

REGULAR ARTICLE

POPULATION DENSITY AND REPRODUCTIVE SEASONALITY OF *TRYONIA CHEATUMI* (GASTROPODA: COCHLIOPIDAE), THE PHANTOM TRYONIA

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ABSTRACT

We studied population density, population size, and reproductive seasonality of the Phantom Tryonia, *Tryonia cheatumi* (Pilsbry, 1935). This endangered freshwater snail is found only in the San Solomon Spring system, a cienega, or karst-based, arid-land freshwater spring system, in western Texas, USA. We sampled populations at seven locations in the system seasonally over a 2-yr period. San Solomon Spring, the system's largest spring and modified into a swimming pool, had the largest population of *T. cheatumi*, with an estimated 49 million individuals and a mean density as high as $23,626 \pm 39,030$ (individuals/m² \pm SD). There were seasonal differences in mean density (up to 25-fold) and median snail size at all sites, but consistent seasonal patterns of mean density or size were not observed. Median snail size among samples was not related to water temperature, and juveniles were present in most samples in all seasons. These results support continuous, aseasonal reproduction, as expected in thermally stable habitats, but differences in median size and mean density among seasons and sites suggest that other factors affect reproduction and seasonal variation in population size of *T. cheatumi*.

KEY WORDS: spring snails, desert springs, life history, snail reproduction, conservation

INTRODUCTION

Many aquatic snails in arid regions are narrowly endemic, usually restricted to one or a few nearby springs, and these species often are of conservation concern (Lydeard et al. 2004; Hershler et al. 2014). Spring snails in the genus *Tryonia* are characteristic of mineral and thermal (hot or warm) springs in the southwestern United States and Mexico, and most are restricted to a single spring or spring system (Hershler 2001; Hershler et al. 2011, 2014). *Tryonia* are small (< 7 mm), obligately aquatic, ovoviviparous snails with separate sexes that graze on periphyton (Brown et al. 2008). *Tryonia* are most

abundant at spring heads, where they are locally dominant members of the invertebrate community (Meffe and Marsh 1983; Hershler 2001). However, *Tryonia* typically occupy narrower microhabitat niches than other spring-dwelling snails (Sada 2008), thereby limiting their spatial distribution and total population size. Furthermore, population size of *Tryonia* and related genera can vary annually, seasonally, or spatially (Taylor 1983; Lang 2001, 2011; Brown et al. 2008; Johnson et al. 2019).

Fishes inhabiting thermally stable spring systems are expected to have largely aseasonal population dynamics, such as continuous or aseasonal reproduction (Winemiller 1989), but freshwater snails may deviate from this pattern (Whelan

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and Strong 2014). For example, spring snails in the genus *Pyrgulopsis* have well-defined, population-specific reproductive seasons that correspond to small, seasonal differences in water temperature (e.g., Mladenka and Minshall 2001; Lysne et al. 2007). Aseasonal or continuous reproduction is proposed for *Tryonia* in warm, thermally stable springs, but seasonal reproduction is proposed in cooler, more thermally variable spring systems (Taylor 1983; Brown et al. 2008). However, these conclusions were based on limited evidence, and detailed life history information is available for only a few species in this group (e.g., Sada 2008). Critically, seasonal temperature variability has not been assessed in springs that support *Tryonia*, and temperature is potentially a primary driver of reproduction (Brown et al. 2008).

The endemic spring snails of Texas, USA, include five species of *Tryonia* (Hershler 2001; Hershler and Liu 2017), all of which are critically imperiled (G1) under NatureServe criteria (Faber-Langendoen et al. 2012; Johnson et al. 2013). *Tryonia cheatumi*, the Phantom *Tryonia*, is listed as endangered under the U.S. Endangered Species Act (USFWS 2013). *Tryonia cheatumi* occurs only in the San Solomon Springs system in arid western Texas within the Chihuahuan Desert ecoregion (Allan 2011; Lang 2011). All known populations are restricted to a 13-km-long series of spring outflows near the town of Balmorhea, including Phantom Lake, Giffin, San Solomon, and East and West Sandia springs, and associated aquatic habitats. Flows from the San Solomon Springs system are declining over the long term due to groundwater extraction for agriculture, and concern exists regarding potential effects from nonconventional oil and gas development in the region (Texas Water Development Board 2005). Limited sampling of *T. cheatumi* from 2001 to 2009 found high densities (thousands per square meter) at Phantom Lake, San Solomon Springs, and East Sandia Springs (Lang 2011). However, effective conservation of the species requires more recent density estimates and life history data to understand population trends over time.

We examined seasonal patterns of population density, population size, and size structure in *T. cheatumi* throughout the San Solomon Spring system over 2 yr. We examined size structure to make inferences about when reproduction occurred and whether reproduction was seasonal or continuous. We also examined temporal patterns of water temperature in different parts of the spring system and how thermal stability was related to size structure.

