatchery strains in use in California, Whitney, Mount Shasta, Coleman, and Hot Creek strains. Indeed, genetic distance analyses ($\delta\mu^2$) supported genetic separation among Alameda Creek trout and hatchery trout with greater than 50% bootstrap values in 1000 replicate neighbor-joining trees. Fish collected for this study from Palo Seco and Sheppard Creeks shared allelic frequencies with both the fish in Alameda Creek and those found in Scott Creek in Santa Cruz County. Fish collected in Horseshoe Creek or San Lorenzo Creek (Alameda County) did not share this unique genetic relationship between Alameda Creek fish and putative wild coastal trout. These two streams had allelic frequencies similar to some hatchery trout strains and to wild trout captured in the Central Valley. These data suggest that there are two possible steelhead ESUs using the tributaries of San Francisco Bay (one coastal and one Central Valley) or that hatchery trout supplementation has impacted some, but not all streams with a subsequent loss of locally adapted genetic characteristics. These data support the implementation of conservation management of rainbow trout in the Alameda Creek drainage as part of the central California coastal steelhead ESU.

Introduction

The status of freshwater rainbow trout (*Oncorhynchus mykiss*) in California's coastal drainages where anadromous steelhead have historically occurred is under considerable scrutiny following the listing of California's steelhead under the Endangered Species Act by the National Marine Fisheries Service (NMFS). The implications of life history in *Oncorhynchus mykiss* under this listing remain controversial. Genetic studies of the anadromous portion of coastal *O. mykiss* populations (fish demonstrating movement into salt water for maturation) have shown a great deal of biogeographic structure throughout their range in California (Nielsen et al 1994a, 1997a; Nielsen 1996a & 1999). California's rainbow trout and steelhead appear to be different life-history morphs of the same species with a great deal of flexibility in timing and structure of anadromy in any one population (Shapovalov and Taft 1954). The anadromous component of this complex life history, however, appears to have great significance in maintaining the genetic diversity for this species in California waters (Nielsen et al. 1997b, 1999).

There has been considerable manipulation of rainbow trout in the hatchery environment since the turn of the century (Busack and Gall 1980) and subsequent planting of hatchery rainbow trout for sport fisheries throughout California. The impacts of hatchery fish on the population genetics of native California trout has only been recently studied (Nielsen 1996a; Nielsen and Fountain 1999; Nielsen et al. 1994b, 1999). Their variation and flexibility in life history structure and the potential implications of hatchery activity on wild stocks has lead to questions about the inclusion of freshwater trout in NMFS's steelhead listings under geographic Evolutionary Significant Units (ESUs).

In this study, we examined genetic diversity found in 10 microsatellite loci amplified from DNA extracted from rainbow trout tissues collected from 12 creeks and streams in Alameda County. Genetic diversity found in trout within this area was compared to results previously analyzed in our laboratory for trout and steelhead populations found throughout California. Based on these data management suggestions are made concerning the status of freshwater trout populations in Alameda County, California.

Material and Methods

Seventy rainbow trout fin tissues sent to our laboratory by Applied Marine Sciences, Inc. from 12 stream populations in Alameda County were amplified successfully for DNA analyses (Table 1). Baseline genetic data on steelhead/rainbow trout previously analyzed in our laboratory included information on two additional collections made on Alameda Creek (1997 California Department of Fish and Game (CDFG) for the National Marine Fisheries Service (NMFS) and 1998 below the dam by Peter Alexander, CA State Parks), nine wild trout populations, and four hatchery rainbow trout strains.

We amplified total genomic DNA from dried fin tissues according to methods in Nielsen et al. 1994a. Amplification of 10 microsatellite (Table 2) followed methods given in Nielsen and Fountain (1999). Microsatellite allele sizes (including the amplified primer) were determined in relation to the Genescan-500 internal size standard (P-E Biosystems, Foster City, CA), *O. mykiss* DNA samples of known size that were rerun on each gel, and a double-stranded reference marker developed in our laboratory showing the most common alleles available for each locus. GENESCAN (v 1.1) and GENOTYPER v 2.1 (P-E Biosystems) DNA fragment analysis software packages were used to score, bin, and output allelic (and genotypic) designations.

