Genetic Influence of Hatchery-Origin Fish to Natural Populations of Rainbow Trout in the Santa Ynez River, California[1]

Glenn M. Greenwald[2] and Donald E. Campton[3]

A synopsis and supplemental evaluation of:

Nielsen, Jennifer L.[4], Christian E. Zimmerman, Jeffrey B. Olson, Talia C. Wiacek, Eric J. Kretschmer, Glenn M. Greenwald, and John K. Wenburg. 2003. ***Population genetic structure of Santa Ynez River rainbow trout – 2001 based on microsatellite and mtDNA analyses***. Final report submitted to U.S. Fish and Wildlife Service, California/Nevada Operations Office, Sacramento, California, November 11, 2003. Intra-agency Agreement No. 11440-1-4000 between the U.S. Fish and Wildlife Service (Ventura, CA) and the U.S. Geological Survey (Anchorage, AK).

February 21, 2005

---

[1] Cite as: Greenwald, G.M. and D.E. Campton. 2005. Genetic influence of hatchery-origin fish to natural populations of rainbow trout in the Santa Ynez River, California. A synopsis and supplemental evaluation of: *Nielsen, Jennifer L., Christian E. Zimmerman, Jeffrey B. Olson, Talia C. Wiacek, Eric J. Kretschmer, Glenn M. Greenwald, and John K. Wenburg. 2003. Population genetic structure of Santa Ynez River rainbow trout – 2001 based on microsatellite and mtDNA analyses. Final report submitted to U.S. Fish and Wildlife Service under Intra-agency Agreement No. 11440-1-4000 between the U.S. Fish and Wildlife Service (Ventura, CA) and the U.S. Geological Survey (Anchorage, AK).* California-Nevada Operations Office (CNO), U.S. Fish and Wildlife Service, Sacramento, California. 20 pp.

[2] U.S. Fish and Wildlife Service, Red Bluff Fish and Wildlife Office, 10950 Tyler Road, Red Bluff, CA 96080 (Glenn_Greenwald@fws.gov)

[3] U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, 1440 Abernathy Creek Road, Longview, WA 98632 (Don_Campton@fws.gov)

[4] Corresponding author: Jennifer L. Nielsen, U.S. Geological Survey, Alaska Science Center, Molecular Ecology Laboratory, 1011 East Tudor Road, Anchorage, AK 99503

Introduction

Nielsen et al. (2003) used multi-locus genotypes at microsatellite, nuclear DNA (nDNA) loci and mitochondrial DNA (mtDNA) to examine the genetic structure of rainbow trout (*Oncorhynchus mykiss*) populations in the upper Santa Ynez River near Santa Barbara, California. They also compared mtDNA haplotype frequencies of those populations to those of hatchery populations and other naturally spawning populations in southern California. This synopsis summarizes those results and provides additional interpretations regarding the potential genetic contributions of introduced rainbow trout from hatchery populations to natural populations currently residing in the Santa Ynez watershed. This synopsis is intended primarily for non-geneticists and individuals responsible for water resource and land use planning in the Santa Ynez River basin.

Background

Rainbow trout, including its anadromous form (steelhead), are native to most coastal drainages of California. This native range includes the Santa Ynez River near Santa Barbara (Fig. 1). Naturally spawning populations of rainbow trout are present throughout much of the Santa Ynez River watershed.

Three mainstem dams impound the Santa Ynez River (Fig. 1). The middle dam, Gibraltar Dam, forming Gibraltar Reservoir, was completed in 1920. The upper dam, Juncal Dam, forming Jameson Reservoir, was completed in 1930. The lower dam, Bradbury Dam, forming Cachuma Reservoir, was completed in 1953. All three dams are barriers to upstream movement of fish.

Rainbow trout of hatchery origin have been stocked in California reservoirs for decades. Most hatchery stocks of rainbow can trace their genetic origins to populations in the upper Sacramento and McCloud River drainages in the Mount Shasta region of northern California. As a result, DNA markers can be used to distinguish hatchery populations of rainbow trout from native populations throughout much of their natural range. Corollary questions regarding whether rainbow trout of native ancestry were anadromous (i.e., steelhead) or non-anadromous (i.e., resident) prior to anthropogenic influences (e.g., prior to dam construction) cannot, in general, be addressed directly with molecular genetic markers.

The extent to which introduced rainbow trout may have reproduced successfully in the upper Santa Ynez River upstream of Gibraltar Dam was unknown until the work of

Nielsen et al. (2003). A major question was: Are rainbow trout populations in the upper Santa Ynez River primarily of "native" origin, hatchery origin, or some genetic admixture of the two sources.

Nielsen et al. (2003) used nuclear DNA (nDNA) markers at 13 microsatellite loci and a 314 base pair (bp) region of mitochondrial DNA (mtDNA) to genetically examine rainbow trout populations in the upper Santa Ynez River. The two types of markers complement one another because mtDNA is inherited clonally from only the female parent (i.e., with no genetic recombination), whereas nDNA markers (e.g., microsatellites) are inherited biparentally in a Mendelian manner. Mitochondrial DNA markers can thus trace genetic lineages (albeit maternal lineages) whereas nuclear DNA markers can assess the breeding structure of populations. Nielsen et al. (2003) also compared mtDNA haplotype profiles for *O. mykiss* in the Santa Ynez River to mtDNA profiles for hatchery and other wild populations in southern California. The degree to which these populations may represent anadromous (i.e., "steelhead") or resident (i.e., "rainbow trout") fish was not evaluated. As noted previously, these latter evaluations are generally not possible with molecular genetic markers because resident and anadromous fish within the same watershed generally share a common ancestry in the absence of a significant hatchery influence.

Historic stocking of trout above Gibraltar Dam

The most comprehensive report about historic stocking of trout in the Santa Ynez River watershed appears to be Entrix (2004). This report provides evidence that Jameson Reservoir was stocked prior to 1934, and that Gibraltar Reservoir was stocked at least nine times from 1932 to 1945. The sources for these fish were reported as being either from an unknown source or from rescued Santa Ynez River basin fish. Rescued Santa Ynez River Basin fish were also reported to have been used to stock Indian Creek in 1945 and Aqua Caliente Creek in 1939 and 1940.

The data presented in Entrix (2004) did not indicate that formally sanctioned trout stocking occurred after 1945 in the Upper Santa Ynez watershed upstream of Gibraltar Dam. However, several years of stocking records were unavailable, and the exact year of the last stocking in this area could not be determined.

Information from the Santa Barbara County Fish and Game Commission indicates that during the 1940's, private individuals were reported to have transferred steelhead in buckets

from “The Narrows” (a location now inundated by Cachuma Reservoir) to a location above Juncal Dam (S. Radom, pers. comm., 2005). Additionally, rainbow trout from the California Department of Fish and Game Fillmore Hatchery were reported to have been used to stock the Santa Ynez River watershed above Gibraltar Dam sometime in the late 1970’s. Although, the exact location(s) of this stocking activity was not determined, this was likely the last official stocking of the Santa Ynez River watershed above Gibraltar Dam (S. Radom, pers. comm., 2005).

None of the fish sampled for this study showed physical evidence (e.g., frayed fins, deformed fins, or missing adipose fins) of hatchery origin (G. M. Greenwald, pers. observ.). Based on the historic data, personal communications, and field observations, we believe all rainbow trout sampled for the Nielsen et al. (2003) study were likely the progeny of wild-spawned fish, rather than hatchery-spawned fish.

