Analyzing the Body Condition of Striped Dolphins (*Stenella coeruleoalba*) Using Histology Methods

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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For my family, John, Sheryl, Sterling, and Jenny Sakuma for helping me pursue my goals and succeed in my undergraduate career. Your continuous support has been my greatest strength, and I am forever grateful.

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ABSTRACT

Dolphins are indicators of marine ecosystem health, and their body condition reflects environmental changes and habitat stability. Striped dolphins (*Stenella coeruleoalba*) inhabit Hawaiian waters and provide an opportunity to assess the relationship between blubber composition, nutritional status, and general well-being. This study compares blubber histology metrics among four striped dolphins, where one was known to be emaciated at the time of death. Specifically, we assessed whether adipocyte area (AA) and adipocyte index (AI) are indicators of nutritional stress and examined variability among individuals. Blubber samples were taken from multiple body locations according to anatomical landmarks that were based on the girth axis and plane and slides were generated for histological examination. Blubber slides were analyzed according to blubber stratification as outer, middle and inner layers in Fiji (ImageJ) where AA and AI were measured. The collected data was statistically analyzed in R Studio. We expect the layers of the blubber to be stratified in all the dolphins, and we expect to see differences between the emaciated and non-emaciated individuals. These findings will contribute to validating blubber histology as a tool to assess cetacean health and may be used to improve marine ecosystem monitoring efforts.

Keywords: *Stenella coeruleoalba*, cetacean, blubber histology, body condition, adipocyte area, adipocyte index, stratification

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1.0 INTRODUCTION

Dolphins play a crucial role in maintaining ocean health, as they are indicators of marine ecosystem stability and reflect the impacts of environmental changes. Among approximately 20 diverse species of cetaceans found in Hawaii, striped dolphins (*Stenella coeruleoalba*) play a key role in the ocean ecosystem and are receptive to environmental stressors. The striped dolphin is a small pelagic dolphin commonly found in warm-temperate to tropical waters around the world (Perrin et al., 2009). While they are not as abundant as other dolphin species in Hawaii, they are relatively common in the deep waters surrounding the main Hawaiian Islands (Baird, 2013). Striped dolphins in Hawaii are not particularly well known despite representing a species that can serve as an indicator of ocean health.

Blubber is a multifunctional tissue that is essential for the survival of cetaceans by providing lipid storage for energy. Mammal behaviors such as mating, reproduction, lactation, and migration rely on blubber stores (Castrillon and Nash, 2020). It also can be mobilized during nutritional stress when energy intake is lower than their energy output (Adamczak et al., 2021). Blubber is highly stratified, with distinct functional layers that contribute to insulation, buoyancy, and energy storage (Khudyakov et al., 2022). The outer blubber layer serves functions such as thermoregulation, buoyancy, and locomotion while the inner layer is more metabolically active (Castrillon and Nash, 2020). These layers also reflect an individual's nutritional status, as changes in lipid composition and thickness can be indicative of overall body condition. When cetaceans lose weight, the subcutaneous fat layer in their blubber diminishes, leading to a thinning of the tissue and eventual emaciation. This reduction in body size and blubber composition can be a direct result of nutritional stress (Albrecht et al., 2024).

Assessing body condition through blubber analysis is a commonly used approach in cetacean biological and ecological research, as changes in blubber thickness and composition are visually apparent and measurable. Furthermore, blubber is readily obtainable during necropsy, making it an accessible tissue for evaluating an individual's energy reserves and overall health (Castrillon and Nash, 2020). Additionally, collection of small blubber biopsies from free-ranging cetaceans is a well-accepted method to study cetaceans (Noren and Mocklin, 2011), providing further opportunities for blubber analysis. Accurately assessing the body condition of cetaceans is crucial to understand individual fitness, survival, and reproductive success. Monitoring changes in body condition of individuals and populations over time provides insights into overall ecosystem health, which is important in the context of climate-induced habitat changes (Castrillon & Nash, 2020).

The purpose of this study is to compare the variability in blubber histology metrics between three dolphins with normal body condition and one dolphin with an emaciated body condition. This study uses metrics such as adipocyte area (AA) and adipocyte index (AI) obtained histologically from blubber samples as quantifiable proxies of body condition. This study aims to evaluate whether blubber histology methods, specifically the analysis of adipocyte area (AA) and adipocyte index (AI), can serve as effective tools to assess the overall body condition and nutritional status of striped dolphins. Additionally, we seek to investigate the potential variability in these metrics amongst individuals with similar body condition at the time of death, focusing on whether differences exist among non-emaciated individuals. This study hypothesizes that there will be variability amongst the four dolphins being analyzed, and that the emaciated dolphin will have smaller AA and larger AI compared to the non-emaciated individuals, indicating a state of nutritional stress.

2.0 METHODS

Necropsies of four striped dolphins (*Stenella coeruleoalba*) that stranded in various locations around the Hawaiian Islands took place at the University of Hawaiʻi at Mānoa Health and Stranding Lab (Table 1). One of the dolphins was determined to be emaciated based on qualitative observations made at the time of necropsy. Qualitative observations included a "peanut head" description of a visible depression behind the blowhole and protruding bones that were apparent through the skin at the time of the external gross examination.

ID Number	Stranding Date	Location	Emaciated	Weight	Length
KW2018006	08/20/2018	Kahului, Maui	No	76.66 kg	197 cm
KW2020007	05/02/2020	Sugar Beach, Maui	No	62.8 kg	185 cm
KW2020009	06/19/2020	Kaʻaʻawa, Oʻahu	Yes	89.1 kg	225 cm
KW2021006	03/18/2021	Waimanalo, Oʻahu	No	97.5 kg	206 cm

Table 1 *Stranding information for 4 striped dolphins*

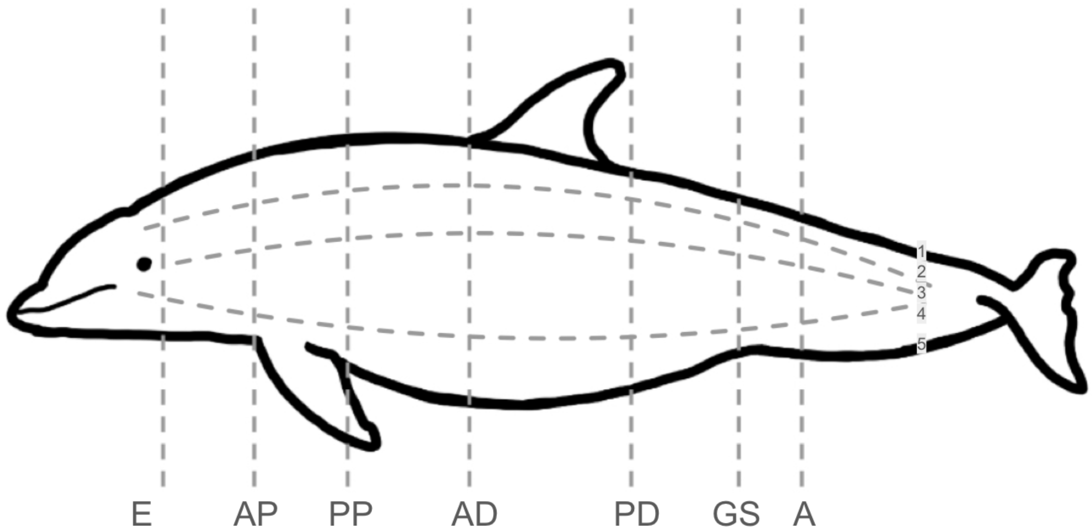

Figure 1 *Full depth blubber core samples were taken at locations where each girth axis and planes intersect. Girth axis: eye (E), anterior pectoral (AP), posterior pectoral (PP), anterior dorsal (AD), posterior dorsal (PD), genital slit (GS), anus (A). Planes: dorsal (1), dorsal-midline (2), midline (3), midline-ventral (4), ventral (5).*

Full-depth blubber core samples were taken at seven girth axes (eye (E), anterior pectoral (AP), posterior pectoral (PP), anterior dorsal (AD), posterior dorsal (PD), genital slit (GS), and anus(A)) across five planes at each axis (dorsal, dorsal midline, midline, midline-ventral, and ventral) (Figure 1). Thin slices of each blubber core from the skin to the muscle-blubber boundary were taken and fixed in 10% buffered formalin. Samples were made into paraffin embedded slides and stained using hematoxylin-eosin (H&E).

2.1 Adipocyte Area (AA)

Images of prepared slides were taken, converted to 8-bit black-and-white images, from all sampled locations. Nine images were taken per slide, consisting of three for the outer layer (closest to the skin), middle layer, and inner layer (closest to the muscle tissue). The computer program Fiji was used to process and measure the images. The AA was first calibrated from pixels to millimeters. The microns per pixel value was set to be 0.3775 with the maximum diameter per cell to be 200. A minimum of 100 cells per image were measured as 100 cells per image for analysis was demonstrated to be similar to results using 300, 500, and 1000 adipocytes in a previous study (Parlee et al., 2014). The program outlined and computed the area of each adipose cell when the user indicated that it was complete. The results were stored as an image as well as a spreadsheet, which provided the average area measurement of each circled adipose cell with each average being recorded (Figure 2). ‘

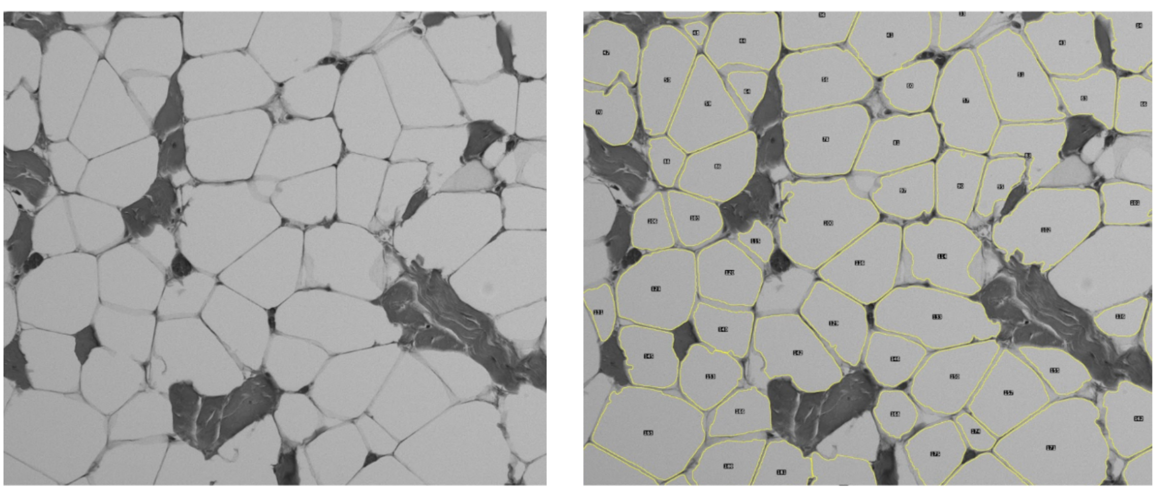

Figure 2 *Image of original blubber sample (left) and image of adipocyte area being measured with ImageJ software (right).*

2.2 Adipocyte Index (AI)

The adipocyte index is the measurement of the ratio of muscle tissue to lipid storage space. It shows the relative amount of lipid stored in the blubber. In ImageJ, after the image was converted to greyscale, the threshold was adjusted using the built-in threshold tool (Figure 3). The threshold created an image that only contains black and white pixels. Values below the set threshold were black, while values above the threshold were white. Lower ratios of connective tissue to lipid storage indicate less lipid stored and more connective tissue in the image.

