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The Relationship Between Cold Stress Syndrome Mortality And Body Shape in Florida Manatees

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ABSTRACT

West Indian manatees (*Trichechus manatus latirostris*) are tropical and subtropical aquatic mammals that can develop cold stress syndrome (CSS) when subjected to water temperatures below 20°C for prolonged periods. This study investigates the potential impact that cold winter water temperatures in Florida may have on manatee body shape as a selective force to reduce surface-area-to-volume ratio (SA:Vol; i.e. Bergmann's and Allen's Rule). Morphometric measurements collected from state-mandated necropsies (1974-2014) were used to calculate surface area, volume, and mass. We found that manatees that died of CSS on average had significantly greater SA:Vol and less mass compared to those that died of other causes, and that for one unit (0.01) increase in SA:Vol, the odds of succumbing to CSS increases by 34.6%.

INTRODUCTION

Florida manatees (*Trichechus manatus latirostris*), a subspecies of the West Indian manatee, are tropical and subtropical aquatic mammals that are currently characterized as endangered according to the International Union for Conservation of Nature (IUCN, 2008). Their negative encounters with humans and habitat-loss has affected their survival rates and mortality numbers, but their efficient immune system allows them to be exceptionally resilient against natural diseases and the repercussions of human-related traumas (Bossart, 1999). An exception to their resistance is the challenges they face with cold weather environments. The manatee thermoregulation involves peripheral circulation and hypodermic fat lining; however, unlike cetaceans and pinnipeds, manatees have a thick skin and lack a "true" or continuous layer of blubber to keep them warm (Gallivan *et al.*, 1983). Consequently, environmental temperatures will most likely act as an impact on their physiological homeostasis.

Prolonged exposure to temperatures below 20°C can lead to the development of a complex disease called cold stress syndrome (CSS) that harms the metabolic, dietary, and immunologic well-being (Irvine, 1983); this can stimulate further opportunistic and idiopathic diseases, potentially leading to mortality (Bossart *et al.*, 2002). Symptoms include thin/weak appearance, fat and lymphoid depletion, skin/heart lesions, and digestive tract inflammation.

The mammals' physiological need to maintain an internal homeostasis, as well as their size and shape being related to morbidity and mortality (O'Shea, 1988), may explain how environmental conditions, especially climate temperature, could potentially act as a natural selection on body structure and morphology, which contributes to survival. A biological principle described by Bergmann states that similar species located in colder habitats tend to be larger, while those in warmer areas tend to be smaller (Ashton *et al.*, 2000). Additionally, Allen's rule extends this prediction to surface-area-to-volume ratio (SA:Vol) in relation to heat dissipation; for endothermic animals, colder climates will select for smaller SA:Vol (short, thick limbs and stocky bodies), while warmer climates will select for larger SA:Vol (long, thin limbs with slender bodies) (Tilkens *et al.*, 2007). Consequently, manatees with a larger surface-area-to-volume ratio and smaller mass are more likely to succumb to CSS, thereby being naturally selected out of the population.

Although this project calls for the determination of the SA:Vol of the manatee body for comparison, many of the recent studies involving manatee morphometrics have only proposed ways to predict Antillean manatee (*T. m. manatus*) body size using craniometrical parameters (Castelblanco *et al.*, 2014), to determine the weight of the Florida manatee with morphometric measurements (Rigney & Flint, 2011), and to estimate the body mass of Amazonian manatees using morphometric data (Amaral *et al.*, 2010). No studies so far have introduced a method or calculation to estimate the body surface area and volume of a manatee, in order to obtain a ratio of those two values.

This study takes on the basis of the predictions from the biological principles of Bergmann and Allen and examines the natural selective force of cold water temperatures on Florida manatee morphology (i.e., shape and size) adaptation in order to aid for survival. We predicted that Florida manatees that died from CSS would have a significantly larger SA:Vol compared to those that died of other causes (human-related & natural; Fig. 1), due to an inability to cope with lower environmental temperatures.

METHODOLOGY

Florida manatee necropsy data, recorded from 1974-2014, were obtained from the Florida Fish & Wildlife Conservation Commission. Those data of 3,459 fresh and slightly decomposed manatees included identification numbers, date of capture, morphometric measurements, and causes of death. In order to address the research question and hypothesis, the methodology of this study requires data extraction and statistical analysis.

