Morphometric pattern in Caretta caretta (Linnaeus, 1758) (Cheloniidae) hatchlings from nests with different embryo development rates

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Abstract
The geometric morphometric analysis of the shell of Caretta caretta hatchlings revealed that morphological variations may be related to incubation duration. Based on the overlapping of anatomical landmarks of the carapace and the plastron, it was possible to discriminate hatchlings from slow and fast developing clutches. Carapace and plastron of hatchlings from nests where incubation lasted less than 55 days are rounder as compared to the hatchlings from nests where incubation took 67 days. The differences observed in shell shape in terms of incubation duration were statistically significant, though carapace and plastron shape overlapping was observed in several individuals. Our results indicate that the incubation duration explains only a small part of the total variation in the shell shape as a whole. Yet, in spite of the low discriminant function coefficient, cross-validation tests indicated that 84.7% and 77.8% of the hatchlings were correctly categorised concerning the carapace and plastron, when the descriptive variable is incubation duration.

Keywords: loggerhead, carapace, plastron, geometric morphometric, shape.

Resumo
A análise da morfometria geométrica do casco de filhotes recém-eclodidos de Caretta caretta revelou que existem variações morfológicas que podem ser relacionadas à duração da incubação. Com base na sobreposição de marcos anatômicos da carapaça e do plastrão, foi possível discriminar filhotes oriundos de ninhos com taxa de desenvolvimento embrionário rápido daqueles filhotes provenientes de ninhos em que o desenvolvimento embrionário foi mais lento. A carapaça e o plastrão de filhotes oriundos de ninhos com duração de incubação inferior a 55 dias são mais arredondados que os dos filhotes provenientes de ninhos com mais de 67 dias de incubação. A variação da forma casco em função da duração da incubação foi estatisticamente significativa, mas ocorreu uma sobreposição na forma da carapaça e do plastrão de numerosos indivíduos. Os nossos resultados indicam que o comprimento da incubação foi capaz de explicar apenas uma pequena parte da variação total do conjunto da forma do casco. Mas, apesar do baixo coeficiente de determinação da função discriminante, testes de validação cruzada indicam que 84.7% dos filhotes foram classificados corretamente em relação à carapaça e 77.8% em relação ao plastrão, considerando a duração da incubação como o descritor.

Palavras-chave: tartaruga marinha, carapaça, plastrão, morfometria geométrica, forma.
1. Introduction

Considering the course of natural selection, shape is one of the most important expressions of the phenotype. As far as sea turtles are concerned — which present high mortality rates in the early life stages and high survival rates in adult life — studies on morphology and the influence of shape in the survival of hatchlings offer an important contribution for the understanding of population biology (Roff, 1992; Myers et al., 2007). In turtles, studies aimed at assessing survival and behaviour of hatchlings normally utilise body size and mass of individuals as variables, and indicate a strong correlation between hatching body and survival (Janzén et al., 2000a,b, 2007, Tucker et al., 2008). Recent studies on freshwater turtles indicate that the ability to swim and therefore to avoid predation correlates with plastron shape, and shorter and wider Trachemys scripta (Schöpff, 1792) swim faster than longer and skinnier individuals (Myers et al., 2007). Since plastron shape is highly heritable, it may constitute a key role in the early life stages of turtles (Myers et al., 2006).

On the other hand, nest site is of crucial importance in embryo development, affecting the offspring phenotype (Glen et al., 2003). Apart from sex, which in all sea turtle species depends on the incubation temperature, the nest environment affects body size, developmental and growth rates and behaviour (O’Steen, 1998; Packard, 1999; Kolbe and Janzén, 2001). Turtle hatchlings, in general, do not exhibit apparent sexual dimorphism — a trait that makes it even more difficult to sort and quantify the effects of phenotypic trait on survival and behaviour against the effects induced exclusively by sex (Congdon et al., 1999).

Incubation duration is defined as the period that starts with oviposition and ends with hatching, and is negatively correlated with the thermal conditions of nests (Marcovaldi et al., 1997). In general, incubation is shorter in nests exposed to higher temperatures, as compared to those exposed to low temperatures (Mrosovsky et al., 2009). In spite of the restrictions concerning temperature oscillations during incubation (Georges et al., 1994, 2004), several studies utilise length of incubation as the indicator of the thermal conditions throughout embryogenesis (Mrosovsky et al., 1999, 2009; Godfrey et al., 1999; Godfrey et al., 2001).

The present work evaluates the influence of incubation duration on the shape of C. caretta hatchlings. The shape of hatchlings was investigated based on the morphometric geometric analysis of carapace and plastron. The relationship between shell shape and sexual dimorphism of hatchlings is discussed later.

