

PATTERNS OF REPRODUCTIVE ALLOCATION: CLUTCH AND EGG SIZE VARIATION  
IN THREE FRESHWATER TURTLES

by

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(Under the Direction of J. Whitfield Gibbons)

ABSTRACT

Understanding the mechanisms and patterns of how energy is allocated into the reproductive components of offspring size and number is central to life-history theory. I examined the utility of X-ray photography for determining offspring size and number in freshwater turtles then used X-ray photographs from a long-term mark recapture study to investigate hypotheses of reproductive allocation in three species. Patterns of reproductive allocation varied markedly within and among species. Overall, egg size varied as a function of maternal body size and age, intra-annual clutch frequency, allocation of continuous resources into integral numbers of offspring, and environmental variation. Clutch size varied with maternal body size, clutch frequency, and environmental variation. I examine how effectively the data support theoretical models of reproductive allocation, identify limitations of fundamental biological findings necessary to address the issues, and suggest empirical research that will enhance future consideration of the topic.

INDEX WORDS: offspring size, life history, reproduction, *Kinosternon subrubrum*, eastern mud turtle, *Pseudemys floridana*, Florida cooter, *Sternotherus odoratus*, musk turtle

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## CHAPTER 1

### INTRODUCTION

Propagule size and number are fundamental traits of great ecological and evolutionary significance. The number of viable offspring an individual produces over its lifetime relative to other individuals is the crux of natural selection. Additionally, offspring size and number can have large fitness consequences for the offspring that are produced. As a result, selection may act through several pathways to modify these traits, leading to a diverse set of interactions and tradeoffs that make discerning causative factors for allocation decisions a difficult undertaking. A wealth of both theoretical and empirical literature exists on the topic; however, the two are often at odds, and a synthetic understanding of reproductive allocation has yet to be reached.

Studies of turtles have and will continue to play an important role in furthering the understanding of life-history evolution and reproductive allocation in particular. In contrast to mammals and birds, which have post-hatching care of offspring, turtles produce eggs that contain all of the energy that a female will allocate to individual hatchlings. Therefore, the entire investment into an offspring can be quantified through examination of the egg. Furthermore, turtles are long-lived and iteroparous (Wilbur and Morin, 1988), are easily given permanent identification marks (Cagle 1939), and often occur in relatively discrete populations at high densities to facilitate mark-recapture study (Congdon and Gibbons, 1990). These combined characteristics make turtles and their eggs excellent subjects for examining parental investment and reproductive allocation.

To investigate reproductive allocation, one must be able to accurately determine both the number of eggs and the allocation of energy to individual offspring. Allocation to offspring is often estimated through examination of egg or offspring size. For turtles, there is plentiful literature indicating that larger eggs produce larger hatchlings (Congdon et al., 1983; Rowe, 1995; Steyermark and Spotila 2001; Roosenburg and Kelley 1996) and a growing body of

evidence that suggests larger hatchlings are more fit (Froese and Burghardt 1974; Swingland and Coe 1979; Morris et al. 1983; Miller et. al. 1987; Janzen 1993; Janzen et al. 2000). Thus, examination of number and size of eggs within and among female turtles may provide adequate information to make inferences about patterns of reproductive allocation.

Long-term mark-recapture studies are ideal for the examination of life-history traits because most populations do not exist in stable resource environments with stable demographic structure, necessitating integration of data over many years of study. One of the longest running turtle mark-recapture studies in the world has been active on the Savannah River Site (SRS) in Aiken, South Carolina under the direction of J. Whitfield Gibbons since 1967 (Gibbons 1990). An exceptional facet of this study is that nearly every gravid turtle captured since 1976 has been X-rayed, allowing determination of egg width and clutch size. In this study, I present 1) an analysis of the efficacy of X-ray photography for estimating offspring size, and 2) a study of reproductive allocation in three species of freshwater turtles based on X-ray photography from the long-term mark-recapture study on the SRS.

## CHAPTER 2

# THE EFFICACY OF X-RAY PHOTOGRAPHY FOR PREDICTING EGG AND HATCHLING SIZE IN TWO FRESHWATER TURTLES

## INTRODUCTION

Determination of offspring size and number is an important facet of many life-history studies. These data can be difficult to collect after parturition, often requiring the sacrifice of animals. However, for freshwater turtles and other egg-laying organisms, X-ray photography is a useful alternative that allows reproductive data to be gathered in a non-destructive manner (Gibbons and Greene, 1979).

Clutch size is directly obtainable from X-ray photographs and thus this technique has been effectively used to evaluate clutch size in turtles (Gibbons, 1982; Gibbons et al., 1982; Iverson, 1991). Measurements of egg length from X-ray photographs vary with the orientation of the egg in the body cavity and are thus unreliable. Egg width, however, can be measured regardless of egg orientation and has been used as an index of egg size in several studies (Congdon and van Loben Sels, 1991; Congdon et al., 2001). Two potential problems with using egg widths as a measure of egg size are 1) differences in egg elongation that might confuse the relationship between egg width and mass and 2) magnification error due to position relative to the X-ray film.

Egg size is often used in life-history studies as an indicator of offspring size. A common assumption made by researchers studying oviparous organisms is that egg size is positively correlated with investment in offspring and offspring size. This assumption has been validated in a number of cases (*Chrysemys picta*, Rowe, 1995; *Malaclemys terrapin*, Roosenburg and Kelley 1996; *Chelydra serpentina*, Steyermark and Spotila 2001), but remains untested in many others. The relationship between egg size and offspring size might also be obscured by the effects of environmental conditions on embryonic development and growth.

In this paper, I evaluate X-ray photography as a technique for estimating hatchling size in *Kinosternon subrubrum* and *Pseudemys floridana* through direct comparison of measurements from X-ray photographs, eggs, and hatchlings.

## MATERIALS AND METHODS

During the spring and summer of 2003, gravid female *K. subrubrum* and *P. floridana* were captured in pitfall traps, along drift fences, or opportunistically by hand on the Savannah River Site in Aiken and Barnwell Counties, South Carolina. Turtles were checked for marks and were given a unique identification via shell notching if not previously marked (Cagle 1939). For each female, plastron length was measured to the nearest mm and mass was measured to the nearest gram. Gravid condition and clutch size were determined by X-ray photography (Gibbons and Greene, 1979). *Kinosternon subrubrum* were X-rayed with a MinXray 300 on 10" x 12" Dupont Cronex detail cassettes at 60 Kv peak for 0.2 seconds at a distance of 80 cm. *Pseudemys floridana* were X-rayed on 14"x 17" Dupont Cronex intensifying cassettes at 70 Kv peak for 0.1 seconds at a distance of 80 cm. Egg widths were determined by measurement of the minor axes of the ellipses formed by eggs on the X-ray photographs (figure 2.1).

To stimulate ovipositioning in gravid turtles, I administered oxytocin, a mammalian hormone that induces smooth muscle contractions in turtle oviducts, via intramuscular injection in the hind legs (Ewert and Legler, 1978). Oxytocin was used exclusively for the first several females, but when it failed to give satisfactory results I used arginine vasotocin, a reptilian hormone that also stimulates oviducal contractions. Many individuals were unresponsive to the first hormone dose and were re-injected up to four times. Some females retained whole or partial clutches after re-dosage, and were released where captured. After injection, the first few individuals were taped to inverted plastic containers with their posterior half extending over the

edge to allow eggs to be deposited on damp paper towels below. However, because the time from injection to ovipositioning was often long, it was not ideal to keep the animals restrained in this manner. Subsequent females were placed in closed containers of either shallow water or a layer of sand and checked frequently so that eggs could be quickly removed without breakage. Two *K. subrubrum* females buried their eggs in the sand as if nesting, but a few eggs were broken as females moved about the container, so I discontinued this method in favor of the shallow water method. After blotting eggs dry, I measured egg length and width to the nearest 0.05 mm with calipers and mass to the nearest 0.01g with a digital scale. Each egg was marked lightly with a pencil on its upper surface so that rotation of the egg could be minimized.

#### *Egg incubation*

Two Percival Scientific environmental growth chambers set at constant temperatures (30° C and 25° C) were used as incubators for collected eggs. Two temperature regimes were chosen in order to produce hatchlings of both sexes, as both species have temperature-dependent sex determination (Ewert and Nelson, 1991), and to determine the effect of incubation temperature on hatchling size. The first egg laid from each clutch was randomly assigned one of the incubation temperatures, with alternating temperatures assigned to subsequent eggs within a clutch, ensuring that each clutch was roughly equally represented at both temperatures. Each egg was partially buried in a mixture of vermiculite and water (1:1 by mass) in an individual chamber (45mm w x 50mm d x 40mm h) of a divided plastic container. Water content in the incubation medium was maintained by misting with water every third day to maintain constant mass. As the term of the incubation period approached, I monitored eggs daily or every other day so accurate pipping date could be recorded. After pipping, hatchlings were given 10 days to emerge, absorb external yolk reserves, and harden their carapaces before they were measured.

