EFFECTS OF A FRESHWATER TURTLE (*TRACHEMYS SCRIPTA ELEGANS)* ON ECOSYSTEM FUNCTIONING IN EXPERIMENTAL PONDS

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Megan Kroeger Lindsay, B.A.

San Marcos, Texas
May 2011

EFFECTS OF A FRESHWATER TURTLE (*TRACHEMYS SCRIPTA ELEGANS*) ON ECOSYSTEM FUNCTIONING IN EXPERIMENTAL PONDS

Committee Members Approved:

_____
Yixin Zhang

_____
Michael Forstner

_____
Dittmar Hahn

Approved:

_____
J. Micheal Willoughby
Dean of Graduate College

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

ACKNOWLEDGEMENTS

I would first like to thank my major advisor, Dr. Yixin Zhang, for his continued passion and excitement for all things science. I would also like to thank my two committee members, Dr. Mike Forstner and Dr. Dittmar Hahn. Dr. Forstner is a never ending source of motivation and inspiration to get things done without excuses. Dr. Hahn, thank you for listening and for all your guidance and support. My three advisors proved to be the right combination of support and guidance I needed in order to be successful. In addition, I would like to thank my lab mates: Frances Lash, Goniela Iskali, Trey Nobles and Mario Sullivan for their support during my time at Texas State University, San Marcos and continued friendship. Thank you to the Boy Scouts of America for the use of the Griffith League Ranch Property, where my study took place. Finally, I would like to thank my husband, Brian, and my parents for their continual love and support each day.

Required Permits: Texas State IACUC (1013 0426 12)**.**

This manuscript was submitted to the committee on April 19, 2011.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

ABSTRACT xi

CHAPTER

I. INTRODUCTION 1

II. OBJECTIVES 6

III. MATERIALS AND METHODS 8

Site Description 8

Leaf Litter Decomposition 13

Periphyton Biomass 14

Invertebrate Community Assemblage Response 14

Sediment Accumulation 15

Water Chemistry 15

Sampling Techniques Used to Determine if Salmonellae was Present or Presented in the System 16

Laboratory Procedures for Examining Salmonellae 16

PCR Amplification 17

Predation on *T. s. elegans* 18

Statistical Analyses 18

IV. RESULTS 21

MANOVA Results 21

Leaf Litter Breakdown Rate 21

Periphyton Biomass Production 27

Invertebrate Community Response 28

Sediment Accumulation 35

Water Chemistry 36

Two-way MANOVA Results 39

Leaf Litter Breakdown Rate 40

Sediment Accumulation 43

Canonical Correspondence Analysis 47

Salmonellae Study 48

V. DISCUSSION 50

LITERATURE CITED 57

LIST OF TABLES

Table **Page**

1. Dominant Plant Species in Experimental Pond Riparian Area.....9

2. Macroinvertebrate Assemblage Found in Experimental Ponds.....34

3. Summary of Multi-factorial MANOVA Results 45

4. Summary of Open Leaf ANCOVA Results.....46

5. Summary of 2-way MANOVA Results.....46

6. Summary of Invertebrate Results.....46

LIST OF FIGURES

Figure **Page**

1. *Trachemys scripta elegans* Rio Grande River Sanderson County August 1995 3
2. Experimental Pond Location System.....10
3. Experimental Pond Layout 11
4. Layout of an Experimental Pond 12
5. Mean Percent Loss of Texas Oak Leaves in Experimental Ponds.....22
6. Mean Percent Loss of Riparian Texas Oak Leaves in Experimental Ponds 23
7. Mean Percent Loss of Open Texas Oak Leaves in Experimental Ponds.....24
8. Mean Percent Loss of Sycamore Leaves in Experimental Ponds.....25
9. Mean Percent Loss of Riparian Sycamore Leaves in Experimental Ponds.....26
10. Mean Percent Loss of Open Sycamore Leaves in Experimental Ponds.....27
11. Mean Chlorophyll *a* Production in Experimental Ponds.....28
12. Mean Number of Macroinvertebrates Found in Experimental Ponds 29
13. Mean Number of Odonata Found in Experimental Ponds.....30
14. Mean Number of Hemiptera Found in Experimental Ponds 31
15. Mean Number of Ephemeroptera Found in Experimental Ponds.....32
16. Mean Number of Diptera Found in Experimental Ponds 33
17. Mean Number of Coleoptera Found in Experimental Ponds.....34
18. Mean Sediment Accumulation in Experimental Ponds 36
19. Mean pH of Experimental Ponds.....37

20. Mean Conductivity (µs/cm) of Experimental Ponds 38
21. Mean Percent Dissolved Oxygen of Experimental Ponds 39
22. Week 6 Percent Loss of Texas Oak Leaves in Closed Packs 40
23. Week 8 Percent Loss of Texas Oak Leaves in Closed Packs 41
24. Week 6 Percent Loss of Sycamore Leaves in Closed Packs 42
25. Week 8 Percent Loss of Sycamore Leaves in Closed Packs 43
26. Week 6 Sediment Accumulation 44
27. Week 8 Sediment Accumulation 45
28. Canonical Correspondence Analysis Bi-Plot.....48
29. 2% Agarose Gel 49

ABSTRACT

EFFECTS OF A FRESHWATER TURTLE (*TRACHEMYS SCRIPTA ELEGANS*) ON ECOSYSTEM FUNCTIONING IN EXPERIMENTAL PONDS

by

Megan Kroeger Lindsay, B.A.

Texas State University-San Marcos
May 2011

SUPERVISING PROFESSOR: YIXIN ZHANG

Ecosystem functioning is a broad term, often used to describe intra- and interspecies interactions of organisms and the resulting effects on the ecosystem, these ecosystem functioning processes can encompass a variety of phenomena, including ecosystem properties, ecosystem goods and ecosystem services (Christenesen et al. 1996). Many aquatic organisms have significant effects on ecosystem functioning and benthic communities. However little is known if freshwater turtles affect ecosystem processes and benthic community assemblage in pond ecosystems. We conducted a study in order to test the direct effects of the red-eared slider *Trachemys scripta elegans* on ecosystem functioning and benthic communities in experimental pond systems that have

never had turtles. The ecosystem processes, biological community and environmental variables we studied were sediment accumulation, leaf litter breakdown rate, periphyton biomass production, invertebrate richness and abundance and water chemistry including pH, conductivity and dissolved oxygen. These processes were measured in the presence or absence of *T . s. elegans* in the experimental ponds. A multivariate analysis of variance (MANOVA), two-way MANOVA's and canonical correspondence analysis (CCA) were used to analyze the treatment effects on biological and environmental variables. Significant treatment effects were found when *T . s. elegans* had been present in the experimental ponds. The pH, conductivity, sediment accumulation, leaf litter breakdown rate and the abundance of invertebrates all averaged higher in ponds that contained *T . s. elegans*. The significant results detected when measuring the ecosystem functioning processes from this study support our hypothesis that the presence of freshwater turtles such as *T. s. elegans* does impact ecosystem functioning by altering ecosystem processes and environmental variables. In addition, our study also investigated the potential of *T. s. elegans* inoculating water or sediment with the bacteria salmonellae. The turtles used in the study, as well as water and sediment from the experimental ponds, were tested using enrichment techniques and polymerase chain reaction (PCR) in order to detect salmonellae. All turtle swabs, water and sediment samples collected did not detect any salmonellae bacteria. The results from this study support our hypothesis that the presence of a freshwater turtle such as *T. s. elegans* doesinfluence ecosystem processes and benthic communities. Overall population sizes of freshwater turtles are down in South Texas due to commercial harvest and habitat loss (Brown et al. 2011). Loss of freshwater turtles in pond ecosystems may affect the productivity due to the decreased amounts of nutrients

provided by the turtles directly and through their activities in ponds. Thus, this study suggests that freshwater turtles can influence pond ecosystem functioning and pond food webs by increasing resource availability for invertebrate communities.

CHAPTER I

INTRODUCTION

Ecosystem functioning is a broad term that includes interactions between organisms and the physical environment, such as nutrient cycling, soil development, water budgeting, and flammability. Ecosystem functioning can also encompass a variety of phenomena, including ecosystem properties, ecosystem goods and ecosystem services (Christensen et al. 1996). Drivers of ecosystem functioning can be biotic or abiotic, and include interactions between species and functional groups (Chapin et al. 1997), as well as resource availability, or modulators such as temperature, pH or disturbance (Hooper et al. 2005). Any changes in these biotic or abiotic factors may result in changes of ecosystem functioning. For example, the loss or addition of species to a community can have substantial impacts on ecosystem functioning including production, respiration, nutrient retention, or decomposition (Gessner et al. 2004). Many biotic and abiotic processes in different ecosystems can link ecosystems to each other. Aquatic and terrestrial ecosystems, for example, can be linked functionally by flows of nutrients and energy that might be mediated by animal movements or wind blowing across habitats, or by water moving through the hydrologic cycle (Kitchell et al. 1979, Polis et al. 1997). The functional traits of community members and the interactions between members can affect and mediate the availability of resources to other consumers (Chapin et al. 1997)

Though spatial subsidies are resource inputs from donor habitats that increase consumer density in recipient habitats; mobile consumers across aquatic-terrestrial habitats can also transport nutrients and detritus when they forage in one habitat and defecate in another (Polis et al. 1997). Many aquatic organisms have significant effects of ecosystem functioning and processes, however little is known how these ecosystem processes are impacted by freshwater turtles in pond ecosystems.

Ponds are an excellent system to study ecosystem processes that are affected by biotic or abiotic factors entering from adjacent systems. Ponds are generally characterized by a depression that holds a small body of water. In the southeastern United States, lakes and ponds are relatively shallow and occasionally dry during periods of drought (Brenner et al. 1991). In Texas more than 800,000 private ponds exist in addition to public ponds (Lock 1993). All species of turtles, fish and benthic invertebrates that live in pond ecosystems can be described as local communities. In the southeastern United States, the biomass and density of turtles in lakes may equal or exceed that of other vertebrates, and the annual productivity of turtles per area is apparently exceeded only by a few fishes (Iverson 1982, Congdon et al. 1986). Another unique characteristic of aquatic turtles in a pond ecosystem in the Southeast is their ability to dominate as the top predator -even though they might be omnivorous- once they have reached adulthood (Aresco 2005). Considering the large density of turtles in pond ecosystems, it is important to recognize the impacts an omnivorous turtle might have in pond ecosystems. Omnivory is a special feature of some animals that is broadly defined as feeding on more than 1 trophic level (Pimm and Lawton 1977, Pimm 1982). Omnivory is an important feature of the life histories of some common aquatic and semi-aquatic turtles, and such omnivory may be

driving the structure of food webs in southeastern lakes (Aresco 2005). In addition, as with large terrestrial herbivores, many turtle populations may be regulated by primary production (bottom up) rather than by predation (top down) in pond systems (Polis and Strong 1996).

Fig.1 TSE Rio Grande River Sanderson Canyon August 1995

The pond slider (*Trachemys scripta*) has one of the most extensive geographic distribution ranges of vertebrate species in the Western hemisphere (Gibbons 1990). We specifically used the red-eared slider (*Trachemys scripta elegans*) for this study (Figure 1). *T. s. elegans* is a semi-aquatic species. Individuals spend most of their lives near submerged or floating vegetation (Gibbons 1990). The turtles take advantage of the riparian areas for nesting sites during spring and for hibernation sites during winter. Basking is a notable characteristic for most species of freshwater turtles; individuals need large woody debris in the shallow depths of ponds, lakes and streams to provide access to sunlight while still in the aquatic environment. *T. s. elegans* is able to flourish in a variety of habitats such as ponds, lakes, slow moving streams and even more developed areas such as ditches near roadways making this turtle species a true habitat generalist (Cagle 1950, Gibbons 1990). Its ability to thrive in a variety of habitats allows this turtle to interact with many species, and therefore it may play an important role in food webs. Aresco and James (2005) stated that a generalist omnivore such as *T. s. elegans* that can easily switch among an herbivory, carnivory and scavenging lifestyle depending on the quality and quantity of

resources, may grow faster and survive better than a more specialized competitor. Studies have shown that slider turtles as juveniles are carnivores and then trend towards omnivory as they mature (Clark and Gibbons 1969, Hart 1983 and Bury 1986). Bouchard and Bjorndal (2006) concluded that although juveniles can process plant material, a carnivorous diet allows for greater juvenile growth, which is linked to higher survivorship and increased future reproductive success in turtles. However, the degree of herbivory of adult *T. scripta* may vary with differences in the availability of plant and animal foods in habitats (Clark and Gibbons 1969, Hart 1983). Many studies (Clark and Gibbons 1969, Hart 1983, Tucker et al. 1998) have examined the habitat, diet and reproduction of *T. s. elegans* however, our understanding of how these freshwater turtles directly impact pond ecosystems by potentially altering ecosystem functioning is still limited.

As large animals, freshwater turtles not only play a role in influencing different trophic levels in aquatic food webs (McCann and Hastings 1997, Duffy 2002), but also can have a relationship with microorganisms, including such pathogens as salmonellae (Gaertner et al. 2008). Salmonellae are enteric pathogens that are typically transmitted to humans via food and drinking water contaminated with feces from vertebrate animals. The relationship between salmonellae and vertebrate animals exists because salmonellae spend a good part of their lives as residents in animal hosts (Winfield and Groisman 2003). The intestinal track of vertebrate animals is presumed to be the native habitat of salmonellae; however, in freshwater turtles salmonellae have been found to persist at other body sites. Two studies from Gaernter et al. (2008) detected salmonellae either in the biofilms, on the carapace or in the cloacae of common musk

turtles (*Sternotherus odoratus*), red-eared sliders (*T. s. elegans*), Texas river cooters (*Psuedemys texana*) and common snapping turtles (*Chelydra serpentina*) indicating salmonellae can persist externally as well. In addition, Gaertner et al. (2009) detected salmonellae in both water and sediment samples from Spring Lake, the pristine headwater of the San Marcos River, Texas. However, positive water and sediment samples only occurred after major precipitation events. Information on the importance of captive turtles as sources of human associated salmonellosis infections is well established (Johnson-Delaney 1996); however, data on the potential of free-ranging turtles as carriers of salmonellae are scarce and contradictory (Brenner et al. 2002, Chambers and Hulse 2006). Some studies have failed to detect salmonellae in all turtles tested. In contrast, Chambers and Hulse (2006) swabbed the cloacae of 10 wild turtles in their study and found all swabs to be positive for *Salmonella enterica*. In this study, we will examine if wild freshwater turtles transfer salmonellae to the experimental pond systems, which have never had turtles. Determining whether freshwater turtles may be capable of inoculating water and sediment with salmonellae will be beneficial in terms of pond management and protecting humans against salmonellosis.

CHAPTER II

OBJECTIVES

The objectives of this study were to examine a) the direct effects of *Trachemys scripta elegans* on ecosystem functioning and invertebrate communities in experimental pond systems and b) the effect of wild *Trachemys scripta elegans* on the presence of salmonellae in these ponds. Twenty-four experimental ponds were used to examine the influence of *T. s. elegans* on invertebrate community structure and ecosystem functioning processes including sediment accumulation, leaf litter breakdown rate and production of periphyton biomass. In addition, we explored the possibility of wild *T. s. elegans* as a vector animal capable of inoculating water or sediment with salmonellae. We hypothesized that freshwater turtles would influence pond ecosystem functioning by affecting the above-mentioned parameters compared to these parameters in ponds without turtles. Also, we hypothesize that freshwater turtles – though often carriers of salmonellae in both pristine and impacted environments- will not inoculate water or sediment with salmonellae to an extent that would allow us to detect them. Further understanding the interactions between freshwater turtles and pond ecosystems will aid in better aquatic ecosystem management strategies for wild turtles and conservation.

Parameters analyzed in this study include:

1) The breakdown rate of leaf litter in ponds and on terrestrial riparian zones with and without turtles present.

2) The rate of algal production to assess the primary production with and without the presence of turtles.

