NUTRIENT DYNAMICS AND ECOLOGICAL ROLES OFFRESHWATER TURTLES

by

SEAN CHRISTOPHER STERRETT

(Under the Direction of John C. Maerz)

ABSTRACT

Nutrient cycling is necessary for the support of ecosystem processes and services. Animals are influential in the storage and transformation of critical nutrients, yet many populations are impacted by a suite of human related activities, including unsustainable harvest. Turtles are a globally imperiled taxa with half of all known species threatened, yet little is known about their influences on ecosystems. Turtles are unique in morphology, physiology, life history and ecology, which suggest that they impact ecosystems in unconventional ways. This dissertation explores the ecological stoichiometry, nutrient dynamics and ecological roles of freshwater turtles using field-collected and experimental research. Research was conducted on four common North American species in three rivers and one collection of ponds in the southeastern USA. The nutrient content of 33 individuals across four focal species indicated that a turtle's skeleton composed 27.5% of total fresh mass and the shell alone composed 93% of turtle skeletal mass. Due to the high Phosphorus (P) content of bone, whole body nutrient content of turtles is the most extreme of any measured organism (%Nitrogen (N):P =1.04). Because bone has such a slow turnover time, this research suggests that adult turtles are in low demand of P, such that their recycling is proportional to their biomass. These results challenge conventional thinking in ecological stoichiometry on nutrient limitation. Turtle standing crop biomass and

nutrients are comparable with estimates of other aquatic taxa, but higher in P per unit of biomass. Further, mass-specific excretion rates of N and P were similar or exceeded estimates of salamanders and fishes. Results from a mesososm experiment suggest that carnivorous juvenile turtles reduce detritivorous macroinvertebrates, thereby reducing invertebrate feeding on nutrient rich leaves. Therefore, juvenile turtles indirectly shift leaf litter nutrient content. These results highlight the complexity of direct and indirect consumer effects on ecosystem processes. Based on our field and experimental results, we would expect juvenile and adult turtles to have contrasting effects on top down and bottom up effects in aquatic ecosystems. Turtles can occur in high abundance in many freshwater ecosystems, making their conservation potentially important in the storage and recycling of nutrients.

INDEX WORDS: Bony Skeleton, Carbon, Chelonian, Consumer-mediated Nutrient Recycling, Detritus, Ecological Stoichiometry, Freshwater ecosystem, Mesocosm, Nitrogen,Phosphorus, Reptile, Trophic Cascade

NUTRIENT DYNAMICS AND ECOLOGICAL ROLES OF FRESHWATER TURTLES

by

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BS, Butler University, 2003

MSc, University of Georgia, 2009

A DissertationSubmitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

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May2014

DEDICATION

This work is dedicated to my grandmother, Kaye Maxwell (Mama K), who never feared asking me questions about what I do. I always appreciated her curiosity and thrill in her eyes when I told her about wrangling turtles. The lessons in those conversations were as great as any I have learned.

ACKNOWLEDGEMENTS

I would like to thank my committee for being an inspirational group of curious and kind scientists. Robert and Nate - thank you dearly for talking me out of the ruts I put myself in.

I am indebted to John Maerz, who has been committed to my personal and professional development since I began my work at the University of Georgia. I decided to stay in John's lab after finishing my master's degree because I knew I had plenty more to learn from him. I hope I had the chance to teach him something about turtle ecology.

The support of my family has made this journey manageable and enjoyable. Thank you Mom, Dad, Celia, Mama K, Lisa, Jim, Katie, Ian, Uncle Ross, John, Judy, Harold, Beverly, Candace, David.Also, thank you to the Liebrich Family for all the opportunities I had to play in the woods when I was a kid.

Many colleagues have helped me with the logistics of completing this work. Thanks to Sonia Hernandez, Bob Bahn, Tom Maddox, Emmy Deng, Ben Atkinson, Peter Pritchard, Max Nickerson, John Jensen, Andrew Mehring, Clint Oakley, Rachel Katz, Vanessa K. Terrell and Cassandra Skaggs. Joe Milanovich was instrumental in helping develop methods for studying the ecological stoichiometry of turtles and I'm very appreciative of the hours we spent on the phone.

My friends have provided enormous support through the years and have put up with my antics. I would especially like to thank Christina Baker,Kyle Barrett, Kristen Cecala, Brian Crawford, Jayna Devore, Katherine Edmonds, Alexa Fritzsche, Sean Graham, Lincoln Larson, Anna McKee,Mia Malloy, Ryan Malloy, Kyle McKay, Joe Milanovich, Drew Neighbors,Julie

Rushmore,Marcia Snyder, Dave Steen and Bill Sutton. Thank you all for the ridiculous times we had and will have.

Athens, Georgia has become one of my favorite places in the world. I need to thank Jittery Joes, Two Story Coffee Shop, The Grit, Barberitos, Terrapin Brewery (even though they would not budge on offering money for my turtle research), Five Points Bottle Shop and Village Package Store for keeping me fueled.

Rachel Katz has been my most fortunate discovery. Thank you for being a remarkable counterpart, colleague and friend. I'm excited to see the world and figure out how it works by your side.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Historically, the field of terrestrial and freshwater biogeochemistry minimized the importance of higher-level consumers in nutrient dynamics. Researchers tended to focus on primary production, and the importance of large detrital, soil, and microbial pools of energy and nutrients (Schlesinger 1997). However, early research into the unique influences of animals on nutrient dynamics (Kitchell et al. 1975, Kitchell et al. 1979, Burton and Likens 1975) is recently and rapidly expanding (Vanni 2002, McIntyre & Flecker 2010, Schmitz et al. 2010, Vanni et al. 2013). Animals can uniquely affect spatial dynamics through the transport of nutrients across system boundaries (Polis et al. 2004, Roe et al. 2010), and temporal dynamics through the capture, retention and recycling of nutrients in high trophic levels (Small et al. 2009). Recent research suggests that vertebrates, especially those species that achieve large body sizes, have greater mobility, greater longevity, and the capacity to regulate the abundance of smaller, lower trophic level species may have unique effects on ecosystem processes (Kitchell et al. 1979, Vanni 2002, Schmitz 2010). Vertebrates are predicted to stabilize ecosystems by maintaining large biotic pools of limiting nutrients (Kitchell et al. 1979, DeAngelis 1989, Vanni et al. 2013), and in flowing systems, long-lived consumers are hypothesized to slow the spiraling of nutrients downstream (Webster & Patten 1979, Small et al. 2009). In lentic systems, vertebrates are predicted to contribute to internal nutrient processing and

reduce sedimentation of nutrients, which increases their availability to other biota (Essington & Carpenter 2000).

This dissertation focuses on the effects of freshwater turtles on nutrient dynamics and decomposition within freshwater ecosystems. Turtles are common constituents of all temperate and tropical freshwater ecosystems (Vitt & Caldwell 2009), and are morphologically and ecologically unique among vertebrates. Nonetheless, we know almost nothing about the importance of turtles to nutrient cycling in freshwater ecosystems. Thus, my dissertation represents the initial attempt to quantify turtle effects on freshwater ecosystem processes. It is my contention that the unique skeleton and life history of turtles, in conjunction with their high potential biomass, will result in unique and strong effects on freshwater nutrient dynamics and decomposition. My work relies heavily on ecological stoichiometry theory (EST), which proposes that elemental demands of consumer tissues besides carbon (C) can limit consumer growth, and that tissues demands and metabolic rates will determine the rates and ratios with which consumers store and recycle nutrients. I hypothesize that the extensive skeleton of turtles creates a high growth demand for phosphorus (P), and that the high biomass of turtles and slow turnover of bone makes turtles important in nutrient storage and recycling. I also hypothesize that turtles alter decomposition rates directly through the recycling of nutrients and indirectly through consumption of aquatic invertebrates. Below I briefly review ecological stoichiometry and our current understanding of vertebrate effects on consumer-mediated nutrient recycling (CMNR) and decomposition to place the chapters of this dissertation in context.

Stoichiometry Theory of Ecology

Ecological stoichiometry (ES) has provided a theoretical basis for studying the roles that organisms play in ecological interactions from biogeochemistry, ecological and evolutionary perspective (Reiners 1986, Sterner & Elser 2002). ES is rooted in the law of conservation of mass and biological homeostasis, and focuses on the relative roles of nutrient ratios that limit biological dynamics (i.e. Liebig's Law of the Minimum). It also focuses on multiple elemental nutrients as currency, as opposed to the single currency of energy, which has been the focus of trophic ecology historically (Lindeman 1942, Sterner & Elser 2002, Hessen et al. 2013). All organisms require some level of homeostatic regulation because all living organisms are made of the same molecular building blocks, which require approximately twenty essential elemental nutrients (referred to as the stoichiometric invariance concept by Allen & Gilooly 2009, Hessen et al. 2013). However, plants are relatively non-homeostatic and have the ability to uptake nutrient proportionally to their availability. In contrast, animals are generally "not what they eat" and, instead, regulate their body nutrient content at some level (Elser 2000, Sterner & Elser 2002). For animals specifically, ES has focused on understanding the trophic consequences of regulating body nutrient content and has led to CMNR. The nutrients that animals do not use for maintenance, growth, or reproduction are recycled back into their habitats as waste that is usable by other parts of food webs (Sterner and Elser 2002). CMNR has become the center of study for various taxa leading to a progression of reviews (Kitchell et al. 1979, Vanni 2002, Sterner & Elser 2002).

Early work in pelagic food webs highlighted the variations in body stoichiometry (C:N:P) of zooplankton driven by different levels of phosphorus (Sterner & Hessen

1994). These findings led to the Growth Rate Hypothesis (GRH), which proposed evolutionary relationships between growth rate, body P content and RNA content in organisms, such that an organism with higher P content, presumably from higher amounts of RNA, would have a higher growth rate (Elser et al. 1996). The GRH has been tested with a wide range of taxa, yet it is most applicable to small organisms, which have lower relative structural complexity (Hessen et al. 2013).

For vertebrates, mechanical structures (e.g. scales, bony skeleton, horns) represent a significant portion of body mass and greatly influence their whole body elemental nutrient composition (Reiners 1986, Elser et al. 1996, Sterner & Elser 2002). Because bone is made of calcium phosphate, a large proportion of body P is contained within their skeleton. Fish ecologists have demonstrated how the variation in skeletal development influences body stoichiometry (Sterner & George 2000, Hendrixson et al. 2007, McIntyre &Flecker 2010). Further, investments in large structures like bony skeletons can change the way that nutrients are stored (Griffiths 2006, Vanni et al. 2013) and recycled (Vanni et al. 2002, Hood et al. 2005).

Consumer-Mediated Nutrient Recycling

Ecosystems are regulated by the transfer of energy and elemental nutrients between biotic and abiotic compartments (also known as biogeochemistry). This process starts as autotrophic or heterotrophic organisms build biomass by using available nutrients to fix carbon through photosynthesis or decomposition, and those materials are transferred through living and detrital pathways to higher trophic levels (Lindeman 1942, DeAngelis 1992). As primary and secondary consumers build biomass, they excrete remineralized nutrients that recycle into the supply for autotrophs and heterotrophs.

Organic nutrients bound in organismal biomass are also liberated when individuals die, and can be imported or exported across system boundaries through animal movements, as famously illustrated with pacific salmon (*Onchorhynchus* spp.: Ben-David et. al. 1998). Therefore, living organisms influence the uptake, retention, recycling and movement of nutrients from organic to inorganic forms and across system boundaries.

The study of animals with nutrient dynamics is due largely to a gradual evolution of ecological understanding that animals act as "engines" or "pumps" by transferring and transforming energy and nutrients back into ecosystems (Lotka 1925, Lindeman 1942, Reiners 1986, Vanni 1996). Animals as small as microcrustaceans (e.g. *Daphnia* spp.) to large grazing vertebrates have measurable effects on nutrient dynamics (reviewed in Vanni 2002). For example, diel vertical migrations of zooplankton, due to nocturnal feeding and predator avoidance, provides a considerable amount of nutrient transportation to the epilimnion of lakes ecosystems (Kitchell et al. 1979). Similarly, large migratory waterfowl (e.g. ducks, geese) move in dense aggregations and are vectors for horizontal nutrient transportation between wetland roosts (Post et al. 1998). In each of these cases, particular behavior and ecological circumstances contribute to the ways in which animals recycle and transport nutrients within and between ecosystems.

Beyond the intrinsic value of studying nutrient cycling, there are practical reasons for humans to understand how organisms and ecosystems change the flow of nutrients. Nutrient cycling is a critical ecosystem service in both natural and human-dominated landscapes, yet increased population pressure often alters the way nutrients are delivered to, cycled and stored within ecosystems (Daily 1997, Chapin et al. 2000). The global economic value of nutrient cycling ranks among the highest ecosystem services (Daily

1997), and the value of these services translates to economic and cultural values (i.e., clean water, waste assimilation; Wilson & Carpenter 1999). It is now well understood that humans are rapidly altering global nutrient cycles (Vitousek et al. 1997, Falkowski et al. 2000, Elser & Bennett 2011). Economic values of freshwater ecosystem services, such as clean drinking water and recreational benefits, are often difficult to assign but are nevertheless directly related to specific freshwater functions (Wilson & Carpenter 1999, Covich et al. 2004). Most ecologists agree loss of biodiversity is a major driver of anthropogenic impacts to ecosystem functions and services (Chapin et al. 2000).

Characteristics of Influential CMNR Species

Common organisms (i.e. widely distributed and abundant) are often the most intensively studied species in ecology, and are often considered fortheir contributions to ecosystem function (Gaston & Fuller 2008, Gaston 2011). In most assemblages, only a few species of any taxonomic group make up the greatest proportion of biomass (Gaston 2010). For instance, in Midwestern reservoirs, the gizzard shad (*Dorosoma cepedianum*) undoubtedly dominate fish biomass and strongly control phytoplankton, planktivorous fishes and even piscivorous fishes (Vanni et al. 2005). Other species have become common, due to invasion, and bring new aspects of nutrient cycling, sometimes to the detriment of a natural ecosystem. For example, the invasive Zebra mussel (*Dresseina polymorpha*) has spread widely across the U.S. since its discovery in the 1980s and has completely altered the biogeochemical cycles of freshwater ecosystems (Strayer 2009). Beyond the direct impacts of *D. polymorpha* (e.g. fouling, competition), it has reduced phytoplankton populations and effectively become a source for P to near toxic levels. Other common species and taxonomic groups are yet to be studied, although common

species often have far reaching impacts on ecosystems due to their high numbers of ecological interactions and are also most likely to be overexploited (Gaston & Fuller 2008, Gaston 2010).

Why Turtles?

Turtles are unique in morphology, physiology, life history and ecology, such that they may add a novel perspective to the discussion on EST. Turtles are the only vertebrate group to have evolutionarily modified their ribs, bringing the pectoral and pelvic girdles backwards resulting in an encased axial skeleton (shell) made of dermal and endochondral bone and covered by keratinized scutes (Gilbert et al. 2008). The turtle shell is an anatomical innovation that can account for up to 27% of a turtle's total wet mass (Iverson 1984, Jackson 2011). While it is thought that the shell primarily evolved to provide protection, it is a metabolically active tissue that has numerous functions in turtle physiology including nutrient storage (Jackson 2011). The high nutrient demand and slow metabolism of the shell is hypothesized to place a significant growth constraint on turtles such that many freshwater turtles undergo an ontogenetic shift from carnivory to a more omnivorous diet that corresponds to a hardening of the shell and decreased growth rate (Clark & Gibbons 1969). Turtles are ectothermic, which contributes to their low metabolic rate, and they exhibit famously negligible adult growth rates and extreme longevity (Gibbons 1987, Jackson 2011, Congdon et al. 2013).

The abundance, widespread distribution, and life-history characteristics of turtle indicate they make major contributions to ecosystem processes (i.e., nutrient cycling). Turtles occur globally in all cool-temperate to tropical environments, including terrestrial, freshwater, marine and, desert ecosystems, where they can naturally achieve remarkable

biomass as high as 877 kg/ha and rivaling many freshwater fish communities (Iverson 1982a, Vitt & Caldwell 2009). Turtles are among the most long-lived and slow-growing vertebrates (Gibbons 1987, Congdon et al. 2013), and in many cases, freshwater turtles live more than 30 years under natural conditions and do not reach sexual maturity for 10 - 15 years (Ernst & Lovich 2009). The temporal scale of turtle life history is vastly different than most freshwater vertebrates and may contribute to unique contributions to ecosystems. Turtle diets are variable within and among species within a community, and some species occupy high trophic positions within aquatic food webs (Aresco & James 2005). Because of these traits, turtles may represent large, stable standing stocks of key nutrients, notably phosphorus, and may alter the availability and stoichiometry of inorganic nutrients through retention and excretion.

Turtles are also among the most globally imperiled vertebrates, largely as a result of overharvesting and habitat loss and degradation (Buhlmann et al. 2009), and their conservation may connect to the management of ecosystem processes. The loss of biodiversity is expected to have a major consequences for freshwater ecosystem function (Dudgeon et al. 2006, Vaughn 2010). Turtles are one of the most threatened groups of vertebrates with 10% of species critically endangered and approximately 50% threatened or in decline (IUCN 2008, TTWG 2010). Freshwater turtles are exposed to many direct and indirect anthropogenic threats including intensive land use and alteration of riparian habitats (Sterrett et al. 2011), road mortality (Steen et al. 2006), overharvest or bycatch (Klemens and Thorbjarnarsen 1995, Grosse et al. 2011), excessive nest predation by mesopredators (Crawford et al. 2014), and disruption of reproductive activity (Moore and Seigel 2006). In addition to population declines, human activities can result in skewed

sex ratios and altered size distributions (Steen et al. 2006, Means 2009). Because body size and diet often varies between sexes, effects on sex ratios and size distributions could have measurable effects on ecosystems processes.

Dissertation Focus

This dissertation represents a first attempt to evaluate the impacts of freshwater turtles on nutrient cycling in freshwater ecosystems (Fig. 1.1). The initial impetus for this research was to fill a knowledge gap for this abundant and diverse taxonomic group, and entertained the notion that understanding their potential role in ecosystem processes might contribute to greater appreciation and improve conservation efforts. However, as this dissertation research developed, the intrinsic and unique characteristics of turtle nutrient dynamics became an increasing motivation and focus. This body of work demonstrates how the unique morphology of turtles also makes them ecologically unique. Specifically, this research shows that turtles are like no other vertebrate group with regards to their body composition and nutrient demands on growth over time. As a result, turtle nutrient dynamics behave in a manner that contradicts existing knowledge and theory but also extend some stoichiometric relationships established for other vertebrate groups. By extending this knowledge, the dissertation then highlights the unique effect of turtles on freshwater nutrient dynamics as reservoirs and recyclers of key nutrients. Finally, this research uses juvenile turtles to examine the complex and compensatory ways in which predators can affect lower trophic levels and ecosystem processes.

Following the introductory chapter, the dissertation is divided into three quantitative chapters and a fifth chapter synthesizing the dissertation and identifying key future directions.

Chapter 2: What can turtles teach us about ecological stoichiometry?provides a descriptive account of the ecological stoichiometry of common freshwater turtles in the Southeastern U.S. and makes direct comparisons with existing knowledge of freshwater fishes. I used field-based excretion experiments, body nutrient analyses and osteological museum specimens to estimate the body stoichiometry and nutrient excretion of freshwater turtles. This information is carried forward to make hypotheses about turtle life history as it pertains to ecological stoichiometry.

Chapter 3: Nutrient dynamics of common freshwater turtles assemblages uses body and excretion stoichiometry data reported in Chapter 2 and mark-recapture data to estimate the composition and biomass, nutrient standing crop, and nutrient recycling of freshwater turtle assemblages in three systems in Georgia. I used mark-recapture methods to estimate turtle densities from ponds and streams sites in Georgia. This study provides the first estimates of excretion stoichiometry for freshwater turtles and highlights the potential for turtles to store and supply nutrients within freshwater ponds and streams.

Finally, ***Chapter 4: Effects of a large consumer on carbon cycling: A mesocosm approach*** used experimental mesocosms to examine the cascading effect of turtle predation and nutrient recycling on leaf litter stoichiometry and decomposition in a simplified detrital food web. This is one of the first studies we are aware of to simultaneously consider the indirect effects of consumption and nutrient recycling via waste on ecosystem processes, and the first that we are aware of to demonstrate evidence of compensatory indirect effects that dampened a predicted trophic cascade.

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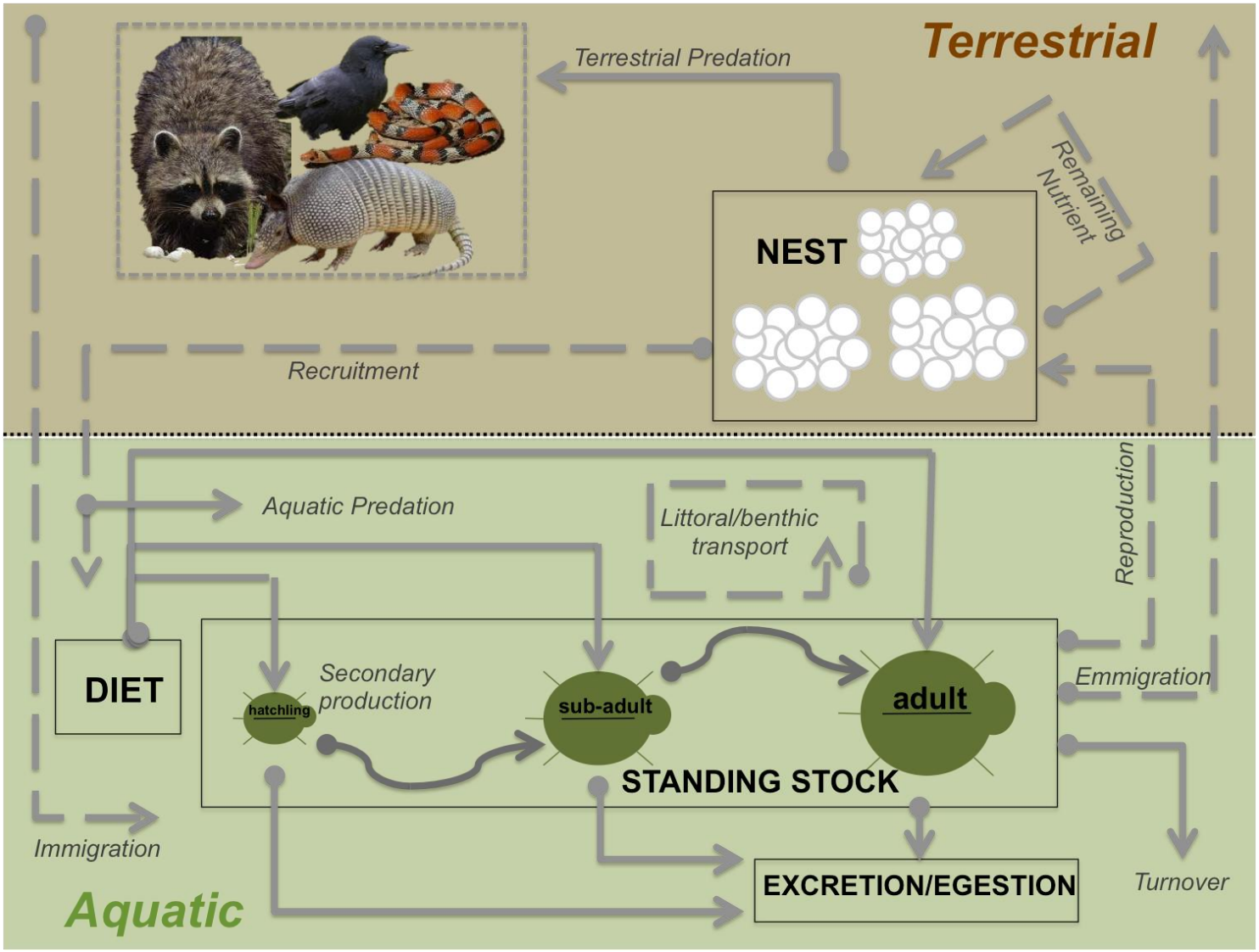

Figure 1.1. Conceptual model of potential nutrient dynamics of freshwater turtles at the interface of aquatic habitats. Boxes represent compartments and fluxes are represented by solid (within ecosystem) and dashed (between ecosystem) lines.

CHAPTER 2

WHAT CAN TURTLES TEACH US ABOUT ECOLOGICAL STOICHIOMETRY THEORY?[1]

[1]Sterrett S.C. and J. C. Maerz *Submitted to *Freshwater Biology*

ABSTRACT

Ecological stoichiometry(EST) is one of several theories proposed to explain variation in organismal growth, life history, and influence on ecosystem processes. EST proposes that an organism's demand for elements other than carbon [energy] drives rates of growth and performance, and that species with high demands for particular nutrients may exert a disproportionate effect on consumer-mediated nutrient recycling (CMNR).To date, EST research is generally limited to invertebrates andfishes.We know very less about the ecological stoichiometry of other vertebrates, including those with reduced (e.g., amphibians) or exaggerated skeletons (e.g., turtles).Turtles are evolutionarily and ecologically distinct among ectothermic vertebrates for their extreme skeletal morphology, delayed maturity, and longevity. Our objective was to describe the ecological stoichiometry of freshwater turtles in relation to ontogeny [size], habitat and diet, and compare patterns with existing knowledge of teleost fishes to determine whether our knowledge of fish stoichiometry is predictive of other ectothermic vertebrates. We measured skeletal investment andestimated body and excretion stoichiometry of four common turtle species representing two families. We also used our results in conjunction with published data on fishes to examine broader relationships between stoichiometry and metabolic rate, adult growth rate, and longevity.Aturtle's skeleton composed 27.5% of total fresh mass and 82% of total dry mass, which is 7.6 times greater than the proportional mass of the skeleton among teleost fishes.The shell composed ~93% of turtle skeletal mass. The concentration of P in turtle bone was an order of magnitude greater than P in soft tissues. As a result,turtle body %N:P was 1.04 (molar N:P =2.3), and 86% of all theP in a turtle resides in theshell. Total body N:P declined and %P increased with increasing turtle mass, consistent with the ontogenetic development of the skeleton. However, we found that N:P content of excreta was

negatively correlated with turtle mass and positively correlated with total body N:P.Consistent with knowledge from fishes, we found that Pconcentration increases ontogenetically with body mass; however, unlike fishes, excreted N:P was negatively correlated with mass and positively correlated with body N:P. Among fishes, increasing %P or lowering N:P would be considered indicative of high P demand. However, our results suggest a lower %P and higher N:P ofjuvenile turtles is indicative of a greater demand for P to build a skeleton. We hypothesize that because turtles show negligible growth after maturity, the slow turnover rate of bone relative to muscle results in low P demand relative to N.As a result and unlike fishes [and invertebrates], turtle effects on CMNR are likely proportionate to their biomass.Turtles are widely distributed and globally imperiled vertebrate taxa. They can occur in high abundance in many freshwater ecosystems, making their conservationpotentially important in the storage and recycling of nutrients.

