Freshwater Mollusk Biology and Conservation Population density and reproductive seasonality of Tryonia cheatumi (Gastropoda; Cochliopidae), the Phantom Tryonia --Manuscript Draft--

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Abstract:	We studied population density and reproductive seasonality of the Phantom Tryonia, Tryonia cheatumi (Pilsbry, 1935), an endangered freshwater snail found in the San Solomon Spring system, a "cienega", or karst-based, aridland freshwater spring system, in western Texas, USA. We sampled seven populations seasonally over a two-year period. Tryonia cheatumi densities were highest in the spring seasons at San Solomon Pool (up to 23,626 ± 39,030 m -2). San Solomon Pool, the main swimming pool at Balmorhea State Park, also has the largest population of T. cheatumi estimated at 57 million individuals (in 2017). There were large interannual differences in density (up to 25-fold) at all sites, but consistent seasonal patterns of density were not observed. Change in temperature did not appear to drive the appearance of juveniles. Instead, in all populations, there appears to be reproduction throughout the year, but more juveniles were observed in some seasons. More juveniles were observed at San Solomon Pool in winter and spring samples, but more juveniles were observed in summer at Phantom Lake Spring.			

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REGULAR ARTICLE

Running head: Life history of Tryonia cheatumi

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

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ABSTRACT

2	We studied population density, population size, and reproductive seasonality of the
3	Phantom Tryonia, Tryonia cheatumi (Pilsbry, 1935). This endangered freshwater snail is
4	found only in the San Solomon Spring system, a cienega, or karst-based, arid-land
5	freshwater spring system, in western Texas, USA. We sampled populations at seven
6	locations in the system seasonally over a two-year period. San Solomon Spring, the
7	system's largest spring and modified into a swimming pool, had the largest population of T.
8	<i>cheatumi</i> with an estimated 49 million individuals and density as high as $23,626 \pm$
9	39,030/m ² . There were seasonal differences in density (up to 25-fold) and median snail size
10	at all sites, but consistent seasonal patterns of density or size were not observed. Median
11	snail size among samples was not related to water temperature, and juveniles were present
12	in most samples in all seasons. These results support continuous, aseasonal reproduction, as
13	expected in thermally stable habitats, but differences in median size and density among
14	seasons and sites suggest that other factors affect reproduction and seasonal variation in
15	population size of <i>T. cheatum</i> i.
16	

KEY WORDS - spring snails, desert springs, life history, snail reproduction

18 INTRODUCTION

19 Many aquatic snails in arid regions are narrowly endemic, usually restricted to one or a 20 few nearby springs, and these species often are of conservation concern (Lydeard et al. 2004; 21 Hershler et al. 2014). The spring snails *Tryonia* are characteristic of mineral and thermal (hot or 22 warm) springs in the southwestern United States and Mexico, and most are restricted to a single 23 spring or spring system (Hershler 2001; Hershler et al. 2011; Hershler et al. 2014). Tryonia are 24 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 25 locally dominant members of the invertebrate community (Meffe and Marsh 1983; Hershler 26 27 2001). However, Tryonia typically occupy narrower microhabitat niches than other springdwelling snails (Sada 2008), limiting their spatial distribution and total population size. 28 Furthermore, population size of *Tryonia* and related genera can vary annually, seasonally, or 29 spatially (Taylor 1983; Lang 2001; Brown et al. 2008; Lang 2011; Johnson et al. 2019). 30 Fishes inhabiting thermally stable spring systems are expected to have largely aseasonal 31 32 population dynamics, such as continuous or aseasonal reproduction (Winemiller 1989), but 33 freshwater snails may deviate from this pattern (Whelan and Strong 2014). For example, spring snails in the genus *Pyrgulopsis* have well-defined, population-specific reproductive seasons that 34 35 correspond to small, seasonal differences in water temperature (e.g. Mladenka and Minshall 36 2001; Lysne et al. 2007). Aseasonal or continuous reproduction is proposed for *Tryonia* in warm, 37 thermally stable springs, but seasonal reproduction is proposed in cooler, more thermally 38 variable spring systems (Taylor 1983; Brown et al. 2008). However, these conclusions were 39 based on limited evidence, and detailed life history information is available for few species in 40 this group (e.g., Sada 2008). Critically, seasonal temperature variability has not been assessed in

41 springs that support *Tryonia*, and temperature is potentially a primary driver of reproduction
42 (Brown et al. 2008).