Analyses of heterozygosity and F_{st} (function of the probability of identity of genes within and between units) were performed using ARLEQUIN (Schneider et al. 1997). Population independence between paired comparisons of allelic frequencies were tested using Fisher's exact tests, based on a Markov chain adaptation of row-by-column contingency tables using GENEPOP (V3.1a: Raymond & Rousset 1997). Statistical significance levels for Fisher's exact analyses were set using sequential Bonferroni tests (Rice 1989).

MICROSAT was used to generate a genetic distance matrix among rainbow trout populations based on $\delta\mu^2$ genetic distance values (Goldstein et al. 1995). The NEIGHBOR and CONSENSE applications from PHYLIP v. 3.572 (Felsenstein 1995) were used to generate mean and consensus neighbor-joining trees from $\delta\mu^2$ genetic distance values

Table 1. Stream locations and number of samples used in the Alameda County trout analyses.

Location	Stream	Year	N
Alameda County	Alameda Creek (this study)	1999	4
	Alameda Creek (below dam)	1998	11
	Alameda Creek (NMFS)	1997	48
	Arroyo Mocho	1999	1
	Horseshoe Creek	1999	11
	Indian Joe Creek	1999	12
	Palo Seco	1999	5
	Pirate Creek	1999	5
	San Leandro Creek	1999	6
	San Lorenzo Creek	1999	3
	Sheppard Creek	1999	1
	Stonybrook Creek	1999	8
	Welch Creek	1999	4
	West Tree Creek	1999	10
San Joaquin River	Mokelumne River	1997	30
	Stanislaus River	1997	41
Sacramento River	Deer Creek	1997	16
	Mill Creek	1997	36
	American River	1997	53
Santa Cruz County	Scott Creek	1997	50
Marin County	Lagunitas Creek	1997	48
Santa Barbara County	Santa Ynez River	1995	11
LA County	Malibu Creek	1993	13
Hatcheries	Whitney Hatchery strain	1997	51
	Mount Shasta Hatchery strain	1997	60
	Coleman Hatchery strain	1997	60
	Hot Creek strain	1997	53

Table 2. List of microsatellite loci and their source publications used to amplify DNA from steelhead rainbow trout (*Oncorhynchus mykiss*) in the Nielsen laboratory. Number in parentheses is the number of alleles found in the Alameda Creek watershed for this study. Mean Hz = mean heterozygosity for this locus in the Alameda Creek drainage.

Locus	Source	Number Alleles	Allelic Size Range (bp)	Mean Hz
Omy27	M. O'Connell pers. comm.*	11 (6)	95 – 117	0.56
Omy77	Morris et al. 1996	18 (13)	93 – 155	0.79
Omy325	M. O'Connell pers. comm.*	27 (19)	87 – 149	0.74
Oneμ2	Schribner et al. 1996	45 (21)	110 – 290	0.74
Oneμ8	Schribner et al. 1996	18 (8)	144 – 190	0.46
Oneμ14	Schribner et al. 1996	14 (11)	145 – 171	0.65
Ots1	Banks et al. 1999	33 (17)	151 – 249	0.71
Ssa14	McConnell et al. 1995	21 (12)	120 – 168	0.65
Ssa85	O'Reilly et al. 1996	14 (11)	105 – 159	0.47
Ssa289	McConnell et al. 1995	14 (7)	104 – 130	0.67

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Results

Allelic size ranges and the number of alleles for the 10 microsatellite loci tested in Alameda County rainbow trout fell within expected values for these loci in *O. mykiss* found throughout the species range (see Table 2). Alameda County *O. mykiss* showed significant diversity in both allelic size, 93 (Omy77) to 284 (Oneμ2) base pairs (bp) and the number of alleles (mean number of alleles per locus = 12.5). Average heterozygosity for all 10 loci combined was Hz = 0.64. Mean F_{IS} for all 10 loci combined equaled 0.17, with most genetic diversity (97%) occurring at the level of individuals within a population in the Alameda Creek drainage. GENEPOP's analysis of population independence was significant for all paired comparisons suggesting differences in population allelic structure for each stream locality. Several populations exhibited low levels of average heterozygosity (Table 3). These characteristics could have resulted from the small sample sizes available for many of the streams analyzed for this study. Reproductive isolation of stream populations, population bottlenecks, founder effects,

Table 3. Mean heterozygosity by population including samples successfully amplified for all ten loci .