Straight centerline plastron length, straight centerline carapace length, maximum carapace width, and maximum height were measured to the nearest 0.05 mm with calipers. Mass was measured to the nearest 0.01 g with a digital scale.

Since it is not possible to match an individual egg in an X-ray photograph with its physical counterpart, analyses of X-ray egg widths were performed using clutch mean values. Because all eggs were not obtained from some females and some eggs did not produce live hatchlings, clutch means of data from eggs and hatchlings represent only a sample of the entire clutch in those cases. For regressions of X-ray egg widths on actual egg width and mass in *K. subrubrum* egg sizes, only clutches for which all eggs were physically measured were included in the analyses. In all other analyses, all the available data were used to maintain adequate sample sizes. Statistical analyses were performed using Statistica, version 5.1 for PC (Statsoft, 1997 unpubl.).

## RESULTS

### *K. subrubrum*

Twenty-one gravid female *Kinosternon subrubrum* were collected during the spring and summer of 2003. Mean plastron length was 86mm (2 SE = 2.2 mm, N = 21) and mean preovipositional mass was 165.2 g (2 SE = 1.02 g, N = 20). Mean clutch size as determined by X-ray was 3.76 (2 SE = 0.5 g, N = 21). Via hormone injection, I obtained complete clutches from thirteen females and partial clutches from the remaining nine females for a total of 66 eggs. Egg and hatchling size statistics are presented in table 2.1.

No measure of maternal body size (plastron length, carapace length, pre-ovipositional mass) was correlated with any measure of egg size (mean egg width, mean egg length, or mean egg mass). Mean clutch size and mean egg mass were negatively correlated ( $r = 0.5$ ,  $p < 0.05$ ).



Incubation temperature did not affect hatchling size relative to egg size (ANCOVA;  $F_{1,23} = 3.48$ , NS) so data from both temperature regimes were pooled in subsequent analyses.

Incubation period was significantly longer at 25 °C (mean = 129.5 days) than at 30 °C (mean = 116.3 days;  $F_{1,23} = 12.97$ ,  $p < 0.01$ ).

Egg width and length were both positively related to egg mass, but egg width accounted for more of the variance in egg mass than did egg length (table 2.2)

Egg mass, egg length, and egg width were all positively related to hatchling mass (table 2.2). Egg width ( $r^2 = 0.48$ ) explained more of the variation in hatchling mass than did egg length ( $r^2 = 0.23$ ).

Mean egg width determined from X-ray photography was positively related to mean egg width, mean egg mass, and mean hatchling mass (table 2.2).

#### *Pseudemys floridana*

Six gravid female *P. floridana* were captured during the study period. Mean plastron length was 246.8 mm (2SE = 24.42 mm, N = 6) and mean preovipositional mass was 2360.4 g (2SE = 622.9 g, N = 5). Mean clutch size was 14.5 (2SE = 3.3, N = 6). I collected complete clutches from three *P. floridana* and partial clutches from the remaining three for a total of 66 eggs. Summary statistics for body size, clutch size, and hatchling size are presented in table 2.1.

Incubation temperature did not affect hatchling size relative to egg size (ANCOVA;  $F_{1,43}$ , NS) so data from both temperature regimes were pooled in subsequent analyses. Increased incubation temperature significantly shortened incubation period (ANOVA;  $F_{1,43} = 1897.96$ ,  $p < 0.01$ ). Eggs at 25 °C averaged 102.3 days from ovipositioning to pipping while those at 30 °C had a mean incubation period of 60.3 days.

Maternal plastron length was not significantly correlated with any measure of egg size (egg length, egg width, egg mass), clutch size, or clutch mass. Clutch size and egg size were not correlated.

Egg mass was positively related to both egg length and egg width, with egg width accounting for a greater proportion of the variance ( $r^2 = 0.86$ ; table 2.2).

Hatchling mass was positively related to egg mass and egg width but not with egg length (table 2.2).

Mean egg width determined from X-ray photography was positively related to actual mean egg width and mean egg mass, but not to mean hatchling mass (table 2.2).

## DISCUSSION

Egg width was a better predictor of egg mass than was egg length for both species. Similar results have been reported for *Chrysemys picta bellii* (Rowe, 1994) and *Kinosternon flavescens* (Iverson, 1991). The cell bodies of macrolecithal eggs (such as those produced by birds and reptiles; characterized by a cell body consisting primarily of yolk) are nearly spherical (Romer and Parsons 1977). Thus an increase in the length of a shelled egg without a concomitant increase in the width of the egg might allow an increase in albumen mass but not an increase in yolk mass. Indeed, egg width was a better predictor of hatchling mass than was egg mass in *P. floridana* and had similar predictive value in *K. subrubrum*.

There was a positive linear relationship between egg mass and hatchling mass in both species, but egg mass explained only 68% of the variance in *K. subrubrum* hatchling mass and 44% of the variance in *P. floridana* hatchling mass. These are substantially weaker relationships than those reported for *Deirochelys reticularia* ( $r^2 = 0.93$ ; Congdon et al. 1983) and *Malaclemys terrapin* ( $r^2 = 0.96-97$ ; Roosenburg and Kelley 1996) but are similar to those reported for

*Chelydra serpentina* ( $r^2 = 0.1-0.57$ ; Steyermark and Spotila 2001). Variability in water content might account for some of the observed disparity between hatchling mass and egg mass. Stronger correlation has been reported between dry masses of eggs and hatchlings than between wet masses of eggs and hatchlings (Rowe 1995). Individual metabolic rates and hydric environments of individual eggs might also account for some of the unexplained variation (Morris et al. 1983). I attempted to control substrate moisture in this study, but variation in water potential substantial enough to affect hatchling size may have occurred.

Egg widths measured from X-ray photographs were strongly linearly related to actual egg widths in both species. X-ray egg widths also predicted most of the observed variation in egg mass. Clutch mean egg width as determined from X-ray photographs was not linearly related to clutch mean hatchling mass in *P. floridana*. However, the clutch mean X-ray widths are based on all of the eggs in each clutch while the sample for hatchling mass was reduced by incomplete ovipositioning by three of the six individuals and by limited hatching success in eggs from some females. Therefore this result should be interpreted with caution. The same limitations apply to the comparison of X-ray egg width and hatchling mass in *K. subrubrum*, but here the sample size was substantially larger and a positive linear relationship was detected. These limitations present a difficulty only for assessment of the validity of the technique and are not an inherent flaw in the technique.

In conclusion, X-ray photography is effective for estimating egg width in these two turtle species. The strong linear relationship between egg width and egg mass also allows mass to be effectively estimated from X-ray photographs. It is difficult to directly establish a relationship between X-ray measured egg width and hatchling mass due to problems in obtaining complete clutches of hatchlings. Yet, egg width predicts a significant portion of the variation in hatchling

mass and can easily be estimated from X-ray photographs. Therefore measurements made from X-ray photographs should be very effective for estimating egg mass and are comparable to direct measurements of eggs for predicting hatchling size, making X-ray photography a valuable tool for addressing patterns of reproductive allocation in freshwater turtles and perhaps other oviparous organisms.

