

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-121-R

Endangered and Threatened Species Conservation

**Native springsnails and the invasive red-rim melania snail (*Melanoides tuberculata*),
species habitat associations and life history investigations in the San Solomon Spring
complex, Texas**

Prepared by:

David Rogowski



Carter Smith
Executive Director

Clayton Wolf
Director, Wildlife

3 October 2012

FINAL REPORT

STATE: Texas GRANT NUMBER: TX E-121-R

GRANT TITLE: Native springsnails and the invasive red-rim melania snail (*Melanoides tuberculata*), species habitat associations and life history investigations in the San Solomon Spring complex, Texas.

REPORTING PERIOD: 17 Sep 09 to 31 May 12

OBJECTIVE(S):

To determine patterns of abundance, distribution, and habitat use of the Phantom Cave snail (*Cochliopa texana*), Phantom Spring tryonia (*Tryonia cheatum*), and the invasive red-rim melania snail (*Melanoides tuberculata*) in San Solomon Springs, and potential interactions.

Segment Objectives:

Task 1. January - February 2010. A reconnaissance visit(s) will be made to the region to investigate the study area and work on specific sampling procedural methods. Visit with TPWD at the Balmorea State Park, as well as meet The Nature Conservancy personnel at Diamond Y and Sandia springs complexes.

Task 2. March 2010– August 2011. Begin sampling. Field sampling will be conducted every 6-8 weeks, over a period of a year and a half. Sampling methods are outlined below stated Tasks.

Task 3. December 2010. Completion of first year of study. With four seasonal samples completed, preliminary data analysis and statistical modeling will begin. Preliminary results will be presented at the Texas Chapter of the American Fisheries Society meeting.

Task 4. January 2011. Laboratory experiments commence. Selection experiments testing specific environmental factors (temperature and/or water velocity).

Task 5. August 2011. Tentative conclusion of field sampling. Data entry

Task 6. September-November 2011. Data analyses and report writing commences.

Significant Deviations:

None.

Summary Of Progress:

Please see attached pdf file.

Location: Reeves and Terrell Counties, Texas.

Cost: Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.

Prepared by: Craig Farquhar

Date: 3 October 12

Approved by:  C. Craig Farquhar **Date:** 3 October 12

ATTACHMENT A

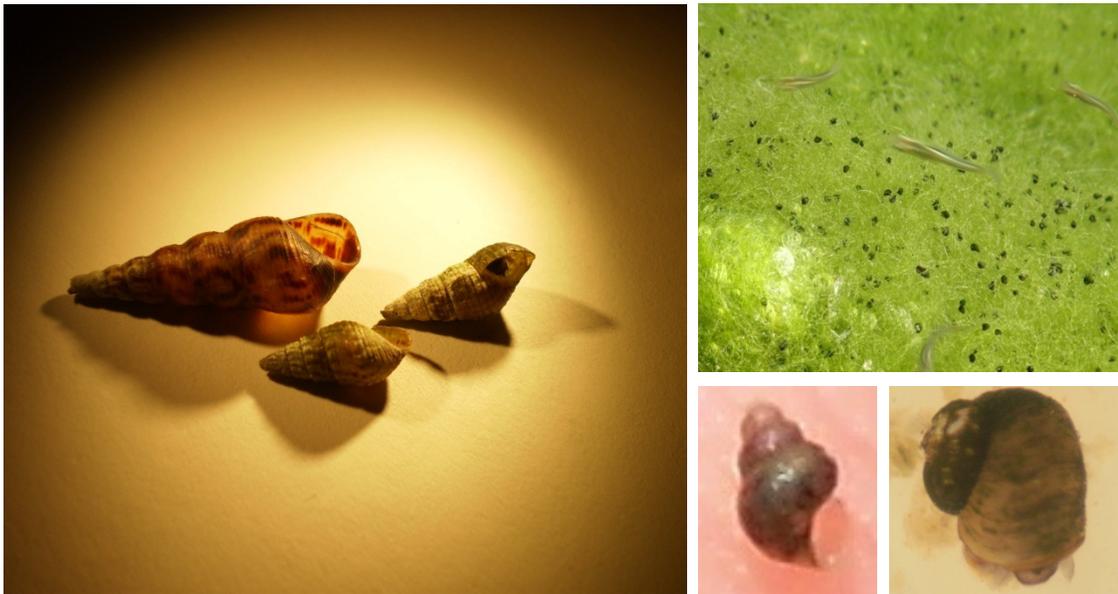
Native springsnails and the invasive red-rim melania snail (*Melanooides tuberculata*), species habitat associations and life history investigations in the San Solomon Spring complex, Texas

Final report for Grant No. TX E121
Prepared for Texas Parks and Wildlife

Prepared by Jeff Bradstreet and Dr. David L Rogowski
j.bradstreet@ttu.edu
david.rogowski@ttu.edu

May 2012

Department of Natural Resources Management
Texas Tech University
Box 42125
Lubbock, Texas 79409-2125 USA
806-742-2841



Melanooides tuberculata (left)
and *Tarebia granifera* (right)

Pyrgulopsis texana and fish
(top); *Tryonia cheatumi*
(bottom left); *Pyrgulopsis*
texana (bottom right)

Preface

This report is primarily based on research conducted by Jeff L. Bradstreet as partial requirement for a M.S. degree in Fisheries Science in the Department of Natural Resources Management at Texas Tech University under the supervision of his major advisor, David L. Rogowski. The objective of this research was to determine patterns of abundance, distribution, and habitat use of the Phantom Cave snail (*Pyrgulopsis texana*), Phantom Spring tryonia (*Tryonia cheatumi*), and the invasive red-rim melania snail (*Melanoides tuberculata*) in San Solomon Springs, and potential interactions. The first four chapters of this report in essence constitute Mr. Bradstreet's formal Thesis. Chapter one provides an introduction to the study system, and chapters two and three report the results of a field study and a laboratory experiment respectively, while chapter four provides a summary of his thesis. Chapters five through seven address additional research that was conducted in relation to this project. Each chapter with the exception of the Introduction and Summary chapter is written in manuscript format.

Acknowledgements

The completion of this project would not have been possible without the help and support of friends and colleagues. We are grateful to Dr. Christopher J. Salice and Dr. Christopher M. Taylor, for providing feedback and editing suggestions. Texas Tech University Department of Natural Resources Management provided a truck for field sampling and use of laboratory equipment and space.

Texas Parks and Wildlife Department provided the funding (Traditional Section 6 grant E121) and research permit (number SPR-0709-302) for this study. The staff at Balmorea State Park provided logistical support. Tom Johnson was in particular very helpful in providing information about the park.

Several volunteers gave their free time to help us out in the field and their contributions are appreciated. Carter Breuning, Cameron Gallegos, Evan Gray, Jimmy Huddleston, Jacob Howard, and Hallie Ladd helped me collect data and provided much needed field and lab assistance.

Table of Contents

Preface and Acknowledgements.....ii

Abstract.....v

List of Tables.....vi

List of Figures.....vii

I. Balmorhea State Park and San Solomon Springs.....1

 Literature cited.....3

II. Density and habitat associations of native and exotic snails in San Solomon Springs.....5

 Abstract.....5

 Introduction.....5

 Methods.....8

 Results.....12

 Discussion.....41

 Literature cited.....49

III. Effects of substrate size on snail distribution.....55

 Abstract.....55

 Introduction.....55

 Methods.....56

 Results.....57

 Discussion.....59

 Literature cited.....60

IV. Summary, management implications, and future directions.....62

 Literature cited.....65

V. Parasite presence in *Melanoides tuberculata* in San Solomon Springs, Balmorhea State Park.....67

 Abstract.....67

 Introduction.....67

 Methods.....68

 Results and discussion.....68

 Literature cited.....69

VI. Does the presence of other snail species affect substrate selection in

<i>Pyrgulopsis texana, Melanoides tuberculata, and Tarebia granifera?</i>	70
Abstract.....	70
Introduction.....	70
Methods.....	71
Results.....	71
Discussion.....	72
Literature cited.....	73
VII. Desiccation resistance of two aquatic invasive snails, <i>Melanoides tuberculata</i>, and <i>Tarebia granifera</i>	75
Abstract.....	75
Introduction.....	75
Methods.....	76
Results.....	77
Discussion.....	77
Literature cited.....	80

Abstract

Isolated springs in West Texas are fragile systems and the two biggest threats to these systems are habitat loss and nonnative species. San Solomon Springs, located in the Chihuahuan Desert ecoregion, is a refuge for native and endangered aquatic species. Two native snail species, which are candidates for federal listing, the Phantom Cave Snail (*Pyrgulopsis texana*) and the Phantom Spring tryonia (*Tryonia cheatumi*), have a very limited distribution and rely on San Solomon Springs located at Balmorhea State Park. Two exotic snails have established populations in San Solomon Springs, the red rim melania (*Melanoides tuberculata*) and the quilted melania (*Tarebia granifera*). Snails are important components of freshwater systems and understanding habitat usage is necessary for recognizing community patterns and improving conservation efforts of native species. We conducted a field study looking at habitat associations of the two native and two exotic snails at San Solomon Springs from May 2010 to May 2011. Our primary goals were to (1) measure habitat parameters, (2) measure snail density, and (3) determine the habitat associations among the four snail species. We also conducted a variety of lab experiments to investigate, substrate selection, parasites, and desiccation resistance.

P. texana was the most abundant snail (3.8 million) in San Solomon Springs, with *T. cheatumi* the least abundant (48,000). *P. texana* was associated with harder substrate, higher temperatures, and faster water velocity. In the lab *P. texana* selected gravel substrate over sand or cobble, even in the presence of the invasive snails. Habitat parameters did not account for much of the variation in *T. cheatumi* density in the field, probably as a result of the few occurrences of this snail in our sampling quadrats. *T. cheatumi* was found in only 20 of 396 quadrats sampled. *M. tuberculata* and *T. granifera* were both associated with deeper water and softer substrate. However, in lab substrate experiments *T. granifera* when tested alone preferred gravel substrates, but when tested with *M. tuberculata* showed no preference, and with *P. texana* it avoided sand substrate. *M. tuberculata* when tested alone or with other snail species showed little preference for sand, gravel or cobble substrates.

One of the main concerns with *M. tuberculata* is that it can host a variety of parasites that can be detrimental to native fish, and humans. We found no trematodes in the 350 *M. tuberculata* collected from the main pool at Balmorhea State Park in March and April of 2012. This does not prove that trematodes are not present in *M. tuberculata*. Prevalence of trematodes can be fairly low and patchily distributed within habitats, making detection difficult.

Understanding the abiotic tolerances of *M. tuberculata* and *T. granifera* may help control and prevent the spread of these two invasive species. Consequently we conducted a laboratory investigation into their resistance to desiccation. There was a significant difference between the two species as well as between spring systems. *M. tuberculata* was more resistant to desiccation than *T. granifera*. In San Solomon Springs *M. tuberculata* could survive losing a greater percentage of body water than *T. granifera*. The predicted percent water weight loss that would result in 50% of the individuals expiring (LD50) was 35% and 20 % for *M. tuberculata* and *T. granifera* respectively.

List of Tables

2.1	Mean concentrations of chemical species in the pool, wetland, and canal. Numbers in parentheses are standard deviations.....	26
2.2	Mean water depth and velocity in the pool, wetland, and canal. Numbers in parentheses are standard deviations.....	27
2.3	Substrate type in each habitat. Numbers are the frequency of a given substrate type in sampled quadrats (multiply by 100 to get percentage). For example, 100% of the pool quadrats contained concrete habitat, 26% contained fine grain sediment, etc. C=concrete, Fg=fine grain (0-0.25 mm), Sa=sand (0.26-2.00 mm), Gr=gravel (2.01-4.00 mm), Pe=pebble (4.00-64 mm), Cob=cobble (65-256 mm), B=boulder (>256 mm), <i>Clad</i> = <i>Cladophora</i> spp., <i>Mac</i> =macrophyte.....	27
2.4	Diatom composition from periphyton samples in the pool, wetland, and canal. Numbers are percent composition of samples. <i>Gom</i> = <i>Gomphonema</i> spp., <i>Dia</i> = <i>Diatoma</i> spp., <i>Nav</i> = <i>Navicula</i> spp., <i>Fra</i> = <i>Fragilaria</i> spp., <i>Nit</i> = <i>Nitzschia</i> spp., <i>Cymb</i> = <i>Cymbella</i> spp., <i>Tab</i> = <i>Tabellaria</i> spp.....	28
2.5	Mean densities (snails/m ²) of four snail species in each habitat. Numbers in parentheses are standard errors.....	33
2.6	Mean abundance estimates for all four snail species at Balmorhea State Park by habitat. Number in parentheses are standard deviations.....	38
2.7	Mean snail length (in mm) for the exotic <i>Melanoides tuberculata</i> and <i>Tarebia granifera</i> in Balmorhea State Park by habitat. Numbers in parentheses are standard deviations.....	38
2.8	Summary of RDA results for the entire park showing significant habitat variables ($P < 0.05$) marked by asterisks.....	42
3.1	Percentage of total individuals of <i>M. tuberculata</i> , <i>T. granifera</i> , and <i>P. texana</i> found in each substrate type.....	57
6.1	Mean (SD) frequency of <i>P. texana</i> , <i>M. tuberculata</i> , and <i>T. granifera</i> occurrence in each substrate type across 16 trials of paired species combinations.....	72
7.1	Experimental design for snail desiccation tolerance experiment.....	76
7.2	Summary results of ANOVA of percent weight loss of <i>M. tuberculata</i> from two spring systems exposed to the atmosphere for one to 16 days.....	77

List of Figures

2.1 Aerial photo of Balmorhea State Park (2008) provided by Google Earth. The pool is the source for water draining into the refuge canal and the reconstructed wetland. The spring head is also located in the pool. Image acquired from Google Earth on 2 April 2011. Date of imagery 31 March 2008. Location 13R 616052.32E 3424092.47N elev. 1028 m.....9

2.2 Stratified random sampling scheme for Balmorhea State Park. The pool (a) was divided into three sections with the south wing having two Sampling sites and the center and north wing sections having 6 and 4 sites respectively. In the wetland (b), each of three sections was 53 m long and contained 4 sampling sites. The refuge canal (c) was divided into 6 sections, each 100 m long and each section had 2 sampling sites. Sampling points were randomly generated so results would represent the entire habitat, therefore, we did not revisit the same sites.....10

2.3 Figure 2.3--Temporal changes in mean temperature from May 2010 to May 2011 in the pool (N=11) from monthly sampling (a) and iButton data loggers (b). Horizontal reference line represents the overall mean (24.82 °C). Data were recovered from iButton data logger set up to record temperature every two hours. Horizontal line represents the mean for the one year sampling period (24.23 °C). *n*=4,394 points.....13

2.4 Temporal changes in mean pH from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (7.09). *n*=11.....14

2.5 Temporal changes in mean specific conductance from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (3.127 mS). *n*=11.....14

2.6 Temporal changes in mean TDS from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (1.93 ppt). *n*=11.....15

2.7 Temporal changes in mean turbidity from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (3.88 NTU). *n*=11.....15

2.8 Figure 2.8--Temporal changes in mean temperature from May 2010 to May 2011 (*n*=11) in the wetland from (a) monthly sampling and (b) iButton data loggers. Horizontal reference line represents the overall mean (23.98 °C). Data were recovered from iButton data loggers set up to record temperature every two hours. Horizontal line in (b) represents the mean for the one year sampling period (19.60 °C). *n*=4,966 points.....17

2.9 Temporal changes in mean pH from May 2010 to May 2011 in the

reconstructed wetland. Horizontal reference line represents the overall mean (7.22). $n=11$17

2.10 Temporal changes in mean specific conductance from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (3.25 mS). $n=11$18

2.11 Temporal changes in mean TDS from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (2.04 ppt). $n=11$18

2.12 Temporal changes in mean turbidity from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (9.12 NTU). $n=11$19

2.13 Figure 2.13--Temporal changes in mean temperature from May 2010 to May 2011 ($N=11$) in the refuge canal from monthly sampling (a) and iButton data loggers (b). Horizontal reference line represents the overall mean (24.82°C). Data were recovered from iButton data loggers set up to record temperature every two hours. Horizontal line represents the mean for the sampling period (24.13°C). $n=3,113$ points.....20

2.14 Temporal changes in mean pH from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (7.03). $n=11$21

2.15 Temporal changes in mean specific conductance from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (3.13 mS).....21

2.16 Temporal changes in mean TDS from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (1.93 ppt). $n=11$22

2.17 Temporal changes in mean turbidity from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (3.60 NTU). $n=11$22

2.18 Boxplot of spatial temperature variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.....23

2.19 Boxplot of spatial pH variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.....24

2.20 Boxplot of specific conductance variation in the pool ($n=132$),

wetland ($n=132$), and canal ($n=132$) from monthly sampling.
Different letters indicate significant differences.....24

2.21 Boxplot of total dissolved solids variation in the pool ($n=132$),
wetland ($n=132$), and canal ($n=132$) from monthly sampling.
Different letters indicate significant differences.....25

2.22 Boxplot of turbidity variation in the pool ($n=132$),
wetland ($n=132$), and canal ($n=132$) from monthly sampling.
Different letters indicate significant differences.....25

2.23 Temporal changes in mean density for *P. texana* (a), *M. tuberculata*
and *T. granifera* (b), and *T. cheatumi* (c) in the pool. We conducted
two sampling events in May.....29

2.24 Temporal differences in mean density for *P. texana* (a) and
M. tuberculata and *T. granifera* (b) in the wetland. Note that
no *P. texana* were observed after June 2010.....31

2.25 Temporal changes in mean density for *P. texana* (a),
M. tuberculata and *T. granifera* (b), and *T. cheatumi* (c) in
the refuge canal.....32

2.26 Bar graph of mean density for each snail species in the pool.
Snail density is being compared among the three pool section.
No individuals of any species were found in the south wing.
Bars represent standard error.....33

2.27 Bar graph of mean density for each snail species in the wetland
by section. No individuals of *T. cheatumi* were found in the
wetland.....34

2.28 Bar graph of mean density for each snail species in the refuge
canal by section. Each section is 100 m in length and therefore
the above figure also represents longitudinal trends.....35

2.29 Regression comparing distance from the spring head and
mean density in *Melanoides tuberculata* over the entire park.
Each dot represents the mean density of a quadrat over
the 11 sampling trips.....36

2.30 Regression comparing distance from the spring head and mean
density in *Tarebia granifera* over the entire park. Each dot represents
the mean density of a quadrat over the 11 sampling trips.....36

2.31 Regression comparing distance from the spring head and mean density

in *P. texana* over the entire park. Each dot represents the mean density of a quadrat over the 11 sampling trips.....37

2.32 Regression comparing distance from the spring head and mean density in *T. cheatumi*. Each dot represents the mean density of a quadrat over the 11 sampling trips.....37

2.33 Size class (in mm) frequencies for *M. tuberculata* across all habitats within Balmorhea State Park organized by season.....39

2.34 Size class (in mm) frequencies for *T. granifera* across all habitats within Balmorhea State Park organized by season.....40

2.35 Joint plot of significant habitat variables associated with variation in snail density in Balmorhea State Park.....41

3.1 Substrate selection results for *P. texana*.....58

3.2 Substrate selection results for *M. tuberculata*.....58

3.3 Substrate selection results for *T. granifera*.....59

6.1 Chamber used in experimental trials, separated into three equally sized sections containing three substrates, sand, gravel, and cobble.....71

6.2 Substrate selection experiments of *P. texana* when tested with *M. tuberculata*. A) Substrate choice of *P. texana* when tested with *T. granifera* B). Values are the mean fraction of snails found on each substrate type across 16 trials and error bars are standard deviations.....72

7.1 Percent weight loss by days of out of water of *M. tuberculata* from Diamond Y (in red) and San Solomon Springs (in blue).....78

7.2 Probability of survival in relation to percent weight loss (water weight) of *M. tuberculata* from San Solomon Springs.....78

7.3 Probability of survival in relation to percent weight loss (water Weight) of *T. granifera* from San Solomon Springs.....79

Chapter I

Balmorhea State Park and San Solomon Springs

San Solomon Springs is part of Balmorhea State Park (Reeves Co., West Texas), a 49.5 acre park in the Chihuahuan Desert ecoregion (Texas Parks and Wildlife Department [TPWD], 2011). The main attraction at the park is the large concrete pool (7.158 m²) built over the springhead of San Solomon Springs. The pool is a popular site for swimmers and SCUBA divers, and the park is visited by birders, campers, and tourists from across the country. In 2007 over 52,000 visitors found their way to this oasis in the desert (TPWD, 2011). Balmorhea State Park provides habitat for several species; the most prominently featured are the endangered Comanche Springs pupfish (*Cyprinodon elegans*) and the endangered Pecos gambusia (*Gambusia nobilis*). The Comanche Springs pupfish also used to occur in Comanche Springs at Fort Stockton (Pecos Co., West Texas), but once the springs dried, this population was extirpated (Hubbs and Howells, 1993) and now the only remaining populations of this fish are found in the waters in Balmorhea. Other fauna found on park grounds include spiny softshell turtles (*Apalone spinifera*), Mexican tetra (*Astyanax mexicanus*), largespring gambusia (*Gambusia geiseri*), water snakes (*Nerodia* spp.) and several bird species with the green heron (*Butorides virescens*) and the belted kingfisher (*Megaceryle alcyon*) being common.

The land where Balmorhea State Park now stands had great importance for farmers and settlers. Spanish explorers visited this area in the 16th century and hundreds of years later settlers arrived and took advantage of the generous flow from San Solomon Springs and constructed irrigation ditches. In 1915, the Reeves County Water District No. 1 was established and instigated the irrigation of nearly 12,000 acres of farmland with water from the springs (TPWD, 2004). In 1934, the State Parks Board acquired the land and the civilian conservation corps began constructing the large swimming pool. The park opened to the public in 1968 and became an important site for visitors and travelers heading to the Davis Mountains (TPWD, 2004). In 1976, TPWD staff constructed a refuge canal for the Comanche Springs pupfish and the Pecos gambusia and in the 1990s a reconstructed desert wetland (also called a ciénega) was built as a refuge for the fish. The water for both is recharged by water flowing from the pool where the spring head is located. A second reconstructed wetland was finished in late 2010 and can be found near the entrance of the park.

The source of the water for San Solomon Springs has a history of contention among scientists. Some state that the majority of the waters come from the nearby Davis Mountains (White et al., 1941; Couch, 1978) while others state that the principal water source are the Apache mountains to the west (Harden, 1972). Based on groundwater chemistry and isotopes, Chowdhury et al. (2004) concluded that the waters originate from both the Delaware and Apache Mountains and also state that during intense rainfall, water may also come from the Davis Mountains. San Solomon Springs is not the only spring found in the Balmorhea area. Phantom Lake, Giffin, Saragosa, East Sandia, and West Sandia springs are also found in this region. However, not all of these springs are suitable habitat due to water loss and Phantom Lake Spring discharge has been declining since the 1930's (Brune and Besse, 2002) and flow ceased completely in 2001 (United

States Bureau of Reclamation, 2011). This makes San Solomon Springs very important habitat for fish due to its stable water levels and water chemistry.

While the fish populations at San Solomon Springs have received attention in the scientific literature (Hubbs et al., 2002; Brannan et al., 2003), less is known about the invertebrate populations. The purpose of our research is to address a gap in knowledge concerning the snail populations at Balmorhea State Park. The park is an important habitat for two endemic freshwater springsnails (Gastropoda:Hydrobiidae) that are candidates for federal listing, the Phantom Cave snail (*Pyrgulopsis texana*) (recently reclassified from the genus *Cochliopa* by Hershler et al. (2010) and the Phantom Spring tryonia (*Tryonia cheatumi*). These two snails are in the family hydrobiidae, a diverse group of 306 known species in 28 genera (Thorp and Covich, 2009). In the American West, hydrobiids are often endemic to isolated springs and seeps (Hershler and Landye, 1988). *P. texana* and *T. cheatumi* only occur in Phantom Lake Spring in Jeff Davis Co., TX and East Sandia Spring and San Solomon Springs in Reeves Co., TX (Hershler et al., 2010; United States Bureau of Reclamation, 2011). San Solomon Springs is the largest spring in the Balmorhea area (Brune and Besse, 2002) and therefore the habitat at Balmorhea State Park is very important for the conservation of these two snail species.

Two exotic invasive snail species are also found in the park (Gastropoda: Thiariidae), the red rim melania (*Melanoides tuberculata*) and the quilted melania (*Tarebia granifera*). The quilted melania was discovered by our lab in 2010. Both snails have invaded many North American freshwater habitats and are common worldwide invaders (Mitchell et al., 2005). The quilted melania (*T. granifera*) is native to Africa (Appleton and Nadasan, 2002), India, Japan, and Hawaii and was introduced to Florida in the 1940s via the aquatic plant and aquarium trade (Abbott, 1952) and populations have been established in Texas since at least 1964 (Karatyev et al., 2009). *T. granifera* and *M. tuberculata* reproduce asexually, which allows the snail to become easily established once a habitat has been colonized (Vargas et al., 1991) and one researcher reported densities exceeding 10,000 snails/m² (Miranda et al., 2010). Red rim melania (*M. tuberculata*) is native to Africa and Asia and was introduced to the U.S. in 1964 via the aquarium trade (Murray, 1964). It is distinguished from *T. granifera* by having a longer adult shell length and a light brown shell color with pronounced red bands rather than ridges (Thompson, 1984; Pointier, 2001). *M. tuberculata* can also become very dense and Freitas and Santos (1995) reported densities of *M. tuberculata* near 16,000 snails/m² during their field study.

Habitat requirements for freshwater snails are wide and varied and some show a high degree of specialization for a particular habitat (Dillon, 2000). Little is known about the habitat parameters (*e.g.* water temperature, pH, substrate type) that influence the distributions of the four above mentioned snails. Our field study at Balmorhea State park had three goals: (1) estimate the density and population size of *M. tuberculata*, *T. granifera*, *P. texana*, and *T. cheatumi*; (2) measure water quality and habitat parameters to serve as a baseline dataset for any future studies; and (3) relate snail abundance to measured habitat parameters to determine if these snails show evidence of non random spatial distribution. These three goals are covered in Chapter II. After we completed the field work, lab experiments were conducted to test the results of our findings in the field. This experiment is covered in Chapter III.

Literature cited

- Abbott, R.T. 1952. A study on an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (*Paragonimus*). Proceedings of the United States National Museum 102:71-116.
- Appleton, C.C., and D.S. Nadasan. 2002. First report of *Tarebia granifera* (Lamarck, 1816) (Gastropoda: Thiaridae) from African Journal of Molluscan Studies 68:399-402.
- Brannan, D.K., C.R. Brannan, and T.E. Lee Jr. 2003. Reproductive and territorial behavior of Comanche Springs pupfish (*Cyprinodon elegans*) in San Solomon Spring pool, Balmorhea State Park, Reeves County, Texas. The Southwestern Naturalist 48(1):85-88.
- Brune, G., and H.C. Besse. 2002. Springs of Texas. Texas A&M University press. 590pp.
- Chowdhury, A. H., C. Ridgeway, and R.E. Mace. 2004. Origin of the waters in the San Solomon Spring system, Trans-Pecos Texas: in Mace, R. E., Angle, E. S., and Mullican, W.F., III, eds., Aquifers of the Edwards Plateau: Texas Water Development Board Report 360, p. 315-344.
- Couch, H.E. 1978. Study of the lower Cretaceous and associated aquifers in the Balmorhea district of Trans-Pecos. Texas: Department of Water Resources, 61 pp.
- Dillon, R.T. 2000. The ecology of freshwater molluscs. The Press Syndicate of the University of Cambridge, United Kingdom, 509pp.
- Freitas J.R., and M.B.L. Santos. 1995. Current advances on the study of snail-snail interactions, with special emphasis on competition process. Memórias do Instituto Oswaldo Cruz 90:261-269.
- Hall, R.O., J.L. Tank, and M.F. Dybdahl. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Research Communications 1(8):407-411.
- Harden, R.W. 1972. Groundwater conditions in the vicinity of Phantom Lake, Giffin, and San Solomon Springs, Reeves and Jeff Davis counties, Texas: consultant report to Texas Water Rights Commission on behalf of the Reeves County Water Improvement District 1.
- Hershler, R., and J.J. Landye. 1988. Arizona hydrobiidae (Prosobranchia: Rissoaca). Smithsonian Contributions in Zoology 459:1-63.
- Hershler, R. 1999. A systematic review of the hydrobiid snails of the Great Basin, western United States. Part II. Genera *Colligyrus*, *Eremopyrgus*, *Fluminicola*, *Pristinicola* and *Tyronia*. The Veliger 42(4):306-337.
- Hershler R., H.P. Liu, and B.K. Lang. 2010. Transfer of *Cochliopa texana* to *Pyrgulopsis* (Hydrobiidae) and description of a third congener from the lower Pecos River basin. *Journal of Molluscan Studies* 76(3):245-256.

- Hubbs, C., and R.G. Howells. 1993. Fishes of the refugium at Balmorhea State Park. Balmorhea State Park, 8pp.
- Hubbs, C., R.J. Edwards, and G.P. Garret. 2002. Threatened fishes of the world: *Gambusia nobilis*, Baird & Girard, 1853 (Poeciliidae). Environmental Biology of Fishes 64(4):428.
- Karatyev, A.Y., L.E. Burlakova, V.A. Karatyev, and D.K. Padilla. 2009. Introduction, spread, and impacts of exotic freshwater gastropods in Texas. Hydrobiologia 619:181-194.
- Miranda N., R. Perissinotto, and C.Appleton. 2010. Salinity and temperature tolerance of the exotic freshwater gastropod *Tarebia granifera*. South African Journal of Science 106(3/4):1-7.
- Mitchell, A.J., R.M. Overstreet, A.E. Goodwin, and T.M. Brandt. 2005. Spread of an exotic fish-gill trematode: a far reaching and complex problem. Fisheries 30(8): 11-16.
- Murray, H.D. 1964. *Tarebia granifera* and *Melanooides tuberculata* in Texas. Annual Reports of the American Malacological Union 1964:15–16.
- Pointier, J.P. 2001. Invading freshwater snails and biological control in Martinique Island, French West Indies. Memórias do Instituto Oswaldo Cruz 96:67-74.
- Texas Parks and Wildlife Department. 2004. Rebirth of a desert wetland: the San Solomon Ciénega. Texas Parks and Wildlife Department. 12pp.
- Texas Parks and Wildlife Department. 2011. Balmorhea State Park. Available from: <http://www.tpwd.state.tx.us/spdest/findadest/parks/balmorhea/>. Accessed on 11 October 2011.
- Thompson, F.G. 1984. The freshwater snails of Florida: a manual for identification. University of Florida Press, Gainesville. 94p.
- Thorp, J.H., and A.P. Covich. 2009. Ecology and Classification of North American Freshwater Invertebrates. Elsevier Science, 1021pp.
- United States Bureau of Reclamation. 2011. Final Environmental Assessment: ciénega wetland rehabilitation at Phantom Lake Spring, Toyavale, Texas. Finding of no significant impact, 47pp. Report number AAO-11-003.
- Vargas, M., J. Gomez, and G. Perera. 1991. Geographic expansion of *Marisa cornuarietis* and *Tarebia granifera* in the Dominican Republic. Journal of Medical & Applied Malacology 3:69-72.
- White, W.N., H.S. Gale, and S.S. Nye. 1941. Geology and groundwater resources of the Balmorhea area, western Texas. U.S. Geological Survey Water-Supply, 50pp.

