

Appendix K

# **California Tiger Salamander Hybridization**



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## Introduction

Introgression, or the incorporation of genes from one species into the gene pool of another as a result of hybridization, occurs through natural processes such as species range expansion and natural selection. It is also sometimes the result of human-related disturbance. Barred tiger salamanders (*Ambystoma tigrinum mavortium*) were introduced to California over 50 years ago and have hybridized with native California tiger salamanders (*Ambystoma californiense*). The number and range of these non-native salamanders and their hybrid progeny have expanded since introduction. This appendix summarizes the most applicable scientific literature to date and outlines the strategy to manage California tiger salamanders in the Santa Clara Valley Habitat Plan (Habitat Plan) through the adaptive management process.

To manage California tiger salamanders populations in a manner that achieves the biological goals and objectives set in the Habitat Plan, we first need to identify the potential negative impacts of hybridization and introgression. Further, we must better understand any ecological impacts that non-native and hybrid salamanders have on aquatic communities in the study area. Rather than assuming that all hybrids should be controlled, we need to determine whether a population with introduced alleles (potential variants of a gene) functions ecologically in a similar manner as the native population (Fitzpatrick et al. 2010).

The Habitat Plan will treat individuals morphologically resembling the threatened Central Distinct Population Segment of the California tiger salamander as if they were the listed entity. Take prohibitions will still apply to all individuals morphologically resembling the listed taxon. The primary reason for the initial management strategy described below is to provide the Habitat Plan with flexibility to deal with the diverse situations resulting from more than 50 years of interbreeding between *Ambystoma californiense* and *Ambystoma tigrinum mavortium* within portions of the California tiger salamander's range (Fitzpatrick and Shaffer 2004). In this case, elimination of all or even most introgressed impure populations is not likely feasible due the extent of the invasion, extent of unsurveyed habitat, longevity of the species, and biphasic life history of the species, which is predominately spent in underground burrows (Fitzpatrick and Shaffer 2007a). The hybrid management plan does not preclude the Wildlife Agencies' ability to approve, on a case by case basis, the Implementing Entity's eradication of introgressed progeny if the presence of

these individuals is determined to interfere with conservation efforts for the listed entity or other covered species.

## Release and Expansion History

Barred tiger salamanders were introduced to specific locations in California starting in the early to mid-20th century by bait dealers and fishermen, who used the larvae as live bait (Fitzpatrick and Shaffer 2007a).<sup>1</sup> This subspecies of barred salamander is native to parts of Texas, eastern New Mexico, Oklahoma, Kansas, Nebraska, eastern Wyoming, and Colorado. It has been found in isolated locations throughout much of California. The barred salamander is much larger than the California tiger salamander (it is the second largest salamander in the United States) and exhibits different behavior and life-history traits than the California tiger salamander. Native California tiger salamanders and introduced barred tiger salamanders have been hybridizing for 50–60 years (Fitzpatrick and Shaffer 2007b). Importantly, barred tiger salamander adults retain juvenile traits such as gills when they breed in aquatic habitats. These individuals, called “paedomorphs,” provide the opportunity to readily distinguish barred salamanders and hybrids from native California tiger salamanders in breeding ponds and wetlands.

Introduced and hybrid tiger salamanders in the south and east San Francisco Bay have likely dispersed there from known introduction sites in the Salinas Valley (Fitzpatrick and Shaffer 2007a). In the Salinas Valley, many sampled tiger salamanders had high frequency of introduced alleles or high hybrid index (Fitzpatrick and Shaffer 2007a). In California, an abrupt transition from high (but variable) to very low introduced allele frequencies occurs at a distance of greater than 7.5 miles (12 kilometers [km]) from release sites (Fitzpatrick and Shaffer 2007a). The Santa Clara County line is approximately 27 miles from the Salinas Valley release sites. More research is required to explain the abrupt change in allele distribution from high frequencies to very low frequencies of introduced alleles at sites greater than 12 km from release sites. One possible explanation for this observation may be the result of a strong selection for certain hybrid characteristics in local populations (e.g., hybrid survival is higher in permanent ponds) (Fitzpatrick and Shaffer 2007a). Another potential explanation is that the spread of the invasion is limited by salamander dispersal capabilities and that the “wave” of hybridization has only reached 12 km to date but continues to spread (Shaffer pers. comm. 2010).

## Santa Clara County Populations

California tiger salamander potential upland and breeding habitat is distributed throughout undeveloped areas of Santa Clara County, due to the presence of stock ponds and other aquatic habitat. Known occurrences are scattered

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<sup>1</sup> The use of any salamander as bait in California and the transport or possession of any salamander in the genus *Ambystoma* is now illegal without a special DFG permit (California Department of Fish and Game 2009).

throughout Santa Clara County, on both sides of the valley, with large clusters of occurrences in Henry W. Coe State Park and Joseph D. Grant County Park (California Natural Diversity Database 2006).

