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RESEARCH ARTICLE

The Role of Sex-specific Plasticity in Shaping Sexual Dimorphism in a Long-lived Vertebrate, the Snapping Turtle *Chelydra serpentina*

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Abstract Sex-specific plasticity, the differential response that the genome of males and females may have to different environments, is a mechanism that can affect the degree of sexual dimorphism. Two adaptive hypotheses have been proposed to explain how sex-specific plasticity affects the evolution of sexual size dimorphism. The adaptive canalization hypothesis states that the larger sex exhibits lesser plasticity compared to the smaller sex due to strong directional selection for a large body size, which penalizes individuals attaining sub-optimal body sizes. The condition-dependence hypothesis states that the larger sex exhibits greater plasticity than the smaller sex due to strong directional selection for a large body size favoring a greater sensitivity as an opportunistic mechanism for growth enhancement under favorable conditions. While the relationship between sex-specific plasticity and sexual dimorphism has been studied mainly in invertebrates, its role in long-lived vertebrates has received little attention. In this study we tested the predictions derived from these two hypotheses by comparing the plastic responses of body size and shape of males and females of the snapping turtle (*Chelydra serpentina*) raised under common garden conditions. Body size was plastic, sexually dimorphic, and the plasticity was also sex-specific, with males exhibiting greater body size plasticity relative to females. Because snapping turtle males are larger than females, sexual size dimorphism in this species appears to be driven by an increased plasticity of the larger sex over the smaller sex as predicted by the condition-dependent hypothesis. However, male body size was enhanced under relatively limited resources, in contrast to expectations from this model. Body shape was also plastic and sexually dimorphic, however no sex by environment interaction was found in this case. Instead, plasticity of sexual shape dimorphism seems to evolve in parallel for males and females as both sexes responded similarly to different environments.

Keywords Phenotypic plasticity · Condition-dependence · Adaptive canalization · Sexual dimorphism · *Chelydra serpentina*

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Introduction

Understanding the processes responsible for the evolution of phenotypic patterns is a major question in evolutionary biology. One fundamental phenotypic pattern found across many sexually reproducing species is sexual dimorphism, the differences in size (sexual size dimorphism, SSD) or shape (sexual shape dimorphism, SShD) of conspecific males and females (Fairbairn et al. 2007). In most invertebrates and ectothermic vertebrates (e.g. spiders, insects, amphibians, reptiles) females are larger than males, but the opposite is true in many endotherms (mammals and birds) and some ectotherms (e.g. some reptiles and amphibians: Abouheif and Fairbairn 1997; Fairbairn et al. 2007). Yet, distinguishing the drivers of this widespread variation in sexual dimorphism, or lack thereof, remains a challenge for many species. Selective forces may act on different fitness components resulting in contrasting patterns of sexual dimorphism among taxa. For instance, sexual selection may favor larger males in species where larger body size increases access to females via male–male competition (Berry and Shine 1980), or favor smaller males in species where smaller body increases mobility and consequently

insemination success (Kelly et al. 2008). Fecundity selection may favor larger females in species where clutch or egg size increases with female size thus conferring higher reproductive success to larger females as compared to smaller ones (Andersson 1994; Valenzuela 2001b; Cox and John-Alder 2007). Ecological selection through niche partitioning may favor sex-specific body size optima associated with foraging strategies that enable each sex to exploit the habitat more efficiently (Schoener 1967). Likewise, the degree of sexual dimorphism observed within a species may vary because the particular selective forces driving sexual dimorphism change throughout ontogeny (Andersson 1994), or due to mechanisms such as sex-specific plasticity as observed mainly in insects (Fairbairn 2005; Bonduriansky 2007; Fernández-Montraveta and Moya-Laraño 2007; Stillwell and Fox 2007; Stillwell et al. 2010). For instance, in the female-larger seed beetle (*Callosobruchus maculatus*), a higher rearing temperature enhances female growth (faster mass gain) as compared to male growth, though at the same time, low temperatures result in female-biased sex ratios perhaps due to a non-random larval mortality of males compared to females (Stillwell and Fox 2007).

Two alternative adaptive hypotheses have been proposed to explain how sex-specific plasticity affects the evolution of SSD, each supported by evidence from a few well-studied invertebrates. (1) The *adaptive canalization hypothesis* states that if the larger sex is under strong directional selection for a large body size (e.g. sexual selection for larger males or fecundity selection for larger females), individuals attaining sub-optimal body sizes will be penalized via a fitness reduction (Fairbairn 2005; Stillwell et al. 2010). Under this hypothesis, the larger sex is predicted to show *reduced* sensitivity to the environment relative to the smaller sex, such that the SSD pattern should change across environments. Evidence for this hypothesis comes from studies of two arthropod species. In water striders (*Aquarius remigis*), which exhibit female-biased SSD, females have longer abdomen length compared to males; however, abdomen length is relatively more plastic in males, suggesting it is adaptively canalized in females (Fairbairn 2005). Likewise, female Mediterranean tarantulas (*Lycosa tarantula*) are larger than males but their body size is not affected by improved feeding conditions while male growth is elevated, such that SSD is no longer detected (Fernández-Montraveta and Moya-Laraño 2007). (2) The *condition-dependence hypothesis* states that if the larger sex is under strong directional selection for a large body size, it will exhibit a greater sensitivity to environmental conditions than the smaller sex as an opportunistic strategy for growth enhancement (Bonduriansky 2007). Under this hypothesis the larger sex is predicted to show an *increased* plasticity relative to the smaller sex. Support for this hypothesis is found in insects, albeit different species. In the fly (*Telostylinus angusticollis*), which exhibits male-biased SSD, sexual and non sexual traits do not differ between males and females under a poor diet, but males grow larger under a rich diet (Bonduriansky 2007). Likewise, a survey across 158 insect species revealed that 80% of them exhibit female-biased SSD coupled with higher female-size plasticity, while the remaining 20% possessing male-biased SSD show no consistent pattern (Teder and Tammaru 2005). It should be noted that other studies are equivocal in their support for either hypothesis. For instance rearing temperatures have no effect on sexual size dimorphism in the beetle (*Stator limbatus*) that exhibits male-biased SSD, but population of origin does, suggesting that a stronger genetic component on body size may exist in this species (Stillwell and Fox 2007).

Because most research on this topic has been addressed in particular invertebrates characterized by having relatively small body size, short generation time, and fast developmental rates, it remains uncertain if the same processes affecting SSD in these taxa apply to species with long generation times such as vertebrates, which may be subject to differing selective pressures (Slack 2006; Jenner and Wills 2007). Indeed, the extent of the role that sex-specific growth plasticity plays in the evolution of sexual dimorphism in long-lived vertebrates such as reptiles, remains understudied, although some valuable insight has been gained in this area, mostly from snakes and lizards (Taylor and Denardo 2005; Cox et al. 2006). For instance, rattlesnake females (*Crotalus atrox*) grow heavier than males under a high-intake diet, but there are no differences in body mass when they are under a low-intake diet (Taylor and Denardo 2005). Likewise, wild lizards (*Sceloporus jarrovii*) exhibit male-biased SSD, however when males and females are raised under identical conditions in captivity males decrease their natural growth rates and consequently SSD is not observed (Cox et al. 2006; Cox and John-Alder 2007).

Here we studied body growth in turtles (*Chelydra serpentina*) to test whether plasticity in growth mediates the sexual dimorphism in a long-lived vertebrate, and whether such plasticity is sex-specific. That is, we tested whether the larger sex exhibits an increased, decreased or equal growth plasticity relative to the smaller sex. Snapping turtles are a good model for this study because they exhibit sexual dimorphism in their carapace and plastron (Mosimann and Bider 1960; Christiansen and Burken 1979; Steyermark et al. 2008). Average carapace length in several populations ranges from 25 to 33.5 cm in adult males and from 22 to 32 cm in adult females and the average adult weight is 3.5 kg for males and 2.9 kg for females (White and Murphy 1973; Gibbons and Lovich 1990). In the wild, males grow at a faster rate and mature at a younger age than females (Christiansen and Burken 1979). Namely, males grow at

about 3 cm/year in plastron length until they reached sexual maturity at carapace length (CL) of 19.1–22.3 cm, and plastron length (PL) of 13.5 cm in their 4–5th year (Christiansen and Burken 1979). Growth rate declines gradually after maturity reaching 0.25 cm/year by the 20th year. Females grow at the same rate as males until their 3rd year, but then growth decreases to 0.6–0.7 cm/year. By the 8th year of life, females reach maturity at PL = 16.2 cm, while already mature males have a larger PL = 22.2 cm (Christiansen and Burken 1979). Males continue growing after 35 years, while in females growth is slight beyond 15–20 years of age (Christiansen and Burken 1979). In addition, snapping turtles lay relatively large clutches (26–55 eggs) (Congdon et al. 2008), making them a good model for a plasticity study as it allows allocation of clutch mates to different experimental treatments. Because snapping turtles posses temperature-dependent sex determination (Yntema 1976) we examined the ontogenetic effect of incubation temperature, as well as posthatching water temperature and resource quality and quantity on growth.

In particular, we examined the existence of sexual size and shape dimorphism (SSD and SShD) of the carapace and plastron. Additionally, we explored the existence of sex-specific plasticity in SSD and SShD. With these data we tested the specific predictions from the adaptive canalization and condition-dependence hypotheses, as follows. If growth of the larger sex is adaptively canalized compared to the smaller sex, it would be expected that snapping turtle males exhibit reduced sensitivity to the environment compared to females. Alternatively, if plasticity mediates sexual dimorphism as a growth-enhancing mechanism of the larger sex, then it would be expected that male snapping turtles exhibit increased sensitivity to the environment compared to females. Alternatively, if both sexes respond equally to the environment it would imply that sex-specific plasticity does not mediate sexual dimorphism in this species. In the case of shape, a trait whose changes have two properties, magnitude and direction [sensu (Collyer and Adams 2007)], the same predictions derived from the adaptive canalization and condition-dependence hypotheses apply as for body size, and were tested using the magnitude component of shape changes.

