Evol Biol (2014) 41:81–98
DOI 10.1007/s11692-013-9249-0

RESEARCH ARTICLE

Divergent Sex-Specific Plasticity in Long-Lived Vertebrates with Contrasting Sexual Dimorphism

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Received: 11 April 2013 / Accepted: 16 July 2013 / Published online: 2 August 2013

Abstract Sex-specific plasticity can profoundly affect sexual size dimorphism (SSD), but its influence in female-larger-SSD vertebrates remains obscure. Theory predicts that sex-specific plasticity may drive SSD evolution if the larger sex benefits from optimal-growth conditions when available (*condition-dependent* hypothesis), or if attaining a suboptimal size is penalized by selection (*adaptive canalization* hypothesis). Sex-specific plasticity enhances the size of the larger sex in male-larger-SSD turtles but whether the same occurs in female-larger species is unknown. Sexual shape dimorphism (SShD) is also widespread in nature but is understudied, and whether SShD derives from sex-specific responses to identical selective pressures or from sex-specific selection remains unclear. Here we tested whether sex-specific growth plasticity underlies the development of sexual size and shape dimorphism in the female-larger-SSD turtle, *Podocnemis expansa*. Individuals hatched from several incubation temperatures and were raised under common-garden conditions with varying temperature and resources. Body size and shape were plastic and sexually dimorphic, but plasticity did not differ between the sexes, opposite to the male-larger turtle *Chelydra serpentina*. Maternal effects (egg size) were significant on size and shape, suggesting that females increase their fitness by allocating greater energy to enhance offspring growth. Results ruled out the sex-specific plasticity hypotheses in *P. expansa*, indicating that SSD and SShD do not derive form differential responses to identical drivers but from sex-specific selective pressures. Our results indicate that differential plasticity does not favor males inherently, nor the larger sex, as would be expected if it was a pervasive driver of macroevolutionary patterns of sexual dimorphism across turtle lineages.

Keywords Evolution of sexual size/shape dimorphism · *Podocnemis expansa* · Turtle development · Rensch's rule · Sexual and natural selection

Electronic supplementary material The online version of this article (doi:10.1007/s11692-013-9249-0) contains supplementary material, which is available to authorized users.

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Introduction

Body size can influence fundamental fitness components such as fecundity, reproductive success, and survival of individuals that affect the population dynamics and evolution of organisms. Sexual size dimorphism (SSD), or the difference in body size between males and females, is widespread in nature, and may result from various ultimate forces, such as sexual selection (via male–male combat or increased mobility of males), fecundity selection, or natural selection (Valenzuela 2001b; Butler et al. 2007; Cox and John-Alder 2007; Kelly et al. 2008; Berry and Shine 1980;

Bonnet et al. 2001). Importantly, extensive research demonstrates that body size can be highly responsive to environmental inputs (Rivera 2008; Starostova et al. 2010; Butler et al. 2007; Packard et al. 1993; Lovich et al. 2010; Rowe 1997). Furthermore, the development and evolution of SSD may be mediated proximately by sex-specific growth plasticity, i.e. the differential growth response of males and females to the same environmental conditions (Fairbairn 2005). Two hypotheses exist about how sex-specific plasticity may shape SSD, which differ in their expectation of the level of plasticity to be exhibited by the larger sex compared to the smaller sex. First, under the *adaptive canalization* hypothesis the larger sex is predicted to be less environmentally sensitive to prevent attaining a sub-optimal body size that will yield lower fitness (Fairbairn 2005). The adaptive canalization model implies that plasticity hinders the body size of the larger sex, and is supported in some insects (Fernández-Montraveta and Moya-Laraño 2007; Fairbairn 2005). Second, under the *condition dependence* hypothesis the larger sex is predicted to be more environmentally sensitive in order to maximize growth rates when optimal growing conditions exist, and thus, to attain a larger body size relative to the opposite sex (Bonduriansky 2007). The condition dependence model implies that plasticity enhances the body size of the larger sex and is supported in other insects (Bonduriansky 2007; Teder and Tammaru 2005; Stillwell et al. 2010; Wyman et al. 2010), as well as a few vertebrates, including reptiles. For instance, differential growth responses to environmental conditions were reported between male and female *Sceloporus jarrovi* (Cox et al. 2006) and *Anolis sagrei* lizards (Cox and Calsbeek 2010), *Paroedura picta* geckos (Starostova et al. 2010), and *Chelydra serpentina* turtles (Ceballos and Valenzuela 2011). However, it remains unclear whether the sex-specific plasticity observed in the aforementioned studies is prevalent in nature irrespective of the direction of SSD. Namely, in all those cases, males are the larger sex and males grew faster than females. Therefore, published accounts to date are not conclusive about whether differential plasticity in vertebrates is a general mechanism that influences the body size of males and not of females (irrespective of the type of SSD), or whether it enhances the body size of the larger sex (irrespective of whether males or females are larger). Elucidating whether plasticity generally favors a particular sex or a relative size is important to understand the development and variability of SSD within species, and may also provide insight into the evolution of macroevoutionary patterns such as Rensch's rule. Rensch's rule describes the positive co-variation of SSD with body size in male-biased related species (Rensch 1950), and negative co-variation in female-biased species (Rensch 1960). Data from some species, such as big-headed geckos (Starostova et al. 2010) and snapping turtles (Ceballos and Valenzuela 2011), are consistent with phenotypic plasticity being a mechanism underlying Rensch's rule by enhancing the size of the larger sex. If true and plasticity enhances the size of the larger sex, it should also enhance female size in female-biased SSD species. But if plasticity is a male-specific enhancer it should have no effect (or have a negative effect) on the size of females in female-biased SSD species. To date, studies of growth plasticity in long-lived vertebrates are scarce, particularly in species with larger females.

Besides dimorphism in size, sexual shape dimorphism (SShD) is also widespread in nature, yet it has been relatively less studied (Andersson 1994; Fairbairn et al. 2007). SShD may stem from the differential response of males and females to the same selective pressures (Butler et al. 2007) or from different selective pressures influencing each sex independently (Bonnet et al. 2001; Mann et al. 2006). For instance, sexual selection may shape SShD in turtles by favoring longer limbs and lighter bodies in males compared to females, as well as larger shell openings that enhance male agility when searching for females (Bonnet et al. 2001). Sexual selection may also favor deeper plastron notches in males than in females, which permits easier movement of the limbs and tail, and enhances mating success (Kaddour et al. 2008). Fecundity selection may also favor a relatively wider carapace in female turtles that permits the storage of larger clutches (Alho and Padua 1982a; Bonnet et al. 2001). Furthermore, turtle shape may respond to environmental factors such as river water velocity (Rivera 2008), or habitat differences (Swingland et al. 1989). Examining plasticity levels provides insight into whether SShD is the result of differential responses to the same selective pressure (reflected in the presence of sex-specific plasticity), or the result of distinct pressures that affect each sex independently (as when both sexes exhibit the same level of plasticity or lack thereof), which is understudied in species where females are the larger sex.

In this study we tested the hypothesis that sex-specific growth plasticity plays a significant role in the development of SSD and SShD in the giant Amazonian river turtle (*Podocnemis expansa*), a long-lived vertebrate exhibiting a marked female-biased SSD. To shed light on the contribution of phenotypic plasticity to the evolution of interspecific patterns of SSD and SShD across taxa with contrasting SSD patterns, we contrast our results with those from a study of the male-larger turtle *C. serpentina* under a similar experimental design (Ceballos and Valenzuela 2011).

Podocnemis expansa is the largest river turtle of South America. Adult females attain an average linear carapace length (LCL) ranging between 50 and 89 cm depending on the geographic area, while males are smaller, with an average LCL ranging between 40 and 50 cm [reviewed in

(Ceballos et al. 2012)]. Clutch size averages 86 eggs at our study site in the Orinoco River (range 26–184 eggs) (Soini 1997), with larger females producing more and larger eggs than small females (Alho and Padua 1982b; Valenzuela 2001b). This turtle also exhibits a marked SShD. As adults, females have an oval carapace, with a shallow anal notch, while males have a circular carapace, with a deeper anal notch in the plastron (Pritchard and Trebbau 1984). A subtle but significant SShD exist at hatching, the carapace of females being constrained in its central region, while it is expanded in males (Valenzuela et al. 2004).