Some level of hybridization is most likely present in a large number of ponds throughout Santa Clara County and may be high in some ponds in the southern portion of the county (e.g., Bluestone Lake, North Fork Pacheco Creek). Of the Santa Clara County populations genotyped, all had some low-level of hybridization (J. Johnson pers. comm. 2009), and one, Bluestone Lake, in southern Santa Clara County, had an average introduced allele frequency of 60% (Fitzpatrick et al. 2009). Three nonnative “superinvasive” alleles have been found in California tiger salamander populations as far north as the Contra Costa/Alameda County line (Fitzpatrick et al. 2009). These three genes represent only about 5% of the genes examined to date (Fitzpatrick et al. 2010). In addition to the Salinas Valley release site, barred salamanders were introduced in Santa Clara County in a perennial pond located east of North Fork Pacheco Creek in the early 1980s and in 1984 to a nearby pond that periodically dried (J. Smith pers. comm. 2010a). As a result, nonnative barred salamanders have been identified during surveys at the North Fork Pacheco Creek (above Pacheco Reservoir) (J. Smith pers. comm. 2010a). The North Fork Pacheco Creek release site is in close proximity of known California tiger salamander locations, such as those found in the southwest corner of Henry Coe Park and throughout the eastern portion of the Plan Area (Belli 2007; J. Smith pers. comm. 2010a, 2010b).

## Factors Affecting Hybridization

Hybridization between California tiger salamander and barred salamander is influenced by both environmental and biological factors. Initially, studies indicated that the survival rates of California tiger salamanders with low levels of introduced alleles may increase relative to native California tiger salamanders, due to a phenomenon called “hybrid vigor” in which cross-breeding produces higher survival rates (Fitzpatrick and Shaffer 2007b). The term “hybrid vigor,” or “heterosis” is a phenomenon where hybrid offspring have higher fitness than either of the pure parentals. For example, hybrid tiger salamanders have higher early larval survival rates (first weeks after hatching) than individuals with mostly native or mostly introduced alleles (Fitzpatrick and Shaffer 2007b).

More recent studies suggest however, that pure native California tiger salamanders are less fit than both hybrids and pure barred tiger salamanders. In a study conducted by Ryan et al., introduced barred tiger salamanders and hybrids appeared to reduce the survival, growth, and development rate of native California tiger salamanders (2009). Most contemporary hybrids in the study were larger than the native California tiger salamanders. Hybrids reduced the survival and growth rate of native California tiger salamanders through cannibalism and competition (Ryan et al. 2009).

Studies have detected a pattern of high introduced allele frequencies in perennial ponds and relatively even native and introduced allele frequencies in seasonal ponds within the introduction zone in the Salinas Valley (Fitzpatrick and Shaffer 2007a). This pattern may be due to overall selection for nonnative alleles leading to high hybrid larval survival in all ponds, particularly for barred tiger salamander characteristics in perennial ponds. Native California tiger salamanders must metamorphose into terrestrial adult salamanders (*metamorphs*) to reproduce, but barred tiger salamanders in perennial ponds often breed prior to metamorphosis, as sexually mature aquatic larval forms (*paedomorphs*) (Fitzpatrick and Shaffer 2004). Paedomorphs often reach sexual maturity earlier than metamorphs, produce larger clutches, and may breed earlier in a given season, leading to higher reproductive success in perennial ponds (Fitzpatrick and Shaffer 2007b). Paedomorphs grow larger than metamorphs, and females produce more eggs (Rose and Armentrout 1976 cited in Fitzpatrick and Shaffer 2004; Petranka 1998 cited in Fitzpatrick and Shaffer 2004). In addition, introduced barred tiger salamanders may be able to take better advantage of perennial ponds by breeding earlier in the fall, thereby giving their larvae a competitive head start over later breeding native California tiger salamanders (Fitzpatrick and Shaffer 2004). Ultimately these factors allow paedomorphs to outcompete metamorphs for the limited resources of a pond ecosystem.

## Ecological Effects of Hybridization

The presence of hybrid tiger salamanders may negatively impact native California tiger salamander population levels. As indicated above, Ryan et al. (2009) observed reduced survival and reduced growth and development rates in native California tiger salamander larvae that co-occurred with non-native salamanders and their back crossed progeny. Native tiger salamanders with slower growth rates because of hybrid salamander competition may have decreased survival in dry years (when ponds are more likely to dry before salamanders reach minimum size to metamorphose) and higher larval predation risk (Werner 1986). Native tiger salamanders with smaller sizes at metamorphosis may have lower adult fitness due to higher desiccation and predation risk (Ryan et al. 2009).

