Evolutionary ecology of stripe-necked musk turtles (*Sternotherus peltifer*) in the Cahaba River drainage

by

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Abstract

Stripe-necked musk turtles (*Sternotherus peltifer*) are one of the least-studied species of the southeastern United States' diverse turtle fauna. Despite this, the environmental variation that exists within their geographic distribution makes them excellent study subjects for addressing questions about how the local environment drives adaptation in aquatic organisms at the population level. In this thesis, I examine variation in shell shape of *S. peltifer* from the Cahaba River drainage basin in central Alabama, USA. I then present the first data on clutch and egg size for the species and conduct a preliminary examination of reproductive allometry. The results of my analyses on both traditional and geometric morphometric measures indicate that *S. peltifer* from above the fall line have flatter shells than those from the coastal plain. This phenotypic pattern aligns with observations from other turtles, suggesting that some aspect of the environment above the fall line, such as flow rate, has led to local adaptation for flat shell shapes in turtles. Since morphological features such as pelvic aperture width or flat shell shapes may present a constraint on reproductive output in small turtles, I examined reproduction in *S. peltifer* from above the fall line. Clutch size and egg width both increased with female body size, suggesting that there is competition for allocation of resources to different aspects of reproduction. Pelvic aperture width also increased with female body size, and did not appear to present a constraint on egg width. These results highlight the importance of the local environment for affecting the morphological and life history evolution of aquatic organisms at the population level.

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List of Abbreviations

3-D	Three-dimensional
ANOVA	Analysis of variance
AUM	Auburn University Museum
CH	Carapace height
CW	Carapace width
CVA	Canonical variate analysis
SCL	Straight carapace length
OLS	Ordinary least squares
PC	Principal component
PCA	Principal component analysis
PL	Plastron length
PW	Plastron width
SMA	Standard major axis

Chapter 1: General introduction

Morphology is important for the ecology of an organism because its shape, size, and other physical characteristics directly impact how it interacts with its environment (Wainwright and Reilly 1994; Koehl 1996). As natural selection acts on phenotypes, morphology is also a critical determinant of an organism's fitness, and therefore is integral to phenotypic evolution (Arnold 1983; Kingsolver and Huey 2003). Variation in aspects of the local environment can lead to divergent selection, causing localized adaptive evolution which results in morphological differentiation between species and populations (Rundle and Nosil 2005; Hendry et al. 2007; Schluter 2009).

Traditional morphometrics involves the use of linear measurements, taken between sets of points. A suite of measures is normally obtained from each specimen, and then often compared using ratios. While this approach can be useful for identifying patterns, it does have some notable weaknesses. For one thing, straight line measurements are not very informative about the actual shape of the organism – it is difficult to describe a 3-dimensional structure using 2-dimensional lines, especially since typically no information was recorded about the geometric relationship between measurements. For example, it is quite difficult to characterize the shape of a round object using only straight lines. Second, as linear measurements are often correlated with body size, patterns detected from analysis of traditional morphometric data can represent variables that are confounded with allometric or ontogenetic shape changes (Adams et al. 2004). Geometric morphometrics (GM) is an alternative statistical and methodological approach to morphology that overcomes these issues (Rohlf and Marcus 1993; Adams et al. 2004,2013). Rather than straight line measures, GM is based on coordinate data of landmarks in a 2 or 3-

dimensional space, which preserves spatial relationships, and thereby solves the problem of trying to characterize rounded shapes. Geometric morphometric methods are also built upon techniques that explicitly characterize shape independent of size, removing the potential confounding factor of allometric shape change. The use of GM methods has revolutionized the field of morphology – and therefore generated new tools for addressing overarching questions in ecology and evolution – by providing insights into such topics as biological symmetry (Mardia et al. 2000), and enabling new approaches to existing fields such as quantitative genetics (Klingenberg and Leamy 2001) and phylogenetics (Catalano and Torres 2017).

Diversity and morphological patterns in southeastern turtles

The environmental heterogeneity of the southeastern United States makes the region an especially interesting place to study morphological evolution. With a humid subtropical climate, multiple distinct river systems, and high geological and physiographical complexity, the area possesses attributes that have led to especially significant diversification of aquatic life, including crayfish, fishes, bivalves, and turtles (Warren et al. 2000; Soltis et al. 2006; Crandall and Buhay 2008; Lopes-Lima et al. 2018; Buhlmann et al. 2009). Especially notable is the regional assemblage of turtle species, which as a global turtle hotspot is rivaled only by parts of southern Asia (Buhlmann et al. 2009; Mittermeier et al. 2015). Southeastern turtle diversity reaches its peak in the Mobile River drainage, where freshwater turtle and tortoise richness can reach at least 18 species at some locations (Buhlmann et al. 2009; Guyer et al. 2015). Naturally, many turtle biologists have elected to work in this hotspot of turtle diversity, especially on groups such as the map turtles and sawbacks (genus *Graptemys*) which peak in diversity in rivers draining into the Gulf of Mexico (Lindeman 2013).

Turtles present a compelling system for studying the relationship between shape and the environment for several reasons. Many of these are due to their unique body plan, which consists of a hard, bony external shell derived from the rib cage and encompassing the pelvic and pectoral girdles (Pritchard 2007). Shell shape influences many aspects of a turtle's life, including mobility (Rivera 2008), reproductive capacity (Gibbons 1982), and even the ease of performing the righting response, an important self-preservation behavior (Stayton 2019). Given the importance of the shell for the ecology of a turtle, it is not surprising that shell shape has been linked to functional trade-offs (e.g., Rivera and Stayton 2011). Another benefit for studying turtle shell shape is that its rigid structure allows measurements to be easily obtained in a consistent manner (Myers et al. 2006).

Despite the attributes that make turtles excellent subjects for studying morphological diversity and interactions with the environment, very few empirical studies have closely examined how shell shape co-varies with habitat characteristics, life history strategy, or performance. Clearly, more work is needed to clarify how the environment may drive morphological and genetic diversification in nature. Additionally, understanding how local adaptation generates patterns of diversity within drainages could be highly relevant to conservation.

Adaptations for coping with flow regimes

The environmental heterogeneity associated with riverine habitats presents opportunities for addressing important questions regarding local adaptation. For instance, flooding events can cause especially intense flow, which might displace turtles from their home range. Additionally, localized differences in topography and geology can greatly affect stream flow (Allan and

Castillo 2007). Aquatic organisms have apparently adapted to cope with natural flow regimes through behavioral, life history, and morphological evolution (Lytle and Poff 2004).

Fishes and turtles both avoid stream reaches where they are more likely to be displaced during flood events (David and Closs 2002; Jones and Sievert 2009), and when displaced some river turtles demonstrate an incredibly keen homing ability (Harding and Bloomer 1979; Andres and Chambers 2006). These behaviors appear to be effective as most available evidence points to emydid turtle populations being highly resilient to extreme flood events (Jergenson et al. 2014; Pitt et al. 2021).

Major disturbances, such as those associated with extreme flow regimes, can have significant influence on life history evolution (Lytle 2001). For example, salmonid fishes time their spawning to coincide with periods of low flow (Fausch et al. 2001), and some aquatic insects employ strategies that time hatching or metamorphosis with either flood or drought (Lytle and Poff 2004). Similar examples are lacking for turtles with regard to flow regime, but intense selection can have significant influence on aspects of turtle life history strategies in only a few generations (Wolak et al. 2010).

Most river turtles appear well-suited for life in large, fast-flowing bodies of water (i.e., are of large body size, strong swimmers, etc.; see Moll and Moll 2004). However, many species occupy a range of habitats, and might therefore be expected to display associated morphological variation. Indeed, a pattern between shell shape and habitat was demonstrated in *Pseudemys* (Aresco and Dobie 2000). This ecomorphological pattern appears to be associated with the fall line, a geological feature that marks the border between the low, sandy coastal plain and the rockier, more mountainous physiographic provinces (Williams et al. 2008). Turtles from above the fall line were found to be flatter and more streamlined than those from the coastal plain, which have high, domed shells. *Pseudemys* shell shape ecomorphology was later revisited using geometric morphometric methods in an integrative framework, which established an important functional basis to the trait – flatter shells experience less drag when moving through flowing water (Rivera 2008). Similar patterns have since been found in several other riverine turtle species (*Graptemys* spp.; Rivera et al. 2014; Ennen et al. 2019).

Based on this body of work, it seems reasonable to expect that heterogeneity of riverine habitats might lead to local adaptation in shell shape in many turtle species. Additional observations of ecomorphological patterns in shell shape can be integrated with the established functional significance and additional types of data (i.e., genetic, reproductive) to address the broad question of how environmental variation influences evolutionary trajectories. This framework has only been addressed in channel-swimming turtles (i.e., emydids), so research on groups that interact with their environment in a different way, such as the bottom-walking kinosternids, should be especially informative.

Study system

The stripe-necked musk turtle, *Sternotherus peltifer* (Smith and Glass 1947), inhabits streams throughout the Pearl, Pascagoula, Tennessee, and Mobile River systems, except for the Black Warrior River above the fall line, where it is replaced by *S. depressus* (Guyer et al. 2015). *Sternotherus peltifer* is a small species, with adult carapace length ranging from 70-124 mm (Williamson 2001; but see Chapter 3). Some populations specialize on eating mollusks and crayfish, and can develop robust jaw musculature and alveolar plates for crushing their prey (Folkerts 1968). Otherwise, there is remarkably little natural history data available for the species.