Population	N	Mean heterozygosity
Stony Brook Creek	8	0.20
Alameda Creek	4	0.53
San Leandro Creek	6	0.55
San Lorenzo Creek	3	0.67
Palo Seco	5	0.40
Horseshoe Creek	11	0.38
Sheppard Creek	1	0.60
Wx Tree Creek	10	0.47
Indian Joe Creek	12	0.38
Arroyo Mocho	1	0.50
Welch Creek	4	0.59
Pirate Creek	5	0.44

and/or very low effective population size within individual streams can not be ruled out, however. The estimated number of migrants (used as a surrogate for gene flow) among Alameda Creek trout populations was $Nm = 1.8$ fish per generation.

The genetic distance matrix for $\delta\mu^2$ genetic distance values calculated among rainbow trout populations are given in Table 4. Genetic distance comparisons are presented as a consensus neighbor-joining tree in Figure 1 for a broad geographic range of rainbow trout/steelhead populations in California. Small sample sizes for many populations prevented NJ analyses of the genetic distance relationships among tributary and mainstem populations of trout on Alameda Creek. However, the inclusion of samples previously collected by NMFS (1997) on the mainstem Alameda Creek above the dam and by State Parks below the dam (1998) in these analyses gives some inference to temporal and spatial population genetic structure within this drainage.

Table 1. Nei's (1978) unbiased distance (Dμ') for rainbow/steelhead populations used in this study.

	ALM	HOR	PAL	SHP	SLE	SLO	AEMb	SSM	MOM	AEMa	SSFB	AMM	CHM	DRM	HCM	NCS	MIM	SCS	SUM
Horseshoe Cr	21.411																		
Palo Seco	9.174	23.827																	
Sheppard Cr	2.162	33.279	8.678																
San Leandro Cr	1.366	41.240	3.655	2.395															
San Lorenzo	39.856	11.134	24.013	30.449	22.414														
Alameda below	4.552	39.999	12.325	8.679	8.894	56.036													
Shasta Hatch	52.204	27.077	45.056	55.142	43.112	9.187	83.396												
Mokelumne R	7.516	24.653	12.583	14.743	7.762	28.162	35.332	40.783											
Alameda above	7.098	33.298	3.748	5.223	3.901	13.878	21.397	32.692	11.474										
SOSFBHAY	1.320	39.548	2.565	2.531	1.626	25.502	8.248	48.550	6.462	3.633									
American R	1.765	12.847	9.098	10.079	3.576	20.488	13.031	37.737	4.328	7.060	3.753								
Coleman Hat	23.057	16.073	25.030	27.952	16.152	8.987	42.034	15.632	15.561	14.361	20.992	11.583							
Deer Creek	42.820	14.306	33.622	48.781	32.783	11.071	65.961	10.985	29.772	29.553	39.822	25.877	3.756						
Hat Cr Steam	21.370	7.253	21.380	26.038	15.557	4.748	41.800	9.956	15.942	12.800	19.500	11.187	1.894	5.409					
NCOAST	1.651	24.473	5.913	6.634	3.743	29.056	6.406	47.155	3.636	9.867	3.162	4.375	21.100	37.177	18.675				
Mill Cr	17.788	10.647	20.995	24.463	13.602	9.563	34.693	16.255	9.745	13.885	17.010	9.094	3.014	6.847	1.898	13.375			
SCOAST	2.273	18.935	5.663	5.778	2.440	24.139	9.478	43.881	2.438	5.400	1.375	2.857	16.766	32.757	15.842	1.651	11.369		
Stanislaus R	26.950	3.833	29.665	31.530	19.011	10.189	47.146	18.767	20.451	16.011	23.390	13.303	1.744	6.101	3.703	26.088	5.937	19.643	
Whitney strain	19.927	2.599	18.748	20.021	12.786	5.019	40.926	17.877	24.168	9.649	17.949	13.761	6.664	13.312	4.628	22.097	8.529	17.472	5.897

ALM (41) = Alameda & Tributaries (1999) = Stony Brook Cr., Alameda Creek, W. Tree Cr., Indian Joe, Arroyo Mocho, Welch Creek, Pirates Creek
HOR (11) = Horseshoe Creek
PAL (31) = Palo Seco
SHP (13) = Sheppard Creek
SLE (11) = San Leandro Creek
SLO (10) = San Lorenzo
AEMb (19) = Alameda Creek below dam (Peter Alexander 1998)
SSM (40) = Mount Shasta Hatchery trout
MOM (30) = Mokelumne River wild trout
AEMa (48) = Alameda Creek above dam (NMFS 1997)
SOSFB (Hay) = Scott Creek, San Lorenzo, Carmel River
AMM (34) = American River wild trout
CHM (10) = Coleman Hatchery rainbow trout
DRM (16) = Deer Creek trout
HCM (34) = Hot Creek hatchery strain
NCS/NCOAST (47) = Lagunitas Creek
MIM (10) = Mill Creek trout
SCS/SCOAST (16) = south coastal steelhead = Hilton Creek & Malibu Creek
SUM (46) = Stanislaus River trout
WHM (51) = Whitney Hatchery strain rainbow trout

