

The origin of tiger salamander (*Ambystoma tigrinum*) populations in California, Oregon, and Nevada: introductions or relicts?

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Abstract Whether intentionally or accidentally introduced, exotic species have the capacity to dramatically disrupt native communities. In central California, tiger salamanders (*Ambystoma tigrinum*) have been introduced as a by-product of the sport fishing bait industry. Some of these introductions are relatively well known and have resulted in the formation of hybrids with the imperiled native California tiger salamander (*A. californiense*). Other populations of *A. tigrinum*, particularly in the northern and eastern parts of the state, remain poorly characterized and are present in regions where relictual amphibian populations of other species have persisted, suggesting that these might be relictual, native *A. tigrinum*. We used genetic sequence data to determine the provenance of all known extralimital *A. tigrinum* populations in California and adjacent Oregon and Nevada through comparison with reference samples from the native range of *A. tigrinum*. Our results suggest that *A. tigrinum* have been introduced

in Northern California, Southern California and the Sierra Nevada, originating from multiple sources across the Great Plains of the US. Furthermore, two populations near the California-Oregon border are most closely related to *A. tigrinum* populations from Washington and Oregon and may represent native tiger salamander lineages.

Keywords Hybridization · Introduced species · Mitochondrial DNA · Non-indigenous species

Introduction

The anthropogenic spread of species beyond their natural ranges has long been recognized as a negative consequence of the globalization of human activities (Elton 1958). Non-indigenous species threaten the biodiversity of native communities and perturb ecosystem function through predation, competition, and habitat alteration (Lodge 1993; Kolar and Lodge 2001). In addition, when introductions bring closely related species into secondary contact, hybridization and “genetic extinction” may occur (Rhymer and Simberloff 1996).

Non-indigenous species have been identified as a major threat to global amphibian diversity (e.g., Blaustein and Wake 1990; Beebe and Griffiths 2005; Kraus 2009) and particularly California amphibian diversity (Bury and Luckenbach 1976; Fisher and Shaffer 1996). Well-known examples of non-indigenous species that negatively affect California’s native amphibians through competition and predation include bullfrogs (*Rana catesbeiana*; Moyle 1973; Kupferberg 1997; Kiesecker and Blaustein 1998; Pearl et al. 2004), crayfishes (Gamradt and Kats 1996) and fishes (Hayes and Jennings 1986; Bradford 1989; Gamradt and Kats 1996; Lawler et al. 1999; Knapp and Matthews

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2000). Additionally, tiger salamanders (*Ambystoma tigrinum*) have been widely introduced into California and throughout the western United States (Collins et al. 1988), leading to hybridization with native populations (Riley et al. 2003; Storfer et al. 2004) and the widespread potential for the spread of disease (Picco and Collins 2008). Hybridization is an often-overlooked consequence of biological invasions because it occurs more rarely and is more difficult to detect than the effects of competition and predation. But the effects of genetic invasions can be great (Allendorf et al. 2001), and there are now several examples of the negative effects of hybridization with non-indigenous amphibian taxa (Arntzen and Thorpe 1999; Storfer et al. 2004; Holsbeek et al. 2008; Ryan et al. 2009; Fitzpatrick et al. 2010).

The tiger salamander is a wide-ranging North American species that occurs from the East Coast of the United States across the Great Plains and into the Colorado, Wyoming, and Utah Rocky Mountains. Tiger salamanders are generally absent from the Great Basin deserts of Utah, Nevada, Oregon, and California, but natural populations occur in the Palouse Prairie region of central and eastern Washington (Nussbaum et al. 1983). The Washington portion of the range is disjunct from more eastern US populations in the shortgrass prairie of Montana, Idaho and Wyoming, with a distributional break along the Snake River Valley of Idaho. *Ambystoma tigrinum* has traditionally been viewed as consisting of six subspecies that exhibit variation in life history characteristics, behavior, and morphology (Petranka 1998). Molecular divergence estimates are high between the eastern and western forms of *A. tigrinum*, and low among the western subspecies (Shaffer and McKnight 1996), leading some authors to recognize two species (*A. tigrinum* in the east, *A. mavortium* in the west) and abandon subspecies designations for the western taxa *A. diabolii*, *A. mavortium*, *A. melanostictum*, and *A. nebulosum* (e.g., Stebbins 2003). Given the considerable uncertainty surrounding the systematics of the group, we continue to use the traditional classification, and refer to all of these taxa as *A. tigrinum*.

Currently, three types of tiger salamanders are recognized in California. (1) The California tiger salamander (*A. californiense*) occupies the Central Valley and inner coast ranges of central and southern California (Shaffer and Trenham 2005) and is distinct from *A. tigrinum* with respect to ecology and life-history characteristics (Petranka 1998). The California tiger salamander is a California endemic species and is listed as “Threatened” (in the central part of its range) or “Endangered” (in Sonoma and Santa Barbara Counties) under the US Endangered Species Act, and is considered a threatened species under the California Endangered Species Act. (2) *A. californiense* × *A. tigrinum* hybrids result from intentional introductions of

A. tigrinum into the Salinas Valley of California (Riley et al. 2003). Hybridization in the Salinas Valley has resulted in the introgression of non-native genes into native populations through a large fraction of *A. californiense*'s native range (Fitzpatrick et al. 2009). (3) Numerous other populations of *A. tigrinum* have been reported in California and nearby Oregon and Nevada. These populations occur outside the range of both *A. californiense* and *A. tigrinum* as isolated populations in Northern California, Southern California and Great Basin habitats east of the Sierra Nevada (Fig. 1). It is on this third set of populations that we focus our attention in this paper.

Based on both morphology (Mullen and Stebbins 1978) and limited mtDNA genotyping (Shaffer and McKnight 1996) these populations are clearly neither *A. californiense*

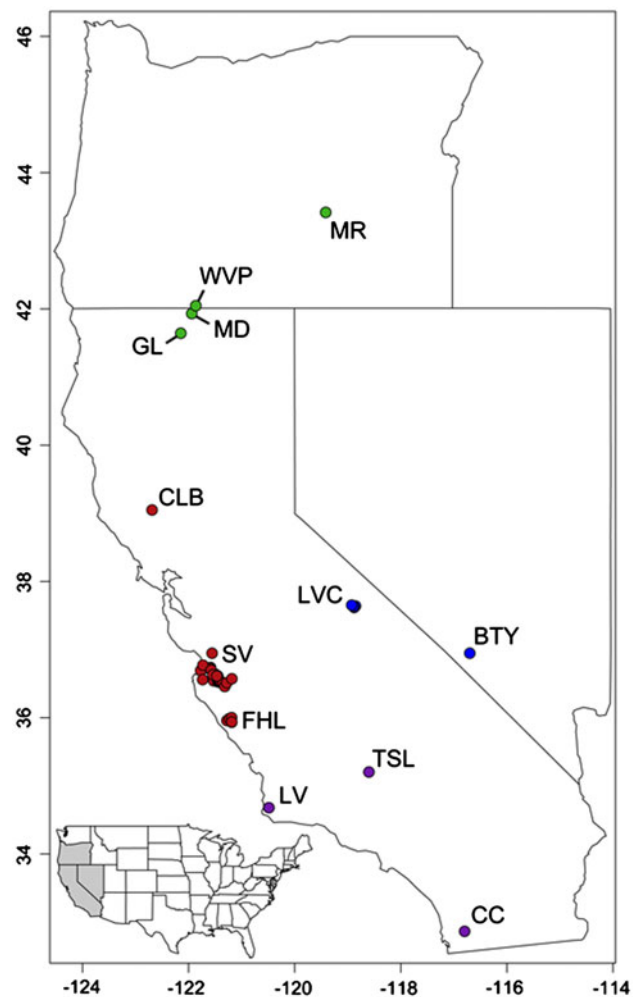


Fig. 1 Map of extralimital populations sampled. *MR* Moon Reservoir; *GL* Grass Lake; *MD* MacDoel Ditch; *WVP* Worden Vernal Pool; *CLB* Clear Lake Basin; *LVC* Long Valley Caldera; *BTY* Beatty; *SV* Salinas Valley; *FHL* Fort Hunter-Liggett; *TSL* Tom Sawyer Lake; *LV* Lompoc Valley; *CC* Chocolate Canyon. Colors represent a priori relatedness hypotheses based on geography and known introductions. See text for discussion of specific hypotheses