Chapter II

Density and habitat associations of native and exotic snails in San Solomon Springs

Abstract

We sampled San Solomon Springs within Balmorhea State Park to investigate the snail community and to determine species habitat correlations. We calculated the densities of two exotic snails: red rim melania (*Melanoides tuberculata*) and the quilted melania (*Tarebia granifera*), and two endemic springsnails: the Phantom Cave springsnail (*Pyrgulopsis texana*) and the Phantom Spring tryonia (*Tryonia cheatumi*). Snails were counted within 396 randomly generated quadrat locations in the springs within Balmorhea State Park from May 2010 to May 2011. Water quality parameters were also measured at each site (water temperature, specific conductance, pH, total dissolved solids, turbidity, and percent dissolved oxygen). We also measured physical characteristics, *i.e.* flow rate and depth. Water samples were taken to measure calcium and magnesium concentrations. Diatoms were identified and enumerated from periphyton samples scraped from substrates. We also measured canopy cover semi-quantitatively using a spherical densiometer. Snail density differed among sites and redundancy analysis showed that physical characteristics such as substrate type, velocity, and depth were the most important variables for explaining snail distribution in San Solomon Springs.

The most common snail in the park was the native *Pyrgulopsis texana*, which had an estimated abundance of 3,853,650 individuals in the park. The least abundant snail was the native *Tryonia cheatumi*, which had an estimated abundance of 47,733 snails. Habitat variables that explained the most variation in snail density for *P. texana* were cobble and *Chara* spp. substrate, temperature, canopy cover, and water velocity. For *T. granifera* and *M. tuberculata* the variables that explained variation in density were depth and fine grain and pebble substrate. *T. granifera* and *M. tuberculata* were negatively associated with distance from the spring head. Based on these results, future management decisions at Balmorhea State Park should include providing larger harder substrate in the two reconstructed wetlands and improving water flow.

Introduction

The Phantom Cave snail (*Pyrgulopsis texana*) and the Phantom Spring tryonia (*Tryonia cheatumi*) are two springsnail species native to the western U.S. and found only in three spring systems in West Texas (Phantom Lake, San Solomon, and East Sandia Springs). They are members of hydrobiidae, the most diverse gastropod family in North America. Many hydrobiid species in the western U.S. are endemic to isolated spring seeps and spring fed rivers (Brown et al., 2008). This local endemism of a couple to a few gastropod species within a spring system is common in the arid American southwest (Pratt, 1977). Hydrobiids have small (1.5-3 mm), smooth shells, though individuals within a population can show high variation in shell morphology. In North America, there are 306 known species in 28 genera. Hydrobiidae is also an imperiled group, with 74% of species in the United States at risk for extinction (Thorp and Covich, 2009). Both

Pyrgulopsis texana and *Tryonia cheatumi* are candidate species for the Federal Endangered Species List and both are high priority species on the Texas Priority Species List (United States Department of the Interior, 2004). *P. texana* and *T. cheatumi* are important components of the stream community, grazing on periphyton and detritus, and are prey items for several *Gambusia* species and the endangered *Cyprinodon elegans* (Brown et al., 2008). *P. texana* is a small snail measuring 1-1.4 mm in length (Dundee and Dundee, 1969). *T. cheatumi* is larger, but still small at 2.9 to 3.6 mm long (Taylor, 1987). The shell of *T. cheatumi* is more conical than the shell of *P. texana*.

Very little is known about life history and microhabitat preferences of *P. texana* and *T. cheatumi*, though other snails in the genus *Pyrgulopsis* and *Tryonia* have been studied. *Pyrgulopsis* spp. prefers habitat with high structural complexity, highly oxygenated waters and is positively correlated with water velocity (Martinez and Thome, 2006). Reproduction is sexual in contrast to the asexual quilled and red rim melania and recruitment occurs year round in thermally stable habitat, with seasonality generally only noticed in colder systems (Martinez and Thome, 2006). *Pyrgulopsis* species are usually oviparous and deposits eggs on hard substrate while *Tryonia* spp. is ovoviviparous (Hershler and Thompson, 1992; Hershler et al., 2005); however, neither of these strategies has been confirmed in *P. texana* or *T. cheatumi*.

Thirty-seven nonnative snail species are currently found in North America (Brown et al., 2008). Field studies and controlled experiments with several of these species show that exotic snails can cause severe negative impacts to ecosystems. The exotic New Zealand mud snail, for example, is easily established in nonnative habitat and may reach densities up to 50,000 snails/m² (Hall et al., 2006). This can affect nutrient cycling and place competitive stress on native snails. Exotic apple snails (*Pomacea insularum*) are also known to negatively affect native predators. In Florida, the endangered snail kite depends on the native Florida apple snail (*Pomacea paludosa*), but populations are being replaced by the exotic apple snail (*Pomacea insularum*) and juvenile snail kites feeding on *P. insularum* are expected to suffer from energetic deficiencies (Cattau et al., 2010). High densities of exotic snails may also increase the presence of avian predators which may also feed on sympatric native snails (Anderson et al., 1984). The effects of exotic snails are not just limited to competitive exclusion and predator effects. The introduction of exotic parasites and disease is another phenomenon associated with some exotic snails. The gill trematode (*Centrocestus formosanus*), a parasite introduced to North America via the invasive red rim melania snail (Scholz and Salgado-Maldonado, 2000) damages fish gills and has decreased populations of cichlids and cyprinids in the southern U.S. (Mitchell et al., 2005). In Texas, the trematode is known to infect the endangered fountain darter (*Etheostoma fonticola*) and researchers have found that fish gills containing encysted trematodes are often inflamed (Mitchell et al., 2005). Exotic snails may also alter carbon and nitrogen cycles by dominating primary consumer biomass (Arango, 2009).

While much is known about the impacts exotic snails have, less is known about their distribution, dispersal ecology, and habitat preferences. Understanding habitat preference may provide insights into the types of habitat exotic snails are able to exploit and how they interact with native snails. The goals of our study were to estimate the

densities and abundances and determine the habitat preferences of two native snails and two exotic snails in San Solomon Springs.

The red rim melania (*Melanooides tuberculata*) and the quilted melania (*Tarebia granifera*) are the two exotic snails well established in our study area. Both snails have invaded many North American freshwater habitats and are common worldwide invaders (Mitchell et al., 2005). The life history and physiological ecology of these snails have been studied (Giovanelli et al., 2005), but little is known regarding habitat associations among the red rim melania, quilted melania and other freshwater gastropods (Giovanelli et al., 2005). Although not much is known about these associations, several authors hypothesize that exotic snails may outcompete and displace native invertebrates (Brown et al., 2008). One researcher observed local extirpation of *Biomphalaria* spp., an air breathing freshwater snail, after the introduction of the red rim melania (Pointier and McCollough, 1989). Generally though, the effect of these exotic two exotic snails on native snails is unknown (Giovanelli et al., 2005).

The quilted melania is native to Africa (Appleton and Nadasan, 2002), India, Japan, and Hawaii and was introduced to Florida in the 1940s via the aquatic plant and aquarium trade (Abbott, 1952). Populations have been established in Texas since at least 1964 (Karatyev et al., 2009) and several other Southern U.S. states also have documented populations. Adults have an average shell length of 25 mm, prefer pebble and cobble substrate, and are generally found in slower moving streams, though some populations do exist in riffle areas (Abbott, 1952; Chaniotis et al., 1980). Most adult shells are distinguishable by the presence of prominent ridges (Thompson, 1984). Most individuals are parthenogenetic and ovoviviparous, meaning most known populations are exclusively female and no mating is necessary for reproduction. Parthenogenesis allows *M. tuberculata* and *T. granifera* to easily establish themselves in newly invaded habitat since one individual can start an entire population (Vargas et al., 1991; Ben-Ami and Heller, 2005). Once established, *T. granifera* can become very dense, and one researcher reported densities exceeding 10,000 snails/m² (Miranda et al., 2010). Generally, once established these snails are impossible to remove. There are few known population control strategies, mostly limited to mechanical removal or removal by hand, but these methods are expensive, labor intensive, and have not been shown effective. (Pointier, 2001).

Red rim melania is native to Africa and Asia and was introduced to the U.S. in 1964 via the aquarium trade (Murray, 1964). It is distinguished from *T. granifera* by having a longer adult shell length (30 mm) and a light brown shell color with pronounced red bands rather than ridges (Thompson, 1984; Pointier, 2001). Adults are completely herbivorous and graze on periphyton and detritus in a similar fashion to the quilted melania (Madsen, 1992). Individuals are most often found in slow or stagnant water of moderate pH and temperature (Duggan, 2002) and they thrive in areas impacted by humans (DeMarco, 1999). Reproductive biology is similar to that of the quilted melania with one parthenogenetic individual capable of colonizing and producing offspring to establish a population. This mode of reproduction and ability to colonize and live in impacted habitat leads to high densities. Freitas and Santos (1995) reported densities of *M. tuberculata* at 16,000 snails/m² during their study. Similar to the quilted melania, red rim melania is thought to out-compete native snail populations (Pointier, 2001). The red

rim melania is also a vector for the human blood fluke and the gill trematode. There are no known effective control methods for the snail and invasion is currently considered an irreversible process (Pointier, 2001).

The purpose of this study is to determine the microhabitat preferences of the exotic and native snails in San Solomon Springs by recording density and habitat parameters at randomly selected sites. We will use data from the field study to determine if the snails at San Solomon Springs have a non random spatial distribution. The results will benefit Texas Parks and Wildlife Department by providing abundance data and habitat preferences of two locally endemic species that are candidates for the federal threatened and endangered species list. This knowledge will help direct future management decisions and conservation efforts in Balmorhea State Park.

Methods

Study Site--Sampling occurred from May 2010 to May 2011 in the San Solomon Springs complex located within Balmorhea State Park, a 45.9 acre park found near the Davis Mountains in Reeves Co., TX. San Solomon is an isolated, thermally stable spring that is a refuge for endangered fish such as the Comanche Springs pupfish (*Cyprinodon elegans*) and the Pecos gambusia (*Gambusia nobilis*). This is an artesian spring system that supports endangered and endemic organisms. Historically, San Solomon has been used as a water source by humans since at least 1583 to cultivate corn and peaches, and in the 19th century used to power mills (Brune, 2002), and today the springs are a popular spot for swimmers and SCUBA divers. We measured snail density and habitat parameters in three habitats in the park: a constructed pool built over the springhead of San Solomon Springs, a reconstructed wetland built to mimic historic natural habitat, and a constructed refuge canal that serves as habitat for *C. elegans* and *G. nobilis* (Figure 2.1). The source of the springs is found near the center of the pool and fills the canal and wetland. Water levels in the latter two can be altered via a series of gates.

We used a stratified random sampling technique and divided each habitat into a number of sections in order to achieve representative samples. We divided the pool and wetland into three sections and the canal into six sections (Figure 2.2) and assigned the number of sites to be sampled within each section based on area. The south wing (max depth 1.22 m) of the pool had the smallest area and had two sampling sites while deep center area (max depth 7.62 m) and north wing (6.40 m max depth) were larger and assigned 6 and 4 sampling sites respectively. We divided the wetland into three 53m long sections and assigned each section four sampling sites. We divided the canal into six 100 m long sections each having two sampling sites. This resulted in 12 sites per habitat. Before we began sampling, we selected the location for these sampling sites pseudo randomly using an online kml point generator (GeoMidpoint, 2007) and Google Earth (© 2011 Google). We conducted sampling events every four to six weeks and after one year we sampled 132 random quadrats in each of the three habitats. Sampling was done between 0800 and 1900 hr. Although snails are more active at night, we did not dive and sample after 1900 hr since the pool is closed at sundown.

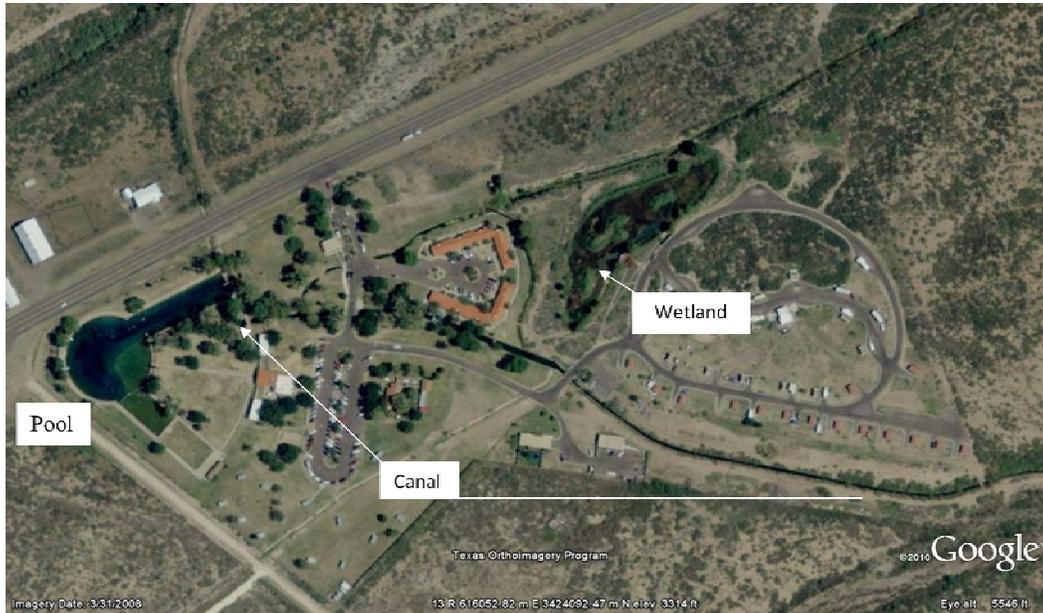


Figure 2.1-- Aerial photo of Balmorhea State Park (2008) provided by Google Earth. The pool is the source for water draining into the refuge canal and the reconstructed wetland. The spring head is also located in the pool. Image acquired from Google Earth on 2 April 2011. Date of imagery 31 March 2008. Location 13R 616052.32E 3424092.47N elev. 1028 m.

Field methods--At each sampling site we recorded the number of snails within a 0.04 m² (20 cm X 20 cm) quadrat and recorded shell lengths for *M. tuberculata* and *T. granifera* using digital calipers. We did not measure shell lengths of native *P. texana* and *T. cheatumi* since we wanted to minimize disturbance and had little confidence in our ability to accurately measure such small snails in the field. We standardized the search time for snails at five minutes for each site. Snail counts were made visually and for sites deeper than 1 m, counts were done underwater using an air line hookah diving system (Hookamax, Three Rivers, Michigan). We also collected two water samples in pre-cleaned glass containers at sampling depth and added 70% nitric acid to one sample (to later determine hardness) and 5.25 N sulfuric acid to another sample (to later determine ammonia, nitrate, and phosphate concentrations) until pH<2. We followed EPA guidelines on water collection and water sample analysis (United States Environmental Protection Agency, 1983). We also scraped periphyton samples from substrate and preserved the samples with 5% Lugol's iodine solution. Water samples and periphyton samples were kept in the dark on ice until returning to the lab at Texas Tech. We later determined hardness (CaCO₃), total hardness (CaCO₃ and Mg), ammonia, nitrate, and phosphate concentrations from these water samples in the lab after neutralizing the pH to 7 with ammonium hydroxide (for samples preserved with nitric acid) and sodium hydroxide (for samples preserved with sulfuric acid).

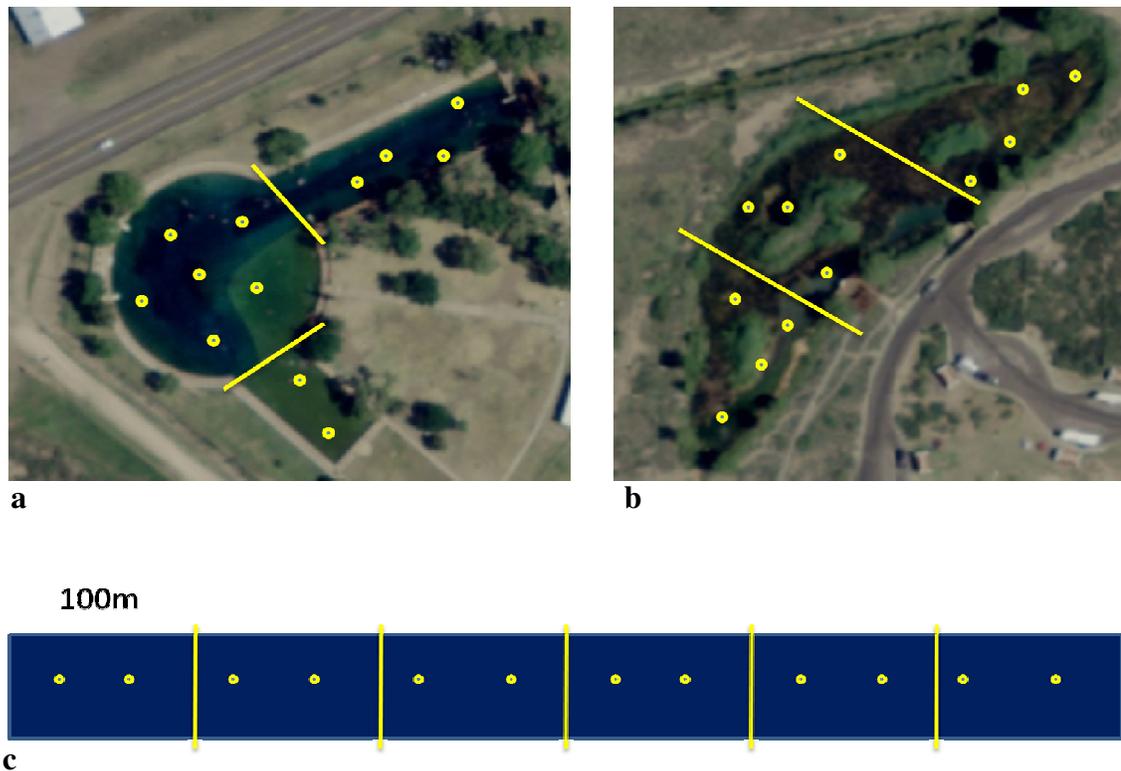


Figure 2.2--Stratified random sampling scheme for Balmorhea State Park. The pool (a) was divided into three sections with the south wing having two sampling sites and the center and north wing sections having 6 and 4 sites respectively. In the wetland (b), each of three sections was 53 m long and contained 4 sampling sites. The refuge canal (c) was divided into 6 sections, each 100 m long and each section had 2 sampling sites. Sampling points were randomly generated so results would represent the entire habitat, therefore, we did not revisit the same sites.

Field methods--At each sampling site we recorded the number of snails within a 0.04 m² (20 cm X 20 cm) quadrat and recorded shell lengths for *M. tuberculata* and *T. granifera* using digital calipers. We did not measure shell lengths of native *P. texana* and *T. cheatumi* since we wanted to minimize disturbance and had little confidence in our ability to accurately measure such small snails in the field. We standardized the search time for snails at five minutes for each site. Snail counts were made visually and for sites deeper than 1 m, counts were done underwater using an air line hookah diving system (Hookamax, Three Rivers, Michigan). We also collected two water samples in pre-cleaned glass containers at sampling depth and added 70% nitric acid to one sample (to later determine hardness) and 5.25 N sulfuric acid to another sample (to later determine ammonia, nitrate, and phosphate concentrations) until pH<2. We followed EPA guidelines on water collection and water sample analysis (United States Environmental Protection Agency, 1983). We also scraped periphyton samples from substrate and preserved the samples with 5% Lugol's iodine solution. Water samples and periphyton samples were kept in the dark on ice until returning to the lab at Texas Tech. We later

determined hardness (CaCO_3), total hardness (CaCO_3 and Mg), ammonia, nitrate, and phosphate concentrations from these water samples in the lab after neutralizing the pH to 7 with ammonium hydroxide (for samples preserved with nitric acid) and sodium hydroxide (for samples preserved with sulfuric acid).

We recorded habitat parameters such as temperature, pH, specific conductance, dissolved oxygen percentage (DO %), and total dissolved solids (TDS) using a portable HANNA multi-parameter meter (HANNA model 9828). We also took measurements of turbidity using a portable fluorometer (Aquafluor handheld fluorometer part no. 8000-010). We recorded canopy cover semi-quantitatively using a spherical densiometer and water velocity using a portable anemometer (MiniAir20, Schiltknecht Messtechnik company, unit model S046). We used a handheld echo sounder (Speedtech, depthmate model SM-5) to record sampling depth and distance from the bank in the pool and canal sites. In the wetland, we used a meter stick and measuring tape for depth and width respectively.

We placed temperature data loggers called iButtons (Maxim Integrated Products, model 1922L#F50) in the pool, wetland, and canal to continuously record temperature over the project year starting in the spring of 2010. Data loggers were attached to rocks about 100 mm in size taken from the study site. We used underwater epoxy to attach the data loggers and based on laboratory trials, epoxy did not affect the functioning of the data loggers. Data loggers were then distributed at randomly generated points using the same method in selecting sampling sites. The pool, wetland, and canal each had six data loggers that were left in place for the duration of the study. Temperature readings were taken every two hours and loggers were retrieved in spring 2011.

Lab methods--In the lab, we determined hardness (and Mg), nitrate, phosphate, and ammonia concentrations (all three in mg/L) within 2 weeks of returning from the field site (samples remain usable for up to 21 days after collection). We neutralized samples fixed with nitric acid using ammonium hydroxide and DI water solution and samples fixed with sulfuric acid with sodium hydroxide. We used HACH titration method 8204 and 8213 (HACH, 2006) to determine hardness as CaCO_3 and total hardness which included Mg. For ammonia, nitrate, and phosphate concentration determination we used HACH methods 8155, 8039, and 8048. We used a spectrophotometer (Bausch & Lomb Spectronic 20) to read % absorbance. The instrument wavelength was set at 655 nm for ammonia, 540 nm for nitrate, and 880 nm for phosphate. Readings were taken as % light absorbance and converted to mg/L using calibration curves we prepared from standard solutions. We used guides and keys by Prescott (1970) and Vinyard (1977) to identify and count diatom valves. Algae and diatom valves were identified by placing periphyton samples on gridded slides and following transects until 150 valves were counted.

Analysis--Density (snails/m²) was estimated by taking the number of snails counted in each quadrat and multiplying by 25. Mean density for each species was calculated by averaging the density across months for each section. For example, pool section 1 (the south wing) was sampled 22 times, so mean density for all snail species was calculated by averaging the density from these 22 sample points. Once the mean densities for each species in each section were determined, these section means were

averaged together to find the mean for the entire habitat. Snail abundance for each species was estimated by multiplying the mean densities for the entire habitat by the bottom area of each habitat. Bottom area was calculated in the canal by taking measured wet widths in each section and multiplying by the length of the section and adding these numbers together to find total area. We used the same method for the wetland. For the north and south wings in the pool, we used measuring tape to find the length and a portable echo sounder to find the depth. We calculated bottom area for section 2 by measuring the length from the center of the pool to the west side (finding the radius) at maximum depth and then plugged those numbers into the equation to find surface area for a hemisphere.

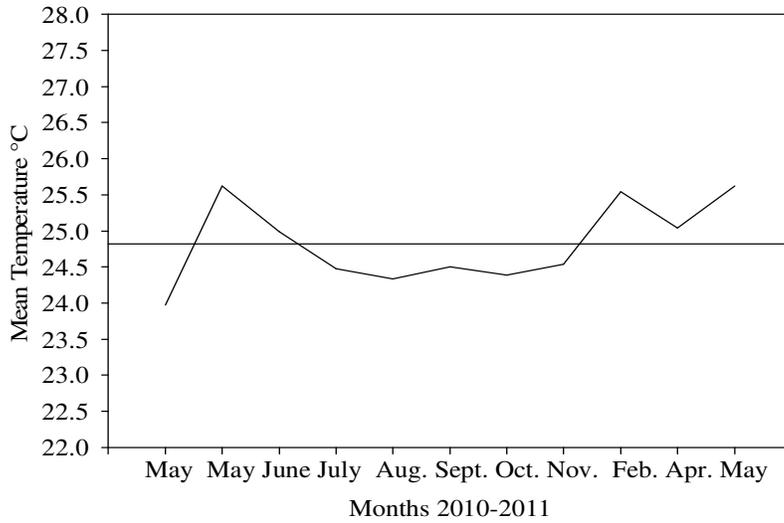
We used the Kruskal-Wallis test to compare differences in density and abundance among the snail species since heteroscedascity was an issue. Post hoc multiple comparisons tests were done in program R using package “pgirmess”. We used regression analysis and ANOVA to determine if changes in density at Balmorhea state park were correlated with distance from the spring head. We used mean densities of each quadrat ($n=12$ for each habitat, but in this analysis we looked at decreases in density over the whole park, so $n=36$).

We used package “vegan” in program R for ordination and ran a redundancy analysis (RDA) for 5000 permutations to develop a model to determine if measured habitats variables were significant in explaining variation in snail density at Balmorhea State Park. Redundancy analysis is a multivariate method of regression that can help find associations between explanatory (habitat measurements) and dependent variables (species abundance) (Makarek and Legendre, 2002). Twelve continuous explanatory variables were used: temperature, specific conductance, pH, percent canopy cover, depth, distance from spring head, total dissolved solids, water velocity, turbidity, calcium concentration, magnesium concentration, and percent dissolved oxygen. Two categorical variables (month and substrate type) were also used. Snail densities were log transformed before inputting them into the RDA models. Program R was used for all statistical analyses and all statistical tests were done at an $\alpha=0.05$.

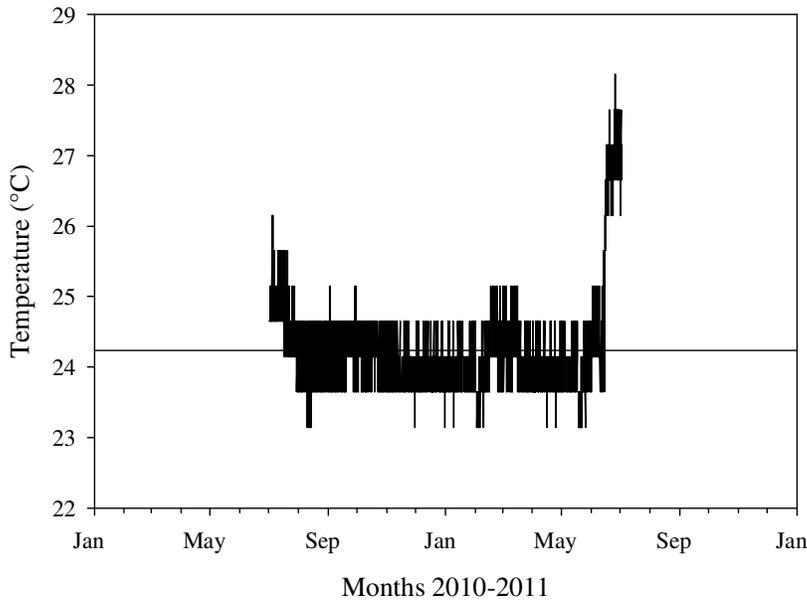
Results

Temporal changes in water quality measurements--Some temporal variation in the measured water quality parameters was observed in the pool wetland and canal. Temperature in the pool ranged from 23.53 °C to 26.92 °C and averaged 24.82 °C (Figure 2.3a). Recovered temperature logger information shows a fluctuation between 23 and 27°C for the entire study period (Figure 2.3b). Thus temperatures from the data logger were similar to data collected in sampling quadrats. Mean temperature was lowest during May 2010 and August 2010 through November 2010. pH ranged from 6.88 to 7.40 and averaged 7.09 (Figure 2.4) with the lowest value occurring in July and the highest value in November. Mean specific conductance in the pool ranged from 1.937 mS to 3.53 mS and reached a peak in June 2010 and decreased in July and August of 2010 (Figure 2.5). TDS ranged from a minimum of 1.75 ppt to 2.95 ppt and had a mean of 1.93 ppt over the sampling period (Figure 2.6). Mean turbidity for the entire sampling period in the pool was 3.88 NTU and ranged from 1.29 to 6.61 NTU. Mean turbidity fluctuated with the

highest levels being found in April and July of 2010 and the lowest in November 2011 which was 2.59 NTU below the mean (Figure 2.7).



a



b

Figure 2.3--Temporal changes in mean temperature from May 2010 to May 2011 in the pool ($n=11$) from monthly sampling (a). Horizontal reference line represents the overall mean (24.82 °C). Data were recovered from iButton data logger set up to record temperature every two hours (b). Horizontal line represents the mean for the one year sampling period (24.23°C). $n=4,394$ points.

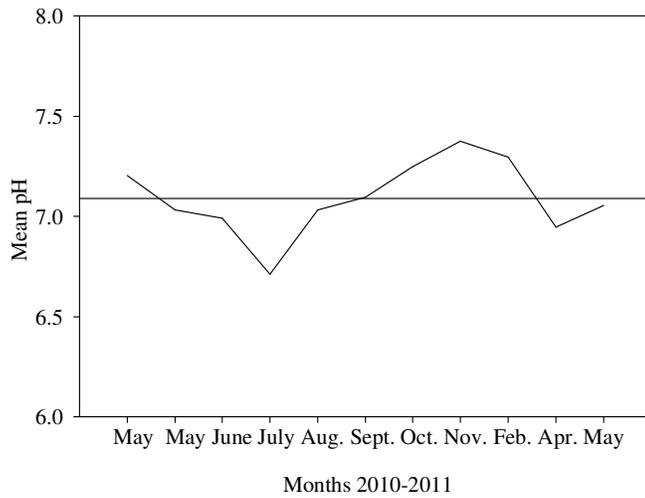


Figure 2.4--Temporal changes in mean pH from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (7.09). $n=11$.

