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Measuring Fluctuating Asymmetry in Plastron Scutes of Yellow-bellied Sliders: the Importance of Gender, Size and Body Location

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ABSTRACT.—The use of fluctuating asymmetry (FA) estimates in animal research is increasing, but most studies thus far have focused on birds, amphibians and insects. Turtles have bilateral shell scutes that can be easily measured with modern imaging techniques and, therefore, should serve as ideal candidates for FA research, although identifying the most appropriate characters to measure must first be determined. With this issue in mind we undertook the current project to assess levels of plastron scute asymmetry in a common freshwater turtle species, specifically examining the level of variation among scutes and the effects of age (size) and gender on our estimates of scute FA. We photographed 86 museum specimens of adult yellow-bellied sliders, *Trachemys scripta* (32 males, 54 females) and used image analysis software to measure their carapace lengths and absolute differences in left-right surface areas of plastron scutes. We found that scutes varied in the degree of FA, but the magnitude of the variation tended to be higher among males and there was higher FA in general in the forward-most plastron scutes. We also found that scute asymmetry increased with carapace size, indicating that turtle shells become increasingly asymmetrical with age. This may be the result of adjacent scutes growing against each other over time, leading to random flux in symmetrical growth, or from age-related bioaccumulation of pollutants, which could interfere with normal symmetrical shell production.

INTRODUCTION

Minor differences in the degree of symmetry of bilateral characters can signal the developmental stability of an organism (Polak, 2003) and the measurement of such 'fluctuating asymmetry' (FA) is being increasingly used to assess the impacts of a variety of stress-inducing conditions. These conditions include disease (Parris and Cornelius, 2004), pollutants (Gallant and Teather, 2001; Bustnes *et al.*, 2002; Lauck, 2006; Söderman *et al.*, 2007), habitat fragmentation (Anciães and Marini, 2000) and nutritional stress (Swaddle and Witter, 1994), and most studies to date have been conducted on bilateral animals such as birds, insects and herpetofauna. However, there has been little work thus far examining FA in turtles, which is surprising, since all turtles carry with them a bilateral shell which prominently displays all major and minor perturbations in growth for the duration of their lives (Lynn and Ullrich, 1950). Furthermore, there is evidence that inbreeding or suboptimal environments can lead to asymmetries in the number of turtle scutes (Fernandez and Rivera, 2004), which further demonstrates the potential of this species in FA research. Moreover, with recent image analysis techniques (Davis and Grayson, 2007; Davis and Maerz, 2007), bilateral scute pairs in turtle shells, especially those on the flat ventral plastrons, can now be easily photographed and measured digitally which should allow researchers to record even subtle deviations in left-right scute sizes. However, before estimates of FA in turtle shells can be obtained, a thorough examination of the possible factors influencing these estimates is warranted, especially given the unique nature of turtle shells among vertebrates.

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One point to consider in estimating FA in any animal is that not all bilateral characters experience the same degree of asymmetrical growth, perhaps because of differing developmental complexity or differing functionality among traits (Aparicio and Bonal, 2002). Thus, selecting the most appropriate trait for measurement is a necessary first step in any asymmetry study. In most cases where FA is assessed in animals, the characters selected for measurement are on opposite sides of the body and grow or develop somewhat independent of each other, such as the legs of frogs (Söderman *et al.*, 2007), wing feathers of birds (Bustnes *et al.*, 2002) or spots on salamander backs (Wright and Zamudio, 2002; Davis and Maerz, 2007). However, the scutes that make up turtle shells are immediately adjacent to each other and the growth of one can impinge on the growth of the other. In a large comparison of many turtle species, Magwene (2001) showed that plastron scutes grow larger by forming new layers of tissue mostly on the anterior and proximate edges of the previous year's growth. In other words, plastron scutes tend to grow larger by forming new tissue partly along the center suture line. This may result in pressure from opposing scutes directed toward the middle. Furthermore, these pressures could eventually cause flux in scute growth as the turtle grows. Evidence for this idea comes from the fact that in older turtles, the suture line running down the center of the plastron tends to deviate from the straight line normally seen in younger turtles (Fig. 1A, B). Given this fact, we could expect shell scutes to become increasingly asymmetrical as turtles age. If this possibility is true, the age of the turtle would need to be accounted for in any future study of shell asymmetry.