METHODS

Study Area

San Solomon Springs is an artesian spring system consisting of three main areas, all of which appear to arise from the same groundwater sources (Chowdhury et al. 2004). The main spring, San Solomon Spring (water source in San Solomon Pool), within Balmorhea State Park, has been modified into a partially concrete-lined swimming pool that

discharges into the concrete-lined San Solomon Canal that, in turn, feeds two restored wetland areas, San Solomon Cienega and Hubbs Cienega. Giffin Spring is on private land approximately 0.2 km northwest of San Solomon Spring (Lang 2011). The system includes two additional spring areas that support *T. cheatumi*. Phantom Lake Spring, approximately 2.4 km southwest of the state park, is upgradient from the main spring and discharges groundwater from a large cavern system (Brune 2002). The spring discharge from the cavern ceased to flow in 1999, but water is now provided to a constructed canal and wetland by a pump located inside the cave. East and West Sandia springs, near Balmorhea, are downgradient of the main spring system and located on property owned by The Nature Conservancy. East Sandia Spring currently consists of a series of isolated pools and marshy areas. In 2000, West Sandia Spring was reduced to a moist soil area, and no aquatic snails were found (J.J. Landye, Arizona Game and Fish Department, retired, personal communication). However, West Sandia Spring was flowing during our study. All springs in the system have been connected intermittently via a series of irrigation canals since the 1940s, but it is unknown whether *T. cheatumi* occurs in the canals.

Field Sampling

We sampled *T. cheatumi* and water temperature at seven sites within the San Solomon Spring system (Fig. 1): (1) East Sandia Spring (30.990978, -103.729036; 603-m² surface area), (2) West Sandia Spring (30.986838, -103.73635; 339 m²), (3) San Solomon Pool (30.944279, -103.788395; 5,556 m²), (4) San Solomon Canal (30.944538, -103.785917; 2,859 m²), (5) Hubbs Cienega (30.945479, -103.786001; 1,050 m²), (6) San Solomon Cienega (30.945138, -103.784405; 4,340 m²), and (7) Phantom Lake Spring (30.935005, -103.849613; 120 m²). These sites encompass all known populations of *T. cheatumi* except Giffin Spring, which we were unable to sample. We sampled most sites in spring (March), summer (June), fall (October), and winter (December) in 2017 and 2018 (Table 1), except for the fall 2018 sample at San Solomon Pool, which was taken in August. Because of limited access to the site, we did not sample Phantom Lake Spring in the fall.

At each site on each sampling event, we estimated *T. cheatumi* density at 10–25 randomly generated points distributed among mesohabitat types found at the sites. Before sampling, we delineated broad mesohabitat types based on benthic substrate composition and macrophyte cover, and we estimated the area of each mesohabitat with a hand-held Trimble GPS unit (Trimble, Inc., Sunnyvale, CA, USA). Based on our delineation, there were one to three mesohabitat types at each site. The number of sampling points at each site was dependent on the size of the site and the number of mesohabitat types present, and we allocated the number of samples in each mesohabitat proportional to the area of the mesohabitat at the site on the date of sampling.

