VARIATION IN MINIMUM TEMPERATION TOLERANCE OF

TWO INVASIVE SNAILS IN CENTRAL

TEXAS, USA

by

Linus Ray Delices, B.A.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Major in Biology
August 2018

Committee Members:

Astrid Schwalb

Weston Nowlin

Benjamin Schwartz

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DEDICATION

This thesis is lovingly dedicated to my parents: Vina Delices and Albert Donaie and to my cousin Tracy Donaie who have supported me all the way since the beginning of my studies. Furthermore, this thesis is dedicated to my best friend Kahla St. Mathe and my loving friend and sister Whitney I. S. Henry who has been a great source of motivation and inspiration. Finally, this thesis is dedicated to all those who believe in the richness of learning and helping students like myself in achieving scientific knowledge.

ACKNOWLEDGEMENTS

Special thanks to the chair of my committee, Dr. Astrid Schwalb, you will always be someone I am thankful to and for taking a chance on me. It goes without saying that the most important person in shaping the scientific abilities of a young graduate student is his adviser, and to have an amazing scientist such as Dr. Astrid Schwalb has been a true honor and privilege for her exquisite attention to detail and for her demand for excellence. I am most grateful to the members of my committee, Dr. Weston Nowlin and Dr. Benjamin Schwartz for their time, encouragement, and expertise throughout this project. There are people in everyone lives who make success both possible and rewarding. So of course, nothing that I have every achieved was without the moral support of the few closest to me, my family and friends. My family has always been the pillar of security in my life. The ones I know will always be there, my parents Vina Delices and Albert Donaie for their encouraging words, love, prayers and countless wakeup calls, kept me on time for exams, classes, appointments and the occasional eat something young man. My sisters Amanda Donaie, Norvella Poleon for always checking on me. My cousin Dwayne Donaie and uncle Gilroy Donaie who steadfastly supported and reinvigorated me. In addition, thank you to Whitney Henry, and Kahla St. Mathe for your incredible friendship, honesty, encouraging words and support throughout this journey. Finally, special thanks to Zachary Mitchell for your time, knowledge, and keeneye in reviewing this document.

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ABSTRACT

Invasive species are a major concern for aquatic ecosystems and tropical freshwater snails (Thiaridae) can be very successful invaders. *Melanoides tuberculata* and *Tarebia granifera* are two invasive snails in Central Texas that serve as intermediate hosts for several Asiatic trematode parasites of fishes, birds, and other organisms including domestic animals and humans. A better understanding of their temperature tolerances is needed to better predict their spread in Texas and to inform management strategies. Therefore, the goal of my study was to determine the critical thermal minimum of these species and to compare temperature tolerances between species, rivers, and different local morphotype. Survival of snails were monitored in environmental tanks in which temperature was decreased by 0.1°C per hour from 23°C to 10°C. In addition, survival was monitored over time in environmental tanks in which temperature was held constant at 17°C, 15°C, 11°C and 10°C after acclimatization. Temperature tolerances differed significantly between snails from different rivers or river segments. There was no significant difference in survival at colder temperatures between *M. tuberculata* and *T. granifera*, nor between local morphotypes of *M. tuberculata* found in Central Texas. My results show that *M. tuberculata* can tolerate colder temperatures down to 11ºC for a few weeks, which will facilitate their dispersal in rivers of Central Texas farther away from thermally stable spring influenced reaches. Future research should examine differences in temperature tolerances between rivers in central Texas and further examine potential role of local adaptation of *M. tuberculata.*

I. INTRODUCTION

There has been a dramatic increase in the introduction of exotic species during the 20th Century into North America, as a result of international trade and travel compared to the 19th century (U.S. Congress, Lacey Act of 1900). In the United States at least 50,000 exotic species have been reported (Pimentel et al., 2005); and a proportion of these exotic species have become invasive (Corn et. al, 1999, i.e., large populations leading to pronounced changes in community composition and ecosystem functions; Chapin et al., 1997), because they are virtually unrestrained without their native predators, competitors, and diseases, and increasing ecological release (Ciruna et al., 2004, Karatayev et al., 2009, Pimentel et al., 2005, Johnson et al., 2009). These invasive species are responsible for causing major economic losses, costing billions (i.e., in 2005 it was reported at $5.4 billion annually USD; Pimentel 2005). In addition, invasive species can simply become a nuisance in these new and novel environments or ecosystems.

Thiarid snails are often successful invaders and are most frequently introduced through the aquarium trade (Padilla & Williams, 2004). In Texas, five invasive snail species are known to have reproducing populations, representing at least 50% of the known exotic invertebrates occurring in the freshwaters of the state (Karatayev et al., 2009). *Melanoides tuberculata* (Müller, 1774) (Fig. 1) and *Tarebia granifera* (Lamarck, 1822) (Fig. 2) are two invasive sub-tropical aquatic snails found in abundance in Texas. *M. tuberculata* species has invaded at least 24 waterbodies in 15 counties in Texas, (Karatayev et al., 2009). In addition, the snails have largely invaded spring-influenced habitats which contain relatively high proportions of endemic and often imperiled native species, i.e., *Pyrgulopsis texana* native snail species found in springs of Texas has become an imperiled group with 74% of species in the Unites States at risk for extinction (Thorp and Covich, 2009), and *Lyrodes cheatumi*, and *Assiminea pecos* are both imperiled (Rogowski et al., 2012). The invasive aquatic snails serve as intermediate host for several Asiatic trematode parasites of fishes, birds, and humans (Mitchell et al., 2000, Tolley-Jordan & Owen, 2008, Pinto and de Melo, 2011, Chen, 1942), and has wreaked havoc with the endangered fountain darter (*Etheostoma fonticola*), in the Comal River since it was introduced, in the early 90's, with their host-parasites that brought its exclusive first intermediate host, *M. tuberculata*, instant infamy in Central Texas (Mitchell et al., 2005).

M. tuberculata originated from Africa, Asia, and Australia (Facon et al., 2003), and *T. granifera* from India, and south-eastern Asia, (Abbott, 1952). *T. granifera* has a recorded distribution wider than *M. tuberculata* across the world, including many of the African countries (Appleton & Nadasan, 2002). Distribution surveys after the 1950s revealed that *M. tuberculata* had invaded regions from their original geographic distribution to sites in South America, the Caribbean, and North America (Facon et al. 2003, Benson and Neilson 2015). It is speculated that *M. tuberculata* invaded, North America sometime prior to 1950 (Karatayev et al., 2009, Murray, 1971), and *T. granifera* invaded as early as the 1935 (Abbott, 1952, Murray & Woopschall, 1965), in southern and western states such as Louisiana (Dundee & Paine, 1977), Florida (Abbott, 1952), Arizona, and Oregon (Murray, 1971).

Females of the viviparous *M. tuberculata* reproduce by apomictic parthenogenesis (Jacob, 1959, Pointier et al., 1993). Parthenogenetic reproduction emerges when a female thiarid produces several unfertilized embryos in a brood pouch above the mantle which develop into viable clones (Livshits et al., 1984). A single female can establish a self-sustaining colony or morph of one genotypic clone in a novel environment in just a few years (Freitas et al., 1987). Females can harbor 10 to 200 young snails after reaching sexual maturity (Berry & Kadri, 1974, Livshits & Fishelson, 1983). The thiarids can also engage in sexual reproduction, resulting in an increase in genetic variation (Facon et al., 2005), although males are usually rare or nonexistent in most populations (Jacob, 1957, Livshits et al., 1984). The operculum in thiarid is an additional mechanism contributing to their invasiveness because it allows snails to survive unfavorable conditions by tightly sealing soft tissues in the shell and serving as a door-like structure. Desiccation tolerance studies show that *T. granifera* survived up to two days in a mud substrate (dehydrated), and *M. tuberculata* survived up to 20 days in an empty tray, (i.e., dewatered completely, Dudgeon, 1982). The study similarly revealed that *M. tuberculata* can survive other chemical treatments that would quickly kill non-operculate snails and the authors found that *M. tuberculata* could survive in undiluted household bleach for 60 minutes (Dudgeon, 1982).

M. tuberculata have a distinct elongated and conical shell with several whorls usually, 6 to 12 and may be as long as 80 mm. While, *T. granifera* has a more robust and conical shell with 6 to 8 whorls (usually), and both snails are highly morphologically variable. *M. tuberculata* exhibits a broad range of shell (phenotypic) variation amongst distinct genetic clones or morphs (Pointier 1989; Pointier et al. 1993). Because various clonal morphotypes can be traced back to their original geographic regions (Facon et al., 2003) with different climates, it could be hypothesized that the various clonal morphs would have different environmental temperature-tolerance profiles. Thus far, morphological patterns can separate amongst the genus of Tarebia species (Isnaningsih, 2017). Previous work in Texas has established three major morphs or haplotypes among *M. tuberculata* (Harding 2016). The most common form is designated TEX and the other two less common haplotypes or morphs are LSMR and OCCR. Harding (2016) presented major distinct phenotypic patterns which allow for the differentiation between haplotypes or morphs by using qualitative external shell morphological characteristics.

