Comparative anatomy of Selachian vertebrae and the correlations with ecological and behavioural traits

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Abstract

Diversity in habitat use and ecological niche seen in sharks should be reflected in the vertebral column. The mechanical constraints of different environments and swimming modes should affect the morphology of vertebral centra allowing sharks to optimally function in their respective habitats. There are few comparative anatomy studies of Chondrichthyan vertebral morphology. Most work on Chondrichthyan vertebrae concerned their use for age and growth studies. This study aims to investigate centra morphology and its relationship with ecological aspects of Selachian species, which includes habitat use, vagility and swimming style. A comparative analysis of vertebrae shape across 12 families (Alopiidae, Carcharhinidae, Carchariidae, Etmopteridae, Galeocerdidae, Lamnidae, Pentanchidae, Pristiophoridae, Pseudocarchariidae, Scyliorhinidae, Sphyrnidae, Squalidae and Trikidae) and 30 species was conducted. Species were compared according to a designated ecological environment of either pelagic (n=13) or benthic (n=17). Vertebrae centra were isolated by removing transverse processes, muscle and other tissue. Centra were bleached for approximately 30 minutes before rinsing in deionized water and left to completely dry on paper towel overnight. Six measurements of the dried centra were measured with digital vernier callipers (mm). A fineness ratio (FR) between the cranial-caudal length and the dorsal-ventral height was used to quantify morphology to compare across shark orders. It was hypothesised that benthic sharks have a larger cranial-caudal length and a smaller dorsal-ventral height in contrast to pelagic sharks. Secondly it was hypothesised that the FR will be greater for benthic sharks resulting in block-shaped centra and lower for pelagic sharks resulting in disc-shaped centra. Benthic sharks had a mean (±SE) FR of 0.98 (±0.05). Pelagic sharks had a mean (±SE) FR of 0.56 (±0.02). A general linear model exposed significant differences between shark orders *(F-value = 4.215,* R^2 *= 0.24, p-*

$\text{value} = 0.015$). FR differed between Squaliformes and Lamniformes and between Squaliforms and Carcharhiniforms. The FR differed between pelagic and benthic sharks ($t = 5.09$, $df = 29$, $p\text{-value} < 0.05$). Multiple Gaussian general linear models showed that swimming mode, centra shape, migration and environment had significant effects on the fineness ratio ($F\text{-value} = 25.91$, $R^2 = 0.88$, $p\text{-value}<0.05$; $F\text{-value} = 36.53$, $R^2 = 0.88$, $p\text{-value}<0.05$; $F\text{-value} = 20.7$, $R^2 = 0.87$, $p\text{-value}<0.05$;). Factor of Mixed Analysis (FAMD) plots formed distinct two distinct clusters of pelagic species with associated pelagic ecological variables and benthic species with associated benthic ecological variables. Species, fineness ratio and centra shape were the largest contributors in explaining the dimensions. The second moment of area (I) was calculated to quantify the stiffness of vertebrae. Values for I differed for benthic and pelagic sharks ($t = -2.36$, $df = 28$, $p\text{-value} = 0.03$). Following beam theory, large fineness ratios were was associated with longer cranial-caudal lengths, fluted cylinder and hourglass vertebral shapes, anguilliform swimming, small second moment of area values, and a flexible vertebral column. Smaller fineness ratios were associated with smaller fineness ratios, shorter cranial-caudal lengths, cylinder vertebral shape, carangiform swimming, large second moment of area values, and a rigid vertebral column. Centra shape is an adaption to different environments and swimming modes and could be used to infer ecological and behavioural traits. These relationships could form a foundation for tools to infer the ecological characteristics of unobserved and extinct species.

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Introduction

Cartilaginous fishes, including sharks, rays, and chimaeras are an ancient yet highly successful lineage of fishes that have undergone many morphological changes since their first appearance in the Palaeozoic era over 250 million years ago (Compagno, 1977, 1990). The class Chondrichthyans is a monophyletic group of specialized predators comprised of two sister taxa: Elasmobronchii (sharks and rays) and Holocephali (chimaeras) (Compagno, 1977; Grogan & Lund, 2004; Helfman et al., 2009; Maisey, 2012). The taxonomic literature divides Elasmobranchii into Neoselachii, a subclass encompassing all extant sharks and rays, and Holocephali, which exist in a single extant order, the Chimaeriformes (Underwood, 2006). Neoselachii is further divided into Selachii (modern sharks spread across eight orders) and Batoidea (rays, skates and sawfish spread across four orders) (Compagno, 2002).

Cartilaginous fish are recognised by their calcified cartilaginous endoskeleton and the presence of claspers, in males, for internal fertilization (Grogan & Lund, 2004; Helfman et al., 2009; Harvey Pough & Janis, 2018). Chondrichthyans differ from bony fish as they lack sutures on their skull, their teeth are embedded in connective tissue on the jaw rather than being fused to the jawbone, and their oily livers are used for buoyancy instead of swim bladders (Helfman et al., 2009).

The dominant extant class of the Chondrichthyans is the Elasmobranchii. Elasmobranchs consist of three infraclasses: Clasoselachimorphs†, Xenacanthimorphs† and Euselachians (modern forms) (Helfman et al., 2009). Approximately 1202 living species of elasmobranchs are considered valid (White, O'Neill & Naylor, 2022). Neoselachii includes 544 extant species across eight orders: Heterodontiformes (bullhead sharks), Orectolobiformes (whale, nurse, and wobbegong sharks), Lamniformes (mackerel sharks), Carcharhiniformes (ground sharks), Hexanchiformes (frill and sevengill sharks), Squaliformes (dogfishes), Squatiniformes (angel sharks) and Pristiophoriformes (sawsharks) (Compagno, 1977a; Ebert, Wintner & Kyne, 2021a; White, O'Neill & Naylor, 2022). The Batoidea includes 658 extant species, divided into four orders: Torpediniformes (electric rays), Myliobatiformes (stingrays), Rhinopristiformes (guitarfishes, wedgefishes, and sawfishes), and Rajiformes (skates) (Nelson, 2006; Ebert, Wintner & Kyne, 2021a; White, O'Neill & Naylor, 2022).

The knowledge of chondrichthyan phylogeny is limited by a lack of fossil skeletal material. Fossils provide us with a valuable window into the chondrichthyan evolution, but the incomplete and inconsistent fossil record hinders a reconstruction of the phylogeny of the group. With the skeleton composed entirely of cartilage, whole-body fossils are rare as they yield low potential for fossilization (Maisey, 2012; Whitenack, Kim & Silbert, 2022). In contrast, fossil teeth and dermal denticles (scales covering shark bodies) are well in abundance as chondrichthyans shed both their teeth and denticles throughout their lifetimes and at a fast rate (Harvey Pough & Janis, 2018). Teeth are the most common fossils as they are extremely durable due to the composition of their enameloid and dentine (Whitenack, Kim & Silbert, 2022). While teeth offer diagnostic features for taxonomic identifications, they provide limited information about the organism's biological traits and life history. Although fossilised vertebrae are less common, they yield important information on the biology and ecology of extinct species.

Understanding these life history traits is important for palaeoecological reconstructions and provides insight into a species perseverance or demise, and an understanding of the extinction patterns in the group (Jambura & Kriwet, 2020). By studying the vertebral morphology in extant forms and its relationship with the habitat and feeding ecology of the species, I hope to interpret and infer biology of extinct taxa in the same way as fossilised teeth, thereby, providing an alternative means of using fossilisation specimens to distinguish life history traits.

With over 400 million years of evolution producing a diversity of biological traits, sharks have always been ecologically important predators across a range of different habitats, niches, and trophic levels (Jambura & Kriwet, 2020). They inhabit most aquatic environments including coastal waters, the open sea, the deep sea, esturaries and lagoons and freshwater riverine (Cailliet et al., 2005). Sharks are represented in all oceans and at all latitudes and are also present across a wide depth gradient. As a result, sharks display a large array of ecomorphotypes due to differences in morphology, habitat, and behaviour. It is important to understand the diversity in the morphology of organisms in relation to their environment to gain insight into how these traits relate to the ecology and life history of the organism. Analysing morphological differences across species and the correlation of morphology with habitat use, aid in the understanding of species adaptation and evolution (Irschick et al., 2017). Furthermore, establishing links between morphology and ecology can lead to predictions about the ecological and life history patterns for taxa where information is lacking.

Our understanding of comparative chondrichthyan biology is the poorest and least understood of all the major marine vertebrate classes (Cailliet et al., 2005). Detailed morphometric, life history and reproductive dynamics information is typically only available for species deemed important for fisheries and ecotourism (Cailliet et al., 2005). Research emphasis has often been placed on charismatic species such as the Great White shark, *Carcharodon carcharias,* and other common surface-dwelling species that are easily accessible and/or are successfully kept in captivity. Low research priority has been placed on elusive cosmopolitan species and those found restricted to deepwater habitats where capture is more challenging (Cailliet et al., 2005). Consequently, many chondrichthyan species remain poorly known to science (Ebert & van Hees, 2015; Livernois et al., 2021).

Shark centra

Neoselachian sharks have a cartilaginous skeleton, completely lacking any true bone (Grogan & Lund, 2004; Ebert, Dando & Fowler, 2021; Stock et al., 2021). This material is much lighter and more flexible than bone because it contains fewer minerals (Ebert, Dando & Fowler, 2021). However, examinations of mineralised cartilage in elasmobranchs have been found to behave similarly to mammalian trabecular bone in material stiffness and ultimate strength (Porter et al., 2006). Because of this similar bone-like stiffness and strength of vertebral cartilage, it is unlikely that the selective force behind the abandonment of bony skeleton by cartilaginous fish was due to decreased functional demands as elasmobranchs perform at functional extremes (Porter et al., 2006). Greater mechanical deformation along the vertebral column allows cartilaginous vertebrae to have an increased elastic energy storage compared to bony fish (Knaub et al., 2024). This change to a cartilaginous skeleton and loss of bone permits centra to undergo large strains and may be an adaptation for the spring-like behaviour of the vertebral column (Porter, Ewoldt & Long, 2024).

The vertebral column is a central axis consisting of a series of successive, incompressible cartilaginous vertebrae spanning from the anterior to the caudal region providing structural support for the animal. Between each vertebrae are intervertebral joints made of two ligaments - one that resists tension and the other resisting compression (Porter et al. 2014). The vertebral column isn't an alternating series of rigid vertebrae and flexible intervertebral joints, rather together the vertebral centra and intervertebral joints act as a spring, absorbing strain when the axial skeleton bends during swimming (Porter et al. 2014). These intervertebral joints, along with centrum length and width, differ both within and among different species and appears to be related to body flexibility (Porter, Rogue & Long, 2009).

Each vertebrae consists of a large middle body mass known as the centrum with projecting neural and haemal arches which attach into two dorsal and ventral foramina respectively (Ridewood, 1921). Each centrum is cylindrical with an hourglass-shaped structure, the corporous calcerea, and both the cranial and caudal walls of the centrum are supported by the intermedalia. Mineralisation of the intermedialia structure varies among species, ontogenetically, and within an individual and in part determines the material properties of centra (Knaub et al., 2024).

In Lamniformes, the intermedialia is less calcified and consists of two dozen or more thin radiating plates (lamaellae). These radiating lamaelle are calcified plates that lie longitudinally in radial planes between the double cone and the centrum surface (Ridewood, 1921). The intermedialia form four sectors; two large groups of lamellae project laterally and are each separated from the smaller dorsal and ventral sectors by gaps within the mineralisation where the vertebral arches insert (Knaub et al., 2024). However, these properties vary along the body and across ontogeny; juvenile shark centra have significantly few lamellae than centra from adult sharks (Knaub et al., 2024). These changes across ontogeny suggest that vertebral morphology and the number of lamellae present shifts across development to support a larger body, caudal fin and greater lateral oscillations during swimming (Knaub et al., 2024). In contrast, Carcharhiniformes possess four highly calcified, laterally obtuse thick wedges with less radial arrangement (Ridewood, 1921; Morse et al., 2022; Park et al., 2022) (Figure 1). Differing intermedialia have developed in various positions in response to different physiological demands and the strengthening of cartilage for resisting these stresses (Ridewood, 1921).

All centra have an areolar mineralised double cone calcification pattern with concentric rings expanding outwards from a central apex, surrounded by the uncalcified cartilage of the neural and haemal arches (Duarte et al., 2001; Dean & Summers, 2006; Porter et al., 2006; Jambura & Kriwet, 2020a). However, the density of mineralisation within a centrum may vary among species, ontogenically and within an individual (Dean & Summers, 2006; Ingle, Natanson & Porter, 2018). The greater the mineralisation, the greater the centrum stiffness and strength (Porter, Koob & Summers, 2007). These differences in vertebral mineralisation have implications for the vertebral column's ability to resist deformation from the loads and bending imposed by swimming (Porter et al., 2006).

The centrum body houses the notochord that passes through its centre. The neural arches hold the dorsal nerve chord, and the haemal arches protect the dorsal aorta and the cardinal vein in the caudal region (Ridewood, 1921; Compagno, 2002; Berio et al., 2022). Consecutive centra are connected by intervertebral joints throughout the vertebral column, and the neural and haemal arches are joined by tracts of white fibrous tissue (Ridewood, 1921). These neural arches are composed of tessellated cartilage, a core of unmineralized hyaline cartilage that’s surrounded by a shell of mineralised blocks called tesserae (Porter & Long, 2010).

Sharks do not have articulating zygapophyseal processes on their vertebrae like those of Teleost fishes (Ridewood, 1921). Each centrum articulates with both the anterior and posterior adjacent centra to combat the compression forces experienced during bending (Lindsey, 1978; Stock et al., 2021). When under compressive forces, the centra arches do not bear appreciable loads. Centra are the primary load-bearing structures of vertebrae in compression and while the arches do not significantly add to load resistance, they may act to alter stress and strain distributions (Porter & Long, 2010; Porter, Ewoldt & Long, 2024). As the body bends, centra bend too, storing and realising mechanical power (Porter, Ewoldt & Long, 2024). Centra faces are concave anteriorly and posteriorly, with a fluid-filled hydrostatic cushion between the hollow space. This cushion receives the forces exerted from one rim to another and helps to distribute the force over the full face of the following vertebrae (Lindsey, 1978).

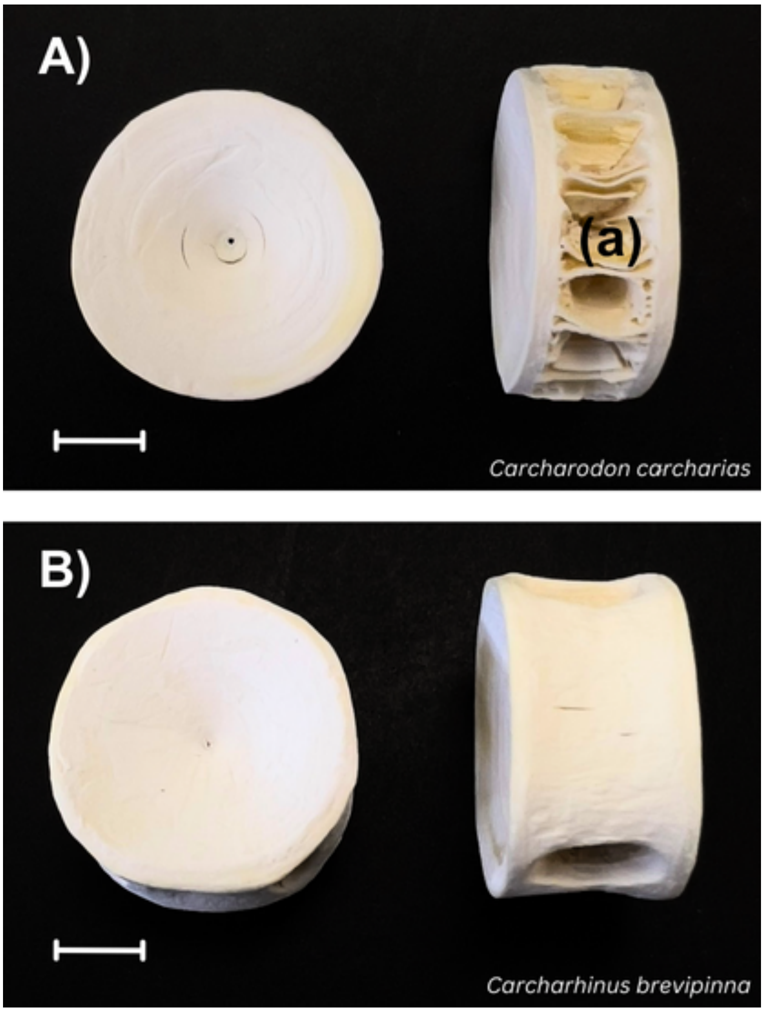

Figure 1: A) Frontal view (left) and sagittal view (right) of Lamniform centra showing radial lamaellae plates (a). B) Frontal (left) and dorsal view (right) of Carcharhiniform centra showing solid wedges. Scale bar = 0.8 mm

Centra lengths and widths vary dramatically along the vertebral column but also between different species (Lindsey, 1978; Porter, Roque & Long, 2009) (Figure 2). The cylindrical centrum body shapes differ by either having concave, straight, or convex lateral sides. Kozuch & Fitzgerald (1989) discussed the four shape differences in centra among species and throughout the vertebral column. These shapes four basic centra shapes were described as follows: 1) Cylinder, a short or medium lengthened centra with flat or slightly concave or convex sides; 2) Modified cylinder, similar to the cylinder shape with concave sides that recurves near the rim; 3) Fluted cylinder, a long slender centrum that flares at either end and lastly, 4) Hourglass, a centrum that is slightly pinched in the centre with strong recurving at the rims.