INTRODUCTION

Severalecological theories have emerged to explain patterns of variation in organism growth, life history and influences on ecosystems (Reiners 1986,Sterner & Elser 2002, Brown et al. 2004). The metabolic theory of ecology (MTE) posits that most of these patterns are best explained by mass-specific energetic constraints on organisms, which can further explain differences in species biomass production and consumer-mediated nutrient recycling(CMNR; Brown et al. 2004). A complement to MTE is ecological stoichiometry theory (EST), whichproposes that patterns of variation in animal growth and CMNR are related to differences in consumer demand or access to elemental nutrients other than carbon[C; energy](Elser et al. 1996, Sterner & Elser 2002). EST assumes that a consumer's demand for certain elements is driven by an imbalance between the consumer's diet and the consumer's need to remain homeostatic with regards to elemental ratios. Consumers that have a high demand for an element relative to its supply may be limited in their growth or excrete other elements disproportionate to elemental ratios. A third set of theories predicts thatbiotic interactions, such as competition and predation, shape life history patterns, growth and the flow of energy and recycling of nutrients(Stearns et al. 2000).Thesetheories are not mutually exclusive. All three theories explainsome of the patterns of variation in animal life histories related to energy and nutrients constraints, which has motivated recent efforts towardsa more unified theory (Allen & Gilooly 2009).

A current constraint on resolving the theories of variation in animal life historiesand CMNR is the limited taxonomic breadth of existing knowledge. Data on a wide array of taxa,includingunicellular organisms, zooplankton, macroinvertebrates, freshwater fishes, birds and mammals are available for testing elements of MTE (Allen & Gilooly 2009). However, data

on a narrower range of taxa are available for exploring aspects of EST and authors have called for exploring a wider taxonomic range (Sterner & Elser 2002). Among consumers, the overwhelming majority ofEST studies focus on zooplankton and macroinvertebrates (Vanni 2002). Among vertebrates, nearly allresearch has been on freshwater fishes, with isolated studies of birds and salamanders (Vanni 2002,Milanovich 2010,Keitzer & Goforth 2013). The reason that stoichiometric data on a wider range of organisms is important to unifyingEST and MTE is related to variation in tissue allocation with increasing mass. MTE is founded on the negative relationshipbetween massandmass-specific energetic demand,while EST is partially founded upon "stoichiometric invariance," which refers to the fact that nutrient concentrations of most tissues are relatively constant across a range of taxa (reviewed by Allen & Gilooly 2009). Stoichiometric invariance means that differences in mass allocation to tissues among taxa should determine variation in nutrient demands and the capacity for nutrients to limit growth. For example, RNA demand increases with growth and mass, leading to the hypothesis that P demand is greater among larger, faster growing taxawith proportionately higher amounts of body P (Sterner& Elser 2002). Support for RNA-based P limitation on growth comes largely from research on microbes and small invertebrates (Elser et al. 2003).However, Allen and Gilooly (2009) note that a significant proportion of P within larger animals is contained in tissues other than ribosomal RNA, and among a wider range of invertebrates, the contribution of RNA to P demand diminishes with increasing mass (Elser et al. 1996, Sterner & Elser 2002,Allen & Gillooly 2009). This suggests that for larger animals, the nutrient demandsand metabolic rates of other tissues will overwhelm P demand of RNA and potentially alter relationships between stoichiometry and mass in explaining life history and CMNR variation (Reiners 1986).

For vertebrates, bone is likely to be the most P demanding tissue driving variation in stoichiometry (Elser et al. 1996). The production of bone among growing individuals should create a high P demand; however, bone has an extremely slow turnover rate relative to other tissues such thatthe maintenance of bone among mature individuals with relatively determinant growth is likely to create little demand for P relative to the consumers P content. Among fishes there is a wide variety of body sizes, growth rates, skeletal investment, and body nutrient content that has allowedresearchers to generate predictions about how vertebrate body stoichiometry are related to growth, life history and CMNR(Tanner et al. 2002, Hendrixson et al. 2007, McIntyre & Flecker 2010). For example, some fish families (e.g. Salmonidae and Cyprinidae) have features such as smooth cycloid scales and modest internal skeletons, resulting in relatively low body P content (Sterner & George 2000,Hendrixson et al. 2007). In contrast, heavily armored catfishes(e.g. Loricariidae or Aspredinidae), whichinvest in bony plates and robust, dorsolaterally-flattened skulls have the highest overall P contentreported for fishes(Vanni et al. 2002, Hoodet al. 2005, Hendrixson et al. 2007). Knowledge of how body stoichiometry varies across fish taxa has led to a greater understanding of fish life history evolution and effects on nutrient recycling (Tanner et al. 2000, McIntyre & Flecker 2010).For example, as predicted by EST, differences in skeletal investment among species or age classes results in corresponding differences in body Nitrogen (N):P ratios, which inversely affect the N:P ratio of excreta. That is, individuals with more bone have lower body N:P and excrete higher N:P due to sequestration of P for maintenance and growth.The question remains whether the stoichiometric relationships established for fishes can be generalized to other vertebrates, particularly those that exhibit limited skeletal development (e.g. amphibians) or species with extreme skeletal investment (e.g. turtles).

Turtles are unique in morphology, physiology, life history and ecology, such that they may add a novel perspective to the discussion on EST. Turtlesare the only vertebrate group to have evolutionarily modified their ribs, bringing the pectoral and pelvic girdles forward resulting in an encased axial skeleton (shell) made of dermal and endochondral bone and covered by keratinized scutes (Gilbert et al. 2008).The turtle shell is an anatomical innovation that can account for up to 27% of a turtle's total wet mass (Iverson 1984, Jackson 2011). While it is thought that the shell primarily evolved to provide protection, it is a metabolically active tissue that has numerous functions in turtle physiology including nutrient storage (Jackson 2011). The high nutrient demand and slow metabolism of the shell is hypothesized to place a significant growth constraint on turtles such that many freshwater turtles undergo an ontogenetic shift from carnivory to a more omnivorous diet that corresponds to a hardening of the shell and decreased growth rate (Clark and Gibbons 1969). Turtles are ectothermic, whichcontributes to their low metabolic rate, and they exhibit famously negligibleadult growth rates and extreme longevity (Gibbons 1987,Congdon et al. 2003,Jackson 2011, Congdon et al. 2013). Turtles occur globally in all cool-temperate to tropical environments, including terrestrial, freshwater, marine and, desert ecosystems, and can achieve remarkable biomass in freshwater ecosystems (Iverson 1982a, Vitt & Caldwell 2009). However, our understanding of their contributions to ecosystem nutrient dynamics is depauperate.We predict that turtles are potentially unique in their contributions to CMNR of freshwater ecosystems, and because turtles are also a globally imperiled taxa, theirconservation may be important for sustaining ecosystem processes.

The objectives of this paper are to A) describe the ecological stoichiometry of freshwater turtles relative toC, N and P and relate that to skeletal mass and stoichiometry; B) contrast turtles with freshwater fishes to determine whether patterns of turtle stoichiometry are consistent with

existing knowledge fromfishes; and C) use our results and published data on fishes to consider the potential role of P limitation in driving vertebrate growth limitation, life history evolution, and CMNR. To this end, we have measured turtle skeletal mass and estimated turtle body and excreta nutrient content. We present data on turtles from two families that vary in their diet, and from lentic and lotic aquatic habitats.We reviewed published data on fish body nutrient content and life history characteristics to compare with our data on turtle body nutrient content and published turtle life history data to illustrate deviations in the application of EST to these two groups of vertebrates.

METHODS

Study system

As part of a larger study focusing on the effects of turtle populations on freshwater nutrient dynamics, we sampled turtles from three habitat types in Georgia, USA. The southeastern U.S. is a global hotspot for turtle diversity, and twelve species of turtles have been documented among stream and pond habitats in Georgia (Sterrett et al. 2011). We focused our studies on four species (Yellow-bellied slider, *Trachemys scripta*; Painted Turtle, *Chrysemys picta*;Common Musk Turtle, *Sternotherus odoratus*;Loggerhead Musk Turtle, *S. minor*), which represent the two most speciose turtle families in North America: Emydidae and Kinosternidae. Three of these turtles (*T. scripta*, *C. picta*, and *S. odoratus*) are also among the most wide-ranging and well-studied turtle species (Ernst & Lovich 2009). *Chrysemys picta* and *S. odoratus* are often associated with slow moving water and *T. scripta* is a habitat generalist found readily in both lentic and lotic habitat. *Sternotherus minor* is a carnivorous riverine specialist, with tendencies towards molluscivory(Ernst & Lovich 2009). Our three focal habitat types weremanmade pondsin theWhitehall Experimental Forest (WEF), stream sites in the North

Oconee River andstream sites in Ichawaynochaway and Spring Creeks. WEF is a 325ha private research area owned by the Warnell School of Forestry and Natural Resources at the University of Georgia, located in the Piedmont Physiographic Region on the outskirts of Athens, Georgia. This research area has a number of partially forested, manmade [impounded] ponds and river floodplain wetlands in a matrix of pine and mixed deciduous forest.The North Oconee River is a 5th-order alluvialPiedmont tributary in the Altamaha River Basin, and characterized by large rocky boulder shoals and sandy pools. The riverflows through Athens, Georgia and along the eastern edge of WEF. Ichawaynochaway and Spring Creeks are 5-order tributaries of the Lower Flint River Basin (LFRB)characterized by rocky, limestone shoals and deep, wide, sandy pools and harbor high turtle diversity (Sterrett et al. 2011). Sites were chosen based on accessibility and knownpresence of turtle populations.

Field Collection and Excretion Collection

Turtles were collected from May to August in 2011and 2012using fish-baited hoop traps (0.9 m dia, three hoops, 3.8 cm mesh; Memphis Net and Twine, Memphis, Tennessee, USA; Legler 1960). Bait in traps was held in a perforated bag that allowed for dispersal of odor but did not allow turtles to feed on the bait.All turtles captured were measured (carapace length, CL; plastron length, PL) and weighed (g) in the field.Individuals not held for excretion collectionwere marked and released at their point of capture following processing [animals were marked as part of a larger study estimating population sizes and biomass].Excretion collection took place in a shaded area near the water at each collection site.Excretion incubationmethods for freshwater turtles were modified following methods for fish excretion estimation(Schaus et al.1997).Immediately following capture, turtles werecleaned by scrubbing off algae and debris from the carapace and plastron and by rinsing debris from inguinal and axillary regions with

filtered water. We removed leeches from all parts of the body. Turtles were placed in individual19-L sterilized (acid washed or autoclaved)polyethylene bins (45.7 x 30.4 x 22.8 cm; Rubbermaid, Atlanta, Georgia, USA) and covered with window screening to block debris. Two liters of pond or stream water were filtered (0.45 μm) to remove suspended particles and added to each bin. The amount of water added to each bin covered most of the carapace of all individuals.In addition, a control bin was added to each set of excretion trials on each date, which was treated in the same manner as those with turtles. At the end of a six-hour incubation period, a 60-mL sample was taken from each container using a new luerlock syringe and immediately filtered (0.45 μm) into a nalgene container and frozen for later analyses. We conducted an hourly collection of excretion measurements from wild turtles in 2011, which illustrated an asymptotic response at six hours for the four focal species for N excretion (S. Sterrett, unpubl. data).Temperature and stress can affect estimates of excretion in wild animals (Vanni 2002, Whiles et al. 2009). Thus, we decreasedhandling time when possible and used shaded containers to decrease temperaturefluctuations that would deviate from surface water temperatures.Excretion samples were analyzed for total dissolved N (NO_3-N) and P (soluble reactive phosphorus - PO_4-P) following a persulfate digestion at the University of Georgia Analytical Chemistry Laboratory. Excretion was estimated as the difference between the excretion and control samples and rates were estimated as the changes in N and Pper volume (2 L), per unit time (6 hrs).

We removed three measurements of NO_3 and five measurements of SRP that were equal to or less than the values of the control measurements. We used linear regression to quantify allometric relationships between wet body mass (log transformed) and mass-specific molar

excretion rates (log transformed). All analyses were completed in Statistica (Version 10, StatSoft©, Inc. 2011, Tulsa, Oklahoma, USA).

Measuring Body Nutrient Content

We retained a subset of individuals used in excretion trials to measure body nutrient composition. Four individuals of each focal species were kept from both years (total of eight individuals per species). One individual (*S. odoratus*) was found recently deceased in a trap and was also used to estimate body nutrients. Turtles were euthanized with intravenous injections of zylazine (1 $mgkg^{-1}$) and decapitated once fully anesthetized and immediately frozen following guidelines of the American Veterinary Medicine Association (AVMA 2007).Tissues were later thawed and dissected into categories: shell (carapace and plastron), organs (included all major organs except gastrointestinal tract), and body (mixture of muscle and bone). Additionally,bone and muscle samples were also dissected for separate analyses. Tissues were dried at 15.5°C to a constant mass, ground in a ball mill grinder and re-dried for storage until analysis.Carbon and Nwere analyzed by subsampling weighed tissues into tins and analyzed by Micro-Dumas Combustion using a Carlo Erba 2NA 1500 CHN analyzer (CarloErba, Milan, Italy). Analysis for total body P was completed by weighing a subsample of each tissue into an acid-washed ceramic crucible, ashing at 500°C, acid digesting and analyzing using the ascorbic acid method of spectrophotometry (Jones et al. 1991). Total body nutrient content was determined as the product of the subsampled nutrient estimateand the contribution of that tissue to the whole body according to its dry mass. We used individual student's T-tests to test for differences in untransformed body nutrient content (C, N, P, C:N, C:P, N:P) between turtle families andecosystem types (stream vs. pond) anda one-way ANOVA to test for differences among species. Tukey's Honestly Significant Difference was used as a *post hoc* test to differentiate

among species in nutrient content.We used linear regression to quantify allometric relationships between wet body mass (log transformed) and whole body molar nutrient content (log transformed). One measurement of body stoichiometry (*S. minor*) was removed from analyses because it deviated from the mean by >2 standard deviations.

Measuring Skeletal Contributions to Body Mass

In addition to measurements from field-collected turtles, museum specimens were used to determine skeletal investment to overall body mass. Osteological museum specimens were solicited from The Florida Museum of Natural History (Gainesville, Florida)and the Chelonian Research Institute (Oviedo, Florida). When complete skeletons existed, the maximumcarapacewas measured to estimatewet mass from regressions based on field-collected animals.Whole or parts of skeletonswere weighed to determine percent skeletal mass (shell, skull, appendicular skeleton, total skeleton).

We used field-collected length and mass data of the focal species fitted to an allometric equation (power function; $Y=aX^b$) to estimate wet mass (Y) from carapace length (X) with *a* and *b* as constants. Mass estimates from these relationships were used to estimate skeletal features as a percent of body mass. Field collected data provided relationships for estimating body mass of osteological specimens in this study (*T. scripta* - n=125, $y=0.0003x^{2.86}$, $r^2=0.99$; C. *picta* - n=23, $y=0.0004x^{2.74}$, $r^2=0.97$; *S. minor* - n=75, $y=0.0002x^{2.96}$, $r^2=0.97$; *S. odoratus* - n=93, $y=0.0048x^{2.40}$, $r^2=0.87$).

Estimating Relationships between Body Stoichiometry and Life History Traits

We usedour measurements and published estimates to examine relationships between species-specific body nutrient content and metabolic rate, adult growth, and longevity among fishes and turtles. Body nutrient content were reviewed fromTanner et al. (2000), Vanni et al.

(2002), Dantras and Attayde (2007) and Hendrixson et al. (2007). We used published estimates of metabolic rate standardized for temperature at 25°C and body size for available fishes that also have estimates of body nutrient content (q25Wkg; Makarieva et al. 2008). Estimates of turtle metabolic rates were converted from standard metabolic rate (mL O^2 hr^{-1}) in Ultsch (2013) to q25Wkg using conversion factors suggested by Makareiva et al. (2008). Growth rates for fishes published in Tanner et al. (2007) were used to compare to adult growth rates of adult turtles that grew over a 10-year period published in Congdon et al. (2013). We used estimates of *Kinosternon subrubrum* and *K. sonoriensis* from Congdon et al. (2013) as proxies for growth of Kinosternidae. We acknowledge that the growth rates of Tanner et al. (2007) are absolute growth rates and do not take into account proportional effects (McIntyre & Flecker 2010). We wanted to examine relationships between body stoichiometry and age at first reproduction; however, we did not have sufficient data on age at first reproduction for most fish species with available nutrient data. Therefore, we used estimates of fish longevity from Carey and Judge (2000) and turtle longevity from reviews in Ernst and Lovich (2009). Longevity is a function of annual adult survival, which is positively related to age a first reproduction (Stearns 1992, Charnov et al. 2001). We recognizethat longevity records do not represent maximal age estimates, but instead are "frames-of-reference" and useful for largercomparisons of taxa(Carey & Judge 2000).We used linear regressions to quantify relationships between body N:P and estimates of metabolism, growth rate, and longevity across fishes and turtles.

RESULTS

We measured excretion rates of 92 individual turtles of the four focal species across three habitat types, and 33 individuals across the four focal species were analyzed for whole body nutrient content. In addition, we measured the skeletons from 151 museum specimens across the

four focal species. Individuals ranged in size within and between families (Table 2.1). Our measurements of Emydidae included a range of juvenile and adult-sized turtles, but our measurements of Kinosternidae were all of adult sized turtles. Total skeleton among turtle species made up 27.5% of total wet body mass and 82% of total dry mass with the shell comprising from82% to 93% of the total skeletal mass acrossfamilies (Table 2.2).The proportion of skeletal mass was 6% to 47% greater for Emydidae compared to Kinosternidae.However, the highest proportion of skeletal mass that was shell occurred in*S. odoratus*and skulls of both Kinosternidae species were 4 to 9 timesthe proportion of body mass compared to Emydidae (Table 2.2). Theskeletal proportion of wet mass of turtles was equal to or greater than 7.6 times the skeletal proportion ofwet mass reported for teleost fishes (Table 2.2). Turtle bone had 9 to 13 times the concentration of P (8.56%) compared to the internal organs (0.66%) or muscle (0.94%) respectively (Table 2.3). As a result of total mass and nutrient content, the shell specifically contained 86% of all P across turtles. In contrast, turtle bone was38% to 60% lower in N (5.1%)compared to internal organs (8.3%) and muscle (12.8%; Table 2.3). Turtle shells had the lowest molar N:P of any tissue (1.34), in contrast to muscle, which had the highest molar N:P (44.68; Table 2.3). As a result, a turtle with its shell had a molar N:P of 2.3 and an overall %N:P of 1.04. This N:P is substantially lower than any value reported among all fishes (Fig. 2.1). Excluding the shell, turtles had a molar N:P of 8.61 (Table 2.3), which is comparable to published values for fishes.

Nutrient concentrations and ratios varied little among turtle speciesor families and did not differ in relation to habitat or diet.Body content (% dry mass) across all species was 29.72 ± 3.69% C (mean ± SD; range 24.46 - 37.65), 6.63 ± 0.50% N (range 5.81 - 7.93) and 6.48 ± 0.84% P (range 4.62 - 8.12).There was no differencein %N and %P nutrient content between

families (df=29, t=-0.98, p=0.33; df=29, t=1.25, p=0.22, respectively) or between species (df=3, MS=0.370, F=1.516, P=0.233; df=3, MS=0.985, F=1.706, P=0.189, respectively).However, there was a difference in mean %C contentbetween families (df=29, t=-2.4, p=0.02) and among species (df= 3, MS=52.31, F=5.82, P=0.003), driven by higher mean C content of *C. picta*(Table 2.1).Pairwise comparisons (Tukey's HSD) suggested that *C. picta* was significantly higher in %C body content than *T. scripta*, *S. minor*, and *S. odoratus*(p=0.03, p=0.002,and p=0.05, respectively). Molar C:P was 12.11 ± 2.81 (7.77-20.85), C:Nwas 5.26 ± 0.80 (4.14-6.70) and N:P was 2.32 ± 0.43 (1.67-3.76).There was no difference in elemental body nutrient content of %N (df=30, t=0.74, p=0.46), %P (df=30, t=-1.31, p=0.197), or molar N:P (df=30, t=0.748, p=0.46)between turtles collected from pond or stream habitats (2/ 2).However, there was a difference in %body C (df=30, t=3.19, p=0.003), molar C:N (df=30, t=2.72, p=0.010) andmolar C:P (df=30, t=2.45, p=0.020) body content of turtlesbetween streams and ponds.

Variation in body nutrient content and mass-specific excretion stoichiometry among turtles was related to body mass, which explained any apparent differences among species or habitats.Because we only had measurements for adult-sized Kinosternidae, our sample lacked sufficient variation in body size to examine ontogenetic relationships with stoichiometry for this family. Therefore, we conducted analyses on all turtles combined and just on Emydidae to draw inferences about the generality of relationships within and between families. Among all turtles, there wasnot a significant relationship between body mass and body molar N:P (p=0.40), although we found this relationshipsignificant among individuals of the two Emydidae species (y=0.85-0.25x, r^2=0.29, p=0.03; Fig. 2.2).Among all turtles and only Emydids, respectively, body mass was negatively correlated withexcretion N:P (all turtles: y=0.5513-0.4008x, r^2=0.05, p=0.03; Emydidae only: y=1.7523-0.8217x, r^2=0.15, p=0.01; Fig. 2.2). Among all turtles, body

mass was negatively correlated with mass-specific molar P excretion rate (y=-1.6572-0.5816x, r^2=0.10, p=0.0036) but not mass-specific N excretion rate (p=0.45). Among the Emydids, there was a significant negative relationships between body mass and mass-specific N excretion (y=-0.0011-0.3976x, r^2=0.10, p=0.05),but not P excretion (p=0.21).There was a positive correlation between body molar N:P and mass-specific molar N:P excretion among all turtles (y=-1.9094+4.3865x, r^2=0.20, p=0.02) and within the family Emydidae (y=-2.3027+5.7136x, r^2=0.52, p=0.0025; Fig. 2.2).

Turtle metabolic rates were similar to the lower range of metabolic rates reported for fish; however, the adult growth estimates were extremely small and longevity estimates extremely long for turtles compared to estimates for fishes (Fig. 2.3).Among these and other published data,there was a positive relationships between body nutrient content and growth and metabolic rates among fish(y=-0.2386+0.0722x, r^2=0.46, p=0.005; y=-1.3185+1.4979x, r^2=0.21, p=0.06, respectively)that was consistent and stronger among turtles and fish combined(y=-0.0358+0.0306x, r=0.81, p=0.00002; y=0.0997+0.0646x, r=0.45, p=0.04, respectively;Fig. 2.3). There was a weak positive, statistically non-significant relationship between body N:P and longevity in fishes (y=2.72+2.41x, r^2=0.11, p=0.10). However, among the broader range of body N:P represented by turtles and fishes,there was a significant negative relationship between body N:P and longevity (y=24.16-2.88x, r=-0.40, p=0.02;Fig. 2.3).

DISCUSSION

Our results extend prior knowledge of the uniqueness of turtle anatomy among vertebrates to the uniqueness of turtles in regards to EST and CMNR. Turtles have a high body P content unprecedented in our knowledge of other vertebrates,resulting in a lowwhole body nutrient %N:P ratio of nearly 1:1(Fig. 2.1). Prior to this study, the lowestpublished estimates of

body N:P among any animals wereapproximately 3:1 forAspredinidaefish species.The obvious reason for the exceptional body nutrient contentof turtles is the high skeletal mass invested in the shelland the associated high P content of bone. Per unit mass, P content in bone was 13 times greater than muscle or internal organs, andbone accounted for 24 to 35% of total wet mass and 82% of total dry mass. Turtle shell alone accounted for 22 to 31% of total wet mass (55 to 75% of dry mass), which accords with earlier estimates of turtle skeletal mass(Iverson 1984) and is>1.75 times higher than the next largest estimateamong animals as diverse as elephants, whales and fishes (reviewed inReynolds & Karlotski 1977,Anderson et al. 1979 and Iverson 1984). Although we were not able to empirically measure the amount of the turtle's total P residing in the whole skeleton (due to homogenization of body), we did estimate that the shell specifically contained 86% of all P across turtles.Excluding the shell,the molar N:P of the remainder of a turtle (8.61)is similar to estimates for fishes(8.4; McIntyre & Flecker 2010).While the skull and appendicular skeleton contribute a much lower mass to the total amount of skeleton, the contribution of the skull was higher in those species that tend towards molluscivory as adults (Table 2.1).

Differences among turtles in body and excretion N:P stoichiometry was largely related ontogenetically to size and, therefore, presumably tosignificant shifts in P demand related to the production of a shell among growing individuals versus the maintenance of a shellamong mature individuals (Fig. 2.2). Among the Emydids, for which we had a sufficient range of juvenile and adult sizes, body mass was negatively correlated with body N:P. That is, larger turtles had a higher mass-specific P content. Under the theory of stoichiometric invariance and assuming that turtles are homeostatic, this would imply that larger turtles have a higher P demand. However, despite the high P demand for the production of bone, once produced, its metabolic turnover is

orders of magnitude slower than other tissues (Chisholm et al. 1982; Daelerum & Angerborn 2005).The pattern of reduced body P content with reduced size is consistent withsmaller turtles having less P relative to body mass because their skeleton is not fully developed. Clark and Gibbons (1969) show a positive relationship between body size and calcium (Ca) in the shell of juvenile *C. picta*, implying greater Ca demand among juvenile turtles. Because Ca and P are linked in the construction ofbone mineral (i.e. Ca hydroxyapatite), it is also implied that smaller turtles have a higher P demand to support skeletal growth, while a mature turtle would have a low P demand despite higher body P content (Fig 2). In other words, we suggest that body P content is ontogeneticallyinversely related to demand in turtles. This hypothesis is supported by the patterns of nutrient excretion.According to EST, if turtles are homeostatic and body P content is positively indicative of P demand, then we would expect larger turtles with higher body P to have excretions lower in N:P than smaller turtles with a lower body P content. Instead, N:P of excretion was negatively correlated with body mass, and body N:P was positively correlated with excretion N:P (Fig. 2.2).These patterns are also consistent with ontogenetic diet shifts (juvenile turtles are more carnivorous while adults are more omnivorous or herbivorous) that suggest a greater P demand among smaller, juvenile turtles. If juveniles had a similar or lower demand for P, the higher P content of their prey should result in decreased N:P of their excretion.