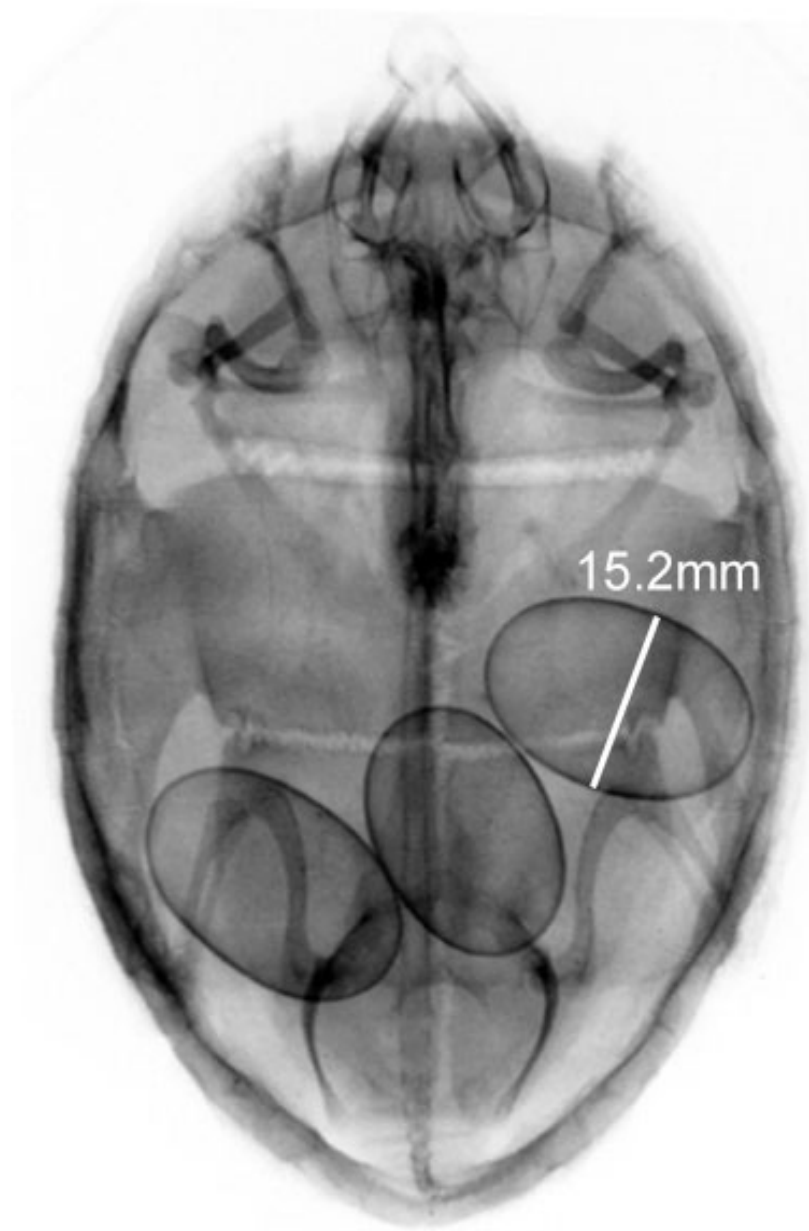


Figure 2.1 X-ray photograph of a gravid *K. subrubrum*. White line indicates position of width measurements (minor axis).

Table 2.1 Summary statistics for body size, clutch size, egg size, and hatchling size parameters. (mean  $\pm$  2SE)

Species	<i>K. subrubrum</i>	<i>P. floridana</i>
N indiv.	21	6
Plastron Length (mm)	86 $\pm$ 2.2	246.8 $\pm$ 24.42
Clutch Size	3.76 $\pm$ 0.5	14.5 $\pm$ 3.3
N Eggs	66	66
Egg Width (mm)	15.2 $\pm$ 0.15	24.44 $\pm$ 0.29
Egg Length (mm)	25.4 $\pm$ 0.336	35.2 $\pm$ 0.49
Egg Mass (g)	3.62 $\pm$ 0.12	12.18 $\pm$ 0.38
N Hatchlings	26	45
Hatchling Carapace Length (mm)	21.14 $\pm$ 0.36	32.79 $\pm$ 0.39
Hatchling Carapace Width (mm)	15.98 $\pm$ 0.36	32.5 $\pm$ .046
Hatchling Plastron Length (mm)	17.98 $\pm$ 0.33	30.7 $\pm$ 0.58
Hatchling Mass (g)	2.52 $\pm$ 0.10	9.02 $\pm$ 0.312

Table 2.2 Results of linear regressions of egg and hatchling size parameters.

Species	Dependent variable	Independent variable	N	slope	intercept	r <sup>2</sup>	p
<i>K. subrubrum</i>	Egg Mass	Egg Width	57	0.58	-5.34	0.73	<0.01
	Egg Mass	Egg Length	57	0.23	-2.35	0.41	<0.01
	Hatchling Mass	Egg Mass	26	0.50	0.68	0.60	<0.01
	Hatchling Mass	Egg Width	26	0.30	-2.30	0.48	<0.01
	Hatchling Mass	Egg Length	26	0.09	0.18	0.23	<0.01
	Mean Egg Width	Mean X-ray Egg Width	12	1.00	-0.47	0.98	<0.01
	Mean Egg Mass	Mean X-ray Egg Width	12	0.62	-5.98	0.75	<0.01
	Mean Hatchling Mass	Mean X-ray Egg Width	13	0.31	-2.30	0.56	<0.01
<i>P. floridana</i>	Egg Mass	Egg Width	64	1.25	-18.37	0.86	<0.01
	Egg Mass	Egg Length	64	0.43	-3.03	0.30	<0.01
	Hatchling Mass	Egg Mass	45	0.51	2.78	0.44	<0.01
	Hatchling Mass	Egg Width	45	0.68	-7.53	0.46	<0.01
	Hatchling Mass	Egg Length	45	0.03	7.78	0.07	NS
	Mean Egg Width	Mean X-ray Egg Width	6	0.92	0.34	0.98	<0.01
	Mean Egg Mass	Mean X-ray Egg Width	6	1.18	-18.75	0.90	<0.01
	Mean Hatchling Mass	Mean X-ray Egg Width	5	0.43	-2.30	0.54	NS

CHAPTER 3

PATTERNS OF REPRODUCTIVE ALLOCATION: CLUTCH AND EGG SIZE VARIATION  
IN THREE FRESHWATER TURTLES



## INTRODUCTION

Propagule size and number are fundamental traits of great ecological and evolutionary significance. The number of viable offspring an individual produces over its lifetime relative to other individuals is the crux of natural selection. Additionally, offspring size and number can have large fitness consequences for the offspring that are produced. As a result, there are several pathways through which selection might act to modify these traits, leading to a diverse set of interactions and tradeoffs that make discerning causative factors for allocation decisions a difficult undertaking. A wealth of both theoretical and empirical literature does exist; however, for certain taxa, including freshwater turtles, theory and observation are at odds and a synthetic understanding of reproductive allocation has yet to be reached.

### *Offspring size and number allocation theories*

Strategies of reproductive allocation into offspring size and number have generally been interpreted via one of several general models of offspring-size evolution. Optimality models are largely founded on the work of Smith and Fretwell (1974) that predicts that offspring size will be optimized at the point where the (maternal) fitness increase from producing larger offspring is equal to the corresponding decrease in fitness from reducing the number of offspring (also see Williams, 1966; Brockelman, 1975; Parker and Begon, 1986). Implicit in this argument are the assumptions that offspring fitness increases with size and that total reproductive output is limited by resources or maternal reproductive capacity, leading to a fundamental tradeoff between offspring size and number. With selection acting to optimize offspring size, these models predict that variation in total reproductive investment will be expressed primarily through changes in offspring number.

Plasticity models predict that in unpredictably fluctuating environments, variation in offspring sizes within or among clutches will increase fitness (Capinera, 1979; Cooper and Kaplan, 1982; Caswell, 1983; Kaplan and Cooper, 1984, McGinley et al. 1987). In environments where phenotype-specific offspring fitness is temporally variable, the optimal size and number of offspring for a single mother to produce will also be variable. If this variability is unpredictable, an individual mother may increase fitness by randomly varying reproductive allocation within a range of strategies. In certain situations, a mixed reproductive strategy (variation in egg size either among clutches or within clutches) will actually decrease the variance in fitness across reproductive bouts (Kaplan and Cooper, 1984). Since long-term fitness is measured as the geometric mean of reproductive success over successive reproductive bouts (Gillespie, 1977), maternal fitness is increased by this reduced variance strategy. The applicability of these models to long-lived organisms has been called into question, however, since increased overlap among generations may reduce the effect of environmental variation on the evolution of variance in offspring size (Schultz, 1989; Schultz 1991).

#### *Reproductive Investment in Turtles*

Turtles are excellent study organisms for investigation of reproductive allocation because they are oviparous and have no post-ovulatory parental care. This means that the entire investment into an offspring can be quantified through examination of the egg. Additionally, individuals are easily given unique identification marks, and reproductive condition can be easily assessed through X-ray photography (Gibbons and Greene, 1979).

I used measurements and X-ray photography from eastern mud turtles (*Kinosternon subrubrum*), common musk turtles (*Sternotherus odoratus*), and Florida cooters (*Pseudemys floridana*) in western South Carolina to evaluate how reproductive output in these species fit

theoretical allocation models and to test hypotheses concerning variation in reproductive allocation due to constraint on egg size, within female variability (within and among clutches), annual heterogeneity, multiple annual clutches, the fractional offspring size problem, and age of females.