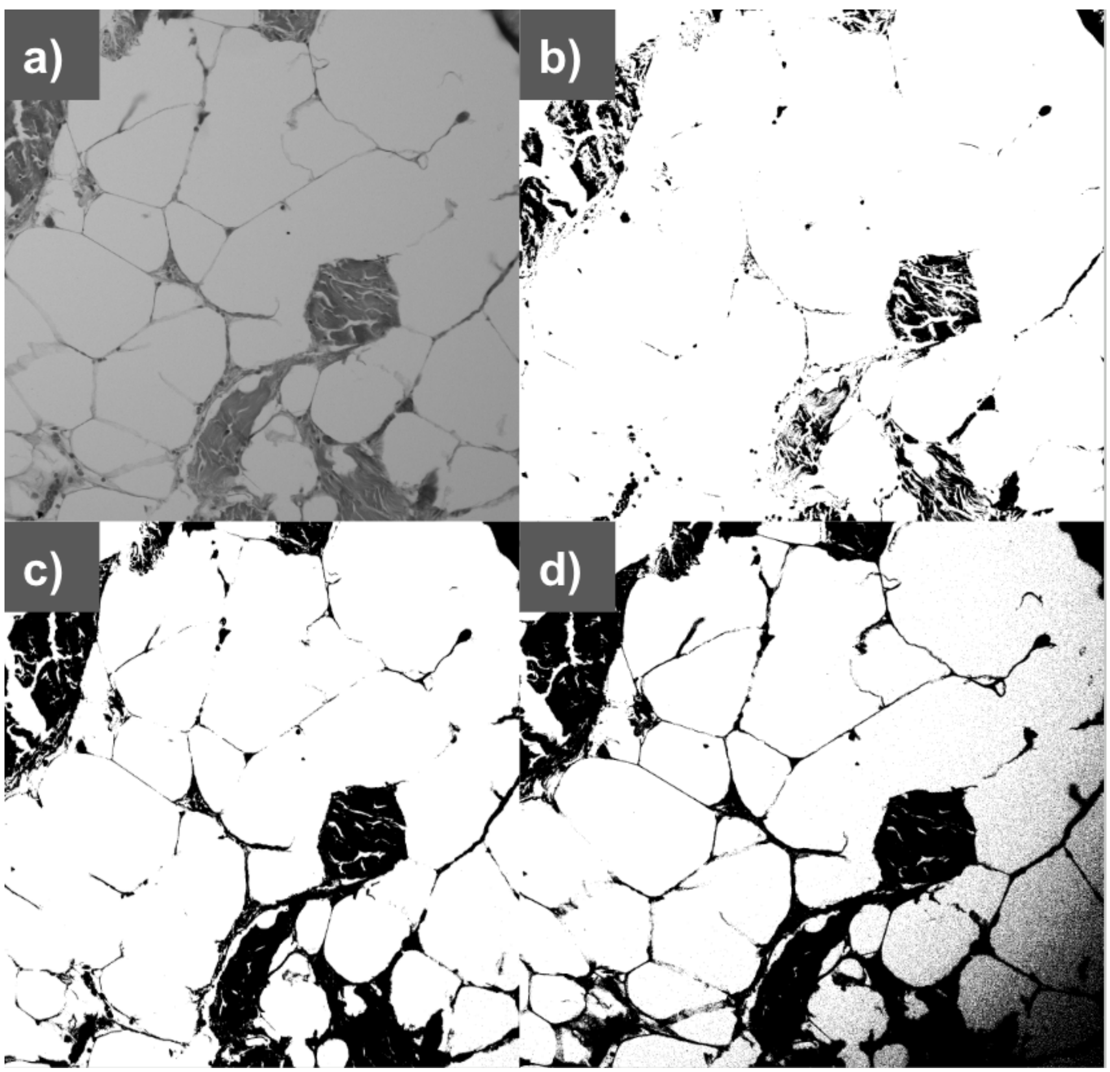

Figure 3 *(a) Image of original blubber slide. Threshold must be set correctly (c) so that there is not too little (b) or not too many (d) pixels in the image.*

2.3 Statistical Analysis

Statistical analyses were conducted using the programming language R using the integrated development environment (IDE) R studio (version 4.4.1). Wallis and Dunn's multiple comparisons tests were used to compare variation between blubber layers, regions of the body, and between individuals. The Kruskal-Wallis was used as the data was nonparametric.

3.0 RESULTS

Blubber images were taken of all slides made from each of the 4 stranded striped dolphins. AA was measured from two of the three images taken of each blubber layer at all sample locations (minimum 15 locations, maximum 35 locations among the four individuals). AI was measured for each of the three images taken at each blubber layer at all sample locations, averaging measurement of 315 images.

The data for the analysis of this project was generated by two undergraduate students at the University of Hawaii. Data from KW2018006 and KW2021006 was generated by Spencer Sakuma and data from KW2020007 and KW2020009 was generated by Camon Paski. Data was combined from the four individuals and analyzed by Spencer Sakuma.

ID Number	**Emaciated**	**Layer**	**r**	**p**
Overall		All Layers	-0.75	< 0.001
		Inner	-0.46	< 0.001
		Middle	-0.43	< 0.001
		Outer	-0.69	< 0.001
KW2018006	No	All Layers	-0.75	< 0.001
		Inner	-0.73	< 0.001
		Middle	-0.54	0.002

		Outer	-0.59	0.0007
KW2020007	No	All Layers	-0.78	< 0.001
		Inner	-0.55	< 0.001
		Middle	-0.43	0.01
		Outer	-0.44	0.008
KW2020009	Yes	All Layers	-0.76	< 0.001
		Inner	-0.68	<0.001
		Middle	-0.52	0.0013
		Outer	-0.22	0.2124
KW2021006	No	All Layers	-0.78	< 0.001
		Inner	-0.71	< 0.001
		Middle	-0.47	< 0.001
		Outer	-0.52	< 0.001

Table 2 *Regression analysis for each animal comparing the correlation coefficient r of AA and AI across the whole body.*

3.1 AA vs AI Correlation

Combined data from all four striped dolphins showed an overall significant inverse linear relationship between the AA and AI metrics (p = < 0.001, r = -0.75) where a high AA value corresponded to a smaller AI value (Table 2; Figure 4).

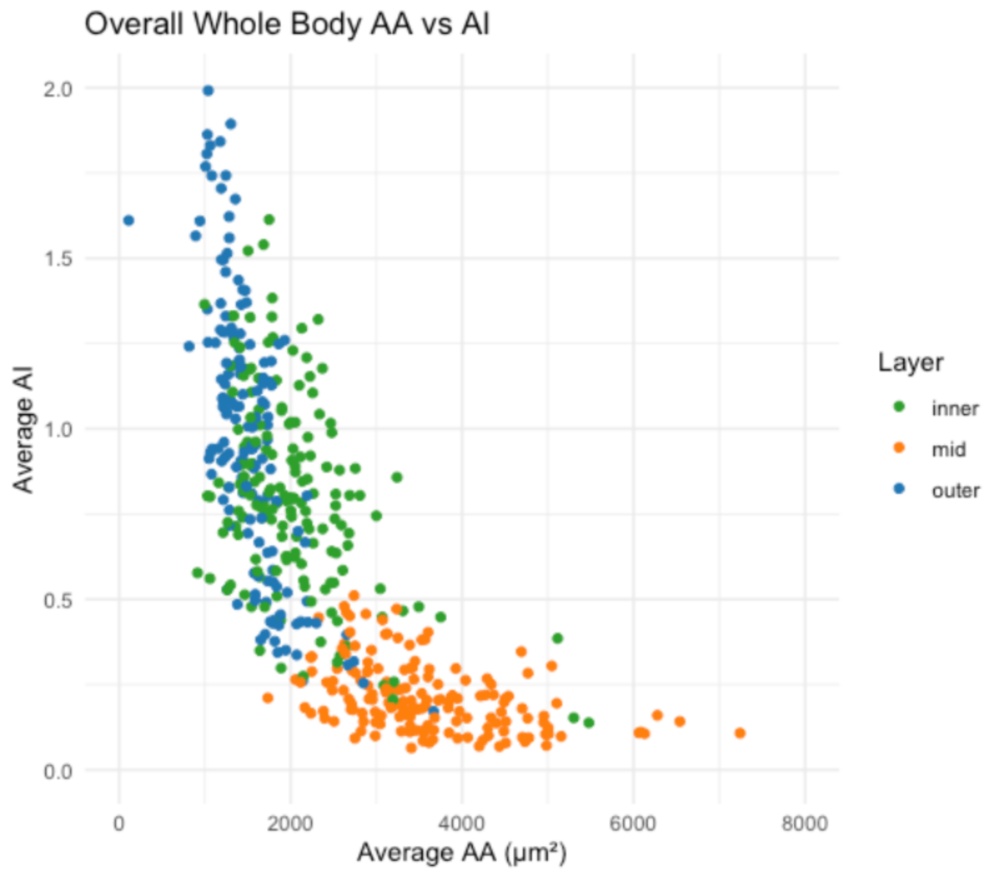

Figure 4 *Combined adipocyte area (AA) and adipocyte index (AI) in all four striped dolphins across 3 layers.*

When the data was divided by animal, the correlation between the AA and AI values was significant across all layers of the blubber and within each layer except in the inner layer of KW2020009 (p = 0.2124) (Table 2; Figure 5).

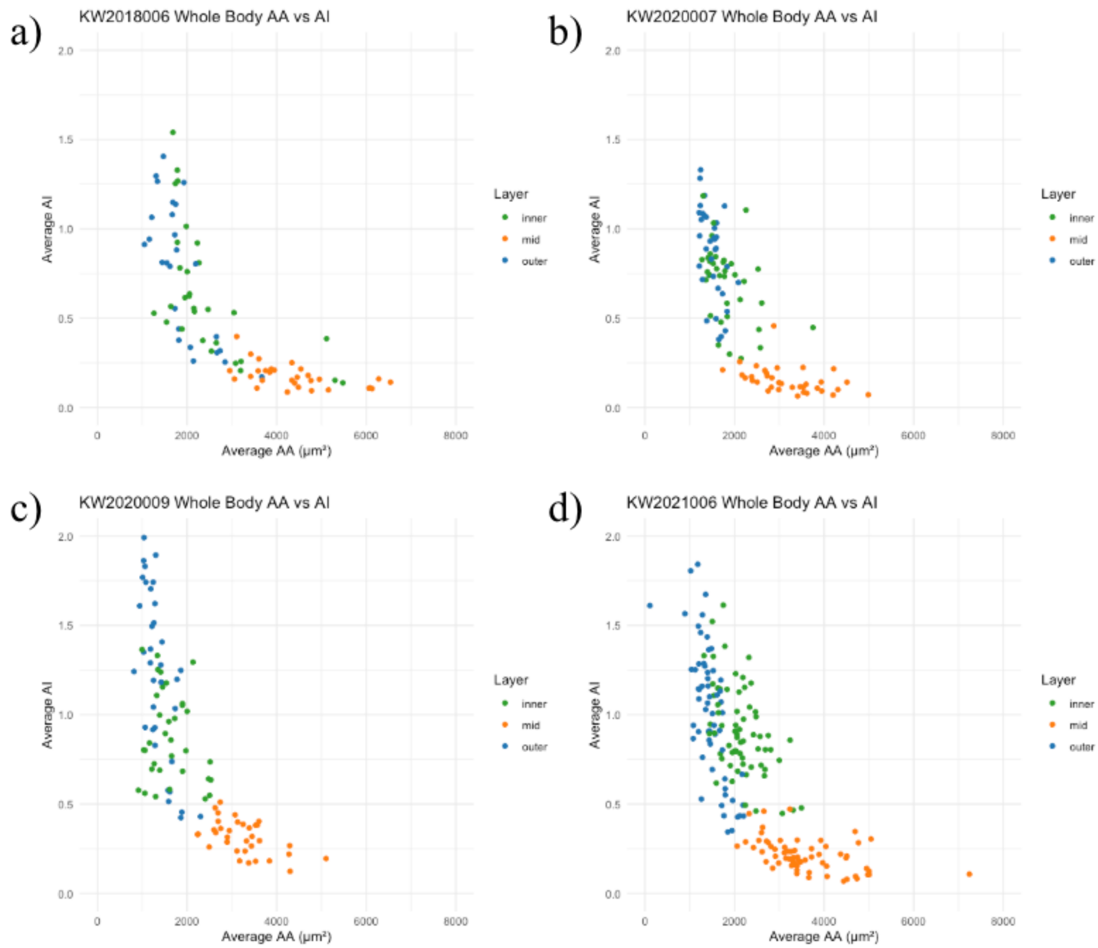

Figure 5 *Individual whole body AA vs AI in a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006.*

3.2 Blubber Stratification

3.2.1 AA Stratification

Overall, when the data from all four striped dolphins were included in the analysis, there were significant differences in the AA values between the 3 layers of blubber. All 3 layers were significantly different from each other with the highest values in the middle layer and the lowest values in the outer layer of blubber. (Figure 6).