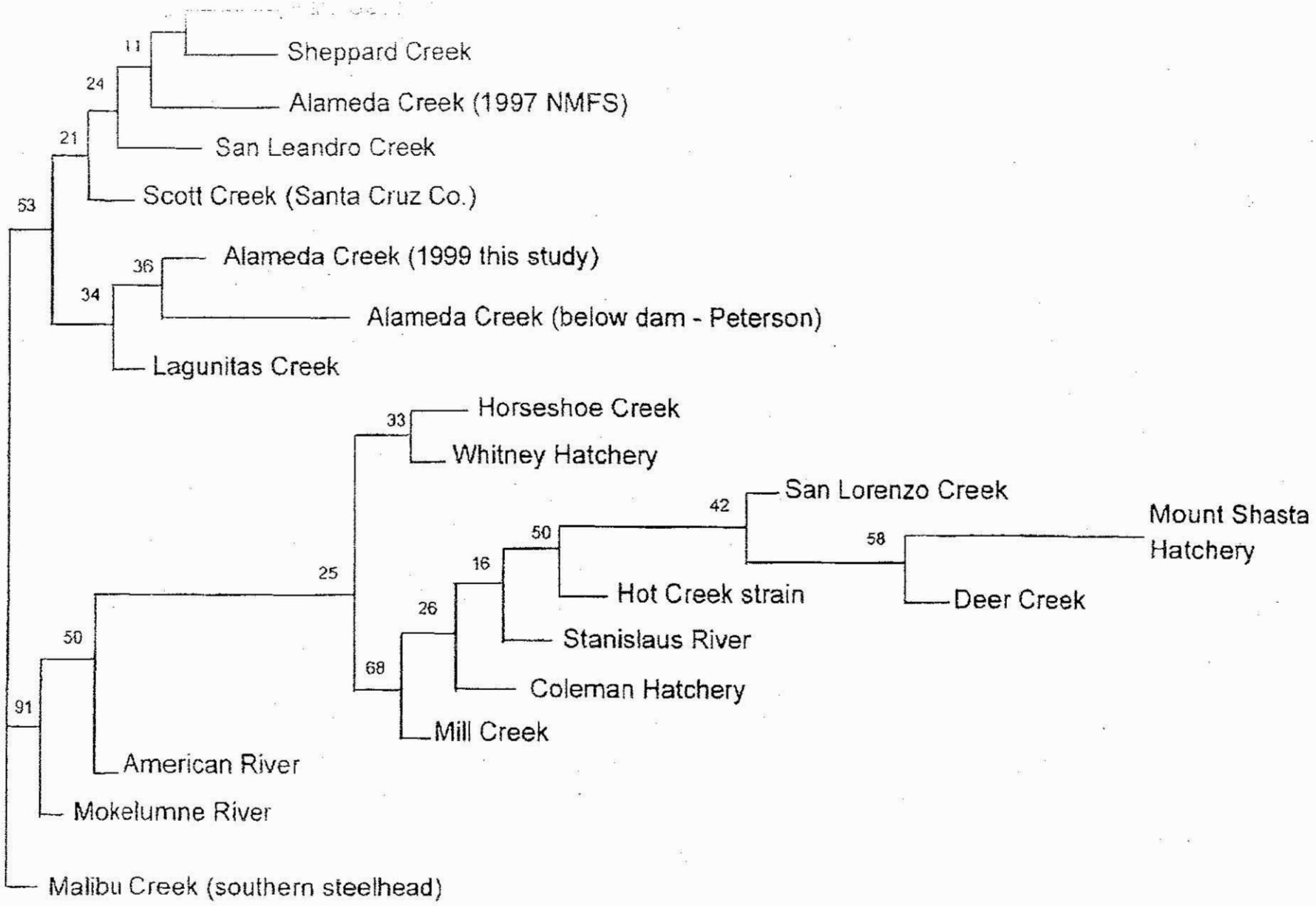

Figure 1. Neighbor-joining tree of genetic distance based on 10 microsatellite loci. Numbers represent the % bootstraps supporting branching patterns to the right of the node from 1000 replicate NJ trees.

