TOOTH WEAR, MICROWEAR AND DIET IN ELASMOBRANCHS

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By

Laura McLennan

Department of Geology

University of Leicester

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Laura McLennan

As abundant and widespread apex predators, elasmobranchs play influential roles in the food-web dynamics of marine communities. This has obvious implications for fisheries management and marine conservation. For successful conservation, the ecology of a species must be known. An understanding of extinct species ecology is also useful. Unfortunately, diet a key component of a species' ecology, is relatively understudied in elasmobranchs. For a majority of elasmobranch species, little or no quantitative dietary data exists. This reflects the limitations of current dietary defining methods.

This thesis presents two alternative methods that can be used to determine the diet of extinct and extant elasmobranchs: meso-style wear analysis and 3D tooth microtextural analysis. These wear techniques can be applied to small sample sizes, and sampled animals with no stomach contents, thus reducing the impact of study on wild elasmobranch populations. The techniques can also be applied to dried and fossil samples, further reducing the impact of study on wild populations and providing a means for the study of extinct species. Furthermore, these wear techniques provide additional advantages over the traditional methods of stomach contents analysis and observation. The wear, measured through the methods outlined in this thesis, accumulates over a longer timescale. The "snapshot bias" associated with traditional methods is thus overcome when analysing diet via meso-style analyses or 3D microtextural analyses.

This thesis also investigates the impact of sediment abrasion to 3D tooth microtextures. Results show that care needs to be taken when comparing fossil specimens originating from deposits with differing sediment compositions.
These findings are applicable to any study using 3D microtextural techniques on fossil specimens of any species, as all have been exposed to sediment abrasion before fossilisation.

This is the first time that these alternative wear methods have been applied to elasmobranchs. They have displayed the potential to be a powerful tool for the dietary analysis of living and extinct elasmobranchs in the future.

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F The F value is a product of an ANOVA/ Welch ANOVA statistical test. It is the variation between sample means and variation within the sample.

d.f. Degrees of Freedom. An ANOVA/Welch ANOVA statistical test produces two d.f. values. The d.f for the numerator are calculated as (k-1), the d.f. for the denominator are calculated as (N-k). Where N= total number of samples and k= number of groups.

P The calculated probability- the probability of finding the observed result, when the null hypothesis is true. When a p-value is less than 0.05 the null hypothesis is rejected.

Table 0.1: Short definitions and categorization of 3D microtextural parameters. For further explanation see Purnell et al. (2013) and ISO 25178-2 (International Orgnisation for Standardization 2012).

parameter	unit	definition	
Sq	µm	Root-Mean-Square height of surface	height
Sp	µm	Maximum peak height of surface	height
Sv	µm	Maximum valley depth of surface	height
Sz	µm	Maximum height of surface	height
Sa	µm	Average height of surface	height
Ssk	-	Skewness of height distribution of surface	height
Sku	-	Kurtosis of height distribution of surface	height
S5z	µm	10 point height of surface	feature
Sdq	-	Root mean square gradient of the surface	hybrid
Sdr	%	Developed interfacial area ratio	hybrid
Sds	$1/mm^2$	Density of summits. Number of summits per unit area making up the surface	hybrid
Ssc	1/µm	Mean summit curvature for peak structures	
Sk	µm	Core roughness depth, height of the core material	material ratio
Spk	µm	Mean height of the peaks above the core material	material ratio
Svk	µm	Mean depth of the valleys below the core material	material ratio
Smr1	%	Surface bearing area ratio (the proportion of the surface which consists of peaks above the core material)	material ratio
Smr2	%	Surface bearing area ratio (the proportion of the surface which would carry the load)	material ratio
Vmp	$µm^3/mm^2$	Material volume of the peaks of the surface	volume
Vmc	$µm^3/mm^2$	Material volume of the core of the surface	volume
Vvc	$µm^3/mm^2$	Void volume of the core of the surface	volume
Vvv	$µm^3/mm^2$	Void volume of the valleys of the surface	volume
Sal	mm	Auto correlation length. Horizontal distance of the auto correlation function (ACF) which has the fastest decay to the value 0.2. Large value: surface dominated by low frequencies. Small value: surface dominated by high frequencies.	spatial

Str	-	Texture aspect ratio (values range 0-1). Ratio from the distance with the fastest to the distance with the slowest decay of the ACF to the value. 0.2-0.3: surface has a strong directional structure. > 0.5: surface has rather uniform texture.	spatial

1.0 Introduction

Current theories of evolution state that for a species, or a group of animals, to survive they must adapt to ever changing environments and/or sexually selected pressures[1]. In recent years selection pressures on elasmobranchs have changed so rapidly that many species are facing extinction. If conservation measures are to be successful, it is important to understand each species' dietary ecology and how elasmobranchs responded to biotic crises in the past.