3) Invertebrate community response within each pond with and without turtles present.

4) Sedimentation accumulation in the presence of turtles.

5) Water chemistry (temperature, DO, pH and conductivity) in the ponds with and without turtles present.

6) The influence of wild turtles on the presence of salmonellae in systems that have never had turtles.

CHAPTER III

MATERIALS AND METHODS

Site Description

In order to study how freshwater turtles may impact pond ecosystem functioning and the bacteria salmonellae we used man-made ponds located on Griffith League Ranch in Bastrop, Texas. Griffith League Ranch lies within the Loblolly pine-oak vegetation series as described by the Texas Parks and Wildlife Department (1992). The experiment was conducted June through August 2010. Griffith League Ranch is one league wide by one league long, forming an approximately 1900 hectare property. Twenty four experimental ponds were constructed in 2000 and have been allowed to naturalize over the last 10 years. In addition, the land has been free of cattle grazing impact since 2000. The dimensions of each pond are 3.6 m × 1.5 m × 0.54 m for an approximate volume of 2.916 m^3. At the beginning of the experiment the approximate depth of each pond was 0.4 m. The depth fluctuated throughout the experiment due to heavy rainfall at week 3 which raised the water level approximately 5 cm. After week 3, a gradual decrease in water depth occurred averaging a total 7.5 - 10 cm for each pond by the end of the experiment. The average water temperature was about 28.0°C for all ponds throughout the experiment. Vegetation extended for 1m from the edge of each pond to the surrounding fence (Table 1). The original fencing that surrounded each pond was

constructed from aluminum sheeting and the dimensions were 4.8 m × 2.9 m × 30 cm. Additional aluminum sheeting was used around the ponds containing turtles increasing the height of the fencing from 30 cm to 60 cm. Figure 2., shows that the first two ponds in the foreground had the preexisting fence and the two ponds in the background have the additional aluminum sheeting. Twelve of the twenty four ponds needed for this experiment have a 10° slope and the other twelve have a 45° slope at one end of the pond. Twelve individuals of red-eared sliders (*T. s. elegans*) were used for the experiment. All turtle individuals used in this experiment were captured either from natural ponds in the Griffith League property or from ponds located on a private property in Guadalupe County, Texas along Long Branch Creek. Hoop nets were used at both property sites from May 24th through June 6th in order to collect the turtles. Turtles were marked and then relocated into the experimental ponds on the Griffith League Ranch property. Figure 3. illustrates the pond layout and indicates if the pond contained a turtle or not. At the end of the experiment, all surviving turtles were released back into the pond from which they were collected.

Table 1. Dominant Plant Species in Experimental Pond Riparian Area

Dominant Plants	Percent Cover
Digitaria (Crabgrass)	50-75%
Hererotheca latifolia	20-50%
Paspalum	20-40%
Cyperaceae (Sedge)	5-10

The dominant trees surrounding the ponds were Loblolly Pines (*Pinus taeda*) and Post Oak (*Quercus stellata*).

Figure 2. Experimental Pond Location System. Each pond was approximately 4.8 m × 2.9 m × 30 cm. 30 cm of existing aluminum sheeting surrounded the ponds. Ponds that contained a turtle had an additional 30 cm of aluminum sheeting added.

Figure 3. Experimental Pond Layout- Green rectangles indicate a *T. s. elegans* was released into an experimental pond and white indicates no turtle was released into the pond.

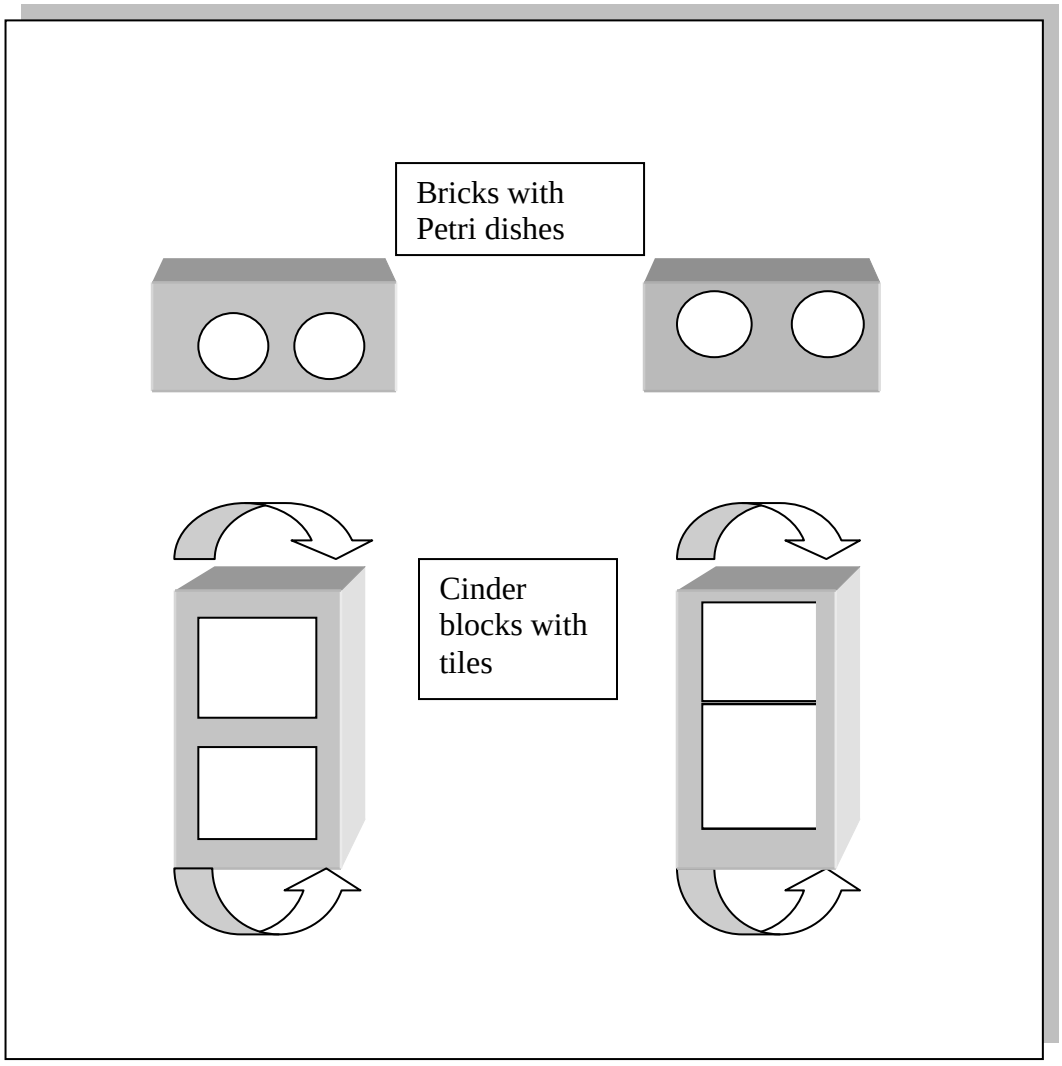

Figure 4. Layout of an Experimental Pond - Two ceramic tiles were secured to each cinder block using Velcro in order to determine algal growth. Leaf packs were also attached to the ends of each cinder block using zip ties. In addition, two Petri dishes were secured to each brick using Velcro in order to determine sediment accumulation.

Leaf Litter Decomposition

Leaf packs were used in order to assess the break down rates of leaf litter in each pond and the riparian bank. We chose Texas Oak (*Quercus texana*) and Sycamore (*Platanus occidentalis*) because both are common in riparian tree species area in this region of Texas and both are present at Griffith League Ranch. The leaves were initially dried at 65°C for at least 48 hours in the lab prior to placing the leaves in the packs. To construct the leaf packs an approximate 8 inches × 8 inches of plastic lawn and garden black mesh was used to secure the leaves and the edges were secured with metal twist ties. One leaf litter pack was placed in each of the twenty four ponds for 24 hours to determine the initial leaching rate of the leaf pack. In addition, four *P. occinedtalis* and four *Q. texana* packs were sunk in the bottom of each of the twenty four ponds. Two *P. occidentalis* and two *Q. texana* packs were placed in the riparian area surrounding each of the twenty four ponds. Over the course of the study, one sycamore and one oak pack was pulled from each pond every two weeks. The packs in the riparian area of each pond were pulled after four weeks and at the end of the experiment. At week 4, two *P. occidentalis* and two *Q. texana* leaves were introduced in the ponds, which were sunk using a metal nut attached by flagging in order to determine leaf breakdown rate without the protective features of the plastic mesh used on the previous leaf packs. One leaf of each species was pulled after two and four weeks and again dried in the lab dryer at 65°C for at least 48 hours to determine mass loss. After each pack or individual leaf is pulled it will be placed in a plastic bag and transported to the lab. Any sediment and fine organic matter accumulated on the leaves was removed by washing with distilled water. In addition, any macroinvertebrates on the leaves were collected and stored in 95%

ethanol for identification to the lowest taxonomic level possible. The remaining leaves left in the packs were dried at 65°C for at least 48 hours, weighed and the total loss in grams recorded. A total of 288 packs and 96 individual leaves were used to assess the terrestrial and aquatic leaf litter breakdown rate of each pond.

Periphyton Biomass

To estimate chlorophyll *a* produced in each pond, four ceramic tiles (14.5 × 14.5 cm) were used. Two tiles were placed on a cinder block in the deepest end of each pond. One tile was collected from the pond every two weeks until the end of the experiment. After each tile was collected it was taken back to the lab and cleaned of algae with a nylon brush and rinsed into an acid-washed HDPE beaker with Milli-Q water. The slurry was filtered onto Pall A-E filters. Chlorophyll *a* was then extracted from the filters using 99% HPLC grade acetone for four hours in aluminum foil covered test tubes and then measured on a Turner Trilogy™ Lab Fluorometer (Turner Designs Inc. Sunnyvale California). 96 tiles were used to determine the periphyton biomass (Chlorophyll *a*) in the 24 ponds.

Invertebrate Community Assemblage Response

Macroinvertebrate community in study ponds was collected in two ways during the experiment. First, macroinvertebrates were collected from the leaf packs by rinsing the leaves with distilled water (DI) and then collecting the macroinvertebrates from either the leaf or in the sieve. Macroinvertebrates in leaf packs were collected at weeks 2, 4, 6 and 8; each time leaves were processed in the lab. All macroinvertebrates collected were

placed in 95% ethanol and identified to the lowest possible taxonomic level. At the end of the experiment, invertebrate community was surveyed using a dip net. One person swept the dip net several times through each pond's substrate and water column in order to capture the total invertebrate community from all microhabitats of the pond. All contents collected were brought back to the lab where the invertebrates were collected and stored in 95% ethanol for identification to the lowest possible taxonomic level.

Sediment Accumulation

Plastic Petri dishes were used in order to determine the amount of sediment accumulation that occurred in each pond. Two dishes were secured to 2 bricks (19.5 × 9.5 × 4 cm) using Velcro and sunk at the bottom of each pond. A dish was collected every two weeks. The sediment collected in each dish was placed in a plastic bag in the field and taken back to the lab. The contents of each plastic bag were removed using distilled water and collected into a sieve. Aluminum weigh boats were used to collect the sediment where it was then placed into a dryer at 65°C for at least 48 hours to dry. The difference in initial mass of the aluminum boat and the mass with the sediment collected yielded the mass of sediment collected. The sediment was then ashed in an oven at 450°C for four hours to determine weights of inorganic and organic matter composing the sediment.

Water Chemistry

Water chemistry was recorded once a week throughout the experiment using a water chemistry meter YSI 556 MPS. The temperature, conductivity, dissolved oxygen

(mg/l), percent dissolved oxygen and pH were recorded. Each measurement was taken in the middle of the deep end of each pond.

Sampling Techniques Used to Determine if Salmonellae was Present or Presented in the System

We examined all twelve *T. s. elegans* used in the experiment for salmonellae. During the initial capture, each turtle was swabbed using two sterilized cotton swabs. One swab was used on the claws and posterior fold of skin connecting leg to body and the second swab was inserted into the cloacae. The swabs were used to determine if any turtles had salmonellae when taken from their natural pond. Water and sediment samples were also collected from each pond where turtles were originally trapped. The water was taken off the top at the same site from which the sediment was collected. The sediment was collected at an approximate depth of twenty inches in the water. The turtles were swabbed again at each of the three body sites at the end of the experiment to determine if salmonellae were present in this system. All turtle swabs, water and sediment samples collected were used to enrich salmonellae in semi-selective media and lysed cells from these enrichments used for analyses by polymerase chain reaction (hereafter referred to as PCR) to determine if salmonellae were present.

Laboratory Procedures for Examining Salmonellae

(1) Enrichment

Swabs taken from the two body sites of each turtle were placed directly into 2-ml cryotubes that contained 1 ml of Buffered Peptone Water (l^{-1}: 10 g peptone, 5 g NaCl, 9 g

Na_2HPO_4, 1.5 g KH_2PO_4, pH 7.2) (International Standard Organization 1993). 100-µl-samples of sediment collected were also transferred into 2-ml cryotubes that contained Buffered Peptone Water. A 40 ml water sample was collected into a 50-ml Falcon tube from each pond and centrifuged in the lab to obtain a pellet. All pellets collected were also transferred into 2-ml cryotubes that contained Buffered Peptone Water. The samples were then incubated at 37°C for 24 hours (International Standard Organization 1993). Next, 100-µl-samples of these pre-enrichment cultures were transferred to 2-ml cryotubes that contained 1 ml of Rappaport-Vassiliadis (RVS) Broth (l^{-1}: 4.5 g peptone (soymeal), 29 g $MgCl_2$ x 7 H_2O, 8 g NaCl, 0.4 g K_2HPO_4, 0.6 g KH_2PO_4, 0.036 g malachite-green, pH 5.2) (Vassiliadis et al. 1981) and incubated at 37°C for 24 hours (Vassiliadis et al. 1981). 100-µl-samples of semi-selective enrichment cultures were used for molecular analyses (i.e. PCR) as well as for isolation.

(2) PCR amplification

For the detection of salmonellae by PCR, 100-µl-samples of the enrichments were centrifuged at 14,000 rpm for 2 minutes. The bacterial pellets were washed once in sterile distilled water, and re-suspended in 100-µl of 50 mM NaOH before being lysed by incubation at 65°C for 30 minutes. 1 µl of this lysate was used as template for PCR amplification with primers 139 ($^{5'}$GTG AAA TTA TCG CCA CGT TCG GGC AA) and 141 ($^{5'}$TCA TCG CAC CGT CAA AGG AAC C) (Rahn et al. 1992) targeting the *inv*A gene that encodes a protein of a type III secretion system, essential for the invasion of epithelial cells by salmonellae (Suárez and Rüssmann 1998, Khan et al. 2000), and

present in all *Salmonella enterica* subspecies as well as in *S. bongori* (Malorny et al. 2003).

PCR was performed in a total volume of 50 µl containing 10 x PCR buffer (500 mM KCl, 25 mM $MgCl_2$, 200 mM Tris/HCl, pH 8.4, 0.1% Triton 100), 1 µl dNTPs (each 10 mM in 10 mM Tris/HCl, pH 7.5), 0.2 µl *Taq* polymerase (5 U μl^{-1}),0.50 µl of each primer (100 ng μl^{-1}), 139 and 141, and 1 µl lysate (Widmer et al. 1999). PCR was performed in a Thermocycler for 35 cycles with denaturation at 96°C, primer annealing at 64°C, and elongation at 72°C, each for 30 seconds (Malorny et al. 2003). The presence of 284-bp-fragments was examined by gel electrophoresis on 2% agarose gels (Sambrook et al. 1989). DNA of *Salmonella typhimurium* ATCC 14028 was always used as positive control.

Predation on T. s. elegans

Only three turtles of the original twelve survived the 9 week experiment. All nine shells were recovered 20-25 meters from the pond site where dense vegetation began. At least one shell showed obvious signs of raccoon predation. Each turtle was given an approximate time of death due to the apparent rate of decomposition and condition of the skeletal shell itself. Due to the decomposition estimate, it appeared a turtle was killed approximately every two weeks.