The patterns we observed among turtles of increasing body size and skeletal development correlating with increased body P is consistent with studies of gizzard shad (*Dorosoma cepedianum*) and zebra fish (*Danio rerio*; Pilati & Vanni 2006). However, Pilati and Vanni (2006) found negative correlationbetween body and excretion N:P of *D. cepedianum*, which is consistent with increased P demand with increasing size and body P content and opposite of what we observed among Emydid turtles. There are a several potential explanations for these

discrepancies. First, the skeleton in fish represents a small proportion of their total body mass, such that a larger proportion of fish P demand may be for other tissues, despite the increased skeletal investment (McIntyre & Flecker 2010). Second, the metabolic rates of bone in fish and turtles may differ, though we know of nodata to support thishypothesis. Third, differences in the degrees to which fish and turtles exhibit indeterminate growth may contribute to differences in ontogenetic P demand. Congdon et al. (2013) used 30-year data sets of 13 turtle populations to show negligible growth of adults over intervals of >10 years.

We found no evidence among fourturtle species that habitat contributed to differences in body and excretion stoichiometry, though we found some evidence between families that diet affects excretion stoichiometry. Emydidae andKinosternidaedid not differ insize-specific excretion stoichiometry; however, the excretion of Kinosternidae was lower in N:P relative to body N:P (Fig. 2.2C), which is likely related to diet. For example, Kinosternidae are omnivorous with tendencies towards carnivory, and are likely ingesting prey with a higher P content. Because the Emydids ontogenetically shift in diet towards predominantly plant material (Parmenter & Avery 1990), we would expect them to excrete more P than smaller turtles that still have a high P demand.

Our results have implications for the way we think about the natural history and life history of turtles. A long-held hypothesis about the natural history of many turtle species is that they are carnivorous earlier in life and omnivorous or herbivorous later in life due to high protein demand for growth (Clark & Gibbons 1969, Parmenter 1980). Turtle muscle is rich in N (Table 2.3), and studies have associated increased protein supply with increased turtle growth (Avery et al. 1993, Bouchard & Bjorndal 2006). Therefore, we do not dismiss the importance of protein in juvenile turtle diets. Rather, wesuggest that the need to produce bone creates a demand for other

nutrients, specifically P andCa, whichcould also significantly limit turtle growth. Because turtles are 82% bone by dry mass, we would go as far as to suggest that the greater ecological limitation of turtle growth may be P and Ca demand, and that juvenile turtles may be the most extreme example of an animal whose growth is limited by elements other than C (Sterner & Elser 2002). Further, we propose that high P demand for the production and strengthening of the shell isa significant driver of juvenile turtle diet selection for P rich prey early in ontogeny, and the relaxation of this P demand facilitates the ontogenetic shift to more herbivorous diets among adults.

We recognize that energetic-metabolic and material processes are not independent (Allen & Gilooly 2009) and that multiple energetic and stoichiometric factors likely influence turtle life histories. Turtles are thermally constrained geographically by the length of growing season and there is evidence of growth enhancements from thermally influenced habitats (Gibbons 1970). Temperature also influencesingestion, digestion, and digestion efficiency ratesof turtles (Kepenis& McManus 1974, Avery et al.1993). However, outside of the cooler times of the year and in natural contexts, turtles are capable of achieving and sustaining high body temperatures behaviorally such that it does not constrain their metabolism (Boyer 1965). We are not aware of any studies of freshwater turtles that demonstrate natural food limitation through density dependence or other means.Moreover, we found that turtles generally ate highly abundant prey (i.e. macroinvertebrates and algae), and we never observed a turtle without substantial prey in its gut. Nonetheless, it is common that turtle growth increases in eutrophic conditions such as turtle farms, agricultural ponds, or urban areas,leading some to conclude that turtles are food limited in growth (Knight & Gibbons 1968). Because food resources in eutrophic systems are likely richer in protein and P, we would argue that evidence of faster growth by turtles in eutrophic systems or

turtle farms does not demonstrate conclusively that food availability, rather than quality, is naturally limiting. Research with fishesdemonstrates that species with large skeletal mass andhighbody P content (family Loricariidae)are seasonally growth limited by P (Hood et al. 2005).We would expect data on turtle consumption and assimilation rates and diet stoichiometry to reveal more about growth limitation and influence on CMNR.We advocate for future studies that manipulate food availability and quality to determine the degree to which juvenile turtles are calorically versus P limited in growth.

We believe that the possibility of P limitation related to growth of a bony skeleton has implications for understanding the life history and evolution of turtles and other vertebrates with extensive skeletons. For example, the available data for only fishes did not suggest a strong relationship between body N:P and longevity. However, the inclusion of data on freshwater turtles suggests a negative relationship may exist between P demand and longevity.We propose that the high P demand and time required to build a skeleton necessitates delayed maturity to achieve a size reasonable for reproduction. For example, the two Kinosternidae species we studied are relatively small species, requiring 4 to 8 years to mature and laying clutches of2 to 4 eggs per year (Ernst & Lovich 2009). One hypothesis is that increased fecundity associated with increased size compensates for delayed maturity; however, long-term data suggests a negligible fecundity advantage for delayed maturity. Moreover, this data indicates that age and size at first reproduction are determined early in life by juvenile growth.This suggests that factors limiting growth and therefore retarding age at maturity requireadaptations for high adult survival and longevity (Gibbons 1987). We would propose that the effects of P limitation on shell growth and delayed maturation has been a strong selective force for high adult survival and the absence of reproductive senescence among turtles.

Finally, our results provide the first glimpse into the roles that turtles play in freshwater nutrient dynamics. Because of their unique morphology, turtles will be, in combination with their biomass, a substantial proportion of the standing stock of P in freshwater ecosystems.Nitrogen and P are often limiting in freshwater and their synergistic effects can greatly influence autotrophic production in lentic and lotic systems(Vanni 2002, Elser et al. 2007). Thus, uptake and turnover of these nutrients by turtles are predicted to be important for freshwater ecosystems. In lotic systems, the high survival and biomass of turtles may be very important in increasing the retention of P (Small et al. 2009, Vanni et al. 2013). EST predicts that a consumer's influence on nutrient supply will be dependent on the nutrient content of the consumer and the resources consumed. Most tertiary consumers have lower C:N, C:P, and N:P than their prey, indicating a potentially higher demand of P relative to supply. In these cases and assuming the consumer is relatively homeostatic, EST predicts an inverse relationship between ratios of the consumer's body and the ratio of nutrients excreted. This relationship has been illustrated in zooplankton, invertebrates and fishes (Sterner 1990, Elser et al. 1996, Dantras & Attayde 2007, McIntyre & Flecker 2010; however, see Milanovich 2010). However, we have illustrated a positive relationship between body N:P and excretion N:P in turtles (Fig. 2.2C) and propose that despite having a low body N:P, adult turtles have a low P demandare excreting N and P proportionate to their body stoichiometry and biomass.

Many of the inferences that we draw from this research rest on hypotheses that need further study. The first and most obvious is that there is a need for research that examines P limitation relative to thermal or food limitations on growth. Additionally, we have not included dietary stoichiometry in our analysis of excretion patterns. Some of our data suggest that diet may affect the nutrient content of turtle excretion. There is limited information on feeding

parameters such as ingestion and digestion rates and assimilation efficiencies for turtles (but see Kepenis& McManus 1974 and Avery et al. 1993), especially adults. Data such as these would allow us to estimate threshold elemental ratios of turtles, which would allow us to better understand the conditions under which nutrients limit turtle growth and effects on CMNR.

There are likely natural history and morphological differences among turtles that will also affect their contributions to CMNR. Similar to variation found in fishes (Hendrixson et al. 2007, McIntyre & Flecker 2010), there is likely variation in body nutrient content among turtle families rooted in morphological modifications. Some turtle groups are known for having shell modifications, which may vary from those in this study (e.g. thin carapaces (e.g. *Deirochelys reticularia*and *Malacochersus tornieri*) or reduced plastrons (e.g. Chelydridae)). These evolutionary modifications may also alter the amount of N and P in whole body stoichiometry. Softshell turtles (e.g.*Apalone* spp.) are a group, which has replaced keratinized scutes with leathery skin and significantly reduced dermal bony elements of the shell (Ernst & Lovich 2009). Iverson (1984) found that some these groups, including *Apalone* spp., have reduced mass in skeletal investment. Finally, several turtle groups have evolved megacephalic heads with increased head musculature for crushing mollusks (*Sternotherus* spp., *Graptemys* spp.; Lindeman 2000). Although the overall contribution of the skull to body mass is modest, the skull mass of molluscivorous species can be significant. For example, the skull proportion of mass for the more molluscivorous species in our studywas 8.7 times greater than that of the more omnivorous/herbivorous species in our study (Table 2.2). In addition to molluscivory in turtles, other trophic specializations exist (i.e. herbivory)and we believe that the proportional contributions to nutrient dynamics may be due in part to the stoichiometry of their diet.

Turtles are among the most widely distributed vertebrates and yet, as a group, they are one of the most globally imperiled taxa (TTWG 2012). As a consequence, the harvest of turtles would have a significant impact on nutrient storage and recycling in freshwater. Because turtles live a long time, retain high biomass, frequently move across landscapes and nest terrestrially (Ernst and Lovich 2009), they are also potentially important conduits for moving nutrients across ecosystems. Bouchard and Bjorndal (2000) demonstrated that nutrients from sea turtle nesting rivaled the inputs of any biological transporter to dune ecosystems. The evolutionary, morphological and ecologically distinctiveness of turtle biology suggest that there are not other groups of organisms that could compensate for the loss of turtles. Thus, the long recognized evolutionary and morphological uniqueness of turtles now needs to be paired with their novelty within ecosystems.

ACKNOWLEDGMENTS

This research was supported in part by a crowdfunded project through the SciFund Challenge. We thank Jai RanganathanandJarrett Byrnes for organizing the SciFund Challenge and all 104 crowdfunding participants, especially Dale Hoyt and Kaye Maxwell, who helped to fund this research. This work was also supported by an innovative and interdisciplinary grant through the University of Georgia. We thank Joe Milanovich for hours of help with developing methods. We thank Vanessa Kinney and Cassandra Skaggs for help in the field and Bob Bahn and the Sonia Hernandez lab for assistance with lab work. Emmy Deng and Tom Maddox processed samples at the UGA Analytical Chemistry Laboratory.We also thank Max Nickerson at the Florida Natural History Museum and Peter Pritchard at the Chelonian Research Institute for their use of museum specimens. The manuscript was improved with suggestions from Rachel Katz.Turtles were collected under a Georgia Department of Natural Resources Scientific

Collecting Permit (#29-WJH-13-191). Procedures in this study were approved by the University of Georgia Institutional Animal Care and Use Committee (Animal Use Protocol #A2011 01-024-Y3-A3).

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Table 2.1. Taxonomy, general diet, longevity, size of animals and body stoichiometry from this study. Plastron length and wet mass are indicated as a mean and range. Turtles are arranged from largest to smallest.

Species	Diet[1]	Longevity (years)[2]	Plastron Length (mm)	Wet Mass (g)	Body Stoichiometry			
Emydidae					%C	%N	%P	N:P (molar)
Yellow-bellied Slider (*Trachemys scripta*)	O (H)	31+	149 (84-223)	767 (132-2050)	29.10 (3.78)	6.93 (0.64)	6.72 (1.04)	2.37 (0.67)
Eastern Painted Turtle (*Chrysemys picta*)	O (H)	40+	111 (81-143)	220 (93-340)	33.51 (1.71)	6.48 (0.54)	6.05 (0.19)	2.37 (0.27)
Kinosternidae								
Loggerhead Musk Turtle (*Sternotherus minor*)	O (C)	21+	75 (63-86)	143 (79-200)	27.22 (1.82)	6.62 (0.26)	6.60 (1.02)	2.27 (0.46)
Eastern Musk Turtle (*Sternotherus odoratus*)	O (O)	28+	63 (56-74)	95 (69-163)	29.33 (4.10)	6.47 (0.47)	6.61 (0.79)	2.24 (0.27)

[1]General diet (primary adult diet in parentheses); O=omnivore; C=carnivore; H=herbivore

[2]Estimates of survival in the wild from mark-recapture studies; reviewed in Ernst and Lovich 2009

Table 2.2. Skeletal investment in teleost fishes and freshwater turtle species as a percentage of wet mass. Standard deviations are shown in parentheses.

	% Shell[1]	% Skull[2]	% Appendicular Skeleton[2]	% Total Skeleton[2]
Yellow-bellied Slider (*Trachemys scripta*)	30.6 (7.9) N=39	0.36 (0.09) N=12	1.4 (0.4) N=11	34.9 (3.8) N=9
Eastern Painted Turtle (*Chrysemys picta*)	23.5 (3.2) N=41	0.37 (0.05) N=7	1.9 (0.4) N=12	28.8 (3.8) N=10
Loggerhead Musk Turtle* (*Sternotherus minor*)	23.6 (3.9) N=48	3.2 (0.7) N=13	2.3 (0.4) N=11	27.2 (3.6) N=10
Eastern Musk Turtle* (*Sternotherus odoratus*)	22.4 (3.8) N= 51	1.45 (0.38) N=17	2.5 (0.9) N=20	23.7 (3.9) N=15
Turtles	25.5 (6.1) N=146	1.5 (1.2) N=49	2.1 (0.7) N=51	27.5 (5.5) N=44
Fishes[3]	-	-	-	3.1 (1.3) N=37

[1]includes estimates from field-collected data and museum specimen estimates.
[2]includes only estimates from museum specimens.
[3]reviewed from Reynolds and Karlotski 1977 and Casadevall et al. 1990 (18 species)
*tendency towards carnivory (and molluscivory) as adults

Table 2.3. Percent nutrient and molar nutrient ratio of body tissues of all turtle species. Standard deviation is in parentheses.

	N	%C	%N	%P	C:N (molar)	C:P (molar)	N:P (molar)
Shell	33	21.85 (3.36)	5.06 (0.74)	8.56 (1.01)	0.79 (0.49)	6.70 (1.49)	1.34 (0.34)
Bone	33	23.28 (5.77)	5.13 (1.59)	8.48 (1.61)	0.0015 (0.001)	7.42 (2.60)	1.40 (0.56)
Muscle	32	47.04 (5.02)	12.77 (1.45)	0.66 (0.13)	0.0099 (0.007)	192.53 (49.99)	44.68 (11.14)
Organs	33	50.67 (6.06)	8.25 (1.30)	0.94 (1.55)	0.15 (0.11)	208.57 (87.81)	28.54 (11.17)
Body*	33	42.91 (4.72)	9.93 (0.99)	2.89 (0.98)	0.67 (0.37)	44.43 (20.77)	8.61 (3.38)

*whole body without shell

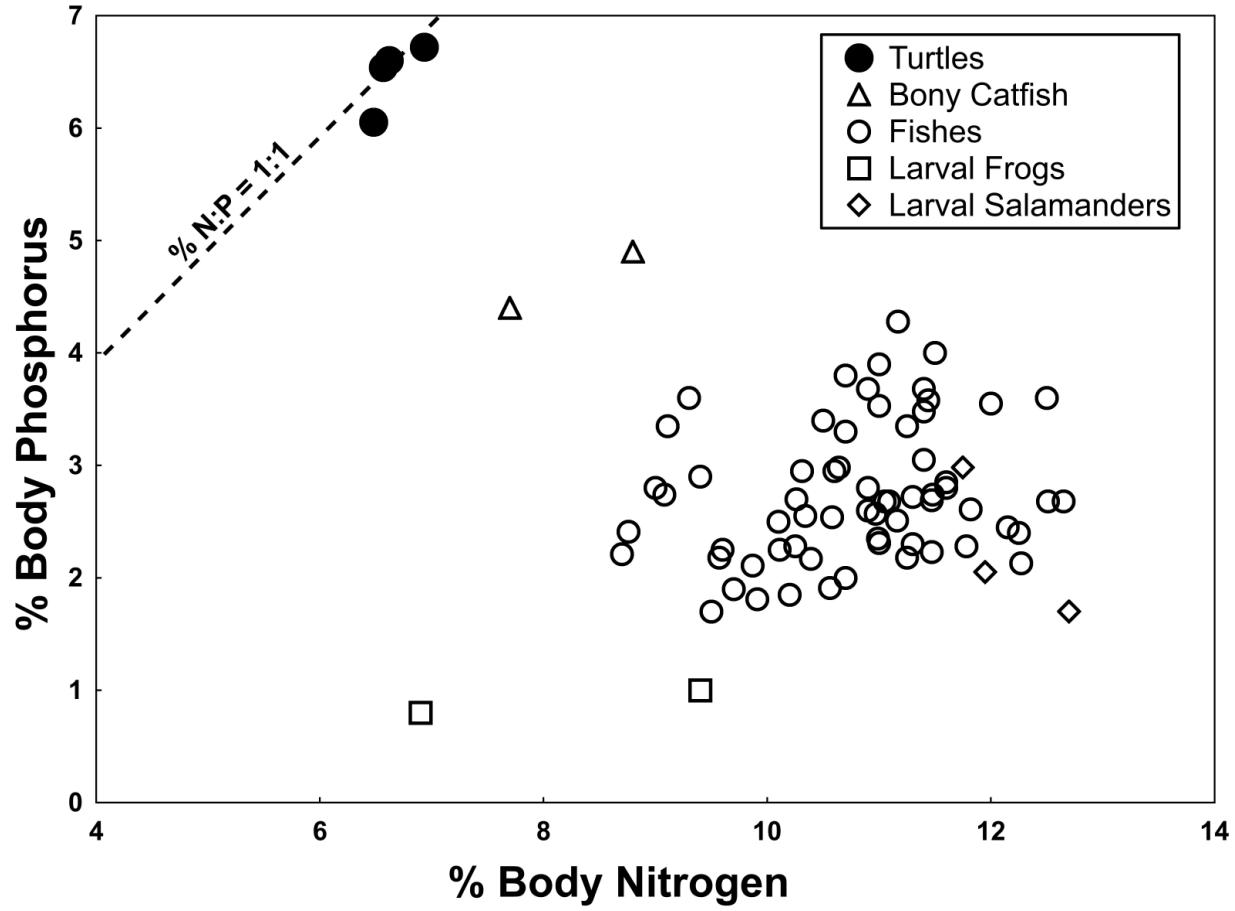

Figure 2.1. A plot of percent body nitrogen and phosphorus of aquatic vertebrates. Each point represents the estimates of a mean of a species or family from a study area as a percentage of dry mass. The dotted line represents a 1:1 isopleth of % N:P. "Bony catfish" represent families Aspredinidae and Loricariidae and are separated from other fishes for illustrative purposes. In addition to the current study, data are compiled from Penczak (1985), Tanner et al. 2000, Vanni et al. 2002, Hendrixson et al. 2007, Dantras and Attayde (2007) and Milanovich (2010).

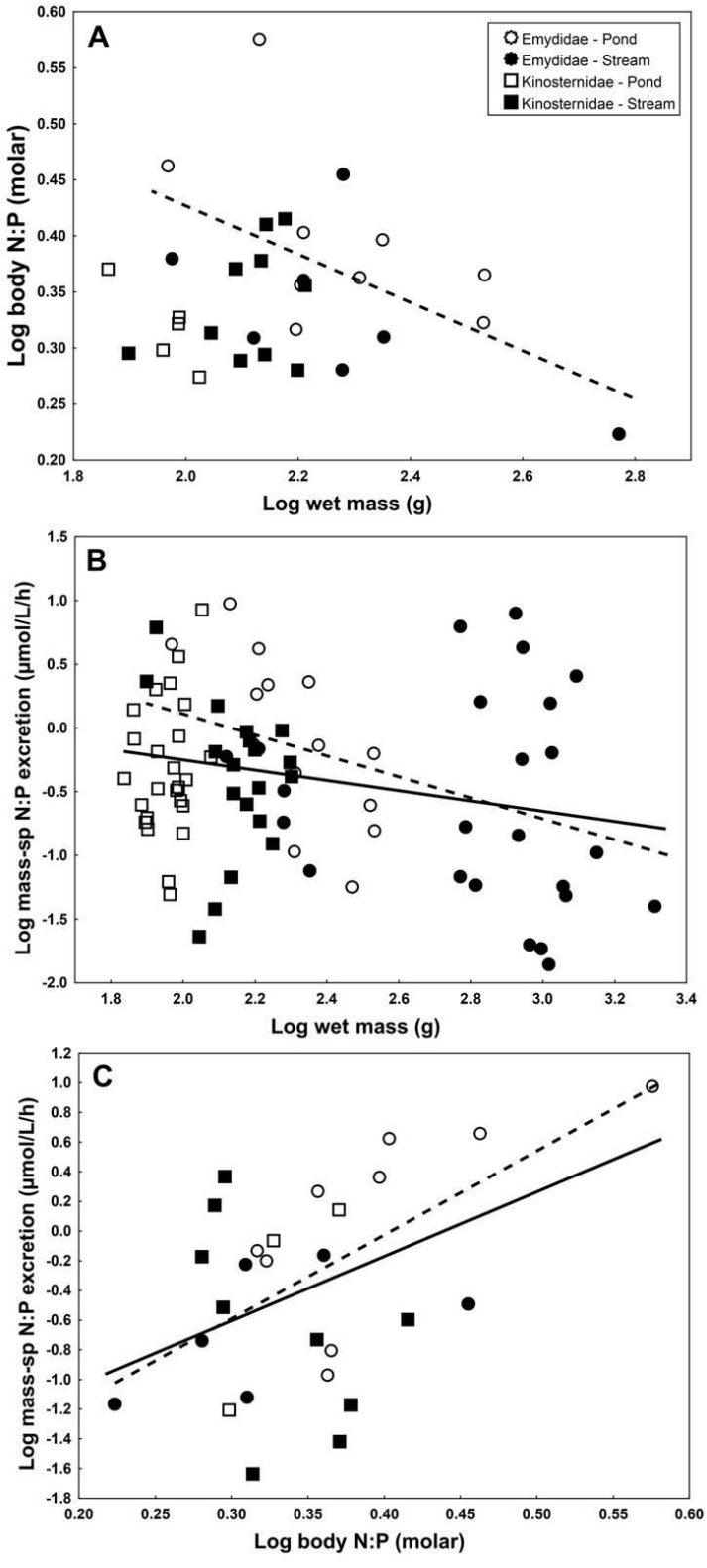
A
Emydidae - Pond
Emydidae - Stream
Kinosternidae - Pond
Kinosternidae - Stream
Log body N:P (molar)
Log wet mass (g)
B
Log mass-sp N:P excretion (µmol/L/h)
Log wet mass (g)
C
Log mass-sp N:P excretion (µmol/L/h)
Log body N:P (molar)

Figure 2.2. Regression of body mass and body N:P versus mass-specific N:P excretion and body NP between family Emydidae (circles) and Kinosternidae (squares) within pond (open symbols) and stream (shaded symbols) ecosystems. Fitted lines for significant relationships are included for all turtles (solid) and the family Emydidae (dotted). Panel A illustrates that as turtles in family Emydidae get larger in body mass, their body N:P decreases, suggesting an increase in body P as a result of skeletal growth (Emydidae: $y=0.85-0.25x$, $r^2=0.29$, $p=0.03$). Panel B illustrates that as turtles get larger in body mass, their mass-specific N:P excretion is reduced, suggesting that larger turtles are excreting more P than smaller turtles (all turtles: $y=0.5513-0.4008x$, $r^2=0.05$, $p=0.03$; Emydidae only: $y=1.7523-0.8217x$, $r^2=0.15$, $p=0.01$). Panel C illustrates a positive correlation between body N:P and excretion N:P (all turtles: $y=-1.9094+4.3865x$, $r^2=0.20$, $p=0.02$; Emydidae: $y=-2.3027+5.7136x$, $r^2=0.52$, $p=0.0025$)

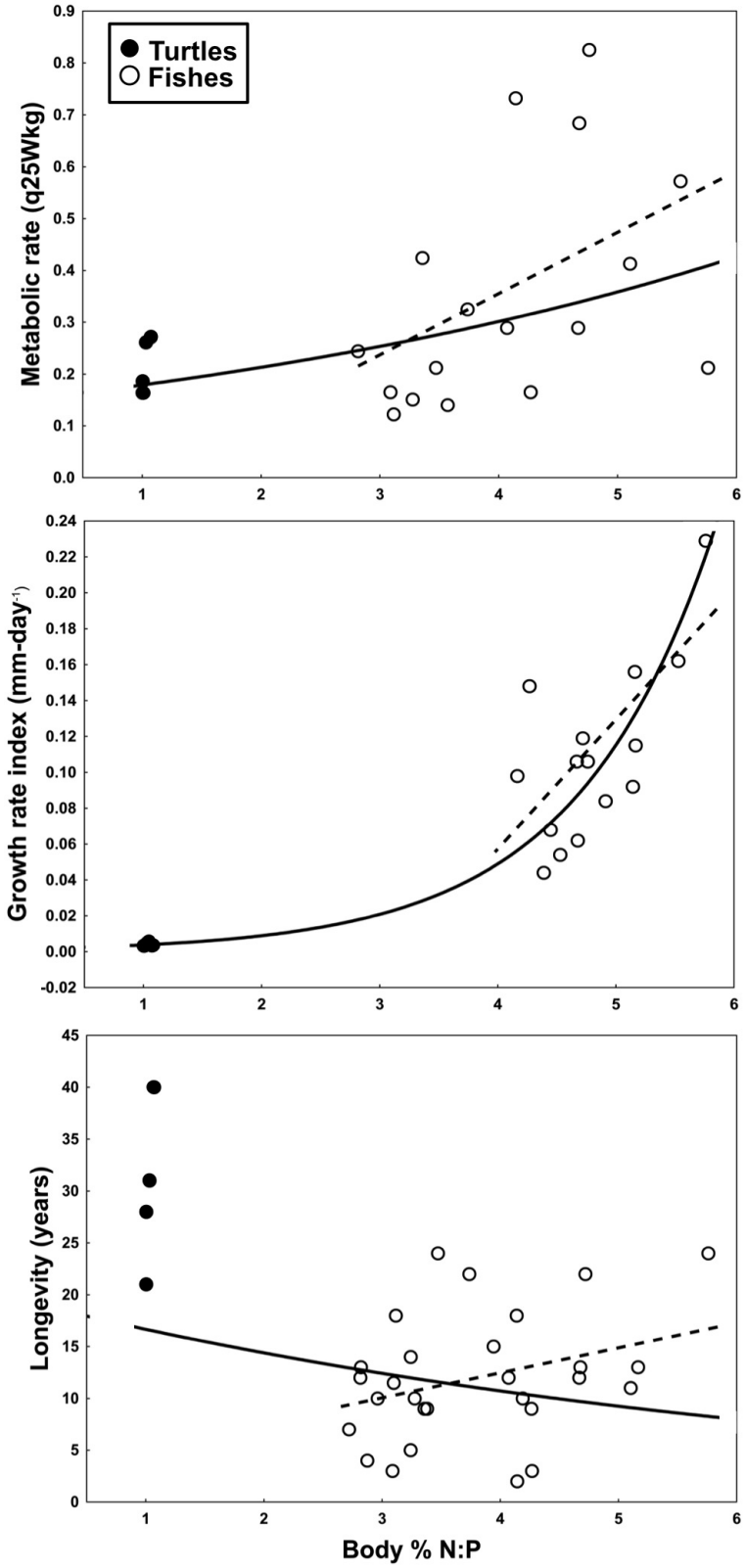
Turtles
Fishes
Metabolic rate (q25Wkg)
Growth rate index (mm-day-1)
Longevity (years)
Body % N:P

Figure 2.3. Relationships of body % N:P and longevity, growth and metabolism. Estimates of % body nutrient N:P content are reviewed from Penczak (1985), Tanner et al. (2000), Vanni et al. (2002), Dantras and Attayde (2007) and Hendrixson (2007). Estimates of metabolic rate for fishes are from Makarieva et al. (2008) and derived for turtles from Ultsch (2013) using conversion suggestions of Makarieva et al. (2008). Metabolic rate (q25Wkg) is standardized for temperature at 25°C and body mass. Estimates of growth are from Tanner et al. (2000) for fishes and Congdon et al. (2013) for turtles. Estimates of longevity for from Carey and Judge (2000) and Ernst and Lovich (2009). Fitted lines are included for all data (exponential; solid line) and only for fish data (linear; dotted line).

