Evol Ecol (2018) 32:29–41
https://doi.org/10.1007/s10682-017-9922-3

ORIGINAL PAPER

A lengthy solution to the optimal propagule size problem in the large-bodied South American freshwater turtle, *Podocnemis unifilis*

Tibisay Escalona[1,2] · **Dean C. Adams**[3,4] · **Nicole Valenzuela**[3]

Received: 18 June 2017 / Accepted: 9 October 2017 / Published online: 16 October 2017
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Abstract In oviparous vertebrates lacking parental care, resource allocation during reproduction is a major maternal effect that may enhance female fitness. In general, resource allocation strategies are expected to follow optimality models to solve the energy trade-offs between egg size and number. Such models predict that natural selection should optimize egg size while egg number is expected to vary with female size, thus maximizing offspring fitness and consequently, maternal fitness. Deviations from optimality predictions are commonly attributed to morphological constraints imposed by female size, such as reported for small-bodied turtle species. However, whether such anatomical constraints exist in smaller-bodied females within large-bodied clades remains unstudied. Here we tested whether resource allocation of the river turtle *Podocnemis unifilis* (a relatively smaller member of the large-bodied Podocnemididae) follows optimality theory, and found a pattern of egg elongation in smaller females that provides evidence of morphological constraints and of a reproductive trade-off with clutch size, whereas egg width supports the existence of an optimal egg size and no trade-off. Moreover, larger females laid larger clutches composed of rounder eggs, while smaller females laid fewer and relatively more elongated eggs. Elongated eggs from smaller females have larger volume relative to female size and to round eggs of equal width. We propose that elongated eggs represent a solution to a potential morphological constraint suffered by small females. Our results suggest that larger females may optimize fitness by increasing the number of eggs, while smaller females do so by producing larger eggs. Our data supports the notion that morphological

✉ Tibisay Escalona
tibisay.escalona@gmail.com

[1] CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, Facultad de Ciencias, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450–208 Porto, Portugal

[2] Department of Biology, Faculty of Sciences, University of Porto, Rua Campo Alegre s/n, 4169-007 Porto, Portugal

[3] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames 50011, IA, USA

[4] Department of Statistics, Iowa State University, Ames 50011, IA, USA

constraints are likely more widespread than previously anticipated, such that they may not be exclusive of small-bodied lineages but may also exist in large-bodied lineages.

Keywords Female fitness · Optimality models · Parental investment · Life history traits · Energy allocation · Morphological constraints

Introduction

Time and optimal maternal allocation of resources that enhance reproduction and survival are at the heart of life history evolution (Roff 1992; Gilbert 2012), and are expected to follow optimality models. Among them, female reproductive strategies may provide solutions to existing energy trade-offs between egg size and number in a given environment to maximize potential fitness. Optimality theory predicts that natural selection should optimize egg size within populations while egg number should co-vary positively with maternal body sizes (Smith and Fretwell 1974). Whether (or how) species reach a balance and attain optimal offspring size and number has been the target of extensive theoretical and empirical research (Bernardo 1996; Hendry et al. 2001). Notably, optimization solutions to the propagule size and number problem differ between oviparous and viviparous species (Cadby et al. 2011) because viviparous parents provide continued supplementation of nutrients and protection to their developing offspring. Similarly, in species with parental care, parents provide protection, help, or extra energy to the offspring post-oviposition/hatching/birth (Bleu et al. 2011) as occurs in some reptiles (Itonoga et al. 2012), birds (Rubolini et al. 2011) and mammals (Maestripieri and Mateo 2009).

Turtles are a lineage of oviparous long-lived ectotherms lacking parental care (see Ferrara et al. 2014), such that most maternal effects are restricted to allocation of nutrients and hormones to the egg (Roosenburg and Dunham 1997), which are suggested to follow optimality models (Brockelman 1975). Nonetheless, whether turtles actually follow optimality models of energy allocation remains debated because no general pattern has been identified across taxa (Rollinson and Brooks 2008). Namely, female body size in turtles is associated with clutch size, egg size, and egg shape (e.g., Rowe 1994; Valenzuela 2001), but the direction of this association varies across species in ways that may violate optimality expectations. First, optimality models predict that egg size should either (a) increase as a function of female size until the optimal egg size is reached, or (b) the same ('optimal') egg size should be produced irrespective of female size (Fig. 1a,b) (Lovich et al. 2012). Thus, positive relationships between female and egg size that do not reach a plateau indicate that no optimal egg size is attained (Fig. 1c) (Ryan and Lindeman 2007; Ennen et al. 2017), and instead, suggest that egg size is constrained by either morphological or by other factors (Bowden et al. 2004; Kern et al. 2015). In some turtles, egg size increases (e.g., egg width) with female size or age without reaching a plateau (Fig. 1c) (e.g., Clark et al. 2001; Valenzuela 2001), while in other species egg size is independent of female size as predicted by optimality models (Fig. 1b) (e.g., Tinkle et al. 1981; Lovich et al. 2012). Furthermore, optimal egg size may be attained in some populations but not others, as detected in *Chrysemys picta* turtles (Iverson and Smith 1993). Overall, deviations from optimality predictions are more common in small-bodied species or small individuals (Rollinson et al. 2013). Second, a negative relationship between egg size and clutch size provide evidence of the reproductive trade-offs (Fig. 1d) (e.g., Rowe 1992) underlying the