Both species are from (sub) tropical origin and have been reported to have invaded (i.e., North American systems with environments which mimic tropical characteristics; these species are found exclusively in in geothermally warmed or thermally stable spring environments, with temperature minimum (above) > 17 °C Mitchell & Brandt, 2005). Whereas, *T. granifera* has been reported to occur in environments with a natural temperature range of 10 - 38˚ C in the United States (Karatayev et al. 2009). Historically, *M. tuberculata* were only recorded from habitats with natural temperature ranges between 27°C to 29 °C in a thermal spring in Morocco (Laamrani et al., 1997), 29°C to 30.4°C in New Zealand (Duggan, 2002), 18°C to 25°C in the United States (Murray, 1971) and between 20°C to 31°C from thermal springs in the Bonneville Basin, UT (Rader et al., 2003). In contrast to previous findings of the cold temperature intolerance of *M. tuberculata*, *T. granifera* have been considered to be less sensitive to temperature variability and can survive seasonal winter cold fronts and high temperature extremes from 0 to 47.5ºC in Africa (Miranda, 2010).

In Texas, the first reports of *M. tuberculata* were from the spring-fed water supply to the San Antonio Zoo, and later both species were found in the spring-fed Comal River, New Braunfels (Murray, 1964). Additionally, both *M. tuberculata* and *T. granifera* may have been introduced to the spring-fed San Marcos River sometime between 1964-1973 (Lindholm, 1979). Comal and San Marcos Springs are the 1st and 2nd largest spring systems in the state, respectively and contain numerous endemic and endangered species. The distribution of both thiarid species was considered restricted to these thermal stable spring runs, since most surface waters in Texas are not thermally stable and are thus unable to maintain reproducing populations that could survive through most winters when water temperatures could drop below the proposed lethal threshold of 18°C (Fleming 2002, Fleming et al. 2011, Mitchell et al., 2005). Though, there is a growing concern between biologist (D.G. Huffman, pers. comm.), that populations of these species may become adapted to local conditions and survive periodic low water temperatures, thereby increasing the likelihood that the snails may spread to environments which are less thermally stable.

In the winter months of 2012 to 2013, water temperature profiles from rivers and streams in the Guadalupe drainage recorded drops (below) < 18°C i.e., at the bypassed channel of the Guadalupe River between Dunlap Dam and Lake McQueeney for 36 consecutive days, (Fig. 3) and during this prolonged cold period, ambient water temperature cooled to 12°C, but it was observed that individuals of *M. tuberculata* survived these lethal temperatures at least 2 km downstream from the upper end of Lake McQueeney, approximately 15 km downstream from the Comal/Guadalupe confluence (D. Huffman, pers. obs). Most of these snails were greater than 30+ mm long and at the abandoned channel between Dunlap Dam and Lake McQueeney snails measured in lengths of 35 to 50+ mm long, buried in sand substrate where water temperatures varied from 14.5°C to 15.5°C (D. Huffman, pers. obs). Additionally, in the winter months of

2014, *M. tuberculata* was also found in the lower San Marcos River near Luling, Texas more than 50 km downstream from the head springs at the upper San Marcos River (D.G. Huffman, pers. comm.) and well outside the river section which is considered to be thermally stable (Groeger et al. 1997). The apparent release of local *M. tuberculata* from the reported thermal restriction could mean that a different or undocumented genotypic clone or morph/haplotype with lower temperature tolerance was introduced into the Comal and the San Marcos Rivers, sometime shortly after Fleming, 2002, 2011) and Mitchell et al., (2005) or that local populations of *M. tuberculata* have adapted to colder temperature regimes. Regardless of the reasons for this apparent tolerance to colder temperatures in the San Marcos and Comal systems, *M. tuberculata* populations in the Guadalupe drainage are now found further from the head springs where colder water temperatures occur for substantial periods of time (Fig. 4).

The objectives of this thesis were to determine the critical thermal minimum (CTmin) of *M. tuberculata* and to examine whether temperature tolerance varied between 1) species (Tarebia vs. Melanoides), 2) morphotypes of *M. tuberculata*, and 3) between different river segments. Based on previous findings in the literature, I predicted that *T. granifera*, would have a greater cold temperature tolerance than *M. tuberculata*. In addition, I predicted that cold temperature tolerance would vary significantly between morphotypes of *M. tuberculata* and between river segments. I assumed that snails from river segments with colder minimum temperatures in central Texas winters would be more tolerant to colder temperatures compared to those from river segments with higher minimum temperatures determined from installed data loggers in 2012-2017. Specifically, I predicted that temperatures at which 50% of the experimental population of snails died should be (1) higher in the upper San Marcos compared to the lower San Marcos River (where minimum temperatures in the upper sediment layer are lower compared to the upper San Marcos); (2) higher in the lower Guadalupe compared to the upper Guadalupe River (where minimum temperatures are lower compared to the lower Guadalupe River, Table 5).

II. MATERIALS AND METHODS

To assess the low-temperature tolerance of the snails, I used both dynamic and static experimental methods. For the dynamic method, water temperatures were continuously decreased from 23°C to 10°C, and the temperature at which snails in the experimental populations died was recorded. For the static method, snails were acclimatized to a certain temperature (i.e., 17°C, 15°C, 11°C, 10°C) and survival were monitored over time (Hoffmann et al., 2003, Lutterschmidt & Hutchison, 1997, Terblanche et al., 2007). A total of 16 experimental trials were performed between 2016 and 2017.

Sampling of snails

Thiarid were collected from a range of sites within the Guadalupe and San Antonio drainages (Fig.5). The upper and lower San Marcos River (San Marcos, Texas) were sampled systematically from the headwater at Spring Lake to the confluence with the surface-fed Blanco River to scull road bridge in Martindale, Texas, and continued 50 km downstream to Luling, Texas. Collection from the Guadalupe River occurred only as flow velocities allowed, including sites upstream and downstream from the confluence of the spring-fed Comal River. The Comal River populations were thoroughly sampled, starting at Landa Lake (the head-springs) to the confluence with the surface-fed Guadalupe River (New Braunfels, Texas). All experiments were performed with freshly collected snails from each of the sites listed in Appendix (Table 9-13).

Snails were collected twice at the beginning, and at the end of each month for 12 months. Except when weather or flooding prohibited snails were collected using dipnets. For each sampling event the following parameters were measured: discharge, exposure to sun (visual estimate), sediment type (Wentworth scale), composition of filamentous algal community: *Rhizoclonium* was the most abundant (visual percent cover estimate), nearby macrophytes: *Hydrilla* sp. and *Ludwigia* sp. were the most abundant, whether snails were on surface or buried, whether snails were near shoreline or in deeper pools, and exact location at which snails were found, including distance in meter (m) out from water line and depth in meter (m). Snails from each local habitat were then placed in small flow-through containers and floated in a 20 L bucket with local river water and with an aerator attached for transport to the Wet Laboratory at Texas State University. Containers were then stored in a large flow-through trough, designed to acclimate the snails in constant well-water at 22-23°C for a minimum of one week. Snails were fed dried *Siprulina* fish food at the beginning of the acclimation period and at the start of each experimental temperature treatment carried out at San Marcos River at the US Fish and Wildlife San Marcos Aquatic Resource Center (SMARC).

Experiments

Thiarids were assessed for viability by gently probing the mantle of the snail, under a dissecting microscope using a blunt thin-tip needle to determine whether the snail would retreat its mantle behind the operculum. Viable snails were placed in 12 Sterlite (330.2 mm x 152.4 mm) containers, aerated and filled with well-water (22-23°C) at SMARC. Viability was determined every 2-3 hours. The number of thiarids per container varied depending upon the availability of snails collected from a given site. Allocating snails to experimental containers was performed randomly on one of the four wire shelves in environmental tanks (Fig. 6). Data loggers were placed randomly in one of the experimental Sterlite containers to monitor the water temperature. The thermal drop/rate was calculated using the starting and end temperatures, including the rate of decline from the data loggers, installed at the Guadalupe and lower San Marcos rivers (Fig. 4), to mimic a winter cold front in Texas (Tables 14-15). The water temperatures of upper San Marcos and the Comal was 23°C at the head-springs (starting temperature), and the lowest temperature drop recorded in the Guadalupe river was 10°C (end temperature). The lowest drop occurred within one day (i.e., ~ 2.3°C per day or 0.1°C per hour). A temperature of (~0.1°C) per hour, were then uploaded to the experimental tanks. Mortality was established when a snail did not recover after 1 to 2 half hours in constant well-water at 23°C. Snails judged as dead were removed from their assigned containers and the temperature, time, date was recorded, and water quality parameters were recorded. The size, distances between apex and the shell aperture were recorded to determine if longer thiarid, survive among species, local morphotype and river segments. Shells were then cleaned for morphometric analyses and assigned an accession number and stored in 95% ethanol. Morphometric identification of morphotypes of M. tuberculata was performed according to Harding, (2016), four broad categories of shell phenotypic variants (pattern), for the thiarid collected at the upper and lower San Marcos, Guadalupe, and the Comal Rivers for the dynamic experiments. The critical thermal minimum was determined by the temperature at which 50% of the snails died at a median survival time (Urquhart et al., 2014).