## **Hypotheses**

### *Constraint*

Most models of offspring size assume that selection can freely modify offspring size with offspring viability and available maternal resources being the only constraints on size. However, there is evidence that morphological or physiological constraints within mothers may set upper limits on egg size that could prevent theoretical optima from being attained. The distance between the ilia (pelvic aperture), an opening through which an egg must pass during ovipositioning, may be a constraining dimension in smaller bodied freshwater turtles (Congdon et al., 1983; Congdon and Gibbons, 1987; Long and Rose, 1989). The posterior opening between the carapace and plastron (caudal gap) is another potentially constraining dimension (Clark et al., 2001; Rose and Judd, 1991). The sizes of both apertures are positively correlated with maternal body size (Congdon and Gibbons, 1987, Clark et al., 2001). Finally, Iverson and Smith (1993) have suggested that egg size increases with maternal body size as a function of increased oviduct size. As a result, I hypothesized that egg size constraint would be present as evidenced by a positive linear relationship between maternal body size and egg size and that evidence of pelvic aperture constraint would be found in smaller bodied species (*K. subrubrum* and *S. odoratus*) via similar slopes of pelvic aperture and maximum egg width on maternal body size.

### *Within and among clutch variation*

Within clutch variability in egg size is not predicted by optimality models but is a major prediction of some plasticity models (Cooper and Kaplan, 1982; Kaplan and Cooper 1984). In addition to the adaptive within clutch egg-size variation predicted by plasticity models, within clutch egg-size variation might also result from differential rates of follicular enlargement or differential rates of nutrient provisioning to ova due to position in the oviducts. I hypothesized that some within-clutch egg-size variability would be detected, but that most egg size variation would occur among clutches.

### *Annual variation*

The proximate and ultimate factors affecting the timing and allocation of reproduction in turtles are not well defined. The total energy allocated to reproduction, however, is tied to resource availability and acquisition, maternal body condition, energy reserves, and maintenance demand, all of which are influenced by extrinsic environmental factors (Gibbons and Greene, 1990; Bernardo, 1996; Kuchling, 1999). Because the ephemeral wetland habitats inhabited by the turtle species examined here are heterogeneous environments with a highly variable water levels and biotic communities (Gibbons 1990), I hypothesized that inter-annual variation would be present in both clutch size and egg size.

### *Multiple annual clutches*

Multiple reproductive bouts within a single nesting season have been observed in several species of freshwater turtles, but the phenomenon remains poorly understood (Iverson, 1979; Congdon and Tinkle, 1982; Gibbons, 1982; Lovich et al., 1983). The production of multiple clutches may be a mechanism for dealing with morphological constraints on the number of shelled eggs the body cavity can accommodate at one time (Kuchling, 1999). Although the exact

timing is unclear, follicles for multiple clutches may mature simultaneously, but in distinct size classes (Moll, 1979). As a result, allocation to first clutches may influence allocation to subsequent clutches through body volume limitations or differential follicle maturation rates due to position in the ovaries. I hypothesized that by comparing subsequent clutches within individuals within a single nesting season, a decrease in clutch size would be observed in later clutches, but that clutches would be composed of similarly sized eggs.

#### *Fractional Offspring Size Problem*

Basic optimality models for offspring size assume that offspring number is a continuous variable (Smith and Fretwell, 1974), yet real organisms produce integral numbers of offspring. Since the total energy allocated to reproduction is continuous and may be imprecisely controlled due to unpredictable resource availability and fluctuations in metabolic rate, an organism may be forced to allocate energy to offspring in a non-optimal manner (Ricklefs, 1968; Ebert, 1994). If a theoretical optimum offspring size exists (as discussed above), a female may have a quantity of energy available for reproduction that cannot be packaged into an integral number of these optimally sized offspring. Therefore she is left with the options of producing a larger number of smaller offspring or producing a smaller number of larger offspring. If the excess energy is distributed over the total number of offspring produced, the absolute gain or loss to an individual egg will decrease as clutch size increases. Therefore, I hypothesized that egg size would be more variable among small clutches relative to larger clutches.

#### *Age*

Two primary hypotheses of aging are the Relative Reproductive Rate Hypothesis (Williams, 1957) that predicts increased reproductive output and/or increased survivorship with age, especially in long-lived organisms, and the senescence hypothesis that predicts declining

reproductive output or survivorship with age (Williams, 1957; Hamilton, 1966). Since reproductive output is correlated with body size in many turtle species (Tucker et al., 1978; Congdon and Gibbons, 1985; Congdon and Gibbons, 1987), increased reproductive output with age might be manifested through continued growth after reproduction. However, variation in juvenile growth rate and size at maturity may lead to variation in adult body size, weakening the relationship between age and body size in older individuals (Carr and Goodman, 1970; Haliday and Verrell, 1988). Increases in reproductive output may also occur independently of size. Congdon et al. (2001) found that older Blanding's turtles (*Emydoidea blandingii*) had increased clutch size and clutch frequency independent of body size. Since evidence for senescence in turtles is weak (Gibbons, 1976; Congdon et al., 2003), I hypothesized that older turtles would have increased reproductive output both through increased body size and other age specific effects, supporting the Relative Reproductive Rate Hypothesis.

## MATERIALS AND METHODS

### *Study Area*

Ellenton Bay on the Savannah River Site in Aiken and Barnwell Counties, South Carolina, is a fluctuating (0-10 ha) natural freshwater wetland that dries completely during drought years and has a maximum depth of 2m at high water (Gibbons, 1990). In all years that turtles were captured, a continuous drift fence with pitfall traps was maintained around the circumference of Ellenton Bay throughout the nesting season, ensuring that nearly all gravid females were captured in the pitfall traps or by hand as they attempted to leave the bay for ovipositioning.

### *Study Species*

Ten freshwater turtle species have been captured in Ellenton Bay, at least six of which persist as viable populations. I chose to examine *Kinosternon subrubrum*, *Sternotherus odoratus*, and *Pseudemys floridana* because these species were relatively abundant and their reproduction less intensively studied than other species common in Ellenton Bay including *Trachemys scripta* (Congdon and Gibbons, 1983; Gibbons and Greene, 1990) and *Deirochelys reticularia* (Congdon et al., 1983).

The basic ecology of the three study species is known from numerous individual studies that have been aptly summarized in general books on turtles (e.g., Ernst et al. 1994).

*Kinosternon subrubrum*, the eastern mud turtle, is a small bodied (up to 12.5 cm carapace length) member of the family Kinosternidae. Its geographic range includes the southeastern United States, extending west to east-central Texas, and north to Long Island, New York. *Kinosternon subrubrum* prefers shallow aquatic habitats where it can easily reach the surface but is also quite terrestrial and makes crepuscular forays onto land from late spring to fall. Individuals often overwinter in self-made terrestrial burrows and also estivate underground when aquatic habitats dry. Primarily a bottom feeder, *K. subrubrum* consumes insects, crustaceans, mollusks, amphibians, carrion, and aquatic vegetation. Terrestrial feeding may also occur during forays on land.

*Sternotherus odoratus*, the stinkpot or common musk turtle, is another small bodied (up to 14 cm carapace length) kinosternid turtle closely related to *K. subrubrum*. *Sternotherus odoratus* ranges throughout most of the eastern United States and into southern Quebec and Ontario. It prefers shallow littoral habitats but may be found at depths up to nine meters (Minton 1972). Rarely found on land, *Sternotherus odoratus* spends extended periods underwater and

rarely basks. Stinkpots often hibernate underwater or near water, buried in mud or beneath rocks, logs, or detritus. The principal food sources for *S. odoratus* are insects, crustaceans, mollusks, amphibians, carrion, and aquatic vegetation.

*Pseudemys floridana*, the cooter, is a large (up to 40 cm carapace length) member of the family Emydidae. Its range is restricted to the Atlantic Coastal Plain. *Pseudemys floridana* may be found in aquatic habitats with slow currents, abundant vegetation, and suitable basking sites. Cooters are frequently observed basking for thermoregulatory purposes, sometimes in aggregations of up to 30 individuals. Individuals may make overland movements when aquatic habitats become unsuitable. As adults *P. floridana* are almost entirely herbivorous and consume a wide variety of aquatic plants and algae.