nor *A. californiense* × *A. tigrinum* hybrids leaving two reasonable alternatives for their distributional status. One possibility is that some or all of these populations are relictual *A. tigrinum* lineages surviving from the wetter Pleistocene. Alternatively, these populations may represent introductions from the “bait-bucket” release of non-indigenous *A. tigrinum* larvae. Both hypotheses are plausible. The Northern Leopard Frog (*Rana pipiens*), Oregon Spotted Frog (*Rana pretiosa*), and Long-Toed Salamander (*Ambystoma macrodactylum*) are pond-breeding amphibians that also have relictual populations in some of these same areas, making it possible that native *A. tigrinum* lineages may have survived in isolated mesic habitat patches in eastern and northern California (Stebbins 2003). Given the extreme sensitivity of these and other isolated relictual amphibian populations (Jennings and Hayes 1994), it is important both from biogeographic and management perspectives to determine whether these extralimital tiger salamander populations are native, sensitive, and potentially endangered, or introduced, exotic populations that should not be protected, and possibly eliminated.

Tiger salamander larvae are frequently used as fish bait and historically have been harvested from wild populations to supply the bait industry (Slater 1934; Mullen and Stebbins 1978; Collins 1981; Collins et al. 1988; Riley et al. 2003), likely altering the natural distributions of tiger salamanders (Collins 1981; Storfer et al. 2004). This confusion was discussed more than three decades ago, when Mullen and Stebbins (1978) proposed that the isolated, extralimital tiger salamander population discovered in far Northern California at Grass Lake (Siskiyou County, CA) might represent a relictual population of tiger salamanders native to California rather than an introduced bait-bucket release. If the conjecture of Mullen and Stebbins (1978) is correct, and native, relictual *A. tigrinum* lineages exist in California, it is important to identify and protect these rare, vulnerable populations.

Our objective in this study was to use DNA sequence data to help determine whether extralimital populations of *A. tigrinum* are representatives of a rare relictual native salamander, or are the result of recent, human-mediated introductions. We collected data for all known *A. tigrinum* populations in California and adjacent Oregon and Nevada, and compared genetic sequence data from each population with reference samples from within the native range of *A. tigrinum*. We sequenced a large (1379 base pair) fragment of mitochondrial DNA from population-level samples of all extralimital populations and a thorough sampling of native lineages from throughout the western range of the species to determine the native/exotic status of each extralimital population of *A. tigrinum*.

Unambiguously determining the status of such populations is challenging, particularly when levels of genetic

divergence are low. In this case, we reasoned that if we found mtDNA haplotypes within an extralimital *A. tigrinum* population that are distinct from all sampled reference populations from the native range of *A. tigrinum* and are phylogenetically similar to a geographically nearby reference population, this would be most consistent with the interpretation that the extralimital population shared a common ancestor with nearby relatives, but has long existed in isolation. In this situation, we would conclude that the extralimital population is likely a native relict. For example, if extralimital populations in northern California are distinct from, but most similar to, reference populations in eastern Washington, then they are probably natural, disjunct populations that were possibly separated from those in Washington during post-Pleistocene climatic drying. Alternatively, if an extralimital population of *A. tigrinum* shares identical haplotypes with a geographically distant reference population (say, from the Great Plains) to the exclusion of nearby reference populations, we would infer that the extralimital population has been recently introduced from that distant location.

The patterns of molecular variation observed may also provide clues on the origin of extralimital populations. For example, if a set of relictual populations were derived from a previously continuous population that was isolated during the drying of the Great Basin, those populations should collectively form a monophyletic group. But if they represent independent human-mediated introductions, there is no reason why all or most extralimital populations should form a monophyletic group, particularly if the introductions were from multiple sites.

Finally, data from known introductions can inform our analysis. Based on extensive genetic analyses and interviews with local bait dealers, it is now firmly established that the widely distributed non-native *A. tigrinum* genotypes in the Salinas Valley of central California were intentionally introduced in the 1950s (Riley et al. 2003; Fitzpatrick and Shaffer 2007). If the mitochondrial variation observed in an extralimital site consists primarily of the same haplotypes as those found in introduced tiger salamanders from the Salinas Valley, we can reasonably conclude that they are also recently introduced, and do not represent a cryptic native Californian *A. tigrinum* lineage.

Our motivation is to use these results to inform management decisions regarding the protection of relictual native California *A. tigrinum*, or alternatively support the eradication of non-indigenous *A. tigrinum* that threaten the persistence of native species, including the California tiger salamander, (*A. californiense*; Fitzpatrick et al. 2009), the Santa Cruz long-toed salamander (*A. macrodactylum croceum*; Ryan et al. 2009), and the Owens Tui Chub (*Siphateles bicolor snyderi*; Chen et al. 2007). Given the predatory habits and ability to rapidly reproduce as large,

paedomorphic and/or terrestrial adults, determining the status of extralimital *A. tigrinum* and initiating either protection or eradication are important management goals both for the salamanders and the vernal pool ecosystems that they inhabit.

Methods

Sampling

We identified four regions where *A. tigrinum* have been documented in California and adjacent Oregon and Nevada (Fig. 1; see Table 1 in Appendix). (1) The Klamath Basin region of Northern California and Oregon includes three collection localities along US Highway 97: Grass Lake, CA, MacDoel, CA, and Worden, OR. Additionally, we included a fourth population in central Oregon from an artificial stock pond near Moon Reservoir (Fig. 1). We group these four populations together based on the a priori hypothesis that they are relictual populations that have been isolated from extant range of *A. tigrinum* in Washington. The Grass Lake population has previously been described tentatively as a native *A. tigrinum* lineage in California (Mullen and Stebbins 1978; Stebbins 2003) or as a non-indigenous population of *A. tigrinum melanostictum* (Bury and Luckenbach 1976). (2) The Long Valley Caldera region includes four collection localities near Mammoth Lakes, CA along Highway 395 and an additional location near Beatty, NV (Fig. 1). These populations have been grouped based on the hypothesis that they have been isolated from the western part of the range of *A. tigrinum* in Utah by the drying of the Great Basin. Little is known regarding the Mammoth Lakes tiger salamander populations except that they potentially have direct negative effects on local endangered native fish populations, and are therefore candidates for eradication if they are in fact non-indigenous (S. Parmenter, pers. comm.). (3) The Salinas Valley region includes 38 collection localities ranging from Gilroy, CA to Soledad, CA along Highway 101, an additional six sites at Fort Hunter Liggett, CA, and a single collection locality in the Clear Lake Basin region near Clearlake Oaks, CA at the—now abandoned—Five Star Fish Farm (Fig. 1). These populations are the result of hybridization between native, resident *A. californiense* and known introductions by Don Green and other fish bait dealers in the 1950s (Riley et al. 2003; Fitzpatrick and Shaffer 2007). We include these populations in our analyses, as they could potentially be the introduction source for the other extralimital populations in California. Based on our extensive previous work on these populations, we included only previously genotyped individuals that contained non-native mitochondrial haplotypes based on single

nucleotide polymorphism (SNP) genotypes obtained for the mtDNA Control Region (CR) marker (Fitzpatrick and Shaffer 2007). We included animals from the Five Star Fish Farm in this region based on verbal confirmation from one of the bait dealers that these animals were derived from his initial introductions into the Salinas Valley. (4) In Southern California, we group three geographically distinct populations based on a lack of a clear biogeographic hypothesis. We have two collection sites in the Lompoc Valley near Lompoc, CA, a single individual collected in Chocolate Canyon near Alpine, CA (Ervin and Burkhardt 2006), and an additional population at Tom Sawyer Lake near Tehachapi, CA. (Fig. 1). The Lompoc populations are believed to be non-indigenous and have previously been subjected to eradication measures (L. Hunt, pers. comm.). In total, we included 58 localities and 344 individuals in our set of extralimital populations (see Table 1 in Appendix). To the best of our knowledge, this is an exhaustive set of extralimital *A. tigrinum* populations in, or adjacent to California.