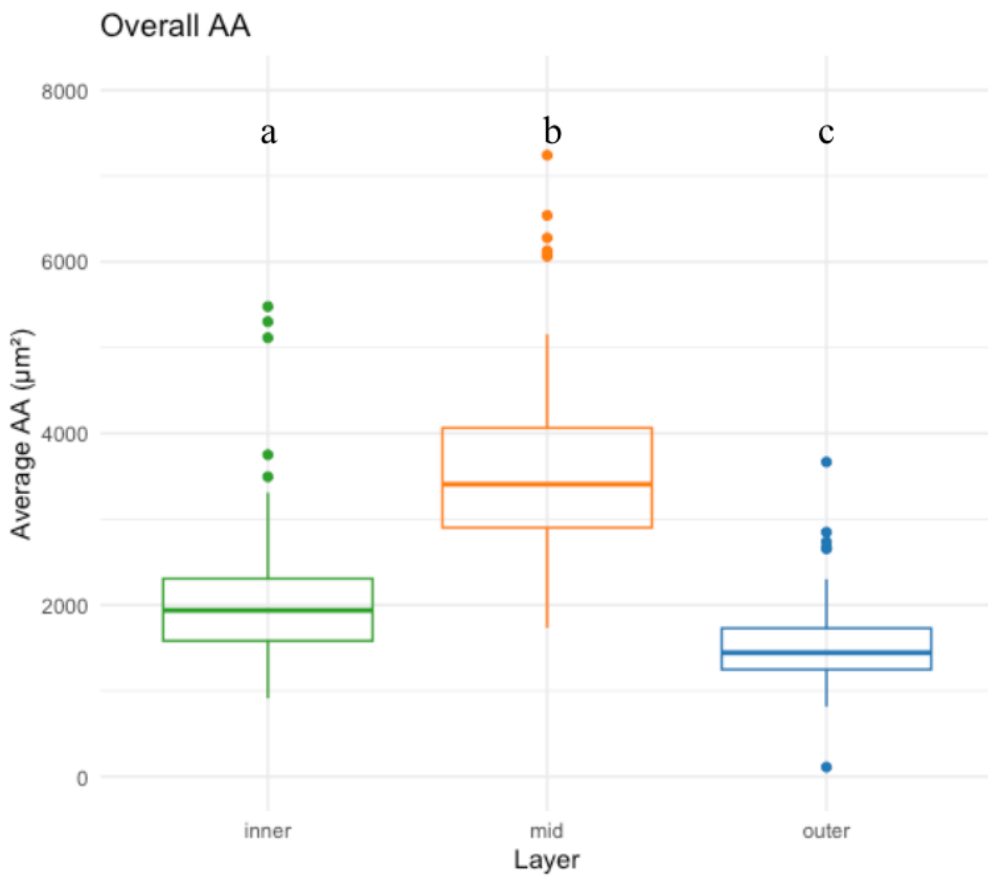

Figure 6 *All four animals' AA combined, separated by layer. Box plots that do not share the same letter are significantly different from each other.*

Additionally, the Kruskal-Wallis tests showed that there were significant differences in the AA between the different layers of the blubber when the data was analyzed for each individual. When considering each animal, the average AA value was highest in the middle layer, smallest in the outer layer and intermediate in the middle layer. Dunn's multiple comparisons showed that the AA values of all 3 layers were significantly different to each other in two of the animals, KW2020007 and KW2021006. In the other two individuals (KW2018006 and KW202009), the middle layer was significantly different compared to the outer and inner layers; however, the outer and inner layers were not significantly different from each other (Figure 7). The inner layer in KW2021006 and KW2020009 had a broader range of AA values, suggesting higher variability in adipocyte size in these two individuals.

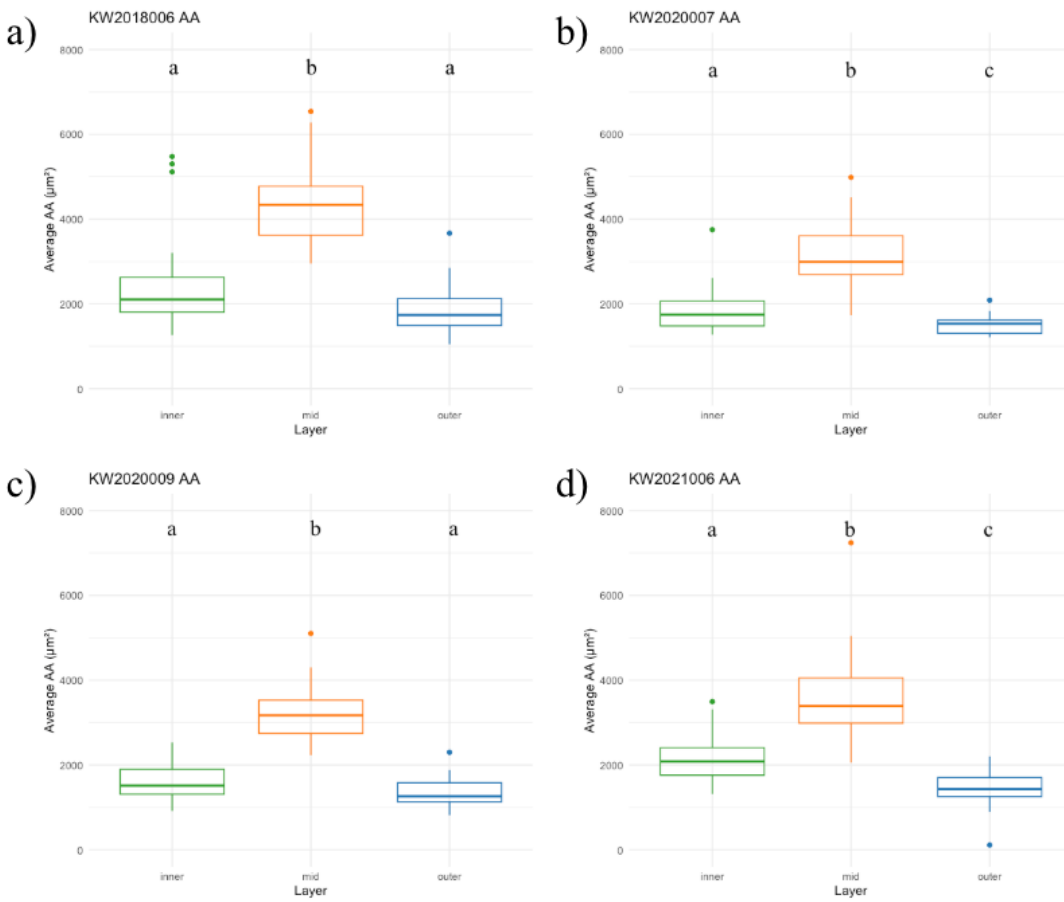

Figure 7 *Separated AA of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006 by layer. Box plots that do not share the same letter are significantly different from each other.*

3.2.2 AI Stratification

Overall, there were significant differences in the 3 layers of blubber. All 3 layers were significantly different from each other (Figure 8). AI values were greatest in the outer layer of blubber and smallest in the middle layer, reflecting an inverse relationship with the overall trend in AA values between the blubber layers.

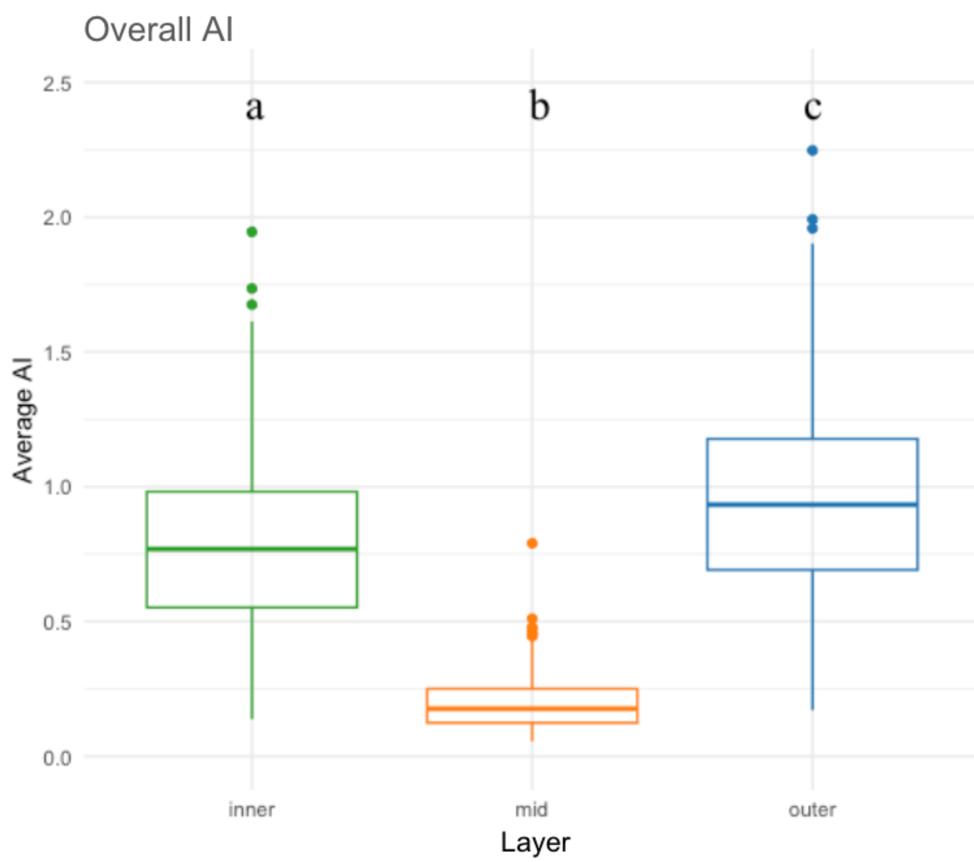

Figure 8 *All four animals' AI combined, separated by layer. Box plots that do not share the same letter are significantly different from each other.*

There were also significant differences in the AI between the different layers of the blubber in each of the 4 individual animals. In each animal, the average AI value was the highest in the outer layer, smallest in the middle layer, and intermediate in the inner layer. Dunn's test showed that all the AI values of all three layers were significantly different to each other in KW2020007, and in the other three animals, the middle layer was significantly different compared to the inner and outer layers. However, the inner and outer layers were not significantly different to each other in all the individuals examined with the exception of KW2020007 (Figure 9).