43 The endemic spring snails of Texas include five species of *Tryonia* (Hershler 2001; 44 Hershler and Liu 2017), all of which are critically imperiled (G1) under NatureServe criteria 45 (Faber-Langendoen et al. 2012; Johnson et al. 2013). Tryonia cheatumi, the Phantom Tryonia, is 46 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 47 48 Desert ecoregion (Allan 2011; Lang 2011). All known populations are restricted to a 13-km-long 49 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 50 51 Solomon Springs system are declining over the long term due to groundwater extraction for agriculture, and concern exists regarding potential effects from non-conventional oil and gas 52 development in the region (Texas Water Development Board 2005). Limited sampling of 53 *Tryonia cheatumi* from 2001-2009 found high densities (thousands/m²) at Phantom Lake, San 54 55 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 56 57 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 two years. 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.

63 METHODS

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65 Study Area

66 San Solomon Springs is an artesian spring system consisting of three main areas, all of 67 which appear to arise from the same groundwater sources (Chowdhury et al. 2004). The main 68 spring, San Solomon Spring (water source in San Solomon Pool), within Balmorhea State Park, has been modified into a partially concrete-lined swimming pool, which discharges into the 69 concrete-lined San Solomon Canal, which in turn feeds two restored wetland areas, San Solomon 70 Cienega and Hubbs Cienega. Giffin Spring is on private land about 0.2 km northwest of San 71 72 Solomon Spring (Lang 2011). The system includes two additional spring areas that support T. cheatumi. Phantom Lake Spring, about 2.4 km southwest of the state park, is upgradient from the 73 74 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 75 canal and wetland by a pump located inside the cave. East and West Sandia springs, near the 76 77 town of Balmorhea, are downgradient of the main spring system and located on property owned 78 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 79 80 snails were found (J.J. Landye pers. comm.). However, West Sandia Spring was flowing during 81 our study. All springs in the system have been connected intermittently via a series of irrigation 82 canals since the 1940s, but it is unknown if *T. cheatumi* occurs in the canals.

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84 Field Sampling

85	We sampled <i>T. cheatumi</i> and water temperature at seven sites within the San Solomon
86	Spring system (Fig. 1): East Sandia Spring (30.990978, -103.729036; 603 m ² surface area), West
87	Sandia Spring (30.986838, -103.73635; 339 m ²), San Solomon Pool (30.944279, -103.788395;
88	5,556 m ²), San Solomon Canal (30.944538, -103.785917; 2,859 m ²), Hubbs Cienega (30.945479,
89	-103.786001; 1,050 m ²), San Solomon Cienega (30.945138, -103.784405; 4,340 m ²), and
90	Phantom Lake Spring (30.935005, -103.849613; 120 m ²). These sites encompass all known
91	populations of <i>T. cheatumi</i> except Giffin Spring, which we were unable to sample. We sampled
92	most sites in spring (March), summer (June), fall (October), and winter (December) in 2017 and
93	2018 (Table 1), except for the fall 2018 sample at San Solomon Pool, which was taken in
94	August. Due to limited access to the site, we did not sample Phantom Lake Spring in the fall.
95	At each site on each sampling event, we estimated <i>T</i> : <i>cheatumi</i> density at 10 to 25
96	randomly generated points distributed among mesohabitat types found at the sites. Prior to
97	sampling, we delineated broad mesohabitat types based on benthic substrate composition and
98	macrophyte cover, and we estimated the area of each mesohabitat with a hand-held Trimble GPS
99	unit. Based on our delineation, there were one to three mesohabitat types at each site. The
100	number of sampling points at each site was dependent on the size of the site and the number of
101	mesohabitat types present, and we allocated the number of samples in each mesohabitat
102	proportional to the area of the mesohabitat at the site on the date of sampling.
103	On each sampling date and prior to sampling for <i>T. cheatumi</i> , we measured water
104	temperature immediately above the bottom at each sampling point using a pre- and post-
105	calibrated Eureka Manta multiprobe (Eureka Water Probes, Austin, TX). We then collected
106	benthic material (containing <i>T. cheatumi</i>) at each point with a 100 -cm ² benthic basket sampler
107	designed for quantitative sampling of spring snail populations (Lang sampler, 10 cm x 10 cm x 3

