EVALUATION OF ORGAN PLACEMENT AS INDICATOR OF ARBOREALITY IN INVASIVE *BOA CONSTRICTOR* SPECIES IN PUERTO RICO

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ABSTRACT

Hemodynamics has been one of the main factors to study the arboreality of snake species since they have shown a direct relationship with the positioning of the heart and gravity. Topographic anatomy is used as a habitat use indicator in snakes by searching for relations between the position and morphology of internal organs and external measurements. The objective of this study was to determine the arboreality of the invasive species *Boa constrictor* in Puerto Rico by studying the positioning of the heart relative to the total body length and observe if there is a difference between the arboreality behavior between adults and sub-adults. We collected and examined data from 86 snakes from a period of three months, in which the specimens were dissected and the relative position of 5 organs to the heart were measured and Total Body Length was calculated using snake size. It was determined that there was no significant difference between the two categories (25% -adults / 27% -subadults), which implied that there was a general arboreality for the species. More detailed analysis revealed that *B. constrictor* is a semi-arboreal species, with sub-adults being more terrestrial and adults more scansorial, that is, the ability to climb trees.

INTRODUCTION

Mainly located on the west side of Puerto Rico, *Boa constrictor* is an invasive species of snakes on the island. Native to the continental neotropics, it is believed to have been introduced primarily by the pet trade (Vega-Ross, 2018). Because of its variety of skin patterns, colors, large size, and relative docility, *B. constrictor* are in high demand in international and domestic trade (Reed et. al, 2009). Once in the wild, its generalist diet and the lack of natural predators favors fast growth and distribution of the population. Although its ecological impact has not been established, it is responsible for eating small mammals, birds, and reptiles, decreasing biodiversity and heterogeneity of native fauna on the island (Reynolds et. al, 2013).

Topographic anatomy is being used as a habitat use indicator in snakes. It focuses on looking for relations between the position and morphology of internal organs and external measurements or structures (Aveiro-Lins, 2006). Regularly, snakes have a characteristic organ topography that is consistent among species. However, variations in heart position have been explained as an adaptation to gravitation environments and phylogeny (Anderson et. al, 2015).

In arboreal and terrestrial snakes, the heart occupies anterior positions whereas in aquatic species it is closer to mid-body (Lillywhite et. al, 2012). Meanwhile, semiaquatic-fossorial and arboreal-terrestrial groups are statistically intermediate (Seymour, 1987). Studies have shown that cardiac overflow and arterial pressure are related to gravity stress on blood circulation and can be a countermeasure to natural selection, morphology, and physiology of snake populations (Lillywhite et. al, 2012; Aveiro-Lins, 2006). Regulation of blood pressure involves barostatic reflexes that over time provokes the shifts of body fluids to preserve the blood volume controlled by mechanoreceptors (Lillywhite, 1987). During movements in a vertical plane, as for the case of arboreal or semi-arboreal species, hydrostatic pressure changes for which the displacement of the heart from a central location represents an advantage as to minimize energy cost of perfusing (Lillywhite, 1987).

There is insufficient data whereas the arboreality of *B. constrictor* is terrestrial or semi-arboreal. Since larger snakes tend to have higher hydrostatic components of blood pressure, it is suggested that young snakes, because of their size, are relatively more arboreal than adults in some species (Seymour, 1987; Lillywhite et. al, 2012). These differences in arboreality are expected to correlate with the differences of positioning of internal organs, especially the heart. This study aims to determine the arboreality of *B. constrictor* through the study of the positioning of organs, with a focus on the heart as to the head. Here, we test the hypothesis that there exists a difference in arboreality between the population of adults and sub-adults of *Boa constrictor*.

OBJECTIVES

- Determine the arboreality for *Boa constrictor* by the study of the position of the heart in relation to the rest of the body.
- Compare the positioning of the heart between two groups: sub-adults and adults.
- Observe if there is a tendency in arboreality between juvenile groups and adult groups.

METHODOLOGY

Sample recollection

Data from 85 snakes (Data collected from September to November 2020) from the species *Boa constrictor* were obtained and examined. The snakes were already euthanized when provided by the Department of Environmental Natural Resources (DRNA).