Field Sampling

Personnel of the U.S. Fish and Wildlife Service used electrofishing and hook-and-line fishing with barbless lures and flies to collect fin clips from 390 rainbow trout from 11 locations upstream of Gibraltar Dam in the upper Santa Ynez River watershed from May 2000 through June 2001 (Table 1; Figs. 2 through 9). Only hook-and-line fishing was used in Jameson Reservoir. Due to an unpredictable and patchy distribution, fish were collected at each water body using a semi-systematic cluster sampling design. Rather than sampling in just one or two locations, we attempted to collect specimens from all sections of each sampled water body that were inhabited by rainbow trout. Our target goal was to collect three or four fish from at least 10 geographically representative sections, and as many as 20 sections, from each sampled water body (Figs. 3 through 8). Attempts were also made to collect specimens from all habitat types in each sampled water body and from all size classes of rainbow trout at each sampling location.

Fork length of each fish was measured to the nearest mm, a GPS location was determined, and a small piece of fin tissue was removed with surgical scissors from the upper lobe of the caudal fin (approximately 4-16 mm^2). Fin clips were placed in labeled 2.0 ml vials filled with 100% ethanol. Fin clips were subsequently divided in half and placed into separate, duplicate numbered vials containing 100% ethanol. The duplicate specimens were mailed on separate dates to two different genetics laboratories in Anchorage, Alaska: the U.S.

Geological Survey (USGS) lab directed by Jennifer Nielsen and the U.S. Fish and Wildlife Service lab, now directed by John Wenburg (Nielsen et al. 2003). A total of 376 fin tissue specimens were successfully extracted for nDNA analysis, and 346 specimens were successfully extracted for mtDNA analysis (Table 1).

Principal Results

Major findings of Nielsen et al. (2003)

Allele frequencies at the nDNA loci differed significantly ($p < 0.05$) among all populations (localities) in the upper watershed above Gibraltar Dam, except between two localities upstream from Juncal Dam: Jameson Reservoir and the North Fork of Juncal Creek.

Overall patterns of genetic structuring were, with one exception, concordant between the mtDNA and nDNA results (Fig. 2 of Nielsen et al. (2003)). As reported by Nielsen et al. (2003), levels of divergence among populations were greater for mtDNA than nDNA, most likely reflecting greater genetic drift effects associated with the former type of markers.

Nielsen et al. (2003) found populations of rainbow trout upstream of Juncal Dam to be diverged genetically from populations downstream from Juncal Dam in the upper watershed above Gibraltar Dam (Fig. 2 of Nielsen et al. 2003). The three sampled populations upstream of Juncal Dam grouped together with 100% bootstrap probability. Similarly, the five sampled populations between Juncal Dam and Gibraltar Dam grouped together with 99% probability.

Detailed examination of Table 6 of Nielsen et al. (2003) suggests that the *relative frequencies* of mtDNA haplotypes *MYS1* and *MYS3* among Santa Ynez populations, and other coastal populations of *O. mykiss* in southern California, most likely reflect the extent to which introduced rainbow trout of hatchery-origin (at least females) have successfully reproduced in those watersheds (see Figs. 3 through 9 of this synopsis). Haplotypes *MYS1* and *MYS3* predominate in California *hatchery* populations of rainbow trout (mean frequency = 0.833 and 0.129, respectively; Table 6 of Nielsen et al. (2003)). Conversely, haplotypes *MYS5* and *MYS8* have never been observed in California hatchery populations and may be unique to *native* populations in southern California (Nielsen et al. 1997b, 1998).

Genetic influence of hatchery-origin fish in the Santa Ynez River

Nielsen et al. (2003) report that haplotype frequencies for mtDNA did not differ significantly ($p > 0.05$) among three natural populations in the lower Santa Ynez River watershed (Cachuma Reservoir, Hilton Creek, and the lower Santa Ynez River) and one or more hatchery strains, suggesting that *O. mykiss* in those latter three populations were largely the descendants of introduced hatchery fish. As noted also by Nielsen et al. (2003), haplotype frequencies for all other populations in the Santa Ynez River, including those upstream of Gibraltar Dam, differed significantly from each of five hatchery populations ($p < 0.05$).

Populations of rainbow trout upstream of Juncal Dam in the upper Santa Ynez River, and in Alder Creek immediately downstream from Juncal Dam, appear to have been influenced genetically by introduced hatchery fish (see Tables 4 and 6 of Nielsen et al. 2003, and Figs. 3 through-5 of this synopsis). The combined frequencies of the *MYS1* and *MYS3* haplotypes ranged from 0.21 to 0.49 for those four populations, suggesting significant hatchery influence. However, those populations also retained haplotypes *MYS5* and *MYS8* at significant frequencies (0.51 to 0.79), thus reflecting also an apparent native genetic component.

On the other hand, rainbow trout inhabiting four sampled tributaries between Juncal Dam and Gibraltar Dam (Gidney Creek, Camuesa Creek, Fox Creek, and Blue Canyon Creek) exhibited little or no mtDNA evidence of a genetic influence from non-native hatchery fish. Combined frequencies of the "native" *MYS5* and *MYS8* haplotypes in those streams were 1.00 for Gidney and Camuesa creeks, 0.98 for Fox Creek, and 0.96 for Blue Canyon Creek (Figs. 5 through 9). Only one fish in Fox Creek and two fish in Blue Canyon Creek showed mtDNA evidence of a potential hatchery influence out of a total of 87 fish analyzed.

Additional mtDNA data not presented by Nielsen et al. (2003)

Results of the mtDNA analysis for 25 rainbow trout from three additional sampling locations were excluded from the population analyses of Nielsen et al. (2003). These specimens (Table 1) included 18 fish from the uppermost fish-bearing reaches of the upper Santa Ynez River (all 18 fish were *MYS5;* Fig. 3), five fish from Indian Creek (three *MYS5* and two *MYS8* individuals; Fig. 2), and two fish from Agua Caliente Creek (Fig. 2), both of

which had haplotype *MYS5*. Hence, all of those 25 fish expressed *native* mtDNA haplotypes *MYS5* or *MYS8*. This slightly reduces the range of frequencies for haplotypes MYS1 + MYS3 upstream of Gibraltar Dam from 0.21-0.49 to 0.15-0.49 (Table 1, Fig. 9).

Results for fish above passage barriers and impediments

Mitochondrial DNA haplotypes were determined for 25 rainbow trout upstream of fish passage barriers or impediments. These 25 rainbow trout are represented by 16 of the 25 fish discussed in the paragraph immediately above, plus nine additional fish, as follows:

Indian Creek. The Indian Creek sample site was located upstream of the 18-foot high Mono Debris Basin, a fish passage barrier (Figs. 2, 9). We sampled several locations of Indian Creek about 4.1 stream miles upstream of the Mono Debris Basin. A total of five rainbow trout were analyzed. Three of these fish had haplotype *MYS8*, and two fish had haplotype *MYS5* (Table 1, Fig. 9). As noted previously, these fish were omitted from the analyses of Nielsen et al. (2003).

Upper Santa Ynez River mainstem. We analyzed 11 rainbow trout specimens collected upstream of the 6-foot high Juncal Road wet crossing at the upper Santa Ynez campground (Fig. 3). This road crossing appears to be a fish passage impediment, rather than a fish passage barrier (G. M. Greenwald, pers. observ.). All 11 analyzed specimens were haplotype *MYS5* (Table 1; Figs. 3, 9). As noted previously, these fish were omitted from the analyses of Nielsen et al. (2003).

Alder Creek. We collected and analyzed nine rainbow trout from locations upstream of the 10-feet high Alder Creek Diversion Dam (Figs. 2, 5). This diversion dam appears to be a fish passage impediment rather than a complete fish passage barrier (G. M. Greenwald, pers. observ.). Three of the specimens upstream from that barrier were haplotype *MYS1*, and six were haplotype *MYS5* (Table 1, Fig. 9). These fish were included in the analyses of Nielsen et al. (2003).