CHAPTER 3

NUTRIENT STORAGE AND EXCRETION BY FRESHWATER TURTLES [1]

[1]Sterrett S.C., R.A. Katz and J. C. Maerz *To be submitted to *Freshwater Science*

ABSTRACT

Turtles are unique among freshwater vertebrates because they achieve remarkably high biomass, have an extensive bony skeleton, and are exceptionally long lived; all of theseare characteristics that contribute to significant influences on freshwater nutrient dynamics. Specifically, we hypothesized that turtle populations represent large, stable pools for freshwater nutrients, especially phosphorus (P); however, unlike other species with high phosphorus body content, we hypothesized that turtles contribute high amounts of phosphorus through excretion. We used data on turtle nutrient composition and excretion in conjunction with capture-mark-recapture to estimate density and biomass to estimate nutrient standing crop nutrients (kg/ha; C, N and P), and excretion rates of four turtles species in three rivers and one collection of ponds in the southeastern USA. Turtle density ranged from 24 to 180 ind/ha and total turtle fresh biomass ranged from 18 to 46 kg/ha with the greatest biomass in the two tributaries of the lower Flint River Basin. As expected, the standing crops of P within the turtle assemblage were high, ranging from 0.2 - 0.45 kg/ha of P. Mass-specific species excretion rates of N and P were similar or exceeded estimates of salamanders and some fish species, though estimates of turtle assemblage N and P excretion estimates were lower than what has been reported for other aquatic organisms.Turtles are globally imperiled due to overharvesting and habitat degradation. Our results suggest that anthropogenic declines in freshwater turtle populations may have consequences for nutrient storage and supply within freshwater ecosystems.

INTRODUCTION

At a time of widespread population declines and shifting species distributions, it is important to understand how changes in biodiversity will alter ecosystem processes (Chapin et al. 2000, Dudgeon et al. 2006, Vaughn 2010). Historically, terrestrial and freshwater biogeochemistry researchers minimized the importance of higher-level consumers in nutrient dynamics. Researchers tended to focus on primary production, and the importance of large detrital, soil, and microbial pools of energy and nutrients (Schlesinger 1997). However, research into the unique influences of animals on nutrient dynamics (Kitchell et al. 1975, Kitchell et al. 1979, Burton and Likens 1975) is rapidly expanding (Vanni 2002, Schmitz et al. 2010, McIntyre & Flecker 2010). Animals can uniquely affect spatial dynamics through the transport of nutrients across system boundaries (Polis et al. 2004, Baxter et al. 2005), and temporal dynamics through the capture and retention of nutrients in high trophic levels (Small et al. 2009). Recent research suggests that vertebrates, especially those species that achieve large body sizes, have greater mobility, greater longevity, and the capacity to regulate the abundance of smaller, lower trophic level species, may have unique effects on ecosystem processes (Kitchell et al. 1979, Vanni 2002, Schmitz 2010). Vertebrates are predicted to stabilize ecosystems by maintaining large biotic pools of limiting nutrients (Kitchell et al. 1979, DeAngelis 1989, Vanni 2013), and in flowing systems, long-lived consumers are hypothesized to slow the spiraling of nutrients downstream (Webster &Patten 1979, Small et al. 2009). In lentic systems, vertebrates (particularly fishes) are predicted to contribute to internal nutrient processing and reducing sedimentation of nutrients, which increases their availability to other biota (Essington & Carpenter 2000, Vanni 2002).

Consumer-mediated nutrient recycling (CMNR) collectively refers to the remineralization of organic matter into usable nutrient forms for the base of food webs (i.e. autotrophs and microbes, Vanni 2002, Sterner & Elser 2002). In aquatic habitats, nutrients excreted by organisms rapidly become available because water can quickly dissolve and disperse nutrients (Carpenter et al. 1992, Schmitz et al. 2010). The direct importance of CMNR can depend on species abundance and biomass, movement behavior, or a species stoichiometry and degree of stoichiometric homeostasis, which are related to dietary or physiological specializations (Gaston 2010, Gaston 2011; Elser et al. 1996, Sterner & Elser 2002). Both experimental and descriptive research has demonstrated the importance of abundant species to CMNR (Vanni et al. 1996, Taylor et al. 2006). For example, the abundance and feeding behavior of gizzard shad (*Dorosoma cepedianum*) suggest their overall contribution to nutrient cycling within reservoirs is high. Among Midwestern U.S. reservoirs, *D. cepedianum* compose a significant portion of standing fish biomass (up to 417 kg ha^{-1} in Acton Lake, Ohio) and support primary production through P excretion (Vanni 1996, Schaus et al. 1997, Vanni et al. 2005). *D. cepedianum* is also a facultative detritivore, and affects nutrient dynamics by translocating nutrients from benthic resources into open waters where those nutrients supply some of the nutrient demands of phytoplankton. Depending on their stoichiometry and degree of homeostasis, some species can have effects on nutrient dynamics that are disproportionate to their biomass (Small et al. 2011, Schmitz et al. 2010). For example, Small et al. (2011) found that *Astyanax aeneus* represented 18% of the total fish biomass but contributed up to 90% of the P recycled by fishes in Costa Rica streams with low ambient P levels. The disproportionate

contribution of P recycled by *A. aeneus* was due in part to terrestrial macroinvertebrate subsidies, which contribute to increased P excretions.

Vertebrates may have relatively unique effects on nutrient storage and recycling. Compared to macroinvertebrates, the high investment in a bony skeleton and other mechanical and protective structures (e.g. scales) strongly affects whole body stoichiometry (Reiners 1986, Sterner & Elser 2002, Sterner & George 2000, Hendrixson et al. 2007, Sterrett & Maerz, in review). Bone is composed of collagen and mineral hydroxyapatite ($Ca_{10}(PO_4)_6(OH_2)$) and has a unique stoichiometry (N:P=~0.8), which accounts for >20% of biomass in some animals (Anderson et al. 1979, Iverson 1984, Sterner& Elser 2002, Sterrett & Maerz in review). The effects of varying levels of bone or other mechanical structure investment on whole body stoichiometry has been illustrated in many fish families (Sterner & George 2000, Vanni et al. 2002, Hendrixson et al. 2007, McIntyre & Flecker 2010) and recently freshwater turtles (Sterrett & Maerz in review). For fishes, variation in whole body %P content between some families (e.g. Characidae and Loricariidae) is attributed to evolutionary investments in armored scales and robust skeletons (Vanni et al. 2002, McIntyre & Flecker 2010), as well as ontogenetic changes in body composition (Vanni et al. 2002, Pilati & Vanni 2007, but see McIntyre & Flecker 2010). Assuming that the prey of most vertebrates has a higher N:P than the consumer, increased skeletal investment with age or size or among fish species is positively correlated to P demand, resulting in species with more bone and a lower body N:P excreting higher N:P (Sterner & Elser 2002, Vanni et al. 2002, Hood et al. 2005, McIntyre & Flecker 2010). However, recent research of freshwater turtles, which have exceptionally large skeletal investment, suggests P excretion

may be positively correlated with body content; resulting in distinctly different effects on CMNR between turtles and fishes.

The objectives of this study were to estimate nutrient storage and recycling of common freshwater turtle species in streams and ponds of the Southeastern United States, and to evaluate the degree to which differences in species composition, abundance, or body sizes among habitats might affect the importance of turtles in nutrient dynamics among different ecosystems. Turtles are common constituents of most temperate and tropical freshwater ecosystems (Vitt & Caldwell 2009). Turtles are morphologically and ecologically unique among vertebrates, most notably because of their highly modified skeleton that constitutes a disproportionate amount of their biomass relative to other vertebrates (Iverson 1984, Gilbert et al. 2011, Sterrett & Maerz in review). The growth of a bony shell likely constrains turtles to slow growth to maturity (Sterrett & Maerz, in review); however, adult turtle survival and longevity is extremely high (Heppel 1998, Gibbons 1987, Congdon et al. 2013) and turtles are capable of achieving a large mass (Pough 1980). As a result, turtle populations are often adult-sized biased (Ernst & Lovich 2009). Many turtles are omnivores, though turtle diets can be strictly carnivorous or predominantly herbivorous depending on the species or an individual's age (Aresco & James 2005). As a result, shifts in the relative abundance of herbivorous, omnivorous, or carnivorous species may alter the importance of turtles to nutrient cycling among systems. Recent evidence also suggests that the slow turnover of bone results in a declining P demand with maturity (Sterrett & Maerz in review). Therefore, unlike fish, turtle populations are likely to represent both large standing stocks of nutrients, notably P, as well as supply high amounts of inorganic P through excretion. Therefore, we hypothesized that turtle populations represent large, stable pools for freshwater

nutrients, especially phosphorus (P), and compared to reports for other freshwater vertebrates, turtles contribute high amounts of phosphorus through excretion relative to their body content.

METHODS

Study Site and Focal Species

Turtle populations were studied in three settings: the North Oconee River (NOR), a complex of manmade ponds within the Whitehall Experimental Forest (WEF) that is bounded by the NOR, and two tributaries (Ichawaynochaway and Spring Creeks) of the Lower Flint River Basin (LFRB; Fig. 3.1; see site descriptions in Sterrett & Maerz in review). The NOR and WEF permit us to compare turtles populations between adjacent pond and river systems, while the NOR and WEF allow us to compare to different rivers systems. The three focal settings have comparable turtle assemblages, but differ in the relative abundance of turtle species. All systems have omnivorous yellow-bellied sliders (Emydidae, *Trachemys scripta*) and a carnivorous musk (Kinosternonidae, *Sternotherus* spp.). All three assemblages also include omnivorous common snapping turtles (Chelydridae, *Chelydra serpentina*). WEF ponds also contain omnivorous painted turtles (Emydidae, *Chrysemys picta*), and NOR and LFRB rivers contain herbivorous river cooters (Emydidae, *Pseudemys* spp.) and carnivorous spiny softshell turtles (Trionychidae, *Apalone spinifera*). The LFRB community also includes carnivorous Barbour's map turtles (Emydidae, *Graptemys barbouri*), which is a molluscivorous specialist, and alligator snapping turtles (Chelydridae, *Macrochelys temminckii*). From prior research, we understand that *T. scripta*, *C. picta*, and *S. odoratus* are the most common species in WEF ponds, and *T. scripta* and *P. concinna* are the most common species in the NOR. *T. scripta*, *S. minor*, and *G. barbouri* are the most common species in the LFRB (Sterrett et al. 2011). For this study, we focused on the

four most common species we encountered and for which we had body and excretion stoichiometry data: *T. scripta*, *C. picta*, *S.odoratus*, and *S. minor*. Though we could not include all species in our estimates, our prior work suggests that differences in body and excretion stoichiometry among turtle species is relatively small and generally related to body size and, to a lesser degree, diet (Sterrett & Maerz in review). Therefore, we believe results from our focal species are generalizable to entire turtle assemblages.

Estimating Turtle Density and Biomass

We used a capture-mark-recapture robust sampling design (Pollock 1982) to estimate the abundance of male and female turtles of each species within each of our three habitat settings. The robust sampling design consists of primary periods that are assumed to open to population additions (births, immigration) and deletions (deaths, emigration) and secondary sampling periods nested within primary periods that are assumed to be closed to population gains and losses. To estimate abundances, we sampled turtles once per year (primary periods) during the summer (May – August). We sampled from 2010-2012 in pond sites within WEF and from 2011-2012 in stream sites within North Oconee River and within Ichawaynochaway and Spring Creeks. The WEF study pond sites were <1 ha in wetted area and sampled areas within streams were approximately 0.5 km in length. At each site, individuals were sampled using hoop traps (0.9 m dia, three hoops, 3.8 cm mesh) baited with sardines (Legler 1960). In streams, 20 traps were set approximately 25 m apart on alternating banks (when water levels allowed) in each 0.5 km stream reach. In ponds, ten traps were set 20 m apart on forested pond margins. Because turtles may only visit a portion of their home range during the sampling event, traps were placed closer than recommended for longer-term studies (Rodda 2012). Traps were set for five

consecutive days in ponds and 3 consecutive days on stream sites (secondary periods) and checked every 24 hours. This resulted in 50 (pond) or 60 (stream) trap nights per trapping session in each habitat type. All turtles captured were identified, sexed using secondary sexual characteristics, measured (maximum carapace length, CL; plastron length, PL), weighed (g) and uniquely marked by notching or drilling the marginal scutes of the carapace (Cagle 1939). This type of unique identification is recognizable many years after first marking (Gibbons 1987). All turtles not held for excretion trials (see Sterrett & Maerz in review) were released at their point of capture following processing.

Turtle abundances (N) were estimated using capture probabilities (*p*) during secondary sampling periods as well as apparent survival and recruitment probabilities between primary sampling periods (Williams et al. 2002, Meader et al. 2011). Because data was not sufficient to estimate *p* for every species-sex combination, we pooled capture histories for all species to evaluate the best model of capture prior to estimating species and sex-specific abundances. Candidate *p* models included: a constant model (i.e., *p* is constant among trapping sessions and years), a primary period model (i.e., *p* is constant among trapping sessions within years), and a secondary period model (i.e., *p* varies among trapping sessions and years; Mt models of Otis 1978). Capture probabilities were estimated for each habitat type within the model and we assumed that all turtles had equal probabilities of capture (i.e., no individual heterogeneity). We fit models using a Markov chain Monte Carlo (MCMC) Bayesian modeling approach using dynamic occupancy formulation of a Jolly-Seber *ad hoc* robust design model (Jolly 1965, Seber 1965, Kery & Schaub 2012), which assumes that all emigration is permanent (i.e., no temporary emigration). We used data augmentation and included 500 unobserved individuals (i.e. null

capture histories; Kery & Schaub 2012), which was considerably more individuals than expected within any single habitat setting. We evaluated the relative support of each candidate *p* model using Akaike's Information Criterion (AIC; Akaike 1973) with a small-sample bias adjustment (AICc, Hurvich & Tsai 1989), with lower AICc values indicating better predicting models (Burnham & Anderson 2002). We calculated Akaike weights (*w*), which ranged from zero to one, with the best approximating model having the highest weight (Burnham & Anderson 2002). Models with Akaike weights within 10% of the best approximating model, which is similar to Royall's 1/8rule for evaluating strength of evidence (Royall 1997), were considered plausible models for capture probabilities.

Using the best-supported *p* model, we then estimated turtle abundances using species- and sex-specific Jolly-Seber *ad hoc* robust design models (14 models). Each model included all habitat types and years where the species was captured. We incorporated the uncertainty in abundance estimates (based on posterior probability distributions) for each species and sex into subsequent density, nutrient and excretion estimates for each habitat type. Within the MCMC model, we calculated the average density of individuals (ind/ha) within each habitat type across all years by dividing abundance by the wetted area. Wetted area of each pond and stream site were estimated using aerial photography (2010 digital ortho quarter-quads) in ArcMap v10.2 (ESRI, Redlands, CA). We understand that the area sampled for streams is assumed and peripheral activity ranges are not taken into account (Rodda 2012). We estimated turtle biomass (wet mass kg/ha) by multiplying density by species-, sex-, and habitat-specific biomasses (g) that were measured in the field. We incorporated uncertainty in biomass estimates by including the

mean and standard deviation of field measurements within the MCMC modeling framework and assumed these values were normally distributed.

Estimating Nutrient Standing Crop and Excretion

We estimated nutrient standing crop and nutrient excretion of each sex of each species within each habitat type within the MCMC modeling framework used to estimate densities and biomass (above). First, nutrient standing crop (C, N, P; kg/ha) was estimated as the product of dry biomass, AFDM (%) and whole body nutrient content (%C, %N, %P; Sterrett and Maerz in review). Ash free dry mass (AFDM) was estimated for turtles similarly to whole body nutrient content because tissues were separated for nutrient analyses. AFDM was only estimated for individuals captured in 2012. A sample of each tissue (shell, miscellaneous organs, body (mixture of muscle and bone) and samples of bone and muscle) was weighed and placed in a ceramic crucible covered with aluminum foil and ashed at 500°C. Crucibles were then reweighed to determine % organic material. Total individual AFDM was calculated as sum of the product of individual tissue % organic material and dry mass divided by total dry mass. Field collected data provided relationships for estimating dry mass from wet mass for each species (*T. scripta*, $y=0.3268x-0.121$, $r^2=0.99$; *C. picta*, $y=0.3563x-0.121$, $r^2=0.99$; *S. odoratus*, $y=0.4482x-10.004$, $r^2=0.92$; *S. minor*, $y=0.3609x+1.1785$, $r^2=0.89$). We obtained estimated of AFDM for each species and mean body mass for each species, sex and habitat type and included uncertainty in our estimates by using the mean and standard deviation of wet and dry masses within the MCMC model framework.

We used the average of whole body %C, %N or %P nutrient content across species (Sterrett & Maerz in review). Nutrient excretion of all focal species (μg/ha/h) was estimated as

the product of density (ind/ha) and individual level nutrient excretion (μg/L/h) of total N and P collected from field experiments (see Sterrett & Maerz in review). Nutrient excretion estimates were taken from species at each habitat type. The average and standard deviation of N and P excretion for *T scripta* across all sites was used to estimate excretion of *T. scripta* in the N. Oconee River because only one individual was measured in the field. We estimated whole body nutrient content (species level) and nutrient excretion (species by habitat type) within the MCMC framework above to incorporate uncertainty in nutrient and extraction estimates.

We estimated the mean and 95% credibility intervals of turtle density (ind/ha), biomass (kg/ha), nutrient standing crop (total C, N and P (kg/ha)) and nutrient excretion (total N and P μg/L/h) at each habitat type within a single MCMC model framework. All models (14) were run using JAGS (R2Jags; v.3.2.0) with RStudio (v.0.97.336). We ran 20,000 iterations, with 5,000 burn-in, thinning rate of 3, and three chains. Since we used sex-specific models, we simulated the total (male plus female) density, biomass, nutrient standing crop and nutrient excretion for each habitat type using the sex-specific mean and standard deviation for each metric and 10,000 simulations in RStudio.

RESULTS

Turtle Capture and Density Estimates

We made 586 captures of 369 individuals of 10 species across all three major habitat types of Georgia. Four common species (*Trachemys scripta, Chrysemys picta, Sternotherus odoratus* and *Sternotherus minor*) made up 86% of the total individuals captured across all sites. In WEF (139 total individuals), *T. scripta*, *C. picta*, and *S. odoratus* made up 21, 16 and 57% of total captures, respectively. Other species captured in WEF include the snapping turtle (*Chelydra*

serpentina; 4%) and Eastern mud turtle (*Kinosternon subrubrum*; 2%). In the NOR (78 total individuals), *T. scripta* and *S. odoratus* made up 46 and 19% of individuals captured, respectively. Other species in the NOR included *C. serpentina* (23%), *S. minor* (5%), and a single individual of *C. picta* and the Eastern spiny softshell (*Apalone spinifera*; 1% of total capture each). In the LFRB (152 total individuals), *T. scripta* and *S. minor* made up 40% and 47% of individuals captured, respectively. Other species captured in the LFRB include the Eastern river cooter (*Pseudemys concinna*, 9%), Alligator snapping turtle (*Macrochelys temminckii*; 2%), *A. spinifera* (2%), and Florida softshell (*Apalone ferox*; 1%). There were differences in dominant species across sites (Fig. 3.2), although *T. scripta* was captured at all sites and was among the most abundant species across all sites making up 34% of all individual captures across sites in Georgia (Fig. 3.2).

Density estimates varied across habitat types with the highest density of focal turtle species in this study being found at WEF (Table 3.1, Fig. 3.2). Total turtle density at WEF was nine times greater than those of NOR, which was dominated by high estimates of male (51.4 ind/ha) and female (82.4 ind/ha) *S. odoratus* (Table 3.1, Fig. 3.2). *Trachemys scripta* had similar density estimates among Piedmont sites: WEF and NOR. There was high variation in estimates of *S. odoratus* in NOR sites (Table 3.1). Among our stream sites, turtles estimates were four times greater than LFRB estimates in NOR. Estimates for *T. scripta* and *S. minor* were similar overall in LFRB. However, density of *T. scripta* in LFRB varied by sex with estimates of males being 4.6 times greater than females. Density estimates for *T. scripta* ranged from ~15 - 40 ind/ha across the range of habitat types, with the highest densities being found in LFRB. We did

not estimate juvenile abundance due to low capture. Capture probability was best predicted when allowed to vary among secondary sampling periods within each site (Table 3.2).

Biomass and Standing Nutrient Crop Estimates

Biomass estimates of focal turtle species were highest in the LFRB (45.7 kg/ha) and WEF (39 kg/ha; Fig. 3.3). Within the Piedmont region, biomass was higher in WEF, but this was driven mostly by high densities of *S. odoratus* and the inclusion of *C. picta* in ponds (Fig. 3.2). Among stream sites, turtle biomass estimates from LFRB were more than two times estimates in the NOR, which was largely due to *T. scripta* (Table 3.3). *Trachemys scripta* had the highest biomass estimates for any species across all habitat types ranging from 16.8 - 39.3 kg/ha., which was 11 and 6 times the amount of *Sternotherus* spp. in the NOR and LFRB, respectively. Due to small body size, family Kinosternidae generally had less biomass than Emydidae across habitats (Fig. 3.2).

Turtle whole body AFDM estimates were 0.43 ± 0.07 (N=5), 0.35 ± 0.09 (N=4), 0.38±0.06 (N=17), and 0.43 ± 0.04 (N=4) for *T. scripta, C. picta*, *S. odoratus* and *S. minor*, respectively. Standing crop nutrient estimates for all turtles were 1.4 ± 0.58 kg C ha^{-1}, 0.33 ± 0.14 kg N ha^{-1}, and 0.33 ± 0.14 kg P ha^{-1} across habitat types. Standing crop C, N and P estimates of turtle biomass was generally highest in WEF and LFRB and doubled estimates of C, N and P in the NOR similar to biomass estimates (Table 3.4, Fig. 3.2). In all habitat types, *T. scripta* made up the highest standing crop of C, N and P (Table 3.4). Standing crop of N and P for *T. scripta* was 2-2.4 times greater in the LFRB than other habitat types (Table 3.4, Fig. 3.3).

The average wet mass of focal species varied by habitat type and sex (Table 3.2). *Trachemys scripta* was the largest of the focal species in this study and was on average 3.3, 6.9

and 7.1 times larger than male *C. picta*, *S. odoratus* and S. *minor* of the same habitat, respectively, and 2.9, 9.1 and 10.3 times larger than females (Table 3.2). The average mass of male and female *T. scripta* was larger in stream sites (Table 3.2). *S. odoratus* was larger in body size in the Oconee than WEF (Table 3.2). Mean mass was similar between sexes in *S. odoratus* and *S. minor* (Table 3.2).

Species and Focal Group Nutrient Excretion

Mean N and P excretion rates varied by species and site (Table 3.5). Excretion rates were generally comparable for turtles between the NOR and LFRB (Table 3.5). Nitrogen and P excretion rates were more similar between turtle species within ponds than between turtles from pond versus stream sites. Mean N excretion of *T. scripta* from streams (NOR and LFRB) was much higher than estimates from ponds (Table 3.5). Mass-specific excretion rates were similar for turtle species within habitat type and were highest for *T. scripta* in NOR (Table 3.5). There was a high amount of individual variation in excretion rates (non-mass-specific; Table 3.5), and larger variations in N:P excretion ratios were found in WEF and LFRB than in the NOR (Table 3.5).

Focal species excretion estimates (combined species) ranged from 28,210-56,474 μg N/ha/h and 1256-3,561μg P/ha/h across sites in Georgia (Fig. 3.3). Estimated N excretion rates by turtles per hectare was similar between WEF and LFRB, and approximately twice the rate estimated for the NOR. *Trachemys scripta* contributed the most to estimates of N across systems, but *S. odoratus* and *S. minor* rivaled P excretion rates for *T. scripta* in WEF and LFRB, respectively. Turtle assemblage P excretion was highest in WEF and lowest in LFRB. In stream sites, N and P excretion rates were dominated by contributions from *T. scripta* (Fig. 3.3).

However, rates of N excretion among all turtles were higher in the LFRB whereas P excretion was higher in the NOR.In WEF, both *T. scripta* and *S. odoratus* contributed equally to overall estimates, whereas *T. scripta* dominated estimates in the NOR (Fig. 3.3). All assemblage level estimates had large amounts of variation due to variation in density- and species- level excretion estimates.

DISCUSSION

Long-lived organisms that attain high biomass can represent stable and long-term pools of nutrients in ecosystems (Kitchell et al. 1975, Frank 2008, Vanni et al. 2013). For example, Kitchell et al. (1975) famously estimated that 74% of the suspended P in Lake Wingra (Wisconsin) was bound to fish biomass. The authors estimated that a lake fish assemblage accounted for 0.26 g/m^3 of P, resulting from 600 kg/ha of fresh biomass. In habitats across Georgia, we estimated that common turtle assemblages make up 18 to 46 kg/ha of biomass and up to 1.9, 0.45, and 0.45 kg/ha of standing crop C, N and P, respectively. The variation in estimates of standing crop nutrients of turtle biomass across habitat was related to differences in species assemblages and relative abundances, turtle density estimates, and differences in body sizes within and among species.