Another important point to consider in estimating FA in turtles is gender. In turtles, males and females of certain species tend to differ in absolute growth rates, with females growing faster than males (Congdon and Gibbons, 1983; Gibbons and Lovich, 1990; Rowe, 1997; Aresco and Dobie, 2000). This difference is either due to physiological differences in metabolism or environmental differences associated with each sex (Gibbons and Lovich, 1990). Regardless of the cause, the difference in growth rates may have implications for the degree of bilateral shell symmetry in either sex. There could be two possible scenarios: female shells could be more asymmetrical than males because their increased growth rates cause more frequent errors in symmetrical growth or females could have more symmetrical shells than males because their faster growth rate is a product of their more efficient use of resources and/or more efficient shell growth (*i.e.*, less prone to errors).

In this study we examined for the first time the degree of fluctuating asymmetry in the shell scutes of a turtle, with the intention of providing information useful for future studies of FA in turtles. We used image analysis software to measure the surface area of all six pairs of plastron scutes from specimens of yellow-bellied sliders, *Trachemys scripta* and derived estimates of FA as the difference in scute area between left and right sides. Using these estimates we examined three possible factors that could influence the estimation of asymmetry in turtles: gender, size and body location (*i.e.*, scute pair). The results of these analyses should provide a framework for future examinations of symmetry in turtle shells.

MATERIALS AND METHODS

Photographing specimens.—We obtained 86 adult yellow-bellied slider specimens from the Georgia Museum of Natural History. Most (67) of the specimens were collected in the state of Georgia, with the remainder from South Carolina (13), Florida (2), North Carolina (2) and Alabama (1). We recorded the sex of the specimen based on tail and forelimb claw lengths (Gibbons, 1990). Photographing the specimens proceeded as follows. Specimens were removed from preservative and air-dried for 20 min. Each specimen was placed on a copystand and photographed from above with a Canon Powershot G6 digital camera

