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Minimum hydroperiod for metamorphosis in the California Tiger Salamander, *Ambystoma californiense* Gray, 1853

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The California Tiger Salamander (CTS), *Ambystoma californiense* Gray, 1853, is a federally- and state listed California endemic species (California Department of Fish and Wildlife [CDFW], 2010; U.S. Fish and Wildlife Service [USFWS], 2015). The foremost historic and present threat to this species is habitat loss associated with agriculture and residential development (Loredo et al., 1996, Cook et al., 2005; Witham, 2021). California Tiger salamanders exhibit a biphasic life history that requires both upland (e.g., grassland, woodland, etc.) and aquatic breeding habitats (vernal pools, ephemeral and perennial stock ponds, and pools within intermittent drainages) (Alvarez et al., 2021).

Past studies have examined the size at metamorphosis of CTS larvae (Loredo and Van Vuren, 1996; Trenham et al., 2000; Wilcox et al., 2015; Alvarez and Wilcox, 2023), which can help gauge cohort fitness success (Whiteman et al., 1996; Searcy et al., 2014) and advise management recommendations for determining suitable and successfully used breeding habitat (CDFW and USFWS, 2003). These studies typically measure the total length (TL, snout to tip of tail) or snout-vent length (SVL, snout to cloaca) of CTS at metamorphosis. Alvarez and Wilcox (2023) combined fieldwork, museum collection data, and a review of available literature to conclude that the size at metamorphosis can range from 56–241 mm TL and 33–122 mm SVL.

In addition to body length, there are minimum hydroperiods required for CTS to complete metamorphosis, but hydroperiod duration varies with exogenous influences, such as water temperature and seasonal rainfall patterns (Edwards 2018). The U.S. Fish and Wildlife Service's (2015) *Recovery Plan for the Central California Distinct Population Segment of the California Tiger Salamander (Ambystoma californiense)* ascertains that the larval stage of Central California Distinct Population Segment of CTS usually lasts three to six months. The current joint CDFW and USFWS (2003) *Interim Guidance on Site Assessment and Field Surveys for Determining Presence or a Negative Finding of the California Tiger Salamander* defines potential CTS breeding habitat as "pools that contain standing water continuously for at least ten weeks" (ten weeks = 70 days). Additionally, CDFW's (2010) *A Status Review of the California Tiger Salamander (Ambystoma californiense)* states that a minimum of ten weeks is required to complete metamorphosis from egg to juvenile referencing Trenham et al. (2000) CTS study on cattle stock ponds of Monterey County. However, two studies set higher and lower developmental times: Feaver (1971) found that 60 days were sufficient in the vernal pool complexes of Madera County; and Searcy et al. (2014) stated a minimum of 88 days were required for a deep vernal pool (Olcott Lake) on Jepson Prairie in Solano County.

During 2018 wet-season surveys, vernal pools were surveyed for CTS larvae at Westervelt Ecological Services' Dutchman Creek Conservation Bank in Merced County (37.1780°N, -120.3974°E) (Fig. 1). Thirteen pools were dry during the day on 26 February due to below average rainfall in the region from December to February. That evening, each of the pools rehydrated from successive rain events and just 53 days later (20 April 2018), all 13 pools were each found to support at least one CTS larva with both TL and SVL greater than the Alvarez and Wilcox (2023) minimum length requirements for metamorphosis (TL $\geq$ 56 mm and SVL $\geq$ 33) (Fig. 2, Table 1). Thirty-four percent (34%) of CTS larvae measured from these pools (106 out of 312 larvae) met the minimum metamorphosis length requirements (Table 1). The largest CTS larva measured had a TL of 76 mm and 44 mm SVL (Table 1). This 53-day calculation assumes that CTS adults bred on the evening or night of 26 February 2018 (the first night of rainfall after habitats were observed dry).