Data Extraction. The initial filter of the study excludes the manatee calves ("perinatals") as well as those with body lengths of 150 cm or less. A young manatee's dependence on its mother's milk serves as a source of energy for metabolism that aids them in coping against energy loss from physical activity and cold temperatures. Furthermore, a manatee's age, size, ability to migrate, and experience are known to be associated with morbidity and mortality, which justifies why adult manatees are more fit to encounter the cold compared to the calves, thereby being the appropriate ones selected for study (O'Shea, 1988).

The manatee morphology was superimposed by two types of cones (right circular cone & circular truncated cone) in order to calculate SA:Vol (Fig. 2); the body representation using those two shapes was considered standard and appropriate, since not every body part can be accurately represented by shape and not all the possible measurements were collected (e.g. true neck region cannot be identified, flipper and tail thickness were not recorded during necropsies). The necropsy data were then filtered for manatee morphometric lengths, shown in Fig. 3, that were needed to determine SA:Vol through the following mathematical formulas:

1. *Manatee Surface Area (SA)*

Right Circular Cone SA = $\pi(\frac{P}{2\pi})[(\frac{P}{2\pi}) + \sqrt{J^2 + (\frac{P}{2\pi})^2}]$

Circular Truncated Cone SA = $\pi[\sqrt{(G - J + L)^2 + ((\frac{P}{2\pi}) - (\frac{N}{2\pi}))^2} \cdot ((\frac{P}{2\pi}) + (\frac{N}{2\pi})) + (\frac{P}{2\pi})^2 + (\frac{P}{2\pi})^2]$

Total SA = Right Circular Cone SA + Circular Truncated Cone SA

2. *Manatee Volume (Vol)*

Right Circular Cone Vol = $\pi(\frac{P}{2\pi})^2(\frac{J}{3})$

Circular Truncated Cone Vol = $\frac{1}{3}\pi(G - J + L)[(\frac{P}{2\pi})^2 + (\frac{N}{2\pi})^2 + (\frac{P}{2\pi})(\frac{N}{2\pi})]$

Total Vol = Right Circular Cone Vol + Circular Truncated Cone Vol

After the determination of SA:Vol for the "adult" manatee counts, the data were organized into cause-of-death (COD) categories "CSS" & "Other". Only the "CSS" deaths during the winter months (Dec-Mar) and the "Other" deaths during non-winter months (Apr-Nov) were selected for this study; this allows observation of manatee carcasses recently exposed to extreme cold snaps, which can give better insight into the impact of acute CSS on these mammals. Furthermore, "Other" deaths that occurred during the winter months were not used because it be argued that they could have died of CSS if they had not died of other unrelated causes first.

Further filtering of data involved removal of any outliers that seem to have both overwhelming and underwhelming morphometric lengths, perhaps due to input error, relative to the known ranges. Additional removal of manatee counts from the sample population included those that were collected in 1984 and prior; because CSS was not officially considered as a cause of death until 1984, any manatee that truly died from CSS could be mistaken for dying from natural causes (Schwarz, 2007).

An additional calculation borrows a formula from Rigney and Flint (2011), which determines the manatee mass using straight length and umbilical girth, two morphometric measurements which are available from the data set in this study; weight was calculated in order to standardize the two COD groups and to take into account the scaling effect, allowing for observation on how body shape can vary independent of mass.

Statistical Analysis. The average SA:Vol and the average mass between "CSS" and "Other" were analyzed with an independent sample t-test to put into perspective the magnitude of difference between the two categories of COD. Then, the mass and SA:Vol values were log-transformed to examine the main effects of mass, COD, and the interaction of the two with a linear model explained by an analysis of covariance (ANCOVA). Finally, the predicted probabilities and odds ratios of succumbing to CSS relative to SA:Vol value were determined using a logistic regression analysis.