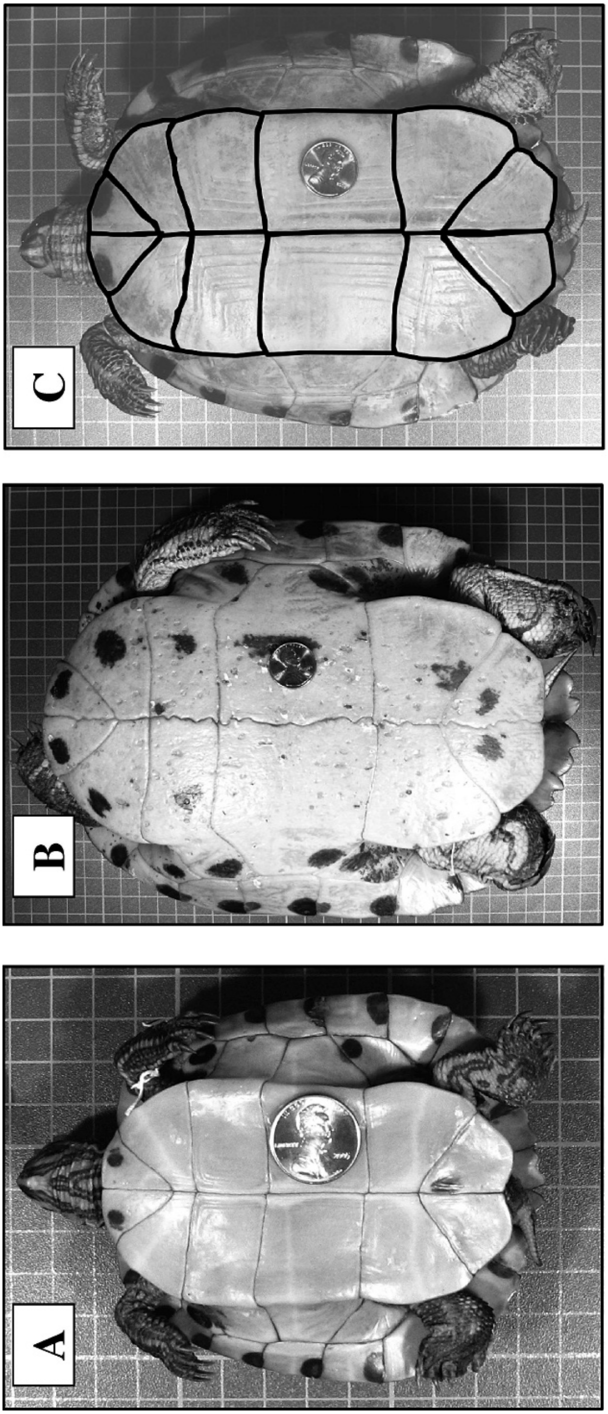

FIG. 1.—Specimens of yellow-bellied sliders shown in ventral photograph position. A penny was photographed with each specimen to provide a common standard to calibrate the image analysis software. The specimen in A is smaller, and presumably younger, than in B. Note the differences in the middle suture line of each specimen. Third image (C) is faded and has scutes outlined in black to demonstrate areas that were measured. Names of scute pairs from anterior to posterior are gular, humeral, pectoral, abdominal, femoral and anal

(Fig. 1). Two pictures were taken of each specimen, dorsal and ventral. Care was taken to ensure that the specimen was centered directly under the camera so that the left and right sides were equally in view in each picture.

Measuring scutes.—We used image analysis procedures to measure the size of the turtles (carapace length) and the 6 plastron scute pairs of each specimen (gular, humeral, pectoral, abdominal, femoral and anal) following digital procedures used for measuring characters of other species (Davis *et al.*, 2004; Davis and Grayson, 2007; Davis and Maerz, 2007). This involved importing the images into Adobe Photoshop with Fovea Pro (Reindeer Graphics, Inc) plugins installed. We then calibrated the image using features in the images that were of known length (1 cm grid squares of the copystand for dorsal pictures and a penny placed on the plastron for ventral). After calibration, our first digital measurement was the size of each specimen, for which we measured the straight-line length of the carapace (in mm) from the dorsal images. From the ventral images we traced the outline of each plastron scute (six scute pairs or 12 total plastron scutes per turtle; Fig. 1C). In most plastron scutes the outer edges were clearly identifiable, however the abdominal (and pectoral to a lesser degree) scutes in turtles have both a flat surface as well as a lateral extension that wraps around the sides of the body, forming the 'bridge' (Ernst *et al.*, 1994). As these bridge sections were not always visible to us in the images, we confined our outlines of these scutes to their flat ventral sections, and if the ventral side of the scute did not have a defined outer edge to trace (frequently in the abdominal scute) we drew a straight line from the point where the adjacent anterior and posterior scutes meet the scute in question (Fig. 1C).

When all scutes were outlined a Fovea Pro measurement routine was initiated that measured the surface area (in mm^2) of each scute outline and saved the information to a text file. From these data we calculated the absolute difference in surface area between the left and right scute for each of the 6 scute pairs. We then transformed the scute asymmetries into percentages of the average scute pair area to account for the fact that larger scutes (or larger turtles) may have larger asymmetries than smaller scutes (or smaller turtles) and to place asymmetry estimates of all turtles on the same scale. We were left with the following variables for each specimen: gender, size (carapace length), and six measures of scute asymmetry (expressed as a percentage, one for each scute pair).