Data collection

Part of the methodology was adapted from Perez, 2019. First, the specimens were taken out of the cold storage, thawed, stretched and laid out on a table. They were identified with the code BOCO, which stands for *Boa constrictor*, the number of the dissection of the day and the date of dissection. Additionally, the snakes' body mass was measured using a handheld digital scale. Using a measuring tape, the distance in cm from the snout to vent, SVL (cloacal opening) was recorded. The length of the tail consisted of the tip of the tail to the vent; adding the tail length and the SVL the value of the Total Body Length (TBL). Height, length, and width of the head were measured too. A dissection was later performed, in which incisions were made across the ventral surface and the integuments at the margins of the incisions were deflected for the exposure of internal organs (Perez, 2019). The relative distance of each organ, (distance between de snout and the middle of the organ of interest) were measured. The positional data was obtained from the distance between snout to the heart, liver, gallbladder, anterior and posterior limits of the stomach, right and left kidneys and intestines of the snake. In addition, the focus of the measurements was the distance from the middle of the heart to the midsection of the organs. It should be noted that for the measurements made from snout to anterior limit of the stomach, the starting point of the stomach was where the tissue of the esophagus became thicker and increased in lumenous circumference; on the other hand, the posterior limit of the stomach measured where the circumference of the valve decreased. Finally, the specimens were discarded according to the regulations of the university.

Statistics Analysis

Within the sample of 85 snakes that were taken, these were divided into the categories of sub-adults (includes neonates and juveniles) and adults, with a sample size of 26 and 59, respectively. This distribution of the samples was based on the minimum SVL of this species for these to be adults, which is 90 cm and 120 cm for the male and female, respectively (Reed & Rodda, 2009). Any value below these parameters is considered sub-adult. A series of statistical tests were performed to measure the distance from the middle of the heart to each of the mentioned organs. These tests include the average distance from mid heart to each organ, ±SD, variance, a Pearson correlation of data recollected. Additionally, an average and ±SD were calculated for the TBL and the heart to snout position in order to use that data and calculate the percentage of the average heart position relative to TBL, $\left(\frac{\textit{Heart to snout distance}}{\textit{Total Body Length}}\right) \times 100$. A T-test was performed to compare sub-adults and adults and represent it in a Box Plot graph. Finally, a linear regression was assembled to examine and determine the influence of positioning of the heart relative to TBL.

RESULTS AND DISCUSSION

In general, a disparity has been noted in the scientific community regarding the arboreality of *Boa constrictor*, since it varies between terrestrial and semi-arboreal (Harrington, et al., 2018). It was established that there is a tendency to different arborealities between sub-adults and adult boas, since both show habitat segregation (Reed & Rodda, 2009). The measured organs distances are presented in Table 1. The Pearson correlation showed that all the distances of the mid organs with respect to the heart gave positive values, which indicates a directly proportional relationship. The present study further supports the fact that while the specimens grow, the position of the organs will appear to move relatively anteriorly in the body cavity (Anderson, et al., 2015).

The heart position and percentage of heart positioning in relation with TBL is shown in **Table 2**. Average TBL was 73.59 (±0.013) and heart position was 19.86 (±4.67) for *Boa constrictor* sub-adults. Also, for the average TBL for adult snakes was 167.28 (±33.86) and heart position was 41.81cm (±8.32). When the percentage of the heart position was calculated and analyzed, it was observed that there was no significant percentage difference between the two groups, since the mean percentages of sub-adults and adults was 27.02% and 25.06%, respectively. Based on the data shown in **Table 2**, the Box Plot graph (**Figure 1**)consisted of the summarized values of the measurements of the position of the heart relative to the total length of the body of the categories sub-adults and adults. Q2 of the box plot shows the median of the positioning of the heart with respect TBL, as also seen in **Table 2**. With the graph, T tests were also performed, and it was shown that there wasn't a significant difference between the means of both groups. No difference was detected when comparing the percentages for adults and sub-adults (T-test, t = 6.565, df= 84, p = < 0.001). It should be noted that according to recent studies, intraspecific variation in the positioning of organs was generally smaller than was the interspecific variation, and measurements were generally consistent within species (Perez, 2019). These results imply that there is no specific arboreality for a certain stage of development, but that its arboreality is the same regardless of the stage of development in which the Boa constrictor is.