Statistical Analyses

A multi-factorial multivariate analysis of variance (MANOVA) was used to determine if the *T.s. elegans* affected the abiotic parameters measured, ecosystem

processes and invertebrate community within each pond. Turtle treatment and sampling time (week) were used as factors while pond conductivity µs/cm, percent dissolved oxygen, pH, sediment accumulation, Oak leaf litter breakdown rate, Sycamore left litter breakdown rate, and production of chlorophyll *a* were used as response variables. Individual ANOVA's were then conducted to determine the relationship of each response variable and both factors. In order to analyze the open leaves that were placed into the ponds and the riparian leaf packs placed around the ponds ANCOVA's were used where turtle was a fixed factor and week was treated as a covariate. For analysis of invertebrate data, all data was transformed by adding 0.5 to each value in order to account for the zeros in the data set. In addition, the square root was then taken for each value in order for the data to meet assumptions for a single factor ANOVA. In some cases assumptions for an ANOVA was still not met and a Kruskal-Wallis analysis was used. All data were analyzed by using R for Windows (R Development Core Team 2005).

In addition to the multi-factorial MANOVA, several two-way MANOVA's were conducted to determine if the length of time the turtles spent in the ponds affected the response variables differently. Four levels representing the length of time the turtles spent in the ponds were devised. Level 0 were the pond treatments in which no *T. s. elegans* was ever present in the pond. Level 1 was turtle treatments in which the turtle was present in the pond for 2 weeks or less. Level 2 was turtle treatments in which the turtle was present for 4-6 weeks of the study and level 3 was the ponds in which each turtle was present throughout the whole study. SPSS v17.0 (SPSS Inc., Chicago, IL, USA) was used to conduct this analysis.

Canonical Correspondence Analysis (CCA) is a direct gradient analysis that can identify the influence of environmental factors on macroinvertebrate assemblages. CCA was used to examine the relationships between turtle treatments, the macroinvertebrate community and the environmental variables measured using the program R (R Development Core Team 2005).

CHAPTER IV

RESULTS

MANOVA Results

All data collected from the 24 ponds were included in the multi-factorial MANOVA analysis in order to illustrate the residual effect that occurred from the turtles being present in the experimental pond system. Table 2 illustrates the combined output for the multivariate and univariate tests.

Leaf Litter Breakdown Rate

A significant difference in the rate of leaf litter breakdown for the closed Texas oak packs was detected between ponds with and without turtles in the water ($F_{1,88}$ = 7.66, P = 0.006) (Table 2). The mean percent leaf litter loss in ponds that contained a turtle was 23.16% and mean percent loss in ponds that did not contain a turtle was 20.78% (Figure 5). A significant difference for oak leaf litter breakdown between weeks was also detected ($F_{3,88}$ = 36.001, P = <0.001) (Table 2). The mean percent loss for all ponds at week 2 was 20.17%; the mean percent loss at week 8 was 32.58%. No significant interaction between ponds and sampling week was detected ($F_{3,88}$ = 1.983, P = 0.129) (Table 2).

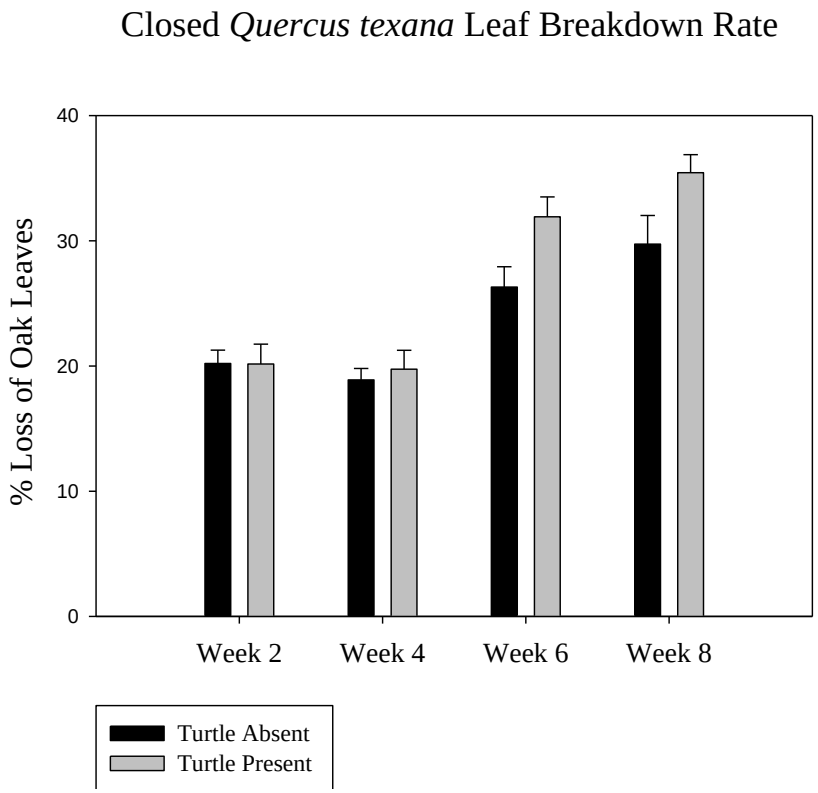

Figure 5. Mean Percent Loss of Texas Oak Leaves in Experimental Ponds. The mean percent loss for ponds that contained a turtle was 23.16% and ponds that did not contain a turtle was 20.78%.

A significant difference in the percent loss of Texas Oak leaves in closed riparian packs was not detected between ponds with and without turtles ($F_{1,45}$ = 0.567, P = 0.455) (Table 2). The mean percent loss for ponds with a turtle was 12.62% and the percent loss for ponds without a turtle was 13.39% (Figure 6). A difference was detected among sampling weeks ($F_{1,45}$ = 79.12, P = <0.001) (Table 2). The mean percent loss at week 4 was 8.4% and the mean percent loss at week 8 was 17.58%.

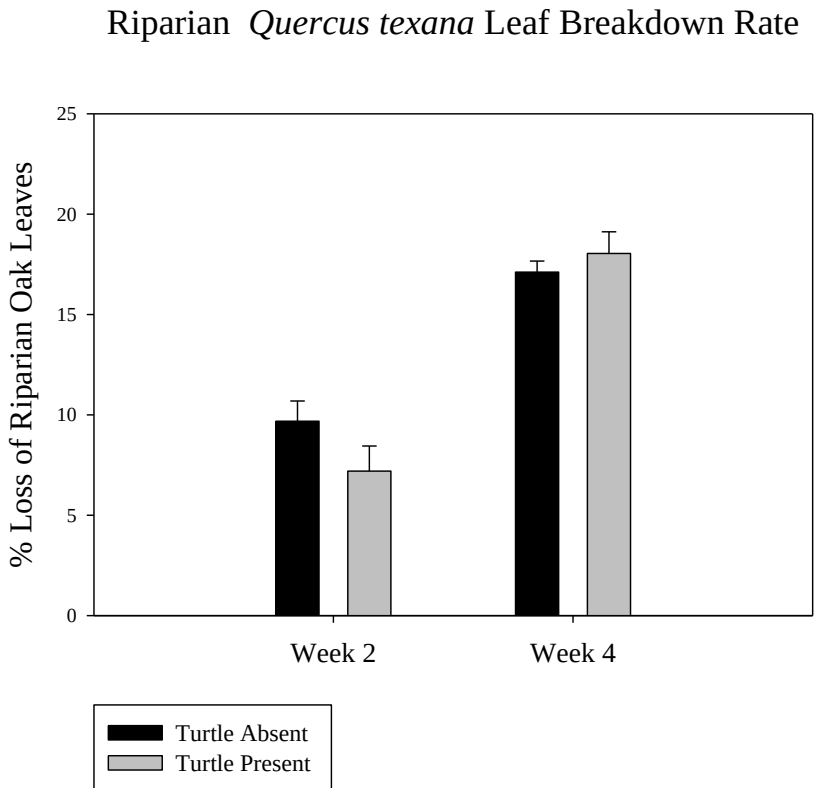

Figure 6. Mean Percent Loss of Riparian Texas Oak Leaves. The mean percent loss for ponds that contained a turtle was 12.62% and the loss for ponds that did not contain a turtle was 13.39%.

A significant difference was not found in the % loss of open Texas Oak leaves between ponds that contained a turtle and those that did not ($F_{1,45}$ = 0.186, P = 0.66) (Table 3). The mean % loss in ponds that contained a turtle was 18.26 % and the mean % loss in ponds without a turtle was 19.55% (Figure 7). There was not a significant difference among weeks either ($F_{1,45}$ = 0.142, P = 0.708) (Table 3). The mean % loss at week 2 was 19.47% and the mean % loss at week 4 was 18.34%.

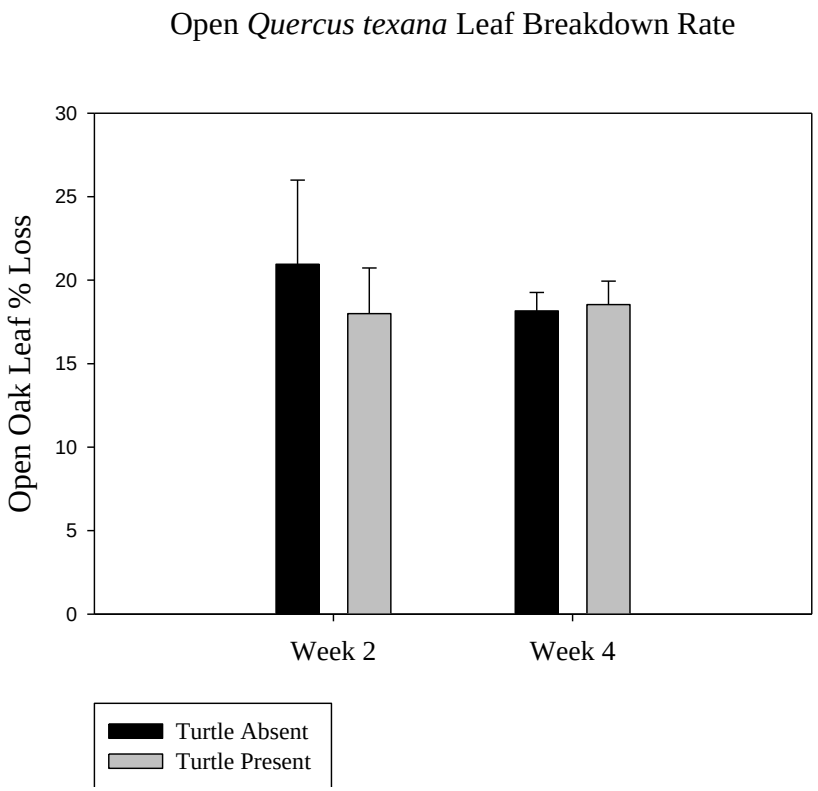

Figure 7. Mean Percent Loss of Open Texas Oak Leaves in Experimental Ponds. The mean % loss for ponds that contained a turtle was 18.26% and the mean % loss for ponds that did not contain a turtle was 19.55%.

A significant difference between ponds with and without turtles was detected in the rate of leaf litter breakdown for the closed Sycamore packs in the water ($F_{1,88}$ = 4.677, P = 0.0332) (Table 2). The mean % loss for ponds that contained a turtle was 26.97% and the mean % loss for ponds without a turtle was 24.98% (Figure 8). A difference among the % loss of Sycamore leaves over the course of the experiment was detected as well ($F_{3,88}$ = 32.856, P = <0.001) (Table 2). The mean % loss for the ponds at week 2 was 22.19%; the mean % loss for the ponds at week 8 was 35.76%. No significant interaction between the ponds and sampling week was detected, although it was close ($F_{3,88}$ = 2.677, P = 0.0519) (Table 2).

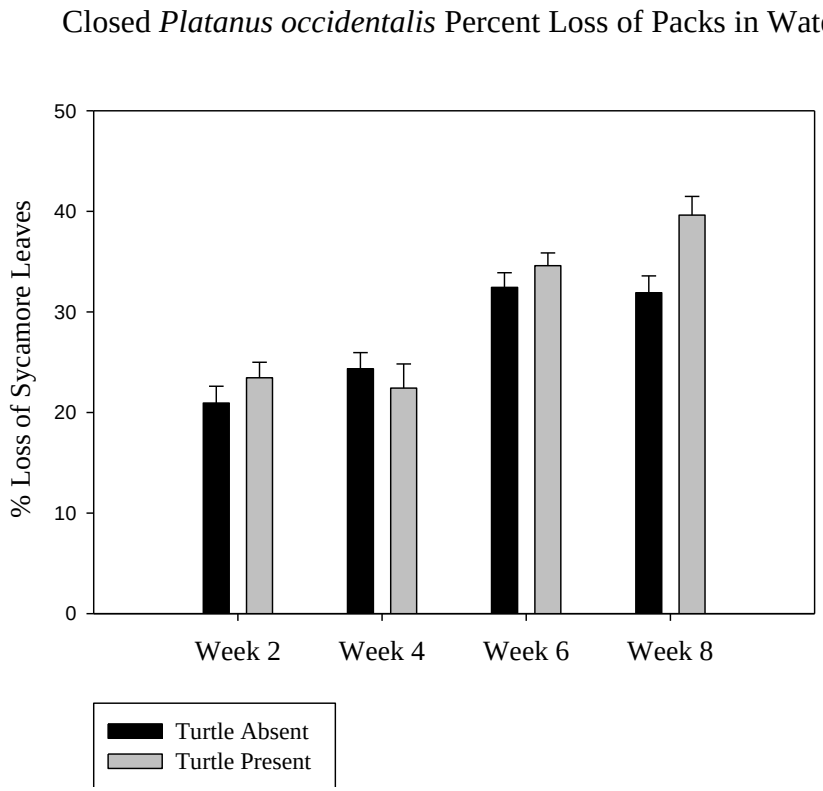

Figure 8. Mean Percent Loss of Sycamore Leaves in Experimental Ponds. The mean % loss for ponds that contained a turtle was 26.97% and the mean % loss for ponds that did not contain a turtle was 24.98%.

A significant difference in the percent loss of riparian Sycamore leaves between ponds with turtles and ponds without turtles was detected ($F_{1,45}$ = 18.45, P = <0.001) (Table 2). The mean percent loss for ponds that contained a turtle was 11.16% and the mean percent loss for ponds that did not contain a turtle was 16.03% (Figure 9). A difference among the sampling weeks was also detected ($F_{1,45}$ = 53.46, P = <0.001) (Table 2). The mean percent loss at week 4 was 9.45% and the mean percent loss at week 8 was 17.73%.

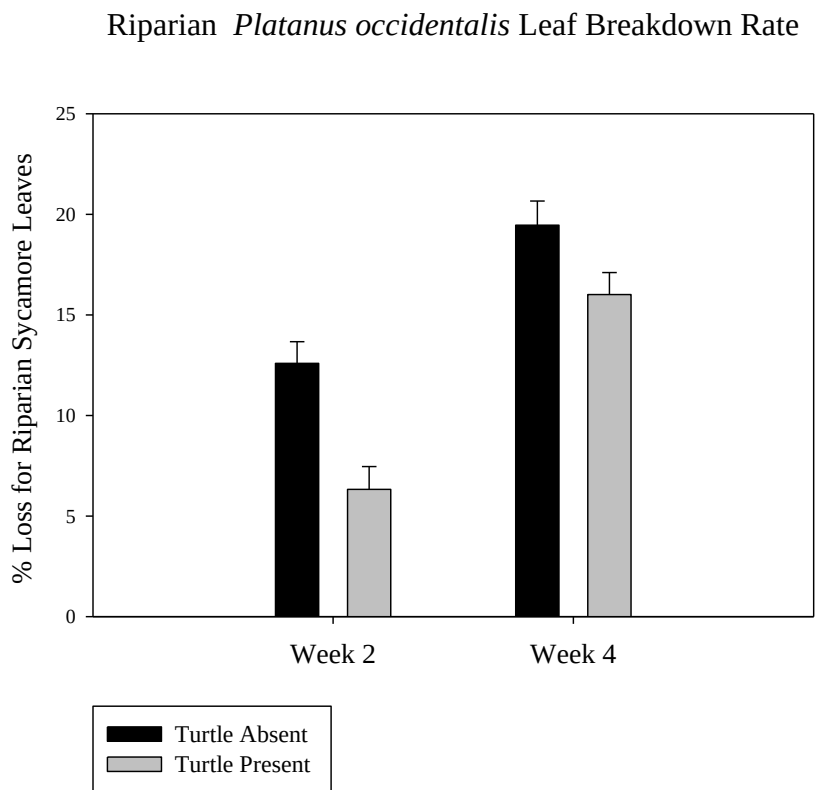

Figure 9. Mean Percent Loss of Riparian Sycamore Leaves in Experimental Ponds. The mean % loss for ponds that contained a turtle was 11.16% and the mean % loss for ponds without a turtle was 16.03%.