As this was our first attempt to measure asymmetry in turtle scutes using image analysis, at the end of the photographing session we conducted a follow-up test to determine if our method produced repeatable estimates of asymmetry. We selected a random set of 20 specimens of varying sizes, and the plastron of each was photographed twice, in non-consecutive, randomized orders, using the same camera setup as before. The scute pairs were then digitally measured from all images as before, but in this case the turtle identification was hidden from the observer. When all measurements were obtained, we compared the estimate of asymmetry (absolute right minus left area) for each scute pair between the first and second photo of each specimen using a series of paired *t*-tests. There were no significant differences in our estimate of asymmetry between the first and second photo for any of the six scute pairs ($P > 0.05$ for all tests). These results gave us confidence in our digital measures of scute areas obtained earlier, and suggest that there was minimal observer error in our estimates of FA.

Data analysis.—We initially tested for possible geographic variation in size using analysis-of-variance with carapace length as the dependent variable and the state where the specimen was collected as the independent variable. Sex as included in this analysis to account for possible gender-related effects. We also graphically examined the distribution of raw asymmetry scores for each scute pair to ensure that their distributions centered around zero, which is a necessary criteria that must be first met for any measure to be considered true fluctuating asymmetry (van Dongen *et al.*, 1999). For the main analyses of FA we initially transformed the percent asymmetry measures (arcsine square root transformation) to approximate a normal distribution. Next we tested for homogeneity of variance in asymmetry scores between sexes using Levene's test on each scute pair, to ensure that the assumptions of homoscedasticity were not violated. Then to determine the variation in asymmetry due to sex, size and scute location, we used repeated measures analysis-of-variance, with scute as the within-subject factor (with six levels), sex as a categorical independent, and carapace length as a continuous covariate. The estimate of asymmetry (transformed) was the dependent variable. All two-way interactions were also included. All tests were conducted using Statistica (2003) software.

RESULTS

General results.—Of the 86 turtle specimens we measured, 32 were males while 54 were females. Males ranged in size from 114–249 mm with an average of 162 mm. Females ranged from 98–314 mm with an average of 180 mm. In our analyses of geographic variation in size we found no significant variation in carapace length among states ($F_{4,84}$ = 1.98, P = 0.105). There was also no significant difference in size between males and females ($F_{1,84}$ = 1.22, P = 0.272). There was no evidence of differing variance in asymmetry between sexes for any of the 6 scute pairs; all P values from the Levene's test of homogeneity of variances were greater than 0.1. Thus, the assumptions of homoscedasticity were not violated in comparisons of asymmetry between sexes. Raw scute asymmetry scores (right scute area minus left scute area, in cm^2) of all 86 specimens were approximately normally-distributed and centered around zero (Fig. 2).

Results of FA analysis.—Results of our repeated-measures ANOVA revealed a significant difference in scute FA between males and females (Table 1) with males showing greater asymmetry than females in all scute pairs except the gular scutes (Fig. 3). The degree of difference between sexes tended to vary among scutes however, reflected by the near-significant interaction between sex and scute (Table 1). Overall, the humeral scute showed the greatest sex-related difference, followed by the femoral. While there was no overall effect of scute on levels of FA (Table 1), it is apparent from Fig. 3 that scutes near the front of the turtle generally showed higher levels of asymmetry than those near the back, for both sexes. Finally, there was a significant effect of carapace length on scute FA in our study (Table 1) and examination of parameter estimates indicated this effect was positive, such that larger turtles tended to have higher levels of scute FA. Further, since the estimate of scute FA was initially corrected for scute size, this relationship was not an artifact of allometry.

