# REPRODUCTIVE FREQUENCY AND SIZE-DEPENDENCE OF FECUNDITY IN THE GIANT GARTERSNAKE (*THAMNOPHIS GIGAS*)

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*Abstract.*—How reproductive output changes with age or size is a key life-history trait that can affect which demographic rates most influence population growth. Although many studies have investigated the reproductive ecology of gartersnakes, we know little about reproduction in the threatened Giant Gartersnake, *Thamnophis gigas*. We used X-radiography to determine reproductive status and estimated fecundity for 73 female *T. gigas* collected from several regions within the range of this species in the Sacramento Valley of California, USA, and synthesize these data with data from litters born in captivity to improve our understanding of reproduction in this species. Average total litter size determined from X-rays (15.9) and captive-born litters (15.5) are within the ranges reported from other gartersnakes, but captive-born litters had high rates of stillbirth. Only 154 of 202 neonates from captive snakes were born alive, and seven of 13 litters contained at least one stillborn neonate. We found that fecundity was positively related to maternal snout-vent length, and some evidence that larger litters contained smaller neonates. The proportion of X-rayed females that were gravid was 0.50 in 2014, 0.47 in and 2015, and 0.64 in 2016. Central California experienced an exceptional drought from 2012–2015, which may have affected the reproductive output and frequency of *T. gigas*. Our estimates of reproductive frequency and size-dependent fecundity in *T. gigas* provide valuable information that can be used in demographic models of this threatened species. Our results demonstrate that X-radiography is a useful, minimally invasive means to study fecundity in wild populations of snakes.

*Key Words.*—California; Central Valley; garter snake; litter size; population ecology; reproductive ecology; stillbirth; X-radiography

## INTRODUCTION

The age or size at which individuals reach maturity, the frequency of reproduction, and how fecundity changes with age and size are fundamental life-history traits. In snakes, fecundity varies among closely related species (Rossman et al. 1996; Gibbons and Dorcas 2004), among populations within species (Bronikowski and Arnold 1999), and among individuals within a population (Aldridge et al. 1995; Weatherhead et al. 1999). In natricine colubrids there is generally a positive relationship between litter size and maternal size, but relationships between neonate size, litter size, and maternal size are more complex (Ford and Killebrew 1983; Ford and Karges 1987; King 1993; Weatherhead et al. 1999). Within a species, there can be variation in the timing of reproduction within the lives of individuals and the amount of energy invested into individual litters. For example, nearby populations of Western Terrestrial Gartersnakes (*Thamnophis elegans*) in different habitats differ in their growth rates, age at maturity, fecundity, and even whether fecundity is related to maternal size or age (Bronikowski and Arnold

1999; Sparkman et al. 2007). Populations of Common Gartersnakes (*T. sirtalis*) in Canada exhibit substantial variability in reproductive characteristics such as litter size and average neonate size (Gregory and Larsen 1993). There can also be inter-annual variation in female fecundity within a population in response to climatic fluctuations (Seigel and Fitch 1985; Tuttle and Gregory 2014). Because of this inter- and intraspecific variation in reproductive characteristics, it may be inappropriate to make assumptions about the life history of a poorly studied species based on closely related species.

Although *Thamnophis* have often been the subject of studies of reproductive biology, little is known about reproduction in the Giant Gartersnake, *T. gigas*. *Thamnophis gigas* (Fig. 1) is a semi-aquatic snake endemic to the Central Valley of California, USA, where it has lost > 90% of its historical wetland habitat (Huber et al. 2010), and consequently has been listed as threatened under the federal Endangered Species Act since 1993 (U.S. Fish and Wildlife Service 1993). As its name suggests, *T. gigas* is the largest of the gartersnakes, historically reaching total lengths of over 1.5 m (Rossman et al. 1996), although existing populations appear to

**Figure 1**. Neonate Giant Gartersnakes, *Thamnophis gigas* from the Sacramento Valley of California, USA (Photographed by Julia Ersan).

have shorter maximum lengths (Wylie et al. 2010; Rose et al. 2018). Previous studies of the reproductive biology of *T. gigas* have been limited by small sample sizes, and did not investigate (Hansen and Hansen 1990) or find evidence of (Halstead et al. 2011) size-dependent fecundity. *Thamnophis gigas* may be expected to differ in its reproductive biology and life history from other *Thamnophis* due to its larger size, and because *T. gigas* inhabits a much warmer climate than populations of *T. sirtalis* and *T. elegans* whose reproductive biology and life history have been thoroughly studied (Gregory and Larsen 1993; Bronikowski and Arnold 1999; Sparkman et al. 2007). A better understanding of the reproductive biology and life history of *Thamnophis gigas* is needed for effective conservation of this species.

We studied reproductive characteristics of female *T. gigas* from six regions distributed throughout its remaining range in the Sacramento Valley of California. We used X-radiography of adult females and litters born in captivity to measure litter size. We tested whether litter size and offspring size were related to maternal size. We also report the proportion of examined females that were reproductive each year, and the incidence of stillbirth in litters born in captivity. Based on studies of other *Thamnophis*, we hypothesize that litter size of *T. gigas* will be positively related to female size. The results of this study not only furthers our understanding of the reproductive biology of this threatened species, but also fills a gap in our knowledge of the reproductive biology of species in the genus *Thamnophis*.