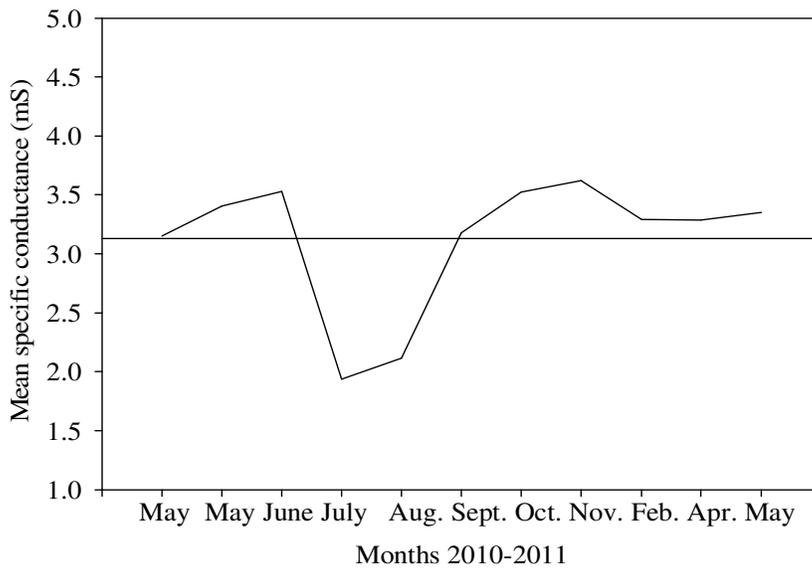


Figure 2.5--Temporal changes in mean specific conductance from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (3.127 mS). $n=11$.

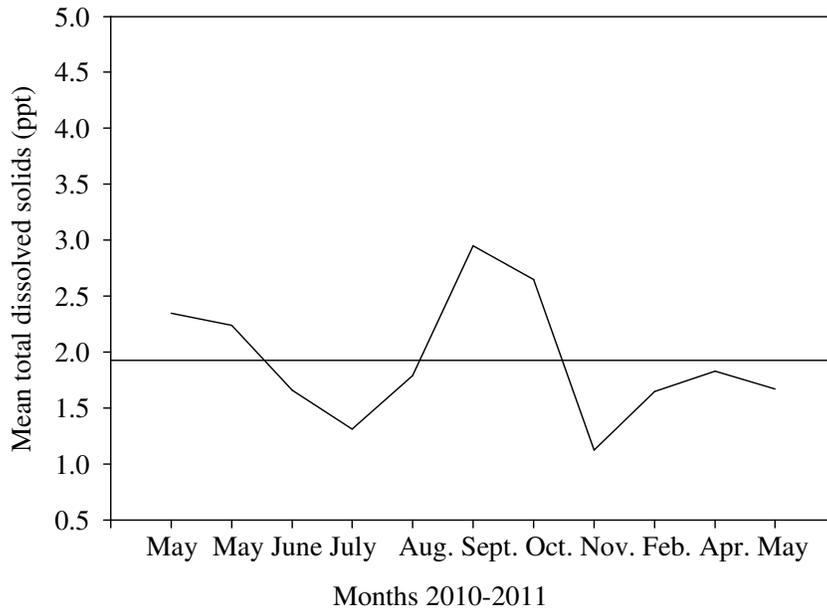


Figure 2.6--Temporal changes in mean TDS from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (1.93 ppt). $n=11$.

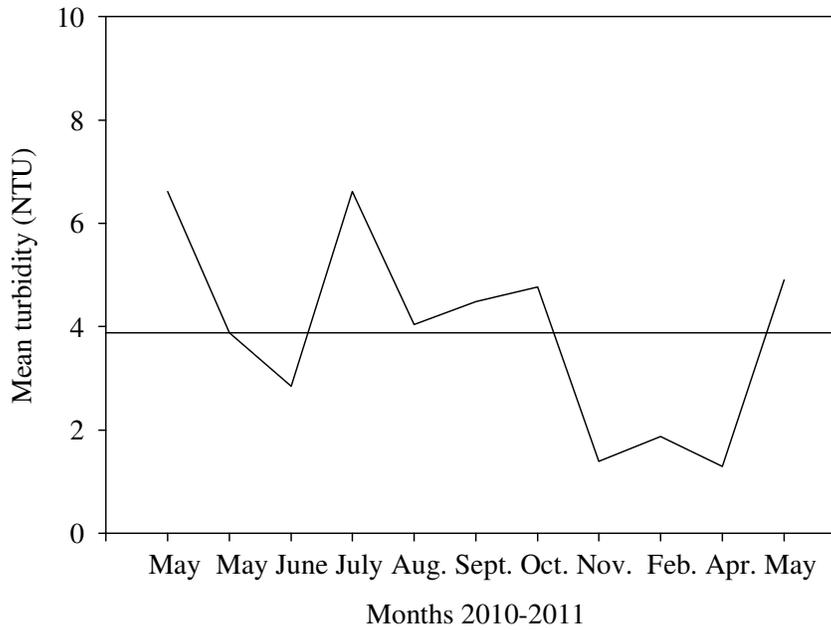
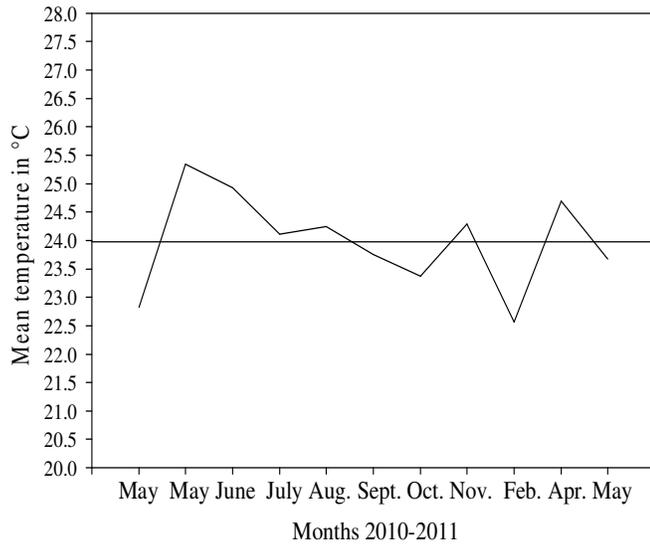
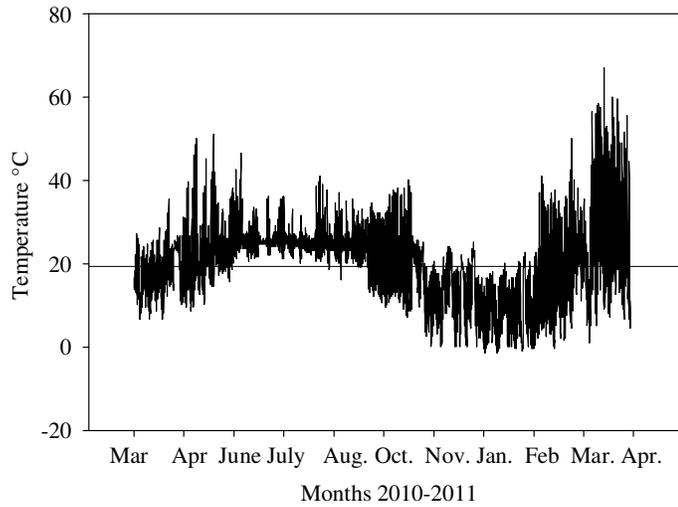


Figure 2.7--Temporal changes in mean turbidity from May 2010 to May 2011 in the pool. Horizontal reference line represents the overall mean (3.88 NTU). $n=11$.

In the wetland, temperature ranged from 22.56 to 25.34 °C and the mean for the entire sampling period was 23.98 °C (Figure 2.8a). Data recovered from iButton data loggers show similar trends to those encountered during field sampling (Figure 2.8b). The recovered data show a peak temperature in May of 46.11 °C. This might have been due to exposure to air conditions since the wetland periodically dried. Temperatures decreased and leveled off in August to around 24.5 °C. Temperatures decreased in October and remained low in November, through February until increasing again in March. Mean pH in the reconstructed wetland ranged from 6.76 to 7.67 with a mean of 7.22 (Figure 2.9). Mean specific conductance ranged from 2.74 mS to 3.64 mS and had a mean of 3.25 mS for the total sampling period (Figure 2.10). Similarly to the pool, mean TDS in the wetland varied throughout the sampling period. TDS ranged from 1.26 ppt to 3.15 ppt with a mean of 2.04 ppt (Figure 2.11). Turbidity in the wetland was higher than the turbidity readings from the pool. Mean turbidity of the wetland ranged from a minimum of 2.86 to 16.68 NTU with an overall mean of 9.12 NTU (Figure 2.12).



a



b

Figure 2.8--Temporal changes in mean temperature from May 2010 to May 2011 ($n=11$) in the wetland from (a) monthly sampling and (b) iButton data loggers. Horizontal reference line represents the overall mean (23.98 °C). Data were recovered from iButton data loggers set up to record temperature every two hours. Horizontal line represents the mean for the one year sampling period (19.60 °C). $n=4,966$ points.

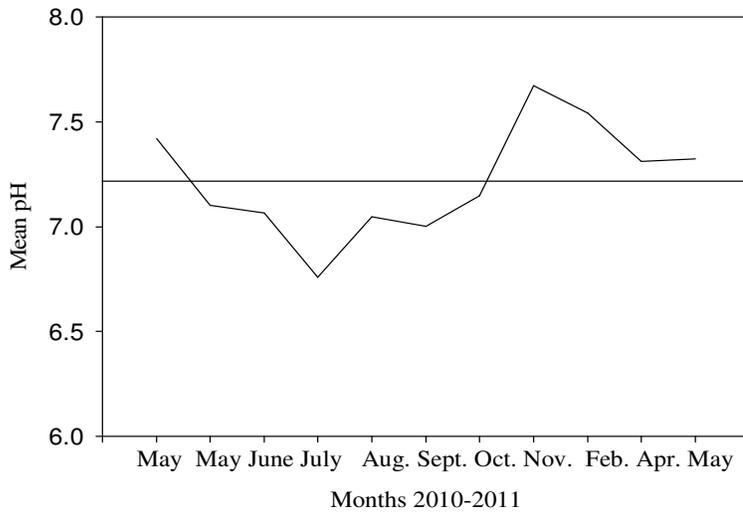


Figure 2.9--Temporal changes in mean pH from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (7.22). $n=11$.

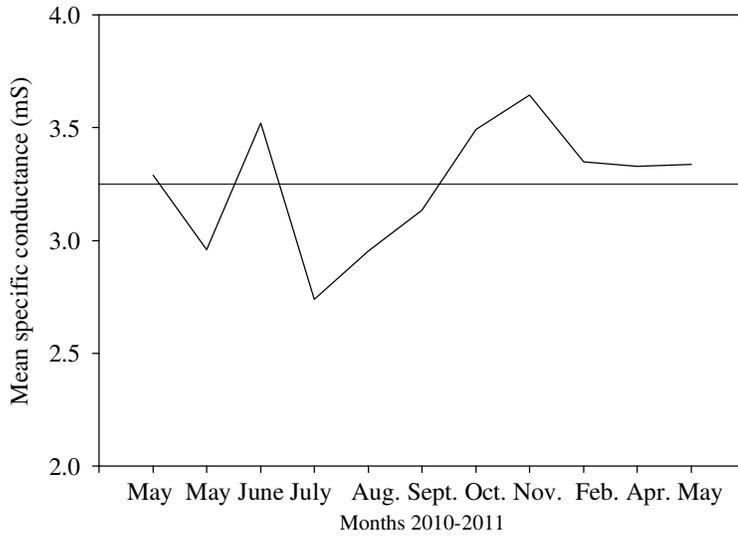


Figure 2.10--Temporal changes in mean specific conductance from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (3.25 mS). *n*=11.

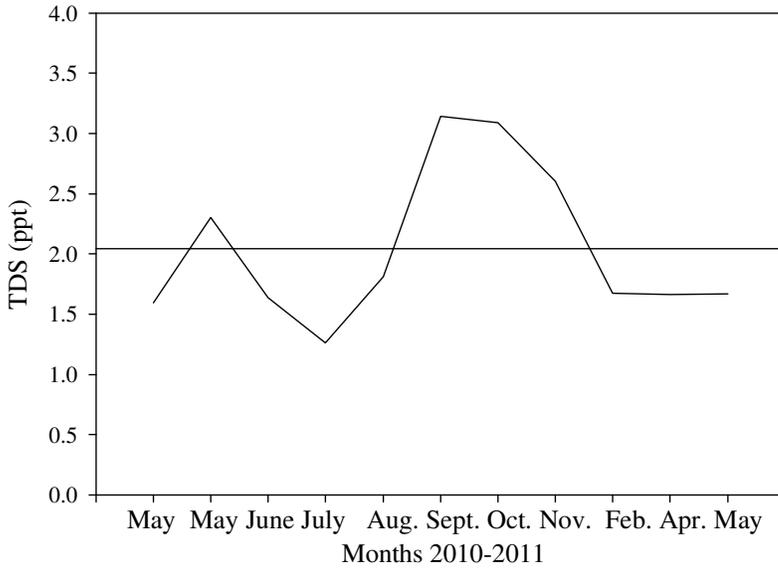


Figure 2.11--Temporal changes in mean TDS from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (2.04 ppt). *n*=11.

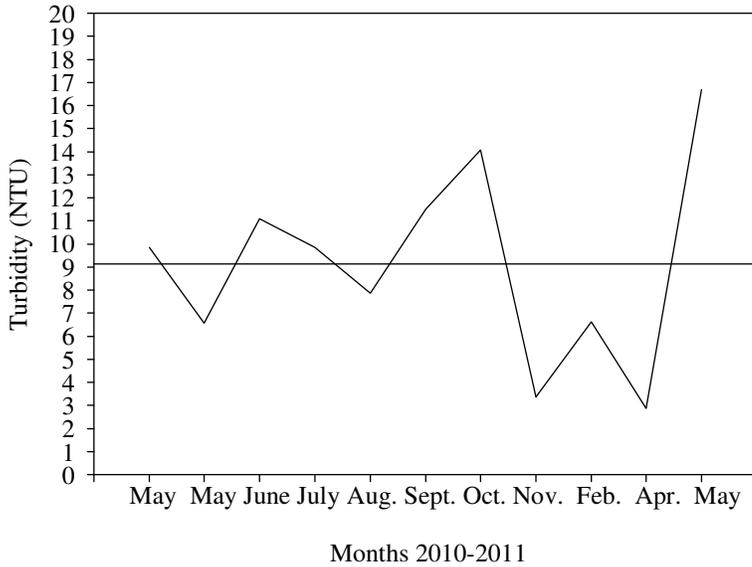
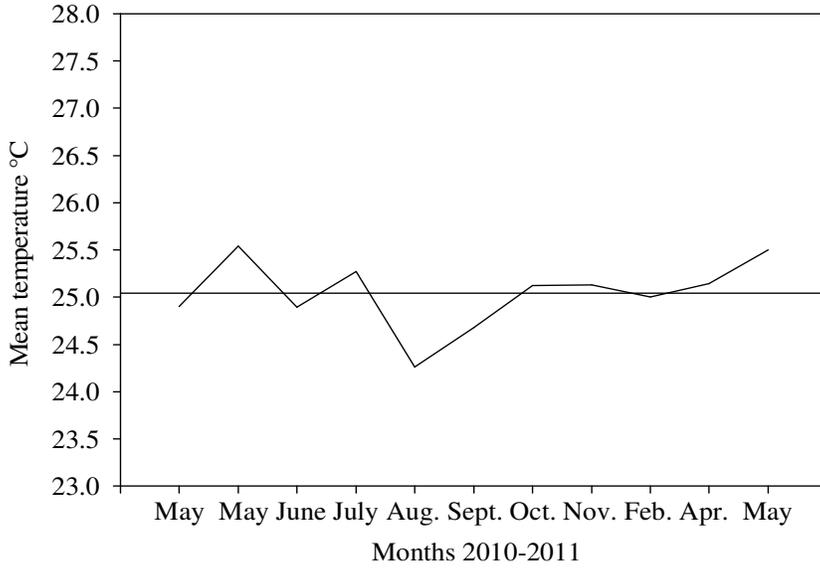
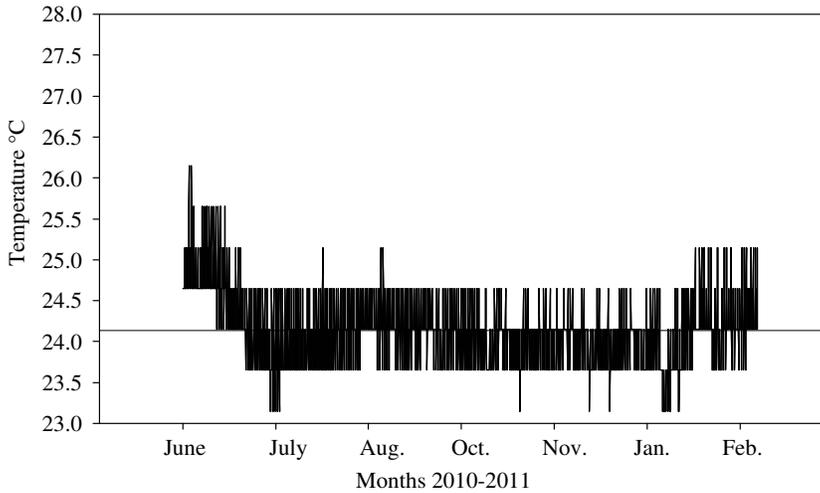


Figure 2.12--Temporal changes in mean turbidity from May 2010 to May 2011 in the reconstructed wetland. Horizontal reference line represents the overall mean (9.12 NTU). *n*=11.

Mean temperature in the canal was 25.05 °C, 1.07 °C warmer than the wetland and 0.23 °C warmer than the pool (Figure 2.13a). Trends from monthly measurements match temperature data recovered from temperature data loggers (Figure 2.13b). Temperature fluctuated between 23 and 25 °C. The mean canal temperature ranged from a minimum of 24.26 °C to a maximum of 25.54 °C over the sampling period. pH of the canal water was the lowest of the three habitats at an overall mean of 7.03 and a range of 6.69 to 7.4 (Figure 2.14). Specific conductance in the canal ranged from 2.10 mS to 3.60 mS and had an overall mean of 3.13mS (Figure 2.15). TDS ranged from 1.28 to 3.12 ppt and had an overall mean of 1.93 ppt (Figure 2.16). Mean turbidity in the canal ranged from 0.60 NTU to 8.21 NTU and the overall mean was 3.60 NTU (Figure 2.17).



a



b

Figure 2.13--Temporal changes in mean temperature from May 2010 to May 2011 ($n=11$) in the refuge canal from monthly sampling (**a**) and iButton data loggers (**b**). Horizontal reference line represents the overall mean (24.82°C). Data were recovered from iButton data loggers set up to record temperature every two hours. Horizontal line represents the mean for the sampling period (24.13°C). $n=3,113$ points.

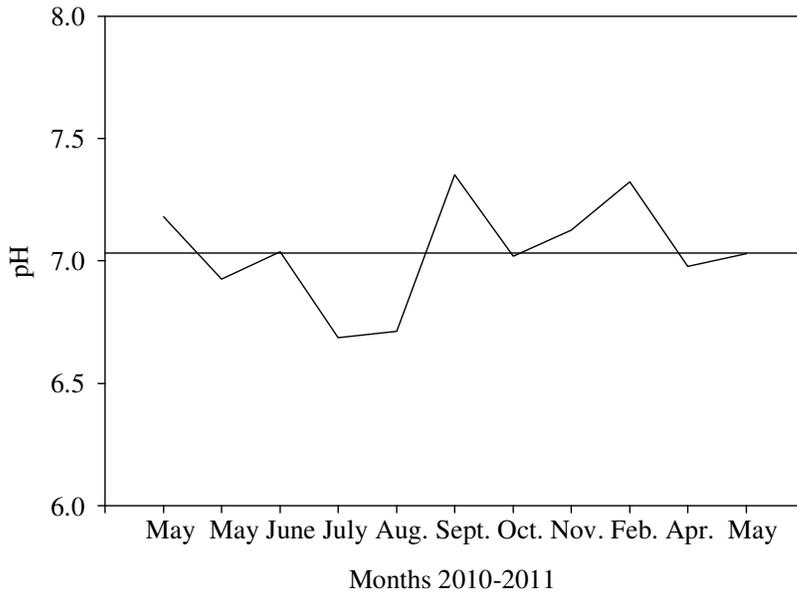


Figure 2.14--Temporal changes in mean pH from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (7.03). $n=11$.

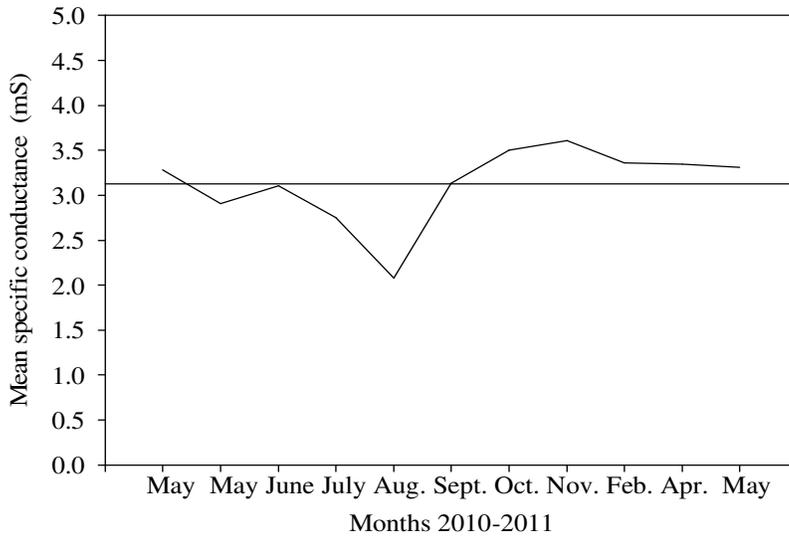


Figure 2.15--Temporal changes in mean specific conductance from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (3.13 mS). $n=11$.

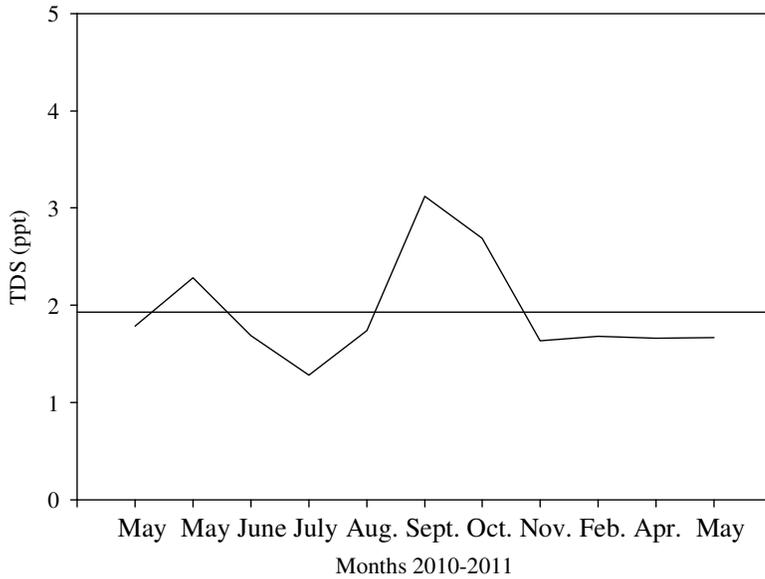


Figure 2.16--Temporal changes in mean TDS from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (1.93 ppt). $n=11$.

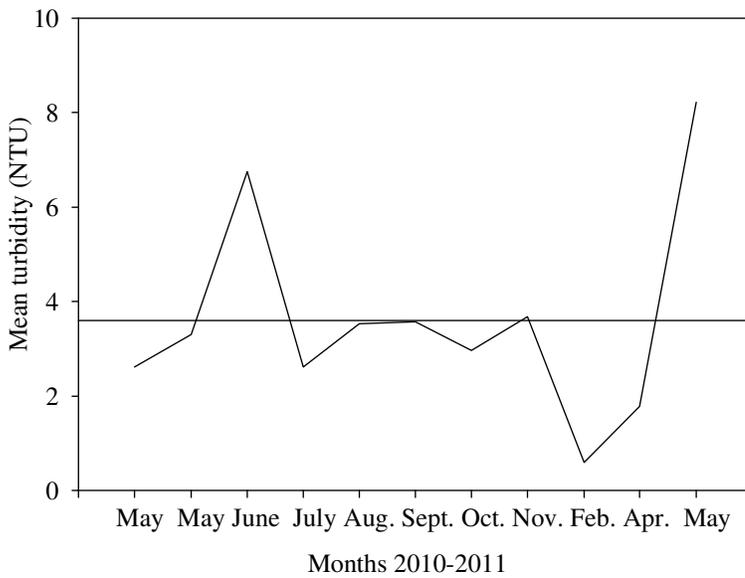


Figure 2.17--Temporal changes in mean turbidity from May 2010 to May 2011 in the refuge canal. Horizontal reference line represents the overall mean (3.60 NTU). $n=11$.

Spatial differences in water quality measurements--Mean temperature, pH, specific conductance, TDS, and turbidity differed among the pool, wetland and canal habitats. An ANOVA revealed that there was a significant difference in temperature

among the groups ($F=64.47$, $P<0.001$, $df=2$). Post hoc Tukey comparisons revealed that wetland mean temperature was significantly lower compared to the pool and canal (adjusted $P<0.001$), but the pool and canal were not significantly different from one another (Figure 2.18). Mean pH for the pool, wetland, and canal were 7.09, 7.22, and 7.03 respectively. Comparisons revealed that the wetland pH was significantly higher than pool pH (adjusted $P=0.002$) and canal pH ($P<0.001$); however, pool and canal pH means were not significantly different from one another ($P=0.20$) (Figure 2.19). Mean specific conductance did not differ significantly between the pool, wetland and canal ($F=0.887$, $P=0.41$, $df=2$) (Figure 20). TDS means for the pool, wetland, and canal were 1.93 ppt, 2.04 ppt, and 1.93 ppt respectively and were not significantly different from one another ($F=1.52$, $P=0.22$, $df=2$) (Figure 2.21). Overall mean turbidity in the wetland, 9.12 NTU, was significantly higher ($P<0.001$) than mean turbidity in the pool (2.59 NTU) and canal (3.60 NTU) (Figure 2.22).

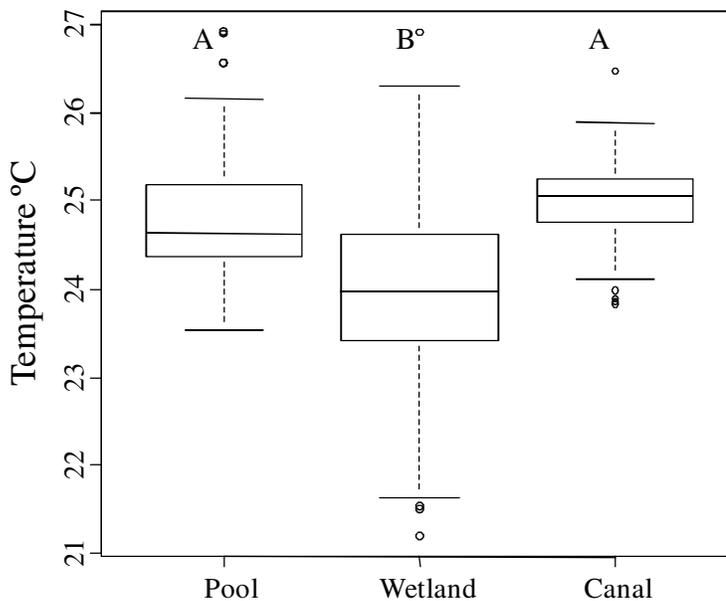


Figure 2.18--Boxplot of spatial temperature variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.

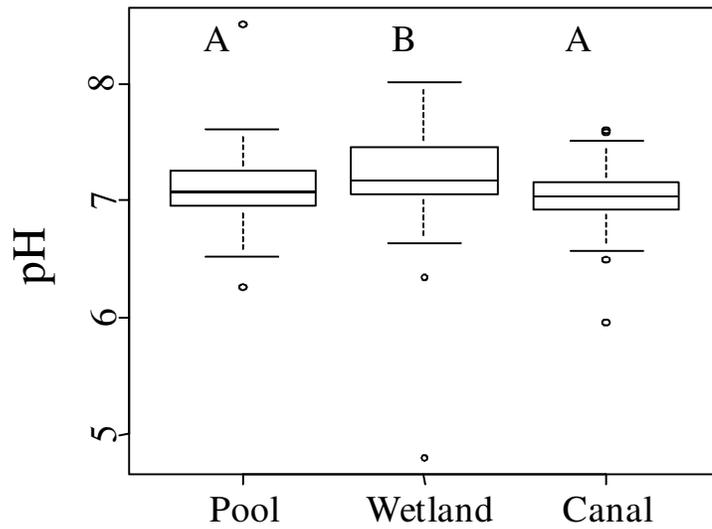


Figure 2.19--Boxplot of spatial pH variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.

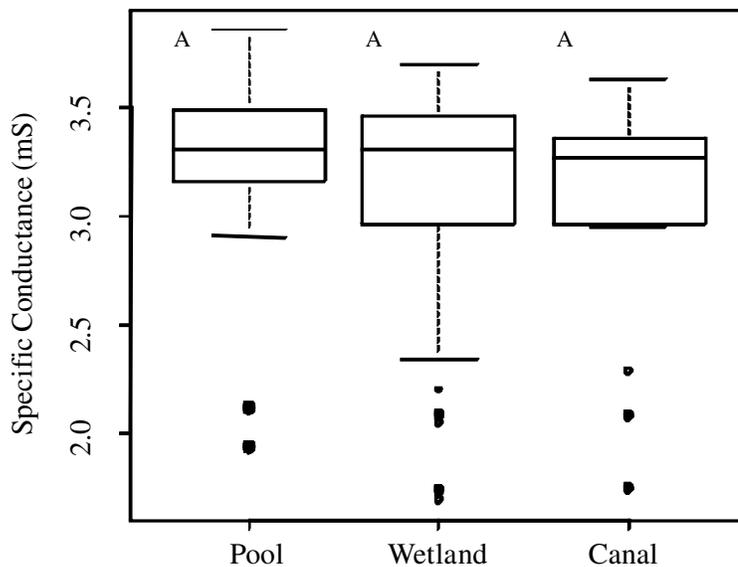


Figure 2.20--Boxplot of specific conductance variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.