Hybrid tiger salamanders with high levels of introduced alleles have higher rates of predation on other native amphibians compared to native California tiger salamanders (Ryan et al. 2009). It is not known whether these high predation levels also occur in populations with only a few introduced alleles (Fitzpatrick et al. 2010). It has been demonstrated that the presence of hybrid tiger salamanders reduces the survival of other pond species. In one study, introduced barred tiger salamanders and hybrids reduced survival, growth and developmental rates of native California tiger salamander, California newt (*Taricha torosa*), and Pacific chorus frog (*Pseudacris regilla*) larvae (Ryan et al. 2009). California red-legged frog (*Rana draytonii draytonii*) and Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*) larvae that reach a large enough size to avoid predation by native California tiger salamanders may not reach a large enough size to avoid predation by large hybrids (Ryan et al. 2009).

Only a small portion of the species' genome has been studied to date. As previously discussed, some studies suggest that hybrid tiger salamanders with low levels of introduced alleles have increased fitness and have the same general ecological traits as native tiger salamanders, although more research is required to be sure that they do not have other negative characteristics (e.g., paedomorphism) (Fitzpatrick and Shaffer 2007b; Fitzpatrick et al. 2010). Determining which non-native alleles are responsible for paedomorphism, overall size, and growth rate needs to be better understood. These physical characteristics appear to have the most significant ecological effect on the California tiger salamander (Shaffer pers. comm. 2010).

## Management of California Tiger Salamander Hybrids

### Initial Management Strategy

The Implementing Entity will adaptively manage California tiger salamanders and hybrids in close coordination with the Wildlife Agencies. Integration of the best available scientific data will be crucial in the adaptive management process as this initial management strategy is based largely upon studies targeting a small fraction of the species' genome and many more superinvasive alleles of unknown effect may have also introgressed into native populations (J. Johnson pers. comm. 2010b). Management will be updated as future studies provide us with better understanding of the functional aspects of the markers initially used to assess admixture proportions.

Rather than focusing management on genetic thresholds based on studies conducted on a relatively small portion of the species' genome, the initial management strategy will focus on restoring and maintaining wetland and pond conditions within the Reserve System that favor California tiger salamanders. Studies suggest that habitat characteristics of native species should be exploited in management strategies to limit hybridization (Riley et al. 2003). Perennial ponds studied in the hybrid zone often contained paedomorphic tiger salamanders, relative to more seasonal aquatic sites like vernal pools (Fitzpatrick and Shaffer 2004). Therefore, initial restoration actions will target sites where paedomorphs have been observed because presence of paedomorphs would indicate presence of non-native alleles in the tiger salamander population. Since different individual tiger salamanders are expected to return to breeding ponds every year, these targeted perennial ponds will be drained on an annual basis. Adaptive management will be used to determine the number of targeted ponds, evaluate effects on grazing, and the use of other available pond restoration techniques. As described in Chapter 5, ponds created for the Reserve System will be designed to rely on passive management (e.g., dry on their own periodically), minimizing the need for artificial draining and management (e.g., stock pond dams fitted with drainage structures). Annual draining of ponds may adversely affect other species covered under this Plan, such as the California red-legged frog or western pond turtle. As such, pond draining will be timed to

minimize effects on other covered species. Initially, a conservative approach may be taken to minimize adverse effects to metamorphosing California red-legged frogs (i.e., ponds should not be drained until late September). More studies would be needed to determine the amount of time paedomorphs require to fully metamorphose in order to disperse from breeding habitat to determine the efficacy of draining ponds and the timing of pond drainage.

Since the final Reserve System will be extensive and include up to 80 acres of wetlands and 104 acres of ponds (**Table 5-13**), the Implementing Entity will prioritize management sites for the purposes of California tiger salamanders unless doing so would preclude the Implementing Entity's ability to fulfill the conservation strategy for other aquatic covered species. Prioritization of management sites will take into consideration factors such as presence of paedomorphs, presence of other covered species, presence of non-native predators/competitors (i.e., fish and bullfrogs), condition of adjacent aquatic sites, and other factors.

Specific management strategies for hybrid salamanders will be incorporated in the applicable reserve unit management plans described in Chapter 5. The California tiger salamander management component of these plans will be updated as necessary to adapt to changing conditions in the reserves and to respond to monitoring or other new data. The Implementing Entity will review and, where biologically appropriate, systematically revise reserve unit management plans at least every 5 years. However, given the dynamic nature of the hybrid salamander situation, this component of the reserve unit management plan may need to be updated more frequently.

Future data may indicate that management of breeding habitat may not be adequate to fulfill the conservation strategy for the California tiger salamander. If that is the case, the Implementing Entity and Wildlife Agencies will evaluate the current management strategy and make appropriate modifications. Modifications could include active eradication of individuals based on scientifically-based genetic thresholds. Since there is a potential that the Plan may need to actively manage individuals later in the permit term, the Plan budget accounts for costs associated with genetic testing of tiger salamanders within the Reserve System, which may be necessary to substantiate future thresholds for management (see *Monitoring* below).