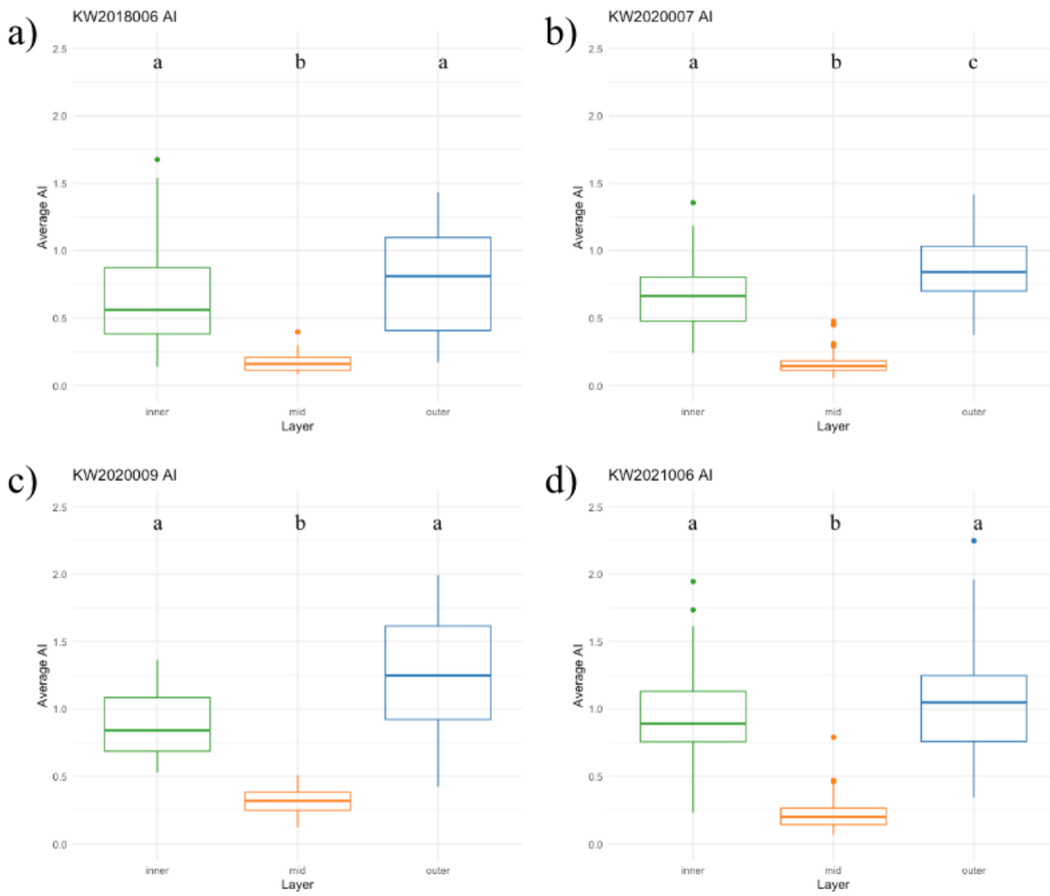

Figure 9 *Separated AI of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006 by layer. Box plots that do not share the same letter are significantly different from each other.*

3.3 AA and AI Across Girth Axis

3.3.1 AA Across Girths

When the data from all four animals were combined and analyzed together, there were no significant differences in the AA of the outer layer. However, there were significant differences in the middle and inner layers and there was a pattern of higher AA towards the head compared to the tail region of the body. (Figure 10).

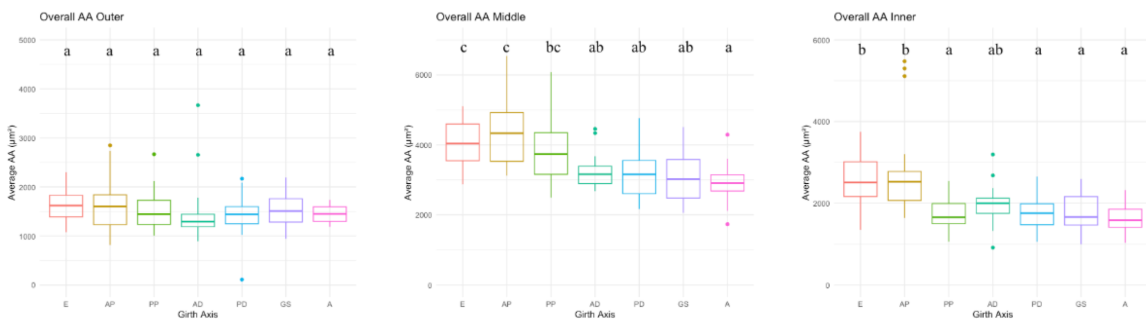

Figure 10 *Overall AA Girth Axis of four animals combined separated by layer. Box plots that do not share the same letter are significantly different from each other. Girth axes: eye (E), anterior pectoral (AP), posterior pectoral (PP), anterior dorsal (AD), posterior dorsal (PD), genital slit (GS), and anal (A).*

When the data was divided by individual animal, there were no significant differences in the outer layer blubber in any of the animals (Figure 11). In the middle layer, there were significant differences in three of the four animals but KW2020007 showed no significant differences (Figure 12). In the inner layer, there were significant differences in three of the four animals, KW2020009 showed no significant differences (Figure 13). Additionally, all animals except the emaciated individual, KW2020009, had a pattern of larger AA values towards the head in the middle and inner layers and significant differences in the inner layer (Figure 12 and 13).

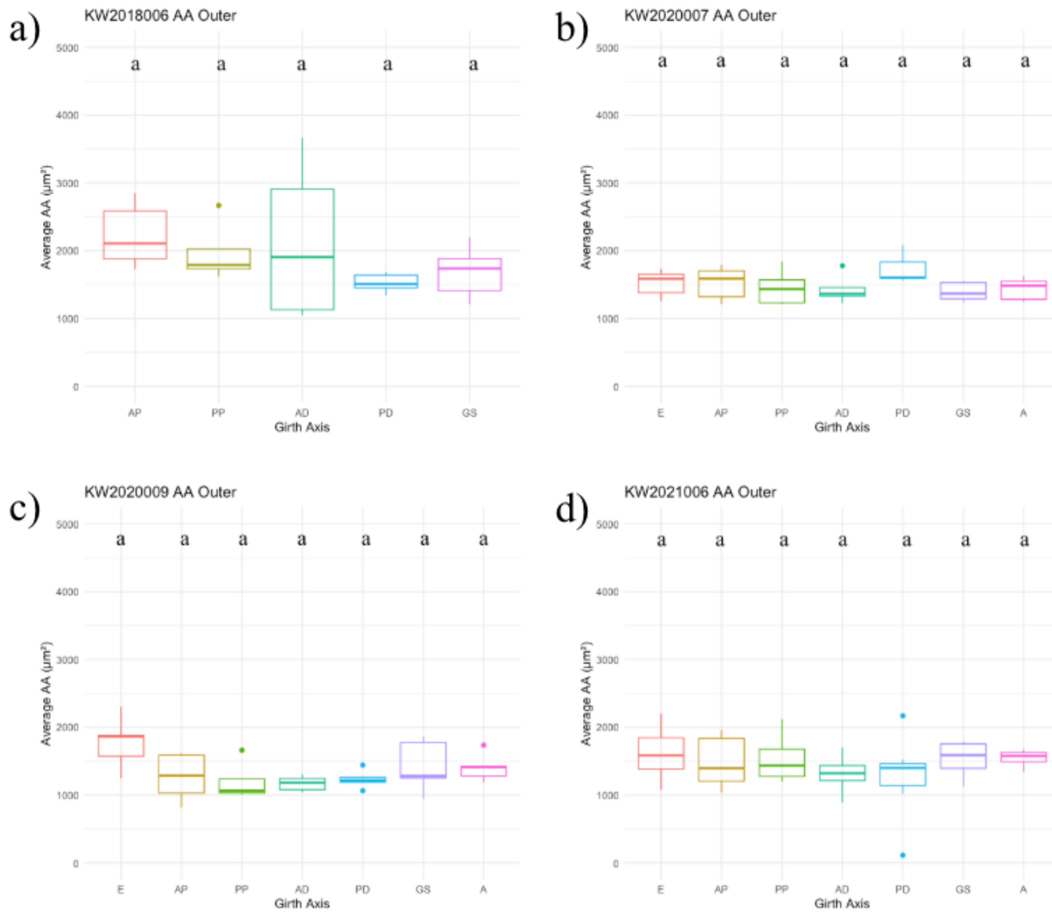

Figure 11 *Outer layer girth axis of each animal in AA a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

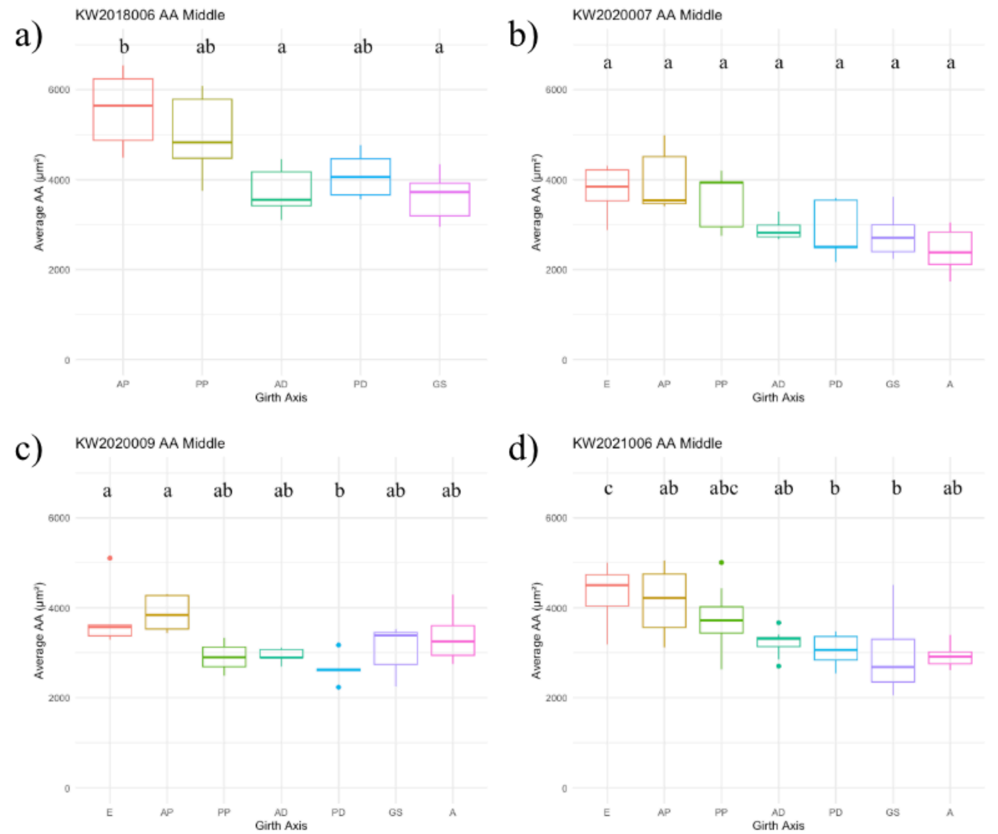

Figure 12 *Middle layer girth axis of each animal in AA a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