The column itself is separated into three regions: anterior, mid-trunk, and caudal, each with significant vertebral morphology and microstructure variation among the regions (Knaub et al., 2024). In general, vertebral size and shape changes along the column and ontogenically in species-specific patterns (Natanson et al., 2018). Centrum sizes increase generally from the head and levels or peaks over the abdominal cavity and then decreases towards the tail region (Natanson et al., 2018). The mid-trunk abdominal region (largest body girth) has the centra with the greatest volume and highest number of band pairs, while the anterior and caudal regions have the smallest volume and number of band pairs (smaller body girths) (Ingle, Natanson & Porter, 2018). As such, vertebrae within a region tend to be homogenous but heterogeneous between regions (Piercy et al., 2006). Smaller sharks have vertebrae of similar size and the same band pair count along the vertebral column whereas for medium or larger sharks, band pair count and vertebral size differentiates along the column with larger centra having higher band pair counts (Natanson et al., 2018).

The centra in the mid-trunk abdominal region have the largest diameter and greatest cranial-caudal length. These vertebrae are also homogeneous with the most distinguishable characteristics, thus making them easier to identify in extant and extinct organisms (Kozuch & Fitzgerald, 1989). Vertebral growth and band pair deposition are both linked to somatic growth, girth in particular, which changes along the vertebral column and as the mid-trunk centra have the highest and clearest number of band pairs, they are frequently used for age and growth studies (Piercy et al., 2006; Natanson et al., 2018). By calculating a fineness ratio (length:width) from this region, the same sample can be used to infer both age and ecological habits of the organism. This approach can prove beneficial when examining newly discovered, rare, or deep-water species, where observing ecological habits can be challenging. Furthermore, when fossilised vertebrae samples are excavated and studied, as the mid-trunk region's distinctiveness are the easiest to identify, inferences on the animal's ecology can be made based on the fineness ratio. In many groups of elongated actinopterygian fishes, the number of vertebrae and the fineness ratio of the vertebral centra were predictors of body morphology (Ward & Brainerd, 2007; Porter, Rogue & Long, 2009).

Shark centra are not easily identifiable to species level in isolation due to their specific uniformity (Kozuch & Fitzgerald, 1989). However, there is still diversity in shark morphology and physiology and variability in the growth, mineralization, and mechanical properties found in their vertebrae within individuals, between individuals, and among species (Ingle, Natanson & Porter, 2018). It is suggested that the additional calcification causing these variations in mineral content and arrangements, develop in a response similar to bone remodelling (Porter et al., 2006). Each shark order has centra that are morphologically distinct. For example, the broadnose sevengill shark (*Notorynchus cepedianus*), a traditionally primitive species, has an unsegmented notochordal vertebral column without highly calcified cartilaginous centra, resulting in a jelly-like consistency (Ebert, 1989). Furthermore, in the tiger shark (*Galeocerdo cuvier*), centra have a modified cylinder shape with distinct concave sides that strongly recurve at the rims. These centra have extremely large pores (the largest of any species) scattered in patterns across the rim, foramina, and lateral walls (Kozuch & Fitzgerald, 1989).

Despite the importance of the vertebral column in all vertebrates, the vertebral column of chondrichthyan species has been the focus of fewer studies than that of osterichthyans. Consequently, the knowledge of morphological differences and development of chondrichthyan vertebrae remains understudied and fragmentary. As the vertebral column is the main skeletal support of the body axis in vertebrates and is shaped by the mode of life of a species, increasing our knowledge of morphological differences in vertebrae and the vertebral column can significantly advance our understanding of ecological functionality and the evolutionary relationships among organisms (Berio et al., 2022).

Locomotion

Shark vertebral columns function as a central spring, absorbing and releasing energy as they swim (Raja Somu et al., 2024). Springs convert kinetic energy into potential energy, this exchange may be used to control body movements and reconfiguration, smooth or delay the delivery of mechanical work as well as combine work from multiple sources (Porter et al., 2014). Vertebrae are moderately rigid with flexible intervertebral joints which together absorb between 3 and 8% of the load generated by body flexing during locomotion (Stock et al., 2021; Park et al., 2022). During straightforward horizontal swimming, not including acceleration, turning, and other manoeuvring, the abdominal centra on the left and right-side experience alternating bending patterns of compression and tension. This bending is translated into backward thrust against the water in various undulations depending on their swimming mode. There is variation in the amount of bending across the shark's body during locomotion. The pre-caudal column between the pectoral fins, has the greatest curvature, almost four times greater than that of the rest of the body (Porter et al., 2014). The mechanical behaviour of the vertebral column changes depending on the locomotion of the shark, as swimming speed (when controlled by tail-beat frequency alone) increases, the vertebral columns get stiffer without more energy loss (Porter, Ewoldt & Long, 2024). Swimming styles are classically categorised into four groups of major modes depending on the head amplitude and the length of the undulating wave during bending; however, recent studies have shown that many fish species are located on a continuum during steady locomotion rather than in distinct modes (Di Santo et al., 2021). The main modes of chondrichthyan swimming are anguilliform and carangiform with the latter containing two sub-categories, sub-carangiform and thunniform swimming.

Anguilliform swimming is indicated when approximately two-thirds of the body length undulates in a relatively large side-to-side wave that increases towards the tail. Characteristically, anguilliform swimming is characterised as the least efficient mode of swimming and is not suited to high-speed swimming, often seen in many species living close to the ocean floor. The benefit of anguilliform swimming, however, is that while swimming speeds are reduced, the large wave undulating throughout the whole body allows for better manoeuvrability and agility. Smaller sharks, such as catsharks or some *Squalus* species, tend towards anguilliform swimming (Lindsey, 1978).

Sub-carangiform swimming is an intermediate between anguilliform and carangiform swimming modes where undulating waves occur in the posterior half of the body resulting in constant and high steady swimming speeds. In carangiform swimming, only the posterior third of the body length undulates, generating thrust through a stiffer tail. The majority of body mass and depth is concentrated towards the anterior region of the body minimizing recoil and energy loss when displacing water. These adaptations make carangiform swimming more efficient and faster than anguilliform swimming.

Finally, thunniform swimming is indicated when lateral thrust movement is exclusively generated by the high aspect (mostly lunate), stiff caudal fin and peduncle and the body is relatively straight (Di Santo et al., 2021). The body is streamlined with a heavily circular anterior, almost torpedo-like in shape (Lindsey, 1978). Thunniform swimming is geared towards continuous fast swimming achieving cruising speeds of 6-10 $L.s^{-1}$ where L is the total length of the fish (Huang et al., 2023). These speeds are considerably faster than the 0,2-2 $L.s^{-1}$ cruising speeds of anguilliform swimmers (Tack, Du Clos & Gemmell, 2021).

Relationship between vertebrae morphology and ecological traits

Previous studies of elasmobranch vertebrae have primarily been focused on utilizing centra as tools for age and growth determination. Hundreds of studies have been published quantifying the age and growth of more than 1200 extant chondrichthyan species (Harry, Smart & Pardo, 2022). The most common method, which involves counting growth zones, has since been deemed unreliable, particularly in larger and older individuals, as it results in large age underestimations (Harry, 2018; Natanson et al., 2018). Furthermore, other vertebral studies have swayed towards focusing on the systematic significance and functionality of the differences in centra calcification patterns across taxa, e.g. Ridewood (1921), Dean & Summers (2006), Porter et al. (2006), Berio et al. (2021) and Morse et al. (2022).

However, fewer studies have focused on the physical morphology of chondrichthyan vertebrae and its ecological implications. The regionalisation of the vertebrate column correlates with many life history traits and functional adaptions (Knaub et al. 2024). The mode of life of a species shapes the vertebrate column (Berio et al., 2022). Porter, Roque & Long (2009) showed that there is a correlation between vertebral morphology and body curvature in sharks while swimming. More studies have since been done examining the mechanical behaviour of centra and its stiffness under the biological strains of swimming (Long et al., 2011; Porter et al., 2014; Ingle, Natanson & Porter, 2018).

Several studies on other marine vertebrates provide evidence for the premise that vertebrae morphology can be used to infer an organism’s ecological habits. Buchholtz (2001) investigated variations in the morphology of the vertebral column in living cetaceans and identified patterns to support predictions of swimming styles in fossil species. Similarly, Donatelli et al. (2021) concluded that in bony fish, internal vertebral morphology can be used to predict habitat, body shape, and swimming modes. Porter, Roque & Long (2009) examined the shape and size of vertebrae, correlating these features with maximum body curvature in five shark species by creating a fineness ratio. They found vertebral morphology to be a better predictor for body curvature than the maximum vertebral number alone. In this study, I aim to contribute to this growing body of studies by exploring the patterns of centra morphology across different niches by calculating a fineness ratio and the second moment of area of vertebral centra.

With parallels in naval architecture and aerospace engineering, a fineness ratio is a measure of how elongated an object is relative to its transverse sectional diameter. This ratio is often used as a baseline for investigating the aerodynamics, speed, and drag forces in fish and marine mammals (Ohlberger, Staaks & Hölker, 2006; Ahlborn, Blake & Chan, 2009; Walker et al., 2013). Shapes with a low fineness ratio are short and wide, while those shapes with a high fineness ratio are long and narrow.

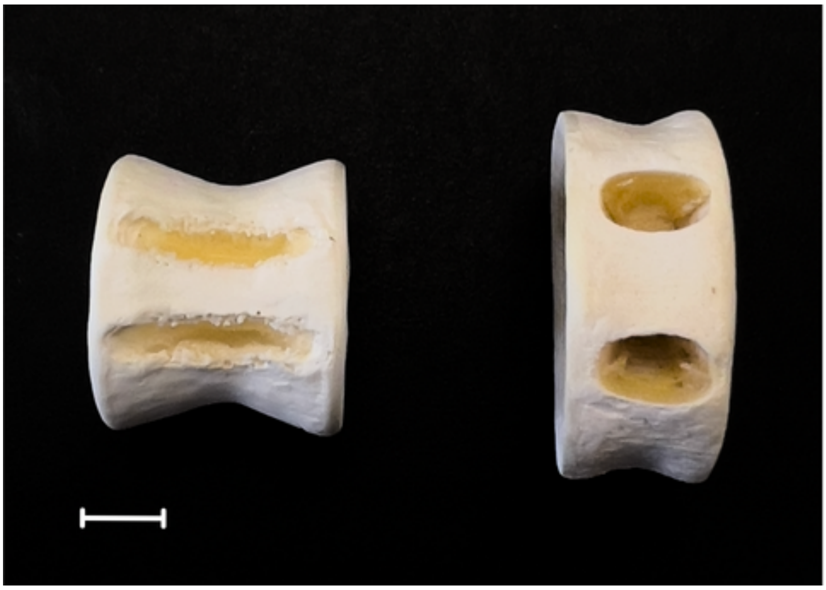

Figure 2: Dorsal view of two shark centra showing differences in cranial-caudal lengths. Left: *M. mustelus* (benthic species). Right: *C. limbatus* (pelagic species)

Scale bar = 0.36 mm*.*

Aims and hypotheses

In this study, I test hypotheses regarding the morphology of the centra across four Selachiani orders: Carcharhiniformes (16 species), Lamniformes (7 species), Squaliformes (6 species), and Pristiophoriformes (1 species). Hexanchiformes were excluded from this study due to their notochordal vertebral column which lacks calcified vertebrae. The remaining orders – Heterondontiformes, Orectolformes, and Squatiformes – were also not included because specimens could not be obtained because either they are very rarely captured or legally protected from capture.

This study aims for a more holistic approach by studying a multitude of species from across most orders available in southern Africa rather a single species in isolation. Examining ecological and life history patterns across multiple shark species provide an understanding of ecosystem influences on body design that cannot be achieved by examining single species (Livernois et al., 2021). Furthermore, a comparative anatomy study is a vital tool for a comprehensive understanding of fish biology and furthers our knowledge of habitat overlap and interactions both within and among species (Gemballa et al., 2006; Andrzejaczek et al., 2022).

This study focuses on benthic and pelagic marine species whose environments encompass open oceans, continental shelves, and slopes, with a few deep-dwelling species. Pelagic environments have lower habitat variability compared to benthic environments which include a vast range of sediment types and reef structure (Livernois et al., 2021). The greatest form diversity (<90%) is amongst those dwelling on continental shelves and slopes (Compagno, 1990). Oceanic species only comprise of 2% of shark diversity.

I aim to test the degree to which centra morphology is explained by the ecological niche and behaviour, including feeding mode, habitat, reproductive mode, swimming mode, and degree of residency. Additionally, I aim to determine the predictive power of centra morphology for ecological niche and behaviour. A comparative analysis noting differences in centra shape and dimensions will be conducted across multiple families. The second moment of area, a nonlinear contribution of the cross-sectional area to the flexural stiffness of the beam, will be calculated to determine vertebral stiffness across species (Porter, Rogue & Long, 2009).

A fineness ratio between the cranial-caudal length and dorsal-ventral height is defined to test the following hypotheses. Firstly, I hypothesise that there are constant differences in centra shape and in fineness ratio among shark orders. Secondly, I hypothesise that the fineness ratio is greater among benthic than pelagic shark species. Lastly, I hypothesise that swimming mode and migration similarly affect the fineness ratio of shark species.

Methods

Vertebrae processing

All samples were kindly provided by the KwaZulu-Natal Sharks Board (KZNSB), the Department of Forestry, Fisheries and Environment (DFFE), and the South African Shark Conservancy (SASC). Samples procured from KZNSB included both dried archival and frozen vertebrae segments obtained from shark nets and drumlines. Samples procured from the DFFE were frozen vertebrae segments from sharks caught as bycatch from trawling vessels. SASC provided frozen vertebrae segments samples caught via handline fishing. There is evidence that the freezing of samples before testing has no effects on the mechanical and material properties of the specimen (Porter et al., 2006).

Vertebrae segments were removed from the mid-trunk region beneath the first dorsal fin. Before vertebral segments were removed, metadata was recorded, and each sample was labelled with the associated standard dissection workup measurements. Due to the various methods and personnel involved in obtaining the vertebrae segments, there was variability in the number of vertebrae collected from each shark. The number of centra in each vertebrae segment ranged from 4 to 20. In total, samples were examined from 177 individual sharks and 1411 centra (30 species, 12 families, and four orders).

Once vertebrae segments were thawed, they were first manually and then chemically cleaned. Neural and haemal arches were excised to isolate the centra so that all centra measurements were recorded accurately. The segments were then separated into individual centra using standard dissection equipment. Once separated, any remaining connective tissue on the face and edges of the centra were removed with a scalpel. Centra were then soaked in 3.5% Sodium Hypochlorite (bleach) for 15 to 30 minutes. For larger centra, a longer soaking time of 30 to 60 minutes was required. If necessary, a second soak in bleach was performed to further eliminate any remaining connective tissue. This procedure was closely monitored to prevent overexposure to the bleach. Chemical alteration of vertebrae is least likely when submerged in 8.25% Sodium Hypochlorite for less than an hour (Mohan et al., 2017). As only 3.5% concentrate of Sodium Hypochlorite was used, the above bleaching times will not result in any chemical alteration of the material. Following the bleaching process, cleaned centra were then rinsed and soaked in distilled water for 10 minutes to remove any residual bleach and left to air-dry overnight.

Centrum morphology

Six measurements were taken from each dried centrum with digital callipers to the nearest 0.01 mm (Figure 3). These measures were:

1. **Dorsal-ventral height (DVH):** The diameter of the centrum vertically across the centre.
2. **Cranial-caudal length (CCL):** The length of the centrum from the anterior face to the posterior face.
3. **Medial-lateral breadth (MLB):** The diameter of the centrum laterally across the centre.
4. **Dorsal length (TOP):** The length of the dorsal edge of the centrum.
5. **Ventral length (BOT):** The length of the ventral edge of the centrum.
6. **Height at centre (CENT):** The height at the centre at the lowest point of the sagittal side of the centrum.