To date, virtually all morphological studies of *Sternotherus peltifer* have centered around systematic questions, the majority of which sought to establish whether *S. depressus* should be regarded as a full species. In one of the first major works on the genus, Tinkle (1958) presented all available information on life history, distribution, systematics, and morphology of *Sternotherus* across the Gulf Coast. In his "taxonomic consideration of species", he notes that there is significant morphological variation across *S. peltifer* populations in different areas, with coastal plain turtles being quite like the holotype (a keeled specimen from Mississippi), and turtles from the Tennessee River and upper Coosa Rivers being much flatter. A decade later, Estridge (1970) collected *Sternotherus* from the Cahaba, Tombigbee, and Black Warrior drainages, again primarily to make recommendations on the taxonomic status of *S. depressus*. Using traditional morphometric techniques, he found that populations in the upper Cahaba drainage and Black Warrior drainage near the fall line were intermediate between the two species, and suggested that they may represent natural *S. depressus x peltifer* hybrids (although he still recommended that *S. depressus* and *S. peltifer* be regarded as separate species). Unfortunately, contemporary inclusion of *S. peltifer* within *S. minor* and small sample sizes seem to have led several analyses to miss or ignore variation on finer geographic scales (Seidel and Lucchino 1981). The popularity of scute length and width ratios in turtle morphology studies also led some authors to not measure carapace height. Interestingly, this resulted in a failure to detect differences between populations (Iverson 1977a). The last of this group of studies found that the "intermediate" population at the fall line in the Black Warrior, hypothesized to be hybrids by Estridge, was not significantly different from typical *S. depressus* (Ernst et al. 1988).

However, recent work has supported Estridge's hypothesis by providing morphological and genomic evidence of hybridization between *S. depressus* and *S. peltifer* in the North River and the Black Warrior River at the fall line (Scott and Rissler 2015; Scott et al. 2019). Despite this, there has still not been an effort to further examine the supposed intermediate populations in the upper Cahaba River.

Questions and thesis objectives

Shell shape variation in *S. peltifer* presents an interesting research topic for several reasons. While ecomorphological variation in shell shape has been demonstrated for several species of emydid turtles (Aresco and Dobie 2000; Lubcke and Wilson 2007; Rivera 2008; Rivera et al. 2014; Ennen et al. 2019), it has not been shown to exist in bottom-walking turtles inhabiting the same habitat. In fact, data on how habitat relates to intraspecific shell shape variation are lacking for Kinosternidae as a whole (Berlant and Stayton 2017). Many bottom-dwelling animals have specialized behaviors and morphologies for dealing with their specific environment, since flow is not constant between the water column and the bottom of a stream (Hart and Finelli 1999). Because of this, *S. peltifer* likely faces different functional challenges than do channel-swimming turtles. Thus, convergent evolution of streamlined shell shapes in these two turtle functional groups would provide evidence of the adaptive significance of the trait (Losos 2011).

Several other questions might follow the documentation of ecomorphological shell shape variation in *Sternotherus*. First – does shell shape variation have consequences for aspects of life history? Environmental aspects have often been found to affect reproductive output in turtles (Tinkle 1961; Gibbons et al. 1982; Fehrenbach et al. 2016; Ennen et al. 2017). Additionally, small females may face morphological constraints that place limits on the size of offspring (Congdon and Gibbons 1987; Clark et al. 2001). Could dorsoventrally flattened shells reduce the volume available for carrying eggs?

Another major question addresses the genetic basis for the shell shape variation. Did historic hybridization between *S. depressus* and *S. peltifer* introduce the genes that code for flattened shell shapes to the Cahaba River *S. peltifer* population? Or did natural selection act on existing genetic variation, causing convergent evolution of the flattened shell phenotype? Either way, if gene flow is occurring across the fall line and there is no contemporary gene flow between *S. depressus* and *S. peltifer*, flattened shell shapes would be unlikely to persist in the upper reaches of the Cahaba River unless they are under positive selection. The hypothesized hybridization events could have caused the flattened shell characteristic of *S. depressus* to become introgressed into local populations of *S. peltifer*. Regardless of the source of the pattern, if the trait is advantageous, selection should cause it to persist locally.

Importantly, patterns of association between shape and the environment cannot on their own be used to determine which evolutionary processes generated those patterns (Radinsky 1985). In fact, an adaptive origin for morphological variation is often an assumption in ecomorphological studies (Feilich and López-Fernández 2019). Discerning which process is responsible for the observed pattern may be impossible without detailed and often long-term experiments. In long-lived groups such as turtles, this presents a significant logistical hurdle. However, stronger evidence for adaptation can be amassed when morphological patterns are integrated with evidence from other sources (i.e., genetic, reproductive, and functional information),

In this thesis, I use morphological and reproductive data to examine how environmental variation has influenced the evolution of *S. peltifer* along the Cahaba River drainage in Alabama. Specifically, I use (1) 3-dimensional geometric morphometrics to address whether shell shape differs between turtles from above and below the fall line, and (2) present baseline reproductive data for the *S. peltifer*, along with an initial test of optimum egg size theory for the species. Together, these approaches begin to address how environmental variation influences evolution in bottom-walking turtles.

Chapter 2: Eco-morphological variation of shell shape in stripe-necked musk turtles (*Sternotherus peltifer*)

Introduction

Detailed examinations of phenotype-environment relationships are important for addressing several major goals of evolutionary ecology, such as identifying the agents and strength of selection (Arnold 1983), determining what drives locally adaptive phenotypic variation (Kawecki and Ebert 2004), and revealing how populations change and adapt in response to environmental change (Boag and Grant 1981). Most of these goals focus on local adaptation, an early stage in speciation through natural selection (Hereford 2009). Many adaptive hypotheses attempt to explain how traits have evolved to better suit an environment, but few of these have been tested. Convergent evolution of a given phenotype in similar ecological contexts is often considered to be evidence of adaptation, since it is unlikely that the same pattern would repeatedly occur among multiple species by chance alone (Wainwright and Reilly 1994; Losos 2011).

Among aquatic organisms, water flow rate is an especially important aspect of the environment (Vogel 1994). Correlated variation in body shape and flow has been repeatedly demonstrated in fishes (Brinsmead and Fox 2002; Langerhans et al. 2003,2007; Blob et al. 2008; Senay et al. 2015; Malato et al. 2017; reviewed by Langerhans 2008). Shape variation has often evolved in response to anthropogenic habitat alteration (Haas et al. 2010; Kern and Langerhans 2018; Akin and Geheber 2020; but see Geladi et al. 2019). Similar ecomorphological patterns exist in algae (Boller and Carrington 2006; Stewart 2008), plants (Boeger and Poulson 2003;

Asaeda et al. 2005), snails (Trussell et al. 1993; Trussell 1997; Holomuzki and Biggs 2006; Kistner and Dybdahl 2013), and tadpoles (Jennings and Scott 1993; Sherratt et al. 2018).

Studies of the effect of flow on fish phenotypes are far more abundant than those focused on other taxa (Langerhans 2008). However, taxonomic variety is important because organisms with vastly divergent morphologies interact differently with each environment. For example, fishes typically create propulsion by moving their bodies, which means that shape reflects dynamic functional demands and not just hydrodynamic efficiency, in contrast with a turtle or snail which has an immobile shell with no direct role in locomotion (Rivera 2008; Mayerl et al. 2019b). In addition, many fish studies implicate a significant role of phenotypic plasticity in generating body shape variation. The rigid nature of invertebrate exoskeletons and snail and turtle shells, in contrast to the flexible bodies of many fishes, may have implications for the potential of phenotypic plasticity to affect body shape within an individual's lifetime. Furthermore, other compensatory mechanisms for dealing with high flow environments, such as habitat selection (Jones and Sievert 2009; Tornabene et al. 2019) or kinematics (Mayerl et al. 2019a), would likely be more plastic than shell shape.

Despite these differences, the limited morphological data available from freshwater turtles parallel those from fishes and other aquatic organisms. Comparisons between stream and pond-living populations of western pond turtles (*Actinemys marmorata*) in California found that turtles in stream populations consisted of turtles with lower and narrower carapaces than those living in ponds (Lubcke and Wilson 2007). In the southeastern United States, a similar pattern has been described within riverine environments, with turtles having more streamlined shells in the faster flowing stretches of the Appalachian foothills, and relatively higher, more domed shells in the coastal plain (Aresco and Dobie 2000; Rivera 2008; Rivera et al. 2014; Ennen et al.

2019). The geographic cutoff in these studies has generally been the fall line, a physiographic feature that demarcates the piedmont and ridge and valley provinces from the coastal plain. The fall line is a biogeographically significant feature for both the presence and abundance of various species, and represents an abrupt change in habitat (Tinkle 1959; Guyer et al. 2015).

Ecomorphological studies on turtles have largely focused on semi-aquatic emydids that move around by swimming (*Pseudemys* - Aresco and Dobie 2000; Rivera 2008; *Actinemys* - Lubcke and Wilson 2007; *Graptemys* - Rivera et al. 2014; Ennen et al. 2019). Functional morphology experiments on a channel-swimming emydid (*P. concinna*) have established that flatter, streamlined carapaces are significantly more hydrodynamic than high, domed carapaces (Rivera 2008). Comparative analyses of shell shape have also found evidence of remarkably consistent morphological convergence between ecologically similar species from two large turtle radiations (Emydidae and Geoemydidae; McLaughlin and Stayton 2016). Other turtle families, such as snapping turtles (Chelydridae) and mud and musk turtles (Kinosternidae) navigate their aquatic environment primarily by walking along the substrate. These turtles may experience flow-induced stressors differently than channel swimming turtles, and it is unclear if the same ecomorphological patterns exist in both groups (Berlant and Stayton 2017).

Most morphological studies on kinosternids have centered on taxonomic questions. This is certainly the case for the *Sternotherus minor* complex, which was the focus of several studies which attempted to determine whether *S. depressus* and *S. (minor) peltifer* should be considered separate species. In his master's thesis, Estridge (1970) found notable populations of *S. peltifer* that had highly flattened shells, reminiscent of *S. depressus*, and suggested that there may be gene flow between the two species. This was confirmed for one of these populations (Scott and Rissler 2015), but the one in the upper Cahaba River was overlooked in follow-up studies.

Subsequent genetic work has solidified relationships in the genus (Scott et al. 2018), but small sample sizes and the popularity of scute length ratios as species delimiting characters in turtle taxonomy have likely led several authors to overlook intraspecific morphological variation (Iverson 1977a; Seidel and Lucchino 1981). Interestingly, there is some evidence for habitat-related shell shape variation in another kinosternid from Costa Rica (Acuña-Mesen 1994).