2. Material and Methods

One hundred and eighty-three C. caretta hatchlings from Comboios beach (19°40’ S and 39°50’ W), in Linhares, Espírito Santo state, Brazil, were used in the geometric morphometric analysis. Embryo development was considered slow or fast, depending on length of incubation. Hatchlings that hatched within 67 and 76 days were considered as slow embryo development individuals, while those that hatched within 50 to 55 days after oviposition were considered as fast development hatchlings. This sorting was conducted based on the pivotal incubation duration, defined as the number of days the nest requires in order to produce 50% of males and 50% of females (Marcovaldi et al., 1997; Godfrey et al., 1999; Mrosovsky et al., 1999, 2009). Pivotal incubation duration for the Comboios beach was estimated by Marcovaldi et al. (1997) to be 59.9 days, and it was confirmed by Mrosovsky et al. (1999) to be 59.2 days. Hatchlings were collected in two stages: the first stage being in December, when 75 specimens that incubated for more than 67 days were photographed; and the second stage being in February, when 108 hatchlings, which hatched in less than 55 incubation days, were photographed. Hatchlings were collected as they emerged from each nest. To reduce the clutch effect, only 10 hatchlings from each nest were used. Hatchlings were photographed alive because it was necessary to hold them for the photos to be taken orthogonally to the carapace and plastron centre. After photography, hatchlings were released on the beach. For the geometric morphometric analysis, the techniques developed by Valenzuela et al. (2004) and Myers et al. (2006) were adopted. To analyse plastron shape, only the external landmarks of scutes were used because the internal limits, which separate left and right sides, were often “malformed” due to the incomplete or recent absorption of the yolk or because the hatchlings had not completely unfolded yet. Because the variation in the internal part of the plastron shape was too high, we chose to use only the external part of scutes (Figure 1a). For the carapace shape, the anatomical landmarks used were the same as those used by Valenzuela et al. (2004). The external limit of the supracaudal scute (landmark 30) was added to the carapace shape (Figure 1b). An image file was created using the software tpsUtil (Rohlf, 2006a), and the anatomical landmarks were digitised in the program TpsDig2 (Rohlf, 2006b). Generalised Procrustes analysis (GPA) was used to superimpose the anatomical landmarks, generating a mean hatching shape (Figure 1c, d). The Procrustes distance represents the distance between two points in Kendall’s multidimensional space, and it is used to measure the similarity between the shapes (Monteiro and Reis, 1999). In this process, the variation in hatching size and the orientation of the photos are overlooked, and the variations found are attributed only to the shape of the shell. After the overlapping of specimens, the average specimen, 56 shape variables as partial warps scores and two other standard uniform components, a linear (U matrix) and a non-linear (W matrix) from the thin-plate spline (TPS), were calculated for the carapace shape. These variables, denominated weight matrix, indicate the necessary change for the shape of each individual to transform into the average specimen generated by the overlapping of every individual’s landmark. For the plastron shape, the same procedure was adopted, in which 20 variables were calculated from the 12 anatomical landmarks. To evaluate whether the carapace and plastron shapes vary with incubation duration, a
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3. Results

The MANOVA results show that the carapace shape of *C. caretta* hatchlings presents significant differences credited to incubation duration. Permutation tests also indicate the significant differences between carapace shapes (*p* < 0.0001). Cross-validation analysis shows that 84.7% of hatchlings were classified correctly, although the differences due to incubation duration explain only 6.75% of the total carapace-shape variation.

The PCA analysis of carapace shape shows an ample overlapping of the carapace shape when fast and slow development hatchlings are compared. The carapace of hatchlings for which incubation lasted more than 67 days is compressed in the cranial region, whereas the anal region, close to the medium scutes, is broader and longer, in comparison to those of hatchlings incubated for less than 55 days. The hatchlings that developed faster have a more rounded carapace, and the rear part of the shell is broader (Figure 2).

The shape of the plastron also differs between hatchlings with distinct embryo development rates, and permutation

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Figure 1. Anatomical landmarks used in the geometric morphometric analysis of a) the carapace; and b) the plastron of *C. caretta* hatchlings. Mean shape of the carapace generated by c) 183 hatchlings; and d) for the plastron created by superimposing 177 hatchlings.
tests show a significant shape variation (p = 0.0001) due to incubation duration. The cross-validation test revealed a correct classification of 77.8% hatchlings for plastron shape, but the differences in plastron shape attributed to length of incubation explain only 2.7% of the variation. The PCA shows an overlap between plastrons of slow and fast growing hatchlings and the two main axes explain 36% of the variation in weight matrix (Figure 3).

The plastrons of hatchlings that developed more slowly are longer, and plastrons of hatchlings that had faster development are shorter and wider. In both cases, there are some similarities in the external geometry of plastrons and carapaces that tend to be more rounded in hatchlings that hatched within 55 days, and longer in those incubated for over 67 days.

4. Discussion

The use of geometric morphometric analysis reveals that there are significant differences in the carapace and plastron shapes of C. caretta hatchlings and these differences are

![Figure 2. Plot of the first axes of the principal components analysis of carapace shape of 183 C. caretta hatchlings. PC1 explains 28% of the carapace shape variation and PC2 explains 14%. The circles represent hatchlings with more than 67 incubation days and squares represent hatchlings with less than 55 days. The carapace thin-plate spline, predicted for negative values of the score average of canonical variable 1 (on the left side), represent hatchlings with fast development and for positive values (on the right side), hatchlings with slow development.]