Of all the available centra, only the measurements from the biggest (largest DVH) five centra from each sample were used in the analysis. In samples where less than five vertebrae were available, all available centra were included. In total, measurements were taken from 880 centra. A mean was calculated for each measurement to obtain the average measurement per individual. To account for the effect of body size when comparing centra measurements within and between species, all measurements were standardized by total length. Standardised measurements are denoted with the subscript 's' and do not have any units attached as it is a ratio. From these standardised calculations, the mean of each measurement was calculated for each species.

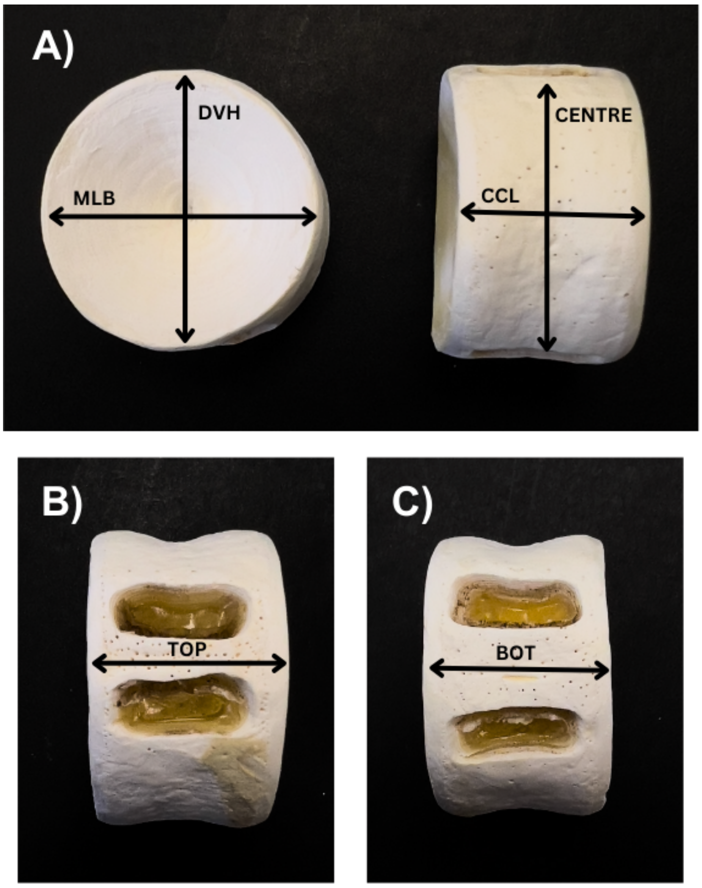

Figure 3: A) Frontal (left) and sagittal (right) view of shark centra with haemal and neural arches removed. Lines represent the dorsal-ventral height (DVH), medial-lateral breadth (MLB), cranial-caudal length (CCL), and height at centre (CENTRE) measurement. B) Dorsal view of shark centrum with haemal arches removed. Lines represent the dorsal length (top). C) Ventral view of shark centrum with neural arches removed. Lines represent ventral length (BOT).

A visual inspection of the centra was conducted to classify the different centra shapes for each species, following the criteria by Kozuch & Fitzgerald (1989). Centrum shape was classified as either cylinder, modified cylinder, fluted cylinder or hourglass (Figure 4). However, this was not applicable to the order: Lamnidae, as the centra in this group exhibit unique radiating lamaellae patterns that do not conform to the aforementioned centra shape criteria (Figure 1). As a result, Lamnidae centra was characterised as its own group and the fifth centra shape.

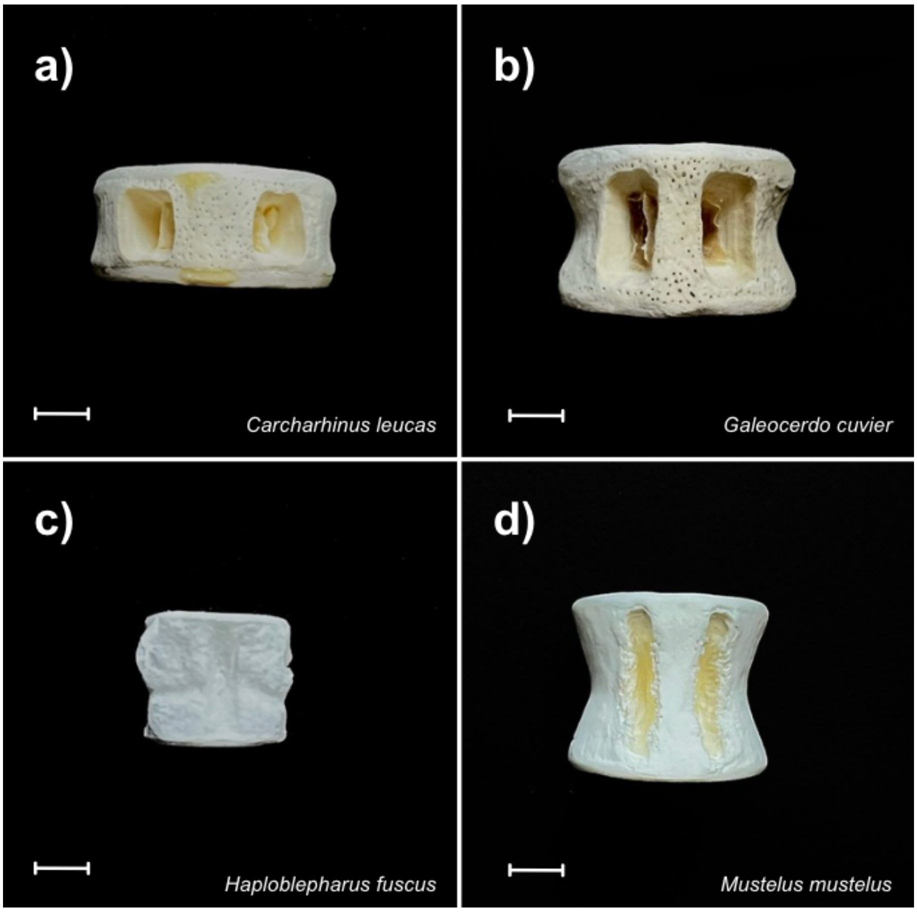

Figure 4: Photographs of all the different centra shapes. a) Cylinder shape (Bull shark, *Carcharhinus leucas*). Scale bar = 4.55 mm. b) Modified cylinder shape (Tiger shark, *Galeocerdo curvier*). Scale bar = 5 mm. c) Hourglass shape (Brown shyshark, *Haploblepharus fuscus*). Scale bar = 1.67 mm. d) Fluted cylinder shape (Smooth hound shark, *Mustelus mustelus*). Scale bar = 4.86 mm.

Environmental variables

For all species presented in this study, data on feeding mode, habitat, reproductive mode, swimming mode, and degree of residency was gathered from published literature, species catalogues and FishBase (Moss, 1977; Compagno, 1984a,b; Froese & Pauly, 2000; Wilga & Lauder, 2004; Cyrino, Bureau & Kapoor, 2008; Ebert, Dando & Fowler, 2021). Each species was also assigned an ecological designated environment of either benthic or pelagic. Benthic species encompass all demersal, benthopelagic, and bathydemersal species while pelagic species include pelagic-oceanic and reef-associated species. For swimming mode, only the categories of carangifrom and anguilliform swimming were used, as thunniform and sub-carangiform swimming are considered subcategories of carangiform swimming. A series of tables showing the distribution of shark species for each migration type and swimming mode for each designated ecological environment, the distribution between migratory type and swimming mode as well as the distribution for shark species designated ecological environment, migration type and swimming mode across the different centra shapes was consolidated.

Statistical analysis

All statistical analyses for this study were performed in R (R Core Team, 2023), and a p-value of 0.05 or less was used to determine statistical significance. A fineness ratio (FR) for each species was calculated from the mean of the means of the standardized measurements for cranial-caudal length (CCL_s) and dorsal-ventral height (DVH_s) shown in Equation 1.

Equation 1:

$$FR = \frac{CCL_s}{DVH_s}$$

A two-sample two-tailed t-test was run to determine whether there is a significant difference in FRs between benthic and pelagic species. To investigate the relationship between the FR between the different shark orders, Carcharhiniform, Lamniform, Squaliform and Pristiophoriform, a general linear model was run. The fineness ratio acted as the dependent variable, while the categorical variable, shark order, was the independent variable. A Tukey Honestly Significant Difference (HSD) post hoc test was then performed to determine the differences in FR means across the orders.

To quantify the resistance of each centrum to bending forces, the centra were modeled as cylinders with circular cross-sections. For each species, the mean of the standardised measurements for dorsal-ventral height (DVH_s) and the medial-lateral breath (MLB_s) were used as the diameter of the centrum. This value was then halved to obtain the radius (r'), which was then used to calculate the second moment of area (I) using Equation 2. As standardised values (centra measurement: total length) were used as the radius, the radius is denoted as r prime (r') as opposed to absolute r and therefore, values for I do not have any units associated with its value.

Equation 2:

$$I = \frac{\pi r'^4}{4}$$

A two-sample two-tailed t-test was run to determine whether there is a significant difference between the values of I between benthic and pelagic species. To examine the relationship between I and FR, a Pearson correlation test was calculated.

Multiple general linear models were run to test the relationship between the fineness ratio and the ecological variables of environment, habitat, migration, feeding style, reproduction, swimming mode and centra shape. Gaussian general linear models were run, assuming normally distributed residuals with the identity link function. No transformations were applied to the dependent variable allowing the resulting equations to be interpreted as linear combinations.

To evaluate model performance and accuracy, resulting p-values (<0.05), adjusted R^2, and Akaike Information Criterion (AIC) values (Akaike, 1973) were used for model selection. As the sample size was less than 200, the *AICcmodavg* package (Mazerolle, 2023) was used to create the AICc, a size-adjusted bias correction for the AIC model selection (Equation 3).

Equation 3:

$$AICc = AIC + \frac{2k(k+1)}{n-k-1}$$

where $AIC = n * log(\sigma^2) + 2k,$ k = total number of parameters, σ^2= variance, and n = sample size.

Using the package *ggplot2* (Wickham, 2016), plots were created to visualise the centra measurements and FR data. Samples were binned into classes and the frequency of the FRs were plotted in a histogram and grouped by their ecologically designated environment. CCL was plotted against DVH in a scatterplot. Additionally, CCL_s was plotted against DVH_s in a scatterplot. A linear regression analysis was conducted to quantify the relationship between CCL_s and DVH_s and fitted to the scatterplot.

To better visualise any emerging patterns between the ecological environment, swimming mode, reproductive mode, feeding mode, migration, centra shape, centra measurements, FR, species, and order, a principal component analysis (PCA) was conducted. The R package *FactoMineR* and *FAMD* command was used to perform a factor analysis of mixed data (FAMD), a PCA method whereby a data set containing both quantitative and qualitative variables is analysed (Lê, Josse & Husson, 2008). This method accounts for the mixed types of variables by acting like a PCA for quantitative variables and a multiple correspondence analysis (MCA) for qualitative variables. By taking into account the mixed types of variables, this analysis allows for the similarities between individuals to be analysed (Kassambara, 2017). For visualisation of results, the package *factoextra* was used to extract plots from the FAMD. The percentage of variance explained by each dimension was displayed in a scree plot. Furthermore, the percentage contribution of each variable to the dimension with the highest variance was also plotted.

However, because of missing data, the Lantern shark species, *Etmopterus brosei* and *Etmopterus compagnoi*, were omitted from the PCA analysis.

Results

Environmental combinations

The tables below present the combinations of coinciding environmental variables for species. The specific environmental data for each species, which includes environment, centra shape, migratory habits, swimming mode, feeding mode and reproduction is available in Table 5.

Table 1: Distribution of shark species by migration type and swimming mode for each environment.

	Environment	
	Pelagic	*Benthic*
Migration		
Migratory	12	5
Resident	1	12
Swimming Mode		
Anguilliform	1	10
Carangiform	12	7

Seventeen sharks were migratory and 13 were resident in Table 1. A total of 11 sharks were anguilliform swimmers and 19 were carangiform swimmers. Only one pelagic shark was an anguilliform swimmer, *Pseudocarcharias kamohari*, and only one pelagic shark was resident, *Carcharhinus amboinensis*.

Table 2: Distribution of shark species by migration type by swimming mode.

	Swimming Mode	
	Anguilliform	*Carangiform*
Migration		
Migratory	3	14
Resident	8	5

Of the 17 migratory shark species in Table 2, the only three anguilliform swimmers were *Pseudocarcharias kamohari, Mustelus mustelus* and *Holohalaelurus regani.*

Table 3: Distribution of shark species by environment, migration and swimming mode for different centra shapes.

	Centra Shape				
	Cylinder	*Modified Cylinder*	*Fluted Cylinder*	*Hourglass*	*Lamnidae*
Environment					
Pelagic	6	3	-	-	4
Benthic	3	1	6	7	-
Migration					
Migratory	7	3	3	-	4
Resident	2	1	3	7	-
Swimming Mode					
Anguilliform	-	1	4	5	1
Carangiform	9	3	2	2	3

Many categories had a distribution of zero among the different centra shapes in Table 3. The were no pelagic sharks with fluted cylinder and hourglass shaped centra and no benthic sharks in the Lamnidae family. No migratory sharks had hourglass shaped centra and there were no resident sharks in the Lamnidae family. Finally, no anguilliform swimmers had cylinder shaped centra. Other extremities were that only one benthic, one resident and one anguilliform swimmer had modified cylinder centra. For all accounts, this was represented by *Pliotrema warreni*. Lastly, there was only one anguilliform swimmer in the Lamnidae family, *Pseudocarcharias kamohari.*

Centra morphology

DVH_s measurements ranged from 6.22 to 14.08 with a mean of 9.76. *P. warreni* had the smallest centrum face and *L. nasus* had the largest face. CCL_s measurements ranged from 4.80 to 11.62 with a mean of 7.28. *A. supercliliosus* had the shortest centrum body and *E. compagnoi* had the longest (Table 4). FR values ranged from 0.42 (*C. carcharias*) to 1.46 (*E. brosei*) with a mean of 0.80 (Table 5). Finally, values for I ranged from 7.86×10^{-11} (*P. warreni*) to 2.12×10^{-9} (*L. nasus*) with a mean of 6.09×10^{-10} (Table 5).

Table 4: Mean (±SE) standardised centra measurements of dorsal-ventral height (DVH_s), cranial-caudal length (CCL_s), medial-lateral breathe (MLB_s), dorsal length (Top_s), ventral length (Bot_s) and height at centre ($Cent_s$) for each species. All measurements are in μm.