#### *Field and Laboratory Procedures*

Gravid female *K. subrubrum*, *S. odoratus*, and *P. floridana* from individually marked populations were captured from Ellenton Bay from 1976 through 1995 and in 2003. Upon capture, all turtles were returned to the laboratory where they were checked for identifying marks and were permanently marked by shell notching (Cagle, 1939) if unmarked. Plastron length was measured to the nearest mm on all females and carapace length and preovipositional mass were measured for many individuals. Age was determined when possible by counts of annuli or from the capture record of previously aged animals. *Kinosternon subrubrum* and *S. odoratus* were X-rayed with a MinXray 300 on 10" x 12" Dupont Cronex detail cassettes at 60 Kv peak for 0.2 seconds at a distance of 80cm. *Pseudemys floridana* were X-rayed on 14"x 17" Dupont Cronex intensifying cassettes at 70 Kv peak for 0.1 seconds at a distance of 80 cm. Clutch size was determined from visual counts of eggs on the resultant X-ray photographs. Each egg was measured to the nearest 0.05 mm using calipers at the minor axis of the near-ellipse seen in the



X-ray photographs. Pelvic aperture was measured as the shortest distance between the ilia on X-ray photographs that presented a symmetrical view of the pelvic girdle. Following processing, turtles were released at the position of capture on the opposite side of the fence.

### *Statistical Analyses*

Statistical analyses were performed using Statistica, Version 5.1 for PC (Statsoft 1997 unpubl.). The relationships between maternal plastron length, age, egg width, and clutch size were evaluated using least-squares linear regression. Pelvic constraint was assessed by testing for homogeneity of slopes between regression equations of pelvic aperture width and maximum egg size on maternal plastron length using Type III sums-of-squares in ANCOVA analysis (Congdon and Gibbons 1987). Within and among clutch variance components were determined using Type II ANOVA or ANCOVA with plastron length as the covariate. Comparisons of egg width and clutch size among the multiple annual clutches of individuals were made using repeated measures ANOVA. Since both egg width and clutch size were linearly related to maternal plastron length, annual variation in egg width and clutch size was assessed using ANCOVA with body size as the covariate. Variation due to the fractional offspring size problem was assessed using Levene's test to compare egg size variance in small clutches versus large clutches. Measures of central tendency are presented as Mean  $\pm$  2SE.

## RESULTS

A total of 255 individual *K. subrubrum* with 635 distinct clutches, 39 *S. odoratus* with 46 clutches, and 46 *P. floridana* with 70 clutches were captured and X-rayed during 13 years of sampling. Summary statistics for maternal body size, mean egg width, and clutch size are presented in table 3.1. *Kinosternon subrubrum* and *S. odoratus* females were similar in size and

egg width, with *S. odoratus* having a slightly larger mean clutch size. *Pseudemys floridana* were roughly three times larger in body size and also produced larger clutches comprising larger eggs.

### *Constraint*

Maternal plastron length was positively related to clutch size in all three species and explained greater than 30% of the variation in *S. odoratus* and *P. floridana* clutch sizes (Table 3.2). Maternal plastron length was also positively related to mean egg width in all species. Regression of plastron length on maximum egg size yielded slightly higher coefficients of determination in *K. subrubrum* and *S. odoratus* but did not increase explanatory power in *P. floridana*.

Simple regression revealed no meaningful relationship between mean egg width and clutch size in *K. subrubrum* and *S. odoratus* but showed a positive relationship between these traits in *P. floridana* (Table 3.2). When the effect of maternal body size was removed by adding maternal plastron length as an independent variable in a multiple regression, no significant relationship was detected between clutch size and mean egg width for any of the three species (Table 3.3).

Differences in the relationships of egg width and pelvic aperture on body size (plastron length) were observed among the three species. In *K. subrubrum* pelvic aperture was slightly larger than maximum egg width and both had similar slopes on plastron length ( $F_{1,136} = 0.9$ ;  $p = 0.34$ ; figure 3.1). In both *S. odoratus* ( $F_{1,54} = 8.2$ ;  $p < 0.05$ ) and *P. floridana* ( $F_{1,72} = 41.02$ ;  $p < 0.05$ ; figure 3.1) pelvic aperture increased with greater slope than maximum egg width. Slope of pelvic aperture width on plastron length was similar across species ( $F_{2,131} = 0.1384$ ; NS).

### *Variation within and among clutches*

Egg size was relatively constant within clutches for all species, with the majority of variation in egg size occurring among clutches.

For *K. subrubrum*, the coefficient of variation of egg size within clutches was 0.01. Analysis of variance confirmed a strong clutch effect on egg size ( $F_{299, 760} = 20.39$ ,  $p < 0.01$ ). Variation within clutches accounted for only 11% of the total variation in egg size. After correction for body size, within clutch variation accounted for 17.6% of the remaining variability.

In *P. floridana* the coefficient of variation of egg size within clutches was 0.03. Egg size differed significantly by clutch ( $F_{78, 804} = 31.93$ ;  $p < 0.01$ ). Variation within clutches accounted for 25% of the variation in egg size. After correction for body size, within clutch variation accounted for 33.8% of the remaining variability.

In *S. odoratus* the coefficient of variation of egg size within clutches was 0.01. Egg size differed significantly by clutch ( $F_{45, 181} = 19.54$ ;  $p < 0.01$ ). Variation within clutch accounted for 17% of the total variation in egg size. After correction for body size, within clutch variation accounted for 22.8% of the remaining variability.

The within female ranges of clutch size and egg size were calculated as the difference between the maximum and minimum values for an individual female among her first annual clutches (Table 3.4). Both egg and clutch size varied among clutches of individual females in *K. subrubrum* and *P. floridana*. *Sternotherus odoratus* had insufficient multiple year nestings for analysis. Changes in egg width and clutches between pairs of successive first annual clutches are presented in table 3.5. In both *K. subrubrum* and *P. floridana* egg width increased with successive clutches more frequently than it decreased while clutch size increased as frequently as

it decreased or stayed the same. No interaction between changes in egg size and changes in clutch size was detected and both reproductive parameters appeared to vary independently.

#### *Annual variation*

Mean body-size-adjusted egg width was variable among years in *K. subrubrum* and *S. odoratus*, but did not vary significantly in *P. floridana* (Table 3.6). Mean adjusted clutch size was variable among years in *K. subrubrum* and *P. floridana*, but did not vary significantly in *S. odoratus*. For *K. subrubrum* the coefficient of variation (CV) among years was 0.08 for clutch size and 0.025 for mean egg width.

Patterns of annual variation in clutch and egg size were highly variable both within and among females. Annual patterns for four *K. subrubrum* are presented in figure 3.2. Individuals varied clutch size and egg width independently, with no evidence of a tradeoff between clutch and egg size within individuals.

#### *Multiple clutches*

*Kinosternon subrubrum* was the only species in this study in which individuals produced multiple annual clutches frequently enough for analysis. Of the 446 individual annual reproductive bouts, 305 were composed of one clutch, 121 of two clutches, and 20 of three clutches. Multiple clutch production was highly variable in frequency from year to year ( $\chi^2 = 61.576$ , d.f. = 12,  $p < 0.01$ ) and ranged from 0% to 57% of annual reproductive bouts. The frequency of multiple clutches in a given year was correlated with both the mean egg size that year (Spearman  $R = 0.59$ ,  $t = 2.337$ ,  $p < 0.05$ ) and the number of gravid females captured in that year (Spearman  $R = 0.71$ ,  $t = 3.23$ ,  $p < 0.01$ ). Within annual reproductive bouts of two clutches, mean egg width was larger in first clutches (mean = 15.8 mm) than in second clutches (mean = 15.1 mm;  $t_{120} = 8.56$ ;  $p < 0.01$ ), but clutch size was not significantly different between clutches

( $t_{120} = 1.47$ ;  $p < 0.14$ ). Repeated measures ANOVA of annual reproductive bouts of three clutches detected differences among clutches in clutch size ( $F_{2,38} = 38.7$ ;  $p < 0.01$ ) and in mean egg width ( $F_{2,38} = 3.73$ ;  $p < 0.05$ ). Post-hoc analysis (Newman Keuls multiple range test) indicated that clutch size in first clutches (mean = 3.9) and second clutches (mean = 3.75) did not differ, but third clutches were significantly smaller (mean = 3.0). Egg width was significantly different in all three clutches and decreased with clutch number (Clutch 1, mean = 15.8; Clutch 2, mean = 15.49; Clutch 3, mean = 15.0).

#### *Fractional Offspring Problem*

Egg size was more variable among individuals producing smaller clutches than among individuals producing larger clutches in *K. subrubrum* and *S. odoratus* according to Levene's test (table 3.7). Egg size variability among individuals was similar among clutch sizes in *P.*

*floridana*.

