2022, Scienceline Publication
World's Veterinary Journal
World Vet J, 12(1): 81-86, March 25, 2022
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2022.wvj11

Relationship between Plastron Color and Nutrition in *Pseudemys nelsoni* Carr, 1938

Pere Miquel Parés-Casanova* **and Albert Martínez-Silvestre**

CRARC, Catalonia Reptiles and Amphibians Rescue Center, Masquefa, Catalonia, Spain

*Corresponding author's Email: pmpares@gencat.cat; ORCID: 0000-0003-1440-6418

ORIGINAL ARTICLE
pii: S232245682200011-12
Received: 21 January 2022
Accepted: 18 March 2022

ABSTRACT

Body coloration of emydids can be triggered by different types of factors. Therefore, the current study aimed to investigate the changes in plastron color of Florida Red-bellied Turtle *Pseudemys nelsoni* (*P. nelsoni* Carr, 1938), an emydid of North America. In the current study, 15 (3 males and 12 females) fresh corpses of captive-reared adult specimens of *P. nelsoni* were analyzed using digital images as well as applying geometric morphometrics and color photo processing techniques. Plastron color had no relationship with size nor fluctuating asymmetry, which could be considered as a negative proxy for stress. Moreover, there were no significant differences between males and females in this regard. It can be suggested that reddish on plastron for *P. nelsoni* was highly related to feeding, compared to other external factors, such as age, size, or stress. In wild *P. nelsoni* populations, reddish plastral coloration was related to body size probably due to ontogenetic differences in the diet, as juveniles are omnivorous. Since adults are herbivores, reddish fading observed in the samples of the current study would be a mere expression of unnatural colors, which can probably be linked to unbalanced feeding. The results of the current research could contribute to the understanding of the ways color changes appear in captive turtles in response to differences in dietary access to carotenoids.

Keywords: Chromatism, Coloration, Diet, Emydidae, Fresh-water turtles, Red-bellied turtle, Shell, Terrapins

INTRODUCTION

Developmental instability (DI) appears when there are disturbances to normal development (Benítez and Parra, 2011). The DI is commonly measured through fluctuating asymmetry (FA, Van Valen, 1962), which represents a deterioration in morphological developmental homeostasis (Coster et al., 2013; Ducos and Tabugo, 2014). Fluctuating asymmetry results from the difference between left and right in bilaterally symmetrical organisms or parts of them, and it provides a measure of how well an individual can buffer its development against genetic or environmental stress during ontogeny (Klingenberg and Mcintyre, 1998).

Animal coloration is a complex morphological trait produced by biochemical and biophysical interactions originating from environmental factors (Brejcha et al., 2019; Surasinghe et al., 2019). The color variation among emydids occurs particularly on the head, limbs, and shell (Surasinghe et al., 2019). The function of the color is not exactly known, but in studies of other vertebrates, chromatic changes are the basis of a visual trait that is sexually selected (Steffen et al., 2019).

Pseudemys is a genus of freshwater turtles (family Emydidae, subfamily Deirochelyinae) from several species distributed throughout the southeastern region of the United States and south into northern Mexico (Cline Dillard, 2017). It is generally accepted that the genus can be broken down into two distinct subgeneric clades, including the red-bellied cooters and the river cooters (Cline Dillard, 2017). Florida Red-bellied Turtle (*Pseudemys nelsoni* Carr, 1938; Figure 1) belongs to the first lineage, the *rubriventris* series, sometimes referred to as subgenus *Pseudemys* (*Ptychemys*, Ward and Jackson, 2008; Jackson, 2010). It is a moderately large turtle (carapace length to 37.5 cm) that is relatively abundant in freshwater wetlands throughout peninsular Florida and extreme southeastern Georgia (Jackson, 2010; Rhodin et al., 2017). The species is one of the largest emydids in North America, with females (to 37.5 cm) typically growing larger than males (to 30 cm). *Pseudemys nelsoni* (*P. nelsoni*) is known for its red-colored ventral shell, or plastron, of juveniles (Jackson, 2010; Rhodin et al., 2021). As animals age, this reddish tint tends to fade and become less distinct (Rhodin et al., 2021).