A linear regression was performed with all the data because there was no difference in heart positioning relative to TBL. According to Lillywhite, the heart positioning in arboreal/ scansorial species occupy around 15% of the Total Body Length, whereas heart positioning in aquatic snake species is nearly 45% of the TBL (Lillywhite; et. al, 2012). In the case of semi arboreal and non-climbing terrestrial snake species, they seem to appear intermediate or closer to the 15% of arboreal species. As seen in **Figure 2**, the result of this analysis showed that as the species increases in size, its percentage of positioning of the heart with respect to the TBL decreases. This shows that smaller snakes tend to move away from 15% and while they increase in size, they are approaching that percentage value of arboreality. Based on the results of the linear regression, we can determine that the subadults show a tendency to be more terrestrial compared to the adults, who have an arboreal tendency. However, regardless of the group, the behavior of the *Boa constrictor* species continues to be closer to semi-arboreality.

Table 1. Pearson Correlation values for the relationship of the position of the organs with respect to the position of the heart in subadults and adults of the *Boa constrictor*.

	Sub-Adults (n=26)					Adults (n=59)				
Organ	Average position (Heart to organ)	SD (Heart to organ)	Variance (Heart to organ)	Pearson Correlation (Heart to organ)	p-Value	Average position (Heart to organ)	SD (Heart to organ)	Variance (Heart to organ)	Pearson Correlation (Heart to organ)	p-Value
Mid Liver	24.642	5.729	32.826	0.975	3.558×10^{-17}	51.967	10.623	112.851	0.867	1.403×10^{-18}
Mid Gallbladder	30.909	7.607	57.862	0.978	5.863×10^{-18}	65.801	13.083	171.163	0.665	1.210×10^{-8}
Anterior Lim. Stomach	27.107	6.514	42.432	0.966	1.393×10^{-15}	57.634	11.552	133.449	0.563	4.230×10^{-6}
Posterior Lim. Stomach	30.911	7.762	60.256	0.973	1.007×10^{-15}	66.267	12.976	168.390	0.672	7.500×10^{-9}
Mid right kidney	34.274	9.093	82.690	0.960	9.652×10^{-15}	76.214	15.548	241.738	0.606	4.760×10^{-7}
Mid left kidney	35.170	9.342	87.275	0.975	4.000×10^{-17}	77.929	15.539	241.474	0.611	3.470×10^{-7}
Mid intestine	34.272	7.586	57.547	0.939	1.170×10^{-12}	71.259	14.361	206.234	0.563	4.200×10^{-6}

Table 2. Heart to snout position and data percentage of heart positioning relative to the Total Body Length values for sub-adults and adults of the *Boa constrictor* in Puerto Rico.

	Sub-Adults	Adults
n	26	59
Average Heart position (cm)	19.86 (±4.67)	41.81 (±8.32)
TBL (cm)	73.59 (±0.013)	167.28 (±33.86)
% Heart position relative to TBL	27.02%	25.06%

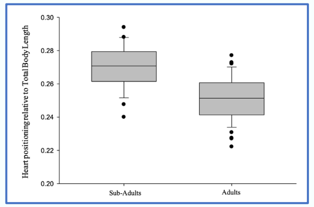

Figure 1. Box-plot distribution of the heart relative to total body length for sub-adults and adults *Boa constrictor.*

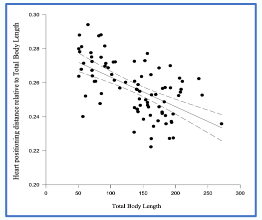

Figure 2. Linear regression representing the distribution position of the heart relative to the Total Body Length to determine the level of arboreality of the species (HtoH-relTBL = 0.281 - (0.000174 * Total Body Length), R^2 = 0.327).

CONCLUSIONS

- The percentage difference between the two groups, sub-adults and adults, did not represent a significant difference, which implies that the species at the intraspecific level show a specific arboreality, which is semi-arboreality behavior .
- Based on our results, sub-adults may be expressing a terrestrial behavior meanwhile the adults showed a more scansorial (i.e., having the capability or adaptation for climbing) behavior.

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