In addition to the dynamic method (see above), a static method was used to determine the length of time (days) thiarid can survive at various cold temperatures. During static experimental trials, the beginning temperature were decreased from 23°C (+/-) by ~ 0.1°C (+/-) per hour until it reached a target temperature of either 17°C, 15°C, 11°C, 10°C and viability was monitored as described above. Differences in survival curves for the static experiments were analyzed with the Kaplan-Meier log-range test (Goel et al., 2010). For the dynamic experiments an analysis of covariance (ANCOVA) was used to determine whether length was a significant co-variable in the model (complete), which was then excluded from further analyses. As assumption of homogeneity of variances were not met for the variable river, a two-way ANOVA with permutation test (Anderson 2001) was used to determine whether temperatures at which snails died differed significantly between species and river segments. A separate ANOVA was done to determine whether there were any significant differences between haplotypes for *M. tuberculata* species. All analyses were conducted in the package R.

III. RESULTS

A total of 3,293 thiarid were collected for laboratory experiments from five rivers within Central Texas with the majority of snails being collected from the Comal River followed by the San Marcos, Guadalupe, San Antonio, and Neches Rivers, respectively (Table 1). Shell length varied across rivers with longer snails collected from the Comal River and smallest snails from the Nueces River (Table 1).

Static temperature experiments

Survival of thiarids significantly varied between temperature treatments, species, and river and as expected, mean survival time of *M. tuberculata* and *T. granifera* decreased significantly with decreasing temperature from 17°C to 10°C (Table 2, Fig. 6). Mean survival time of *M. tuberculata* was significantly higher compared to *T. granifera* at all temperature treatments, except 15°C in which no *T. granifera* were used due to low sample sizes (Table 3). Snail survival differed significant between rivers (Log - Rank TS: 64.4, df = 6, P < 0.001; Table 4), but results were equivocal in that there were no consistent patterns among relative differences between rivers at each temperature treatment (Table 5, Table 10 and Fig. 9).

Dynamic temperature experiments

The experimentally-determined critical thermal minimum of *M. tuberculata* was 11°C, at which 50% of the snails died within 15 days (Table 5 and Fig. 7). The ANCOVA showed that shell length was not a significant covariate ($F_{1, 1840}$ = 0.10, P = 0.80). There was no significant difference between haplotypes ($F_{1, 931}$ = 0.0085, P = 0.93). The two-way ANOVA with permutation test detected that differences between species were not statistically significant (P = 0.24), but there was a significant interaction between species and rivers ($P < 0.001$). For some river segments survival of *M. tuberculata* was higher and for others river segment *T. granifera* was higher (Table 5). As predicted the temperature at which 50% of the snails died in different river segment was higher in the upper San Marcos compared to the lower San Marcos River, and higher in the lower Guadalupe compared to the upper Guadalupe River (Table 5 and Fig. 8). The permutation test also showed that differences between rivers were statistically significant ($P < 0.001$).

IV. DISCUSSION

This study found a substantially lower tolerance to colder temperatures in M. tuberculata than previously found by Mitchell and Brandt (2005) who reported a critical thermal minimum of 18°C. In the present study, I found a critical thermal minimum at 11°C. Mitchell and Brandt (2005) experimentally determined that 10% of their experimental *M. tuberculata* population died within 27 days after exposure to 17°C and that all individuals died within 8 days when exposed to 11°C. In contrast, this study found that very few *M. tuberculata* died (80% of the experimental population) within 50 days of exposure to 17°C and that all individuals survived for 14 days when exposed to 11°C. The contrast in results between Mitchell and Brandt (2005) and the current study may be due to differences in experimental approaches (i.e., static and dynamic methods). In addition, Mitchell and Brandt (2005) did not have a period of acclimatization for individuals of *M. tuberculata*, when they were first brought into the lab and moved to experimental temperature treatments; such acclimatization periods are needed for individuals to recover or adjust to temperature shifts (Fechhelm, 1982) and can reduces stress to the snails. Regardless of the differences between the two studies, an 11°C critical thermal minimum for *M. tuberculata* suggests that the assumption that its distribution is largely restricted to thermal stable spring systems is not appropriate.
The current findings are also, in accordance with our assumption that snails from river segments with colder temperatures in winter would be more tolerant to colder temperatures compared to those from river segments with warmer temperatures during winter (Fig. 9). This is also in general agreement with other studies of other invertebrates and fish that individuals adapt to local conditions. For example, apple snails

(*P. canaliculate*) morphs showed enhanced tolerance to colder temperatures after cold acclimation regiments (Yoshida et al., 2014).

Overall in this study the survival of *T. granifera* at constant 10°C for 72 hours compared to the studies used from other regions show *T. granifera* can generally survive colder temperatures, for example Miranda et al. (2011) found that 75% of tested T. granifera survived exposure to temperatures as low as 0 °C for 32 hours and in the study by Chaniotis et al. (1980), the investigators found 100% of tested *T. granifera* died when exposed to 7 °C for 24 hours. Although the studies end temperatures were colder than this study, it is reasonable to assume local *T. granifera* snails would survive longer at constant 10°C. Given the results of this study, it is reasonable to assume that populations of *M. tuberculata* are impacted during winter months in less spring influenced systems compared to the warmer months when snails are found in high densities. Overall this study can assume there is an increased mortality for snails at lower temperatures, but the study was limited to the thermal minimum indicating that as along as temperatures are (above) >11°C for most of the year, at various sites/rivers thiarid will likely, continue to have reproducing (asexually or sexually) populations.

In contrast to my predictions, no differences in thermal tolerance were found between morphotypes, which is consistent with the anecdotal observation that both local morphotypes in the study seem to spread farther downstream (Table 7). Thiarid from the upper Guadalupe and lower San Marcos river segment, survived lower experimental temperature minimum compared to the thiarid at the thermal stable springs of the Comal and upper San Marcos.

Further research is needed to examine differences in temperature tolerances of morphs between rivers segment (e.g., Neches vs. Guadalupe rivers). A more balanced experimental design with defined sample sizes, and control tanks in which temperature are not manipulated and replicates, may have detected more consistent differences in temperature tolerances between rivers, and future research should investigate that further. Particularly, since it is apparent that *M. tuberculata* can survive at colder temperatures within the lower San Marcos River and at the confluence of the Comal River into the surface-fed Guadalupe River. Future studies should also study the temperature preferenda and likewise, test for difference in snail length from different river segment at colder temperatures.

TABLES

Table 1. Descriptive statistics of snails collected at five major rivers and the mean shell length collected.

Rivers	**Sites**	***Melanoides tuberculata* (N)**	***Tarebia granifera* (N)**	**Mean shell length Max-Min (mm)**
Upper San Marcos	12	128	333	20.2 42.9-9.9
Lower San Marcos	6	257	13	20.23 31.6-7.6
Comal	14	1395	512	26.4 58.7-10.0
Upper Guadalupe	5	36	4	23.8 47.6-10.4
Lower Guadalupe	4	152	42	21.1 48.7-10.5
San Antonio	2	192	135	19.2 35.1-9.8
Neches	2	94	0	14.9 24.7:9.4

Table 2. Kaplan-Meier survival analysis table comparing *M. tuberculata* and *T. granifera* at constant temperatures of 17°C, 15°C, 11°C, and 10°C

	Survival Time (Days)	**Std. Error**	**95% Conf. Lower**	**95% Conf. Upper**
17°C *M. tuberculata* (N = 163)	55.6	±0.9	53.7	57.5
17°C *T. granifera* (N = 34)	42.6	±2.705	37.3	47.9
11°C *M. tuberculata* (N = 153)	17.5	±0.292	16.9	18.1
11°C *T. granifera* (N = 24)	20	±0	20	20
10°C *M. tuberculata* (N = 454)	3.8	±0.1	3.6	3.9
10°C *T. granifera* (N = 32)	2.8	±0.3	2.3	3.3
15°C *M. tuberculata* (N = 143)	17.9	±0.7	16.5	19.4
Log-Rank Test:	**Statistic**	**DF**	**P Value**	
17°C	62.2	1	<0.001	
11°C	24.8	1	<0.001	
10°C	14.3	1	<0.001	
M. tuberculata all temp. (N = 913)	1051.8	3	<0.001	
T. granifera all temp. (N = 90)	105.2	2	<0.001	
Comparisons	**Statistic**	**P Value**	**Significant**	
17°C vs. 10°C	519.2	<0.001	Yes	*M. tuberculata*
10°C vs. 11°C	434.3	<0.001	Yes	*M. tuberculata*
10°C vs. 15°C	346.8	<0.001	Yes	*M. tuberculata*
17°C vs. 15°C	284.6	<0.001	Yes	*M. tuberculata*
17°C vs. 11°C	270.8	<0.001	Yes	*M. tuberculata*
15°C vs. 11°C	73.4	<0.001	Yes	*M. tuberculata*
17°C vs. 10°C	64.8	<0.001	Yes	*T. granifera*
11°C vs. 10°C	50.6	<0.001	Yes	*T. granifera*
17°C vs. 11°C	14.1	<0.001	Yes	*T. granifera*
Summary: The log rank statistic for the survival curves is greater that would be expected by chance; there is a statistically significant difference between survival curves (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure. All Pairwise Multiple Comparison Procedures (Holm-Sidak method): Overall significance level = 0.05				

