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Decadal change in a population of western pond turtles at an isolated agricultural site in the San Joaquin Valley, California, USA

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Abstract. Although necessary for humans, agriculture can negatively affect populations of other species through direct and indirect means. This is true even for aquatic turtles, which require terrestrial areas in which to nest. The western pond turtle (*Actinemys marmorata*; *sensu lato*) has lost most of its habitat in the Central Valley of California, USA, to agricultural activities, flood control, and urbanisation. Although a few areas still support this turtle, most habitats are now altered by humans. In 1999 and 2009, I trapped western pond turtles at a remnant slough surrounded by irrigated agricultural fields in the San Joaquin Valley portion of the Central Valley. In 1999, I caught 123 turtles in 2 days of trapping (4.39 turtles/trap night) and in 2009 I caught 216 turtles in 4 days of trapping (4.15 turtles/trap night). Both sexes grew fast, similar to other sites in the San Joaquin Valley. I caught significantly more turtles ≤5 years of age in 2009 than in 1999, but significantly fewer large adults. In 2009, I X-rayed females and the mean clutch size was 7.4 ($n = 7$). Although activities and vegetative cover associated with agriculture can impact aquatic turtles, the population of western pond turtles at the slough were reproducing well, but the marked decline in the number of large adults is of concern for the long-term sustainability of this population.

Keywords: *Actinemys marmorata*, age structure, agriculture, clutch size, growth, reproduction, sex ratios, size structure, western pond turtle.

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Introduction

As the human population of the world continues to increase, the need for agricultural land to feed people rises. Although necessary for human survival, agriculture negatively affects aquatic ecosystems. Nearby water systems are often used to irrigate fields, but daily and seasonal use can cause water levels to fluctuate. Fluctuating water levels can impact littoral zones, necessary for associated wildlife species (see review by Carmignani and Roy 2017). Also, excess water used for irrigating plants can run off into aquatic systems, carrying with it sediments, organics, and chemical pollutants that may harm organisms (Willis and McDowell 1982; Carlson 1985; Lacher *et al.* 1999; de Sola *et al.* 2002; Neumann and Didgeon 2002).

Aquatic turtles are vulnerable to additional hazards posed by agriculture. Because turtles need terrestrial areas in which to nest, females that leave the water may be killed by farm machinery, and eggs in nests could be destroyed by tilling (Saumure *et al.* 2007; Tingley *et al.* 2009). Also, nest thermal conditions can change over time because nests laid in barren fields become deeply shaded when crops mature, reducing the reproductive output of nests (Mui *et al.* 2016; Thompson *et al.* 2018). Agricultural activities also can interfere with terrestrial activity not associated with nesting, such as dispersal and terrestrial aestivation (Saumure and Bider 1998; Tingley *et al.* 2009).

Agriculture has been widespread in the Central Valley of California, USA, for more than a century leading to the endangerment of many endemic species (U.S. Fish and Wildlife Service 1998; Kelly *et al.* 2006) and has led to the loss of populations of the western pond turtle (*Actinemys marmorata*; *sensu lato*) in this part of their range (Jennings and Hayes 1994; Germano and Bury 2001). The western pond turtle is considered a Species of Special Concern in California, although after more than a century of habitat loss in the Central Valley, the species still occurs at a number of sites (Germano and Bury 2001). In the San Joaquin Valley (southern part of the Central Valley), loss of habitat has been particularly severe (Jennings and Hayes 1994; Bury and Germano 2008). This valley once had abundant habitat for western pond turtles in the form of wetlands and three large lakes and associated marshes, which have been drained and much of the land converted to agriculture (Griggs *et al.* 1992; U.S. Fish and Wildlife Service 1998; Germano *et al.* 2011).