An important consequence of studying shell shape variation in many emydid turtles is that sexual shape dimorphism is difficult to address in many taxa due to a high degree of sexual size dimorphism. The most-studied turtle taxa in aquatic ecomorphology, *Pseudemys* and *Graptemys*, both possess high female-biased sexual size dimorphism (Cox et al. 2007; Ceballos et al. 2013). This has required that sexes be analyzed separately, making direct shape comparison between sexes difficult (Rivera et al. 2014; Ennen et al. 2019), which is important because selective pressures associated with sex-specific aspects of life history can cause consistent shape differences between sexes (Oke et al. 2019). For instance, discovering why an optimum egg size seems to exist for some, but not all species is an important question in turtle life history. Morphological constraints have been implicated as a size-limiting factor for eggs of small-bodied species (Congdon and Gibbons 1987), and body cavity volume limits clutch size (Shine 1992; Qualls and Shine 1995; Du et al. 2005). Sexual size dimorphism is far less extreme in *Sternotherus* than in *Pseudemys* or *Graptemys*, enabling direct examination of sexual shape variation (Ceballos and Iverson 2014).

Many studies on turtle shell morphology, including virtually all studies on *Sternotherus*, have examined variation using traditional straight-line measures. This is suboptimal because it is difficult to characterize 3-dimensional shape using 2-dimensional measurements. Geometric morphometric methods have revolutionized morphological studies by making it possible to account for actual shape variation, limited only by the number of sites suitable for landmarks (Zelditch et al. 2004). Because scute and bone intersections are ideal landmark locations, these methods have increasingly been used in ecological and evolutionary research on turtle shells (Valenzuela et al. 2004; Rivera 2008; Hawkshaw et al. 2019; Dziomber et al. 2020; Horváth et al. 2021).

I hypothesized that stream flow conditions are a significant driver of local adaptation in turtle shell morphology, and predicted that *S. peltifer* from above the fall line would be flatter than turtles from below the fall line, because of selection favoring shell shapes that are efficient in higher flow environments. I also hypothesized that differences in life history between sexes would lead to females and males experiencing different selective pressures. I predicted that females from above the fall line would not be as flat as males from the same area, due to the need to have space for eggs within the shell.

Methods

Data collection

An ideal natural system for studying habitat-associated shell shape variation in aquatic turtles has significant variation in river characteristics, is large and accessible enough to sample from many locations, and is relatively unaltered by human activity. The Cahaba River flows through central Alabama, from its headwaters/source northeast of Birmingham to its confluence with the Alabama River near Selma. The river is bisected by the fall line, which divides the drainage almost evenly between the ridge and valley province to the north and the gulf coastal plain to the south (Williams et al. 2008; Guyer et al. 2015). Above the fall line, the Cahaba flows swiftly over rocky substrates, eventually slowing down as it enters the sandy coastal plain (Fig.

2.1; Ward et al. 2005). Unlike many other rivers in the southeastern US, the Cahaba has been allowed to flow unimpeded by large dams. I surveyed for *S. peltifer* at sites throughout the Cahaba River drainage (n = 19; Fig. 2.1; Appendix 1) using collapsible mesh crawfish traps (12" diameter, 36" long, 5" opening; Promar, USA) baited with sardines in soybean oil (Beachcliff, USA), or by hand while snorkeling or wading. Most sites were located at bridge crossings, parks, or public boat ramps. I set traps near the bank, usually at large root masses, along aggregations of submerged woody debris, or near riprap. An air-filled plastic bottle was placed in the upstream-facing end of each trap as a flotation device, and the downstream-facing ends were allowed to sink towards the substrate. I checked traps for turtles at least every 12 hours.

For each turtle captured (n = 93), I recorded a suite of straight-line shell measurements with a pair of digital calipers. These included: midline straight carapace length (SCL); midline plastron length (PL); carapace width, measured at the junction of the 6th and 7th marginal scutes (CW); carapace height, measured behind the 2nd vertebral scute (CH); and plastron width (PW). Using a triangular file, I marked the marginal scutes of most turtles with a unique sequence to enable identification in case of recapture (Cagle 1939). I also collected the same set of measurements from 29 preserved specimens of *S. peltifer* housed in the Auburn University Museum of Natural History (AUMNH). All museum specimens were collected from the Cahaba River watershed between 1963 and 2009 (Appendix 2).

I used stereophotography to obtain sets of images for use in 3-D geometric morphometric analyses (Olsen and Westneat 2015). The photography setup consisted of two digital SLR cameras positioned on tripods so that they were facing the same point on a surface at a 45- 90° angle in the horizontal plane, and a 45° angle in the vertical plane, as described in the documentation for the StereoMorph method (Olsen and Westneat 2015). Preliminary set up for this method involves the use of a calibration grid, which is used by the program to determine the precise spatial and angular positions of the two cameras in relation to each other. I took photographs of the plastron and carapace of each turtle from multiple aspects, with at least three landmarks visible across all images. Following stereophotography, I promptly released all turtles at the site of capture.

There are many options for data collection for 3-D geometric morphometric studies, but this one is particularly well-suited to field studies on turtles. One important aspect of stereophotography is that it does not require euthanasia – once the equipment is in place, photographs can be taken quickly enough that escape attempts by the specimen are not problematic. Methods such as computed tomography require the specimen to be still for much longer. StereoMorph is also better for data collection in the field than photo-based 2-D geometric morphometrics. Each StereoMorph session has its own calibration, so variation in the angle and distance of the camera relative to the subject is a non-issue (Olsen and Westneat 2015). In contrast, 2-D geometric morphometrics requires consistent angles and distances of the camera relative to the subject, which can be difficult to achieve when collecting data across many different locations and sessions.

I assigned 44 landmarks on the right side of the shell of each turtle using the StereoMorph package in R (Fig. 2.2) (Olsen and Westneat 2015). Most landmarks were placed at the intersection of two or more scutes, similar to the scheme used in a previous GM study of kinosternid turtles (Berlant and Stayton 2017).

Data preparation and shell shape characterization

As there is significant allometric shape change in *S. peltifer* and my juvenile sample size was small (n = 13), I only included adult-sized turtles (≥ 70 mm SCL; n = 78) in analyses (Ernst et al. 1988). I used a principal component analysis to reduce dimensionality in the straight-line measures to several main axes of variation.

Using the 'gpagen' function in the R package geomorph (Adams and Otárola-Castillo 2013), I conducted a Generalized Procrustes Fit on the landmark dataset, which scaled and aligned the landmarks from all turtles so that only shape variation remained (Zelditch et al. 2004).

Statistical analyses

As I included five straight-line variables in the PCA, some of the PCs may not be easily interpretable. Following Rivera (2008), I retained the principal components that explained the most variation, up to 95% of the total variation. To identify the effects of location, sex, and their interaction on shell size and shape, I used two-way ANOVA on each retained principal component.

I used the 'prcomp.lm' function to conduct a Procrustes ANOVA, modeling landmark-based shape variation against sex, location relative to the fall line, and their interaction as with the straight-line measurements (Adams and Otárola-Castillo 2013). To identify and visualize the principal axis of shape variation between groups, I conducted a canonical variate analysis (CVA) using the 'CVA' function in the morpho R package (Schlager 2017). CVAs are often used to identify traits unique to a given group because they emphasize between-group variation above within-group variation (Zelditch et al. 2004). The CVA also included a jackknife cross-validation test, in which individuals were assigned back to groups to test how well these groups fit the data.

Results

Straight-line measurement analyses

I captured a total of 93 turtles throughout the Cahaba River system. Wading was most effective at sites above the fall line, where the water is usually relatively clear. Most of these turtles were also collected at night, though some were also encountered during the day. All turtles sampled below the fall line were captured in traps. Of the turtles captured, 78 were adults ($n = 49$ from above fall line; $n = 29$ from below; Table 2.1) of sufficient size to fit criteria for inclusion in traditional morphometric analyses with an additional 13 museum specimens (Appendix 2). The magnitude and sign of the loadings of each variable within a given principal component were used to interpret whether that principal component represented size or shape variation (Jolicoeur and Mosimann 1960). The first two principal components explained 94.29% of the total variation (Table 2.2). The first principal component (85.01% of total variation) has the same sign for all included measures, so that an increase in PC1 represents an increase in all included measures. So, PC1 can be interpreted to represent variation in size. Female turtles had significantly higher values of PC1 than males ($F = 16.045$, $p = 0.00013$; Table 2.3). Neither location relative to the fall line nor the interaction between sex and location were statistically significant (Fall line: $p = 0.169$; Interaction: $p = 0.105$).

The second principal component (representing 9.28% of the total variation) has carapace height (CH) in strong opposition to all other included measures, reflecting an increase in shell height while all other straight-line shell measures decrease. I interpret this PC to represent how domed (positive values of PC2) or flat (negative values) a turtle is for its size (Fig. 2.3). Turtles from above the fall line had significantly lower values of PC2 ($F = 42.951$, $p = 3.82e^{-9}$; Table 2.4; Fig. 2.4) than turtles from below the fall line. Neither sex nor the interaction between sex and location relative to the fall line were statistically significant (Sex: p = 0.727; Interaction: p = 0.149).

Geometric morphometric analyses

Of the total sample (n = 91), 40 individuals were excluded from geometric morphometric analysis because of either small size or the presence of significant damage to the marginal scutes that prevented all landmarks from being placed on every individual. The final sample included in GM analyses consisted of 51 individuals (Table 2.1). Procrustes ANOVA found that there was significant shape variation associated with sex (F = 3.4384, p = 0.001) and location relative to the fall line (F = 1.8134, p = 0.036), but the interaction between those terms was not significant (p = 0.732; Table 2.5). On average, turtles from above the fall line had flatter carapaces and slightly more elongate plastrons than did turtles from below the fall line. The CVA cross-validation test correctly assigned individuals to their location of origin 84.31% of the time, but was slightly more accurate for turtles from below the fall line (88.89% correct) than for those from above the fall line (79.17% correct; Fig. 2.5). Males had smaller plastrons than females, especially regarding the rear lobe. The rear of the carapace, including the rear marginal scutes, was also more elongate and flared out in males. Turtles were assigned to the correct sex more accurately, 96.08% on average. Females were assigned correctly every time, while males were assigned to the correct sex 92.59% of the time.