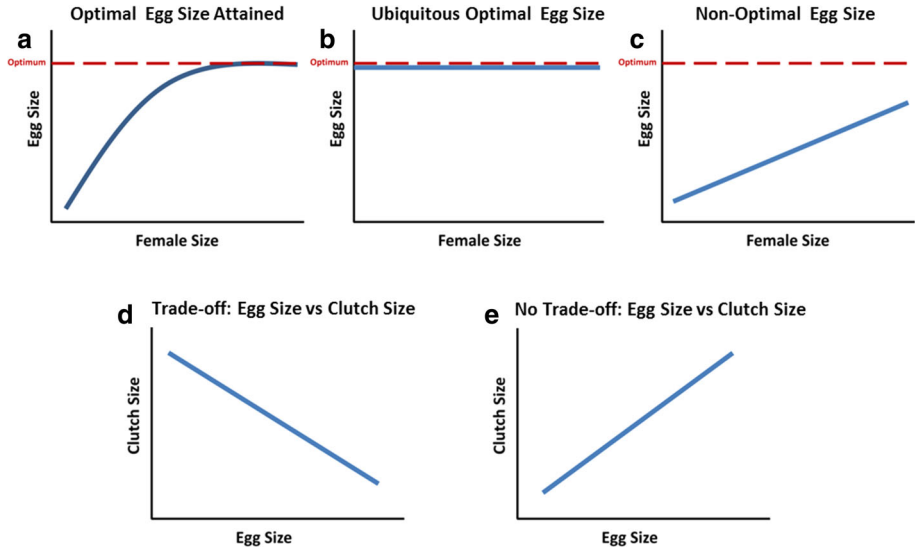


Fig. 1 Patterns of egg size and clutch size predicted by optimality theory and energy trade-offs. **a** Optimal egg size is attained by the larger females. **b** Females of all sizes produce eggs of optimal size. **c** Optimal egg size does not exist or its attainment is constrained. **d** A trade-off exists between egg size and clutch size. **e** No trade-off exists between egg size and clutch size. See text for exemplar evidence of each pattern reported in turtles

optimal egg size problem. Such negative association between egg and clutch size is observed in some turtles (Rowe 1992; Iverson et al. 1993), whereas reproductive trade-offs appear absent in others whose egg number and egg size increases with female size (Fig. 1e) (e.g., Valenzuela 2001; Litzgus et al. 2008).

Besides energy trade-offs, physical constraints may also exist in turtles that preclude the evolutionary attainment of an optimal egg size. For instance, turtle eggs must fit through the female pelvic aperture opening which they pass on their smallest axis (the egg width axis), just as humans must fit through the birth canal for their successful delivery in ways that imposes strong selection on neonate size. Accordingly, the 'anatomical-constraint' hypothesis (Congdon and Gibbons 1987), states that the width of the female's pelvic aperture may limit egg width in smaller females, who consequently would lay eggs of suboptimal size (Macip-Ríos et al. 2012). Such pelvic constraints are documented in some turtles such as in *C. picta* and *Deirochelys reticularia* (Congdon and Gibbons 1987; Rollinson and Brooks 2008), but are absent in others (Naimi et al. 2012; Macip-Ríos et al. 2013). Interestingly, populations of *C. picta* whose individuals are larger-bodied appear to escape such anatomical constraint (Iverson and Smith 1993). Notably, in some turtles egg width (but not egg length) increases with female size (Rasmussen and Litzgus 2010), such that smaller females lay relatively more elongated eggs than larger females (Brooks et al. 1992; Rowe 1994; Litzgus and Mosseau 2006). This pattern may represent a solution to the restriction imposed by a small aperture on egg width, as round eggs have lesser volume (i.e., are smaller) than elongated eggs of equal width. Thus, some small-body turtle species or smaller-bodied populations appear to show evidence of anatomical constraints on egg size, while such constraints are absent in the larger-bodied individuals of relatively small species. But whether smaller-bodied species (or individuals) from large-bodied turtle lineages are also anatomically constrained has not been evaluated. *Podocnemis* is a genus of

relatively large riverine turtles (Ceballos et al. 2012), whose largest member is the giant Amazonian river turtle *P. expansa,* a species purportedly lacking a trade-off between egg size and number, or any anatomical constraint (Valenzuela 2001). Here we studied *Podocnemis unifilis*, a congener whose females are half the size of *P. expansa* females on average (Ceballos et al. 2012), and found evidence suggestive of the existence of anatomical constraints in egg length and shape.

P. unifilis is endemic to northern South America, inhabiting the Amazon and Orinoco river basins. Reproductive female size ranges from 27 to 51.8 cm in carapace length (Escalona et al. 2012). Thus, while *P. unifilis* is a relatively smaller member of *Podocnemis*, it is still a large-bodied turtle overall. Life history traits other than egg and clutch size, including energy allocation decisions are poorly known in this species (Vanzolini 2003). Our study represents a data collection effort over 3 years on female reproductive traits from a single population of *P. unifilis* whose nesting behavior was previously reported (Escalona et al. 2009). We examine maternal effects derived from female size on clutch size, and egg dimensions (egg length, egg width, egg mass) including egg shape (elongation) and egg volume, to test whether energy allocation in this species follows expectations from optimality criteria.

Materials and methods

The study was conducted by daily monitoring 11 nesting beaches in a pristine area of the Orinoco basin of Venezuela over three nesting seasons (late January through mid-March of 1999–2001). Nesting forays initiated when the river levels dropped exposing the nesting grounds. Nests constructed each night were easily located the following morning by inspecting the beaches systematically along lengthwise transects 2 m apart throughout each beach, and following fresh female tracks which are highly visible.