Most sloughs (linear courses in wetlands that transport water from marsh to marsh) in the San Joaquin Valley no longer support western pond turtles because water captured for irrigation has made them permanently, or semi-permanently, dry; however, there are exceptions (Germano and Bury 2001). In 1999, western pond turtles were caught at a remnant of a slough that appeared to retain water because of nearby irrigated

Fig. 1. The study site located west of Fresno in the San Joaquin Valley of California, USA. The site was a remnant freshwater slough surrounded by irrigated agriculture and abutted a two-lane paved road (bottom of the image). Note the non-ploughed habitat in the upper part of the system that could support turtle nests. (Image from Google Earth, http://earth.google.com/, taken in 2018).

agriculture (Germano and Bury 2001). The site was surrounded by crop fields, but it was not certain that the population could survive the effects of agriculture in the long term. I returned in 2009 to determine if the population persisted after a decade and to measure reproduction in the population. These data add to our knowledge of the tolerance of this species for less than pristine habitats and the effect that irrigated agriculture might have on this species.

Materials and methods

Study area

The study site is in the San Joaquin Valley of California, USA, and was a remnant of a freshwater slough that was surrounded by irrigated agriculture with a paved road on the south end (Fig. 1). The site is in western Fresno County, west of the city of Fresno and east of Interstate 5, the major transportation route on the west side of the valley. Climate of the area is Mediterranean with cool, wet winters (although low rainfall) and a long period of drought from May to November. Using Fresno, California, as the nearest location with climate data, the yearly mean air temperature is 16.3–17.3°C, monthly maximum mean temperatures is 35.5–37.0°C in July, and minimum averages in December are 1.0°–2.8°C (www.worldclimate.com). The 24-h monthly average temperatures vary greatly across the year, from 6.5°C in December to 27.7°C in July. Most rainfall (82.8–88.6%) occurs November–April and averages 269 mm/year (www.worldclimate.com).

The site was used as a water holding facility for adjacent agriculture and received water runoff from fields. It was long and narrow, with two segments because of a dirt road that bisected the slough (Fig. 1). The site was about 1235 m long and the width varied from about 20 to 50 m (measured from aerial photographs at Google Earth, http://earth.google.com/). There was little emergent vegetation, but grass and herbaceous annual plants grew along the edge of the water. I saw turtles basking either on the mud banks of the slough or on logs from dead trees that were in the water.

Study methods

I originally caught western pond turtles at the site 20 August and 10 September 1999 as part of a larger study of the distribution of this species in the Central Valley of California (Germano and Bury 2001). I returned in 2009 to reassess the population structure after 10 years and to collect reproductive information. I captured turtles in commercial folding nylon-net traps (models FT-D and FT-FA, Nylon Net Company, Memphis, TN, USA) and in wire-mesh traps with double funnels that I constructed (Iverson 1979). On 19 August 1999, I set eight traps that I checked the next day and on 9 September, I set 20 traps and checked the next day. In 2009, I set 12 traps on 29 May that I checked on 30–31 May and I set 14 traps on 1 July and they were checked on 2–3 July. I baited traps with canned sardines. For any turtle captured, I recorded bodyweight (with a digital scale, in g), carapace length (CL; in mm), sex, age, and general condition.

I determined age using annuli on scutes from the carapace and plastron (cf. Bury and Germano 1998; Germano and Bury 1998; Germano 2016). Although there is some controversy about using scute rings to determine ages of turtles (Wilson *et al.* 2003), the technique is reliable when one ring on individual scutes forms annually and upper limits are established for estimating age. Only one complete ring forms in a year in western pond turtles based on recaptures of individuals 1–7 years later (Bury and Germano 1998; Germano 2016), but false rings occasionally occur and must be discounted (for criteria see Germano and Bury 1998). Scute annuli match the age of western pond turtle individuals up to about 10–12 years in the southern portion of the species range (Bury and Germano 1998; Germano 2010, 2016). Scute annuli do not form much past sexual maturity in all species reviewed or studied (Germano and Bury 1998), and some biologists have incorrectly tried to determine ages of turtles beyond this limit (Wilson *et al.* 2003). At this agricultural site, I could only classify some western pond turtles as older than 15 years because scute rings were worn and edges of scutes were bevelled; these animals were no longer depositing discernible rings (Germano and Bury 1998). Even though the technique cannot be used to determine the age of adult turtles >15 years when first captured, it allows comparisons of age structure of a large segment of individuals among populations and for determining growth rates. Ages >10–15 years can be determined when marked turtles are recaptured in later years.