Order	Species	DVH_s	CCL_s	MLB_s	Top_s	Bot_s	$Cent_s$
Squaliformes							
	Etmopterus brosei	7.08	10.33				
	Etmopterus compagnoi	8.66	11.62				
	Squalus acutipinnis	8.11(±0.24)	7.51(±0.46)	8.07(±0.34)	7.51(±0.46)	7.45(±0.49)	5.43(±0.43)
Carcharhiniformes							
	Carcharhinus limbatus	11.84(±0.20)	5.73(±0.08)	12.30(±0.19)	5.62(±0.08)	5.69(±0.09)	11.62(±0.26)
	Carcharhinus brachyurus	10.46(±0.24)	5.94(±0.16)	11.27(±0.26)	5.91(±0.17)	5.87(±0.16)	10.48(±0.24)
	Carcharhinus obscurus	11.18(±0.20)	6.22(±0.09)	11.70(±0.23)	6.19(±0.10)	6.12(±0.09)	10.86(±0.28)
	Carcharhinus amboinensis	10.18(±1.90)	5.64(±1.00)	10.32(±2.01)	5.62(±1.03)	5.57(±0.97)	9.35(±2.82)

Carcharhinus plumbeus	11.89(±0.83)	6.35(±0.44)	11.94(±0.87)	6.29(±0.45)	6.27(±0.45)	11.91(±0.83)
Carcharhinus brevipinna	11.57(±0.36)	6.15(±0.28)	11.73(±0.39)	6.09(±0.27)	6.09(±0.27)	11.08(±0.38)
Carcharhinus leucas	11.50(±0.28)	5.19(±0.13)	11.63(±0.26)	5.15(±0.13)	5.18(±0.16)	11.28(±0.29)
Galeocerdo cuvier	9.92(±0.12)	6.15(±0.09)	10.17(±0.14)	6.09(±0.09)	5.99(±0.10)	9.77(±0.15)
Haploblepharus fuscus	8.33(±0.34)	8.77(±0.06)	9.38(±0.29)	8.59(±0.15)	8.61(±0.07)	7.31(±0.47)
Haploblepharus pictus	7.92(±0.16)	9.44(±0.34)	9.52(±0.12)	9.45(±0.20)	9.49(±0.23)	7.18(±0.16)
Haploblepharus edwardsii	7.20(±0.17)	9.27(±0.29)	8.32(±0.31)	9.17(±0.28)	9.09(±0.28)	6.36(±0.47)
Holohalaelurus regani	7.30	8.80	7.88	8.80	8.64	4.98
Apristurus saldanha	7.17	7.39	7.12	7.38	7.30	5.82
Poroderma africanum	8.11	7.51	8.06	7.51	7.45	5.43
Poroderma pantherinum	7.69(±0.19)	8.47(±0.23)	8.43(±0.39)	8.38(±0.23)	8.36(±0.23)	5.65(±0.53)
Sphyrna mokarran	10.83(±0.55)	6.29(±0.46)	11.00(±0.55)	6.24(±0.46)	6.28(±0.47)	10..43(±0.47)
Sphyrna lewini	10.43(±0.20)	5.91(±0.10)	10.06(±0.23)	5.84(±0.20)	5.87(±0.09)	10.10(±0.31)
Sphyrna zygaena	10.20(±0.34)	5.68(±0.23)	9.95(±0.36)	6.23(±0.69)	6.23(±0.71)	9.96(±1.56)
Mustelus mustelus	8.93(±0.51)	8.22(±0.69)	9.28(±0.66)	7.48(±0.48)	8.11(±0.61)	7.91(±0.45)
Mustelus palumbes	8.25(±0.17)	9.13(±0.29)	8.42(±0.28)	8.95(±0.34)	8.97(±0.32)	6.86(±0.29)
Galeorhinus galeus	9.70(±0.18)	8.43(±0.31)	10.41(±0.27)	8.24(±0.29)	8.23(±0.30)	8.89(±0.16)
Lamniformes						
Alopias superciliosus	9.17	4.80	9.11	4.79	4.77	9.00
Lamna nasus	14.08	6.61	14.76	6.53	6.64	13.92
Carcharias taurus	13.78(±0.16)	6.55(±0.10)	14.19(±0.13)	6.56(±0.10)	6.36(±0.11)	13.72(±0.16)
Carcharodon carcharias	13.52(±0.22)	5.62(±0.10)	13.68(±0.26)	5.62(±0.10)	5.48(±0.09)	13.48(±0.23)
Pseudocarcharias kamoharai	9.89	8.22	10.52	8.18	8.14	9.56
Pristiophoriformes						
Pliotrema warreni	6.22(±0.17)	5,07(±0,18)	6.44(±0.24)	5.07(±0.17)	5.04(±0.17)	5.09(±0.16)

* *n* = 1 for measurements without standard error values.

** missing values for vertebrae that were too damaged for all measurements

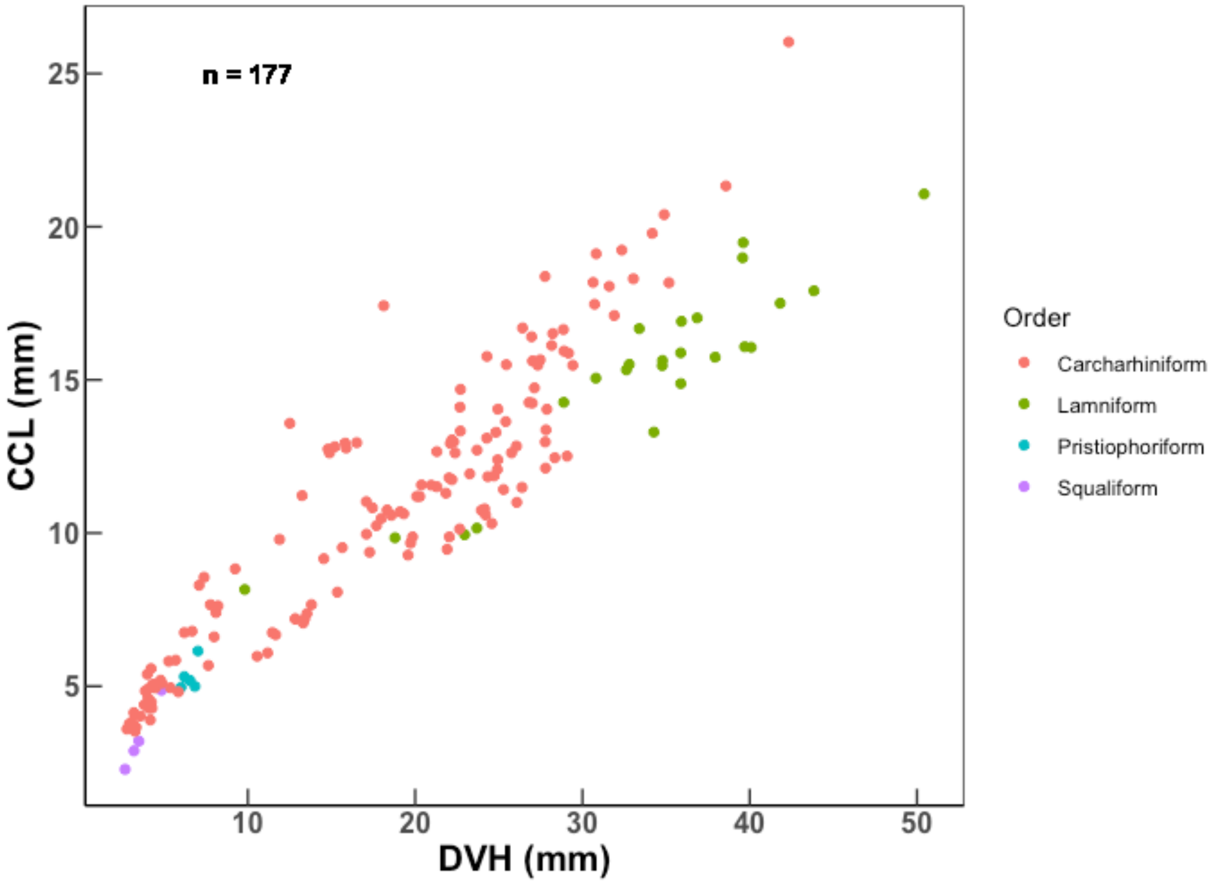

Figure 5: Scatterplot between the dorsal-ventral height (DVH) and the cranial-caudal length (CCL) of vertebrae

Squaliformes, typically smaller species, are located in the bottom left corner in Figure 5 displaying the smallest centra. In contrast, Lamniformes, generally larger species, have some of the largest centra. Carcharhinformes exhibit the largest diversity of species evident by the more dispersive points which highlight the variation in the centra sizes across this order.

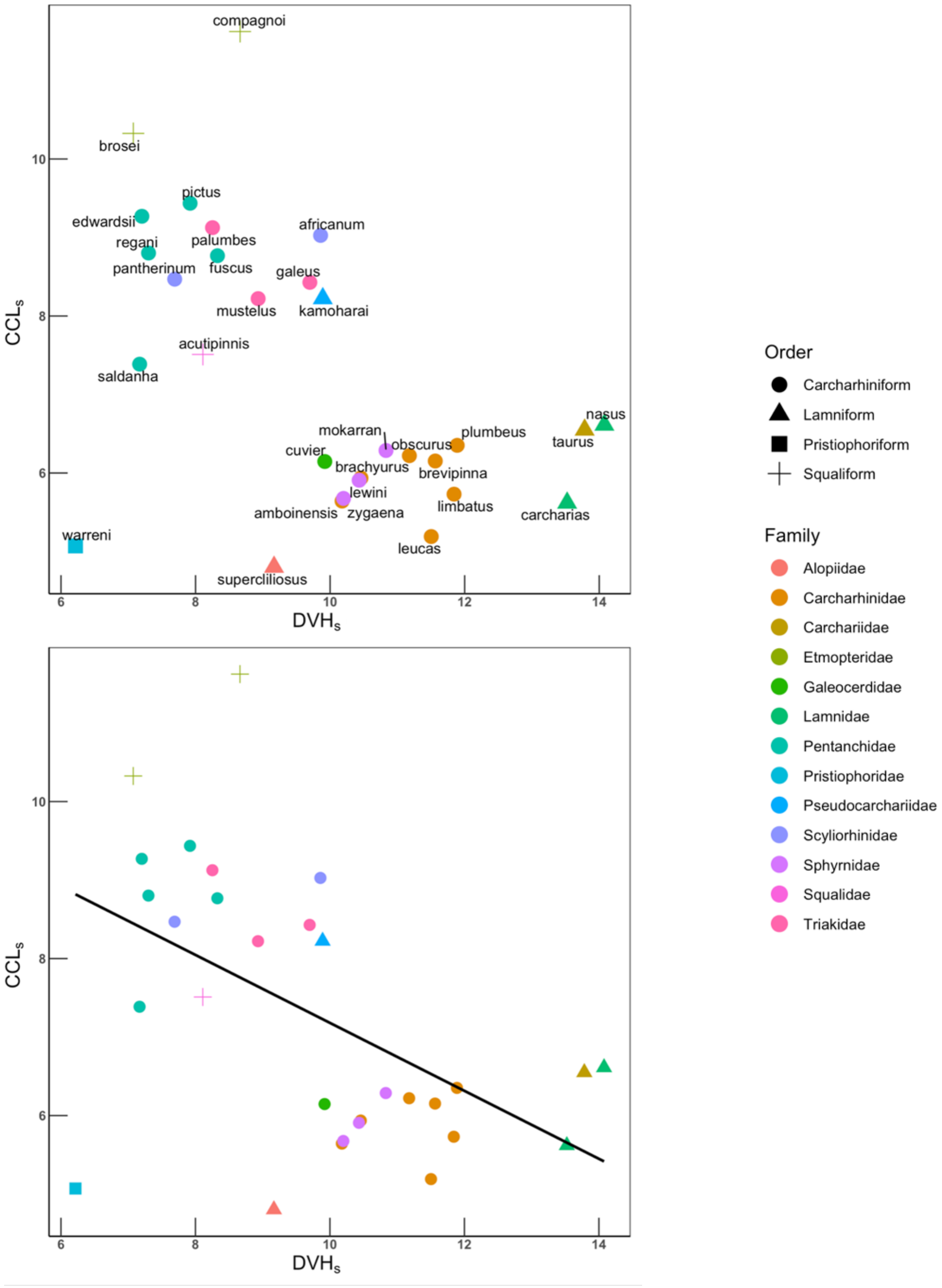

Figure 6: (Top) Scatterplot of the standardised measurements of dorsal-ventral height (*DVH$_s$*) and standardised measurements of cranial-caudal length (*CCL$_s$*) of vertebrae for each species. (Bottom) Identical plot with a fitted linear regression line. Point shape and colour represent Order and Family, respectively

Four Orders are represented in Figure 6: Carcharhiniform (n=21), Lamniform (n=5), Pristiophoriform (n=1) and Squaliform (n=3), along with thirteen Families: Alopiidae (n=1), Carcharhinidae (n=7), Carchariidae (n=1), Etmopteridae (n=2), Galeocerdidae (n=1), Lamnidae (n=2), Pentanchidae (n=5), Psuedocarchariidae (n=1), Scyliorhinidae (n=2), Squalidae (n=1) and Triakidae (n=3). Pelagic species cluster in the bottom right corner of Figure 6, characterized by large DVH_s (indicating a bigger centra face) and smaller CCL_s (indicating a shorter centra length). Benthic species form a cluster on the left characterized by both a large DVH_s and a large CCL_s, indicating that their centra face and length are similar in size. Squaliform species, *E. brosei* and *E. compagnoi*, and Pristiphoriform, *P. warreni*, are positioned outside the two main clusters at the top left and bottom left of Figure 6 and are outliers. Squaliformes have a larger CCL_s (longer centra length) and a smaller DVH_s (smaller centra face). *P. warreni* has both a small CCL_s (shorter centra length) and a small DVH_s (smaller centra face).

The linear regression equation line for the relationship between CCL_s and DVH_s in Figure 6 was $CCL_s = 11.51 – 0.43DVH_s$ (F-value = 10.08, $R^2 = 0.24$, p-value = 0.0036). As DVH_s increases, CCL_s decreases indicating there is a negative linear relationship between the two measurements. However, despite the model being significant (p-value = 0.0036), the low F statistic indicates a weak correlation and associated low R^2 value only explains 24% of the variability in CCL_s. *Carcharhinus plumbeus* and *Carcharodon carcharias* were the only two species to fall directly on the regression line. *P. warreni, E. compagnoi* and *A. supercliliosus* were the three species furthest away from the regression line with the remaining species spread sporadically above and below the upper and lower ends of the regression line.

Table 5: Sample size, fineness ratio (FR), second moment of area (I), habitat use, ecological designation, centra shape, swimming mode, feeding style and reproduction style of neoselachian species.

Order	**n**	**Species**	**FR**	I	**Habitat**	**Ecological Designation**	**Centra shape**	**Migratory? (Y/N)**	**Swimming Mode**	**Feeding Style**	**Reproduction**
Squaliformes											
	6	*Etmopterus brosei*	1.46	1.23×10^{-10}	pelagic-oceanic	benthic	hourglass	N	anguilliform	cutting	
	2	*Etmopterus compagnoi*	1.34	2.76×10^{-10}	pelagic-oceanic	benthic	hourglass	N	carangiform	cutting	ovoviviparous
	19	*Squalus acutipinnis*	0.93	2.10×10^{-10}	demersal	benthic	fluted cylinder	N	carangiform	-	ovoviviparous
Carcharhiniformes											
	55	*Carcharhinus limbatus*	0.48	1.04×10^{-9}	reef-associated	pelagic	cylinder	Y	carangiform	gouging	viviparous
	33	*Carcharhinus brachyurus*	0.57	6.84×10^{-10}	reef-associated	pelagic	cylinder	Y	carangiform	gouging	viviparous
	30	*Carcharhinus obscurus*	0.56	8.41×10^{-10}	reef-associated	pelagic	cylinder	Y	carangiform	gouging	viviparous
	10	*Carcharhinus amboinensis*	0.55	5.42×10^{-10}	reef-associated	pelagic	cylinder	N	carangiform	gouging	viviparous
	50	*Carcharhinus plumbeus*	0.53	9.90×10^{-10}	benthopelagic	benthic	cylinder	Y	carangiform	gouging	viviparous
	55	*Carcharhinus brevipinna*	0.53	9.03×10^{-10}	reef-associated	pelagic	cylinder	Y	carangiform	gouging	viviparous
	59	*Carcharhinus leucas*	0.45	8.78×10^{-10}	reef-associated	benthic	cylinder	Y	carangiform	gouging	viviparous
	70	*Galeocerdo cuvier*	0.62	5.00×10^{-10}	benthopelagic	pelagic	modified cylinder	Y	carangiform	gouging	ovoviviparous
	10	*Haploblepharus fuscus*	1.05	3.02×10^{-10}	demersal	benthic	hourglass	N	anguilliform	gouging	oviparous
	27	*Haploblepharus pictus*	1.19	2.84×10^{-10}	demersal	benthic	hourglass	N	anguilliform	gouging	oviparous
	20	*Haploblepharus edwardsii*	1.29	1.78×10^{-10}	demersal	benthic	hourglass	N	anguilliform	gouging	oviparous
	5	*Holohalaelurus regani*	1.21	1.63×10^{-10}	bathydemersal	benthic	fluted cylinder	N	anguilliform	gouging	oviparous
	5	*Apristurus saldanha*	1.03	1.28×10^{-10}	bathydemersal	benthic	fluted cylinder	N	anguilliform	gouging	oviparous
	5	*Poroderma africanum*	0.92	5.50×10^{-10}	demersal	benthic	hourglass	N	anguilliform	gouging	oviparous
	25	*Poroderma pantherinum*	1.10	2.07×10^{-10}	reef-associated	benthic	hourglass	N	anguilliform	gouging	oviparous
	30	*Sphyrna mokarran*	0.58	6.96×10^{-10}	pelagic-oceanic	pelagic	cylinder	Y	carangiform	gouging	viviparous