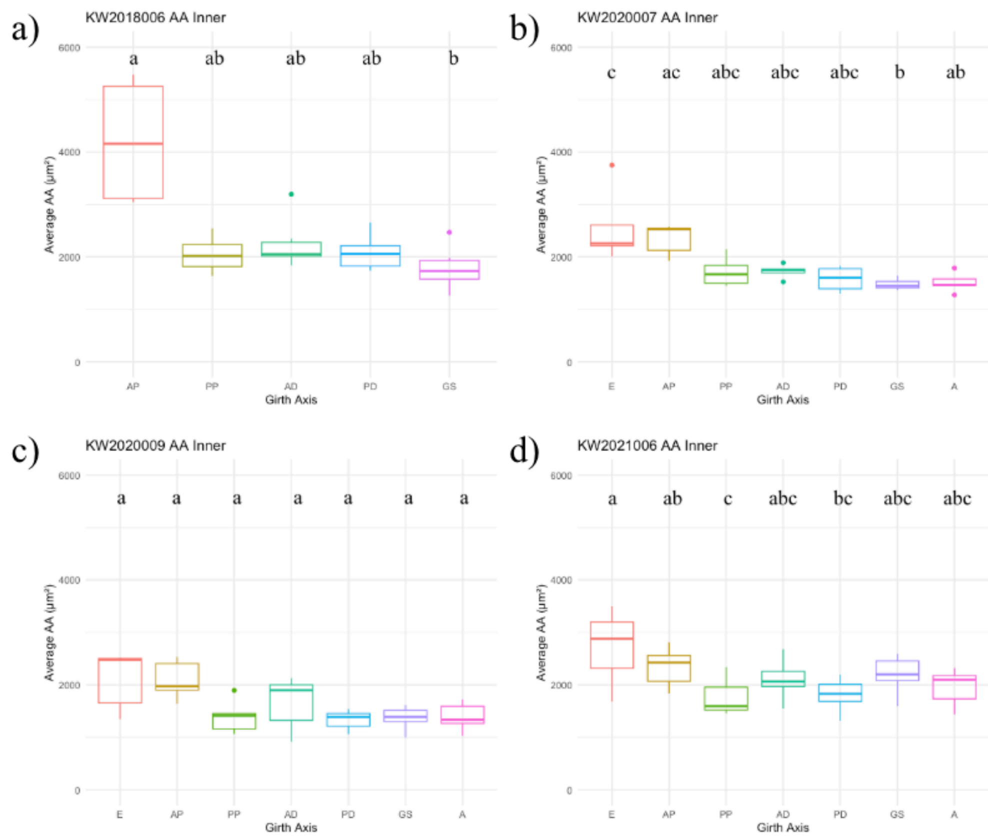

Figure 13 *Inner layer girth axis of each animal in AA a) KW2018006, b) KW2020007, c) KW2020009, and KW2021006. Box plots that do not share the same letter are significantly different from each other.*

3.3.2 AI Across Girths

When the data from all four animals were combined and analyzed together, there were significant differences in the AI of the outer and middle layers but no significant differences across girths in the inner layer (Figure 14).

When the data was divided by animal, there were significant differences between girths in all animals in the outer layer (Figure 15). In the middle layer, KW2018006 and KW2020007 had significant differences, while the other two did not. Additionally, in the middle layer, all animals except KW2020009 and KW202007 had a pattern of larger AI towards the tail (Figure 16). In the inner layer, KW2018006 and KW2021006 had significant differences, but the other two animals did not (Figure 17).

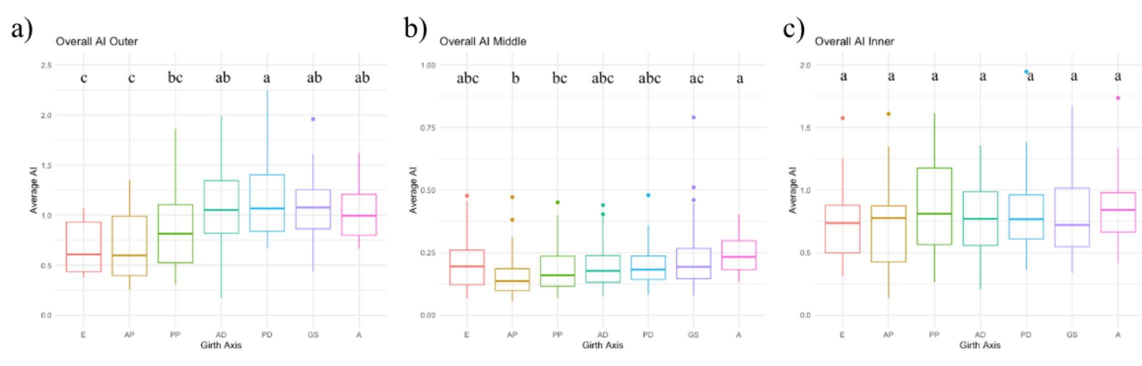

Figure 14 *Overall AI Girth Axis of four animals combined separated by layer. Box plots that do not share the same letter are significantly different from each other.*

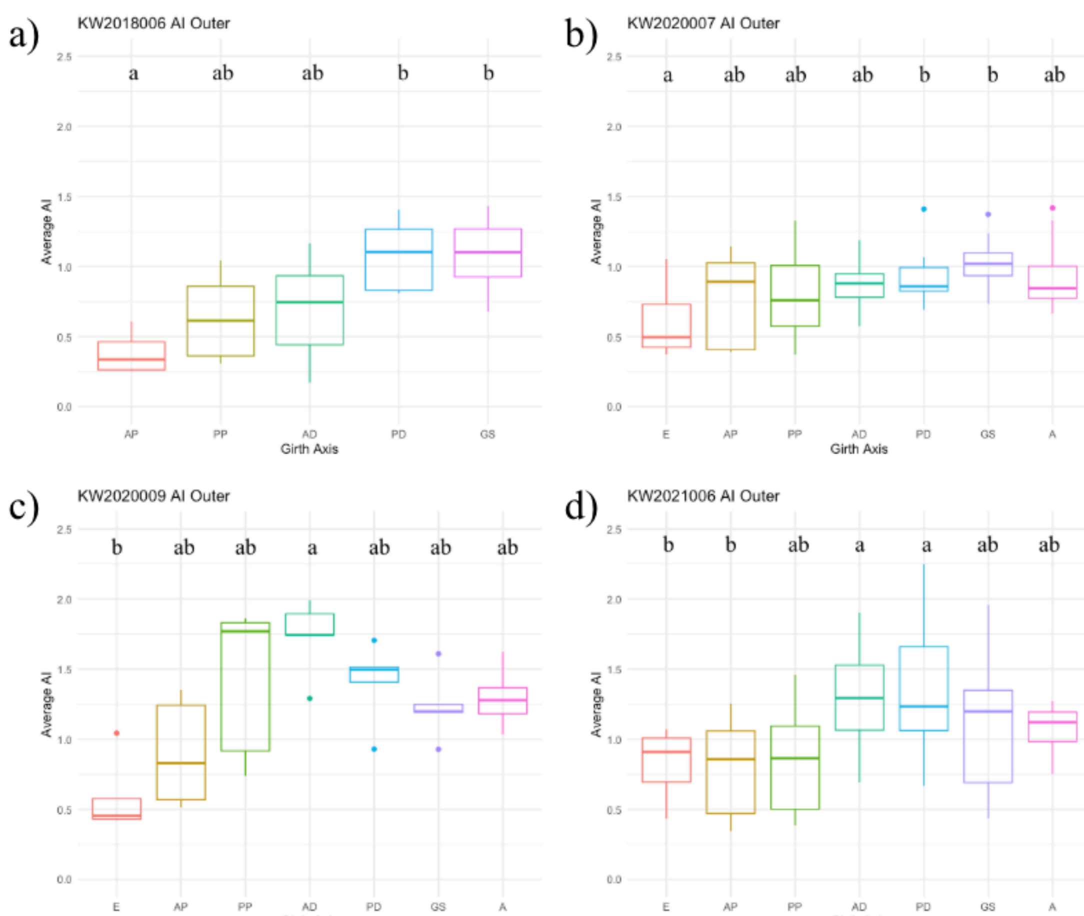

Figure 15 *AI Outer layer girth axis of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

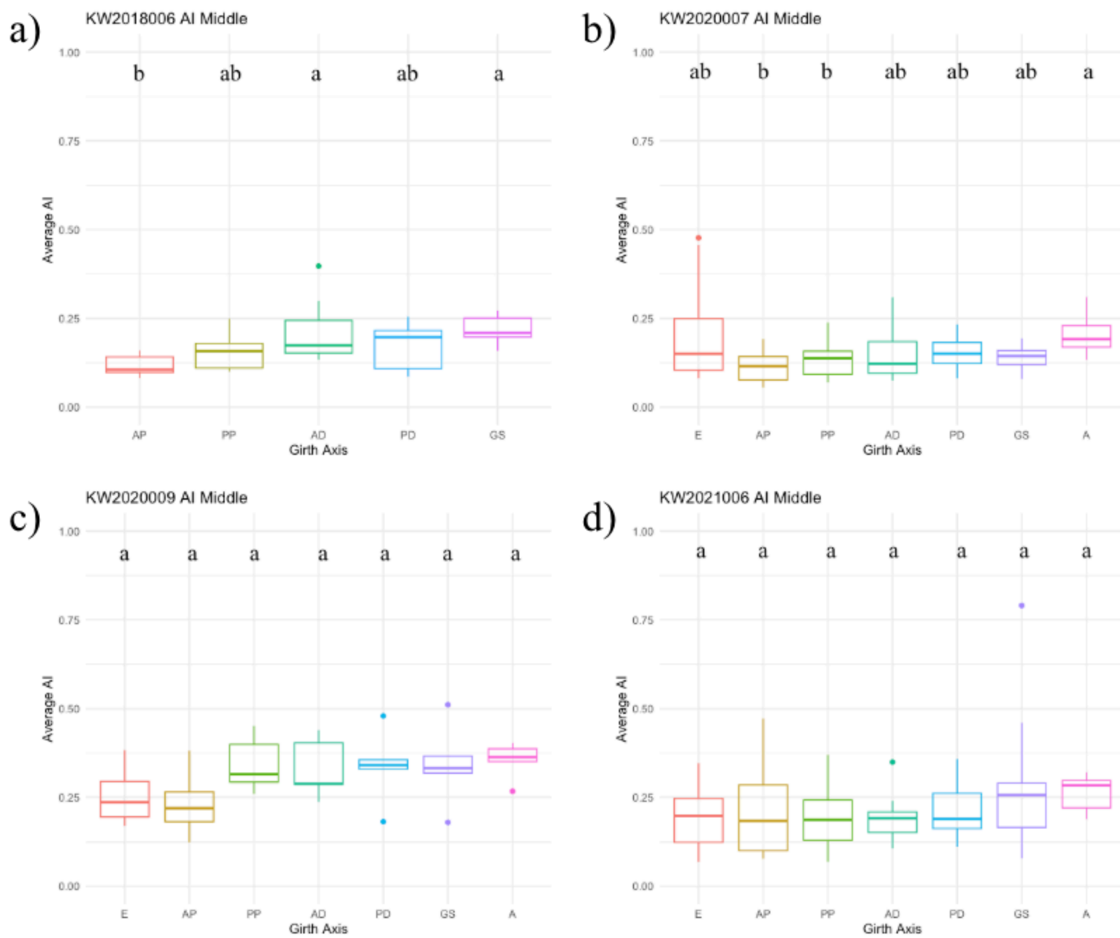

Figure 16 *AI Middle layer girth axis of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