Discussion

I found strong evidence for shell shape variation between *S. peltifer* from opposite sides of the fall line in the Cahaba River drainage, as well as evidence of sexual shape dimorphism. My findings are similar to Estridge's (1970), who suggested that the flattened phenotype of *S.*

peltifer in the Black Warrior and upper Cahaba drainage may be due to hybridization with *S. depressus*. His suspicions about the Black Warrior turtles have since been validated (Scott et al. 2019), but there are currently no data that support the hypothesized genetic exchange between *S. depressus* and Cahaba *S. peltifer*. Similar morphological examinations of *S. peltifer* in other stretches of river would be helpful for examining the phenotype-environment relationship in greater detail. Anecdotal reports suggest that the pattern observed in this study may be widespread in the greater Mobile River drainage (Tinkle 1958) (J. Jenkins, pers. comm.). The spatial scale and modern methods used here were able to find significant variation where it was missed by several earlier studies (Iverson 1977a; Seidel and Lucchino 1981; Ernst et al. 1988). My findings are similar to those from other phenotype-environment studies in turtles, which also found turtles to be relatively more streamlined in areas of higher flow (Lubcke and Wilson 2007; Rivera 2008; Rivera et al. 2014; Ennen et al. 2019).

Many fish ecomorphology studies aim to test adaptive hypotheses, but often invoke phenotypic plasticity as an alternative explanation for shape variation (e.g., Franssen et al. 2013). The observed variation in turtle shell shape could reasonably be interpreted as the product of either spatially divergent natural selection (Rivera 2008), contemporary gene flow (Scott et al. 2019), or phenotypic plasticity (Aresco and Dobie 2000; Ennen et al. 2019). There is strong evidence for the existence of some degree of habitat-influenced phenotypic plasticity to traits related to body shape in fishes (Fischer-Rousseau et al. 2010; Kelley et al. 2017). Plasticity in shell shape, as a skeletal feature, could be labile or non-labile. Few studies have addressed potential plasticity in turtle shell shape, but forces associated with crushing mollusk prey does occasionally induce plasticity in the cranial morphology of durophagous populations of *Sternotherus* (Pfaller et al. 2010; Iverson 2020). The results of one study on hatchling European pond turtles (*Emys orbicularis*) have been used to suggest that habitat-associated phenotypic variation is best explained by plasticity, since individuals raised in their "pond" treatment reached greater sizes over the study period than those kept in their "river" treatment (Zuffi et al. 2017; Ennen et al. 2019). However, since sample sizes were small and the experiment only lasted a year, it is unclear whether the observed differences would persist into adulthood.

So, while it is possible that there is some degree of plasticity to shell shape, for a variety of reasons it seems more likely that the observed variation is the result of adaptive evolutionary change. While few studies have addressed the topic, there is not much evidence for flow-induced plasticity in turtle shell shape, and the forces exerted by flow on bottom-walking turtles are likely less intense than those felt by fishes occupying the water column (Hart and Finelli 1999). Additionally, turtle shell shape is heritable, implying a genetic rather than environmental basis for phenotypic variation (Myers et al. 2006). Genomic analyses of selection in the *Sternotherus* hybrid zone in the Black Warrior drainage also found that there are reduced levels of gene flow in some regions of the genome, accompanied by variation in shell shape, leaving the possibility that selection is acting on shell morphology across the local environmental gradient (Scott and Rissler 2015; Scott et al. 2019). Common garden experiments stretching across many generations would be required to fully evaluate whether adaptation, plasticity, or both lead to the shell shape variation that I observed in Cahaba River *S. peltifer*, which would be extremely difficult to do with turtles.

My results also provide insight into the origins of flattened shell shapes in other turtle species. Most notably among these is *S. depressus*, a very close relative of *S. peltifer* (Scott et al. 2018). The existence of multiple species exhibiting the same phenotypic pattern is suggestive of convergent evolution – though it is not a definitive test (Losos 2011). A classic example is the convergent radiations of anole ecomorphs on islands in the Caribbean (Williams 1972; Jackman et al. 1997; Losos 2009). A definitive test of convergent evolution will require a phylogenetic comparative study of morphological data. As Estridge (1970) pointed out, it is possible that hybridization between *S. peltifer* and *S. depressus* has led to genes coding for flattened shell shapes spreading throughout the upper Cahaba River, but genetic analyses will be needed to know for sure. Hybridization between these species has occurred along the fall line in the neighboring Black Warrior drainage (Scott et al. 2019). It is possible that a similar situation has occurred between the Cahaba and Black Warrior, either through overland migration or stream capture.

Flattened shell shapes may have evolved in parallel in stream-dwelling turtles from around the world (e.g., *Notochelys platynota, Platysternon megacephalum*). In addition, depressed shell shapes are not exclusive to aquatic turtle species, having been documented in tortoises and terrestrial geoemydids (Ireland and Gans 1972; Xiao et al. 2020). In these species, flattened shells are likely adaptations for wedging into rock crevices. Some authors have applied the same thinking to *S. depressus*, suggesting that the flat shell characteristic of the species had evolved to enable turtles to wedge themselves in cracks between layers of bedrock substrate (Jackson 1988). This hypothesis would be very difficult to test in *S. depressus*, but it seems unlikely this is the evolutionary driver that has led to shell shape variation in Cahaba drainage *S. peltifer*. Bedrock, while a major component of the substrate at some sites along the Cahaba, is not as ubiquitous as it is in the Black Warrior drainage (Williams et al. 2008).

Habitat associated shape variation in turtle shells may have a significant impact on reproductive output. Results from studies on lizards clearly show that reduced body volume is associated with smaller clutches (Vitt and Congdon 1978; Shine 1992; Qualls and Shine 1995;

Du et al. 2005). Despite the relatively robust body of literature on turtle reproductive ecology, apparently few researchers have examined relationships between shell shape (or height) and clutch size. A few studies on emydid turtles also show that an increase in relative shell height can correlate with larger clutches (Tucker et al. 1998; Zuffi et al. 1999). These studies build on a growing body of literature that has established that for small female turtles, morphological features can act as constraints on egg size (Congdon and Gibbons 1987; Clark et al. 2001; Lindeman 2020). Usually, the morphologically constraining feature is thought to be either the width of the pelvic aperture or the size of the gap between the rear carapace and plastron (Congdon and Gibbons 1987; Clark et al. 2001). Still, flattened shell shapes could act as another morphological constraint on reproductive characteristics in kinosternids and other small turtles.

The observed pattern of shell shape variation may have some important conservation implications. Many large cities in the southeastern US are situated along rivers either at or above the fall line (i.e., Birmingham, Atlanta, Chattanooga, Charlotte, etc.). Pollution and alteration of riverine habitat have been implicated in population declines for aquatic organisms. As populations continue to grow, it is likely that resource extraction and recreation use will increase, potentially subjecting turtles to flow regimes unlike those they have been living in and adapting to for generations. For example, *S. depressus* does not do well in parts of its historical distribution that have been dammed and inundated (Holmes 2005). Similarly, many conservation organizations have begun captive breeding endangered turtle species with the hope of one day releasing them back into the wild. Those managing these programs should be aware of the specific environment of the populations from which they source their animals, and keep that in mind if offspring are eventually released.

In this chapter, I have provided the first evidence of convergent phenotypic patterns of variance in shell shape between channel swimming and bottom-walking turtles, likely in response to habitat variation, which is suggestive of parallel evolution. In long-lived organisms like most aquatic turtles, it is very difficult to collect enough data to associate phenotypic variation with changes in fitness. However, if other sources of data (i.e., life history, genetics, behavior) corroborate the observed relationship between habitat and shell shape, there would be good evidence of an adaptive value to shell shape variation of *S. peltifer* in the Cahaba River drainage.

Table 2.1: Sample size of wild-caught turtles used in morphological analyses. The total sample is indicated first, followed by the sample used in geometric morphometrics in parentheses. Museum specimens were not included in geometric morphometric analyses.

Fall Line	Sex	Wild	Museum	Total
Upper Cahaba	M	25 (11)	10	35 (11)
	F	24 (13)	2	26 (13)
	All	**49 (24)**	**12**	**61 (24)**
Lower Cahaba	M	16 (16)	0	16 (16)
	F	13 (11)	1	14 (11)
	All	**29 (27)**	**1**	**30 (27)**
Combined	M	41 (27)	10	51 (27)
	F	37 (24)	3	40 (24)
	All	**78 (51)**	**13**	**91 (51)**

Table 2.2: Loadings and variation explained by first five principal components of PCA on straight-line measures of straight carapace length (SCL), plastron length (PL), plastron width (PW), carapace height (CH), and carapace width (CW).

	PC1	PC2	PC3	PC4	PC5
SCL	0.4605	-0.2021	-0.6655	0.1168	-0.5390
PL	0.4657	-0.2095	-0.1875	-0.6104	0.5757
PW	0.4623	-0.1151	0.6914	-0.2665	-0.4733
CH	0.3770	0.9228	-0.0528	0.0327	0.0483
CW	0.4639	-0.2244	0.2028	0.7360	0.3895
% variation explained	0.8501	0.0928	0.0274	0.0173	0.0124

Table 2.3: Summary table for ANOVA on PC1, sex, and location relative to the fall line.