55	*Sphyrna lewini*	0.57	5.42×10^{-10}	pelagic-oceanic	pelagic	modified cylinder	Y	carangiform	gouging	viviparous
50	*Sphyrna zygaena*	0.56	5.06×10^{-10}	pelagic-oceanic	pelagic	modified cylinder	Y	carangiform	gouging	viviparous
4	*Mustelus mustelus*	0.92	3.37×10^{-10}	demersal	benthic	fluted cylinder	Y	anguilliform	crushing	viviparous
29	*Mustelus palumbes*	1.11	2.37×10^{-10}	demersal	benthic	fluted cylinder	N	anguilliform	crushing	ovoviviparous
64	*Galeorhinus galeus*	0.87	5.02×10^{-10}	benthopelagic	benthic	fluted cylinder	Y	carangiform	gouging	ovoviviparous
Lamniformes										
5	*Alopias superciliosus*	0.52	3.42×10^{-10}	pelagic-oceanic	pelagic		Y	carangiform	gouging	ovoviviparous
4	*Lamna nasus*	0.47	2.12×10^{-9}	pelagic-oceanic	pelagic		Y	carangiform	gouging	ovoviviparous
49	*Carcharias taurus*	0.48	1.88×10^{-9}	reef-associated	benthic		Y	carangiform	gouging	ovoviviparous
54	*Carcharodon carcharias*	0.42	1.68×10^{-9}	pelagic-oceanic	pelagic		Y	carangiform	gouging	ovoviviparous
5	*Pseudocarcharias kamoharai*	0.83	5.33×10^{-10}	pelagic-oceanic	pelagic		Y	anguiliform	gouging	ovoviviparous
Pristiophoriformes										
25	*Pliotrema warreni*	0.82	7.86×10^{-11}	demersal	benthic	modified cylinder	N	anguilliform	suction-crushing	ovoviviparous

Fineness ratio

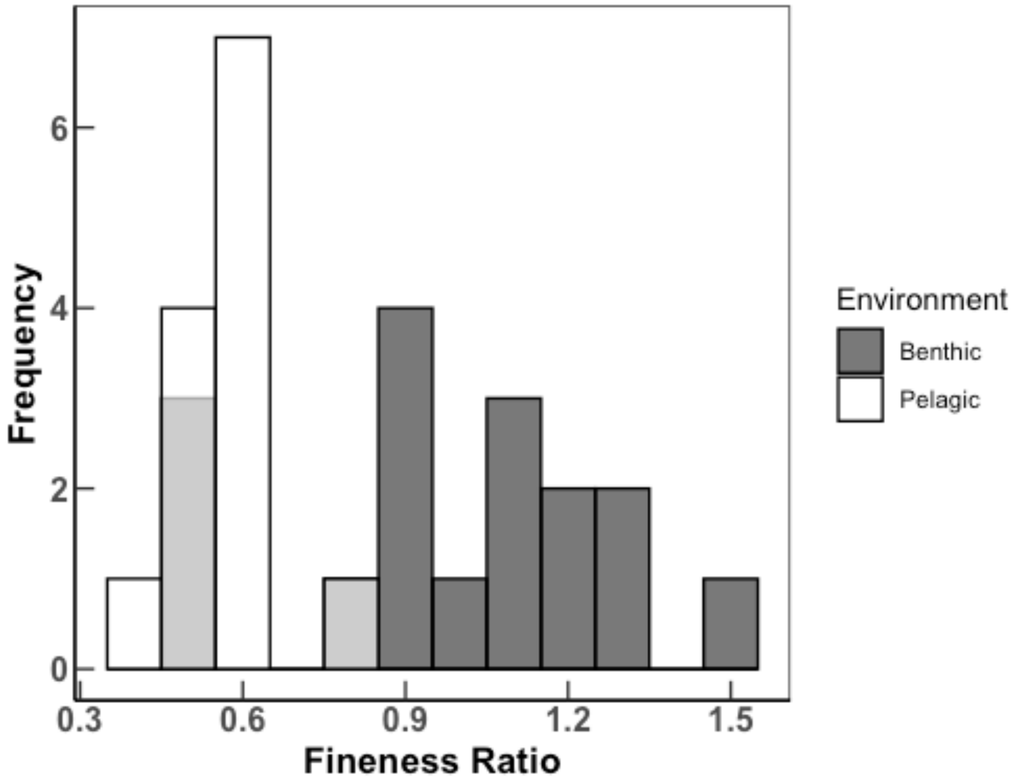

Figure 7: Histogram showing the frequency distribution of the Fineness Ratio for each species, grouped according to their environment (*White* = Pelagic species, *Black* = Benthic species*, Grey* = Overlap between groups)

The higher end of the graph in Figure 7 is exclusively occupied by benthic species with FR values greater than 0.9. The lower end of the graph consists mainly of pelagic species with FR values of 0.8 and lower. However, there four benthic species with a FR of less than 0.9 (one with 0.8 and three with 0.4) and overlapping with some pelagic species. The highest frequency of pelagic species had a FR of 0.6, while the highest frequency of benthic species peak at a FR of 0.9. A two-sample two-tailed t-test showed a significant difference in the mean FRs for benthic and pelagic species (*t* = 5.09, *df* = 29*, p-value* < 0.05). The mean (±SE) FR for benthic species was 0.98 (± 0.05) (n=17) and 0.56 (±0.02) for pelagic species (n=13).

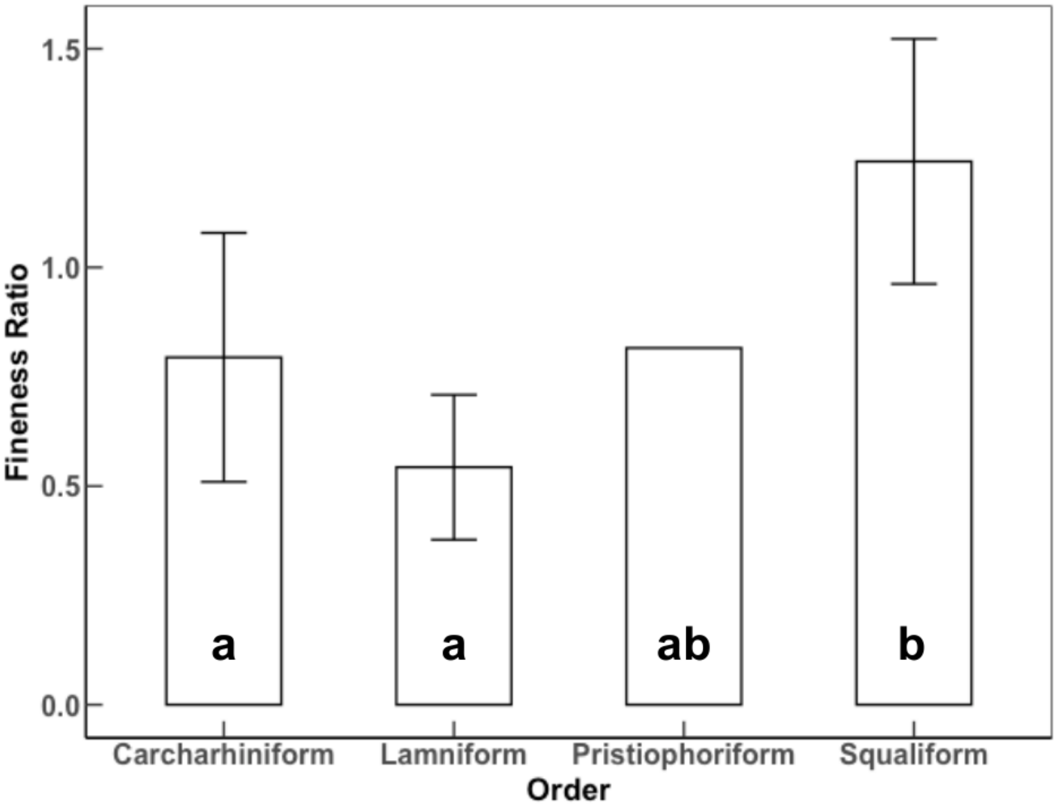

Figure 8: Plot showing the mean (±SD) fineness ratio for each order sampled. Small letters indicate statistical differences between groups.

The mean (±SD) FR for Carcharhiniforms was 0.79 (±0.28) (*n*=21) and 0.54 (±0.17) for Lamniformes (*n*=5) in Figure 8. Pristiophoriforms were represented by a single species and had a FR of 0.82. Squaliforms, a highly benthic order had the highest mean FR of 1.24 (±0.28) (*n*=3). Pristiophoriforms, a dorsoventrally flattened species that spends most of its time on the sea floor had the next highest FR. In contrast, Lamniforms, consisting of mostly pelagic species, had the lowest mean FR. The order Carcharhiniform has a high diversity of both benthic and pelagic species resulting in more median FR due to this variability. The small letters within each bar for each category indicates statistical differences between groups in Figure 8. Any two groups that share a letter are not statistically different, and any two groups that do not share a letter are statistically different.

Table 6: Summary of the results for the general linear model for fineness ratio and shark orders showing coefficients, standard error (*std error*), t statistic (*t-value*) and p-values. Significant p-values < 0.05 are highlighted in bold.

	Coefficients	**Std Error**	**t-value**	**p-value**
(Intercept)	0.79	0.059	13.512	**0.0000065**
Lamniform	-0.25	0.134	-1.874	0.072
Pristiophoriform	0.02	0.276	0.078	0.939
Squaliform	0.45	0.166	2.694	**0.012**

The baseline (intercept), representing Carcharhiniform was significant (p-value < 0.05) as well as the coefficient for Squaliforms (0.45), which also showed a positive significant effect on the FR (p-value < 0.05) (Table 6). While Lamniformes show a slight significance with a p-value close to 0.05 (p-value = 0.072), the effect is not strong enough to be considered statistically significant. The effect of Pristiophoriforms on the FR was not statistically significant. Although shark order was shown to have some influence on FR, only 25% of the variance in FR was explained by the model, F-value = 4.215, $R^2 = 0.25$, p-value = 0.015.

Table 7: Summary of pairwise comparison results for Tukey HSD post hoc tests showing the mean difference between orders (*mean diff*), 95% upper and lower confidence intervals and the p-value. Significant p-values < 0.05 are highlighted in bold.

Comparison	**Mean diff**	**Upper**	**Lower**	**p-value**
Carcharhiniform – Lamniform	0.25	-0.12	0.62	0.26
Pristiophoriform – Lamniform	0.27	-0.54	1.08	0.79
Squaliform – Lamniform	0.70	0.16	1.24	**0.0076**
Pristiophoriform – Carcharhiniform	0.02	-0.74	0.78	1.00
Squaliform – Carcharhiniform	0.45	-0.01	0.90	**0.056**
Squaliform – Pristiophoriform	0.43	-0.43	1.28	0.53

In the pairwise comparisons of FR across the four different shark orders, only the comparisons between Squaliformes and Lamniformes, as well as between Squaliformes and Carcharhiniformes were significantly different (p-value <0.05) (Table 7). Squaliformes had a higher FR than both Lamniformes and Carcharhiniforms, with mean differences of 0.70 and 0.45, respectively. Pristiophoriformes showed no significant differences compared to the other three orders, nor was there a significant difference between Lamniformes and Carcharhiniformes.

From the multiple general linear models run to test the relationship between the FR and the chosen environmental variables, the best models were 1, 2 and 3 (Table 8). As the sample size was quite small (n=30), models 1 and 2 were the simplest as they included the fewest parameters which reduced model complexity and improved accuracy. Both models explained 88% of the variance in FR and were associated with the smallest AICc values (-35.72 and -33.72 respectively). Models 1 and 2 had less than two AICc units (the known standard indicating significance) between them (ΔAICc = 1.99 units) meaning that either model could have the likelihood of being the model to explain the data best.

Model 3 was also included as one of the best models due to its few parameters and extremely low p-values (p-value = 0.000099) despite having an AICc value greater than two units from model 1 (ΔAICc = 3.52 units). The inclusion of the environment variable is essential for the testing of the hypotheses. The remaining models were rejected as the inclusion of too many parameters caused unnecessary model complexity and inaccurate results. Although models 4, 5, 7, 8 and 9 all have high adjusted R^2 values greater than 0.88, because of their associated high AICc values and low Akaike weights, these models were still less supported and may be overfitting the data.

Table 8: Summary of model variables, number of parameters (*k*), AICc values, difference in AICc values (*∆AICc*), AICc weights (*w*), p-values and Adjusted R^2 (R^2) values for the candidate models

Model		**k**	**AICc**	**∆AICc**	**w (%)**	**p-value**	R^2
1	FR ~ Swimming mode + Centra Shape	7	-35.72	0.00	0.63	0.000057	0.88
2	FR ~ Migration + Centra Shape + Swimming Mode	8	-33.72	1.99	0.23	0.000057	0.88
3	FR ~ Environment + Swimming Mode + Centra Shape	8	-32.20	3.52	0.11	0.000099	0.87
4	FR ~ Environment + Centra Shape + Swimming Mode + Migration	9	-29.64	6.08	0.03	0.00035	0.88
5	FR ~ Environment + Centra Shape + Swimming Mode + Migration + Feeding Style + Order	13	-23.66	12.06	0.00	0.00065	0.91
6	FR ~ Environment + Centra Shape	7	-1.47	34.24	0.00	0.014	0.62
7	FR ~ Environment + Habitat + Centra Shape + Swimming Mode + Migration + Feeding Style + Reproduction + Order	20	35.07	70.78	0.00	0.0048	0.94
8	FR ~ Environment * Habitat * Centra Shape * Swimming Mode * Migration * Feeding Style * Reproduction * Order	23	122.80	158.51	0.00	0.048	0.94
9	FR ~ Environment * Habitat * Centra Shape * Swimming Mode * Migration * Feeding Style * Reproduction + Order	23	122.80	158.51	0.00	0.048	0.94

Table 9: Summary of the results from model 1, 2 and 3 selected using AICc showing the degrees of freedom (*df*), sum of squares (*Sum sq*), F statistic (*F-value*) and p-values for each model. Significant p-values < 0.05 are highlighted in bold.

	Model	**Predictor**	**df**	**Sum sq**	**F-value**	**p-value**
1	*FR ~ Swimming Mode + Centra Shape*	Swimming Mode	1	1.03	87.51	**0.00022**
		Centra Shape	4	1.49	31.68	**0.00036**
		Residuals	24	0.28		
2	*FR ~ Migration + Centra Shape + Swimming Mode*	Migration	1	1.12	96.72	**0.00013**
		Centra Shape	4	0.98	21.08	**0.0019**
		Swimming Mode	1	0.44	38.14	**0.0066**
		Residuals	23	0.27		
3	*FR ~ Environment + Swimming Mode + Centra Shape*	Environment	1	1.32	108.20	**0.00016**
		Swimming Mode	1	0.23	19.19	**0.00022**
		Centra Shape	4	0.97	19.95	**0.0030**
		Residuals	23	0.28		

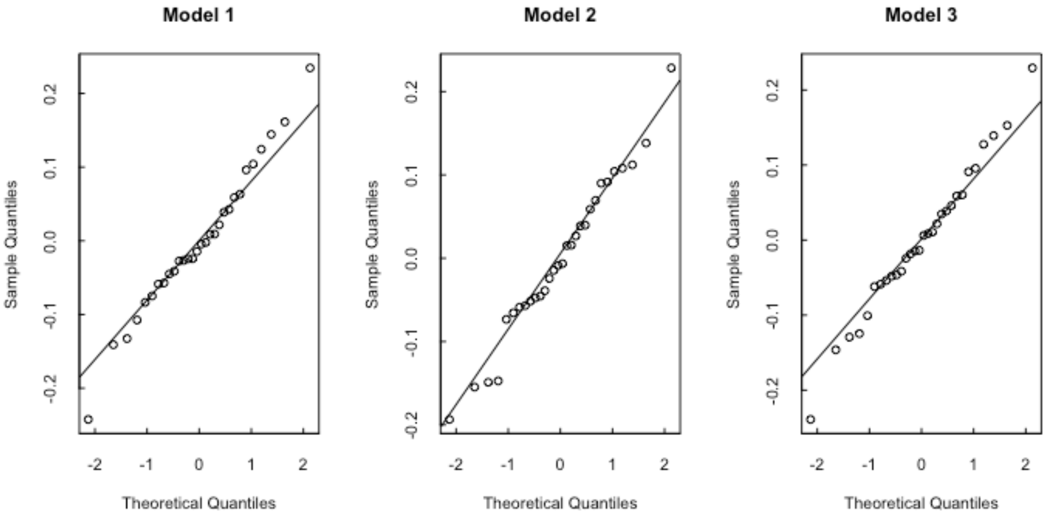

Figure 9: Normal Q-Q plots for the residuals of models 1, 2 and 3

The residuals in the QQ plots for models 1, 2 and 3 predominantly fell along the diagonal reference line in Figure 9. This indicated that the assumptions of normality were met and validating the use of models 1, 2 and 3 for statistical analysis.

Model 1 had the fewest parameters and the lowest cumulative p-values of the three models. Both swimming mode and centra shape had significant effects on the FR (p-values < 0.05). However, the effect of swimming mode had a stronger effect because of the larger associated F statistic (F-value = 87.51) (Table 9). Centra shape had the largest sum of squares value (Sum sq = 1.49) and explained more variance than swimming mode. The low sum squares residual value (Sum sq = 0.28), indicated that very little variance is left unexplained by this model.