RESULTS

The mean SA:Vol for manatees that died of CSS ($\bar{x}$=0.152, SD=0.0286) was significantly larger than manatees that died of Other causes ($\bar{x}$=0.127, SD=0.0269; t(251.78)=-9.123, p=2.2 $\times 10^{-16}$; see Fig. 4). In addition, the mean mass for manatees that died of CSS ($\bar{x}$=216.3, SD=156.3) was significantly smaller than manatees that died of Other causes ($\bar{x}$=360.9, SD=181.3; t(306.38)=9.063, p=2.2$\times 10^{-16}$; see Fig. 5).

The values of SA:Vol and mass for CSS and Other (Graph 1) were log transformed into a linear regression (Graph 2) for the ANCOVA test. From the graph, the regression for CSS was defined by $y=-0.339x-0.0617$ and $R^2=0.9898$, while the regression for Other was defined by $y=-0.343x-0.0499$ and $R^2=0.9879$. The results of the ANCOVA test are shown in Table 3; the factors of mass, COD, and the interaction of the two all significantly impacted SA:Vol ratio, $p=2\times10^{-16}$, 0.0001, and 0.002 respectively.

An analysis of logistic regression predicts the probability of manatees succumbing to CSS with SA:Vol as the predictor. A test of the full model against a constant-only model showed statistical significance ($X^2(1) = 74.92, p<0.0001$). Furthermore, the odds ratio of 1.346 indicates that for every increase of 0.01 in SA:Vol value, the probability of succumbing to cold stress increases by 34.6%.

DISCUSSION

Independent of mass, manatees that died of CSS on average had a significantly greater SA:Vol compared to those that died of other causes, which aligns with the prediction of our study. A greater SA:Vol would mean greater energy expenditure to deal with heat dissipation, which is a likely explanation for CSS death due to the inability to cope with cold water temperatures. Additionally, CSS manatees also had on average significantly less mass than Other manatees. Taking a look at mass is to account for the scaling effect, which means that having larger SA:Vol would be due to a smaller size.

After comparing the predictions of SA:Vol vs Mass (Graph 1) with the actual data (Graph 2), we can infer that natural selection appears to have narrowly restricted the Florida manatee body shape to a stockier form (i.e. the most reduced SA:Vol at any given mass). Therefore, rather than differences between in SA:Vol due to body shape at any given mass, smaller Florida manatees are the most vulnerable to CSS due to the scaling effect. Future study can test this hypothesis by applying the methods of this study to the Antillean manatee subspecies, which lives in water habitats that are known to not drop below 20°C, and comparing the SA:Vol results.

In regards to the linear regression (Graph 2), both of the R^2 values tell us that the line almost perfectly fits the data (0.99) and that the variability of logMass can be explained by logSA:Vol, and vice versa. Furthermore, the ANCOVA test shows us that mass, COD, and more importantly their interaction have significant effects on SA:Vol.

The logistic regression plot visually demonstrates that as the SA:Vol increases, the probability of CSS mortality also increases. Moreover, the analysis of the regression serves to quantify how much the variable of SA:Vol can act as a predictor for the probability of the outcome variable of CSS death. The model X^2 value tells us that the influence of SA:Vol on probability of CSS is not a coincidence or by chance when compared to a constant condition. Conclusively, for every unit (0.01) increase in SA:Vol, the odds of succumbing to CSS increases by 35%; Florida manatee morphology is related to CSS mortality.

An improvement to this project could involve having a more complete morphometric data that allows the study to account for the surface area and volume of manatee flippers and tail, since appendages are the optimal specimens for observing the effects of adaptation mentioned in the study-related biological rules.

In addition to examining the biological principles of adaptation and natural selection on Florida manatees beyond the scientific text and seeing them applied in the natural world, such a research about the cold water temperature effects on manatees adds on to the conversation for developing future management strategies for this species. The phasing out of power plants, which are man-made sources of warm water that Florida manatees depend on during the winter, can increase CSS-related mortality and decrease manatee population (Laist & Reynolds, 2005);

this study's findings can perhaps contribute in the urge for long-term possible management options to establish warm-water refuges. Lastly, after examining the relationship between CSS mortality and manatees, it holds even more importance to lower the creatures' rates of watercraft injury and mortality and other human causes, and regulate the loss of natural habitat to coastal establishments.

