

Shaffer *et al.*'s (1993) allozyme work did not reveal much variation (only 9 of 26 loci were variable) and indicate that Sonoma County California tiger salamanders are not distinct from other western populations of California tiger salamander from Yolo County to San Luis Obispo County.

*Response:* A variety of genetic tools are available to assess genetic variation. These tools are often referred to as "genetic markers." All are indicators of genetic variation, but none is considered determinative. Which genetic marker is most useful depends on the question being asked and the organism being studied (Haig 1998, Parker *et al.* 1998).

Allozymes are proteins which are used as genetic markers because DNA contains information that is used by cells to build protein. Allozymes have been used to assess genetic variation for many years. Allozyme studies have the advantage of being relatively inexpensive and straightforward, once the basic technique is developed for a group. However, drawbacks of using allozymes include the limited number of proteins that can be screened (Parker *et al.* 1998) and the fact that they often detect little variability (Haig 1998). On average across taxa, less than half of all loci are variable. It is not uncommon for population biologists to encounter species for which allozymes cannot be used as genetic markers because they lack variation (Parker *et al.* 1998).

Molecular techniques, such as mtDNA, allow biologists to examine variation in DNA directly, rather than looking at the product derived from DNA (*i.e.*, proteins) (Parker *et al.* 1998). Analysis of animal mtDNA is the most commonly used technique for examining phylogenetic relationships among populations of the same species and among closely related species (Taberlet 1996). One advantage of mtDNA in particular is its high rate of evolution (*i.e.*, rate of nucleotide substitution) compared to other DNA (Taberlet 1996, Parker *et al.* 1998). The D-loop (which Shaffer and colleagues examined for their tiger salamander studies) is especially variable, making it useful to study recently divergent populations or species. Different genetic techniques are expected to resolve different amounts of variation because the genetic markers used have different evolutionary characteristics (Parker *et al.* 1998). The observation that some characters (in this case, allozymes) are not variable does not diminish the utility of other data (in this case mtDNA) in describing relationships among groups.

*Issue 6:* Several commenters felt that our finding that California tiger

salamanders in Sonoma County qualified as a DPS was based on an isolated, and dated, report (*i.e.*, Shaffer *et al.* (1993)). One commenter noted several times that Shaffer *et al.* (1993), the source of mtDNA data for California tiger salamanders, is an unpublished report.

*Response:* We are required to use the best available scientific data. In this case, the data were in an unpublished report. During the comment period, we received a second report (Shaffer and Trenham 2002) that contained findings similar to Shaffer *et al.* 1993 but which was based on more extensive data collection. The publication of Shaffer and McKnight (1996) using mtDNA techniques for California and other tiger salamanders and the publication of mtDNA work by Shaffer *et al.* (2000) on Yosemite toad (*Bufo canorus*) gives us confidence that Shaffer's work is scientifically defensible.

*Issue 7:* Several commenters noted that recent aerial photos and a map that is based on the photos show 515 or more pools located within, or in the vicinity of, the Santa Rosa Plain. They believe these could potentially provide habitat for the Sonoma County California tiger salamander. They stated that many of the pools have not been surveyed and, therefore, the species could be more widespread in Sonoma County than is currently known.

*Our Response:* The map submitted displayed 515 water bodies and was based on interpretation of aerial photography with little on-the-ground verification. We compared the map of potential habitat for the California tiger salamander to information and data we obtained and have determined 360 water bodies can be eliminated as potential habitat for the California tiger salamander due to a variety of factors including: unsuitable soils, unsuitable vegetation, high elevation, presence of aquatic predators, agricultural development (row crops, vineyards, etc.), urbanization, and unsuitable hydrology. One hundred and fifty-five water bodies remained within the suitable habitat area.

Of the 155 remaining water bodies, 53 were characterized as "man-made long" and "natural long" ponds/wetlands, which hold water for too long and/or harbor aquatic predators, and were eliminated as potential habitat for the California tiger salamander. Another set of water bodies were "man-made short" and "natural short" ponds/wetland (12 in total) which do not hold water long enough to be a source of potential habitat for the California tiger salamander. Consequently only "man-made moderate" and "natural

moderate" mapped water bodies were considered potential suitable habitat (90 in total).

Of the "man-made moderate" and "natural moderate" mapped water bodies, four were formerly known breeding sites that have been eliminated and eight are currently identified as existing breeding sites. Some of the mapped water bodies are anticipated to have aquatic predators given their location on the Laguna de Santa Rosa floodplain, which would limit California tiger salamander utilization. Others contain habitat with sightings near the ponds, but these ponds have been repeatedly surveyed by experts, with results indicating they do not support breeding populations of California tiger salamanders.

The determination that some of the mapped water bodies contain potential habitat is solely based on aerial photographs; however, the majority of these are on private property and inaccessible to surveying without landowner permission. Several recognized salamander biologists have conducted repeated road surveys in Sonoma County along areas where the California tiger salamander is known to exist or where suitable habitat appears to exist (D. Cook, The Wildlife Society, pers. comm., 2002; P. Northen, California State University, Sonoma, pers. comm., 2002; J. Seifers, Santa Rosa, California, pers. comm., 2002; H. B. Shaffer, pers. comm., 2002; P. C. Trenham, UCD, pers. comm., 2002). Night driving is a standard technique for surveying for reptiles and amphibians (Shaffer and Juterbock 1994; Parris 1999). The locations where these biologists found breeding sites, migrating adult salamanders, subadults, larvae, and egg masses in roadside ditches were entered into the CNDDDB. This data is considered essential (D. McGriff, CDFG, pers. comm., 2002) and the data was utilized in our analysis of the status of the California tiger salamander in Sonoma County. Several of the experts indicated that there are likely to be a few small breeding sites or potential habitat for California tiger salamanders on private lands containing grassland areas and suitable soils on the Santa Rosa Plain, including stock ponds (P. Northen, pers. comm., 2002; H. B. Shaffer, pers. comm., 2002; P. C. Trenham, pers. comm., 2002); however, these private lands were inaccessible during their survey efforts.

*Issue 8:* One commenter believed that two sites not specifically mentioned in the proposed rule should be included as breeding sites for California tiger salamanders.