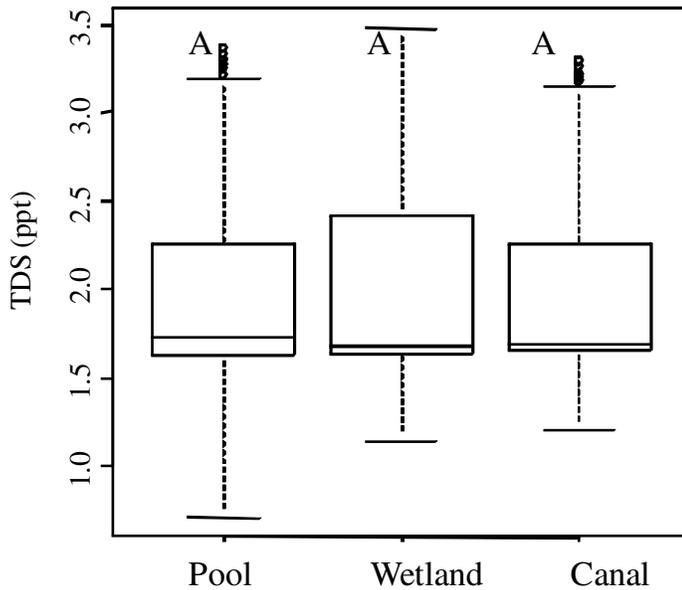


Figure 2.21-- Boxplot of total dissolved solids variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.

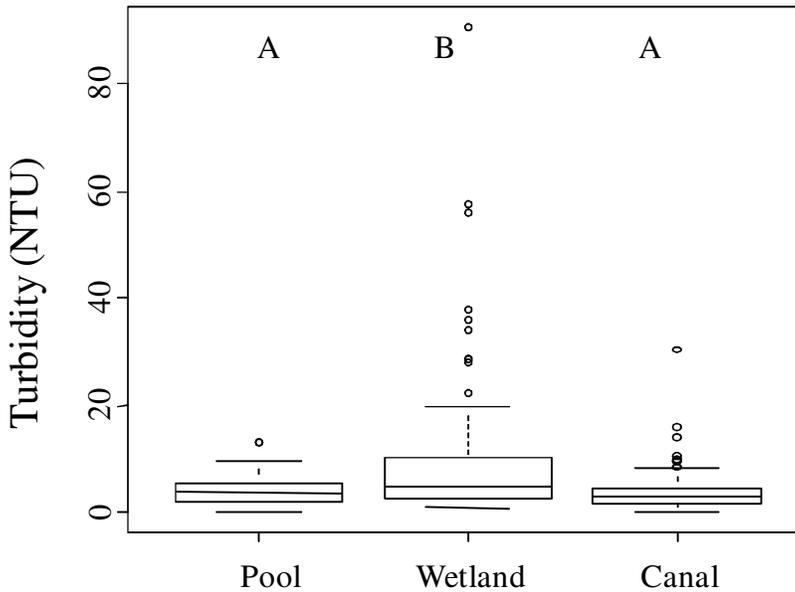


Figure 2.22-- Boxplot of turbidity variation in the pool ($n=132$), wetland ($n=132$), and canal ($n=132$) from monthly sampling. Different letters indicate significant differences.

Chemical species concentrations and spatial differences (Ca, Mg, NH₃, NO₃, PO₄)--Differences in mean calcium concentrations among the three habitat sites were significant ($F_2=3.23$, $P=0.039$, $df=2$) (Table 2.1). The pool had a significantly higher mean calcium concentration than the wetland ($P=0.034$), but differences between the

canal and the pool were not significant. The calcium concentration in the wetland, 426.05 mg/L, was the lowest of the three sites. Mean magnesium concentration also differed significantly among habitats ($F=3.28$, $P=0.038$, $df=2$). Mean magnesium concentration was significantly lower in the canal when compared to the wetland and pool ($P=0.045$). Differences in mean concentration between the pool and wetland were not significant ($P>0.05$). Ammonia (NH_3), nitrate (NO_3), and phosphate (PO_4) did not differ significantly among the pool, wetland, and canal ($P>0.05$). Mean ammonia concentrations were all below 1 mg/L and above the 0.02 mg/L detection limit. Nitrate concentrations all exceeded 1mg/L with the wetland having the highest mean nitrate concentration (1.69 mg/L). These readings were above the detection limit of 0.5mg/L. Phosphate concentrations were all less than nitrate concentrations with the highest found in the wetland at 0.44 mg/L and all samples were above the 0.07mg/L detection limit.

Table 2.1--Mean concentrations of chemical species in the pool, wetland, and canal. Numbers in parentheses are standard deviations.

Habitat	Ca (mg/L)	Mg (mg/L)	NH_3 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
Pool	450.06	273.71	0.074	1.63	0.39
$n=132$	(28.69)	(117.95)	(0.024)	(0.33)	(0.06)
Wetland	426.05	274.50	0.081	1.69	0.44
$n=132$	(21.29)	(21.29)	(0.019)	(0.19)	(0.11)
Canal	442.59	249.68	0.068	1.60	0.31
$n=132$	(9.72)	(10.37)	(0.036)	(0.10)	(0.04)

Physical parameters (depth, current velocity, substrate)--Mean depth was found to differ significantly among sites ($F=355.38$, $P<0.001$, $df=2$) and pair wise comparisons provide evidence that mean depth and mean water current velocity in the pool, wetland, and canal were significantly different from one another. Mean depth of the sampled sites at the pool (4.02 m) was significantly greater than mean depth of sampled sites in the wetland and canal (0.47 m and 0.85 m respectively). The omnibus F test also showed that water velocity significantly differed among habitats ($F=355.38$, $P=0.001$, $df=2$). Mean water velocity in the canal, 0.30m/s, was significantly higher than the pool or wetland (multiple comparisons $P<0.001$). Mean water velocity for the pool and wetland however were not significantly different. Table 2.2 summarizes the depth and velocity results.

Substrate varied among habitats (summarized in Table 2.3). Substrate in the pool overall consisted mostly of concrete, sand, and cobble. In the pool concrete appeared in all of the sampled quadrats, fine grain in 26%, and sand in 47% of sampled quadrats. Boulder appeared in the fewest samples (5% of the quadrats) in the pool. The south wing in the pool contained only concrete substrate. The dominant substrate in the wetland was fine grain sediment, which appeared in 84% of the sampled quadrats. Detritus and litter appeared in 43% of the quadrats, and cobble in 13.6% of quadrats. Gravel and pebble were found in 3.8% and 6% of the quadrats, respectively. Cobble substrate often had a surface layer of *Cladophora* spp. (18.9% of the samples).

Table 2.2--Mean water depth and velocity in the pool, wetland, and canal. Numbers in parentheses are standard deviations

Habitat	<i>n</i>	Mean depth (m)	Mean current velocity (m/s)
Pool	132	4.02 (1.46)	<0.01
Wetland	132	0.47 (0.10)	0.01 (0.008)
Canal	132	0.85 (0.06)	0.30 (0.08)

In the wetland, substrate type shifted from dominantly cobble and *Cladophora* spp. mats to dominantly fine grain and detritus 53 m downstream of the wetland's inflow. Dominant substrate then remained mostly fine grain until the end of the wetland. Substrate composition of the canal was similar to that of the pool. The majority of the sampled substrate was concrete (appeared in all sampled quadrats) and sand (appeared in 48% of the sampled quadrats). Cobble was also very common (34.8%) as were submerged macrophyte shoots (17.4% of sampled quadrats). Concrete appeared in all quadrats in the canal. The most common sediment type was sand followed by cobble, and then pebble

Table 2.3-- Substrate type in each habitat. Numbers are the frequency of a given substrate type in sampled quadrats (multiply by 100 to get percentage). For example, 100% of the pool quadrats contained concrete habitat, 26% contained fine grain sediment, etc. C=concrete, Fg=fine grain (0-0.25 mm), Sa=sand (0.26-2.00 mm), Gr=gravel (2.01-4.00 mm), Pe=pebble (4.00-64 mm), Cob=cobble (65-256 mm), B=boulder (>256 mm), *Clad*=*Cladophora* spp., *Mac*=macrophyte.

Habitat	<i>n</i>	C	Fg	Sa	Gr	Pe	Cob	B	<i>Clad</i>	<i>Mac</i>
Pool	132	1.00	0.26	0.47	0.09	0.27	0.30	0.05	0.00	0.00
Wetland	132	0.00	0.84	0.11	0.04	0.06	0.14	0.00	0.19	0.00
Canal	132	1.00	0.01	0.48	0.09	0.32	0.35	0.01	0.00	0.17

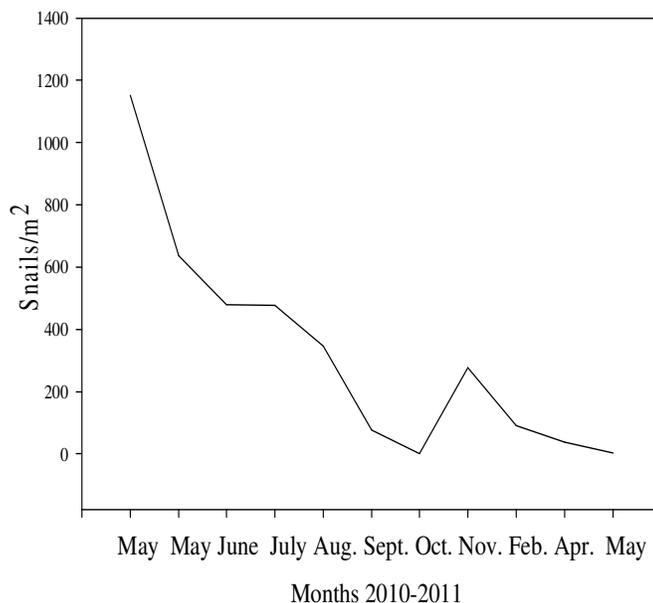
Biotic parameters (periphyton species, canopy cover)--In the pool, *Gomphonema* spp. valves comprised 23.9% of all diatom valves followed by *Navicula* spp. which made up 23.1% of the diatom community (Table 2.4). *Terpsinoe musica* made up the lowest percentage of the periphyton community at 1.7%. In the wetland, *Terpsinoe musica* was the most abundant diatom species and made up 38% of the sampled periphyton (Table 2.4). *Gomphonema* was also prevalent and comprised 20% of sampled diatoms. *Gomphonema* spp., *Navicula* spp., and *Cymbella* spp. were the three most abundant diatom taxa in the canal, representing 22%, 19%, and 18% of the sampled periphyton. *Nitzschia* spp. was the least abundant and only made up 3% of periphyton samples. Canopy cover also differed among sites. No canopy cover was present in any of our quadrats in the pool and canopy cover percentage for the wetland was 0.01%. Mean

canopy cover for the canal was 14.03% and was higher than the percentage for the other two habitats.

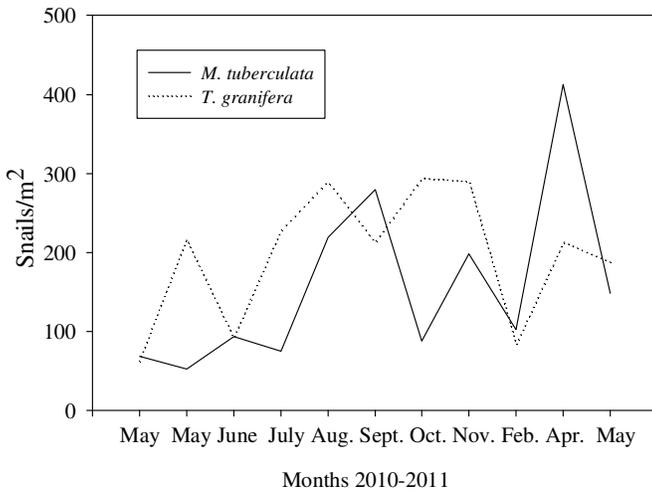
Table 2.4 --Diatom composition from periphyton samples in the pool, wetland, and canal. Numbers are fraction composition of samples. For example, in the pool, *T. musica* was present in 3% of sampled quadrats (N=132). *Gom*=*Gomphonema* spp., *Dia*=*Diatoma* spp., *Nav*=*Navicula* spp., *Fra*=*Fragilaria* spp., *Nit*=*Nitzschia* spp., *Cymb*=*Cymbella* spp., *Tab*=*Tabellaria* spp.

Habitat	<i>T. musica</i>	<i>Gom</i>	<i>Dia</i>	<i>Nav</i>	<i>Fra</i>	<i>Nit</i>	<i>Cymb</i>	<i>Tab</i>
Pool	0.03	0.24	0.12	0.23	0.11	0.11	0.08	0.08
Wetland	0.38	0.20	0.09	0.13	0.08	0.04	0.05	0.03
Canal	0.10	0.22	0.14	0.19	0.08	0.03	0.18	0.06

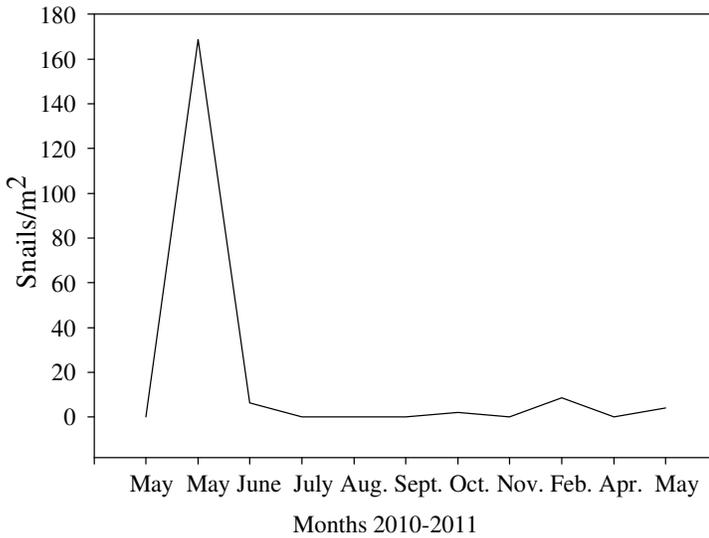
Snail density--*M. tuberculata* mean density in the pool was lowest in May 2010 with 52 snails/m² and highest in April 2011 with 412 snails/m² (Figure 2.23a). *T. granifera* density was lowest in May 2010 with 62 snails/m² and highest in October 2010 with 293 snails/m². *P. texana* density decreased over time (Figure 2.23b). The highest density was 1152 snails/m² in May 2010 and the lowest was zero snails/m² in October. *T. cheatumi* mean density was zero individuals/m² for the months of May 2010, July-September 2010, November 2010, and March 2011. The highest mean density of *T. cheatumi* was 168 snails/m², which we observed during May 2010 (Figure 2.23c)



a



b



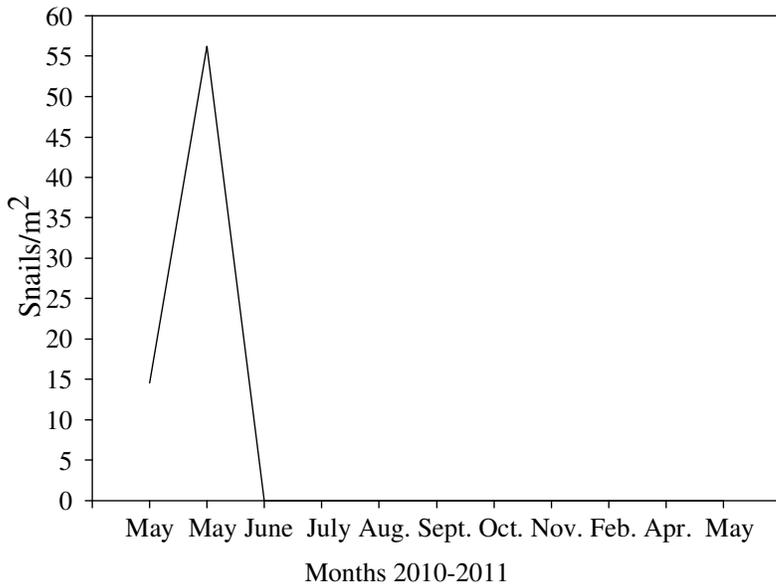
c

Figure 2.23--Temporal changes in mean density for *P. texana* (a), *M. tuberculata* and *T. granifera* (b), and *T. cheatumi* (c) in the pool. We conducted two sampling events in May.

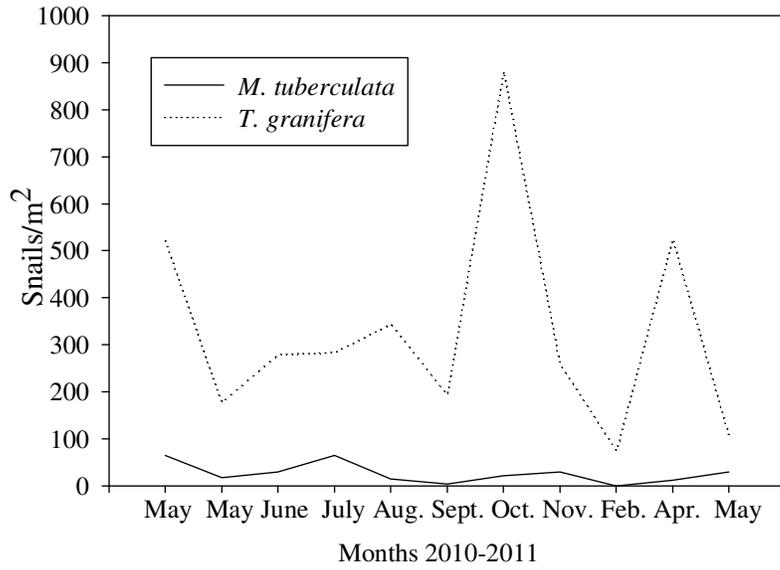
In the wetland, *M. tuberculata* mean density was highest at 64 snails/m² in May 2010 and July 2010 and lowest in February 2011 with a density of zero snails/m² (Figure 2.24a). *T. granifera* mean density was highest in October 2010 with 881 snails/m² and

lowest in February 2011 with 75 snails/m². *P. texana* density was highest in May 2010 (56 snails/m²) and from June 2010 to April 2011 density was zero snails/m² (Figure 2.24b).

M. tuberculata mean density in the canal ranged from zero snails/m² in August 2010 to a maximum of 33 snails/m² in May 2010. *T. granifera* density also had a minimum of zero snails/m² but this occurred in November 2010 (Figure 2.25a). The maximum value of *T. granifera* was 187 snails/m² in April 2011. *P. texana* minimum density was 2,993 snails/m² in September 2010 and *P. texana* maximum mean density was 14,133 snails/m² in March 2011 (Figure 2.25b). *T. cheatumi* had a minimum of zero snails/m² mean density from June to August 2010 and in October 2010. The maximum density of *M. tuberculata* observed, 34 snails/m², occurred in February 2011 (Figure 2.25c).

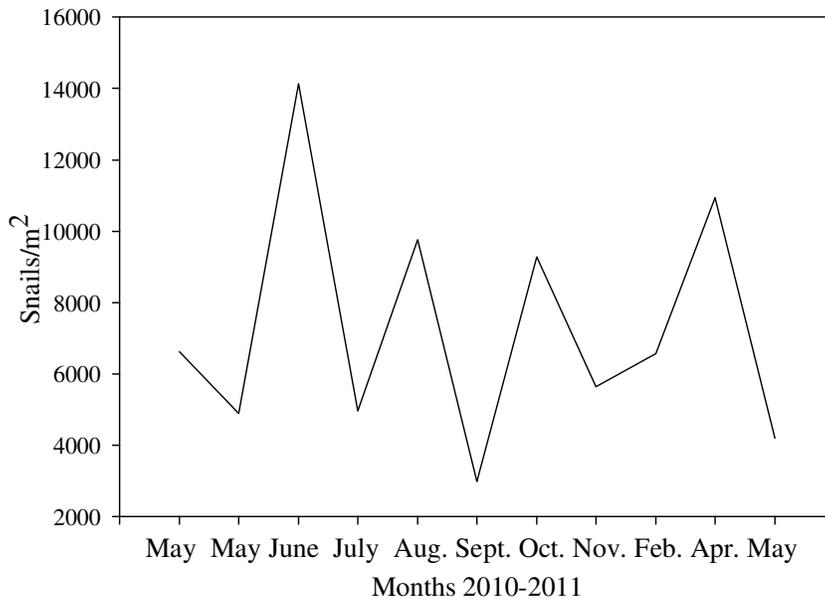


a

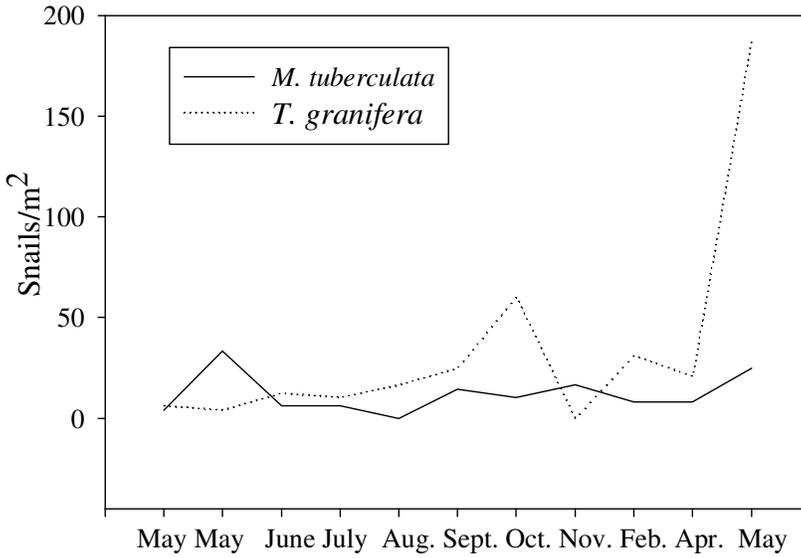


b

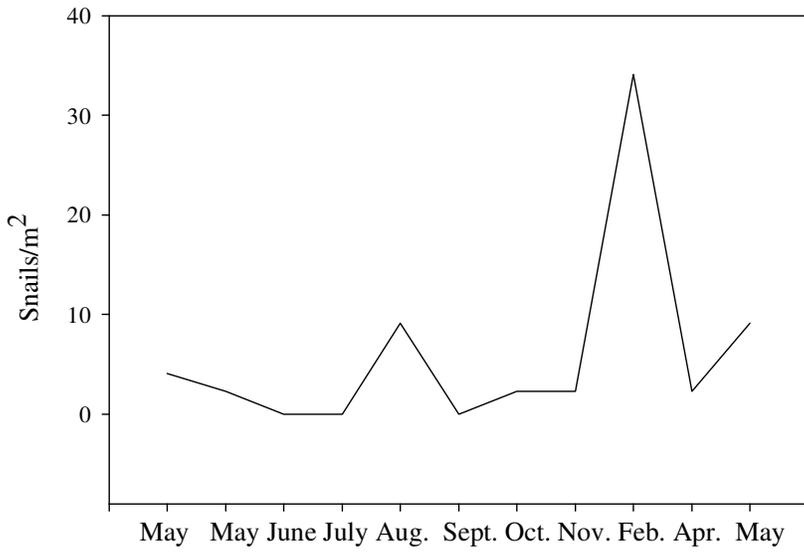
Figure 2.24--Temporal differences in mean density for *P. texana* (a) and *M. tuberculata* and *T. granifera* (b) in the wetland. Note that no *P. texana* were observed after June 2010.



a



b Months 2010-2011



c Months 2010-2011

Figure 2.25--Temporal changes in mean density for *P. texana* (a), *M. tuberculata* and *T. granifera* (b), and *T. cheatumi* (c) in the refuge canal.

Spatial differences within habitat--Snail density varied among the different habitats (Table 2.5). In the pool, no snails were observed in the south wing during the entire sampling period. In the center pool section, *P. texana* had a higher mean density

than *T. granifera*, *M. tuberculata*, or *T. cheatumi* ($\chi^2=54.71$, $P<0.001$, $df=3$) with an observed 599 snails/m² (Figure 2.26). In the north wing, *M. tuberculata* had the highest mean density, 264 snails/m² ($\chi^2=81.19$, $P<0.001$, $df=3$). When averaging all sections in the pool, the highest mean snail density observed was for *P. texana*, at 326 snails/m². *T. cheatumi* had the lowest mean density with 11 snails/m².

Table 2.5--Mean densities (snails/m²) of four snail species from San Solomon Springs in three habitats. Numbers in parentheses are standard errors.

Species	Pool	Wetland	Canal
<i>M. tuberculata</i>	134 (76)	26 (15)	12 (5)
<i>T. granifera</i>	142 (97)	331 (237)	34 (17)
<i>P. texana</i>	326 (188)	6 (6)	9,139 (2,025)
<i>T. cheatumi</i>	11 (11)	0 (0)	5 (2)

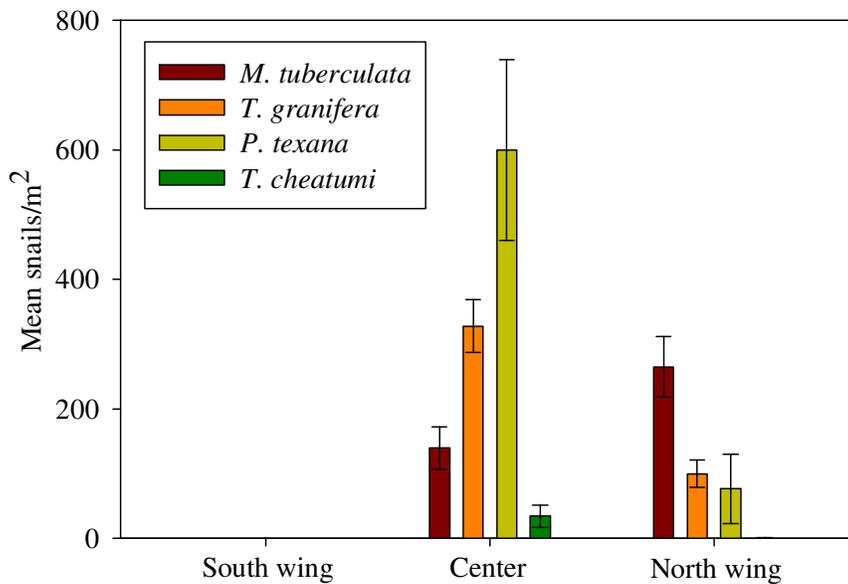


Figure 2.26--Bar graph of mean density for each snail species in the pool. Snail density is being compared among the three pool sections. No individuals of any species were found in the south wing. Bars represent standard error

In the wetland, *T. granifera* was the most abundant snail in all sections. In section 1, *T. granifera* density was significantly higher ($\chi^2=101.13$, $P<0.001$, $df=2$) than the other snail species. Mean density of *T. granifera* in section one was 805 snails/m² and mean densities of *M. tuberculata* and *P. texana* were both nine snails/m². *T. granifera* mean density decreased from 805 snails/m² in section one to 130 snails/m² in section two, but

density was still higher than the other snail species ($\chi^2=43.32$, $P<0.001$, $df=2$) (Figure 2.27). *M. tuberculata* and *P. texana* mean densities in section two were 56 and zero snails/m² respectively. In section three, *T. granifera* mean density was higher than the mean density of *P. texana* and *M. tuberculata* ($\chi^2=32.43$, $P<0.001$, $df=2$).

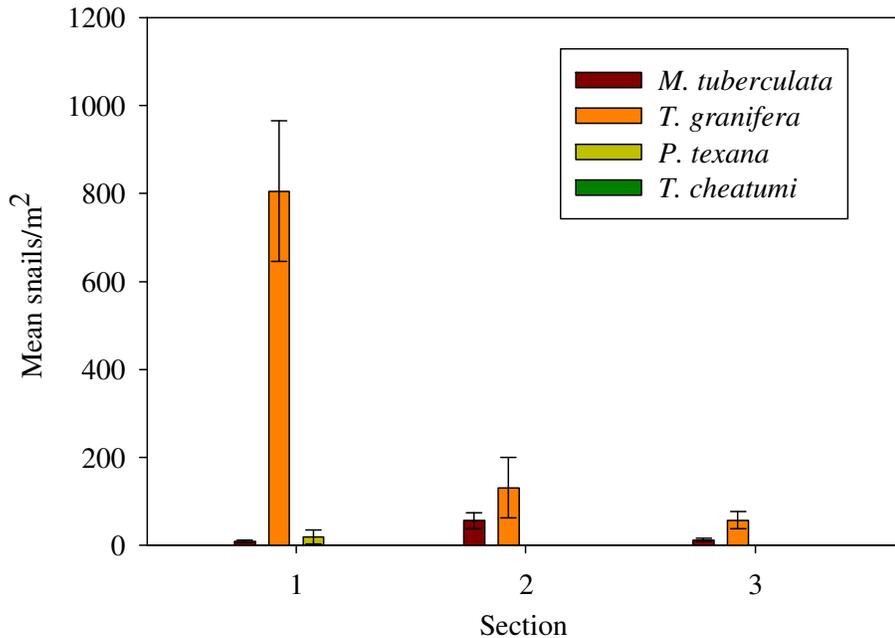


Figure 2.27--Bar graph of mean density for each snail species in the wetland at Balmorhea State Park, by section. No individuals of *T. cheatumi* were found in the wetland.

In the canal, significant differences of mean density among snail species was observed ($\chi^2=32.43$, $P<0.001$, $df=3$) (Figure 2.28). Highest *P. texana* density occurred in section one with 17,834 snails/m². The highest mean densities in the canal for *M. tuberculata* and *Tarebia granifera* were observed in section three, at 31 individuals/m² and 109 snails/m² respectively. In section four, densities for *P. texana*, *T. granifera*, and *T. cheatumi* were 7,057 snails/m², two snails/m², and two snails/m² respectively, with no individuals of *M. tuberculata* found. Densities for *T. granifera* and *T. cheatumi* were four snails/m² and 18 snails/m², the highest mean density of *T. cheatumi* in the canal. No *M. tuberculata* were found in section six and *T. granifera* mean density was very low at two snails/m². This section had the lowest density of *P. texana* in the canal with 4,172 snails/m².

Snail mean density longitudinal trends—*M. tuberculata* mean density was negatively correlated with distance from the spring head over the entire park ($F=21.8$, $P<0.001$, $df=35$) (Figure 2.29). Associations between distance from the spring head and mean density were not significant for *T. granifera* (Figure 2.30) or *P. texana* (Figure 2.31). Distance from the spring head did have a significant association with a decrease in mean density in *T. cheatumi* (Figure 2.32).