On each sampling date and before sampling for *T.*

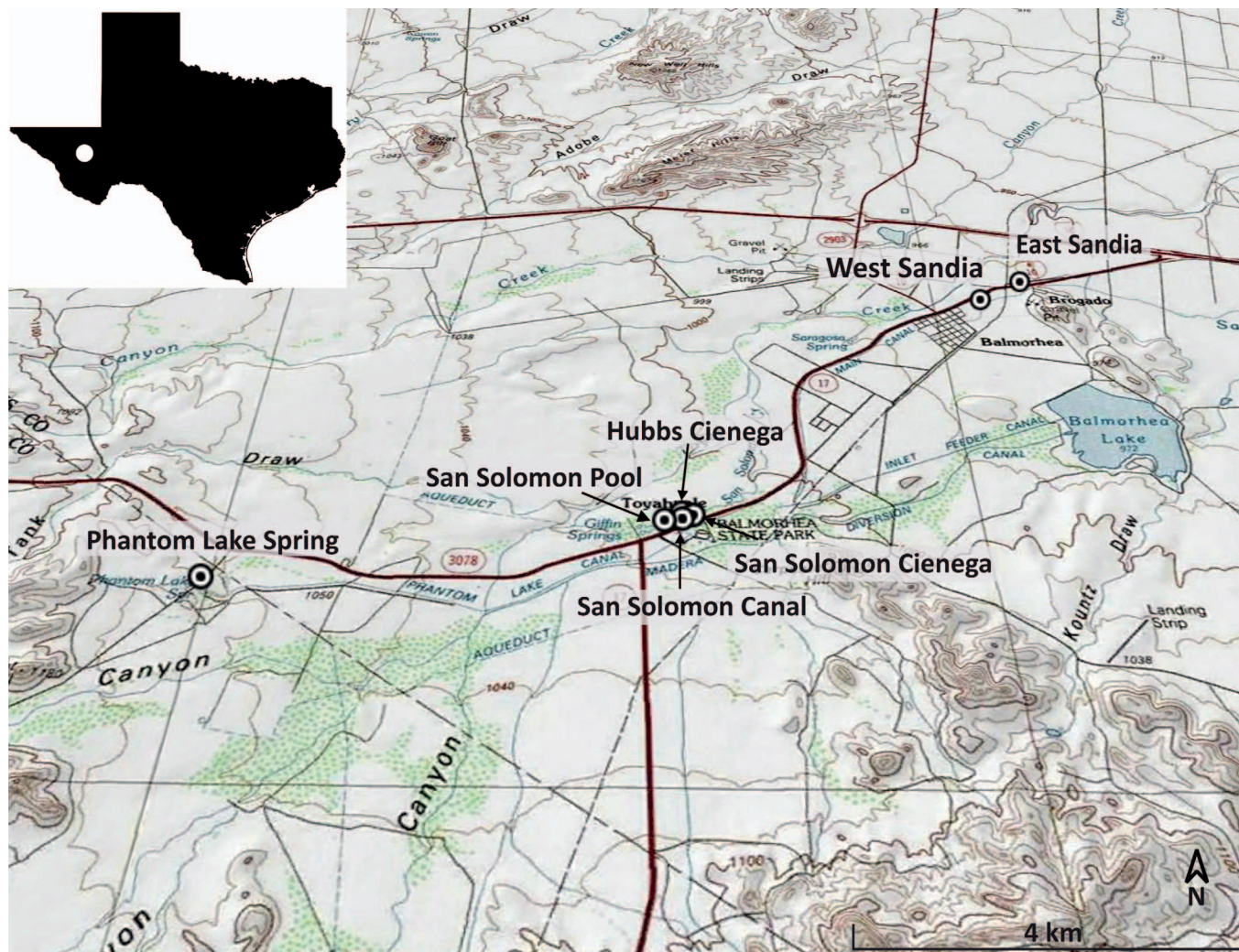


Figure 1. Map showing the location of sampling sites in the San Solomon Spring system (imagery from USGS 2013). Inset shows the location of the study area in Texas, USA.

Table 1. Parameter estimates for the generalized linear model examining the effect of site and season on mean density of *Tryonia cheatumi* at seven sites in the San Solomon Spring system. b is the slope and W_T is the Wald chi-squared test statistic. Degrees of freedom for all parameters = 1. Redundant parameters were set to zero and are not included. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

Parameter	b	SE	95% Confidence Interval	W_T	P
Intercept	0.04	1.00	-1.91 to 1.99	0.002	0.966
CAN	6.65	1.02	4.65 to 8.66	42.34	< 0.001
ES	5.78	1.13	3.56 to 8.00	26.04	< 0.001
HC	6.23	1.09	4.10 to 8.37	32.71	< 0.001
PHA	8.67	1.08	6.56 to 10.78	64.75	< 0.001
SSC	2.40	1.10	0.20 to 4.57	4.74	0.03
SSP	8.80	1.06	6.72 to 10.87	68.90	< 0.001
Fall	0.76	0.64	-0.48 to 2.00	1.45	0.23
Spring	0.23	0.45	-0.65 to 1.12	0.27	0.60
Summer	0.42	0.44	-0.45 to 1.29	0.88	0.35

cheatumi, we measured water temperature immediately above the bottom at each sampling point by using a pre- and postcalibrated Manta multiprobe (Eureka Water Probes, Austin, TX, USA). We then collected benthic material (containing *T. cheatumi*) at each point with a 100-cm² benthic basket sampler designed for quantitative sampling of spring snail populations (Lang sampler, 10 cm × 10 cm × 3 cm, 500-μm mesh; Lang 1999, 2001; Johnson et al. 2019). The number of samples taken on each date at each site was as follows: East Sandia Spring, $N = 10$ –16; West Sandia Spring, $N = 10$ –11; San Solomon Pool, $N = 18$ –20; San Solomon Canal, $N = 15$ –25; Hubbs Cienega, $N = 5$ –6; San Solomon Cienega, $N = 14$ –16; and Phantom Lake Spring, $N = 5$ –20. We preserved samples in the field in 95% ethanol and later sorted, counted, and identified the contents under a dissecting microscope at the San Marcos Aquatic Resource Center, U.S. Fish and Wildlife Service and the Aquatic Ecology Laboratory in the Freeman Aquatic Station at Texas State University, San Marcos. Samples contained two invasive snail species, *Melanoides tuberculata* and *Thiara granifera*, that we distinguished from *T. cheatumi* by shell coloration and sculpture.

We obtained samples of *T. cheatumi* large enough for size structure analysis (> 50 individuals) from three sites: San Solomon Pool ($N = 1,260$), San Solomon Canal ($N = 645$), and Phantom Lake Spring ($N = 524$). We measured shell height (maximum shell height parallel to the axis of coiling) with a stereoscopic microscope and attached Infinity-1 camera (Teledyne Lumenera, Ottawa, Ontario, Canada). Infinity Analyze (Teledyne Lumenera, Ottawa, Ontario, Canada) was used to calibrate measurement by using a stage micrometer (Meiji Techno America, San Jose, CA, USA; 1 mm with 0.01-mm divisions). Each shell was placed on clay in the same orientation for measurement, conforming to a standardized shell photography guide (Callomon 2019).

Data Analysis

We tested for differences in median water temperature across seasons at each site by using a Kruskal–Wallis test for independent samples and pairwise comparisons (two-sided tests), due to heterogenous variance and nonnormal error structure. Analyses were conducted in SPSS 27 (IBM, Armonk, New York, United States). We tested for differences in the variance around median water temperature among sites with Levene’s test (Zar 1999).

We examined whether *T. cheatumi* density differed across sites or seasons by using a generalized linear model (GzLM). We used GzLM because our data contained many zero observations, exhibited substantial nonnormal error structure, and had heteroscedastic variance (Maindonald and Braun 2007; O’Hara and Kotze 2010). We modeled density by using a negative binomial distribution and a log link function. Site (San Solomon Canal, East Sandia Spring, Hubbs Cienega, Phantom Lake Spring, San Solomon Cienega, San Solomon Pool, and West Sandia Spring) and season (fall, winter,

summer, and spring) were categorical variables, and we applied the Huber–Sandwich estimator procedure to account for heterogeneous variance. We assessed overall model significance by comparing the fitted model to an intercept-only model, and pairwise post hoc comparisons among sites and seasons were performed with a sequential Bonferroni procedure. These analyses were performed in R 3.6.3.

