



United States Department of the Interior



FISH AND WILDLIFE SERVICE
Ventura Fish and Wildlife Office
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IN REPLY REFER TO:
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June 15, 2007

Robert J. Uram
Sheppard, Mullin, Richter, and Hampton, LLP
Four Embarcadero Center, 17th Floor
San Francisco, California 94111-4106

Subject: Regulatory Status of the Tiger Salamander Population at Cielo Grande Ranch
(Assessor's Parcel Numbers 014-030-029 through 014-030-032; 014-030-034
through 014-030-039; and 014-030-056) in Gonzales, Monterey County, California

Dear Mr. Uram:

I am writing in response to your letter, dated March 9, 2007, and received in our office on March 12, 2007, requesting our concurrence that the population of tiger salamanders currently occupying ponds on the subject project site is not subject to protection under the Federal Endangered Species Act of 1973, as amended (Act). On March 19, 2007, we requested clarification of several issues related to your March 9, 2007, letter, including a characterization of aquatic habitats in the vicinity of the project site and details of the genetic research conducted on salamanders from the subject property. You responded to our request with a letter and additional information, dated May 11, 2007, which we received in our office on May 14, 2007.

The U.S. Fish and Wildlife Service's (Service) responsibilities include administering the Act, including sections 7, 9, and 10. Section 9 of the Act prohibits the taking of any federally listed endangered or threatened species. Section 3(18) of the Act defines take to mean to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct. Service regulations (50 CFR 17.3) define harm to include significant habitat modification or degradation which actually kills or injures wildlife by significantly impairing essential behavioral patterns, including breeding, feeding or sheltering. Harassment is defined by the Service as an intentional or negligent action that creates the likelihood of injury to wildlife by annoying it to such an extent as to significantly disrupt normal behavioral patterns which include, but are not limited to, breeding, feeding, or sheltering. The Act provides for civil and criminal penalties for the unlawful taking of listed species. Exemptions to the prohibitions against take may be obtained through coordination with the Service in two ways: through interagency consultation for projects with Federal involvement pursuant to section 7 or through the issuance of an incidental take permit under section 10(a)(1)(B) of the Act.

On August 3, 2004, the Service published a final rule listing the California tiger salamander (*Ambystoma californiense*) as threatened range-wide, which included down-listing the previously federally endangered Sonoma and Santa Barbara distinct population segments (69 Federal Register

(FR) 47212). On August 19, 2005, U.S. District Judge William Alsup vacated the Service's down-listing of the Sonoma and Santa Barbara populations from endangered to threatened. Therefore, the Sonoma and Santa Barbara populations of the California tiger salamander are listed as endangered, and the Central California populations (including those occurring in Monterey County) are listed as threatened.

In the 1940s and 1950s, bait dealers from the Salinas Valley in Monterey County imported thousands of barred tiger salamander (*Ambystoma tigrinum mavortium*) larvae from Texas and other parts of the southwestern United States (Riley et al. 2003). Many of the non-native barred tiger salamanders were released in the hope of establishing harvestable populations in central California because they attain larger size prior to metamorphosis and can be available further into the summer than native California tiger salamanders. As a result of these introductions, non-native tiger salamanders established reproductive populations within dispersal distance of populations of California tiger salamanders.

One of the primary threats to the California tiger salamander is hybridization with non-native tiger salamanders in areas where the non-native salamanders were introduced and established viable populations (69 FR 47212). Hybridization between California tiger salamanders and non-native tiger salamanders results in introgression (i.e., the exchange of genetic material between different species or sub-species). Depending on the degree and extent of introgression (i.e., the number of reproductive generations between hybrid salamanders), certain populations of California tiger salamanders may become populations of salamanders with primarily non-native genes. Such genetic change has been characterized as a kind of extinction (Rhymer and Simberloff 1996), and may result in a population with fundamentally altered ecological function (Ellstrand and Schierenbeck 2000).

The August 3, 2004, listing rule for the California tiger salamander identified hybridization with non-native salamanders as a serious threat to the species in the Central Coast region of California (69 FR 47239). Research indicates that within this region, many Monterey County populations of the California tiger salamander are compromised by non-native genes to varying degrees (Fitzpatrick and Shaffer 2007).