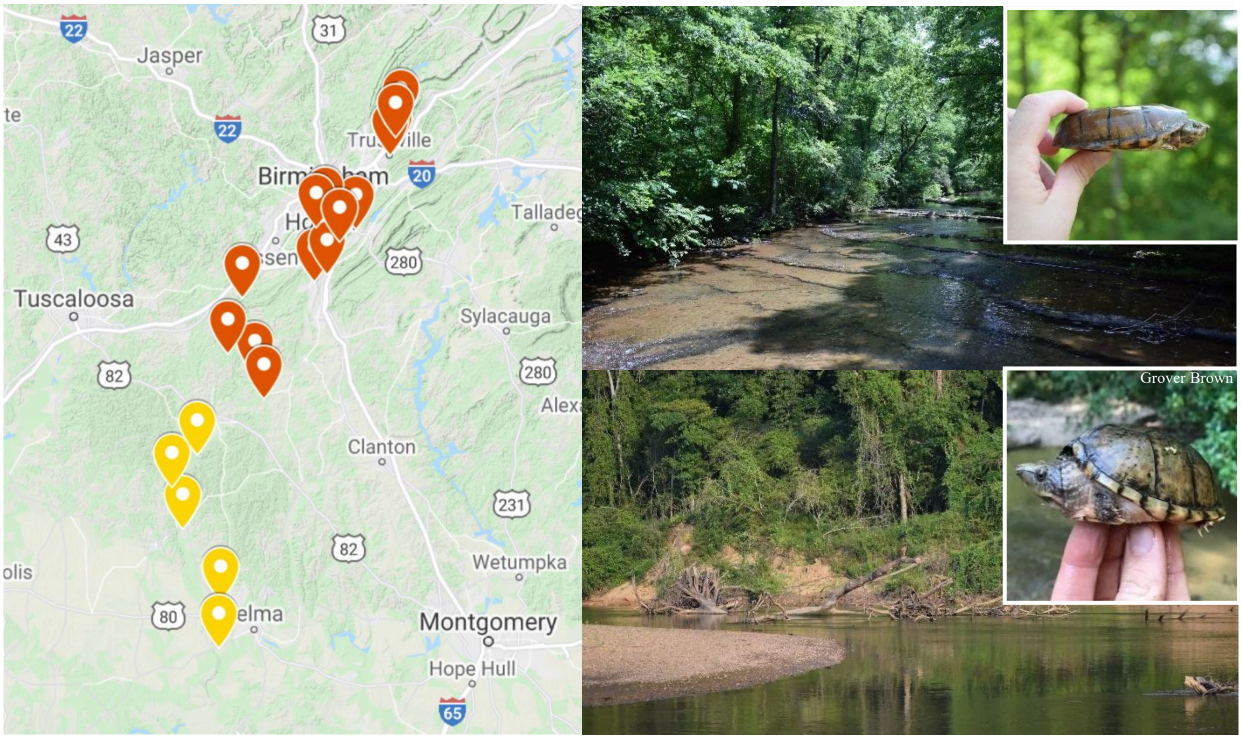
	Df	Sum Sq	Mean Sq	F value	P
Fall line	1	6.83	6.83	1.921	0.169
Sex	1	57.02	57.02	16.045	0.00013*
Interaction	1	9.56	9.56	2.691	0.105
Residuals	87	309.16	3.55		

Table 2.4: Summary table for ANOVA on PC2, sex, and location relative to the fall line.

	Df	Sum Sq	Mean Sq	F value	P
Fall line	1	13.565	13.565	42.951	$3.82e^{-9}$*
Sex	1	0.039	0.039	0.2123	0.727
Interaction	1	0.670	0.670	2.121	0.149
Residuals	87	27.478	0.316		

Table 2.5: Summary table with results of Procrustes ANOVA on geometric morphometric data, sex, and location relative to the fall line.

	Df	SS	MS	R sq	F	Z	P
Fall line	1	0.005973	0.0059725	0.03248	1.8134	1.8924	0.036*
Sex	1	0.011324	0.0113242	0.06158	3.4384	3.5363	0.001*
Interaction	1	0.002275	0.0022750	0.01237	0.6908	-0.6522	0.732
Residuals	47	0.154794	0.0032935	0.84178			
Total	50	0.183889					



Grover Brown

Figure 2.1. Map of sample locations, with images of typical habitat and *S. peltifer* phenotypes from the ridge and valley and coastal plain. Orange and yellow pins indicate sampling locations above (n = 14) and below the fall line (n = 5), respectively. The top image features typical habitat above the fall line, with local *S. peltifer* phenotype in the top insert. The bottom image is representative of habitat below the fall line, with local *S. peltifer* phenotype in the bottom insert.

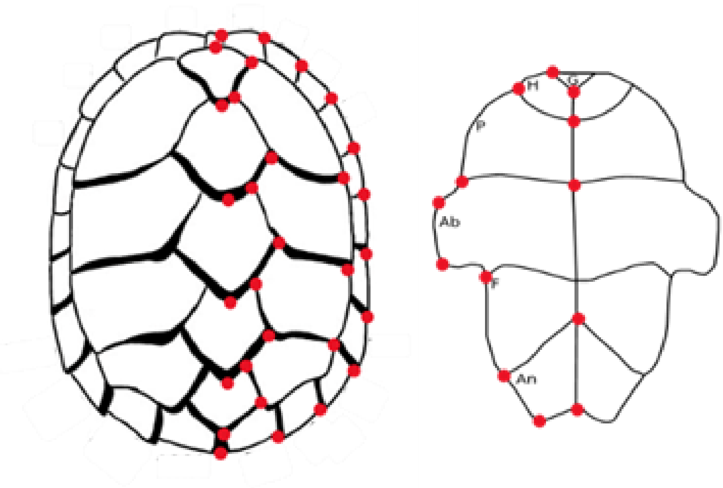


Figure 2.2. Diagram of carapace (left) and plastron (right) of *S. peltifer* included in this study. Red dots represent landmarks used in geometric morphometric analyses. Anterior end of the shell is facing up.

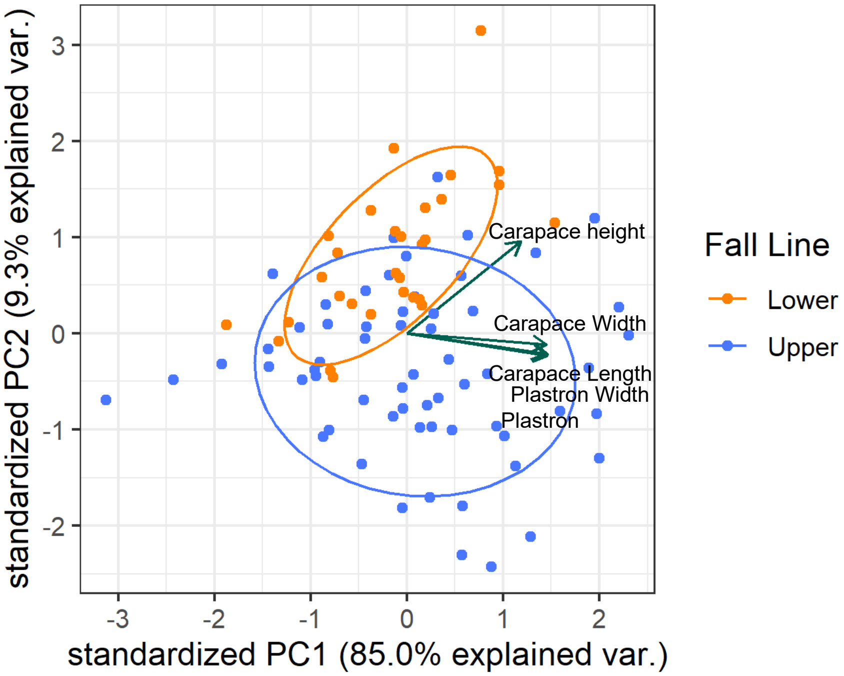


Figure 2.3. Biplot of principal component analysis of traditional morphometric measures, showing the distributions of Principal Component 1 (PC1) and Principal Component 2 (PC2) among turtles from above (blue) and below (red) the fall line. Arrows represent the original variable loadings among the first two PCs. High values of PC1 indicate larger body size, and high values of PC2 indicate a taller or more domed carapace.

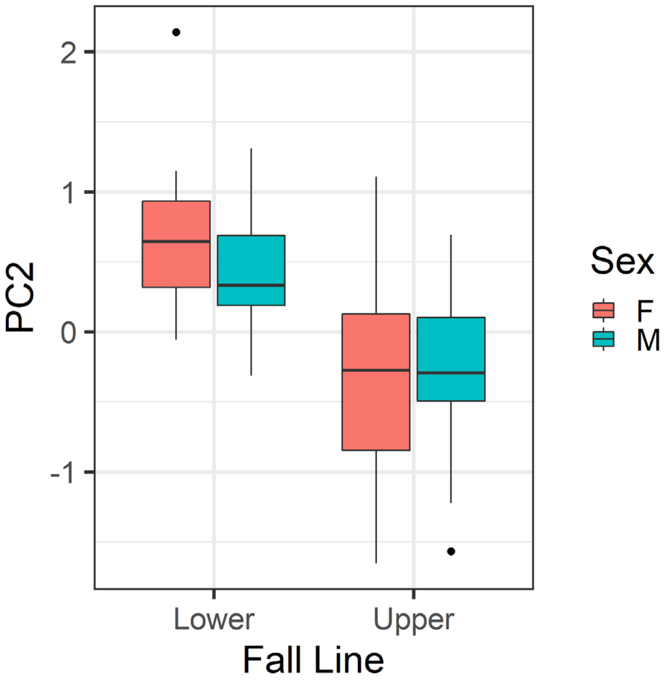


Figure 2.4: Boxplot showing difference in PC2 values relative to sex and location relative to the fall line. Turtles from above the fall line have lower PC2 values than those from below the fall line, representing flatter shells for a given size. The black line in each box represents the median value. Upper and lower hinges (boxes) correspond to the first and third quartiles, and whiskers extend to most extreme value up to 1.5*IQR. Black dots represent outliers.