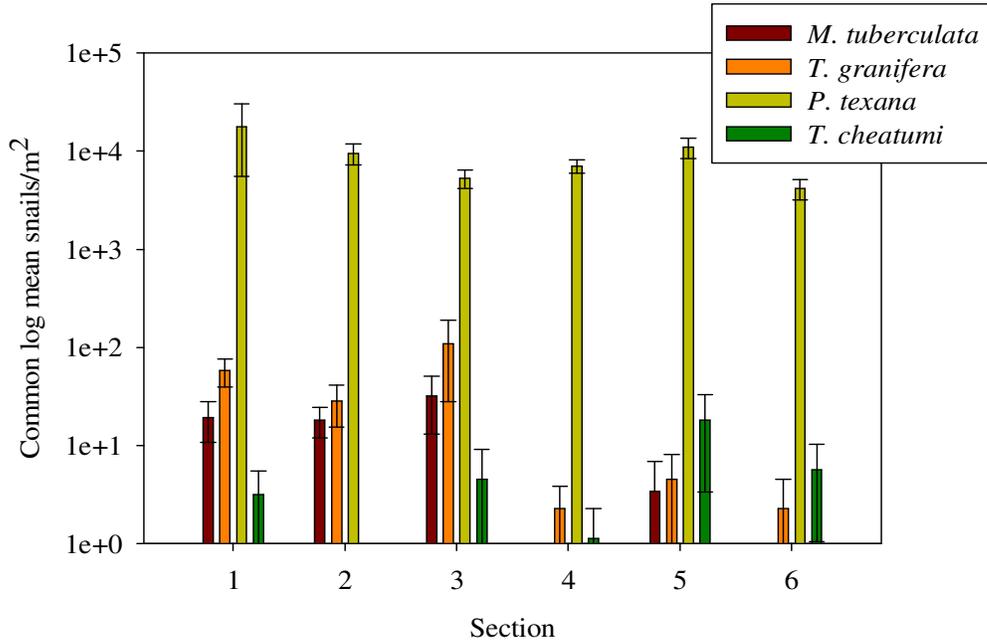


Figure 2.28--Bar graph of mean density for each snail species in the refuge canal at Balmorhea State Park, by section. Each section is 100 m in length and therefore the above figure also represents longitudinal trends.

Snail Abundance--In the pool, *P. texana* was the most abundant snail with an estimated population of 2,995,021 total individuals (Table 2.3). The least abundant snail in the pool was *T. cheatumi* which had an estimated population of 46,304 individuals. In the wetland the dominant snail species shifted from *P. texana* to *T. granifera*, which had an estimated 322,154 individuals. No *T. cheatumi* individuals were found in the wetland. In the canal, to the population of *P. texana* was estimated at 2,995,021 individuals. Standard deviations are large due to the highly clumped distribution of snails

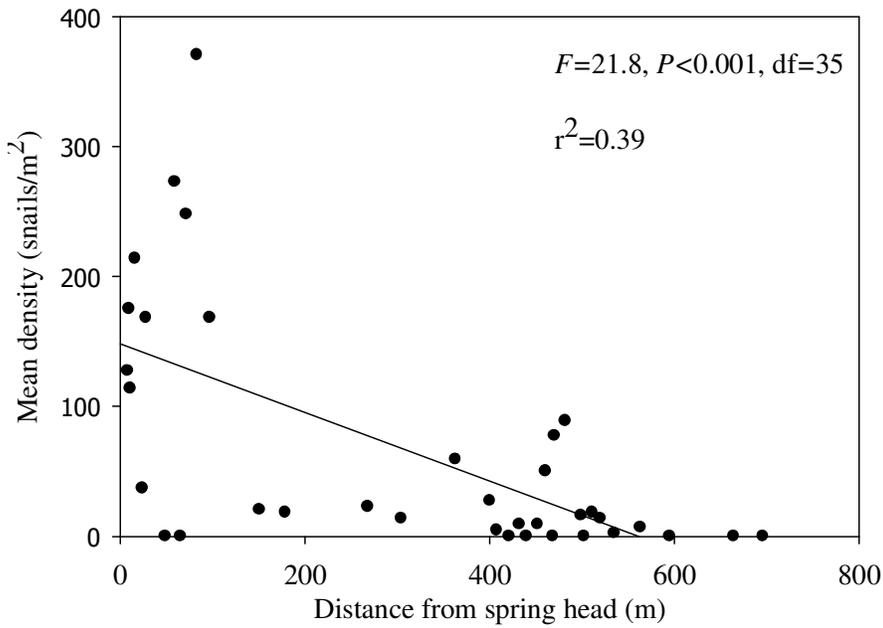


Figure 2.29--Regression comparing distance from the spring head and mean density in *Melanoides tuberculata* over the entire park. Each dot represents the mean density of a quadrat.

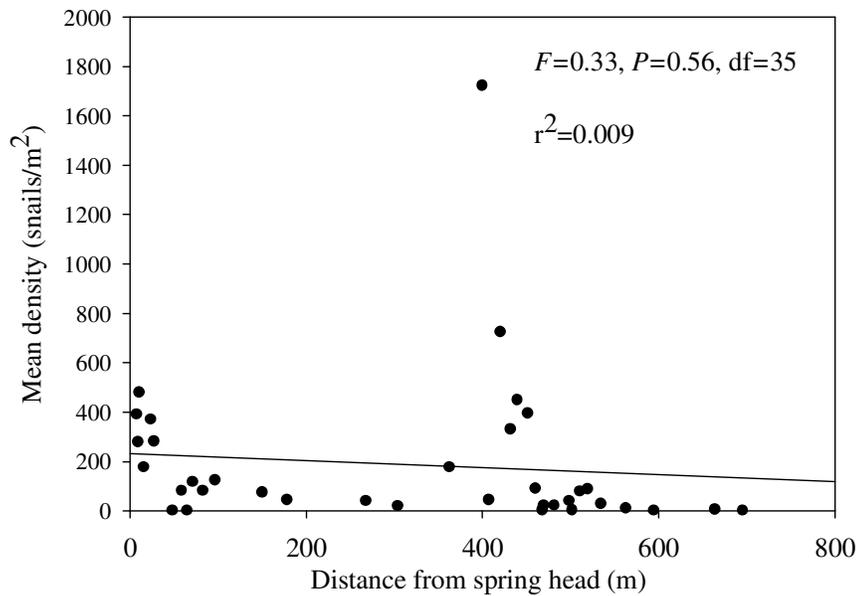


Figure 2.30--Regression comparing distance from the spring head and mean density in *Tarebia granifera* over the entire park. Each dot represents the mean density of a quadrat over the 11 sampling trips.

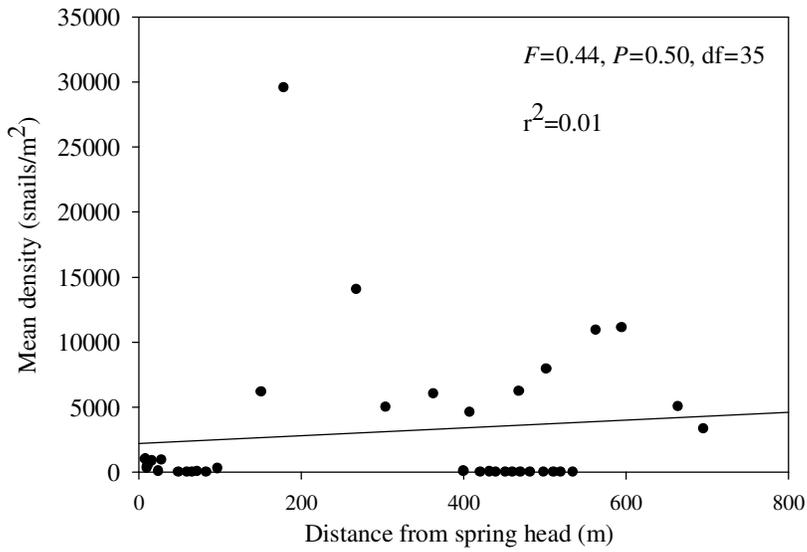


Figure 2.31--Regression comparing distance from the spring head and mean density in *P. texana* over the entire park. Each dot represents the mean density of a quadrat over the 11 sampling trips.

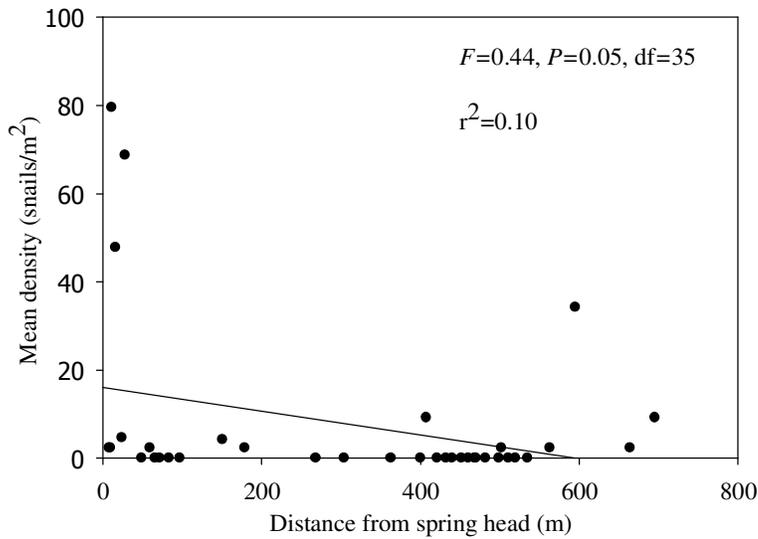


Figure 2.32--Regression comparing distance from the spring head and mean density in *T. cheatumi*. Each dot represents the mean density of a quadrat over the 11 sampling trips.

Table 2.6—Mean abundance estimates for all four snail species at Balmorhea State Park by habitat. Numbers in parentheses are standard deviations.

Habitat	<i>M. tuberculata</i>	<i>T. granifera</i>	<i>P. texana</i>	<i>T. cheatumi</i>
Pool	352,438 (306,887)	502,116 (714,885)	852,367 (1,354,532)	46,304 (78,812)
Wetland	34,878 (39,670)	322,154 (337,127)	6,262 (10,846.1)	0 (0)
Canal	4,864 (5,736)	13,881 (19,090)	2,995,021 (1,834,018)	1,469 (1,397)

Exotic snail species length--Mean lengths of *M. tuberculata* and *T. granifera* varied with the habitat (Table 2.7). *M. tuberculata* mean length was greatest (and the most variable) in the wetland (19.38 mm) while *T. granifera* mean length (9.67 mm) was the least in the wetland. In the canal, mean lengths for *M. tuberculata* and *T. granifera* were 14.23 mm and 11.66 mm. Mean lengths in the pool were similar to those in the canal with *M. tuberculata* and *T. granifera* having mean lengths of 15.25 mm and 10.00 mm.

We divided the lengths into eight size classes for both *M. tuberculata* and *T. granifera* and determined the frequency of all size classes per season. For *M. tuberculata*, the 1-6 mm size class was most frequent during spring 2011 and least abundant during summer 2010 (Figure 2.33). For *T. granifera*, frequency of the smallest size class was similar across all seasons (Figure 2.34). The most frequent size class for each season was 7-12 mm.

Table 2.7--Mean snail length (in mm) for the exotic *Melanoides tuberculata* and *Tarebia granifera* in Balmorhea State Park by habitat. Numbers in parentheses are standard deviations.

Habitat	<i>M. tuberculata</i>	<i>T. granifera</i>
Pool	15.26 (7.27)	10.00 (2.87)
Wetland	19.38 (9.39)	9.67 (4.32)
Canal	14.23 (5.31)	11.66 (5.34)

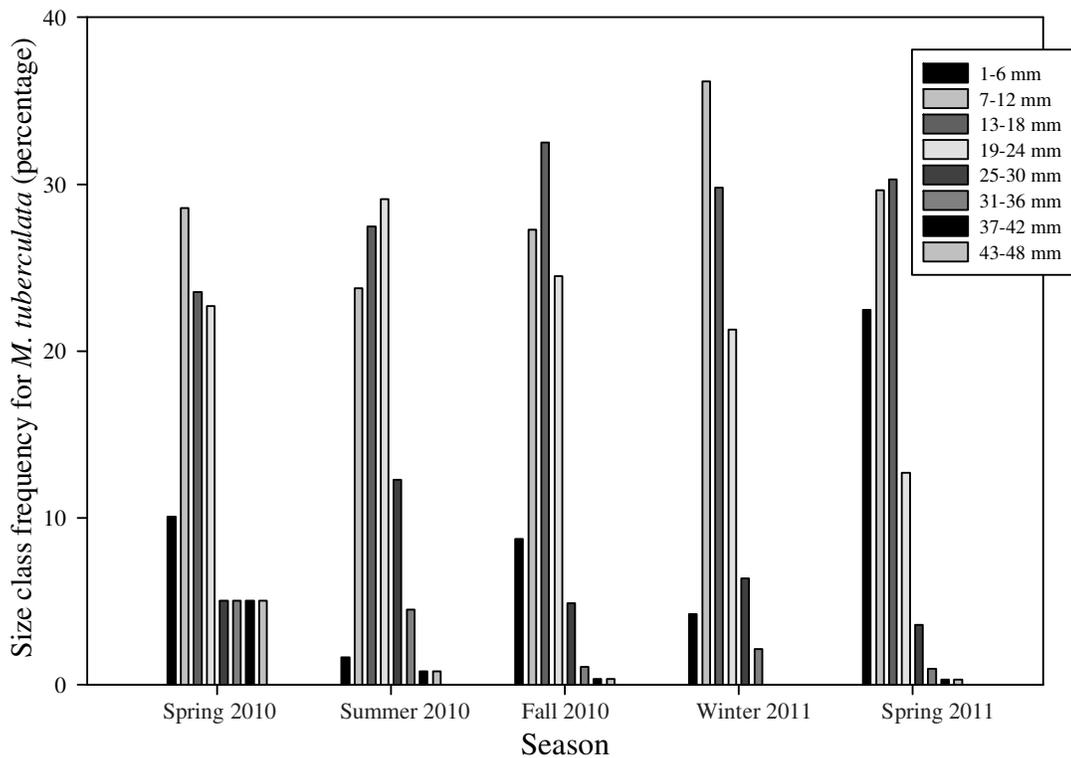


Figure 2.33--Size class (in mm) frequencies for *M. tuberculata* across all habitats within Balmorhea State Park organized by season

Differences in mean lengths were spatially significant in *M. tuberculata* ($F=19.01$, $P<0.001$, $df=2$) with the mean shell length in the wetland (19.38 mm) significantly higher than mean shell lengths in the pool or canal ($P<0.001$ in pair-wise comparisons). Differences between mean shell length in the pool and wetland were not significant ($P=0.51$). Spatial differences in mean shell length were also significant for *T. granifera* ($F=22.51$, $P<0.001$, $df=2$). Mean length in all habitats were significantly different from one another ($P<0.001$) with mean length in the wetland being the lowest and mean length in the canal being the greatest.

Throughout the entire park, snail lengths in *M. tuberculata* and *T. granifera* showed temporal variation. Among the 11 sampling trips, differences in mean shell lengths for *M. tuberculata* were significant ($F=11.02$, $P<0.001$, $df=10$). Post hoc Tukey comparisons showed that mean length in April 2010, June 2010, and August 2010 was higher than mean shell length in all other sampling months. Mean shell length was lowest in February 2011, March 2011, and April 2011. Difference in mean shell length for *T. granifera* among sampling months was also significant ($F=8.19$, $P<0.001$, $df=10$). Mean shell length in July 2010, March 2011, and April 2011 was lowest among the months while mean length of shells in May 2010, June 2010, and August 2010 was highest.

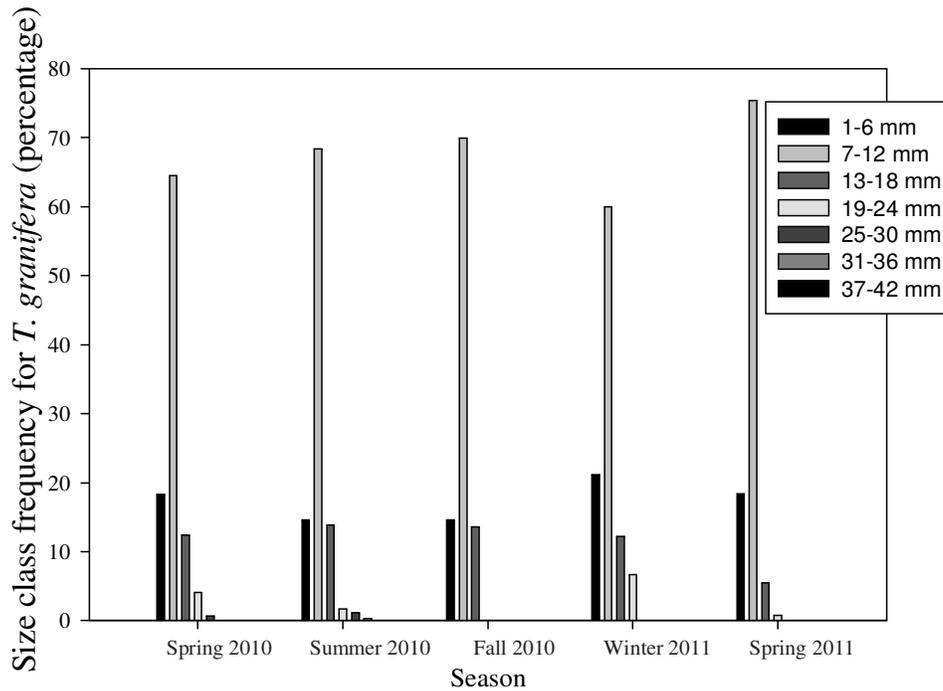


Figure 2.34--Size class (in mm) frequencies for *T. granifera* across all habitats within Balmorhea State Park organized by season

Snail habitat associations--In the RDA the first two principle components accounted for 84% of the variation in snail density. Temperature, percent canopy cover, water velocity, distance from the spring head, pH, turbidity, and depth were significant environmental variables ($P < 0.05$). Month was not significant ($P = 0.66$) (Table 2.8, Figure 2.34). *M. tuberculata* and *T. granifera* densities were positively associated with depth and fine grain, sand, and pebble substrates. Exotic snail densities were also positively associated with each other. Both were negatively associated with increasing distance from the spring head. *P. texana* density was positively associated with temperature, canopy cover, water velocity, and cobble and *Chara* spp. substrates. *P. texana* density was negatively associated with turbidity, depth, and distance from the spring source. *P. texana* density was also negatively associated with *T. granifera* and *M. tuberculata* density. The environmental variables did not account for much variation in *T. cheatumi* density.

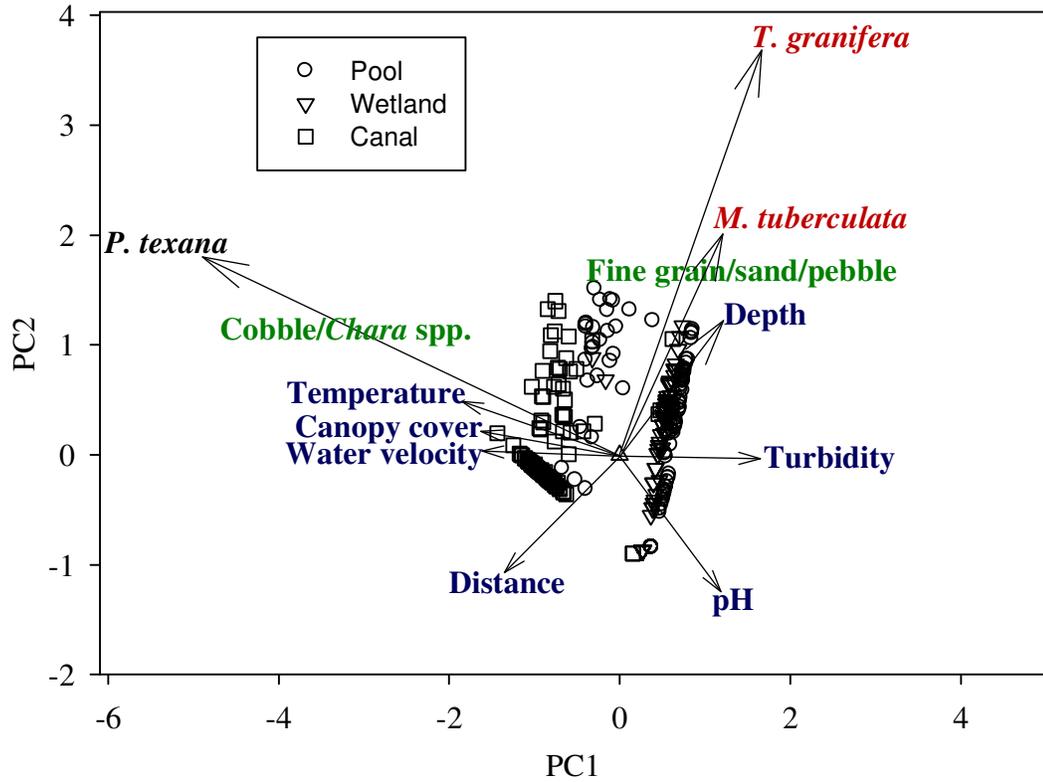


Figure 2.35--Joint plot of significant habitat variables associated with variation in snail density in Balmorhea State Park.

Discussion

San Solomon Springs in Balmorhea State Park is a unique habitat that is home to a variety of endemic aquatic species, despite extensive historical modifications of the spring system. In recent years there has been a variety of efforts to improve habitat for the native fishes via a created wetland and refuge canal. However, prior to this study little regard has been given to the management of invertebrates, native or invasive. While we cannot say whether the invasive or the native snails are increasing or decreasing, we at least now know their distributions within the spring system and have an idea of habitat preferences.

Table 2.8--Summary of RDA results for the entire park showing significant habitat variables ($P < 0.05$) marked by asterisks.

	PC1	PC2	
<u>Importance of components</u>			
Eigenvalue	3.3283	1.4318	
Proportion Explained	0.5877	0.2528	
Cumulative proportion	0.5877	0.8406	
<u>Species scores</u>			
<i>M. tuberculata</i>	1.28543	2.08039	
<i>T. granifera</i>	1.7034	4.06576	
<i>P. texana</i>	-5.62935	1.70175	
<i>T. cheatumi</i>	-0.12128	0.16538	
<u>Environmental Vectors</u>			
Distance from springhead	-0.71112	-0.70307	*
Depth	0.631778	0.775149	*
Specific conductance	0.594727	-0.80393	
TDS	0.783426	-0.62148	
pH	0.703155	-0.71104	*
Temperature	-0.90716	0.420782	*
Turbidity	0.99946	0.03286	*
Dissolved oxygen %	-0.94035	-0.34021	
Ca	-0.39967	-0.91666	
Mg	0.961577	0.274536	
Water velocity	-0.99998	0.006145	*
Canopy cover %	-0.9954	0.09576	*
<u>Categorical Factors</u>			
	r^2	P value	
Month	0.0214	0.6629	
Substrate	0.5632	0.0002	*

Water quality parameters varied temporally and spatially. Mean temperature in the pool fluctuated between 24 and 26.92 °C. This falls within the expected range quoted by the Texas Parks and Wildlife Department (TPWD) as being between approximately 22 °C to 24 °C (Texas Parks and Wildlife Department [TPWD], 2011). What we did not expect was the difference we observed between the temperature ranges in the pool and the canal. According to temperature logger data, minimum temperature in the pool (Figure 2.3) was lower than the minimum temperature in the canal (Figure 2.13) and the temperature range was wider in the pool (22.7 °C to 26.7 °C) than in the canal (23.2 °C to 26.1 °C). Understanding why these temperature ranges are different is important since temperature can be a limiting factor in aquatic ecosystems that affects species distribution. Typically, shallow lotic systems are known for large seasonal fluctuations in water temperature whereas deeper lentic systems tend to be more thermally stable, but the deep pool showed more fluctuation than the shallower canal. One reason for this unexpected difference may be the multiple suspected origins for the water flowing into the pool. Based on groundwater chemistry and isotopes, Chowdhury et al. (2004) concluded that the waters originate from both the Delaware and Apache Mountains and also state that during intense rainfall, water may also come from the Davis Mountains. The amount of water contributed by these sources may vary temporally, which would influence water temperature.

Spatially, mean temperature from 2010 to 2011 was not significantly different between the pool and canal. Mean temperature in the wetland, however, was significantly lower than the mean temperature of the pool and canal. This was expected because mean depth in the wetland is the lowest at 0.47 m compared to the 4.02 m and 0.85 m depth in the pool and canal. This lower depth means that water in the wetland is subject to more dynamic changes caused by weather conditions. Also, depth in the wetland varies throughout the year more than depth in the pool and the canal, which would also contribute to greater temperature variability.

Spatially, mean pH differed significantly among habitats with the wetland pH (7.22) being significantly higher than the pool and canal pH (7.09 and 7.03 respectively). Since pH is on a logarithmic scale, these are large differences. Since the water in all three habitats comes from the same source (from the spring head found at the bottom of the circular section in the pool), we initially expected to see no differences in water chemistry parameters such as pH. The pool and canal, both being lined in concrete and containing similar substrate, had similar pH. The pH in the wetland was higher than the pH in the canal and pool and we found this unusual because the pool and canal are lined with concrete. Presumably the limestone in the concrete would make pH in the pool and canal higher than the wetland. Higher pH in the wetland may be the result of less CO₂ in the wetland compared to the pool and canal. Based on visual observation the wetland seemed to have more vegetation with the community dominated by thick *Cladophora* spp. mats and *Typha* spp. Temporally, pH among the three habitats was similar with low values occurring in the summer and high values in the fall. pH fluctuations might be the result of changes in evaporation and precipitation. Chowdhury et al. (2004) concluded that the waters at San Solomon Springs originate from three different sources, so variation in aqueous carbon contributed from these three sources might also explain the variation in pH.

Differences in mean specific conductance among habitats were not statistically significant. This falls within expectations since the water for all three habitats is coming from the same source. Temporal differences were also similar in all three habitats with minimum mean specific conductance occurring in the fall of 2010 and maximum during the winter of 2010. TDS means were also not significantly different from one another, which is expected since TDS concentrations are calculated using specific conductance values. Mean turbidity was significantly higher in the wetland (9.12 NTU) than in the pool and canal (2.59 NTU and 3.60 NTU respectively). These differences are due to substrate type. While the pool and canal are characterized by concrete, cobble, and pebble, the wetland is predominantly silt.

Chemical species concentrations were similar between habitats except for calcium concentration. Mean calcium concentration in the wetland was significantly lower ($P=0.034$) than mean concentration in the pool and canal. This decrease in calcium concentration may be due to higher biological demand upstream in the pool from *Chara* spp. which is known to affect levels of calcium (Pentecost, 1984). The other chemical species fit within levels typically associated with oligotrophic or mesotrophic systems (low nitrate, phosphate, and ammonia concentrations).

We determined the composition of diatoms in periphyton samples from each habitat to see if it could help explain native snail distribution. Since periphyton samples were not taken from each quadrat (often due to substrate being mud and thus scraping periphyton was not possible) diatom abundance was not included as an environmental variable in the redundancy analysis. However, we were able to determine future research directions and possible questions to investigate based on these data. In the wetland, *Terpsinoe musica* was the most abundant diatom and *T. granifera* was the most abundant snail. Whether *T. granifera* shows preference for a particular diatom type nutritionally is not reported in the literature. In the canal and pool, where abundances of native snails are highest, diatom composition is not dominated by one taxon as in the wetland. Hydrobiid spring snails are herbivores that graze on diatoms (Jensen and Siegismund, 1980) so knowing what types of algae or diatoms *P. texana* and *T. cheatumi* prefer could be valuable in future management decisions and developing protocol for breeding these snails in the lab. Future lab experiments that include testing preferences in microalgae communities should be coupled with future monitoring efforts.

Currently there are no data from previous studies to which our snail density and distribution results can be compared. Therefore, we have no way of knowing how imperiled the native snails are or how their distribution within the park has changed. The available literature on the native *P. texana* describe the morphometrics, taxonomy, and anatomy (Hershler et al., 2010) and some of the characteristics of the habitat (Dundee and Dundee, 1969), but nobody has empirically assessed the population, distribution, or habitat associations of *P. texana* or *T. cheatumi*. Even less is known about the other native snail in San Solomon Springs, *T. cheatumi*. Given that these snails are candidates for federal listing under the U.S. Endangered Species Act, we felt that it was necessary to start collecting abundance and habitat data. Also, habitat degradation in western U.S. desert artesian springs has resulted in the loss of endemic species (Keleher and Rader,

2008), so understanding how abundances of the two native snails at San Solomon Springs are changing may contribute to understanding of the current viability of native benthic invertebrates in these western desert systems. Further investigation into the ecology of *P. texana* and *T. cheatumi* is necessary if managers are to address conservation concerns.

P. texana and *T. cheatumi* mean density in the pool showed an overall decrease from May 2010 to May 2011 and *T. cheatumi* had a noticeably sharp decrease between May and June 2010. Since sampling was done at different locations in the pool each month this decrease could be the result of spatial variation, but there may be underlying temporal characteristics that influence changes in snail density from month to month. The decrease in density from May 2010 to June 2010 might be the result of annual pool drainage. Every year for a week in the late spring, the staff at Balmorhea State Park close the pool and drain the water so the south wing is completely dry, the water in the center area is about 2.0 m deep, and the water in the north wing is about 0.70 m deep. The staff drains the water so they can clean the algae (using power washers instead of chemicals) off the sides of the pool to protect swimmers from injury via slippage. In 2010 this was done in early May (in 2011 it was done in March). Drying out the south wing probably has little effect on the mean snail density between May and June since no snails were ever found in the south wing. *P. texana* and *T. cheatumi* were most abundant in the center section in which the maximum depth was 2.0 m after drainage.

There are a few possible explanations regarding the potential link between this draining and the decrease in mean density of native snails in the pool from May 2010 to June 2010. First, the *Chara* spp. and periphyton along the periphery are exposed to direct sunlight and atmospheric conditions rather than being under water, which could kill a portion of the available forage for snails once the pool fills. Second, snails might become stuck in these dry patches and die of desiccation before finding refuge. Third, this might concentrate the density of Mexican tetra into an area where snail density is high, resulting in increased predation of *P. texana* and *T. cheatumi*. The same arguments can be made for the north wing as well. In summary, the decrease in available habitat resulting from the sudden decrease in water depth during the spring could result in increased mortality, which would decrease the mean density in the following month. *T. granifera* mean density also decreased from May to June; however, *M. tuberculata* mean density increased during this period which could indicate that *M. tuberculata* is more tolerant to desiccation than the other snails.