A significant difference was detected in the % loss of open Sycamore leaves in ponds that contained a turtle and ponds that did not ($F_{1,45} = 5.46$, $P = 0.024$) (Table 3). The mean % loss of a Sycamore leaf in a pond that had a turtle was 20.80% and the mean % loss in ponds without turtles was 16.52% (Figure 10). A difference among the sampling weeks was also detected ($F_{1,45} = 10.89$, $P = 0.002$) (Table 3). The mean % loss at week 2 was 15.63% and the mean % loss at week 4 was 21.68%.

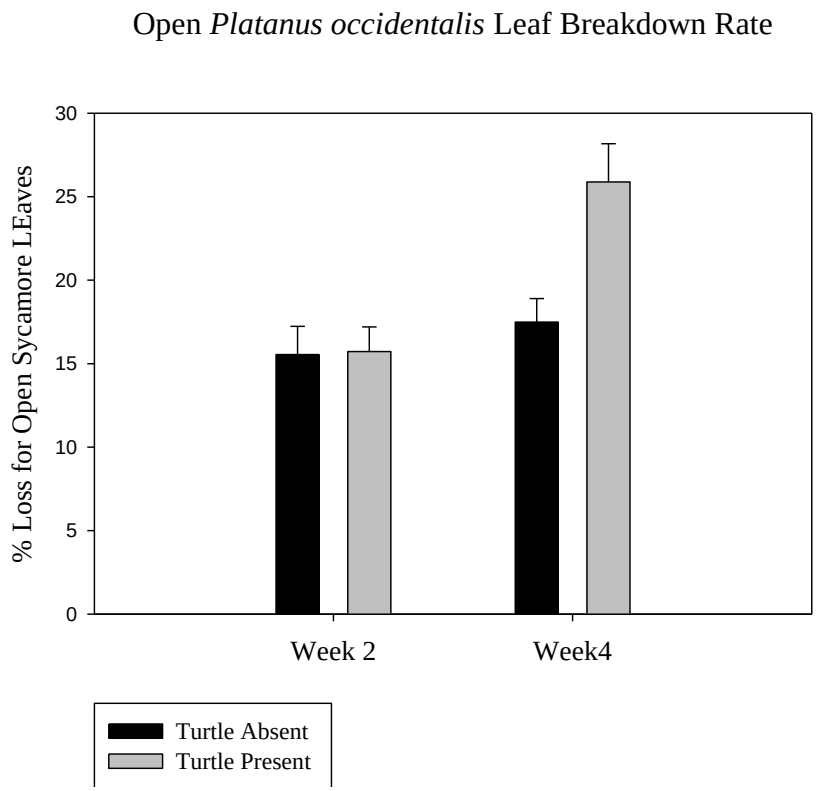

Figure 10. Mean Percent Loss of Open Sycamore Leaves in Experimental Ponds. The mean percent loss for ponds that contained a turtle was 20.8% and the mean % loss for ponds without a turtle was 16.52%.

Periphyton Biomass Production

There were no significant differences detected in the amount of chlorophyll *a* produced among the ponds ($F_{1,88} = 0.236$, $P = 0.6283$) (Table 2). The mean amount of chlorophyll *a* produced in ponds that contained turtles was 1025.12 µg/L; the mean amount of chlorophyll *a* produced in ponds without turtles was 948.18 µg/L (Figure 11). Also, no differences were detected among sampling weeks ($F_{3,88} = 1.6002$, $P = 0.1951$) (Table 2). The mean amount of chlorophyll *a* produced at week 2 was 689.91 µg/L; the mean amount of chlorophyll *a* produced at week 8 was 1062.85µg/L. No interactions were detected among ponds and sampling week ($F_{3,88} = 0.1811$, $P = 0.9089$) (Table 2).

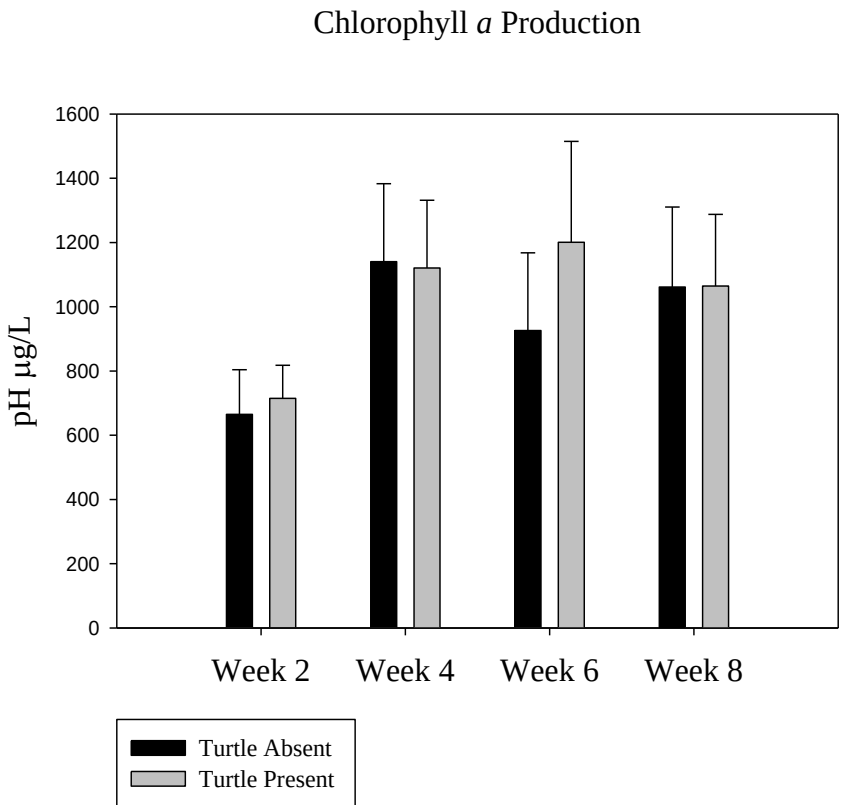

Figure 11. Mean Chlorophyll *a* Production in Experimental Ponds. Mean pH at week two was 6.6 and mean pH at week 8 was 7.15.

Invertebrate Community Response

A total of 3,613 invertebrates were collected from the 24 ponds either from the leaf packs or the dip net sampling. A total of 2,784 invertebrates were found in ponds that contained turtles; while 829 invertebrates were found in the ponds that did not contain turtles. The mean number of macroinvertebrates is listed by order in Figure 12. All individuals were from the following orders: Odonata, Hemiptera, Ephemeroptera, Diptera or Coleoptera. The difference in total number of invertebrates among ponds with and without turtles was not significant (KW $x^2_1 = 3.14$, $P = 0.076$) (Table 5).

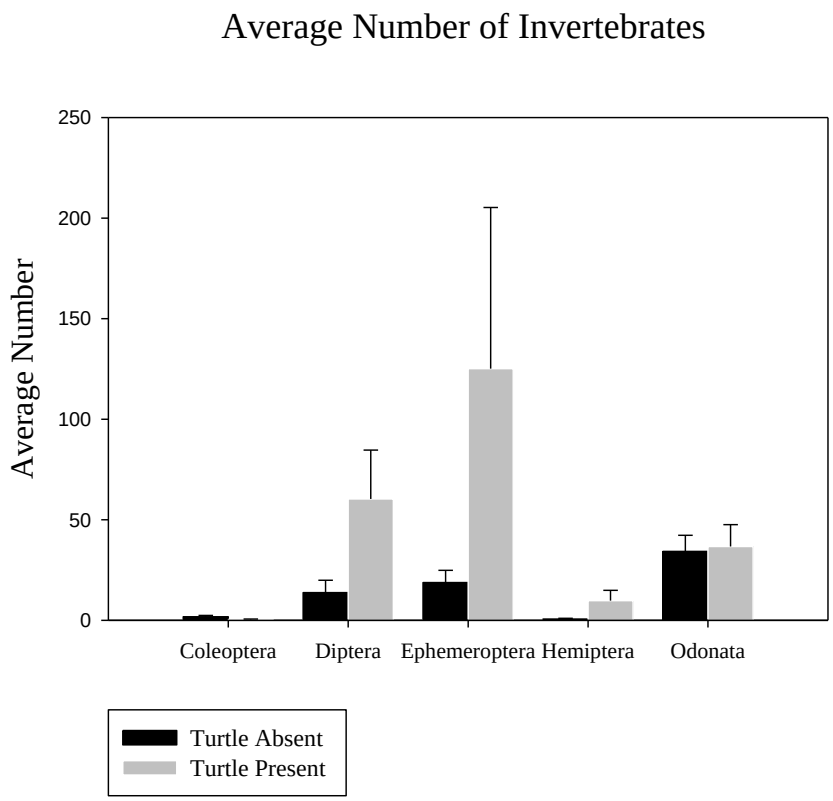

Figure 12. Mean Number of Macroinvertebrates in Experimental Ponds. A significant difference was not detected; although 2,784 invertebrates were found in ponds that contained a turtle and 829 invertebrates were found in ponds without a turtle.

A total of 848 Odonates were found; individuals were from one of the following families: Libbellulidae (505), Cordullidae (286), Coenagrionidae (50) or Ashnidae (5). There were not significant differences detected among the ponds that contained a turtle and ponds that did not ($F_{1,22} = 0.035$, $P = 0.85$) (Table 5). The mean number of Odonates found in ponds that contained a turtle was 36.42 and the mean number of Odonates in ponds that did not contain a turtle was 34.25 (Figure 13).

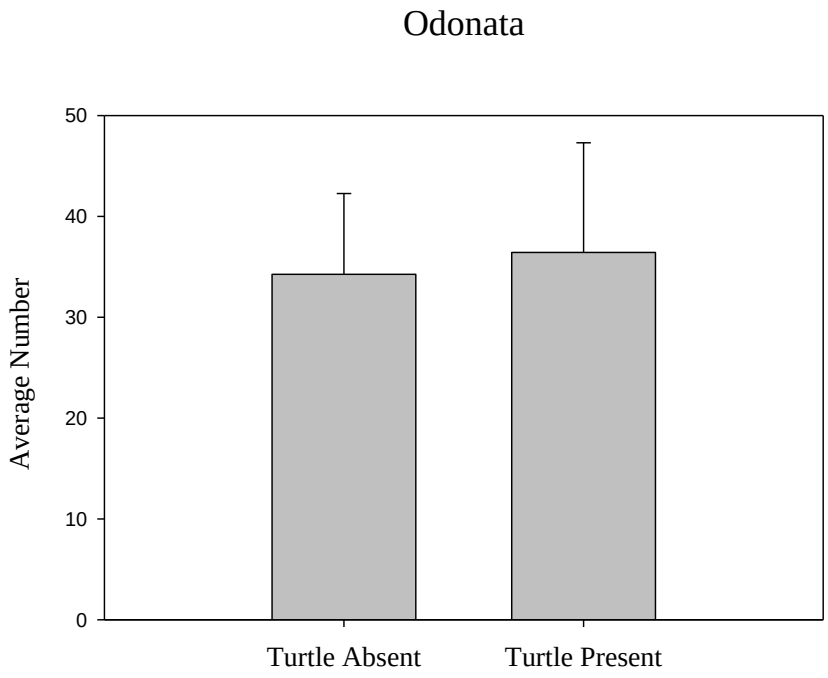

Figure 13. Mean Number of Odonata Found in Experimental Ponds. The mean number of Odonates found in a pond that contained a turtle was 36.42 and the mean found in ponds without a turtle was 34.25.

A total of 124 Hemipterans were found; 117 were in ponds that contained turtles, 7 were found in the ponds that did not contain turtles. Each individual was from one of the following families: Notonectidae (117), Pleidae (3), Nacoridae (2) or Gelastocoridae (2). A significant difference between the two treatments was detected ($F_{1,22} = 4.98$, $P = 0.036$) (Table 5). The mean number of Hemipterans in ponds that contained a turtle was 9.75 and the mean in ponds that did not contain a turtle was 0.58 (Figure 14).

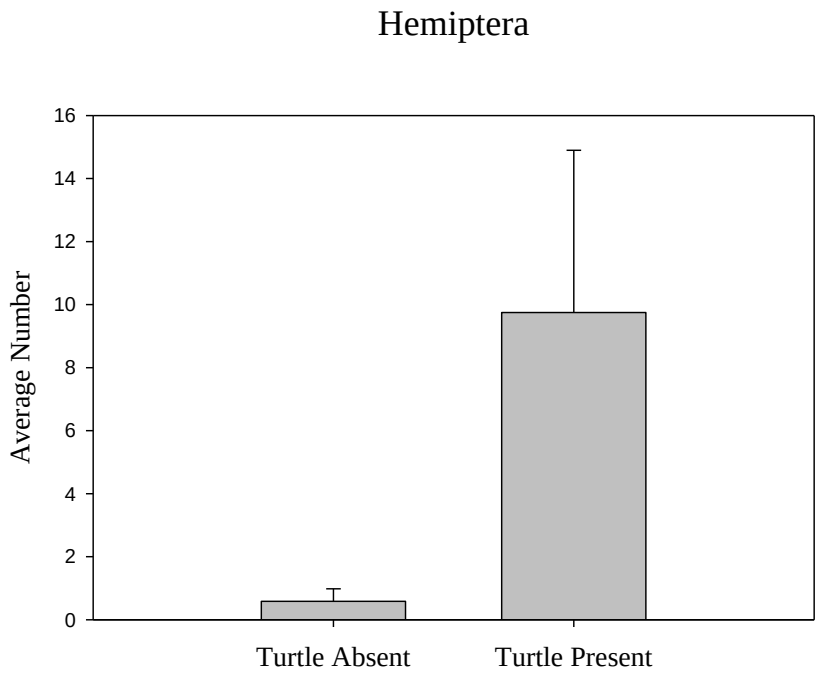

Figure 14. Mean Number of Hemiptera Found in Experimental Ponds. The average number of Hemipterans found in a pond that contained a turtle was 9.75 and the mean found in ponds without a turtle was 0.58.

A total of 1,726 Ephemeropterans were found in the ponds. 1,501 were found in the ponds that contained a turtle; 225 were found in ponds that did not contain a turtle. All individuals were from the family Baetidae. A significant difference was detected between the two treatments ($F_{1,22} = 4.046$, $P = 0.057$) (Table 5). The mean number of Baetidae found in ponds that contained a turtle was 125.08 and the mean number of Baetidae found in ponds that did not contain a turtle was 18.75 (Figure 15).