Animal body coloration is a complex trait resulting from the interplay of multiple color-producing mechanisms (Brejcha et al., 2019). Protein excess and low calcium-phosphorus ratios can lead to the development of pyramidal-shaped scutes among some Chelonia species (Gerlach, 2004). Integument color changes in response to differences in

To cite this paper: Parés-Casanova PM and Martínez-Silvestre A (2022). Relationship between Plastron Color and Nutrition in *Pseudemys nelsoni* Carr, 1938. *World Vet. J.*, 12 (1): 81-86. DOI: https://dx.doi.org/10.54203/scil.2022.wvj11

dietary carotenoid access of Emydid turtles have been relatively unstudied and to date, no research has been done to document objectively the changes in plastron color of captive-reared *P. nelsoni*. It is interesting to see how aspects of health can interact with the exogenous provision of carotenoids. Therefore, the current study aimed to investigate if color plastron in *P. nelsoni* is correlated to size (interpreted as age) and gender, however also to FA, among captive animals.

Figure 1. Florida Red-bellied Turtle *Pseudemys nelsoni* (*P. nelsoni* Carr, 1938). Picture: Albert Martínez-Silvestre.

MATERIALS AND METHODS

Ethical approval

Corpses were from animals whom private keepers had released into the wild. They had been captured and euthanized, according to the official control of invasive species in Spain, therefore no specific ethical approval was necessary for the use of these collection specimens.

Samples

A total of 15 euthanized corpses of adult specimens (3 males and 12 females) of *P. nelsoni* were obtained from Catalonia Reptiles and Amphibians Rescue Center (CRARC) Catalonia, Spain, collection. None of the corpses presented malformations or lesions that could influence the symmetry of the studied region. All animals were healthy and in good corporal condition.

Imaging

Each turtle was leveled dorsally in accordance with a horizontal plane. Image capture was performed with a Nikon® D70 (Japan) digital camera (image resolution of 2.240 × 1.488 pixels) equipped with a Nikon AF Nikkor® 28-200 mm telephoto lens. The camera was placed on a stand so that the focal axis of the camera was parallel to the horizontal plane and centered on the plastral (ventral) aspect. A scale was put over each specimen.

Geometric morphometrics

Each picture (including a total of 15 images) was then transported to TPSUtil (Rohlf, 2015) to convert the files. The digitation process was followed utilizing TPSDig2 (Rohlf, 2015). A set of 13 landmarks (3 mid-sagittal and 5 per side) were located on each plastron (Figure 2) on intersections of different scutes. This process was carried out twice to estimate the measurement error (Fruciano, 2016).

A Generalized Procrustes Analysis (GPA) eliminated scale, translational, and rotational differences of the coordinate data of the landmarks (Webster and Sheets, 2010). The coordinate data of each specimen was then scaled by Centroid Size (CS), a dimensionless parameter computed as the square root of the sum of squared distance between each landmark and the plastron centroid (Bookstein, 1991). The CS and GPA-scaled coordinates represent surrogates of size and shape, respectively (Webster and Sheets, 2010). Centroid size was considered as an age proxy for the interpretation of results.

To cite this paper: Parés-Casanova PM and Martínez-Silvestre A (2022). Relationship between Plastron Color and Nutrition in *Pseudemys nelsoni* Carr, 1938. *World Vet. J.*, 12 (1): 81-86. DOI: https://dx.doi.org/10.54203/scil.2022.wvj11

To detect the components of variances and deviations, a Procrustes ANOVA was used. In Procrustes ANOVA, the individual effect denotes the individual variations of shape and size, the main effect of sides indicates the variation between sides and is considered as the measure of directional asymmetry (DA), the individuals x sides is the mixed effect, indicating FA in the data, and the measurement error represents the variation due to measurement error in taking landmarks of the same individual in separate sessions (Klingenberg and McIntyre, 1998).