DISCUSSION

Our data indicate that estimations of FA in plastron scutes of yellow-bellied sliders depend on the turtle's gender, size, and which scute is measured. The influence of gender was such that female had more symmetrical scutes than males (Fig. 3). Females of this species, like many other turtle species, have a faster absolute growth rate than males (Congdon and Gibbons, 1983; Gibbons and Lovich, 1990; Rowe, 1997; Aresco and Dobie, 2000), thus it could be argued that faster growth in turtles is indicative of more 'efficient' (*i.e.*, symmetrical) growth, with fewer bilateral errors. Interestingly, higher levels of FA in males than females has also recently been found in frogs (Söderman *et al.*, 2007) and was attributed to the possibility of growth interference by steroid hormones, such that males were considered under more developmental 'stress' than females. This possibility can not be

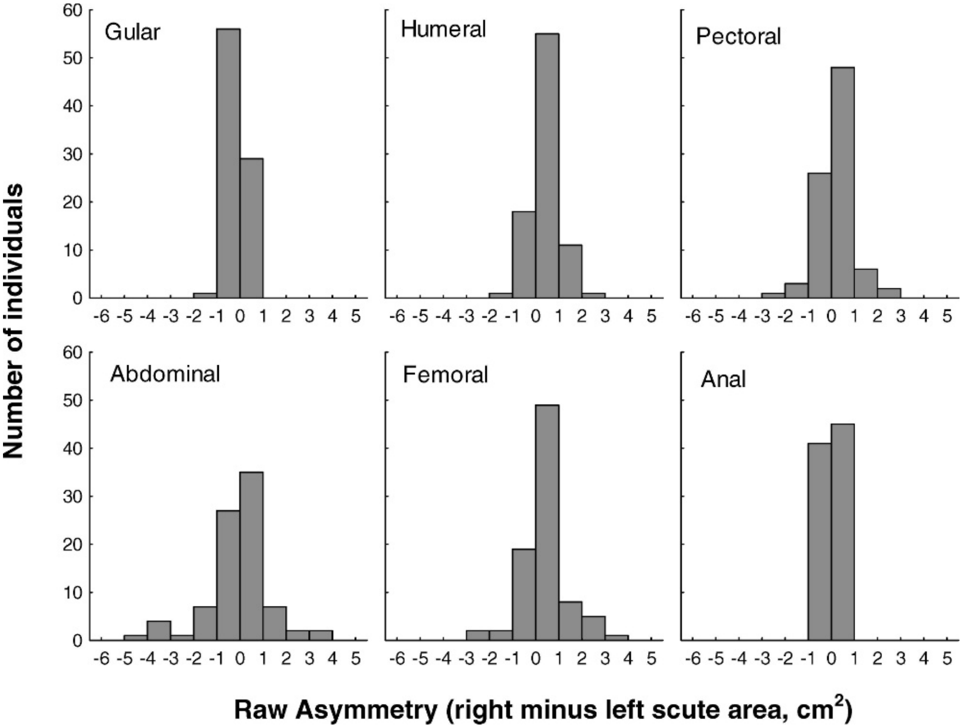

FIG. 2.—Distribution of raw scute asymmetry scores (right minus left scute area, in cm^2) for all 86 specimens in this study

discounted in the turtles examined here, although we can only speculate as to the exact cause. Nevertheless, this sex-related difference is an important component of shell growth that must be taken into account in future studies of FA in turtles.

We also found that as turtles grow their degree of plastron scute asymmetry increases, a result not unexpected based on the nature of scute growth. Magwene (2001) showed that plastron scutes of many turtle species grow larger by forming new layers of tissue mostly on the anterior and proximate edges of the previous year's growth. In other words, plastron

TABLE 1.—Results of repeated measures ANOVA examining effects of scute location (within-subject factor with 6 levels), sex and size (carapace length) on levels of fluctuating asymmetry in plastron scutes of yellow-bellied sliders. The between-subject interaction of sex*carapace length was initially included in the model but removed when found not significant ($P > 0.1$)