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FIGURES & TABLES

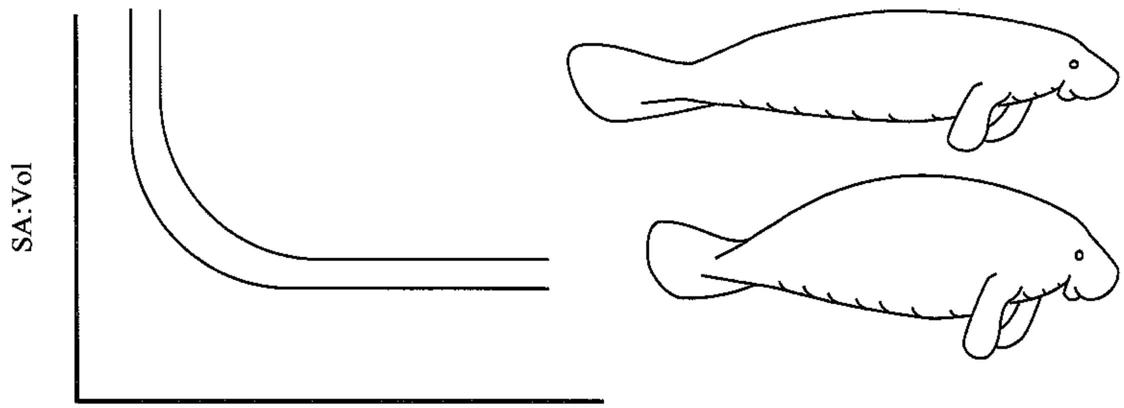


Figure 1: Illustration of Research Hypothesis. Manatees succumbed to CSS will have a significantly larger SA:Vol (blue) than those that died of other unrelated causes (red).

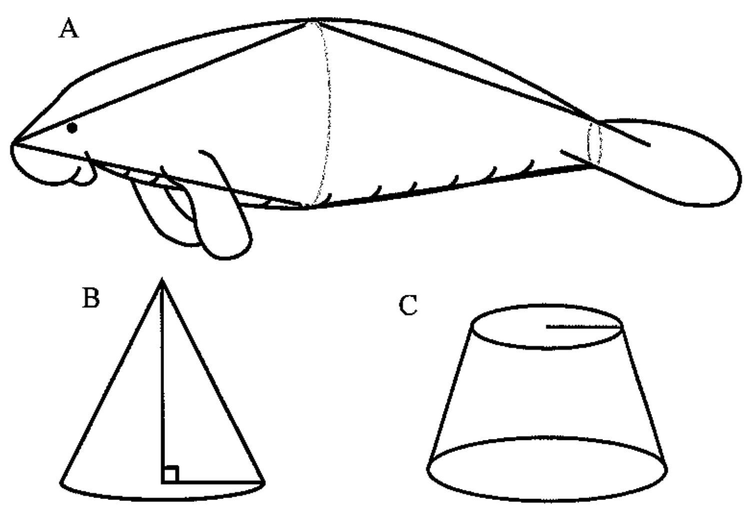


Figure 2: Manatee Body Representation by Cones. (A) Manatee illustration superimposed by two cones: (B) right circular cone, used to determine SA & Vol from nose to umbilicus, and (C) circular truncated cone, used to determine SA & Vol from umbilicus to tail fluke base.