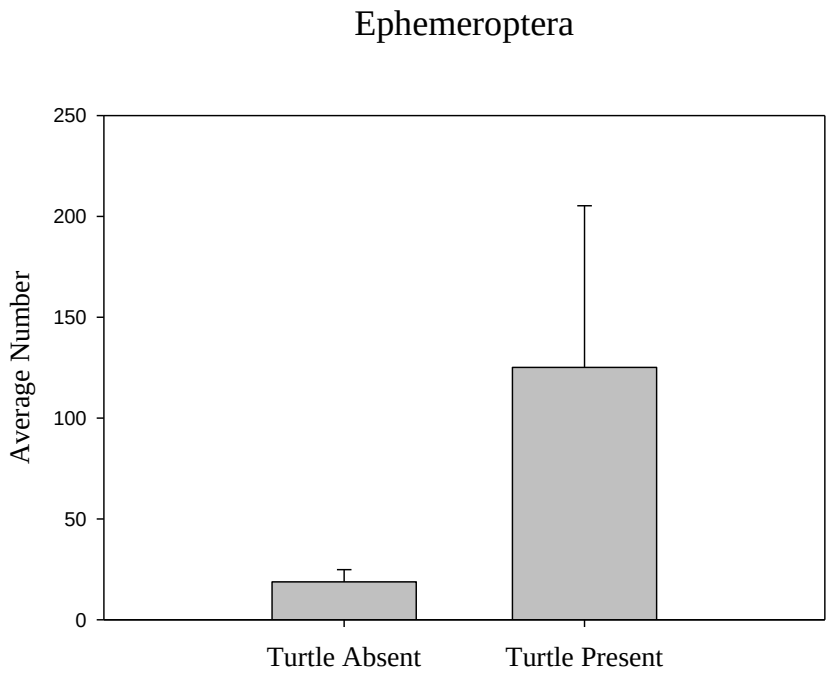

Figure 15. Mean Number of Ephemeroptera Found in Experimental Ponds. The mean number of Baetidae found in ponds that contained a turtle was 125.08 and the mean number found in ponds without a turtle was 18.75.

A total of 889 Diptera were found. 724 were found in ponds that contained turtles; 165 were found in ponds that did not contain turtles. All individuals were from the family Chironomidae. A significant difference was not detected between the two treatments (X^2_1 = 1.70, P = 0.192) (Table 5). The mean number of Diptera found in ponds that contained a turtle was 60.33 and the mean number of Diptera found in ponds without a turtle was 13.75 (Figure 16).

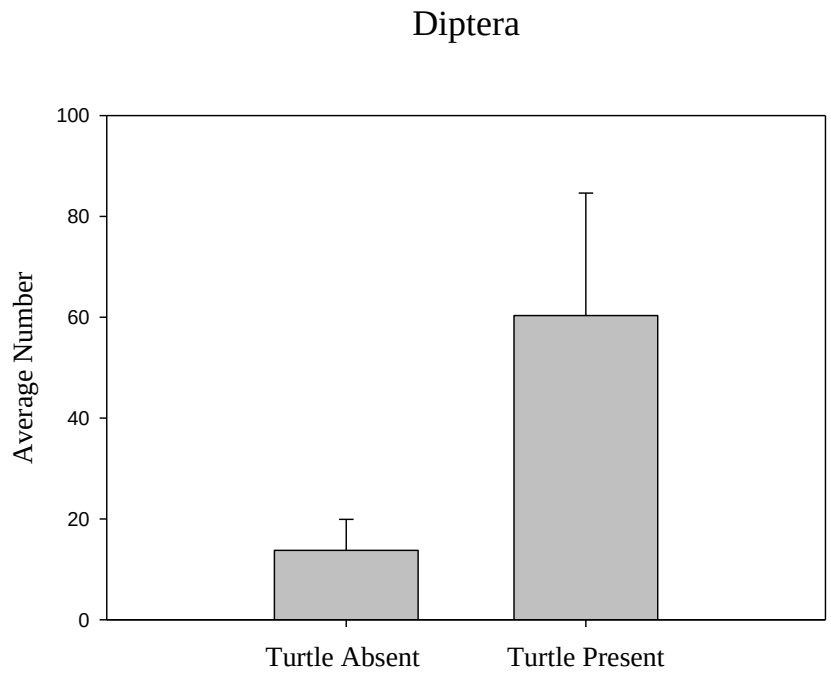

Figure 16. Mean Number of Diptera Found in Experimental Ponds. The mean number of Diptera found in ponds that contained a turtle was 60.33 and the mean number of Diptera found in ponds without a turtle was 13.75.

A total of 26 Coleopteran were found. 5 individuals were found in ponds that contained turtles; 21 were found in ponds that did not contain turtles. All individuals were from one of the following families: Dytiscidae (23), Halipidae (2), or Hydrophilidae (1). A significant difference between the two treatments was not detected ($X^2_1 = 1.13$, $P = 0.287$) (Table 5). The mean number of Coleoptera found in ponds that a contained turtle was 0.42 and the mean number of Coleoptera found in ponds that did not contain turtles was 1.75 (Figure 17).

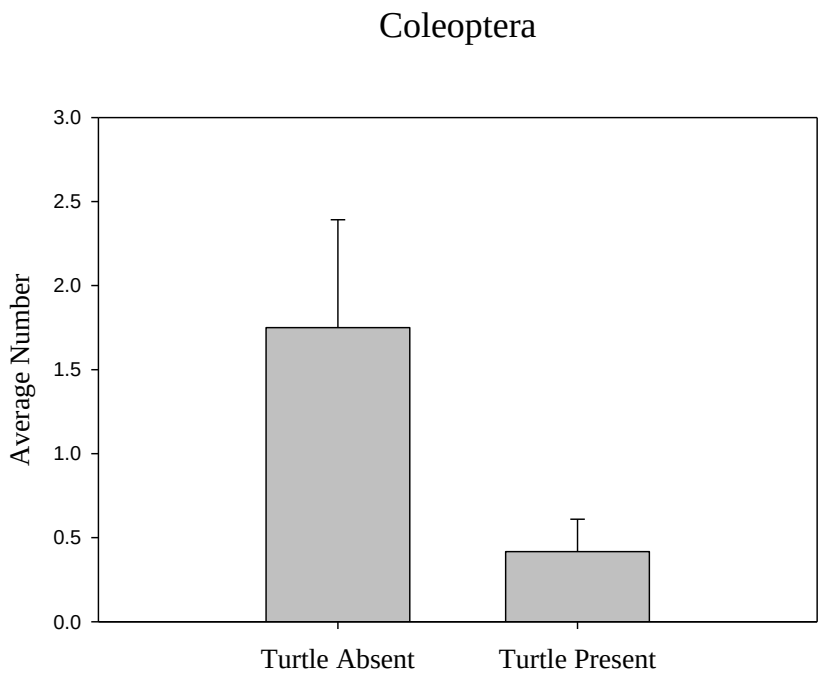

Figure 17. Mean Number of Coleoptera Found in Experimental Ponds. The average number of Coleopterans found in a pond that contained a turtle was 0.42 and the average found in ponds without a turtle was 1.75.

Table 2. Macroinvertebrate Assemblage Found in Experimental Ponds. Order, Family and the number found in either turtle absent or present treatments.

Order	**Total Number Found**	**Turtle Present**	**Turtle Absent**
Coleoptera	26	5	21
Diptera	889	724	165
Ephemeroptera	1,726	1,501	225
Hemiptera	124	117	7
Odonata	848	437	411
Coeloptera	**Total Number Found**	**Turtle Present**	**Turtle Absent**
Dytiscidae	23	4	19
Halipidae	2	0	2

Hydrophilidae	1	1	0
Diptera	**Total Number Found**	**Turtle Present**	**Turtle Absent**
Chironomidae	889	724	165
Ephemeroptera			
Baetidae	1,726	1,501	225
Hemiptera	**Total Number Found**	**Turtle Present**	**Turtle Absent**
Notonectidae	117	112	5
Pleidae	3	3	0
Nacordiae	2	2	0
Gelastocoridae	2	0	2
Odonata	**Total Number Found**	**Turtle Present**	**Turtle Absent**
Anisoptera	798	398	400
Zygoptera	50	39	11
Libellulidae	505	241	264
Codullidae	286	152	134
Coenagrionidae	50	39	11
Ashnidae	5	0	5

Sediment Accumulation

Significant differences in sediment accumulation between ponds that contained and turtle and those that did not were found ($F_{1,88} = 5.023$, $P = 0.0275$) (Table 2). The mean sediment accumulation for those ponds that contained a turtle was 2.09 g and the

mean sediment accumulation for ponds that did not contain a turtle was 1.26 g (Figure 18). Significant differences of sediment accumulation were found among weeks as well ($F_{3,88}$ = 6.34, P = <0.001) (Table 2). Mean sediment accumulation at week 2 of the experiment was 0.418 g and mean sediment accumulation at the end of the experiment was 2.61 g. No significant interaction between ponds and sampling week was detected ($F_{3,88}$ = 6.34, P = 0.905) (Table 2).

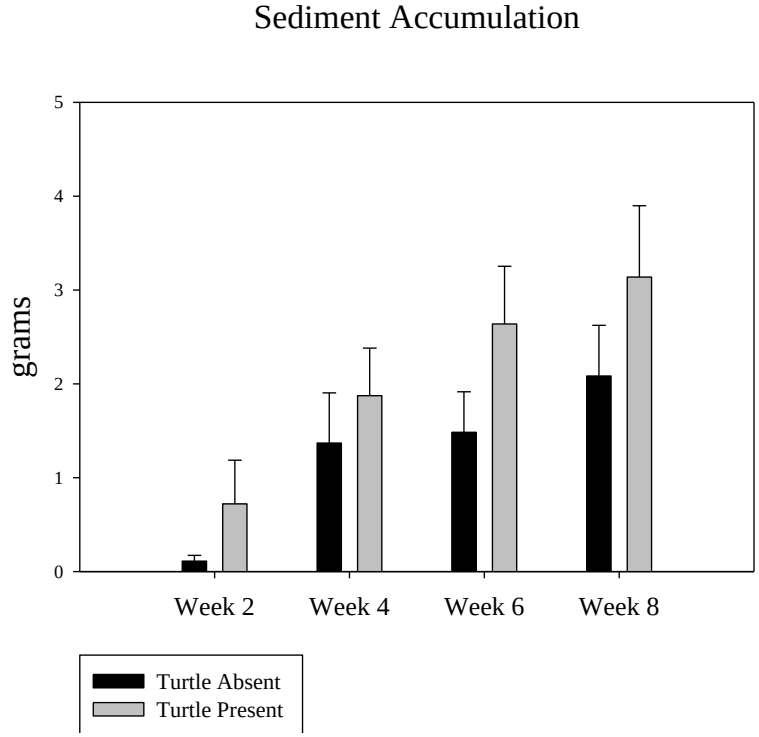

Figure 18. Mean Sediment Accumulation in Experimental Ponds. Mean sediment accumulation for ponds that contained a turtle was 2.09 g and the mean sediment accumulation in ponds without a turtle was 1.26 g.

Water Chemistry

Significant differences were found in pH values between ponds with and without turtles ($F_{1,88}$ = 70.41, P = <0.001) (Table 2). The mean pH for ponds that a contained turtle was 7.19 and ponds that did not contain turtles was 6.77 (Figure 19). Pond pH also differed among weeks as expected ($F_{3,88}$ = 27.87, P = <0.001) (Table 2). The mean pH of

the ponds at week 2 of the experiment was 6.6 and the mean pH of the ponds at the end of the experiment was 7.15. No significant interaction between ponds and sampling week was detected ($F_{3,88}$ = 1.27, P = 0.286) (Table 2).

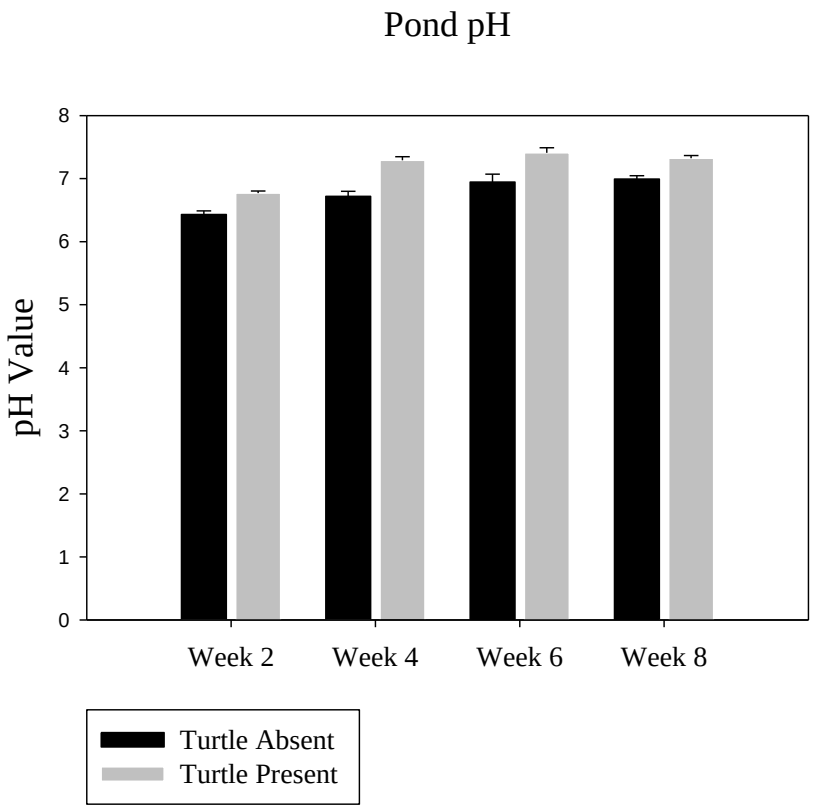

Figure 19. Mean pH of Experimental Ponds**.** The mean pH of ponds that contained a turtle was 7.19 and the mean pH for ponds without a turtle was 6.77.

Significant differences in conductivity (µs/cm) were found between ponds that contained a turtle and those that did not ($F_{1,88}$ = 103.63, P = <0.001) (Table 2). The mean conductivity for ponds that contained a turtle was 190.81 µs/cm and the mean conductivity for ponds that did not contain a turtle was 116.81 µs/cm (Figure 20). Conductivity also differed among weeks as expected ($F_{3,88}$ = 26.74, P = <0.001) (Table 2). The mean conductivity at week 2 for the ponds was 123.37 and the mean conductivity

of the ponds at the end of the experiment was 206.58. No significant interaction between ponds and sampling week was detected ($F_{3,88} = 0.746$, $P = 0.527$) (Table 2).

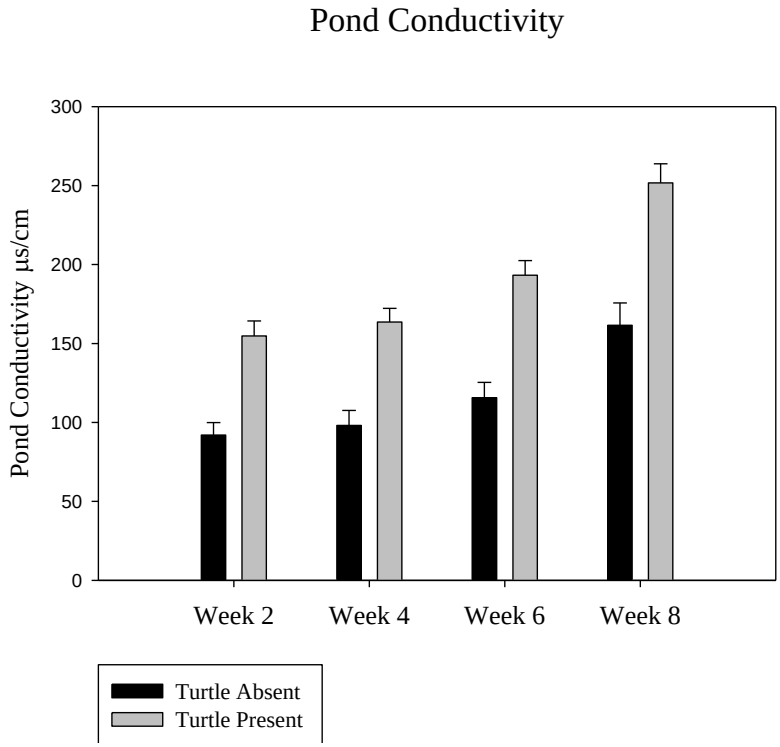

Figure 20. Mean Conductivity (µs/cm) of Experimental Ponds. Mean conductivity for ponds that contained a turtle was 190.81 µs/cm and the mean conductivity for ponds without a turtle was 116.81 µs/cm.