Source of variation	Variable	df	MS	F	P
Between-subject	Sex	1	0.089	8.44	0.005
	Carapace Length	1	0.048	4.56	0.036
	Error	83	0.010		
Within-subject	Scute Location	5	0.014	1.81	0.109
	Scute Location*Sex	5	0.017	2.23	0.051
	Scute Location *Carapace Length	5	0.016	2.03	0.073
	Error	415	0.008		

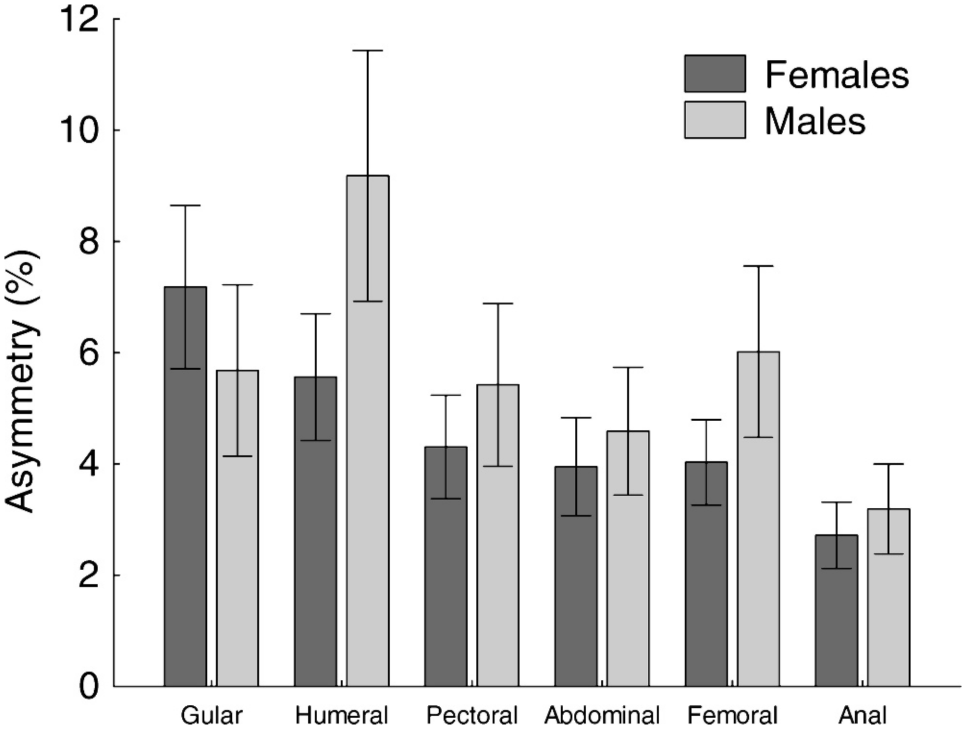

FIG. 3.—Level of scute area asymmetry (absolute difference in left-right scute area, as a percentage of the average scute area) of all scute pairs for male (n = 32) and female (n = 53) sliders in this study. Error bars represent 95% confidence intervals

scutes tend to grow larger in part by forming new tissue along the turtle's center suture line. This may result in pressure from opposing scutes directed toward the middle and would explain the frequent observations of 'squiggly' center suture lines in the plastrons of older individuals (Fig. 1A, B). This pressure could in time cause random flux in the normally symmetrical growth of scute pairs, and based on this idea we expected scute asymmetries to increase with turtle size (as a proxy for age). However, it is also possible that turtle scutes may become increasingly asymmetrical with age, not necessarily because of increasing pressures between scutes, but because longer lived individuals simply have more opportunities for erroneous growth. Still another possibility is that older specimens may bioaccumulate toxins over time, which have been found in slider turtles (Tryfonas *et al.*, 2006), and which may interfere with normal physiological processes such as tissue growth. Regardless of the reason, these data point to the importance of also accounting for the effect of age (or size) in future studies of scute symmetry.