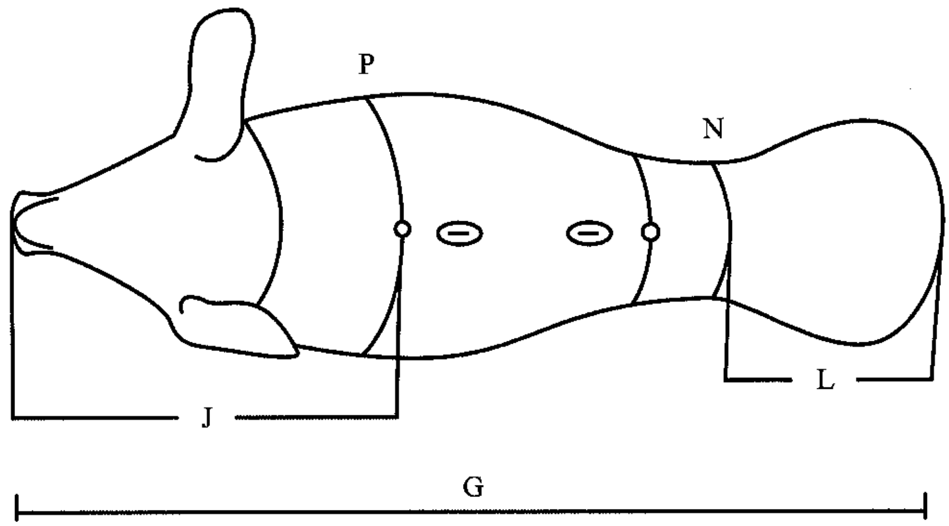


Figure 3: Manatee Morphometrics. Standard morphometric measurements of Total Length (G), Snout to Umbilicus (J), Fluke Base Length (L), Girth at Umbilicus (P), and Girth at Fluke Base (N) were all required to calculate SA and Vol. The least amount of morphometric lengths were used to maximize the sample size for study.

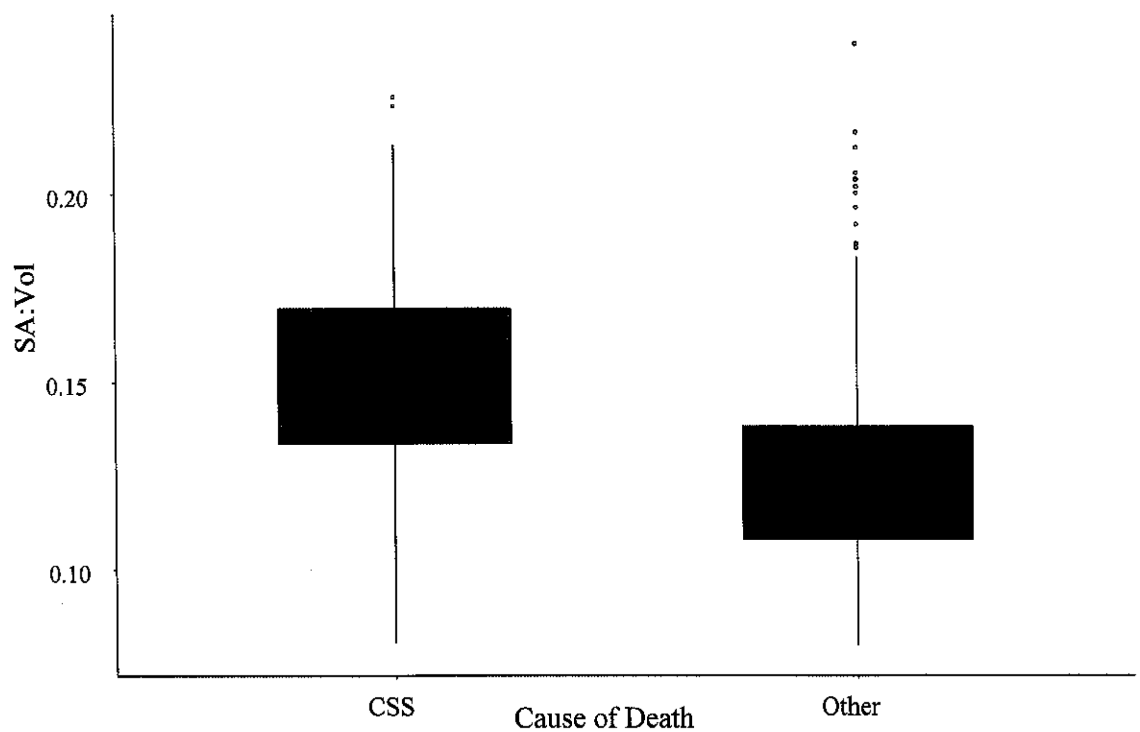


Figure 4: Boxplots of mean SA:Vol relative to cause of death. The average SA:Vol categorized into "CSS" and "Other" causes of death.