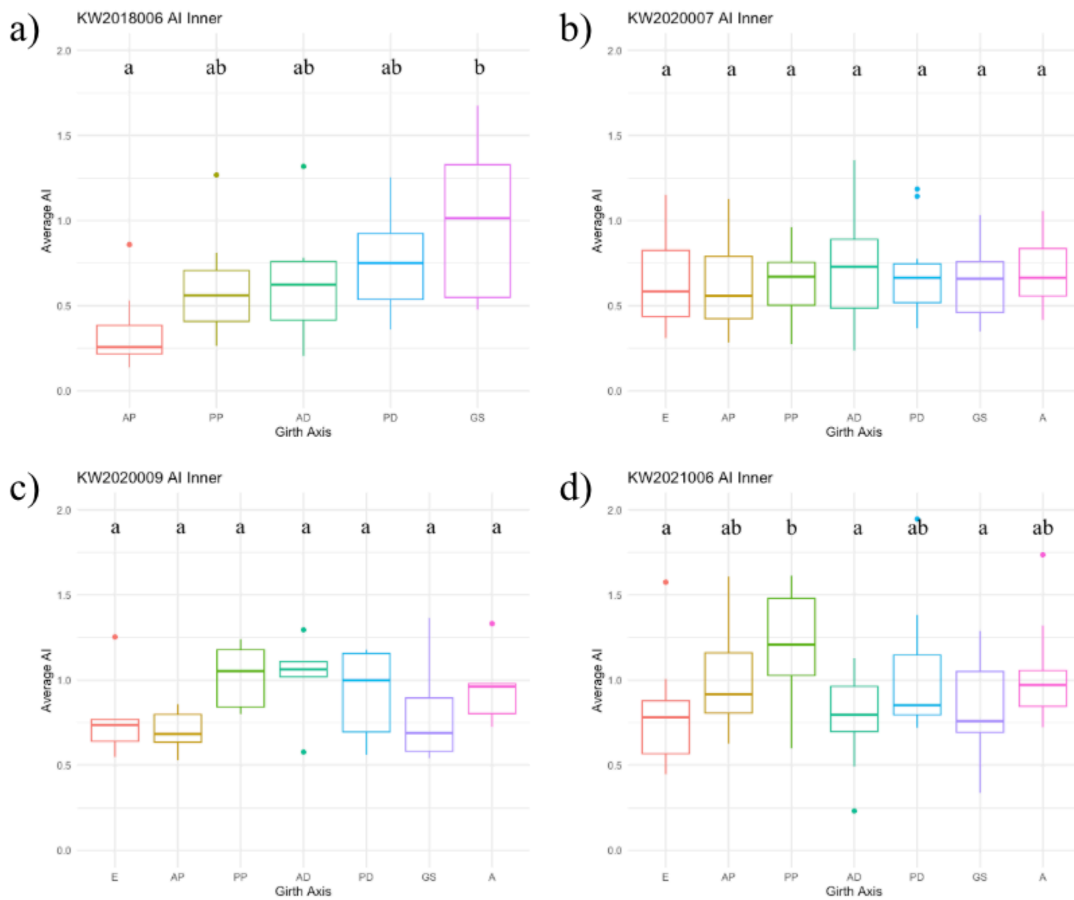

Figure 17 *AI Inner layer girth axis of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

3.4 AA and AI Across Planes

3.4.1 AA Across Planes

When the data from all four animals were combined and analyzed together, there were only significant differences across the sampling planes in the middle layer blubber (Figure 18). When the data was divided by animal there were no significant differences in AA across the three blubber layers in all 4 animals. However, there was a pattern of larger

AA values towards the ventral plane in the middle and outer layers except in KW2018006 (Figure 19 and 20).

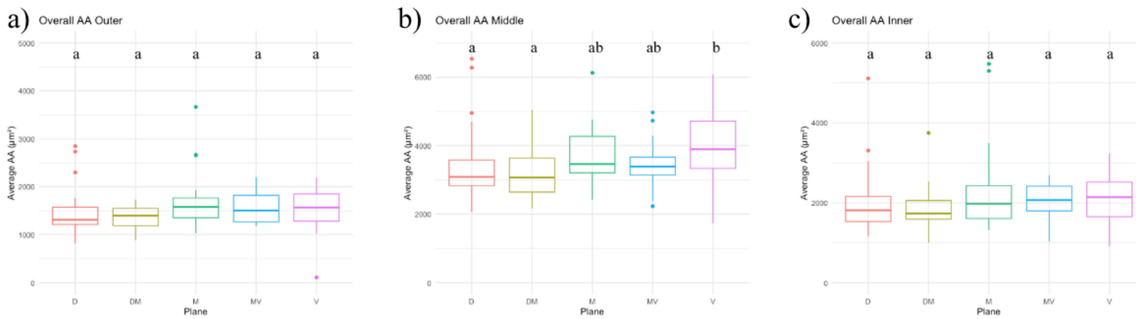

Figure 18 *Overall AA Planes of four animals combined separated by layer. Box plots that do not share the same letter are significantly different from each other. Planes: dorsal (D), dorsal midline (DM), midline (M), midline ventral (MV), and ventral (V).*

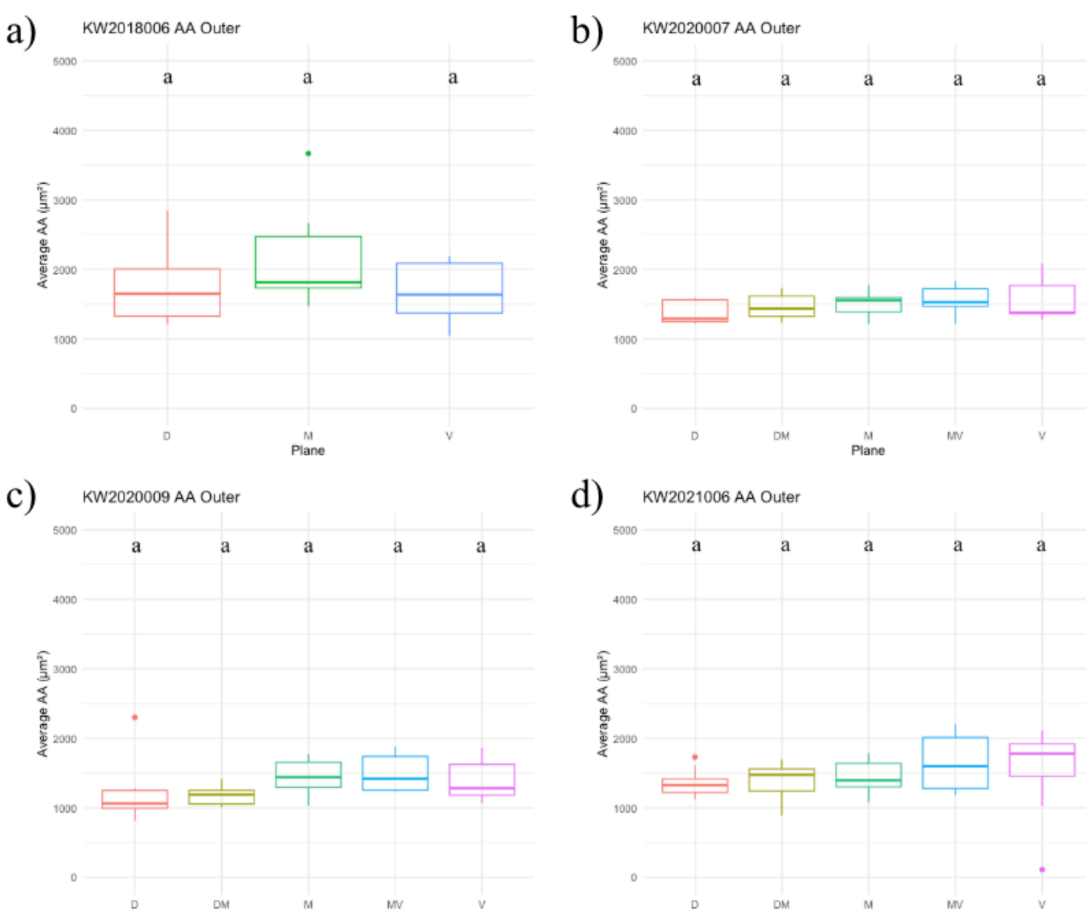

Figure 19 *AA outer layer planes of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

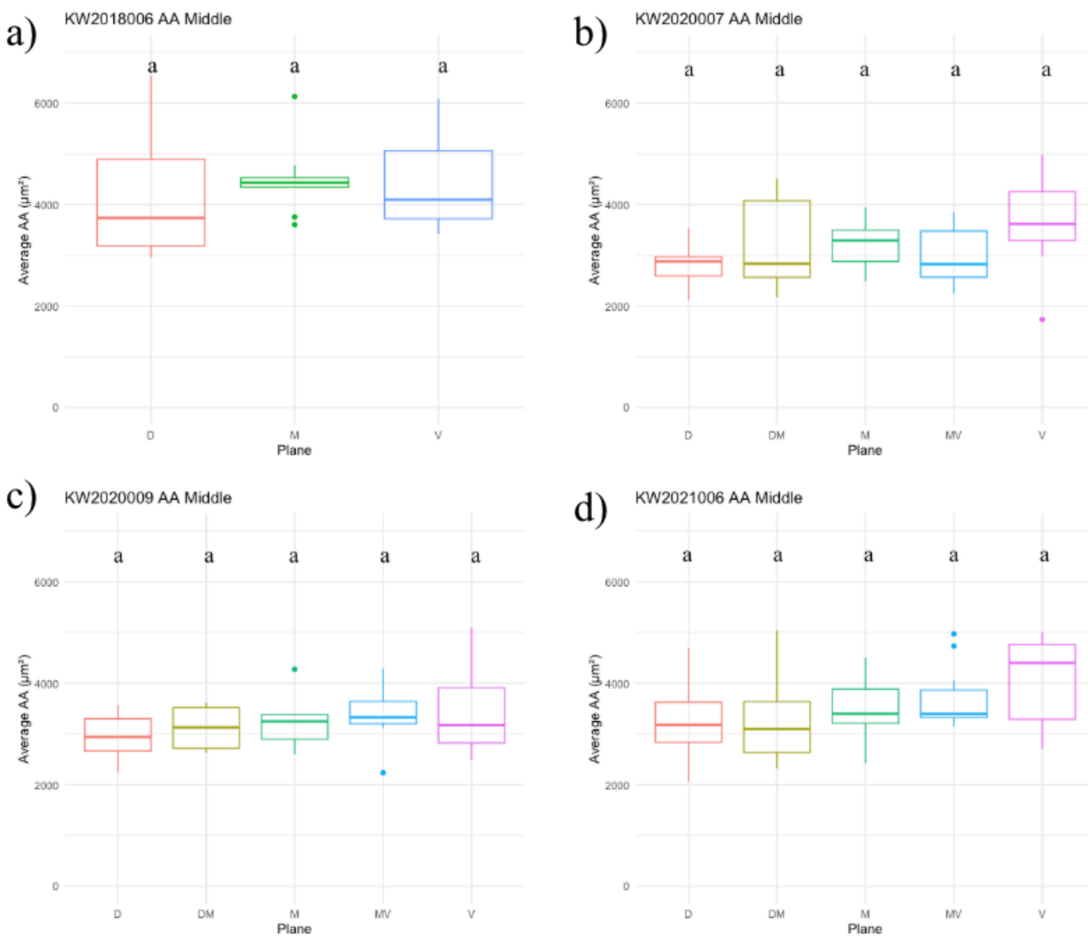

Figure 20 *AA middle layer planes of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