108	cm, 500 μ m mesh; Lang 1999, 2001; Johnson et al. 2019). The number of samples taken on each
109	date at each site was as follows: East Sandia Spring, $N = 10-16$; West Sandia Spring, $N = 10-11$;
110	San Solomon Pool, $N = 18-20$; San Solomon Canal, $N = 15-25$; Hubbs Cienega, $N = 5-6$; San
111	Solomon Cienega, $N = 14-16$; Phantom Lake Spring, $N = 5-20$. We preserved samples in the
112	field in 95% ethanol and later sorted, counted, and identified the contents under a dissecting
113	microscope at the US Fish and Wildlife Service San Marcos Aquatic Resource Center and the
114	Aquatic Ecology Laboratory in the Freeman Aquatic Station at Texas State University. Samples
115	contained two invasive snail species, Melanoides tuberculata and Thiara granifera, which we
116	distinguished from <i>T. cheatumi</i> by shell coloration and sculpture.
117	We obtained samples of <i>T. cheatumi</i> large enough for size structure analysis (>50
118	individuals) from three sites, San Solomon Pool ($N = 1,260$), San Solomon Canal ($N = 645$), and
119	Phantom Lake Spring ($N = 524$). We measured shell height (maximum shell height parallel to the
120	axis of coiling) with a stereoscopic microscope and attached Luminera Infinity-1 camera
121	(Teledyne Lumenera, Ottawa, Canada). Infinity Analyze was used to calibrate measurement
122	using a stage micrometer (Meiji Techno America, San Jose, CA, 1 mm with 0.01 mm divisions).
123	Each shell was placed on clay in the same orientation for measurement, conforming to a
124	standardized shell photography guide (Callomon 2019).

126 Data Analysis

We tested for differences in median water temperature across seasons at each site using a
Kruskal-Wallis test for independent samples and pair-wise comparisons (2-sided tests), due to
heterogenous variance and non-normal error structure. Analyses were conducted in SPSS

130 (Version 27). We tested for differences in the variance around median water temperature among131 sites with Levene's test (Zar 1999).

132 We examined if *T. cheatumi* density differed across sites or seasons using a generalized 133 linear model (GzLM). We used GzLM because our data contained many zero observations, 134 exhibited substantial non-normal error structure, and had heteroscedastic variance (Maindonald 135 and Braun 2007; O'Hara and Kotze 2010). We modeled density using a negative binomial 136 distribution and a log link function. Site (San Solomon Canal, East Sandia Spring, Hubbs 137 Cienega, Phantom Lake Spring, San Solomon Cienega, San Solomon Pool, and West Sandia 138 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 139 140 overall model significance by comparing the fitted model to an intercept-only model, and pairwise post hoc comparisons among sites and seasons was performed with a sequential Bonferroni 141 procedure. These analyses were performed in R version 3.6.3. 142

To assess the relationship between reproduction of snails and water temperature, we 143 examined two-tailed Pearson correlation coefficients (R, version 3.6.3) between median shell 144 145 height of snails at each site on each sampling date and mean water temperature measured at that 146 site. Shell height was not normally distributed in most samples (Shapiro-Wilk Test, W =147 0.860856-0.979127, P = 0.4063-<0.001). We tested for differences in shell size among sites 148 and seasons using Kruskal-Wallis tests followed by comparison of each pair with the Wilcoxon 149 method including a Bonferroni correction for multiple comparisons when needed. These analyses 150 were conducted in JMP Pro 15.0.0 (SAS Institute).

152 **RESULTS**

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154 **Density and Population Size**

155 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 156 East and West Sandia springs did not differ from each other (P = 0.703), but both had 157 consistently lower temperatures (~20.5°C) throughout the study period than the other sites (P <158 0.001 for all comparisons). San Solomon Cienega had lower water temperatures than San 159 160 Solomon Pool and San Solomon Canal, and Phantom Lake Spring had slightly lower temperatures than San Solomon Canal (P < 0.001 for all comparisons). 161 Water temperatures varied seasonally across sites (Kruskal-Wallis test: $H_3 = 184.94$; P <162 163 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 164 lower than those in the summer and fall (P < 0.001 for both comparisons); and fall temperatures 165 166 were lower than those in the summer (P < 0.001). Seasonal variation in water temperatures

167 differed among sites (Levene's statistic = 9.32; df = 6, 46; P < 0.001), with San Solomon

168 Cienega having the greatest seasonal variation in water temperature, differing >9°C between the

169 winter minimum and the summer maximum (Figure 2a). All other sites showed limited variation

170 in water temperature across seasons, with a range of variation of <4°C annually.