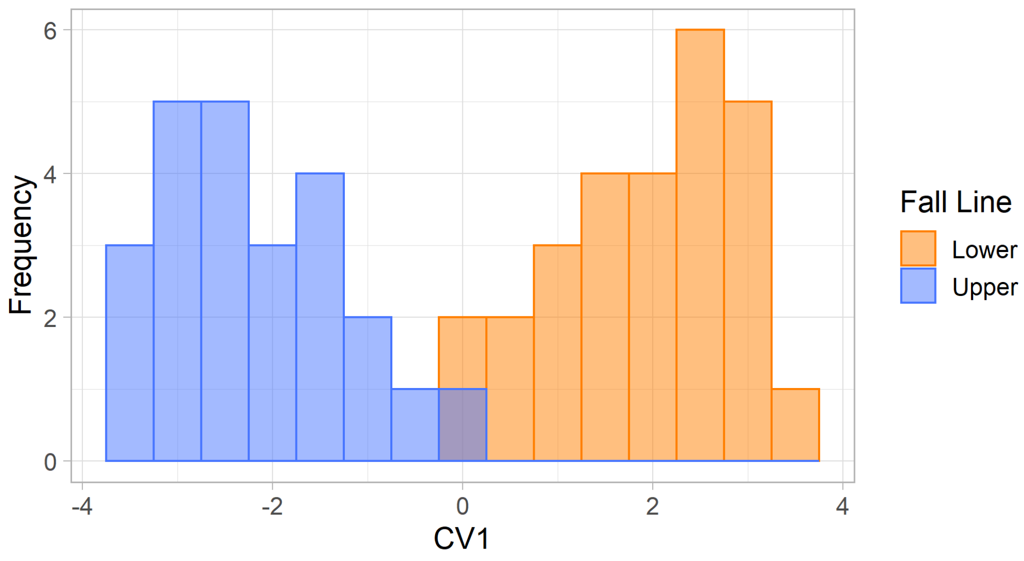


Figure 2.5. Frequency plot relating canonical variate (CV) scores with assigned groups representing location relative to the fall line.

Chapter 3: Reproductive patterns and preliminary test of optimal egg size theory in central Alabama stripe-necked musk turtles (*Sternotherus peltifer*)

Introduction

Although turtle research has experienced a lot of progress in recent decades (Lovich and Ennen 2013), there is still a surprising lack of basic natural history information for many species. For instance, many musk turtles (genus *Sternotherus*) are understudied relative to other North American turtle groups, perhaps due in part to their small size and somewhat restricted geographic distributions (Lovich and Ennen 2013). This is compounded by recent discovery of cryptic diversity using genome-wide sequencing, which has altered our understanding of relationships within the genus (Scott et al. 2018). Following the resulting taxonomic changes, the genus Sternotherus consists of six species in two clades, with the *S. minor* clade consisting of *S. depressus*, *S. intermedius*, *S. minor*, and *S. peltifer*. Previously, *S. peltifer* was usually considered a subspecies of *S. minor*, and *S. intermedius* was thought to be a large intergrade population between *S. minor* and *S. peltifer*. Much of the natural history information on the *S. minor* clade comes from the abundant springs in peninsular Florida, within the distribution of the new, restricted concept of *S. minor*. Far fewer studies have been conducted to the north and west, leaving significant gaps in our understanding of the basic ecology of *S. peltifer*. For example, though reproduction and life history have been examined in *S. minor* at multiple sites in Florida (Table 3.1; e.g., Iverson 1977,1978; Cox and Marion 1978; Etchberger and Ehrhart 1987), there is essentially no data available for *S. peltifer* aside from a few anecdotes mentioned in Tinkle's thesis over 60 years ago (Tinkle 1958).

Basic reproductive data are important for laying the groundwork for conservation planning (Purvis et al. 2000; Litzgus and Mousseau 2006; Hamann et al. 2010) and for informing more comprehensive life history studies (Iverson 1992). Because the resources available for reproduction are finite, aspects of life history should evolve to maximize offspring and parental fitness (Stearns 1989; Bernardo 1996). A prominent question in life history evolution surrounds optimum egg size (OES) theory, which suggests that because egg size has been optimized by natural selection, variation in reproductive output should occur as a change in the number of eggs produced (Smith and Fretwell 1974; Brockelman 1975). Under OES theory, egg size is predicted to be constant within a population. Since egg size does not vary, females that have more resources available to reproduction (i.e., large body size, access to abundant food source) should produce more offspring than those with fewer resources. However, many turtle species seem to deviate from OES theory, with both egg and clutch size increasing with female body size (e.g., Congdon and Gibbons 1985). A possible explanation for this phenomenon is that in small turtles, skeletal features such as the size of the pelvic aperture or caudal gap (distance between the rear plastron and carapace) might constrain egg size, although at least one tortoise species lays eggs wider than the pelvic aperture (Congdon and Gibbons 1987; Hofmeyr et al. 2005). Shell shape – which along with size determines shell volume – is yet another potential morphological constraint on reproductive output, though this has not yet been explored either in detail or very widely (Chapter 2). The question of whether OES theory or the morphological constraint hypothesis best explains patterns of turtle reproduction has been addressed in several kinosternids, so far with inconsistent results (Long and Rose 1989; Clark et al. 2001; Macip-Ríos et al. 2012,2013). Continued work across turtle species and populations will hopefully clarify how aspects of the environment (e.g., latitude, elevation, seasonality) and morphology (e.g., female body size, body shape, PAW) might explain deviations from OES theory.

Under OES theory, clutch size should increase isometrically with body size and egg size should be unrelated to body size, whereas both clutch and egg size should increase hypoallometrically with female body size under the morphological constraint hypothesis (Ryan and Lindeman 2007). Therefore, the appropriate way to test OES predictions is to investigate allometric relationships, which is done by evaluating slopes generated by regression of log-transformed variables (King 2000). The expected slope under isometry is 1.0 when modeling a linear size measurement against a linear reproductive characteristic (e.g., carapace length against egg width), and a slope of 3.0 is expected under isometry when regressing a linear size measurement against a volumetric reproductive characteristic (e.g., carapace length against clutch size; King 2000). In a recent review of turtle reproductive allometry, Iverson et al. (2019) found consistent overall patterns of hypoallometry between female body size and reproductive characteristics. Lindeman (2020) examined reproductive allometry in a small sample of *S. odoratus* from Texas with a nearly fixed clutch size, and found an isometric relationship between body size and egg size. Since clutch size variation was extremely low in his sample, he suggested that this result supports the idea that females experience a functional trade-off in allocation of resources to clutch and egg sizes.

In Chapter 2, I demonstrated variation in shell shape that might be relevant for determining egg and clutch size, since body cavity volume can constrain reproductive output (Du et al. 2005). Therefore, you might expect within-population variation in either egg sizes or clutch sizes, depending on which morphological features, if any, constrain reproduction in that population (i.e., shell volume, shell height, PAW). Thus, the objectives of this study were: 1) to generate baseline information on the reproductive ecology for *S. peltifer* for future comparisons between populations and across species; and 2) to conduct initial analyses assessing relationships between egg and clutch characteristics and female body size.

Methods

Data collection

I captured *S. peltifer* in the Cahaba River throughout May and June of 2020 as part of an ecomorphological study (see capture details in Chapter 2). For each turtle captured, I measured straight carapace length (SCL), plastron length (PL), and head width (HW) using digital calipers, and mass using a 600g spring scale (Pesola). I also recorded information on any injuries or other abnormalities, and assessed reproductive status in females by manually palpating the inguinal region (Donini et al. 2017).

After verifying reproductive status by inguinal palpation, I took all gravid female turtles to a nearby veterinary clinic for digital radiography (Fig. 3.1; Alford Avenue Animal Hospital, Birmingham, AL). Because radiographs do not require euthanizing individuals, they have become a popular tool for studies of turtle reproductive ecology (Gibbons and Greene 1979; Donini et al. 2017). Turtles were arranged with their plastrons lying on the surface of the x-ray machine (i.e., “right side up”). I included a coin of known size, placed atop a piece of foam (6.25 mm tall), in all but one of the radiographs to use as a reference for later measurements (Graham and Petokas 1989). For the radiograph that did not include a size reference object, I instead scaled based on maximum HW. From these I recorded the number of eggs in each clutch (CS) and, using Adobe Photoshop software, measured egg widths (EW) and pelvic aperture widths (PAW). I defined EW as the maximum distance along the minor axis of the egg, and PAW as the widest point between the two ilia (for a diagram of the pelvis of *Sternotherus*, see Cordero 2018). Egg length, though a potentially important mechanism for coping with morphological constraints (Escalona et al. 2018), cannot be accurately measured from radiographs because eggs are often not oriented horizontally within the body cavity. Following radiography all individuals were observed for at least an hour to ensure that they did not display any significant signs of stress from radiography, before being promptly released at the original site of capture.

Statistical analyses

I $\log_{10}$ transformed all variables (SCL, CS, EW, PAW) prior to analysis. This is necessary in order to generate slopes that can be used to test allometric hypotheses (King 2000) . Log-transformation has several additional benefits, such as helping data meet assumptions of normality, reducing heteroscedasticity of variables, and enabling comparisons among taxa with varied body sizes (Warton et al. 2006; Iverson et al. 2019).

All analyses were conducted in R version 4.0.4 (R Core Team 2021). I employed linear models to evaluate the allometric relationships between clutch attributes (average egg width, clutch size) and female body size (SCL). Standard major axis (SMA, sometimes called reduced major axis), may be a more appropriate line-fitting method than ordinary least squares (OLS) regression for studies of reproductive allometry (LaBarbera 1989; Warton et al. 2006; Warne and Charnov 2008). Previous authors have also used slopes from regressions of log-transformed data to evaluate relationships between female size and reproductive characteristics. However, linear regression often underestimates slope compared to the true population distribution. The SMA is a preferable analytical technique for generating slopes for this purpose, but has not seen much use in the reptile reproductive allometry literature. However, as no authors have used SMA in turtle studies yet, I ran each model with both SMA and OLS regression to facilitate comparisons to previous studies (e.g., Iverson et al. 2019). To obtain slope estimates, 95% confidence intervals, and test whether these slopes differed from those expected under isometry, I fit SMA models using the smatr package in R (Warton et al. 2012). OLS models and 95% confidence intervals were also fitted using the 'lm' and 'confint' functions in R (R Core Team 2021). Because my sample size was small, the goal of these analyses was to obtain and report effect size and error, rather than an emphasis on hypothesis testing.