We expected to see decreases in all four snail species rather than decreases in three of the four. One reason might be the difference in desiccation tolerance between *M. tuberculata* and *T. granifera*. Freshwater snails with larger shells are known to resist desiccation better than snails with smaller shells (Gerard, 2001; Facon et al., 2004) so the larger mean shell length of *M. tuberculata* (Table 2.5) could result in greater desiccation tolerance. While differences in desiccation tolerance between *M. tuberculata* and *T. cheatumi* has not been tested in a single experiment, separate experiments for *M. tuberculata* and *T. granifera* do provide insight as to how desiccation tolerance might differ. Dudgeon (1989) found that *M. tuberculata* adults could survive up to 20 days in dry conditions, while Chaniotis et al. (1980) noticed high mortality in *T. granifera* after only four days. Differences in food preferences might also explain why *M. tuberculata* mean density increased whereas mean density in other species fell. Whereas periphyton

grazing is the feeding strategy for *T. granifera* (Chaniotis et al., 1980) *M. tuberculata* in addition to grazing periphyton is also a voracious detritus consumer (Madsen, 1992) allowing it to survive in locations where vegetation quality has decreased as the result of the pool drainage. Although the feeding preferences of *P. texana* and *T. cheatumi* have not been investigated, the family they belong to is recognized as periphyton grazers (Brown et al., 2008).

After June 2010, *P. texana* mean density in the pool continued to decrease until November 2010. We do not know if this decrease during the summer and fall is a yearly trend followed by a spike in density the following spring or if this decrease is an anomaly brought about by environmental conditions. Recruitment tends to be continuous for hydrobiids in thermally stable springs (Hershler, 1984) so this decrease could indicate that there is something influencing the decrease of *P. texana* mean density. *P. texana* may also reproduce seasonally since observations of snails in the lab do not indicate continuous recruitment throughout the year. After the sharp decrease recorded in the June sampling, mean density of *T. cheatumi* remained consistently low until the field study concluded. Since we found so few *T. cheatumi* individuals, we do not know if the sharp decrease after June indicates any sort of reproductive pattern.

Temporal patterns for *P. texana* mean density were different in the wetland. The decrease from May to June 2010 was similar to the decrease in the pool, but instead of mean density generally decreasing, mean density in the wetland after June 2010 remained at 0 snails/m² (Figure 2.24a). This could be the result of the random sampling technique that we used. Since the majority of the sampling sites had mud or silt substrate, sampling events that consisted of sites only containing silt substrate would yield very low densities of *P. texana*. A second reason might be that stochastic events in the wetland affected *P. texana* density more than *M. tuberculata* and *T. granifera* density. The two invasive snails are regarded as habitat generalists, and in environments where they both occur, *M. tuberculata* is often the most common snail (Haynes, 1990). Parts of the wetland were dry at different times during the field sampling, so snails such as *M. tuberculata* and *T. granifera* that can reproduce quickly and are more resistant to desiccation than *P. texana* might prevent *P. texana* from becoming abundant after a disturbance. As stated previously, competitive exclusion of native snails by *M. tuberculata* (Pointier, 2001) and *T. granifera* (Karatyev et al., 2008) is known to occur and Rader et al. (2003) speculate that *M. tuberculata* could extirpate native snails in isolated spring systems. Therefore, high invasive snail densities, especially in the case of *T. granifera* with a mean density over 800 snails/m² in October 2010, could drive down mean *P. texana* density.

In the canal, *Pyrgulopsis texana* mean density was highest in June 2010 and mean density was higher in the canal than in the pool or wetland. High density in freshwater spring snails (family Hydrobiidae) found in isolated spring systems has been reported by other researchers. In New Mexico, from 1995 to 1996 the state listed and endangered Koster's springsnail (*Juturnia kosteri*) maximum density was 89,472 snails/m² in the Bitter Lake National Wildlife Refuge, but these numbers have since declined (New Mexico Department of Game and Fish, 2005). In general, members of hydrobiidae are known to occur in high densities and snails of the genus *Pyrgulopsis* are known to be found at mean densities near 10,000 snails/m² (Brown et al., 2008). Ergo, the mean

densities of *P. texana* found in the park are similar to mean densities of other species in the genus *Pyrgulopsis*.

Tryonia cheatumi, the second native springsnail at Balmorhea State Park, was the least abundant snail with only a mean of 11 snails/m² in the pool, five snails/m² in the canal, and no individuals found in the wetland. Total abundance of this snail at the park was estimated to be under 50,000 total snails, much lower than the total abundance of *P. texana*. Information on *Tryonia* snails is scarce, so less is known about typical density and abundance in thermally stable springs and no density information on *Tryonia cheatumi* was provided in the 2010 Review of Native Species that are Candidates for Listing by the U.S. Fish and Wildlife Service. We do not know if the absence of *T. cheatumi* in the wetland is an aberration, a temporal trend, if they once occurred but are now absent, or if they never were present in the wetland.

There is much more information on the two exotic species, *M. tuberculata* and *T. granifera* than with the two aforementioned native species due to their wider distribution and human health concerns. Total abundance of *Tarebia granifera* at Balmorhea State Park was estimated at 838,151 snails, making it the second most abundant snail after *P. texana* and outnumbering the native *T. cheatumi* by more than 18 times. Before our study, *T. granifera* was not known to occur in Balmorhea State Park until it was noticed by our lab. In habitats containing both *M. tuberculata* and *T. granifera*, *T. granifera* is known to outnumber *M. tuberculata* (Pointier et al., 1998), which we found to be the case at Balmorhea (Table 2.5. *M. tuberculata* was the third most abundant snail at Balmorhea. *M. tuberculata* density can also get very high and it has been known to displace native snail species (Guimarães, 2001).

According to other studies, shell length and age are positively correlated in *M. tuberculata* (Pointier, 1993; Elkarmi and Ismail, 2007) and *T. granifera* (Chaniotis et al., 1980). Thus, we measured the shell length of both exotic snails to see if there were any differences in age distribution. Spatially, the smallest mean shell lengths for *M. tuberculata* were in the pool and the largest were in the wetland. This may indicate that *M. tuberculata* individuals are moving downstream as they age, or that they live longer outside the pool or that reproduction is also lower outside the pool habitat. We observed the opposite in *T. granifera* individuals where smaller lengths in the wetland might indicate movement upstream. Upstream movement in *T. granifera* was documented in a study done by Prentice (1983) in the West Indies, where Prentice observed *T. granifera* individuals moving upstream at a rate of 100 m per month. The movement of *M. tuberculata* adults downstream could be passive via movement on floating plant matter or avian transport, but movement could also be deliberate. *T. granifera* individuals might also show a deliberate movement and Appleton et al. (2009) report that *T. granifera* upstream movement might be explained by preference for greater habitat heterogeneity.

The temporal variation in snail size is important since it indicates when *M. tuberculata* and *T. granifera* are reproducing. Results from other studies (Elkarmi and Ismail 2007; Garner and Haggerty, 2010) support that the frequencies of length classes can provide information on the frequencies of age classes and cohorts. Elkarmi and Ismail (2007) were able to determine age structure and life expectancy (five years) of a *M. tuberculata* population theoretically using known lengths, growth curves, and variable

metric methods. Knowing how the age structure of the invasive snail populations at Balmorhea change over time might help us understand fecundity, birth rate, generation time, and life expectancy. These parameters can differ for separate populations of the same species based on differences in habitat characteristics such as water temperature, season, and water quality (Chaniotis et al., 1980). These parameters are important in creating population models which can help us assess how feasible control methods might be at San Solomon Springs. In a way, population age and size structure may be a better way to characterize the snail populations at Balmorhea State Park rather than focusing solely on the total abundance or total biomass of snails. Data on size and age class frequencies could also show how predation pressure is affecting *M. tuberculata* and *T. granifera*.

For *M. tuberculata* the most frequently observed size classes were 7-12 mm and 13-18 mm. Young *M. tuberculata* are held in a brood pouch, which can hold as many as 70 individuals (Livshits and Fishelson, 1983), until they are released at around 0.07-2.33 mm in length with a complete shell (Ben-Ami and Hodgson, 2005). An increase in the frequency of the 1-6mm size class from 4.2% in fall 2010 to 22.5% in February 2011 indicates an increase in recruitment during the late fall and winter of 2010; also, a juvenile size class was present in all months which provides support for year round recruitment in thermally stable waters (Berry and Kadri, 1974). The increase in recruitment during winter might be due to decreases in predation from fish on 7-12 mm sexually mature snails from summer to winter. Also, members of thiaridae are nocturnal and shorter day lengths in the winter may mean that *M. tuberculata* reproduction depends on photoperiod. In a lab study, Berry and Kadri (1974) found that sexually mature *M. tuberculata* individuals kept in constant darkness produced more juveniles. Also, timing of release from the brood pouch was positively associated with decreasing temperature in their study, which could mean that more juveniles were found at Balmorhea State Park in the winter because adults might be releasing more juveniles in the winter. Ideally, future field research at Balmorhea lasting multiple years will help in supporting the recruitment patterns that we noticed in our field study.

Tarebia granifera size structure was dominated by the 7-12 mm size class, which made up at least 60% of the snails measured in any given season. Spikes in the 1-6 mm class were not present in *T. granifera* as they were in *M. tuberculata* and this class was present year round which could indicate year round recruitment. Year round recruitment for *T. granifera* was reported by Miranda et al. (2011) for a population in a South African estuary. The juveniles develop inside a brood pouch, similar to juvenile development in *M. tuberculata* and up to 77 juveniles were recorded in the brood pouches of adult *T. granifera* by Appleton et al. (2009). Aside from consistent physical and chemical conditions year round, the brood pouch might explain the consistent reproduction we observed in both invasive snails (conceptually the brood pouch would protect juveniles against stressors such as drought and desiccation). Stochastic events at San Solomon Springs are mostly limited to periodic drying of the wetland, so the importance of the role of the brood pouch in maintaining year round recruitment may not be as great as the effect of the stable habitat on recruitment. Understanding size structure may help us with future management goals regarding these two invasive species. Also, San Solomon Springs provides a rare opportunity to examine how temporal differences in *P. texana*

and *T. cheatumi* densities change as recruitment in the two invasive snails change. For example, the decrease in *P. texana* density in the pool during the spring months (March and April) of 2011 might be partially explained by the increase in recruitment in *M. tuberculata* during spring 2011. Since *M. tuberculata* is known to displace other aquatic snails via competition (Guimarães, 2001) fluxes in exotic snail recruitment could be affecting *P. texana* and *T. cheatumi*. We cannot establish that recruitment in both exotics is affecting temporal variation in density for the native snails, but these data are a starting point for future field and lab investigations.

P. texana is associated with habitat having high current velocity, more canopy cover, and higher temperatures than habitats associated with *T. granifera* or *M. tuberculata*. Substrate types associated with *P. texana* were cobble, gravel, pebble, and macrophyte. Studies have found that other springsnail species such as the page springsnail (*Pyrgulopsis morrisoni*) are also associated with swift moving water (Martinez and Thome, 2006). *T. cheatumi* was not associated with any explanatory variables. This may be due to finding very few *T. cheatumi* species since quadrats where they were found often only contained one or two individuals (indicated by the low density seen in Table 1). *M. tuberculata* and *T. granifera* were associated with depth and fine grain substrate. *M. tuberculata* is known to bury itself in substrate (Cohen, 1986) which may indicate its association with softer substrate. Overall though, given the wide range of substrate and little association with explanatory variables other than depth in the RDA model, *M. tuberculata* and *T. granifera* are mostly habitat generalists while the native *P. texana* has narrower habitat requirements. Habitat parameters did not explain variation in *T. cheatumi* density.

This field study was successful in providing data on species abundances and habitat associations of the native *P. texana* and *T. cheatumi* and these data will be valuable in establishing future field studies at Balmorhea State park and future lab experiments. Paramount in these future endeavors is determining husbandry techniques to get reliable reproduction in both native species in the lab. Given their nature as a candidate species and their limited range and dispersal capabilities, being able to supplement natural populations with individuals from the lab could prove valuable. Also, data on the life history of these species are unknown and this information is necessary in modeling which can help us determine the viability of the native snail populations with respect to time. These data may also influence management decisions, and management implications are presented in Chapter IV.

Literature Cited

- Abbott, R.T. 1952. A study on an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (*Paragonimus*). Proceedings of The United States National Museum 102:71-116.
- Anderson, R., I. Hogarth, R. Pickard, and C. Ogle. 1984. Loss of wildlife habitat in Northland, 1978–83, with notes on recently identified wildlife values. New Zealand Wildlife Service Technical Report 6.

- Appleton, C.C., and D.S. Nadasan. 2002. First report of *Tarebia granifera* (Lamarck, 1816) (Gastropoda: Thiaridae) from African Journal of Molluscan Studies 68:399-402.
- Appleton, C.C., Forbes, A.T. and Demetriades, N.T. 2009. The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zoologische Mededelingen 83:525-536.
- Arango, C.P., L.A. Riley, J.L. Tank, and R.O. Hall. 2009. Herbivory by an exotic snail increases nitrogen fixation in a nitrogen limited stream. Canadian Journal of Fisheries and Aquatic Sciences 66:1309-1317.
- Ben-Ami, F., and J. Heller. 2005. Spatial and temporal patterns of parthenogenesis and parasitism in the freshwater snail *Melanooides tuberculata*. Journal of Evolutionary Biology 18(1):138-146.
- Ben-Ami, F., and A.N. Hodgson. 2005. Ovoviviparity and the structure of the brood pouch in *Melanooides tuberculata* (Gastropoda: Prosobranchia: Thiaridae). Journal of Morphology 263:322-329.
- Berry A. J. and Kadri A. H. 1974. Reproduction in the Malaysian freshwater cerithiacean gastropod *Melanooides tuberculata*. Journal of the Zoological Society of London 172:369–381.
- Brown, K.M., B. Lang, and K.E. Perez. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. Journal of the North American Benthological Society 27:484-495.
- Brune, G., and H.C. Besse. 2002. Springs of Texas. Texas A&M University press. 590pp.
- Cattau, C.E., J. Martin, and W.M. Kitchens. 2010. Effects of an exotic prey species on a native specialist: Example of the snail kite. Biological Conservation 143:513–520.
- Chanotis, B. N., J.M. Butler, F.F. Ferguson, and W.R. Jobin. 1980. Thermal limits, desiccation tolerance, and humidity reactions of *Thiara (Tarebia) granifera muiensis* (Gastropoda: Thiaridae) host of the Asiatic lung fluke disease. Caribbean Journal of Science 16:91-93.
- Chowdhury, A. H., R.E. Mace, R. Anaya, and T. Way. 2000. A numerical groundwater flow model for the Trinity aquifer of south-central Texas- A tool to estimate water availability. Southwest Focus Ground Water Conference, National Ground Water Association, p. 72.
- Chowdhury, A.H., C. Ridgeway, and R.E. Mace. 2004. Origin of the waters in the San Solomon Spring system, Trans-Pecos Texas: in Mace, R. E., Angle, E. S., and Mullican, W.F., III, eds., Aquifers of the Edwards Plateau: Texas Water Development Board Report 360, p. 315-344.
- Cohen, A.S. 1986. Distribution and faunal associations of benthic invertebrates at Lake Turkana, Kenya. Hydrobiologia 141:179-197.

- DeMarco, P. Jr. 1999. Invasion by the introduced aquatic snail *Melanoides tuberculata* (Muller, 1774) (Gastropoda: Prosobranchia: Thiaridae) of the Rio Doce State Park, Minas Gerais, Brazil. *Studies of Neotropical Fauna and Environment* 34:186-189.
- Dudgeon, D. 1989. Ecological strategies of Hong Kong Thiaridae (Gastropoda:Prosobranchia). *Malacological Review* 22:39-53.
- Duggan, I.C. 2002. First record of a wild population of the tropical snail *Melanoides tuberculata* in New Zealand natural waters. *New Zealand Journal of Marine and Freshwater Research* 36:825-829.
- Dundee, D., and H. Dundee. 1969. Notes concerning two Texas molluscs, *Cochliopa texana* Pilsbry and *Lyrodes cheatumi* Pilsbry (Mollusca: Hydrobiidae). *Transactions of the American Microscopical Society* 88(2):205-210.
- Dillon, R.T. 2000. *The ecology of freshwater molluscs*. The Press Syndicate of the University of Cambridge, United Kingdom, 509p.
- Elkarmi, A.Z., and N.S. Ismail. 2007. Growth models and shell morphometrics of two populations of *Melanoides tuberculata* (Thiaridae) living in hot springs and freshwater pools. *Journal of Limnology* 66(2):90-96.
- Facon, B., E. Machline, J. P. Pointier, and P. David. 2004. Variation in desiccation tolerance in freshwater snails and its consequences for invasion ability. *Biological Invasions* 6:283-293.
- Freitas J.R., and M.B.L. Santos. 1995. Current advances on the study of snail-snail interactions, with special emphasis on competition process. *Memórias do Instituto Oswaldo Cruz* 90:261-269.
- Garner, J. T., and T. M. Haggerty. 2010. Distribution, density, and population dynamics of the Anthony Riversnail (*Athearnia anthonyi*) in Limestone Creek, Limestone County, Alabama. *American Malacological Bulletin* 28:121-126.
- Geo Midpoint. 2007. Random Point Generator. Available from: <http://www.geomidpoint.com/random/>. Last accessed 20 May 2011.
- Gerard, C. 2001. Consequences of drought on freshwater gastropod and trematode communities. *Hydrobiologia* 459:9-18.
- Giovanelli, A., M.V. Vieira, and C. Silva. 2005. Interaction between the intermediate host of schistosomiasis in Brazil, *Biomphalaria glabrata* (Say, 1818) and a possible competitor, *Melanoides tuberculata* (Muller, 1774): A field study. *Journal of Molluscan Studies* 71:7-13.
- Guimarães, C.T. 2001 Possible displacement of planorbids by *Melanoides tuberculata* in Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz* 96:173-176.
- HACH company. 2006. Digital titrator model 16900 manual. HACH company, 24th ed. 195pp.
- Hall, R.O., M.F. Dybdahl, and M.C. VanderLoop. 2006. Extremely high secondary production of introduced snails in rivers. *Ecological Applications* 16:1121–1131.

- Haynes, A. 1990. The numbers of freshwater gastropods on Pacific Islands and the theory of island biogeography. *Malacologia* 31:237–248
- Hershler, R.. 1984. The hydrobiid snails (Gastropoda: Rissoacea) of the Cuatro Cienegas basin: systematic relationships and ecology of a unique fauna. *Journal of the Arizona–Nevada Academy of Science* 19:61–76.
- Hershler, R., and F.G. Thompson. 1992. A review of the aquatic gastropod subfamily Cochloppinae(Prosobranchia: Hydrobiidae). *Malacological Review Supplements* 5:1-140.
- Hershler, R. 1999. A systematic review of the hydrobiid snails of the Great Basin, western United States. Part II. Genera *Colligyus*, *Eremopyrgus*, *Fluminicola*, *Pristinicola* and *Tyronia*. *The Veliger* 42(4):306-337.
- Hershler, R., M. Mulvey, and P. Liu. 2005. Genetic variation in the Desert Springsnail (*Tryonia porrecta*): implications for reproductive mode and dispersal. *Molecular Ecology*, 14:1755-1765.
- Hershler R., H.P. Liu, and B.K. Lang. 2010. Transfer of *Cochliopa texana* to *Pyrgulopsis* (Hydrobiidae) and description of a third congener from the lower Pecos River basin. *Journal of Molluscan Studies* 76(3):245-256.
- Jensen, K. T., and H.R. Siegismund. (1980). The importance of diatoms and bacteria in the diet of Hydrobia-species. *Ophelia* 1 (Suppl.):193-199.
- Karatyev, A.Y., L.E. Burlakova, V.A. Karatyev, and D.K. Padilla. 2009. Introduction, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia* 619:181-194.
- Keleher, M. J., and R. Rader. 2008. Bioassessment of Artesian Springs in the Bonneville Basin, Utah, USA. *Wetlands* 28:1048-1059.
- Livshits, G., and L. Fishelson. 1983. Biology and reproduction of the freshwater snail *Melanoides tuberculata* (Gastropoda: Prosobranchia) in Israel. *Israel Journal of Zoology* 32:21-35.
- Makarek, V., and P. Legendre. 2002. Nonlinear redundancy analysis and canonical correspondence analysis based on polynomial regression. *Ecology* 83(4):1146-1161.
- Malcom, J.W., W.R. Radke, and B.K. Lang. 2005. Habitat associations of the San Bernardino springsnail, *Pyrgulopsis bernardina* (Hydrobiidae). *Journal of Freshwater Ecology* 20(1):71-77.
- Madsen, H. 1992. Food selection by freshwater snails in the Gezira irrigation canals, Sudan. *Hydrobiologia* 228:203-217.
- Martinez, M.A., and D.M. Thome. 2006. Habitat usage by the Page Springsnail, *Pyrgulopsis morrisoni* (Gastropoda:Hydrobiidae) from central Arizona. *The Veliger* 48(8):8-16.

- Miranda N., R. Perissinotto, and C.Appleton. 2010. Salinity and temperature tolerance of the exotic freshwater gastropod *Tarebia granifera*. South African Journal of Science 106(3/4):1-7.
- Miranda, N., R. Perissinotto, and C.Appleton. 2011. Population structure of an invasive parthenogenic gastropod in coastal lakes and estuaries of Northern KwaZulu-Natal, South Africa. PLoS One 6(8):p. e24337.
- Mitchell, A.J., R.M. Overstreet, A.E. Goodwin, and T.M. Brandt. 2005. Spread of an exotic fish-gill trematode: a far reaching and complex problem. Fisheries 30(8):11-16.
- Murray, H.D. 1964. *Tarebia granifera* and *Melanoides tuberculata* in Texas. Annual Reports of the American Malacological Union 1964:15–16.
- New Mexico Department of Game and Fish. 2005. Recovery and conservation plan for four Invertebrate species: Noel's amphipod (*Gammarus desperatus*), Pecos assiminea (*Assiminea pecos*), Koster's springsnail (*Juturnia kosteri*), and Roswell springsnail (*Pyrgulopsis roswellensis*). 81pp.
- Pentecost, A. 1984. The growth of *Chara globularis* and its relationship to calcium carbonate deposition in Malham Tarn. Field Studies 6:53-58.
- Pointier, J.P., and F. McCollough. 1989. Biological control of the snail hosts of *Schistosoma mansoni* in the Caribbean area using *Thiara* spp. Acta Tropica 46:147-155.
- Pointier, J.P. 1993. Ecology of the introduced snail *Melanoides tuberculata* (Gastropoda, Thiariidae) in relation to *Biomphalaria glabrata* in the marshy forest zone of Guadeloupe French West Indies. Journal of Molluscan Studies, 59:421-428.
- Pointier J.P., S. Samadi, P. Jarne, and B. Delay. 1998. Introduction and spread of *Thiara granifera* (Lamarck, 1822) into Martinique island, French West Indies. Biodiversity and Conservation 7:1277-1290.
- Pointier, J.P. 2001. Invading freshwater snails and biological control in Martinique Island, French West Indies. Memórias do Instituto Oswaldo Cruz 96:67-74
- Pratt, W. L. 1977. Hydrobiid snails of the Moapa warm spring complex, Nevada. Western Society of Malacologists, Annual Report 1977 10:7.
- Prentice, M.A. 1983. Displacement of *Biomphalaria glabrata* by the snail *Thiara granifera* in field habitats in Santa Lucia, West Indies. Annals of Tropical Medicine and Parasitology 77:51-59.
- Prescott, G.W. 1970. How to know the freshwater algae. WMC Brown Company publishers, Dubuque Iowa. 304pp.
- Pung, O.J., C.B. Grinstead, K. Kersten, and C.L. Edenfield. Spatial distribution of hydrobiid snails in salt marsh along the Skidaway River in southeastern Georgia with notes on their larval trematodes. 2008. Southeastern naturalist 7(4):717-728.

- Rader, R.B., M. Belk and K. Keleher 2003. The Introduction of an Invasive Snail (*Melanoides tuberculata*) to Spring Ecosystems of the Bonneville basin, Utah. *Journal of Freshwater Ecology* 18(4):647-657.
- Scholz, T., and G. Salgado-Maldonado. 2000. The introduction and dispersal of *Centrocestus formosanus* (Nishigori, 1924) (Digenea:Heterophyidae) in Mexico: A Review. *The American Midland Naturalist* 143(1):185-200.
- Taylor, D.W. 1987. Fresh-water molluscs from New Mexico and vicinity. Bulletin 116, New Mexico Bureau of Mines and Mineral Resources, 50pp.
- Thompson, F.G. 1984. The freshwater snails of Florida: a manual for identification. University of Florida Press, Gainesville. 94p.
- Thorp, J.H., and A.P. Covich. 2009. Ecology and Classification of North American Freshwater Invertebrates. Elsevier Science, 1021pp.
- Texas Parks and Wildlife Department. 2011. Balmorhea State Park. Available from: <http://www.tpwd.state.tx.us/spdest/findadest/parks/balmorhea/>. Accessed on 11 October 2011.
- United States Department of the Interior. 2004. Biological opinion on construction of a dam and pumping system at Phantom Lake Spring. Memorandum 29pp.
- United States Environmental Protection Agency. 1983. Methods for Chemical Analysis of Water and Wastes. U.S.E.P.A., Cincinnati, OH, pp. xv-xx.
- Vargas, M., J. Gomez, and G. Perera. 1991. Geographic expansion of *Marisa cornuarietis* and *Tarebia granifera* in the Dominican Republic. *Journal of Medical & Applied Malacology* 3:69-72.
- Vinyard W. 1977. Diatoms of North America. Mad River Printing. 119pp.

Chapter III

Effects of substrate size on snail distribution

Abstract

Several biotic and abiotic factors are important in influencing snail distributions and habitat associations. Our field results show that substrate size and type are important in explaining variation in density of *Pyrgulopsis texana* and *Tryonia cheatumi*. To test our field results, we conducted a controlled laboratory experiment on substrate preference. We used *Melanoides tuberculata*, *Tarebia granifera*, and *Pyrgulopsis texana* individuals. Individuals were placed in containers divided into three sections, each having a different substrate type (sand, gravel, or pebble). *P. texana* and *T. granifera* both selected gravel substrates while *M. tuberculata* had not substrate preference. From these results we can infer that *M. tuberculata* is more of a habitat generalist than *P. texana* and that substrate type is an important component in shaping aquatic snail distribution among *M. tuberculata*, *P. texana*, and *T. cheatumi*.

Introduction

Microhabitat usage in freshwater aquatic snails is often species specific and depends on several environmental factors. Substrate size and type has been recognized by several scientists as a useful explanatory variable in assessing habitat associations. For hydrobiid snails (Gastropoda: Hydrobiidae), sediments provide protection from fish predators and substrate for grazing (Barnes, 2003). Periphyton thickness is also an important component of substrate use in snails with some species preferring dense algal mats and others associating more with bare substrate (Holomuzki and Biggs, 2007). Sediment type is also critical for fecundity and growth in some snail species. Smaller snails, such as those in hydrobiidae, show higher growth rates on smaller fine grain sediment than on coarser sand (Forbes and Lopez, 1990). In general though, most snail species prefer cobble substrate since it has large surface area for periphyton growth, and it is available year round. Freshwater gastropods are mainly herbivores and thus prefer large substrate that can support periphyton growth (Thorp and Covich, 2009).

The chemical parameters among our sites did not significantly differ or contribute strongly to habitat selection. The field data suggest that substrate size might be the most important physical parameter in determining habitat selection in our study species. Thus, we decided to conduct a controlled laboratory experiment to determine if substrate type might contribute to habitat selection in *Melanoides tuberculata*, *Tarebia granifera*, and *Pyrgulopsis texana*.

The purpose of our study was to examine the effects of substrate size on snail activity and distribution in a controlled laboratory setting. These studies were conducted based on results of the RDA model from the field data (Figure 2.34 and Table 2.8). The field data suggest that substrate size might be the most important physical parameter in determining habitat selection in our study species. The importance of substrate size in microhabitat selection has been explored in other freshwater snail species in both lentic and lotic systems. Greenwood and Thorp (2001) found that differences in distribution between two species of snails in the Ohio River were primarily determined by substrate.

Another researcher stated that substrate size was the most important factor affecting the distribution of another springsnail, *Pyrgulopsis morrisoni* (Martinez and Thome, 2006).

While the impacts of exotic snails on habitat functioning is well known, less is known about their distribution, dispersal ecology, and habitat preferences. Understanding habitat preference may provide insights into the types of habitat exotic snails are able to exploit and how these exotics interact with native snails. Characterizing the physical and chemical environments that snails inhabit is important for understanding habitat selection and dispersal. Since the chemical parameters among our sites did not significantly differ or contribute strongly to habitat selection, we conducted controlled laboratory experiments to determine if substrate might contribute to snail habitat selection. We limited our focus to testing substrate size selection in *Melanoides tuberculata*, *Tarebia granifera*, and *Pyrgulopsis texana*.

Methods

Snails were collected from Balmorhea State Park and transported in 1.5L containers and placed in 10 gallon aquaria immediately upon returning to the lab. Tanks were filled with 25% water from the collection site and 75% de-chlorinated tap water. We fed snails once per week using ground algae pellets and set up aquarium lights and a 12 hour light/dark cycle to stimulate algal growth. No *T. cheatumi* individuals were collected since we did not want to exacerbate population decline of a snail with low densities in San Solomon Springs.

We tested if microhabitat selection in *M. tuberculata*, *T. granifera*, and *P. texana* exhibited a non random pattern under controlled laboratory conditions. We conducted the experiment at Texas Tech University from 0900 to 1900 hr during June and July 2011 under controlled air temperatures near 22.2°C. Two weeks before the experiment, snails were moved from their stock tanks to aerated tanks with specific conductivity near 3.32 mS and pH 7.22. We did this so snails could acclimate to water conditions that mimic those at Balmorhea State Park. Four cylindrical plastic containers 31.50cm in diameter were used as experimental chambers. Each chamber had 3 equally sized sections delineated that had one of three substrate types: sand (labeled as section 1), gravel (labeled as section 2), or pebble (labeled as section 3). Sand was defined as particle size 0-1mm, gravel was defined as particle size 1-4mm, and pebble as size 5-60mm. This was the treatment group. If snails were found on the container wall, we recorded them as such and included them in the analysis. Each trial consisted of four containers, two of which had a substrate type assigned to each section and two of which had no substrate which served as the control.

We ran enough trials to have 20 control and 20 treatment replicates for each species. Between trials, substrates were placed in sieves and cleaned with de-ionized water and placed in the chambers at random. Snails were placed in the center of each container with opercula facing upward and trials ran for four hours. After the four hour trial, we counted the number of snails in each section. We initially did the snail counts every 30 minutes, but digging was required in order to locate *P. texana* individuals due to their small size and disturbing the substrate this often would have affected the results.