Native populations that we used for comparative purposes (hereafter referred to as reference samples) included numerous collection localities from within the known native range of *A. tigrinum* in the United States (Fig. 2; see Table 1 in Appendix). We included 1–4 individuals ($N = 128$) from each of 82 collection localities spanning the native range of *A. tigrinum* except the geographically restricted and endangered Sonoran Tiger Salamander, *A. t. stebbinsi*.

DNA extraction and amplification

We extracted DNA from tail or liver tissue that was either preserved in 95% ethanol or frozen at -80°C using a standard salt extraction protocol (Sambrook and Russell 2001). Given the very low levels of nuclear divergence among members of the tiger salamander complex, including some of our target taxa (Weisrock et al. 2006), we focused exclusively on mitochondrial DNA in this study. We designed oligonucleotide primers for the mitochondrial control region (CR), and NADH dehydrogenase subunit 2 (ND2) using Primer3 (Rozen and Skaletsky 2000) and sequences downloaded from GenBank. ND2 was chosen because of its high variability in *Ambystoma* (Samuels et al. 2005) and CR was used to build on previously available comparative data (Shaffer and McKnight 1996; Storfer et al. 2004). Mitochondrial DNA (mtDNA) was amplified using the polymerase chain reaction (PCR) on Eppendorf Mastercycler Ep gradient thermal cyclers. Our PCR protocol was as follows: initial denaturation at 94°C for 7 min, 20 cycles of 30 s 94°C denaturation, 30 s 60°C annealing, and 1 min 72°C elongation, and a single 72°C final elongation for 10 min. Successful amplification

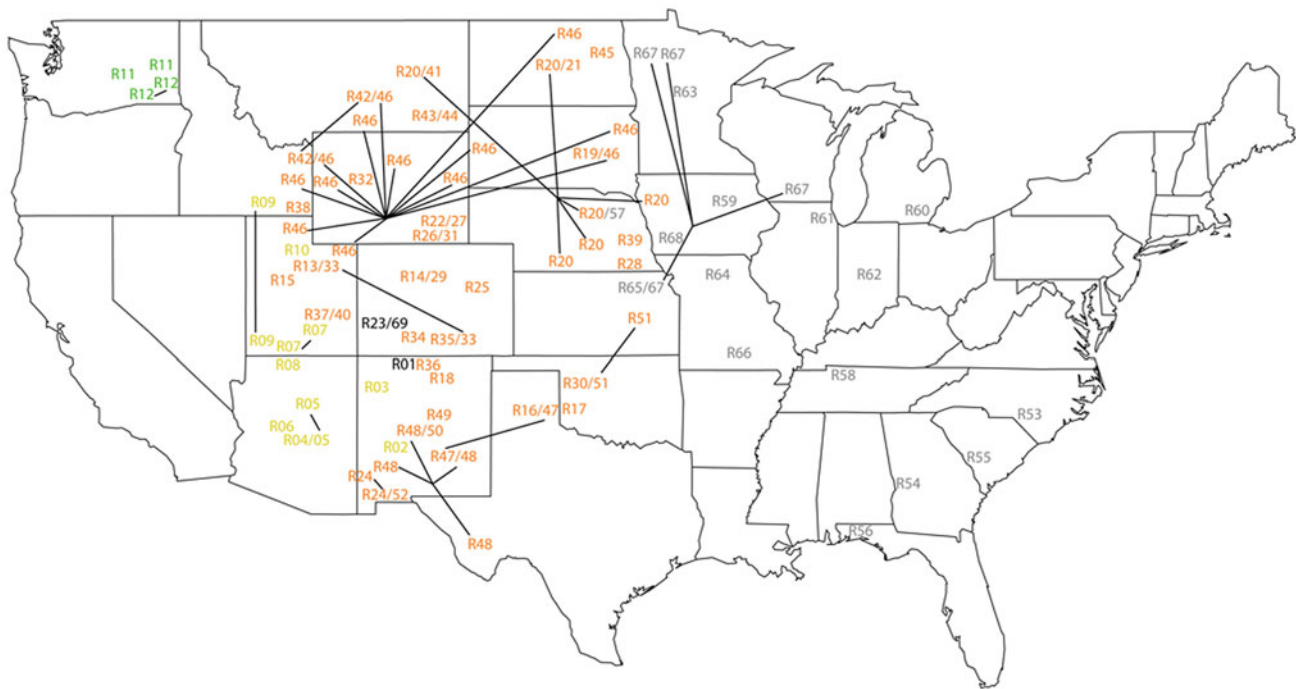


Fig. 2 Map of reference populations sampled for *A. tigrinum*. Haplotype numbers are placed at collecting localities. Lines connecting haplotypes are for reference only and are intended to improve the ability to locate localities sharing a given haplotype. *Green* denotes

the Pacific Northwest clade, *brown* denotes the Northern Great Plains clade, and *orange* represents the Southern Great Plains clade. *Gray* represents Eastern *A. tigrinum* (not associated with any extralimital populations)

of PCR product was visualized on 1% agarose gels, and the size of the PCR fragment was assessed with Low Mass DNA Ladder.

Sequencing and phylogenetic analysis

PCR product was purified and sequenced using Agencourt’s Single Pass Sequencing service. This service includes template purification of PCR amplicons, DNA sequencing reactions with BigDye Terminator v3.1, and sequence delineation on an ABI PRISM 3730xl. Sequences for each gene were verified by GenBank BLAST searches and alignment with GenBank sequences. We aligned all sequences using MUSCLE (Edgar 2004) and concatenated data from each gene. Alignments were checked and adjusted by eye, verifying that the ND2 data translated properly.

Our strategy was to first characterize the haplotype pool present at each of the extralimital *A. tigrinum* populations, as well as the geographic distribution of haplotypes in the known native range. We identified the unique haplotypes from all of the extralimital populations and from the reference set separately, and analyzed this complete set of haplotypes. Thus, the same haplotype could occur twice on the resulting phylogenetic tree if it was present in both the reference and extralimital populations. Using this combined set of haplotypes we inferred a single phylogenetic

gene tree by partitioning the alignment by gene and carrying out a maximum-likelihood bootstrapping search in RAXML (Stamatakis et al. 2008). We then attempted to phylogenetically assign the extralimital haplotypes to known native haplotypes (or haplotype groups if it fell within a native cluster but was not identical to any native haplotype) based on the resulting topology.

We also generated a phylogenetic network of our concatenated data using SplitsTree version 4.10 (Huson and Bryant 2006) with uncorrected “p” genetic distances and the NeighborNet algorithm (Bryant and Moulton 2004). We also performed a bootstrap analysis with 1000 replicates implemented in SplitsTree. Phylogenetic networks may provide additional insight into the complexities of the relationships present in data such as ours that are obscured when using a single phylogenetic tree (Huson 1998). We used both the network and phylogenetic information in combination with the geographic proximity of native and extralimital sites, to infer the most likely origin of each set of extralimital populations.

