

**Population Genetic Structure of Alameda Creek
Rainbow/Steelhead Trout - 2002**

By

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Abstract

Genetic diversity was analyzed in rainbow trout/steelhead on Alameda Creek, a tributary to San Francisco Bay, California. Trout samples were collected from four sub-basins within the Alameda Creek drainage in 2002. Mitochondrial DNA sequence (D-loop) and 14 microsatellite loci were amplified from 175 fish. Four haplotypes - MYS1 (24%), MYS3 (16%), MYS5 (57%) and MYS12 (3%) - were found in this collection on Alameda Creek. Pairwise comparisons using microsatellite data showed significant differences in allelic frequency among the four populations. With the exception of fish collected from Arroyo Mocho, genetic analyses of the Alameda Creek fish were similar to previous analyses done in this drainage in 1999 (Nielsen and Fountain 1999). Three sample locations – Arroyo Hondo, upper Alameda Creek and San Antonio Reservoir – were more closely related to each other than to any reference collection used in these analyses and their closest genetic relationship was found with fish collected in Alameda Creek 1997-1999, including steelhead captured in Alameda Creek downstream of the BART weir analyzed in 1999. The closest out-of-basin genetic relationship for all year classes of Alameda Creek trout (excluding Arroyo Mocho) was with steelhead collected from Lagunitas Creek, Marin County. Microsatellite analyses showed Arroyo Mocho trout to be more closely related to hatchery fish from the Whitney Hatchery strain. A Neighbor-Joining bootstrap value of 71% (based on 2000 replicate trees) separated the branch containing Arroyo Mocho and the Whitney Hatchery strain from the rest of the Alameda Creek populations in these analyses.

Introduction

There is significant controversy over the conservation status of freshwater rainbow trout (*Oncorhynchus mykiss*) in California's coastal drainages blocked by dams or other anthropomorphic barriers in relationship to anadromous steelhead (fish demonstrating movement into salt water for migration) listed under the U.S. Endangered Species Act. Anadromous steelhead occurred historically in streams flowing into the eastern edge of San Francisco Bay (Gall et al. 1990; Behnke 2002). However, since urban development has encompassed most of the Bay Area they have been rarely found in these drainages. Recent documentations of anadromous steelhead found at the mouths of these streams have increased the controversy over the status of trout found in this area (Scott 1997). Central California steelhead have been recently listed by the National Marine Fisheries Service (NMFS) under the U.S. Endangered Species Act as a threatened Evolutionarily Significant Unit (ESU; Federal Register Vol. 62, No. 159, 50 CFR Part 222 and 227), affording significant protections to the marine migrating component of *O. mykiss*. Under the jurisdiction of the U.S. Fish and Wildlife Service, similar protection remains illusive for the freshwater component of this species. Highly flexible life history strategies in *O. mykiss* (Shapovalov and Taft 1954), otolith microchemistry (Rybock et al. 1975; Zimmerman and Reeves 2000), and genetic studies (Gall et al. 1990; Nielsen et al. 1997a) suggest that freshwater habitats may contain relic, non-anadromous components of the *O. mykiss* gene pool found in geographically proximate anadromous populations.

There has been considerable manipulation of rainbow trout in the hatchery environment since the early 1800's (Busack and Gall 1980). Impacts of hatchery supplementation of *O. mykiss* on wild stocks in streams and reservoirs throughout North America over the last 200 years has been the subject of many studies (see reviews in Reisenbichler and McIntyre 1977, Waples and Do 1994, Campton 1995, and Nielsen 1999). The early findings of Gall et al. (1990) suggested that anadromous steelhead populations have residualized as freshwater fish behind man-made structures and dams throughout the San

Francisco Bay area. It is argued that if these residual populations reflect similar genetic population structure to their anadromous progenitors, they should be considered part of NMFS's ESU and retain equal protection under the ESA.

There is significant public concern over what fragments of the freshwater component of this species are part of the evolutionary legacy of the species, and therefore, part of the ESU, and what parts have been influenced by hatchery stocks that are not considered part of the ESU. This question has been studied previously in Alameda County when rainbow trout collected above the dam on Alameda Creek were most closely related genetically to fish collected below the dam and trout found in Lagunitas Creek in Marin County (Nielsen and Fountain 1999). Recent occurrences of anadromous steelhead at the mouth of Alameda Creek also suggested that part of the anadromous evolutionary legacy may survive in this area. This study represents a genetic analyses of four new samples of trout from the Alameda Creek drainage collected in 2002 (Arroyo Mocho, Arroyo Hondo, San Antonio Reservoir and upper Alameda Creek) that were analyzed in isolation and in comparison with the Alameda Creek samples collected 1997-1999 and several hatchery and wild populations taken from our 1999 study.