Snails that did not move after the four hour period were not used in the analysis. This methodology is similar to the substrate usage experiments conducted by Greenwood and Thorp (2001) who removed snails that did not move along the substrate from their analysis. We used only adult *M. tuberculata* and *T. granifera* individuals with mean shell lengths of 14.32 mm (SD 1.41) and 12.16 mm (SD 1.84) respectively. We ran each species in separate trials and each container either had 10 *M. tuberculata*, 10 *Tarebia granifera*, or 20 *P. texana* individuals. *M. tuberculata* and *T. granifera* individuals were not re-used but some *P. texana* individuals were used in more than 1 trial since the number of snails we were able to keep in the lab was restricted. The number of snails in each section after four hours was used in the analysis. Data were analyzed using the non parametric Kruskal-Wallis test for significance.

Results

Thirty-four percent of all tested *M. tuberculata* species were found in gravel substrate and 24% and 34% were found in sand and pebble (Table 3.1). For *T. granifera*, 40% were found in gravel and for *P. texana*, 58% were found in gravel substrate. *M. tuberculata* did not prefer one substrate type over the others ($\chi^2= 3.07$, $P=0.21$, $df=2$) (Figure 3.1) and in the control groups *M. tuberculata* did not show any statistically significant preference for any section (Figure 3.2). Mean number of *T. granifera* individuals was significantly higher ($\chi^2= 11.1$ $P=0.003$, $df=2$) in section 2 (gravel) than in the other treatment sections (Figure 3.2ab). *P. texana* also had significantly higher ($\chi^2=43.51$, $P<0.001$, $df=2$) mean number of individuals in the gravel section when compared to the sand and pebble sections (Figure 3.3a and b).

Table 3.1--Percentage of total individuals of *M. tuberculata*, *T. granifera*, and *P. texana* found in each substrate type.

Species	Sand	Gravel	Pebble	Container wall
<i>M. tuberculata</i>	0.24	0.34	0.34	0.08
<i>T. granifera</i>	0.25	0.40	0.26	0.09
<i>P. texana</i>	0.15	0.58	0.19	0.08

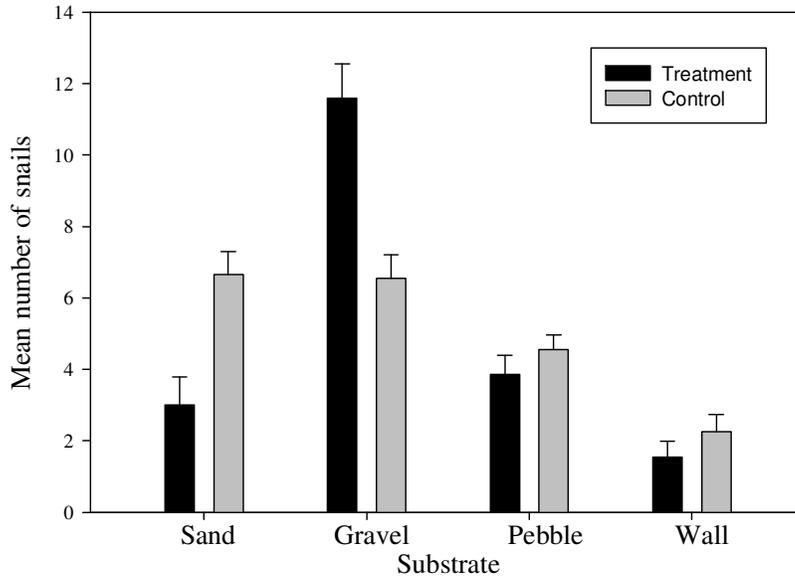


Figure 3.1--Substrate selection results for *P. texana*.

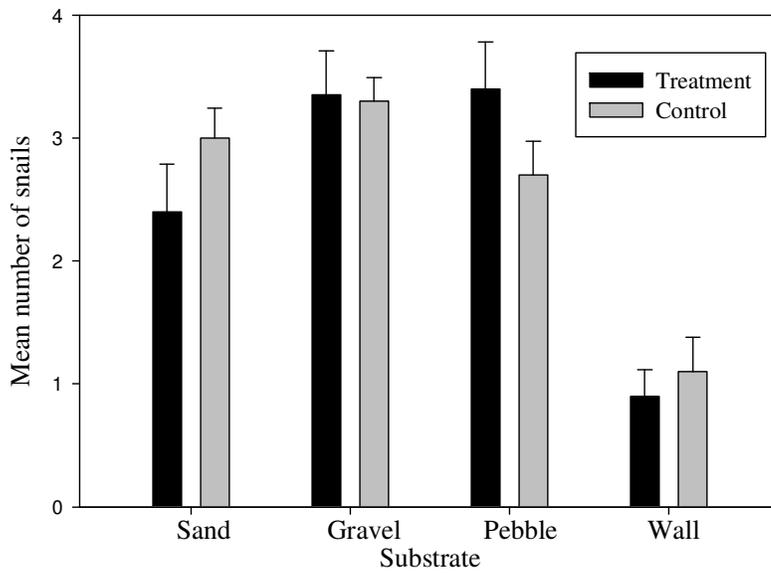


Figure 3.2--Substrate selection results for *M. tuberculata*.

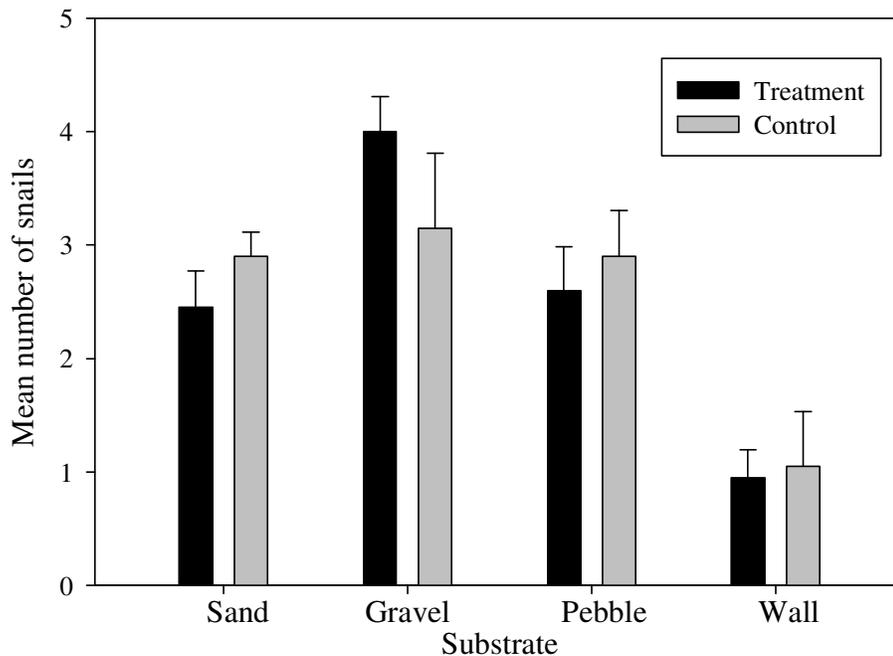


Figure 3.3--Substrate selection results for *T. granifera*.

Discussion

Depth, substrate, and stream flow are widely recognized habitat variables that affect freshwater snail distribution and habitat selection (Dillon, 2000). Crowl and Schnell (1990) reported that water flow and substrate particle size were the two most important factors in explaining differences in *Physella virgata* density (Crowl and Schnell, 1990). Another field study (Stewart and Garcia, 2002) found that substrate was the primary cause of variation in snail density of *Leptoxis carinata*, a freshwater snail, in central Virginia. Physical factors such as water velocity can contribute to substrate selection in some snail species. There are a few hypotheses as to why snails prefer one substrate type over another. Some snail species require hard substrates for oviposition (Cranford, 1988), others need harder substrate since it is more likely to have a thick periphyton layer than soft substrate (Thorp and Covich, 2009). In general, substrate is an important component of the aquatic system that can explain snail microhabitat selection and distribution.

Our experiment supports the results of the field study, that the native snail *P. texana* prefers harder and larger substrate. No snails showed a preference for a particular section in the control groups. Other studies on habitat selection in hydrobiidae have confirmed our findings. Martinez and Thome (2006) found that substrate particle size was an important determinant of snail distribution and density and they found that their study organism, the Page springsnail (*Pyrgulopsis morrisoni*), prefers hard substrate.

Cobble was not included in this study since particle size was limited by container size, but field data (Chapter II) suggest that cobble is a highly utilized substrate in *P. texana*. Other variables to consider when looking at substrate selection are periphyton thickness and temperature. Therefore, an investigation looking at how periphyton thickness or diatom composition affects snail habitat selection may be beneficial. Also, our field data show that depth may also explain variations in snail density at Balmorhea State Park and a lab experiment designed to test snail preference for a particular depth would help support our findings in the field.

M. tuberculata in particular is known for using a range of habitats from permanent springs to ephemeral and manmade habitats (Brown, 1994). Kock and Wolmarans (2009) studied the distribution and habitat preferences of *Melanoides tuberculata* by looking at data from other studies. They concluded that *M. tuberculata* distribution depends largely on substrate type and temperature. *T. granifera* in our lab study showed a preference for gravel habitat which contrasts with their classification as habitat generalists. Our field data from chapter II show that they are associated with sand, pebble and fine grain substrates.

Literature cited

- Barnes, R.S.K. 2003. Feeding rates of continually submerged *Hydrobia ulvae* vary during the daylight hours. *Journal of Marine Biology* 83:1273-1275.
- Bobo, T., Y.W. Chien, K. Schaffer, A. Tam. 2001. Substrate preferences of *Campeloma decisum* and *Elimia livescens*: influence of species interaction and substrate tenacity.
- Brown, D.S. 1994. *Freshwater snails of Africa and their Medical Importance*. 2nd edition. 608pp.
- Cranford, P.J. 1988. Behavior and ecological importance of a mud snail (*Ilyanassa obsoleta*) population in a temperate macrotidal estuary. *Canadian Journal of Zoology* 66(2):459-466.
- Crowl, T.A. and G.D. Schnell. 1990. Factors determining population density and size distribution of a freshwater snail in streams: effects of spatial scale. *Oikos* 69:359-367.
- Dillon, R.T. 2000. *The ecology of freshwater molluscs*. The Press Syndicate of the University of Cambridge, United Kingdom, 509p.
- Forbes, V.E., and G.R. Lopez. 1990. The role of sediment type in growth and fecundity of mud snails (Hydrobiidae). *Oecologia* 83:53-61.
- Greenwood, K.S., and J.H. Thorp. 2001. Aspects of ecology and conservation of sympatric, Prosobranch snails in a large river. *Hydrobiologia* 455:229-236.
- Holomuzki, J.R., and B.J. Biggs. 2007. Physical microhabitat effects on 3-dimensional spatial variability of the hydrobiid snail, *Potamopyrgus antipodarum*. *New Zealand Journal of Marine and Freshwater Research* 41:357-367.

- Kock, K.N., and C.T. Wolmarans. 2009. Distribution and habitats of *Melanoides tuberculata* (Müller, 1774) and *M. victoriae* (Dohrn, 1865) (Molluska:Prosobranchia:Thiaridae) In South Africa. *Water SA* 35(5):713-720.
- Martinez, M.A., and D.M. Thome. 2006. Habitat usage by the Page Springsnail, *Pyrgulopsis morrisoni* (Gastropoda:Hydrobiidae) from central Arizona. *The Veliger* 48(8):8-16.
- Stewart, T.W., and J.E. Garcia. 2002. Environmental factors causing local variation in density and biomass of the snail *Leptoxis carinata* in Fishpond Creek, Virginia. *American Midland Naturalist* 148:172-180.
- Thorp, J.H., and A.P. Covich. 2009. *Ecology and Classification of North American Freshwater Invertebrates*. Elsevier Science, 1021pp.

Chapter IV

Summary, management implications, and future directions

Summary--The San Solomon Springs complex at Balmorhea State Park is a unique habitat containing a variety of endemic and endangered species. Two invasive exotic snails, *Melanoides tuberculata* and *Tarebia granifera*, and two endemic springsnails, *Pyrgulopsis texana* and *Tryonia cheatumi*, are found in the San Solomon Springs complex. There are a few reasons as to why understanding the spatial patterns, population sizes, and habitat associations of these four snail species is important. First, there is a gap in knowledge when it comes to habitat associations among snail species. While the life histories of several snail species are known, habitat associations are commonly overlooked. Secondly, understanding habitat associations is necessary in order to direct conservation efforts, especially for endemic hydrobiid springsnails found in isolated spring systems like San Solomon Springs. In general, basic research in aquatic snails is lacking although the need and importance of this type of research has been expressed by other researchers (Brown et al., 2008). Interest in these organisms is increasing and recent studies have been published examining how water quality parameters affect hydrobiid snail distribution (Malcom et al., 2005; Tsai et al., 2007). Understanding how exotic gastropods are related to habitat is also important for understanding how populations are established and perhaps assessing invasion risk. Based on the results of our field research, we think that *T. granifera* has potential to spread to other thermally stable springs in the Balmorhea area such as East Sandia Spring and West Sandia Spring. Invasions may be especially dangerous to isolated springs ecosystems in the Chihuahuan Desert ecoregion since these springs often have high levels of endemism.

Our results supported expectations that individual species would have a clumped distribution throughout the spring system. In the pool, the endemic *P. texana* was most common in the center rather than the south wing or north wing. We did not find any snail species in the south wing of the pool, which was unexpected. While this area of the pool receives high foot traffic from swimmers as it is shallow, water quality parameters indicate that it is still suitable snail habitat. Depth may explain the lack of snail species in the south wing since there is a positive relationship between the two exotic snails and increasing depth. In the north wing the dominant snail species changes from *P. texana* to *M. tuberculata*. This may be due to a difference in substrate. Substrate in the center pool consists of concrete, pebble, and *Chara* spp. while in the north wing it is fine grain and cobble.

T. granifera and *M. tuberculata* densities were positively associated with depth and negatively with distance from the springhead. *P. texana* was associated with areas of high current velocity, increased canopy cover, and higher temperatures. This agrees with what is currently known about other hydrobiid springsnails in isolated spring systems. While these field data are useful in determining what factors may influence snail distribution, the interactions among these variables is hard to tease apart. In order to examine how substrate might influence snail distribution, we conducted a controlled lab experiment in which we found that *P. texana* individuals had a preference for gravel over sand and cobble substrates, while *M. tuberculata* was more of a generalist species that did

not prefer any particular type of substrate. This suggests that the distribution of *M. tuberculata* in the field may be more strongly associated with water depth.

We were primarily interested with the influence of physical and chemical factors, rather than biological factors, on snail distribution and abundance. However, biological factors such as predation and food availability are important determinants of snail distribution (Aldridge, 1983). We did not investigate if predators influenced snail population or distribution; however, predators are likely having an impact on the snail distribution in San Solomon Springs. In hard water, crayfish and molluscivorous fish heavily influence the abundance and distribution of aquatic snails (Lodge et al., 1987). One field study examined if differences in fish community structure were related to benthic invertebrate community structure. After examining 44 ponds containing either no fish, primarily molluscivorous fish, or primarily piscivorous fish, the authors reported that ponds containing molluscivorous fish had lower numbers of aquatic snails than the other pond types (Brönmark and Weisner, 1996). Pressure from molluscivores also influences how snails utilize microhabitat, which affects distribution (Nyström and Pérez, 1998). Weber and Lodge (1990) found that freshwater snails abandon preferred substrate (large and hard substrate) and seek shelter in macrophyte assemblages in the presence of crayfish. Molluscivorous fish (Stein et al., 1984) and crayfish (Alexander and Covich, 1991) are also known to affect size structure of snail communities. The abundance of the northern crayfish (*Orconectes virilis*) was negatively associated with snail abundance but positively associated with snail size (Crowl and Schnell, 1990). Because snail shell size positively correlated with age in many snail species (Graham, 2003) predators may also influence aquatic snail age structure and distribution.

Snail predators at Balmorhea State Park include Mexican tetra (*Astyanax mexicanus*), headwater catfish (*Ictalurus lupus*), and a species of crayfish. Crayfish did not occur in any of the sampling quadrats, but individuals were present throughout the park and most noticeable in the wetland. The presence of crayfish in the wetland may help explain the low density of *P. texana* and *T. cheatumi* when compared to the two exotic snails. Small snails typically have thinner shells than larger aquatic snail species and are less resistant to crushing (Brönmark and Weisner, 1996). The smaller size of *P. texana* and *T. cheatumi* may result in them being selected as prey items by Mexican tetra and crayfish over the larger *M. tuberculata* and *T. granifera*.

Management implications--From our field investigation and laboratory experiment we found that the native *P. texana* is positively associated with gravel/cobble substrate and *Chara* spp. (for feeding, cover, or possibly both). *P. texana* is negatively associated with turbidity, and pH. Also, while *P. texana* is more abundant in terms of numbers of individuals, the biomass of *P. texana* is likely much less than *M. tuberculata* or *T. granifera* which are much larger in size. *T. cheatumi* abundance is the lowest of all known snail species at Balmorhea. Both native snails show little association with the wetland habitat and more specifically the fine grain sediments which dominate most of the wetland bottom area. Any future habitat reconstruction/restoration should incorporate larger and harder substrate types.

This field study was the first attempt to empirically determine snail abundance and distribution of the native *Pyrgulopsis texana* and *Tryonia cheatumi* at Balmorhea

State Park. This dataset can serve as a baseline for future studies and monitoring efforts directed at native snail conservation in San Solomon Springs.

Future directions and current research--Future studies at Balmorhea State Park should continue monitoring snail density with emphasis on *Pyrgulopsis texana* and *Tryonia cheatumi*. These snails are grazers and feed on periphyton, and while we counted the number of diatoms in periphyton samples, a better method might be to measure periphyton thickness or biomass and also measure the surface area of large substrates (cobble and boulder). Laamrani et al. (2011) included periphyton thickness as a parameter in their *M. tuberculata* distribution study, so it might be a worthwhile parameter to include in future studies of the springs at Balmorhea State Park.

According to our substrate selection experiment results (Chapter III) and the results of our redundancy analysis (Chapter II), *P. texana* and *T. cheatumi* are associated with habitat containing large cobble substrate and negatively associated with fine grain silt or mud. Thus we would expect that the new wetland that was created at Balmorhea State Park in 2010, whose substrate consists mostly of silt would not be ideal habitat for the native snails. Future studies should also investigate the status of *P. texana* and *T. cheatumi* density at Giffin Springs and East Sandia Springs since little is known about the distribution and habitat use of the snail communities in these springs.

While we measured shell length of *M. tuberculata* and *T. granifera* in the field, a better method would be to measure shell girth (of the first whorl) along with shell length. This is prudent, since the apex of the shell is fragile, prone to weathering, and often breaks off naturally or during handling, resulting in a size bias. Future experiments should focus on food preference, life history, and habitat selection for *Pyrgulopsis texana*, and *Tryonia cheatum* only if their abundance increases.

Regarding reproduction, given the status of both native snails as candidate species and their limited range and dispersal capabilities, being able to supplement natural populations with captive bred individuals could prove valuable. Also, data on the life history of these species are unknown and this information is necessary in modeling which can help us determine the longterm viability of native snail populations. With respect to *M. tuberculata* and *T. granifera*, finding a way to reliably age these snails would be beneficial in population modeling and determining if these invasive snails are affecting the abundance of *P. texana* and *T. cheatumi*. Our field results revealed little association between the invasive snails and *P. texana* currently, but densities of *M. tuberculata* and *T. granifera* might become high enough in the pool and canal to eventually affect habitat use of the native snails. Additionally we do not know what the habitat associations or densities of the native snails were prior to the introduction of the two invasives. Thus, we do not know if the invasives have affected habitat associations, density, or distribution within San Solomons Springs.

From our research we can make some management recommendations that we believe would be beneficial to the native snails. Placing larger and harder substrate in the current wetlands should provide habitat for the native snails. Although the wetlands were built with the Pecos gambusia and Comanche Springs pupfish in mind, *P. texana* and *T. cheatumi* were found only on large hard substrate or near *Chara* spp. and macrophytes in

the wetland, thus the addition of more appropriate substrate should be beneficial to the native snails.

It might be worthwhile to further investigate the presence of parasites at Balmorhea State Park, particularly since there was a recent (Spring 2012) outbreak of “swimmer’s itch” within the pool area. While we did not detect any trematodes in our small sample, we can not state that *Centrocestus formosanus* or any other trematodes are not present within the spring. Exotic species along with their associated parasites can negatively affect native vertebrates (Font, 2003). *C. formosanus* metacercariae have been reported in numerous fish species in Texas (Mitchell et al., 2002) and pathologies such as hemorrhaging, destruction of gill lamellae, and edema are common results of infection in fish (Fleming et al., 2011). The presence of both *M. tuberculata* (first intermediate host) and the green heron (definitive host) indicate that *C. formosanus* could exist in Balmorhea State Park. Given the possibility of *C. formosanus* presence and the potential detrimental effects on the native Pecos gambusia (*Gambusia nobilis*) and Comanche Springs pupfish (*Cyprinodon elegans*), it might be prudent to include investigations into parasite presence in future studies.

We do not know the potential of *P. texana*, *T. cheatumi*, *M. tuberculata*, or *T. granifera* for active dispersal, especially in regards to upstream movement in the refuge canal. Kappes and Haase (2012) note that there is a lack of knowledge about active dispersal in bivalves and gastropods and understanding dispersal may help researchers predict how invasive species might affect the distribution of native species. The best way to investigate movement may be to construct artificial flumes and use pumps to alter the water flow rate. While a field study would be beneficial, it would be difficult and time consuming to use mark recaptures methods with *P. texana* due to their small size. Preliminary laboratory investigations we have conducted show that flow rate does affect upstream movement in *P. texana*.

Our current research (as of March 2012) is focused on propagation of viable reproductive *P. texana* individuals. Given the potential status of *P. texana* as a federally listed species and its small habitat range, having a standardized method for propagating *P. texana* under laboratory conditions could be important for future conservation efforts. We are examining the effects of water chemistry, light cycles, food, and density of *M. tuberculata* and *T. granifera* on *P. texana* reproduction. We also plan to investigate the occurrence of trematode parasites utilizing snails in San Solomon Springs, at Balmorhea State Park.

Literature Cited

- Aldridge, D. W. 1983. Physical ecology of freshwater prosobranchs. In Russell-Hunter, W. D. (editor), *The Mollusca*, Vol. 6: Ecology. Academic Press, Orlando, Florida, USA:329-358.
- Alexander, J. E., and A. P. Covich. 1991. Predation risk and avoidance behavior in two freshwater snails. *Biological Bulletin* 180:387–393.

- Brönmark, C., and S. E. B. Weisner. 1996. Decoupling of cascading trophic interactions in a freshwater, benthic food chain. *Oecologia* 108:534–541.
- Brown, K.M., B. Lang, and K.E. Perez. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. *Journal of the North American Benthological Society* 27:484-495.
- Crowl, T. A., and G. D. Schnell. 1990. Factors determining population density and size of a freshwater snail in streams: effects of spatial scale. *Oikos* 59:359–367.
- Fleming, P.B., D.G. Huffman, T.H. Bonner, T.M. Brandt. 2011. Metacercarial distribution of *Centrocestus formosanus* among fish hosts in the Guadalupe River drainage of Texas. *Journal of Aquatic Animal Health* 23(3):117-124.
- Font, W.F. 2003. The global spread of parasites: What do Hawaiian streams tell us? *Bioscience* 53:1061-1067.
- Graham, A.L. 2003. Effects of snail size and age on the prevalence and intensity of avian schistosome infection: relating laboratory to field studies. *Journal of Parasitology* 89(3):458-463.
- Kappes, H., and P. Haase. 2012. Slow, but steady: dispersal of freshwater mollusks. *Aquatic sciences* 74(1):1-14.
- Laamrani, H., K. Khallayoune, B. Delay, and J.P. Pointier. 2011. Factors affecting the distribution and abundance of two prosobranch snails in a thermal spring. *Journal of Freshwater Ecology* 12(1):75-79.
- Lodge, D. M., K. M. Brown, S. P. Klosiewski, R. A. Stein, A. P. Covich, B. K. Leathers, and C. Brönmark. 1987. Distribution of freshwater snails: Spatial scale and the relative importance of physicochemical and biotic factors. *American Malacological Bulletin* 5:73–84.
- Malcom, J.W., W.R. Radke, and B.K. Lang. 2005. Habitat associations of the San Bernardino springsnail, *Pyrgulopsis bernardina* (Hydrobiidae). *Journal of Freshwater Ecology* 20(1):71-77.
- Mitchell, A.J., A.E. Goodwin, M.J. Salmon, and T.M. Brandt. 2002. Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. *North American Journal of Aquaculture* 64:55-59.
- Nyström, P., and J.R. Pérez. 1998. Crayfish predation on the common pond snail (*Lymnaea stagnalis*): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobiologia* 368:201-208.
- Stein, R. A., C. G. Goodman, and E. A. Marschall. 1984. Using time and energetic measures of cost in estimating prey value for fish predators. *Ecology* 65:702–715.
- Tsai, Y.J., K. Maloney, and A.E. Arnold. 2007. Biotic and abiotic factors influencing the distribution of the Huachuca springsnail (*Pyrgulopsis thompsoni*). *Journal of Freshwater Ecology* 22:213-218.
- Weber, L.M., and D.M. Lodge. 1990. Periphytic food and predatory crayfish: relative roles in determining snail distribution. *Oecologia* 82:33-39.

Chapter V

Parasite presence in *Melanoides tuberculata* in San Solomon Springs, Balmorhea State Park

Abstract

In addition to altering nutrient cycling and energy flow within a system, *Melanoides tuberculata* can impact native species by introducing exotic parasites. We investigated the presence of trematode parasites in *M. tuberculata* using non lethal means (heat and light) and dissection. After examining 300 individuals using the non lethal method, we dissected 50 snails by crushing the shells at the first body whorl and smearing the gonads and digestive tract on a microscope slide. We examined tissues using a dissecting scope at 100 total magnification. We did not find any rediae or cercariae in the 350 examined snails; however, there may be trematodes present at Balmorhea State Park based on other empirical evidence.

Introduction

Melanoides tuberculata is an exotic snail species (Thiaridae: Gastropoda) native to Africa and Asia and introduced to the United States via the aquatic plant and aquarium trade (Murray, 1964). Individuals are most often found in slow or stagnant water of moderate pH and temperature (Duggan, 2002) and they thrive in areas impacted by humans (DeMarco, 1999). Adults reproduce asexually via parthenogenesis, which means density can be very high. Freitas and Santos (1995) reported densities of *M. tuberculata* at 16,000 snails/m² during a field study.

These high densities not only confer a competitive advantage over native snails, but may also facilitate the spread of parasites to vertebrates. A gill trematode, *Centrocestus formanus* is a digenetic trematode widely found in Asia (Scholz and Salgado-Maldonado, 2000) that uses *M. tuberculata* as its first intermediate host. After developing in *M. tuberculata*, cercariae emerge from snails and encyst on fish. Several species of fish in the families Cyprinidae, Ictaluridae, and Poeciliidae are hosts to *C. formosanus* (Scholz and Salgado-Maldonado, 2000). Significant damage to gills can occur from *C. formosanus* infections which can be disastrous for endemic fish species such as the fountain darter (*Etheostoma fonticola*) (Mcdermott, 2000). Balmorhea State Park supports two endangered fish, the Pecos gambusia (*Gambusia nobilis*) and the Comanche Springs pupfish (*Cyprinodon elegans*), that could be impacted by *C. formosanus*. The final hosts are piscivorous wading birds such as herons, pelicans, and cormorants.

M. tuberculata can also spread parasites other than *C. formosanus* such as the Chinese liver fluke (*Clonochris sinensis*) and the oriental lung fluke (*Paragonimus westermani*), both of which can infect humans and are reported to occur in the United States (Guimarães et al., 2001). *M. tuberculata* may also introduce other species of *Centrocestus* into habitats (Ben-Ami and Heller, 2004). The purpose of this study was to

investigate the presence of trematode parasites in *Melanoides tuberculata* collected from the pool at Balmorhea State Park.

Methods

We investigated parasite presence using lethal and non-lethal methods during March and April 2012. All snails were collected from the pool at Balmorhea State Park. The non lethal method was adapted from protocol established by Lo and Lee (1996) and the lethal method from Tolley-Jordan and Owen (2008) and Ladd and Rogowski (2011). We preferred using the non-lethal method and tested 300 snails by placing 50 vials, each with a single snail in 40 mL of de-chlorinated tap water, under 60W incandescent light bulbs for 24 hours. Heat from the lamps increased the water temperature to around 34°C, which stimulates the emergence of cercariae. We looked for cercariae by pipetting 10 mL aliquots from each vial into Petri dishes and scanned dishes using a dissecting scope.

Fifty snails were investigated using the lethal method, wherein we crushed the snail between the first and second body whorl using scissor handles. The digestive tract and gonads were removed from the shell and spread on a microscope slide using a cover slip. We examined the tissues under a dissecting scope at 100 total magnification.

Results and discussion

Out of 350 *M. tuberculata* investigated, we found no instances of parasites from snails collected March 2012 from the Balmorhea State Park swimming pool. Even though we did not find any evidence of *C. formosanus* trematode larvae in the *M. tuberculata* snails that were examined, parasite infections may still exist within snails or other invertebrates in San Solomons Springs at Balmorhea State Park. For *C. formosanus* to be present within a system, the intermediate hosts (snails and fish) as well as the avian definitive host need to be present. A variety of potential hosts exist at Balmorhea, notably the red rim melania (*Melanoides tuberculata*), quilted melania (*Tarebia granifera*), Mexican tetra (*Astyanax mexicanus*), Largespring gambusia (*Gambusia geiseri*), and green heron (*Butorides virescens*). Therefore, future monitoring efforts should be conducted to determine parasite presence. Infection rate of *C. formosanus* in *M. tuberculata* is generally low and occurs in patches (Tolley-Jordan and Owen, 2008) thus confirming that *C. formosanus* is present within infected snails at Balmorhea may be difficult to determine.

Tolley-Jordan and Owen (2008) found 6.1% of *M. tuberculata* individuals and 4.8% of *Tarebia granifera* individuals sampled from the Comal River were infected with parasitic trematodes. We did not assess parasite presence in *T. granifera*, since we were focused on looking for *C. formosanus*. We also do not know if the two native snails at Balmorhea, the Phantom Cave snail (*Pyrgulopsis texana*) and the Phantom Spring tryonia (*Tryonia cheatumi*), carry any parasitic trematodes. Given the potential of trematodes to damage not only the native snail populations but also the endangered fish populations at Balmorhea, finding a non-lethal method to assess infection rates of *P. texana* and *T. cheatumi* may be warranted. A similar non-lethal method to what we used could be applied to the native snails, but water temperature should only be increased a few degrees

over the average water temperature at Balmorhea (24°C) since these native springsnails probably have a narrower tolerable temperature range than *M. tuberculata*.