I defined most adults as those ≥120 mm CL; the size at which most males develop secondary sexual characteristics in their shells and tails (Bury and Germano 2008). Because of distinct secondary sexual characteristics, however, I classified 13 turtles in 2009 as males with CLs 112–119 mm. I individually marked turtles by notching marginal scutes (Cagle 1939) before releasing turtles the day after capture. In 2009, I radiographed turtles that I could determine were females (≥120 mm CL) using a portable X-ray machine to determine if they were gravid and how many eggs were present.

Analyses

To determine if sex ratios differed significantly, I used Chi-square analysis with Yates correction for continuity. I compared the percentages of adults (≥120 mm CL) to juveniles between years using a 2 × 2 contingency table, and I used the same test to compare the percentage of turtles in the population ≤5 years old between years. Because the data were not normal, even after transformation, I compared mean CL for sex and year using the Kruskal–Wallis test. I used the method of Legendre and Legendre (1998) to adjust *P* values of subsequent multiple Mann–Whitney tests comparing groups (sex by year). The data for upper-decile CL had equal variances and were distributed normally, so I used a two-way ANOVA to make comparisons between sexes and year, and the interaction of sex with year. To determine if the overall distribution of CLs and ages differed significantly between years, I used a Kolmogorov–Smirnov test. I compared mean bodyweight of males to females by year using ANCOVA with CL as the covariate. Although samples sizes were small ($n = 4$–5), upper-decile bodyweights were normal, and variances were equal. After removing gravid females from analyses, I used a two-way ANOVA to make comparisons by sex, year, and the interaction of sex and year. I used Tukey's honestly significant differences (HSD) test to make *post-hoc* comparisons. For all tests, $\alpha = 0.05$.

I used the Richards growth model (Richards 1959) to construct individual curves where three parameters are estimated using the asymptotic size (CL) and age: M, shape of growth curve; K, growth constant; and I, the point at which inflection of the curve begins. The model uses the general formula:

$$\mathrm{CL} = \text{asymptotic size}\left(1 + (\mathrm{M} - 1)\mathrm{e}^{(-\mathrm{K}\times(\mathrm{Age}-\mathrm{I})}\right)^{(1/(1-\mathrm{M})}.$$

I did not use ages of turtles that I judged to be >15 years old. For those turtles that I could estimate age, I used continuous estimates (Lindeman 1997) based on a yearly period of 1 April to 30 October that could support growth. As an example, I would list a turtle caught with two scute rings 30 May as 1.28 years old (one full year of growth and an additional 0.28 of a year of growth when caught). Precision of the estimate of growth period is not critical but estimating age to a decimal fraction of a year improves fit of the curve (Lindeman 1997). I used mean upper decile sizes of each sex as the starting value in the model, but the model determined asymptotic sizes. To anchor growth curves, I also used the mean size of hatchlings from a site in San Joaquin Valley (CL = 26.0 mm, $n = 3$; Hill 2006) and a site in the Mojave Desert (CL = 26.4 mm, $n = 3$; Lovich and Meyer 2002).