![Figure 3. Plot of the first axes of the principal components analysis of plastron shape of 177 C. caretta hatchlings. PC1 explains 13.9% of the carapace-shape variation and PC2 explains 10.4%. The circles represent hatchlings with more than 67 incubation days and squares represent hatchlings with less than 55 days. The carapace thin-plate spline, predicted for negative values of the score average of canonical variable 1 (on the left side), represent hatchlings with fast development and for positive values (on the right side), hatchlings with slow development.]

associated to length of incubation. The results are similar to those found by Valenzuela et al. (2004) study that evaluated the relationship between shell shape and sex of hatching of freshwater turtles Podocnemis expansa (Schweigge, 1812) and Chrysemys picta (Schneider, 1783). This method is more effective and easier to perform than the method that uses linear measurements to identify morphological differences in carapace and plastron shapes (Hildebrand et al., 1997; Michel-Morfin et al., 2001; Glen et al., 2003). Nevertheless, a very high percentage of variation in shape (92.7% for the carapace and 97.2% for the plastron) cannot be attributed to the development-rate group (fast or slow). This number is too high to be overlooked in the analyses. In the permutation and cross-validation tests that support discriminant functions for the total data, the results from cross-validation tests are actually more important than the coefficient of determination in this case, because if these results had any practical importance, the technique to be used would be that of discriminant analysis, not multivariate regression. Overlapping carapace and plastron shapes in a number of specimens are easily explained. Other effects, such as multiple paternity of nests (Harry and Briscoe, 1988; Moore and Ball Jr., 2002), laying more than once during a nesting season (Hawkes et al., 2005), maternal characteristics (Myers et al., 2006, 2007), like temperature oscillations during incubation, embryo development rate (Georges et al., 1994, 2004), and sex (Reece et al., 2002) may explain the mosaic of factors that interact to create the final shape of the hatchlings’ shells.

However, some methodological questions that might have influenced the results should be highlighted. The most recommended procedures indicated to identify sex of hatchlings are radiouimmunoaassay (RIA) and histological inspection (Wyneken et al., 2007). In this study, pivotal incubation duration (Marcovaldi et al., 1997; Mrososky et al., 1999) was used to predict the two groups with fast and slow development rates, possibly formed by female and male hatchlings, in that order. This method is based on the length of embryonic development, and it is recommended for a large number of nests so that the effects of climatic variations and the nest microenvironments are weakened (Mrososky et al., 2009). The length of duration is an indicator of thermal environment and of embryo development rate. There is a strong correlation between incubation temperature and embryo development rate, with consequences for incubation duration and the sex ratio of hatchlings. In eggs of C. caretta incubated in a laboratory under constant temperatures above 29 °C (called pivotal temperature, yielding 50% offspring of each sex), the embryos developed faster, and more females were produced. Below 29 °C embryo development is slower and the sex ratio of hatchlings reveals the predominance of males (Marcovaldi et al., 1997). Embryo development rate is affected by temperature throughout incubation, including those temperatures the nest is exposed to before and after the thermosensitive period, after which sex is irreversibly defined. Temperature oscillations during incubation and the respective effects on embryo development rate, incubation
length and sex of hatchlings are the main limitations against using incubation pivotal duration as a tool to predict sex ratio in natural nests (Georges et al., 1994, 2004). Since sex of hatchlings was not histologically identified in the present study, overlapping shell shapes may in part be explained by sex difference.

In some cases, when the temperature oscillates during incubation, male hatchlings occur in high temperature and female hatchlings in low temperature (Pieau, 1982; Gross et al., 1995; Valenzuela, 2001). To minimise this effect, we used hatchings from nests that hatched at least five days beyond the pivotal incubation duration. Even though a difference of over 12 days was initially considered between the group of hatchlings with fast (< 55 days) and slow (> 67 days) development, the use of incubation length may not have been a criterion efficient enough to sort hatchlings by sex. Even so, cross-validation analysis results show high values for both carapace (84.7% correctly classified) and plastron (77.0% correctly classified) shapes which compare well with Valenzuela et al. (2004), which showed a correct classification of 85% of sex of P. expansa hatchlings. The results obtained in this study should be an encouragement to perform more thorough studies involving the direct determination of the sex of hatchlings to test if the discriminant function obtained can be extrapolated to predict the sex of other hatchlings.

Complementary studies may show the place, the meaning, and the intensity of the carapace allometric variation between hatching, juvenile, and adult turtles (Monteiro et al., 1997; Myers et al., 2006). Furthermore, other species and populations should be investigated because it is possible to individualise turtle populations in geographically close areas and (Myers et al., 2006) to quantify the variability in shape as a heredity function (Monteiro et al., 2002). Experiments relating shape to body size, locomotor performance and behaviour may help understand the importance of the phenotype in the survival of hatchlings in the early life stages of sea turtles. These examples show the possibilities and amplitude of geometric morphometric analysis in ecological studies.

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