Literature cited

- Ben-Ami, F., and J. Heller. 2005. Spatial and temporal patterns of parthenogenesis and parasitism in the freshwater snail *Melanoides tuberculata*. *Journal of Evolutionary Biology* 18(1):138-146.
- DeMarco, P. Jr. 1999. Invasion by the introduced aquatic snail *Melanoides tuberculata* (Muller, 1774) (Gastropoda: Prosobranchia: Thiaridae) of the Rio Doce State Park, Minas Gerais, Brazil. *Studies of Neotropical Fauna and Environment* 34:186-189.
- Duggan, I.C. 2002. First record of a wild population of the tropical snail *Melanoides tuberculata* in New Zealand natural waters. *New Zealand Journal of Marine and Freshwater Research* 36:825-829.
- Freitas J.R., and M.B.L. Santos. 1995. Current advances on the study of snail-snail interactions, with special emphasis on competition process. *Memórias do Instituto Oswaldo Cruz* 90:261-269.
- Guimarães, C.T. 2001 Possible displacement of planorbids by *Melanoides tuberculata* in Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz* 96:173-176.
- Ladd, H.A., and D.L. Rogowski. 2012. Egg predation and parasite prevalence in the invasive freshwater snail, *Melanoides tuberculata* (Müller, 1774) in a west Texas spring system. *Aquatic Invasions* 7:287-290.
- Lo C.T., and K.M. Lee. 1996. Pattern of emergence and the effects of temperature and light on the emergence and survival of heterophyid cercariae (*Centrocestus formosanus* and *Haplorchis pumilio*). *The Journal of Parasitology* 82:347-350.
- Martinez, M.A., and D.M. Thome. 2006. Habitat usage by the Page Springsnail, *Pyrgulopsis morrisoni* (Gastropoda:Hydrobiidae) from central Arizona. *The Veliger* 48(8):8-16.
- McDermott, K.S. (2000). Distribution and infection relationships of an undescribed digenetic trematode, its exotic intermediate host, and endangered fishes in springs of west Texas. MS Thesis, Texas State University, San Marcos, Texas, 50 pp.
- Murray, H.D. 1964. *Tarebia granifera* and *Melanoides tuberculata* in Texas. *Annual Reports of the American Malacological Union* 1964:15-16.
- Scholz, T., and G. Salgado-Maldonado. 2000. The introduction and dispersal of *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae) in Mexico: A review. *American Midland Naturalist* 143(1):185-200.
- Tolley-Jordan, L.R., and J.M. Owen. 2008. Habitat influences snail community structure and trematode infection levels in a spring-fed river, Texas, USA. *Hydrobiologia* 600:29-40.

Chapter VI

Does the presence of other snail species affect substrate selection in *Pyrgulopsis texana*, *Melanoides tuberculata*, and *Tarebia granifera*?

Abstract

Habitat use can be influenced by abiotic and biotic factors. Substrate has been an important habitat factor for snails, however, habitat choices may be modified as a result of food availability, predation, and competition. Previously (Chapter III) we have shown that substrate type affects habitat choice. Here we examine if the presence of other snail species affects substrate selection (sand, gravel, pebble). Two invasive snails, *Melanoides tuberculata* and *Tarebia granifera*, and a native snail *Pyrgulopsis texana*, were used in this experiment. All snails were collected from San Solomon Springs, Balmorhea State Park, Texas. Trials were conducted during afternoon hours from November 2011 to February 2012. Two snail species were added to each 31.50cm diameter container, and location in the experimental arena was recorded after four hours. The preference of *P. texana* for gravel substrate established in the first substrate selection experiment did not change in the presence of the two invasive species. Thus, the invasive snails do not directly affect habitat selection of *P. texana*.

Introduction

The Phantom Cave snail (*Pyrgulopsis texana*) is a species of conservation interest found in the San Solomon Spring system in Balmorhea State Park, TX.). *P. texana* is a member of the hydrobiidae (also known as springsnails), the most diverse gastropod family in North America (Brown et al., 2008). Hydrobiidae is also an imperiled group, with 74% of species in the United States at risk for extinction (Thorp and Covich, 2009). *Pyrgulopsis texana* is a candidate species for the Federal Endangered Species List and a high priority species on the Texas Priority Species List (United States Department of the Interior, 2004). *P. texana* is an important member of the stream community as a consumer of periphyton and a prey item for fishes. Despite their importance in the food web and conservation status, little is known about the ecology or behavior of *P. texana*. This is not surprising as the ecology of hydrobiids in the western United States is lacking (Hershler, 1999; Brown et al., 2008).

Two exotic snails, the red rim melania (*Melanoides tuberculata*) and the quilted melania (*Tarebia granifera*) have been introduced into San Solomon Springs. How *M. tuberculata* and *T. granifera* affect habitat and resource usage of native springsnails is not well understood. Given that *M. tuberculata* has spread throughout the United States since its introduction in the 1930's (Murray 1971) and our discovery of *T. granifera* in San Solomon Springs, understanding how exotic invasive snails affect native snail habitat use may be important for conservation efforts. These two invasives may threaten native snails through competition, predation (Ladd and Rogowski 2012), or other indirect effects. Competitive exclusion of native snails by *M. tuberculata* (Pointier, 2001) and *T. granifera* (Karatyev et al., 2008) is known to occur and Rader et al. (2003) speculate that *M. tuberculata* could extirpate native snails in isolated spring systems. The purpose of

this experiment was to determine if *M. tuberculata* and *T. granifera* affect habitat selection by *P. texana*.

Methods

Snails were collected within Balmorhea State Park (Reeves Co., Texas), and maintained in a 42 liter aquarium at 22° C within the Aquatic Ecology laboratory at TTU. Snails were transferred to a separate aquarium with conditions closely replicating those of San Solomon Spring (pH = 7.09, specific conductance =3.2mS) 48 hours before conducting experimental trials. Two species of snails were then added to the center of the experimental 31.5 cm diameter circular container. Each section contained one of three substrate types: sand (0-2 mm), gravel (2-4 mm), or pebble (>5mm) (Figure 6.1). Control chambers were marked in the same way as experimental chambers, but were not filled with any substrate. Snails were allowed to move freely among the sections for four hours. After completion of each trial, snail locations were recorded and shell lengths of *M. tuberculata* and *T. granifera* were recorded.



Figure 6.1--Chamber used in experimental trials, separated into three equally sized sections, with the three substrates, sand, gravel, and cobble.

Results

P. texana showed a preference for gravel substrate when *M. tuberculata* was present ($\chi^2 = 5.4$, $df = 2$, $P = 0.065$) or *T. granifera* ($\chi^2 = 23.0$, $df = 2$, $P < 0.0001$) (Figure 6.2). In the presence of *P. texana*, *M. tuberculata* preferred pebble substrate ($\chi^2 = 6.17$, $df = 2$, $P = 0.046$). *T. granifera* had no substrate preference when paired with *M. tuberculata* but *T. granifera* did show a preference for gravel and pebble when *P. texana* was present ($\chi^2 = 12.7$, $df = 2$, $P = 0.0017$) (Table 6.1).

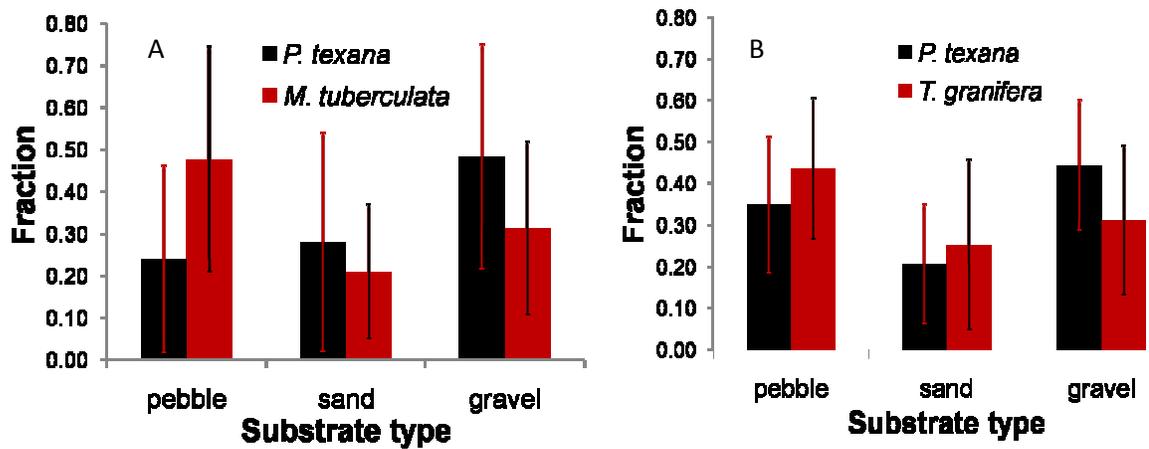


Figure 6.2--Substrate selection experiments of *P. texana* when tested with *M. tuberculata*. A) Substrate choice of *P. texana* when tested with *T. granifera* B). Values are the mean fraction of snails found on each substrate type across 16 trials and error bars are standard deviations.

Table 6.1--Mean (SD) frequency of *P. texana*, *M. tuberculata*, and *T. granifera* occurrence in each substrate type across 16 trials of paired species combinations.

Species	<i>M. tuberculata</i> and <i>P. texana</i>			<i>T. granifera</i> and <i>P. texana</i>			<i>M. tuberculata</i> and <i>T. granifera</i>		
	Sand	Gravel	Pebble	Sand	Gravel	Pebble	Sand	Gravel	Pebble
<i>P. texana</i>	0.28 (0.26)	0.48 (0.27)	0.24 (0.22)	0.21 (0.14)	0.44 (0.16)	0.35 (0.16)	N/A		
<i>M. tuberculata</i>	0.21 (0.16)	0.31 (0.20)	0.48 (0.27)	N/A			0.29 (0.15)	0.23 (0.13)	0.43 (0.16)
<i>T. granifera</i>	N/A			0.25 (0.20)	0.31 (0.18)	0.44 (0.17)	0.40 (0.16)	0.34 (0.15)	0.36 (0.22)

Discussion

P. texana preferred gravel substrate both in the presence of exotic snails and when exotic snails were absent (data in chapter III of report). Thus, the exotic snails do not appear to directly influence substrate size selection of *P. texana*. We were surprised to see that *M. tuberculata* and *T. granifera* had no effect on *P. texana* substrate choice in our experiment. This may indicate that substrate size has a stronger influence on habitat selection in *P. texana* than the presence of exotic snails. Our results are supported by the redundancy analysis (Figure 2.34) which shows that *P. texana* density is not strongly associated with *M. tuberculata* or *T. granifera* density. One should not infer that the exotic snails have no effect on the distribution of *P. texana* in the field, there are other direct and indirect actions that we did not test.

The two exotic snails may be competing for the same resources as the native snails. They may even be feeding on eggs or juveniles of native snails (e.g. Ladd and Rogowski 2011). It is also possible that the density of the invasives in our experiments were not high enough to affect *P. texana*.

Other factors such as density dependent effects in *P. texana*, available periphyton, water velocity, and quality of periphyton forage could affect *P. texana* habitat selection in San Solomon Springs. For hydrobiid snails (Gastropoda: Hydrobiidae), sediments provide protection from fish predators and substrate for grazing (Barnes, 2003). Periphyton thickness is also an important component of substrate use in snails with some species preferring dense algal mats and others bare substrate (Holomuzki and Biggs, 2007). Therefore, the results of this experiment alone do not explain aquatic snail habitat selection at San Solomon Springs.

The results of this experiment do indicate that substrate type is important in habitat selection for *P. texana*. Substrate size has been recognized as very important for habitat selection in other aquatic snails such as *Pyrgulopsis morrisoni* (Martinez and Thome, 2006). Therefore, future habitat restoration projects should consider substrate type and for *P. texana* in particular, substrate types smaller than sand should be avoided.

Literature cited

- Barnes, R.S.K. 2003. Feeding rates of continually submerged *Hydrobia ulvae* vary during the daylight hours. *Journal of Marine Biology* 83:1273-1275.
- Brown, K.M., B. Lang, and K.E. Perez. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. *Journal of the North American Benthological Society* 27:484-495.
- Hershler, R. 1999. A systematic review of the hydrobiid snails of the Great Basin, western United States. Part II. Genera *Colligyrus*, *Eremopyrgus*, *Fluminicola*, *Pristinicola* and *Tyronia*. *The Veliger* 42(4):306-337.
- Holomuzki, J.R., and B.J. Biggs. 2007. Physical microhabitat effects on 3-dimensional spatial variability of the hydrobiid snail, *Potamopyrgus antipodarum*. *New Zealand Journal of Marine and Freshwater Research* 41:357-367.
- Karatyev, A.Y., L.E. Burlakova, V.A. Karatyev, and D.K. Padilla. 2009. Introduction, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia* 619:181-194.
- Ladd, H.A., and D.L. Rogowski. 2012. Egg predation and parasite prevalence in the invasive freshwater snail, *Melanoides tuberculata* (Müller, 1774) in a west Texas spring system. *Aquatic Invasions* 7:287-290.
- Martinez, M.A., and D.M. Thome. 2006. Habitat usage by the Page springsnail, *Pyrgulopsis morrisoni* (Gastropoda:Hydrobiidae) from central Arizona. *The Veliger* 48(8):8-16.

- Murray, H.D. 1971. The introduction and spread of thiarids in the United States. *The Biologist* 3:133–135.
- Pointier, J.P. 2001. Invading freshwater snails and biological control in Martinique Island, French West Indies. *Memórias do Instituto Oswaldo Cruz* 96:67-74.
- Rader, R.B., M. Belk and K. Keleher 2003. The Introduction of an Invasive Snail (*Melanoides tuberculata*) to Spring Ecosystems of the Bonneville basin, Utah. *Journal of Freshwater Ecology* 18(4):647-657.
- Thorp, J.H., and A.P. Covich. 2009. *Ecology and Classification of North American Freshwater Invertebrates*. Elsevier Science, 1021pp.
- United States Department of the Interior. 2004. Biological opinion on construction of a dam and pumping system at Phantom Lake Spring. Memorandum 29 pp.

Chapter VII

Desiccation resistance of two aquatic invasive snails, *Melanoides tuberculata*, and *Tarebia granifera*

Abstract

The red rimmed melania (*Melanoides tuberculata*) and the quilted melania (*Tarebia granifera*) are exotic aquatic snails invasive in many spring systems. Understanding their tolerance for abiotic stressors such as temperature, pH, salinity, and desiccation may provide insight into their dispersal capabilities and explain their success in invaded systems. We investigated the survival of *M. tuberculata* and *T. granifera* when removed from water to determine desiccation tolerance. *M. tuberculata* from two sites, San Solomon Springs and Diamond Y springs, and *T. granifera* from one site, San Solomon Springs were tested for desiccation resistance. Percent water weight loss was measured, and survival monitored for snails exposed to air from one to 15 days. Snails from Diamond Y Springs were more tolerant to desiccation than snails from San Solomon Springs, we believe this is primarily due to the larger size of *M. tuberculata* in Diamond Y Springs. *M. tuberculata* had a greater tolerance for desiccation than *T. granifera*. For 50% probability of survival, weight loss percentage was 35% in *M. tuberculata* and near 20% in *T. granifera*.

Introduction

The invasive red-rimmed melania snail (*Melanoides tuberculata*) and the quilted melania (*Tarebia granifera*) are aquatic snails that have proven to be a nuisance throughout the world (Karatayev et al., 2009). These snails have a number of traits that have enhanced their population growth, allowed them to become invasive, and detrimentally impact local fauna, including endangered fish and native snails (Guimarães et al., 2001; Phillips et al., 2010). These snails have a life span of 3-5 years, are parthenogenic, brood their young internally and give birth to free living juveniles (Ben-Ami and Hodgson, 2005). Thus only one snail is needed to establish a population.

M. tuberculata appears to be tolerant of levels of salinity approaching seawater, but is restricted by cold temperatures (Mitchell and Brandt, 2005) as is *T. granifera* (Chaniotis et al., 1980). They rely on a gill to breathe as well as probably having supplemental respiration through exposed skin. These snails have an operculum that allows them to retract within their shell for protection from predators or to survive periods of desiccation.

Very little research has been conducted in regards to controlling or eradicating populations of red-rimmed melania or the quilted melania. In the interest of control or eradication it would be beneficial to know their desiccation tolerance. There has been some preliminary research into melanooides desiccation resistance (Dudgeon, 1982; Facon et al., 2004), however, those investigations were not for the purpose of control or

eradication. The Facon study was done at high humidity (80%) and individuals were exposed to increasing periods of desiccation followed by water immersion for 48 hours. Humidity levels in the southwest are rarely that high.

Many springs have been renovated to remove unwanted species (e.g. fish, vegetation) via water diversions (e.g. Scopetonne et al., 2005). If a site/area can be dewatered, how long should it remain dry to ensure the removal of all red-rimmed melania or quilted melania? Specifically we want to know how long can these snails survive out of water, or what percentage of water weight must these species suffer to ensure mortality. We investigated the desiccation resistance of two populations of melanoides from spring systems in west Texas (San Solomon Spring and Diamond Y springs), and of the quilted melania from San Solomon Spring.

Methods

M. tuberculata from two sites, San Solomon Springs and Diamond Y springs, and *Tarebia* from one site, San Solomon Springs, were tested for desiccation resistance. From each population and species we collected 180 snails and measured length and wet weight (patted dry) prior to placement in 50 ml individual glass vials. Water was added to a random selection of 12 snails at 24 hour intervals for 15 days (Table 7.1). Prior to adding water snail dry weight was measured. Due to logistical problems we conducted two separate dessication trials. The two *M. tuberculata* populations were tested first followed by a trial with *T. granifera*. Room percent humidity levels and temperature were recorded each day of the experiment. The average humidity and temperature in the lab during the experiment for *M. tuberculata* was 51.0 % (SD 13.7) and 18.6 °C (SD 0.69) respectively, and for *T. granifera* it was 63.8 % (SD 6.9) and 18.8 °C (SD 0.24) respectively.

Table 7.1--Experimental design for snail desiccation tolerance experiment

Spring	Species	Days															total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Diamond Y	<i>M. tuberculata</i>	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	180
San Solomon	<i>M. tuberculata</i>	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	180
	<i>T. granifera</i>	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	180

Results

There was a significant difference in percent water loss of *M. tuberculata* between the two spring sites, with snails from San Solomon Springs desiccating more rapidly than those from Diamond Y (Table 7.2 and Figure 7.1). *M. tuberculata* from Balmorhea (13.5 mm, SD=2.85) were significantly smaller in length than snails collected from Diamond Y spring (23.37 mm, SD=1.76) (ANOVA: $SS_{1,358}=9230$, $F=1644$, $p<0.001$). Probability of survival in *M. tuberculata* decreased as percent weight loss increased (Figure 7.2) with the predicted LD50 (desiccation tolerance) for *M. tuberculata* from Balmorhea and Diamond Y springs at 35% and 25% water weight loss respectively. With a loss of 42% of water weight there is a predicted 95% mortality for *M. tuberculata* from Balmorhea. Probability of survival in *T. granifera* also decreased as water loss increased, and the LD50 was at 20% weight loss (Figure 7.3). For *T. granifera* the predicted percent of weight loss for 95% mortality was 30%.

Table 7.2--Summary results of ANOVA of percent weight loss of *M. tuberculata* from two spring systems exposed to the atmosphere for one to 16 days.

Source	DF	Sum of Squares	F Ration	Prob > F
Overall model	17	2.33	79.9	<0.001
Days (desiccation)	14	1.42	59.1	<0.001
Spring	1	0.0506	29.5	<0.001
Length	1	0.0191	11.1	0.0009
Spring x length	1	0.0127	7.43	0.0067

Discussion

Understanding the desiccation tolerance of *M. tuberculata* and *T. granifera* will help researchers and managers predict how these snails may spread and the effectiveness of potential control measures. *M. tuberculata* in particular is known to survive for days on dry fisheries equipment due to its operculum (Mitchell et al., 2007) and Dudgeon (1982) determined that *M. tuberculata* survived up to eight days out of water. *T. granifera* is also known to survive desiccation well. Miranda et al. (2011) recorded that *T. granifera* populations were able to survive in estuaries despite long desiccation events that should have eliminated most of the population. Populations of these invasive snails are also likely resistant to desiccation due to their high fecundity and asexual life history, which allows one surviving adult after a drying event to re-populate the system. Data on how desiccation affects the population ecology of *M. tuberculata* and *T. granifera* are lacking and more research into how desiccation affects reproduction may also be necessary.

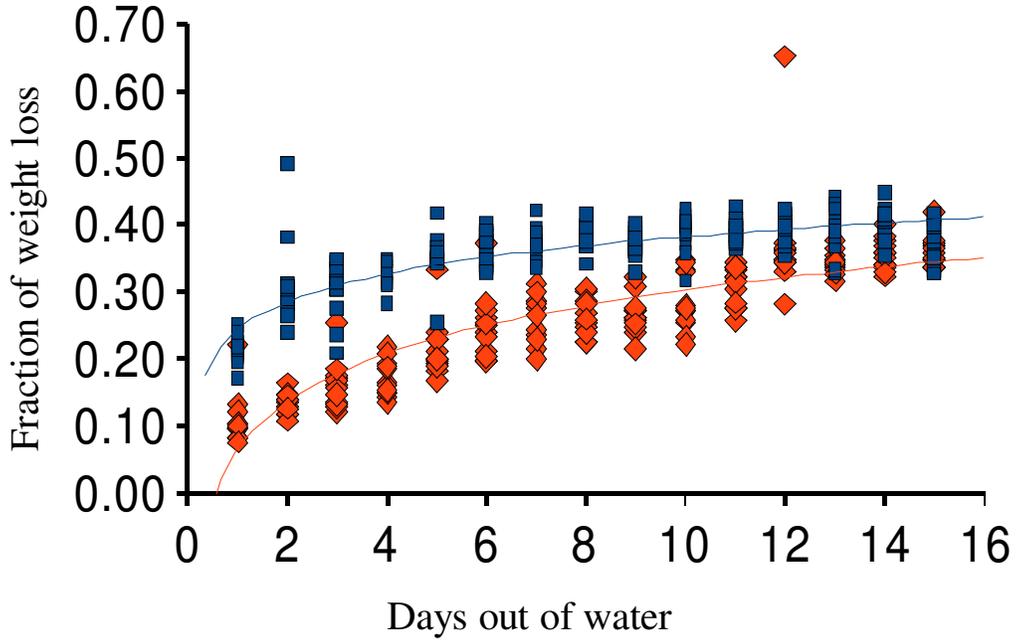


Figure 7.1--Percent weight loss by days of out of water of *M. tuberculata* from Diamond Y (in red) and San Solomon Springs (in blue).

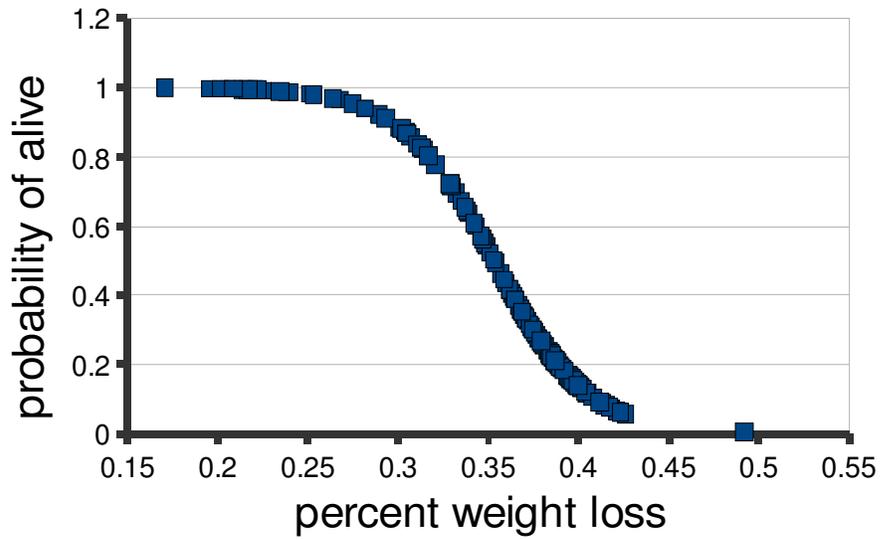


Figure 7.2--Probability of survival in relation to percent weight loss (water weight) of *M. tuberculata* from San Solomon Springs.

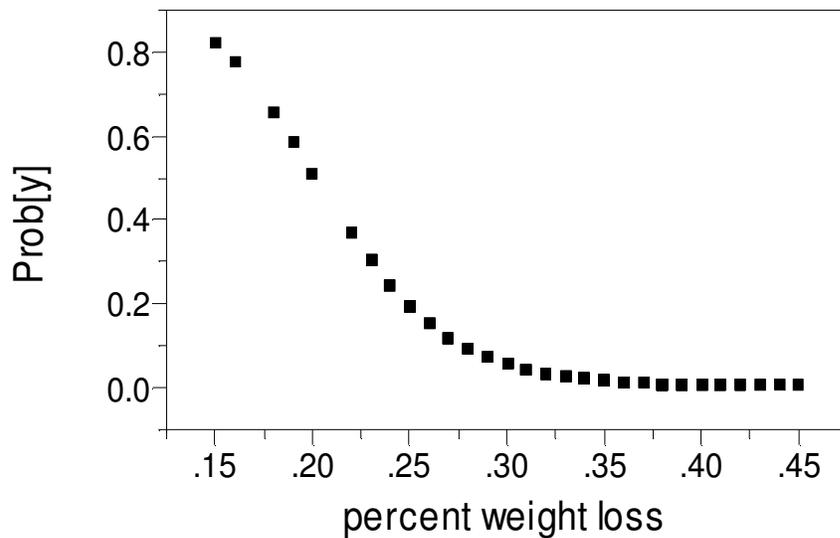


Figure 7.3--Probability of survival in relation to percent weight loss (water weight) of *T. granifera* from San Solomon Springs.

Investigations into measures to control and eradicate *M. tuberculata* and *T. granifera* populations have not been successful. Generally, once established these snails are impossible to remove. There are few known population control strategies, mostly limited to mechanical removal or removal by hand, but these methods are expensive, labor intensive, and have not been shown effective (Pointier, 2001). As humidity levels vary spatially and temporally by site, we measured percent water loss to determine thresholds of tolerance. Our results show that 50% of *M. tuberculata* snails from San Solomon Springs died at 35% weight loss, which corresponds to around 9 days out of water. Shallow parts of the reconstructed wetland fed by San Solomon Springs do dry periodically, but we are not sure what effect this has on the invasive snail population. Figure 2.24 shows that *M. tuberculata* and *T. granifera* mean density were lower in the summer months in the wetland when humidity and precipitation were low and air temperatures were high, but we do not know if this indicates a significant die off in the population. Future lab experiments examining desiccation tolerance under different air temperatures, and population modeling may help in determining the effects of desiccation at the population level.

Whether or not dewatering could be a successful invasive snail control method is currently unknown. There are a number of factors that make dewatering large areas of San Solomon Springs unattractive, such as the presence of endangered fish and endemic springsnails. Based on our results, periodically dewatering areas of the wetland at San Solomon Springs for 8-9 days could help control invasive snail numbers and allow native species to proliferate, but eradication seems unlikely. Given the high tolerance to desiccation in these invasive snails, parthenogenesis, and their high fecundity,

populations could be easily established in other isolated desert spring systems, thus as with many invasive species, prevention is the best method of control.

Literature cited

- Chaniotis, B. N., J. M. Butler, JR., F. F. Ferguson, and W. R. Jobin. 1980. Thermal limits, desiccation tolerance, and humidity reactions of *Thiara (Tarebia) granifera mauiensis* (Gastropoda: Thiaridae) host of the Asiatic lung fluke disease. *Caribbean Journal of Science* 16:91-93.
- Dudgeon, D. 1982. Aspects of the desiccation tolerance of four species of benthic mollusca from Plover Cove Reservoir, Hong Kong. *The Veliger* 24:267-271.
- Facon, B., E. Machline, J. P. Pointier, and P. David. 2004. Variation in desiccation tolerance in freshwater snails and its consequences on invasion ability. *Biological Invasions* 6:283-293.
- Ben-Ami, F., and A. N. Hodgson. 2005. Ovoviviparity and the structure of the brood pouch in *Melanooides tuberculata* (Gastropoda: Prosobranchia: Thiaridae). *Journal of Morphology* 263:322-329.
- Guimarães, C. T., C. P. d. Souza, and D. d. M. Soares. 2001. Possible competitive displacement of planorbids by *Melanooides tuberculata* in Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz* 96:173-176.
- Karatayev, A. Y., L. E. Burlakova, V. A. Karatayev, and D. K. Padilla. 2009. Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia* 619:181-194.
- Miranda, N., R. Perissinotto, and C. Appleton. 2011. Population structure of an invasive parthenogenic gastropod in coastal lakes and estuaries of Northern KwaZulu-Natal, South Africa. *PLoS One* 6(8):p. e24337.
- Mitchell, A. J., and T. M. Brandt. 2005. Temperature tolerance of red-rim melania *Melanooides tuberculatus*, an exotic aquatic snail established in the United States. *Transactions of the American Fisheries Society* 134:126-131.
- Mitchell, A.J., M.S. Hobbs, and T.M. Brandt. 2007. The effect of chemical treatments on red-rim melania *Melanooides tuberculata*, an exotic aquatic snail that serves as a vector of trematodes to fish and other species in the USA. *North American Journal of Fisheries Management* 27:1287-1293.
- Phillips, C. T., M. L. Alexander, and R. Howard 2010. Consumption of eggs of the endangered fountain darter (*Etheostoma fonticola*) by native and nonnative snails. *The Southwestern Naturalist* 55:115-117.
- Pointier, J.P. 2001. Invading freshwater snails and biological control in Martinique Island, French West Indies. *Memórias do Instituto Oswaldo Cruz* 96:67-74.
- Scopettone, G. G., P. H. Rissler, C. Gourley, and C. Martinez. 2005. Habitat restoration as a means of controlling non-native fish in a Mojave Desert oasis. *Restoration Ecology* 13:247-256.

Wingard, G. L., J. B. Murray, W. B. Schill, and E. C. Phillips. 2008. Red-rimmed melania (*Melanoides tuberculatus*) - A snail in Biscayne National Park, Florida - harmful invader or just a nuisance. Page 6. U.S. Geological Survey, Reston, VA.