Because *P. unifilis* is a nocturnal nester such that most nesting females were unavailable for direct measurement, we estimated female size by measuring the width of the tracks left by females in the sand which serves as a proxy for carapace length (e.g., Soini 1981; Valenzuela 2001). For this purpose, the best print along each track was selected and the track width was measured as the distance between the outermost sides of the hind feet. We validated the accuracy of this approach by calculating the correlation between track width and carapace length of a large set of females captured along the river that were measured directly ($n = 248$).

For each nest we measured clutch size and egg size. After counting the number of eggs per nest (clutch size), we randomly selected 10 eggs per nest (average clutch size = 22 eggs, range = 3–28, see results) to measure egg width (mm), egg length (mm), egg mass (g), and egg shape (the ratio of egg length to egg width) which are sufficient to accurately assess egg dimensions (Escalona 1995). Finally, we calculated egg volume using the formula for an ellipsoid (Iverson and Ewert 1991; Litzgus and Mousseau 2006) as $V = (\pi/6000) \times LW^2$, where L = egg length, W = egg width, and $\pi = 3.14$.

We used linear regression analysis to examine the functional relationship between female size and the reproductive traits measured above (clutch size, egg width, egg length, egg mass, and egg shape). We also used Pearson correlation to test for the strength of association among the egg dimensions (i.e., length, width, and mass). Variables were natural-log-transformed prior to their analysis following King (2000). Additionally, we controlled for maternal body size when testing for a trade-off between the number and size

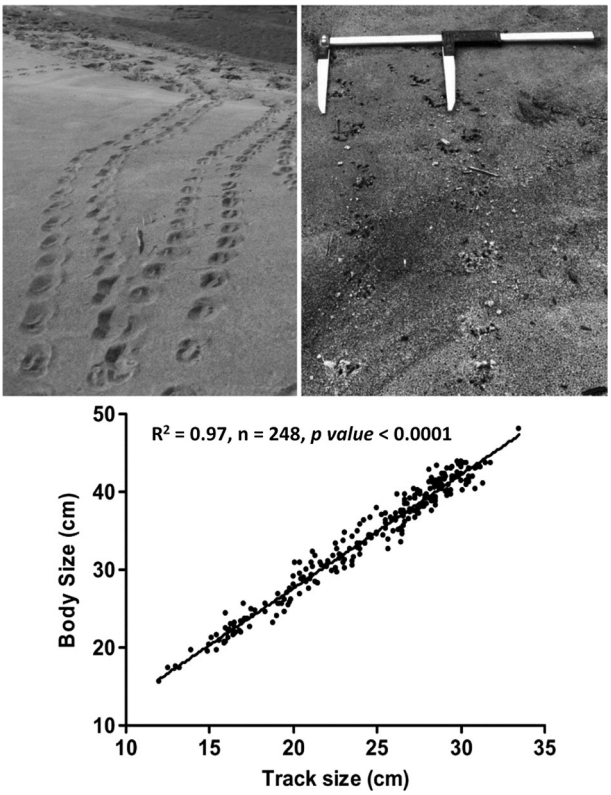


Fig. 2 Tracks left on the beach by female *Podocnemis unifilis* during their nesting forays (top panels) and relationship between female track size and body size (linear carapace length) (bottom panel)

of eggs by performing different regression analysis in the following manner. First, we carried out separate multiple regression analysis between clutch size versus female and egg size (except egg shape that was examined by linear regression). Second, we performed separate linear regressions of clutch size versus female size, and of egg size (i.e., length, width, mass and shape) versus female size to obtain the residuals. Then, the relationship between egg size and clutch size residuals was examined by linear regression (Rowe 1992; Valenzuela 2001). All analyses were performed in JMP Pro 13 (SAS Institute Inc. 2016). Finally, we tested for optimal egg size by regressing egg volume onto egg shape or female size.

Results

A strong and significant positive relationship was found between female track width and carapace length for the set of captured females ($R^2 = 0.97$, $n = 248$, p *value* < 0.0001; Fig. 2), indicating that track width can be used with confidence to estimate female size in this population. In the study area, mean female linear length of carapace (LLC) was 37.4 ± 2.92 cm ($n = 901$), and average clutch size was 22.1 ± 4.67 eggs ($n = 841$). As expected, female size was positively associated with clutch size, showing that larger

females tended to lay larger clutches (Table 1; Fig. 3a), although a large amount of variation in clutch size remained unexplained by female size ($R^2 = 0.023$, n = 363, *p value* = 0.002). The average egg dimensions from 279 nests (n = 3144 eggs) were

Table 1 Regression analyses between female size (track width), clutch size (number of eggs) and various egg dimensions (length, width, mass), including egg shape or elongation (the ratio between egg length to egg width)

Predictor variable	Response variable	R^2	F value	*df*	*p value*	Slope
Female size	Clutch size	0.023	9.502	1361	0.002	0.396
Female size	Egg length	0.159	46.621	1245	< 0.001	0.373
Female size	Egg width	0.013	3.162	1245	0.076 ns	0.074
Female size	Egg mass	0.001	0.218	1245	0.641 ns	0.063
Female size	Egg shape	0.204	62.800	1245	< 0.001	0.447
Res. egg length	Res. clutch size	0.020	5.008	1245	0.026	0.353
Res. egg width	Res. clutch size	0.064	16.734	1245	0.001	0.821
Res. egg mass	Res. clutch size	0.041	10.481	1245	0.001	0.439
Res. egg shape	Res. clutch size	0.110	28.831	1245	< 0.001	0.782