Results

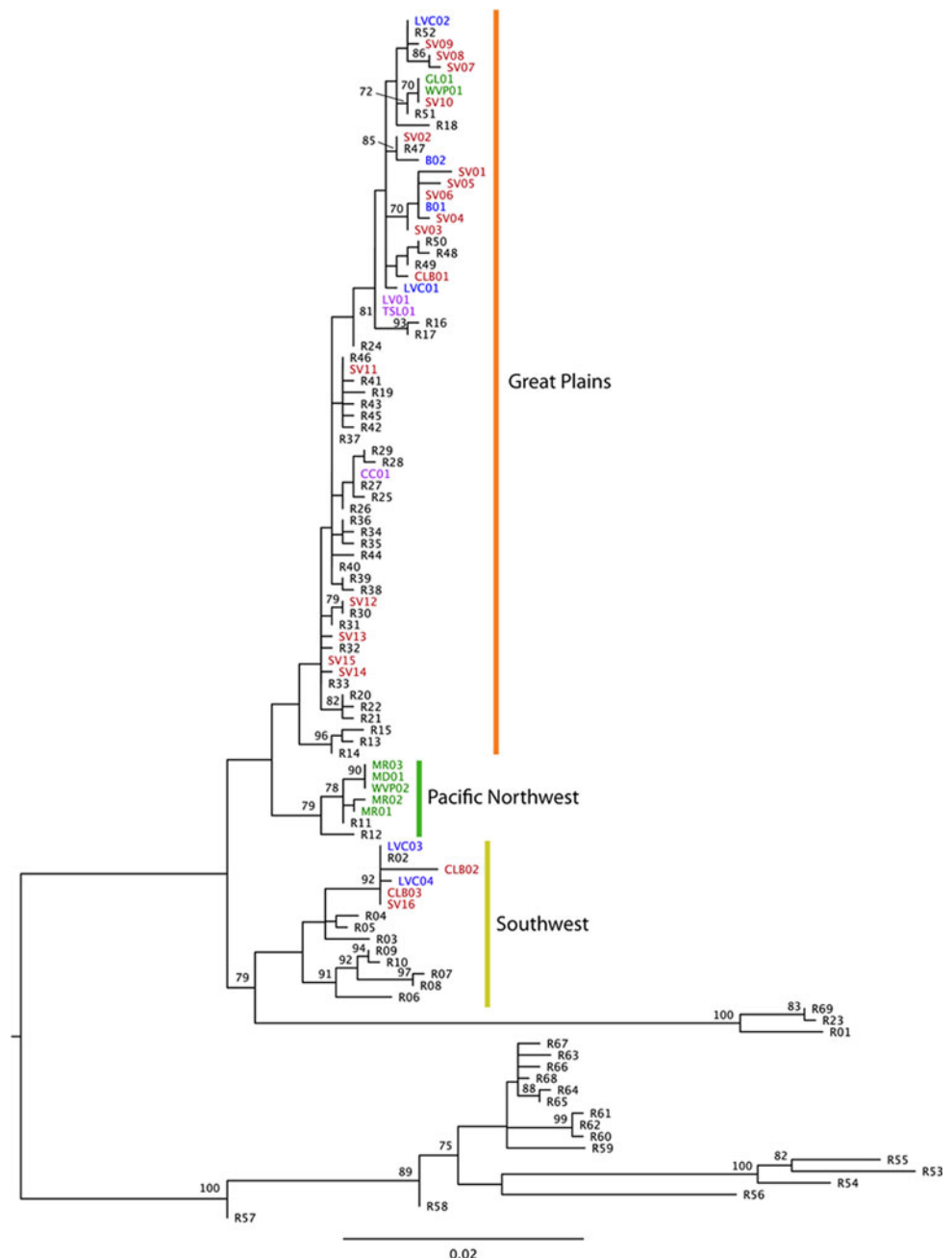
We amplified 725 bp of CR and 655 bp of ND2 for a total of 1379 bp of mtDNA sequence for each individual. We

recovered 69 unique reference sample haplotypes of *A. tigrinum* subspecies (see Table 1 in Appendix). Haplotype codes and catalogue numbers used in this study are annotated in the sequence information uploaded to GenBank (HM544136–HM545077). Across the individuals sampled from extralimital populations of *A. tigrinum* (including *A. californiense* × *A. tigrinum* hybrids), we identified 35 unique extralimital haplotypes (see Table 1 in Appendix). As expected, there was relatively little variation available for phylogenetic analysis, and ML bootstrap values were often low (Fig. 3). Our goal, however, was to

compare extralimital haplotypes with reference native sites, rather than to infer a robust phylogeny of *A. tigrinum*, and the data were generally variable enough for this purpose. The major splits of the phylogenetic network (Fig. 4) mirrored the well-supported clades present in the phylogenetic tree (Fig. 3).

From the Klamath Basin, we sequenced 17 individuals from Grass Lake, 19 individuals from MacDoel Ditch, and seven individuals from Worden Vernal Pool. The Klamath Basin populations display an interesting pattern in which the southernmost site (Grass Lake) is entirely composed of

Fig. 3 Maximum-likelihood phylogram of haplotypes. Numbers at nodes are bootstrap proportions >70. Color and haplotype name refers to position in Fig. 2. See Table 1 in Appendix for more locality information. Highlighted clades contain extralimital haplotypes and correspond with colored labels on Fig. 2



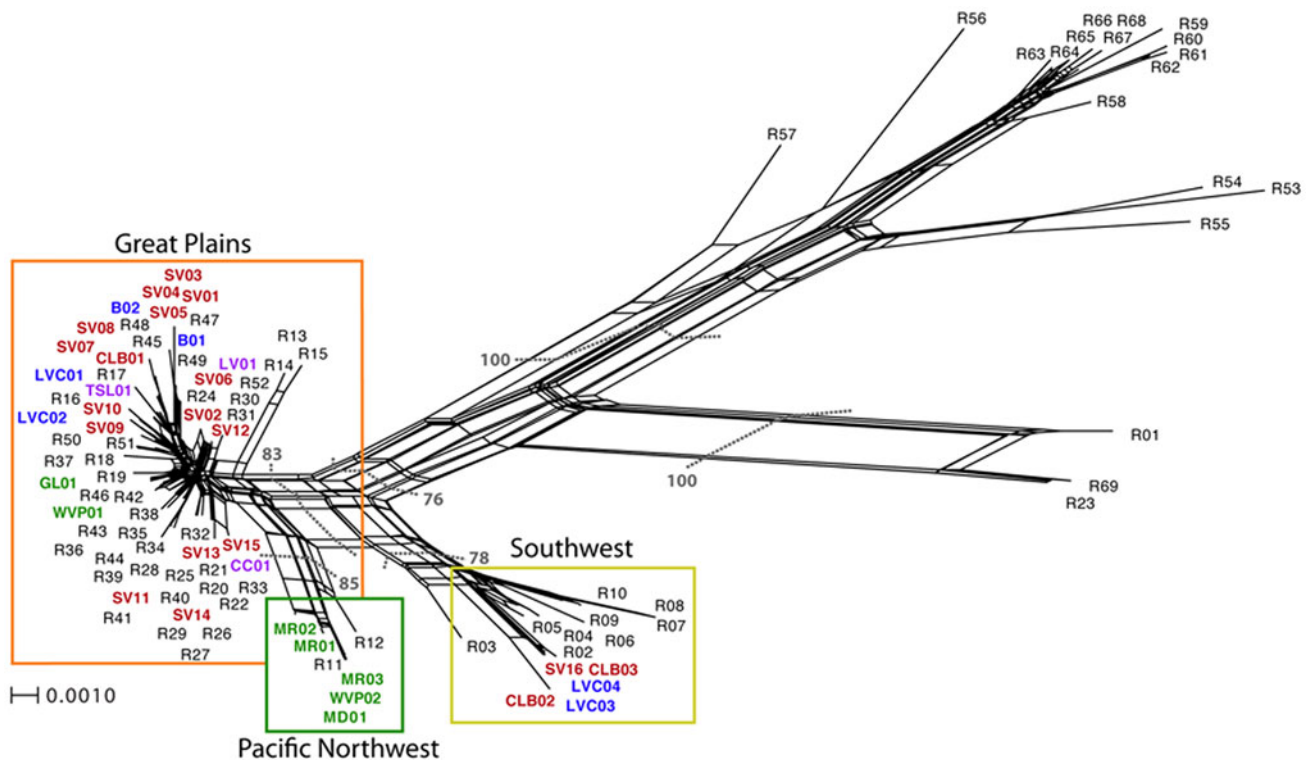


Fig. 4 Phylogenetic network of haplotypes. *Color* and haplotype name refers to position in Fig. 2. See Table 1 in Appendix for more locality information. *Highlighted* clades contain extralimital haplotypes and correspond with *colored* labels on Fig. 2. Bootstrap support