As outlined in your March 9, 2007, and May 11, 2007, letters, Dr. H. Bradley Shaffer of the University of California at Davis conducted genetic analysis of larval salamanders from four ponds on the subject property and from seven additional aquatic sites in the vicinity of the subject property. To evaluate the genetics of the salamander population at the subject property, Dr. Shaffer and a colleague analyzed tissue from 21 larval salamanders at each of four ponds on the site (Johnson and Shaffer 2006). A fifth pond occurs on the site; however, Dr. Shaffer did not detect any larvae in this pond despite extensive sampling with a 15-foot long seine. Dr. Shaffer then genotyped individual tissue samples for one mitochondrial single nucleotide polymorphism (SNP) locus (Dloop) and up to seven nuclear SNP loci (FoxG1b, Slc4a4, Dlx3, Contig325, HoxD8, Gnat2, and Gnat1 (Voss et al. 2001)). Through previous research, Dr. Shaffer and his colleagues have identified diagnostic differences between *Ambystoma tigrinum* and *A. californiense* at each of these loci (Fitzpatrick and Shaffer 2004).

At each SNP locus, each individual was scored as 'aa' if it was homozygous for native alleles, 'gg' if it was homozygous for introduced alleles, or 'ga' if it was heterozygous, with one copy each of a native and introduced allele. These data were summarized, for each gene at each pond, as the total frequencies of each genotype. Dr. Shaffer then calculated a Hybrid Index score for each pond by tallying the proportion of alleles (pooled across individuals and genes) that are native for each pond,

using the formula Hybrid Index = (total number of native alleles)/(total number of alleles). This Hybrid Index score is one way of summarizing the overall level of "nativeness" of a sample of animals from a pond (Johnson and Shaffer 2006).

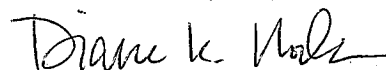
Using these methods, Dr. Shaffer calculated Hybrid Index scores of 0.0798, 0.1132, 0.0649, and 0.1166 for the four ponds on the project site. In other words, for the 9 genetic loci analyzed, none of the ponds contained less than 88.34 percent non-native alleles. In addition, none of the 84 larval salamanders that Dr. Shaffer sampled were found to be pure California tiger salamanders. As a result of these investigations, Dr. Shaffer concluded that "all of the animals in all ponds [on the proposed project site] contain primarily non-native gene copies" (Johnson and Shaffer 2006).

In addition to the genetic analysis conducted on tiger salamanders from the ponds on the subject property, Dr. Shaffer and his colleagues conducted genetic analyses between 1997 and 2003 on tiger salamanders from seven additional aquatic sites within 1.2 miles (the maximum known dispersal distance for California tiger salamanders) of the subject property. These earlier genetic analyses were not as advanced or rigorous as the techniques used in 2006 to evaluate the genetics of salamanders from the subject property. Despite these limitations, Dr. Shaffer and his colleagues detected considerable levels of introgression in salamanders sampled from the seven aquatic sites within dispersal distance of the subject property. Specifically, researchers determined (based on evaluating 1 mitochondrial and 4 nuclear genetic loci) that the mean introduced allele frequencies for the seven sites were 0.487, 0.782, 0.697, 0.972, 0.55, 0.76, and 0.518. Considering that between 4 and 10 reproductive seasons have passed since several of these sites were sampled (i.e., since 1997 (10) and since 2003 (4)), the level of genetic introgression at all of these 7 sites may have increased since these allele frequencies were calculated.

We have carefully reviewed the information you provided with your March 9, 2007, and May 11, 2007, letters, including the results of genetic investigations conducted on tiger salamanders from aquatic sites on, adjacent to, and near the subject property. Following our review, we conclude that none of the individual tiger salamanders which comprise the salamander population at the subject property are the listed entity under the Act (i.e., California tiger salamanders). Therefore, tiger salamanders utilizing the ponds on the subject property are not afforded the protections of the Act.

If you have any questions regarding this letter, please contact Roger Root of my staff at (805) 644-1766, extension 336.

Sincerely,



Diane K. Noda
Field Supervisor

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