Variables were Ln-transformed

Res Residuals, R^2 R squared, *df* degrees of freedom, *ns* non-significant

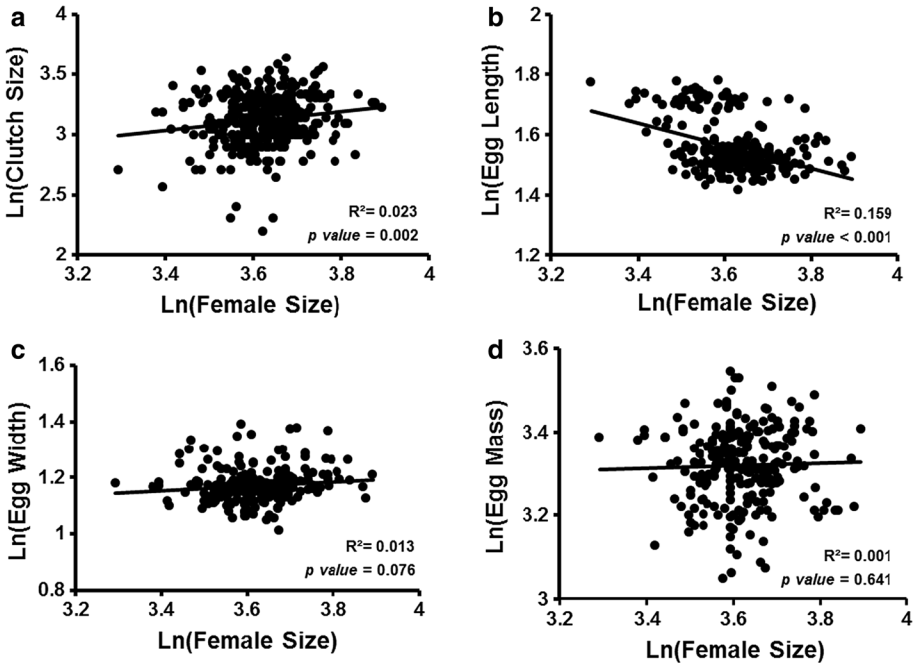


Fig. 3 Clutch size (**a**) and Egg dimensions (Length (**b**), width (**c**) and mass (**d**)) as a function of female size in *Podocnemis unifilis*

4.7 ± 0.42 cm of length, 3.2 ± 0.21 cm of width and 27.9 ± 2.65 g of mass. All egg dimensions (i.e., length, width and mass) were positively correlated with each other (Fig. 4). Egg length was the only egg dimension significantly associated with female size (Table 1, Fig. 3). Namely, egg length (and egg shape—i.e. the ratio of egg length to egg width) was negatively correlated with female size (Table 1; Figs. 3b and 5c), indicating that larger females laid relatively shorter eggs (i.e., more spherical) than smaller females, but of relatively similar width and mass. This relationship was consistent from year to year. We detected no change in clutch size or female size as the nesting season progressed (results not shown).

Results from the multiple regression analysis revealed significant but weak support for a trade-off between clutch size and egg size (Table 1). Namely, when the effect of female body size was held constant, clutch size was negatively associated with egg length (slope = − 0.353, t value = − 2.233, p value = 0.006, $R^2 = 0.041$). In contrast, clutch size increased with egg width (slope = 0.821, t value = 4.082, p value < 0.001, $R^2 = 0.084$) and egg mass (slope = 0.439, t value = 3.230, p value < 0.001, $R^2 = 0.062$) when female size was held constant. The regression of egg shape on clutch size was also significant and negative (slope = − 0.162, F value = 34.799, p value < 0.001, $R^2 = 0.124$; Fig. 5a), while the regression of egg shape on egg mass detected no significant change (slope = − 0.070, F value = 1.203, p value = 0.274, $R^2 = 0.005$; Fig. 5b). Thus, eggs from larger clutches were relatively shorter, wider, and heavier (that is, eggs were rounder and relatively bigger) than those from smaller clutches. These data reveal that a trade-off is only evident between clutch size and egg length (and consequently egg shape). Results are identical when regressing the residuals of the regression of clutch size onto female size against the residuals of the regression of egg dimensions and egg shape on female size (Table 1). However, very little variation was explained ($R^2 < 0.11$ in all cases), indicating that while significant, the relationship was very weak. Finally, the regression of egg volume on egg shape (slope = 0.004, F value = 2.634, p value = 0.106, $R^2 = 0.011$), or female size (slope = − 0.0002, F value = 2.915, p value = 0.088, $R^2 = 0.012$), was not significant, indicating that egg volume remain fairly consistent irrespective of egg shape or maternal body size.

Discussion

In oviparous vertebrates lacking parental care, energy allocation to eggs is a major mechanism females employ to enhance their reproductive success (Mousseau and Fox 1998) by affecting a variety of propagule traits, including egg size and number (Warner et al. 2010). Energy allocation strategies in these organisms such as in turtles are predicted to follow optimality theory, yet empirical data often appear to counter expectations derived from these models. Deviations from optimality predictions in turtles have been explained as resulting from morphological constraints in small-bodied species (Rollinson et al. 2013).