#### *Age*

*Kinosternon subrubrum* was the only species in the study with great enough numbers of known-age individuals for analysis. I collected data from 115 clutches from known-age turtles ranging from 3 to 20 years old with a mean age of 7.5 years. Age was positively related to plastron length ( $n = 115$ ,  $r^2 = 0.244$ ,  $p < 0.05$ ). With maternal body size held constant, age was positively correlated with mean egg width ( $n=114$ , Spearman  $R = 0.63$ ,  $t = 8.6$ ,  $p < 0.01$ ), but not with clutch size ( $n=114$ , Spearman  $R = 0.02$ ,  $t = 0.19$ ,  $p = 0.84$ ). Clutch frequency was not greater in older turtles ( $F_{1,18} = 0.82$ ,  $p = 0.366$ )

## DISCUSSION

### *Constraint*

A positive relationship between maternal body size and reproductive parameters (clutch size and egg size) in turtles has been documented within populations (Iverson 1977; Iverson, 1979; Congdon and Gibbons, 1983; Congdon et al., 1983; Rowe, 1994; Valenzuela 2001), among populations (Congdon and Gibbons, 1983; Iverson, 1992), and among species (Congdon and Gibbons, 1985; Elgar and Heaphy, 1989; Iverson, 1992). However, numerous studies have found no relationship between maternal plastron length and egg size (*P. floridana*, *T. carolina*, and *S. odoratus*, Congdon and Gibbons, 1985; *M. terrapin*, Roosenburg and Dunham, 1997; *E. blandingii*, Congdon 2001).

I found a positive relationship between clutch size and maternal body size (plastron length) in all three species in this study; however, the linear model did not explain more than 36% of the variability in any species. This may be due in part to the fact that body size may impose an upper limit on clutch size due to turtles' rigid body cavities, but larger females might still produce small clutches due to resource limitation or other constraints (Gibbons et al., 1982).

I found a positive relationship between egg width and maternal body size (plastron length) in all three species. In *K. subrubrum*, there is evidence that this relationship is due to pelvic aperture constraint. The similar slopes between pelvic aperture and maximum egg width on body size suggest that selection for larger eggs is limited by the expansion of the pelvic girdle with growth (Congdon and Gibbons, 1987). The relationships between egg width and body size observed in *S. odoratus* and *P. floridana* do not appear to be due to pelvic aperture constraint. This is in agreement with Clark et al. (2001) who found the caudal gap to be a more constraining dimension than pelvic aperture in *S. odoratus* (caudal gap was not measured in this study as it is

inaccessible from X-ray photographs). Similarly, Congdon and Gibbons (1987) did not find evidence of pelvic aperture constraint in *P. scripta*, a turtle with similar body size to *P. floridana*, concluding that at larger body sizes the pelvic aperture size allowed by locomotion, limb retraction, and support requirement was larger than the width of an optimally sized egg.

#### *Variation within and among clutches*

Egg width did vary within clutches in all three species, but clutch identity explained the majority of the variability in all cases. A portion of the clutch effect was the result of maternal body size, but significant among clutch variability remained after body size was corrected for. It is not possible to discern whether the within clutch variability is adaptive (as predicted by plasticity models) or a non-adaptive result of physiology.

Individual female *P. floridana* and *K. subrubrum* altered both clutch size and egg width among clutches produced during different reproductive seasons. The variability of both parameters and the lack of correlation between the two suggest that the proximate factors influencing clutch size are independent from those influencing egg size. In most turtle species, follicle maturation begins in late summer or fall (Harless and Morlock, 1979) with vitellogenesis continuing until hibernation in some species (Dobie, 1971; White and Murphy, 1973) and continuing into the spring up to ovulation in other species (Christiansen and Dunham, 1972; Mahmoud and Klicka, 1972, Iverson 1979). Thus clutch size might be determined by the number of follicles enlarged in late summer based on body condition and environmental factors, while egg size is dependent on resource availability and metabolic rate through fall and spring. When environmental parameters such as resource availability and temperature are temporally variable and unpredictable, clutch and egg size decisions may become decoupled.

### *Annual Variation*

The reproductive response to annual environmental variation varied by species. In *K. subrubrum*, both egg size and clutch size appear to be plastic at a population level. In *S. odoratus* only egg width was variable, and in *P. floridana* only clutch size varied among years. These results provide conflicting evidence for optimal egg size theory (Smith and Fretwell, 1974; Brockelman, 1975), which predicts changes in reproductive output manifested primarily through changes in clutch size. The lack of annual change in mean egg size in *P. floridana* supports this prediction; however, the reversed pattern in *S. odoratus* is more consistent with a plasticity model (Cooper and Kaplan 1982). The observed variation in *K. subrubrum* is ambivalent since substantial variation was observed in both traits. The pattern of variation observed in *K. subrubrum* (greater variability [CV] in clutch size among years than in egg size) has been interpreted as evidence for optimal egg size theories (Rowe, 1994; Roosenburg and Dunham, 1997); yet, these theories make no specific predictions about acceptable levels of offspring size variation (Congdon, 1989).

The highly variable patterns of among year variation in *K. subrubrum* (Fig. 3.2), suggest that response to annual variability is manifested differently dependent on female identity. Individual characteristics such as body size, body condition, previous reproductive output, age, and microhabitat selection may augment the response of an individual to larger scale environmental heterogeneity making the effects of environmental parameters on reproductive allocation difficult to determine.

### *Multiple clutches*

Production of multiple clutches was a common phenomenon only in *K. subrubrum*. The frequency of production of multiple clutches was highly variable in frequency from year to year



and was correlated with egg width and number of nesting females, suggesting that increasing clutch frequency is a mechanism for increasing total reproductive investment in favorable seasons.

Egg size declines were apparent in both second and third clutches, but clutch size declines were only detected in third clutches. Gibbons et al. (1982) reported that clutches laid later in the season were composed of fewer eggs in *P. scripta* and *P. floridana* and that similar but non-significant decreases in egg number were observed in *K. subrubrum*. Seasonally decreasing clutch size has also been reported in *Mauremys japonica* (Fukada, 1965), *Terrapene ornata* (Legler, 1960), and *Caretta caretta* (Caldwell et al., 1959). These clutch size declines may be the result of reduced reproductive investment in later clutches as body fat reserves are depleted. Reduction in egg size may also be a manifestation of reduced reproductive investment, but might also have adaptive explanations related to the different environmental regime experienced by developing eggs later in the season (i.e., colder mean temperatures, greater temperature variability, altered hydric environment).

#### *Fractional offspring size problem*

As discussed above, clutch size decisions may be made months before the resources that will determine egg size have been amassed. It follows that attainment of an optimal offspring size based on a clutch size determined some months before would require accurate forecasting of the total annual reproductive investment by females. Thus, in a temporally heterogeneous environment with unpredictable and limited resources, deviations in offspring size from theoretical optima will inevitably occur. The magnitude of these deviations for a single offspring will decrease with clutch size, since the variance from the projected total reproductive investment will be distributed over all offspring in the clutch. The greater variation in egg size

among small clutches than in larger clutches detected in *K. subrubrum* and *S. odoratus* is evidence that partitioning continuous resources into discrete offspring contributes to offspring size variation in these species. The absence of such a pattern in *P. floridana* may be due to the larger clutch sizes produced. Variation due to fractional resource allocation would be less apparent since such variation is distributed over a larger number of eggs, making detection difficult when other sources of egg size variation exist. Offspring size variation associated with the fractional offspring size problem has rarely been addressed in the literature and virtually no analyses similar to those presented here have been performed for freshwater turtles.

### *Age*

*Kinosternon subrubrum* continue to grow past sexual maturity and thus may produce larger clutches and larger eggs as a function of increased body size. In addition, older *K. subrubrum* produced larger eggs than younger turtles of similar body sizes, without reduction in clutch size or frequency. Increased egg size in older turtles might be the result of improved prediction of resource availability, better resource acquisition efficiency, or physiological changes associated with aging. These data support the relative rate hypothesis in that *K. subrubrum* appear to increase reproductive output with age through increases in egg size and clutch size mediated through indeterminate growth and increases in egg size through unidentified age-specific mechanisms. Other age-specific traits, such as survivorship, nest predation rates, or embryo development success should be considered in future studies to further understand the interaction between reproductive investment and aging in these turtle species.

### *Conclusions*

Numerous factors, both intrinsic and extrinsic, affect allocation of energy to reproductive products and processes in *K. subrubrum*, *S. odoratus*, and *P. floridana*. This study documents

that female body size and age, within clutch variation, annual variation, clutch number within a season, and fractional resource partitioning contribute to egg size variability. Important sources of variation for clutch size are body size, annual variation, and clutch number within a season.