values for the major clades are labeled in *gray* with *dotted* lines denoting the splits. We omitted support values for all smaller clades and larger clades with values <70

a single haplotype (GL01) that is shared with an individual from the Salinas Valley region (SV10) and is associated with a reference haplotype (R51) that was found in Kansas and Oklahoma and are part of a diverse Great Plains clade (Fig. 3). We also found a haplotype identical to GL01 at Worden Vernal Pool (WVP01). The MacDoel haplotype (MD01) and common Worden Vernal Pool haplotype (WVP02) from populations 36 and 50 km to the north of Grass Lake (straddling the California/Oregon border) are identical to each other and quite different (18 substitutions) from GL01. MD01 and WVP02 are also identical to one of three haplotypes found at the isolated Moon Reservoir (MR03) approximately 250 km to the northeast (Fig. 1). We sequenced eight individuals from Moon Reservoir, and found that the three haplotypes are distinct from, but most closely related to, two reference haplotypes (R11 and R12) from eastern Washington (Fig. 3). The Washington reference haplotypes and the haplotypes from MacDoel, Worden, and Moon Reservoir, but not from Grass Lake, form a strongly supported Pacific Northwest clade (Fig. 3).

In the Long Valley Caldera, we sequenced two individuals from the Hot Creek Hatchery, eight individuals from Mammoth Vernal Pool (a now-extirpated population), 16 individuals from Sherwin Creek Pond, and 49

individuals from Laurel Pond, a large, artificial pond. The Long Valley Caldera region revealed four haplotypes, two that were common (LVC01 and LVC02), one that was rare but present at three of four sites (LVC03) and the last (LV04) represented by a single individual. The LVC03 haplotype is identical to other non-indigenous haplotypes (CLB03 and SV16) and reference haplotype R02 from New Mexico (Fig. 3). LVC02 falls within a clade with several Salinas Valley haplotypes (SV07–09), and is identical to reference haplotype R52 from New Mexico. LVC01 is not strongly associated with any particular reference haplotype but falls within the large Great Plains clade that includes the Salinas Valley and other extralimital haplotypes, as well as a collection of reference haplotypes from New Mexico, Oklahoma, and Texas (Fig. 3). The four individuals sampled from the Beatty, NV population revealed two haplotypes (B01 and B02). B01 was identical to SV06 from the Salinas Valley, and both B01 and B02 are nested within the Great Plains clade containing many known non-indigenous Salinas Valley haplotypes.

We sequenced up to five individuals from each of the 44 Salinas Valley region localities. The Salinas Valley region contained almost half of the haplotype diversity present in all of the extralimital populations (16/36). Several Salinas

Valley haplotypes were identical to reference haplotypes. For example, the “Bluestone Quarry” haplotype (SV02) and a reference haplotype from New Mexico and Texas (R47), and a “Fort Hunter-Liggett” haplotype (SV12) and a reference haplotype from Oklahoma (R30) form two such pairs (Fig. 3). Many Salinas Valley haplotypes (SV11–14) are associated with reference haplotypes from the Great Plains but lack strong bootstrap support (Fig. 3). The most common Salinas Valley haplotype (SV16) was associated with a Southwest clade containing reference haplotypes from Arizona, Idaho, New Mexico, and Utah (Fig. 2). The somewhat isolated Fort Hunter-Liggett populations are ~60 km south of the upper Salinas Valley populations and contain two haplotypes (SV07 and SV08) that were distinct from the rest of the Salinas Valley, but remain nested within the Great Plains clade (Fig. 3). The Fort Hunter-Liggett populations also shared the most common Salinas Valley haplotype (SV16) with the upper Salinas Valley populations (Fig. 3). We sequenced 52 individuals from the Five-Star Fish Farm in the Clear Lake Basin. This population revealed only three haplotypes despite extensive sampling over multiple years, and one of these haplotypes (CLB02) was only present in a single individual. Of the remaining two common Clear Lake Basin haplotypes, CLB01 clustered with haplotypes in the Great Plains clade from New Mexico and Texas (R48–50), while CLB03 was identical to haplotypes R02 from New Mexico, SV16 from the Salinas Valley, and LVC03 from the Long Valley Caldera in the Southwest clade (Fig. 3).

The Lompoc Valley region of southern California revealed a single haplotype (LV01) that was shared only with the two individuals sequenced from the Tom Sawyer Lake (TSL01) population. This haplotype was unique and not found in any of our reference samples, but fell within the large Great Plains reference haplotype clade containing most of the non-indigenous Salinas Valley samples (Fig. 3). The single Chocolate Canyon individual from extreme southern California contained a haplotype (CC01) that is identical to reference haplotype R27 from Wyoming (Fig. 3).

Discussion

Our sequence data shed new light on the question of whether extralimital *A. tigrinum* populations in California and adjacent Oregon and Nevada harbor relict native lineages or are the result of recent, human-mediated introductions. Given the broad distribution, and the potential for separate histories of each set of extralimital populations, we discuss the evidence for or against human-mediated introduction for each region separately.

The Northern California Klamath Basin populations are likely the result of two separate and recent introductions

into California. Our data suggest that the Grass Lake population has likely resulted from a single human-mediated introduction from stock similar to that released into the Salinas Valley. This conclusion is in contrast to an earlier study that used morphological measurements to evaluate the taxonomic relationship of Grass Lake salamanders with other subspecies of *A. tigrinum* (Mullen and Stebbins 1978). Our reasoning for this conflicting conclusion is simple; the lone Grass Lake haplotype is identical to a haplotype detected in the Salinas Valley where we know that human-mediated introductions occurred, and similar to a reference haplotype found in Oklahoma and Kansas. We conclude that only a single introduction event may be responsible for this population based on the lack of haplotype diversity despite extensive sampling over multiple years. Alternatively, the low haplotype diversity can be explained by repeated population bottlenecks due to the isolated location of Grass Lake and the presumed low population sizes that would be necessary to allow the population to have gone undetected prior to 1969 (Mullen and Stebbins 1978). The sequence identity of the Grass Lake haplotype with a known non-indigenous haplotype from the Salinas Valley (SV10) and an Oklahoma/Kansas reference haplotype (R51), to the exclusion of more geographically proximate native *A. tigrinum* haplotypes from eastern Washington (R11–12) is compelling evidence for our conclusion that the Grass Lake population is non-indigenous. Additionally, the discovery of tiger salamanders at Grass Lake in 1969 (Mullen and Stebbins 1978) roughly 10–20 years after the large-scale introductions into the Salinas Valley and subsequent transfer to other areas in California (e.g., Clear Lake Basin), further supports our conclusion that the Grass Lake population is non-indigenous.

The other two Klamath Basin populations in MacDoel, CA and Worden, OR also exhibit low haplotype diversity. Twenty five of 26 individuals shared a single haplotype, which was also shared with the Moon Reservoir population in Oregon. Ecologically, the Moon Reservoir population seems like a strong candidate for being non-indigenous (Stebbins 2003); it occurs in a highly modified habitat (to our knowledge, a single, artificial stock pond), and there is a history of fish stocking (and sport fishing) in the vicinity (Bowers et al. 1999). However, genetically the Moon Reservoir population is closely associated with haplotypes from the native range of *A. tigrinum* from eastern Washington and with the geographically proximal populations from MacDoel Ditch and Worden Vernal Pool. From the genetic data alone, we conclude that the Moon Reservoir, MacDoel Ditch, and Worden Vernal Pool populations represent either the expansion of salamanders from the native range of *A. tigrinum* in Washington (either naturally or via human intervention), or remnant native *A. tigrinum*

populations from a once large metapopulation that became isolated following Pleistocene drying. We cannot distinguish between these two alternative hypotheses with our data. Furthermore, we detected a shared haplotype between Grass Lake (GL01) and Worden Vernal Pool (WVP01) suggesting that either natural or human-mediated movements could be resulting in the admixture of these two distinct salamander lineages. If the MacDoel Ditch and Worden Vernal Pool populations are native, hybridization with lineages from Grass Lake presents an immediate risk of biodiversity loss via hybridization. Based on our data, it remains possible that the *A. tigrinum* populations at MacDoel Ditch in far-northern California and Worden Vernal Pool in extreme-southern Oregon are native. Until additional data are obtained, we suggest that these populations should be protected from immigration of non-indigenous tiger salamander lineages and considered potential candidates for management as threatened populations.