Reptilian growth and sexual dimorphism are influenced by temperature and resource availability (Taylor and Denardo 2005; Cox et al. 2006; Cox and John-Alder 2007; Ceballos and Valenzuela 2011). Additionally, embryonic growth and sex determination are affected by temperature in *P. expansa* (Alho et al. 1985; Valenzuela 2001a). Thus, here we explored growth plasticity of *P. expansa* due to incubation temperature as well as to post-hatching water temperature, food quantity and quality, using a common-garden setting following Ceballos and Valenzuela (2011). Under the adaptive canalization hypothesis, we would expect the larger sex (females) to respond less to environmental variation. Alternatively, under the condition dependence hypothesis, we would expect females to be more plastic and attain larger size under optimal environmental conditions for growth (warmer temperature, higher food quality and quantity). On the contrary, if no plasticity is found or if both sexes exhibit identical patterns of plasticity, it could be concluded that differential plasticity does not play a role in the development of SSD in *P. expansa*. The same predictions apply to SShD and can be tested by exploring the magnitude and direction of shape changes exhibited by males and females [sensu (Collyer and Adams 2007)] (Ceballos and Valenzuela 2011).

Materials and Methods

Eggs Collection and Incubation

On 16 February 2007, a total of 570 eggs from 10 clutches were collected at "La Playita" sand beach (6°36′N, 67°7′W) within the Arrau Turtle Wildlife Refuge, on the Orinoco River, Venezuela. Nests were located by following the trackways left by females in the sand the night before, and eggs were immediately transported to the Experimental Field Station of the Foundation for the Development of Physical and Natural Sciences (FUDECI) in the city of Puerto Ayacucho. Eggs were cleaned, measured, and weighed (Table 1). Eggs from each clutch were randomly but evenly assigned to one of three incubation temperatures in 9 incubators (3 per treatment): 30.5, 32.5 and 34.5 °C, which produce 100 % males, 1:1 males and females, and 100 % females, respectively (Valenzuela 2001a, b). These incubation treatments permitted disentangling the effect of sex and incubation temperature on post-hatching growth. Eggs were incubated in boxes 4/5 filled with sand from the nesting beach, to provide an air space of ~3 cm above the substrate. Each box contained 10 eggs (1 per clutch). Sand moisture was maintained by weekly replacing any lost weight from the existing level at the onset of incubation using distilled water (Valenzuela 2001a, b). Boxes were rotated daily within the incubator to minimize the potential effects of thermoclines. Temperature was monitored hourly per box using 2–3 dataloggers (Dallas Semiconductor iButton®) with 0.5 °C precision. Because temperature within incubators varied somewhat from their set value (30.5, 32.5, and 34.5 °C), we refer to the incubation treatments hereafter by the mean temperature that the eggs actually experienced (30.9, 32.2, 33.7 °C), instead of the set values (see mean incubation time and hatching success in Table 1).

Hatchlings remained in their boxes and incubators for 5–8 days after hatching until residual yolk was internalized. Hatchlings were then marked by notching of their carapace (Cagle 1939), and identification was reinforced 5 months later using a numbered 5/8-inch-long monel tag (National Band and Tag Co.). Hatchlings were then raised in a common-garden setting as explained below [modified from (Ceballos and Valenzuela 2011)].

Posthatching Common-Garden Experiment

Hatchlings were distributed among eight post-hatching treatments (Fig. 1) obtained by the combination of (A) two water temperatures: colder and warmer, (B) two food qualities: higher and lower protein content, and (C) two food quantities: higher and lower amount as described below. This design permitted the comparison of two contrasting levels (high/low) from three environmental variables (temperature, resource quality and resource quantity) purported to affect growth significantly. Hatchlings were raised inside plastic-mesh enclosures (3 m × 3 m × 1 m), placed inside two outdoor cylindrical cattle water tanks (11.2 m of diameter × 1.5 m height, and filled with 98,000 l of water). The cool water temperature treatment was obtained by partially shading an enclosure with a polyethylene cloth. The second enclosure was exposed to direct sunlight to obtain a warmer temperature treatment. Water temperature was recorded every 3 h for a year using 4 dataloggers per enclosure. Differences in thermal conditions between treatments were assessed by calculating the cumulative temperature units (CTUs), which measure the amount of heat accumulated above the developmental threshold for *P. expansa* (28 °C) (Valenzuela 2001a).

Table 1 Summary statistics of the clutches and incubation experiments used in this study

Clutch (n)	Number of eggs incubated in the lab	Mean egg weight in grams (min, max)	Mean incubation time (days until piping) per incubation treatment			Hatching success (number of hatchlings)
			30.9 °C (mo = 30.5, SD = 0.83)	32.2 °C (mo = 32.5, SD = 0.91)	33.7 °C (mo = 34, SD = 1.03)	
A (97)	57	41.5 (39.3–43.4)	54	48.1	43.9	57.9 % (33)
B (111)	57	42.9 (37.9–46.4)	54.4	47.8	44	89.5 % (51)
C (84)	57	34.0 (31.3–43.3)	54.5	48	43.7	80.7 % (46)
D (84)	57	44.0 (41.6–47.7)	55.5	48.3	44.1	64.9 % (37)
E (101)	57	43.5 (41–45.8)	NA	NA	NA	0 % (0)
F (100)	57	42.5 (40–45.1)	54	48	44.1	56.1 % (32)
G (100)	56	40.3 (38.3–41.8)	54.5	48.8	44.2	21.4 % (12)
H (103)	61	42.5 (39.4–47)	53.5	47.7	43.9	77 % (47)
I (86)	54	45.1 (39.5–50.6)	53.8	47.7	44.2	64.8 % (35)
J (108)	57	42.3 (38.0–45)	53.8	48.2	44.5	71.9 % (41)
Total (974)	570	41.9 (31.3–50.6)	54.2	48.1	44.1	65.1 % (334)
Hatching success			62 %	70 %	63 %	
Sex ratio (% male)			95 % (n = 94)	88 % (n = 99)	22 % (n = 92)	
Sex ratio juveniles at the end of study			97 % (n = 68)	82 % (n = 82)	19 % (n = 72)	

n = clutch size, x = mean, mo = mode, SD = standard deviation. Incubation treatment temperature values refer to the average temperature recorded with dataloggers inserted in the incubation boxes (see "Materials and Methods" section for details). These average temperatures were used in subsequent analyses and discussion

Clutch size includes so called "oil eggs" which are smaller, yellow-colored, deflated, and considered infertile (Alho and Padua 1982b). No egg from clutch E hatched such that this clutch was considered infertile as was excluded from the calculations of hatching success. Incubation temperature had no effect on hatching success, as there were no differences in the number of hatchlings across temperatures ($P > 0.6861$, $df = 2$). However, increasing temperature had a significant accelerating effect on embryo development and reduced total incubation time ($r = 0.93$, $P < 0.0001$)

Enclosures exposed to direct sunlight accumulated 50 % more CTUs such that animals experienced conditions more conducive for growth (CTU = 7,045.4/year, average temperature = 29.3 °C, SD = 2.8 °C, min = 20.5 °C, max = 45.5 °C), compared to the shaded enclosure (CTU = 4,627.0/year, average = 28.9 °C, SD = 1.77 °C, min = 22 °C, max = 38.5 °C).

Each enclosure was divided into 4 units (1.5 m × 1.5 m × 1 m) using a plastic mesh that allowed water exchange but not food exchange. Two diet qualities were offered: a lower quality diet using commercial food "Cachamarina C" with 21 % protein, and a higher quality diet using commercial food "Trucharina 40" with 40 % protein (Protinal Lab; see nutritional composition in Table S1). Within each food-quality treatment two levels of food-quantity were used: lower and higher food amount corresponding to 2 and 8 % of the total body weight of the group, respectively. During the second year the abundant food treatment was reduced from 8 to 4 % of their body weight as it was noticed that 8 % greatly exceeded ad libitum conditions. Animals in the 2 % food treatment ate food readily with no leftovers. All animals were usually fed 6 days per week, and water was replaced weekly. The combination of pre-hatching (3 incubation temperatures) and post-hatching (2 water temperatures, 2 food qualities and 2 food quantities) treatments resulted in a total of 24 environmental treatments (Fig. 1). To account for potential maternal effects, hatchlings from each clutch were randomly distributed among these 24 treatments following an incomplete randomized block design (Montgomery 1997) such that all experimental variables could be tested with the sample sizes allotted per treatment.