Here we found evidence suggestive of the existence of such constraints in *P. unifilis,* a member of a lineage of relatively large-bodied turtles. Our data indicate that *P. unifilis* exhibits a subtle but significant trade-off between egg length and clutch size (Table 1), and eggs from larger clutches were shorter and rounder than those from smaller clutches, supporting the notion that rounder eggs in large clutches are favored to maximize oviductal packing of eggs (Iverson and Ewert 1991). Furthermore, egg length and egg shape (length/width) decreases with female size in *P. unifilis*, indicating that smaller females lay

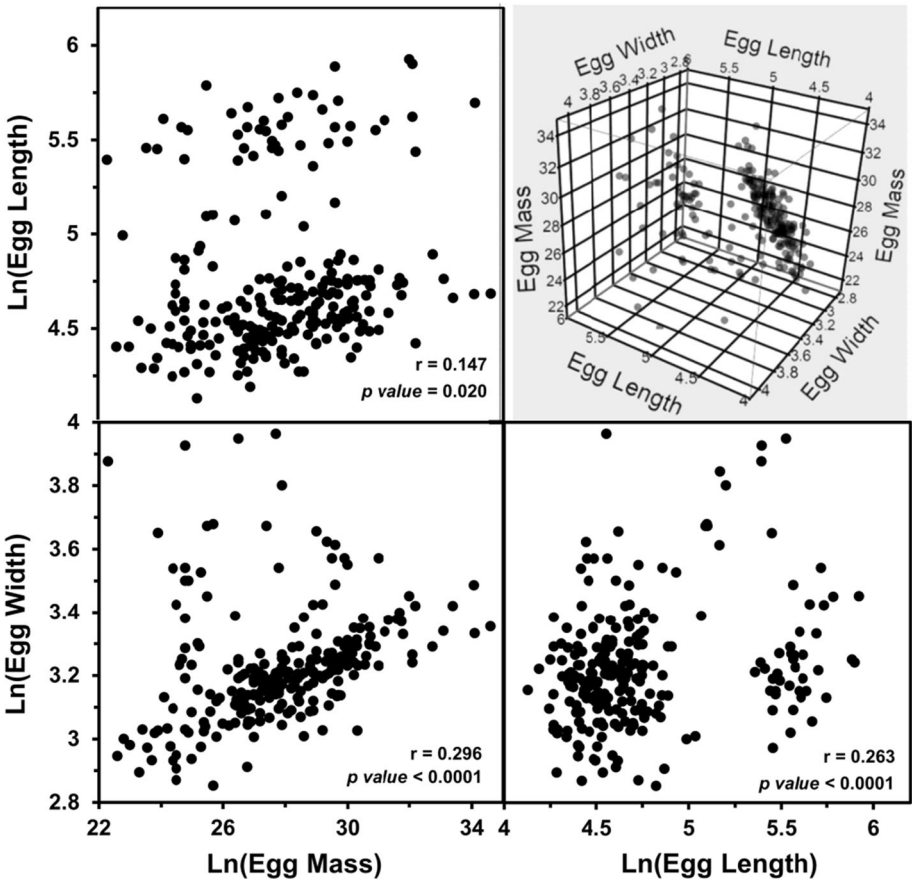


Fig. 4 Egg dimensions (length, width, mass) Pairwise correlation and 3D plot in *Podocnemis unifilis*

relatively more elongated eggs (Figs. 3b and 5c), which allows smaller females to produce a larger egg for any given egg width than if they produced a round egg whose diameter is equal to the width of the elongated egg. We hypothesize that the production of elongated eggs in general, and of eggs of increased length in smaller females in particular, may be a solution to the morphological restriction suffered by smaller females in *P. unifilis*. The pattern observed here has been detected in distantly related turtles (e.g., Wilkinson and Gibbons 2005; Rollinson and Brooks 2008). In contrast, the congener *P. expansa* produces round eggs that increase both in size and number with female size, showing no sign of a trade-off (Valenzuela 2001). Further research is needed to test this hypothesis directly by empirically measuring the pelvic aperture size of nesting females using X-ray imaging which was precluded in our study due to logistical reasons. Such analysis will permit assessing additional potential constraints of the pelvic architectural design that may exist (see Zug 1971; Long and Rose 1989; Lovich et al. 2012).

When viewed in terms of classical optimality theory, our results in *P. unifilis* appear paradoxical, because a negative association of female size and egg length could be interpreted as indication that larger females are not provisioning their offspring maximally. The pattern is better explained when viewed from the perspective of maternal allocation by