Table 3. 3. Kaplan-Meier Survival Analysis comparing rivers. Survival Log-Rank (mean days +/- SE, (95% CI))

	Survival Time	**Std. Error**	**95% Conf. Lower**	**95% Conf. Upper**
Lower San Marcos River N=113	12.84	1.34	10.22	15.47
Comal River N=610	19.84	0.88	18.10	21.57
Lower Guadalupe River N=58	16.75	2.95	10.96	22.54
San Antonio River N= 73	17.64	0.70	16.26	19.02
Upper San Marcos River N=110	17.64	0.70	16.26	19.02
Upper Guadalupe River N=11	17.64	0.70	16.26	19.02
Nueces N=30	4	0	4	4
Log-Rank Test:	**Statistic**	**DF**	**P Value**	
	64.3	6	<0.001	
Comparisons	**Statistic**	**P Value**	**Significant?**	
UpGuR vs. Nueces	40	5.33E-09	Yes	
LoSMR vs. ComaR	26.72	4.70E-06	Yes	
ComaR vs. Nueces	21.56	6.52E-05	Yes	
UpSMR vs. Nueces	21.26	7.20E-05	Yes	
LoSMR vs. UpSMR	20.79	8.71E-05	Yes	
SaAnR vs. Nueces	17.96	3.61E-04	Yes	
LoSMR vs. Nueces	17.80	3.68E-04	Yes	
ComaR vs. LoGuR	11.33	1.06E-02	Yes	
UpSMR vs. UpGuR	7.27	8.73E-02	No	
ComaR vs. SaAnR	6.13	1.48E-01	No	
SaAnR vs. UpSMR	4.38	3.34E-01	No	
ComaR vs. UpSMR	3.99	3.74E-01	No	
LoSMR vs. SaAnR	3.62	4.11E-01	No	
LoSMR vs. UpGuR	3.53	3.91E-01	No	
LoGuR vs. Nueces	2.22	6.41E-01	No	
ComaR vs. UpGuR	1.37	8.11E-01	No	
LoGuR vs. UpSMR	1.28	7.74E-01	No	
LoGuR vs. UpGuR	1.27	6.99E-01	No	
SaAnR vs. UpGuR	0.59	8.26E-01	No	
LoSMR vs. LoGuR	0.13	9.20E-01	No	
LoGuR vs. SaAnR	0.0000012	9.99E-01	No	

Table 4. Descriptive Statistics for *M. tuberculata* survival times held at constant temperatures of 17°C, 15°C, 11°C and 10°C across rivers. Survival Time (mean days +/- SE).

Species	**Rivers**	**10°C (N = 486)**	**11°C (N =179)**	**15°C (N =143)**	**17°C (N = 197)**
M. tuberculata	(All)	3.8±0.1	17.5±0.29	17.8±0.7	55.7±1
M. tuberculata	upper SMR	2.83± 0.30	19.5± 0.4	NA	NA
M. tuberculata	lower SMR	4.4± 0.8	17.1± 0.5	NA	44.8± 7.1
M. tuberculata	Comal	2.6±0.2	17.9± 0.4	18.2± 0.9	56.1± 0.9
M. tuberculata	upper Gaudalupe	NA	17.6±0.7	NA	NA
M. tuberculata	lower Gaudalupe	3.1±0.2	14.6±1.6	NA	61.5±0
M. tuberculata	San Anotonio	4±0	NA	17.4±1.3	NA
M. tuberculata	Nueces	4±0	NA	NA	NA
T. granifera	(All)	2.8± 0.3	20±0	NA	42.1± 2.7
T. granifera	Comal	4±0	NA	NA	NA
T. granifera	upper SMR	2.5± 0.3	20±0	NA	42.6± 2.7
T. granifera	Nueces	4.0± 0	NA	NA	NA

Table 5. Descriptive Statistics. The temperatures at which 50% of snails died (median values), morphotype and minimum temperature from data loggers (HOBO) from river segments in the dynamic experiments.

	M. tuberculata	*T. granifera*	LSMR	TEX	Minimum temperature from data loggers (HOBO)
Upper Guadalupe	11.2°C	11.2°C	11.2°C	11.2°C	10.5°C
Lower Guadalupe	15°C	11.8°C	14.4°C	15°C	12.6-15.6˚ C
Upper San Marcos	15°C	10.2°C	17°C	15°C	15.7˚ C
Lower San Marcos	10°C	10.2°C	10.2°C	10°C	11.8˚ C
Comal	11°C	11°C	10.2°C	11°C	NA
San Antonio	11.6°C	11.6°C	NA	NA	NA
Nueces	15°C	NA	NA	NA	NA

FIGURES

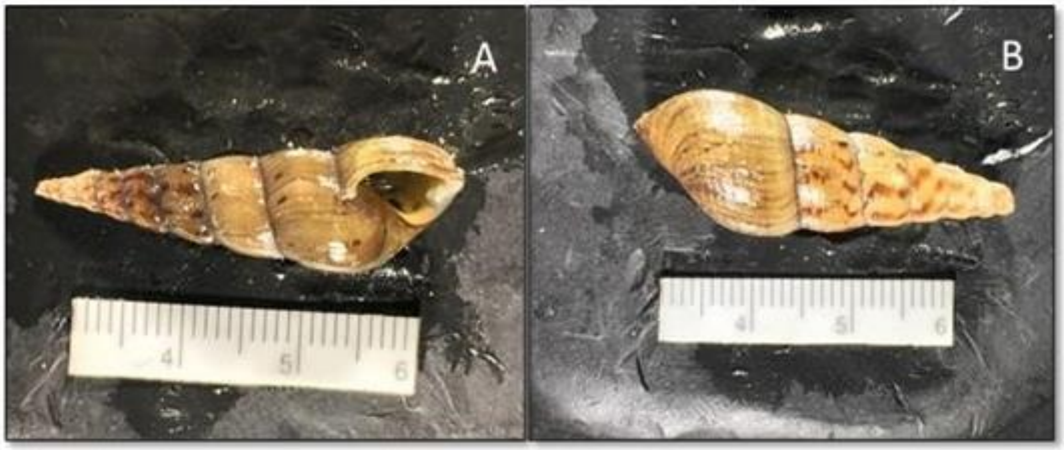

Figure 1. Photos of living local *M. tuberculata*, the red-rimmed melania, also known as the Malaysian trumpet snail in the pet trade. Collected from the Comal and San Marcos River and measuring 30+ mm in shell length. A – Ventral view under water (operculum retracted); B – Dorsal view of same snail in air.

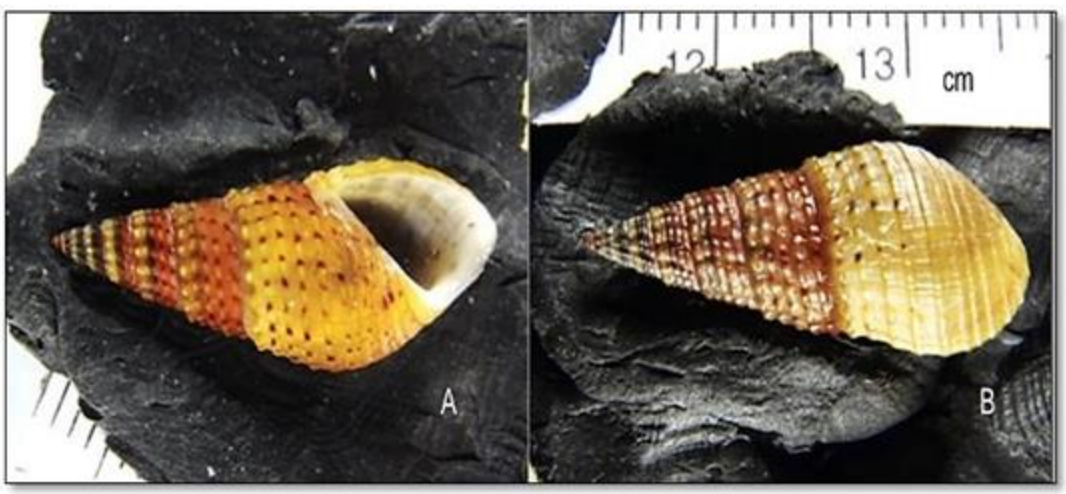

Figure 2. Photos of living local *Tarebia granifera*, the quilted melania, also known as the Thiara-snails. A - Ventral view under water (operculum retracted); B - Dorsal view of same snail in air.