Material and Methods

Rainbow trout samples collected in 2002 (N = 175) were amplified successfully for DNA analyses (Table 1). Baseline genetic data on steelhead/rainbow trout included in these analyses contained three additional collections from Alameda Creek: 1) 1997 California Department of Fish and Game (CDFG) for the National Marine Fisheries Service (NMFS); 2) 1998 collections made below the BART weir by Peter Alexander, East Bay Regional Park District and Jeff Miller, Alameda Creek Alliance; 3) 1999 Alameda Creek collections (N = 70) previously reported in Nielsen and Fountain (1999). We compared these data to genetic analyses done on four hatchery rainbow trout strains (Coleman, Whitney, Hot Creek and Mount Shasta Hatchery strains) and

three wild steelhead populations from the geographically proximate area (Sacramento River's Mill Creek, American River, and coastal Lagunitas Creek).

We amplified total genomic DNA from dried fin tissues according to methods in Nielsen et al. 1994a. Total genomic DNA was extracted using Chelex-100 resin (BioRad) or Purgene (Gentra Systems, Inc) following methods given in Nielsen et al. (1994a) and from the manufacturer. Amplification of mitochondrial DNA sequence (N = 162) and 14 microsatellite loci (Table 2) followed methods given in Nielsen et al. (1998) and Nielsen and Sage (2001). We used a LI-COR Long Reader 4200 automatic sequencer and V3.00 Gene ImagIR software to visualize and size microsatellite alleles. Allelic standardizations between results obtained from an ABI 373 automatic sequencer (used in previous genetic analyses of Alameda Creek samples) and the LI-COR were performed in our laboratory for all loci. These results are available from the author upon request.

Analyses of heterozygosity, genetic disequilibrium, and Fisher's exact tests for Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP version 3.1a (Raymond and Rousset 1997). HWE tests were performed for all trout populations independently and combined. GENEPOP (Fisher's Exact Tests) and ARLEQUIN version 1.1 (FSTAT pairwise comparisons; Schneider et al. 1997) were used to test for differences in allelic frequencies between all possible population pairs. Statistical significance levels for allelic frequency comparisons were set using sequential Bonferroni tests (Rice 1989). Genetic distance values or the proportion of shared alleles between individuals and groups of individuals can be used to graphically depict genetic relationships. Pairwise genetic distance values based on Cavalli-Sforza and Edwards chord distance (1967) generated using BIOSYS version 1.1 (Swofford & Selander 1981) were plotted as a neighbor-joining (NJ) tree using the NEIGHBOR and CONSENSE applications from PHYLIP version 3.57c (Felsenstein 1993). Genetic relationships depicted in our consensus NJ tree were tested using random bootstrap replications (n = 2000) to assess the reproducibility of branching patterns (Felsenstein 1985).

Table 1. Stream locations and number of samples used in the Alameda County trout analyses 1997-2002.

| Location | Stream | Year | N |
|-------------------|---------------------------------|------|----|
| Alameda County | Alameda Creek (this study) | 2002 | 28 |
| | Arroyo Hondo | 2002 | 29 |
| | San Antonio (Reservoir) | 2002 | 87 |
| | Arroyo Mocho | 2002 | 31 |
| | Alameda Creek (1999 study) | 1999 | 70 |
| | Alameda Creek (below BART weir) | 1998 | 11 |
| | Alameda Creek (NMFS) | 1997 | 48 |
| Sacramento River | Mill Creek | 1997 | 36 |
| | American River | 1997 | 53 |
| Santa Cruz County | Lagunitas Creek | 1997 | 48 |
| Hatcheries | Whiney 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 used to amplify DNA from steelhead/rainbow trout (*Oncorhynchus mykiss*). 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 (2002).