I compared rates of growth among habitats and sites using the G statistic, which represents the time required to grow 10–90% of asymptotic size and is an indicator of the duration of primary growth (Bradley *et al.* 1984). It is defined as:

$$\mathrm{G} = \ln((1 - 0.10^{1-\mathrm{M}})/(1 - 0.90^{1-\mathrm{M}}))/\mathrm{K}.$$

The raw parameters K and M are closely linked in determining growth curves and neither is useful for comparing growth between populations (Bradley *et al.* 1984). The best overall measure of growth is G because it is less affected by instability of the non-linear fit than either K or M, and it produces values on an easily interpreted scale (Bradley *et al.* 1984); in my case, years. I also made comparisons of rates of growth between sexes using the mean and the 95% confidence interval of CL from the growth analyses of adults as a way of determining approximate time of divergence of growth. I calculated CL by 2-year intervals from ages 2 to 10 years. I determined calculated CL to be significantly different between sexes if the mean of one sex did not intersect the 95% confidence interval of the other.

Results

In 1999, I caught 123 western pond turtles in 28 trap nights (4.39 turtles/trap night) and in 2009, I made 216 captures of 200 western pond turtles in 52 trap nights (4.15 turtles/trap night), of which only one turtle was a recapture from 1999. I neither saw nor caught any other species of turtle at the site in either year. The sex ratio of turtles in 1999 was 46 males to 35 females (1.31 M:1.00 F), which was not significantly different from 1:1 ($\chi^2 = 1.235$, d.f. = 1, $P = 0.267$). In 2009, I caught 46 males and 41 females, and the sex ratio (1.12 M:1.00 F) also was not significantly different from 1:1 ($\chi^2 = 0.184$, d.f. = 1, $P = 0.668$).