No differences in percent dissolved oxygen was found between ponds that contained turtles and those that did not ($F_{1,88} = 0.3097$, $P = 0.579$) (Table 2). The mean percent dissolved oxygen for ponds that contained a turtle was 18.91% and the mean percent dissolved oxygen for ponds that did not contain a turtle was 20.92% (Figure 21). A significant difference in percent dissolved oxygen was found among weeks ($F_{3,88} = 2.75$, $P = 0.046$) (Table 2). The mean percent dissolved oxygen for the ponds at week 2 of the experiment was 27.34% and the mean percent dissolved oxygen at the end of the

experiment was 12.55%. No significant interaction between ponds and sampling week was detected ($F_{3,88} = 0.057$, $P = 0.982$) (Table 2).

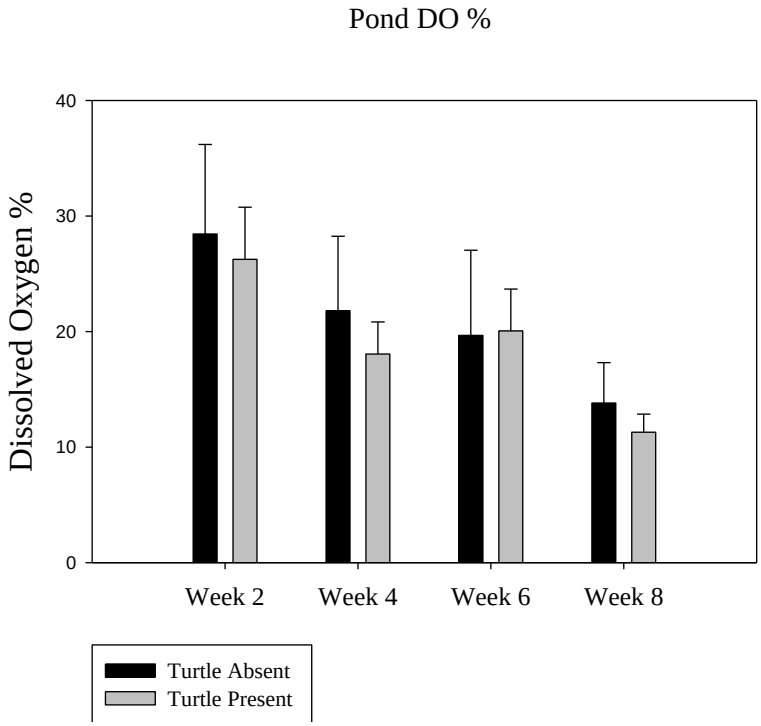

Figure 21. Mean Percent Dissolved Oxygen of Experimental Ponds. The mean % DO for ponds that contained a turtle was 18.91% and the mean % DO for ponds without a turtle was 20.92%.

Two-way MANOVA Results

In order to determine if stronger effects existed according to how long the turtle was present in the pond, a two-way MANOVA was used. Four categories were devised according to the length of time the turtle was present in the pond. Level 0 contained the twelve control ponds where no turtle was ever present. Level 1 contained the ponds in which a turtle was present for 0 – 2 weeks, level 2 contained ponds in which a turtle was present 4 – 6 weeks and level 3 contained ponds in which a turtle was present for the entire eight week study. Table 4 illustrates the combined output from the two-way MANOVA.

Leaf Litter Breakdown Rate

A significant difference was detected in the leaf breakdown rate of the Oak leaves among the ponds at week 6 of the study ($F_{3,80}$ = 2.984, P = 0.04) (Table 4). The mean percent loss at week 6 was 29.10%. Level 1 had the highest percentage of loss at 34.93% (Figure 22).

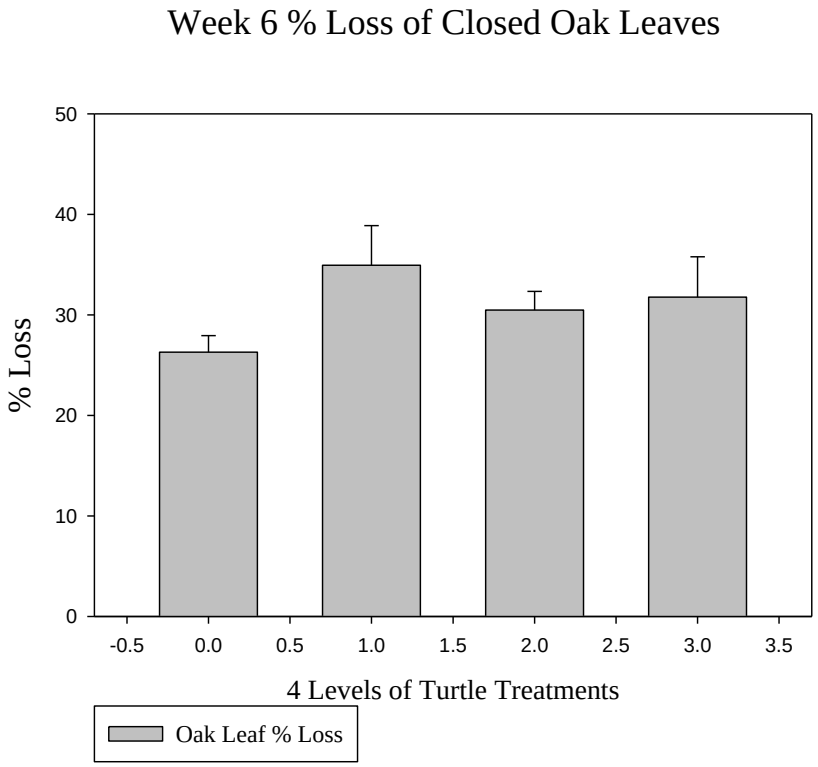

Figure 22. Week 6 Percent Loss of Texas Oak Leaves in Closed Packs. Level 1(turtle present for 2 weeks or less) had the highest % of loss at 34.93%. The presence of turtles and week were both significant (Table 4).

A significant difference was detected in the leaf breakdown rate of the Oak leaves among the ponds at week 8 of the study ($F_{3,80}$ = 2.984, P = 0.04) (Table 4). The mean percent loss at week 8 was 32.58 %. Level 3 had the highest percentage of loss at 36.43% (Figure 23).

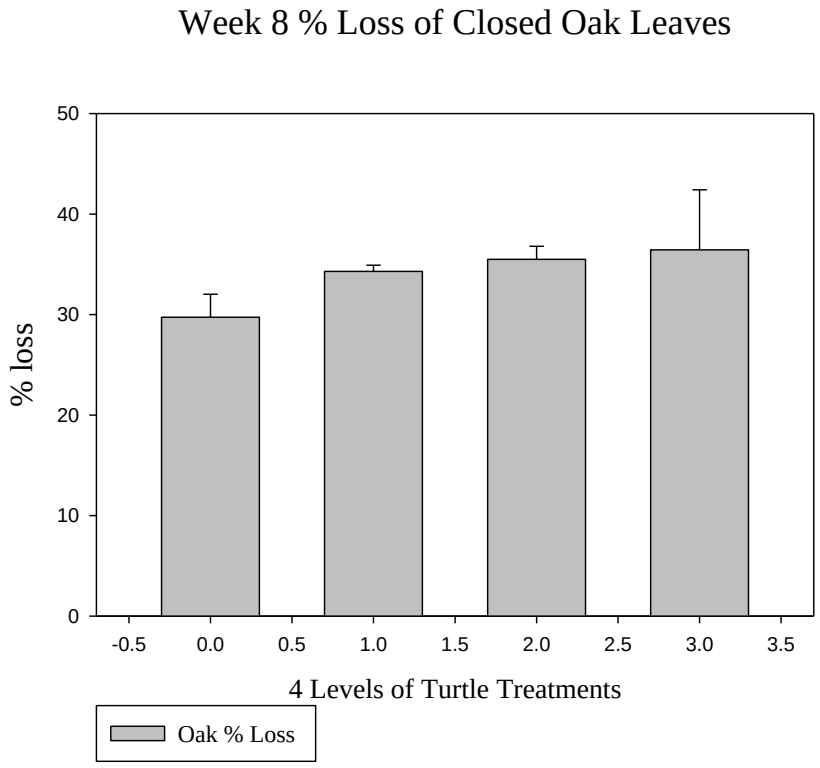

Figure 23. Week 8 Percent Loss of Texas Oak Leaves in Closed Packs. Level 3(turtle present the entire study) had the highest % loss at 36.43%. The presence of turtles and week were both significant (Table 4).

A significant difference was not detected in the leaf breakdown rate of the Sycamore leaves among the ponds at week 6 of the study ($F_{3,80} = 1.972$, $P = 0.125$) (Table 4). The mean percent loss at week 6 was 33.52 %. Level 2 had the highest percentage of loss at 36.23% (Figure 24).

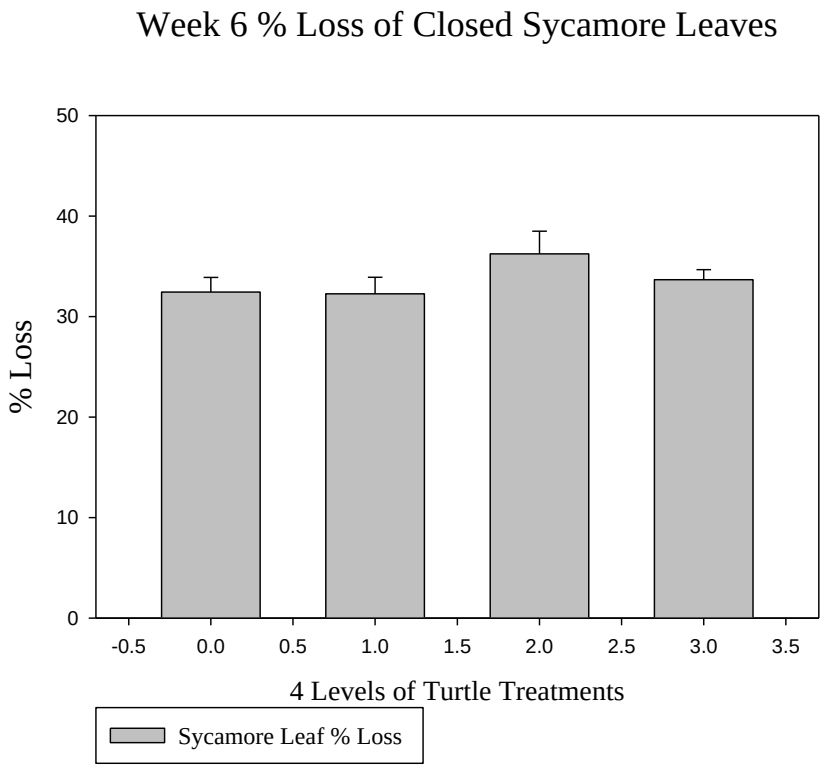

Figure 24. Week 6 Percent Loss of Sycamore Leaves in Closed Packs. Level 2(turtle present for 4-6 weeks) had the highest % loss at 36.23%. A significant difference was not detected for turtle treatments; a significant difference was detected for week (Table 4).

A significant difference was not detected in the leaf breakdown rate of the Sycamore leaves among the ponds at week 8 of the study ($F_{3,80} = 1.972$, $P = 0.125$) (Table 4). The mean percent loss at week 8 was 35.77 %. Level 2 had the highest percentage of loss at 41.96% (Figure 25).

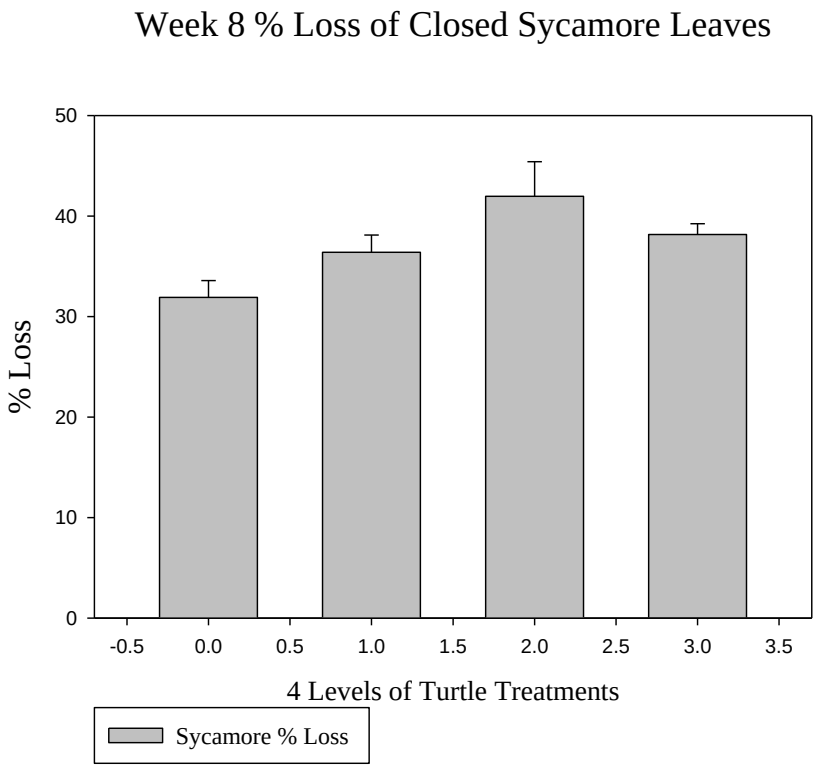

Figure 25. Week 8 Percent Loss of Sycamore Leaves in Closed Packs. Level 2(turtle present for 4-6 weeks) had the highest % loss at 41.96%. A significant difference was not detected for turtle treatments; a significant difference was detected for week (Table 4).

Sediment Accumulation

A significant difference was detected in the sediment accumulation among the ponds at week 6 of the study ($F_{3,80} = 5.729$, $P = 0.001$) (Table 4). The mean sediment accumulation in grams at week 6 was 2.066. Level 1 had the highest accumulation of sediment at 4.36 grams (Figure 26).

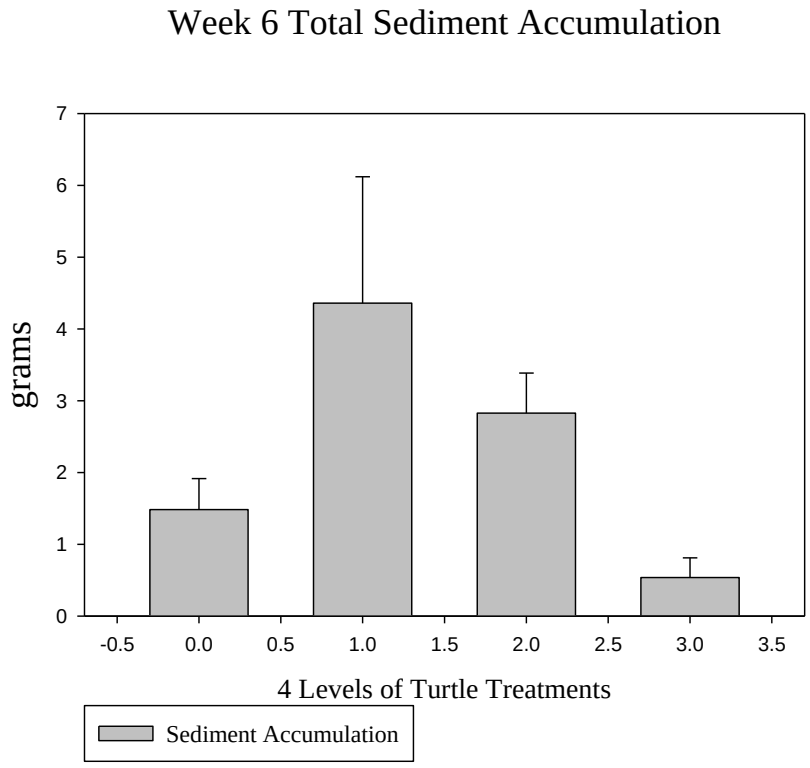

Figure 26. Week 6 Sediment Accumulation. Level 1(turtle present for 2 weeks or less) had the highest accumulation at 4.36 grams. The presence of turtles and week were both significant (Table 4.).