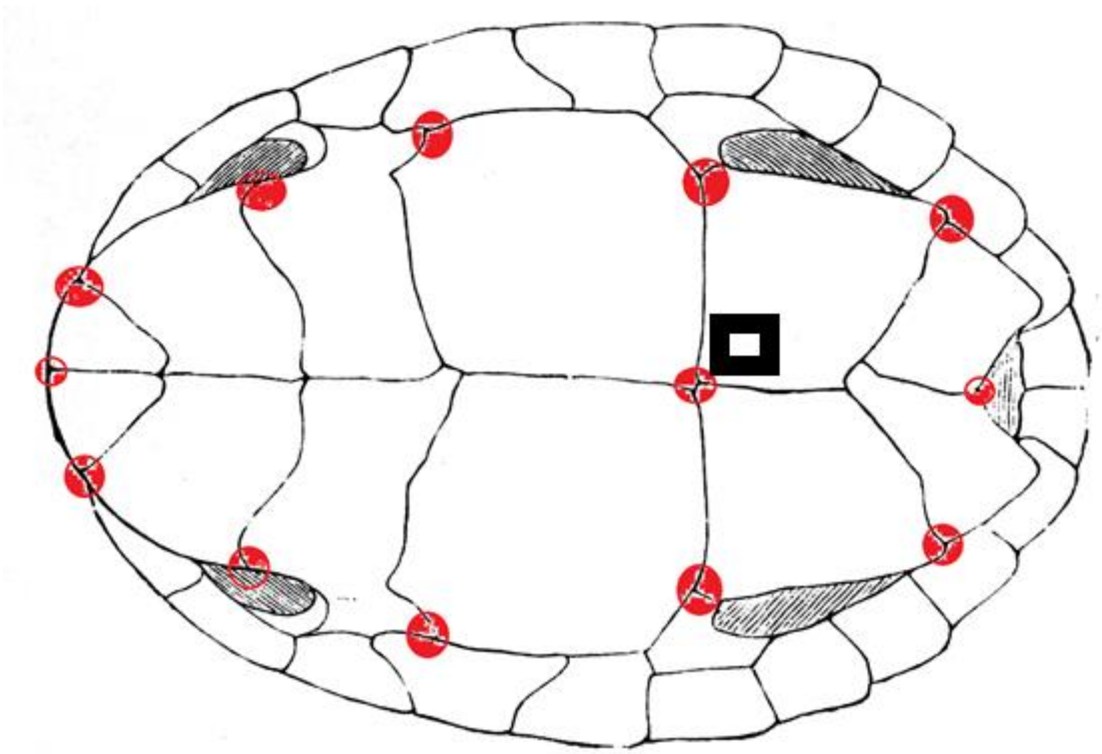

Figure 2. Landmark configuration used in the study composed of 13 discrete on 2D pictures of *Pseudemys nelsoni* plastron (3 mid-sagittal and 5 pairs). The right side corresponds to gular scutes. Color was analyzed on the squared area, close to the midsagittal line of the left femoral scute.

Color analysis

Plastron color was represented by a combination of RGB values for red, green, and blue color channels, having percentual values, which were registered individually on the same scute area for each animal. A linear fit tested the correlation between reddish and size. Finally, multivariate regression was performed on reddish color on asymmetric values. Values of colors were $\log_{10}$-transformed for analyses.

Gender differences

Since *P. nelsoni* is sexually dimorphic, differences in terms of gender were previously determined. An NPMANOVA (Non-Parametric Multivariate Analysis of Variance) test was applied to detect differences between genders for three colors and for asymmetry (using Euclidean distances) and a Mann-Whitney *U* test was applied to detect size differences between genders.

For all statistical analysis, MorphoJ software version 1.07a (Klingenberg, 2011) and PAST software v. 2.17c (Hammer et al., 2001) were used. P values less than 0.05 were considered statistically significant. For color analysis, GIMP 2.6.11 software was utilized (Reinke et al., 2018). As no normal distribution across individuals was expected, permutation tests were used to sample from 10,000 random iterations without replacement.

RESULTS

The variance associated with asymmetry was significantly greater than the variance produced by measurement error, which represented a 3% (Table 1) so data allowed precise estimation of asymmetries. Wilcoxon paired test reflected no statistical differences between two color replicas, which presented on average a 6.7% of the difference. As gender had no significant effect on colors, asymmetry, and size ($p > 0.05$), males and females were clustered for ulterior analysis. Procrustes ANOVA for shape showed a significant effect of FA, however not of DA (Table 1). The Pillai trace confirmed these results (0.69 and 7.78 for DA and FA, respectively). Reddish color ranged from 53 to 89% (average 71.9%), not being correlated with size ($p > 0.05$). Individual asymmetric values were not correlated to reddish color (multiple $R^2 = 0.083$; Wilk' $\lambda = 0.317$; $F_{23,16} = 0.559$; $p < 0.05$).