Materials and Methods

Eggs Collection and Incubation

A total of 671 eggs of *Chelydra serpentina* from 0 to 2 day old nests were collected from a turtle farm in Birmingham, Iowa (n = 19 clutches), and the Horticulture Station of Iowa State University in Ames, Iowa (n = 2 clutches). We estimate that clutches were laid by different females because eggs were collected within a 7-day period, giving no time for a female to lay more than one clutch. Eggs were measured, weighed, individually marked, and distributed uniformly but randomly into 3 incubators set at 26°C, 28°C and 31°C, which are viable temperatures expected to produce 100, 50 and 0% males, respectively, based on previous studies (Dimond 1983; Gutzke and Bull 1986; Packard et al. 1987; O'Steen 1998; Steyermark et al. 2008). Egg weight was measured because it is a maternal effect that can affect growth in reptiles (Rhen and Lang 1995; Valenzuela 2001a, b; Zhu et al. 2006). Incubation started within 12 h of egg collection. Eggs were completely buried in plastic boxes 4/5-filled with moist sand set at 4% water content (Lott 1998). Substrate moisture was maintained by replacing lost weight with water once a week to avoid the confounding effect that humidity conditions during incubation can have on hatchling size in this species (Packard et al. 1999; Ackerman et al. 2008). Sand boxes were rotated to avoid any systematic effect of potential thermal clines within incubators. Additionally, substrate temperature was recorded hourly with up to 4 dataloggers per box (Dallas Semiconductor iButton) with 0.5°C precision. Eggs were placed into individual plastic cups upon pipping to follow the identity of each individual. Upon emergence from the shell and after yolk absorption was completed (approximately at 2 weeks of age), hatchlings were uniquely tagged to keep track of individual identity by attaching a combination of colored beads to the posterior edge of the carapace (Galbraith and Brooks 1984).

Incubators fluctuated from their set temperatures, such that the experimental design was modified as follows. The incubator set at 31°C malfunctioned and overheated, resulting in only 15.2% hatching success (Table 1) and all these hatchlings were deformed and died by the 2nd month of age. All these individuals were excluded from the study. The remaining hatchlings obtained from the other two incubators were reassigned to three new experimental groups encompassing relatively even incubation thermal ranges as determined by the temperature recorded inside their individual incubation boxes. The *26°C-bin* group (n = 105 hatchlings) experienced temperatures ranging between 25.63 and 26.33°C, the *27°C-bin* group (n = 74 hatchlings) with temperatures between 26.71 and 27.39°C, and the *28°C-bin* group (n = 73 hatchlings) with temperatures between 27.53 and 28.19°C. We refer to these groups simply as the 26, 27 and 28°C groups hereafter. Hatchlings were distributed evenly and randomly among the posthatching treatments as described below.

Common-garden Experiment and Sexing Procedure

Common garden experiments were conducted at the indoor Aquatic Research Campus Facility at Iowa State

Table 1 Summary statistics of incubation temperatures, hatching success and sex ratios (% male) of *Chelydra serpentina* obtained in this study

Incubator setting (°C)	Actual temp average (°C)	Actual temp range (°C)	Initial egg number	Hatching success % (n)	Sex ratio (% male) (n)	Number of hatchlings with undetermined sex
26	26.03	25.6–26.7	223	68.6% (153)	85% (117)	36
28	27.49	26.9–28.2	222	77.0% (171)	33% (135)	36
31	31.97	27.0–35.5	225	13.8% (31[†])	100% (2)	29
Total			670	53% (355)	(254)	101

Variation in incubation temperatures from set values caused high egg mortality at the 31°C treatment due to overheating and sex ratio deviations from those expected under constant temperature conditions in the other two treatments

[†] All hatchlings from 31°C were deformed, died before 2 months, and were excluded from the study. Hatchlings from the 26°C and 28°C incubation treatments (n = 252) were re-classified into three new experimental groups based on the actual mean temperature they experienced, referred to as 26-, 27- and 28°C-bin (see "Methods" section for details)

University. Individuals from each clutch were randomly assigned to at least 3 of the 8 posthatching treatments (Fig. 1), such that each treatment contained hatchlings from at least 7 clutches. Eight posthatching treatments were set up by combining 3 environmental factors with 2 contrasting conditions each, as follows. (1) Water temperature: colder (20°C) and warmer (25°C), achieved by using a dechlorinated water flow-through system controlled by a thermostat for each temperature (temperatures could vary up to ±1.5°C before triggering an alarm system). At 10 months of age, the warmer water temperature was decreased from 25 to 23°C to reduce observed signs of cannibalism. (2) Food quality: higher (43%) and lower (35%) protein-content diets corresponding to commercial "Hatchling Aquatic Turtle" and "Growth Aquatic Turtle" food from Zoo Med Lab (see list of ingredients and nutritional composition in Appendix). (3) Food quantity: lower and higher food quantities corresponding to 2 and 8% of total body mass respectively. The experiment followed a partially-balanced incomplete block design (Montgomery 1997) such that all experimental variables could be tested with the sample sizes allotted without requiring each clutch to be represented in all treatment combinations.

A total of four 5ft-diameter tanks were used, two of which were set at the colder temperature and the other two at the warmer temperature. All four tanks were divided in half by using a plastic net that separated the high from the low food-quantity treatments within each tank. Turtles were fed 6 days a week after their tanks were vacuum-cleaned. Quantification of the amount of food eaten by each individual was precluded by the use of shared tanks per treatment. However, turtles in the 2% treatment consumed all food provided, while turtles in the 8% treatment ate ad libitum with minimum leftover food observed the following day. Turtles were provided daily with 12 h of UVA/UVB and full spectrum light. The experiment was terminated when turtles were 15.5 months old.

Turtles were sexed by laparoscopy (Kuchling 2006) at approximately 15 months of age using a 2.7-mm-diameter rigid Storz Hopkins 308 endoscope with a battery-operated Storz cold-light source, or by gonadal inspection under a dissecting microscope for individuals that died before the end of the experiment (n = 65). Sex of individuals whose gonads were not fully observable by laparoscopy was confirmed by radioimmunoassay of their circulating testosterone after an FSH challenge (Lance et al. 1992) validated by gonadal inspection of a subset of individuals (concentration of testosterone in females: mean = 0.259 ng/ml, SD = 0.84, n = 54; and in males: mean = 1.372, SD = 1.22, n = 88). The sex ratios (% males) of the 252 hatchlings ($n_{males} = 118$, $n_{females} = 92$) obtained from the 26, 27, and 28°C incubation groups were 94, 45, and 26% respectively. A non-infectious skin condition that produced excessive-shedding afflicted the hatchlings during their second month of age and caused a high mortality across all treatments. By 15.5 months of age sex ratios of the 149 surviving juveniles ($n_{males} = 92$, $n_{females} = 57$) in these groups were 95, 48, and 19% respectively.

Geometric-morphometric Shape and Size Quantification

To quantify carapace and plastron size and shape, individuals were photographed with a digital camera (Olympus SP-500 Ultra Zoom) at 2 weeks after hatching, and every 6 weeks until the end of the experiment at 15.5 months posthatching. A total of 25 landmarks were digitized on the photographs at the intersection of scutes on the carapace, and 18 on the plastron (Fig. 2). The configuration of landmarks represents the shape of an object as a single complex, multi-dimensional trait [(Klingenberg and Gidaszewski 2010; Adams et al. 2011) and references therein]. Individuals whose carapace or plastron contained extra-numerary or fused scutes (n = 7) were excluded from further analysis. To obtain shape and size variables,