Using these ten microsatellite loci the collection of Alameda Creek tributary and mainstem rainbow trout sent to us by Applied Marine Sciences, Inc. appear most closely related to fish collected below the dam and trout found in Lagunitas Creek in Marin County (Figure 1). However, bootstrap values based on 1000 replicate trees supporting this relationship were low with only 34% of the trees supporting this relationship in our consensus genetic distance analysis. Genetic similarity found among the NMFS samples (1997) from Alameda Creek, Applied Marine Sciences' collections from Palo Seco, San Leandro and Sheppard Creek (1999), and Scott Creek trout from Santa Cruz County is more difficult to explain. The NMFS samples were collected by California Department of Fish and Game (CDFG) by electrofishing several areas of the mainstem above the dam (Peter Alexander, State Parks, personal communications).

The Alameda Creek, Scott Creek, and Lagunitas Creek trout were separable with high bootstrap values (>50%) from four common hatchery rainbow trout strains (Whitney, Mount Shasta, Coleman, and Hot Creek) used for supplementation in streams and reservoirs throughout California (Figure 1). This was not true for collections made by Applied Marine Sciences in Horseshoe Creek and San Lorenzo Creek. Horseshoe Creek trout showed a close genetic association in allelic frequency to the Whitney Hatchery strain, while the San Lorenzo Creek samples from Alameda County were most closely related to the Hot Creek strain. These associations may be the result of artificial stocking practices in this area or alternatively, they may represent historic evolutionary relationships among the stream populations and the founder populations for the different hatchery strains. The Mount Shasta Hatchery strain, most commonly used for stocking streams and creeks in the San Francisco Bay area was found to be significantly different in allelic frequencies (Fisher's exact $P < 0.02$ in all comparisons) for all ten loci in comparisons with the Alameda Creek trout.

Discussion

With the listing of California's steelhead populations as threatened or endangered evolutionarily significant units (ESUs) under the Endangered Species Act, the question of local stock integrity and historic impacts of stocking rainbow trout in the creeks and streams surrounding San Francisco Bay area has risen to the surface of public interest

(Scott 1997). The California Department of Fish and Game ceased management of steelhead in Alameda Creek and other S.F. Bay tributaries during the 1950's. At that time urban development with it subsequent environmental damage and the construction of water barriers and dams prevented steelhead from migrating and spawning in many of the major streams and tributaries in this area (W. Jones, CDFG, personal communications).

Only recently have anadromous steelhead begun to appear in the mouths of these rivers again. The origins of these anadromous fish are in question. Are they part of the original evolutionary components of S. F. Bay's wild steelhead runs? Are they descendents of the landlocked populations of steelhead captured by the construction of dams and reservoirs in the 1950's that have somehow passed the gauntlet of dams and obstructions and made their way to the sea? Are they simply hatchery strays that have lost their way and ended up by chance in Alameda Creek? All of these hypotheses have legitimate support from the scientific literature as strong possibilities, and each creates its own set of priorities and actions in resource management for the area.

Hatchery fish could be contributing to the Alameda Creek stock in two different ways, as anadromous adults or as rainbow trout introduced into the system for sport harvest. Hatchery steelhead stocks are produced in large numbers on the Sacramento River. Strays in S. F. Bay could be derived from steelhead produced by the Coleman Hatchery, but our genetic analyses show significant allelic differentiation among the trout collected below the dam on Alameda Creek and the Coleman Hatchery strain. The Feather River Hatchery and to a lesser extent the Merced River Hatchery also produce steelhead smolts for release into this system, but we currently have no genetics data on these stocks for these analyses.[1] We did sample and have microsatellite data for wild trout from several of the major rivers in the Sacramento-San Joaquin system, including the American, Stanislaus, and Mokelumne rivers. None of the trout populations on these rivers had close microsatellite allelic associations with the Alameda Creek collection made for this study (see Figure 1).

[1] Pending CALFED contract between my laboratory and CDFG for genetic analyses of Central Valley steelhead populations includes these populations. Expected start date is early 2000.