During the first month posthatching, 24 hatchlings escaped the enclosures and another 25 with atypical number of scutes in their carapace or plastron were excluded from further analysis. Three hatchlings died from unknown causes and 62 were stolen at 16 months of age (the latter were included in all analyses up to that age). Thus, 285 out of the 334 individuals that hatched started the posthatching experiment, and 222 juveniles reached the end of the experiment (25 months), and were released into the Orinoco river as part of FUDECI's head-start conservation program.

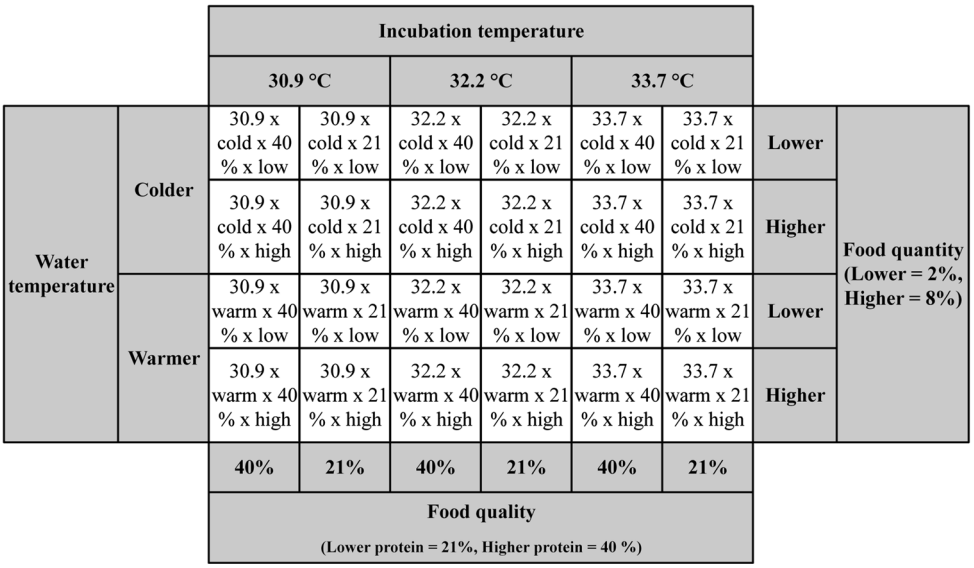


Fig. 1 Experimental design used in this study. Hatchlings from three incubation temperatures were distributed among 24 treatments generated by the combination of three variables (water temperature, food quality and food quantity). Food quantity = percent of body weight

Shape and Size Quantification

Carapace and plastron growth was monitored because these components may exhibit different levels of variation and ontogenetic trajectories among habitats and between sexes (Ceballos and Valenzuela 2011; Rivera 2008; Mosimann and Bider 1960). Size and shape were monitored at hatching and every 4 months thereafter until 25 months of age. The carapace and plastron of each individual were photographed using an Olympus SP-500 UZ digital camera, and a metric tape was included for scaling. A geometric morphometric approach was followed to quantify shell morphology and size, to estimate hatchling sex (Valenzuela et al. 2004), and also to assess the effect of sex-specific phenotypic plasticity (if present) on shape. For this purpose, 29 and 21 fixed landmarks were digitized on the carapace and plastron respectively (Fig. 2). Landmarks were subjected to a Generalized Procrustes Analysis (GPA) which superimposes all configurations of landmarks to a common coordinate system while holding mathematically-constant the effects of position, orientation and scale (Rohlf and Slice 1990). We then obtained a set of multivariate shape variables (partial warp scores and uniform components), as well as a centroid size, the average of the distances from each landmark to its center of gravity. Because centroid size contains the information on carapace and plastron size of each individual, it was used as a surrogate of carapace and plastron size (Bookstein 1991). To estimate individual sex, 7 additional fixed landmarks and 12 sliding landmarks were digitized along the anal notch on the posterior edge of the plastron (Fig. 2), a sexually dimorphic region in adults (Pritchard and Trebbau 1984). Fixed landmarks are positioned on repeatable anatomical points (intersection of scutes), and are not allowed to move during the GPA. Sliding landmarks are digitized anywhere along the curve contour of the plastron, and are allowed to move between adjacent landmarks such that they can capture better the shape of curved lines (Bookstein 1997). After digitizing the landmarks, three independent GPA's were performed, one for each set of carapace, plastron and anal notch landmarks. Shape variables (54 for the carapace, 38 for the plastron, and 34 for the anal notch), and a centroid size variable (1 for each body part) were obtained from each analysis. All geometric morphometric analyses were performed using TpsDig, TpsRelW, TpsUtil software (Rohlf 2001, 2003).

Sexing Technique

Individuals were sexed using a geometric morphometric approach based on the shape of the anal notch (modified from Valenzuela et al. 2004). First, a two-factor MANOVA was used to assess if significant differences existed in the shape of the anal notch of males and females in a sample of 92 individuals for which sex was determined by gonadal inspection under a 40×-dissecting microscope. If significant, we then used discriminant function analysis on 80 % of these individuals using the shape variables as independent variables to obtain the maximal discrimination between the sexes. For cross-validation, the sex of the remaining 20 % of the individuals was estimated using the discriminant function (see Valenzuela et al. 2004). The function analysis and cross-validation were repeated at 7 days, and at 5, 9, 13, 17, 21, and 25 months of age.

Data Analysis

We first determined if plasticity of body size existed in *P. expansa* and whether it was sex-specific. Secondarily, we determined whether the size plasticity of males was greater

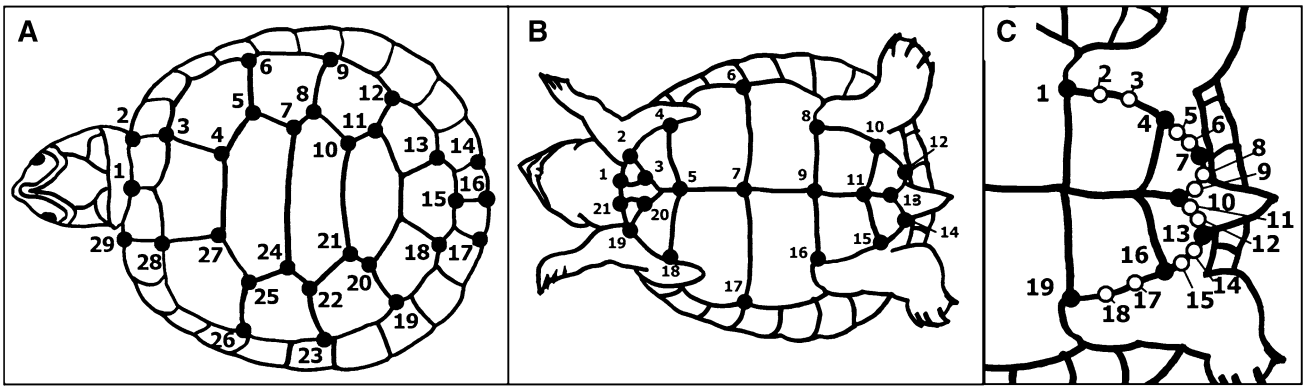


Fig. 2 Landmark location on the carapace (*left*), plastron (*center*), and anal notch (*right*) of hatchlings. *Solid circles* indicate fixed landmarks and *open circles* indicate sliding landmarks (see text for details)

(Pm > Pf), lesser (Pm < Pf), or equal (Pm = Pf) to that of females. For this purpose we performed several univariate and multivariate analyses of variance (Sokal and Rohlf 1995). Potential maternal effects were accounted for by including egg weight (transformed to the cubic root of its natural log) as a covariate in all models (Valenzuela 2001b; Ceballos and Valenzuela 2011). The main factors (independent variables) were considered in the full model in the following order: incubation temperature, water temperature, food quality and food quantity, plus all their two-, three-, four-, and five-way interactions. Size was treated as the response or dependent variable. We used two models in R software v. 2.9.1: a linear model (lm in package stats) (R_Development_Core_Team 2010) and a mixed effects model (lme in package nlme) (Pinheiro and Bates. 2000) which treats the environmental variables as fixed effects, and clutch as a random effect (Valenzuela 2001b; Rhen and Lang 1995). Models were run at all ages independently (7 days, 5, 9, 13, 17, 21, and 25 months of age). The same procedure was applied to the analysis of shape, treating carapace or plastron shape data as response variables.