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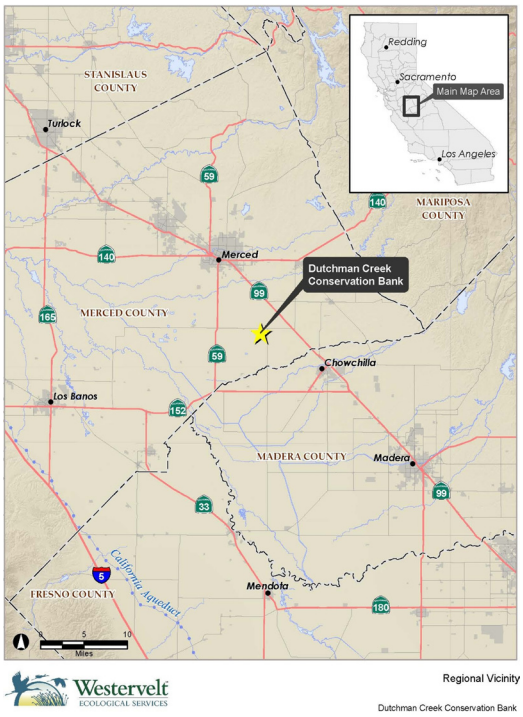

Figure 1. Regional Vicinity of Dutchman Creek Conservation Bank, Merced County, California.

However, a few days likely passed for the habitats to sufficiently inundate such that CTS adults would breed within them; therefore 53 days may be an overestimate of the time period. CTS larvae displayed fully developed front and rear limbs; however, gill resorption was not yet observed, indicating that metamorphosis had not yet begun due to sufficient water remaining in breeding ponds. Evidence suggests that larvae can transform from fully aquatic to fully terrestrial in as few as four days (Alvarez and Wilcox, 2023).

These findings suggest that CTS larvae may be capable of undergoing metamorphosis 17 days less than the current habitat hydroperiod requirements suggested by CDFW and USFWS (2003). Perhaps the 70-day minimum hydroperiod length requirement is based upon the results of CTS population studies from regions with lower average temperatures, higher average rainfall, and in breeding habitats with longer hydroperiods (Trenham et al., 2000; Searcy et al., 2014). Our findings appear to be closer to the 60-day minimum from Feaver (1971) who studied similar habitats (i.e., natural vernal pools) in a similar climatic region in Madera County, located just 37 miles southeast of the Dutchman Creek Conservation Bank. We acknowledge that the observation of a fully metamorphosed salamander within this time period would give more definitive support.

More detailed studies (e.g., drift fence) within this hotter and drier portion of the CTS range (e.g., Merced, Madera, Fresno, and Tulare Counties) in more ephemeral habitats (i.e., vernal pools) should be conducted to determine if the minimum time required to complete CTS's metamorphic cycle is similar to, less than, or greater than what was presented here. However, we suggest that interannual variation in the variables