Results

I captured and measured eight gravid turtles over a period spanning from 12 May to 12 June of 2020. Of those, two were captured in traps and the other six were encountered during wading surveys. Mean clutch size was 3.25 ± 1.16 eggs, and average egg width ($n = 26$) was 17.3 ± 1.06 mm. Body (SCL, PL, mass) and pelvic aperture size measurements are presented in Table 3.2.

Linear regression indicated positive relationships between female body size (SCL) and pelvic aperture width, mean egg width, and clutch size (Table 3.3). The slopes of the relationships between SCL and both clutch size and pelvic aperture width did not deviate from the expectation under isometry (3 and 1, respectively). However, there was a hypoallometric relationship between SCL and mean egg width (slope < 1) that trended toward significance ($p = 0.07$; Table 3.3; Fig. 3.2).

Discussion

This study represents the first examination of the reproductive ecology of *S. peltifer*, and will hopefully lay the groundwork for future geographic comparisons of life history, and potentially inform conservation planning. A larger sample will be required to appreciate the species' reproduction more fully, but my results include important natural history information and suggest that *S. peltifer* may not conform to OES theory.

Tinkle (1958) suggested that female *S. peltifer* probably mature between 90–100 mm SCL, based on his examination of the ovaries of a small number of specimens. My results generally agree, though data presented here cannot address minimum female reproductive size. I did obtain a radiograph of a small female *S. peltifer* (SCL = 70.89 mm), which did not reveal any calcified eggs. However, several larger individuals (97–118 mm SCL) were also radiographed during the study period and found to lack oviductal eggs. Tinkle was unable to provide data on clutch size, as none of these females contained oviductal eggs, but suggested that annual reproductive output might reach 10 based on enlarged follicles (Tinkle 1956,1958). Maximum clutch size in this study was five eggs, which implies that some females might have multiple clutches in a year. Several of the females that I examined in this study were larger than the previous record maximum adult size, so it seems unlikely that many clutches have more than five eggs. There do not appear to be especially large differences in clutch size and egg width between *S. peltifer* and other lotic *Sternotherus* species (Table 3.1). In all cases, the pelvic aperture was at least ~4 mm wider than the widest egg. The pelvic aperture of *S. odoratus* and *Kinosternon flavescens* increases with body size and is sexually dimorphic, with females possessing larger pelvic openings than males (Long and Rose 1989; Cordero 2018). It is unclear if this dimorphism exists in *S. peltifer* also, as I did not radiograph any males.

My results do not follow the expectation under OES theory, as SCL and EW appear to be correlated. Rather, the positive relationships between SCL and both EW and CS suggest that there is some competition for resources between the number and size of eggs in a clutch (Smith and Fretwell 1974; Brockelman 1975). A possible explanation for the observed deviation from

OES theory is that some aspect of the skeletal morphology of *S. peltifer* prevents females from carrying and laying optimally-sized eggs (Congdon and Gibbons 1987). Multiple approaches have been applied to evaluate support for the morphological constraint hypothesis. For instance, some authors have suggested that the slopes of EW and PAW should be parallel with each other under this hypothesis (Lovich et al. 2012), while others have compared the largest EW and smallest PAW values (Macip-Ríos et al. 2013). Following both methods, my results do not appear to implicate pelvic aperture width as a morphological constraint on egg size. The width of the pelvic aperture increased more quickly with body size than did egg width, which does not support the conclusion that PAW constrains egg size. Importantly, my sample size was small and likely did not span the entire range of reproductive female sizes, so my allometric slope estimates might not be representative of the population (Iverson et al. 2019). Furthermore, my samples came from several locations along the Cahaba River (Table 3.4), where there is considerable variation in both environmental characteristics and shell shape (and perhaps shell volume, see Chapter 2).

The slope estimates returned by my models were greater from SMA compared to OLS regression methods. While the 95% confidence intervals associated with my OLS models encompass a considerable amount of uncertainty, the OLS slope estimates themselves are more suggestive of hypoallometric relationships when compared to the SMA models. As explained by Warton et al. (2006), OLS is known to generate slope estimates that underestimate the true relationship, which is not ideal for testing model slopes against allometric hypotheses (see also - Fairbairn 1997). In a recent analysis using data from studies of numerous turtle species and populations, Iverson et al. (2019), using slopes generated from OLS regressions of log transformed variables, found hypoallometric relationships between female body size and most of the examined reproductive measures. They provided several compelling biological explanations, but it seems possible that the line-fitting method that they used might have been biased downward for some of these reproductive variables.

Additional studies are needed to investigate other aspects of reproduction in this species. For instance, both the length of the reproductive season and number of clutches that a female lays each year are unknown. This is important because, along with clutch size and egg size, clutch frequency is a major determinant of reproductive success and fitness (Congdon and Gibbons 1990). Based off my sampling dates, *S. peltifer* nests in the spring and early summer, but it is unclear whether individuals have multiple clutches in a calendar year. The reproductive season is extensive in *S. minor*, with females in some populations laying as many as four clutches between October and early July (Etchberger and Ehrhart 1987). I did not detect eggs in any *S. peltifer* by palpating after June, but the beginning of the reproductive season is unknown. Nesting behavior is also relatively unknown, with a single observation recorded for the species from Virginia, at its northern range limit (Pinder et al. 1995). Turtle reproductive ecology often varies clinally in species that occupy broad distributions, as seen in *S. odoratus* (Tinkle 1961; Iverson et al. 1993). Seeing as *S. peltifer* inhabits the most latitudinally and environmentally variable distribution of any species in the *S. minor* group, it would be interesting to compare both timing of reproduction and egg and clutch characteristics from across the species range. Future studies will hopefully explore the relationship between clutch characteristics and shell shape, which varies with the local environment in *S. peltifer* (Chapter 2).

Table 3.1. Statistics summarizing available reproductive data for lotic *Sternotherus* species, including location, clutch size (CS), female size as straight carapace length or plastron length (SCL/PL), and egg width (EW). Length measurements (female size, egg width) are in millimeters.

Species	State	Mean CS (Range)	Mode CS	Mean female size (Range)	SCL or PL	Mean EW[1] (Range)	*N*[2]	Source
S. peltifer	AL	3.25 ± 1.16 (2–5)	2, 4	108.9 (98.3–125.4)	SCL	17.3 ± 1.06 (14.55–19.0)	8	Current study
S. depressus	AL	– (1–3)	–	–	–	–	–	Close 1982
S. minor	FL	2.6 ± 0.99 (1–5)	3	77.18 ± 9.36	PL	17.2 ± 1.3 (14.85–19.2)	35	Iverson 1977,1978
		2.4 (1–4)	3	94.4 (71–118)	SCL	17.1 ± 1.2 (14.3–19.7)	38	Cox and Marion 1978
		3.0 ± 0.7 (1–5)	3	77.2 ± 7.2	PL	17.5 ± 1.1 (12.7–19.5)	33	Etchberger and Ehrhart 1987
S. carinatus	OK	5 (4–6)	–	–	–	17.8 (14.4–18.0)	11	Mahmoud and Klicka 1972
		2.3 ± 0.5 (1–3)	–	102 ± 8 (86–121)	SCL	16.6 ± 0.8 (14.6–17.8)	34	Iverson 2002
	AR	3.8 ± 1.5 (2–7)	–	117 ± 12 (95–155)	SCL	17.2 ± 0.9 (15.1–18.2)	21	

1. Mean EW is the mean of all eggs measured, rather than the grand mean among clutches.
2. *N* refers to the number of clutches examined. Where possible, reported figures are for clutches of calcified oviductal eggs, but some data from previous studies include individuals that were not in a reproductive state, or which contained only enlarged follicles or corpora lutea. For instance, Etchberger and Ehrhart (1987) and Iverson (2002) presented data on more females than clutches.

Table 3.2. Morphological measurements of gravid female *S. peltifer* included in this study, including straight midline carapace length (SCL), midline plastron length (PL), mass, and pelvic aperture width (PAW). Pelvic aperture width was measured from digital radiographs using Adobe Photoshop.

Female ID	SCL (mm)	PL (mm)	Mass (g)	PAW (mm)
Sp54	104.32	74.5	145	21.7
Sp55	120.11	92.36	260	25.5
Sp56	98.49	70.96	155	24.5
Sp59	124.0	92.57	272.5	26.0
Sp60	125.44	96.01	287.5	23.5
Sp63	98.71	73.0	170	21.4
Sp66	98.32	73.77	135	20.1
Sp69	101.63	79.42	155	22.5

Table 3.3. Summaries of linear models run on log transformed straight carapace length versus egg and clutch variables. P-values for ordinary least squares (OLS) models indicate whether the R-squared was > 0 (a), and those for standard major axis (SMA) models indicate whether the observed slope differs from the expected slope (b).

Model	Type	Slope (95% CI)	Isometric expectation	P (a)	P (b)
Clutch size	OLS	2.37 (-0.078 – 4.90)	–	0.055	–
	SMA	3.47 (1.78 – 6.76)	3.0	–	0.636
Mean egg width	OLS	0.377 (-0.019 – 0.773)	–	0.059	–
	SMA	0.547 (0.279 – 1.07)	1.0	–	0.074
Pelvic aperture	OLS	0.575 (-0.024 – 1.17)	–	0.057	–
width	SMA	0.831 (0.425 – 1.62)	1.0	–	0.550

Table 3.4. Locations, elevations, and sample sizes of gravid females included in this study. Recorded elevations are meters above sea level. Latitude and longitude are in decimal degrees format, based on WGS84 datum.

