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## NATURAL HISTORY NOTE

# Further Evidence of Range-wide Overwintering in the Larvae of the California Tiger Salamander (*Ambystoma californiense*)

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With some exceptions, the larval form of biphasic amphibians transition through a larval phase lasting one to three seasons, or more, following hatching from the egg (Gilbert and Frieden 1981, Duellman and Trueb 1994, Dodd 2013, Petranks 1998). Metamorphosis normally follows the larval phase and can be highly variable among amphibians in timing and duration, becoming extreme in the urodels—permanently remaining in the larval form (i.e., neoteny, paedogenesis, and paedomorphosis; Gould 1977, Alberch et al. 1979). Facultatively paedomorphic salamander populations vary both locally and annually in their frequency of metamorphs and paedomorphs (Eagleson 1976, Patterson 1978, Sexton and Bizer 1978, Collins 1981, Semlitsch 1985). Semlitsch (1985) and Whitman (1994) reported that facultatively paedomorphic individuals retain the ability to metamorphose, suggesting that populations can be a mix of terrestrial adult forms as well as paedomorphs. In California, members of the Ambystomidae show signs of paedomorphosis with members of the genus *Dicamptodon* known to

be paedomorphic (Stebbins and Cohen 1995). Other Ambystomids in California, particularly the non-native Barred Tiger Salamander (*Ambystoma mavortium*) also shows signs of paedomorphosis (Petranks 1998). Additionally, the California Tiger Salamander (*A. californiense*) has been reported to overwinter as larvae, but the behavior was thought to be isolated to areas of the eastern San Francisco Bay Area (i.e., Alameda, Contra Costa, Alameda, and Santa Clara counties; Alvarez 2004, Wilcox et al. 2015). Here in we report on overwintering larvae of the California Tiger Salamander in a disparate area relative to initial reports, and remark on management implications.

Focal surveys for California Tiger Salamander larvae in eastern Merced County were conducted for five consecutive years from 2017 to 2021 within the range of the Central Valley Recovery Unit of the California Tiger Salamander (management unit determined by the U.S. Fish and Wildlife Service [USFWS 2017]). Surveys were conducted in vernal pool grassland habitat, specifically in aquatic features, including natural playa pools, as well as human-made cattle

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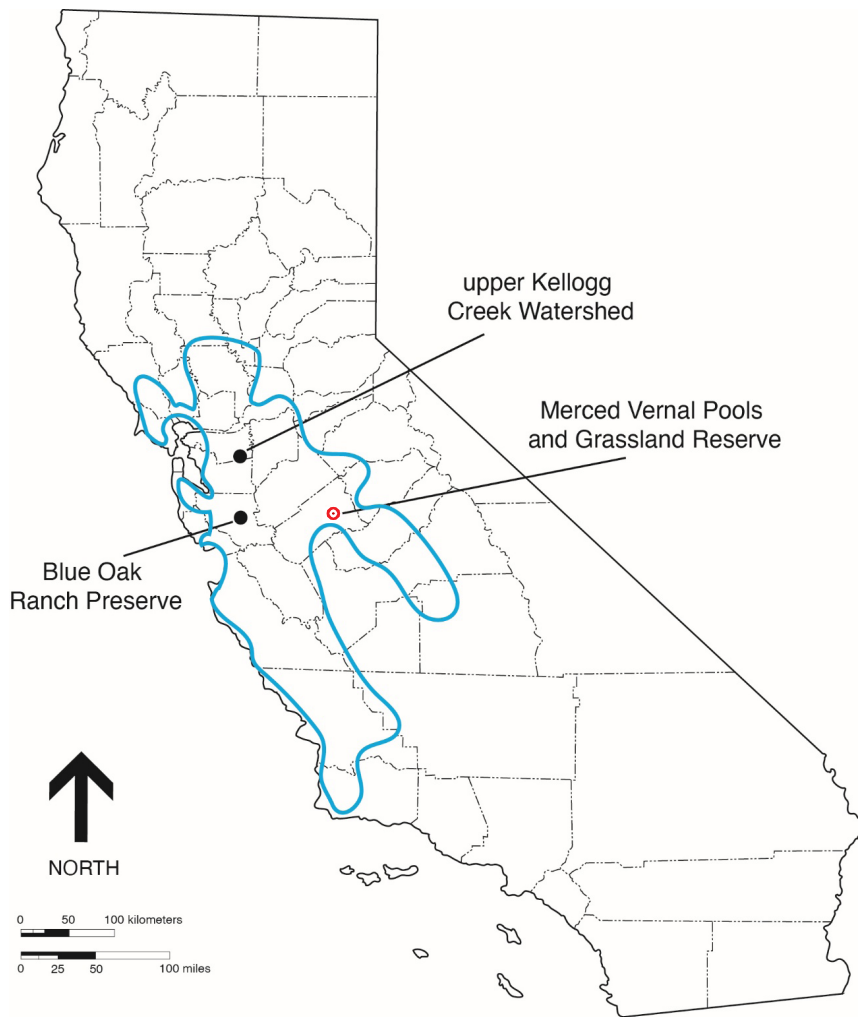
**Fig. 1.** Overwintering California Tiger Salamander (*Ambystoma californiense*) larvae found in Avocet Pond, Merced County, on 12 March 2020. Morphological features suggest the individual is in the process of metamorphosis (i.e., robust leg development, reduced gills [right side], distinct opaque coloration changes). Photographed by Francesca Cannizzo and Robert Shields.