To assess the relationship between reproduction of snails and water temperature, we examined two-tailed Pearson correlation coefficients (R 3.6.3; R Core Team 2022) between median shell height of snails at each site on each sampling date and mean water temperature measured at that site. Shell height was not normally distributed in most samples (Shapiro–Wilk test: $W = 0.860856$ – 0.979127 , $P = 0.4063$ to < 0.001). We tested for differences in shell size among sites and seasons using Kruskal–Wallis tests followed by comparison of each pair with the Wilcoxon method including a Bonferroni correction for multiple comparisons when needed. These analyses were conducted in JMP Pro 15.0.0 (SAS Institute, Cary, NC, USA).

RESULTS

Density and Population Size

Water temperatures differed among sites (Fig. 2a). Water temperatures differed among sites across the entire study period (Kruskal–Wallis test: $H_6 = 350.06$, $P < 0.001$). Temperature at East and West Sandia springs did not differ from each other ($P = 0.703$), but both had consistently lower temperatures ($\sim 20.5^\circ\text{C}$) throughout the study period than the other sites ($P < 0.001$ for all comparisons). San Solomon Cienega had lower water temperatures than San Solomon Pool and San Solomon Canal, and Phantom Lake Spring had slightly lower temperatures than San Solomon Canal ($P < 0.001$ for all comparisons).

Water temperatures varied seasonally across sites (Kruskal–Wallis test: $H_3 = 184.94$, $P < 0.001$) following expected seasonal patterns (Fig. 2b). Winter temperatures were lower than those in the spring, summer, and fall ($P < 0.006$ for all comparisons); spring temperatures were lower than those in the summer and fall ($P < 0.001$ for both comparisons); and fall temperatures were lower than those in the summer ($P < 0.001$). Seasonal variation in water temperatures differed among sites (Levene’s statistic = 9.32; $df = 6, 46$; $P < 0.001$), with San Solomon Cienega having the greatest seasonal variation in water temperature, differing $> 9^\circ\text{C}$ between the winter minimum and the summer maximum (Fig. 2a). All other sites showed limited variation in water temperature across seasons, with a range of variation of $< 4^\circ\text{C}$ annually.

Mean density of *T. cheatumi* differed significantly among sites (Table 1). The highest mean density of *T. cheatumi* was observed in San Solomon Pool (mean density over all sampling events = $8,976$ individuals/m² \pm $19,900$ [SD]; Table 2 and Fig. 3). Phantom Lake Spring also had high mean density ($7,438/\text{m}^2 \pm 12,963$ SD), but mean density at this site

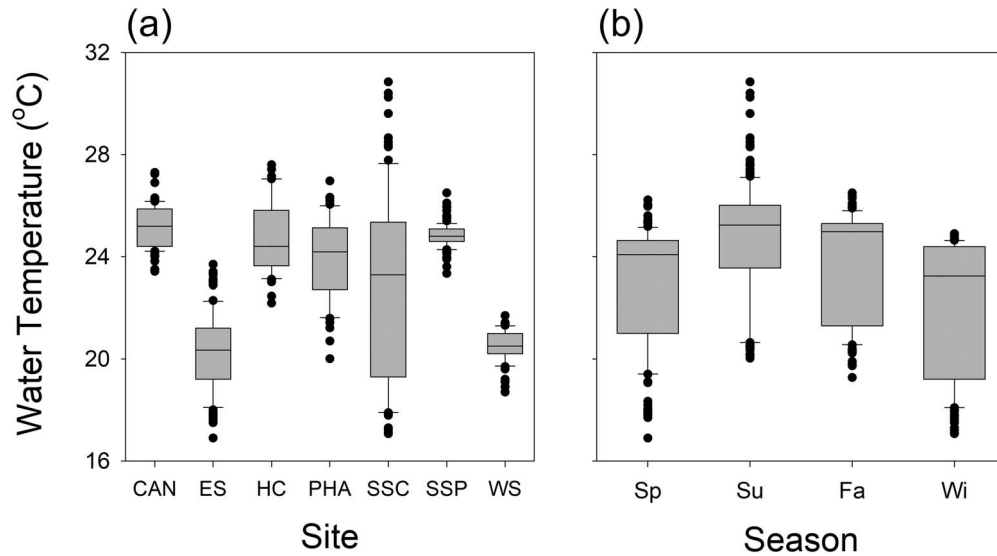


Figure 2. Box-and-whisker plots showing variation in water temperature among (a) sites and (b) seasons in the San Solomon Spring system. Boxes represent the 25% and 75% quartiles, lines within boxes are the median, whiskers are $1.5 \times$ the interquartile range, and dots are outliers. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring. Season abbreviations are as follows: Sp = spring, Su = summer, Fa = fall, and Wi = winter.

did not differ significantly from the other sites except San Solomon Pool due to extremely high seasonal variation. We observed *T. cheatumi* at West Sandia Spring in only one sample, where it was present at low density (spring 2018, $9/\text{m}^2 \pm 29$ SD; mean density across sampling events = $1/\text{m}^2 \pm 12$ SD). Mean density at the other four sites ranged from 7 to $2,099/\text{m}^2$. Mean estimates of density appeared to vary widely among seasons at all sites, particularly San Solomon Pool and Phantom Lake Spring, but confidence intervals (CIs) around these estimates overlapped widely, and season was not a significant factor in the GzLM model (Table 2). Population size appeared to vary annually at some sites, but CIs overlapped between years at all sites except San Solomon Canal, where estimated population size in 2017 was nearly 20 times higher than in 2018 (Table 3).