The biomass estimates in this study are comparable to the lower end of those reported for the same species in other habitats across the species' ranges. Our results are consistent with other studies that commonly report *T. scripta* and *C. picta* as having the highest density of any turtle species within suitable habitats (Ernst & Lovich 2009). We estimated *T. scripta* biomass between 18 to 46 kg/ha, whereas estimates from Michigan to Pennsylvania range from 40.6 to 877.3 kg/ha (Table 3.5). Though we estimated low *C. picta* biomass within WEF ponds, estimates of *C.*

picta biomass in Midwestern states range from 28.2 to 106.4 kg/ha (Iverson 1982, Ernst & Lovich 2009). Therefore, our estimates of nutrient standing stocks and excretion rates are likely representative but conservative for similar freshwater habitats. Moreover, our estimates do not fully account for the less abundant species or those species less amenable to trapping (Sterrett et al. 2010), and are therefore probably low estimates of nutrient standing stocks and excretion rates for turtle assemblages. Because most estimates of turtle abundance or biomass are for ponded habitats (Iverson 1982, but see Dreslik & Phillips 2005), we are unable to compare estimates from our two river systems to other studies.

Freshwater biodiversity is extremely high in the southeastern United States (Lydeard & Mayden 1995), particularly in the Coastal Plain physiographic region. However, surprisingly few estimates of biomass exist for the ecosystems, making it difficult to place turtle contributions to nutrient dynamics in proper ecological context. Estimates of fish standing stocks in freshwater habitats range from 0.2 - 27.6 g dry mass m^{-2} across a large range of global habitats(reviewed in Turner et al. 1999).In comparison, our estimates of total turtle assemblage dry mass were 0.6, 1.3 and 1.5 g dry mass m^{-2} in the NOR, WEF and LFRB, respectively(converted from wet mass/ha to g/m^{-2} using species dry mass conversions derived from Table 3.3 area corrected). Within the LFRB, estimated biomass of introduced Flathead catfish (*Pylodictis olivaris*) were 3.8 and 6.6 kg/ha in the main stem Flint River and Ichawaynochaway Creek (Kaeser et al. 2011; with a reduction in the Flint River from 32.1 kg/ha in 1985), which is 86% less than our conservative estimate of freshwater turtle biomass in tributaries of the LFBR. Therefore, it is likely that turtles represent a sizeable component of freshwater biomass and are influential in nutrient dynamics

within the LFRB, but we will need information on other vertebrate assemblages to confirm that hypothesis.

In the Piedmont physiographic region, more studies exist for plants and invertebrate stream fauna, but are limited for vertebrates. In the Middle Oconee River (in proximity to the NOR), Grubaugh and Wallace (1995) estimate biomass of a dominant aquatic macrophyte (*Podostemum ceratophylum*) to vary seasonally between 430-700 g AFDM m^{-2} and the benthic macroinvertebrate assemblage to vary between 12.7-27.8 g AFDM m^{-2}, which are several orders of magnitude higher than our estimates of turtle biomass within the NOR (~0.25 g AFDM m^{-2}, converted from kg wet mass/ha to g AFDM m^{-2} dry mass using reported values for AFDM and dry mass, Table 3.3). While estimates of standing crop nutrients for macroinvertebrates do not exist in the Oconee River Basin, the body stoichiometry of macroinvertebrate from a variety of stream ecosystems is much higher than what we have reported for turtles (~20-73 %N:P, 0.6 %P across a range of benthic macroinvertebrate taxa (Cross et al. 2003, Evans-White et al. 2005, Bowman et al. 2005) vs. 1.04 %N:P, 6.48 %P across four species, Sterrett & Maerz in review). This suggests that while turtles may be lower in standing crop biomass compared to macroinvertebrates, they may still be influential in P storage, considering their high body P content (Oconee: turtles - 0.02 g P m^2 vs. macroinvertebrates - 0.08-0.17 g P m^2). In addition, a key function of vertebrates can be to slow the downstream spiraling of nutrients (Small et al 2009), and turtles may be important in this regard within the NOR. Small et al. (2009) proposed that consumers slow the flow of nutrients in stream by increasing the uptake length (S_w), thus increasing the time nutrients are bound in biomass. Phosphorus composes many biological components (e.g. ATP, nucleic acids), yet the majority of body P in higher animals is bound to

mechanical structure such as a bony skeleton (Reiners 1986, Elser et al. 1996). We are unaware of published biomass estimates for fish in the Oconee River Basin (Mary Freeman and Megan Hagler, pers. comm.).

Turtle excretion may be valuable to stream ecosystems, although influences likely vary by site and conditions. It is unlikely that turtle nutrient recycling is valuable to freshwater nutrient budgets in tributaries of the LFRB because of significant nutrient loading and seasonally high hydrologically dependent nutrient concentrations (Fu & Winchester 1994, Golladay & Battle 2002, McEntire 2009). The LFRB stream sites in this study are heavily influenced by natural (e.g. atmospheric deposition, weathering) and anthropogenic (e.g. agricultural) inputs (Fu &Winchester 1994, Golladay & Battle 2002). The NOR is also influenced by urban and poultry farming, but agriculture does not greatly influence water quality in the Upper Oconee Watershed (Fisher et al. 2000). Subsequently, there are large differences in measured N and P concentrations between the LFRB (reported median value 441.4-665.1 μg/L NO_3, 16.3-57.5 μg/L NH_4, 2.1-4.0 μg/L SRP, Upper Ichawaynochaway Creek, Golladay & Battle 2002) and the N. Oconee River (0.00053 μg/L Total N, $4.8e^{-5}$ μg/L Total P, NOR, Fisher et al. 2000). Despite greater biomass in the LFRB of focal species (Fig. 3.2), P excretion rates in the NOR were nearly double the estimates of turtles in the LFRB (Fig. 3.3). Additionally, the rates of measured turtle assemblage excretion for the NOR (28210.2 μg/L N, 2736.3 μg/L P) greatly exceed estimates of background nutrient concentrations for the NOR. However, nutrient uptake of nutrients in rivers is greatly dependent upon flow velocity (Gibson & Meyer 2007, Withers & Jarvie 2008). Uptake rates for P in fifth order agricultural streams are relatively large (16,175-367,000 m, SRP uptake

length, Withers & Jarvie 2008) and suggest lower efficiency of P retention and less P cycling than smaller streams, a result likely due to high seasonal or overall flows.

Nutrient recycling by turtles may have a different influence in lentic systems where internal nutrient cycling controls productivity and hydrologic flushing is reduced (Essington & Carpenter 2000). Turtle assemblage N and P excretion rate estimates which take into account density and species level excretion rate for ponds in WEF (56,354 μg/ha/h N, ~3500 μg/ha/h P, Fig. 3.3) were relatively high compared to estimates of measured N and P concentrations from WEF impoundments (0.004-0.022 Total N mg/L, 0.009-0.02 Total P mg/L, Lewis et al. 1986). Turtle N and P excretion estimates from WEF were also higher than those estimates of NOR. Furthermore, turtle biomass in WEF ponds was twice that of similar species in the NOR (Fig. 3.2). Gido (2002) determined that nutrient loading of three abundant fish species was greater than measured tributary nutrient loading for N and P (43% and 12% of days modeled, respectively) in a large Texas impoundment. We do not have data on volume of WEF or nutrient loading to make direct comparisons with the turtle excretion data in this study. In lentic systems, fish specifically influence nutrient dynamics through benthic feeding behaviors and nutrient translocation into pelagic zones (Kitchell et al. 1975, Andersson et al.1988, Vanni et al. 2005). Similar to fish, freshwater turtles inhabit and feed in littoral areas of lentic ecosystems (Bulte & Blouin-Demers 2008). Because turtles derive their nutrients from littoral areas but are constrained by the ability to bask, it is possible that turtle influences in translocating nutrients are greatest on the margins of these ecosystems.

One common aspect of all ecosystems in this study was that each contained a ubiquitous and abundant omnivorous member of the family Emydidae and a carnivorous member of the

Kinosternidae: *T. scripta* or *C. picta* and *S. odoratus* in WEF and NOR; and *T. scripta* and *S. minor* in the LFRB. Though not universal, this pattern is common across habitat types and a wide geographic portion of the U.S. (Dreslik & Phillips 2005, Conner et al. 2005, Sterrett et al. 2011). Body size and densities of these species were important in determining their importance in overall turtle contributions. Despite high density estimates of *S. odoratus* (133 ind/ha) and *S. minor* (48 ind/ha) in WEF and LFRB, the abundance and large body size of *T. scripta* contributed to higher biomass and standing crop nutrient (C, N and P) estimates in both habitats (Table 3.2, Fig. 3.3). The larger mass and higher proportion of skeleton (Sterrett & Maerz in review) contributes to higher amounts of stored nutrients within stable tissues (Hendrixson et al. 2007). Though the dietary differences of the two families has an effect on nutrient excretion rates (Sterrett & Maerz in review), this difference has a minor effect compared to the large differences in body size in determining the relative influence of species on nutrient dynamics.

Several emergent results may indicate potential sources of variations in turtle biomass and standing crop nutrients between habitats. For example, we found differences in body size distributions between pond and stream sites for *T. scripta* and *S. odoratus* (Table 3.3). These differences are small, yet may accumulate to meaningful differences in nutrient standing crops represented by turtle populations. Additionally we found differences in sex ratios of *T. scripta* between sites (Table 3.1). These differences in sex ratios are important because in large sexually dimorphic species like *T. scripta* and many others (Gibbons & Whitfield 1970), body size makes a significant difference in standing crop nutrients and excretion rates and ratios (Fig. 3.2). Additionally, these changes may be particularly relevant in situations when size-specific selection occurs as a result of road mortality or harvest (Steen & Gibbs 2004, Mali et al. 2014)

Nutrient excretion in vertebrates is positively related to ingested nutrients and constrained, or negatively related to demand to grow and maintain body tissues (Sterner & Elser 2002, Vanni 2002, Sterrett & Maerz in review). *Trachemys scripta* had the highest molar N:P excretion estimate, which was 12 times larger than *Sternotherus* spp. (Table 3.5). Because all turtle species in this study were relatively similar in body C, N and P stoichiometry (%N:P=1.04, Sterrett & Maerz in review), the differences we observed in excretion rate and ratio between these species are likely a result of diet (see also Chapter 2, Fig. 3.2C). Both *T. scripta* and *C. picta* undergo an ontogenetic shift from carnivory to a diet of mostly plant material (Bouchard & Bjorndal 2006). The nutritional quality (C:P, N:P) of plant material is lower than animal material, creating a stoichiometric imbalance. Therefore, animals compensate for this deficiency by eating at higher rates (Sterner & Elser 2002, Grimm 1988).

The N and P excretion rate estimates for turtles per hectare (non-mass-specific) are much lower than estimates of zooplankton, benthic macroinvertebrates and fishes (reviewed in Vanni 2002). Turtle assemblage estimates range from 0.06-0.14 mg N/m^2/day and 0.003-0.008 mg P/m^2/day (rescaled to day and area-corrected from data in Fig. 3.3). However, turtle species mass-specific excretion rates (Table 3.5) are generally similar or higher than estimates of salamanders (Milanovich 2010, Keitzer & Goforth 2013) and freshwater fishes (Vanni et al. 1996, Hood et al. 2005). Low assemblage level estimates compared to other taxa may be due to differences in density. Turtles assemblage estimates are likely overestimated because we assume excretion over a 24 hour period, which is unlikely for turtles. Gido (2002) used a diel conversion factor to account for reductions in excretion rates at night.

The inferences made about nutrient excretion estimates in this study may have been influenced by temperature and capture methods. Therefore, research is needed to test whether our results are consistent across a wider range of conditions. Temperature and handling stress can affect measurements of excretion from wild animals in an experimental arena (Vanni 2002, Whiles et al. 2009). Temperature can be particularly problematic for measuring excretion in ectothermic organisms due to temperature dependent metabolic physiology (Vanni 2002, Whiles et al. 2009). Consumption, assimilation and digestion rates are temperature dependent in turtles with warmer temperatures increasing rates of processing (Kapenis & McManus 1974, Avery et al. 1993). Annual air temperatures were at their highest in Georgia during this study (May - September), regularly reaching 32°C. While we shaded experimental containers to decrease temperature fluctuations and minimize handling times in field excretion measurements, it is possible that this impacted the rates we measured. Other researchers have used devices (i.e. finger cot, condom) fitted to the rear carapace to measure egested materials in a laboratory setting (Bjorndal 1991, Avery et al. 1993). Because the goal of the current study was to measure dissolved nutrients from wild caught turtles, these methods were infeasible, therefore modifications were made to existing CMNR methods (Vanni 2002). Furthermore, the large variation in species and assemblage level estimates could be a result of a number of factors. Assemblage level estimates take into account variation associated with density estimates (Table 3.1) and variation in species excretion rates from each site (Table 3.5). Species level excretion rates may be influenced by feeding prior to measurements, trapping stress effects or a number of other issues. Turtles were potentially in traps for >8 hours prior to measuring excretion. Further

research should compare our excretion results with excretion rates of turtles captured using direct capture methods (i.e. dipnet, snorkeling, Sterrett et al. 2010).

Our estimates of turtle assemblage biomass, standing crop nutrients and nutrient recycling are conservative considering our methods used to capture turtles. Several large and locally abundant species from both ponds and streams were not accounted for in this study due to our methods of capture and logistics of measuring excretion. For example, in the N. Oconee River, the northern snapping turtle (*Chelydra serpentina*) is a common inhabitant and accounted for 23% of captures. This species is among the largest freshwater species and most extensive geographic ranges in North America (Steyermark et al. 2008, Ernst & Lovich 2009). Although the capture of this species is among the lowest of any species in WEF in this study, the large body size of *C. serpentina* suggests that they may also constitute large pools of nutrients in areas where they are common.

In the LFRB, two other species, Eastern river cooter (*Pseudemys concinna*) and Barbour's map turtle (*Graptemys barbouri*), are locally abundant (Sterrett et al. 2011), but were not considered in the current study due to sampling constraints. That is, other capture methods (i.e. snorkeling, basking traps) produce higher detection rates and are needed to sample these species in the LFRB (Sterrett et al. 2010). Both *P. concinna* and *G. barbouri* are large bodied species and comparable to *T. scripta* in morphology, with the exception of differences in skull morphology. While *P. concinna* has a similarly proportionate skull to *T. scripta*, *G. barbouri* has an exceptionally large skull due to sexually dimorphic traits related to molluscivory (Lindeman 2000). *Pseudemys concinna* and *G. barbouri* made up 16% and 15% of captures in a survey of river turtles in the LFRB (Sterrett et al. 2011). While these species are often locally abundant in

certain areas, their density estimates rarely rival estimates of the focal species in this study where they overlap. However, biomass estimates of female *P. concinna* can range from 48 to 390 kg/ha in stream systems (Jackson & Walker 1997). Herbivorous turtle species tend to have the highest standing crop biomass estimates among others turtles (Iverson 1982). Accounting for these species would increase estimates of biomass and nutrient standing crop.

Nutrients bound in animal biomass have potentially long-term impact on nutrient dynamics in freshwater, yet their role as true sinks or sources remain a point of discussion. Vanni et al. (2013) suggested that fish may be nutrient sinks when their body nutrients do not become readily available to other organisms during decomposition. When organisms die, the nutrients in their bodies become liberated and act as sources of limiting nutrients (Kitchell et al. 1979). An extreme example of a nutrient pulse from decomposition is anadromous Atlantic salmon (*Salmo salar*), which grows to adulthood in marine systems but breed and die in freshwater leaving behind enormous amounts of marine-derived N and P subsidies (Mathisen et al. 1988, Jonsson & Jonsson 2003). Parmenter and Lamarra (1991) illustrated experimentally that rainbow trout (*Oncorhynchus mykiss*) lost only 60% of the original P bound in freshwater decomposition over a 10 month period and noted that bone decomposition is likely a much slower process than breakdown of other tissues. It has been suggested that the P in bones and scales may become unavailable to biotic use and even buried by sediments (Kitchell et al. 1975, Parmenter & Lamarra 1991). As far as we are aware, the temporal limit of bone decomposition has yet to be tested. However, whale-falls (i.e. marine whale decomposition) create long-term sources of limiting nutrients in marine ecosystems that last years (Smith et al. 1989). Little is known about the circumstances of turtle death (i.e. predation vs. physiological; Gibbons 1987). If turtles die in

aquatic habitats, their large bony skeleton may become a source or sink of limiting resources such as P and Ca.

Common organisms, or those that are highly abundant and widespread, shape ecosystems and comprise the bulk of biomass, yet they are often overlooked for their roles in ecosystem function (Gaston & Fuller 2008, Gaston 2011). For example, Burton and Likens (1975) found that 93% of salamander biomass in stream at Hubbard Brook was dominated by one species (*Plethodon cinereus*) which was responsible for most of the assemblage level nutrient recycling. The turtle species highlighted in the current study are among the most common and well-studied species of North America, and arguably the world. In particular, *T. scripta, S. odoratus*, and *C. picta* have wide geographic native ranges in the U.S. and use a variety of freshwater habitats, frequently moving between habitats (Burke et al. 1995, Buhlmann et al. 2009, Ernst & Lovich 2009). Although species abundance generally declines with increases in body size among animals (Gaston 2011), *T. scripta* is an example of a species that both attains a large body size and also is found at high relative density.

The contributions of turtles standing crop and remineralized nutrients has conservation and global change implications. Red-eared sliders (*T. scripta elegans)* are a globally wide-spread and potentially invasive species (Cadi & Jolly 2004, Polo-Cavia et al. 2009, Rodder et al. 2009). In habitats where they are non-native, *T.s. elegans* amass high densities and potentially outcompete native species for food and basking sites (Cadi & Jolly 2004, Polo-Cavia et al. 2008). Invasive species also have the ability to alter ecosystem nutrient cycling (Crooks 2002, Dukes & Mooney 2004). For example, non-native catfish, (*Pterygoplichthys* spp., Family Loricariidae) can create nutrient hotspots because they feed in high-density aggregations and

attain biomass greater than native species (Capps & Flecker 2013). Turtles are also among the most exploited and imperiled vertebrates around the globe (Lydeard & Mayden 1995, Dudgeon et al. 2006). Half of the turtle species are threatened or endangered (TTWG 2012). The largest and most common species are also the most overexploited (Gaston 2010). From 2002 to 2009, over 126 million turtles were exported from the U.S., with an estimated 19% (~24 million) harvested from wild populations. Females and particular genera (e.g. *Trachemys, Pseudemys, Chelydra*) are targeted due to their large sizes (Reed & Gibbons 2003, Mali et al. 2014). This level of harvest is unsustainable and leads to local and regional population declines (Klemens & Thorbjarnarson 2000, Heppel 1998). Our results suggest that, in addition to the negative effects on populations, the harvest of freshwater turtles should be seen as both an extraction of key nutrients such as P, as well as the loss of an important biotic capture and retention pool within freshwater ecosystems. Similar arguments have been made about the large scale harvest of other animals on nutrient cycling (Barraclough & Robinson 1972, Maranger et al. 2008), and experimental manipulation of commonly harvested species results in measurable changes in ecosystem function (Solan et al. 2004, Taylor et al. 2006).

Our results suggest that turtle biomass, in combination with their high tissue nutrient concentrations (Sterrett & Maerz, in review), are potentially a substantial portion of standing stock nutrients, especially P, in freshwater. However, more research is needed to evaluate the ecological relevance of turtle excretion in freshwater nutrient budgets. Turtles are abundant consumers in freshwater ecosystems (Aresco & James 2005) and represent long-term storage units for critical limiting nutrients. Future research should focus on the role of turtle diets and resource availability toward the contributions of turtles to nutrient recycling, and studies of other

vertebrate and invertebrate fauna within freshwater ponds and rivers are needed to determine the collective and unique effects species may have on ecosystem processes.

ACKNOWLEDGMENTS

This research was supported in part by a crowdfunded project through the SciFund Challenge. We thank Jai Ranganathan and Jarrett Byrnes for organizing the SciFund Challenge and all 104 crowdfunding participants, especially Mary Freeman,Dale Hoyt, Linda & Donald Hudson, Kaye Maxwell, and Lisa Maxwell, who helped to fund this research. This work was also supported by an innovative and interdisciplinary grant through the University of Georgia. We thank Vanessa Kinney and Cassandra Skaggs for help with field work and Katie Bentley and William Booker for help with lab work. Turtles were collected under a Georgia Department of Natural Resources Scientific Collecting Permit (#29-WJH-13-191). Procedures in this study were approved by the University of Georgia Institutional Animal Care and Use Committee (Animal Use Protocol #A2011 01-024-Y3-A3).

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Table 3.1. Density estimates for four focal turtle species (*Trachemys scripta, Chrysemys picta, Sternotherus odoratus, Sternotherus minor*) by sex and habitat type (Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) with standard deviation and upper and lower 95% credibility intervals.

	Estimate	SD	Lower CI	Upper CI
WEF				
T. scripta - male	9.6	2.0	7.6	14.1
- female	14.2	1.6	11.9	18.1
-total	23.8	2.5		
C. picta - male	11.8	1.7	9.8	15.9
- female	10.3	1.8	7.8	15.0
-total	22.0	2.5		
S. odoratus - male	51.4	6.5	41.4	66.6
- female	82.4	8.9	67.5	102.8
- total	133.8	11.3		
WEF total turtle	~180			
NOR				
T. scripta - male	9.4	2.8	6.7	15.7
- female	5.4	2.3	2.9	12.3
- total	14.8	3.9		
S. odoratus - male	6.8	11.6	2.7	23.5
- female	2.6	1.6	1.2	6.7
- total	9.4	5.6		
N. Oconee total turtle	~24			
LFRB				
T. scripta - male	32.5	13.2	16.7	79.64
- female	7.1	3.3	3.8	15.8
- total	39.5	13.6		
S. minor - male	24.4	8.9	14.1	47.14
- female	24.4	9.4	14.5	44.5
- total	48.8	12.9		
LFRB total turtle	~88			

Table 3.2. Number of parameters (K), Akaike's information criterion (AIC_c), ΔAIC_c, and model weights (w_i) for candidate models predicting capture probabilities for all turtle species (n=4) across all sites (n=9) in the Whitehall Experimental Forest, North Oconee River and tributaries of the Lower Flint River Basin from May 2010 through August 2012.

Candidate model	Interpretation	K	AIC_c	ΔAIC_c	w_i
secondary period	Capture probabilities vary among secondary sampling periods within each site	81	3214.6	0.0	1.000
primary period	Capture probabilities vary among each primary period within each site	21	3718.0	503.4	0.000
constant	Capture probabilities are constant across each site	9	3820.0	605.4	0.000

Table 3.3. Mean wet and dry mass (and standard deviation) of all individuals of four focal species (*Trachemys scripta, Chrysemys picta, Sternotherus odoratus, Sternotherus minor*) by sex across three habitat types (Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) across Georgia.

	wet mass (g)	dry mass (g)
T. scripta		
WEF - male	697.0 (337.9)	224.0 (110.4)
WEF - female	919.2 (449.1)	296.6 (146.8)
NOR - male	892.2 (344.3)	287.7 (112.5)
NOR- female	1575.5 (638.8)	511.0 (208.8)
LFRB - male	899.6 (288.5)	290.2 (94.3)
LFRB - female	1405.4 (588.0)	455.5 (192.2)
C. picta		
WEF - male	210.8 (54.86)	75.23 (19.5)
WEF - female	321.9 (57.8)	114.8 (20.6)
S. odoratus		
WEF - male	100.3 (29.2)	34.9 (13.1)
WEF - female	100.6 (23.1)	35.09 (10.4)
NOR - male	153.5 (30.0)	58.8 (13.4)
NOR - female	161.8 (29.7)	62.51 (13.3)
S. minor		
LFRB - male	126.5 (28.2)	46.8 (10.2)
LFRB - female	136.6 (39.9)	50.5 (14.4)

Table 3.4. Mean standing crop estimates of Carbon, Nitrogen, and Phosphorus (kg/ha; with standard deviation) of focal turtle species (*Trachemys scripta, Chrysemys picta, Sternotherus odoratus, Sternotherus minor*) across three habitat types (Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) in Georgia.

	WEF			**NOR**			**LFRB**		
	C	N	P	C	N	P	C	N	P
T. scripta	0.80 (0.34)	0.19 (0.08)	0.19 (0.08)	0.67 (0.32)	0.16 (0.08)	0.16 (0.08)	1.58 (0.74)	0.38 (0.18)	0.38 (0.18)
C. picta	0.25 (0.1)	0.04 (0.01)	0.04 (0.01)	-	-	-	-	-	-
S. odoratus	0.48 (0.14)	0.12 (0.04)	0.12 (0.04)	0.07 (0.04)	0.02 (0.001)	0.02 (0.001)	-	-	-
S. minor	-	-	-	-	-	-	0.29 (0.1)	0.07 (0.02)	0.07 (0.02)
Total	1.53	0.35	0.35	0.74	0.18	0.18	1.87	0.45	0.45

Table 3.5. Focal turtle species (*Trachemys scripta, Chrysemys picta, Sternotherus odoratus, Sternotherus minor*) N and P mean excretion rate (μg L^{-1}· h^{-1}; standard deviation) and mean mass-specific excretion rate (μg L^{-1}· g dry mass · h^{-1}) and molar N:P excretion in Whitehall Experimental Forest (WEF), N. Oconee River (NOR) and the Lower Flint River Basin (LFRB) in Georgia.

