“Adaptive skull shape changes in bottlenose dolphins (*Tursiops* spp.): inference from 3D morphological analyses”

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Geometric morphometrics (GM) is a powerful tool for analysing shape variation, offering insights into evolutionary relationships, ecological processes, and developmental changes. Advancements in computing and 3D imaging have broadened GM's applications, allowing complex structures and subtle variations to be studied in greater detail. To analyse skull shape variations, researchers often use three-dimensional geometric morphometrics (3DGM), which captures complex shapes and spatial relationships from 3D models. This mitigates biases associated with 2D image capture, such as perspective and lens distortion, and improves landmark placement by preserving surface features such as contours and curves that are lost in 2D projections. This enables more accurate shape analysis and enhances the ability to detect subtle shape variations, which is particularly useful in intraspecific comparisons. Additionally, GM data can be integrated with biological and environmental datasets, adding ecological and functional context to shape analysis. This integration, in turn, provides a more comprehensive understanding of the relationships between shape variation, function, adaptation, and evolutionary history.

The diverse habitats occupied by odontocetes, ranging from rivers to open oceans, makes them a suitable model for studying morphological shape, as this diversity drives the development of unique morphological features across different species. For example, skull shape in odontocetes is closely related to differences in developmental timing, especially differences in growth rates between species, which is thought to drive diversification and shape changes across the clade. In raptorial dolphins, faster growth of the rostrum results in distinct adult morphologies compared to suction feeders. A similar pattern is observed in riverine environments, where accelerated growth of the face and rostrum (leading to elongation of these structures) may be associated with feeding adaptations in shallow waters. Morphological studies identified an association between skull shape variations in structures involved in key functional aspects of odontocete life, including feeding, echolocation, breathing and swimming or diving behaviours. For example, variations in the rostrum, squamosal, parietal and zygomatic bones are associated with jaw musculature, which may facilitate jaw mechanics associated with specific feeding strategies. Similarly, variation in skull asymmetry, concavity of the frontal region and rostrum shape are associated with echolocating structures, suggesting they may influence echolocation properties. Finally, the overall skull profile (slender versus stout) and the orientation of the foramen magnum and rostrum are associated with swimming mechanics and efficiency.

Furthermore, studies using 3DGM have also identified skull shape sexual dimorphism (SD) in some species, including the bottlenose dolphin. SD appears to be influenced by differences in growth rates and durations, reflecting differences in local ecological conditions. This suggests that the degree and nature of SD in skull shape can vary between different populations of the same species. This variation may initially arise from phenotypic plasticity in response to local environmental conditions, often manifested through differences in size scaling relationships. Despite advances in understanding odontocete evolution at the species level, the drivers of intraspecific skull shape variation remain relatively understudied. However, investigating intraspecific skull shape variation could reveal underlying ecological and evolutionary processes essential for understanding dolphin species' diversification. The genus *Tursiops,* a well studied member of the family Delphinidae, comprises three distinct species: the common bottlenose dolphin *(Tursiops truncatus)*, the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) and the Tamamend's dolphin (*Tursiops erebennus*). *T. truncatus* in particular, exhibits substantial intraspecific variation, making it a valuable model for studying skull shape. This variation is associated with the species' wide distribution, including diverse habitats and a range of ecological and behavioural characteristics. A common pattern is a coastal versus offshore differentiation, observed in multiple regions worldwide. Dolphins in coastal regions typically inhabit shallow, prey-rich areas, while offshore dolphins live in deeper waters with lower biodiversity. These habitat differences were suggested to provide distinct selective pressures, which contribute to observed regional differentiation. Multiple studies have identified genetically unique populations of common bottlenose dolphins with distinct cranial shapes, which in some cases led to the denomination of subspecies, namely *Tursiops truncatus gephyreus* in the Atlantic coast of South America, and *Tursiops truncatus ponticus* in the Black Sea. This combination of ecological, genetic, and morphological diversity makes *Tursiops* an ideal system for investigating the evolutionary forces shaping skull morphology, particularly by allowing comparisons across well defined operational taxonomic units.

Skull shape variations appear to reflect population differentiation within the genus, and several coastal operational units have been suggested. However, further research is needed to determine the exact processes driving these coastal versus offshore skull differences. Namely, it is unclear whether these differences reflect a consistent pattern of adaptation to a coastal environment, or if the observed variations are unique to individual units. If the variations are unique to specific units, it suggests other evolutionary processes, such as genetic drift or other forms of selection, are also likely driving the observed differences. A key obstacle to understanding the drivers of intraspecific skull shape variation relate to methodological challenges, including inconsistent landmarking methods, which reduce comparability and replicability. Additionally, placing landmarks manually is time-consuming, hindering the analysis of large datasets and complex structures. Finally, challenges persist in linking skull shape data to environmental factors (e.g., depth, water temperature), making it difficult to infer potential drivers or test associated functional implications.