North Fork Juncal Creek. We conducted visual and electrofishing surveys on the reach approximately 600 yards upstream of the 25-foot waterfall on North Fork Juncal Creek (Figs. 2 and 4). No fish of any species were detected in this reach.

Discussion and Conclusions

The mtDNA results presented by Nielsen et al. (2003) are consistent with the hypothesis that introduced rainbow trout of hatchery origin have made a greater mean genetic contribution to sampled populations upstream of Juncal Dam (North Fork Juncal Creek, Jameson Reservoir, upper Santa Ynez River above Jameson Reservoir) and Alder Creek (located about 3.1 stream miles downstream from Juncal Dam) than to the other sampled populations upstream of Gibraltar Dam (i.e., Gidney Creek, Camuesa Creek, Blue Canyon Creek, Fox Creek. Indeed, those latter four populations, including fish from Indian Creek and Aqua Caliente Creek, showed little evidence for a hatchery genetic influence (Table 1, Fig. 9). The general consistency between the mtDNA and microsatellite trees, except for Fox and Alder creeks (Fig. 2 of Nielsen et al. 2003), further suggests that nuclear genetic complements – with respect to native versus introduced genes in the Santa Ynez River - are most likely similar to those suggested by the mtDNA data.

Despite the suspected genetic introgression from introduced rainbow trout upstream of Juncal Dam and in Alder Creek, those populations and others throughout the upper Santa Ynez River still retain significant, native genetic complements as evidenced by the presence of haplotypes *MYS5* and *MYS8* (Table 1, Fig. 9). Overall, 317 of 391 rainbow trout (81.1%) sampled for the current study (n = 346), and previously in Fox and Alder creeks (n = 45), throughout the upper Santa Ynez River watershed (i.e., upstream from Gibraltar Dam) retained mtDNA haplotypes of presumed native origin (*MYS5* or *MYS8*) that are not known from hatchery strains of rainbow trout (Nielsen et al. 2003). Overall, 55 of 346 fish (15.9%) analyzed for the present study had mtDNA haplotypes *MYS1* or *MYS3,* suggestive of a hatchery influence, but those fish were concentrated upstream of Juncal Dam and in Alder Creek immediately downstream from the dam (Fig. 9).

The mtDNA data from several studies were collated in Table 6 of Nielsen et al. (2003). Those data show that 129 of 536 *O. mykiss* (24%) analyzed for mtDNA from the Santa Ynez River watershed had haplotype *MYS1* or *MYS3* characteristic of hatchery strains in California.

Despite the preceding interpretations, we cannot exclude the possibility that the *MYS1* and *MYS3* haplotypes occurred naturally at low frequencies among southern California populations of *O. mykiss* prior to any hatchery fish introductions. Consequently, the mere presence of those haplotypes at low frequencies is not necessarily direct evidence that

hatchery-origin rainbow trout have successfully reproduced. However, the significantly *higher* frequencies of those haplotypes in particular drainages and subdrainages relative to other populations in the same or adjacent watersheds (e.g., upstream of Juncal Dam and in Alder Creek versus elsewhere in the upper Santa Ynez River) is strong evidence for a significant genetic influence by hatchery-origin rainbow trout in those former areas.

As noted previously, most hatchery strains of nonanadromous rainbow trout were founded in the mid-to-late 1800's from wild fish taken from the upper Sacramento River region of northern California. The results of this long-term aquaculture are reflected today in the Mount Shasta Hatchery rainbow trout strain. This hatchery strain is dominated by two mtDNA haplotypes, *MYS1* and *MYS3* (Table 6 of Nielsen et al. 2003). Those haplotypes are found in many wild populations throughout California, and populations with those haplotypes remain ambiguous as to hatchery or wild origins. However, the general absence or low frequencies of those haplotypes among several natural populations in southern California, including their absence or very low frequencies in populations upstream from known natural barriers, further support the hypothesis that these haplotypes were naturally rare among native populations of rainbow trout (and steelhead) in southern California. Thus, the presence of those haplotypes at significant frequencies most likely reflects a hatchery influence.

Based on historical stocking records, rainbow trout were stocked in Jameson Lake upstream of Juncal Dam at least once in the early 1930's (CDFG 1934, as cited by Entrix 2004). According to the Entrix (2004) report, "rainbow trout had been stocked in the past [in Jameson Lake] with fair success (CDFG 1934)." Unfortunately, "the year of planting or number of fish planted are not known. It is only known that rainbow trout were planted in Jameson Lake before 1934" (Entrix 2004). As noted previously, Juncal Dam was completed in 1930, thus narrowing the documented rainbow trout introductions to the early 1930's.

In summary, rainbow trout in the upper Santa Ynez River upstream of Gibraltar Dam appear to have largely been derived genetically from native populations. However, hatchery-origin fish appear to have also made significant genetic contributions (20 - 50%) to populations upstream of Juncal Dam and in Alder Creek immediately downstream from that dam.

Acknowledgements

Bill Spearman (USFWS, Anchorage, Alaska) provided assistance with the initial project design. Genetic analyses of nDNA and mtDNA were performed in the laboratories of Jennifer Nielsen (U.S. Geological Survey [USGS], Anchorage, Alaska) and John Wenburg (USFWS, Anchorage, Alaska), respectively. Genetic analyses were conducted under a transfer fund agreement from the USFWS, Ventura Fish and Wildlife Office, Ventura, California. Review of this synopsis was provided by William Ardren, Meredith Bartron, Tom Kisanuki, and John Wenburg. We also wish to acknowledge the following people for their support with this project: Jim Adams, Louis Anadaloro, Terri Austin, Ted Bachurin, Michele Berry, Roger Bloom, Valary Bloom, Sandra Britton, Maurice Cardenas, Cindy Carpanzano, Josh Chinn, Frank Dealy, Steven Delongfield, Scott Engblom, Nikolai Ferrel, Maeton Freel, Grace McLaughlin, Judy Hohman, Stuart Hurlbert, Korie Johnson, Marian Kadota, Steve Kirkland, Chris Lento, Kelley Lewelling, Steve Miller, Kevin Niemela, Bob Null, Stan Radom, Moises Lopez Rodarte, Kevin Sage, Jim Smith, John Southwick, Ed Toves, Jamie Uyehara, Scott Volan, and Kirk Waln. We thank the following agencies for granting permission to access sampling locations: Los Padres National Forest, Montecito Water District, and City of Santa Barbara Water Resources Division. This project was partially funded by a grant (Transfer Fund Agreement No. 11440-9600-0000) from the USFWS Federal Aid in Sport Fishing Restoration Act program (reverted Dingell-Johnson Act funds) to the Ventura Fish and Wildlife Office.

References

California Department of Fish and Game (CDFG). 1934. Lake Survey for Jameson Lake. July 17, 1934. (As cited by Entrix 2004.)

Entrix. 2004. Historical rainbow trout/steelhead stocking in the Santa Ynez River above Bradbury Dam. Prepared for Cachuma Project Adaptive Management Committee, Santa Barbara, California. Prepared by Entrix, Inc., Walnut Creek California. Project No. 3080802. January 20, 2004.

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.

Nielsen, J. L., C. E. Zimmerman, J. B. Olson, T.C. Wiacek, E. J. Kretschmer, G. M. Greenwald, and J. K. Wenburg. 2003. Population genetic structure of Santa Ynez River rainbow trout – 2001 based on microsatellite and mtDNA analyses. Final report submitted to U.S. Fish and Wildlife Service, California/Nevada Operations Office, Sacramento, California, November 11, 2003.