		N				P				N:P (molar)
	n	mean	sd	mass-sp mean	mass-sp sd	mean	sd	mass-sp mean	mass-sp sd	
WEF										
T. scripta	1	172	-	4.6	-	0.3	-	0.01	-	1279.2
C. picta	14	331.8	187.5	4.3	1.6	9.9	14	0.1	0.12	226.8
S. odoratus	30	104.3	52.5	3.3	1.7	10	14.3	0.3	0.44	102.4
NOR										
T. scripta	7	1607.6	1211.4	10.3	9.8	156	163.2	1.4	1.5	33.2
S. odoratus	2	335.8	214.2	6	4.6	20.9	10.5	0.37	0.24	34.2
LFRB										
T. scripta	18	1138.6	892.2	6.5	8.3	14.3	22.3	0.11	0.21	1294.9
S. minor	19	184.1	130.4	3.7	2.8	9.6	12.7	0.2	0.3	107.3

Table 3.6. Review of density and biomass estimates for four focal species (*Trachemys scripta*, *Chrysemys picta*, *Sternotherus odoratus*, *Sternotherus minor*) across habitats in the United States, including estimates from this study ((Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB))

Species	Habitat Type	Location	Density (ind/ha)	Biomass estimates (kg/ha)	Reference
Chrysemys picta	Lake	Indiana	48.8	11.2	Wade and Gifford 1965
	Pond	Illinois	0.14	-	Dreslik et al. 2005
	Pond	Michigan	576	28.2	Gibbons 1968
	Pond	Pennsylvania	591	106.4	Ernst 1971
	Pond	Michigan	89.5	16.6	Congdon et al. 1986
	Pond	Michigan	39.9	7.2	Congdon et al. 1986
	Pond	Michigan	41.6	7.4	Congdon et al. 1986
	Pond	**Georgia**	**22.0**	**5.8**	**This study (WEF)**
Trachemys scripta	Pond	South Carolina	61.5	33.6	Congdon et al. 1986
	Pond	Michigan	41.8	37.1	Congdon et al. 1986
	Pond	South Carolina	353	877.3	Congdon et al. 1986
	Pond	Illinois	40.2	26.14	Dreslik et al. 2005
	Pond	Florida	361.4	282.6	Auth 1975
	Pond	South Carolina	88	40.6	Gibbons 1970
	Lake	Alabama	148.5	10.6	Dodd 1989
	Pond	**Georgia**	**23.8**	**19.8**	**This study (WEF)**
	Stream	**Georgia**	**14.7**	**16.8**	**This study (NOR)**
	Stream	**Georgia**	**39.7**	**39.3**	**This study (LFRB)**
Sternotherus odoratus	Creek	Oklahoma	150	10.2	Mahmoud 1969
	Lake	Virginia	194	13.6	Mitchell 1988
	Pond	Illinois	2.67	0.42	Dreslik 2005
	Lake	Indiana	79.5	8.35	Wade and Gifford 1965
	Pond	South Carolina	7.5	1.2	Congdon et al. 1986
	Pond	South Carolina	21.8	1.4	Congdon et al. 1986
	Pond	Florida	700	41.7	*derived in Iverson 1982
	Pond	Illinois	_	10	Reehl et al. 2006
	Pond	**Georgia**	**133.3**	**13.4**	**This study (WEF)**
	Stream	**Georgia**	**11.0**	**1.5**	**This study (NOR)**
Sternotherus minor	Spring	Florida	2857	45.7	Iverson 1982 (estimated from Cox and Marion 1979)
	Spring	Florida	-	12.5	Meylan et al. (1992)
	Stream	**Georgia**	**48.0**	**6.42**	**This study (LFRB)**

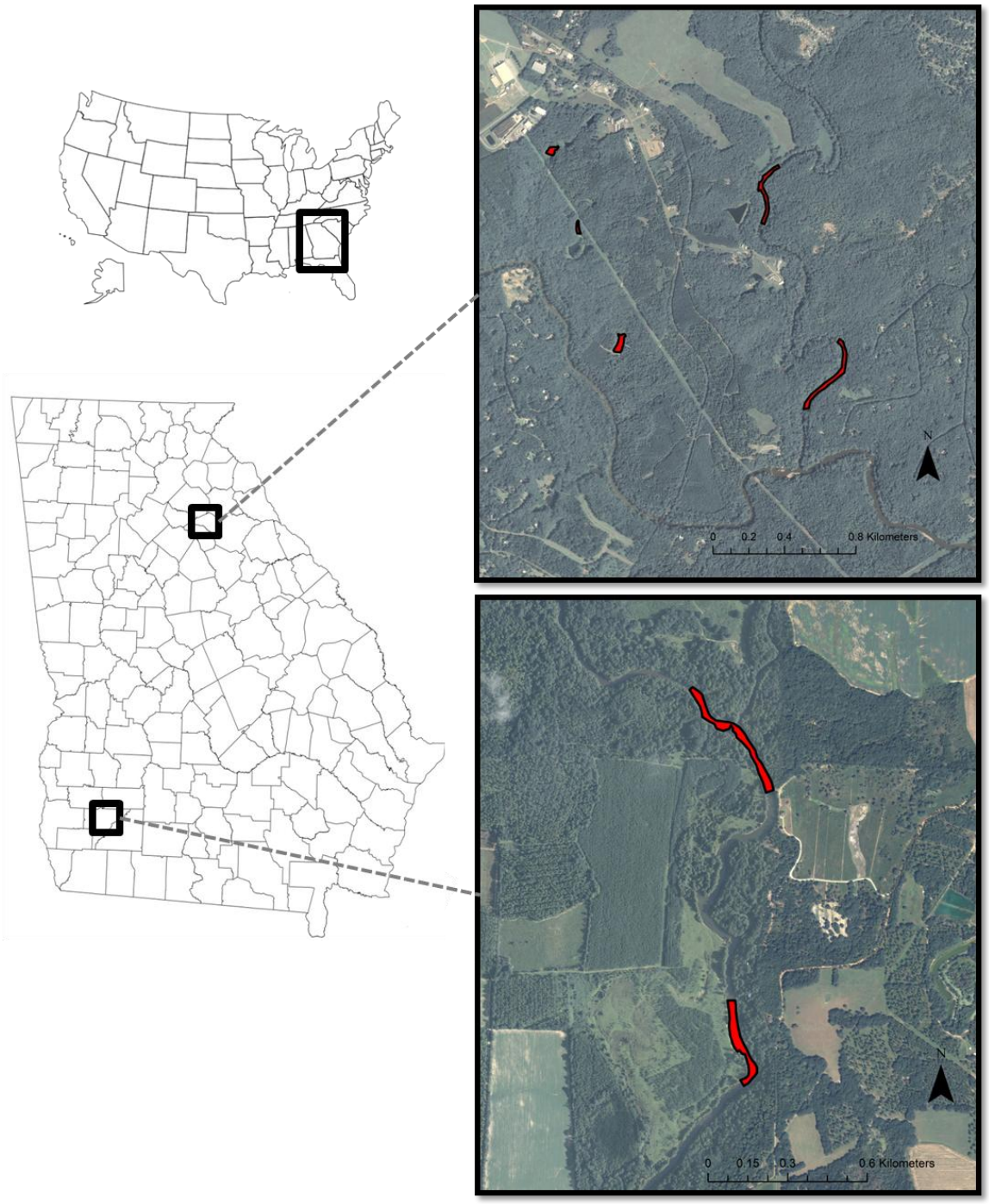

Figure 3.1. Study sites (in red) from Whitehall Experimental Forest and North Oconee River (top panel) and Lower Flint River Basin (bottom panel) in Georgia, USA, which were sampled for turtles to estimate density, biomass and standing crop nutrients and aggregate turtle excretion. One site from N. Oconee River and Spring Creek not pictured.

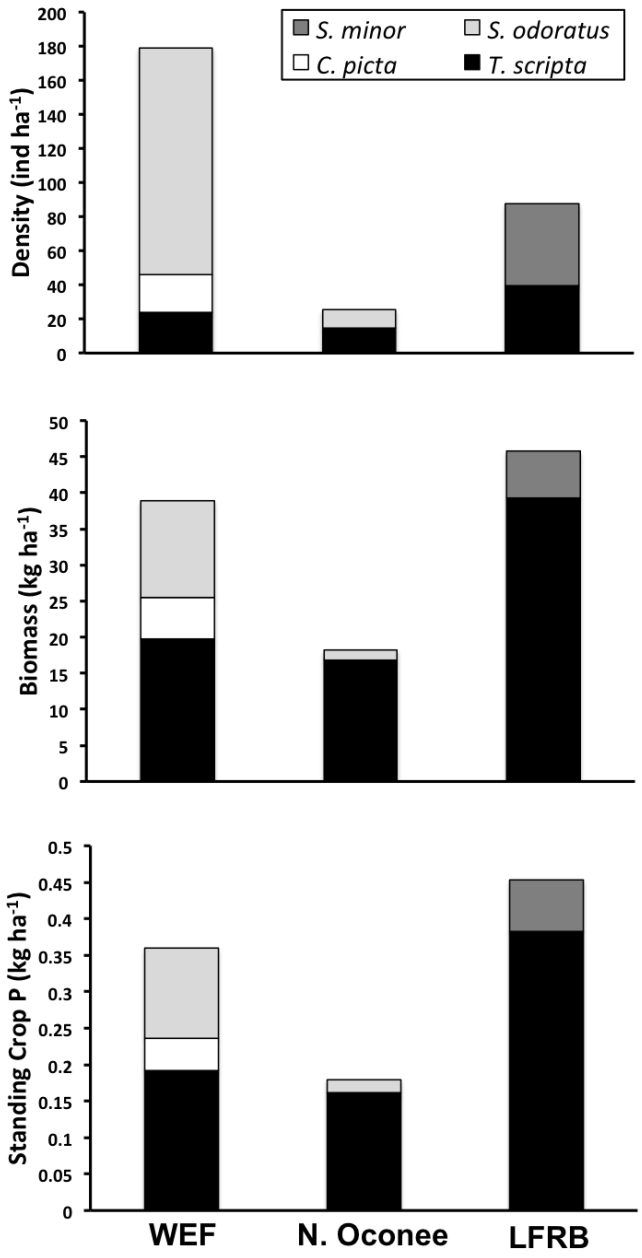

Figure 3.2. Density (ind ha^{-1}), biomass estimates (kg ha^{-1}) and standing crop P (kg ha^{-1}) of focal turtle species (*Trachemys scripta*, *Chrysemys picta*, *Sternotherus odoratus*, *Sternotherus minor*) as a percentage of the total across three habitat types ((Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) in Georgia, USA.

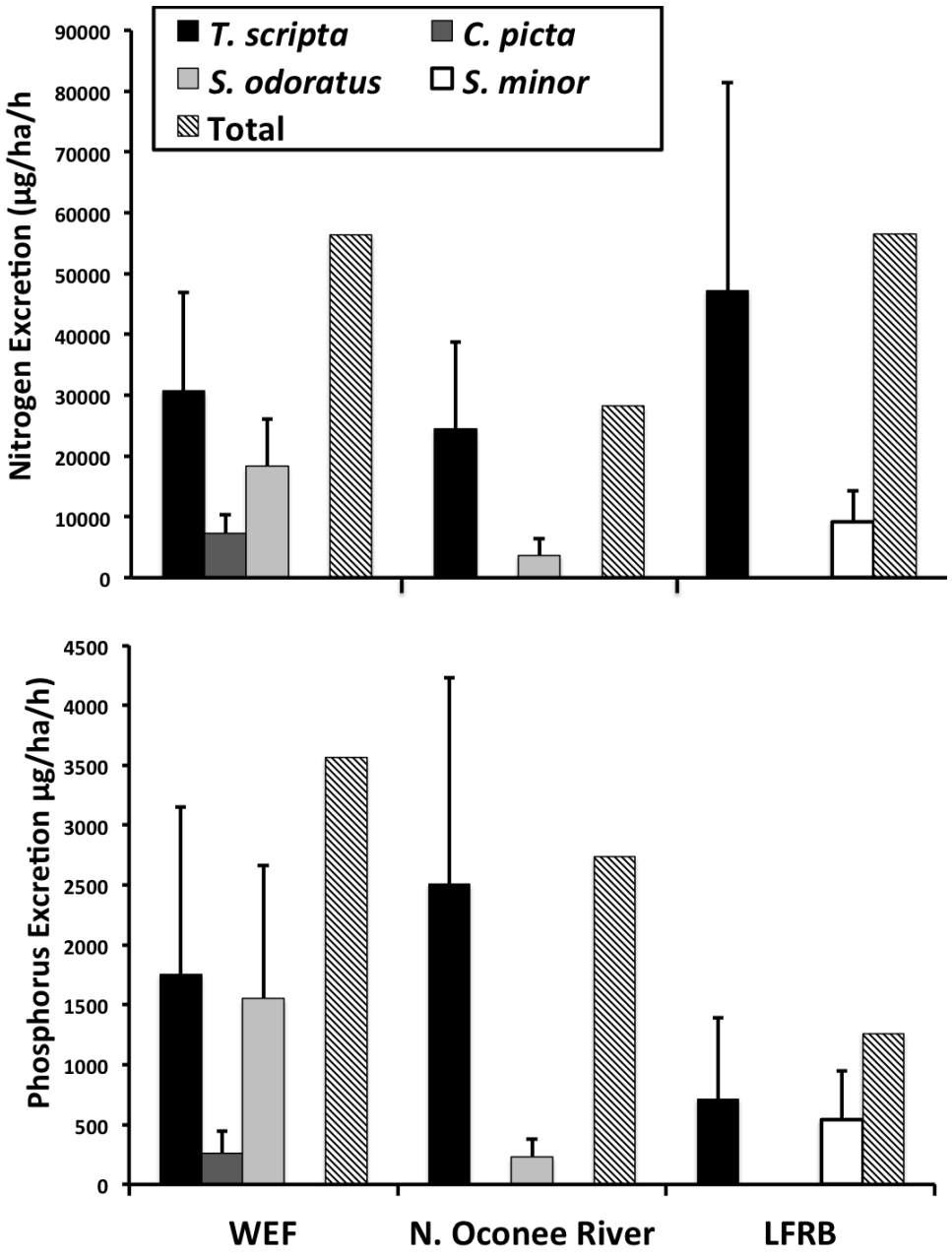

Figure 3.3. Assemblage level nutrient excretion rates (µg ha^{-1} h^{-1}; mean ± standard deviation) of turtles (*Trachemys scripta, Chrysemys picta, Sternotherus odoratus, Sternotherus minor*) at different habitat types ((Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) across Georgia, USA.

CHAPTER 4

AN EXPERIMENTAL TEST OF A STOICHIOMETRIC CASCADE OF JUVENILE PAINTED TURTLES ON DECOMPOSING LEAF LITTER[1]

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ABSTRACT

Trophic cascades where a lower level producer is positively affected by predator consumption of secondary grazers can be uncommon or dampened in many ecosystems. One potential reason for this may be the counteractive effects of higher level consumers on resource stoichiometry. In the case of decomposition, predator recycling of nutrients may stimulate microbial production, resulting in accelerated decomposition despite predator reductions of detritivore abundance. Juvenile freshwater turtles are abundant predators of macroinvertebrates, and turtles are unique in their stoichiometry, potentially affecting ecosystem processes through nutrient recycling. We used mesocosms to test the hypotheses that turtle consumption of invertebrates would indirectly lead to reduced leaf litter decomposition; however, turtles would increase concentrations of remineralized nutrients, leading to altered leaf litter stoichiometry, which would be correlated with leaf litter decomposition. As predicted, the presence of a juvenile turtle reduced macroinvertebrates by 94%; however, turtle presence had no measurable effect on DIN or PO_4 concentrations in the water. We found no difference in % leaf mass loss between mesocosms with and without turtles; however, the presence of turtles did increase leaf concentrations of N and P by 76% and 200% respectively. Three factors explained 92% of the variation in % leaf mass loss among mesocosms. DIN at two weeks was positively correlated with leaf mass loss, while the concentration of macroinvertebrates and leaf concentration of P were negatively correlated with leaf mass loss. These results indicate that macroinvertebrates were preferentially consuming N and P rich portions of leaves. Turtle consumption of invertebrates did not yield a reduction in decomposition because the increased N and P in leaves likely increased microbial breakdown, resulting in a

compensatory mode of decomposition. Juvenile turtles did not appear to affect decomposition via nutrient recycling, probably because the high P demand of juvenile turtles to develop their shell resulted in very low P excretion, which may differ from the effects of adult turtles. This study demonstrates that, in addition to effects on primary consumers, accounting for direct and indirect effects of predators on available nutrients and resource stoichiometry may explain the lack or dampening of ecological cascades.

INTRODUCTION

Consumers alter their environments by changing nutrient availability through predation and nutrient recycling (Kitchell et al. 1979, Vanni 2002, Schmitz et al. 2010). This reality challenges the conventional characterization of consumers as exerting strictly "top down" effects on ecosystem processes. By directly or indirectly affecting nutrient recycling, higher level consumers may also have "bottom up" effects on ecosystem processes (Hunter & Price 1992, Dodds 2009, Schmitz 2010). Separating the effects of top consumers on prey regulation versus nutrient recycling is challenging and often requires a series of complex experiments (Rosemond et al. 1993, Stiling & Rossi 1997). Nonetheless, alarming rates of consumer losses, particularly larger species, demands that we understand the effects of those consumers on ecosystem processes (Naeem et al. 1994, Srivastava & Vellend 2005, Estes et al 2011, Grimm et al. 2003).

Predators can regulate lower trophic levels through consumptive and non-consumptive effects, which can lead to impacts on ecosystem processes (Schmitz 2006, Schmitz et al. 2010). For example, predatory fishes can reduce benthic macroinvertebrate populations, thereby indirectly decreasing leaf litter decomposition rates (Konishi et al. 2001, Ruetz et al. 2002, Mancinelli et al. 2002, Boyero et al. 2008). This type of indirect top down regulation creates a classic trophic cascade, which has been demonstrated in various ecosystems to influence primary production and decomposition (Carpenter et al. 1985, Pace et al. 1999, Shurin et al. 2002, Moore et al. 2004, Schmitz 2010). Trophic cascade effects are generally stronger in aquatic ecosystems, compared to terrestrial ecosystems (Schmitz et al. 2000, Shurin et al. 2002). However, there are many hypotheses that exist to explain the variation in magnitude or dampening effects on

trophic cascades (e.g. food web diversity or ecosystem productivity; Polis & Strong 1996, Shurin et al. 2002). Dampening effects may also be a result of predators having compensatory impacts on primary production or decomposition (i.e. bottom up effects) by increasing available nutrients, a indirect result of consumption (Vanni et al. 1997).

Consumers enhance and alter nutrient availability by transforming organic materials into bioavailable nutrients via excretion and egestion processes (Consumer-mediated nutrient recycling, CMNR; Sterner & Elser 2002, Vanni 2002). The nutrients that an organism recycles are a product of their diet and body stoichiometry, such that nutrients not sequestered for growth or reproduction are recycled and made available to other organisms (Sterner & Elser 2002, Vanni 2002). There are many examples of predator effects on nutrient dynamics including shrimp, fishes, and amphibians (Crowl et al. 2001, Schaus et al. 2002, Beard et al. 2007, Iwai & Kagaya 2007). In aquatic systems, the role of consumer-mediated nutrient recycling is particularly compelling because some freshwater systems support high biomass of large consumers and water can quickly distribute nutrients for uptake (Shurin et al. 2006, Schmitz et al. 2010).

Nutrient availability and ratios are particularly influential on decomposition in freshwater ecosystems. Many freshwater systems are supported by the decomposition of allochthonous resources (Moore et al. 2004, Tank et al. 2010). Detritus ontogeny depends on the actions of other taxa within the food web (Moore et al. 2004). The nutritional qualities of leaf detritus increase with the colonization of bacteria and fungi, subsequently increasing the potential for decomposition (Arsuffi & Suberkropp 1985, Cummins 1974). Macroinvertebrate grazers and shredders consume microbial biofilms, and in doing so, often fragment leaf litter and further accelerate decomposition. Increasing the availability

of dissolved nutrients such as nitrogen (N) and phosphorus (P) can stimulate microbial growth, which can also accelerate decomposition (Gulis & Suberkropp 2003, Gulis et al. 2004, Benstead et al. 2009). Though the realization that consumers could affect nutrient availability through recycling is not new (Kitchell et al. 1979); it is only within the last decade that there has been expanded attention to the ways consumers affect nutrient dynamics within and across ecosystem boundaries (Vanni 2002, Schmitz 2010, Schmitz et al. 2010).

Turtles are abundant consumers in many freshwater ecosystems (Aresco & James 2005). Their extreme morphology and life history suggest turtles may have unique effects on freshwater processes (Sterrett & Maerz in review, Sterrett et al. chapter 3). Turtles can have high abundance and biomass, and high skeletal mass makes turtles significant standing stocks of nutrients, notably phosphorus. Because turtles are extremely long-lived (Iverson 1982a, Congdon et al. 1986, Gibbons 1987), they likely represent stable reservoirs of nutrients barring significant mortality or harvest. Turtles are relatively voracious consumers of plants and invertebrates (Iverson 1984, Ernst & Lovich 2009). Many turtles undergo an ontogenetic shift from a carnivorous diet as a juvenile to an omnivorous or herbivorous diet as an adult (Clark & Gibbons 1969, Bouchard & Bjorndal 2006). This diet shift usually corresponds with an ontogenetic shift from shallower margins of habitats to deeper areas (Congdon et al. 1992). Recent evidence indicates that juvenile turtles have a high P demand for the production of their shell, and that this P demand likely declines with maturation (Sterrett & Maerz in review). As a result, adult turtles can excrete high amounts of N and P, while juvenile turtles may limit P excretion,though this hypothesis remains untested.

Turtles are among the most globally imperiled vertebrates, largely as a result of overharvesting and habitat loss and degradation (Buhlmann et al. 2009). Understanding the effects of turtles on ecosystem processes is important for understanding the wider consequences of their declines. In the only study we are aware of to date, Lindsay et al. (2013) manipulated the presence of red-eared sliders (*Trachemys scripta elegans*) in small, artificial ponds and found turtle presence increased water pH, conductivity, sedimentation, macroinvertebrate abundance, and leaf litter breakdown. However, the authors did not report whether they stocked their artificial ponds with adult or juvenile turtles, and they did not examine the mechanisms of turtle effects on ponds. Therefore, the objectives of our study were to quantify the direct (e.g. predation) and indirect (e.g. nutrient remineralization) effects of turtles on leaf litter decomposition in a simplified detritus-based food web. For this study we focused on the effects of juvenile turtles, which allowed us to create small mesocosms with turtle biomass per unit area comparable to natural ponds and to focus on the highly carnivorous juvenile life stage of turtles. We hypothesized that juvenile turtles would directly reduce the abundance of aquatic macroinvertebrates and that macroinvertebrate concentration would be positively correlated with leaf decomposition, and therefore, the presence of turtles would reduce leaf mass loss via detritivore processing. However, we also hypothesized that turtles would sustain higher water column concentrations of inorganic N and P, which would increase leaf litter concentrations of N and P. We hypothesized that leaf litter N and P would be correlated with leaf litter mass loss, and therefore, the presence of turtles would accelerate leaf mass loss via microbial decomposition. Depending on the relative compensatory influences of turtles on leaf stoichiometry and detritivore concentrations,

the presence of turtles might have a marginal effect on leaf litter decomposition though decomposition pathways may differ in the presence versus absence of turtles.

METHODS

Experimental design

We established 12 total replicate mesocosms in the Whitehall Experimental Forest, Clarke County, Georgia from 21 May - 26 June 2012. We used ultra-violet resistant polyethylene aquaculture tanks (152 x 86cm, 1900L at full capacity, Rubbermaid®) as mesocosms. Tanks were set up within 40 m of forested habitat and approximately 300 m from a permanent water body. A clean, concrete cinder block was positioned in the center of each mesocosm to act as both refuge and basking substrate for turtles, and served as a reference point for changes in water level. Seventeen days prior to the start of the experiment, tanks were filled with tap water to a depth of 13 cm and allowed to age for 4 days. Water from a local pond was filtered through a 250 μm mesh to remove macroinvertebrates, and added to each tank to bring the volume of water to the top of the cinderblock (final volume ~354 L). During the experiment, two large rain events (5/29/12 and 6/11/12) from tropical storms raised the water level in all mesocosms approximately 5 cm from standard water level. Approximately 20-40 L of surface water was removed from each tank the morning after the rain to return mesocosms to standard level. Mesh covers were placed on top of all mesocosms to prevent trespass by tree frogs and large predatory macroinvertebrates (i.e. Odonata), but allow other potential macroinvertebrates to colonize tanks. Temperature loggers (Hobo®, Onset Computer Corporation) were placed in six tanks to monitor temperature changes over time.

We added Ogeechee tupelo (*Nyssa ogeche*) leaves collected during Fall 2011

from Tift County, Georgia. Leaves were collected directly following abscission, and air dried and stored in large, paper bags. We added 270 g of leaves to each mesocosm on 18 May 2012, three days prior to adding macroinvertebrates and turtles.

On "Day 0", we added cultured *Hyalella azteca* (Amphipoda) composed of two size classes to each mesocosm. *H. azteca* are a widespread, abundant and tolerant epibenthic amphipod that inhabits primarily lentic habitats. They are known for being habitat generalists with an affinity for organic detritus (Doig & Liber 2010). We added ninety juvenile (1-3 days old) and 50 subadult (13-14 days old; *H. azteca* mature at 19-21 days and produces 3-17 offspring, (per. comm. Pete Lasier; Othman &Pascoe 2001) amphipods to each mesocosm. We randomly assigned one juvenile Eastern painted turtle (*Chrysemys picta*) to half of the mesocosms to act as a predator treatment (6 turtle present and 6 turtle absent replicates). *C. picta* are widespread in the United States and they inhabit areas with slow moving water, such as ponds, swamps and streams. *C. picta* are carnivorous as juveniles and omnivorous as adult (Clark & Gibbons 1969). We captured some juvenile *C. picta* from nearby wetlands (Eastern painted turtle, *C. p. picta*), and purchased some (Southern painted turtle, *C. p. dorsalis*) from an animal supplier (ExoticReptiles, Charleston, South Carolina). All turtles were less than 30 mm carapace length (CL), suggesting they were one year old (Ernst & Lovich 2009). Turtles were kept in a climate-controlled laboratory and fed floating reptile sticks (Reptomin) *ad libitum* prior to the beginning of the experiment. One turtle was found dead in week 3, so this mesocosm was removed from the experiment (5 turtle present replicates).

Water and leaf nutrient sampling

Leaves and water were sampled on days 0, 14, and 35 (hereafter, weeks 0, 2 and

5). Whole leaves were rinsed of macroinvertebrates over a 250-µm-mesh sieve, placed in foil pouches and stored in 60°F drying oven. Samples of dissolved organic carbon (DOC) and water nutrients (NO_3^-, NH_4^+, PO_4^{3-}) were collected by sampling 60 mL from the water column using an acid washed luer lock syringe (BD®, New Jersey), filtering (0.45 µm) transferring to a plastic bottle (Thermo Fisher Scientific®, Massachusetts) and freezing. Leaves were ground and homogenized in a ball mill grinder and re-dried for storage. Leaf subsamples were weighed and the total C and N were analyzed by Micro-Dumas Combustion using a Carlo Erba 2NA 1500 CHN analyzer (Carlo Erba, Milan, Italy). Analysis for total P was completed by weighing a leaf subsample into an acid-washed ceramic crucible, ashing at 500°C, acid digesting and analyzing using the ascorbic acid method of spectrophotometry (Jones et al. 1991). Water samples were analyzed for DOC using the High Combustion Infrared Method on Shimadzu Total Organic Carbon 5000A Analyzer (Kyoto, Japan). Water nutrients (NO_3^-, NH_4^+, PO_4^{3-}) were analyzed with a Alpkem 300 Series Continuous Flow Analyzer (Clackamas, Oregon) using methods published in Standard Methods for the Examination of Water and Wastewater (APHA 2006). We refer to the combination of NO_3^- and NH_4^+ as total dissolved inorganic N (DIN) in this study (which differs from measurements of Total N for turtle excretion - see below), with the understanding that other dissolved N compounds may be present, albeit in smaller quantities. All nutrient samples were analyzed at the University of Georgia Analytical Chemistry Laboratory.