The Clear Lake Basin haplotypes are genetically in agreement with the statements of bait dealers that animals introduced into the Five Star Fish Farm were of the same stock introduced into the Salinas Valley in the 1950s. Furthermore, the Salinas Valley haplotype diversity likely reflects the diversity encountered by bait dealers in the native range of *A. tigrinum* from which the stock was collected for the initial large-scale introductions. While we cannot make precise conclusions from our phylogeny, it is interesting that we have recovered a strongly supported Southwest clade containing Salinas Valley haplotypes that are distinct from the Great Plains clade. The variation observed among introduced Salinas Valley haplotypes makes sense because the bait-dealers stopped at multiple locations during their collections. Interviews with one of the individuals involved in the introductions (Don Green) indicated that animals were collected from a variety of locations including Arizona, Colorado, and Texas (Riley et al. 2003), and our genetic results are consistent with this verbal account. In total, we have found that the salamander stock introduced into the Salinas Valley overlaps with haplotypes from throughout the western native range of *A. tigrinum*. However, based on these genetic results, it appears that the primary source of the introductions was closely related to populations in central New Mexico (R02 and R47–50; Fig. 2).

The populations at Fort Hunter-Liggett may represent the combination of introductions from the native range of *A. tigrinum* and transfer of individuals from the Salinas Valley. A previous investigation of the Fort Hunter-Liggett sites revealed that these populations were composed primarily of *A. tigrinum* genes, with low levels of native *A. californiense* also present (Fitzpatrick and Shaffer 2007). Our data suggest that some of the non-indigenous haplotypes encountered at Fort Hunter-Liggett were potentially

transferred from the upper Salinas Valley populations, as several haplotypes are shared between the two regions. However, we cannot say whether the native haplotypes identified by Fitzpatrick and Shaffer (2007) were similarly transferred from the Salinas Valley or present before the introductions.

The four Long Valley Caldera populations demonstrated high within-site haplotype diversity relative to other heavily sampled population (e.g., Clear Lake Basin, Klamath Valley). Each of the four Long Valley Caldera breeding site shared haplotypes with other sites in the region, which is not surprising given their close geographic association (our four sampling sites are within 6.5 km of each other). The haplotypes of the Long Valley Caldera region were placed in the phylogeny in both the Great Plains and Southwest clades, containing much of the Salinas Valley non-indigenous haplotype diversity. Thus, the phylogenetic placement of the Long Valley Caldera haplotypes mimics the distribution of Salinas Valley haplotypes, suggesting either a common pattern of introductions or sequential introductions from one region to another. Specifically, the two most common Long Valley Caldera haplotypes are highly associated with the “Christiansen Agricultural Pond” (see Table 1 in Appendix), which is almost entirely non-native at nuclear SNP markers (JR Johnson and HB Shaffer, unpublished data), and the Five Star Fish Farm introduction site, as well as reference populations from New Mexico which we believe were near the actual collection sites. The Long Valley Caldera region shows no evidence of relict *A. tigrinum* lineages despite heavy sampling effort over multiple years and all known breeding sites. These *A. tigrinum* populations frequently produce paedomorphic (i.e., large, sexually mature, gilled adults) individuals that have the potential to negatively impact endangered fishes through direct predation (Steve Parmenter pers. comm.). Because the available evidence is consistent with the interpretation that the salamander populations in the Long Valley Caldera region are the result of introductions of exotic *A. tigrinum*, we feel that management action in accordance with preventing negative interactions with the Owens Valley fishes is appropriate. The Beatty, NV haplotypes are strongly associated with Salinas Valley haplotypes and a reference haplotype from New Mexico contained within the large Great Plains clade, confirming that this population is also non-indigenous.

No reference haplotypes were identical to those found in the Lompoc Valley and Tom Sawyer Lake extralimital populations. This suggests that the Lompoc and Tom Sawyer Lake populations are probably distinct introductions from those that occurred in the Salinas Valley. Given their distribution outside of the Great Basin and within the range of the California tiger salamander and their recent discovery in areas of high human population density, they

must represent non-indigenous populations of *A. tigrinum*. We apparently did not sample the source region, or at least an identical representative haplotype in our reference samples and cannot conclusively show that these populations are non-indigenous based on the genetic data. However, the placement of these populations in the Great Plains clade containing many Salinas Valley haplotypes (Fig. 3) indicates that they are likely non-indigenous (if not from the same stock), and that further sampling from the native range of *A. tigrinum* might reveal the details of their source.

If we can assume the Lompoc Valley populations are non-indigenous, they represent a direct threat to the persistence of a native endangered species, the Santa Barbara distinct population segment of *A. californiense*. Using nuclear SNP markers we have recently identified the first known hybrid individual between these two taxa in Santa Barbara County, CA, approximately 15 km from the sampled Lompoc Valley populations (JR Johnson and HB Shaffer, unpublished data). The spread of non-indigenous genes through native populations of *A. californiense* in the Salinas Valley has occurred broadly and rapidly (Fitzpatrick and Shaffer 2007; Fitzpatrick et al. 2009) and raises many conservation concerns regarding the management and protection of the *A. californiense* in the face of hybridization (Ryan et al. 2009; Fitzpatrick et al. 2010). Given that contact and hybridization have just occurred in the Santa Barbara/Lompoc region, it is critical to take measures to eliminate these populations and prevent the spread of individuals/haplotypes out of the Lompoc Valley into the range of the endangered Santa Barbara distinct population segment. We view this as a critical conservation action in need of immediate attention.

Lastly, the Chocolate Canyon haplotype is contained within the Great Plains clade and grouped with reference haplotypes from Wyoming, Colorado, and Nebraska, but with no obvious association with other extralimital haplotypes. However, this population was represented by only a single individual, so shared haplotypes with the Salinas Valley may have gone undetected. Future tissue collections from Chocolate Canyon should lend more insight into the status of the population.

Conclusions

We did not find clear evidence for relictual native tiger salamander lineages in California's extralimital *A. tigrinum* populations, but were unable to eliminate the possibility for some sites. For the extralimital haplotypes encountered in the Clear Lake Basin, Long Valley Caldera, Salinas Valley, Lompoc Valley, Chocolate Canyon, and Tom Sawyer Lake

regions, we found similar or identical haplotypes from Great Plains reference populations in the native range of *A. tigrinum*, indicating that these populations are the result of introductions. Our genetic data fit with anecdotal and published reports of bait dealers repeatedly importing *A. tigrinum* from New Mexico and Texas into California 50–60 years ago. In our opinion, conservation of native species such as the Santa Barbara subpopulation of the California tiger salamander and the Owens Valley fish assemblage should proceed under the assumption that these extralimital tiger salamander populations are non-indigenous.

Only in the Klamath Basin populations is there a realistic possibility of native, disjunct *A. tigrinum* populations. Haplotypes from Grass Lake in the southern Klamath Basin are clearly introduced and share a haplotype with the known non-indigenous Salinas Valley populations. However, the more northerly ones from Worden Vernal Pool (in Southern Oregon), MacDoel Ditch (in extreme Northern California), and Moon Reservoir (in Central Oregon) form a unique, monophyletic group of haplotypes that is most closely related to, but distinct from, those farther north in eastern Washington. Perhaps additional sampling in the Pacific Northwest will reveal that the extralimital populations in Oregon are the result of introductions from these native Washington sites, but based on our data, the possibility remains that these are relictual populations, potentially in need of protection. Our data suggest the occurrence of gene flow between the introduced Grass Lake population and at least one nearby population, and it therefore remains possible that movement of individuals from Grass Lake to the other Klamath Basin populations could result in the genetic extinction of a relict native tiger salamander in California.