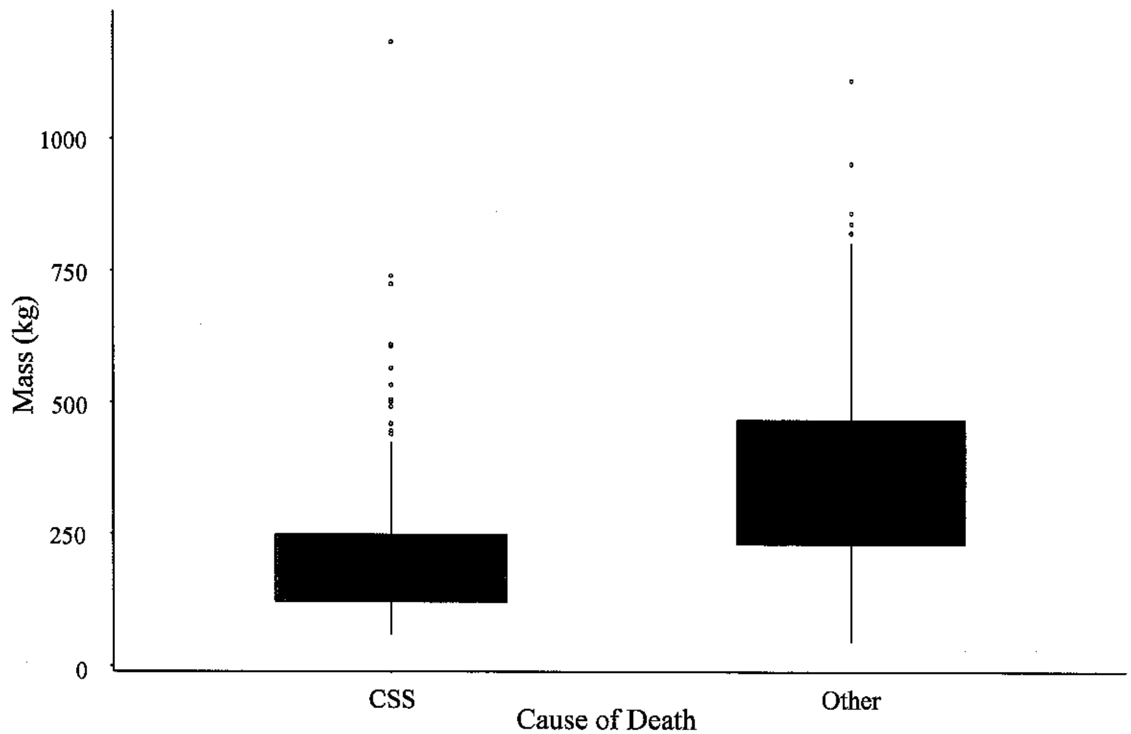


Figure 5: Boxplots of mean manatee mass relative to cause of death. The average mass categorized by "CSS" and "Other" causes of death.

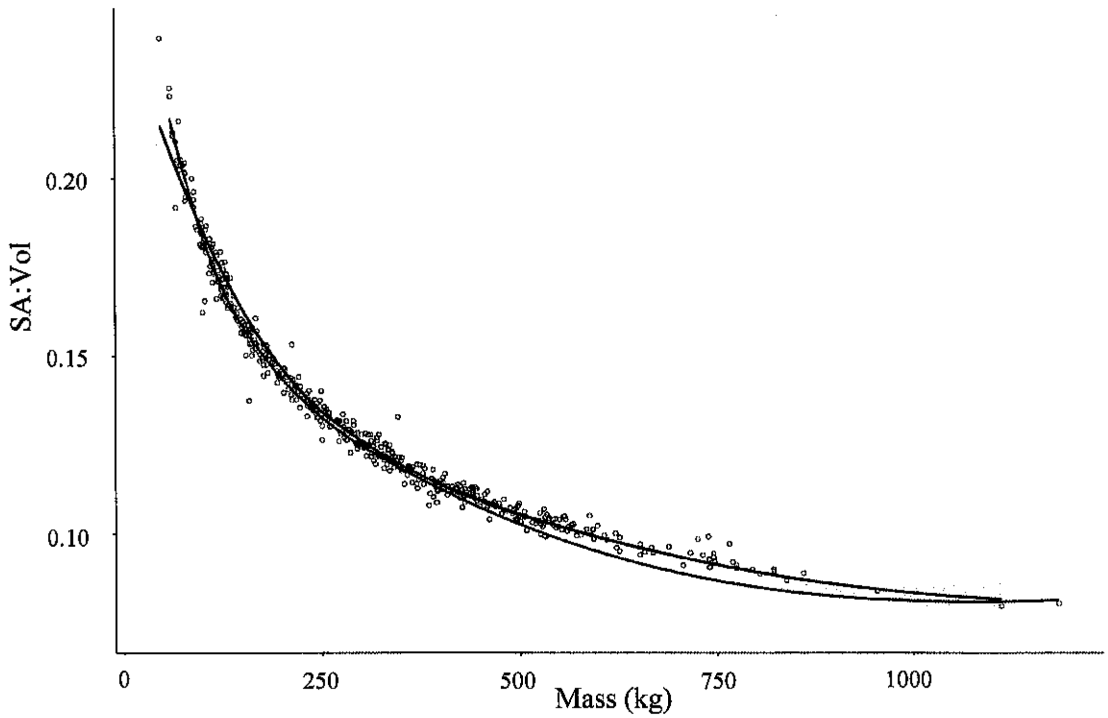
Table 1: Descriptive statistics of CSS & Other. The table below provides a descriptive statistics of the Florida manatees categorized by "CSS" and "Other" causes of death. The table displays the number of manatees for each category (n), as well as the mean SA:Vol and mass with their standard deviations.