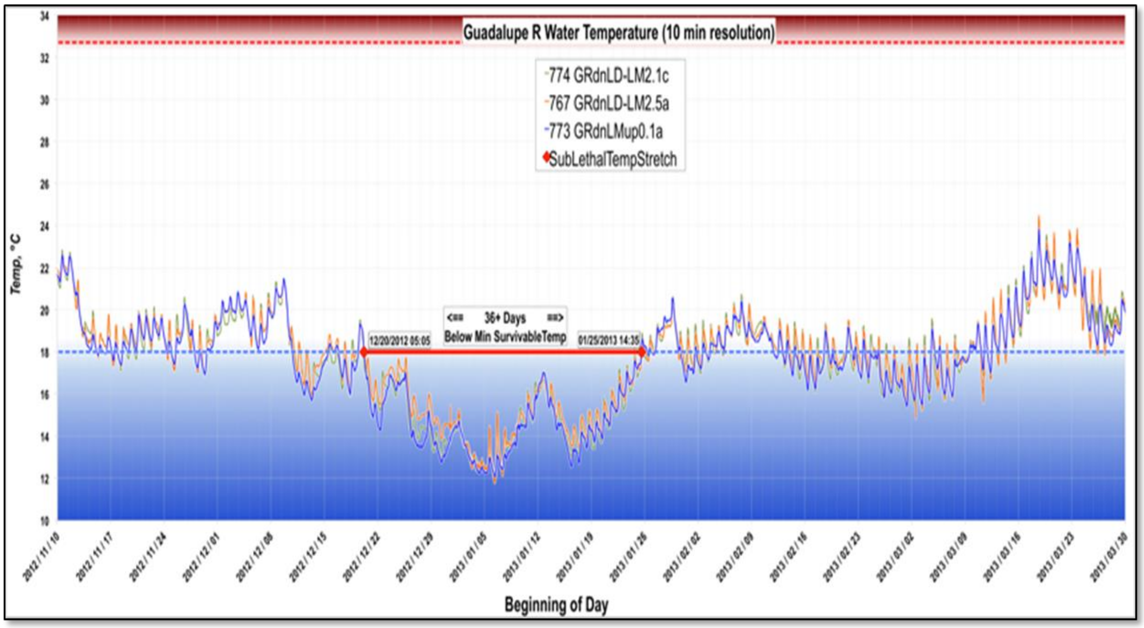

Figure 3. Water temperature profile from the Guadalupe River at the bypassed channel of Guadalupe River between Dunlap Dam and Lake McQueeney sites. Ambient water temperature drops to 12 °C. GPS location for the Guadalupe River (29.710627, -98.134565), showing seasonal (winter) swing from installed (HOBO) data loggers in 2012-13. Two data loggers installed one suspended in the water column (774GRdnLD-LM2.1c) and the other (767GRdnLD-LM2.5a) was buried approximately 15 cm into the benthos, a third logger suspended in the water column (773 GRdnLMup0.1a) was installed at the Guadalupe dam site. In 2012/11 to 2013/02 temperatures were recorded below <18°C for 36d and <15°C for 14 days where *M. tuberculata* were found surviving.

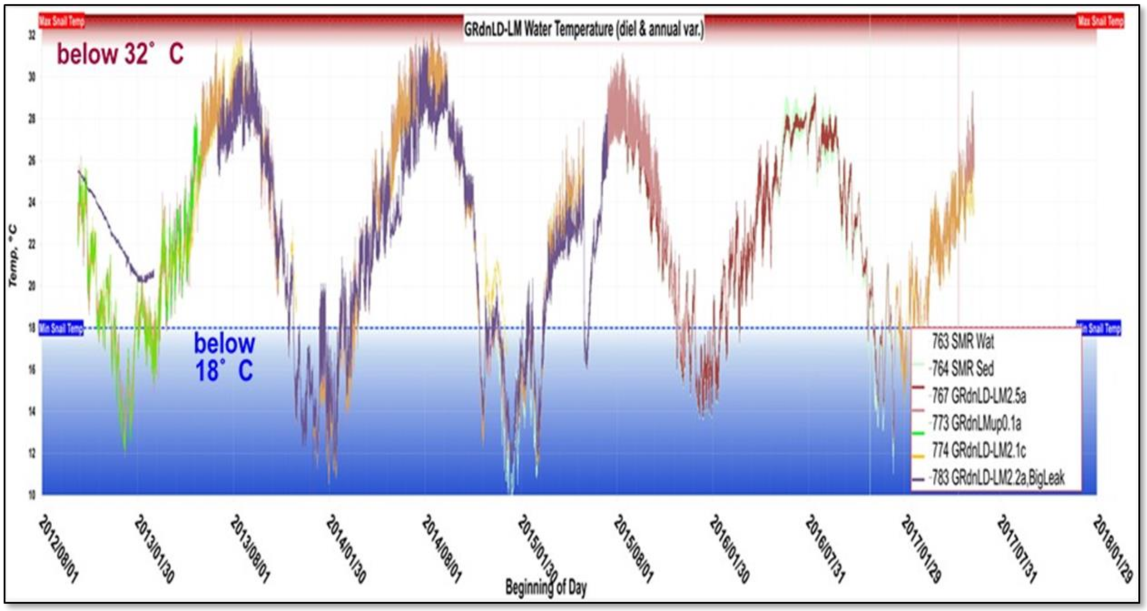

Figure 4. Water temperature profile from the Guadalupe River at the bypassed channel of Guadalupe River between Dunlap Dam and Lake McQueeney and at the lower San Marcos Rivers sites. Ambient water temperature drops to 10 °C. GPS location for the Guadalupe River (29.710627, -98.134565), and lower San Marcos River (29.668734, -97.700645), showing seasonal (winter) swing from installed (HOBO) data loggers in 2012-2017. Data loggers were installed in the water column (763SMRWat), (773 GRdnLMup0.1a), (767 GRdnLD-LM), (774 GRdnLD-LM Wat), and buried approximately 15 cm into the benthos (764SMR Sed), (783 GRdnLD-LM,BigLeak Sed).

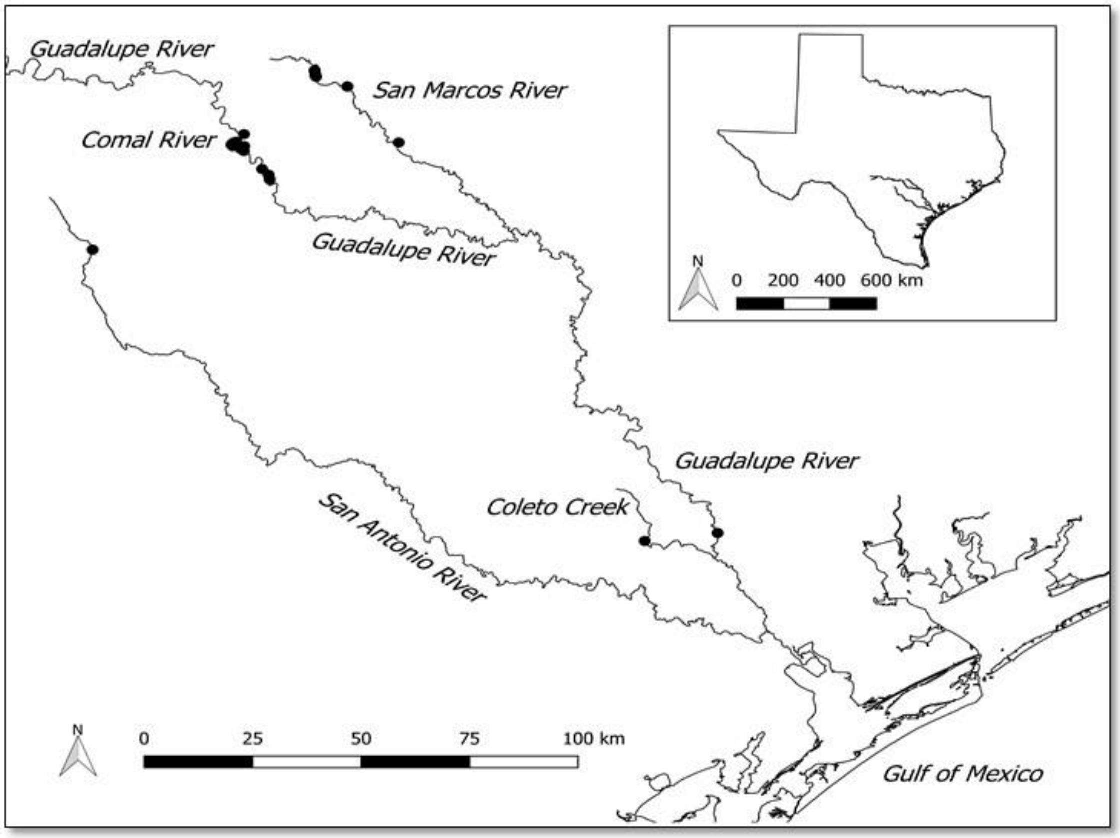

Figure 5. Map of the sampling areas. Rivers associated to sites surveyed where reproducing population of thiarids (black-dot) were found in Texas. Five major rivers were sampled, the San Marcos, Comal, the Guadalupe, Nueces (not show above) and San Antonio Rivers. The San Marcos and the Guadalupe Rivers were further divided into two sections: upper and lower sections. Sites/reaches were plotted using ArcGIS platform. Sources: Esri, USGS and the GIS User Community. Created by Zachary Mitchell.