## MATERIALS AND METHODS

*Data collection*.—We captured female *T. gigas* in six regions of the Sacramento Valley of California, USA, from 2013–2016 (Table 1). Study sites comprising the Sutter Basin, Central Colusa Basin, and Natomas Basin regions include both canals associated with rice agriculture and restored wetlands managed for *T. gigas* and other aquatic wildlife. The habitat in the Southern Colusa Basin and Northern and Southern Butte basins consists of canals adjacent to rice agriculture (Fig. 2). We captured snakes by hand and by using modified aquatic funnel traps (Casazza et al. 2000; Halstead et al. 2013) placed along the edges of wetlands or emergent vegetation. We uniquely marked all captured snakes by both branding ventral scales (Winne et al. 2006) and inserting passive integrated transponder (PIT) tags. We determined the sex and measured the snout-vent-length (SVL; by straightening snakes along a meter stick) and mass of each snake. We examined females from three primary sources for this study. First, 56 females were part of a radio-telemetry study in the Sutter, Central Colusa, Southern Colusa, and Northern and Southern Butte basins from 2014–2016, and between April and early September were X-rayed prior to having surgery to implant or remove radio-transmitters. We chose these females based on being large enough to accommodate radio-transmitters ($\geq$ 200 g), and their putative reproductive status did not influence their collection. We used data from these females in the analysis of litter
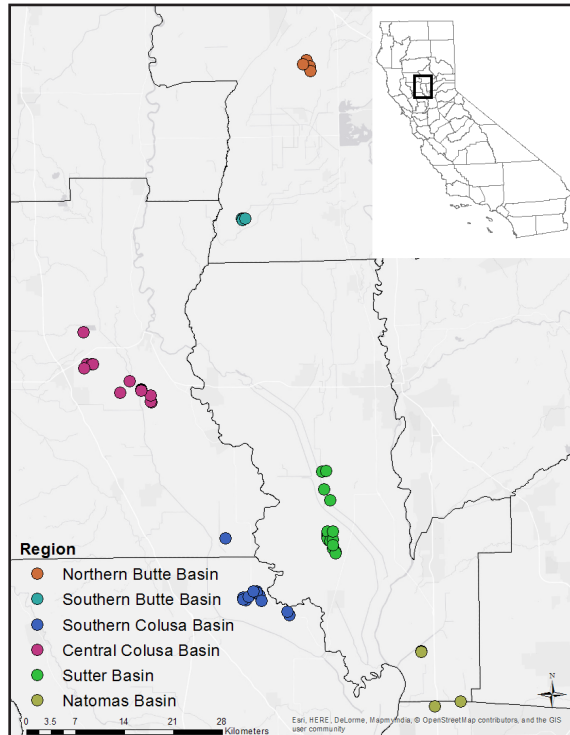
**FIGURE 2.** Location of study sites of Giant Gartersnakes (*Thamnophis gigas*) in the Sacramento Valley of California, USA.

veterinary staff performed X-radiography to count the number of ovulated eggs or developing embryos in the oviducts of females. X-rays were taken using a MinXray HF 100 generator (MinXray, Northbrook, Illinois, USA) paired with an EDR3 Rapid Study Portable Digital Radiography System (Eklin Medical Systems, Sunnyvale, California, USA). We examined 73 individuals, and we examined some females in more than one year, resulting in a total of 104 X-rays. In addition to the X-rayed snakes, we collected 13 gravid females from the Central Colusa Basin (n = 4), Sutter Basin (n = 4), and Natomas Basin (n = 5) from 2013–2014 and kept them in captivity until parturition for a study on neonate prey preference (Ersan 2015). After parturition, we counted the number of live and stillborn neonates in these captive-born litters and weighed and measured the SVL of all live neonates within 4 d of their birth. We used data from these captive-born litters in our analysis of litter size and neonate size. We also included records of litter size, maternal size, and neonate SVL from captive-born litters in two previous studies of reproduction in *T. gigas* in some of our analyses (Hansen and Hansen 1990; Halstead et al. 2011).

***Statistical analysis.***—We compared the SVL and mass of X-rayed females and females that gave birth in captivity using a linear model with a fixed effect of data source on female size. We also compared the SVL of females that gave birth in captivity in this study to the SVL of females that gave birth in captivity in Halstead et al. (2011) and Hansen and Hansen (1990) using a linear model with a fixed effect of study on female SVL. We analyzed the fecundity data using Bayesian hierarchical models. We standardized all covariates by subtracting the mean and dividing by the standard deviation to

size and reproductive frequency. Second, in July and August 2016, we collected an additional 17 putatively gravid females that were captured in traps. To determine if these selected females were gravid, we first palpated the abdomen in the field to feel for the presence of embryos or ovulated eggs. We only used data from these putatively gravid females in the analysis of litter size. We took all females to the Sacramento Zoo, where

**TABLE 1.** Time period sampled, number of individuals sampled, number of X-rays taken, number of gravid females, number of captive litters, and sources for each study region of Giant Gartersnakes (*Thamnophis gigas*) from the Sacramento Valley of California, USA. Note that gravid refers to the number of X-rays in which the individuals were found to carry eggs. Some individuals were X-rayed in multiple years. Individuals includes both snakes that were X-rayed and snakes that gave birth to litters in captivity. The abbreviation NWR is National Wildlife Refuge.