Radom, S. 2005. Personal communication between Stan Radom, Santa Barbara Fish and Game Commission, Santa Barbara, California, and Glenn Greenwald, U.S. Fish and Wildlife Service, on February 21, 2005.

Stoecker, M.W. 2004. Steelhead migration barrier inventory and recovery opportunities for the Santa Ynez River, CA. Prepared for the Community Environmental Council, Santa Barbara, California. January 29, 2004. 228 pp., plus appendices.

Fig. 1. Regional map for Upper Santa Ynez Trout Genetics Study, Santa Barbara County, California.

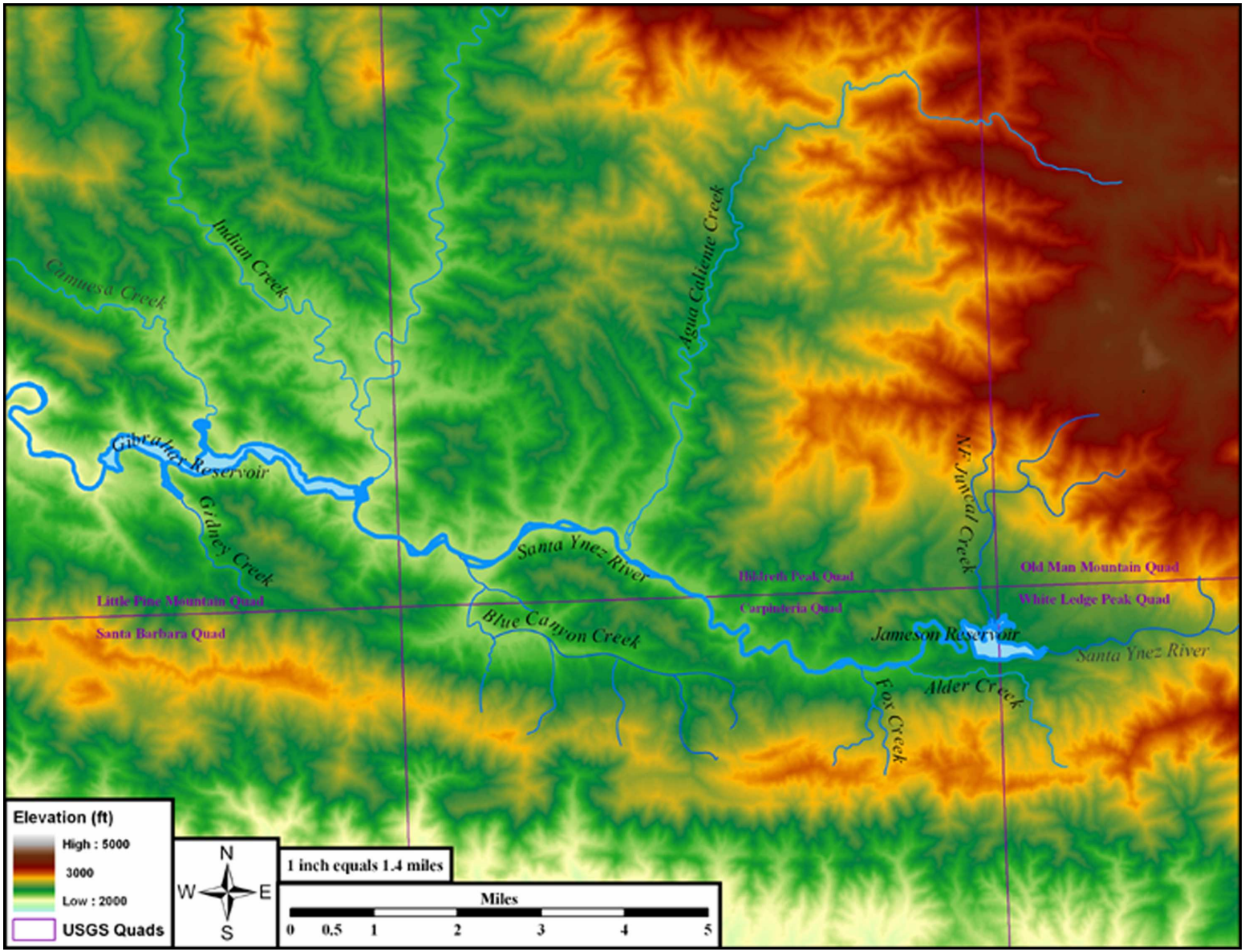

Fig. 2. Digital elevation model (DEM) map of Upper Santa Ynez Trout Genetics Study project area, Santa Barbara County, California.