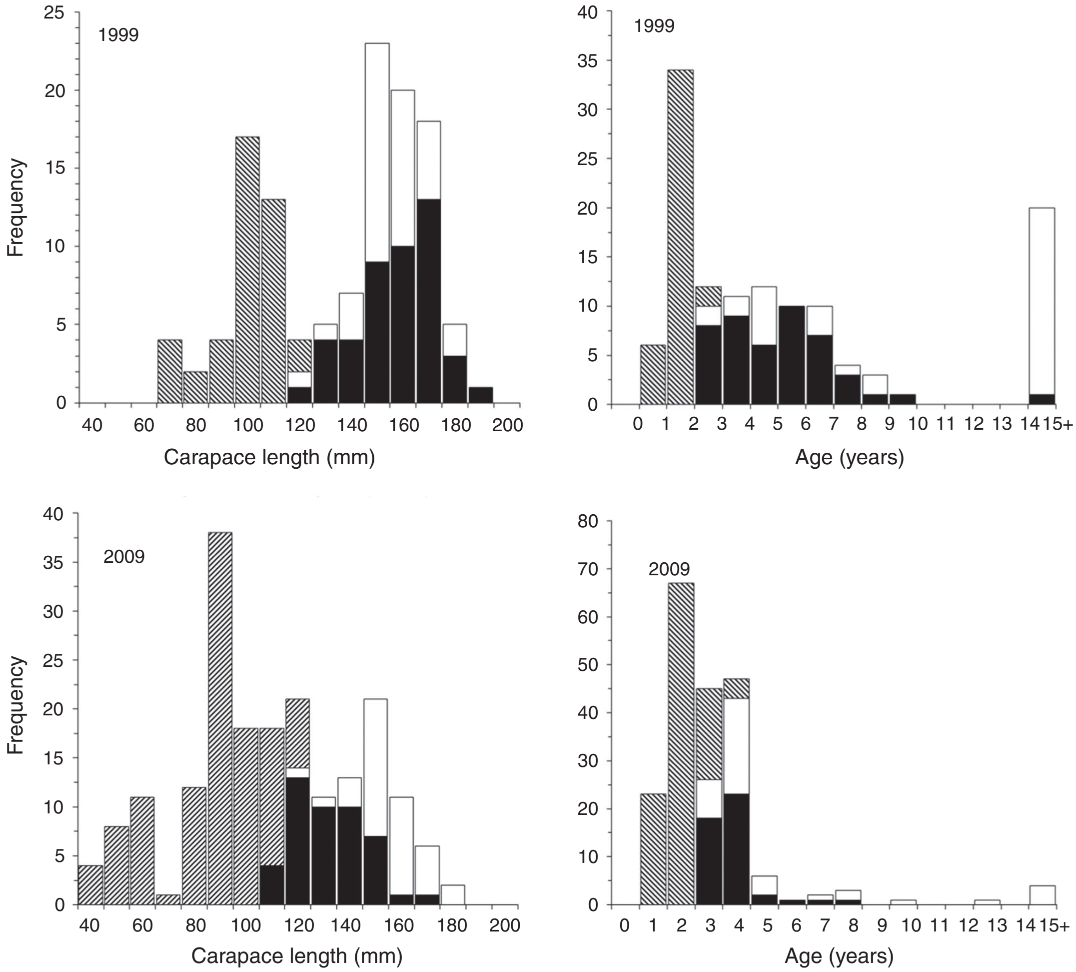

Fig. 2. Distributions of carapace lengths (mm) and ages (years) of western pond turtles (*Actinemys marmorata*) in 1999 and 2009 from a remnant slough west of Fresno in the San Joaquin Valley of California, USA. Black bars are males, open bars are females, and grey bars are juveniles (sex not determined).

By size, adults (≥120 mm CL) comprised 65.9% (81 of 123) of the turtles captured in 1999 and juveniles were 34.1% (42 of 123) of captures (Fig. 2). In 2009, of the 200 individuals I caught, 37.0% (74 of 200) were adults and 63.0% (126 of 200) were juveniles (Fig. 3), which was a significantly higher percentage of juveniles than in 1999 ($\chi^2 = 25.40$, d.f. $= 1$, $P < 0.001$). The overall distribution of sizes, however, did not differ significantly between years ($D = 0.294$, $P = 0.387$). Based on estimated ages, 61.0% ($n = 75$) of the turtles I caught in 1999 were ≤5 years old and 59.3% ($n = 73$) were ≤2 years old, whereas in 2009, 94.0% ($n = 188$) of the turtles I caught were ≤5 years old and 45.0% were ≤2 years old (Fig. 2). The number of turtles I caught that were ≤5 years in 2009 was significantly greater than the number caught in 1999 ($\chi^2 = 54.92$, d.f. $= 1$, $P < 0.001$), but the overall age distributions were not significantly different ($D = 0.188$, $P = 0.912$).

Average CLs were significantly different by year ($H = 71.40$, d.f. $= 3$, $P < 0.001$) with males and females in 1999 being significantly larger (Table 1) than males and females in 2009 ($W = 1836.5$–2967.5, all $P < 0.001$), although neither sex differed significantly in size within years (1999: $W = 1318.5$, $P = 0.269$; 2009: $W = 2048.0$, $P = 0.076$). There was a significant interaction between sex and year in upper-decile CL ($F_{1,14} = 35.97$, $P < 0.001$). In 1999, males were larger than females, but in 2009, males were smaller than females (Fig. 3).

Average weight (with CL as a covariate) was significantly different by sex and year ($F_{3,160} = 20.62$, $P < 0.001$), with males in 1999 significantly heavier (Table 1) than females in either year, or males in 2009 (slope: qs $= 5.439$–10.23, all P-values ≤ 0.05). Males in 2009 were significantly lighter than females in either year (elevations: qs $= 6.812$–6.831, both P-values ≤ 0.05), but females did not differ significantly in weight between years (elevations: $q = 0.691$, $P > 0.05$). Upper decile weights (Table 1) differed significantly by year ($F_{1,14} = 54.07$, $P < 0.001$), but not by sex ($F_{1,14} = 0.020$, $P = 0.882$) or the interaction of sex and year ($F_{1,14} = 2.58$, $P = 0.131$). Turtles of both sexes were significantly heavier in 1999 than in 2009 (Tukey HSD, $P \leq 0.05$).