The degree of fluctuating asymmetry varied among the six plastron scute pairs in our study with greater asymmetries toward the front of the turtles (Fig. 3). Meanwhile, anal scutes varied the least, both in raw asymmetry (Fig. 2) and size-corrected asymmetry (Fig. 3). Since we knew that turtle scutes can vary in growth rates (Ernst *et al.*, 1998), we were unsurprised at this variability. However we had no *a priori* expectations of which scutes would be most variable in terms of FA. Part of the difficulty in making predictions on this variation stems from the paucity of studies examining growth patterns in plastron scutes in turtles, but it also is because we did not envision possible differences in scute functionality. In other words, plastron scutes serve to protect the lower portion of the turtle and we suspected that all scutes have this same function and no others. However, since our result indicate a degree of variation in FA among scutes, it is, therefore, possible that this variation is driven simply by flux in shell growth. If so, this variation would make scutes ideal for estimation of FA in turtles, although our data suggest that more anterior scutes show the highest levels of FA and would be most appropriate for measurement.

Fluctuating asymmetry has been shown to be an important predictor of fitness in multiple species of organisms (*e.g.*, Møller, 1996; Møller, 1999; Møller and Manning, 2003; Johnson *et al.*, 2004; Davis and Maerz, 2007). Because of this, measures of fluctuating asymmetry at the individual or population level are well-recognized as important tools in animal research and conservation (Leary and Allendorf, 1989; Clarke, 1995). As long-lived animals that can face lengthy exposures to pollutants and toxins, we suggest that future examinations of FA in turtles are warranted, especially given the ease at which turtle shells can be measured using current image analysis software. The methodology used here should provide a framework for such studies, but our results highlight the importance of accounting for size, gender and body location in future work.

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LITERATURE CITED

ANCIÃES, M. AND M. Â. MARINI. 2000. The effects of fragmentation on fluctuating asymmetry in passerine birds of Brazillian tropical forests. *Journal of Applied Ecology*, **37**:1013–1028.

APARICIO, J. M. AND R. BONAL. 2002. Why do some traits show higher fluctuating asymmetry than others? A test of hypotheses with tail feathers of birds. *Heredity*, **89**:139–144.

ARESCO, M. J. AND J. L. DOBIE. 2000. Variation in shell arching and sexual size dimorphism of river cooters, *Pseudemys concinna*, from two river systems in Alabama. *Journal of Herpetology*, **34**:313–317.

BUSTNES, J. O., I. FOLSTAD, K. E. ERIKSTAD, M. FJELD, Ø. O. MILAND AND J. U. SKAARE. 2002. Blood concentration of organochlorine pollutants and wing feather asymmetry in Glaucous Gulls. *Functional Ecology*, **16**:617–622.

CLARKE, G. M. 1995. Relationships between developmental stability and fitness: application for conservation biology. *Conservation Biology*, **9**:18–24.

CONGDON, J. D. AND J. W. GIBBONS. 1983. Relationships of reproductive characteristics to body size in *Pseudemys scripta*. *Herpetologica*, **39**:147–151.

DAVIS, A. K. AND K. L. GRAYSON. 2007. Improving natural history research with image analysis: the relationship between skin color, sex, size and stage in adult red-spotted newts (*Notophthalmus viridescens viridescens*). *Herpetological Conservation and Biology*, **2**:67–72.

——— AND J. C. MAERZ. 2007. Spot symmetry predicts body condition in spotted salamanders, *Ambystoma maculatum*. *Applied Herpetology*, **4**:195–205.

———, B. FARREY AND S. ALTIZER. 2004. Quantifying monarch butterfly larval pigmentation using digital image analysis. *Entomologia Experimentalis et Applicata*, **113**:145–147.

ERNST, C. H., J. E. LOVICH AND R. W. BARBOUR. 1994. Turtles of the United States and Canada. Smithsonian Institution Press, Washington, DC.

———, J. C. WILGENBUSCH, T. P. BOUCHER AND S. W. SEKSCIENSKI. 1998. Growth, allometry and sexual dimorphism in the Florida box turtle, *Terrapene carolina bauri*. *Herpetological Journal*, **8**:72–78.