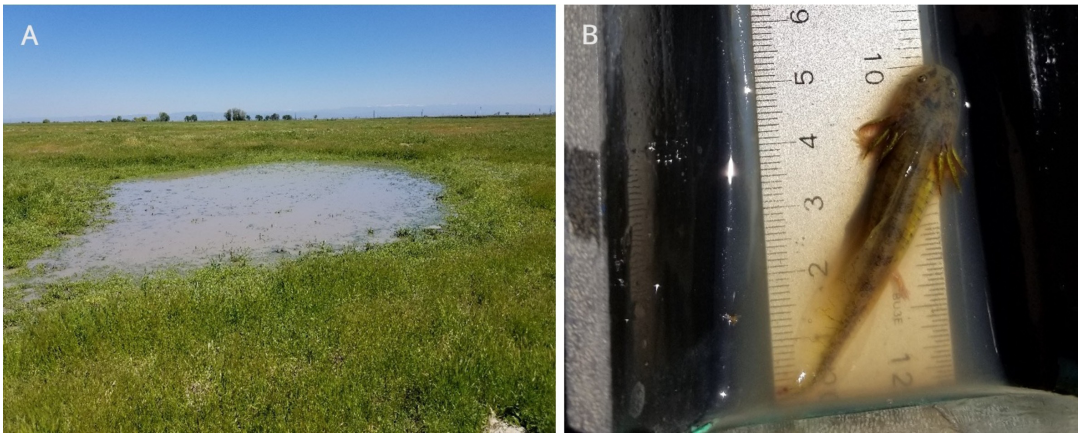

Figure 2. (A) Vernal pool at Dutchman Creek Conservation Bank on 20 April 2018, inundated after being observed dry 53 days prior on 26 February 2018; (B) California Tiger Salamander larva with a total length (TL, 60 mm) and (SVL, 34 mm), greater than minimum metamorphosis requirements (TL ≥ 56 mm and SVL ≥ 33 mm; Alvarez and Wilcox, 2023). Photos by Sean O'Brien.

Table 1. Results of California Tiger Salamander (CTS) larvae monitoring from 20 April 2018, at Dutchman Creek Conservation Bank, Merced County, California. TL = total length, SVL = snout-vent length.

Pool No.	Habitat Measurements			# of CTS Larvae Measured	TL or SVL	Descriptive Statistics for Measurements (mm)			Minimum Length Requirement for Metamorphosis	
	Water Temp (°F)	Max Depth (in)	Ave Depth (in)			Min	Max	Average (StDev)	Number	Percent
1	72	8	4	24	**TL**	50	71	59.3 (5.1)	6	25.0%
					SVL	21	40	31.0 (3.9)		
2	78	6	4	24	**TL**	39	62	50.9 (5.6)	1	4.2%
					SVL	20	36	27.7 (3.6)		
3	73	7	3	24	**TL**	50	66	58.1 (3.9)	6	25.0%
					SVL	27	39	31.5 (2.5)		
4	75	14	4	24	**TL**	40	61	52.8 (6.1)	4	16.7%
					SVL	22	36	29.6 (3.6)		
5	72	14	5	24	**TL**	52	74	62.8 (5.3)	18	75.0%
					SVL	28	44	34.6 (3.6)		
6	69	7	4	24	**TL**	52	73	61 (5.3)	9	37.5%
					SVL	23	39	31.8 (3.3)		
7	59	5	3	24	**TL**	51	67	58.5 (4.8)	12	50.0%
					SVL	29	41	33.1 (2.8)		
8	59	6	2	24	**TL**	46	64	56.6 (4.6)	9	37.5%
					SVL	25	37	31.7 (2.7)		
9	78	10	6	24	**TL**	42	61	52.3 (4.2)	2	8.3%
					SVL	23	35	30 (2.7)		
10	77	6	3.5	24	**TL**	40	69	55.5 (6.4)	10	41.7%
					SVL	23	41	31.6 (4.3)		
11	56	12	6	24	**TL**	54	76	64 (5.9)	16	66.7%
					SVL	30	43	35.3 (4.0)		
12	59	4	2	24	**TL**	36	76	57.3 (9.5)	8	33.3%
					SVL	20	44	30.7 (5.3)		
13	74	3	1.5	24	**TL**	45	62	54.9 (4.4)	5	20.8%
					SVL	27	35	30.5 (2.1)		
Totals				**312**		**Totals**			**106**	**34.0%**

that affect growth, and development (e.g., temperature, precipitation, prey availability) may necessitate conducting studies at multiple sites over multiple years.

Lastly, we suggest that CDFW and USFWS recommendations on minimum ponding durations for aquatic breeding habitats to be considered suitable for CTS be reduced from 70 days (ten weeks) to at least 56 days (eight weeks). This suggestion considers our observation of CTS larvae of adequate size to metamorphose in 53 days and an additional four days that CTS larvae require to transform from fully aquatic to fully terrestrial. This recommendation will increase the number of aquatic habitats considered potential to support CTS breeding, which has implications for pre-construction surveys and mitigation/conservation bank establishment.

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