Based on radiographs I took in 2009, 17.9% (seven of 39) females were gravid; however, many of these females were

Table 1. Mean (range), sample size (*n*), and standard error (s.e.) of carapace length (CL) and weight, and mean upper-decile CL (UDCL) and weight (UDW) of adult western pond turtles (*Actinemys marmorata*) captured in 1999 and 2009 at a remnant slough in Fresno County, California
Weight of females in 2009 excludes those that were gravid

	CL (mm)				Weight (g)			
	n	Mean	s.e.	UDCL	*n*	Mean	s.e.	UDW
				1999				
Males	46	157.0 (120–185)	2.32	180.2	46	622.5 (283–1035)	26.7	917.0
Females	35	154.1 (120–175)	1.86	170.5	35	639.2 (286–900)	22.8	897.5
				2009				
Males	46	130.8 (112–165)	1.92	153.6	46	355.9 (221–684)	14.9	549.6
Females	41	136.9 (120–167)	1.99	163.0	34	397.2 (285–770)	17.6	609.3

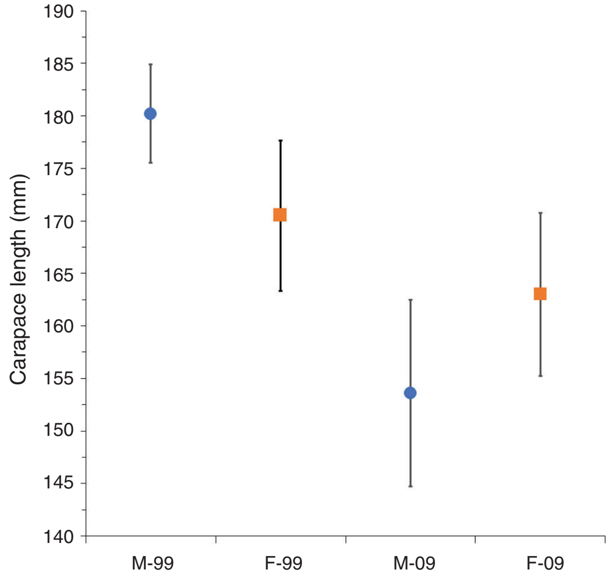

Fig. 3. Mean upper-decile carapace lengths (mm) of male (M) and female (F) western pond turtles (*Actinemys marmorata*) in 1999 and 2009 from a remnant slough west of Fresno in the San Joaquin Valley of California, USA. Symbols are the means (blue are males and orange are females) and vertical lines are the 95% confidence intervals.

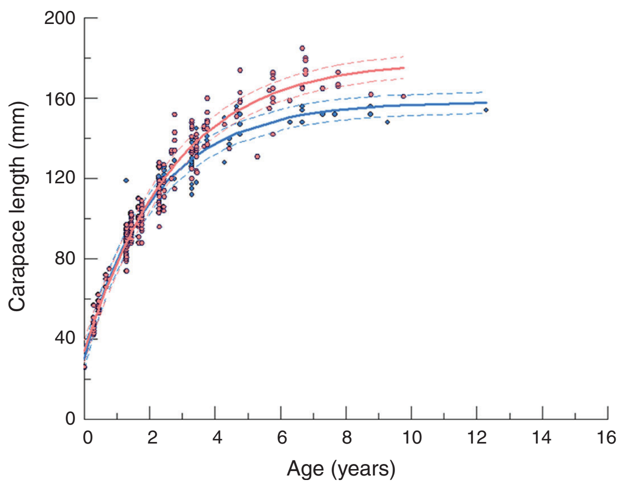

Fig. 4. Growth curves of female (triangle, red colour) and male (closed circles, blue colour) of western pond turtles (*Actinemys marmorata*) from a remnant slough west of Fresno in the San Joaquin Valley of California, USA. The Coefficient of Determination (r^2) for males is 0.953 and for females 0.949.

small. The smallest females with eggs were two females at 148 mm CL and were 6.29 and 9.28 years old. The percentage of females >145 mm CL that were gravid was 77.8% (7 of 9). The average size of a clutch was 7.4 eggs (standard error = 0.48; range, 6–10). The youngest female with eggs was 4.29 years old and was 150 mm CL (with six eggs).