| <u>Locus</u> | <u>Source</u> | <u>Number Alleles</u> | <u>Allelic Size Range (bp)</u> | <u>Mean Hz</u> |
|--------------|-------------------------|-----------------------|--------------------------------|----------------|
| Omy27 | Heath et al. 2001 | 11 (5) | 95 – 117 | 0.52 |
| Omy77 | Morris et al. 1996 | 18 (12) | 93 – 155 | 0.70 |
| Omy207 | O'Connell et al. 1997 | 24 (22) | 97 – 161 | 0.54 |
| Omy325 | O'Connell et al. 1997 | 27 (16) | 97 – 149 | 0.69 |
| Ogo1a | Olsen et al. 1998 | 8 (6) | 124 – 162 | 0.49 |
| Ogo4 | Olsen et al. 1998 | 12 (10) | 118 – 148 | 0.54 |
| One μ 8 | Schribner et al. 1996 | 18 (8) | 144 – 190 | 0.46 |
| One μ 10 | Schribner et al. 1996 | 5 (4) | 121 – 131 | 0.57 |
| One μ 11 | Schribner et al. 1996 | 9 (4) | 138 – 154 | 0.51 |
| One μ 14 | Schribner et al. 1996 | 14 (11) | 145 – 171 | 0.31 |
| Ots1 | Banks et al. 1999 | 33 (17) | 151 – 249 | 0.66 |
| Ots3 | Banks et al. 1999 | 5 (5) | 151 – 249 | 0.45 |
| Ots4 | Banks et al. 1999 | 7 (6) | 108 – 150 | 0.44 |
| Ots100 | Nelson and Beacham 1999 | 13 (13) | 167 – 225 | 0.72 |

Results

Mitochondrial DNA (mtDNA) sequence revealed four haplotypes (MYS1, MYS3, MYS5 and MYS12; see Nielsen et al. 1994b for sequence variation by haplotype) in the new samples collected from the Alameda Creek drainage. The distribution of haplotypes - MYS1 (24%), MYS3 (16%), MYS5 (57%) and MYS12 (3%) - is given by sub-basin in Table 3. Allelic size ranges and the number of alleles for the 14 microsatellite loci tested on Alameda Creek rainbow trout fell within expected values for these loci in *O. mykiss* found throughout the species range (see Table 2). Alameda Creek *O. mykiss* showed significant diversity in allelic size and the number of alleles (mean number of alleles per locus = 9.93). Average heterozygosity for all 14 loci combined was Hz = 0.54. All microsatellite loci were found to be in Hardy-Wienberg equilibrium in the four Alameda Creek

populations ($p = 0.048$ (Ogo4) to $p = 0.89$ (Omy27) ARLEQUIN Markov chain exact tests).

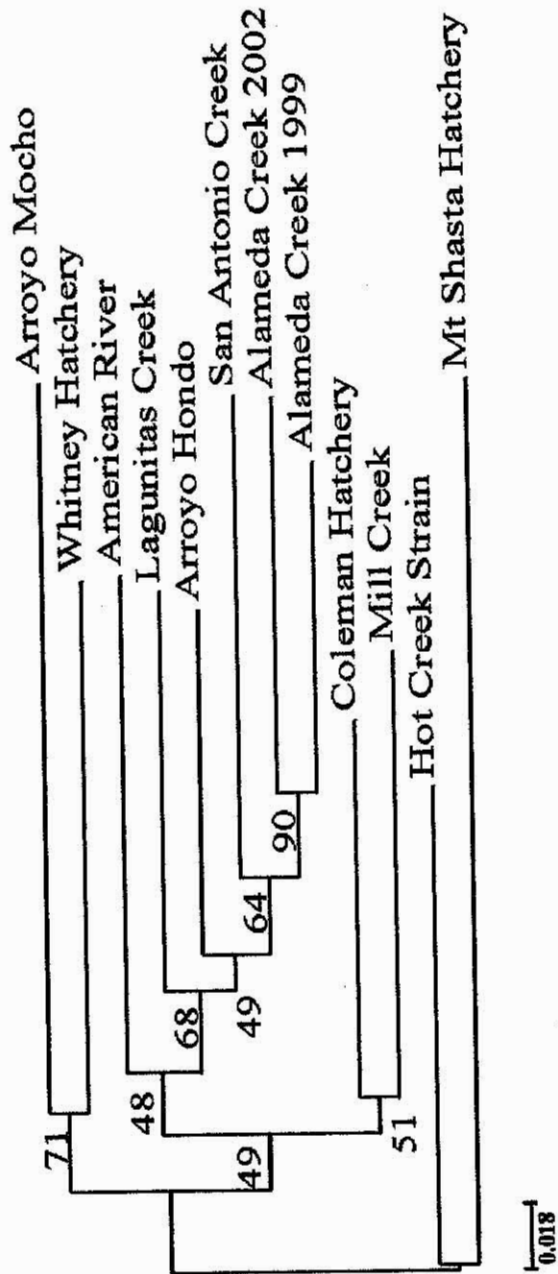
Table 3. Distribution of mtDNA haplotypes in Alameda Creek rainbow trout samples collected 2002.