| Site | Years Sampled | Individuals | X-rays | Gravid | Captive Litters | Source |
|---|---|---|---|---|---|---|
| Northern Butte Basin | 2015–2016 | 7 | 9 | 6 | 0 | This study |
| Southern Butte Basin | 2015–2016 | 6 | 7 | 5 | 0 | This study |
| Central Colusa Basin | 2014–2016 | 20 | 24 | 9 | 4 | This study |
| Southern Colusa Basin | 2014–2016 | 21 | 31 | 19 | 0 | This study |
| Sutter Basin | 2013–2016 | 26 | 33 | 11 | 4 | This study |
| Natomas Basin | 2013–2014 | 5 | 0 | 0 | 5 | This study |
| Badger Creek | 1997 | 2 | 0 | 0 | 2 | Halstead et al. (2011) |
| Colusa NWR | 1997 | 1 | 0 | 0 | 1 | Halstead et al. (2011) |
| Gilsizer Slough | 1995–1996 | 6 | 0 | 0 | 6 | Halstead et al. (2011) |
| Sacramento NWR | 1995–1997 | 2 | 0 | 0 | 2 | Halstead et al. (2011) |
| Fresno County | 1978 | 4 | 0 | 0 | 4 | Hansen and Hansen (1990) |
| Sacramento Co. | 1987–1990 | 12 | 0 | 0 | 12 | Hansen and Hansen (1990) |

improve model convergence and ease interpretation of model coefficients. We modeled the number of neonates produced by a female (litter size) using a Poisson response with a log link function. We included both live and stillborn offspring in our measure of litter size, following the recommendations of Gregory et al. (1992). Some of our fecundity data came from X-rays of gravid females and we did not know how many of the eggs or embryos counted were carried to term and born alive. Thus, including counts of dead young in litter size made litter size data from these two sources comparable and allowed them to be pooled for some analyses. In addition, the number of live and stillborn offspring includes all female investment in reproduction. We tested for a relationship between female SVL and litter size using X-rayed females and captive-born litters from the current study only, with a fixed effect of data source on the intercept. We also fit a model to a pooled dataset that combined litters from the current study with litters born in captivity from 1995–1997 (Halstead et al. 2011) and from 1978–1990 (Hansen and Hansen 1990). We included random effects of region, year, and individual on the intercept in all models, to reflect the geographical clustering of regions, temporal variability, and repeated measures of fecundity from some snakes. We initially included a fixed effect of study on litter size for this pooled dataset, but preliminary models showed no difference in the intercept among studies, so our final model included no effect of study. To generate a model with parameters comparable to earlier studies of maternal size-fecundity relationships in natricine snakes, we also fit a simple linear regression of litter size on maternal SVL without transforming either variable.

We tested for relationships between neonate size (SVL and mass) and maternal SVL and litter size by fitting a linear model to data from 154 neonates from 12 captive litters in this study (one litter was lacking morphology data from the sole live neonate) and 168 neonates from 11 captive litters reported by Halstead et al. (2011). The model included a fixed effect of study to estimate potential differences in neonate size between the two studies, and a random effect of litter to account for the non-independence among neonates from the same litter. We also compared the SVL of neonates observed in this study to the litter averages reported by Hansen and Hansen (1990) using a linear model with fixed effects of study, maternal SVL, and litter size on neonate SVL. We tested for effects of maternal SVL and litter size on the probability a litter contained stillborn offspring by fitting a binomial model to pooled data from the 13 captive-born litters in this study with 11 captive-born litters reported by Halstead et al. (2011).

For our analysis of the proportion of reproductive females and frequency of reproduction, we only used data from the radio-telemetry females, to ensure our results were not biased by the 17 females selectively examined in 2016 based on their putative reproductive status. We estimated the proportion of examined females that were reproductive in a given year, and the frequency of reproduction for individuals that were examined in more than one year. Note that because the putative reproductive status of females did not influence whether they were chosen for the radio-telemetry study, this sample is not biased towards gravid females. We limited this analysis to females that were X-rayed during the pre-parturition period between 1 May, when females are likely to have enlarged eggs that are visible on radiographs, and 11 July, the earliest recorded date of parturition in *T. gigas* (Halstead et al. 2011). We fit a binomial model with a fixed effect of year on the proportion of females that were reproductive ($p$). We tested for differences in the proportion of reproductive females each year by calculating the difference between the estimates of $p$ for each year. We also included a fixed effect of female SVL and linear and quadratic effects of Julian date on $p$ to account for potential differences in the size of females and timing of examinations each year.

For 12 females, we did not measure their SVL for one year in which they were captured and examined, but had a measure of SVL from the previous year. We imputed their SVL for the missing year using Bayesian imputation (Bonner et al. 2010) and a von Bertalanffy growth curve for *T. gigas* (Rose et al. 2018). This allowed us to include these individuals in our analysis, while accounting for uncertainty in their size in years in which they were examined but not measured.