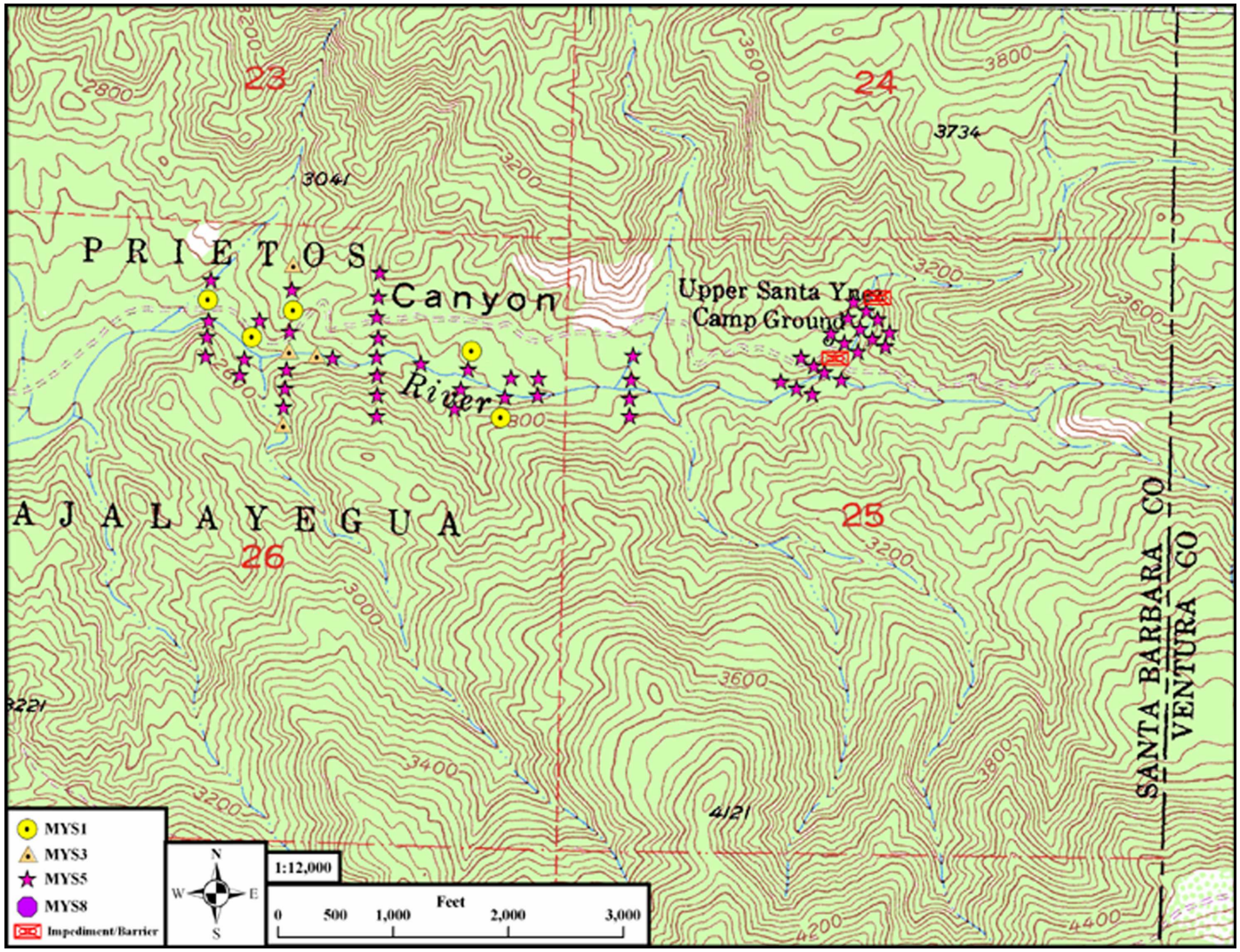

Fig. 3. Map of portion of White Ledge Peak USGS 7.5' Quad, Santa Barbara County, California. The recorded GPS location and mitochondrial DNA haplotype are plotted for individual rainbow trout collected in the upper Santa Ynez River mainstem above Jameson Reservoir (n = 60) by the USFWS during 2000 – 2001. Collection locations are approximate, and symbols from locations with multiple specimens have been artificially dispersed to facilitate differentiation. Specimen location symbols were artificially dispersed in lines that run approximately perpendicular to the creek bed. Sample locations with more than four specimens indicated represent a 25 - 100 yard long section of stream that was sampled at multiple locations, with only one waypoint being recorded.

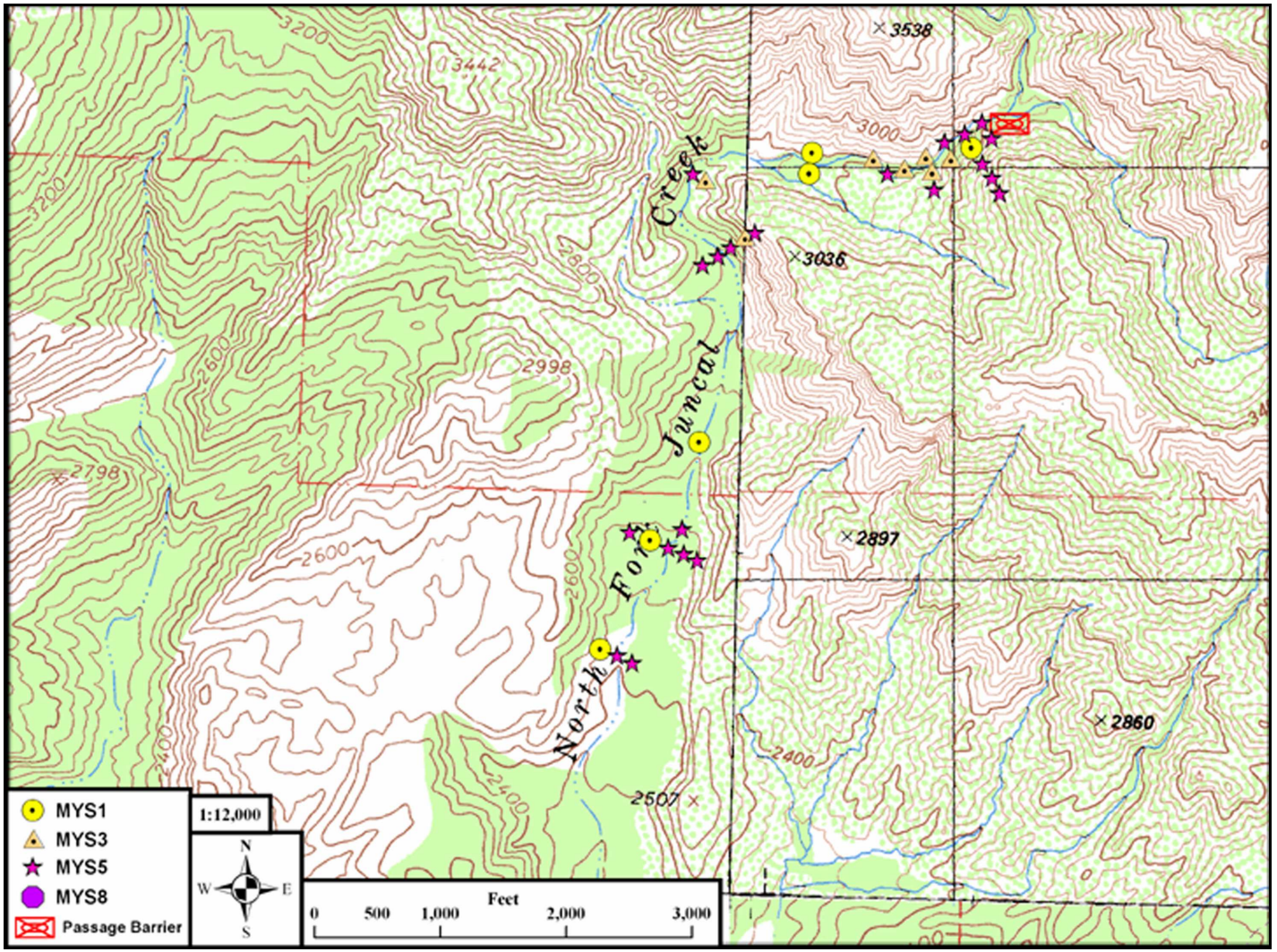

Fig. 4. Map of portions of Hildreth Peak and Old Man Mountain 7.5 minute USGS quads, Santa Barbara County, California. The recorded GPS location and mitochondrial DNA haplotype are plotted for individual rainbow trout collected in North Fork Juncal Creek (n = 34) by the USFWS during 2000 – 2001. Collection locations are approximate, and symbols from locations with multiple specimens have been artificially dispersed to facilitate differentiation. Specimen location symbols were artificially dispersed in lines that run approximately perpendicular to the creek bed. Sample locations with more than four specimens indicated represent a 25 - 100 yard long section of stream that was sampled at multiple locations, with only one waypoint being recorded.