| Population | MYS1 | MYS3 | MYS5 | MYS12 |
|---------------|------|------|------|-------|
| Upper Alameda | 0 | 7 | 14 | 5 |
| San Antonio | 9 | 0 | 68 | 0 |
| Arroyo Hondo | 0 | 19 | 10 | 0 |
| Arroyo Mocho | 30 | 0 | 0 | 0 |
| Total | 39 | 26 | 92 | 5 |

Mean F_{st} for all 14 loci combined equaled 0.14, with most genetic diversity (98%) occurring at the level of individuals within a population in the Alameda Creek drainage. GENEPOP's analysis of population independence was significant ($p < 0.0001$) for all paired comparisons suggesting differences in population allelic structure for each locality based on microsatellite allele frequencies. ARLEQUIN's F_{st} pairwise genetic structure analyses are given in Table 4. No significant differences in microsatellite allelic variation were found between the Alameda Creek collection analyzed in 1999 (1997-1999 collections combined) and the collection made in 2002 (excluding Arroyo Mocho). ARLEQUIN's estimated number of migrants (used as a surrogate for gene flow) among Alameda Creek trout populations was $Nm = 1.16$ fish per generation.

Genetic distance comparisons are presented as a consensus Neighbor-Joining (NJ) tree (Figure 1) for a broad geographic range of hatchery and wild rainbow trout/steelhead populations from central California. Inclusion of samples previously collected in 1997–1999 (combined sample listed as "Alameda 1999" in the tree) in these analyses provides inference to temporal and spatial population genetic structure within this drainage.

Neighbor Joining Tree



Neighbor Joining Tree Generated Using Cavalli-Sforza and Edwards (1967) Chord Distances
 Bootstrap Values Generated from NJBP2 Program

Figure 1. Neighbor joining tree generated using Cavalli-Sforza and Edwards (1967) chord distance with bootstrap values calculated for each branch based on 2000 replicate trees.

Table 4. Population pairwise distance measures based on F_{st} analyses for the 2002 Alameda Creek sample populations. All comparisons were statistically significant ($p < 0.0001$).

| Population | Alameda Creek | Arroyo Hondo | Arroyo Mocho |
|------------------|---------------|--------------|--------------|
| Arroyo Hondo | 0.08880 | | |
| Arroyo Mocho | 0.22452 | 0.17460 | |
| San Antonio Res. | 0.13037 | 0.07480 | 0.17668 |

Results

With few exceptions, microsatellite analyses of land locked rainbow trout collected from four locations on Alameda Creek (2002) gave similar results to those presented for fish collected in 1997-1999 within the same drainage (Nielsen and Fountain 1999). Microsatellite diversity supported the same close genetic relationship between Alameda Creek rainbow trout (excluding Arroyo Mocho), previously evaluated steelhead captured in Alameda Creek downstream of the BART weir (Nielsen and Fountain 1999) and coastal steelhead found in Lagunitas Creek, Marin County, California. Microsatellite diversity in Alameda Creek rainbow trout was not significantly different from that found in rainbow trout from geographically proximate San Francisquito Creek (see Nielsen 2000).

Microsatellite allelic frequencies and F_{st} analyses showed that all four sub-basins sampled in 2002 were genetically unique, but that three of the four areas (upper Alameda Creek, Arroyo Hondo and San Antonio Reservoir) were more closely related to each other than any of them were to any other sample taken from outside of the watershed. Their closest genetic relationship was with samples taken from Alameda Creek in 1997-1999 (Nielsen and Fountain 1999). We found no significant year-to-year variation among samples collected from Alameda Creek, 1997-2002.