My data provide conflicting, equivocal support for optimal egg size models and plasticity models. The observed egg-size variability within clutches could be interpreted as evidence for developmental plasticity, but may also have non-adaptive explanations. Basic research on size-specific hatchling fitness under different environmental regimes and additional investigation of turtle reproductive physiology will be required to distinguish between the two interpretations. The pattern of annual variability in *S. odoratus* (greater variability in egg size) seems to support a plasticity model, but the relative variability of egg and offspring size observed in *P. floridana* and *K. subrubrum* (greater variability in clutch size) are more consistent with an optimality model. Again, data on hatchling fitness curves will be necessary to provide cogent arguments in support of either interpretation, as will be an improved understanding of the proximate environmental factors influencing allocation.

The observed patterns of variation in reproductive allocation in this study highlight several shortcomings of the existing theoretical paradigms. Neither optimality nor plasticity models account for a temporal disconnect between allocation decisions for clutch size and decisions for egg size. Yet, as discussed above, the biology of temperate zone turtles may not allow allocation decisions to be made optimally because of seasonal constraints that impose time lags between allocation decisions. The lack of concordance between egg size and clutch size observed in all three species, combined with decreasing variance in mean egg size among larger clutches in *K. subrubrum* and *S. odoratus*, suggests that a theoretical optimum offspring size may exist, but that in many situations environmental unpredictability may prevent it from being

achieved. Physiological constraints may also prevent optimal allocation from occurring. The decline in egg size with clutch number is possible evidence that ovarian or oviducal conditions impact egg size. Additionally, neither model paradigm accounts for morphological constraint on egg size. The positive relationship between egg size and body size is evidence that some form of constraint (pelvic aperture for *K. subrubrum*) does exist in some species of freshwater turtles. These animals may trade the ability to produce optimally sized eggs in order to mature at a smaller size and younger age, allowing lifetime fecundity to be increased. Finally, neither model incorporates age specific traits, yet egg size increased with age in *K. subrubrum* independently of body size.

In conclusion, my findings with freshwater turtles do not adequately support either of the two major models that attempt to explain the allocation of energy to reproductive products and processes. The relatively simplistic nature of the models appears inadequate to address the full complement of factors affecting egg size and egg number in long-lived, intra-annually iteroparous species with inherently complex biology, as is characteristic of many turtles.

Table 3.1. Summary statistics for body size and reproductive parameters. Mean  $\pm$  2SE.

Species	N	Mean Plastron length (mm)	Mean Clutch Size	Mean Egg Width (mm)
<i>K. subrubrum</i>	635	87.49 $\pm$ 0.19	3.4 $\pm$ 0.08	15.17 $\pm$ 0.07
<i>S. odoratus</i>	46	78.2 $\pm$ 2.08	4.98 $\pm$ 0.51	15.48 $\pm$ 0.17
<i>P. floridana</i>	79	234.92 $\pm$ 3.98	11.53 $\pm$ 0.76	24.57 $\pm$ 0.29

Table 3.2. Results of linear regressions of reproductive and body size parameters  
p < 0.05 except where noted (NS).

	Dependent variable	Independent variable	slope	intercept	r <sup>2</sup>
<i>K. subrubrum</i> N = 635	Clutch Size	Plastron Length	0.06	-1.48	0.07
	Egg Width	Plastron Length	0.08	8.01	0.19
	Maximum Egg Width	Plastron Length	0.08	7.86	0.20
	Egg Width	Clutch Size	0.07	14.92	0.01
<i>S. odoratus</i> N = 46	Clutch Size	Plastron Length	0.14	-6.03	0.32
	Egg Width	Plastron Length	0.04	12.62	0.19
	Maximum Egg Width	Plastron Length	0.04	12.48	0.22
	Egg Width	Clutch Size	0.09	15.01	0.08 NS
<i>P. floridana</i> N = 79	Clutch Size	Plastron Length	0.11	-15.09	0.36
	Egg Width	Plastron Length	0.04	14.84	0.34
	Maximum Egg Width	Plastron Length	0.05	14.96	0.34
	Egg Width	Clutch Size	0.09	23.58	0.05

Table 3.3. Multiple regression results for plastron length and clutch size versus mean egg width.

Species	Dependent variable	Independent Variables	$\beta$	partial corr.	p
<i>K. subrubrum</i>	Egg Width	Plastron Length	0.45	0.44	<0.05
		Clutch Size	-0.04	-0.04	NS
<i>S. odoratus</i>	Egg Width	Plastron Length	0.42	0.36	<0.05
		Clutch Size	0.04	0.03	NS
<i>P. floridana</i>	Egg Width	Plastron Length	0.69	0.57	<0.05
		Clutch Size	-0.18	-0.18	NS

Table 3.4 Summary statistics for range of reproductive parameters across years in first annual clutches of individual females that produced eggs in two or more years. (Maximum-Minimum Value for each female). Data are pooled across years.

Species	Variable	N	Min	Max	Mean	2SE
<i>K. subrubrum</i>	Egg Width (mm)	121	0.008	2.92	0.96	0.11
	Clutch Size	121	0	4	1.17	0.01
<i>P. floridana</i>	Egg Width (mm)	15	0.08	2.72	0.97	0.43
	Clutch Size	15	0	7	2.13	0.93

Table 3.5 Frequency of occurrence of differences in mean egg width and clutch size between successive pairs of clutches. (Only first annual clutches included). + indicates an increase in value in the later clutch. - indicates a decrease in value in the later clutch.

Species	Mean Egg Width	Clutch Size	N
<i>K. subrubrum</i>	+	+	44
	+	-	53
	+	same	48
	-	+	27
	-	-	23
	-	same	32
<i>P. floridana</i>	+	+	8
	+	-	6
	+	same	5
	-	+	4
	-	-	1
	-	same	0

Table 3.6. Results of ANCOVA testing for differences in mean clutch size and mean egg width among years.

Species	Clutch size			Egg width		
	F	P	df	F	P	df
<i>K. subrubrum</i>	2.1	<0.05	12, 621	10.3	<0.01	12, 621
<i>S. odoratus</i>	0.5	NS	7, 37	2.97	<0.05	7, 37
<i>P. floridana</i>	2.4	<0.05	12, 65	1.22	NS	12, 65

Table 3.7. Results of Levene's test

Species	Clutch Size	N	SD	F	p	df
<i>K. subrubrum</i>	$\geq 4$	271	0.80	9.64	<0.01	1, 633
	$\leq 3$	364	0.93			
<i>S. odoratus</i>	$\geq 5$	28	0.45	4.832	<0.05	1, 44
	$\leq 4$	18	0.67			
<i>P. floridana</i>	$\geq 12$	41	1.22	0.32	NS	1, 77
	$\leq 11$	38	1.29			