Second, we tested for the presence of SSD and SShD using analyses of variance with sex as the main factor, and size and shape of carapace and plastron as the response variable. Third, to determine if one sex exhibits greater plasticity than the other requires finding a significant interaction between sex and environment (scenarios IV *a–d* in Fig. S1), plus a significant difference between males and females in the magnitude of change in size or of the component of shape across two environments (Collyer and Adams 2007; Ceballos and Valenzuela 2011). Thus, we tested for interactions between sex and all the environmental factors (e.g., incubation temperature, water temperature, and food variables), and tested for significant differences in the slopes of the reaction norm (Ceballos and Valenzuela 2011; Sokal and Rohlf 1995).

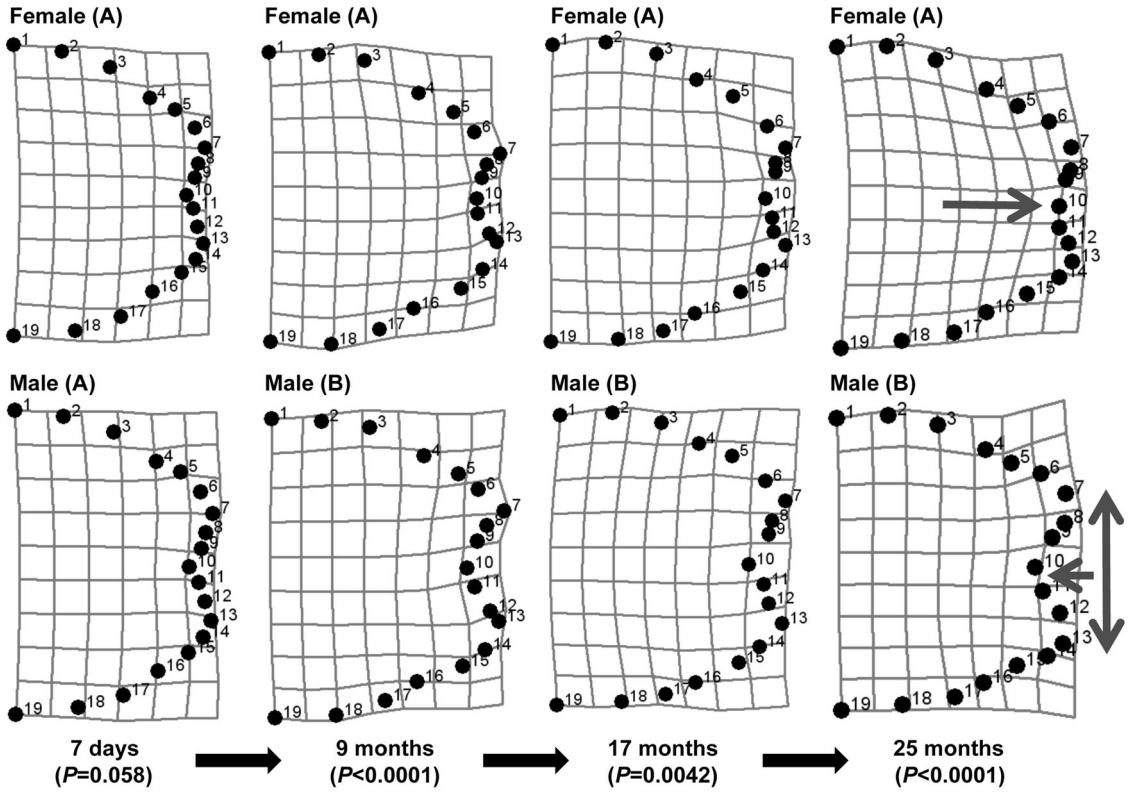
Fourth, when significant interactions between environmental factors existed, post hoc pairwise comparisons were performed using a residual randomization procedure (Collyer and Adams 2007; Adams and Collyer 2009). This procedure allows testing for the joint effect of the factors by holding constant the residuals of the main factors while randomizing the residuals of the interaction. Significance was assessed with Bonferroni correction for multiple comparisons (Sokal and Rohlf 1995).

Finally, to visualize differences in growth due to sexual dimorphism or phenotypic plasticity, shapes of each group were depicted using thin-plate spline (TPS) deformation grids from the overall average shape to the average shape of each group (Ceballos and Valenzuela 2011) using TpsSpline software (Rohlf 2001, 2003).

Results

Sexing and Sex Ratios

Starting at 5 months of age, the anal notch of the plastron became increasingly sexually dimorphic (deeper in males than in females), and by 25 months it could be used as a reliable diagnostic trait to assess individual sex (Fig. 3). Indeed, at 25 months the dimorphism of the anal notch permitted the highest discrimination between males and females (98.9 %), the highest correct classification rates to sex for individuals with known sex (99.4 %), and the highest cross-validation with individuals of known sex (75 %) compared to all other ages examined (classification rates for some exemplary ages are shown in Fig. 3). Using this sexing method, we estimated that the sex ratios (% male) of hatchlings at the beginning of the common garden experiment (n = 285) were: 95 % from 30.9 °C, 88 % from 32.2 °C, and 22 % from 33.7 °C (Table 1). The sex ratios of juveniles at the end of the study (n = 222) remained very similar to the sex ratios at hatching (Table 1). Clutch size, egg weight, incubation time, incubation temperature, and hatching success are summarized in Table 1.



	7 days	9 months	17 months	25 months
Sexually dimorphic?	No	Yes	Yes	Yes
Sex discrimination?	n/a	94.60 %	91.20 %	98.90 %
Correct classification	n/a	96.80 %	94.10 %	99.40 %
Cross-validation	n/a	72.20 %	74.50 %	75.00 %

Fig. 3 Thin-plate spline deformation grids illustrating the sexual dimorphism of the plastral anal notch during 2 years posthatching, and evaluation of its discriminant power to assess individual sex. Grids are magnified ×3 for visualization purposes. *Gray arrows* indicate the direction of deformation from the overall average shape to the group mean per sex and age. *Different letters* for males and females (*A*, *B*) indicate significant sexual dimorphism. The head is located on the *left* as in Fig. 2

Maternal and Environmental Effects

The effects of egg weight, incubation temperature, food quality and food quantity on carapace and plastron shape and size varied across the ages evaluated (Tables 2, 3, 4) as described below. Heavier eggs produced larger and heavier hatchlings ($r = 0.7597$, slope $= 0.6045$, $P < 0.0001$, $n = 334$). The effect of egg weight on body size lasted from hatching until the 5th month of age, while the effect on shape was present from hatching until the end of the study. Lighter eggs produced a shorter carapace with narrower shoulders and wider anal region; while heavier eggs produced a more elongated carapace with wider shoulders and narrower anal region. Egg weight had the opposite effect on plastron shape. Namely, lighter eggs produced a more elongated and narrow plastron, while heavier eggs produced shorter and wider plastrons.

As with egg weight, the influence of incubation temperature was longer-lasting and stronger on body shape than on size. This incubation temperature effect was independent of sex as the interaction between sex and incubation temperature on shape or size was not significant. In terms of size, warmer incubation produced larger individuals relative to the intermediate and lower temperatures. However, this effect was intermittent for both carapace and plastron (significance of pairwise comparisons in Table 2). In terms of shape, the coldest incubation produced a relatively shorter carapace with a wider pectoral region, while the highest temperature produced an elongated carapace with a widened anal region (Table 3; Fig. S2), and the intermediate treatment produced the widest carapace overall. The effect of incubation temperature on plastron shape (Table 4) was similar to that on carapace shape, but even more pronounced and longer-lasting (pairwise comparisons in Tables 3, 4; Fig. S2).