## Potential Management Strategy Modifications

Rather than establishing a threshold number of introduced alleles, above which an individual is considered a hybrid, it is necessary to determine whether the population with introduced alleles functions ecologically in a similar manner as the native population (Fitzpatrick et al. 2010). California tiger salamanders in Santa Clara County have been documented carrying three fixed alleles (called "superinvasive alleles") from the introduced barred salamander. If those salamanders still function in a way similar to native California tiger salamanders it may be appropriate to treat them as the native species (Fitzpatrick et al. 2010).

Currently, little is known regarding the effects of these three alleles. Researchers are proposing a set of experiments to examine these effects.

In the Salinas Valley to San Francisco Bay hybrid zone, approximately 5% (3 alleles) of genotyped native alleles have been almost completely replaced by the superinvasive alleles (Fitzpatrick et al. 2009). Since we do not yet know the morphological and ecological consequences of the fixation of introduced alleles, Fitzpatrick et al. (2009) recommend treating these the same as native California tiger salamanders.

Eradication of introduced tiger salamander alleles may not be feasible because adults may disperse over large distances and live for up to 11 years in underground burrows (Trenham et al. 2000). Further, eradication efforts under the Habitat Plan can only occur on Habitat Plan reserves. Most hybrids likely occur on private land, where access and management options are limited (Fitzpatrick and Shaffer 2007a).

Introduction of a few non-native alleles may change genetic similarity sufficiently to affect the legal status of California tiger salamander populations (Fitzpatrick et al. 2009). However, changes in morphological, ecological, or behavioral characteristics (that could potentially arise through introgression of even small amounts of genomic material may justify a change in legal status (Fitzpatrick et al. 2009; Fitzpatrick et al. 2010). For example, if future studies indicate that tiger salamanders with introduced alleles exhibit increased numbers of paedomorphs or increased predation rates on native pond species, new management actions, including eradication of hybrids, may be required to ensure that hybrids do not preclude the recovery of California tiger salamanders or adversely affect other native species.

It may therefore become necessary in the future to manage a subset of the population with allele frequencies beyond a set threshold as “not protected,” rather than verifying the genotype of each individual in the pond (Fitzpatrick and Shaffer 2007a).

The Implementing Entity will work with the Wildlife Agencies through the adaptive management process if monitoring data indicates that the initial management strategy is not adequate to meet the goals and objectives of the Plan.

## Monitoring

Hybrid index studies conducted by the University of California Davis in tiger salamander populations in California provide some baseline data for the study area. A baseline study will identify the distribution and level of hybridization in ponds and the presence of barred salamanders in the Reserve System (STUDIES-8). Baseline surveys will document presence of paedomorphs and identify non-native alleles and the frequency of those alleles present in a representative sample of salamander populations within the Reserve System. Future studies will focus

on how each non-native allele is physically expressed and the subsequent ecological impact of these alleles.

In years following baseline data collection, monitoring California tiger salamander level of hybridization will take place annually for a representative sample of wetlands and ponds within the Reserve System. Monitoring frequency could be modified with the approval of the Wildlife Agencies through the adaptive management process.

The results of monitoring will inform future management decisions made by the Implementing Entity and the Wildlife Agencies. The Plan will closely monitor the effects of this strategy on all affected covered species, not just the California tiger salamander. California red-legged frogs and western pond turtles commonly co-occur with California tiger salamanders and may be affected by habitat management proposed to address the California tiger salamander hybridization issue. Furthermore, grazing will play an important role in achieving the biological goals and objectives of many of the covered species (i.e., bay checkerspot butterfly). Draining of ponds for the benefit of California tiger salamanders has the potential of affecting the feasibility of grazing some portions of the Reserve System. If monitoring results indicate that the hybrid management strategy for California tiger salamanders is adversely affecting the conservation strategy for other covered species, the management plan will be modified through the adaptive management process with Wildlife Agency approval.

## Education and Research

The Implementing Entity will conduct education and outreach, provide technical assistance, and inform landowners of regulatory incentives (e.g., Safe Harbor Agreement) to restore, create, and maintain breeding habitat conditions on their land that favor native California tiger salamanders (POND-11). New nonnative salamander introductions are caused by humans and therefore could be decreased with a public education campaign. Public education will also be conducted to inform the public that the use of any salamander as bait in the State of California is illegal (POND-12).

The Plan will fund research to determine the distribution of, and ecological effects resulting from, introgression and interbreeding of native and non-native tiger salamanders (STUDIES-8). These studies will be coordinated with, and be complementary to, similar studies conducted outside of the purview of the Habitat Plan. With Wildlife Agency approval, the Implementing Entity will incorporate specific management prescriptions supported by this research, and research conducted by others, in the reserve unit management plans.

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