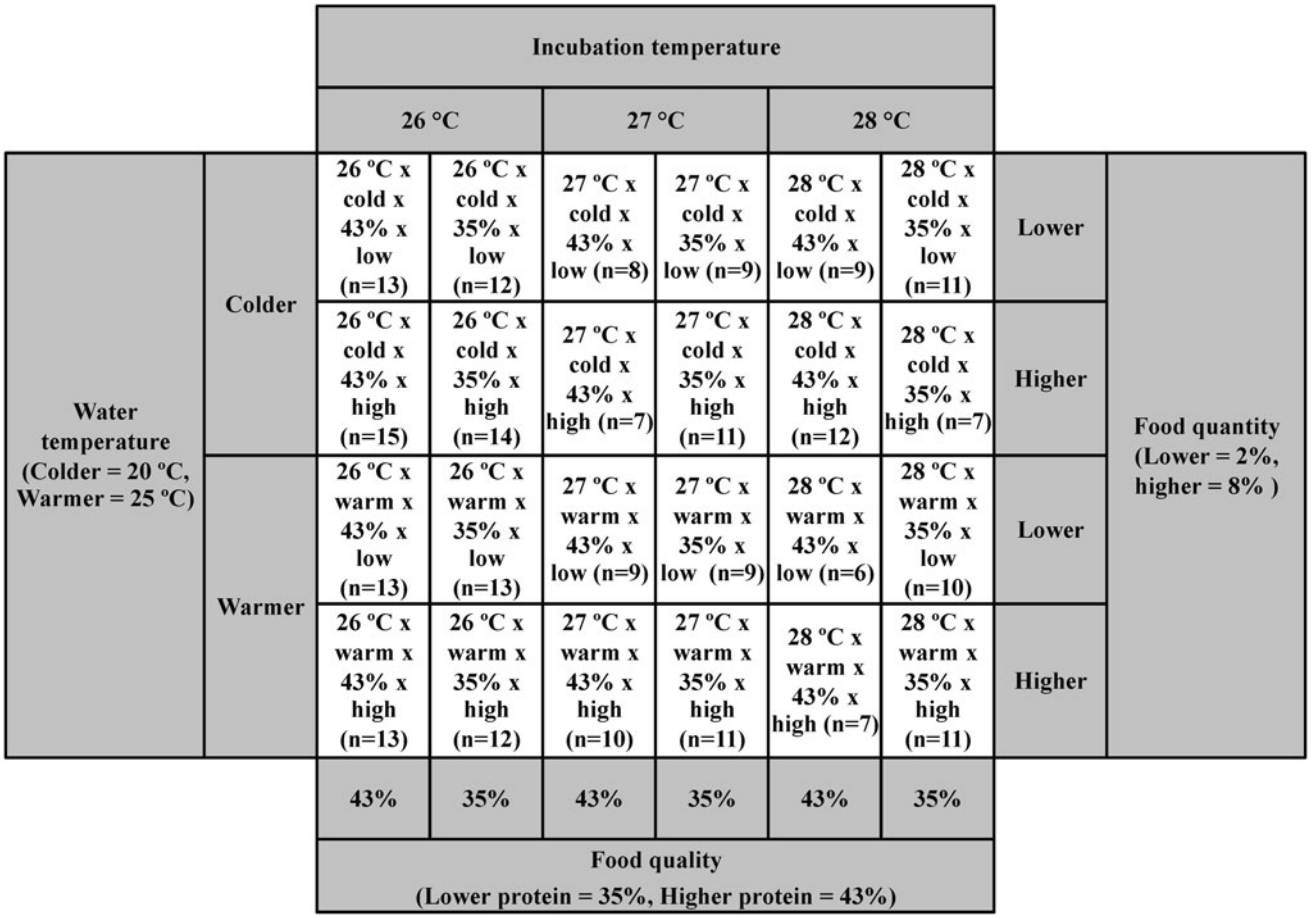

Fig. 1 Experimental design used in this study of *Chelydra serpentina*. Treatments (24 in total) were obtained by the combination of three incubation temperatures (as measured from dataloggers during incubation), two water temperatures, two food qualities and two food quantities (% body weight). n = sample size as described in the text

landmarks were subjected to a Generalized Procrustes Analysis (GPA) which, briefly, superimposes all configurations of landmarks to a common coordinate system, by holding mathematically-constant the effects of position, orientation and scale (Rohlf and Slice 1990). Two independent GPA were performed, one with carapace landmarks of all individuals at all ages evaluated, and a second for landmark configurations from plastrons. Both body components were studied independently because the turtle carapace can exhibit more variation among habitats than the plastron (Rivera 2008), and because the precloacal length of the plastron in snapping turtles grows relatively faster than the carapace length in males, while carapace and plastron grow at the same rate in females (Mosimann and Bider 1960).

For each GPA, partial warp scores and uniform components were obtained, along with the centroid size. Partial warps and its uniform components were used as shape variables, which provide a multivariate description of the configuration of the anatomical set of landmarks (Bookstein 1991). Centroid size describes an average distance from the center of gravity of all landmarks to every landmark, thus it was used as a general measure of size. In summary, for each individual at each age we obtained 46 shape variables and one centroid size variable for their carapace, and 32 shape variables and one centroid size variable for their plastron. Turtles were also weighed periodically and the carapace and plastron measured with a 12″-digital-electronic caliper (Brown and Sharpe) to record the traditional linear-medial length and width.

Data Analysis

To test if plasticity was sex-specific, namely if males exhibited a greater, lesser or equal growth plasticity than females ($Pm > Pf$, $Pm < Pf$, $Pm = Pf$, respectively) we tested for the effects of sex, environment, and their interaction on size and shape of carapace and plastron (growth and allometric changes). We performed several univariate and multivariate analyses of variance (Sokal and Rohlf 2001) with egg size (egg weight transformed to the cubic root of its natural log) as covariate to account for potential

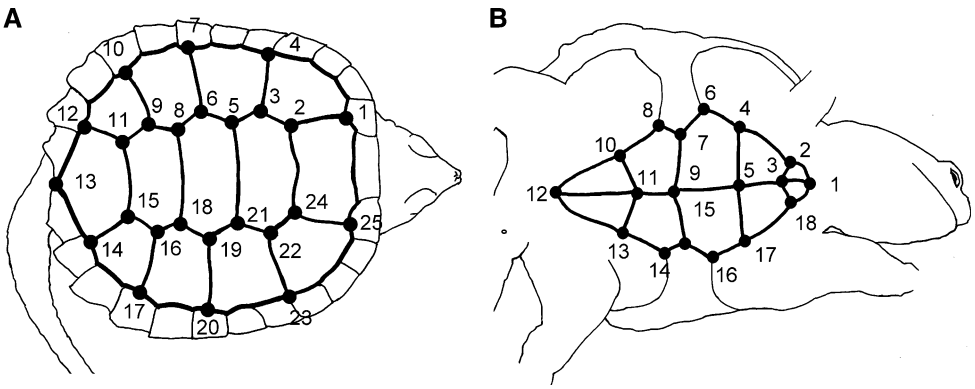

Fig. 2 Location of 25 landmarks (filled circles) digitized on the carapace (**a**) and 18 landmarks digitized on the plastron (**b**) of *Chelydra serpentina*

maternal effects (Steyermark 2008) (ANCOVAs and MANCOVA's respectively, denoted as M/ANCOVA's). Analyses were performed for two time points: at hatching to account for effects of sex and incubation temperature during embryonic growth, and at the end of the experiment (15.5 months of age) to account for effects of sex, incubation temperature, food quantity and food quality post-hatching. All pairwise comparisons were Bonferroni-corrected while maintaining an experiment-wise alpha = 0.05 (Sokal and Rohlf 2001).

Using this approach, several possible biological patterns can be identified, which allow the evaluation of predictions that emerge from the hypotheses of phenotypic plasticity. First, a significant effect of sex would indicate the presence of sexual dimorphism (Fig. 3, panel I), and a significant effect of any environmental factor would indicate that growth is environment-specific or plastic (Fig. 3, panel II). If the effects of sex and environment are found to be additive (i.e., no significant interaction), this would indicate that growth is plastic but not sexually dimorphic, such that plasticity is the same in both sexes (Pm = Pf) (Fig. 3, panel III). Lastly a significant interaction between sex and environment would indicate that growth plasticity is sex-specific, i.e., that the sexes differ in their plastic response (either $Pm > Pf$ or $Pm < Pf$) (Fig. 3, panel IV), and examining the magnitude of the average change among treatments for males and females would reveal which sex exhibits a relatively stronger response (i.e., greater plasticity).

For those traits displaying a significant interaction between sex and environment, an additional analysis was conducted to explore the ontogeny of such effects (Schlichting and Pigliucci 1998; Stillwell et al. 2007). In this case, Euclidean distances were calculated at multiple ages (0.5, 2, 3, 4, 6.5, 8.5, 10, 12, and 15.5 months) as described above. The Euclidean distances of each sex through time were used to estimate slopes for each sex, and their difference assessed through a test of slopes using the library Smart (Warton and Weber 2002) in R software.

All M/ANCOVAs were performed using a linear model (lm function in R). To test for the possibility that phenotypic differences were due to maternal effects, analyses were repeated using a mixed effect model (lme in nlme library) (Pinheiro and Bates 2000) in R software (v. 2.9.1). The mixed effect model treats the environmental factors as fixed effects and clutch as a random effect. If the environmental effect is significant in both models, it would imply that the observed differences are due to phenotypic plasticity. On the other hand, if the environmental effect is not significant in the lme model, it would imply that the observed differences are due to the clutch of origin, and thus more likely due to maternal effects (genetic or non-genetic basis).

Finally, for the purpose of visualizing shape differences among groups, graphical representations of mean carapace and plastron group shapes were reconstructed using thin-plate spline deformation grids from the overall average to the average of each focal group (Zelditch et al. 2004). Procrustes analyses were performed using TpsDig, TpsRelw and TpsUtil software, and mean shapes were calculated with TpsSpline software (Rohlf 2001, 2003).

Results

A summary of incubation temperature statistics, hatching success and sex ratios, are found in Table 1. At hatching, individuals differed in size and shape of their carapace and plastron by incubation treatment. The carapace shape of hatchlings from the highest temperature (28°C) were more flared anteriorly and plastrons were relatively constricted from head to tail ("chubby plastrons") when compared to the shape of hatchlings from the intermediate (27°C) and lowest temperatures (26°C) (Table 2, Fig. 4). In contrast, plastron (centroid) size was the largest in the 28°C group compared to the 26°C group, while there were no statistical differences in size between the 26 and 27°C groups, or between the 27 and 28°C groups (Table 3).