stock ponds. Larval sampling used three methods: seine, aquatic dip net, and ski net (Sweet 2017). Some features were entirely surveyed, while only a subset of larger features, over an acre in size, were sampled. All California Tiger Salamanders captured during sampling were measured snout to vent (SVL) and total length (TL) and recorded as one of five size classes. Typically, larval surveys were conducted multiple times during the year. The first survey was conducted approximately one month after a potential breeding feature held a minimum of 15 cm of water, to document whether breeding was attempted. A second survey was typically conducted in the same features to assess the likelihood that larvae would metamorphose before the feature dried.

On 12 March 2020, a California Tiger Salamander larva was found in a stock pond referred to as Avocet Pond (37.411445687 N, 120.369990063 W), with gills, fully developed digits, and evident intercostal grooves (Fig. 1). The larvae measured 89 mm SVL and 177 mm TL. Stock ponds surveyed on the same date—less than 1.5 miles from Avocet Pond—had California Tiger Salamander larvae ranging from 48 mm to 89 mm in TL.

Avocet Pond is atypically a perennial water body and did not dry in 2019—the year preceding our survey and discovery of overwintering larvae. We speculated that, based on size of larvae and developmental stage when detected in March, it is likely that California Tiger Salamanders had overwintered as larvae at this site. We add that evidence of overwintering was noted in the maturity and use of legs in the larvae in Avocet Pond. Alvarez and Wilcox (in prep.) indicate that the use of legs for locomotion signifies that metamorphosis is progressing imminently (see Fig. 1). Data from similar habitats surveyed in the surrounding area during March 2020, included larvae that were 49–72% smaller than those found in Avocet Pond, and noted legs of individuals were not well developed or used for locomotion, indicating that these individuals were likely hatched during late 2019–2020. Growth rates of California Tiger Salamanders—following hatching at approximately 12 mm (Storer 1925)—that are under 100 mm TL are believed to be 1 mm/day (D. Cook, Sonoma County Water Agency, pers. comm.). Based on this reporting, California Tiger Salamanders in adjacent features were likely hatched between 25

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**Fig. 2.** Known locations of overwintering larvae of California Tiger Salamander (*Ambystoma californiense*) in California (black dots; Alvarez 2003, Wilcox et al. 2015), and the newly reported location (red dot) in Merced County, California. Blue line represents approximate distributional range of California Tiger Salamander according to USFWS (2002).

December 2019 and 3 February 2020 (i.e., within 2.6 months of sampling).

Despite early reports that California Tiger Salamanders do not overwinter as larvae (Stebbins and Cohen 1995), both Alvarez (2004) and Wilcox et al. (2015) found individuals that indeed overwintered as larvae in no fewer than 17 different water bodies in three counties. Further, both researchers speculated that the species likely overwintered as larvae in other parts of California. The observation reported here appears to extend the geographic range of this phenotypic trait 110 km eastward into the lower foothills of the Sierra Nevada. Although the same Distinct Population Segment (DPS; DPS determined by the USFWS [USFWS 2017]), overwintering larvae reported by Alvarez (2004) were found to be in a separate genetically distinct population from those Wilcox et al. (2015) reported from Santa Clara County. This additional population of overwintering larvae are among the same genetically distinct population as those found by Alvarez (2004), which were found in Alameda and Contra Costa counties

(Shaffer et al. 2004). However, Shaffer et al. (2013) found that there was a fundamental genetic split between the Bay Area/Central Coast Range and Central Valley, and considered it a “very deep” split, that was consistent across mitochondrial DNA and single nucleotide polymorphisms data sets.

We note that perennial aquatic features in the native range of the California Tiger Salamander were likely rare—cattle stock ponds are a relatively rare feature on the landscape (<https://bit.ly/3H0l9sc>). Nevertheless, California Tiger Salamanders appear to be phenotypically plastic in that individuals from some populations appear to overwinter when aquatic habitats support favorable conditions. Overwintering as larvae likely confers a higher level of fitness at metamorphosis—larger larvae metamorphose at a larger size (Semlitsch and Pechmann 1988).

We feel that overwintering larvae of the California Tiger Salamander in disparate areas reflects plasticity as an overwintering larval trait that may be ubiquitous but under reported. Regardless of the underlying mechanisms that manifest its expression, the trait does

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appear more widespread than previously believed. We find these observations to be significant, particularly in the context of management recommendations from the U.S. Fish and Wildlife Service (2002, 2017) to manually draw down managed ponds to control invasive species. Many authors are now suggesting that land managers strongly consider the potential presence of over-summering (also noted at this site) and overwintering larvae of this species during planning and implementation of habitat enhancement or management activities in the range of California Tiger Salamanders (Alvarez 2004, Wilcox et al. 2015, this study). These observations extend the range of overwintering in the larvae of California Tiger Salamanders, suggesting that the behavior may be range wide. Thus, management consideration of this phenotypic trait should be integrated into management strategies range wide.

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