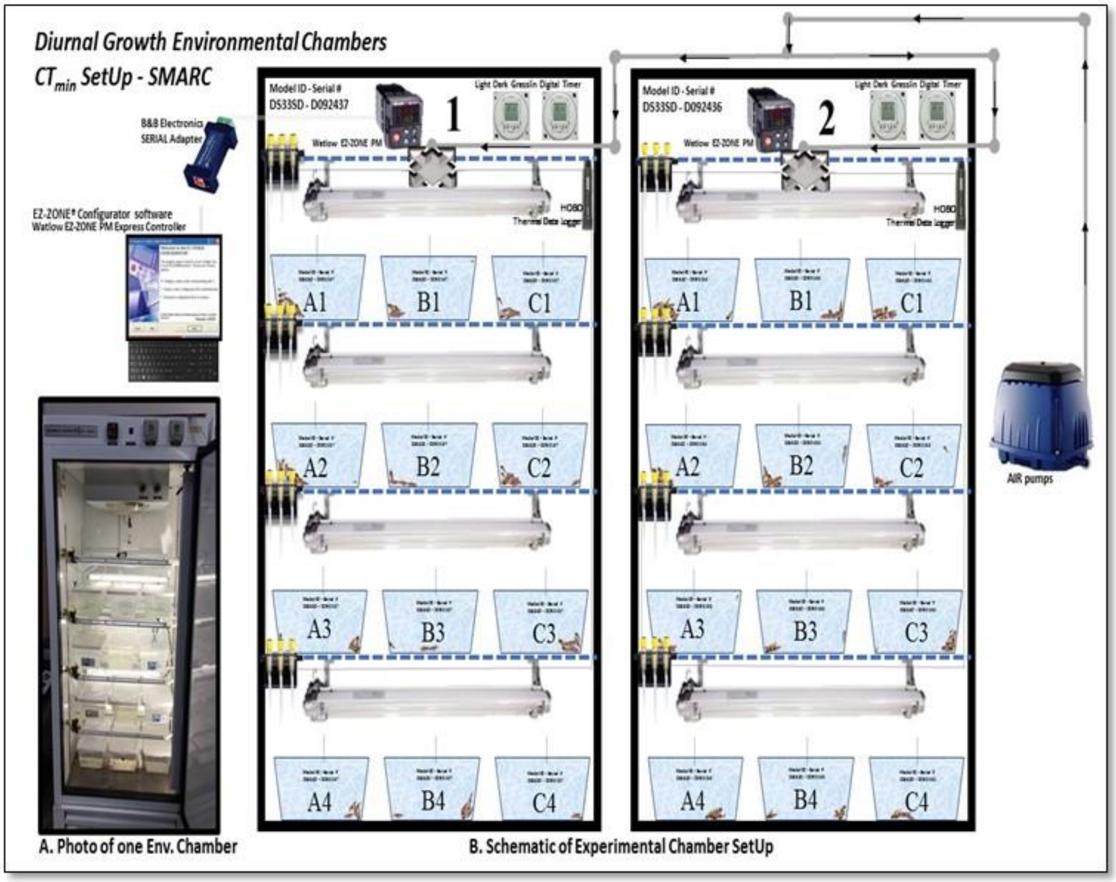

Figure 6. Environmental tanks set up. (A) Photo of the experimental layout in the unit on the front panel (above the door) has a standard Watlow EZ Zone PM temperature control, and two Grasslin (temperature and light) control clocks. Two chambers is connected to a personal computer through a USB interface serial port to the Watlow EZ-Zone PM controllers. (B) Schematic illustration of the unit's layout with 12 (330.2 mm x 152.4 mm) containers labeled (A1-4, B1-4, and C1-4) filled with well-water connected to a pump placed outside the unit for aerating the containers. A total of three containers are placed on each of the 4 wire shelves. Attached to each shelve (below) is a florescence light, controlled by a Grasslin clock on the front panel of the chambers.

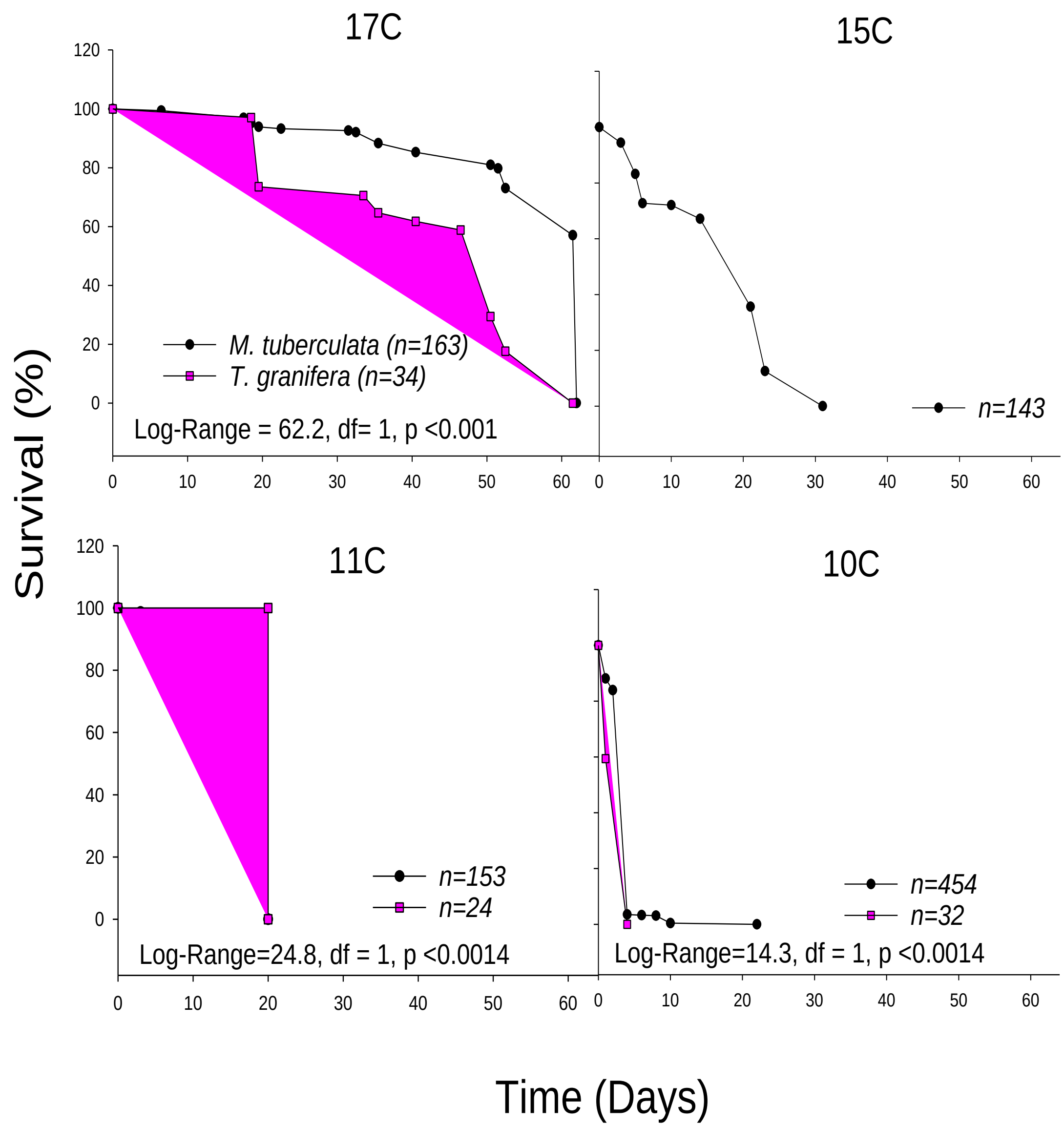

Figure 7. Survival over time at different constant temperatures of 17°C for *M. tuberculata and T. granifera* species. Control snails held in 22°C well water for 120d.