In model 2, migration, centra shape and swimming mode all had a significant effect on FR (p-value < 0.5). Migration had the largest F statistic and lowest p-value in the model, thus having the greatest effect on the FR (F-value = 96.72, p-value = 0.00013) (Table 9). The associated F statistic for the added variable, migration, is larger than the largest F statistic of swimming mode in model 1 and may indicate a higher importance in explaining the variance of the FR then that of swimming mode and centra shape alone in model 1. Swimming mode and centra shape continued to have a significant effect on the FR with large F statistics, however, centra shape had a lower corresponding p-value despite having the lowest F statistic. Model 2 had the lowest residual sum of squares (sum sq = 0.27) and therefore had the smallest portion of unexplained variance of the three models.

Model 3 removed migration as a predictor variable and instead included environment. Again, swimming mode and centra shape continued to have a significant effect on the FR (p-values < 0.05) (Table 9). However, their F statistics decreased and were the lowest of the three models. Environment had the largest F statistic with the smallest p-value of all three models (F-value = 108.20, p-value = 0.00016) implying the strongest effect on the FR. Again, residuals had a small sum of squares value (Sum sq = 0.28) supporting the effectiveness of the model.

All three models effectively captured the relationships between the dependent and independent models evident by p-values < 0.05 and low residual values. Centra shape and swimming mode were significant predictors in all three models (p-values < 0.05). However, environment and migration had the two largest F statistics and lowest p-values across all three models and therefore the strongest effects. These results provide evidence that environmental conditions, centra morphology, swimming style and migratory habits all significantly influence the FR of sharks.

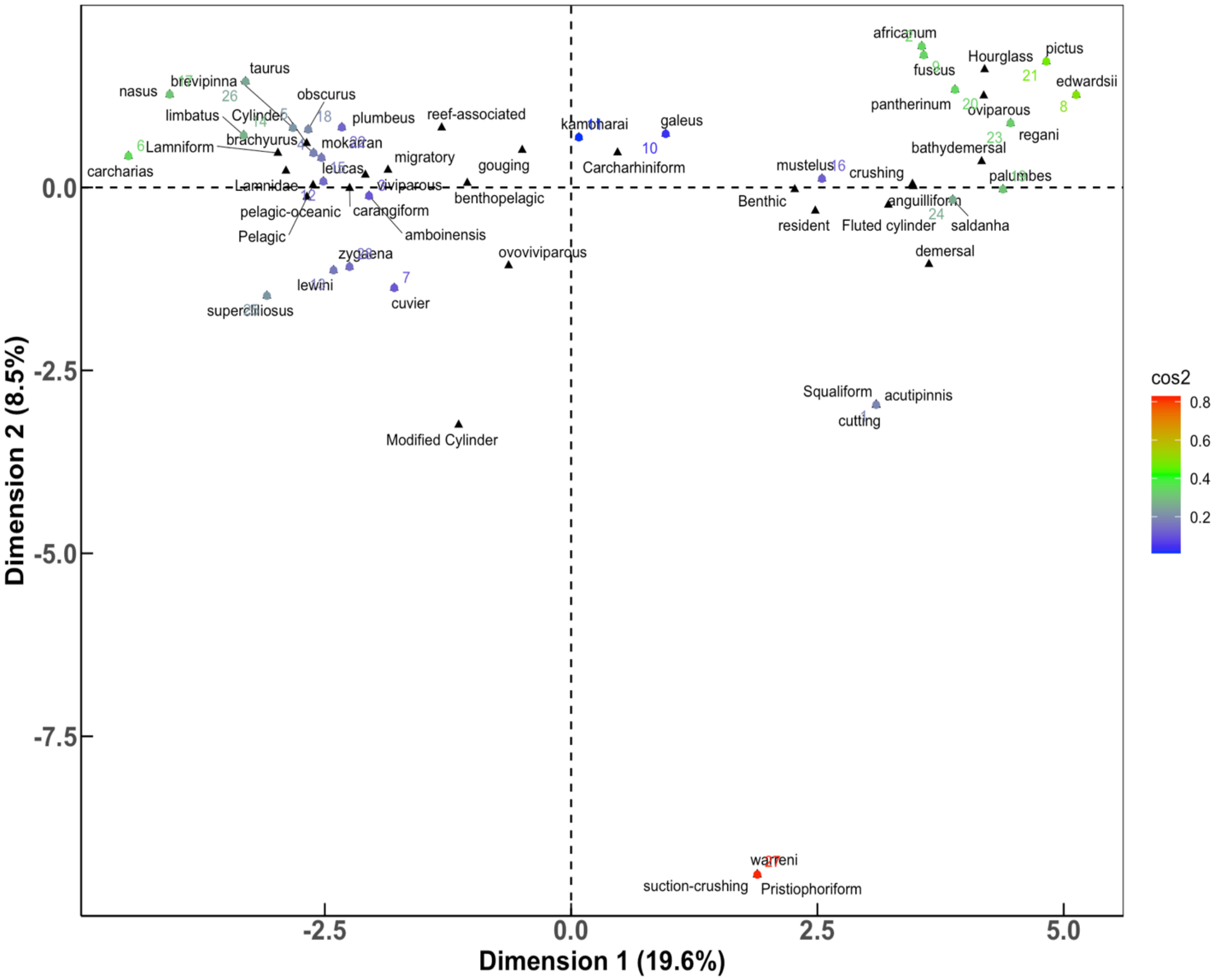

Figure 10: Factor analysis of mixed data (FAMD) plot of individuals for the first two dimensions

Two main clusters are distinguished based on my ecological designation of benthic and pelagic species in Figure 10. *P. warreni* (order: Pristiophoriform) and *S. acutipinnis* (order: Squaliform) are outliers. Points that are closer together in the plot represent individuals with similarities across the mixed data variables (quantitative and qualitative). Dimension 1 (D1), on the x-axis, explains the largest proportion of variance (19.6%), followed by Dimension 2 (D2) on the y axis (8.5%).

On the right side of Figure 10, benthic species (Family: *Scyliorhinidae*, *Pentachidae,* and *Triakidae*) are clustered together along with variables associated with benthic niches, such as demersal environment, oviparous reproduction, residency, crushing dentition. In contrast, the left side of Figure 10 features more pelagic and coastal species (Family: *Carcharhinidae, Lamnidae, Galeocerdidae, Alopiidae,* and *Sphymidae*) associated with pelagic ecological variables such as pelagic-oceanic environment, viviparous reproduction, migratory and gouging feeding. The morphologically distinct *P. warreni* is located on the bottom right of Figure 10 highlighting its unique characteristics compared to the other species.

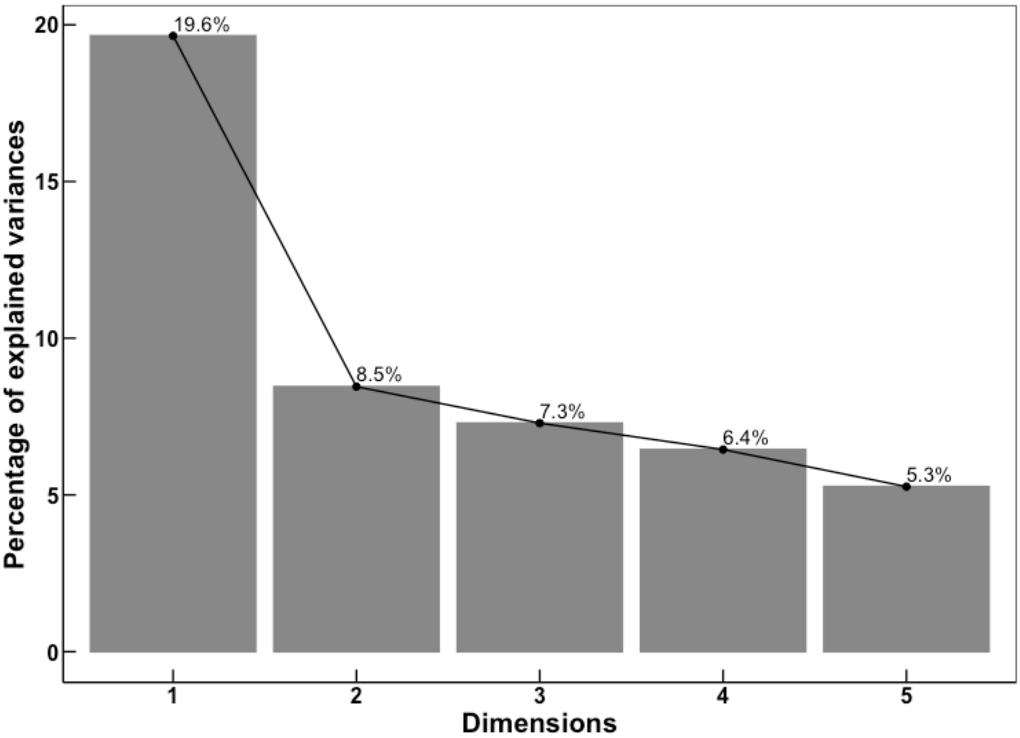

Figure 11: Scree plot of the percentage (%) of explained variances for the dimensions of the FAMD analysis in descending order.

The five dimensions cumulatively explain 47.1% of the total variation of the centra morphology and ecological variables of sharks in Figure 11. D1 accounts for largest proportion of the variation, explaining 19.6% of the total variance. D2 explains 8.5% of the variance, a notable decrease compared to D1. This decreasing trend continues for D3 (7.3%), D4 (6.4%), and D5 (5.3%) with each subsequent dimension explaining progressively less variance.

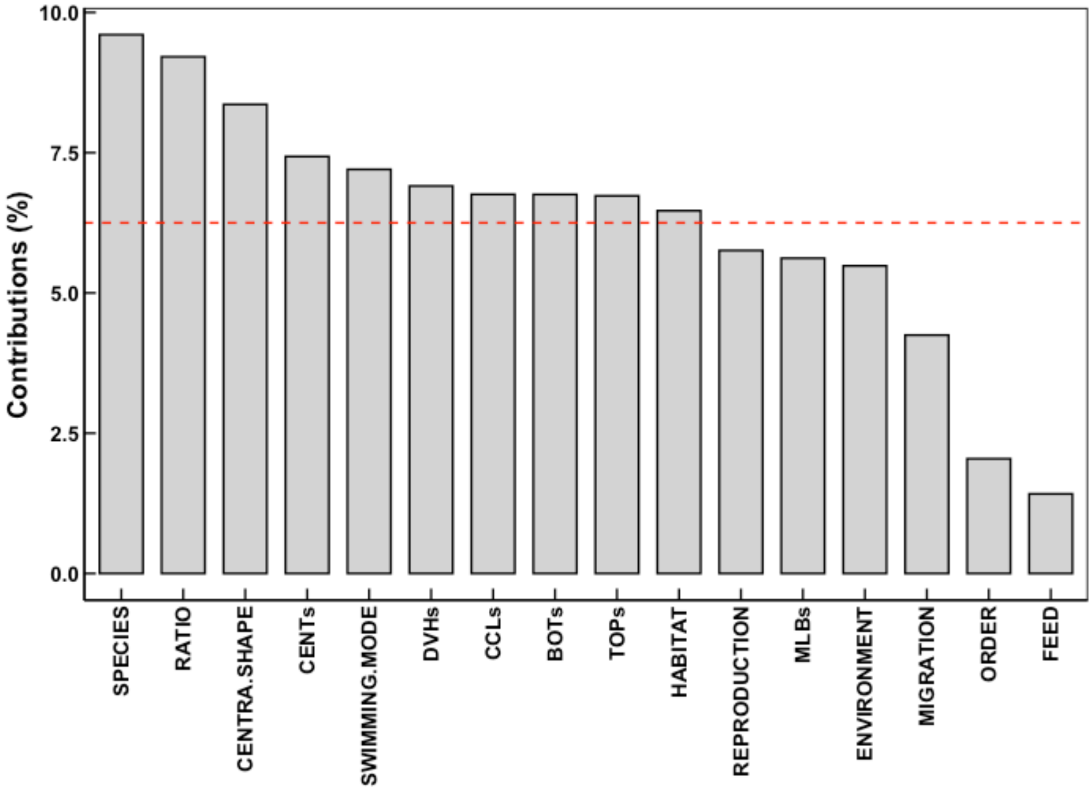

Figure 12: Contribution of each variable to Dimension 1. Dotted red line indicates the expected average value if contributions were uniform.

Shark species (9.6%), fineness ratio (9.2%) and centra shape (8.4%) are the three highest contributors to D1 in Figure 12. Ten of the sixteen total variables have contributions above the expected average contribution value of 6.4%. The six variables with contributions below the average include reproduction strategy (reproduction) (5.8%), MLB_s (5.6%), ecological designated environment (environment) (5.5%), migration (4.2%), order (2.0%) and feeding mechanics (feed) (1.4%).

When calculating values for I using equation 2, the values for r' were extremely small because all measurements were standardized by total length. Raising these already small values for r' to the fourth power further reduced their magnitude, resulting in very small numerical outputs. Therefore, all values for I are reported in scientific notation. The two-sample, two-tailed t-test showed a significant difference in the mean value of I between benthic and pelagic species (*t* = -2.36, *df* = 28, *p-value* = 0.03). Benthic sharks had a mean I of 4.31×10^{-10} (n = 17), whereas pelagic sharks had a mean of 8.41×10^{-10} (n = 13). The twelve sharks with the lowest I values were exclusively benthic. The values of I for pelagic sharks were almost twice those of benthic species. The Pearson correlation analysis of the relationship between second moment of area (I) and fineness ratio (FR) indicated a strong, significant negative relationship (r = 0.69, n = 30, p = 0.00002).

Discussion

Previous studies on other marine vertebrates provide evidence that vertebrae morphology can be used to infer an organism’s ecological habits (Buchholtz, 2001; Donatelli et al., 2021). Porter, Roque & Long (2009) used a fineness ratio to determine that centra morphology was a better predictor of body curvature during turning manoeuvres in sharks rather than the maximum vertebral number alone. In this study, I build on these findings by investigating the variations in centra morphology among different shark species and explore how these variations relate to their environments. The results showed significant differences in centra morphology, that correlate with environments, swimming modes and respective migratory habits.

Environmental combinations

Ecological trends were observed between environment, migratory habits, swimming mode and centra shape. Certain ecological trends are known as the narrative in shark ecology. For example, pelagic sharks tend to be fast swimming carangiform swimmers and anguilliform swimmers tend to be slower moving and benthic (Lindsey, 1978; Ebert, Dando & Fowler, 2021). Furthermore, carangiform swimmers are more migratory and anguilliform swimmers are more resident.

For centra shape, the influence of the trend of pelagic carangiform swimmers and benthic anguilliform can be seen. No pelagic organisms had fluted cylinder or hourglass shaped centra and no anguilliform swimmers had cylinder shaped centra. Fluted cylinder and hourglass shape are associated with benthic anguilliform swimmers whereas pelagic carangiform swimmers are associated with cylinder, modified cylinder and Lamnidae centra shapes. Similar observations were identified by Kozuch & Fitzgerald (1989). It is theorised that fluted cylinder and hourglass shaped centra are too flexible to withstand the large mechanical strain associated with high-speed swimming needed for pelagic swimmers. Instead, fluted cylinder and hourglass shapes prioritize manoeuvrability rather than speed. Conversely, cylinder shaped centra prioritise faster swimming rather than manoeuvrability making them not suitable for agile movements during anguilliform swimming.

Centra morphology

A general negative linear relationship was seen between $\mathrm{CCL_s}$ measurements and $\mathrm{DVH_s}$ measurements – as $\mathrm{DVH_s}$ increases, $\mathrm{CCL_s}$ decreased. Larger sharks had lower $\mathrm{CCL_s}$ measurements compared to $\mathrm{DVH_s}$ measurements and smaller sharks had higher $\mathrm{CCL_s}$ measurements compared to $\mathrm{DVH_s}$ measurements. Centra growth is proportionally related to somatic growth (Piercy et al., 2006; Natanson et al., 2018), the bigger the shark, bigger the centra face. However, this proportional growth doesn't necessarily extend to the centra length. Larger sharks had smaller $\mathrm{CCL_s}$ measurements than $\mathrm{DVH_s}$ measurements. Therefore, the decrease in $\mathrm{CCL_s}$ measurements as $\mathrm{DVH_s}$ measurements decrease, was expected.

The centra dimension measurements reveal morphological differences among the shark orders but also similarities among species within the same family. Phylogenetically, Squaliformes and Pristiophoriformes fall within the Squalomorph superorder. They are sister groups and have derived their centra morphology from ancestors with well-developed centra (Compagno, 1977). The remaining orders, Lamniformes and Carcharhiniformes, are sister groups in the Galeomorph superorder. All shark orders apart from Squaliformes are strongly supported as monophyletic (Naylor et al., 2012). However, it is still unclear as to whether the characteristics of these sharks are primitive or derived and whether they are formed from a common ancestry or parallel evolution (Compagno, 1977). The identified centra morphological patterns are described below.