Site	Latitude	Longitude	Elevation	# of gravid females
Trussville (Cahaba)	33.63013	-86.60294	204	4
Shades Creek	33.45428	-86.80757	188	2
Birmingham (Cahaba)	33.43164	-86.71405	130	1
Bulldog Bend (Little Cahaba)	33.05803	-87.02365	87	1

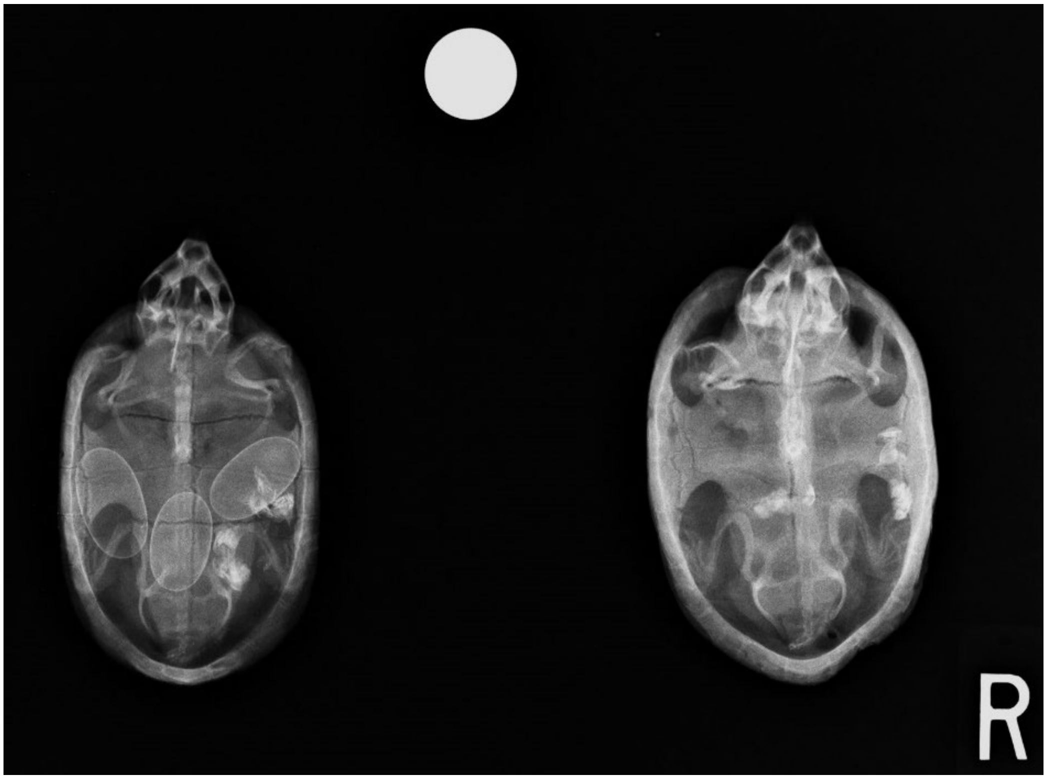


Figure 3.1. Radiograph of two stripe-necked musk turtles (*S. peltifer*) from the Cahaba River in central Alabama. The female on the left is gravid with three eggs, the female on the right does not have any shelled eggs, but has recently consumed some mollusks (likely *Corbicula*). The white circle at the top of the image is a standard US quarter, and was used to scale egg measurements.

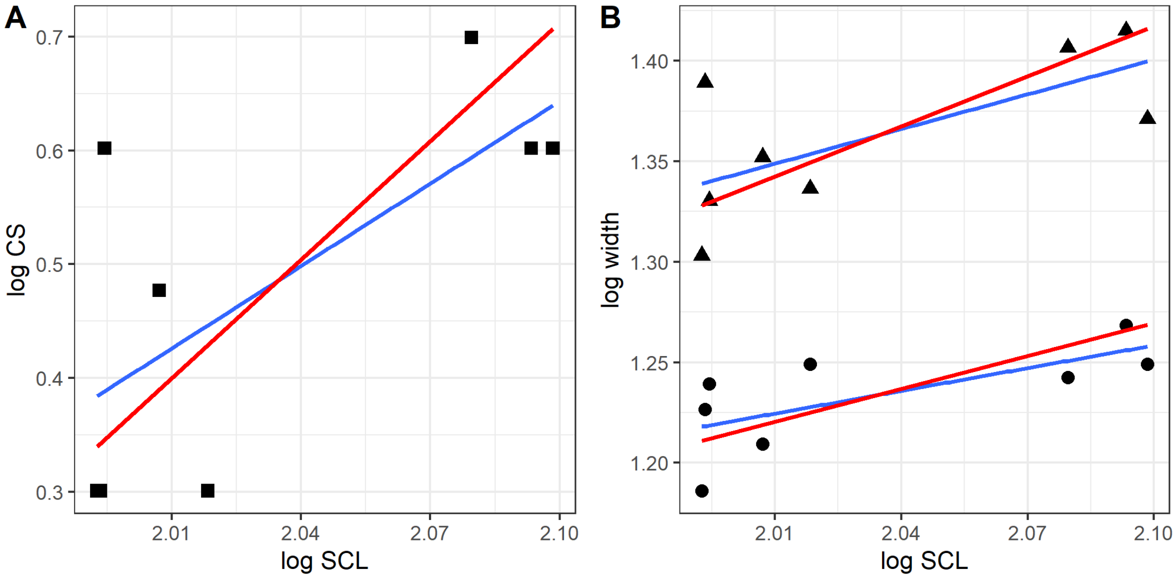


Figure 3.2. Scatter plots showing log-log relationships between (A) straight carapace length (SCL) vs clutch size (CS, squares); and (B) SCL vs clutch mean egg width (circles) and pelvic aperture width (triangles). The most extreme points represent untransformed measures of SCL (98.3–125.4 mm), clutch size (2–5), mean egg width (15.4–18.6 mm), and pelvic aperture width (20.1–26.0 mm). Blue and red lines represent ordinary least squares and standard major axis regression fits, respectively.

Chapter 4: Conclusions

In this thesis, I set out to investigate how aspects of the environment co-varied with shell shape in Cahaba River *S. peltifer*, and to evaluate potential reproductive trade-offs with shell shape. Both traditional and geometric morphometric data indicate that turtles from above the fall line are significantly flatter than those from below it, supporting the hypothesis that aspects of riverine habitat such as flow regime have led to local adaptation in shell shape. Many of the reproductive characteristics of Cahaba River drainage *S. peltifer* appear to be like those of *S. minor* in Florida. My sample did not follow the predictions of optimum egg size theory, nor did I find evidence that the width of the pelvic aperture constrains egg width in *S. peltifer*. Additional sampling from throughout the species' range will be of interest for revealing how environmental and morphological variation affect life history evolution at the population level. These results highlight the importance of environmental factors for shaping the evolutionary trajectories of turtle populations. This work also highlights the importance of natural history information for sparking future research, as many aspects of this study followed directly from comments recorded many decades ago by other graduate students.

My results raise some interesting questions about the evolutionary ecology of *S. peltifer*. First, it remains to be seen if the shell shape-environment pattern observed in this study is unique to the Cahaba, or if it also exists in the Coosa and Tennessee drainages. It would also be very interesting to know whether female turtles with flatter shells produce fewer or smaller eggs than females with more domed shells, as such a pattern would demonstrate an evolutionary trade-off and therefore be evidence of the adaptive origin of shell shape variation. As *S. peltifer* occupy varied environments across their geographic distribution, reproductive and morphological data from many populations should help to reveal what factors best explain deviations from optimum egg size theory in turtles. Genomic data will help to clarify several points, namely whether the fall line is a major barrier to gene flow, the degree to which phenotypic plasticity and adaptive evolution contribute to shell shape variation between populations, and whether historical hybridization with *S. depressus* may have contributed to the existence of flattened shell shapes in *S. peltifer* populations. Exploration of these questions will help to broaden our knowledge of how the local environment acts on phenotypes to influence the evolution of populations.

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Appendix 1. List of sampling sites

Site	Location relative to Fall Line	Latitude	Longitude
Goodner Mtn Rd	Above	33.7035	-86.5785
HTHS	Above	33.6632	-86.5904
Civitan Park	Above	33.6285	-86.6022
Pinchgut Creek	Above	33.6177	-86.6078
Hwy 280	Above	33.4318	-86.7145
Coshatt Dr	Above	33.3972	-86.7619
Cahaba Valley Creek	Above	33.3176	-86.8049
Buck Creek (Helena)	Above	33.2974	-86.8435
Shades Creek (Homewood)	Above	33.4505	-86.8107
Shades Creek (Huntington)	Above	33.4361	-86.8369
Mill Creek (Tannehill SP)	Above	33.2568	-87.0618
Caffee Creek	Above	33.1150	-87.1063
Bulldog Bend (Little Cahaba)	Above	33.0577	-87.0241
Sixmile Creek (6 Mile Run)	Above	32.9993	-86.9941
Harrisburg Rd	Below	32.8556	-87.1986
Heiberger	Below	32.7747	-87.2734
Sprott	Below	32.6683	-87.2419
Oakmulgee Creek (Hwy 14)	Below	32.4804	-87.1270
Elam Holley Park	Below	32.3591	-87.1333

Appendix 2. List of specimens examined

Specimen ID	Year collected	County	Latitude	Longitude
AUM 3650	1963	Shelby	33.09533	-86.88375
AUM 3652	1965	Bibb	32.90547	-87.29260
AUM 3651	1966	Shelby	33.10345	-86.85910
AUM 9479	1968	Jefferson	33.43161	-86.71437
AUM 32699	1972	Shelby	33.41548	-86.74031
AUM 32695	1972	Shelby	33.41548	-86.74031
AUM 32700	1972	Shelby	33.41548	-86.74031
AUM 32701	1972	Shelby	33.41548	-86.74031
AUM 32697	1972	Shelby	33.41548	-86.74031
AUM 32698	1972	Shelby	33.41548	-86.74031
AUM 32702	1972	Shelby	33.41548	-86.74031
AUM 32704	1972	Shelby	33.41548	-86.74031
AUM 38952	2009	Bibb	33.05242	-87.03410