Elasmobranchs(Sharks, rays and skates) evolved during the Late Ordovician/ early Silurian. They diversified and radiated rapidly after the end Devonian mass extinction to become the dominant oceanic predators in the Carboniferous[2-4]. Since this time elasmobranch dominance in the oceans has fluctuated[4]. Modern sharks, selachimorphs, evolved during the early Jurassic, living alongside hybodont sharks for nearly 140 million years. By the Late Cretaceous, nearly all selachimorph (shark) genera, alive today, had evolved[2].

Having successfully navigated the last 400 million years, elasmobranchs are now showing population declines[5-6]. In the last 35 years some species have recorded population declines as high as 99%[5]. Many species are now identified as endangered and many others too rare to classify. This is particularly concerning given the documented importance of elasmobranchs within marine ecosystems. As elasmobranch populations decline, the effects are being recorded in lower trophic levels, which are having knock-on impacts to fisheries and other industries that rely on the ocean[5-6]. To counter these declines, various conservation measures are being conducted, such as the creation of marine reserves.

For conservation measures to be effective, it is necessary to understand the ecology of the species being conserved. For a majority of selachimorphs species very little is known of their ecology. Data on dietary preference is particularly elusive for many species. The migratory nature of many selachimorphs poses additional problems. In most cases it is not possible to create a marine reserve that protects the entire range of the species in

question. It is possible, however, to protect important, non-migratory, prey types and fundamental locations such as feeding grounds and nursery areas. Marine reserves protecting key prey types can only be effective, however, if prey species are known.

Determining the diet of species has been a topic of interest for decades. Within elasmobranchs, dietary defining techniques usually take the form of stomach contents analyses, observation and, more recently, isotopic analyses. Within fossil elasmobranchs, the previous techniques are impractical. As a result, tooth morphology and evidence of predation, typically on bones, are more commonly used to assign diet. Each of these methods has certain limitations which render them inappropriate in the study of some species. This thesis proposes two new dietary defining techniques, applied to elasmobranchs for the first time, and discusses some of the considerations required in the process of application. Each of these methods has the potential to be powerful dietary discriminators for elasmobranchs, overcoming many of the limitations posed by traditional techniques.

1.1 Aims and objectives

This project aimed to investigate two alternative measures of dietary discrimination to be used on elasmobranchs. These measures are microtextural analyses of tooth surfaces and meso-style wear on teeth.

This shall be achieved by completing the following objectives:

- Investigating mesowear techniques and applying these to modern elasmobranchs (Chapter 2).
- Investigating the use of microtextural analyses as a tool for dietary discrimination in elasmobranchs through the application of techniques to *Carcharias taurus* (Rafinesque, 1810) individuals (Chapter 3).
- Investigation of taphonomic impacts upon the dietary defining tooth microtextures of elasmobranchs (Chapter 4).
- Investigating the use of microtextural analyses as a tool for dietary discrimination in fossil elasmobranchs, through the application of

techniques to the investigation of dietary competition between *Carcharocles megalodon* (Agassiz,1843) and *Carcharodon carcharias* (Linneaus, 1758) (Chapter 5).

1.2 Background literature

1.2.1 Classic methods of dietary discrimination in elasmobranchs

Determining the dietary preferences of species and individuals has always interested scientists. Dietary defining techniques for elasmobranchs, usually involve one of the following: stomach contents analyses, observation and, more recently, isotopic analyses. Fossil elasmobranchs pose more of a problem, with many of the above techniques proving impractical. As a result, for extinct species, tooth morphology and evidence of predation, typically on bones, are more commonly used to assign diet. Each of the above methods have strengths and weaknesses, which make them more or less applicable to different elasmobranch species and situations.

1.2.1.1 Observation

Observation of feeding is commonly adopted when studying diet in extant elasmobranchs. This method is particularly common for those species that feed close to the shore[7-8]. There are two particularly significant drawbacks of this method however. Firstly, the ocean is a very large area, it is thus impossible to know whether the observations made are representative of all feeding activity for that species. As an example, *Carcharodon carcharias* is well known for its recorded attacks on seals which occur near the coast and are easily observable[7]. These observations do not however provide explanation for the consumption of other elasmobranchs, birds and teleosts known to form part of this species diet (Appendix 8.1.1.7). In addition to the possibility of missing certain behaviours, there is also documented evidence that behaviours alter with the presence of human observers[161]. Dietary preference of *Heterodontus portjacksoni* determined through observation[9] does not tally with the dietary preference obtained from stomach contents[10].