A significant difference was detected in the sediment accumulation among the ponds at week 8 of the study ($F_{3,80} = 5.729$, $P = 0.001$) (Table 4). The mean sediment accumulation in grams at week 8 was 2.61. Level 1 had the highest accumulation of sediment at 4.63 grams (Figure 27).

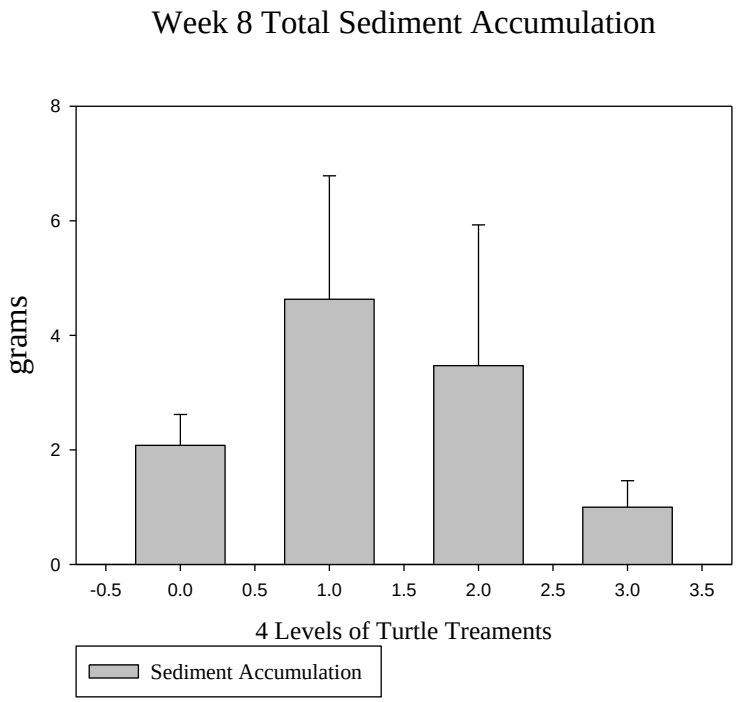

Figure 27. Week 8 Sediment Accumulation. Level 1again had the highest accumulation at 4.63 grams. The presence of turtles and week were both significant (Table 4.).

Table 3. Summary of Multi-factorial MANOVA Results. The table shows the (a) multivariate test on overall effects of turtle treatments and week on ecosystem response variables and (b) univariate tests to determine if the response variables were significant. Invertebrate data is excluded. Turtle and week are both significant in overall model while the interaction is not. (n=96).

(a) Multivariate test	DF	Pillai Trace	P value
Turtle	1	0.790	<0.001
Week	3	1.381	<0.001
Turtle × Week	3	0.303	0.1456

(b) Univariate tests	Turtle F ($df_{1,88}$)	Week F ($df_{3,88}$)	Turtle × Week F ($df_{3,88}$)
pH	70.41***	27.88***	1.27
Conductivity	103.64***	26.75***	0.74
% DO	0.31	2.76*	0.57
Sediment	5.02*	6.35***	0.18

Oak % Loss (Water)	7.67**	36.0***	1.93
Riparian Oak % Loss	0.56	79.12***	0.89
Sycamore % Loss (Water)	4.68*	32.86***	2.67
Riparian Sycamore % Loss	18.45***	53.46***	1.24
Chlorophyll *a*	0.24	1.60	0.18

Probability levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4. Summary of Open Leaf ANCOVA Results. ANCOVA's were used to determine if the open Oak and Sycamore % loss was different between turtle treatment and the covariate time (n= 48).

ANCOVA	Turtle F ($df_{1,45}$)	Week F ($df_{1,45}$)
Open Oak % Loss	0.567	79.12***
Open Sycamore % Loss	18.45***	53.46***

Probability levels: * $p < 0.05$, ** $p < 0.01$, *** $p <0.001$

Table 5. Summary of 2-way MANOVA Results. 2-way MANOVA's were used to examine the length of time turtles were present in the ponds on the response variables: Oak and Sycamore leaf litter breakdown rates and sediment accumulation. Week was detected to be significant in all models; Oak leaf breakdown rate and sediment accumulation were significantly higher in ponds that contained turtles; no interactions were detected.

Response Variable	Turtle F ($df_{3,80}$)	Week F ($df_{3,80}$)	Turtle × Week F ($df_{9,80}$)
Oak Leaf Breakdown Rate	2.894*	30.257***	0.960
Sycamore Leaf Breakdown Rate	1.972	25.899***	1.387
Sediment Accumulation	5.729**	5.945**	0.886

Probability levels: * $p < 0.05$, ** $p <0.01$, *** $p < 0.001$

Table 6. Summary of Invertebrate Results. Both (a) parametric and (b) non-parametric tests were conducted to determine if number of macroinvertebrates found was significant between ponds with and without turtles (n=24).

(a) Parametric	F (df = 1, 22)	P- value
Odonata	0.035	0.854
Ephemeroptera	4.05	0.056*
Hemiptera	4.98	0.036*
(b) Non-Parametric	KW x^2_1	P- value
Total Invertebrates	3.14	0.076

Coleoptera	1.13	0.287
Diptera	1.70	0.192

Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) produced a significant model illustrating certain pond parameters measured were associated with the five orders of macroinvertebrates found and the turtle treatments. 26.4% of the variation was explained by the first two axes in our model (total inertia = 1.4297). The model was significant as determined by a Monte Carlo permutation test (999 permutations) (F = 3.27, P = 0.025*). Axis one explained 15.0 % of the total variation while, axis two explained 11.4% of the total variation. The loadings for the macroinvertebrate on CCA 1 are as follows: -0.629 (Coleoptera), -0.331 (Diptera), 0.488 (Ephemeroptera), -0.819 (Hemiptera) and -0.364 (Odonata). The loadings for the macroinvertebrates on CCA 2 are as follows: 0.631 (Coleoptera), -0.657 (Diptera), 0.0483 (Ephemeroptera), 0.387 (Hemiptera) and 0.446 (Odonata). The CCA bi-plot show the orders Odonata, Hemiptera and Coleoptera clustered around % dissolved oxygen. Instead of associating these orders and % dissolved oxygen, these three orders were not statistically significant in the turtle treatments and therefore should appear to have an inverse relationship to the turtle treatments as shown on the bi-plot. Ephemeroptera appears to be closely related to both Sycamore and Texas Oak leaf litter breakdown rates. This is intuitive because Ephemeroptera are filter feeders that consume a variety of algae and detritus and should help to accelerate leaf litter breakdown rates in the ponds. Finally, Diptera is seems to be associated with sediment accumulation. Distributions of Diptera larvae are directly associated with sediment

composition which is also supported by other studies (Ali et al. 2002, Lobinske et al. 2007).

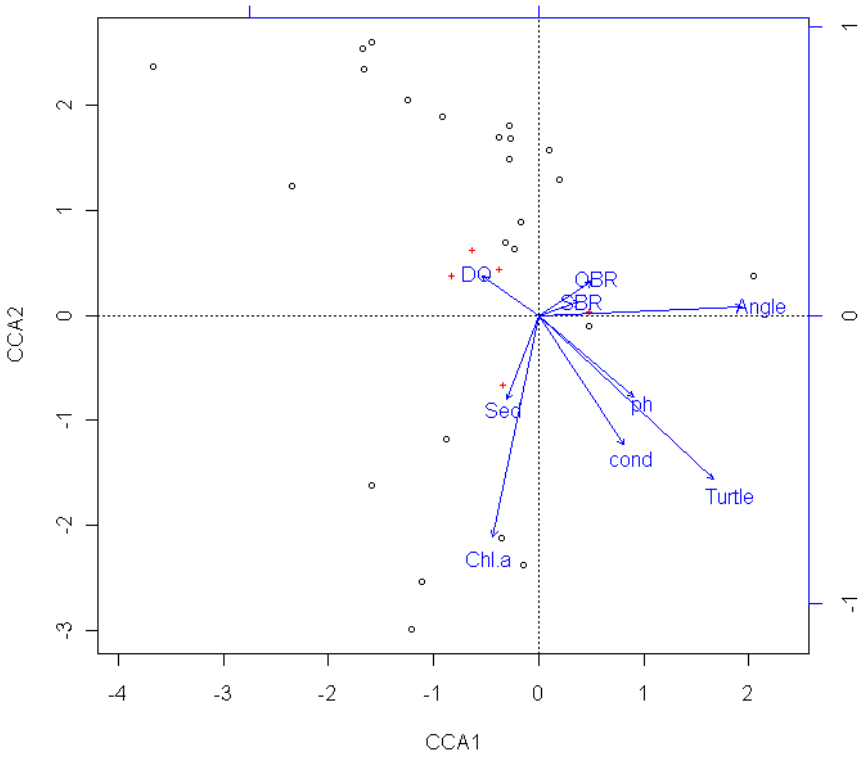

Figure 28. Canonical Correspondence Analysis Bi-Plot. The first two axes explained 26.4% of the total variance from the model. The symbols on the bi-plot stand for: DO = dissolved oxygen, OBR = Oak breakdown rate, SBR = Sycamore breakdown rate, Angle = the slope of the pond, Sed = sediment accumulation, Chl a = chlorophyll *a* production, cond = conductivity (µs/cm), ph = pH and turtle = presence or absence treatments.

Salmonellae Study

All initial body swab sites (claws, posterior fold of the leg or cloacae) were negative for all 12 *T. s. elegans*. All experimental ponds used at the Griffith League Ranch property tested negative for salmonellae for both the water and sediment samples that were taken at the start of the study. At week 9, the remaining turtles were removed from their ponds and swabs were again taken from the three body sites. All swabs from

the turtles' body sites tested negative for salmonellae. In addition, all water and sediment samples collected from the 24 ponds again tested negative for salmonellae.

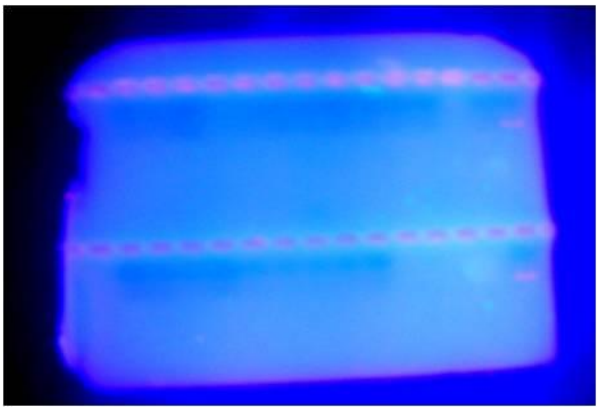

Figure 29. 2% Agarose Gel. The gels shows all negative results for water samples from ponds 1-12 collected at the end of the study and two positive controls for the bacteria, salmonellae.

CHAPTER V

DISCUSSION

In this study, the aim was to determine if and how a common freshwater turtle (*T .s. elegans*) influences pond abiotic conditions and biotic processes, in terms of water chemistry, sediment accumulation, leaf litter breakdown rate, production of chlorophyll *a*, invertebrate assemblage and the bacteria, salmonellae in experimental pond systems. We found the values of pH and conductivity to be significantly higher in ponds that had contained a turtle. In addition the closed leaf packs placed in the water had a significantly higher percent loss and sediment accumulation was higher in ponds that contained a turtle. No significant differences were detected in the production of chlorophyll *a* or the total number of macroinvertebrates found. The significant differences we did find indicate freshwater turtles may act as a driver for pond ecosystems due to their daily activities which increase nutrients indirectly by resuspension of organic matter in the water column and their direct input of nutrients through excretion of feces

Leaf litter breakdown was higher in turtle ponds for both leaf species in the closed packs placed in the water. A study conducted by Cross et al. (2006) found increased levels of nitrogen and phosphorus released into North Carolina headwater streams increased the decomposition rate of benthic leaf litter. The turtles would have increased nutrient levels through excretion of feces and sediment dispersal and in turn increase the leaf litter decomposition. However, it should be noted that both leaf species

only differed by 3% in the percent loss between the ponds that contained turtles and those that did not. No significant difference was detected in either the Texas Oak leaf packs in the riparian area or the open Texas Oak leaves that were placed in the water. A higher leaf litter breakdown rate was found for the Sycamore open leaves in ponds that contained a turtle. Again, there was no significant difference in the rate of leaf decomposition as ponds with turtles only had a 3% higher loss than ponds that did not contain a turtle. The Sycamore riparian packs had a higher loss rate in treatments that did not contain a turtle. The varying results from all packs and open leaves did not produce a clear pattern of how the presence of *T. s. elegans* affected leaf litter breakdown rate. This type of study needs to be repeated to better determine the relationship between freshwater turtles and leaf breakdown rates.

The total number of invertebrates collected from each pond did not statistically differ between treatments. However, the difference in the raw numbers accumulated from the different treatment ponds is still important to be considered which 2,784 were in ponds that contained a turtle as compared to 829 invertebrates found in ponds that did not contain turtles. Odonates did not differ between the two treatments while Diptera, Hemiptera, and Ephemeroptera were significantly higher in ponds that contained turtles. Hemiptera and Ephemeroptera were statistically higher in turtle ponds and even though Diptera was not; 724 Diptera larvae were found in ponds that contained turtles and only 165 were found in ponds that did not contain turtles. Chironomidae and Baetidae were the most numerous invertebrates found. The Chironomidae, which are often the most abundant organism in both number and biomass, can be especially significant in ecosystem functioning (Merritt et al. 1998). Ephemeroptera and Baetidae nymphs are

mostly collector or scrapers and feed on a variety of detritus and algae, and some macrophyte and animal material (Merritt et al. 1998). In our experimental pond system, Hemiptera are basically predators which would feed off the Ephemeroptera and Diptera in the ponds fueling the pond food web. Cross et al. (2006) also found the nitrogen and phosphorus enrichment treatment had a significant positive effect on total invertebrate density and biomass in a mixed substrate habitat. The presence of turtles in the experimental ponds would have increased the amount of nutrients present in the ponds from the excretion of feces, dispersing sediment and increase the production of biofilms and in turn given the invertebrates a preference for the turtle ponds. Evidence showing that the number of invertebrates was higher in ponds that contained turtles, illustrates *T. s. elegans* can act as a driver for pond food webs. In contrast to our study, Perrson and Svensson (2006) found the presence of the bethivorous fish to significantly reduce the density and community composition of the benthic invertebrates. The reduction in numbers was attributed to direct predation upon the invertebrates.

Sediment accumulation was higher in ponds that contained turtles which was expected. Schindler et al. (1996) hypothesized that benthivorous fish translocate nutrients from the sediment to the water by their feeding activities and by excreting nutrients derived from the benthic habitat into the water. I believe we can assume freshwater turtles, as a large animal with high biomass, through their movement and foraging influences aquatic systems, which is the same as hypothesized by Schindler et al. (1996). As the turtles moved throughout each pond, activities such as coming up to the surface to bask, searching for food or burying within the substrate, sediment can be stirred up, resuspended in the water column and then resettles at the benthos. The resuspension of

sediment in the ponds impacted several aspects of our study such as changes in water chemistry, leaf litter breakdown rates and invertebrate community. Perrson and Svensson (2006) conducted a similar study to ours in order to determine how benthivorous fish alter ecosystem functioning in pond ecosystems. They detected significantly different nutrient levels of nitrogen, ammonium and phosphorus levels in treatments that contained a benthivorous fish as opposed to treatments that did not contain a fish. Persson and Svensson (2006) concluded the higher concentrations were the result of direct effects of the benthivorous fish, such as excretion of nutrients with benthic origin of resuspension of sediment.