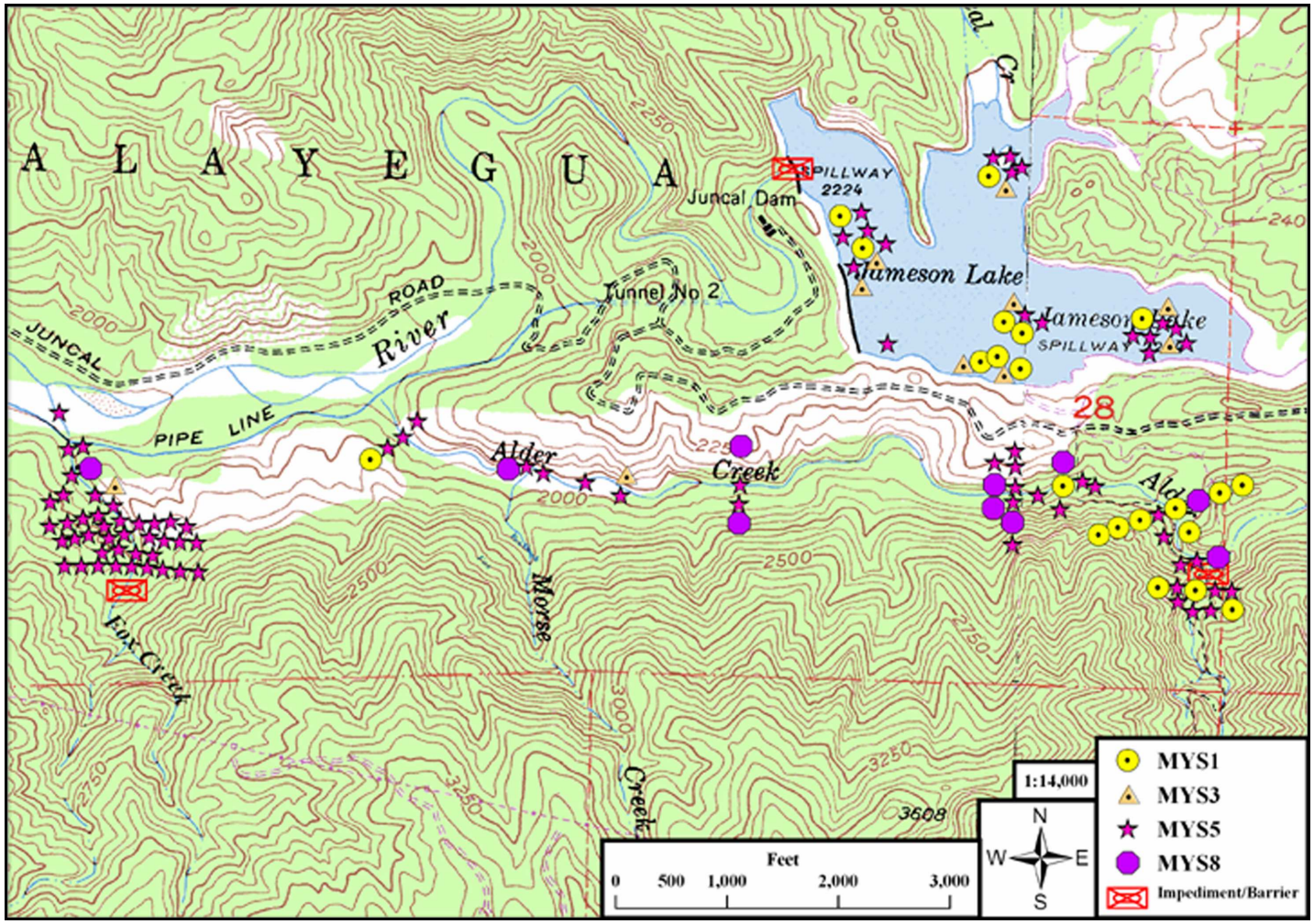

Fig. 5. Map of portions of Carpinteria and White Ledge 7.5 minute USGS quads, Santa Barbara County, California. The recorded GPS location and mitochondrial DNA haplotype are plotted for individual rainbow trout collected in Jameson Reservoir (n = 35), Alder Creek (n = 51), and Fox Creek (n = 42, far left tributary) by the USFWS during 2000 – 2001. Collection locations are approximate, and symbols from locations with multiple specimens have been artificially dispersed to facilitate differentiation. Sample locations with multiple specimens in Jameson Reservoir were artificially dispersed in a cluster around the actual collection point. Sample locations with multiple specimens from Alder and Fox creeks were artificially dispersed in lines that run approximately perpendicular to the creek beds. Creek sample locations with more than four specimens indicated represent a 10 - 50 yard long section of stream that was sampled at multiple locations, with only one waypoint being recorded.

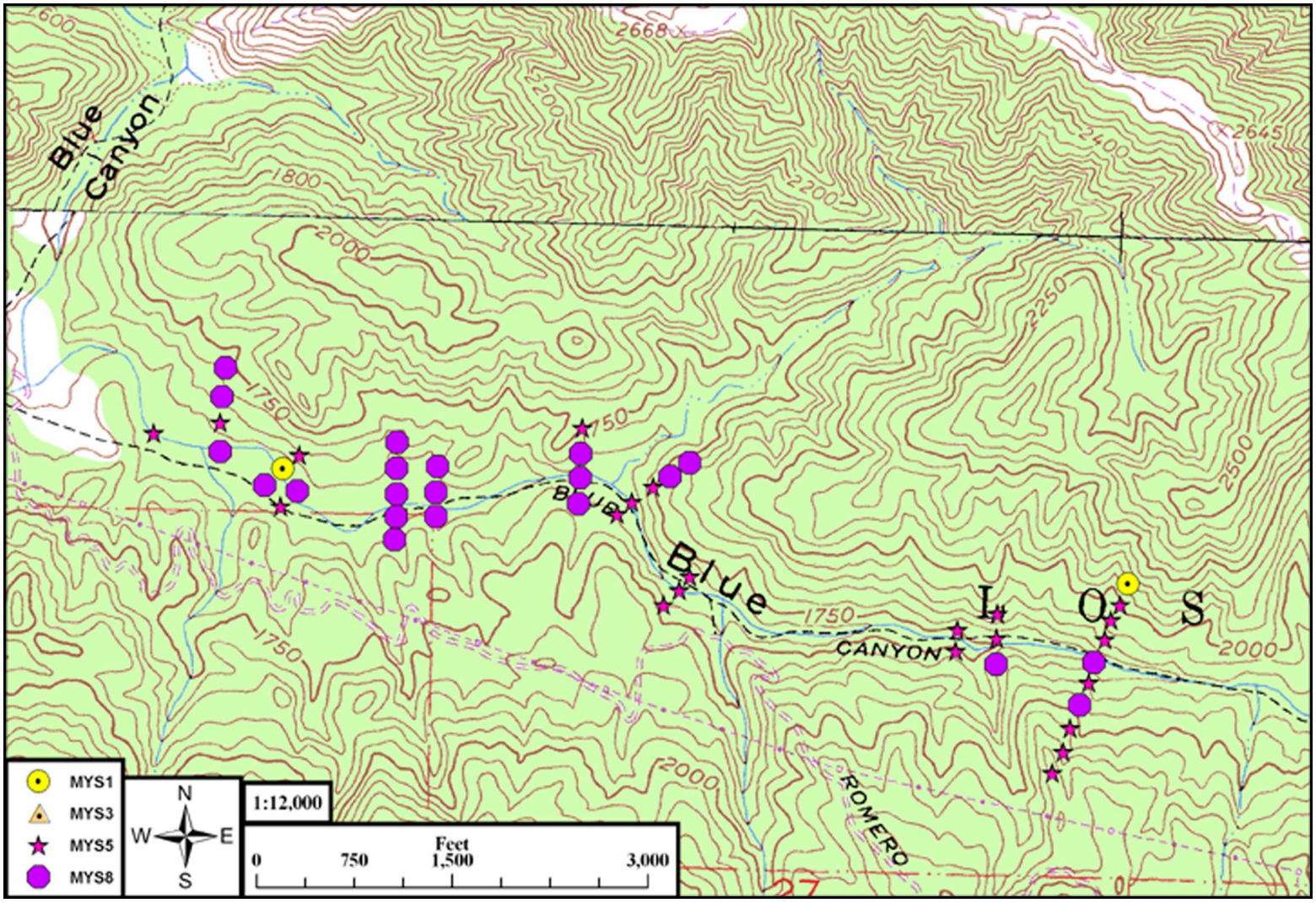

Fig. 6. Map of portions of Carpinteria and Hildreth Peak 7.5' USGS quads, Santa Barbara County, California. The recorded GPS location and mitochondrial DNA haplotype are plotted for individual rainbow trout collected in Blue Canyon Creek (n = 45) by the USFWS during 2000 – 2001. Collection locations are approximate, and symbols from locations with multiple samples have been artificially dispersed to facilitate differentiation. Specimen location symbols were artificially dispersed in lines that run approximately perpendicular to the creek bed. Sample locations with more than four specimens indicated represent a 25 - 100 yard long section of stream that was sampled at multiple locations, with only one waypoint being recorded.