To cite this paper: Parés-Casanova PM and Martínez-Silvestre A (2022). Relationship between Plastron Color and Nutrition in *Pseudemys nelsoni* Carr, 1938. *World Vet. J.*, 12 (1): 81-86. DOI: https://dx.doi.org/10.54203/scil.2022.wvj11

Table 1. Summary of Procrustes ANOVA for the shape of plastral scute asymmetry for *Pseudemys nelsoni*. Values are dimensionless and were taken from males and females.

Effect		SS	MS	Df	F	P values
Size	Individuals	253973.49	18140.96	14	4.16	0.0048
	Error	65419.98	4361.33	15		
Shape	Individuals	0.0423	0.0002	154	7.24	<0.0001
	DA	0.0002	0.00002	11	0.6	0.825
	FA	0.0058	0.00003	154	3.31	<0.0001
	Error	0.0037	0.00001	330		

*FA: Fluctuating asymmetry for size and shape. DA: Directional asymmetry, FA: Fluctuating asymmetry, SS: Sums of squares, MS: Mean squares. Units are in Procrustes distances (dimensionless). Df: Degrees of freedom. The individuals effect denotes the individual variations, the main effect of DA indicates the variation between sides. FA is the mixed effect. The error represents the variation due to measurement error in taking landmarks of the same individual in two separate sessions. P values less than 0.05 were considered statistically significant.

DISCUSSION

Although normally undetectable by visual inspection, a perfect balance between opposing sides is not the reality of bilaterally symmetric biological bodies. It is widely thought that FA is a useful proxy of wellbeing, as it indicates developmental stress (Niemeier et al., 2019). Fluctuating asymmetry has been widely used to detect stress among turtles (Rivera and Claude, 2008; Parés-Casanova et al., 2019; Ibrahim, 2020). As an initial hypothesis, it was thought that reddish plastron and bony exoskeletal FA for the ventral side of the turtle in *P. nelsoni* would be related to fitness. The reason is that animals with the lower corporal condition would absorb less light and thus were unable to deposit carotenoids. In other words, since plastron coloration is phenotypically plastic (Ibáñez et al., 2017; Steffen et al., 2019; Surasinghe et al., 2019), less stressed animals would increase plastral reddish chroma. However, according to the obtained results, although FA in adult *P. nelsoni* was significant, asymmetry and size were not correlated with reddish plastral color. Therefore, reddish plastral color was not either correlated to gender and size (aging).

Colorful dots, patches, and stripes of many chelonians are made up, at least in part, of carotenoids (Ibáñez e al., 2017). Animals cannot synthesize carotenoids, therefore the production of carotenoid-based coloration is ultimately linked to its ability to obtain dietary carotenoids (Surasinghe et al., 2019) by eating algae and a variety of aquatic plants from their environment (Steffen et al., 2019). Integument color changes in response to differences in dietary carotenoid access in other wild species (Steffen et al., 2019). In wild *P. nelsoni* populations, reddish plastral coloration is related to body size (age) probably due to ontogenetic differences in the diet (Ibáñez et al., 2017), as juveniles' diet is omnivorous, while adults are vegetarians (Jackson, 2010). This observation suggests that natural feeding promotes carapace pigment production, however, the causal factor (food) is not found in the captive environment. It is suggested that feeding affects this condition. Carotenoid-based pigmentation has been suggested for other species, such as European pond turtles (*Emys orbicularis*, Ibáñez et al., 2017) and Eastern painted turtles (*Chrysemys picta*, Surasinghe et al., 2019). Plastron reddish color seems to vary considerably within captive animals of the current study regardless of their gender and age. Red chromatism appeared to be under the same cause for both genders. Red-bellied turtles are predominately herbivorous, but juveniles tend to primarily consume small insects (Jackson, 2010). According to the study of De Vosjoli (1991), loss of color in captive box turtles would be partially due to a diet lacking plant pigments so supplementing the diet with plant pigments helps maintain color therefore reddish fading observed in the samples from captive animals would be a mere expression of unnatural colors, probably be linked to unbalanced feeding.

CONCLUSION

In Red-bellied turtles, *Pseudemys nelsoni*, the absence of related-age reddish fading on plastron would be an expression of unnatural colors when animals are reared in captivity, probably linked to artificial feeding. Studies correlating terrapins color and sexual dichromatism with artificial management are yet in their infancy. The results of our research can contribute to the understanding of how emydids show an integumentary color change in response to differences in dietary access.

DECLARATION

Authors' contributions

Pere M. Parés-Casanova conceived and designed the experiment, analyzed the data, wrote the first draft of the paper. Albert Martínez-Silvestre contributed to the discussion of results. Authors checked and approved the final draft of the manuscript before submission to this Journal.