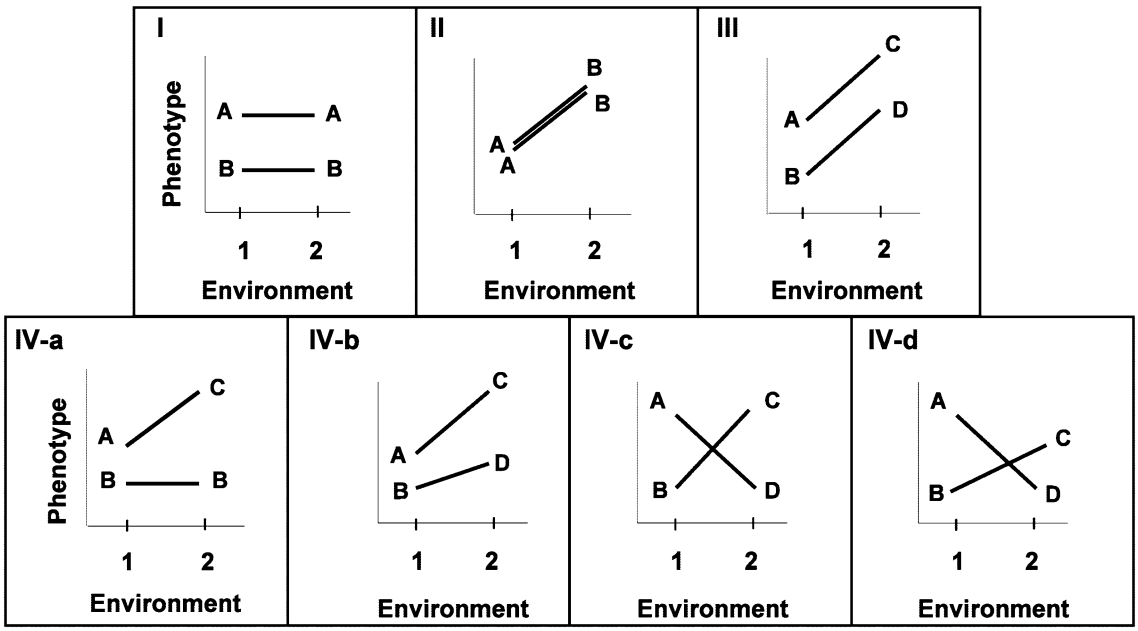

Fig. 3 Combination of reaction norms illustrating the potential responses of the two sexes (lines) to two hypothetical environments (*1* and *2*). *Different letters* (*A*, *B*, *C*, *D*) indicate different phenotypes. Body size can vary only in magnitude, while body shape can vary in magnitude and direction (different slopes). *I* Growth is sex-specific but not plastic (sexes have different phenotypes which do not change with environment); *II* Growth is plastic but not sex-specific (males and females have the same phenotype and it changes equally with environment); *III* Growth is plastic and sex-specific (sexes have different phenotypes and they change equally with environment). Scenarios *IV a–d* represent interactions between sex and environment, that is, when growth is plastic and sex-specific, and the phenotype of each sex changes in different ways depending on the environment (slopes are different). Namely, *IV-a* one sex exhibits a plastic phenotypic response but the other does not, *IV-b* both sexes exhibit a plastic response which in differs in magnitude and/or direction, *IV-c* both sexes exhibit a plastic response of identical magnitude but in opposite directions which reverses the SSD or SShD pattern but maintains its magnitude; and *IV-d* both sexes exhibit a plastic response which differs in magnitude and is in opposite directions which reverses the SSD or SShD pattern and changes the magnitude of the sexual dimorphism

Analyzing traits at hatching and 15.5 months of age revealed that body size and body shape were highly plastic to all environmental factors tested: incubation temperature, water temperature, food quality, and food quantity (Tables 2, 3). Incubation temperature had a very distinct effect on shape at hatching compared to its effect at 15.5 months of age (Fig. 4). In general, mean carapace from the 26 and 27°C groups were relatively more elongated and wider compared to the shorter and narrower mean carapace from the 28°C group (Fig. 4). On the contrary, mean plastron in the 28°C group was relatively wider than the other 2 groups (Fig. 4). Incubation temperature also affected body size of juveniles. The intermediate incubation temperature produced the largest carapace and plastron, and the lowest temperature the smallest (Table 3).

Water temperature was the environmental factor with the strongest effect on body shape and size posthatching. Overall, warmer water enhanced body elongation, making the carapace and plastron narrower (Fig. 5). Warmer temperature also enhanced carapace and plastron size by 55% when compared to the colder water treatment (Table 3). Moreover, the influence of water temperature differed among incubation groups as the interaction between these two factors in the linear model was significant (Table 3). However, when clutch was treated as a random-effect term using the mixed-effect model, this interaction became non-significant. This suggests that the size phenotype resulting from this interaction more likely has a maternal influence (genetic or non-genetic) beyond that imparted by egg size alone, rather than being a plasticity effect.

Resource availability affected growth plasticity in a differential manner: food quality had a significant effect on shape, while food quantity affected size (Tables 2, 3). Namely, individuals raised under the lower-protein food exhibited a more elongated carapace than those under the higher-protein diets (Fig. 6). Furthermore, food quality had a significant effect on plastron shape of juveniles that differed among the water temperature treatments as indicated by the significant water temperature by food quality interaction (Table 2). However, after pairwise analyses and Bonferroni-correction for multiple comparisons all significant differences disappeared ($P > 0.03$ in all instances), indicating that the effect was weak.

Table 2 MANCOVA results of final models of sex, environmental, and maternal effects on carapace and plastron shape of hatchlings and juveniles of *Chelydra serpentina*

Factor/interaction	*df*	Wilks	Approx. F	Df (num, den)	*P* values	Pairwise comparisons
Hatchlings						
Carapace shape						
Sex	1	0.7648	1.3171	46, 197	0.1024	
IncTemp (°C)	2	0.5174	1.671	92, 394	0.0004	26 ≠ 28 ($P = 0.0001$), 27 ≠ 28 ($P = 0.0035$), 26 = 27 ($P = 0.0560$)
Egg weight	1	0.5269	3.8449	46, 197	<0.0001	
Residuals	242					
Plastron shape						
Sex	1	0.744	2.2684	32, 213	0.0003	
IncTemp (°C)	2	0.5731	2.1329	64, 426	<0.0001[†]	26 = 28 ($P = 0.0384$), 27 = 28 ($P = 0.0932$), 26 = 27 ($P = 0.1204$)
Egg weight	1	0.6492	3.596	32, 213	<0.0001	
Residuals	244					
Juveniles:						
Carapace shape						
Sex	1	0.5195	1.9099	46, 95	0.0041	
IncTemp (°C)	2	0.3072	1.6611	92, 190	0.0018	26 ≠ 28 ($P = 0.0017$), 27 ≠ 28 ($P = 0.0010$), 26 = 27 ($P = 0.7925$)
WaterTemp	1	0.349	3.8521	46, 95	<0.0001	
FoodQl	1	0.3746	3.4482	46, 95	<0.0001	
Egg weight	1	0.5573	1.6408	46, 95	0.0217	
Residuals	140					
Plastron shape						
Sex	1	0.6016	2.2349	32, 108	0.0011	
IncTemp (°C)	2	0.4722	1.5364	64, 216	0.0124	26 = 28 ($P = 0.2711$), 27 ≠ 28 ($P = 0.0150$), 26 = 27 ($P = 0.1274$)
WaterTemp	1	0.4149	4.7596	32, 108	<0.0001	
FoodQl	1	0.7018	1.4341	32, 108	0.0881	
Egg weight	1	0.4574	4.0032	32, 108	<0.0001	
WaterTemp × FoodQl	1	0.6583	1.7516	32, 108	0.0176	All $P > 0.03$
Residuals	139					

FoodQl food quality, *IncTemp* incubation temperature, *WaterTemp* water temperature, *df* degrees of freedom, *num* numerator, *den* denominator

[†] Because incubation temperature had a highly significant effect on plastron shape, but none of the pairwise comparisons were significant at the Bonferroni-corrected alpha, significance was reassessed via a non-parametric (permutational) MANCOVA using the Vegan library in R software ($P = 0.044$). This method is capable of identifying a significant effect even when there is a large number of dependent variables (Anderson 2001). This result implies that there is a weak signal for the effect of incubation temperature on plastron shape and explains why pairwise comparisons found no differences among groups

Sexual Shape and Size Dimorphism

Hatchlings were sexually dimorphic in their plastron shape and carapace size, but not in their carapace shape or plastron size. The plastron of males was relatively longer in its anteroposterior axis, compared to females (Fig. 7). In terms of size, the carapace of males was relatively larger than that of females (Table 3). Sexual dimorphism at hatching did not vary by environment as the interaction between sex and incubation temperature was not significant on either carapace shape, plastron shape, carapace size or plastron size ($F_{\text{carapace shape, 92, 394}} = 0.66$, $P = 0.60$; $F_{\text{plastron shape, 64, 422}} = 0.78$, $P = 0.82$; $F_{\text{carapace size, 2}} = 0.65$, $P = 0.51$; and $F_{\text{plastron size, 2}} = 0.97$, $P = 0.37$).

A similar analysis was performed posthatching. At 15.5 months of age, snapping turtles exhibited sexual shape dimorphism of both the carapace and plastron (Tables 2, 3). Specifically, males had a relatively narrower and more elongated carapace and plastron than did females (Fig. 7). This sexual shape dimorphism was not plastic as evidenced