Table 2 ANCOVA tests of the influence of sex, environment and maternal effects on the size of the carapace and plastron overtime

Model by age	Carapace size				Plastron size			
	df	*F*	*P*	Pairwise comparisons and group means	*df*	*F*	*P*	Pairwise comparisons and group means
7 days								
Sex	1	79	<0.0001	Female = 108.20 (A)	1	28.4	<0.0001	Female = 93.31 (A)
				Male = 107.10 (B)				Male = 92.17 (B)
IncT°	2	65	0.0015	30.9 °C = 106.57 (A)	2	13.63	<0.0001	30.9 °C = 92.22 °C (A)
				32.2 °C = 107.63 (B)				32.2 °C = 91.92 °C (A)
				33.7 °C = 108.26 (B)				33.7 °C = 93.64 °C (B)
EggWeight	1	916	<0.0001		1	183.55	<0.0001	
Residuals	279				291			
5 months								
WaterT°	1	545	0.0036	Warm = 145.71 (A)	1	10.78	0.0012	Warm = 122.5 (A)
				Cold = 142.93 (B)				Cold = 119.8 (B)
FoodQl					1	0.6	0.4401	
FoodQtt	1	795	0.0005	HQtt = 145.93 (A)	1	12.73	0.0004	HQtt = 122.5 (A)
				LQtt = 142.67 (B)				LQtt = 119.8 (B)
EggWeight	1	1,112	<0.0001		1	18.89	<0.0001	
FoodQl × FoodQtt					1	17	<0.0001	HQl–HQtt = 125.04 (A)
								LQl–HQtt = 120.65 (B)
								HQl–LQtt = 118.62 (B)
Residuals	281				288			
9 months								
IncT°					2	4.96	0.0076	30.9 °C = 158.5 (A)
								32.2 °C = 156.6 (A)
								33.7 °C = 162.5 (B)
WaterT°	1	11,169	<0.0001	Warm = 199.86 (A)	1	11.65	0.0007	Warm = 162 (A)
				Cold = 187.24 (B)				Cold = 156.7 (B)
FoodQl	1	943	0.0087	HQl = 194.57 (A)	1	3.49	0.0629	HQl = 160.7 (A)
				LQl = 191.47 (B)				LQl = 157.8 (B)
FoodQtt	1	3,342	<0.0001	HQtt = 196.33 (A)	1	50.32	<0.0001	HQtt = 164.8 (A)
				LQtt = 190.04 (B)				LQtt = 154.1 (B)
FoodQl × FoodQtt					1	16.86	<0.0001	HQl–HQtt = 170.83 (A)
								LQl–HQtt = 160.34 (B)
								HQl–LQtt = 153.01 (B)
Residuals	279				285			
13 months								
Sex	1	1,339	0.0376	Female = 233.78 (A)	1	5.07	0.025	Female = 196.6 (A)
				Male = 229.23 (B)				Male = 191.7 (B)
IncT°	2	2,505	0.0179	30.9 °C = 229.99 (A)	2	4.58	0.011	30.9 °C = 192.3 (AB)
				32.2 °C = 227.02 (A)				32.2 °C = 189.6 (A)
				33.7 °C = 235.86 (A)				33.7 °C = 198.8 (B)
WaterT°	1	11,225	<0.0001	Warm = 237.64 (A)	1	31.4	<0.0001	Warm = 199.6 (A)
				Cold = 225.22 (B)				Cold = 188.2 (B)
FoodQl	1	6,583	<0.0001	HQl = 235.82 (A)	1	6.99	0.0087	HQl = 196.2 (A)
				LQl = 226.30 (B)				LQl = 191.1 (B)
FoodQtt	1	28,500	<0.0001	HQtt = 241.17 (A)	1	69.96	<0.0001	HQtt = 202.1 (A)
				LQtt = 221.93 (B)				LQtt = 185.8 (B)

Table 2 continued

Model by age	Carapace size				Plastron size			
	df	*F*	*P*	Pairwise comparisons and group means	*df*	*F*	*P*	Pairwise comparisons and group means
FoodQl × FoodQtt	1	6,414	<0.0001	HQl–HQtt = 252.71 (A) HQl–LQtt = 222.7 (B) LQl–HQtt = 231.72 (B) LQl–LQtt = 221.16 (AB)	1	25.66	<0.0001	HQl–HQtt = 212.19 (A) LQl–HQtt = 194.67 (B) HQl–LQtt = 184.35 (B)
Residuals	275				287			
17 months								
Sex					1	5.95	0.0153	Female = 237.8 (A) Male = 231.8 (B)
IncT°	2	6,123	0.0007	30.9 °C = 266.49 (A) 32.2 °C = 263.13 (A) 33.7 °C = 274.32 (B)	2	5.92	0.003	30.9 °C = 232.4 (AB) 32.2 °C = 229.1 (A) 33.7 °C = 240.8 (B)
WaterT°	1	23,471	<0.0001	Warm = 277.86 (A) Cold = 259.63 (B)	1	34.11	<0.0001	Warm = 241.1 (A) Cold = 227.6 (B)
FoodQl	1	5,414	0.0003	HQl = 272.26 (A) LQl = 263.71 (B)	1	12.25	0.0005	HQl = 230.2 (A) LQl = 238.2 (B)
FoodQtt	1	67,099	<0.0001	HQtt = 284.11 (A) LQtt = 253.93 (B)	1	130.81	<0.0001	HQtt = 247.6 (A) LQtt = 221.8 (B)
FoodQl × FoodQtt	1	11,423	<0.0001	HQl–HQtt = 296.99 (A) HQl–LQtt = 253.06 (B) LQl–HQtt = 273.25 (B) LQl–LQtt = 254.8 (AB)	1	40.62	<0.0001	HQl–HQtt = 262.93 (A) LQl–HQtt = 236.41 (B) HQl–LQtt = 220.22 (B)
Residuals	273				283			
21 months								
IncT°	2	3,908	0.0214	30.9 °C = 286.06 (AB) 32.2 °C = 285.61 (A) 33.7 °C = 294.78 (B)	2	6.62	0.0016	30.9 °C = 248.9 (A) 32.2 °C = 248.2 (A) 33.7 °C = 260.4 (B)
WaterT°	1	4,720	0.0024	Warm = 293.83 (A) Cold = 285.04 (B)	1	4.39	0.0373	Warm = 255.6 (A) Cold = 250 (B)
FoodQl	1	3,478	0.0089	HQl = 292.15 (A) LQl = 286.47 (B)	1	7.01	0.0087	HQl = 256.4 (A) LQl = 249.8 (B)
FoodQtt	1	48,874	<0.0001	HQtt = 306.13 (A) LQtt = 276.47 (B)	1	63.7	<0.0001	HQtt = 266.3 (A) LQtt = 242.1 (B)
FoodQl × FoodQtt	1	6,079	0.0006	HQl–HQtt = 296.99 (A) HQl–LQtt = 253.06 (B) LQl–HQtt = 273.25 (B) LQl–LQtt = 254.8 (AB)	1	16.44	<0.0001	HQl–HQtt = 284.37 (A) LQl–HQtt = 258.34 (B) HQl–LQtt = 242.15 (B)
Residuals	217				225			
25 months								
WaterT°	1	6,482	0.003	Warm = 321 (A) Cold = 309.97 (B)	1	7.94	0.0053	Warm = 282.5 (A) Cold = 272 (B)
FoodQl	1	1,809	0.1139	HQl = 316.28 (A) LQl = 313.2 (B)				
FoodQtt	1	19,795	<0.0001	HQtt = 325.66 (A) LQtt = 306.56 (B)	1	18.86	<0.0001	HQtt = 285.9 (A) LQtt = 269.3 (B)

Table 2 continued

Model by age	Carapace size				Plastron size			
	df	*F*	*P*	Pairwise comparisons and group means	*df*	*F*	*P*	Pairwise comparisons and group means
FoodQl × FoodQtt	1	12,515	<0.0001	HQl–HQtt = 341.67 (A) HQl–LQtt = 302.67 (B) LQl–HQtt = 317.78 (B) LQl–LQtt = 309.47 (AB)				
Residuals	217				228			

All 2nd to 5th order interactions were tested, and non-significant interactions were removed from the model, as well as any significant terms for which post hoc pairwise comparisons were not significant at Bonferroni-corrected-α. Thus, the final models presented here include exclusively significant factors and interactions (with the single exception of when a higher level interaction was significant which requires all factors to be included in the model even if non-significant)

EggWeight egg weight, *IncTemp* incubation temperature, *WaterTemp* water temperature, *Warm* warmer water temperature, *Cold* colder water temperature, *FoodQtt* food quantity, *FoodQl* food quality, *HQtt* higher food quantity, *LQtt* lower food quantity, *HQl* higher food quality, *LQl* lower food quality, *df* degrees of freedom, *num* numerator, *den* denominator

Water temperature post-hatching significantly affected body size and shape (Tables 2, 3, 4). Animals in warmer water grew larger, more elongated, and had a thinner carapace and plastron compared to the colder-water treatment (Fig. S3).