FERNANDEZ, C. A. AND A. C. RIVERA. 2004. Asymmetries and accessory scutes in *Emys orbicularis* from Northwest Spain. *Biologia*, **59**:85–88.

GALLANT, N. AND K. TEATHER. 2001. Differences in size, pigmentation, and fluctuating asymmetry in stressed and nonstressed northern leopard frogs (*Rana pipiens*). *Ecoscience*, **8**:430–436.

GIBBONS, J. W. 1990. Life History and Ecology of the Slider Turtle. Smithsonian Institution Press, Washington, DC.

——— AND J. E. LOVICH. 1990. Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). *Herpetological Monographs*, **4**:1–29.

JOHNSON, O., K. NEELY AND R. WAPLES. 2004. Lopsided fish in the Snake River Basin - fluctuating asymmetry as a way of assessing impact of hatchery supplementation in chinook salmon, *Oncorhynchus tshawytscha*. *Environmental Biology of Fishes*, **69**:379–393.

LAUCK, B. 2006. Fluctuating asymmetry of the frog *Crinia signifera* in response to logging. *Wildlife Research*, **33**:313–320.

LEARY, R. F. AND F. W. ALLENDORF. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology and Evolution*, **4**:214–217.

LYNN, W. G. AND M. C. ULLRICH. 1950. Experimental production of shell abnormalities in turtles. *Copeia*, **1950**:253–262.

MAGWENE, P. M. 2001. Comparing ontogenetic trajectories using growth process data. *Systematic Biology*, **50**:640–656.

MØLLER, A. P. 1996. Parasitism and developmental instability of hosts: a review. *Oikos*, **77**:189–196.

———1999. Asymmetry as a predictor of growth, fecundity and survival. *Ecology Letters*, **2**:149–156.

——— AND J. MANNING. 2003. Growth and developmental instability. *Veterinary Journal*, **166**:19–27.

PARRIS, M. J. AND T. O. CORNELIUS. 2004. Fungal pathogen causes competitive and developmental stress in larval amphibian communities. *Ecology*, **85**:3385–3395.

POLAK, M. (ed.). 2003. Developmental Instability: causes and Consequences. Oxford University Press, New York, NY.

ROWE, J. W. 1997. Growth rate, body size, sexual dimorphism and morphometric variation in four populations of painted turtles (*Chrysemys picta bellii*) from Nebraska. *American Midland Naturalist*, **138**:174–188.

SÖDERMAN, F., S. VAN DONGEN, S. PAKKASMAA AND J. MERILÄ. 2007. Environmental stress increases skeletal fluctuating asymmetry in the moor frog *Rana arvalis*. *Oecologia*, **151**:593–604.

STATISTICA. 2003. Statistica version 6.1. Statsoft Inc.

SWADDLE, J. P. AND M. S. WITTER. 1994. Food, feathers and fluctuating asymmetries. *Proceedings of the Royal Society of London Series B*, **255**:147–152.

TRYFONAS, A. E., J. K. TUCKER, P. E. BRUNKOW, K. A. JOHNSON, H. S. HUSSEIN AND Z. Q. LIN. 2006. Metal accumulation in eggs of the red-eared slider (*Trachemys scripta elegans*) in the Lower Illinois River. *Chemosphere*, **63**:39–48.

VAN DONGEN, S., L. LENS AND G. MOLENBERGHS. 1999. Mixture analysis of asymmetry: modelling directional asymmetry, antisymmetry and heterogeneity in fluctuating asymmetry. *Ecology Letters*, **2**:387–396.

WRIGHT, A. N. AND K. R. ZAMUDIO. 2002. Color pattern asymmetry as a correlate of habitat disturbance in spotted salamanders (*Ambystoma maculatum*). *Journal of Herpetology*, **36**:129–133.

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