We analyzed all regression models using JAGS (Just Another Gibbs Sampler) version 4.2.0 (Plummer 2003), accessed through R version 3.3.1 (R Core Team 2016) using the R2jags package (Su, Y.-S., and M. Yajima. 2015. R2jags: Using R to run "JAGS." Available at http://cran.r-project.org/package=R2jags. [Accessed 15 June 2016]). We used Uniform(0,1) priors on all probability parameters, and a weakly informative Normal($\mu = 0$, $\sigma = 10$) prior for the effect of maternal SVL on litter size, neonate SVL, and neonate mass. We used half-Cauchy priors for the standard deviation of random effects (Gelman 2006). We ran models on seven chains for 400,000 iterations after a burn-in of 100,000 and thinned the resulting chains by a factor of 40, resulting in a total of 70,000 iterations. We visually inspected trace plots to ensure chains were well-mixed, and evaluated convergence using the $\hat{R}$ statistic: all $\hat{R}$ values were < 1.01 (Brooks and Gelman 1998). To assess the evidence for a relationship between a covariate and the response variable, we measured the posterior probability that the coefficient for the covariate had the same sign as its median value. Unless otherwise noted, we report this posterior probability, along with posterior median

TABLE 2. Sample size (n), mean, standard deviation (SD), and range of maternal snout-vent length (SVL) and mass, litter size, neonate SVL and mass, and date of parturition of female Giant Gartersnakes (*Thamnophis gigas*) from the Sacramento Valley of California, USA.

| Parameter | n | Mean | SD | Range | Source |
|---|---|---|---|---|---|
| Maternal SVL (mm) | 104 | 790 | 88.6 | 514–1040 | X-rays |
| | 13 | 731.5 | 90.5 | 640–910 | Captive females |
| Maternal mass (g) | 104 | 317 | 114.3 | 99–815 | X-rays |
| | 13 | 293.2 | 143.6 | 186–664 | Captive females |
| Litter size | 50 | 15.9 | 4.6 | 8–33 | X-rays |
| | 13 | 15.5 | 7.9 | 5–35 | Captive litter (Total) |
| | 13 | 11.8 | 6.3 | 1–23 | Captive litter (Live) |
| Neonate SVL (mm) | 154 | 187.7 | 10.1 | 146–207 | Captive litter (Live) |
| Neonate mass (g) | 154 | 4.3 | 1 | 2–7.3 | Captive litter (Live) |
| Date of parturition | 13 | 5 August | 19 days | 12 July to 12 September | Captive litter |

estimates and 95% credible intervals (CI) for all model parameters. When summarizing raw data, we report means ± 1 SD.

## RESULTS

We made 104 X-rays on 73 female *T. gigas* from 2014–2016; 47 individuals were examined in only one year, 21 were examined in two years, and five were examined in three years. X-rayed females ranged from 514–1040 mm SVL ($\bar{x}$ = 790 mm ± 88.6 SD) and 99–815 g mass (317 g ± 114.3; Table 2). Of the 104 X-ray examinations, 50 snakes were gravid and we counted the number of eggs or embryos. The smallest gravid female was 541 mm SVL. The earliest a female was determined to be gravid from an X-ray was 8 April, and the latest was 1 September. The 13 females that gave birth to litters in captivity had parturition dates from 12 July to 12 September. Females that gave birth in captivity were significantly shorter ($\bar{x}$ = 731.5 mm ± 90.5; $P$(diff < 0) = 0.98) but did not differ in mass ($\bar{x}$ = 293.2 g ± 143.6; $P$(diff < 0) = 0.73) compared to the X-rayed females (Table 2). Females that gave birth in captivity in this study were significantly shorter than females that gave birth in captivity in Halstead et al. (2011; $\bar{x}$ = 815.0 mm ± 89.0; $P$(diff < 0) = 0.98) and Hansen and Hansen (1990; $\bar{x}$ = 910.4 mm ± 102.8; $P$(diff < 0) > 0.99).

***Litter size, neonate size, and maternal size.***—We counted an average of 15.9 offspring (± 4.6; range = 8–33) from X-rayed females. The 13 litters born in captivity had an average total litter size of 15.5 offspring (± 7.9; range = 5–35), of which an average of 11.8 individuals (± 6.3; range = 1–23) were born alive. We observed at least one dead neonate in seven of 13 litters, and of 202 neonates, 48 were stillborn. At least one litter from each of the three regions had dead neonates. The number of stillborn offspring in a litter ranged from 0 to 22, with

an average of 3.7 stillborn offspring (± 6.5) per litter. When we pooled data on stillbirth from this study with 11 captive-born litters from Halstead et al. (2011), 13 of 24 litters had at least one stillborn neonate. We found some evidence for a positive effect of maternal SVL on the probability a litter contained at least one stillborn neonate, although the credible interval overlapped zero ($\beta_{SVL}$ = 0.73, -0.40–2.01; $P(\beta_{SVL} > 0)$ = 0.89). Litter size was not related to the incidence of stillbirth ($\beta_{Fec}$ = 0.02, -0.12–0.17; $P(\beta_{Fec} > 0)$ = 0.62).

The Natomas Basin (n = 5 litters) had the smallest average litter size with 12.2 offspring ± 5.1, and the largest average litter size was 17.3 offspring ± 6.9 in the Sutter Basin (n = 15 litters). Average litter size was consistent among years, ranging from 15 offspring ± 10.5 in 2013 (n = 3 litters) to 16.9 ± 5.4 in 2015 (n = 17 litters). The variance in litter size attributable to region and year was minimal after accounting for maternal size in a regression model (Table 3).