Density of *T. cheatumi* differed significantly among sites (Table 1). The highest density
of *T. cheatumi* was observed in San Solomon Pool (mean density over all sampling events =

8,976 individuals/ $m^2 \pm 19,900$ SD; Table 2, Fig. 3). Phantom Lake Spring also had high mean 173 174 density $(7,438/m^2 \pm 12,963 \text{ SD})$, but mean density at this site did not differ significantly from the 175 other sites except San Solomon Pool due to extremely high seasonal variation. We observed T. 176 *cheatumi* at West Sandia Spring in only one sample, where it was present at low density (spring 2018, $9/m^2 \pm 29$ SD; mean density across sampling events = $1/m^2 \pm 12$). Mean density at the 177 other four sites ranged from $7-2,099/m^2$. Mean estimates of density appeared to vary widely 178 179 among seasons at all sites, particularly San Solomon Pool and Phantom Lake Spring, but confidence intervals around these estimates overlapped widely, and season was not a significant 180 factor in the GzLM model (Table 2). Population size appeared to vary annually at some sites, but 181 182 confidence intervals 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). 183

The two invasive snail species were present at all study sites except East and West Sandia 184 springs. At Phantom Lake Spring, *Terebia* mean density was $175/m^2 \pm 228$ SD and *Melanoides* 185 density was $4,793/m^2 \pm 6,397$ SD. At San Solomon Pool, *Terebia* mean density was $3,846/m^2 \pm$ 186 766 SD and *Melanoides* density was $1,593/m^2 \pm 1,262$ SD. At San Solomon Canal, *Terebia* mean 187 density was $15.445/m^2 \pm 6.482$ SD and *Melanoides* density was $1.245/m^2 \pm 834$ SD. At San 188 Solomon Cienega, *Terebia* mean density was $19,504/m^2 \pm 25,203$ SD and *Melanoides* density 189 190 was $8.495/m^2 \pm 6.989$ SD. At Hubbs Cienega, *Terebia* mean density was $9.535/m^2 \pm 12.102$ SD 191 and *Melanoides* density was $12,060/m^2 \pm 5,809$ SD.

San Solomon Pool had the highest estimated population size of *T. cheatumi* with 49,477,642 individuals (25,520,917–73,434,367, 95% CI) (Table 3), followed by San Solomon Canal, Phantom Lake Spring, Hubbs Cienega, San Solomon Cienega, and East Sandia Spring. Estimated population size at West Sandia Spring was small (478 individuals, 0–1,413).

197 Size Structure

198 Snail size differed among seasons at all three sites where we examined size structure 199 (Fig. 4). There was a significant difference in shell height among seasons at San Solomon Pool 200 (Kruskal-Wallis test: $\gamma 2 = 183.2108$, df = 3, P < 0.0001; Wilcoxon paired comparisons: P < 0.0001201 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 202 significant difference in shell height among seasons at San Solomon Canal (Kruskal-Wallis test: 203 $\gamma 2 = 16.7944$, df = 3, $P = \langle 0.0001;$ Wilcoxon paired comparisons; $P = \langle 0.0001 - 0.0005 \rangle$; 204 median shell height was highest in the spring (1.90 mm) and lowest in the fall (1.49 mm). Two 205 of six pairwise comparisons were significantly different. There was a significant difference in 206 shell height between summer and winter at Phantom Lake (Kruskal-Wallis test: $\chi 2 = 84.6952$, df 207 = 3, P < 0.0001); median shell height was higher in the winter (2.27 mm) than in summer (1.41 208 209 mm).

Shell height differed among sites in all seasons except spring (Fig. 5). Median shell 210 211 height did not differ between San Solomon Pool and San Solomon Canal in the spring (Kruskal-212 Wallis test: $\chi 2 = 0.6710$, df = 1, P = 0.4127), but size appeared to be more variable in San 213 Solomon Pool. There was a significant difference in shell height among all three sites in the 214 summer (Kruskal-Wallis test: $\gamma 2 = 236.9808$, df = 2, P < 0.0001, all pairwise comparisons 215 significantly different); median shell height was highest in San Solomon Pool (2.57 mm) and 216 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: $\gamma 2 = 160.8383$, df = 217 218 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).

225

226 **DISCUSSION**

We found substantial populations of *T. cheatumi* at San Solomon Springs and Phantom 227 Lake Spring and smaller populations at the other sites. We found large changes in density (up to 228 25-fold) of *T. cheatumi* across years and sampling periods. San Solomon Pool had high densities 229 in both spring seasons, summer 2017 and winter 2018, and lower densities in winter 2017, 230 summer 2018, and both fall seasons. At Phantom Lake Spring, peak densities occurred in 231 summer 2017 and winter 2018. San Solomon Spring supports the largest population of T. 232 cheatumi in the spring system despite the site's heavy recreational use and modification as a 233 swimming pool (~2000 people/day during the summer). The potential decline in density between 234 235 spring and summer in both years may be related to annual draw-down and cleaning of the pool in 236 late spring. However, we were unable to detect statistical differences in density between spring 237 and summer, and we do not know the extent of snail mortality that occurs coincident with pool 238 cleaning.