Food availability also affected body size and shape but in a more complex manner (Tables 2, 3, 4). Individuals reared under the high food quantity regime grew a larger carapace and plastron, and developed a more elongated shape than individuals in the scarcer food regime (Fig. S3). Food quality had a similar effect. Individuals consuming higher protein developed a more elongated carapace and plastron and grew larger in size, than individuals under lower protein (Fig. S3). However, these effects were not permanent. While effects on carapace shape were statistically significant through the second year of age, those on plastron shape disappeared and reappeared intermittently during the second year.

All food effects were independent of sex as no interaction between sex and food quality or food quantity was detected. However, the interaction between food quality and food quantity had a significant effect on shape and size at some ages (Tables 2, 3, 4). Namely, when food was abundant the high protein diet increased carapace and plastron size, but when food was scarce food-quality had no effect (Fig. S4). Regarding shape, individuals in the higher food quality and quantity diet were more elongated compared to individuals in the lower quality and quantity diet (Fig. S5). A significant interaction was also detected between food quantity and water temperature on carapace shape at 17 months of age. Specifically, individuals from colder water and fed more food were more elongated with a flared posterior edge, while those from colder water but fed less food had a wider carapace and were caudally constrained (Fig. S5).

Interestingly, during the first year of life shape changed disproportionately more than size, while during the second year size changed more than shape (Fig. S6). This ontogenetic effect on allometry was stronger on the plastron than on the carapace (Fig. S6).

Sexual Size and Shape Dimorphism

SSD and SShD were evident in carapace and plastron from hatching through the end of the study (Tables 2, 3, 4). Female hatchlings had a larger and more elongated carapace, with a narrower mid region (Fig. 4), and wider anal region. Contrastingly, males exhibited a shorter carapace, with a wider pectoral region, wider mid region, and narrower anal region. Such SShD changed slowly with age as individuals progressed towards the male and female adult morphology overtime. By 25 months female had more elongated carapaces with a compressed anal region, and males were wider with a flared anal region (Fig. 4). Similar to incubation temperature, SShD was more evident in the plastron than in the carapace. Female plastron was larger and wider in its anterior regions, but pointier in the anal region rendering the anal notch small and shallow. In males, the pectoral region of the plastron was not as developed as in females, but the anal region was relatively wider and deeper than in females.

Sex-Specific Plasticity

Overall, *P. expansa* showed high growth plasticity to all environmental factors, and high sexual shape and size

Table 3 MANCOVA tests of the influence of sex, environment and maternal effects on carapace shape at different ages

Model by age	Carapace shape					
	df	Wilks'	*F*	*df* num, den	*P*	Pairwise comparisons
7 days						
Sex	1	0.4066	6.1343	54, 227	<0.0001	
IncT°	2	0.2592	4.0539	108, 454	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0062$)
						30.9 °C ≠ 33.7 °C ($P = 0.0003$)
						32.2 °C ≠ 33.7 °C ($P = 0.0064$)
EggWeight	1	0.4494	5.1501	54, 227	<0.0001	
Residuals	280					
5 months						
Sex	1	0.4606	4.8802	54, 225	<0.0001	
IncT°	2	0.3126	3.2852	108, 450	<0.0001	30.9 °C = 32.2 °C ($P = 0.0675$)
						30.9 °C ≠ 33.7 °C ($P = 0.0003$)
						32.2 °C ≠ 33.7 °C ($P = 0.0007$)
WaterT°	1	0.608	2.6866	54, 225	<0.0001	
FoodQtt	1	0.6407	2.3362	54, 225	<0.0001	
EggWeight	1	0.4892	4.3513	54, 225	<0.0001	
Residuals	278					
9 months						
Sex	1	0.527	3.706	54, 223	<0.0001	
IncT°	2	0.3505	2.8457	108, 446	<0.0001	30.9 °C = 32.2 °C ($P = 0.0969$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0016$)
WaterT°	1	0.5263	3.7173	54, 223	<0.0001	
FoodQtt	1	0.6646	2.0836	54, 223	0.0001	
EggWeight	1	0.4717	4.6252	54, 223	<0.0001	
Residuals	276					
13 months						
Sex	1	0.5562	3.2806	54, 222	<0.0001	
IncT°	2	0.4071	2.3319	108, 444	<0.0001	30.9 °C = 32.2 °C ($P = 0.0745$)
						30.9 °C ≠ 33.7 °C ($P = 0.0004$)
						32.2 °C ≠ 33.7 °C ($P = 0.0117$)
WaterT°	1	0.5374	3.5395	54, 222	<0.0001	
FoodQl	1	0.6007	2.7333	54, 222	<0.0001	
FoodQtt	1	0.6125	2.6009	54, 222	<0.0001	
EggWeight	1	0.5024	4.072	54, 222	<0.0001	
Residuals	275					
17 months						
Sex	1	0.4751	4.4392	54, 217	<0.0001	
IncT°	2	0.3536	2.7398	108, 434	<0.0001	30.9 °C = 32.2 °C ($P = 0.0360$)
						30.9 °C ≠ 33.7 °C ($P = 0.0003$)
						32.2 °C ≠ 33.7 °C ($P = 0.0024$)
WaterT°	1	0.4494	4.9227	54, 217	<0.0001	
FoodQl	1	0.5604	3.1529	54, 217	<0.0001	
FoodQtt	1	0.5374	3.4593	54, 217	<0.0001	
EggWeight	1	0.4577	4.7604	54, 217	<0.0001	
WaterT° × FoodQtt	1	0.7335	1.4598	54, 217	0.0312	Cold–LQtt ≠ Cold–HQtt ($P = 0.0068$)
FoodQl × FoodQtt	1	0.6626	2.046	54, 217	0.0002	HQl–HQtt ≠ LQl–HQtt ($P = 0.0057$)
Residuals	270					

Table 3 continued

Model by age	Carapace shape					
	df	Wilks'	*F*	*df* num, den	*P*	Pairwise comparisons
21 months						
Sex	1	0.5076	2.9279	54, 163	<0.0001	
IncT°	2	0.2957	2.5326	108, 326	<0.0001	30.9 °C = 32.2 °C ($P = 0.0326$)
						30.9 °C ≠ 33.7 °C ($P = 0.0029$)
						32.2 °C = 33.7 °C ($P = 0.0217$)
WaterT°	1	0.4868	3.1818	54, 163	<0.0001	
FoodQl	1	0.6322	1.7557	54, 163	0.0038	
FoodQtt	1	0.5417	2.5534	54, 163	<0.0001	
EggWeight	1	0.5155	2.8369	54, 163	<0.0001	
Residuals	216					
25 months						
Sex	1	0.4627	3.4628	54, 161	<0.0001	
IncT°	2	0.306	2.4085	108, 322	<0.0001	30.9 °C = 32.2 °C ($P = 0.0745$)
						30.9 °C ≠ 33.7 °C ($P = 0.0230$)
						32.2 °C ≠ 33.7 °C ($P = 0.0221$)
WaterT°	1	0.4052	4.3774	54, 161	<0.0001	
FoodQl	1	0.5183	2.7707	54, 161	<0.0001	
FoodQtt	1	0.4362	3.854	54, 161	<0.0001	
EggWeight	1	0.5009	2.9713	54, 161	<0.0001	
Residuals	214					