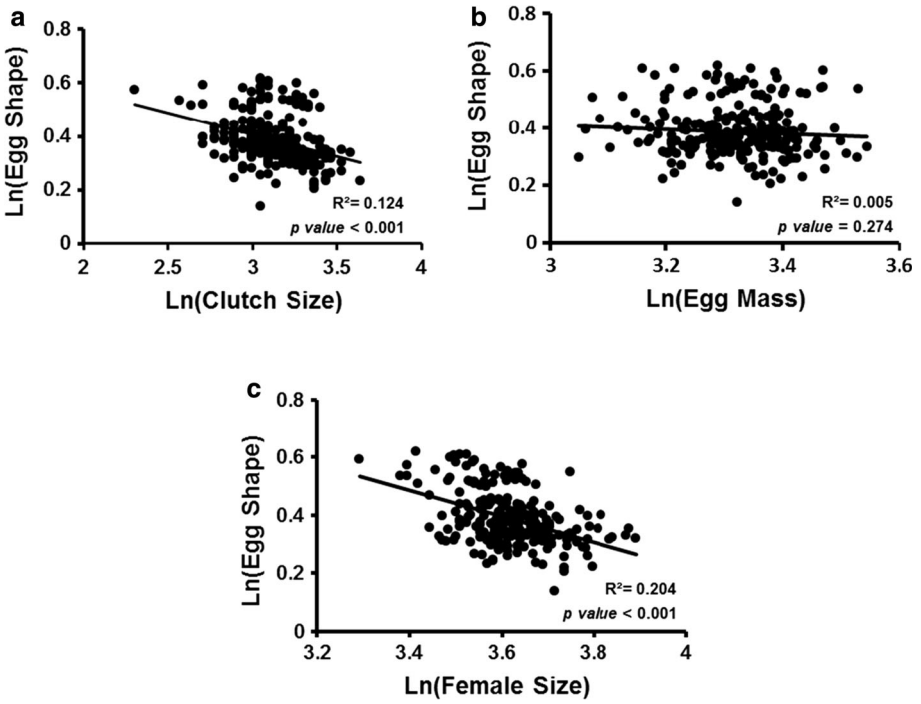


Fig. 5 Egg shape (i.e., the ratio of egg length to egg width) as a function of clutch size (**a**), egg mass (**b**) and female size (**c**), in *Podocnemis unifilis*

small females. For small females, the morphological constraints would reduce the number of options available for offspring apportionment: they can either make longer (i.e., larger) eggs, or produce more eggs of equal width to larger females. Based on our findings and assuming a morphological constraint is corroborated in this species, we hypothesize that smaller females may maximize their fitness by producing relatively larger eggs compared to larger females via the elongation of their eggs, because offspring survival is positively correlated with egg size in turtles (e.g., Rollinson and Brooks 2008) including the congeneric *P. expansa* (Valenzuela 2001). The variation in egg size we observed is concordant with the variance in egg volume detected in multiple populations of *P. unifilis* (Vansolini 2003), which was interpreted as evidence of a lack of optimal egg size in this species. Yet, our data revealed that egg volume did not vary with egg shape or female size, which would instead support the existence of an optimal egg size in *P. unifilis*. We propose that the strategy of smaller females in *P. unifilis* to produce relatively longer eggs might be a solution to the propagule size problem that brings their allocation closer to optimal values than would otherwise be attained by producing rounder eggs of the same width. This is consistent with observations in a few other small-bodied turtles, i.e. *C. picta*, *Glyptemys insculpta* and *Clemmys guttata* (Brooks et al. 1992; Rowe 1994; Litzgus and Mousseau 2006; Rasmussen and Litzgus 2010). Future studies that assess the size and the architectural design of the pelvic aperture directly in *P. unifilis* will be needed to test this hypothesis. We also note that as embryonic development progresses, elongated eggs become rounder (T.E. personal observation), such that no differential effects are expected

in embryonic folding, development or survivorship due to the initial elongated egg shape *per se*.

Furthermore, when combined with findings on *P. expansa* (Valenzuela 2001), we speculate that there may be a minimum viable egg size for survival in the genus *Podocnemis*, consistent with optimality theory that predicts a minimum propagule size threshold for offspring survival. When compared to the eggs of *P. expansa* (a considerably larger turtle), the eggs of *P. unifilis* are much larger relative to overall female size, a pattern that is accentuated in smaller females compared to larger females within *P. unifilis*. Nonetheless, a large amount of variation in clutch size in *P. unifilis* remained unexplained by female size, raising the question of what other forces might affect these life history traits. Possible factors include body condition (i.e., energetic reserves, density of dermal bone of carapace, degree of hydration) (Willemsen and Hailey 2002; Litzgus et al. 2008; Rasmussen and Litzgus 2010), sexual maturity (i.e., age and size of first reproduction), resource availability (i.e., food quantity and quality) and climatic conditions (i.e., temperature), which may cause a particular female to produce a large or a small clutch in any given year irrespective of her body size. Moreover, these conditions can also affect the number of clutches laid in a reproductive season (Gibbons and Greene 1990; Iverson and Smith 193; Dodd 1997) or over multiple nesting seasons (Litzgus et al. 2008). It is unclear whether other factors such as lipid content may vary among eggs in ways that explain some of the variation in egg mass that we observed here. Additionally, physiological and other morphological constraints (i.e., senescence, endocrine regulation of vitellogenesis, uterine compression of the ovum/albumen mass, ovarian follicle size, oviductal length and diameter, maternal body-volume capacity and shape, anal gap) can also influence clutch size, egg size and egg shape (Iverson and Ewert 1991; Congdon et al. 2003; Bowden et al. 2004; Hofmeyr et al. 2005; Wilkinson and Gibbons 2005; Litzgus et al. 2008; Rasmussen and Litzgus 2010; Bowden et al. 2011; Lovich et al. 2012). Therefore, even in years when food is plentiful there are limitations in the number of eggs a female can fit in her oviduct and the size of eggs she can pass through her pelvic aperture.

In summary, our data show that in *P. unifilis* both large and small females may be attempting to maximize their fitness, but do so in very different ways, suggesting mixed strategies in terms of optimality models. Namely, larger females increase the number of eggs, while smaller females produce relatively larger eggs via egg elongation, thus countering the morphological limitation to egg size imposed by their smaller body size. Our findings support the presence of maternal effects in *P. unifilis* derived from female size, which operate more strongly on the offspring from smaller females.

Further research is needed to determine the relationship between egg length and shape versus female abdominal size and shape, the influence of the anatomy and physiology of the female reproductive tract on egg parameters, and as well as the relative survival of offspring from smaller versus larger females.

Acknowledgements This study was partially funded by grants from the Wildlife Conservation Society, Sustainable Aquatic Resources Center, Saint Louis Zoo, Jersey Zoo, Organization of Tropical Studies, International Center for Tropical Ecology, Scott Neotropical Fund and the Graduate School-Dissertation Fellowship of the University of Missouri St Louis (all to TE). Field work was enabled by invaluable help from Cesar Escalona, Maria Edilia Varela, Miguel Estaba (and his family), Felix Daza (and his family), Conrad Vispo and Claudia Knab-Vispo, Andres Rosenschein and Ivonne Monge. We thank two anonymous reviewers for their thoughtful and valuable comments on our manuscript.