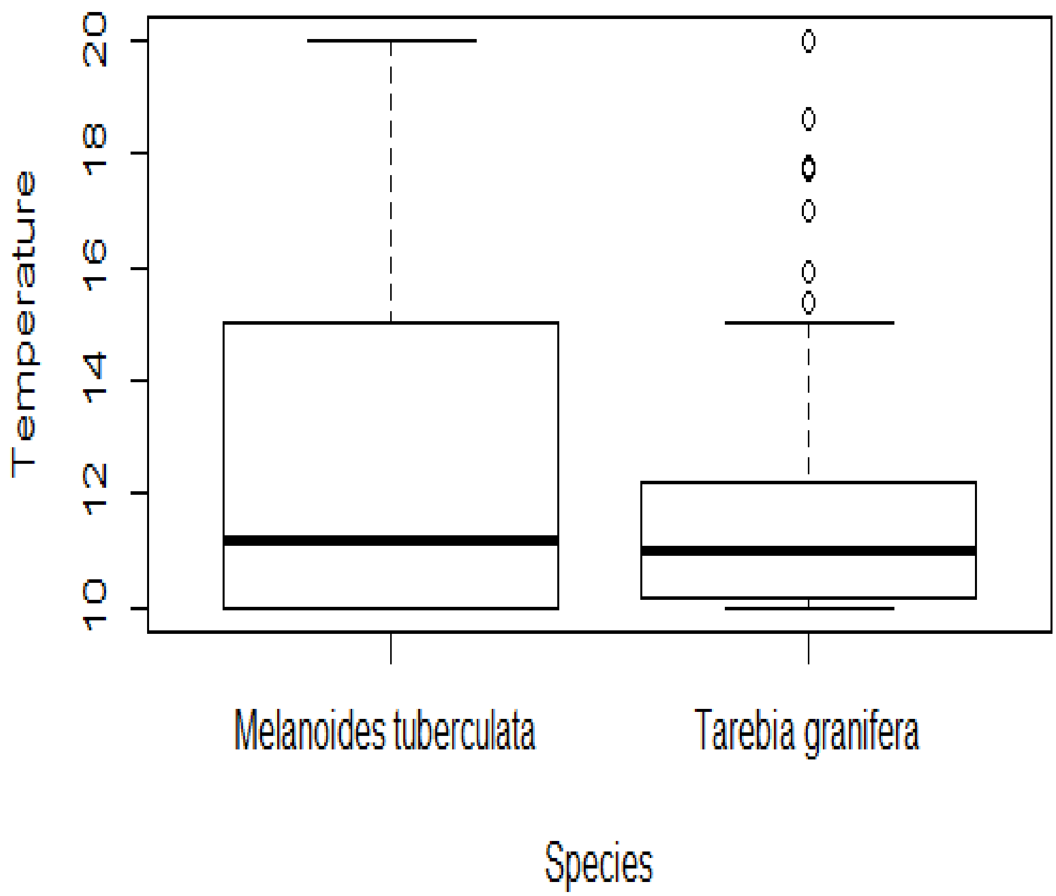

Figure 8. Boxplot of survival median temperatures for *M. tuberculata* and *T. granifera* species in the dynimic experiments. *M. tuberculata* (N= 1339) and *T. granifera* (N= 949) were collected in Texas. The median survival temperature of *M. tuberculata* (11.2°C) *and T. granifera* (11°C) at 50% mortality.

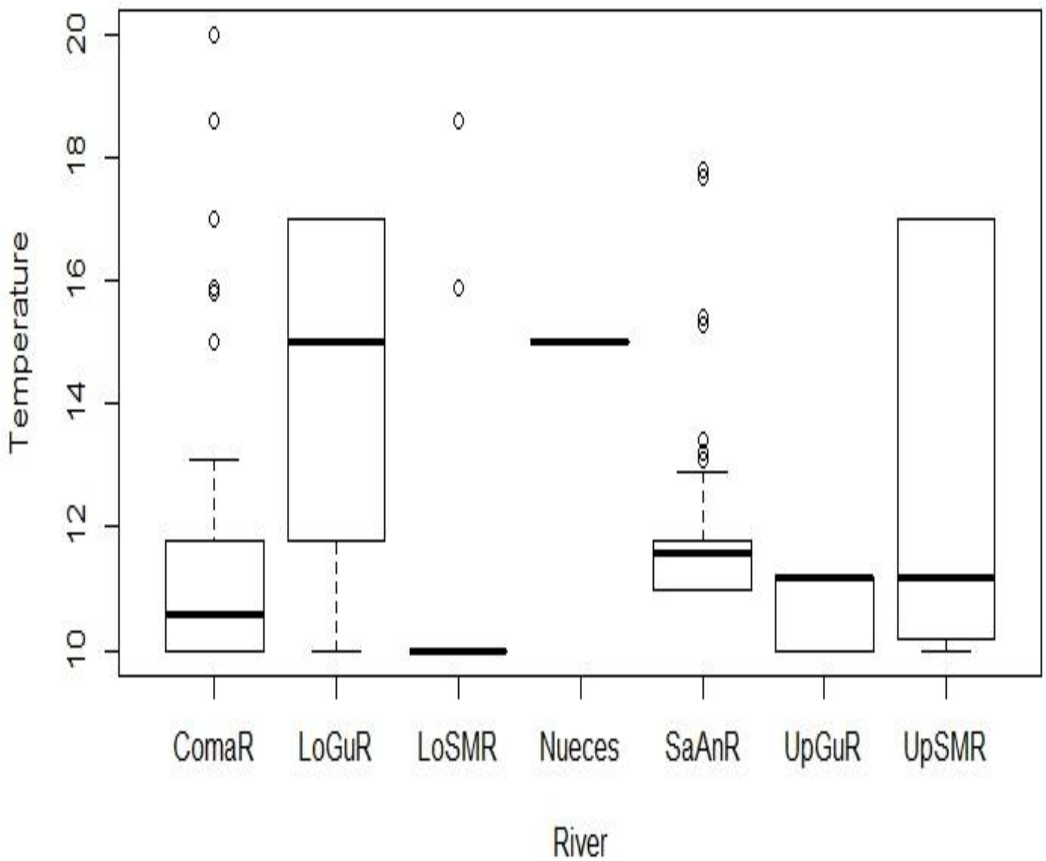

Figure 9. Boxplot of survival mean temperature when 50% of snails die from the five major rivers snails are collected.

APPENDIX SECTION

Table 9. List of Rivers associated to sampling sites and corresponding GPS coordinates on the Comal River Stations exact GPS coordinates from Google Earth

Comal River Stations	
Reach/sites	**GPS Location: (latitude, longitude)**
Main Channel	29.709300, -98.133240
Old Channel	29.710720, -98.129552
New Channel	29.709300, -98.133240
Landa Lake 'Paddle Boats Dock' Site	29.711800, -98.135551
Landa Lake 'South Paddle boat dock' Site	29.710610, -98.135170
Landa Lake 'South Slough' Site	29.715866, -98.134300
West Pecan Island 'Drainage Pipe' Site	29.715012, -98.134201
Landa Lake 'Duck Area' Site	29.712877, -98.136688
Landa Lake 'Bridge Crossing' Site	29.713175, -98.136162
Landa Lake 'Fishing Pier' Site	29.713583, -98.135757
Landa Lake 'Kids Train Railway' Site	29.710800, -98.134560
Landa Lake 'Sensitive Environment' Site	29.714323, -98.136093
Spring Island Site	29.718250, -98.131271
E Kingemann St	29.720404, -98.125244

Table 10. List of Rivers associated to sampling sites and corresponding GPS coordinates on the Guadalupe River Stations exact GPS coordinates from Google Earth

Guadalupe River Stations	
Reach/sites	**GPS/Location:**
Faust St. 'Bridge' LoGuad. Site	29.697620, -98.107544
Gruene River Crossing UpGuad. Site	29.738199, -98.106415
Abandoned channel 'Dunlap Dam' LoGuad. Site	29.653147, -98.064888
Coleto "Boat Pier" LoGuad. Site	28.722446, -97.170296
Coleto "Cross-Power Station" LoGuad. Site	28.731300, -97.164940
Cypress Bend 'Waterway' Site	29.709253, -98.106461
Cypress Bend 'Park' Site	29.712852, -98.105629
Lake McQueeney Site I ‘upper pool riffle’ Site	29.639189, -98.049706
Lake McQueeney Site II ‘power plant’ Site	29.627926, -98.047325

Table 11. List of Rivers associated to sampling sites and corresponding GPS coordinates on the San Antonio River Stations exact GPS coordinates from Google Earth

San Antonio River Stations	
Reach/sites	**GPS/Location:**
San Antonio Zoo 'Bird/Pool' Site	29.463764, -98.471797
San Antonio River ' Bridge crossing out the Zoo ' Site	29.463574, -98.471858

Table 12. List of Rivers associated to sampling sites and corresponding GPS coordinates on the Blanco River Stations exact GPS coordinates from Google Earth. *No Alive Snails Collected