Lamniformes

Lamniformes are a small group of sharks with various specialised forms and habits. With the exception of the smaller Crocodile shark (*Pseudocarcharias kamoharai*), all lamniform species are medium-sized to gigantic (Compagno, 2002). Since larger body size and girth are correlated with centra length (Natanson et al., 2018; Jambura & Kriwet, 2020), the large dorsal-ventral heights (DVH) are expected.

Lamna nasus, *Carcharias taurus,* and *Carcharodon carcharias* had the largest DVH_s respectively, and therefore, the largest centra faces. In contrast, *Alopias supercliliosus* (big eye thresher shark) had the shortest CCL_s among all the species examined. Except for *P. kamoharai,* all lamniformes had DVH_s measurements greater than their respective CCL_s. The centra faces of *A. supercliliosus* and *C. carcharias* were more rounded and circular ($DVH_s \sim MLB_s$), whereas those of *L. nasus*, *P. kamoharai* and *C. taurus* were more elliptical shaped ($MLB_s > DVH_s$).

Most lamnoids are strong, fast swimmers and highly migratory, adaptations essential for their life as pelagic predators (Gemballa et al., 2006). For example, *C. carcharias* has a quick model swimming speed ranging from 0.80 to 1.31 $m.s^{-1}$ (Watanabe et al., 2019) The increased surface area provided by larger DVH_s may be required for greater muscle attachment, increased structural support, and shock absorption to support their active lifestyles.

Squaliformes

Squaliformes are generally a smaller-sized order of sharks, barring the much larger Sleeper sharks. Squaliform species are found on continental and insular shelves and slopes but are strongly associated with the bottom, often reaching depths of 1000 m (Ebert, Dando & Fowler, 2021). They are generally a slower swimming species with swimming speeds ranging approximately from 0.26 to 0.29 m.s^{-1} (Domenici, Standen & Levine 2004). *Squalus acutipinnis* (Family: Squalidae) is a local species known to inhabit inshore to upper shelves areas close to the bottom at depths up to 450 m (Viana & de Carvalho, 2016). *Etmopterus brosei* and *Etmopterus compagnoi*, both members of the Etmopteridae family, are also bottom dwelling species found in depths ranging from 200 to 1500 m (Ebert, Dando & Fowler, 2021). Squalidae species are an older group compared to Etmopteridae who branched out much later (Naylor et al., 2012).

S. acutipinnis, E. brosei and *E. compagnoi* had the smallest centra measurements reflecting both their small body size and girth. Despite having some of the smallest centra faces, both *Etmopterus brosei* and *Etmopterus compagnoi* (lantern sharks) had the two largest CCL_s measurements of the species studied. These longer cranial-caudal lengths are characteristic of their hourglass centrum shape (Kozuch & Fitzgerald, 1989). In contrast, *S. acutipinnis*, which occupies shallower waters, had a fluted cylinder centra shape. Its centra were slightly shorter, with larger DVH_s measurements than CCL_s. The centra faces of both families – Squalidae and Etmopteridae – were rounded and circular, with deep concave walls that result in a pinched centre. In comparison with other sharks, the centra of Squaliformes were relatively fragile and brittle, with thin cartilaginous walls.

Pristiophoriformes

Pristiophoriformes, commonly known as sawsharks, are small, slender sharks characterized by flattened heads, long saw-like snouts (rostrum) and cylindrical bodies (Weigmann et al., 2020). Very little is known about this order, which consists of only one family and two genera: *Pliotrema* and *Pristiophorus*. Endemic to the Southwest Indian Ocean, *Pliotrema* *warreni* occur on or near the bottom of offshore continental shelves and upper slopes, at depths from 10 to 430 m with the possibility of deeper occurrences. *Pliotrema* species have relatively restricted distributions in the western Indian Ocean, although a few vagrants have been recorded in the south Atlantic oceans off the Namibian coast (Weigmann et al., 2020; Ebert, Dando & Fowler, 2021).

The centra of *Pliotrema warreni* were circular with the DVH_s only slightly larger than CCL_s. *P. warreni* had the smallest DVH_s of all the species examined, with the DVH_s and CCL_s being approximately equal. The centra were shaped like modified cylinders, with a slight recurving of the rims near the centrum face. Visually, *P. warreni* is distinct from other typical shark species. It has a highly restricted distribution, occurs at deeper depths on or near the ocean floor, and is one of the few species with a modified cylinder centrum shape. Additionally, *P. warreni* is the only species in this study with suction-crushing feeding mechanics. As a result, these aforementioned unique traits differing them from conventional sharks may explain their outlier positions.

Carcharhiniformes

This order is the largest, most widespread and most diverse group of sharks (Compagno, 1990; White, O'Neill & Naylor, 2022). In this study, six of the ten families were represented: Pentanchidae (5 spp), Scyliorhinidae (2 spp), Carcharhinidae (7 spp), Galeocerdidae (1 spp), Sphyrnidae (3 spp), and Triakidae (3 spp). Members of this order have a wide range of appearances, from small, bottom-dwelling catsharks to larger, predatory requiem sharks. Species can be found worldwide in all oceanic environments. While some are poor swimmers with limited distribution, others are highly migratory and strong swimmers (Helfman et al., 2009; Ebert, Dando & Fowler, 2021).

Pentachidae, also known as deepsea catsharks, are generally small sharks (<80cm long) with elongated bodies, typically found at depths beyond 200 m. However, this excludes two shallower-occurring genera, *Halaelurus* and *Haploblepharus*, (Ebert, Dando & Fowler, 2021). This study includes five species from this family: *Haploblepharus pictus, Haploblepharus edwardsii. Haploblepharus fuscus* (shysharks), *Holohalaelurus regani* and *Apristurus saldanha*.

Centra morphological similarities were strongly evident within this family, especially among the shysharks. *H. pictus, H. edwardsii* and *H. fuscus* are endemic to South Africa, where they inhabit close inshore shallow waters, including kelp forests, sandy areas, and rocky bottoms (Ebert, Dando & Fowler, 2021; Ebert, Wintner & Kyne, 2021). These three species had an hourglass centra shape with elliptical centra faces ($DVH_s < MLB_s$), and their DVH_s never exceeded their CCL_s. These similarities further extend to *H. regani*, an Izak catshark, which had a fluted cylinder centra shape, an elliptical centra face, and longer CCL_s. Both hourglass and fluted cylinder shapes are associated with longer cranial-caudal lengths and strongly pinched centrum walls (Kozuch & Fitzgerald, 1989).

In comparison to the shallower species*, A. saldanha* is a deepwater catshark inhabiting depths between 300 and 1000 m along the continental slope (Ebert, Dando & Fowler, 2021). Its centra were fluted cylinder in shape, with a rounded circular face ($DVH_s \sim MLB_s$) and longer CCL_s. Despite sharing taxonomic similarities, the centra morphology of *A. saldanha* seemed to be more comparable to that of the deepwater shark, *S. acutipinnis*, as both exhibit fluted cylinder centra shapes with round centra faces. *A. saldanha* was clustered closer to *S. acutipinnis* than the other members of the Pentanchidae, reiterating the similarities between the two species.

Similar to Pentanchidae, Scyliorhinidae are small catsharks with elongated bodies, typically found on or near the seabed. These species are also restricted to relatively small geographic ranges (Ebert, Dando & Fowler, 2021). This family is not monophyletic, rather it consists of three distinct paraphyletic lineages (Naylor et al., 2012). The Poroderma family are grouped together in one group.

Endemic to South Africa, *Poroderma pantherinum* and *Poroderma africanum* are nocturnal species found from the surfzone to the intertidal zone, usually on or near the bottom (Compagno, 1984b). Both species have elongated hourglass shaped centra. However, *P. africanum* had a more rounded centra face, with DVH_s approximately equal to MLB_s, while *P. pantherinum* had a more elliptical centra face with DVH_s smaller than MLB_s.

Carcharhinidae, commonly known as requiem sharks, are among the largest species in the Carcharhiniformes order. This family is well supported as monophyletic (Naylor et al., 2012). They are typically found in tropical continental shelf waters, often closely associated with coral reefs and oceanic islands. Carcharhinids are active strong swimmers, some solitary while others are schooling species (Compagno, 1984b). These sharks are highly predatory and dominate environments with their biodiversity, abundance and biomass (Ebert, Dando & Fowler, 2021). This family includes the highest number of species investigated by this study: *Carcharhinus leucas, Carcharhinus limbatus, Carcharhinus plumbeus, Carcharhinus brevipinna, Carcharhinus brachyurus, Carcharhinus obscurus,* and *Carcharhinus amboinesis.*

All species had cylinder shaped centra with short, straight (sometimes convex) walls. CCL_s were significantly shorter relative to DVH_s, giving their centra a disc-like appearance. *C. leucas, C. plumbeus, C. brevipinna,* and *C. amboinesis* all had rounded centra faces ($DVH_s \sim MLB_s$), whereas *C. limbatus, C. brachyurus* and to a lesser extent *C. obscurus*, had more elliptical shaped centra faces ($DVH_s < MLB_s$). Centra sizes were relatively consistent across all species, with DVH_s ranging from 10.18 to 11.89 and CCL_s ranging from 5.19 to 6.35. These similarities are likely a result of many shared environmental and ecological factors among the species driving the same morphological adaptions to arise.

Galeocerdidae consists solely of *Galeocerdo curvier* (Tiger shark). This family was recently resurrected due to distinct morphological, biological and genetic differences from other members of the Carcharhinidae family (Naylor et al., 2012; Ebert, Dando & Fowler, 2021; Balanin et al., 2023). Tiger sharks are large powerful sharks with broad, blunt snouts, typically occurring on or near continental and insular shelves. They are known to travel long distances between various habitats (Ebert, Dando & Fowler, 2021).

G. curvier centra are among the most easily recognised centra among the Carcharhiniformes (Kozuch & Fitzgerald, 1989). These centra had a distinct modified cylinder centra shapes with strongly recurving at the rims and extremely large encircling pores along centra walls. The rims were particularly robust at both the posterior and anterior ends. The centra walls were concave and medium in length, with CCL_s still less than DVH_s. The centra faces were mostly round with MLB_s only slightly exceeding DVH_s. Despite unique centrum features, the centra dimensions of *G. cuvier* are similar to that of Sphyrnidae and some Carcharhinidae species. These centra similarities support the indication of Galeocerdidae being a sister group of Sphyrnidae proposed by Naylor et al. (2012).

Sphyrnidae, or hammerhead sharks, are distinguished by their uniquely shaped heads which enhance manoeuvrability and increase sensory capacity (Farrell, 2011; Ebert, Dando & Fowler, 2021). Three of the larger species from this family are represented here: *Sphyrna zygaena, Sphyrna lewini,* and *Sphyrna mokarran*. Sphyrna centra are typically cylinders (*S. mokarran*) or modified cylinders (*S. lewini* and *S. zygaena*) with slightly concave walls. The centra walls were medium in length with CCL_s never exceeding DVH_s. Sphyrnidae centra were typically shorter than that of Carcharhinids.

Lastly, Triakidae are a large group of bottom swimming sharks, most of which are found on continental and insular shelves, including sandy, muddy and rocky inshore habitats (Ebert, Dando & Fowler, 2021). These species tend to have restricted distributions, often endemic to specific regions. Many species are highly active, continuous swimmers, while others rest or swim close to the ocean floor (White, O'Neill & Naylor, 2022). All genera within the Triakidae family are considered monophyletic (Naylor et al., 2012).Three species from this family are represented in this study: *Mustelus mustelus, Mustelus palumbes*, and *Galeorhinus galeus*. Proportionally, the centra lengths of Triakidae were among the longest in the Carcharhiniformes, along with bottom-dwelling Pentanchidae genera, *Haploblepharus.*

Mustelus palumbes, the smallest shark of the three species, had the longest CCL_s. The centra walls of Triakidae species were deeply concave, with a strong pinched centre that flared at the ends, which gave them a fluted cylinder shape. The MLB_s was greater than DVH_s resulting in an elliptical shaped centrum face. Among the three species, *G. galeus* had the most robust centra, with the largest and widest centra face.

Fineness ratio

The centra of benthic species had longer cranial-caudal lengths in comparison to their dorsal-ventral heights resulting in a block-shaped centrum. Centra of pelagic species had a shorter cranial-caudal length in comparison to their dorsal-ventral height resulting in a much shorter disc shaped centrum. The FR of benthic and pelagic sharks were significantly different with benthic sharks having a FR 75% greater than that of pelagic sharks.

The FR between some shark orders were also significantly different from each other. The benthic Squaliform sharks had the largest mean FR, and the pelagic Lamniform sharks had the smallest. Squaliformes are a much older lineage compared to Lamniformes that derived later. These evolutionary differences coupled with the stark difference in habitat occupation, might explain the extremities in centra length differences and further support the significant differences of the FR for benthic and pelagic environments. The FR between Squaliforms and Carcharhiniformes were also different but to a lesser extent. Seeing that there are phylogenetic similarities between Carcharhiiniformes and Lamniformes, one would also assume that there would be significant differences between Carcharhiniformes and Squaliformes. Carcharhiniforms is a large order encompassing both pelagic and benthic sharks as opposed to the benthic Squaliformes. The presence of both pelagic and benthic shark species may create a median FR with the low FR of pelagic sharks balancing out the high FR of benthic sharks.

This distinctly high FR for Squaliform species may assist in the easy identification of unknown centra as a Squaliform species if an extremely large FR is present. Conversely, if there is a distinctly low FR of an unknown centra, there is a large likelihood that it is a Lamniform species.

The three best general linear models showed that environment, migration, swimming mode and centra shape significantly influenced the FR. Specifically, the environment had the strongest influence on the FR followed by migration habits then swimming mode and centra shape. These results support the notion that variation in centra morphology is strongly associated with environment complexity and the demand of transversing through in these environments – be it covering vast distances in an empty water column or maneuvering through complex substrates. Previous ecological assessment of the shark fossils based on maneuverability, body shape, mouth gape and other morphological features indicated significant ecological portioning of species across both environmental gradients and within taxonomic lineages (Whitenack, Kim & Silbert, 2022).

As benthic environments are often associated with anguilliform swimmers and pelagic environments with carangiform swimmers (Sternes & Shimada, 2020), the strong influence of swimming mode on the FR is expected given the significant effect of environment. The same holds true for migratory patterns and its association with swimming mode. Carangiform swimmers tend to be migratory and anguilliform swimmers, resident

(Hoffmann, Buser & Porter, 2020). Because of the strong interconnectivity between these known patterns between environment, swimming mode and migration, the significant effect each variable had on the FR was expected.

Centra shape also had a significant effect on the FR meaning species with cylinder, modified cylinder, fluted cylinder, hourglass shaped centra and the Lamnidae family had different fineness ratios. The use of different centrum shapes may be an adaption for a species ecological and functional requirement in their respective environments. In turning maneuvers, centra shape was also a strong predictor of flexibility in extant sharks (Kajiura, Forni & Summers, 2003; Porter, Roque & Long, 2009).

All pelagic species had a FR of less than 0.6 with the exception of the Crocodile shark, *Pseudocarcharias kamoharai,* with an FR of 0.83. The Crocodile shark, a deep-water oceanic species, has a depth range between 0 and 600 m (Ebert, Dando & Fowler, 2021). This FR is higher than expected and did not conform the pattern observed with other Lamnids who all had an FR within similar ranges.

Majority of benthic species had a FR greater than 0.9 with the exception of the Zambezi shark, *Carcharhinus leucas* (FR = 0.45), Ragged-tooth shark, *Carcharias taurus* (FR = 0.48), Sandbar shark, *Carcharhinus plumbeus* (FR = 0.53), and Soupfin shark, *Galeorhinus galeus* (FR = 0.87). Both the *C. leucas* and *C. taurus* are considered to be “reef associated” species and *G. galeus* and *C. plumbeus* are benthopelagic species (Froese & Pauly, 2000).

These four species could be seen as transitional species that are neither completely benthic nor completely pelagic. *G. galeus* and *C. plumbeus* are coastal pelagic species commonly found on continental and insular shelves but are usually associated with ocean floors lacking heterogeneous features such as coral reefs and rough bottoms (Compagno, Ebert & Cowley, 1991; Ebert, Dando & Fowler, 2021). Therefore, their vertebrae columns would not require the same level of flexibility needed to better maneuver and navigate a topographically complex ocean floor.