There have been several prior efforts to document snail density at San Solomon Springs. Bradstreet (2012) found an average density of *T. cheatumi* of $168/m^2$ in May 2010 and $0/m^2$ throughout the rest of the year, values that are far lower than our estimates. However, that 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 *Melanoides* and *Thiara*. Because of these issues, it is difficult to compare our results to those of Bradstreet
(2012).

Lang (2011) sampled San Solomon Pool, San Solomon Canal, Phantom Lake Spring, and 248 East Sandia Spring in summer 2001, 2003, and 2009 (Table 4) using the same benthic sampling 249 device that we used. That study found much higher T. cheatumi density at East Sandia Spring, 250 San Solomon Canal, and Phantom Lake Spring than we found in our summer samples, but Lang 251 252 (2011) found much lower density at San Solomon Pool. However, Lang (2011) took only three samples/site at targeted locations with known snail presence, compared with the 10-25 randomly 253 selected points/site that we sampled. Consequently, the values reported by Lang (2011) represent 254 255 estimates only from high snail-density areas, while our values represent estimates of site-wide 256 mean density. Snail density showed high spatial variation in our study, and many samples had no 257 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 258 259 of T. cheatumi we observed at East Sandia Spring and Phantom Lake Spring in 2017 and 2018 260 are potentially concerning. However, because of the fundamental differences in sampling design 261 between our study and Lang's (2011), it is impossible to conclude whether T. cheatumi density 262 has changed at any sites since 2001–2009. Sampling approaches such as stratified sampling are 263 needed to account for the high spatial variability of *T. cheatumi* and to provide more precise 264 density estimates needed to detect temporal changes in abundance. Furthermore, the high and

unpredictable seasonal variation we observed means that single, annual samples may beinsufficient for monitoring long-term changes in density.

267 Discharge from San Solomon Spring has not changed appreciably between 2009 and the 268 present (USGS Gage 08427500, https://waterdata.usgs.gov/tx/nwis/rt, accessed March 9, 2022). 269 Although all habitats that support T. cheatumi are influenced by the same groundwater source, 270 we do not know if flows have changed at East Sandia and Phantom Lake springs and whether 271 flow may be related to the potential decline of the species at those sites. Recent changes in the 272 area have the potential to affect T. cheatumi populations. Oil and gas extraction has boomed in 273 the last 10 years, 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 274 275 and Phantom Lake Spring) have undergone major reconstruction in the last few years. Repair of the pool at San Solomon Springs (2018 and 2020) and construction of a cienega at Phantom Lake 276 Springs (2010) have increased available habitat for T. cheatumi, but we do not know if these 277 changes are associated with changes in snail population size. 278

Our study partially supported the hypothesis of continuous, aseasonal reproduction, 279 280 which is expected in thermally stable habitats (Winemiller 1989). As expected by this 281 hypothesis, we found no relationship between snail size and water temperature, size structure 282 showed no obvious seasonal pattern, and juvenile individuals were present in most samples in all 283 seasons. However, mean size differed significantly among most seasons and sites, which 284 suggests that other, unknown factors have some influence on reproductive cycles. A more 285 detailed analysis of population dynamics, including estimation of seasonal patterns of individual 286 growth and survival, could help explain the seasonal and spatial variation 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*.

289

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297

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- **383 FIGURE LEGENDS**
- 384
- Figure 1. Map showing the location of sampling sites in the San Solomon Spring system
- 386 (imagery from USGS 2013). Inset shows the location of the study area in Texas, USA.
- 387 Figure 2. Box-and-whisker plots showing variation in water temperature among (a) sites and (b)
- 388 seasons in the San Solomon Spring system. Boxes represent the 25% and 75% quartiles, lines
- 389 within boxes are the median, whiskers are $1.5 \times$ the interquartile range, and dots are outliers. Site
- 390 abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs
- 391 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,
- 393 Fa = fall, and Wi = winter.
- 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 pair-wise 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.
- 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.
 - 20