We found a significant positive relationship between female SVL and litter size: longer females produced

TABLE 3. Parameter estimates (medians and 95% credible intervals) from a Poisson regression model of the effect of maternal snout-vent length (SVL) on litter size of Giant Gartersnakes (*Thamnophis gigas*) from the Sacramento Valley of California, USA. Data include captive-born litters ($\alpha_{cap}$, n = 13) and X-ray embryo counts ($\alpha_{X-ray}$, n = 50). The parameters $\alpha_{cap}$ and $\alpha_{X-ray}$ represent the intercepts for the captive-born litters and X-ray embryo counts respectively. The parameter $\beta_{SVL}$ represents the slope of the litter-size maternal SVL relationship, and $\sigma$ represents the standard deviation of random effects. All parameters are on the natural log scale.
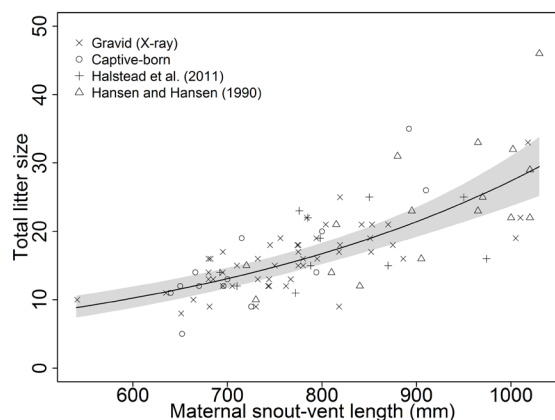
| Parameter | Median | 2.5% | 97.5% |
|---|---|---|---|
| $\alpha_{cap}$ | 2.85 | 2.63 | 3.06 |
| $\alpha_{X-ray}$ | 2.77 | 2.58 | 2.93 |
| $\beta_{SVL}$ | 0.22 | 0.16 | 0.28 |
| $\sigma_{ind}$ | 0.046 | 0.0018 | 0.15 |
| $\sigma_{site}$ | 0.047 | 0.0021 | 0.21 |
| $\sigma_{year}$ | 0.054 | 0.0025 | 0.38 |

TABLE 4. Parameter estimates (medians and 95% credible intervals) from a regression model of the effects of maternal snout-vent length (SVL) and litter size on neonate mass and SVL of Giant Gartersnakes (*Thamnophis gigas*) from the Sacramento Valley of California, USA. Data are captive-born litters from this study ($\alpha_R$, n = 12) and Halstead et al. (2011; $\alpha_H$, n = 11). The parameter $\beta_{Fec}$ represents the effect of litter size on neonate size, and $\beta_{SVL}$ represents the effect of maternal SVL on neonate size. The parameter $\sigma_{litter}$ represents the standard deviation of the litter random effect. The parameters $\sigma_{mass}$ and $\sigma_{SVL}$ represent the standard deviation of neonate mass and SVL around the mean values of each. The statistic $P(\beta<0)$ represents the posterior probability a slope parameter is negative; values near 1 indicate high probability of a negative effect, values near 0 indicate high probability for a positive effect.

| Response | Parameter | Median | 2.5% | 97.5% | $P(\beta<0)$ |
|---|---|---|---|---|---|
| Neonate mass | $\alpha_R$ | 4.31 | 3.75 | 4.87 | |
| | $\alpha_H$ | 4.51 | 3.95 | 5.06 | |
| | $\beta_{Fec}$ | -0.25 | -0.62 | 0.12 | 0.92 |
| | $\beta_{SVL}$ | -0.09 | -0.18 | 0.00 | 0.97 |
| | $\sigma_{mass}$ | 0.43 | 0.39 | 0.46 | |
| | $\sigma_{litter}$ | 0.90 | 0.67 | 1.28 | |
| Neonate SVL | $\alpha_R$ | 202.4 | 194.8 | 210.1 | |
| | $\alpha_H$ | 188.4 | 180.7 | 196.0 | |
| | $\beta_{Fec}$ | -4.19 | -10.11 | 1.92 | 0.91 |
| | $\beta_{SVL}$ | 3.41 | -3.15 | 9.72 | 0.14 |
| | $\sigma_{SVL}$ | 7.37 | 6.82 | 8.00 | |
| | $\sigma_{litter}$ | 11.71 | 8.72 | 16.73 | |



FIGURE 3. The relationship of litter size to maternal snout-vent length (SVL) of female Giant Gartersnakes (*Thamnophis gigas*) from this study, from Halstead et al. (2011), and from Hansen and Hansen (1990). The black line represents the median relationship, and the gray shading represents the 95% credible interval from a hierarchical regression model of log(litter size) on maternal SVL.

larger litters (Table 3; Fig. 3). We found no significant difference in the average litter size between X-rayed females and captive-born litters (0.08, -0.12–0.27; $P(\alpha_{cap} > \alpha_{X-ray}) = 0.79$). After accounting for maternal SVL, the variation in litter size attributable to year, region, and individual was minimal. The standard deviation for year, region, and individual random effects were < 2% of the log(litter size) for a female of average SVL (Table 3). We also fit a model that pooled data from this study with 11 captive-born litters from 1995–1997 (Halstead et al. 2011), and 16 captive-born litters from 1978–1990 (Hansen and Hansen 1990), resulting in 90 total litters. The estimated intercept ($\alpha_0$ = 2.79, 2.70–2.89) and slope ($\beta_{SVL}$ = 0.22, 0.17–0.27) of the size-fecundity relationship when all four data sources were pooled were nearly equal to the estimates from the model of the current study alone (Table 3). When we fit a simple linear regression of litter size on untransformed maternal SVL, we again found a strong positive relationship ($\beta_{SVL}$ = 0.039, 0.029–0.048).