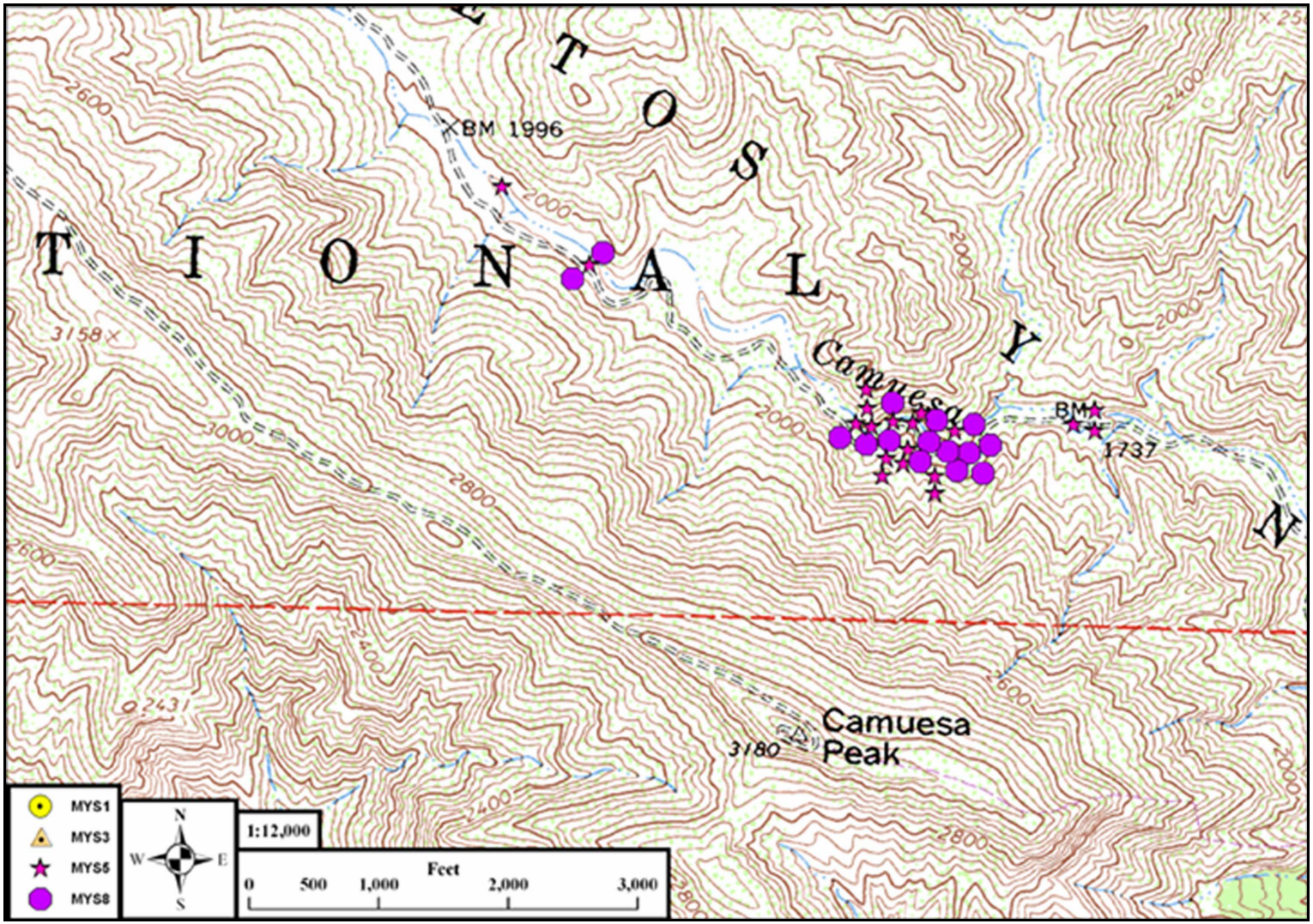

Fig. 7. Map of portion of Little Pine Mountain 7.5' USGS Quad, Santa Barbara County, California. The recorded GPS location and mitochondrial DNA haplotype are plotted for individual rainbow trout collected in Camuesa Creek (n = 34) by the USFWS during 2000 – 2001. Collection locations are approximate, and symbols from locations with multiple specimens have been artificially dispersed to facilitate differentiation. Specimen location symbols were artificially dispersed in lines that run approximately perpendicular to the creek bed. Sample locations with more than four specimens indicated represent a 25 - 100 yard long section of stream that was sampled at multiple locations, with only one waypoint being recorded.