The Richards growth equations described well the growth of western pond turtles at the slough for each sex with Coefficient of Determination (r^2) values of 0.953 for males and 0.949 for females, indicating good fit of the models. Turtles grew quickly, and males grew faster than females (Fig. 4), which was evident by age four based on calculated CL from the growth model (Table 2). Using the growth equations, the time it took to reach 120 mm CL (start of adult size) was 2.06 years for males and 2.50 years for females. To reach 150 mm CL took 3.90 years for males and 5.91 years for females.

Discussion

The highly modified and isolated slough in the middle of agricultural fields in the San Joaquin Valley that I studied supported a large population of western pond turtles, many of which were young. In the 10 years between sampling this population, I found that the number of turtles remained high, but the percentage of small (juvenile) turtles increased. I did find that there were fewer large (and old) turtles in 2009 than in 1999. I do not know why the percentage of large turtles decreased in the population in 2009, but it is possible that workers in the agricultural fields surrounding this site may be directly affecting adult turtles. In July 2009 I had several traps stolen that were in the slough, which caused me to stop trapping the population sooner than I had planned. It is possible that farm workers took the traps. If so, they may also preferentially catch adult turtles at other times, perhaps to eat, and that numbers of large and older turtles may have been diminishing for many years because of capture by humans. Turtles have been eaten by people perhaps for as long as humans have

Table 2. Calculated carapace lengths (95% confidence interval) in mm of male and female western pond turtles (*Actinemys marmorata*) at a remnant slough in Fresno County, California

Calculated carapace lengths at various ages were determined from growth equations for each sex (Fig. 4). Significant differences ($P < 0.05$) between the sexes, as determined by non-overlap of mean with confidence intervals, are indicated (*)

Age (years)	Males	Females	Combined
2	109.6 (104.2–115.0)	108.2 (102.9–113.4)	109.6 (104.8–114.5)
4	146.4* (141.0–151.8)	137.4* (132.2–142.6)	143.8 (139.0–148.5)
6	163.8* (158.4–169.2)	149.4* (140.4–154.1)	158.2 (153.4–163.0)
8	171.8* (166.4–177.1)	154.5* (149.0–159.2)	164.5 (159.7–169.3)
10	–	156.1 (151.3–161.8)	165.9 (161.5–171.7)

hunted for food. In the late 19th and early 20th centuries, there was a thriving export market of western pond turtles in the San Joaquin Valley, most of which were sold for food in San Francisco (summarised in Bury and Germano 2008). Many rural people in countries south of the U.S. border include turtles and their eggs in their diet (Schneider *et al.* 2011; Legler and Vogt 2013). Most farm workers in the Central Valley of California are migrants from Mexico and Central America countries and it is likely they continue the practice of finding extra protein where they can. I do not know why farm workers did not seemingly have an impact on this turtle population in 1999, but if farm workers have recently discovered turtles at this site and are exploiting them, turtles at this site may not persist, despite apparent nesting success.

Besides direct removal of turtles as a food source by farm workers, agriculture has long-posed dispersal and nesting problems for aquatic turtles. Studies on wood turtles (*Glyptemys insculpta*) have found that farm machinery sometimes kills turtles that venture into fields (Saumure and Bider 1998; Saumure *et al.* 2007; Tingley *et al.* 2009), and that wood turtles whose aquatic habitat abutted hay fields grew more slowly in later years and there were fewer young in the population than in a forested habitat, although predation rates were lower in the hay field site (Saumure and Bider 1998). Hatchling snapping turtles (*Chelydra serpentina*) that came from nests in agricultural fields had delayed times to hatch, hatched at a smaller size, lost more mass, and had lower post-hatching growth rates than turtles in control sites in open areas (Thompson *et al.* 2018). These authors found that nest temperatures were the same between control nests and nest in fields when nest were made in the spring and fields were bare, but nests in fields averaged 2.5°C cooler than control nests as crops grew.