The two invasive snail species were present at all study sites except East and West Sandia springs. At Phantom Lake

Spring, *Terebia* mean density was $175/\text{m}^2 \pm 228$ SD and *Melanoidea* mean density was $4,793/\text{m}^2 \pm 6,397$ SD. At San Solomon Pool, *Terebia* mean density was $3,846/\text{m}^2 \pm 766$ SD and *Melanoidea* mean density was $1,593/\text{m}^2 \pm 1,262$ SD. At San Solomon Canal, *Terebia* mean density was $15,445/\text{m}^2 \pm 6,482$ SD and *Melanoidea* mean density was $1,245/\text{m}^2 \pm 834$ SD. At San Solomon Cienega, *Terebia* mean density was $19,504/\text{m}^2 \pm 25,203$ SD and *Melanoidea* mean density was $8,495/\text{m}^2 \pm 6,989$ SD. At Hubbs Cienega, *Terebia* mean density was $9,535/\text{m}^2 \pm 12,102$ SD and *Melanoidea* mean density was $12,060/\text{m}^2 \pm 5,809$ SD.

San Solomon Pool had the highest estimated population size of *T. cheatumi*, with 49,477,642 individuals (95% CI = 25,520,917–73,434,367) (Table 3), followed by San Solomon Canal, Phantom Lake Spring, Hubbs Cienega, San Solomon Cienega, and East Sandia Spring. Estimated population size at

Table 2. Mean density (individuals/ $\text{m}^2 \pm$ SD) of *Tryonia cheatumi* at seven sites in the San Solomon Spring system. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

Year	2017				2018			
Site	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
ES	318 ± 737	$283 \pm 1,050$	29 ± 70	156 ± 641	156 ± 641	67 ± 249	0 ± 0	9 ± 29
WS	— ^a	0 ± 0	0 ± 0	0 ± 0	9 ± 29	0 ± 0	0 ± 0	0 ± 0
SSP	$23,626 \pm 39,030$	$15,425 \pm 26,782$	789 ± 976	$1,505 \pm 2,367$	$15,512 \pm 47,354$	$4,530 \pm 8,854$	$1,200 \pm 2,781$	$9,221 \pm 31,056$
CAN	305 ± 714	$2,116 \pm 7,228$	$3,735 \pm 13,763$	$3,243 \pm 8,415$	242 ± 721	55 ± 172	55 ± 218	300 ± 980
HC	0 ± 0	167 ± 197	83 ± 186	400 ± 563	$1,000 \pm 1,501$	$1,020 \pm 1,235$	200 ± 352	$2,240 \pm 4,232$
SSC	0 ± 0	21 ± 77	0 ± 0	7 ± 26	0 ± 0	77 ± 266	54 ± 187	0 ± 0
PHA	$3,050 \pm 3,825$	$12,989 \pm 21,828$	—	$1,917 \pm 2,623$	325 ± 507	$2,980 \pm 2,213$	—	$23,367 \pm 46,780$

^aDashes indicate that no sample was taken.

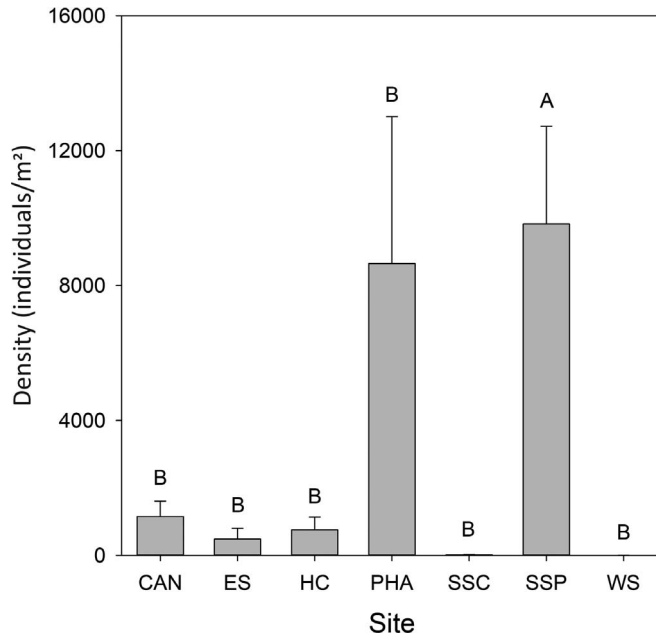


Figure 3. Estimated marginal mean density of *Tryonia cheatumi* across seasons at seven sites in the San Solomon Spring system. Error bars are ± 1 SE. Means with different letters are significantly different (post hoc pairwise comparisons, $\alpha = 0.05$). Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

West Sandia Spring was small (478 individuals, 95% CI = 0–1,413).