Live neonates (n = 154) averaged 187.7 mm SVL (± 10.1; range = 146–207) and 4.3 g mass (± 1, range = 2–7.3). The average SVL of neonates varied among litters, from a low of 174.8 mm ± 8.4 (n = 5 neonates) to a high of 199.3 mm ± 6.6 (n = 12 neonates). Neonates in

the current study were significantly shorter on average than neonates from Halstead et al. (2011; -14.1 mm, -25.3 to -2.9; $P(\text{diff} < 0) = 0.99$), but average neonate mass did not differ between the two studies (-0.19 g, -0.99–0.59; $P(\text{diff} < 0) = 0.69$). Neonates in the current study were also significantly shorter than neonates reported by Hansen and Hansen (1990; -9.9 mm, -21.0 to 1.3; $P(\text{diff} < 0) = 0.96$). Neonate size (mass and SVL) was negatively related to total litter size, although credible intervals overlapped zero (Fig. 4, Table 4). Similarly, we found support for a negative relationship between neonate mass and maternal SVL and weak evidence of a positive relationship between neonate SVL and maternal SVL (Fig. 4, Table 4).

***Annual proportion of gravid females and reproductive frequency.***—We made 65 X-rays on 46 radio-telemetry snakes during the pre-parturition period; these snakes averaged 793.1 mm SVL (± 79.2; range 664–1018 mm). The proportion of examined females that were gravid during the pre-parturition period was highest in 2016 (0.64), followed by 2014 (0.50) and 2015 (0.47). We examined the most snakes in June in 2014, and in May in 2015 and 2016 (Table 5). When we included year, Julian date (linear and quadratic), and maternal SVL as fixed effects in a binomial model of reproductive status, the estimated probability that a female was gravid was slightly higher in 2016 than 2015 (0.16, -0.12–0.41; $P(2016 > 2015) = 0.88$) and 2014 (0.25, -0.09–0.55; $P(2016 > 2014) = 0.92$), but there was no difference between 2014 and 2015 (0.09, -0.20–0.36; $P(2015 > 2014) = 0.73$).

To get an estimate of the frequency with which individual females reproduce, we looked at a subset of 17 females that were examined with X-rays in two or
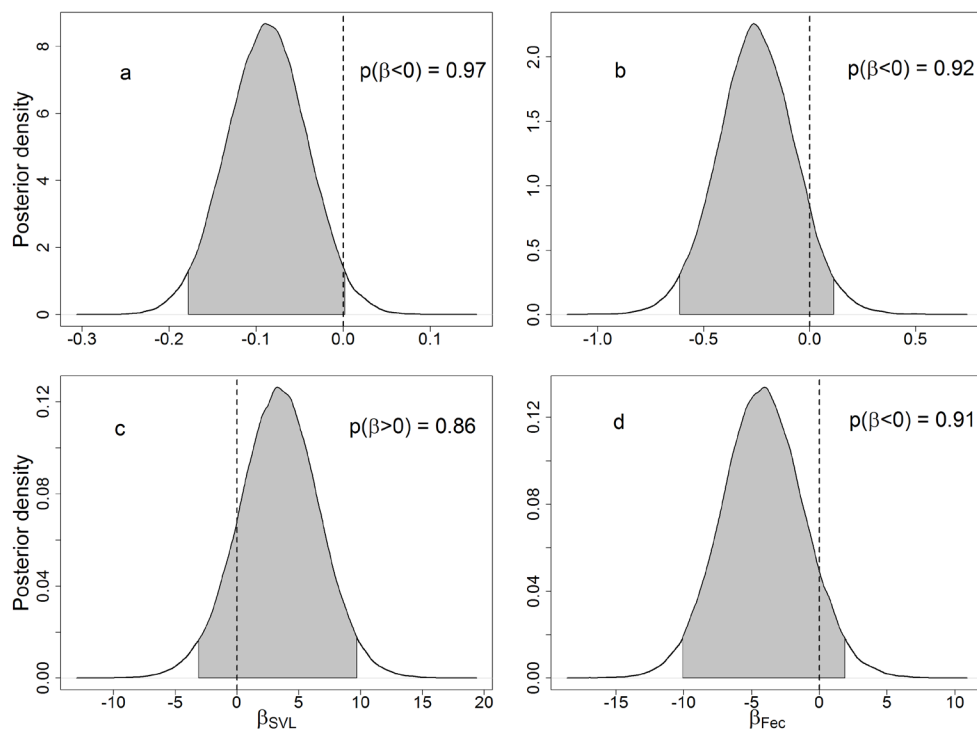
**Figure 4**. Posterior probability of slope parameters for the effect of maternal snout-vent length (SVL) of female Giant Gartersnakes (*Thamnophis gigas*) from the Sacramento Valley, California, USA, on (a) neonate mass or (c) neonate SVL, and for the effect of total litter size on (b) neonate mass or (d) neonate SVL. The vertical dashed lines represent a value of 0, the gray shading is the 95% credible interval of the parameter estimate, and the value in the upper right corner represents the posterior probability that the parameter has the same sign as its median estimate. The parameter $\beta_{Fec}$ represents the effect of litter size on neonate size, and $\beta_{SVL}$ represents the effect of maternal SVL on neonate size.

more years during the pre-parturition period. Of two females examined in three years, one was gravid all three years, and one was gravid in only one of three years. Of the 15 females that were examined in two years, four were gravid in both years, five were gravid in one of two years, and six did not reproduce in either year. Overall, the annual frequency of reproduction among 17 females examined in more than one year was 46%.