Despite this site being surrounded by crop lands, females seem to have found nesting habitat. If the high percentage of young turtles in my slough population indicates nesting success, then females may have avoided nesting in crop fields and instead used the thin strip of non-ploughed habitat surrounding the slough and open areas. There was a patch of tree-covered native ground within the road boundary at the north end of the slough as well as a patch of native scrub habitat along the west side of the slough. These areas may support nests that are not disturbed by ploughing and irrigation. Females also may nest on, or on the edge of, the dirt road that surrounds the slough. At the Fresno wastewater treatment facility, I found evidence of turtles nesting on dirt roads (Germano 2010), and Blanding's turtles (*Emydoidea blandingii*) in Ontario, Canada, nested in gravel logging roads and dirt shoulders of paved roads (Mui *et al.* 2016).

The mean number of eggs in a clutch (7.4) was lower than the average clutch sizes at the Fresno wastewater treatment site (8.2) and the Hanford wastewater treatment site (8.5; Germano 2010), which are about 35 and 75 km, respectively, from the slough. Mean clutch sizes (n of clutches >5) at these two sewage treatment facilities are the highest in the range of the western pond turtle (Germano 2010, 2016). Further south in the San Joaquin Valley, the mean clutch size at Goose Lake was 7.0 (Germano 2016). This relatively high clutch size at the slough might counteract some of the loss of large adults, especially if this adult loss diminishes in the future.

Although I did not chemically test the water of the site, it is likely that the water is polluted by agricultural chemicals that drain into the site from surrounding fields. Western pond turtles are found in many types of contaminated water, however, either from livestock, logging operations, sewage, or eutrophication as pools dry in the summer (Storer 1930; Buskirk 2002; Bury and Germano 2008; D. Germano, pers. obs.). If the water was polluted, it did not seem to affect the growth rate of turtles because growth was high at the slough, similar to western pond turtles in other parts of the San Joaquin Valley (using the statistic G as a comparison; Table 3). The growth rates of turtles at this site and other sites in the San Joaquin Valley are higher than in other parts of the range of western pond turtles (Germano and Rathbun 2008; Germano and Bury 2009; Bury *et al.* 2010; Germano and Riedle 2015).

Despite high growth rates, relatively high clutch sizes, and large numbers of young turtles, western pond turtles at this site might not persist if the unexplained loss of large turtles I found in 2009 does not change. As of 23 August 2018, the water system of the site seems as it did when I trapped (Google Earth, http://earth.google.com/). A new round of trapping needs to occur at the site, but because of the exposed nature of the site, it could be hard to determine the current state of the population without bringing undue attention to the turtles. Although not nearly as accurate, visual surveys of basking turtles using binoculars may be the best method to estimate the current status of this population while minimising exposure of turtles to being captured.

Table 3. Growth parameters from Richards' growth curves for western pond turtles (*Actinemys marmorata*) from four sites in the San Joaquin Valley of California

Parameters describing model fit and growth curves are shape of curve (M), growth constant (K), inflection point of curve (I), and time required to grow from 10–90% of asymptotic size (G) in years. FWTP, Fresno wastewater treatment plant; HWTP, Hanford wastewater treatment plant

Site	M	K	I	G	Reference
Slough	0.1788	0.4482	–0.2644	5.19	Present study
FWTP	0.0468	0.5582	–0.2355	4.00	Germano (2010)
HWTP	0.4232	0.6653	0.2031	3.79	Germano (2010)
Goose Lake	1.470	0.5440	0.9220	6.58	Germano (2016)

Conflicts of interest

The author declares no conflicts of interest.

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