Cause of Death	n	$\bar{x}$ SA:Vol ±SD	$\bar{x}$ Mass ±SD
CSS	146	0.152 ± 0.0286	216.3 ± 156.3
Other	374	0.127 ± 0.0269	360.9 ± 181.3

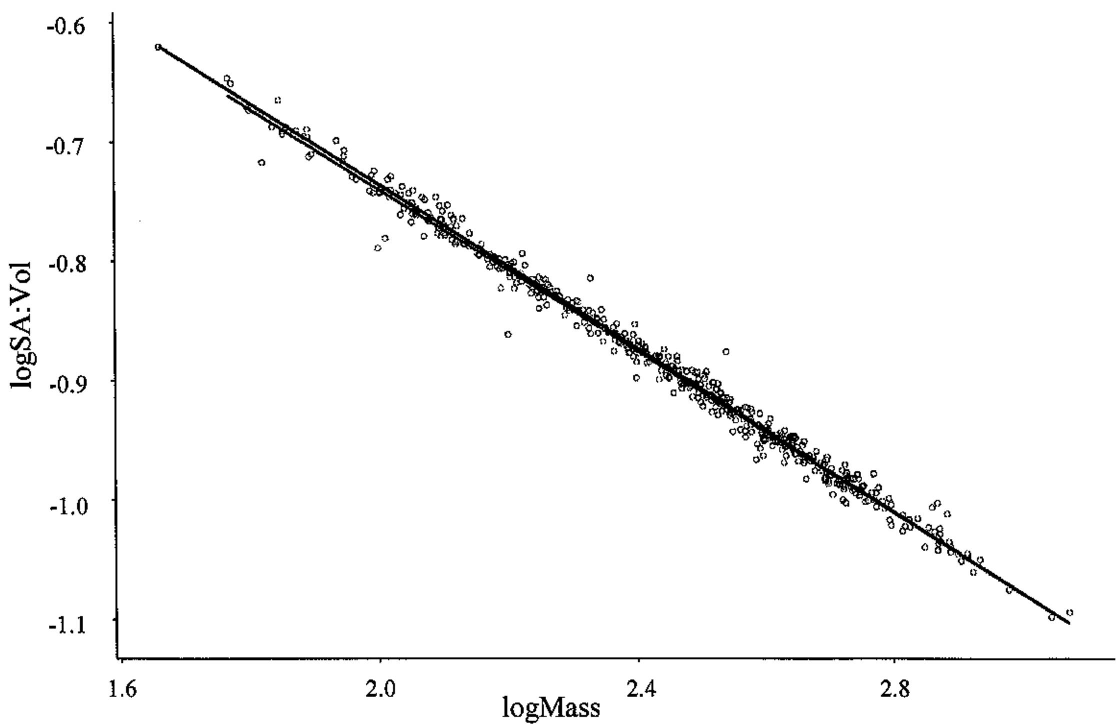
Table 2: Independent sample t-test results for mean SA:Vol & Mass in relation to cause of death. The t-test compares the mean SA:Vol as well as mass of "CSS" and "Other" in order to detect any significant differences in the scores for the two categories.

	t	*df*	Mean difference	*p*-value
SA:Vol	-9.123	251.78	-0.0251	2.2×10^{-16}***
Mass	9.063	306.38	145.1	2.2×10^{-16}***

*Sig 0.05; ** Sig 0.01; *** Sig 0.001



Graph 1: Regression Plot of Data. A graph of SA:Vol vs. Mass for "CSS" (blue) & "Other" (red).