Size Structure

Snail size differed among seasons at all three sites where we examined size structure (Fig. 4). There was a significant difference in shell height among seasons at San Solomon Pool (Kruskal–Wallis test: $\chi^2 = 183.2108$, $df = 3$, $P < 0.0001$; Wilcoxon paired comparisons: $P < 0.0001$); median shell height was highest in the fall (2.76 mm) and lowest in the spring (1.77 mm). Four of six pairwise comparisons were significantly different (Fig. 4). There was a significant difference in shell height among seasons at San Solomon Canal (Kruskal–Wallis test: $\chi^2 = 16.7944$, $df = 3$, $P < 0.0001$; Wilcoxon paired comparisons: $P < 0.0001$ – 0.0005); median

shell height was highest in the spring (1.90 mm) and lowest in the fall (1.49 mm). Two of six pairwise comparisons were significantly different. There was a significant difference in shell height between summer and winter at Phantom Lake (Kruskal–Wallis test: $\chi^2 = 84.6952$, $df = 3$, $P < 0.0001$); median shell height was higher in the winter (2.27 mm) than in summer (1.41 mm).

Shell height differed among sites in all seasons except spring (Fig. 5). Median shell height did not differ between San Solomon Pool and San Solomon Canal in the spring (Kruskal–Wallis test: $\chi^2 = 0.6710$, $df = 1$, $P = 0.4127$), but size appeared to be more variable in San Solomon Pool. There was a significant difference in shell height among all three sites in the summer (Kruskal–Wallis test: $\chi^2 = 236.9808$, $df = 2$, $P < 0.0001$; all pairwise comparisons significantly different); median shell height was highest in San Solomon Pool (2.57 mm) and lowest at Phantom Lake (1.41 mm). There was a significant difference in shell height between San Solomon Pool and San Solomon Canal in the fall (Kruskal–Wallis test: $\chi^2 = 160.8383$, $df = 1$, $P < 0.0001$); median shell height was higher in San Solomon Pool (2.76 mm) than in San Solomon Canal (1.49 mm). There was a significant difference in shell height among all three sites in the winter (Kruskal–Wallis test: $\chi^2 = 57.0120$, $df = 2$, $P < 0.0001$; all pairwise comparisons significantly different); median shell height was highest at Phantom Lake (2.20 mm) and lowest at San Solomon Canal (1.57 mm). There was no correlation between median shell height and water temperature across all sample events that yielded > 50 individuals ($r = -0.10$, $P = 0.654$; $N = 19$).

DISCUSSION

We found substantial populations of *T. cheatumi* at San Solomon Springs and Phantom Lake Spring and smaller populations at the other sites. We found large changes in density (up to 25-fold) of *T. cheatumi* across years and sampling periods. San Solomon Pool had high densities in both spring seasons, summer 2017, and winter 2018 and lower densities in winter 2017, summer 2018, and both fall seasons. At Phantom Lake Spring, peak densities occurred in summer 2017 and winter 2018. San Solomon Spring supports the largest population of *T. cheatumi* in the spring system despite

Table 3. Population size estimates (95% confidence interval) for *Tryonia cheatumi* at seven sites in the San Solomon Spring system in 2017 and 2018. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

Site	2017	2018	Mean across Both Years
ES	129,533 (1,155–257,912)	639,611 (0–1,867,644)	389,209 (0–1,016,600)
WS	0 (—)	827 (0–2,447)	478 (0–1,413)
SSP	57,162,692 (25,559,251–88,766,133)	41,377,184 (5,027,369–77,726,998)	49,477,642 (25,520,917–73,434,367)
CAN	6,154,181 (712,138–11,596,224)	320,672 (51,342–590,001)	3,333,041 (559,177–6,106,905)
HC	186,136 (25,389–346,884)	1,170,750 (15,867–2,325,633)	655,000 (85,865–1,224,135)
SSC	31,000 (0–78,772)	131,750 (0–315,577)	81,375 (0–176,376)
PHA	686,448 (69,342–1,303,554)	925,020 (0–1,303,554)	792,480 (115,830–1,469,130)