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Appendix

See Table 1

Table 1 Specimens examined

Region	State	County	Locality	LAT	LON	Hap	N	Catalogue Number			
Clear Lake Basin	CA	Lake	Five Star Fish Farm	39.048	−122.687	CLB01	13	HBS 26702, 26719–20, 26730, 38114, 38118, 38148, 38156, 109680–1, 109683, 109685, 109692			
						CLB02	1	HBS 26715			
						CLB03	38	HBS 26703–4, 26707, 26709, 26712, 26718, 26722–3, 26725–6, 26729, 38115–7, 38119–21, 38145–6, 38149, 38151–5, 38157, 109682, 109686–91, 109693–4, 109696–8			
Klamath Basin	CA	Siskiyou	Grass Lake	41.642	−122.146	GL01	17	HBS 8974, 9082, 108881–95			
			MacDoel Ditch	41.931	−121.939	MD01	19	HBS 8925, 8928–30, 8936, 8938–41, 9026–35			
	OR	Klamath	Worden Vernal Pool	42.045	−121.863	WVP01	1	HBS 8922			
						WVP02	6	HBS 8906, 8917–18, 8920–1, 8924			
Long Valley Caldera	CA	Mono	ML—Hot Creek	37.639	−118.854	LVC01	1	HBS 21369			
						LVC03	1	HBS 21368			
			ML—Laurel Pond	37.618	−118.878	LVC01	28	HBS 117476–9, 117481–5, 117487, 117489, 117491–5, 117498–500, 117508, 117512–5, 117517, 117522, 117525, 117527			
						LVC02	14	HBS 117486, 117488, 117490, 117497, 117502–3, 117506–7, 117511, 117516, 117523–4, 117526, 117530			
						LVC03	6	HBS 117496, 117501, 117509–10, 117518–19			
			ML—Sherwin Creek	37.633	−118.892	LVC02	16	HBS 117529			
						LVC02	16	HBS 28070–5, 28077–8, 28081–4, 28086–9			
						ML—Vernal Pool	37.652	−118.925	LVC01	5	HBS 9002–3, 9005, 9007, 9020
									LVC02	2	HBS 9006, 9010
						LVC03	1	HBS 9009			
Salinas Valley	CA	Monterey	Barnwell 1	36.606	−121.464	SV13	1	HBS 28805			
						SV16	2	HBS 28806–7			
			Barnwell 2	36.608	−121.462	SV12	1	HBS 28975			
						SV16	1	HBS 28976			
						SV16	3	HBS 30147–8, 30150			
			Christiansen	36.720	−121.621	SV09	4	HBS 109633–6			
			Chualar Lake	36.540	−121.526	SV16	4	HBS 25862–5			
			Chualar Tank 2	36.603	−121.447	SV14	1	HBS 29296			
						SV16	2	HBS 29293, 29294			
			Chualar Vernal Pool	36.598	−121.453	SV16	3	HBS 25805, 25808, 25810			
			Costa N3	36.613	−121.451	SV16	4	HBS 28704–7			
			Costa Tire	36.618	−121.461	SV02	1	HBS 29032			
SV10	1	HBS 29039									

Table 1 continued

Region	State	County	Locality	LAT	LON	Hap	<i>N</i>	Catalogue Number
			Costa Weedy	36.618	−121.465	SV16	4	HBS 28717–9, 28721
			Fanoe 1	36.525	−121.444	SV16	2	HBS 107352, 107360
			Fanoe 2	36.531	−121.448	SV16	1	HBS 107292
						SV06	3	HBS 107290–1, 107293
			Fanoe 3	36.533	−121.440	SV06	3	HBS 107380–2
			Fanoe 4	36.528	−121.429	SV05	1	HBS 107409
						SV06	1	HBS 107407
						SV11	1	HBS 107406
			FHL—8j	35.958	−121.277	SV07	3	HBS 31153–5
			FHL—b-9	35.988	−121.222	SV16	4	HBS 30964–7
			FHL—LTV	35.933	−121.186	SV12	3	HBS 31128, 31130–1
			FHL—MPRC	35.986	−121.234	SV07	2	HBS 31043–4
						SV16	2	HBS 31042, 31045
			FHL—Oat Hill	36.001	−121.188	SV03	3	HBS 31063, 31065–6
			FHL—VD	35.937	−121.185	SV07	3	HBS 31017–8, 31020
						SV08	1	HBS 31019
			Iverson 1	36.529	−121.420	SV04	1	HBS 25576
						SV06	2	HBS 25574, 25577
			JCL—Pond 2	36.528	−121.406	SV16	5	HBS 22052–5, 25435
			JCL—Pond 2A	36.529	−121.405	SV06	2	HBS 21831–2
						SV16	2	HBS 21833–4
			JCL—Pond A	36.525	−121.404	SV06	1	HBS 21900
			JCL—Pond C	36.522	−121.409	SV06	2	HBS 21724, 21726
						SV16	1	HBS 21723
			JCL—Pond D	36.519	−121.407	SV06	2	HBS 22107–8
			JCL—Pond F	36.534	−121.401	SV06	1	HBS 21781
						SV16	2	HBS 21778–9
			JCL—Pond G	36.531	−121.388	SV06	3	HBS 28942–4
			JCL—Pond H	36.528	−121.388	SV06	3	HBS 25724–5, 25731
			LaMacchia 1	36.502	−121.349	SV01	1	HBS 25784
						SV06	2	HBS 25782, 25785
			LaMacchia 2	36.503	−121.349	SV06	3	HBS 111050, 111055, 111058
			Marina Ag Pond	36.692	−121.772	SV16	4	HBS 108796–9
			Miravale III	36.457	−121.317	SV16	2	HBS 108899, 108903
			Natividad	36.736	−121.586	SV09	3	HBS 38582–4
			Nightpipe	36.717	−121.586	SV16	2	HBS 38540, 38542
			Prunedale 2	36.771	−121.729	SV16	4	HBS 107452, 107454, 107457–8
			Red Shack	36.641	−121.502	SV02	2	HBS 38623–4
						SV16	2	HBS 38622, 38625
			Sal Annex 1	36.698	−121.574	SV16	2	HBS 102539, 102542
			Spence	36.629	−121.539	SV02	2	HBS 27000, 27003
						SV16	2	HBS 27001–2
			Sycamore	36.612	−121.467	SV02	1	HBS 29085
						SV16	2	HBS 29083, 29086
			Toro Lake	36.559	−121.735	SV15	3	HBS 103685–7
		San Benito	Gloria Lake	36.512	−121.280	SV06	4	HBS 12311–2, 12564, 12567
						SV16	2	HBS 12313, 12566
			Melindy	36.572	−121.184	SV06	2	HBS 31804–5