- 406 Figure 5. Comparison of size distributions of *Tyronia cheatumi* populations among three sites in
- 407 the San Solomon Spring system within seasons. Boxes represent the 25% and 75% quartiles,
- 408 lines within boxes are the median, whiskers are $1.5 \times$ the interquartile range, and dots are
- 409 outliers. Data for 2017 and 2018 are combined for each season. Site abbreviations are as follows:
- 410 CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom
- 411 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. 412

413 **Tables**

414

- 415 Table 1. Parameter estimates for the generalized linear model examining the effect of site and season on density of *Tryonia cheatumi*
- 416 at seven sites in the San Solomon Spring system. b is the slope and W_T is the Wald c2 test statistic. Degrees of freedom for all
- 417 parameters = 1. Redundant parameters were set to zero and are not included. Site abbreviations are as follows: CAN = San Solomon
- 418 Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC San Solomon Cienega, SSP = San
- 419 Solomon Pool, and WS = West Sandia Spring.

Parameter	b	SE	95% CI	W_T	Р	
Intercept	0.04	1.00	-1.91 - 1.99	0.002	0.966	J.
CAN	6.65	1.02	4.65 - 8.66	42.34	< 0.001	
ES	5.78	1.13	3.56 - 8.00	26.04	< 0.001	
HC	6.23	1.09	4.10 - 8.37	32.71	< 0.001	XX
PHA	8.67	1.08	6.56 - 10.78	64.75	< 0.001	
SSC	2.40	1.10	0.20 - 4.57	4.74	0.03	
SSP	8.80	1.06	6.72 - 10.87	68.90	< 0.001	
Fall	0.76	0.64	-0.48 - 2.00	1.45	0.23	
Spring	0.23	0.45	-0.65 - 1.12	0.27	0.60	
Summer	0.42	0.44	-0.45 - 1.29	0.88	0.35	
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420

424 Table 2. Mean density (individuals/ $m^2 \pm SD$) of *Tryonia cheatumi* at seven sites in the San Solomon Spring system. Site abbreviations

425 are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San

426 Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

						X		
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	_	0 ± 0	0 ± 0	0 ± 0	9 ± 29	0 ± 0	0 ± 0	0 ± 0
SSP	$23,\!626 \pm 39,\!030$	$15,\!425\pm26,\!782$	789 ± 976	$1,505 \pm 2,367$	$15,512 \pm 47,354$	$4{,}530 \pm 8{,}854$	$1{,}200\pm2{,}781$	$9,221 \pm 31,056$
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	$1,000 \pm 1,501$	$1,\!020\pm1,\!235$	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	$2,\!980 \pm 2,\!213$	_	$23,367 \pm 46,780$
Uncorre								

431 Table 3. Population size estimates (95% confidence interval) for *Tryonia cheatumi* at seven sites in the San Solomon Spring system in

- 432 2017 and 2018. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA =
- 433 Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.
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Site	2017	2018	Mean across both years	435
ES	129,533 (1,155–257,912)	639,611 (0–1,867,644)	389,209 (0–1,016,600)	436
WS	0 ()	827 (0–2447)	478 (0–1413)	100
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–	437
CAN	6.154.181 (712.138–11.596.224)	320.672 (51.342–590.001)	3.333.041 (559.177–6.106.905	430)
HC	186,136 (25,389–346,884)	1,170,750 (15,867–2,325,633)	655,000 (85,865–1,224,135)	´439
SSC	31,000 (0-78,772)	131,750 (0–315,577)	81,375 (0-176,376)	440
PHA	686,448 (69,342–1,303,554)	925,020 (0–130,3554)	792,480 (115,830–1,469,130)	441
	Ś	Correcte		

443 Table 4. Mean density (individuals/m² \pm 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	$42,\!206.6\pm36,\!846.1$	$65{,}844.5 \pm 60{,}961.9$	$4,187.6 \pm 5,859.0$	283 ± 1050	67 ± 249
	SSP	$3,195.5 \pm 910.1$		-	$15,\!425\pm26,\!782$	$4{,}530 \pm 8{,}854$
	CAN	$14,\!215.8\pm7,\!434.8$	$348,\!617.7\pm24,\!624.7$	$11,681.2 \pm 11,924.9$	$2,116 \pm 7,228$	55 ± 172
	PHA	$47,\!661.5\pm24,\!537.1$	$46{,}284.0 \pm 51{,}526.7$	$31,462.1 \pm 20,251.3$	$12,\!989 \pm 21,\!828$	$2,980 \pm 2,213$
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448				XX		
449				20		
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