Significant differences were found in the water chemistry aspects in which pH and conductivity averaged higher in ponds containing turtles, while no difference in dissolved oxygen was detected between pond treatments. The combined factors of the turtles, decreasing water level and higher temperatures as the experiment progressed contributed to the water chemistry changing. pH could be affected by a higher rate of photosynthesis as the aquatic plants in the ponds grew throughout the summer. In addition, the water level dropped increasing the alkalinity of the ponds. Higher conductivity in the turtle ponds is attributed to the turtle's activities resuspending sediment in the water column and the decrease in pond water level throughout the study. This is also supported by conductivity increasing in all ponds as the summer progressed and the water level dropped. None of the turtles caught for this experiment were carrying salmonellae externally or at the cloacae. This is surprising because approximately 50% of wild freshwater turtles carry salmonellae at one of the three sites (Hahn et al. 2007, Gaertner et al. 2008). Specifically, Gaertner et al. (2008) trapped 21 wild red-eared

sliders and 38% tested positive for salmonellae at both their carapace and cloacae; in addition two more red-eared sliders tested positive only on their carapace. Here, 12 red-eared sliders were trapped, predicting at least four turtles to be carrying salmonellae, but none were positive for the infection. One reason for this phenomenon may be a lack of rainfall prior to the time the turtles were trapped. Gaertner et al. (2009) conducted a study to determine if salmonellae may inoculate water or sediment samples after a heavy precipitation event. They found all water samples from the 9 sites tested at Spring Lake positive for salmonellae at least one of the 4 times they sampled. This demonstrates salmonellae that is harbored in the feces of terrestrial animals may be washed into aquatic systems and inoculate bodies of water with salmonellae. Two months prior to the trapping of the turtles used for this study no large precipitation event occurred. March and April 2010 only produced three precipitation days that had above 0.50 inches of rain at one time and one day where rainfall accumulation was above one inch in Hays and Bastrop Counties (NOAA accessed 15 March 2011). The three turtles that survived the entire experiment also tested negative at both body sites. Again, May, June, July and August 2010 only had four days when precipitation during that day was above 1 inch (NOAA accessed 15 March 2011). All pond water and sediment tested negative for salmonellae at the beginning and end of the experiment suggesting no salmonellae bacteria was present initially in the system or was washed into the pond system because no large precipitation event occurred. PCR analysis also indicated no salmonellae were present on any turtle, so the water and sediment samples would be expected to remain negative throughout the study. This study does support that aquatic systems are being

inoculated with salmonellae after a large precipitation event where the bacteria is flushed into the water from terrestrial systems.

Turtles are sometimes seen as a nuisance in pond and lake management when fisheries are the main goal for management. This is because it is well known and has been that slider turtles are omnivorous and may eat small fish or fish eggs as a part of their diet (Cagle 1950, Gibbons 1990). However, it is only part of their diet and the amount of consumption is not enough to truly have a negative effect on fish populations and in fact may help fish populations by removing smaller or older fish. In Texas, overall numbers of freshwater turtles are down due to commercial harvest (Brown et al. 2011). The removal of freshwater turtles from aquatic systems would remove one source of nutrient input that helps to drive aquatic systems. Our results indicate that freshwater turtles such as *T. s. elegans* can actually help to drive pond ecosystems by increasing nutrient input and in turn increase the net biomass of invertebrates. This concept could potentially be extrapolated to fishery dependent regions where the ponds are so used nutrient inputs are low and the presence of turtles could help drive the pond system to produce more fish. Ecosystem functioning is not something current fishery managers consider but as science progresses and complex interactions are better understood these connections are pertinent.

This study should be repeated if there is any chance in the future and efforts to prevent predation upon the turtles should be considered. I believe we found certain patterns that were expected such as the altered water chemistry, sediment accumulation increase and increased leaf litter breakdown rates. An additional another study with predation prevention would probably reveal stronger effects from the turtle treatments.

In conclusion, this study indicated that the effects we did find support that the freshwater turtle, *T. s. elegans*, can act as a driver for pond ecosystems by increasing nutrient input directly through excretion of feces and by increased sediment dispersal in the water column. A reduced number turtles in pond systems could lower the productivity in terms of the plant and invertebrate community and limit ecosystem functioning processes. Overall population sizes of freshwater turtles are down in South Texas due to commercial harvest and habitat loss (Brown et al. 2011). Loss of freshwater turtles in pond ecosystems may affect the productivity due to the decreased amounts of nutrients provided by the turtles directly and through their activities in ponds. Thus, this study suggests that freshwater turtles can influence pond ecosystem functioning and pond food webs by increasing resource availability for invertebrate communities.

LITERATURE CITED

Ali, A., J. Frouz and R.J. Lobinske. 2002. Spatio-temporal Effects of Selected Physio-chemical Variables of Water, Algae and Sediment Chemistry on the Larval Community of Nuisance Chironomidae (Diptera) in a Natural and a Man-Made Lake in Central Florida. Hydrobiologia 470: 181-193.

Aresco, M.J. and F.C. James. 2005. Ecological Relationships of Turtles in Northern Florida Lakes: A Study of Omnivory and the Structure of a Lake Food Web. Florida Fish and Wildlife Conservation Commission Report.

Bouchard, S S. and K.A. Bjorndal. 2006. Non-additive Interactions between Animal and Plant Diet Items in an Omnivorous Freshwater Turtle *Trachemys scripta*. Comparative Biochemistry and Physiology 144: 77-85.

____ and K.A. Bjorndal. 2005. Ontogenetic Diet Shifts and Digestive Constraints in the Omnivorous Freshwater Turtle *Trachemys scripta*. Physiological and Biochemical Zoology 79: 150-158.

Brenner, M., M.W. Binford and E.S. Deevey. 1991. Lakes. Pages 364-391 in R.L. Meyers and J.J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, Orlando, Florida, USA.

Brenner, D., G.A. Lewbart, M. Stebbins, and D.W. Herman. 2002. Health Survey of Wild and Captive Bog Turtles (*Clemmys muhlenbergii*) in North Carolina and Virginia. Journal of Zoo and Wildlife Medicine 33:311-316.

Brown, D.J., V.R. Farallo, J.R. Dixon, J.T. Baccus, T.R. Simpson and M.R.J. Forstner. 2011. Freshwater Turtle Conservation in Texas: Harvest Effects and Efficacy of the Current Management Regime. Journal of Wildlife Management In Press.

Bury, R.B. 1986. Feeding Ecology of the Turtle, Clemmys marmorata. Journal of Herpetology 20:515-521.

Cagle, F.R. 1950. The Life History of the Slider Turtle, *Psuedemys scripta troostii* (Holbrook). Ecological Monographs 20: 31-54.

Chambers, D.L. and A.C. Hulse. 2006. *Salmonella* Serovars in the Herpetofauna of Indiana County, Pennsylvania. Applied and Environmental Microbiology 3771-3773.

Chapin, F.S., B.H. Walker, R.J. Hobbs, D.U. Hooper, J.H. Lawton, O.E. Sala, and D. Tilman. 1997. Biotic Control Over the Functioning of Ecosystems. Science 277: 500-503.

Christensen, N.L., et al. 1996. The Report of the Ecological Society of America Committee on the Scientific Basis for Ecosystem Management. Ecological Applications 6: 665-691.

Clark, D.B. and J.W. Gibbons. 1969. Dietary Shift in the Turtle *Pseudemys scripta* (Schoepff) from Youth to Maturity. Copeia 1969: 704-706.

Congdon, J., L. Greene, and J.W. Gibbons. 1986. Biomass of Freshwater Turtles: A Geographic Comparison. American Midland Naturalist 115: 165-173.

Cross, W.F., J.B. Wallace, A.D. Rosemond and S.L. Eggert. 2006. Whole-System Nutrient Enrichment Increases Secondary Production in a Detritus-Based Ecosystem. Ecology 87: 1556-1565.

Duffy, J.E. 2002. Biodiversity and Ecosystem Function: The Consumer Connection. Oikos 99: 201-219.

Gaertner, J.P., T. Garres, J.C. Becker, M.L. Jimenez, M.J. Forstner and D. Hahn. 2008. Temporal Analyses of Salmonellae in a Headwater Spring Ecosystem Reveals the Effects of Precipitation and Runoff Events. Journal of Water and Health 7.1: 115-121.

_____, D. Hahn, J. Jackson, M.J. Forstner and F.L. Rose. 2008. Detection of Salmonellae in Captive and Free-Ranging Turtles Using Enrichment Culture and Polymerase Chain Reaction. Journal of Herpetology 42: 223-231.

_____, D. Hahn, F.L. Rose, and M.J. Forstner. 2008. Detection of Salmonellae in Different Turtle Species within a Headwater Spring Ecosystem. Journal of Wildlife Diseases 44: 519-526.

Gibbons, J.W. 1990. Life History and Ecology of the Slider Turtle. Smithsonian Institution, Washington, DC, U.S.A.

Grosmaire, E.K. 1977. Aspects of the Natural History of Freshwater Turtles within the Lower Rio Grande Valley of Texas. Thesis, Texas A&M University, College Station, Texas, USA.

Hahn, D., J.P. Gaertner, M.J. Forstner, and F.L. Rose. 2007. High Resolution Analysis of Salmonellae from Turtles within a Headwater Spring Ecosystem. FEMS Microbial Ecology 60: 148-155.

Hart, D.R. 1983. Dietary and Habitat Shift with Size of Red-eared Turtles (*Pseudemys scripta*) in a Southern Louisiana Population. Herpetologica 39: 285-290.

Hooper, D.U., F.S. Chapin, III, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H. Lawton, D.M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer and D.A. Wardle et al. 2005. Effects of Biodiversity of Ecosystem Functioning: A Consensus of Current Knowledge. Ecological Monographs 75: 3-35.

Iverson, J.B. 1982. Biomass in Turtle Populations: A Neglected Subject. Oecologia 55: 69-76.

Kitchell, J.F., R.V. O'Neill, D. Webb, G.W. Gallepp, S.M. Bartell, J.F. Koonce and B.S. Ausmus. 1979. Consumer Regulation of Nutrient Cycling. Bioscience 29: 28-34.

Khan, A.A., M.S. Navaz, S.A. Khan and C.E. Cerniglia 2000. Detection of Multidrug-resistant *Salmonella typhimurium* DT104 by Multiplex Polymerase Chain Reaction. FEMS Microbiology. Letter 182:355-360.

Leibold, M.A., M. Holyoak, N. Mouquet, P. Amarasekare, J.M. Chase, M.F. Hoopes, R.D. Holt, J.B. Lock, J.T. 1993. Management of Recreational Fish Ponds in Texas. Texas Agricultural Extension Service. www.aces.edu/dept/fisheries/rec_fishing/pdf/**texas**fish**ponds**.pdf. Accessed 29 August 2010.

Lobinske, R.J., A. Ali, R.J. Leckel Jr. and J. Frouz. 2007. Influence of Selected Sediment Physical Parameters of Spatial Distribution in Three Central Florida Lakes. The Florida Entomologist 90: 593-604.

Malorny, B., J. Hoorfar, C. Bunge and R. Helmuth. 2003. Multicenter Validation of the Analytical Accuracy of *Salmonella* PCR: Towards and International Standard. Applied and Environment Microbiology 69: 290-296.

Martinez-Urtaza, J., M. Saco, J. Novoa, P. Perez-Pineiro, J. Peiteado, A. Lozano-Leon and O. Garcia-Martin. 2004. Influence of Environmental Factors and Human Activity on the Presence of *Salmonella* Serovars in a Marine Environment. Applied and Environmental Microbiology 70: 2089-2097.

McCann, K. and A. Hastings. 1997. Re-evaluating the Omnivory-Stability Relationship in Food Webs. Proceedings: Biological Sciences 264: 1249-1254.

Merritt, R.W.ed., K.W. Cummins, ed., and M.B. Berg, ed. An Introduction to the Aquatic Insects of North America. 4th ed. Dubuque: Kendall-Hunt, 1998.

National Oceanic and Atmospheric Administration. 2011. United States Government. 15 March 2011. www.noaa.gov.

Perrson, A. and J.M. Svensson. 2006. Effects of Benthivorous Fish on Biogeochemical Processes in Lake Sediments. Freshwater Biology 51: 1298-1309.

Pimm, S.L. 1982. Food Webs. Chapman and Hall, London, U.K.

____, and J.H. Lawton. 1977. Number of Trophic Levels in Ecological Communities. Nature 268: 329-331.

Polis, G.A., W.B. Anderson, and R.D. Holt. 1997. Toward an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs. Annual Review of Ecology and Systematics 28: 289-316.

_____ and D.R. Strong. 1996. Food Web Complexity and Community Dynamics. American Naturalist 147: 813-846.

Rahn K. et al. 1992. Amplification of an *invA* Gene Sequence of *Salmonella typhimurium* by Polymerase Chain Reaction as a Specific Method of Detection of *Salmonella*. Molecular and Cellular Probes 6: 271-279.

R Development Core Team. 2005. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing, www.r-project.org.

Sambrook J., E.F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

Schindler D.E., S.R. Carpenter, K.L. Cottingham, X. He, J.R. Hodgson, J.F. Kitchell and P.A. Soranno. 1996. Food Web Structure and Littoral Zone Coupling to Pelagic Trophic Cascades. In: Food Webs: Integration of Pattern and Dynamics. Eds. G.A. Polis and K.O. Winemiller, p. 96-105. Chapman & Hall, New York.

SPSS for Windows, Rel. 11.0.1. 2001. Chicago: SPSS Inc.

Suárez M. and H. Rüssmann. 1998. Molecular Mechanisms of *Salmonella* Invasion: The Type III Secretion System of the Pathogenicity Island 1. International Microbiology 1: 197-204.

Texas Parks and Wildlife Department. 1992. Plant communities of Texas (Series level): February 1992. Austin, TX: Texas Parks and Wildlife Department, Texas Natural Heritage Program.

Tucker, J.K, G.L. Paukstis and F.J. Janzen. 1998. Annual and Local Variation in Reproduction in the Red-eared Slider, *Trachemys scripta elegans*. Journal of Herpetology 32: 515-526.

Vassiliadis, P., V. Kalapothaki, D. Trichopoulos, C. Mavrommatti and C. Serie. 1981. Improved Isolation of Salmonellae from Naturally Contaminated Meat Products by using Rappaport-Vassiliadis Enrichment Broth. Applied and Environmental Microbiology 42: 615-618.

Vaughn, C.C. Biodiversity Losses and Ecosystem Function in Freshwaters: Emerging Conclusions and Research Directions. 2010. BioScience 60: 25-35.

Widmer, F., B.T. Shaffer, L.A. Porteous and R.J. Seidler. 1999. Analysis of *nif*H Gene Pool Complexity in Soil and Litter at a Douglas Fir Forest Site in the Oregon Cascade Mountain Range. Applied and Environmental Microbiology 65: 374-380.

Winfield, M.D. and E.A. Groisman. 2003. Role of Non-host Environments in the Lifestyles of *Salmonella* and *Escherichia coli*. Applied and Environmental Microbiology 69: 3687-3694.

VITA

Megan Kroeger Lindsay was born to Jim and Janie Kroeger in Chattanooga, Tennessee. Megan earned her Bachelor's degree in Environmental Studies from Brevard College in Brevard, North Carolina as a Cum Laude graduate in May 2005. In 2007, Megan received an internship opportunity at Bon Secour National Wildlife Refuge in Gulf Shores, Alabama. This experience propelled Megan to pursue her Master's degree in order to obtain a position as a federal biologist. Therefore, Megan enrolled in Texas State University, San Marcos in the fall of 2009 and works in the Stream Ecology Lab under Dr. Zhang. Permanent address: 221 Bunny Trail Kyle, Texas 78640. Email: meg16p@hotmail.com.