Model factors and abbreviations as described in Table 2

Table 4 MANCOVA tests of the influence of sex, environment and maternal effects on plastron shape at different ages

Model by age	Plastron shape					
	df	Wilks'	*F*	*df* num, den	*P*	Pairwise comparisons
7 days						
Sex	1	0.457	7.932	38, 254	<0.0001	
IncT°	2	0.286	5.822	76, 508	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0001$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0038$)
EggWeight	1	0.415	9.424	38, 254	<0.0001	
Residuals	291					
5 months						
Sex	1	0.379	10.753	38, 249	<0.0001	
IncT°	2	0.243	6.733	76, 498	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0001$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0021$)
WaterT°	1	0.741	2.288	38, 249	<0.0001	
FoodQl	1	0.791	1.727	38, 249	<0.0077	
FoodQtt	1	0.797	1.668	38, 249	<0.0119	
EggWeight	1	0.473	7.301	38, 249	<0.0001	
Residuals	286					

Table 4 continued

Model by age	Plastron shape					
	df	Wilks'	*F*	*df* num, den	*P*	Pairwise comparisons
9 months						
Sex	1	0.389	10.194	38, 247	<0.0001	
IncT°	2	0.329	4.835	76, 494	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0001$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0013$)
WaterT°	1	0.642	3.625	38, 247	<0.0001	
FoodQl	1	0.817	1.455	38, 247	0.0497	
FoodQtt	1	0.679	3.075	38, 247	<0.0001	
EggWeight	1	0.467	7.411	38, 247	<0.0001	
Residuals	284					
13 months						
Sex	1	0.316	14.163	38, 249	<0.0001	
IncT°	2	0.333	4.812	76, 498	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0001$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0032$)
WaterT°	1	0.586	4.627	38, 249	<0.0001	
FoodQl	1	0.807	1.570	38, 249	0.0234	
FoodQtt	1	0.663	3.330	38, 249	<0.0001	
EggWeight	1	0.469	7.414	38, 249	<0.0001	
FoodQl × FoodQtt	1	0.757	2.099	38, 249	<0.0005	HQl–HQtt ≠ LQl–LQtt ($P = 0.0027$)
Residuals	286					
17 months						
Sex	1	0.322	13.564	38, 245	<0.0001	
IncT°	2	0.320	4.957	76, 490	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0001$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0006$)
WaterT°	1	0.667	3.226	38, 245	<0.0001	
FoodQl	1	0.770	1.929	38, 245	0.0017	
FoodQtt	1	0.590	4.481	38, 245	<0.0001	
EggWeight	1	0.484	6.882	38, 245	<0.0001	
FoodQl × FoodQtt	1	0.684	2.985	38, 245	<0.0001	HQl–HQtt ≠ LQl–Htt ($P = 0.0001$)
						HQl–HQtt ≠ HQl–LQtt ($P = 0.0001$)
Residuals	282					
21 months						
Sex	1	0.354	9.017	38, 188	<0.0001	
IncT°	2	0.369	3.199	76, 376	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0002$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0016$)
WaterT°	1	0.649	2.676	38, 188	<0.0001	
FoodQtt	1	0.676	2.368	38, 188	<0.0001	
EggWeight	1	0.483	5.303	38, 188	<0.0001	
Residuals	225					
25 months						
Sex	1	0.288	12.099	38, 186	<0.0001	

Table 4 continued

Model by age	Plastron shape					
	df	Wilks'	*F*	*df* num, den	*P*	Pairwise comparisons
IncT°	2	0.316	3.813	76, 372	<0.0001	30.9 °C ≠ 32.2 °C ($P = 0.0001$)
						30.9 °C ≠ 33.7 °C ($P = 0.0001$)
						32.2 °C ≠ 33.7 °C ($P = 0.0009$)
WaterT°	1	0.700	2.103	38, 186	<0.0007	
FoodQl	1	0.757	1.571	38, 186	0.0267	
FoodQtt	1	0.569	3.702	38, 186	<0.0001	
EggWeight	1	0.514	4.633	38, 186	<0.0001	
Residuals	223					

Model factors and abbreviations as described in Table 2

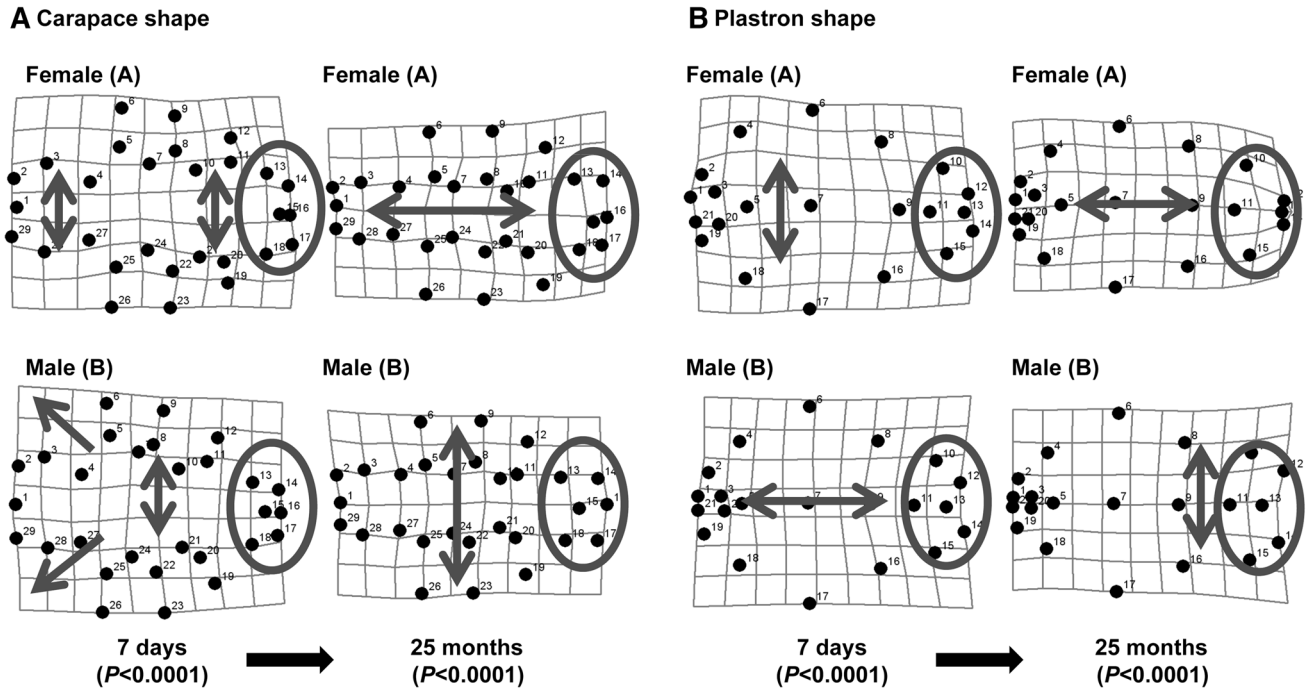


Fig. 4 Thin-plate spline deformation grids illustrating the sexual dimorphism of the carapace and plastron shape of *P. expansa* (n = 100 females, 186 males) at 7 days and 25 months of age. *Magnification*, *symbols* and *letters* are as in Fig. 3

dimorphism in the carapace and plastron at almost all ages evaluated, yet this plasticity was not sex-specific as no interaction between sex and any environmental variable was found. This finding implies that the plasticity of males and females was similar for size and shape (Pm = Pf) (Scenario III in Fig. S1). Thus, no evidence was found that differential plasticity is a mechanism underlying SSD or SShD in *P. expansa*.