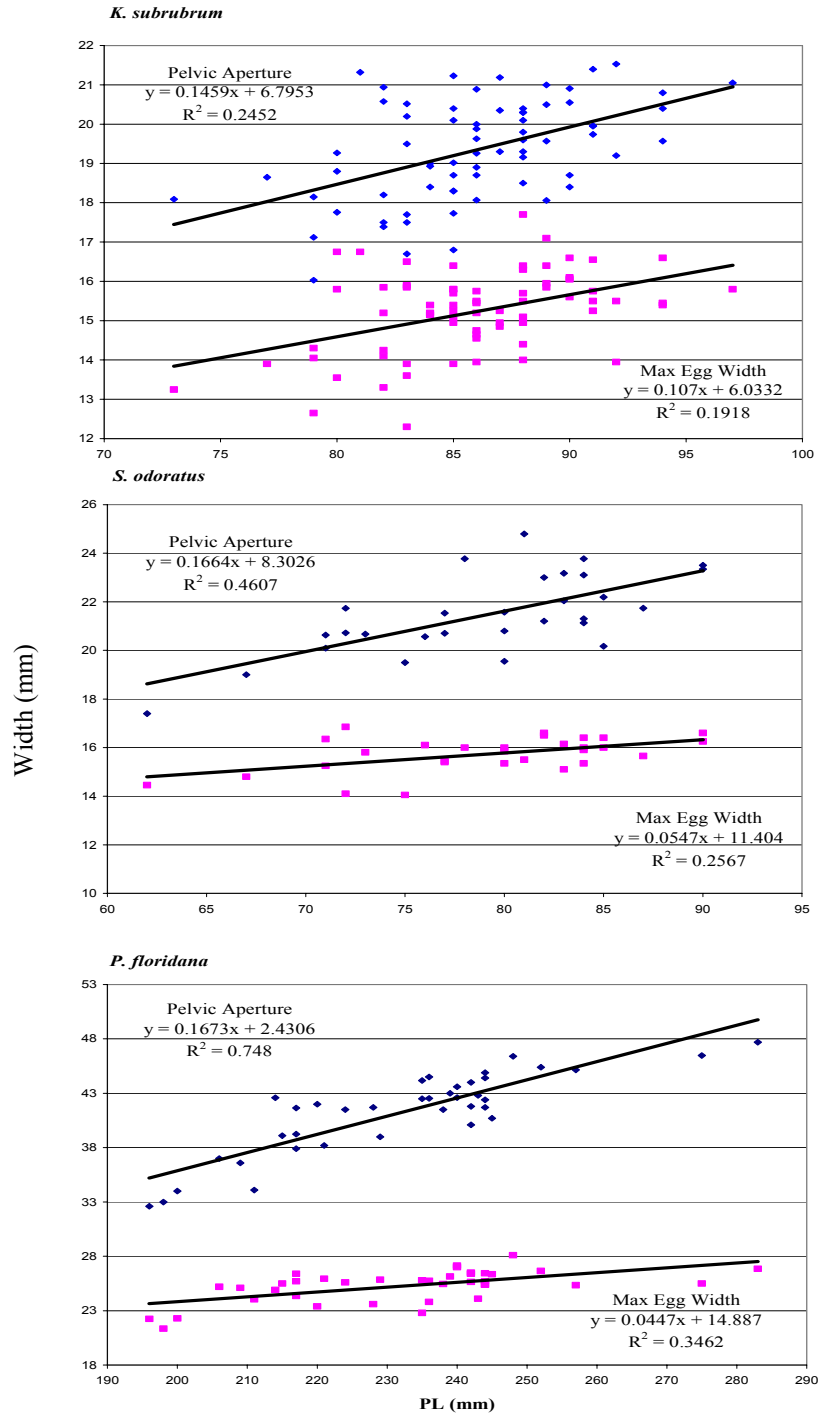


Figure 3.1. Linear regressions of pelvic aperture and maximum egg width on plastron length for all three species. Slopes for pelvic aperture and maximum egg width are similar only in *K. subrubrum*. Slopes of pelvic aperture on plastron length are similar across species.

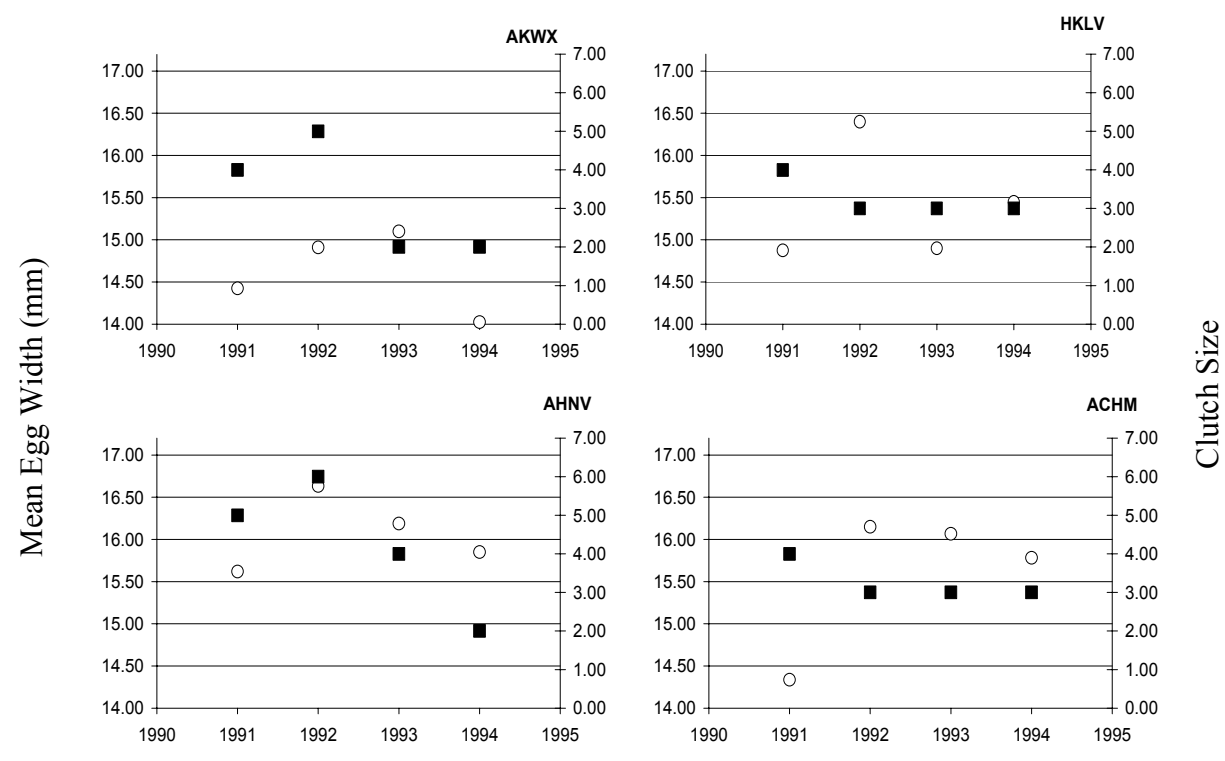


Figure 3.2 Variation in mean egg width (O) and clutch size (■) among first clutches in four consecutive years for four selected *K. subrubrum*. Patterns of variation are variable by individual and show no clear correspondence between clutch size and egg size.

CHAPTER 4

CONCLUSIONS

Reproductive allocation decisions are influenced by a large number of intrinsic and extrinsic factors. I examined egg size and egg number within and among three species of freshwater turtles to identify patterns of reproductive allocation. In general, egg size varied as a function of female body size and age, clutch number within a season, allocation of resources into integral numbers of offspring, and environmental variation. Clutch size varied with female body size, clutch frequency, and environmental variation. However, life-history models for reproductive allocation were found to be largely simplistic and, because they were too generalized, poorly founded on the particular biologies of the organisms they attempted to describe. Incorporating the effects of body-size, reproductive physiology, age, and disconnected allocation decisions into models of reproductive allocation will be essential before a holistic approach to understanding fundamental life-history questions can be properly developed for long-lived species like turtles.

Several avenues of basic research must also be developed to establish more complete understanding of reproductive allocation and life-history theory based on empirical evidence, particularly for freshwater turtles.

- 1) *Determination of the timing and sequence of allocation decisions.* Although many studies have documented selected aspects of reproduction, few have considered the entire female cycle from energy acquisition through final offspring production. Issues such as the timing and duration of follicular enlargement, proportion of reproductive investment from resources gathered and sequestered during egg production versus previously stored resources, and the ability of females to modify clutch size through additional follicle enlargement or follicular atresia remain unresolved or poorly understood for most species of turtles and other reptiles. Empirical research must be directed toward acquiring a basic understanding of each of these

phenomena to validate key assumptions of life-history theory and to allow the development of models founded on the biology of these organisms.

2) *Experimental studies.* Attempts to discern causal relationships in allocation decisions through the study of natural systems can be thwarted by the sheer number of potentially influencing factors and the inability of the investigator to effectively measure or control environmental variation. As a result, the proximate and ultimate factors that contribute to variation in clutch and egg size are largely unknown. Controlled studies, such as mesocosm experiments that manipulate resources and other environmental parameters at various stages of the reproductive cycle and that simultaneously make assessments of maternal condition will be essential to accurately determine how environmental and physiological factors influence allocation decisions.

3) *Offspring fitness.* The effects of environmental heterogeneity on offspring fitness have not been explored for freshwater turtles. Several studies have investigated hatchling fitness as a function of size, but none has addressed how this relationship is altered under different environmental conditions that might realistically be applicable to particular populations. Without this information, specific predictions for offspring-size variability cannot be made under any theoretical paradigm.

4) *Comparative studies.* Several strong comparative studies of reproductive investment exist; however, basic data on patterns of reproductive investment and allocation are not available for most species, limiting the scope and value of comparative investigations. Continued collection of basic life-history data will be important for developing a more cohesive, fundamental understanding of turtle reproduction that incorporates phylogeny and ecology.

5) *Long-term studies.* The value of long-term ecological studies is widely recognized, yet logistical and monetary constraints have limited the number of such studies. Long-term studies can be powerful for detecting and understanding ecological and evolutionary phenomena that often do not become apparent through short-term investigation. Long-term studies on long-lived species like turtles also allow investigators to examine age-specific traits, allowing reproductive investment and allocation to be interpreted in light of more general life history parameters such as age at maturity, longevity, and growth past maturity.

Until a fundamental knowledge of the basic biological processes of energy acquisition, processing, and distribution for a species is available within the context of natural environmental variability and influence, simplistic reproductive allocation models will be unlikely to be applied effectively. The situation with turtles underscores the necessity of acquiring basic ecological and physiological understanding of a variety of reproductive processes if a complete understanding of reproductive allocation of energy is to be forthcoming.

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