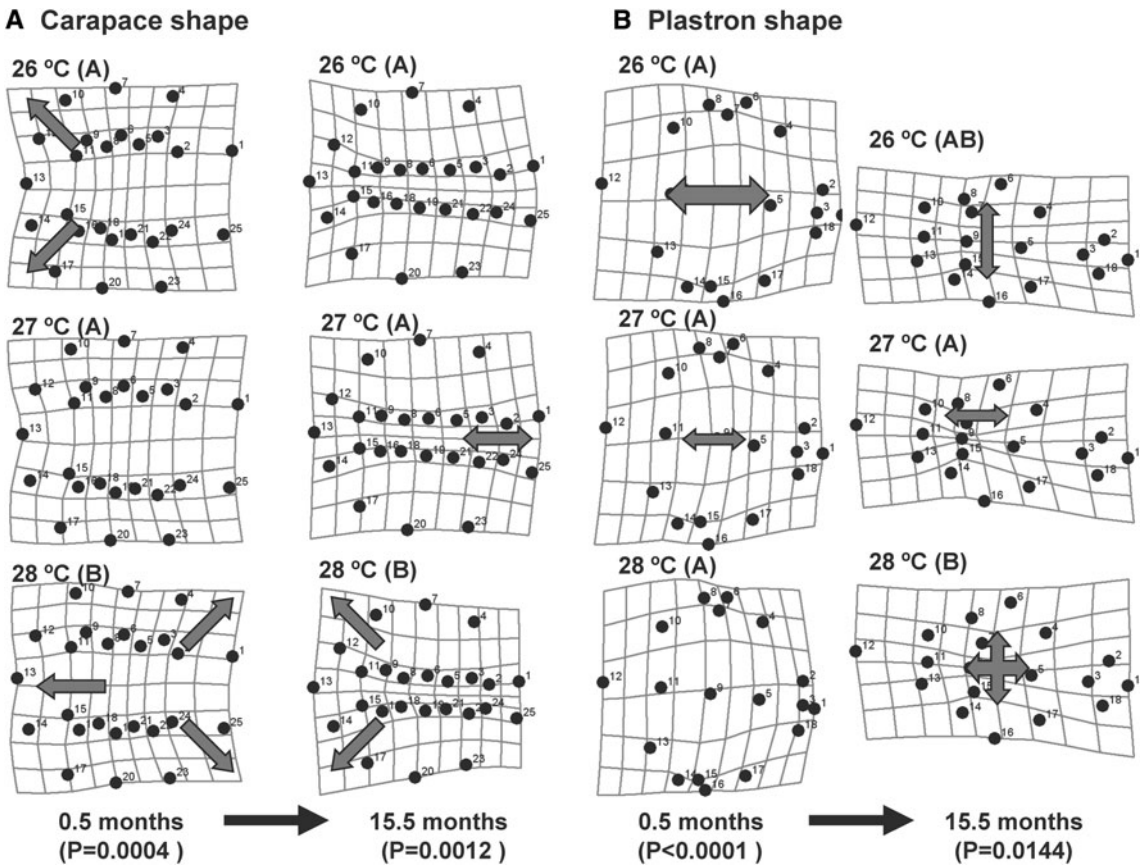

Fig. 4 Thin-plate spline deformation grids illustrating the effect of incubation temperature on carapace (*panel A*) and plastron (*panel B*) shape of *Chelydra serpentina* at 0.5 and 15.5 months of age. Deformation grids were magnified 5× for visualization purposes. Gray arrows indicate direction of change. $P = P$ values corresponding to the significance of the differences among incubation temperatures at each age. Groups that differ significantly are denoted by *different letters*. Individual's heads are located on the right side of each grid

by the fact that the interaction between sex and all environmental factors were not significant (Table 2). However, a significant interaction between sex and food quantity detected in carapace and plastron size (Table 3), indicated that sexual size dimorphism was both present, and was plastic. Together, these results revealed that males and females responded differently to the low and high food quantity treatments, indicating sex-specific differential plasticity.

Sex-specific Plasticity on Sexual Shape and Size Dimorphism

Furthermore, the absolute value of the difference in average size (absolute Euclidean distance) between low and high food quantity treatments was 6.54 for males and 3.1 for females across all ages, and 15.9 and 10.7 respectively at 15.5 months of age (Table 3, Fig. 8), revealing that males exhibited greater plasticity than females overall. Unexpectedly, we found that under lower food quantity conditions males attained larger carapace and plastron size than females. In contrast, under higher food quantity males had a smaller carapace and plastron than females (Fig. 8). Results did not change when using the traditional linear measurements (e.g. linear carapace length) instead of centroid size (Table 3).

We also detected a significant interaction of age by sex by food-quantity on carapace and plastron size using centroid size ($P_{\text{carapace}} < 0.0001$, $P_{\text{plastron}} < 0.0001$, Table 4), indicating that the sex-specific plasticity changed over time. To explore the ontogeny of this interaction given that the environment can affect phenotypes at various life stages, we tested for differences in sex-specific growth plasticity by comparing body size plasticity of males and females at additional ages of 0.5, 2, 3, 4, 6.5, 8.5, 10, and 12 months as described above. We calculated the average mean of all groups, as well as the Euclidean distances between the low and high food treatment of males and females that indicate the magnitude of plasticity of each sex through time (Table 5). These values were used to build an ontogenic profile of the reaction norms that revealed a trend of increasing body size plasticity of both

Table 3 ANCOVA results of final models of sex, environmental, and maternal effects on carapace and plastron size of *Chelydra serpentina*

Factor/interaction	*df*	Sum sq	Mean sq	F value	*P*	Mean group	Pairwise comparisons
Hatchlings							
Carapace size							
Sex	1	41.07	41.07	7.9	0.0054	F: 54.534, M: 55.362	
IncTemp (°C)	2	9.62	4.81	0.9	0.3992		
EggWeight	1	1,904.29	1,904.29	365	<0.0001		
Residuals	244	1,272.73	5.22				
Plastron size							
Sex	1	0.3	0.3	0.2	0.6698		
IncTemp (°C)	2	32.31	16.16	9.9	<0.0001	26: 34.96, 27: 34.88, 28: 35.68	26 ≠ 28 ($P = 0.003$), 27 = 28 ($P = 0.0012$), 26 = 27 ($P = 0.6851$)
EggWeight	1	986.02	986.02	603.4	<0.0001		
Residuals	244	398.7	1.63				
Juveniles							
Carapace size							
Sex	1	762	762	2.0	0.1604		
IncTemp (°C)	2	12,086	6,043	15.8	<0.0001	26: 116.86, 27: 131.05, 28: 105.15	26 = 28 ($P = 0.5029$), 27 = 28 ($P = 0.0312$), 26 ≠ 27 ($P = 0.0125$)
WaterTemp	1	88,527	88,527	231.4	<0.0001	W: 144.57, C: 92.67	
FoodQtt	1	330	330	0.9	0.3549		
IncTemp × WaterTemp	2	2,705	1,352	3.5	0.0318[†]		All $P > 0.02$
Sex × FoodQtt[§]	1	2,219	2,219	5.8	0.0173		
Residuals	138	52,801	383				
Plastron size							
Sex	1	315	315	2.1	0.1513		
IncTemp (°C)	2	4,508	2,254	14.9	<0.0001	26: 70.75, 27: 79.24, 28: 63.81	26 = 28 ($P = 0.5667$), 27 = 28 ($P = 0.0476$), 26 ≠ 27 ($P = 0.0140$)
WaterTemp	1	34,314	34,314	227.1	<0.0001	W: 88.40, C: 56.31	
FoodQtt	1	77	77	0.5	0.4752		
IncTemp × WaterTemp	2	1,120	560	3.7	0.027[‡]		All $P > 0.01$
Sex × FoodQtt	1	1,092	1,092	7.2	0.0081		
Residuals	138	20,848	151				

Mean size of the groups are shown if the main factor is significant. *FoodQtt* food quantity; Water Temp: *C* cold, *W* warm; Sex: *F* female, *M* male; *H* high food quantity, *L* low food quantity. Other abbreviations as in Table 2

When analyses were repeated with mixed effect model two *P* values were non-significant at alpha = 0.05: [†] $P = 0.0798$, [‡] $P = 0.0557$. [§] The interaction Sex×FoodQtt was also tested using linear carapace length (mm) and it was also significant ($P = 0.0228$), with group means (means with different letters are significantly different): male-low = 75.7 (A), female-low = 65.4 (B), male-high = 66.6 (B), female-high = 71.7 (A, B)

sexes through time (Fig. 9). The magnitude of plasticity from the low- to the high-food treatment of each sex was plotted at ages 6.5–15.5 months, and their respective slopes were calculated (slope males = 1.463, $P = 0.0096$; slope females = 1.0105, $P = 0.0608$). A test of slopes revealed that the slope for males was higher than that for females but only in a marginally significant way ($P = 0.0796$). This trend indicates that plasticity increases with age overall, and that males tend to exhibit a relatively higher body size plasticity compared to females, a difference that appears to be accentuated with age. This trend is in the same direction and thus consistent with the significant result from the analysis of the sex by food quantity interaction and strengthen the notion that males exhibit a relative increased body size plasticity when compared to females (Pm > Pf).

Ontogeny of Maternal Effects on Hatchlings and Juveniles

Embryonic growth was also influenced by maternal allocation, as initial egg weight had a highly significant effect on carapace and plastron shape and carapace and plastron (centroid) size of hatchlings (Tables 2, 3). By 15.5 months of age, the effect of egg weight on body size was no longer

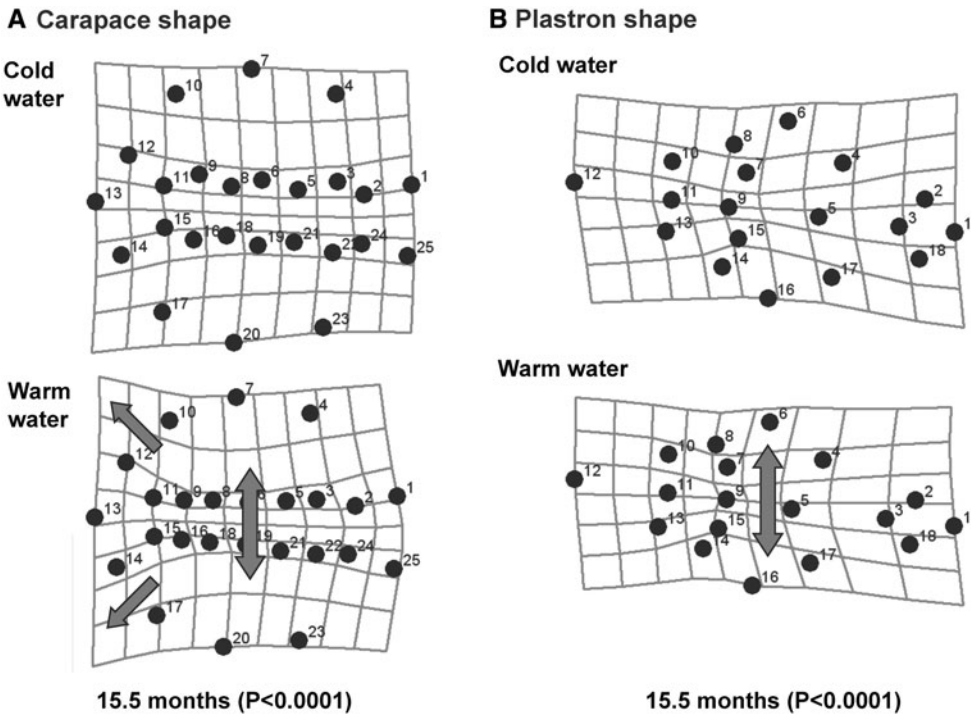

Fig. 5 Deformation grids depicting the carapace (*panel A*) and plastron (*panel B*) shape at 15.5 months of *Chelydra serpentina* juveniles reared under cold and warm water. *Gray arrows* indicate differences in the direction of growth, $P = P$ values corresponding to the significance of the differences between treatments. Deformation grids were magnified 5× for visualization purposes. Individual's heads are located on the right side of each grid