Discussion

Sex-Specific Plasticity Does Not Explain SSD or SShD

Differential phenotypic plasticity between males and females is purportedly an important mechanism affecting SSD (Bonduriansky 2007; Fairbairn 2005; Ceballos and Valenzuela 2011) but its role in shaping interspecific

differences in long-lived vertebrates remains unclear. Here we studied the plasticity in size and shape of the female-larger turtle *P. expansa*, to test if plasticity is: (a) an enhancer of male size, or (b) an enhancer of body size of the larger sex. We found that *P. expansa* exhibits a highly plastic response of body size and shape to temperature and to resource availability and quality. Additionally, *P. expansa* showed a high level of sexual dimorphism in the carapace and plastron that became more pronounced and less variable with age. Notably however, the patterns of plasticity across environments were the same in males and females (Pm = Pf), in contrast to previous findings in the male-larger turtle *C. serpentina* (Pm > Pf) (Ceballos and Valenzuela 2011) under a similar experimental design. The lack of differential plasticity in *P. expansa* indicates that the *condition dependent* hypothesis (Bonduriansky 2007) does not explain SSD in this species because this model implies that phenotypic plasticity enhances growth and that the larger sex takes greater advantage of this effect by being more plastic. Our data also rule out the *adaptive canalization* hypothesis (Fairbairn 2005) in *P. expansa* because this model implies that phenotypic plasticity inhibits growth and that the larger sex avoids this detrimental effect by being less plastic than the smaller sex. Consequently, our findings have important implications at the macroevolutionary level as they indicate that differential plasticity does not favor males inherently nor the larger sex in turtles, yet general effects across taxa should be expected if differential plasticity were an important mechanism shaping patterns across species. Instead, we suggest that differential plasticity is not a pervasive mechanism responsible for shaping interspecific SSD patterns across lineages.

The divergent pattern of differential plasticity between *P. expansa* and *C. serpentina* suggests that different forces have shaped SSD in these two lineages. Consistently, the macroevolutionary patterns of co-variation between body size and SSD are opposite in these two families. In particular, in Chelydridae (to which *Chelydra* belongs) SSD is accentuated with body size consistent with Rensch's rule, while Podocnemididae (to which *Podocnemis* belongs) is the only chelonian family whose pattern is opposite to Rensch's rule (Ceballos et al. 2013). These observations support the notion that Chelydridae takes advantage of an enhancing effect of differential plasticity on male size, and that Podocnemididae circumvents the negative effect that such enhancing effect on males would have for female-biased SSD by not displaying differential plasticity altogether. Thus, we propose that differential plasticity when present, enhances male size and thus it contributes to the evolution of patterns consistent with Rensch's rule. Such differential plasticity must therefore be absent in lineages that evolve patterns opposite to Rensch's rule. Further studies in additional taxa from lineages that display positive and negative co-variation of body size and SSD are needed to test this hypothesis.

We also found no differences in the plasticity of shape between males and females in *P. expansa*, indicating that sex-specific selective pressures must be responsible for the marked SShD present in this species, rather than being the result of differential responses to the same drivers (Bonduriansky 2007; Fairbairn 2005). Our results are consistent with macroevolutionary analyses indicating that fecundity selection on females and sexual or ecological selection on males are important drivers of sexual dimorphism in turtles (Ceballos et al. 2013).

Ontogeny of Sexual Size and Shape Dimorphism

The development of SSD in *P. expansa* occurred intermittently early in life (at 7 days, 13 and 17 months of age), while SShD was pervasive from hatching. Therefore, the pronounced SSD present in adult *P. expansa* is not entirely the result of differential growth trajectories of males and females during early life, but must develop at a more advanced age. Our study comprised over 2 years, which represent ~18–29 % of the juvenile period of *P. expansa*, a species that matures at 7–11 years of age (Mogollones et al. 2010; Soini 1997). Such delay in the development of SSD is concordant with observations in *C. serpentina* where males and females exhibit similar growth trajectories in the first years of life and a differential growth decline thereafter (Christiansen and Burken 1979). However, because captive conditions can affect growth rates between sexes (John-Alder et al. 2007; Taylor and Denardo 2005) we cannot rule out completely the possibility (albeit unlikely) that size differences between males and females due to differential growth exist in natural populations of *P. expansa* during the first 2 years of life.

On the other hand, and while several turtle species exhibit SSD and SShD at hatching (e.g. Ceballos and Valenzuela 2011; Valenzuela et al. 2004; Myers et al. 2007), the adaptive significance of SShD at these early life stages is unknown. Certain body size or shape may be linked to traits that increase survivorship, but no sex-specific advantages of such traits have been reported. For example, larger hatchlings may survive better than smaller ones (Janzen 1993), although other studies have detected no survival differences by size in hatchlings or juveniles of the same species before reaching maturity (Congdon et al. 1999). Likewise, hatchlings with shorter and wider plastron may swim faster (Myers et al. 2007). In our study, as hatchlings aged, their SShD changed slowly to resemble more the adult morphology (Pritchard and Trebbau 1984). While the adult SShD is likely adaptive as it may increase female fecundity and male mating ability (Kaddour et al.

2008), the SShD detected in our study may simply be a precursor of such adult SShD without conferring an advantage at this early life stage.

The difference in the development of SSD and SShD was also reflected in the ontogenetic allometry (Fig. S6) because shape changed faster than size during the first year and slower in the second year. This pattern was stronger in the plastron as the allometry reached a shape plateau at an earlier age than the carapace. Consequently, for practical applications, the earlier onset of sexual shape dimorphism in the plastron, and particularly around the anal notch, makes this morphological region a better sex-diagnostic trait in *P. expansa* than the carapace (Valenzuela et al. 2004; Lubiana and Ferreira 2009). Whether the allometric growth detected between the plastron and carapace has any adaptive value remains an open question worthy of further study.

Maternal Effects

Maternal energy allocation affected body size significantly, but the effect was not permanent. Heavier eggs yielded a larger carapace and plastron from hatching until 5 months, and this effect disappeared after 9 months when environmental effects on body size became prevalent. Such fading or intermittent maternal effects and the increased environmental influence overtime has been reported for this and other species For instance, protein levels in the diet influenced *P. expansa* weight starting at 8 months of age (Sa et al. 2004). In *C. serpentina* the effect of egg weight on body size was sustained until 8 months of age when it disappeared (Ceballos and Valenzuela 2011). These results indicate that maternal allocation is important for body size during the neonatal stage period, which is estimated as 10 % of the time to maturity (Morafka et al. 2000), or the first 7–10 months of age in *P. expansa* (Mogollones et al. 2010; Soini 1997). The more unpredictable the resource availability is during this period, the stronger would be the importance of this maternal allocation, particularly because early nutrition can have lasting effects in reptiles (Massot and Aragón 2013). Because in *P. expansa*, larger females produce more and larger eggs per clutch (Valenzuela 2001b), our data suggest that females may increase their fitness by producing larger eggs as greater allocation enhances offspring size and growth early in life (this study), as well as hatchling survival (Valenzuela 2001b).

In summary, *P. expansa* displays high plasticity of body size and shape, along with SSD and SShD. However, no difference in body size plasticity between the sexes was detected in *P. expansa*, in direct contrast with our previous observations in *C. serpentina*. These two studies indicate that sex-specific plasticity is species-specific (and perhaps lineage-specific) and does not constitute a pervasive driver of macroevolutionary patterns of sexual dimorphism across vertebrate lineages. However, while the contrasting results from two turtles with opposing patterns of SSD are provocative, more data across a variety of taxa are needed to test the generality of the patterns and processes associated with the evolution of sexual dimorphism. Finally, body shape is equally plastic in males and females in both *P. expansa* and *C. serpentina*. Thus we hypothesize that sex-specific selective pressures drive the marked patterns of SShD present in these species, and are not likely generated from differential responses to the same drivers such as resource availability or quality as examined here.

Acknowledgments We thank R. Espín, F. Torres, and FUDECI staff in Puerto Ayacucho (Venezuela) for their logistic support throughout this study; A. Navarro and A. Siegle from the Iowa Turtle Army from the Valenzuela Lab for helping with the geometric morphometric analyses; P. von Hildebrand and B. Jeffrey from the Fundación Puerto Rastrojo, Colombia, for donating some of the incubators; and D. C. Adams for statistical advise. Funding was provided by: P.E.O. International Peace Scholarship to CC, Turtle Conservation Fund and NSF DDIG DEB-0808047 Grants to NV and CC, NSF IOS 0743284 plus REU and RET supplements to NV, and the EEOB Department at Iowa State University to CC. Work was carried out under scientific permit 609 from the Ministry of the Environment of Venezuela and the IACUC of Iowa State University (5-07-6366-J).

Conflict of interest None.

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