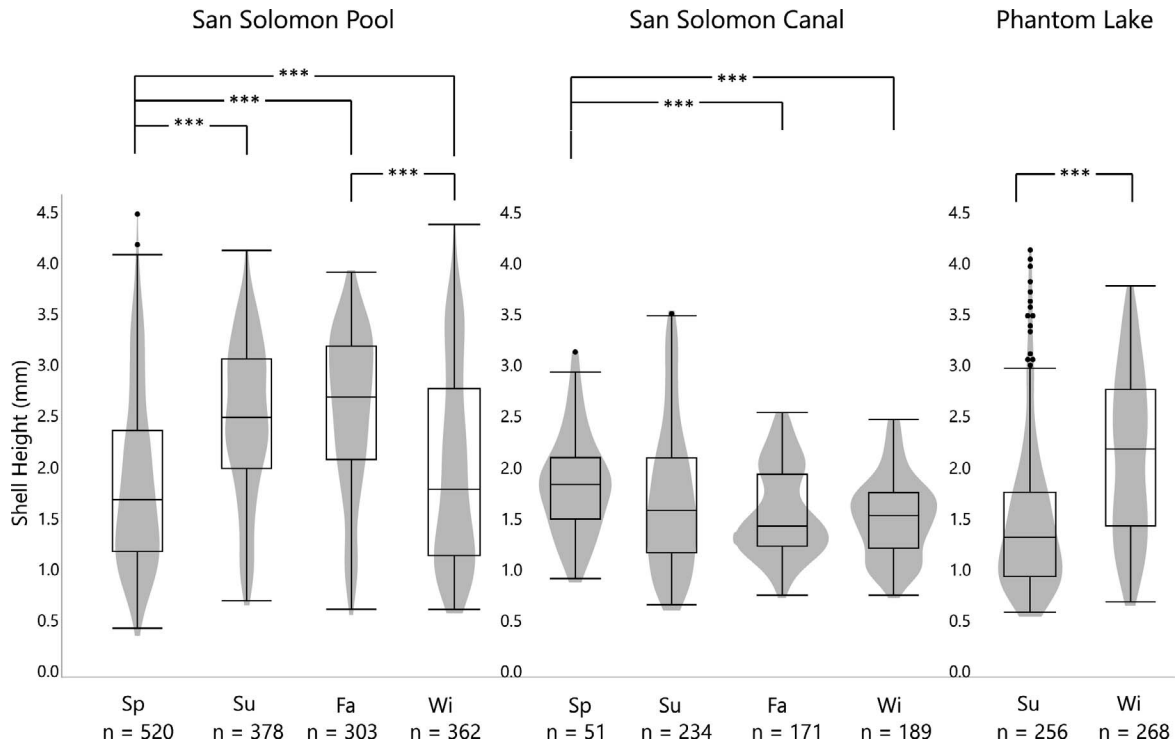


Figure 4. Comparison of size distributions of *Tyronia cheatumi* populations among seasons at three sites in the San Solomon Spring system. Data for 2017 and 2018 are combined for each season. Boxes represent the 25% and 75% quartiles, lines within boxes are the median, whiskers are $1.5 \times$ the interquartile range, and dots are outliers. Season abbreviations are as follows: Sp = spring, Su = summer, Fa = fall, and Wi = winter. All comparisons denoted by *** were significant at $P < 0.001$; comparisons without a bracket were not significant.

the site's heavy recreational use and modification as a swimming pool ($\sim 2,000$ people/d during the summer). The potential decline in density between spring and summer in both years may be related to annual drawdown and cleaning of

the pool in late spring. However, we were unable to detect statistical differences in density between spring and summer, and we do not know the extent of snail mortality that occurs coincident with pool cleaning.

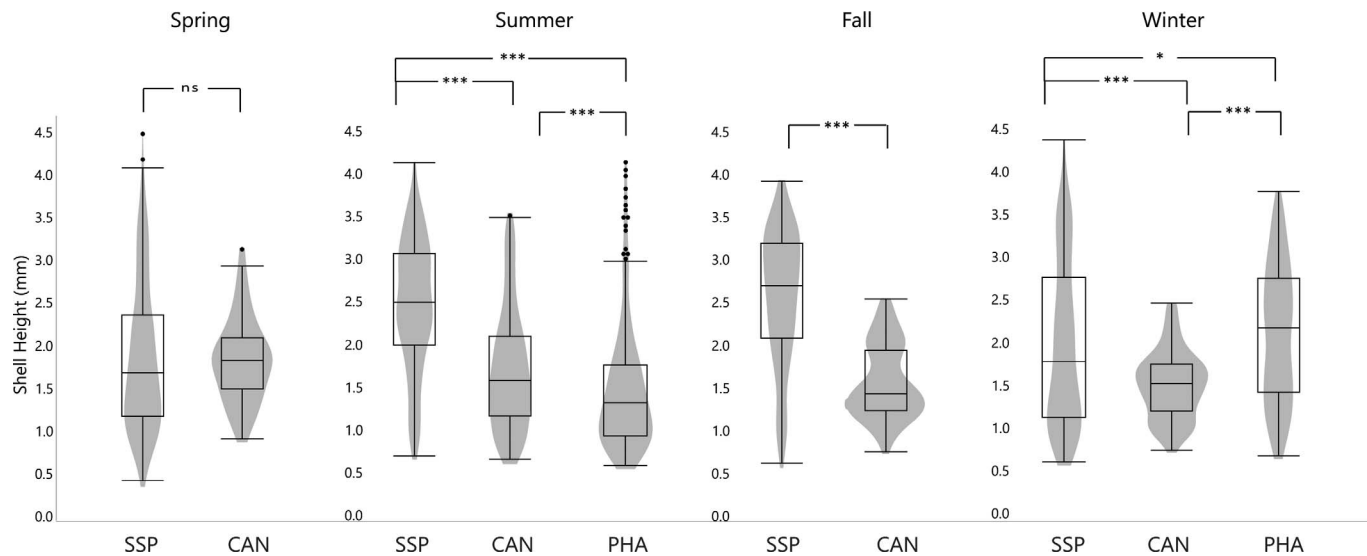


Figure 5. Comparison of size distributions of *Tyronia cheatumi* populations among three sites in the San Solomon Spring system within seasons. Boxes represent the 25% and 75% quartiles, lines within boxes are the median, whiskers are $1.5 \times$ the interquartile range, and dots are outliers. Data for 2017 and 2018 are combined for each season. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring. Significance codes: * $P < 0.05$; *** $P < 0.001$; ns, not significant.