The 2002 sample taken from Arroyo Mocho fell outside of the general Alameda Creek clade in our genetic distance Neighbor-Joining tree. Mitochondrial analyses of the 2002 Arroyo Mocho rainbow trout showed all fish carried haplotype MYS1, the most common haplotype for rainbow trout in Californian hatchery strains and a common haplotype for wild steelhead and rainbow trout in northern California (Nielsen et al. 1994a). Only one fish from Arroyo Mocho was included in our 1999 analyses and that fish also carried the MYS1 haplotype (JLN unpublished data).

Microsatellite data also put Arroyo Mocho at greater genetic distance in comparisons with the other three sub-basin collections from Alameda Creek. Neighbor-Joining analyses placed Arroyo Mocho rainbow trout on a well-supported branch (71% bootstraps), as sister taxa to rainbow trout from the Whitney Hatchery, a strain commonly used in supplementation programs in central and southern California. There are two possible reasons for the unique genetic differentiation found in this population. These fish may represent a population of rainbow trout that are introgressed with hatchery fish due to historic stocking in the drainage, or these fish may be a natural population of resident rainbow trout that were never associated with the anadromous population thought to have founded the other sample regions.

The putative adfluvial population of rainbow trout using the Arroyo Hondo tributary of Calaveras Reservoir held unique genetic signatures based on both mtDNA and microsatellite allelic diversity. Fish amplified for mtDNA in this collection carried haplotypes MYS3 and MYS5, haplotypes previously identified as important genotypes found to be specific in a central and southern California range distribution (Nielsen et al. 1994a; Nielsen 1999). Haplotype MYS3 is found at low frequencies in the Hot Creek Hatchery strain, but to date haplotype MYS5 has not been found in any of California's rainbow trout hatchery strains (Nielsen et al. 1997a and 1999; Nielsen 1999). Microsatellite F_{st} and genetic distance analyses showed the closest sub-basin genetic relationship between Arroyo Hondo and adfluvial fish from the San Antonio Reservoir.

Adfluvial rainbow trout sampled from San Antonio Creek and San Antonio Reservoir were mixed for mtDNA haplotypes MYS1 (12%) and MYS5 (88%). Both haplotypes were discussed above. Microsatellite DNA analyses placed this sub-basin population as closely related to Arroyo Hondo and at similar genetic distance between upper Alameda Creek and Arroyo Mocho. Upper Alameda Creek samples were most closely related to Arroyo Hondo fish in the 2002 sample set. Upper Alameda Creek samples were the only rainbow trout to carry mitochondrial haplotype MYS12 in this study. MYS12 has been found at low frequencies in wild steelhead populations throughout California (Nielsen et al. 1994a) and is phylogenetically similar to other unique *O. mykiss* haplotypes found in central and southern California including MYS5 (Nielsen et al. 1997b).

These data add to the accumulating evidence that rainbow trout trapped behind dams and water diversions represent significant genetic components of the native, wild *Oncorhynchus mykiss* resource found in central and southern California. The amazing adaptable life history capacity of this species with an ability to adapt to freshwater resident populations when ocean access was closed during the construction of these dams and diversions has contributed to their evolutionary viability. Despite significant hatchery supplementation throughout the small coastal drainages of California, there appears to be genetic integrity in landlocked populations of rainbow trout that are closely related to their anadromous progenitors. The evolutionary consequences of plasticity in an environmentally heterogenous metapopulation of steelhead/rainbow trout seems to be lost on the regulatory community where jurisdiction for conservation is divided between freshwater and marine resources.

One important question is whether or not the current resident stocks that show genetic identity to anadromous populations would or could contribute increased population viability for local anadromous runs, most of which have declined to the point of listings under the U.S. Endangered Species Act. Heterogeneous life history strategies in metapopulation models have show that migration of a plastic phenotype between sites is favored over local specialists across a broad range of parameter space (Kingsolver and Huey 1998; Sultan

and Spencer 2002). These models and our genetic data suggest that exchange among life history types through dispersal may play an important role in adaptive population differentiation for this species (see also Scheiner 1998 and Tufto 2000). Based on metapopulation models fitness costs to plasticity will reduce the range of conditions in which the plastic type will prevail only if the cost is both large and global rather than environmentally specific (Moran 1992; Sultan 2000).