Macroinvertebrate and leaf mass loss

At the end of the experiment, all contents of each tank were filtered separately through a 250-µm-mesh net to separate leaves from macroinvertebrates and to determine

leaf mass loss. Leaves were hand sorted, rinsed, and stored in paper bags and dried in a 60° F oven to a constant mass. We accounted for leaves removed during prior samples when determining % mass loss at week 5. Macroinvertebrates were captured in a sieve and preserved in 10%Phosphate-buffered formalin and later identified to the lowest possible taxonomic level (usually family, although Chironomidae were split into Tanypodinae and non-Tanypodinae). Macroinvertebrates within each taxonomic group were counted to determine abundance, and expressed as a concentration (individuals per liter) for comparison to other studies.

Turtle excretion sampling

At the end of the experiment, turtles were removed from each mesocosm to measure growth and estimate nutrient excretion rate. Turtles were cleaned by scrubbing off algae and debris from the carapace and plastron and by rinsing debris from inguinal and axillary regions with 0.45 μm filtered tank water. Each turtle was transferred to an individual, acid-washed container (Rubbermaid®, 29.5x17x12cm). A liter of water from the turtle's mesocosm was filtered (0.45 μm) into plastic container. After turtles were held in the containers for 10 hours, 60 mL of water was subsampled, filtered (0.45μm) and immediately frozen for analysis.In addition, control excretion containers (no turtle) were included and treated the same as those with turtles. Samples were analyzed for total dissolved N (NO_3-N) and soluble reactive phosphorus (PO_4-P) following a persulfate digestion at the University of Georgia Analytical Chemistry Laboratory. Persulfate oxidizes any organic N (i.e. urea) or inorganic N (i.e. ammonium) to NO_3 for measurement as total N in solution. Excretion was estimated as the difference between the excretion and control samples and rates were estimated as the changes in N and P per

volume (1 L), per unit time (10 hrs). Mass-specific excretion was estimated using dry mass, which was derived from field collected relationship between wet and dry mass (Sterrett & Maerz in review).

Statistical analyses

We used repeated measures ANOVA to test for the effects of turtle presence on water chemistry (DOC, NO_3, NH_4, PO_4) and leaf litter nutrients (%C, %N, and %P) among weeks 0, 2 and 5. We used a t-test to test for differences in mean macroinvertebrate concentrations (individuals/L) and mean % leaf mass loss between mesocosms with and without a turtle present. We used general linear model and Akaike Information Criterion corrected for small sample sizes (AICc) to compare competing linear regression models with factors of turtle presence, macroinvertebrate concentration, water nutrient concentration (DIN, PO_4 at weeks 2 and 5) and leaf nutrient content (%N and %P at weeks 2 or 5) on % leaf mass loss. We checked for normality in dependent variables using Shapiro-Wilk tests. All analyses were considered significant at $\alpha=0.05$. All analyses were completed in Statistica (Version 8, StatSoft©, Inc. 2008, Tulsa, Oklahoma, USA).

RESULTS

Mean macroinvertebrate concentration in tanks with turtles present (mean ± 1 sd = 0.8 ± 0.9 ind./L) was 94% lower than in tanks with turtles absent (12.5 ± 5.8 ind./L; t= 4.434. df=9, p=0.002). In addition to amphipods that were originally stocked in tanks, we found Chironomidae (Tadypodinae (predators) and non-Tadypodinae (detritivores)), Chaboridae, Ceratopogonidae, Arachnida, Belostomatidae and Culicidae. The majority of macroinvertebrates found at the end of the experiment were non-predatory amphipods

and non-Tadypodinae Chironomidae, with a small percentage composed of predatory macroinvertebrates (Table 4.1).

Dissolved nutrient concentrations changed over time, but the presence of a turtle had no effect (DOC, NO_3, NH_4, PO_4; Table 4.2, Fig. 4.1). By week 5, DOC declined by 66%, NO_3 increased by 49% and PO_4 declined by 88% irrespective of the presence of a turtle (Table 4.2). There was no significant change in DIN ($NO_3 + NH_4$) over the five weeks; however, there was a change in the composition, with an increase in NO_3 and decline in NH_4 over the 5 weeks regardless of treatment (Fig. 4.2).

The presence of turtles had an effect on %C, %N, and %P leaf litter content (Table 4.3). Leaf %C content declined ~7% over the five week period with greater C loss in the presence of turtles (Table 4.3, Fig. 4.3). Among all mesocosms, mean leaf %N increased over the five weeks by ~51%. Though the effect of turtle presence on leaf %N was not statistically significant (Table 4.3), there was a 76% increase in mean leaf %N in the presence of turtles and only a 35% increase? in mean leaf N in the absence of turtles (Fig. 4.3). A post hoc comparison of the difference in mean leaf %N at week 5 between mesocosms with versus without turtles was statistically significant. Consistent with leaf %N, mean leaf %P increased over time and increased more in the presence of turtles (Table 4.3, Fig. 4.3). Leaf P increased 200% in the presence of turtles compared to only 45% in the absence of turtles (Table 4.3., Fig. 4.3). Among all treatments, there was a positive correlation between leaf %N and %P content (r^2=0.569, p=0.007, Fig. 4.4). Among all treatments, there was a negative correlation between macroinvertebrate concentration and leaf %P at week 5 (r^2=0.557, p=0.008, Fig. 4.5).

Overall, there was no significant difference in mean % mass leaf loss between mesocosms with (mean ± 1 sd = 42.9 ± 3.2) and without turtles (44.6% ± 4.9; t=0.656, df=9, *p*=0.528). A single top model that included total DIN (NO_3 + NH_4) water concentration at week 2, and macroinvertebrate concentration and %P leaf content at week 5 explained 91.8% of the variance in % leaf mass loss ($F_{3,7}$=26.025, p<0.0004, r^2=0.918). All three factors in the top model were statistically significant. Total DIN at week two was positively correlated with % mass loss (β=0.692, T=5.452, df=7, P<0.001), macroinvertebrate concentration was negatively correlated with % leaf mass loss (β =-0.536, T=-3.268, df=7, p=0.014), and leaf %P content was also negatively correlated with % leaf mass loss (β=-0.672, T=-3.715, df=7, p=0.008; Fig. 4.6). The second highest ranked model was greater than 3.4 ΔAICc from the top model and included the three factors in the top model in addition to PO_4 at week 5.

During the five-week experiment, turtles grew an average of 3.2 mm carapace length (sd=1.7, range 1.28-5.21mm; 0.09 mm/day; n=5), 1.8 mm plastron length (sd=1.2, 0.76-3.13, 0.05 mm/day) and added 1.4 g (sd=0.7, 0.73-2.24 g, 0.04g/day). Turtles excreted an average of 75.5 µg N/L/h (sd=32.0, n=5) and 0.9 µg P/L/h (sd=1.7, n=5). Mass-specific excretion rates for turtles were 27.6 µg N/g dry mass/L/h (sd=8.8) and 0.35 µg P/g dry mass/L/h (sd=0.7). Molar excretion rate was 5.4 µmol N/L/h (sd=2.3) and 0.0291 µmol P/L/h (sd=0.1). Turtles excreted at a molar N:P ratio of 539.9 µmol/L/h (sd=362.8). Assuming that the majority of P was a result of turtles as PO_4, we estimate that turtles contributed up to 0.3% of dissolved P. Daily temperature in the mesocosms throughout the experiment was 25.7°C (3.4 sd, N = 6) and fluctuated between 17.8 and 34.85°C.

DISCUSSION

As expected, juvenile turtles substantially reduced macroinvertebrate abundance and subsequently increased the concentration of N and P of leaves; however, the presence of juvenile turtles did not have a measureable effect on dissolved nutrient concentrations or % leaf mass loss over five weeks. These results partially support our hypotheses that turtles can indirectly affect leaf decomposition through consumption of invertebrate detritivores, but does not support our hypothesis that recycling of N and P by turtles would increase decomposition rates. The effects of turtles on macroinvertebrate abundance is consistent with other studies (Thorp & Bergey 1991, Williams 2011, but see Lindsay et al. 2013). Thorp and Bergey (1991) found a 32% reduction in *H. azteca* between predator (presumed fish and turtles) and non-predator treatments in a pond cage experiment (however, see Lindsay et al. 2013). Mesocosm conditions in this study offered suitable temperature ranges for consumption and digestion by *C. picta* (Parmenter 1980, Avery et al. 1993, Kapenis & McManus 1974), as was evident from turtle growth during our experiment that was comparable to estimates from turtles measured from natural populations (Ernst & Lovich 2009). The absence of any measurable effect of turtles on dissolved nutrient concentrations coupled with low amounts of excreted P by juvenile turtles indicates juvenile turtles were not important in supplying nutrients to microbial decomposers. Though the positive correlation between DIN and % leaf mass loss was consistent with known effects of nutrient enrichment on decomposition (Gulis & Suberkropp 2003, Greenwood et al. 2007), the negative relationships between macroinvertebrate concentrations, leaf %P and % mass loss appear paradoxical and indicate a complex indirect effect of turtle predation on decomposition. It is well

understood that microbial colonization increases the nutrient content of leaf litter (Cummins 1974). Additionally, detritivores will preferentially feed on nutrient rich leaf litter conditioned with microbial growth (Arsuffi & Suberkropp 1985). In the absence of turtles, macroinvertebrate abundance was high and leaf %N and %P were lower, suggesting that macroinvertebrates preferentially grazed N and P rich portions of leaves and likely drove decomposition through litter shredding and fragmentation. Macroinvertebrates have been shown to selectively feed on patches of leaf litter higher in fungal biomass (Arsuffi & Suberkopp 1985), thereby lowering fungal biomass in leaf litter (Mehring & Maret 2011), and potentially lowering litter nutrient content. In the presence of turtles, macroinvertebrate abundance was low and leaf %N and %P were higher, likely reflecting the absence of grazing on nutrient rich biofilms. We would hypothesize that the lack of macroinvertebrate grazing on microbial biofilms appears to result in compensatory leaf litter breakdown via a different mode (i.e. primarily microbial) when turtles were present.

These results are particularly interesting when considering the cascading effects of predators on detritus decomposition. Our results demonstrate the necessity of considering predator nutrient recycling in addition to the indirect trophic effects via predation on grazers and shredders. However, our results also demonstrate that we must consider predator indirect trophic effects on resource stoichiometry. Selective grazing on the more nutrient rich resources is common among herbivores and detritivores (Arsuffi & Suberkropp 1985, Hanlon & Anderson 1979, Hobbs 1996, Katayama et al. 2013), such that stoichiometric effects of predators on basal resources should be common. Few studies have connected the ability of a consumer to directly or indirectly change detritus

nutrient content through nutrient excretion (see Beard et al. 2002) or by reducing selective grazing by intermediate consumers.

There are two important and unexplained results in our study. We are unable to explain the random variation in DIN among mesocosms at week 2, or why turtles had no measurable effects on DIN despite excreting relatively high amounts of N. Consistent with the known importance of DIN on microbial growth and decomposition (Gulis & Suberkropp 2003, Greenwood et al. 2007), DIN in week 2 was a significant predictor of leaf decomposition by week 5. DIN likely primed microbial growth, as was evident in conversion from NH_4 to NO_3between weeks 2 and 5. We used a common water source to establish all mesocosms, so we hypothesize that variation in DIN stemmed from random variation in %N among tupelo leaves used in our study. We estimated that juvenile turtles excreted inorganic N at a daily rate equal to 15% of DIN within mesocosms. This rate was consistent among turtles and reflected rates measured over a 10 hour period. Further, the rates for juvenile turtles were significantly lower than previous estimates (Sterrett et al. chapter 3). Therefore, we are confident that juvenile turtles did excrete significant amounts of N recycled from their consumption of macroinvertebrates. Nonetheless, this did not result in measurable increases in DIN within mesocosms. It is possible that microbial or algal uptake of N was sufficient to offset turtle supply, which would be consistent with the higher leaf %N we observed in the presence of turtles; however, we did not measure microbial or plant N uptake and it is not clear that N availability was limiting in our study.

We caution against extrapolating the results of this experiment to the effects of turtle populations on processes within larger natural systems. Juvenile *C. picta* are

carnivorous and have a high P demand to support the growth of their shell (Clarke & Gibbons 1969, Sterrett & Maerz in review). Therefore, we expected and observed that juveniles sequester P and excrete N disproportionately (Sterrett & Maerz in review). However, juvenile turtle mortality is significantly higher than adult mortality such that most turtle populations and biomass are significantly adult biased (Ernst & Lovich 2009). Adult turtles exhibit limited growth and have a lower P demand due to the slow turnover of bone, and are more omnivorous (Congdon et al. 2013, Bouchard & Bjorndal 2006, Sterrett & Maerz in review). Our prior research shows that larger, adult turtles excrete 4.4 time more N and 11 times more P than juveniles (Chapter 3). Therefore, we would expect natural, adult biased turtle populations to have smaller effects on macroinvertebrate abundance and much greater effects on nutrient recycling (Chapter 3). In the case of species with carnivorous adults (e.g., common musk turtle, *Sternotherus odoratus*), we might expect both a negative effect on macroinvertebrate abundance and a large positive effect on nutrient recycling. Future studies should contrast the effects of juvenile and adult turtles of both common omnivorous and carnivorous species to develop a more holistic estimate of turtles on decomposition within natural systems.

This experiment adds to a growing body of evidence that vertebrate consumers can indirectly influence detrital processing (Wyman 1998, Konishi et al. 2001, Mancinelli et al. 2002, Ruetz et al. 2002, Persson & Svenson 2006); however, this study illustrates the potentially complex and compensatory effects of predators even in a highly simplified food web. Our results suggest that predation effects that appear as strictly "top down" effectsignore the direct and indirect effects of predators on resource nutrient content, which is classically viewed as a "bottom up" driver of ecosystem processes. Though we

found evidence that juvenile turtles could control factors that affect decomposition via consumption of macroinvertebrates, we hypothesize that adult turtles may have a greater influence through consumer mediated nutrient recycling in natural ecosystems.

ACKNOWLEDGMENTS

The project was funded by a University of Georgia Innovative and Interdisciplinary research grant and successfully crowdfunded through SciFund Challenge. We would like to thank Bess Brown for help collecting turtles and Jay Shelton, Peter Lasier and Matt Urich for help in acquiring and rearing amphipod. Casey Harris, Dustin Kemp, Fern Lehman, Bill McDowell, Meredith Myers and Daniel Streicker helped with collecting leaf litter and Kyle Barrett helped with acquiring pond water. Emmy Deng and Tom Maddox conducted chemical analyses at the University of Georgia Analytical Laboratory. All procedures in this study were approved by the Georgia Animal Care and Use Committee (AUP #A2011 08-030-Y2-A1) and turtles were collected under a Georgia Department of Natural Resources Scientific Collecting Permit (#29-WJH-13-191).

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Table 4.1. Macroinvertebrates groups as a percentage of total remaining at the end of experiment from each treatment. The predator macroinvertebrate guild included Chaboridae, Ceratopogonidae, Arachnida, Belostomatidae and Chironomidae (Tadypodinae). Value in parentheses are standard error.

Turtle treatment	% Amphipoda	%Non-tanypodinae Chironomidae	%Predator	Mean total abundance
present (n=5)	7.1 (3.1)	82.2 (4.0)	6.2 (3.4)	269.0 (135.1)
absent (n=6)	6.4 (2.6)	87.8 (4.6)	2.5 (1.4)	4423.2 (840.3)

Table 4.2. One-way repeated measures ANOVA of soluble nutrient concentrations.

Variable	Factor	Df	MS	F	P
DOC					
	Intercept	1	64022	1017.023	<0.001
	Turtle	1	1	0.016	0.903
	Error	9	63		
	Week	2	9759	275.952	<0.001
	Turtle X Week	2	52	1.467	0.257
	Error	18	18		
DIN					
	Intercept	1	35890.76	305.5380	<0.001
	Turtle	1	0.03	0.0002	0.988
	Error	9	117.47		
	Week	2	223.42	2.570	0.104
	Turtle X Week	2	83.64	0.962	0.401
	Error	18	86.92		
NH_4					
	Intercept	1	17344	219.521	<0.001
	Turtle	1	5	0.063	0.807
	Error	9	79		
	Week	2	755	14.247	<0.001
	Turtle X Week	2	42	0.791	0.469
	Error	18	53		
NO_3					
	Intercept	1	3335	195.347	<0.001
	Turtle	1	6	0.337	0.567
	Error	9	17		
	Week	2	170	9.607	0.001
	Turtle X Week	2	9	0.528	0.599
	Error	18	18		
PO_4					
	Intercept	1	9872	99.922	<0.001
	Turtle	1	70	0.710	0.421
	Error	9	99		
	Week	2	2273	14.234	<0.001
	Turtle X Week	2	355	2.226	0.137
	Error	18	160		

Table 4.3. One-way repeated measures ANOVA of percent dry mass of nutrients in leaf litter.

Variable	Factor	Df	MS	F	P
C					
	Intercept	1	74019	129173.9	<0.001
	Turtle	1	4	6.2	0.035
	Error	9	<1		
	Week	2	42	55.5	<0.001
	Turtle X Week	2	<1	1.1	0.358
	Error	18	18		
N					
	Intercept	1	34.898	364.064	<0.001
	Turtle	1	0.168	1.747	0.219
	Error	9	0.096		
	Week	2	0.580	7.808	0.004
	Turtle X Week	2	0.084	1.132	0.344
	Error	18	0.074		
P					
	Intercept	1	0.0418	536.376	<0.001
	Turtle	1	0.0007	8.893	0.015
	Error	9	<0.0001		
	Week	2	0.0010	6.587	0.007
	Turtle X Week	2	0.0002	1.376	0.278
	Error	18	<0.0002		

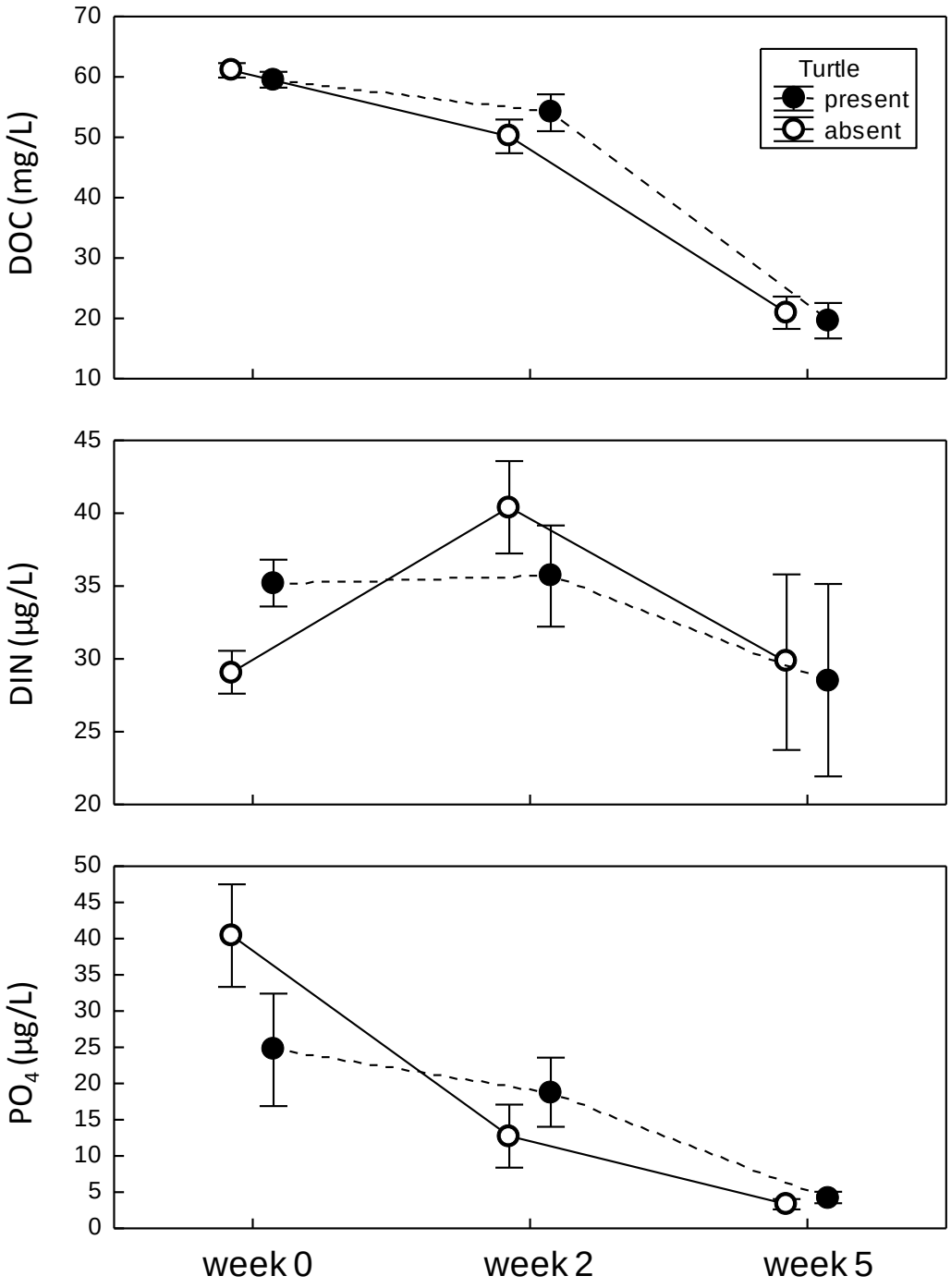

Figure 4.1. Dissolved organic carbon (DOC: mg/L), dissolved inorganic N (DIN; µg/L) and PO_4 (µg/L) between turtles present and absent in weeks 0, 2, 5. Bars represent standard error. There were no statistical differences between treatments with and without turtles.

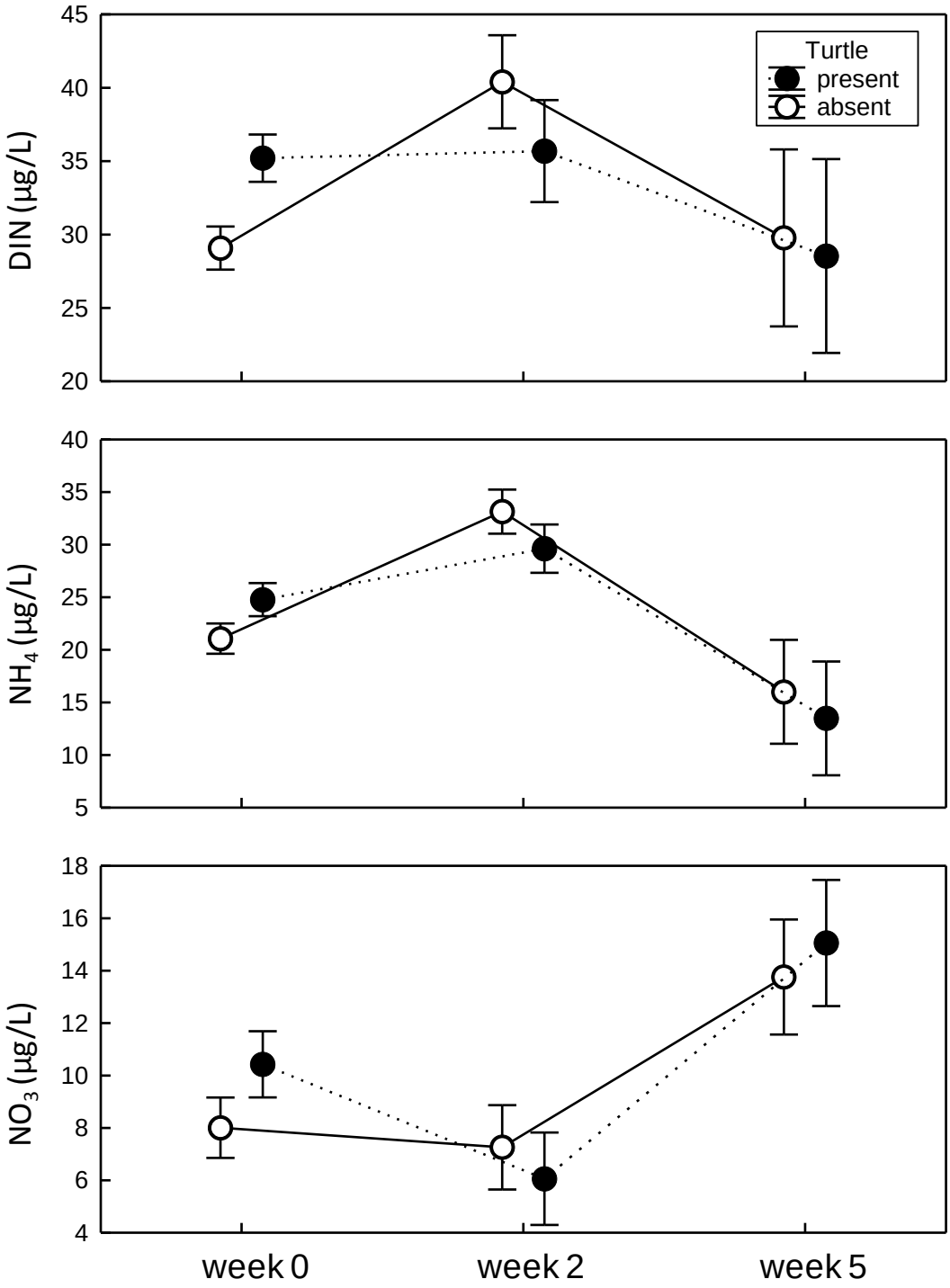

Figure 4.2. Dissolved inorganic N (DIN; µg/L), NH_4 (µg/L) and NO_3 (µg/L) between turtle present and absent treatments on weeks 0, 2, 5. Bars represent standard error. There were no statistical differences between treatments with and without turtles.