Table 4. Mean density (individuals/m² ± SD) of *Tryonia cheatumi* at four sites in the San Solomon Spring system in summer samples from 2001 to 2018. Values from 2001, 2003, and 2009 are from Lang (2011); values from 2017 and 2018 are from this study. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, PHA = Phantom Lake Spring, and SSP = San Solomon Pool.

Site	May 2001	May 2003	June 2009	June 2017	June 2018
ES	42,206.6 ± 36,846.1	65,844.5 ± 60,961.9	4,187.6 ± 5,859.0	283 ± 1,050	67 ± 249
SSP	3,195.5 ± 910.1	— ^a	—	15,425 ± 26,782	4,530 ± 8,854
CAN	14,215.8 ± 7,434.8	348,617.7 ± 24,624.7	11,681.2 ± 11,924.9	2,116 ± 7,228	55 ± 172
PHA	47,661.5 ± 24,537.1	46,284.0 ± 51,526.7	31,462.1 ± 20,251.3	12,989 ± 21,828	2,980 ± 2,213

^aDashes indicate that no sample was taken.

There have been several prior efforts to document snail density at San Solomon Springs. Bradstreet (2012) found an average density of *T. cheatumi* of 168/m² in May 2010 and 0/m² throughout the rest of the year, values that are far lower than our estimates. However, their study sampled quadrats visually, instead of collecting and sorting benthic material. Juvenile *T. cheatumi* are < 1 mm in height and are difficult to find in underwater visual surveys, particularly in areas with vegetation. Juveniles comprised most of our samples in some seasons, and they are similar in appearance when unmagnified to juveniles of the invasive snails *Melanoidea* and *Thiara*. Because of these issues, it is difficult to compare our results with those of Bradstreet (2012).

Lang (2011) sampled San Solomon Pool, San Solomon Canal, Phantom Lake Spring, and East Sandia Spring in summers 2001, 2003, and 2009 (Table 4) by using the same benthic sampling device that we used. That study found much higher *T. cheatumi* density at East Sandia Spring, San Solomon Canal, and Phantom Lake Spring than we found in our summer samples, but Lang (2011) found much lower density at San Solomon Pool. However, Lang (2011) took only three samples per site at targeted locations with known snail presence, compared with the 10–25 randomly selected points per site that we sampled. Consequently, the values reported by Lang (2011) represent estimates only from high snail-density areas, whereas our values represent estimates of site-wide mean density. Snail density showed high spatial variation in our study, and many samples had no snails. This resulted in very high error around our estimates of mean density; in contrast, the targeted sampling of Lang (2011) produced lower (but still high) error. The much lower densities of *T. cheatumi* that we observed at East Sandia Spring and Phantom Lake Spring in 2017 and 2018 are potentially concerning. However, because of the fundamental differences in sampling design between our study and that of Lang (2011), it is impossible to conclude whether *T. cheatumi* density has changed at any sites since 2001–2009. Sampling approaches such as stratified sampling are needed to account for the high spatial variability of *T. cheatumi* and to provide more precise density estimates needed to detect temporal changes in abundance. Furthermore, the high and unpredictable seasonal variation we observed means that single, annual samples may be insufficient for monitoring long-term changes in density.

Discharge from San Solomon Spring has not changed appreciably between 2009 and the present (U.S. Geological Survey Gage 08427500; <https://waterdata.usgs.gov/tx/nwis/rt>, accessed March 9, 2022). Although all habitats that support *T. cheatumi* are influenced by the same groundwater source, we do not know whether flows have changed at East Sandia and Phantom Lake springs and whether flow may be related to the potential decline of the species at those sites. Recent changes in the area have the potential to affect *T. cheatumi* populations. Oil and gas extraction has boomed in the past 10 yr, but the effects of this activity on groundwater flow in the San Solomon Spring system are unknown. Two sites that support large populations of *T. cheatumi*—San Solomon Pool and Phantom Lake Spring—have undergone major reconstruction in the past few years. Repair of the pool at San Solomon Springs (2018 and 2020) and construction of a cienega at Phantom Lake Springs (2010) have increased available habitat for *T. cheatumi*, but we do not know whether these changes are associated with changes in snail population size.

Our study partially supported the hypothesis of continuous, aseasonal reproduction, which is expected in thermally stable habitats (Winemiller 1989). As expected by this hypothesis, we found no relationship between snail size and water temperature, size structure showed no obvious seasonal pattern, and juvenile individuals were present in most samples in all seasons. However, size differed significantly among most seasons and sites, suggesting that other, unknown factors have some influence on reproductive cycles. A more detailed analysis of population dynamics, including estimation of seasonal patterns of individual growth and survival, could help explain the seasonal and spatial variation that we observed in *T. cheatumi*. A better understanding of the factors that influence reproduction and population size is needed for effective conservation of *T. cheatumi*.

ACKNOWLEDGMENTS

Specimens were collected under Texas Parks and Wildlife Department permit SPR-0111-003 and U.S. Fish and Wildlife Service permit TE676811-9. The U.S. Bureau of Reclamation, Dustin Armstrong, Pete Diaz, Randy Gibson, and the University of Texas Rio Grande Valley College of Sciences assisted with various aspects of the study. Dominique Alvear

assisted with sample curation. The suggestions of editors and reviewers for *Freshwater Mollusk Biology and Conservation* improved the manuscript substantially.

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