Table 1 continued

Region	State	County	Locality	LAT	LON	Hap	<i>N</i>	Catalogue Number			
Lompoc Valley	CA	Santa Clara	Bluestone Quarry	36.946	−121.556	SV02	3	HBS 26983, 26985–6			
		Santa Barbara	Burton Mesa 2	34.679	−120.487	SV16	1	HBS 26984			
		Burton Mesa 3	34.678	−120.484	LV01	8	HBS 28305–12				
		Burton Mesa 3	34.678	−120.484	LV01	9	HBS 28350, 28352, 28353, 28355–60				
Other populations	CA	Kern	Tom Sawyer Lake	35.150	−118.492	TSL01	2	HBS 108767–8			
		San Diego	Chocolate Canyon	32.864	−116.797	CC01	1	HBS 107276			
	NV	Nye	Beatty	36.940	−116.707	B01	2	HBS 14564–5			
Reference sites	OR	Harney	Moon Reservoir	43.415	−119.414	MR01	6	HBS 9037–8, 9041–2, 9043, 9046			
						MR02	1	HBS 9045			
						MR03	1	HBS 8911			
	AZ	Coconino	Clint’s Well	34.454	−111.396	R04	1	HBS 1466			
						R05	1	HBS 1467			
						R08	2	HBS 7832–3			
				Fracas Lake	36.630	−112.238	R05	2	HBS 1491, 1496		
							R05	2	HBS 1491, 1496		
							R06	1	HBS 110812		
			CO	Alamosa	Crown King	34.205	−112.338	R06	1	HBS 110812	
					Blanca Wetlands	37.549	−105.416	R33	1	HBS 15164	
					Blanca Wetlands	37.549	−105.416	R35	1	HBS 15163	
				Lincoln	Limon	39.417	−103.676	R25	1	HBS 6593	
					Rio Grande	South Fork	37.649	−106.649	R34	1	HBS 15142
					San Juan	Silverton	37.747	−107.682	R23	1	HBS 7124
	Summit	Ryan Gulch	39.656	−106.078	R69	1	HBS 7125				
					R14	1	HBS 7163				
					R29	1	HBS 7169				
FL	Santa Rosa	Jay	30.951	−87.082	R56	1	HBS 6187				
GA	Marion	Box Springs	32.427	−84.654	R54	1	HBS 6645				
IA	Cass	Cumberland	41.216	−94.872	R68	1	HBS 10388				
		Grundy	New Hartford	42.566	−92.622	R59	1	HBS 5986			
		Woodbury	Lawton	42.475	−96.229	R20	1	HBS 5960			
ID	Bonneville	Ririe	43.607	−111.667	R46	1	HBS 7629				
		Oneida	Dammed Spring	42.210	−112.692	R09	2	HBS 7656–7			
		Oneida	Weston Canyon	42.120	−112.118	R38	2	HBS 7679–80			
IL	Cook	Kennicott Grove	42.081	−87.864	R61	1	HBS 4469				
IN	Hamilton	Carmel	39.955	−86.157	R62	1	HBS 8108				
KS	Chase	Emporia	38.276	−96.367	R51	2	HBS 5190–1				
		Doniphan	Troy	39.792	−95.073	R68	1	HBS 10245			
MI	Washtenaw	Goss Pond	42.295	−83.678	R60	1	HBS 5766				
MN	Cass	Motley	46.430	−94.654	R63	1	HBS 5587				
		Clearwater	Bagley	47.651	−95.425	R67	1	HBS 14606			
		Polk	Winger	47.587	−95.978	R68	1	HBS 14630			
MO	Clay	Liberty	39.289	−94.394	R65	1	HBS 5175				
		Linn	Brookfield	39.878	−93.075	R64	1	HBS 10011			
		Texas	Cabool	37.089	−92.060	R66	1	HBS 6074			

Table 1 continued

Region	State	County	Locality	LAT	LON	Hap	<i>N</i>	Catalogue Number
	MT	Carbon	Bridger	45.113	−108.741	R46	2	HBS 7483–4
		Custer	Miles City	46.132	−105.563	R20	1	HBS 7357
						R41	1	HBS 7356
		Garfield	Hillside	46.887	−106.357	R43	1	HBS 7394
						R44	1	HBS 7398
		Musselshell	Roundup	46.340	−108.479	R42	1	HBS 7448
						R46	1	HBS 7447
	NC	Scotland	Scotland	34.826	−79.460	R53	1	HBS 5995
	ND	Nelson	Kloten	47.744	−98.078	R45	1	HBS 14738
		Ramsey	Webster	48.297	−98.927	R46	2	HBS 5424–5
		Sheridan	Goodrich	47.472	−100.118	R20	1	HBS 5535
						R21	1	HBS 5536
	NE	Antelope	Oakdale	42.064	−98.010	R20	1	HBS 5862
						R57	1	HBS 5861
		Furnas	Holbrook	40.307	−100.010	R20	1	HBS 7933
		Gage	Beatrice	40.237	−96.797	R28	1	HBS 10281
		Hall	Cairo	41.000	−98.702	R20	2	HBS 13506–7
		Saunders	Touhy	41.133	−96.854	R39	1	HBS 10442
	NM	Colfax	Eagle's Nest	36.513	−105.282	R18	1	HBS 15233
		Grant	Corners Ranch	32.641	−108.572	R24	1	HBS 6916
						R52	1	HBS 6917
			Silver City	32.784	−108.231	R48	2	HBS 6950–1
			Tyrone	32.809	−108.736	R24	2	HBS 6934–5
		Lincoln	Capitan	33.541	−105.601	R47	1	HBS 7011
						R48	1	HBS 7012
		McKinley	Thoreau	35.885	−108.239	R03	1	HBS 7102
		Rio Arriba	Brazos	36.754	−106.554	R01	1	HBS 24702
			Tres Piedras	36.649	−105.983	R36	1	HBS 18442
		Socorro	San Antonio	33.883	−106.682	R02	2	HBS 6957–8
			White Sands	33.825	−106.261	R48	1	HBS 7004
						R50	1	HBS 7003
		Torrance	Encino	34.876	−105.482	R49	1	HBS 7060
	OK	Beckham	Sandy Sanders	35.068	−99.836	R17	2	OMNH 40368–9
		Ellis	Packsaddle	35.897	−99.698	R30	1	OMNH 41728
						R51	3	OMNH 41727, 41729–30
	SC	Aiken	Ellenton Bay	33.221	−81.747	R55	1	HBS 6652
	SD	Beadle	Virgil	44.295	−98.486	R19	1	HBS 14789
						R46	1	HBS 14788
		Butte	Belle Fourche	44.680	−103.815	R46	2	HBS 7300, 7304
		Deuel	Altamont	44.846	−96.688	R46	2	HBS 14818–9
	TN	Montgomery	Palmyra	36.429	−87.471	R58	1	HBS 5615
	TX	Collingsworth	Marilla	35.013	−100.379	R16	1	TNHC 72507
						R47	1	TNHC 72506
		Jeff Davis	Fort Davis	30.565	−103.940	R48	2	HBS 7926–7
	UT	Cache	Porcupine Reservoir	41.518	−111.736	R46	2	HBS 16004–5
		Garfield	Boulder	37.922	−111.424	R07	2	HBS 7827–8
		Kane	Kanab Creek	37.102	−112.548	R07	2	HBS 7868–9

Table 1 continued

Region	State	County	Locality	LAT	LON	Hap	N	Catalogue Number
		Salt Lake	Salamander Lake	40.701	-111.607	R10	2	HBS 7712–3
		Uintah	Vernal	40.455	-109.538	R46	1	HBS 20205
		Utah	Thistle	39.994	-111.493	R15	1	HBS 7763
		Wasatch	Fruitland	40.193	-110.933	R13	1	HBS 20183
						R33	1	HBS 20181
		Washington	Lucky D Ranch	37.216	-112.974	R09	2	HBS 7877–8
		Wayne	Hanksville	38.374	-110.714	R37	1	HBS 18162
						R40	1	HBS 18161
	WA	Adams	4th of July Lake	47.243	-117.986	R11	1	HBS 6883
			Wildcat Lake	46.727	-118.157	R12	1	HBS 6822
		Grant	Potholes Reservoir	46.960	-119.263	R11	1	HBS 6792
		Whitman	Lake Chinook	46.726	-117.153	R12	2	HBS–2
	WI	Dane	Oregon	42.951	-89.378	R68	1	HBS 5154
	WY	Fremont	Diversion Dam	48.217	-98.961	R32	1	HBS 7569
			Togwatee Pass	43.585	-109.783	R46	2	HBS 7584–5
		Goshen	Hawk Springs	41.723	-104.264	R22	1	HBS 7242
						R27	1	HBS 7241
		Hot Springs	Black Mountain	43.740	-108.048	R46	2	HBS 7525, 7530
		Laramie	Midway	41.365	-104.463	R26	1	HBS 7200
						R31	1	HBS 7199
		Niobrara	Boner Road	43.083	-104.364	R46	2	HBS 7277, 7281
		Teton	Wilson	43.488	-110.828	R42	1	HBS 7602
						R46	1	HBS 7603

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