Pascual et al. 2001 have demonstrated the establishment of anadromous populations of steelhead in Argentina from freshwater adapted parental stocks of rainbow trout (see also Behnke 2002). According to Behnke (2002) the diversity of ancestral life history forms found in most rainbow trout strain in husbandry today could have provided the hereditary basis for the development of a steelhead-like population in Argentina. Zimmerman and Reeves (2000) used otolith microchemistry to demonstrate that a small proportion (4 – 22%) of individuals in sympatric populations of steelhead and resident rainbow trout appear to have maternal contribution from their opposite life history type. These arguments suggest that landlocked rainbow trout derived from anadromous populations before urban development and stream blockage may retain significant adaptive behavior for anadromy long after adfluvial adaptation to freshwater habitats.

In this study one interesting aspect of the genetic analyses is the status of the Arroyo Mocho trout. Since these fish appear distinct from the rest of the sub-basin populations on Alameda Creek it will be important to address how and why they are so. It seems unlikely that these fish represent a population of rainbow trout that are introgressed with hatchery fish unless this sub-basin has a unique hatchery supplementation history. Further genetic study may reveal aspects of their hatchery co-ancestry. However, if these fish represent a natural population of resident rainbow trout in the Bay Area, how have they retained reproductive isolation, life history specificity and genetic integrity? If their population structure is unique to the basin and has not been influenced by hatchery stocks do they deserve any less protection and conservation efforts than the land locked anadromous derived stocks? These are questions that demand a closer

examination using an integrated approach with ecology, behavior and genetics data. Hopefully such studies will progress the thinking of the regulatory community to adjust their perspective to the true biology of this species instead of making rules based on simple jurisdictional boundaries between freshwater and marine habitats.

Literature

- Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity* 90: 281-288.
- Behnke, R. J. 2002. Comment: First documented case of anadromy in a population of introduced rainbow trout in Patagonia, Argentina. *Trans. Am. Fish. Soc.* 131: 582-585.
- Busack, C. A. and G. A. E. Gall. 1980. Ancestry of artificial propagated California rainbow trout strains. *California Fish and Game* 66: 17-24.
- Campton, D. E. 1995. Genetic effects of hatchery fish on wild populations of Pacific salmon and steelhead: what do we really know? *In: Schramm, H. L. and Piper, R. G. (eds.) Uses and Effects of Cultured Fishes in Aquatic Ecosystems*. American Fisheries Society Symposium Number 15, Bethesda, MD, pp. 337-353.
- Cavalli-Sforza, L. L. and A. W. F. Edwards, 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 32: 550-570.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 39: 783-791.
- Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package), version 3.57c. Department of genetics, University of Washington. Box 357360, Seattle, WA. 98105, U.S.A.

- Gall, G. A. E., B. Bentley and R. C. Nuzum. 1990. Genetic isolation of steelhead rainbow trout in Kaiser and Redwood creeks, California. *California Fish and Game* 76:216-223.
- Heath, D. D., S. Pollard and C. Herbinger. 2001. Genetic structure and relationships among steelhead trout (*Oncorhynchus mykiss*) populations in British Columbia. *Heredity* 86: 618-627.
- Kingsolver, J. G. and R. B. Huey 1998. Evolutionary analysis of morphological and physiological plasticity in thermally variable environments. *A. Zool.* 38: 545-560.
- Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. *Am. Nat.* 139: 971-989.
- Morris, D. B., K. R. Richard, J. M. Wright (1996). Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. *Canadian Journal Fisheries Aquatic Sciences* 53: 120-126.
- Nelson, R. J. and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. *Animal Genetics* 30: 228-229.
- Nielsen, J. L. 1999. The evolutionary history of steelhead (*Oncorhynchus mykiss*) along the US Pacific Coast: developing a conservation strategy using genetic diversity. *ICES Journal of Marine Science* 56: 449-458.
- Nielsen, J. L. 2000. Microsatellite analyses of San Francisquito Creek rainbow trout. Technical Report submitted to the Center for Conservation, Stanford University, Stanford, CA, January 12, 2000.
- Nielsen, J. L. and M. C. Fountain. 1999. Microsatellite analyses of Alameda Creek rainbow/steelhead trout. Technical Report submitted to Applied Marine Sciences, Inc. Livermore, CA. October 7, 1999.
- Nielsen, J. L. and G. K. Sage. 2001. Microsatellite analyses of the trout of northwest Mexico. *Genetica* 111: 269-278.
- Nielsen, J. L., C. A. Gan, J. M. Wright and W. K. Thomas. 1994a. Biogeographic distributions of mitochondrial and nuclear markers for southern steelhead. *Molecular Marine Biology and Biotechnology* 3: 281-293.