To cite this paper: Parés-Casanova PM and Martínez-Silvestre A (2022). Relationship between Plastron Color and Nutrition in *Pseudemys nelsoni* Carr, 1938. *World Vet. J.*, 12 (1): 81-86. DOI: https://dx.doi.org/10.54203/scil.2022.wvj11

Competing interests

Authors declare no competing interests.

Ethical consideration

Ethical issues including plagiarism index, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked and confirmed by the authors.

Acknowledgments

Joaquim Soler, from the CRARC, Catalonia Reptiles, and Amphibians Rescue Center, Masquefa, Catalonia, Spain, provided the corpses. We are also grateful to anonymous reviewers for their constructive comments.

REFERENCES

Benítez HA, and Parra LE (2011). Fluctuating asymmetry: A morpho-functional tool to measure development stability. International Journal of Morphology, 29(4): 1459-1469. DOI: https://www.doi.org/10.4067/s0717-95022011000400066

Bookstein FL (1991). Morphometric tools for landmark data: Geometry and Biology. In Morphometric tools for landmark data: Geometry and biology. Cambridge University Press, 35: 512. DOI: https://www.doi.org/10.1002/bimj.4710350416

Brejcha J, Bataller JV, Bosáková Z, Geryk J, Havlíková M, Kleisner K, Maršík P, and Font E (2019). Body coloration and mechanisms of color production in Archelosauria: The case of deirocheline turtles. Royal Society Open Science, 6: 1-34. DOI: https://www.dx.doi.org/10.1098/rsos.190319

Cline Dillard K (2017). A comparative analysis of geometric morphometrics across two Pseudemys turtle species in East central Virginia. Thesis. Virginia Commonwealth University. DOI: https://www.doi.org/10.25772/TA0F-XX11

Coster G, de Dongen S, van Malaki P, Muchane M, Alcántara-Exposito A, Matheve H, and Lens L (2013). Fluctuating asymmetry and environmental stress: Understanding the role of trait history. PLoS One, 8(3): 1-9. DOI: https://www.doi.org/10.1371/journal.pone.0057966

De Vosjoli P (1991). The general care and maintenance of box turtles: Including sections on chinese and malayan box turtles. Advanced Vivarium Systems. Available at: https://www.abebooks.com/9781882770113/General-Care-Maintenance-Box-Turtles-1882770110/plp

Ducos MB, and Tabugo SRM (2014). Fluctuating asymmetry as an indicator of ecological stress and developmental instability of Gafrarium tumidum (ribbed venus clam) from Maak and Lagoon Camiguin Island, Philippines. International Journal of the Bioflux Society, 7(6): 516-523. Available at: http://www.bioflux.com.ro/docs/2014.516-523.pdf

Fruciano C (2016). Measurement error in geometric morphometrics. Development Genes and Evolution, 226(3): 139-158. DOI: https://www.doi.org/10.1007/s00427-016-0537-4

Gerlach J (2004). Effects of diet on the systematic utility of the tortoise carapace. Journal of the Herpetological Association of Africa, 53(1): 77-85. DOI: https://www.doi.org/10.1080/21564574.2004.9635499

Hammer Ø, Harper DAT, and Ryan PD (2001). Past: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1): 229. Available at: https://palaeo-electronica.org/2001_1/past/past.pdf

Ibáñez A, Alfonso Marzal JM, and Bertolero A (2017). The effect of growth rate and ageing on color variation of European pond turtles. The Science of Nature, 104: 49. DOI: https://www.doi.org/10.1007/s00114-017-1469-1

Ibrahim RW (2020). Conformal geometry of the turtle shell. Journal of King Saud University-Science, 32(3): 2202-2206. DOI: https://www.doi.org/10.1016/j.jksus.2020.02.024

Jackson D (2010). Pseudemys nelsoni carr 1938- florida red-bellied turtle. Conservation Biology of Freshwater Turtles and Tortoises, 1894: 041.1-041.8. DOI: https://www.doi.org/10.3854/crm.5.041.nelsoni.v1.2010

Klingenberg CP (2011). MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources, 11(2): 353-357. DOI: https://www.doi.org/10.1111/j.1755-0998.2010.02924.x