The stocking of hatchery raised rainbow trout in streams and rivers throughout the U. S. has caused concern about potential introgressive hybridization, genetic swamping, or competitive exclusion with native trout (Reisenbichler and McIntyre 1977; Dowlings and Childs 1992; Campton 1995). The role of hatcheries in the preservation and conservation of native fish stocks remains controversial (Hindar et al. 1991; Waples and Do 1994; Busby 1996). The Sacramento River genetic origins of most of California's hatchery rainbow trout strains complicates our ability to discriminate genetically among trout of hatchery and wild origins in central California (Busack and Gall 1980; Gall 1993). Recent DNA technologies, however, give significant resolution for genetics questions at this scale that were previously not available (Nielsen 1996a).

It has been shown, however, that bringing a species into artificial propagation in the hatchery environment can reduce overall effective population size and lead to reduction in genetic diversity (Waples 1990; Waples and Do 1994; Hedrick et al. 1995). Small population size with subsequent inbreeding among closely related individuals increases the likelihood that genetic variation will be lost over time due to genetic drift (Tajima 1989). This appears to be the case California's rainbow trout strains (Nielsen 1996a & b; Nielsen et al. 1997b). The concern is that if a supplementation of hatchery rainbow trout has been successful, a large fraction of the individuals in the post supplementation population will be derived from a relatively small number of "founders" from the initial population and result in a further erosion of genetic diversity (called the Ryman-Laikre effect). It is questionable if a population subject to the Ryman-Laikre effect can actually be considered as part of an evolutionary significant unit for the conservation of the species.

Genetic analyses done for this study show no significant relationships among trout populations collected on Alameda Creek and fish collected from the four primary rainbow trout hatchery strains used for supplementation in California by CDFG suggesting a low probability of the Ryman-Laikré effect on these streams. These data support the inclusion of these fish within the same ESU as those found in Lagunitas Creek. However, at this time NMFS's ESA listings only afford protection to populations of *O. mykiss* found below man-made barriers. This represents the evident confusion between ESA policy positions and the natural biology and genetics of this fish species.

The picture is further complicated by the fact that the genetic separation from common hatchery rainbow strains was not evident on San Lorenzo and Horseshoe Creeks leading to the question of direct genetic effects of hatchery supplementation in these streams. Low levels of genetic diversity and/or heterozygosities at several microsatellite loci suggesting small effective population sizes may have had an equally significant impact on these trout populations. These data support two possible steelhead ESUs using the streams and tributaries of San Francisco Bay (coastal and Central Valley) with the Central Valley stocks providing the founding populations for the hatchery strains in use today. In either case, genetics analyses using microsatellites do not support the inclusion of trout from these streams in the same ESU as those found in Alameda and Lagunitas creeks simply because trout in these streams can be found below dams or barriers.

One important factor thought to derive from the loss of natural genetic diversity is a drop in "fitness" resulting from in the loss of locally adaptive genetic variation not found in any other group or population (Allendorf and Leary 1988). The markers used in this study, i.e. microsatellites, are considered genetically neutral and are fixed within a population by migration or drift over long periods of time (Jarne and Logda 1996). However, markers with no selective adaptive function can "hitchhike" with markers linked to selective forces when they are closely linked to a beneficial allele and are therefore carried along as the gene that is selected for increases in frequency (Kaplan et al. 1989). If the trout in small tributaries of San Francisco Bay have lost adaptive fitness for important life-history traits, such as the propensity for anadromy, cannot be predicted from population genetics data such as those presented here.

In our rush to conserve genetic signatures across the landscape, it is also important to take into account the sustainability of increased abundance that may result from any conservation activities. Still genotypic and allelic frequencies in a population do reflect a significant contribution from the past that may hold important implications for the future of the species and locally adaptive stocks. At best, a successful conservation program will have little permanent genetic impacts on the natural diversity remaining within an ESU and its evolution through time; at worst, it will once again shift the balance of nature toward our own illusion of genetic "purity" and retain genotypes in isolation from the vagaries of evolution.

Because of the complex nature of the life history of *O. mykiss*, it seems incredibly naïve to not afford protection to a portion of the population simply because anthropomorphic artifacts such as dams and water diversions block ocean access. On the other hand, protection for all systems with rainbow trout below such artifacts is equally unjust. Despite our limited knowledge of the mechanisms of evolution, if we are to protect "evolutionarily significant units" they should be considered with informed judgement and the best scientific information available. These data make just such a contribution to our understanding of genetic population structure of steelhead/rainbow trout in Alameda Creek.

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