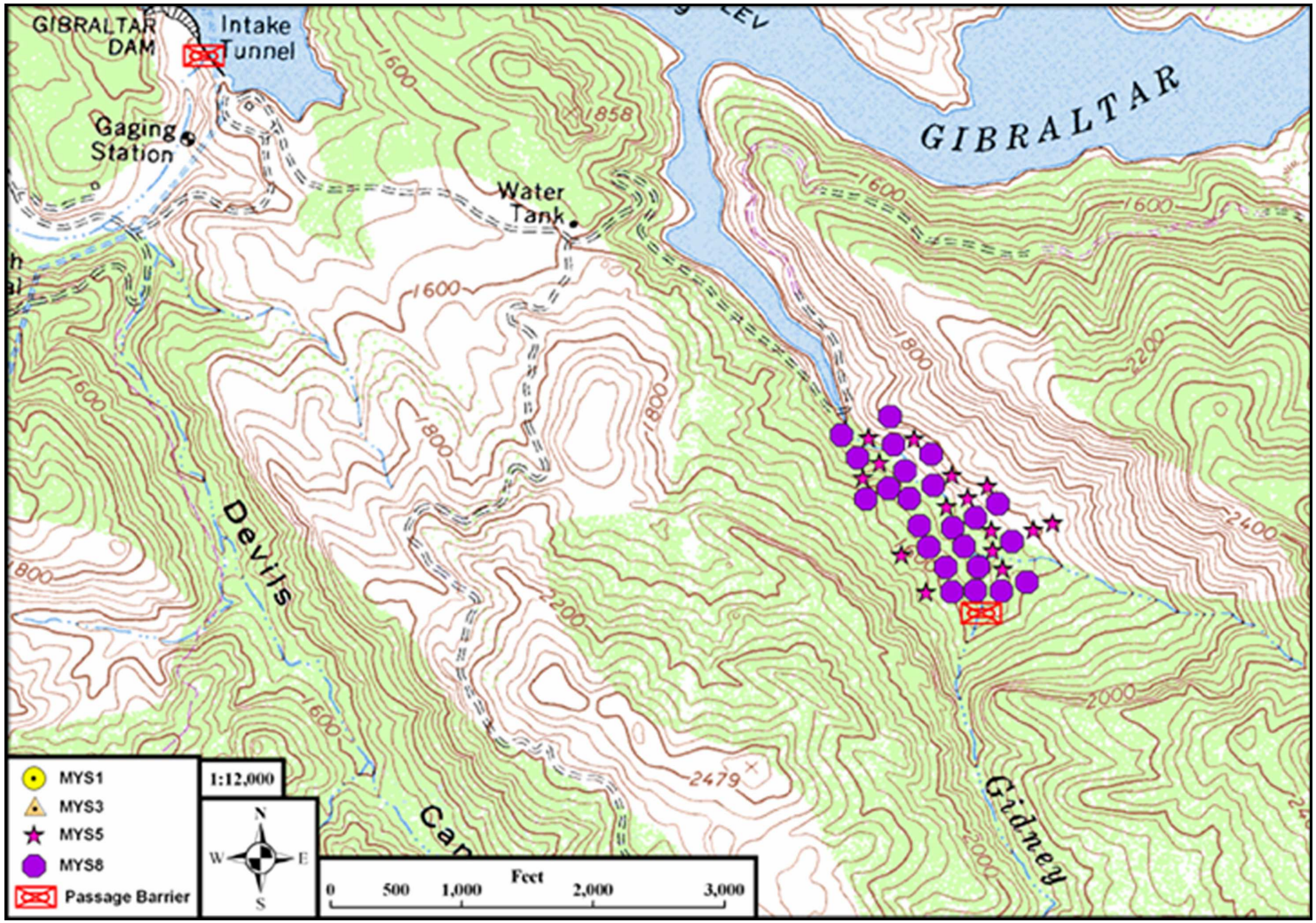

Fig. 8. Map of portion of Little Pine Mountain 7.5' USGS Quad, Santa Barbara County, California. The recorded GPS location and mitochondrial DNA haplotype are plotted for individual rainbow trout collected in Gidney Creek (n = 38) by the USFWS during 2000 – 2001. Collection locations are approximate, and symbols from locations with multiple specimens have been artificially dispersed to facilitate differentiation. Specimen location symbols were artificially dispersed in lines that run approximately perpendicular to the creek bed. Sample locations with more than four specimens indicated represent a 25 – 50 yard long section of stream that was sampled at multiple locations, with only one waypoint being recorded.

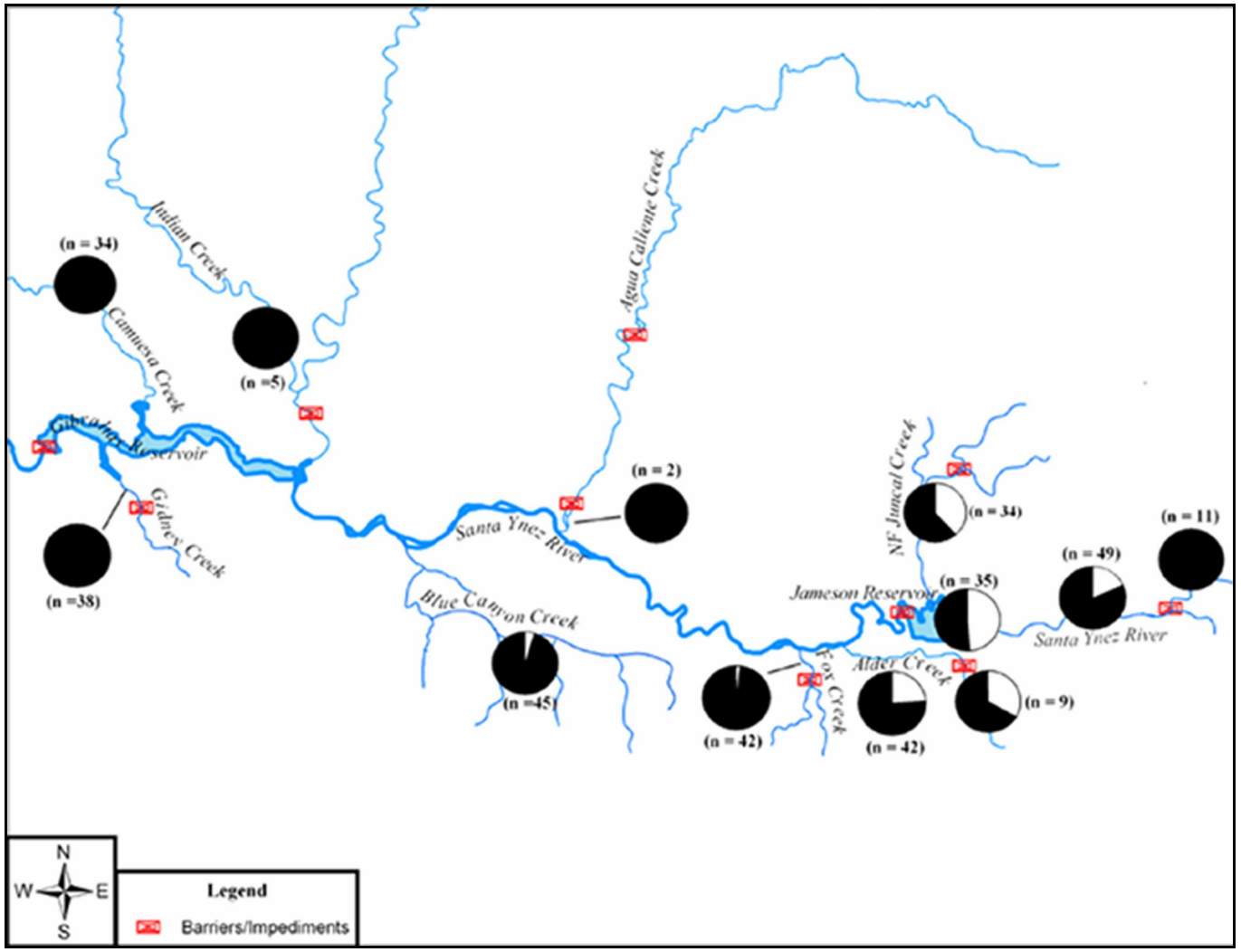

Fig. 9. Mitochondrial DNA (mtDNA) haplotype frequencies for rainbow trout sampled by the U.S Fish and Wildlife Service from water bodies in the Upper Santa Ynez River watershed, Santa Barbara County, California during 2000 and 2001. Pie charts indicate the relative proportion of mtDNA haplotypes found in proportion to all specimens analyzed for each water body. White portion of pie charts indicates combined haplotypes MYS1 and MYS3. Black portion of pie charts indicates combined haplotypes MYS5 and MYS8. Numbers next to pie charts indicate total number of specimens analyzed for mtDNA for each sampled water body. All fish passage barriers and impediments are not indicated.

Table 1. Summary of rainbow trout fin clip specimens conducted by the U.S. Fish and Wildlife Service during 2000 and 2001 in the upper Santa Ynez River watershed, Santa Barbara County, California. The sample locations are geographically listed from the most upstream (SYR above Jameson) to the most downstream (Gidney Creek). Abbreviations: nDNA = nuclear DNA, NF = North Fork, mtDNA = mitochondrial DNA, SYR = Santa Ynez River mainstem. *Specimens from Agua Caliente Creek and Indian Creek were not included in the statistical analyses of Nielsen et al. (2003). Also, 18 specimens (all haplotype *MYS5*) from the upper Santa Ynez River mainstem above Jameson Reservoir were not included in Nielsen et al. (2003).

Sample Location	**Total # Fin Clips Collected**	**# Specimens Completed for nDNA Analysis**		**# Specimens Completed for mtDNA Analysis**	**# Haplotype *MYS1***	**# Haplotype *MYS3***	**# Haplotype *MYS5***	**# Haplotype *MYS8***
SYR above Jameson*	64	59		60	5	4	51	0
Jameson Reservoir	38	38		35	9	8	18	0
NF Juncal Creek	37	37		34	6	7	21	0
SYR below Jameson	2	0		0	---	---	---	---
Alder Creek	54	54		51	12	1	29	9
Fox Creek	56	56		42	0	1	40	1
Aqua Caliente Creek*	2	0		2	0	0	2	0
Blue Canyon Creek	48	48		45	2	0	22	21
Indian Creek*	5	0		5	0	0	3	2
Camuesa Creek	41	41		34	0	0	19	15
Gidney Creek	43	43		38	0	0	15	23
Totals:	**390**	**376**		**346**	**34**	**21**	**220**	**71**