Klingenberg CP, and Mcintyre GS (1998). Geometric morphometrics of developmental instability: Analyzing. Evolution, 52(5): 1363-1375. DOI: https://www.doi.org/10.2307/2411306

Niemeier S, Müller J, and Rödel MO (2019). Fluctuating asymmetry – Appearances are deceptive. Comparison of methods for assessing developmental instability in european common frogs (Rana temporaria). Salamandra, 55(1): 14-26. Available at: https://www.medsci.cn/sci/show_paper.asp?id=c5aed119e25967e9

Parés-Casanova PM, Cladera M, and Martínez-Silvestre A (2019). Adaptative directional asymmetric shape in Testudo hermanni hermanni Gmelin, 1789 (Reptilia: Testudines: Testudinidae). Herpetology Notes, 12: 743-747. Available at: https://www.biotaxa.org/hn/article/view/45074

Reinke B, Pearson S, and Selman W (2018). Plastron pigmentation variation in a coastal turtle species of conservation concern (Malaclemys terrapin). Herpetologica, 74(2): 141-145. DOI: https://www.doi.org/10.1655/HERPETOLOGICA-D-17-00046.1

Rhodin AGJ, Iverson JB, Bour R, Fritz U, Geogres AR, Shaffer B, and van Dijk PP (2017). Turtles of the world. Annotated checklist and atlas of taxonomy, syninymy, distribution, and conservation satatus. 8th edition. Chelonian Research Foundation and Turtle Conservancy. Available at: http://georges.biomatix.org/storage/app/uploads/public/598/525/d26/598525d266c50880989684.pdf

Rhodin AGJ, Iverson JB, Bour R, Fritz U, Georges A, Shaffer HB, and van Dijk PP (2021). Turtles of the world. annotated checklist and atlas of taxonomy, synonymy, distribution, and conservation status. In R. A. Rhodin, A.G.J., Iverson, J.B., van Dijk, P.P., Stanford, C.B., Goode, E.V., Buhlmann, K.A., and Mittermeier (Ed.), Conservation Biology of Freshwater Turtles and Tortoises:

A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group, 9th edition. Chelonian Research Foundation and Turtle Conservancy. DOI: https://www.doi.org/10.3854/crm.8.checklist. atlas.v9.2021

Rivera G, and Claude J (2008). Environmental media and shape asymmetry: A case study on turtle shells. Biological Journal of the Linnean Society, 94(1993): 483-489. DOI: https://www.doi.org/10.1111/j.1095-8312.2008.01008.x

Rohlf FJ (2015). The tps series of software. Hystrix, 26: 9-12. DOI: http://www.dx.doi.org/10.4404/hystrix-26.1-11264

Steffen JE, Hultberg J, and Drozda S (2019). The effect of dietary carotenoid increase on painted turtle spot and stripe color. Comparative Biochemistry and Physiology Part-B: Biochemistry and Molecular Biology, 229: 10-17. DOI: https://www.doi.org/10.1016/j.cbpb.2018.12.002

Surasinghe T, Christen R, Dewey A, Gouthro A, Tocchio K, Sheehan B, McCulley T, and Dobeib Y (2019). Variable plastron coloration of the eastern painted turtles Chrysemys picta picta in a single locality of South-Eastern Massachusetts, USA. Herpetological Bulletin, 150: 23-25. DOI: https://www.doi.org/10.33256/hb150.2325

Van Valen L (1962). A study of fluctuating asymmetry. Evolution, 16: 125-142. Available at: https://www.jstor.org/stable/2406192

Ward J, and Jackson D (2008). Pseudemys concinna (Le Conte 1830)-River cooter. conservation biology of freshwater turtles and tortoises. Chelonian Research Monographs, pp. 2-7. DOI: https://www.doi.org/10.3854/crm.5.006.concinna.v1.2008

Webster M, and Sheets HD (2010). A practical introduction to landmark-based geometric morphometrics. In J. A. and G. Hunt, editors, quantitative methods in paleobiology. The Paleontological Society, pp. 163-188. Available at: https://geosci.uchicago.edu/~mwebster/Webster_and_Sheets_2010.pdf

To cite this paper: Parés-Casanova PM and Martínez-Silvestre A (2022). Relationship between Plastron Color and Nutrition in *Pseudemys nelsoni* Carr, 1938. *World Vet. J.*, 12 (1): 81-86. DOI: https://dx.doi.org/10.54203/scil.2022.wvj11