## DISCUSSION

Despite its large size, many reproductive traits of *Thamnophis gigas* appear to be similar to those of other *Thamnophis* and ecologically similar watersnakes in the genus *Nerodia*. The average litter sizes for *T. gigas* estimated from X-rays (15.9) and captive litters (15.5)

in this study are larger than that reported for Checkered Gartersnakes (*T. marcianus*; 9.5) from Texas, USA (Ford and Karges 1987), and six populations of *T. elegans* from California (5.2–10.5; Miller et al. 2011). Litter size, however, is within the range of average litter sizes (7.6–23) reported from 11 populations of *T. sirtalis* distributed throughout Canada (Gregory and Larsen 1993) and close to that reported for Northwestern Gartersnakes (*T. ordinoides*) from Oregon, USA (16.6; Stewart et al. 1990). Female Northern Watersnakes (*Nerodia s. sipedon*) in Ontario, Canada, and Lake Erie Watersnakes (*N. s. insularum*) in Lake Erie of North America reach similar maximum lengths (asymptotic SVL = 920 mm, 1015 mm SVL) as *T. gigas* and produce larger litters on average (20.0 and 22.9, respectively; King 1986; Brown and Weatherhead 1999; Weatherhead et al.

**TABLE 5**. Number of female Giant Gartersnakes (*Thamnophis gigas*) from the Sacramento Valley of California, USA, examined by X-ray and number gravid by month, 2014–2016. Only females examined from 1 May to 11 July are included.

| Year | May | | June | | July | | Total | | Proportion |
|------|-----|-----|------|-----|------|-----|-------|-----|-----|
| | Examined | Gravid | Examined | Gravid | Examined | Gravid | Examined | Gravid | reproductive |
| 2014 | 0 | 0 | 17 | 8 | 5 | 3 | 22 | 11 | 0.50 |
| 2015 | 16 | 7 | 10 | 6 | 6 | 2 | 32 | 15 | 0.47 |
| 2016 | 7 | 3 | 4 | 4 | 0 | 0 | 11 | 7 | 0.64 |

1999; King et al. 2016). Larger female *T. gigas* produce larger litters than smaller individuals, and the slope of the maternal size-fecundity relationship from our linear regression model with untransformed maternal SVL (0.039) was similar to that reported for other new-world natricine snakes: *N. s. insularum* (0.041; King 1986), *T. marcianus* (0.038; Ford & Karges 1987), *T. ordinoides* (0.060; Stewart et al. 1990), *T. sirtalis* (0.054; Larsen et al. 1993), and *T. elegans* (0.035–0.047; Bronikowski & Arnold 1999).

In contrast to the clear maternal size-fecundity relationship, our small sample of captive-born litters made it difficult to disentangle the effects of maternal size and litter size on neonate size. Our results suggest that neonates from larger litters were shorter and lighter than neonates from smaller litters. Surprisingly, larger females appeared to produce lighter neonates, after accounting for the effect of female size on fecundity. Studies of natricine snakes have generally found a positive relationship between maternal size and neonate size, after accounting for negative effects of litter size on neonate size (King 1993; Weatherhead et al. 1999). Clarifying how maternal size interacts with litter size to influence neonate length and mass in *T. gigas* will require data on neonate size from a larger sample of litters.

The smallest gravid female we recorded was 541 mm SVL, and 18 of 63 gravid females were < 700 mm SVL. Using 541 mm SVL as a minimum size at first reproduction, and based on growth trajectories of *T. gigas*, it is likely that, on average, females first reproduce at three years of age (Rose et al. 2018). Female *T. radix* and *T. sirtalis* have been reported to first reproduce when two or three years old (Fitch 1965; Stanford and King 2004; Tuttle and Gregory 2012), and populations of *T. elegans* vary in their age at maturity from three to five years old (Bronikowski and Arnold 1999). Therefore, the life history of our study populations appears to fit the general pattern seen in many *Thamnophis* species, and the large size of *T. gigas* is not necessarily indicative of later maturity and higher fecundity than other gartersnakes.