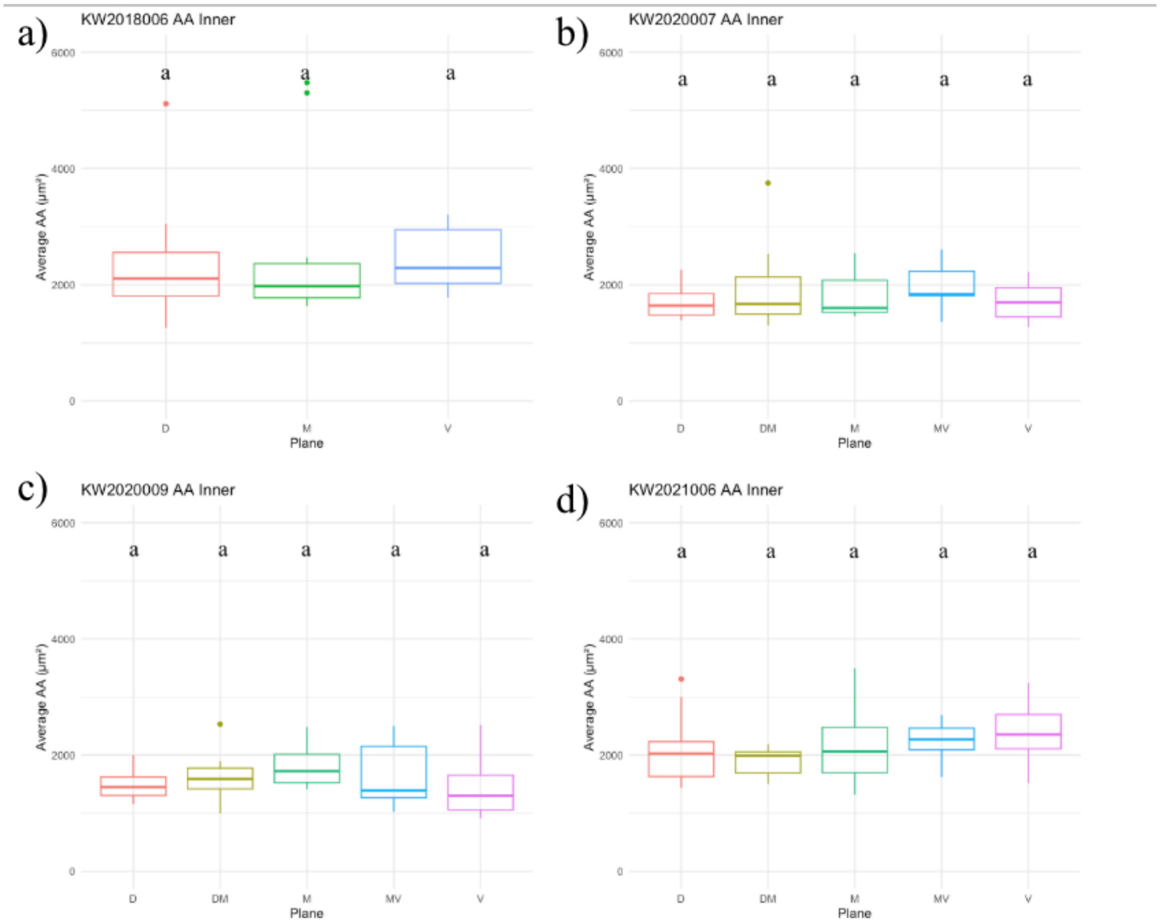

Figure 21 *AA inner layer planes of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

3.4.2 AI Across Planes

When the data from all four animals were combined and analyzed together, AI varied across planes in the outer and middle layers with a pattern of lower AI in the ventral plane in those layers (Figure 22). When the data was divided and analyzed by animal, the outer layer, KW2020007 and KW2021006 had significant differences, but the other two animals did not (Figure 23). All 4 animals in the outer layer exhibited a pattern of a higher index in the dorsal and dorsal-midline planes compared to the ventral-most planes. There were significant differences in the middle layer in all 4 animals, with a pattern of a higher index in the dorsal and dorsal-midline planes (Figure 24). In the inner layer, KW2020007 had significant differences, but the other three animals were not significantly different with no distinct patterns (Figure 25).

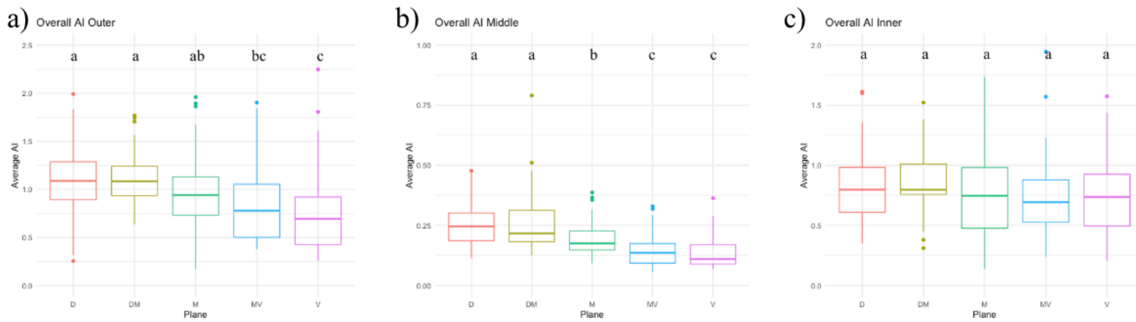

Figure 22 *Overall AI Planes of four animals combined separated by layer. Box plots that do not share the same letter are significantly different from each other.*

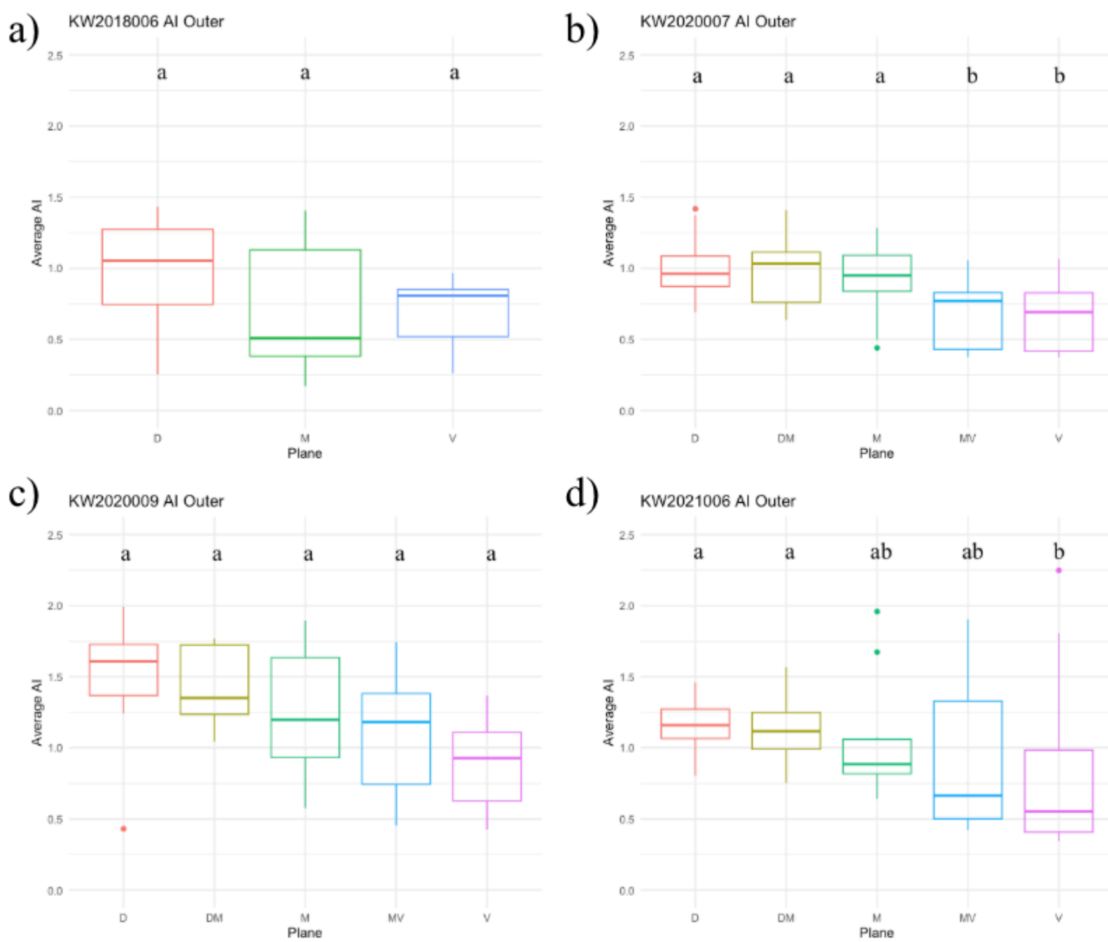

Figure 23 *AI outer layer planes of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

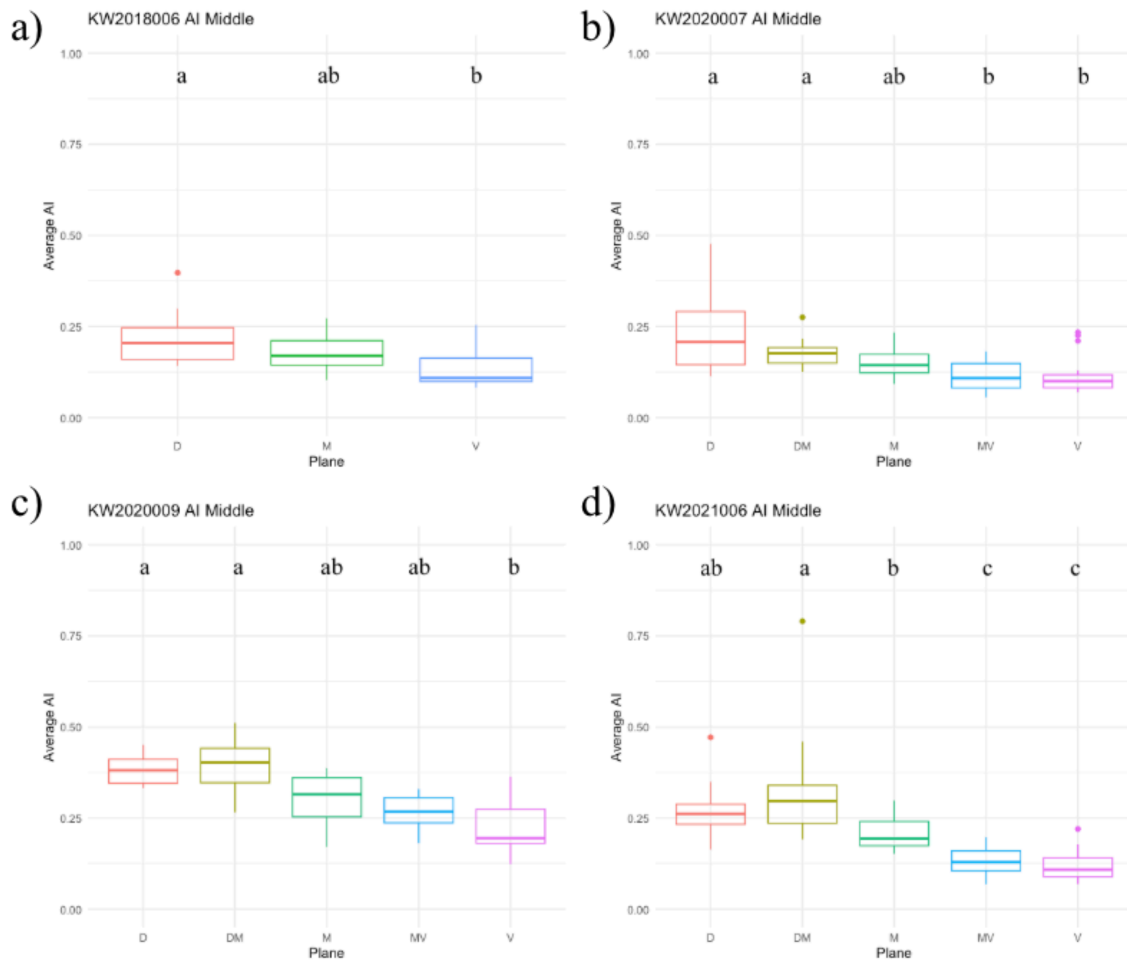

Figure 24 *AI middle layer planes of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