References

Bernardo J (1996) The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Am Zool 36(2):216–236

Bleu J, Massot M, Haussy C, Meylan S (2011) Experimental litter size reduction reveals costs of gestation and delayed effects on offspring in a viviparous lizard. Proc Biol Soc B 279(1728):489–498

Bowden RM, Harms HK, Paitz RT, Janzen FJ (2004) Does optimal egg size vary with demographic stage because of a physiological constraint? Funct Ecol 18(4):522–529

Bowden RM, Paitz RT, Janzen FJ (2011) The ontogeny of postmaturation resource allocation in turtles. Physiol Biochem Zool 84(2):204–211

Brockelman WY (1975) Competition, the fitness of offspring, and optimal clutch size. Am Nat 109 (970):677–699

Brooks RJ, Shilton CM, Brown GP, Quinn NWS (1992) Body size, age distribution, and reproduction in a northern population of wood turtles (*Clemmys insculpta*). Can J Zool 70:462–469

Cadby C, Jones S, Wapstra E (2011) Potentially adaptive effects of maternal nutrition during gestation on offspring phenotype of a viviparous reptile. J Exp Biol 214(Pt 24):4234–4239

Ceballos CP, Adams DC, Iverson JB, Valenzuela N (2012) Phylogenetic patterns of sexual size dimorphism in turtles and their implications for Rensch´s rule. Evol Biol 40:194–208

Clark PJ, Ewert MA, Nelson CE (2001) Physical apertures as constraints on egg size and shape in the Common Musk Turtle Sternotherus odoratus. Funct Ecol 15(1):70–77

Congdon JD, Gibbons JW (1987) Morphological constraint on egg size: a challenge to optimal egg size theory. Proc Natl Acad Sci USA 84:4145–4147

Congdon JD, Nagle RD, Kinney OM, Sels RCV (2003) Testing hypotheses of aging in long-lived painted turtles (*Chrysemys picta*). Exp Gerontol 38(7):765–772

Dodd C (1997) Clutch size and frequency in Florida box turtles (*Terrapene carolina bauri)*: implications for conservation. Chelonian Conserv Biol 2:370–377

Ennen JR, Lovich JE, Averill-Murray RC, Yackulic CB, Agha M, Loughran C, Tennan L, Sinervo B (2017) The evolution of different maternal investment strategies in two closely related desert vertebrates. Ecol Evol 7(9):3177–3189

Escalona T (1995) Evaluation of factors affecting nests of an exploited turtle (*Podocnemis unifilis*) in the Nichare river, Venezuela. Master, University of Kent at Canterbury, Canterbury

Escalona T, Adams D, Valenzuela N (2009) Nesting ecology in the freshwater turtle *Podocnemis unifilis*: spatiotemporal patterns and inferred explanations. Funct Ecol 23(4):826–835

Escalona T, Conway-Gomez K, Morales-Betancourt M, Arbelaez F, Antelo R (2012) *Podocnemis unifilis*. In: Páez VP, Morales-Betancourt MA, Lasso CA, Castaño-Mora OV, Bock BC (eds) Biología y conservación de las tortugas continentales de Colombia. Vol. Series recursos hidrobiológicos y pesqueros continentales de Colombia. Instituto de Investigación de los Recursos Biológicos Alexander von Humboldt, Bogota, pp 386–398

Ferrara CR, Vogt RC, Sousa-Lima RS, Tardio BMR, Diniz Bernardes VC (2014) Sound communication and social behavior in an amazonian river turtle (*Podocnemis expansa*). Herpetologica 70(2):149–156

Gibbons W, Greene J (1990) Reproduction in the slider and other species of turtles. In: Gibbons W (ed) Life history and ecology of the slider turtle. Smithonian Institution Press, Washington DC, pp 24–134

Gilbert JDJ (2012) Reproductive allocation in animals. In: Gibson DJ (ed) Oxford Bibliographies online: Ecology. Invited contribution. Oxford University Press, New York

Hendry AP, Day T, Cooper AB (2001) Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. Am Nat 157(4):387–407

Hofmeyr MD, Henen BT, Loehr VJT (2005) Overcoming environmental and morphological constraints: egg size and pelvic kinesis in the smallest tortoise, *Homopus signatus*. Can J Zool 83:1343–1352

Itonaga K, Jones S, Wapstra E (2012) Effects of maternal basking and food quantity during gestation provide evidence for the selective advantage of matrotrophy in a viviparous lizard. PLoS ONE 7(7): e41835

Iverson JB, Ewert MA (1991) Physical characteristics of reptilian eggs and a comparison with avian eggs. In: Deeming DC, Ferguson MWJ (eds) Egg Incubation Its effects on embryonic development in birds and reptiles, 1st edn. Cambridge University Press, Cambridge, pp 87–100

Iverson JB, Smith GR (1993) Reproductive ecology of the painted turtle (*Chrysemys picta*) in the Nebraska sandhills and across its range. Copeia 1:1–21

Iverson JB, Christine C, Byrd K, Lydan K (1993) Latitudinal variation in egg and clutch size in turtles. Can J Zool 71:2448–2461