One area in need of further exploration is variation in reproductive traits throughout the range of *T. gigas*. The average litter size of *T. gigas* in this study was similar to the average of 17 neonates (± 5.1; range 13–21) reported by Halstead et al. (2011), but our litter sizes were smaller than the average litter size of 23 neonates ($\bar{x}$ = 23.4 ± 9.3; range 10–46) reported by Hansen and Hansen (1990). The average size of neonates in this study (187.7 mm SVL) was smaller than that reported by both Halstead et al. (208.8 mm SVL) and Hansen and Hansen (206.4 mm SVL). Measurements of snake length can vary among observers (Cundall et al. 2016), but neonate SVL was measured by multiple observers

in the current study, and we do not think a consistent measurement bias is the source of the observed difference in neonate SVL between this and previous studies. One potential explanation for the smaller litter sizes and smaller neonate size in our study is that the adult females that gave birth in captivity were smaller than those in Halstead et al. (2011) and Hansen and Hansen (1990). Halstead et al. (2011) collected females from four sites in the Sacramento Valley, including two sites also sampled in this study (Central Colusa Basin included Colusa National Wildlife Refuge and Sutter Basin included Gilsizer Slough), but Hansen and Hansen (1990) measured captive-born litters from populations in Sacramento County, the southern limit of our study area, and from farther south, in the San Joaquin Valley. Unfortunately, because *T. gigas* have been almost extirpated from the San Joaquin Valley (U.S. Fish and Wildlife Service 2017), it is unlikely we will be able to collect reproductive data from southern populations for comparison. Likewise, few historical reproductive data are available from populations in the Sacramento Valley. Therefore, we are unable to evaluate whether the smaller neonate size and litter size found in this study represent a trend or natural spatial and temporal variability. Future studies should sample a variety of sites within the remaining distribution of *T. gigas* to further our understanding of variation in reproductive traits. Because *T. gigas* inhabits natural and restored wetlands, as well as agricultural canals, another potentially fruitful avenue for future work is to evaluate whether the reproduction and life history of this species varies among habitat types.

We collected data during a period of drought (2013–2015) and the first average water year following this prolonged drought (2016). Some reproductive characteristics could be different during a period of normal or wet years. For example, a study in Kansas, USA, found that clutch size was higher in years with higher rainfall for three snake species (Seigel and Fitch 1985), and a study of Plains Gartersnakes (*T. radix*) in Alberta, Canada, reported that litter size was negatively related to rainfall and positively related to temperature the previous year (Tuttle and Gregory 2014). The proportion of females that reproduce each year is a key component of demographic models; failure to accurately estimate this parameter could lead to over- or under-estimates of productivity. Indeed, a study of *T. elegans* found that the proportion of females that are gravid each year, and annual variation in this parameter, greatly influence the population growth rate (Miller et al. 2011). Our estimates of the proportion of female *T. gigas* that are reproductive each year may not be representative of a natural population because our data came from snakes that were part of a radio-telemetry study. Because only large females (≥ 200 g mass) could

accommodate radio-transmitters, we did not X-ray smaller but potentially sexually mature females. Thus, our estimates of the proportion of adult females that are reproductive could be positively biased compared to the wider population. Alternatively, the stress associated with surgery and carrying a radio-transmitter could influence the reproductive status of a female. In the future, it would be useful to examine a random sample of female *T. gigas* to obtain an unbiased estimate of the proportion of females that are reproductive in a given year. Also, future studies during periods of normal or above-average rainfall would provide useful information on the natural variability in the reproductive ecology of this species.

Our study shows the usefulness of X-radiography as a non-invasive method for counting litter size in snakes. To validate this method, it would be valuable to compare embryo counts from X-rays to observed litter sizes in the same individuals. An advantage of X-radiography for measuring litter size is that it does not require females be kept in captivity for an extended period of time. One limitation of X-radiography is that we cannot determine how many embryos are non-viable or how many neonates will be stillborn. Stillbirth might be a general phenomenon in *T. gigas*, as it has been documented in multiple populations and in studies separated by nearly 20 y (Halstead et al. 2011). The proportion of live vs. stillborn neonates in *T. gigas* varied greatly among litters, but on average only approximately 80% of neonates were born alive. Also, we found some evidence for a positive relationship between maternal size and the incidence of stillbirth in a litter, although we caution that our small sample limits our inference into this relationship. The frequency of stillbirth in *T. gigas* litters could be a reason for concern; increased incidence of stillborn offspring was linked to inbreeding in an isolated population of Adders (*Vipera berus*; Madsen et al. 1996). On the other hand, stillbirth has also been found in common, non-threatened natricine snakes such as *T. elegans*, *T. sirtalis*, and *N. sipedon* (Gregory et al. 1992; Weatherhead et al. 1998; Sparkman et al. 2007). For demographic studies of *T. gigas* and other snakes in which stillbirth is common, estimates of fecundity based on X-rays should be adjusted to account for the fact that not all embryos are likely to be born alive. More data on the frequency of stillbirth in the wild, and variation in stillbirth among populations could help elucidate drivers of this phenomenon and whether it represents a cause for concern in this threatened species.

*Conservation implications*.—Our study is the first to document size-dependent fecundity and report reproductive frequency in female *T. gigas*, and provides valuable information on reproduction in this threatened species. The relationship between fecundity and female size in *T. gigas*, and the indeterminate growth in this species (Rose et al. 2018), suggests that the largest females have the greatest reproductive output. To increase recruitment in *T. gigas*, it might be important to manage habitats to improve the survival of large adult females. Females spend > 50% of their time in terrestrial habitat, primarily taking refuge under cover, even during the active season (Halstead et al. 2015). Survival of large adult female *T. gigas* is higher in terrestrial habitat than in aquatic habitat (Halstead et al. 2012). Ensuring that suitable terrestrial habitat is available near wetlands and canals, and limiting disturbance to terrestrial habitat when *T. gigas* are likely to be present, could be important management actions to ensure that females can survive to older ages and produce large litters.

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