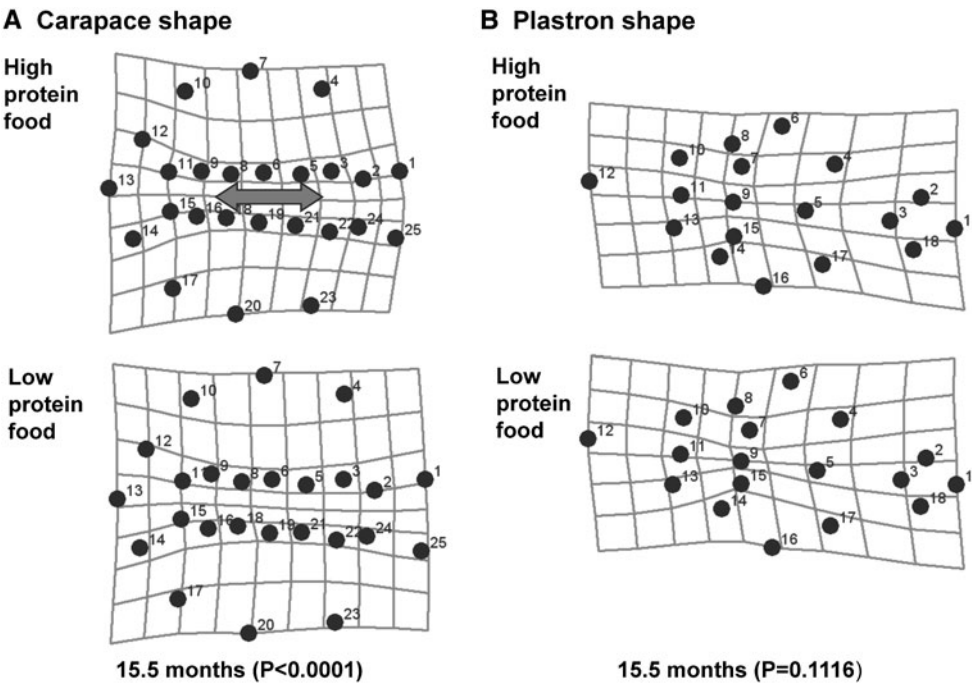

Fig. 6 Deformation grids depicting the carapace (*panel A*) and plastron (*panel B*) shape at 15.5 months of *Chelydra serpentina* juveniles provisioned with two food quality diets. *Gray arrows* indicate differences in direction of growth, $P = P$ values corresponding to the significance of the differences between treatments. Deformation grids were magnified 5× for visualization purposes. Individual's heads are located on the right side of each grid

significant, but it was slightly significant on carapace shape, and highly significant on plastron shape. To understand at what specific age this effect on body size disappeared, additional M/ANCOVAs were carried out at 2, 3, 4, 7, 8, 10, and 12 months of age. Egg weight had a strongly significant effect on carapace and plastron shape at all ages, but the significant effect on carapace and plastron size vanished after the 8th month ($P < 0.0001$ from 0.5 to 6.5 months for carapace and plastron size, and $P > 0.1$ from 8.5 to 15.5 months on carapace and plastron size).

Discussion

Deciphering the proximate and ultimate processes that generate the diversity in sexually dimorphic patterns

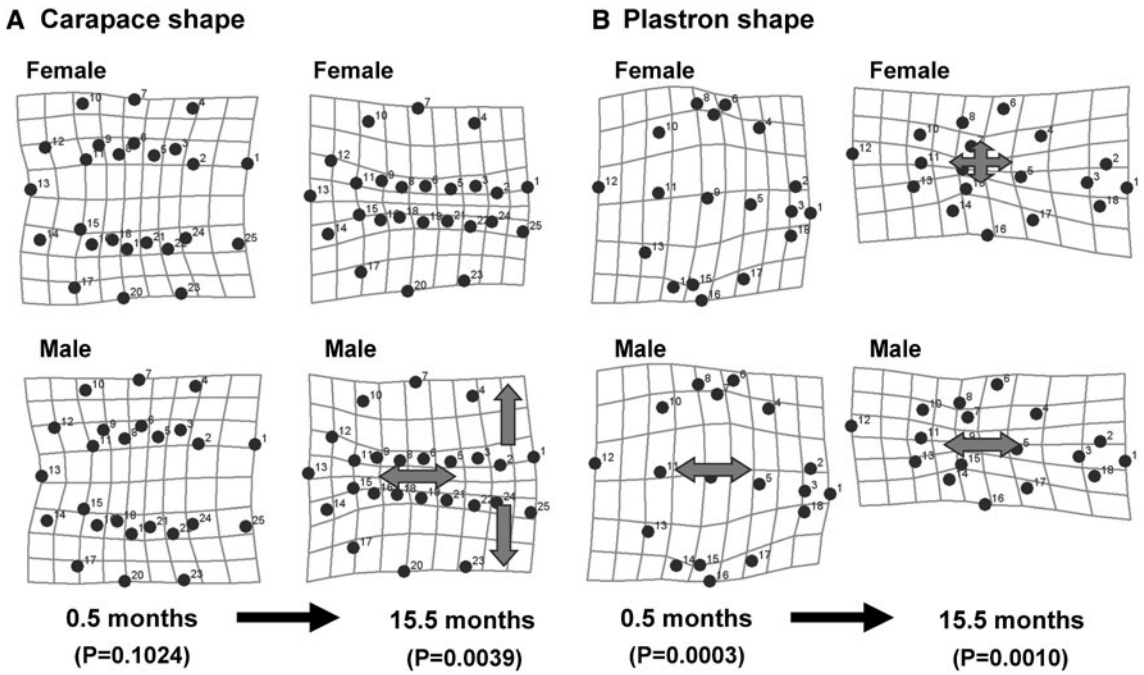

Fig. 7 Deformation grids depicting the carapace (*panel A*) and plastron (*panel B*) shape changes of *Chelydra serpentina* males and females between 0.5 and 15.5 months of age. *Gray arrows* indicate differences in direction of growth, $P = P$ values corresponding to the significance of the differences between males and females at each age. Deformation grids were magnified 5× for visualization purposes. Individual's heads are located on the right side of each grid

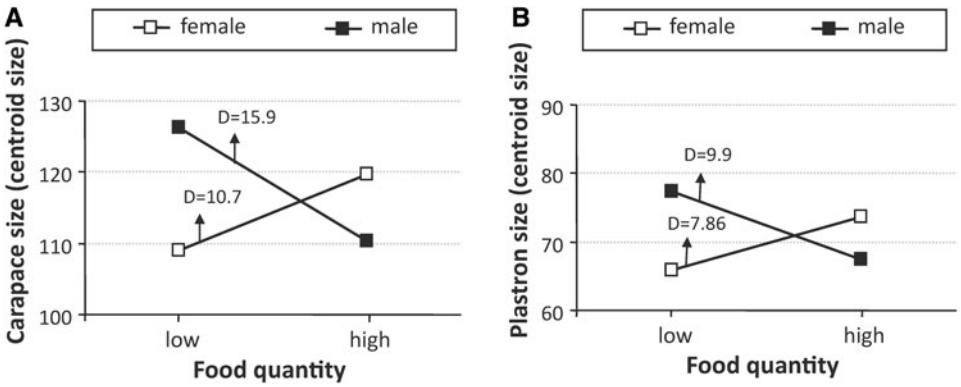

Fig. 8 Reaction norm illustrating the response of carapace size (*panel A*) and plastron size (*panel B*) of 15.5 month-old *Chelydra serpentina* males and females to two food quantity treatments. D = Euclidean distance (magnitude of vector)

observed in nature is a prevailing question in evolutionary biology. Recent studies have shown that growth plasticity can be sex-specific (Bonduriansky 2007; Stillwell and Fox 2007; Stillwell et al. 2010), however the particular role of such differential environmental sensitivity of the sexes in shaping SSD/SShD is still being elucidated, particularly in vertebrates (Taylor and Denardo 2005; Cox et al. 2006). In this study we found that growth of snapping turtles was highly plastic, sexually dimorphic, and also that growth plasticity was sex-specific, with the larger sex (males) exhibiting a greater plasticity overall and a trend of proportionally greater plasticity with age than the smaller sex (females). This supports the hypothesis that growth of the larger sex (males in this case) is under directional selection for increased body size, while the smaller sex exhibits a comparatively more canalized (i.e., less plastic) growth pattern, in partial accordance with the predictions from the condition dependent-hypothesis (Bonduriansky 2007) as detailed below.