- Nielsen, J. L., C. A. Gan, W. K. Thomas. 1994b. Differences in genetic diversity for mtDNA between hatchery and wild populations of *Oncorhynchus*. Can. J. Fish. Aquat. Sci. 51(Suppl.1): 290-297.
- Nielsen, J. L., C. Carpanzano, M. C. Fountain and C. A. Gan. 1997a. Mitochondrial DNA and nuclear microsatellite diversity in hatchery and wild *Oncorhynchus mykiss* from freshwater habitats in southern California. Transactions of the American Fisheries Society 126: 397-417.
- Nielsen, J. L., M. C. Fountain & J. M. Wright, 1997b. Biogeographic analysis of Pacific trout (*Oncorhynchus mykiss*) in California and Mexico based on mtDNA and nuclear microsatellites, pp. 53-73 in Molecular Systematics of Fishes, edited by T. Kocher & C.A. Stepien, Academic Press, San Diego, CA.
- Nielsen, J. L., M. C. Fountain, J. C. Favela, K. Cobble, and B. L. Jensen. 1998. *Oncorhynchus* at the southern extent of their range: a study of mtDNA control-region sequence with special reference to an undescribed subspecies of *O. mykiss* from Mexico. Envir. Biol. Fish. 51: 7-23.
- O'Connell, M., R. G. Danzmann, J.-M. Cornuet, J. M. Wright and M. M. Ferguson. 1997. Differentiation of rainbow trout (*Oncorhynchus mykiss*) populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Can. J. Fish. Aquat. Sci. 54: 1391-1399.
- Olsen, J. B., P. Bentzen and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Mol. Ecol. 7: 1087-1089.
- Pascual, M., P. Bentzen, C. R. Rossi, G. Mackey, M. T. Kinnison and R. Walker. 2001. First documented case of anadromy in a population of introduced rainbow trout in Patagonia, Argentina. Trans. Am. Fish. Soc. 130: 53-67.
- Raymond, M. and F. Rousset (1997). GENEPOP version 3.1a, August 1997. Univ. Montpellier II, 34095 Montpellier cedex 05, France (available through anonymous login at <ftp.cefe.cnrs-mop.fr> or e-mail Raymond@isem.univ-montp2.fr), 30 pp.

- Reisenbichler, R. R. and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile and wild steelhead, *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada 34: 123-128.
- Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.
- Rybock, J. T., H. F. Horton and J. L. Fessler. 1975. The use of otoliths to separate juvenile steelhead trout from juvenile rainbow trout. U. S. National Marine Fisheries Service Fishery Bulletin 73: 654-659.
- Scheiner, S. M. 1998. The genetics of phenotype plasticity. VII. Evolution in a spatially structured environment. J. Evol. Biol. 11: 303-320.
- Schneider S., J.-M. Kueffer, D. Roessli and L. Excoffier. 1997. ARLEQUIN: An exploratory population genetics software environment. Available: <http://anthropologie.unige.ch/arlequin>.
- Scott, T. C. 1997. Trout's reappearance spawns controversy: significance of fish found in Alameda Creek is disputed. San Francisco Chronicle 12/26/97.
- Scribner, K. T., J. R. Gust, R. L. Fields. 1996. Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53: 685-693.
- Shapovalov, , L. and A. C. Taft. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California and recommendations for their management. State of California Department of Fish and Game Fish Bulletin No. 98.
- Sultan, S. E. 2000. Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5: 537-542.
- Sultan, S. E. and H. G. Spencer. 2002. Metapopulation structure favors plasticity over local adaptation. Am. Nat. 160: 271-283
- Swofford, D. L. & R. B. Selander, 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Heredity 72: 281-283.

- Tufto, J. 2000. The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *Am. Nat.* 156: 121-130.
- Waples, R. S. and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. *Canadian Journal of Fisheries and Aquatic Sciences* 51(Supp.1): 310-329.
- Zimmerman, C. E. and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* 57: 2152-2162.