Blanco River Stations	
Reach/sites	**GPS/Location:**
Old Martindale Rd crossing ‘upstream’ Site	29.871281, -97.915634
Blanco Confluence Site*	29.856148, -97.907669
Blanco Confluence ‘Old Bastrop Rd’ LoSMR Site	29.857243, -97.897148

Table 13. List of Rivers associated to sampling sites and corresponding GPS coordinates on the San Marcos River Stations exact GPS coordinates from Google Earth. *No Alive Snails Collected

San Marcos River Stations	
Reach/sites	**GPS/Location:**
Loop 82 bridge 'Salt Grass' UpSMR Site	29.889774, -97.934418
Loop 82 bridge 'Island' UpSMR Site	29.889311, -97.934235
Freeman Building 'Sessom Creek Run' UpSMR Site	29.889311, -97.934235
Freeman Building 'Ponds' UpSMR Site	29.889427, -97.936310
Rio Vista Park Island UpSMR Site	29.880157, -97.932777
Rio Vista Park 'Walk Path' UpSMR Site	29.879965, -97.933128
Rio Vista Park 'Rocks' UpSMR Site	29.879608, -97.932693
Rio Vista Park 'Bridge' UpSMR Site	29.877609, -97.933144
Rio Vista Park 'Playing Field Pie' UpSMR Site	29.876541, -97.931831
IH-35 'Woods Apt' UpSMR Site	29.874205, -97.931572
IH-35 'Frontage Rd' UpSMR Site	29.874464, -97.931152
Highway 299 'Cape St' UpSMR Site	29.869091, -97.929092
A. E. Woods State Fish Hatchery UpSMR Site*	29.868841, -97.931007
Waste Management 'Animal Shelter Rd' UpSMR Site*	29.866301, -97.926514
Blanco Confluence Site*	29.856148, -97.907669
Blanco Confluence 'Old Bastrop Rd' LoSMR Site	29.857243, -97.897148
Martindale 'Scull Rd Bridge' LoSMR Site	29.849556, -97.856934

Table 13. Continues List of Rivers associated to sampling sites and corresponding GPS coordinates on the San Marcos River Stations exact GPS coordinates from Google Earth. *No Alive Snails Collected

Martindale ‘Scull Rd Island’ LoSMR Site	29.849789, -97.857193
Martindale ‘Deviney Rd Bridge’ LoSMR Site	29.839605, -97.845436
Fentress Bridge LoSMR Site	29.752644, -97.780876
Stair town Rd LoSMR LoSMR Site	29.711945, -97.737801
Luling H-90 LoSMR Site	29.668734, -97.700645
Palmetto State Park H-2091 Site*	29.590922, -97.586559
Spring Lake ‘Meadows Center’ UpSMR Site*	29.893898, -97.930351
Spring Lake ‘Glass Bottom Boats Pier’ UpSMR Site*	29.893620, -97.930267
Spring Lake ‘Driving Pie’ UpSMR Site*	29.893147, -97.931366
Spring Lake ‘Wet Lands’ UpSMR Site*	29.892189, -97.932114
Spring Lake ‘Wet Lands Boat House Pier’ UpSMR Site*	29.891710, -97.931725
Martindale ‘Scull Rd Rock Area’ LoSMR Site	29.849144, -97.857376

Table 14. Descriptive statistics of data loggers installed in the Guadalupe Basin (Temperatures) from 2012 to 2017.

River Segment	Mean	Min	Max	Var	SD
UpSMR	23.3°C	15.7°C	32.3°C	10.1	3.2
LoSMR	21.5°**C**	11.8°C	29.4°C	21.3	4.6
LoSMR	21.3°C	8.9°C	20.5	18.3	4.3
Comal	23.2°C	15.4°C	31.2	10.8	3.2
UpGudR	21.9°C	10.5°C	32.4	23.1	4.8
UpGudR	21.9°C	10.1°C	32.2	19.4	4.4
UpGudR	22.2°C	10.5°C	32.2	24.1	4.9
LoGudR	19.9°C	16.9°C	24.9	1.2	1.1
LoGudR	19.0°C	15.0°C	22.0	2.5	1.6
LoGudR	19.6°C	15.6°C	23.0	2.5	1.6
LoGudR	18.8°C	12.6°C	27.3	8.3	2.9
LoGudR	18.7°C	15.0°C	22.0	2.5	1.6

Table 15. Number of days below temperatures of 17˚ C, 15 ˚ C, 11 ˚ C and 10 ˚ C from previously installed data loggers at the Guadalupe watershed from 2012-2017**.**

	Days below constant temperatures			
Year	**<10˚ C**	**<11˚ C**	**<15˚ C**	**<17˚ C**
2012	0	0	21	36
2013	2	7	58	72
2014	4	11	51	84
2015	1	28	52	72
2016	1	2	21	32
2017	0	0	14	30

Table 16. Morphotypes found in major rivers for dynamic experiment.

Rivers	**LSMR**	**TEX**	**Grand Total**
ComaR	17	774	791
LoGuR	16	78	94
LoSMR	59	85	144
UpGuR	4	21	25
UpSMR	16	86	102
Grand Total	112	1044	1156

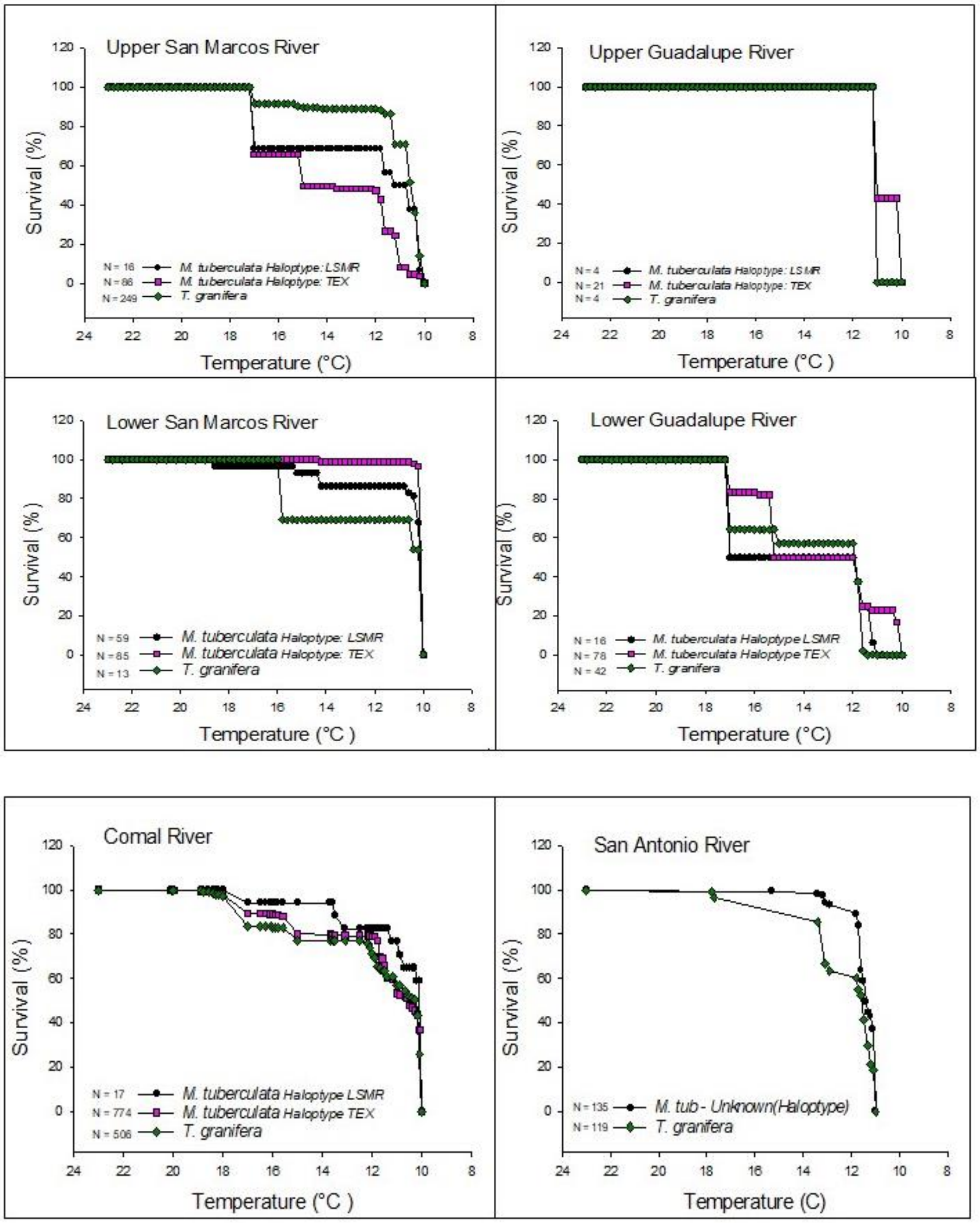

Figure 10. Survival curves for River segments for (*M. tuberculata and T. granifera)* species. Each point is a date snail die.

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