Table 4 Ontogeny of the interaction between sex and food quantity on carapace and plastron size of *Chelydra serpentina* at 0.5, 2, 3, 4, 6.5, 8.5, 10, 12 and 15.5 months of age

Factor/interaction	F value	*df*	*P*
Carapace size			
Age	426.26	8	<0.0001
Sex	6.87		0.0297
FoodQtt	6.40		0.0500
Age × sex	0.24	8	0.9828
Age × FoodQtt	0.84	8	0.5906
Sex × FoodQtt	25.96		<0.0001
Age × Sex × FoodQtt	5.20	8	<0.0001
Residuals		729	
Plastron size			
Age	357.4068	8	<0.0001
Sex	5.8945	1	0.0153
FoodQtt	4.5356	1	0.0333
Age × Sex	0.4016	8	0.9201
Age × FoodQtt	0.8945	8	0.5202
Sex × FoodQtt	24.8621	1	<0.0001
Age × Sex × FoodQtt	5.0268	8	<0.0001
Residuals		1,779	

FoodQtt food quantity

Sex-specific Plasticity on Sexual Size Dimorphism

Our results are consistent with other empirical studies that reported varying levels of SSD in changing environments, including geographic variation in other turtles (Iverson 1985; Lovich et al. 2010; Wolak et al. in press). In the mud turtle (*Kinosternon hirtipes*) with male-biased SSD, differences in SSD observed across three different river basins were associated with changes in male body size, which were attributed to a differential impact of food limitation on males (decreased male size under limited food) (Iverson 1985). Additionally, the brown anole lizard (*Anolis sagrei*) displayed different magnitudes of male-biased SSD across two islands in the Bahamas (Cox and Calsbeek 2010), a variability associated with environmental factors that differentially enhanced growth of males and females, potentially via sex-specific plasticity (Cox and Calsbeek 2010). Furthermore, in geckos (*Paroedura picta*) that exhibit male-biased SSD (Starostova et al. 2010), the larger sex exhibits greater body size plasticity (snout vent length) than the smaller sex at certain experimental temperatures and results in an accentuated SSD.

Our study in turtles is consistent with previous findings across different taxa, which support the differential plasticity hypothesis via condition-dependence as a potential mechanism enhancing body size of the larger sex (Bonduriansky 2007), except that the conditions enhancing growth in *C. serpentina* are unexpectedly those of lower food availability as detailed in the next section. On the one hand, other studies suggest that differential plasticity may operate in species in which females are the larger sex. For example, in insects most species have larger females than males, and females increase size relatively faster than males when environmental conditions are better (Teder and Tammaru 2005). Likewise, SSD of the Moroccan turtle (*Mauremys leprosa*) varied across 3 populations found in low- medium-, and high-productivity rivers (Lovich et al. 2010) and it was un-biased, female-biased, and extremely female-biased, respectively. Interestingly, male body size remain the same while, female size varied across sites and resulted in the contrasting SSD patterns. Thus in this turtle in which females are typically the larger sex, females are responsible for the SSD variation which was associated with the interaction of natural and sexual selection (Lovich et al. 2010) but the role of plasticity in shaping this pattern is uncertain. Therefore, the question remains open as to whether such greater plasticity is an intrinsic function of being the larger sex or of maleness. Discerning between these alternatives requires examining plasticity patterns as done here but in a species where females are the larger sex. Indeed, if in female-biased SSD species females exhibit increased plasticity it would indicate that the larger sex is the one driving SSD evolution (Bonduriansky 2007) irrespective of which sex is larger. On the other hand, if males of female-larger species exhibit increased plasticity, it would indicate that males drive the evolution of SSD irrespective of the SSD pattern, as has been previously suggested (Fairbairn and Preziosi 1994). Alternatively, if there are no differences in growth plasticity between the sexes in female-larger species, sex-specific plasticity would be ruled out as a mediator of sexual dimorphism in female-larger species. Thus, further experimentation on sex-specific plasticity of female-biased species is warranted.

On the other hand, it is worth noting that in our study we found that male snapping turtles grew larger under the scarce-food treatment as compared to the abundant-food treatment. This result is unexpected since greater food availability is generally more optimal for growth, and is the condition that the larger sex is assumed to take advantage of (Bonduriansky 2007), in contrast with our findings. Other species may exhibit a similar pattern. For instance, the Yarrow's spiny lizard (*Sceloporus jarrovi*) exhibits male-biased SSD in the wild, but the level of dimorphism is reduced under common garden conditions where resources are likely less limiting than in the wild (Cox et al. 2006). In our case, we hypothesize that the enhanced male growth under lower resource availability may stem from higher acquisition rates by males, perhaps derived from a more aggressive behavior under food scarcity than in females, or from lower allocation tradeoffs suffered by males as compared to females under limited resources

Table 5 Post hoc pairwise comparisons of the interaction of age by sex by food quantity on carapace and plastron size of *Chelydra serpentina* at 0.5, 2, 3, 4, 6.5, 8.5, 10, 12 and 15.5 months of age (Fig. 9)

Group (mean size)	Group (mean size)	Euclidean distance between groups	*P*
Carapace size			
0.5-fem-high (54.10)	0.5-fem-low (54.88)	0.78	0.9155
0.5-male-high (55.09)	0.5-male-low (55.37)	0.28	0.9698
2-fem-high (56.05)	2-fem-low (56.66)	0.60	0.9249
2-male-high (57.04)	2-male-low (57.88)	0.84	0.9055
3-fem-high (55.15)	3-fem-low (55.80)	0.65	0.9234
3-male-high (56.19)	3-male-low (57.02)	0.83	0.9034
4-fem-high (55.74)	4-fem-low (55.85)	0.11	0.9891
4-male-high (56.98)	4-male-low (57.60)	0.62	0.9135
6.5-fem-high (66.86)	6.5-fem-low (65.24)	1.62	0.7698
6.5-male-high (66.47)	6.5-male-low (69.61)	3.14	0.6622
8.5-fem-high (82.87)	8.5-fem-low (75.26)	7.61	0.1862
8.5-male-high (78.83)	8.5-male-low (84.01)	5.19	0.2630
10-fem-high (97.30)	10-fem-low (89.02)	8.28	0.0465
10-male-high (89.21)	10-male-low (100.20)	10.99	0.0794
12-fem-high (103.82)	12-fem-low (92.56)	11.26	0.0125
12-male-high (95.74)	12-male-low (106.65)	10.91	0.0505
15.5-fem-high (120.61)	15.5-fem-low (109.91)	10.70	0.0167
15.5-male-high (111.29)	15.5-male-low (126.84)	15.55	0.0173
Plastron size			
0.5-fem-low (35.28)	0.5-fem-high (34.84)	0.43	0.9169
0.5-male-low (35.31)	0.5-male-high (34.90)	0.41	0.9298
2-fem-low (35.99)	2-fem-high (35.67)	0.32	0.9369
2-male-low (36.65)	2-male-high (35.87)	0.78	0.8699
3-fem-low (35.78)	3-fem-high (35.18)	0.59	0.8603
3-male-low (36.44)	3-male-high (35.55)	0.89	0.8613
4-fem-low (35.68)	4-fem-high (35.62)	0.06	0.9872
4-male-low (36.74)	4-male-high (36.0)	0.74	0.8629
6.5-fem-low (41.05)	6.5-fem-high (40.23)	0.82	0.7705
6.5-male-low (43.25)	6.5-male-high (41.26)	2.00	0.7824
8.5-fem-low (50.97)	8.5-fem-high (57.17)	6.19	0.1619
8.5-male-low (56.50)	8.5-male-high (54.28)	2.22	0.2798
10-fem-low (51.64)	10-fem-high (59.72)	8.08	0.0187
10-male-low (60.31)	10-male-high (55.23)	5.08	0.0571
12-fem-low (55.85)	12-fem-high (61.75)	5.90	0.0360
12-male-low (63.43)	12-male-high (57.31)	6.12	0.0960
15.5-fem-low (67.54)	15.5-fem-high (74.26)	6.72	0.0262
15.5-male-low (77.95)	15.5-male-high (67.54)	10.41	0.0156

Fem female, *high* high food quantity, *low* low food quantity

(John-Alder and Cox 2007). In terms of a proximate physiological mechanism, our observations in snapping turtles and those in *Sceloporus jarrovi* are consistent with the hypothesis that testosterone, a hormonal sex-specific developmental regulator (Badyaev 2002), enhances growth of males in male-biased species (Cox and John-Alder 2005; Cox et al. 2009). Food abundance may override the effect of testosterone in some taxa (John-Alder et al. 2007), and it may explain the difference detected in snapping turtles between lower- and higher-food conditions. This hypothesis requires further testing. On the other hand, female snapping turtles grew larger under the abundant-food treatment. Likewise, females of the male-larger diamondback rattlesnake (*Crotalus atrox*) grew larger than males under a high-intake treatment in captivity (Taylor and Denardo 2005). We hypothesize that this may reflect a more efficient food utilization by snapping turtle females than by males, perhaps resulting from fecundity selection (Andersson 1994) given that the number of eggs a female turtle produces increases with body size in snapping turtles

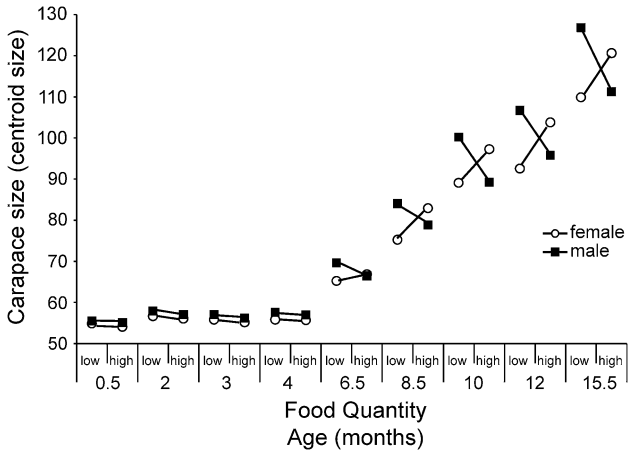

Fig. 9 Ontogeny of the response of carapace size of *Chelydra serpentina* males and females to two food quantity treatments during the first 15.5 months of life. Euclidean distances (magnitude of each vector) and their significance are presented in Table 5

(Congdon et al. 2008), other turtle species (Iverson and Moler 1997; Valenzuela 2001b), and numerous other vertebrates and invertebrates (reviewed in Andersson 1994). Such higher female efficiency in food utilization could be precluded when resources are limited and particularly more if it were true that males do indeed outcompete females under lower food conditions. These hypotheses are speculative at this point in the absence of additional data and further experiments are required to test them directly.