Kern MM, Guzy CJ, Lovich JE, Gibbons JW, Dorcas ME (2015) Relationships of maternal body size and morphology with egg and clutch size in the diamondback terrapin, *Malaclemys terrapin* (Testudines: Emydidae). Biol J Linn Soc 117:295–304

King RB (2000) Analyzing the relationship between clutch size and female body size in reptiles. J Herpetol 34(1):148–150

Litzgus JD, Mousseau TA (2006) Geographic variation in reproduction in a freshwater turtle (*Clemmys guttata*). Herpetologica 62:132–140

Litzgus J, Bolton F, Schulte-Hostedde A (2008) Reproductive output depends on body condition in spotted turtles (*Clemmys Guttata*). Copeia 1:86–92

Long DR, Rose FL (1989) Pelvic girdle size relationships in three turtle species. J Herpetol 23(3):315–318

Lovich JE, Madrak SV, Drost CA, Monatesti AJ, Casper D, Znari M (2012) Optimal egg size in a sub-optimal environment: reproductive ecology of female Sonora mud turtles (*Kinosternon sonoriense*) in central Arizona USA. Amphib-reptil 33(2):161–170

Macip-Ríos R, Brauer-Robleda P, Casas-Andreu G, Arias-Cisneros M, Sustaita-Rodriguez V (2012) Evidence for the morphological constraint hypothesis and optimal offspring size theory in the Mexican mud turtle (*Kinosternon integrum*). Zool Sci 29(1):60–65

Macip-Ríos R, Sustaita-Rodriguez V, Casas-Andreu G (2013) Evidence of pelvic and nonpelvic constraint on egg size in two species of *Kinosternon* from Mexico. Chelonian Conserv Biol 12(2):218–226

Maestripieri D, Mateo J (2009) Maternal effects in mammals. The University of Chicago Press, Chicago

Mousseau T, Fox C (1998) The adaptive significance of maternal effects. Trends Ecol Evolut 13(10):403–407

Naimi M, Znari M, Lovich J, Feddaci Y, Baamrane M (2012) Clutch and egg allometry of the turtle *Mauremys leprosa* (Chelonia: Geoemydidae) from a polluted peri-urban river in west-central Morocco. Herpetol J 22:43–49

Rasmussen M, Litzgus J (2010) Patterns of maternal investment in spotted turtles (*Clemmys guttata*): implications of trade-offs, scales of analyses, and incubation substrates. Ecoscience 17(1):47–58

Roff D (1992) Evolution of life histories: theory and analysis. Chapman and Hall press, New York

Rollinson N, Brooks RJ (2008) Optimal offspring provisioning when egg size is "constrained": a case study with the painted turtle *Chrysemys picta*. Oikos 117(1):144–151

Rollinson N, Edge CB, Brooks RJ (2013) Recurrent violations of invariant rules for offspring size: evidence from turtles and the implications for small clutch size models. Oecologia 172(4):973–982

Roosenburg W, Dunham A (1997) Allocation of reproductive output: egg and clutch size variation in the diamondback terrapin. Copeia 2:290–297

Rowe J (1992) Comparative life histories of the painted turtle *(Chrysemys picta)* from Western Nebraska. Dissertation, University of Nebraska-Lincoln, University of Nebraska-Lincoln

Rowe J (1994) Egg size and shape variation within and among Nebraskan painted turtle (*Chrysemys picta*) population: relationships to clutch size and maternal body size. Copeia 4:1034–1040

Rubolini D, Romano M, Navara K, Karadas F, Ambrosini R, Caprioli M, Saino M (2011) Maternal effects mediated by egg quality in the yellow-legged gull *Larus michahellis* in relation laying order and embryo sex. Front Zool 8(1):24

Ryan K, Lindeman P (2007) Reproductive allometry in the common map turtle. Graptemys geographica. Am Midl Nat 158(1):49–59

SAS Institute Inc (2016) Using JMP 13. (13.0.1 ed) SAS Institute Inc. Cary

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108 (962):499–506

Soini P (1981) Estudio, reproduccion y manejo de los quelonios acuaticos del genero *Podocnemis* (Charapa, Cupiso y Taricaya) en la Cuenca del Rio Pacaya. In: Gonzales G (ed) Seminario sobre proyectos de investigación ecológica para el bosque tropical húmedo. Instituto de Investigaciones de la Amazonia Peruana, Dirección General Forestal y de Fauna, Lima, pp 124–143

Tinkle D, Congdon J, Rosen P (1981) Nesting frequency and success: implications for the demography of painted turtles. Ecology 62:1432–1436

Valenzuela N (2001) Maternal effects on life history traits in the Amazonian giant river turtle *Podocnemis expansa*. J Herpetol 35(3):368–378

Vanzolini P (2003) On clutch size and hatching success of the South American turtles *Podocnemis expansa* (Schweigger, 1812) and *P. unifilis* Troschel, 1848 (Testudines, Podocnemididae). An Acad Bras Ciênc 75(4):415–430

Warner D, Jorgensen C, Janzen F (2010) Maternal and abiotic effects on egg mortality and hatchling size of turtles: temporal variation in selection over seven years. Funct Ecol 24(4):857–866

Wilkinson L, Gibbons J (2005) Patterns of reproductive allocation: clutch and egg size variation in three freshwater turtles. Copeia 4:868–879

Willemsen R, Hailey A (2002) Body mass condition in Greek tortoises: regional and interspecific variation. Herpetol J 12:105–114
Zug GR (1971) Buoyancy, locomotion, morphology of the pelvic girdle and hindlimb, and systematics of cryptodiran turtles. Misc publ Mus Zool Univ Mich 142:1–98