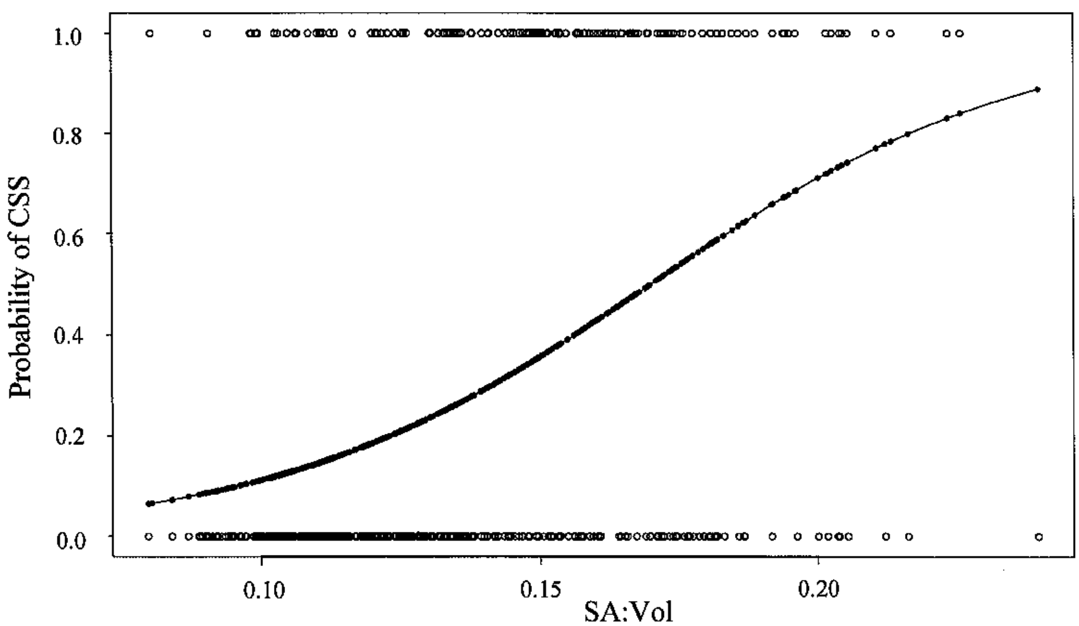


Graph 2: Linear Regression of Log-Transformed Data. A graph of logMass vs. logSA:Vol for CSS" (blue) & "Other" (red) with corresponding regression lines.

Table 3. ANCOVA test for factors significantly affecting SA:Vol: The table below reports the degrees of freedom (*df*), the sum of squares, the associated mean square, the equality of effect's group means (F-value), and the significance of the results (*p*-value).

	df	Sum sq	Mean sq	F value	*p*-value
Mass	1	0.3586	0.3586	2076.530	2×10^{-16}***
COD	1	0.0025	0.0025	14.225	0.0001***
Mass:COD	1	0.0016	0.0016	9.269	0.002**
Residuals	511	0.0882	0.0002		

*Sig 0.05; ** Sig 0.01; *** Sig 0.001



Graph 3: Logistic regression of effect of SA:Vol on probability of CSS. A binomial outcome variable is presented in this graph of Probability of CSS vs. SA:Vol, where "1" is succumbing to CSS and "0" is dying of other causes.

Table 4: Logistic regression analysis for effect of SA:Vol on probability of CSS mortality. The table below reports the beta coefficient (B), the standard error (SE), and the odds ratio in terms of comparing a constant and a SA:Vol treatment on the probability of CSS mortality.

	B (SE)	Odds Ratio
Constant	-5.043 (0.53)	
SA:Vol	29.703*** (3.7118)	1.346

Model $X^2(1) = 74.92$, $p<0.0001$***