Sex-specific Plasticity on Sexual Shape Dimorphism

Interestingly, we found evidence of differential plasticity for sexual size dimorphism but not for sexual shape dimorphism, indicating that sex-specific responses to different environments can be divergent for a phenotypic trait (e.g. body size) but parallel for another phenotype (e.g. body shape). Variation in the degree of the plastic response has also been observed in other systems (Stillwell et al. 2007). Parallel responses as seen for shape in our study may be due to shared selective pressures between the sexes, or to genetic or functional constraints (Hendry et al. 2006). The monomorphic response of sexual shape dimorphism we observed contrasts with reports for wild-caught guppies (*Poecilia reticulata*) (Hendry et al. 2006), though it was unknown if in that case the interactions between sex and environments were the result of genetic variation or a plastic response.

Growth Plasticity

Water temperature and incubation temperature did not affect growth differentially in male and female snapping turtles (our study) or in other taxa (Stillwell et al. 2007; Arendt 2006), while they had a significant effect on body size in other species (Janzen 1993; Valenzuela 2001b; Arendt and Hoang 2005). Yet, water temperature produced the strongest effect on body size, with colder temperatures retarding growth rates and warmer temperatures accelerating growth rates. Although snapping turtles do not regularly bask in the wild, they do thermoregulate by selecting a preferred water temperature between 24 and 28°C both in the wild and in captivity (Spotila and Bell 2008), which encompas the warmer-water treatment temperature used in our study. Consistent with our findings, there is a positive relationship between temperature and the rate of food ingestion and digestion, which enhances growth (reviewed in Spotila and Bell 2008). Relative lower growth rate as seen in our colder-water treatment is also observed in the wild where snapping turtles barely grow during the winter (Christiansen and Burken 1979).

Interestingly, the effect of incubation temperature on growth was strong, yet it was reversed with time. While the warmest incubation temperature (28°C) was associated with larger plastrons at hatching, these individuals became the smallest by 15 months of age. Similar compensatory growth responses by smaller hatchlings were reported in giant Amazonian turtles (*Podocnemis expansa*) (Valenzuela 2001b), and alligator snapping turtles (*Macrochelys temminckii*) (Ligon and Lovern 2009). The effect of incubation temperature on the growth of *C. serpentina* is not as clear as results from different studies are mixed. For example, in one study low-temperature (24°C) hatchlings were smaller and exhibited higher growth rates during the first 6 months of age than high-temperature hatchlings (29°C) (Rhen and Lang 1995), consistent with our findings. However, in another study hatchlings from 25.5°C were heavier and exhibited higher growth rates during the first 10 months of life than those incubated at 22 and 29°C (Bobyn and Brooks 1994). Discrepancies may reflect differences in the parameters used to measure growth between studies (plastron size vs. body mass), population differences, or potential interactions among specific incubation temperatures, hydric incubation conditions, clutch of origin, or other maternal effects (Bobyn and Brooks 1994).

Food quality and quantity differed in their effect on growth. Food quantity affected body size (differentially between sexes) but not body shape, while food quality strongly affected carapace shape, but not size. This is consistent with the notion that resource availability is one of major environmental factors affecting SSD plasticity (Stillwell et al. 2007; Cox and Calsbeek 2010; Stillwell et al. 2010), as shown empirically in some species where food availability had a positive effect on growth (e.g. Madsen and Shine 1993), but contrasts with other studies in which growth was enhanced by greater food quality [e.g.

(Davidowitz et al. 2004; Quezada-Euan et al. 2011)]. Snapping turtles are carnivorous and scavengers in the wild (Ernst et al. 1994). Assuming that fish carrion has a protein content of about 20% (Parmenter and Avery 1990), then our diets may have had a higher protein content (35 and 43%) than those experienced in the wild. Nevertheless, in this study snapping turtles grew below and above the growth rates experienced in the wild (Christiansen and Burken 1979). For instance, in the colder-water treatments turtles grew to an average of 40 g/year, and in the warmer-water treatments they grew to an average of 160 g/year, while in the wild juveniles grow at a rate of 145 g/year (Congdon 1989).

Sexual Size and Shape Dimorphism

Males were larger than females at hatching and this effect was independent of incubation temperature, as indicated by the non-significant sex by incubation temperature interaction. Furthermore, although sex and incubation temperature effects are potentially confounded in our experiments, previous work on *Chelydra serpentina* in which the effects of temperature and sex were experimentally disentangled by hormonal manipulation demonstrated that temperature, and not sex, affected growth in this species (Rhen and Lang 1994; Rhen et al. 1996). However, we detected no sexual size dimorphism in juveniles, similar to what occurs in nature, where males and females grow at the same rate during the first 3 years of life, after which males grow faster relative to females (Christiansen and Burken 1979). Larger body size in hatchling snapping turtles has been associated with greater survivorship, supporting the "bigger is better" hypothesis (Janzen 1993). Other studies however, have not detected advantages of larger body size in hatchlings or juveniles before reaching maturity (Congdon et al. 1999).

On the other hand, sexual shape dimorphism was evident at hatching and at 15.5-months of age in our study. The potential adaptive significance of SShD at these early life stages is less clear than during adulthood. For example, adult snapping turtles have sexually dimorphic activities (Brown and Brooks 1993). Males engage in male–male combat (Brown and Brooks 1993), for which carapace would be expected to be wider than in females (Mann et al. 2006) as we found here mainly at the shoulder level (Fig. 7A). Likewise, females engage in nesting for which plastron would be expected to be wider than in males (Bonnet et al. 2001), as observed in juveniles in our study (Fig. 7B). Thus, we propose that the sexual shape dimorphism detected in hatchlings is consistent with that found in adults and may simply be present as a precursor to the adult dimorphism without conferring an advantage at early life stages. However, other studies have found that *Trachemys scripta* turtle hatchlings with shorter and wider plastron swim faster (Myers et al. 2007). Further studies on the functional significance of sexual shape dimorphism in *C. serpentina* hatchlings would be useful. From an applied perspective, plastron shape should provide a more powerful signal for sexing *C. serpentina* hatchlings using geometric morphometrics as done in other turtles (Valenzuela et al. 2004) given that sexual shape dimorphism was evident in the plastron, but not in the carapace of hatchlings.

Finally, the effect of initial egg weight on body size lasted until the 8th month, while the effect of initial egg size on body shape was still present by the end of this experiment. Though food intake was not individually measured, this observation suggests that the hatchlings' reliance on yolk reserves is crucial for hatchling growth and females may affect offspring body size early in life via maternal allocation, but after the 8th month juveniles rely increasingly on environmental resources and maternal effects fade (Fig. 8). The effects of initial egg size on body shape on the other hand, appear to be longer-lasting, at least in the plastron.

In conclusion, we have found evidence to support the condition-dependence hypothesis in which body size of the larger sex, males in snapping turtles, is enhanced compared to the smaller sex. Thus differential plasticity appears to be a proximate mechanism underlying SSD evolution, perhaps by sexual selection. Alternatively, if greater plasticity is an inherent property of the larger sex, then the differential plasticity may also be a proximate mechanism underlying SSD evolution, perhaps by fecundity selection. Further parallel studies in female-larger species are required to discern between who exactly is being favored (individuals of a particular sex or of a particular size) under this condition-dependence scenario. Notably, the disparity of sex-specific responses observed here under differing environments indicates that caution is needed when interpreting results from a single treatment in captivity as such uniform environments may obscure plastic responses existing in the wild.

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Appendix

See Table 6.

Table 6 Ingredients and nutritional composition of the two types of turtle food (Zoo Med Lab) with relatively higher and lower quality used in this study (source: http://www.zoomed.com downloaded on September 15, 2010)

	Higher quality food (Hatchling food®)	Lower quality food (Growth food®)
Ingredients	Fish Meal, Blood Meal (flash dried, non-ruminant), Poultry By-Product Meal, Soybean Meal, Wheat, Wheat Flour, Fish Oil, Soy Lecithin, L-Ascorbyl-2-Polyphosphate (source of Vitamin C), Choline Chloride, Vitamin E Supplement, Niacin Supplement, Calcium Pantothenate, Riboflavin, Thiamine Mononitrate, Biotin, Pyridoxine Hydrochloride, Folic Acid, Vitamin A Acetate, Vitamin D3 Supplement, Vitamin B12 Supplement, Manganese Sulfate, Zinc Sulfate, Ferrous Sulfate, Copper Sulfate, Sodium Selenite, Potassium Iodate, Propionic Acid (a natural preservative).	Soybean Meal, Wheat Flour, Fish Meal, Wheat Middlings, Corn Gluten Meal, Spirulina, Fish Oil, Soy Lecithin, Dicalcium Phosphate, L-Ascorbyl-2-Polyphosphate (source of Vitamin C), Choline Chloride, Vitamin E Supplement, Niacin Supplement, Calcium Pantothenate, Riboflavin, Thiamine Mononitrate, Biotin, Pyridoxine Hydrochloride, Folic Acid, Vitamin A Acetate, Vitamin D3 Supplement, Vitamin B12 Supplement, Manganese Sulfate, Zinc Sulfate, Ferrous Sulfate, Copper Sulfate, Sodium Selenite, Potassium Iodate, Propionic Acid (a natural preservative).
Composition (%)		
Crude Protein (Min)	43	35
Crude Fat (Min)	10	5
Crude Fiber (Max)	3	5
Moisture (Max)	11	11
Ash (Max)	13	10
Phosphorus (Min)	1	1

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