This thesis represents the first effort towards investigating worldwide trends in bottlenose dolphins' skull shape variation using 3DGM. Employing automated surface semi-landmarking, this study links skull shape to environmental data and explores associated allometric patterns at fine geographical scale. This approach aims to determine whether skull shape divergence is a global phenomenon or region-specific, enhancing our understanding of the adaptive nature of cetacean skull changes, the mechanisms driving cranial diversification in bottlenose dolphins, and the potential identification of new coastal operational units. Specifically, this research investigates whether skull shape variations are driven by local adaptation, stochastic events, or biogeographic history. Addressing these questions will provide insights into the factors initiating diversification and reveal functional implications associated with ecological processes within the clade.

This study addresses four key objectives: 1) Quantify 3D skull shape differences between well-described coastal and offshore operational units of bottlenose dolphins in a worldwide context. 2) Investigate the correlation between these skull shapes and environmental variables. 3) Investigate fine-scale skull shape variations within the operational unit inhabiting the Western North Atlantic (WNA). 4) Investigate allometric patterns associated with different populations on a fine regional scale (WNA).

To achieve these objectives, Chapter 2 presents the development of a standardized protocol for creating 3D models using photogrammetry. This protocol provides step-by-step guidelines for constructing accurate and replicable 3D models, to be used in geometric morphometric (GM) analysis.

In Chapter 3, Surface Semi-Landmarking (SSL) techniques are tested to address the limitations associated with manual landmarking, by comparing the skull shapes of coastal populations from the Gulf of Guayaquil (Ecuador) and the Mediterranean Sea with those of offshore specimens. The results show that SSL can be effective at showing that both coastal populations exhibit distinct shape patterns, which not only differ from the offshore ecotype but also from each other. In comparison to manual landmarking, SSL provides enhanced surface coverage, thereby improving the accuracy and efficiency of skull shape analysis. These advancements facilitate more robust intraspecific comparisons and enable the analysis of large-scale datasets with greater efficiency.

In Chapter 4 the first objective was addressed by comparing the skull shapes of 10 coastal regions with their offshore counterparts at a worldwide scale. While skull shape variations between coastal and offshore operational units are well documented, no previous 3DGM studies have directly compared multiple coastal units to offshore specimens. While ecological conditions have been proposed as potential drivers of these variations, this hypothesis has not been extensively tested using environmental data. Therefore, in this study, the relationship between skull shape and these environmental variables was tested to identify which factors best correlate with skull shape. The results revealed consistent patterns of skull shape differentiation between the coastal units, with the offshore ecotype standing as an average skull shape. Skull shape was found to correlate with several environmental variables which represented characteristics of coastal and offshore habitats. This chapter provides new insights into the bottlenose dolphin phenotypic diversity, identifies the drivers of phenotypic variation and improves our understanding of the evolutionary and ecological processes shaping diversity in this species

In Chapter 5, objectives three and four were addressed by performing a fine-scale analysis of skull shape variations in the WNA, investigating potential allometric patterns related to skull shape. While genetic partitioning has been observed between several coastal populations along the U.S. coast and in the Gulf of Mexico and the Caribbean, skull shape variation across these populations remains underexplored. Additionally, although broad-scale allometric patterns between offshore and coastal bottlenose dolphins have been identified, fine-scale geographic analyses of these patterns are lacking. The results reveal distinct skull shape patterns between locations, with diagnostic skull shapes identified in Florida, the Gulf of Mexico and Delaware Bay. Furthermore, skull shape variations observed across different locations were found to be associated with allometric differences, suggesting that ecological plasticity partially accounts for the observed shape differences. As such, both shape variations and static allometry are effective criteria for distinguishing between bottlenose dolphin populations. Additionally, our allometric analysis reveals distinct male and female patterns in some populations, suggesting that local environmental conditions may influence shape variation and sexual dimorphism in dolphins. These differences may arise due to the varying ecological needs of males and females in these populations.

Chapter 6 summarises the key findings of this PhD research. Coastal bottlenose dolphin units exhibit distinct skull shapes associated with local environments, while offshore dolphins show more consistent skull shapes, likely due to stabilising selection. Therefore, coastal skull variations also likely reflect non-adaptive processes such as genetic drift, historical events such as glacial cycles, and possibly phenotypic plasticity. Some skull shape variations in bottlenose dolphins mirror those seen across Delphinidae, which often correlate with local environmental characteristics. Furthermore, morphological variation often occurs in skull traits that can be related to feeding, communication, and respiration. This likely reflects local habitat functional demands, with potential trade-offs due to the structural integration of the various skull features. Niche partitioning, driven by competition for food resources is also suggested to be an important driver of skull shape variation in bottlenose dolphins, possibly reinforced by social behaviours. Finally, the study findings support known species/sub-species classification (*T. aduncus*, *T. erebennus*, *T. t. gephyreus*) and suggest the potential for additional distinct coastal operational taxonomic units in the North Sea, Japan, West Africa, and Western South America. The Southeast Pacific shows especially strong population differentiation, likely driven by greater ecological heterogeneity and historical founder effects.