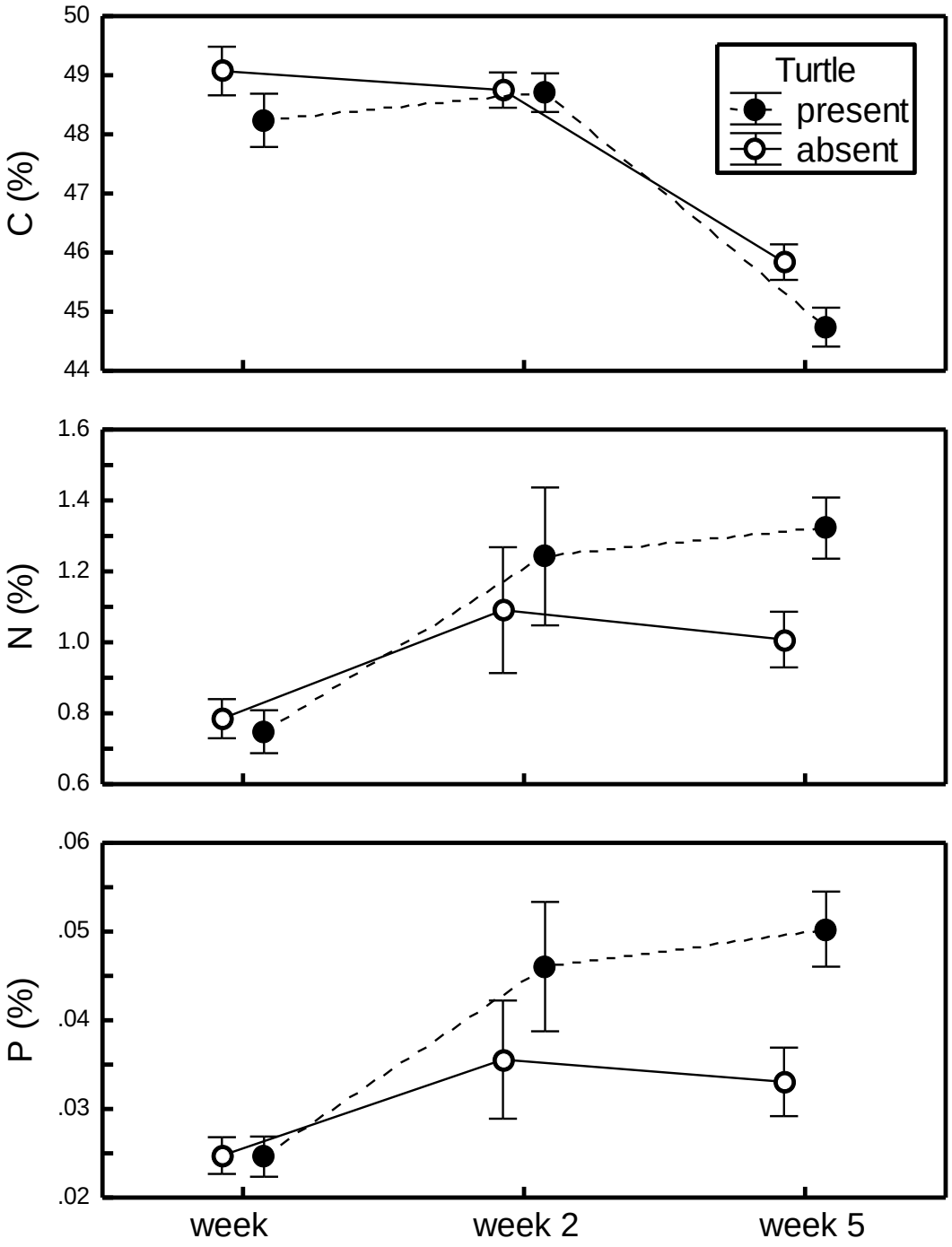

Figure 4.3. Leaf percent Carbon (C), Nitrogen (N) and Phosphorus (P) content from tanks with turtles absent and present across the duration of the experiment. Bars represent standard error. There was a statistical difference between treatments with a decrease in leaf %C content and an increase in %N and %P leaf content.

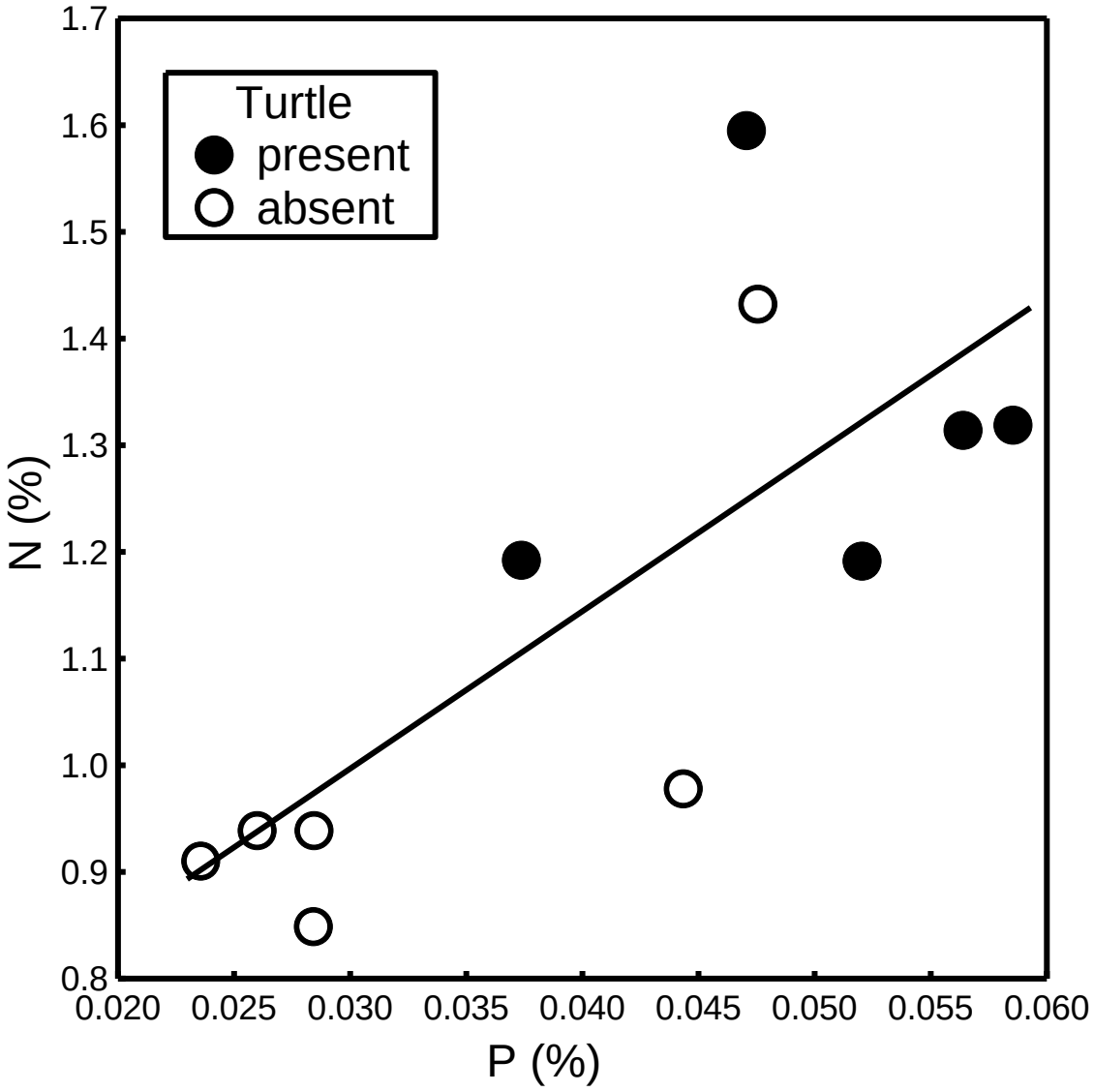

Figure 4.4. Relationship between %Nitrogen (N) and %Phosphorus (P) leaf content at week 5 in tanks with turtles present and absent (y=-0.004+0.039x, r^2=0.569, p=0.007). This relationship suggests that the effects of macroinvertebrates was similar on %N and %P leaf content.

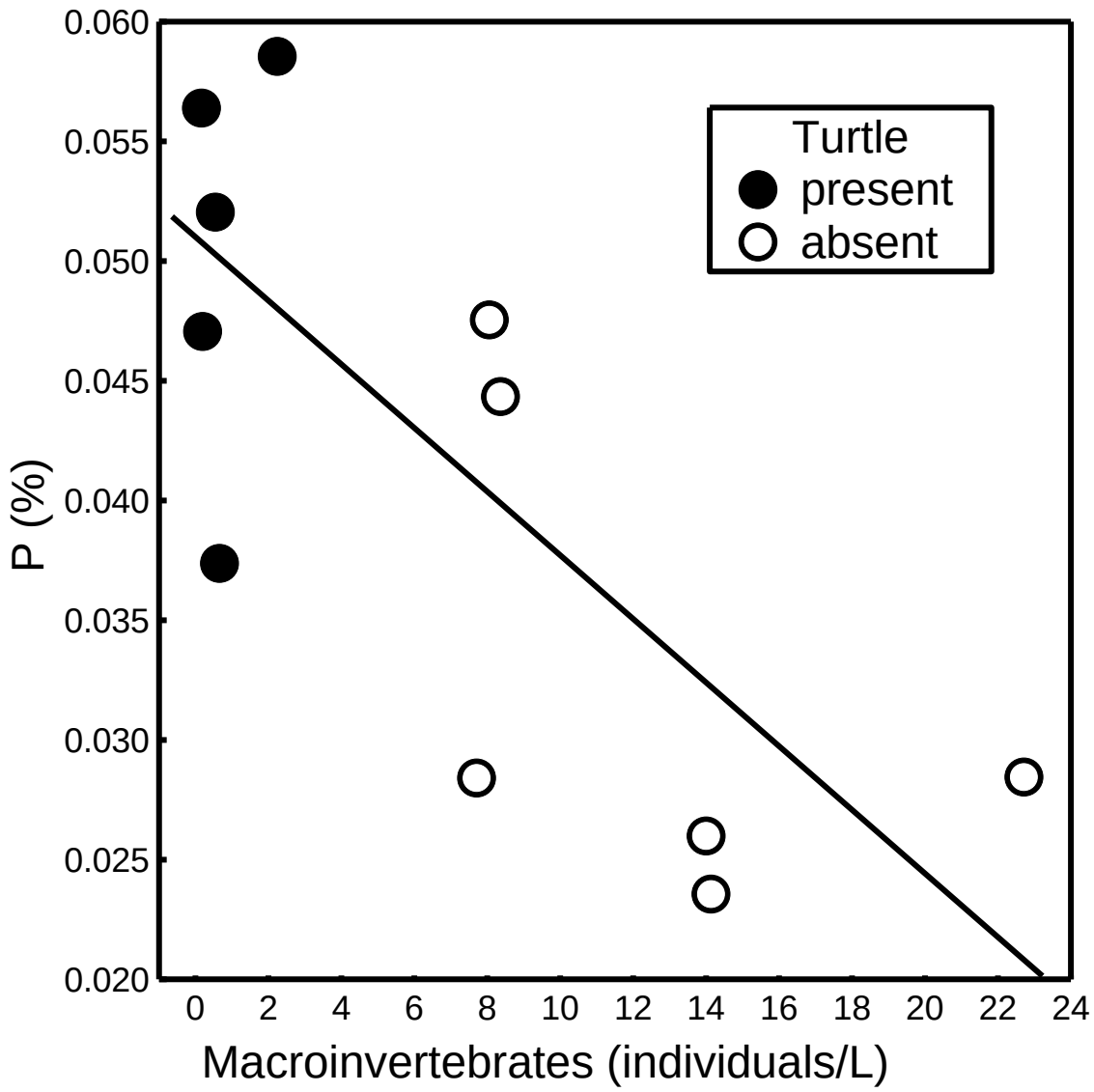

Figure 4.5. Relationship between macroinvertebrate concentration (individual/L) and %Phosphorus (P) leaf content at week 5 in tanks with turtles present and absent (y=0.0501-0.0013x, r^2=0.557, p=0.008) in food web mesososm experiment.

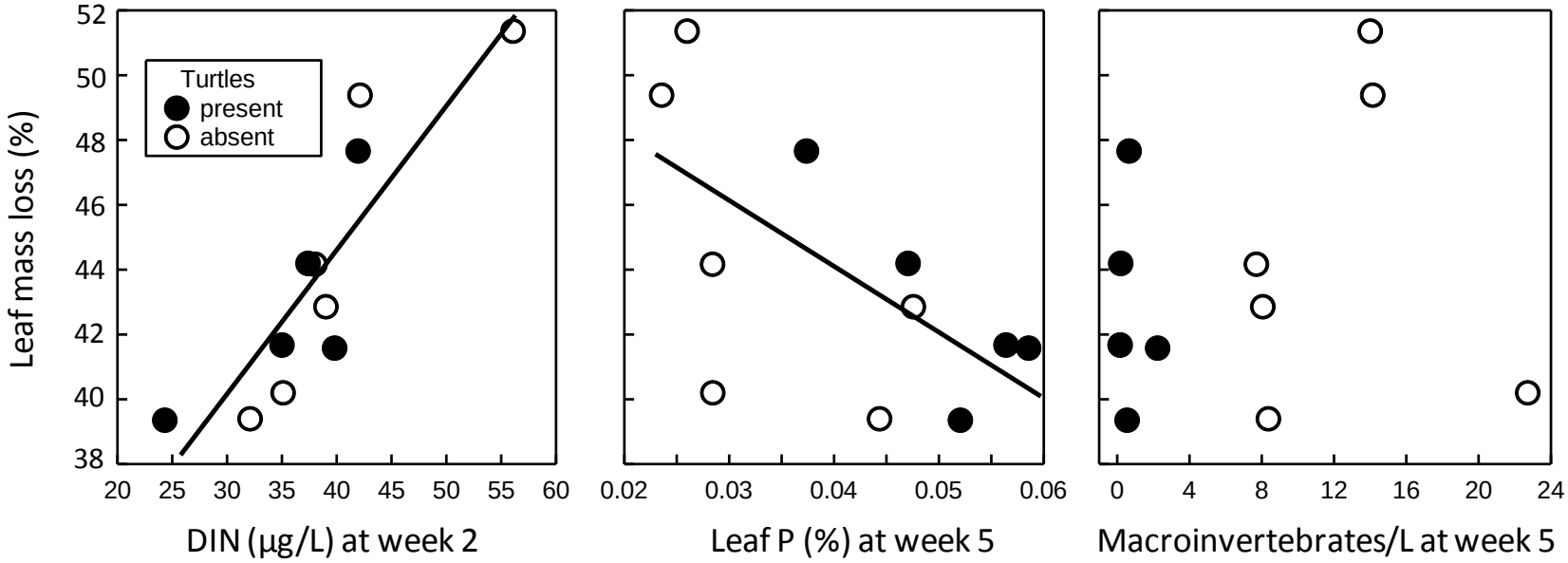

Figure 4.6. Relationships between predictor variables and % leaf mass loss between turtle present and absent treatments (DIN: y=26.46+0.45x, r^2=0.75, p=0.0006; Leaf %Phosphorus (P) at week 5: y=51.99-200.28x, r^2=0.39, p=0.03; macroinvertebrate concentration; not significant) used in final general linear model to predict % leaf mass loss in mesocosm experiment.

CHAPTER 5

GENERAL CONCLUSIONS

This dissertation represents an attempt to understand how unique traits specific to turtle evolution and ecology relate to nutrient cycling contributions in aquatic ecosystems. The ideas and studies herein are grounded in decades of experimental and long-term ecological research, which have foreshadowed how turtles influence nutrient dynamics (including work from K. Bjorndal, J.D. Congdon, J.W. Gibbons, J.B Iverson, E. Moll, D. Moll, among many others). Despite our knowledge of their unique morphology, contributions to biomass, food webs and energy flow (Iverson 1982, Iverson 1984, Congdon et al. 1986, Aresco &James 2005), turtles are largely unrecognized for their contributions to nutrient cycling and other ecosystem processes. An exception to this was a whole ecosystem study of community metabolism in Florida's Silver Spring (Odum 1957). A photo from H.T. Odum's paper showed researchers posing underwater with river cooters (*Pseudemys* spp.), the species with the highest standing crop biomass within the spring run ecosystem.

The objectives of this dissertation were to A) describe the ecological stoichiometry of freshwater turtles relative to C, N and P, and contrast turtles with patterns of existing stoichiometry knowledge from other vertebrate groups (Chapter 2), B) estimate the nutrient storage and recycling of common freshwater turtle species in streams and ponds of the Southeastern United States (Chapter 3), and C) experimentally study the indirect effects of

predation and nutrient remineralization of turtles on leaf litter decomposition in a simplified detrital food web (Chapter 4).

The impetus for studying the ecological stoichiometry of turtles was the obvious mechanical structure that makes turtles unique among all other animals: the shell. In Chapter 2, we illustrated that turtles are unique among all other taxa in body nutrient content due to the extreme mass associated with their bony skeleton (~82% of dry mass). Due to the amount of bone in the body, and the high P content of bone, turtles are distinct from other organisms in having low body %N:P. Most of our results concerning body size, body nutrient stoichiometry and are consistent with those of other taxa. However, the positive relationship between body stoichiometry N:P and excretion N:P is novel and intriguing in ecological stoichiometry theory. Our results suggest that turtles have a high P demand as juveniles, when there is a need to build the shell, but demand decreases with maturity because of negligible adult growth and turnover of bone. In contrast to what has been found for other taxa, this also suggests that turtles recycle nutrients in positive proportion to their biomass. The unique morphology, life history and ecology of turtles offers a novel perspective for ecological stoichiometry theory. Potentially as interesting are the implications for turtle life history. We hypothesize that juvenile turtles are one of the most nutrient limited organisms.

The third chapter built on the description of turtle ecological stoichiometry to estimate nutrient storage and excretion of freshwater turtles and compared these estimates among three freshwater habitats in Georgia. Although mass-specific excretion rates were comparable to measurements of fishes and salamanders, excretion of turtle assemblage (focal species) was lower than estimates of other aquatic organisms. We found that turtle standing crop biomass and

nutrients (N and P) were highest in the Lower Flint River Basin of the Coastal Plain compared to Piedmont ponds and streams. Despite density estimates of eastern musk turtle (*Sternotherus odoratus*) and loggerhead musk turtles (*Sternotherus minor*), which were equal to or higher than yellow-bellied slider (*Trachemys scripta*) across habitat types, the large body size and density of *T. scripta* resulted in higher biomass and standing crop nutrient estimates of our focal species in all habitats. Overall, our results suggest that turtle biomass, in combination with their high tissue nutrient concentrations (Sterrett & Maerz, in review), are potentially a significant portion of standing stock nutrients, especially P, in freshwater ecosystems. Further research will be needed to determine whether our results indicating the importance of turtle nutrient recycling in ponds and streams hold up to a broader range of conditions.

The fourth chapter described an experimental mesocosm study of the presence of juvenile turtles in a simple detrital food web. This study ties in with our expectations of a shifting body nutrient content of juvenile turtles associated with building the shell (Sterrett & Maerz in review). We predicted that juvenile turtles would have a cascading effect on leaf litter decomposition by eating detritivores, but also change the stoichiometry of leaf litter through nutrient excretion. Because turtles were likely sequestering nutrients for growth, we found no effect of turtle presence on dissolved nutrient availability. However, as expected, the juvenile turtles reduced macroinvertebrate concentrations, which were feeding on N and P rich portions of leaves. Thus, turtles were having an indirect cascading effect on leaf litter nutrient content and consequently compensatory effects on decomposition. These results are intriguing because they highlight the potential complexity of top-down effects.

Limitations and further research

The unique body stoichiometry of turtles and low variation between species in this study suggest that turtle life history may be limited by other factors than have been considered so far. Several studies have focused on the importance of dietary protein in the growth of juvenile turtles (Parmenter 1980, Avery et al. 1993, Bouchard & Bjorndal 2006). We advocate for further research on turtle growth in response to P limitation. Phosphorus is necessary for construction of major biomolecules (e.g. nucleic acids, ATP) and is critical for building mineral hydroxyapatite needed to build bone (Elser et al. 1996). Given that turtles need to build bone for protection, we would hypothesize that juvenile turtle would have a high P demand, which would limit their growth early in life. This idea is supported by the ecology of juveniles, which exclusively eat animal foods rich in N and P early in life followed by a switch to omnivory.

There are several obvious limitations to understanding turtle contributions to nutrient storage and excretion in our study. First, our inability to account for all turtle species in each of our focal habitat types underestimates the total nutrient storage and excretion by the turtle assemblage. We used baited hoop traps to capture turtles, which reduced our ability to capture certain species not attracted to bait, mostly notably Barbour's map turtle (*Graptemys barbouri*) and Eastern River Cooter (*Pseudemys concinna*; Sterrett et al. 2010). Further, we focused on estimating body nutrient content and excretion estimates of four focal species, which represented two families, and made up the majority of our total capture at each site. However, this did not include the snapping turtle (*Chelydra serpentina*), which made up 23% of individual captures in the North Oconee River. The logistics of performing excretion trials with *C. serpentina* (i.e. demeanor) were not considered in this study. These three species (*C. serpentina*, *G. barbouri*, *P.*

concinna), are locally common in aquatic habitats of Georgia and throughout the southeastern U.S. (Sterrett et al. 2011). While other species exist that were not accounted for, these species in particular may be important because they are larger in body size in body size than our focal species, suggesting that they would significantly add to estimates of turtle standing crop biomass and nutrients. Additionally, differences in morphology and diet suggest that their contributions would vary compared to turtles in this study. For example, *C. serpentina* has a reduced plastron and thin carapace compared to most species of North American hard-shelled turtles, suggesting that these characteristics may influence body nutrient content. The morphology of *P. concinna* and *G. barbouri* are comparable with the exception of jaw size and morphology (i.e. female *G. barbouri* exhibits megacephaly). However, a major difference between *P concinna* and *G. barbouri* is diet, which suggest a contrast in their potential nutrient cycling. *P. concinna* is herbivorous, whereas *G. barbouri* is carnivorous and highly sexually dimorphic; males feed on macroinvertebrates and females exclusively eat mollusks. Further research should include large bodied turtles in estimates of standing crop nutrient, as well as contrast how diets influence nutrient recycling contributions.

A second limitation to our study is the ability to assess the relevance of turtle storage and excretion in aquatic ecosystem. Little information exists for biomass of organisms living in medium-sized rivers in the southeastern U.S., which would allow for contributions of turtles to have some context. Additionally, we did not concurrently collect data on nutrient loading, limitations or uptake rates, which would allow for assessing if turtle assemblage recycling rates are important (Vanni et al. 2005, Keitzer & Goforth 2013).

Further research is needed to confirm our estimates of turtle excretion. Issues related to temperature, behavior and fasting can influence excretion rates (Whiles et al 2009). Experimental approaches in lab and field settings should be used to find optimal incubation time period for turtles, which would allow for more confidence in estimating turtle excretion (Whiles et al. 2009). In particular, the methods used to acquire turtles in this study (trapping) allowed for a window of time when turtles could excrete or egest materials in response to stress. Future research should use methods such as snorkeling to capture turtles for incubation methods.

A limitation to our experimental approach in chapter 4 is that we were unable to assess the effects of adult and juvenile painted turtles on detrital food webs. Our decision to use juveniles was based on logistics of scaling mesocosms to a realistic density of painted turtle biomass. However, we would also expect adult turtles to have a much lower effect on macroinvertebrates due to ontogenetic diet changes toward vegetation and inefficiency in capturing small prey (Ernst & Lovich 2009). We hypothesize that adults would have a greater effect on nutrient cycling because they are in lower P demand, and would recycle nutrients at higher rates (Sterrett & Maerz in review). To test the effects of adults, large mesocosms would be needed (e.g. swimming pools).

It will also be important to compare different turtle species effects on CMNR and decomposition. For example, *S. odoratus* were as abundant as *T. scripta* and *C. picta* in pond habitats and remain carnivorous as adults. We might expect *S. odoratus* to have effects on macroinvertebrate abundance and nutrient recycling. Modifications to enclosure methods used for studying fishes in lake system seem promising for studying the effects of turtles on ecosystem processes in lentic habitats (Vanni et al. 1997).

A number of questions arose in this dissertation research related to the intersection of turtle ecology and consumer mediated nutrient cycling (Fig. 1.1). In addition to turtle nutrient recycling, freshwater turtles also transport aquatically-derived nutrients into upland habitats through nesting (*sensu* Bouchard & Bjorndal 2000, Fig. 1.1). Because all North American turtles nest terrestrially and nest predation is high, turtles may contribute nutrient subsidies to riparian ecosystems. Second, turtles feed in benthic and littoral areas of lakes and streams similar to fishes and likely transport and transform nutrients to other parts of aquatic habitats (Vanni et al. 2005, Bulte & Blouin-Demers 2008). Research that includes information about diet and feeding behavior, daily movements and excretion estimates may allow for studying how turtles contribute to nutrient availability. Finally, some carnivorous turtles (e.g. *Sternotherus* spp. *Graptemys* spp.) have recently switched their diets from gastropods, a group of algae grazers, to invasive siphon and pedal feeding clams (e.g. *Corbicula fluminea*, *Dresseina polymorpha*). These recent changes in diets have led to one study illustrating the change of energy flow through this diet shift (Bulte & Blouin-Demers 2008). There are likely changes to ecosystem processes associated with these turtle mediated shifts in energetic and nutrient pathways.

It remains unclear if turtle effects on nutrient contributions in freshwater ecosystem are unique among other freshwater consumers. This dissertation has made many comparisons of turtles to freshwater fishes in nutrient cycling, for which a large body of literature exists (McIntyre & Flecker 2010). Both turtles and fishes constitute high biomass in freshwater ecosystems and likely overlap across a wide spectrum of dietary preferences (Iverson 1982, Ernst & Lovich 2009, McIntyre & Flecker 2010). Fish have been considered to be the dominant pools of N and P in lakes and streams when they are abundant (Griffiths 2006, McIntyre & Flecker

2010), although turtle populations suggest that they may also make up a substantial proportion of nutrients, especially P (Chapter 3). However, there are particular characteristics of turtle biology,life history and body nutrient content, which suggest they influence nutrient cycling differently than most fishes. First, the turnover rates of fishes aremuch faster than turtles, suggesting that the storage of nutrients remain locked in turtle body tissuesover a longer time scale. Again, it is unclear if this storage constitutes a nutrient sink, although this topic has recently been considered in fishes (Vanni et al. 2013). Second, the extreme amount of bone in turtles and the slow turnover of bone suggest that turtles may be in low demand following maturity and subsequently recycle P at higher ratesthan fishes per unit biomass. However, turtle excretion rates and ratios likely shift ontogenetically with changes in diet, similar to fishes (Pilati & Vanni 2006).Third, turtles have the ability to export nutrients through emmigration and nesting, whereas fish nutrient cycling (e.g. recycling, egg laying, body decomposition) is confined to aquatic areas.The aquatically derived subsidies of turtles into upland habitats or between wetlands may be a characteristic, which distinguishes turtle nutrient cycling from fishes. The functional redundancy of turtles and fishes as consumers in aquatic food webs is intriguing and will require further research, which should focus on comparisons of trophic position, standing crop nutrients and aggregate nutrient recycling.

Turtle conservation and consideration of common species

The species highlighted in this dissertation (yellow-bellied slider, painted turtle, Eastern musk turtle) are among the most common and well-understood species in North America (Ernst & Lovich 2009). Common species shape and influence ecosystems and consequently are most impacted by anthropogenic effects, including overexploitation (Gaston & Fuller 2008, Gaston

2010). For turtles, unsustainable harvest is a global issue affecting both rare and common species (Klemens 2000, Mali et al. 2014). The number of turtles reportedly removed from wild populations (~24 million from 2002 to 2009) impacts population viability (Heppel 1998, Mali et al. 2014). This dissertation has initiated research into how turtles contribute to ecosystem processes. Now it is time to incorporate their influences on ecosystem processes into conservation efforts.

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