Carcharhinus leucas and *C. taurus* commonly dwell in shallow bays with the *C. leucas* also known to be amphidromous, occurring in freshwater and brackish environments (Ebert, Dando & Fowler, 2021; Livernois et al., 2021). However, despite often being on or near the bottom, both species are highly migratory and capable of covering great distances contrary to other benthic species. Due to these migration habits, these shark species need to be able to swim at different speeds during these movement patterns. For example, during transiency periods, *C. leucas* swim at a mean speed of 0.77 $m.s^{-1}$ (Niella, Afonso & Hazin, 2017). However, during residency periods, swimming speeds were considerably slower with a mean speed of 0.18 $m.s^{-1}$ (Niella, Afonso & Hazin, 2017). These differences in ecological habits may account for their deviation from the observed pattern of the FR between pelagic and benthic species.

The relationship between FR and I has a strong, negative correlation. Sharks with a higher FR (benthic species) tend to have substantially lower I values, whereas sharks with a lower FR (pelagic species) have much larger I values. The larger the value for I, the greater the stiffness of the vertebrae and its resistance to bending. This pattern is expected as benthic sharks experience more bending throughout its body thus requiring less stiffness and resistance. Whereas pelagic sharks have minimal body bending thus requiring stiffer vertebrae to resist bending undulations. Large, robust bodied sharks i.e. *L. nasus, C. taurus, C. carcharias*, had the largest I values and the smaller, slender bodied Squaliformes, Carcharhiniformes (Pentachidae, Scyliorhinidae, Triakidae) and Pristiophoriformes had the smallest I values.

Benthic traits and species were clustered together, whereas pelagic traits and species formed another cluster. Fluted cylinder and hourglass centra shapes were evidence of a benthic occurring species, and cylinder centra shapes were associated with pelagic species as the respective shapes fell within the corresponding clusters. Additionally, the relationship between anguilliform swimmers and benthic environments as well as carangiform swimmers and pelagic environments is further emphasized and evident as each swimming mode was located in their respective clusters. These correlations between benthic environments and anguilliform swimmers and pelagic environments and carangiform swimmers is a well-established pattern within the literature (Lindsey, 1978; Wilga & Lauder, 2004; Sternes & Shimada, 2020).

Between the two clusters were the order and species that was not entirely benthic nor pelagic. These were the Carcharhiniform order, *P. kamohari,* and *G. galeus.* Due to Carcharhiniformes large diversity of both pelagic and benthic species, the median position is expected. Both *P. kamohari* and *G. galeus* deviate from the pelagic and benthic clusters respectively. As previously discussed, *P. kamohari* has centra measurements differing from the other Lamniformes. In addition, this species is the only pelagic anguilliform swimmer.

These deviations from the observed trends of pelagic species account for the deviation away from the pelagic cluster and explain the median position between the two clusters as *P. kamohari* exhibits traits of both pelagic and benthic species. Similarly, *G. galeus* also shares traits of both pelagic and benthic shark species as it is highly fast swimming benthopelagic shark.

Squalus acutipinnis was set apart from the main benthic cluster. This deviation from the cluster could be a feature of the Squaliform order, however, definitive conclusions cannot be drawn as the other Squaliform sharks, *E. brosei* and *E. compagnoi*, were not included in the analysis.

Situated further from the benthic cluster in an outlier position is the morphologically distinct sawshark, *P. warreni*. While benthic, *P. warreni* has a unique body shape and environmental variables contrary to those typically exhibited by benthic organisms. Including by not limited to distinct body shape, suction-crushing feeding mechanics and modified cylinder shaped centra. However, as *P. warreni* was the only Pristiophoriform species, more species data is required to draw definitive conclusions on the outlier position of this order and species.

Dimension 1 explained most of the variation with the variables of species, FR and centra shape contributing the most. Despite having had a strong influence on the FR in the general linear model results, migration and environment were low contributors to Dimension 1. Although Dimension 1 explained the most variation, the variation was still less than 20%. The first five dimensions cumulatively explained less than 50% of the total variance of the data. This is relatively low explanatory power and although these components may explain some portion of dataset’s variability, more than half of the variance is left unexplained. Even though some visual patterns were evident, other components and further testing may be necessary to fully capture the structure and explain more variance to strengthen the significance of the observed patterns.

Locomotion

Body curvature is highly variable among species and during swimming, these variations are influenced by the mechanical behaviour of the vertebral column (Paig-Tran et al., 2022). The majority of pelagic species are carangiform swimmers, while most benthic species tend to use anguilliform swimming (Sternes & Shimada, 2020). Pelagic species occupy oceanic open waters devoid of structures, namely swimming through an empty water column over great distances (Farrell, 2011). Benthic environments are more structurally complex with many obstacles to avoid. Therefore, benthic species need a flexible body to navigate these environments, while that same flexibility is not required in pelagic environments. These two swimming modes differ in the magnitude and location of the undulating wave produced through the body during swimming.

Using the beam theory, commonly applied in structural engineering, we can understand how a shark’s body responds to the external forces experienced during swimming (Jimenez & Camp, 2023). Assuming all else is equal, the longer the beam, the more flexible it is. Vertebrae of sharks are cartilaginous, which admits a degree of bending not seen in bone. The longer the vertebrae, for any given thickness, the greater it will flex under a given lateral force. The fluted cylinder and hourglass shape of anguilliform swimmers will allow even greater flexibility by being thinner in the center. Additionally, fluted cylinder and hourglass shaped centra had among the smallest values for I confirming the flexibility of these centra shapes with its low stiffness and high affinity for bending. For this example, the shark's vertebral column acts as the beam receiving the propulsion forces generated by the tail. The body of fish are heterogenous and a complex network of connective tissues, muscle fibers, nerves and blood vessels. Despite the assumptions of homogeneous materials in beam theory, Jimenez & Camp (2023) found strong support for beam-like bending in fish and the use of beam theory to describe vertebral flexion.

Anguilliform swimming mode

During anguilliform swimming, the undulating wave travels through approximately two thirds of the shark's body with the head region having limited movement (Lindsey, 1978). Therefore, a longer beam from the mid-section to the tail is created. Because the majority of the body undulates resulting in a longer beam, according to beam theory, the vertebrae column of anguilliform swimmers is therefore highly flexible allowing the bending load to be distributed along the body. This concurs with the results presented here, as benthic species tend towards anguilliform swimming, have a higher fineness ratio and lower second moment of area.

Benthic species within structurally complex environments such as coral reefs, rocky bottoms and kelp forests, need to be highly flexible swimmers allowing for effective maneuvering to avoid obstacles and propulsion in tight spaces (Friedman et al., 2020). The longer beam and reduced bending stiffness in anguilliform swimmers, allows for greater bending and the flexibility required for these environments. Furthermore, if you consider each centrum along the vertebral column to each be its own beam, the same can be said. The vertebral column is not a continuous rod, rather it consists of sequential individual blocks i.e. centra held together by intervertebral joints. Benthic species possess longer cranial-caudal lengths and therefore have longer centra. These longer centra act as long beams distributing the oscillating forces along the centra and allowing greater flexibility of the beam. Additionally, centra are also a lot smaller and slender which in turn further increases the flexibility of the beam.

An example of this can be seen within the genera *Haploblepharus*, the shysharks. Endemic to the southern South African coastline, Haploblepharids occupy a labyrinthine environment, kelp forests (Ebert, Dando & Fowler, 2021). Being anguilliform swimmers, these sharks have a highly flexible vertebral column as well as extremely small, long centra making them most efficient in manoeuvring throughout the crevices in their environment. Moreover, a unique behavioural trait of shysharks is to curl up with its tail covering their eyes when captured or stressed. These actions allude once again to the extreme flexibility of their vertebral columns. Shysharks have restricted distributions and do not migrate, therefore their vertebrae are not designed for continuous swimming to cover far distances.

The exceptions to this pattern are the deep-water sharks, *E. compagnoi* and *S. acutipinnis* and benthopelagic sharks, *C. plumbeus, C. leucas, G. galeus* and *C. taurus*. Although these species are considered benthic, they are carangiform swimmers rather than anguilliform swimmers. The benthic environments they tend to occupy do not have high structure variability and therefore their vertebral columns might not require the same flexibility.

Carangiform swimming mode

Carangiform swimming is a faster and more efficient mode of swimming than anguilliform swimming (Tey & Che Sidik, 2015). Carangiform swimmers tend to be fast, active continuous swimmers as less energy is lost by displacing water laterally from propulsion forces. In carangiform swimmers, only the posterior region, the last third of the body undulates, and the anterior region remains relatively rigid (Lindsey, 1978). Therefore, the vertebral column needs to withstand strong lateral forces withing bending. The vertebrae have a low fineness ratio, high second moment of area and cylinder shape, further preventing flex within the vertebrae. Bending is localised near the tail, allowing for rapid and powerful thrusts for faster swimming. The rigidity of the anterior vertebral column is essential for the absorption of these strong thrust forces and providing stability during swimming.

In this study, only vertebrae from beneath the first dorsal was investigated. During carangiform swimming, this region does not undulate. Carangiform swimmers tend to occupy pelagic environments and have large but short centra. These shorter centra act as shorter rigid beams resulting in a less flexible vertebral column. Higher values of second moment of area for carangiform swimmers confirm this pattern of rigidity as centra are considerably stiffer than anguilliform swimmers. Furthermore, the larger centra creates a thicker stocky cylinder reducing bending capacity further. This rigidity created by these centra dampens the undulating waves generated during thrust allowing the body to remain still and keeping the animal more streamlined to reduce drag. This reduces energy loss as less energy is lost through the displacement of water of the anterior region ultimately allowing the shark to move more easily through the water (Lindsey, 1978).

Pelagic environments refer to free waters of the open ocean away from constraining land masses making it easier to navigate. Here, high maneuvering flexibility is not necessary as there are not any obstacles to avoid. Instead, a stiff and stable vertebral column is preferred to efficiently absorb the pressures of faster and continuous swimming while optimising energy usage across vast distances.

Thunniform swimming is similar to carangiform swimming, however, it is more extreme. A subcategory of carangiform swimming, thunniform swimming is the fastest swimming mode achieving high velocities, fast acceleration, and continuous swimming but lack maneuverability (Newbrey et al., 2013). Thunniform swimming is popular among Lamnid sharks and in this study *L. nasus* and *C. carcharias* are thunniform swimmers. During this swimming mode, undulation is localized in the stiff lunate-shaped tail limiting bending in the entire body. As a result, a very short rigid beam is created in the vertebral column from the caudal peduncle to the tail. The rigidity created in the mid-section region is essential in absorbing high-powered thrust delivered by the tail (Crofts, Shehata & Flammang, 2019).

Thunniform swimmers have a fusiform torpedo-shaped body with a pointed head and a thick girth that narrows towards the tail (Lindsey, 1978). Vertebrae in the mid-section are extremely large and robust but very short. Once again, these large vertebrae create a thick short beam with high resistance to bending keeping the body still and streamlined as the tail propels the shark through the water column.

An example of these centra extremities is the Great White shark, *Carcharodon carcharias.* Known as one of the largest apex predators, *C. carcharias* is capable of short powerful bursts of speed, used to ambush prey as well as continuous swimming covering vast distances during seasonal migrations. *C. carcharias* had the lowest FR and the third largest I with extremely large but short centra that acts as rigid beams absorbing the strong forces generated by the tail that efficiently maximise propulsion while minimising drag.

Contrary to my results, Porter, Roque & Long (2009) said that shorter centra lengths correlates to higher body curvature during turning maneuvers. Crofts, Shehata & Flammang (2019) show similar patterns in the flexibility of heterocercal tails. Centra closer to the caudal peduncle were shorter and more flexible then the longer centra closer to the end of the stiff tail. Newbrey et al. (2013) said vertebral columns with longer centra and low flexure is a characteristic of thunniform swimming lamnid sharks.

However, absolute centra measurements were used in each of these studies to reach said conclusions. In this study, when looking at the standardised measurements of centra instead of the absolute measurements, the reverse is seen as longer centra lengths are more flexible and shorter centra lengths are more rigid. Despite these discrepancies, results from this study, Porter, Roque & Long (2009) and Crofts, Shehata & Flammang (2019), ultimately say the same thing. When looking at the absolute measurements, Lamniformes would have longer centra lengths as the centra are physically bigger and longer. However, when total length is considered and the measurements are standardised, the shorter centra lengths of Squaliforms are actually longer than the shorter Lamniform centra lengths that would otherwise be considered long.

Furthermore, when looking at the angles formed by the double-cone apex of a sagittal slice of vertebrae, larger angles resulted in lower stiffness (Paig-Tran et al., 2022). This supports my results as the longer the centra, the larger the double-cone apex angle and therefore, the lower the stiffness of centra. Regardless of which centra measurements were used, carangiform swimmers are shown to have more rigid vertebral columns and anguilliform swimmers a more flexible vertebral column.

In this study body undulation and flexibility in swimming sharks was determined using a fineness ratio, second moment of area and beam theory. Previous studies have concluded that instead, body undulation and flexibility is determined by the number of vertebrae within the vertebral column, the flexion between the intervertebral joints and the angle of flexion (Brainerd & Patek, 1998). However, in a study by Porter, Roque & Long (2009) it was statistically shown that morphological features such as second moment of area, cranial-caudal length and dorsal-ventral height were better predictors of maximal body curvature than vertebral number alone. Therefore, this study places less focus on the influence of vertebral number on body flexure and instead focuses on morphological traits of centra.

Applications

Centra of sharks have previously been useful in estimating body size and growth rates, therefore allowing the reconstruction of biological and ecological traits as many of these aspects are correlated with body size (Jambura & Kriwet, 2020).The notion that centra morphology can infer ecological environment and locomotory habits furthers this reconstruction and has many beneficial applications when studying both extant and extinct species. As new shark species are constantly being discovered, observing these organisms in-situ can be challenging leaving many gaps within ecological data. Additionally, many oceanic and deep-water species are also difficult to observe (Cotton & Grubbs, 2015).

Once these rare species are eventually caught and dissected, inferences can be made from their vertebral column to determine environment and locomotion without the need for other costly and logistically challenging methods. Thus, a single organism can provide insight into much more ecological data than solely diet, age and growth, reproduction, and anatomy. This ultimately fills knowledge gaps of ecological functionality and furthers the understanding of the evolutionary trends.

Similarly, the same applies to extinct species. As the observation of extinct species is impossible, measuring morphological differences in fossilised vertebrae may provide insight into how the organism may have lived and allows for possible comparisons to be made with living sister taxa. Overall, this study provides a different method of increasing the information yielded during organism dissections and providing more insight into the ecological functionality associated with different anatomical morphologies.

Limitations and future studies

There are several limitations to this study, primarily the range of data available. Future studies should seek to increase taxonomic coverage, both extant and extinct. Many species and notable orders were excluded from this study due to logistical difficulties regarding their acquisition. Increasing the range of species studied should strengthen and affirm the notable trends and allow for stronger generalisations across groups.

Additionally, another limitation is the restricted portion of the vertebral column examined. Expanding the studied vertebral area to the entire vertebral column from the head to the tail would be beneficial in determining possible emerging trends at different locations throughout the vertebral column. Are the trends identified here consistent across different functional regions? If not, how would the trends change throughout the vertebral column?

Biomechanical testing of centra would also be beneficial in gaining deeper insight and understanding of how these morphological differences influence functional performance. Introducing the use of biomechanical data along with morphological measures would allow further testing of hypotheses concerning form-function relationships. It should be noted that all the above conclusions are hypotheses subject to revision with the incorporation of additional data.

Conclusions

This study showed distinct differences in fineness ratio among shark orders and yet the environment and the mode of swimming accounted for a substantial degree of the variation regardless of phylogeny. The mean fineness ratio for benthic sharks was 75% greater than that of pelagic sharks. Centra morphologies correspond to the optimisation of body flexure for maximum energy output during swimming and migrations. Beam theory explains how the vertebral column acts as a beam during swimming and flexes in reaction to undulating waves and propulsion by the tail. In benthic sharks, the wave runs along the entire body length whereas in pelagic sharks, only the posterior third of the vertebral column is typically involved in flexing.

The fineness ratio was influenced by shark environment, swimming mode and migratory habits. Combinations of life-history traits among sharks resulted in swimming mode and migratory patterns independently explaining some of the variation. Fineness ratios were positively influenced by anguilliform swimming (as opposed to carangiform) and by resident behaviour. Carangiform swimming and long-distance migrations are two behaviours that demand efficiency.

There is a degree of consistency in habitat and behaviour within orders which likely explains the explanatory power of the phylogenetic groups. Nevertheless, the effects of environment and swimming behaviour were consistent across all orders which suggest that centra morphology can be used as a reliable indicator of shark habits among extant and extinct forms.

The correlations between morphology and environment provide a vital tool for analysing shark ecology. This is especially relevant to the study of species where observation in-situ is difficult but also to palaeobiological interpretations of fossilised centra. While further investigations are still needed, the hypotheses developed in this study are accepted.

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