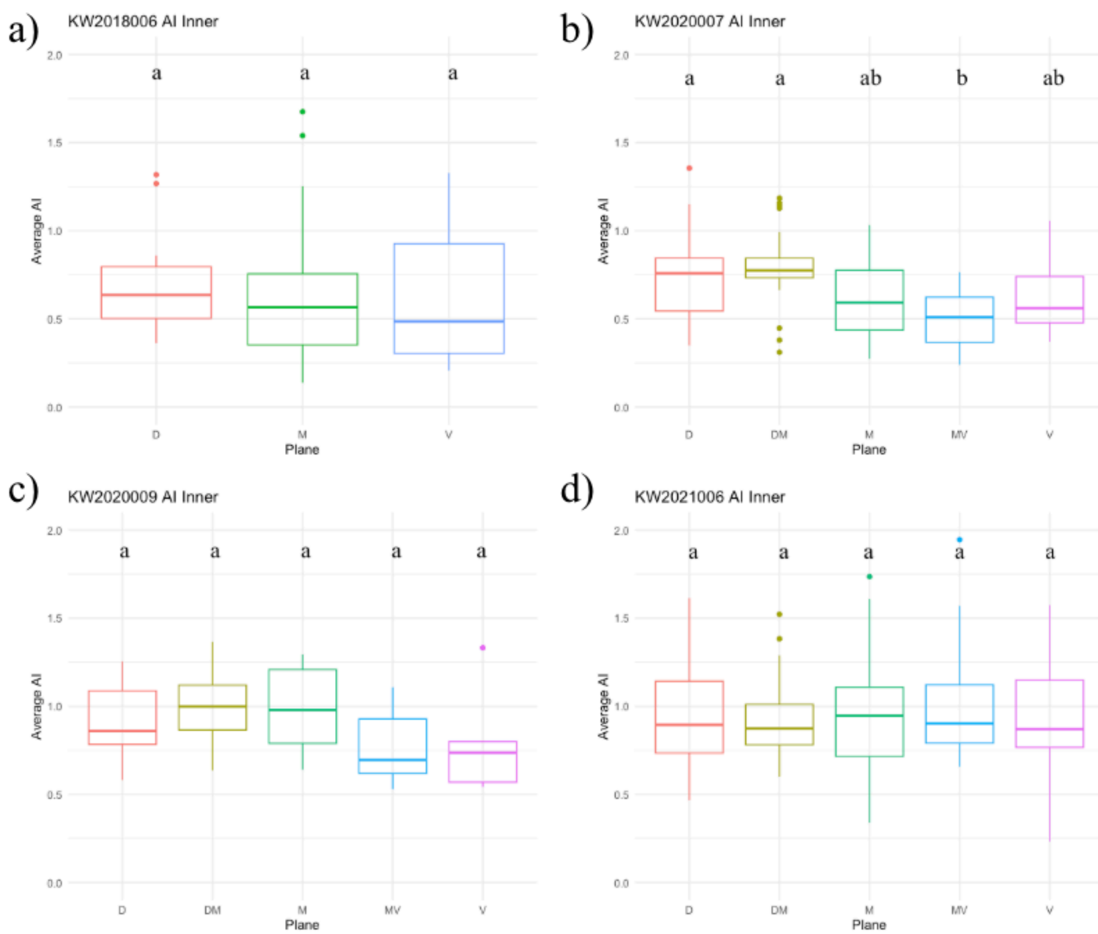

Figure 25 *AI inner layer planes of each animal a) KW2018006, b) KW2020007, c) KW2020009, and d) KW2021006. Box plots that do not share the same letter are significantly different from each other.*

3.5 AA and AI Across Animals

3.5.1 AA Across Animals

When AA was compared between each animal there were significant differences seen in each layer. KW2020009 had the smallest average AA in the outer and inner layers, and KW2018006 had the highest values in all three layers. Across the three layers, AA values had significant differences in all animals. In the outer layer KW2020009 and

KW2021006 had significantly smaller AA than KW2018006. In the middle layer, KW2021006 was significantly different compared to the other three individuals, and in the inner layer, KW2018006 and KW2021006 AA values were significantly different from KW2020007 and KW2020009 (Figure 26).

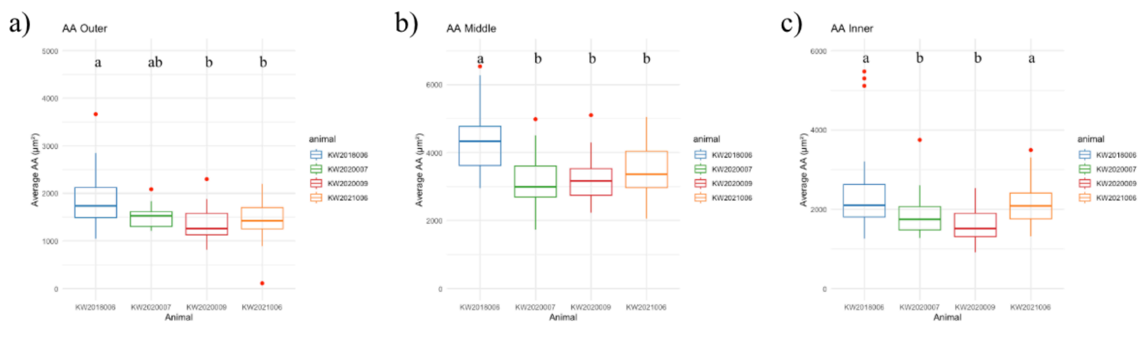

Figure 26 *Box plots of AA for each animal distinguished by layer, a) outer, b) middle, c) inner. Significance determined by the Kruskal-Wallis and Dunn's test when $p < 0.05$. Box plots that do not share the same letter are significantly different from each other.*

3.5.2 AI Across Animals

There were significant differences across all layers in the AI data compared between individuals. KW2020009 had the highest average AI value in the outer and middle layers and KW2018006 had the lowest AI values in the outer and inner layers. All layers had significant differences in the 4 animals. In the inner and outer layers, KW2018006 and KW2020007 were significantly different from KW2020009 and KW2021006 (Figure 27). In the middle layer, KW2020009 had significantly larger AI compared to all other individuals, and KW2021006 was significantly different to KW2020007.

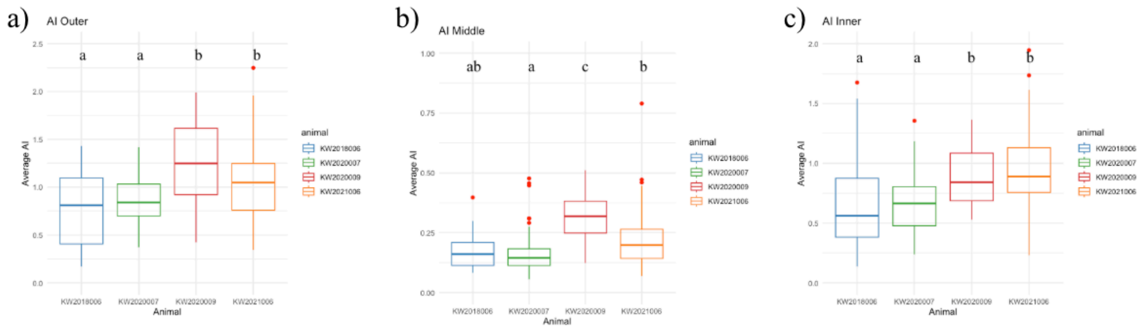

Figure 27 *Box plots of AI for each animal distinguished by layer, a) outer, b) middle, c) inner. Significance determined by the Kruskal-Wallis and Dunn's test when p < 0.05. Box plots that do not share the same letter are significantly different from each other.*

4.0 DISCUSSION

4.1 AA vs AI Correlation

The correlation between AA and AI for all four animals was evident by linear regressions, suggesting that it's an inverse relationship, where a decrease in AA correlates with an increase in AI, indicating a depletion of lipid reserves with an increase in connective tissue, consistent with patterns observed in malnourished marine mammals (Adamczak et al., 2021). The four dolphins had an $R^2 = 0.55$ which indicates a weak relationship between AA and AI. This is similar to the R^2 found in the false killer whale middle layer ($R^2 = 0.49$) (Phipps et al., 2023) and the R^2 found in humpback whales ($R^2 = 0.614$) (Castrillon et al., 2017). This relationship aligns with previous research that has identified blubber histology as an effective tool in assessing cetacean nutritional status (Castrillon and Nash, 2020).

4.2 Blubber Stratification

The results agreed with previous studies that blubber in striped dolphins is stratified into distinct layers with significant differences in adipocyte area (AA) and adipocyte index (AI) across the different layers (Castrillon et al., 2017). This is also an indicator that the different blubber layers serve different purposes. The inner layer may be more important to nutritional status than the other layers, as it has been more responsive to fluctuations in body condition (Castrillon and Nash, 2020). KW2020007 and KW2021006 showed a significant difference in all three layers. In the emaciated animal and KW2018006, the inner and outer layers were significantly different from the middle layer, but not from each other. This may be because as the inner layer is metabolized, it reduces adipocyte size making the measurements more similar to the outer layer, which had the smallest adipocytes in all the animals. When examining AI, all three layers of KW2020007 were significantly different to each other, but the inner and outer layers were significantly different compared to the middle layer, but not to each other in the other three individuals. Overall, it was found that the adipocyte area is largest in the middle layer, while smallest in the outer layer and intermediate in the middle layer, and the inverse was true for the adipocyte index. These findings agree with what was described in a previous study of striped dolphin blubber by Gómez-Campos et al., (2015).

4.3 Blubber Variation Across the Body

Furthermore, variation was observed across the girth axes and body planes of the animal, indicating that blubber serves several roles across the body such as metabolism and thermal insulation (Koopman et al., 1998). There was a general pattern of larger AA values towards the head. This may indicate that certain areas of the body are a priority of energy storage. Additionally, there was smaller AI in the ventral region, reinforcing its function as a main energy storage (Gómez-Campos et al., 2015). There were also less significant differences in KW2020009, which may be related to its emaciated condition. If all adipocytes are smaller and more uniformed due to nutritional depletion, differences between regions would not be expected.

4.4 AA and AI Compared Across Animals

Adipocyte area (AA) and adipocyte index (AI) were significantly different across the four animals. However, KW2020009, the emaciated individual, didn't have the smallest AA or largest AI consistently which was expected. This suggests that the individual variation in AA and AI is likely a result of factors, and not only reflective of observable body condition. KW2020009 was not significantly different to all of the other individuals except in the middle layer AI. This could indicate that even though the average AA was smaller, and AI was larger, the differences may not be enough to be seen statistically. The variation across the body may make the range too broad to specify the differences due to nutritional status. Future studies should include determining which areas of the body are most variable between individuals of different nutritional status to identify what region is most informative of overall body condition. This may provide a clearer picture of how nutritional status impacts blubber structure in cetaceans.

5.0 CONCLUSION

This study concludes that blubber of the striped dolphin *(S. coeruleoalba)* had variability amongst the four dolphins being analyzed. The ability to assess body condition through blubber histology can provide insights to the health of marine ecosystems. Continued research in analyzing blubber histology methods will be crucial to improve conservation strategies and the overall health of marine ecosystems.

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