1.2.1.2 Stomach contents

Analysis of stomach contents is currently the standard approach to understanding diet in elasmobranchs. Whilst it has been used to successfully determine diet in many elasmobranch species[10-18], the method does have drawbacks. Firstly, this form of analysis only provides a snap-shot view into the diet of a captured individual; it does not provide a comprehensive view of the individual's diet[19]. This in itself creates biases, as the last meal of an individual caught is hugely dependent upon season[16, 20], gastric acid secretion[21-22] and migratory patterns of both predator and prey[11].

There is also scientific evidence that hungry elasmobranchs, with empty stomachs, are more likely to be caught[23-24]. This leads to a large number of elasmobranchs being captured that cannot provide data on diet for the species[21-24]. Elasmobranchs also possess the ability to evert their stomachs. During the stress of capture, many elasmobranch species will evert the contents of their stomachs. This exacerbates the problem of individuals being caught with empty stomachs. The consequence of empty stomach captures and stomach eviction is the sacrifice of large numbers of individuals in order to understand the diet of the species[21-24].

Finally, stomach contents analysis is only applicable to extant species. Fossilisation of stomach contents is extremely rare, providing little insight into the diets of extinct elasmobranchs.

As a result, stomach contents analysis is unsuitable as a tool for understanding alteration to feeding strategies during biotic crises in the fossil record. It is also unsuitable in the study of dietary discrimination of rare and endangered species.

1.2.1.3 Isotopes

The use of isotopic analyses in the study of dietary discrimination of elasmobranchs is being increasingly implemented. Trophic level and foraging location can be determined through the study of the stable isotopes

$\delta^{13}C$ and $\delta^{15}N$. Isotopic signals accumulate over long time periods within the tissues of elasmobranchs. This method thus avoids the problems of "snap-shot" diet experienced by stomach contents analyses. The method can also be non-lethal, so can safely be used on endangered species. Isotopic analyses do not however, record actual diet[25-27]. This makes comparison of dietary preferences between individuals and species difficult unless the isotopic composition of feed items in the immediate habitat is known. In addition to this, multiple dietary combinations can yield the same $\delta^{13}C$ and $\delta^{15}N$ values, again making comparison difficult[25-27]. At present it is necessary to combine stable isotope analyses with stomach contents analyses to generate an accurate measure of diet for each species.

1.2.1.4 Tooth morphology and bite marks

Within the fossil record tooth morphology is commonly used to determine the diet of extinct species[28-29]. This method typically produces a broad picture of dietary habit within a species; it is incapable however of recording geographical or ontogenetic differences within a species which are commonly observed in elasmobranchs today. Individuals of the same

Figure 1.1: representative dental morphologies of the species *Carcharodon carcharias, Galeocerdo cuvier, Carcharhinus leucas* and *Prionace glauca.* A) Anterior tooth from a 5.18m *C. carcharias*. The tooth is broad, cuspate and possesses a coarse, serrated cutting edge. Cusp height (tooth tip to parallel lowest tooth/root junction) is 35 mm. B) tooth originates from a 3.0m *G. cuvier* individual. The tooth displays a strongly recurved cusp and very coarse serrations along the cutting edges. Cusp height is 14mm C) Tooth belongs to a 2.0m *C. leucas* individual. The tooth is broad and cuspate with strongly serrated cutting edges and mild inflections half way up each cutting edge. Cusp height is estimated at 14mm.D) tooth from a 2.5m *P. glauca.* The tooth displays a cuspate morphology with an inflection along the distal edge and a coarsely serrated cutting edge. Cusp height is approximately 11mm.

species can have identical tooth morphology but consume a different diet. In addition to this, it is observed in modern elasmobranchs that the same diet can be consumed by species with very different dental morphologies. *Carcharodon carcharias* (Appendix 8.1.1.7)*, Galeocerdo cuvier* (Appendix 8.1.1.8) *and Carcharhinus leucas* (Appendix 8.1.1.3), are a good example of this (Figure 1.1 A-C). The largest individuals of these species are all documented to consume a similar diet [30-32], yet their dental morphology is quite different. The singular similarity in dental morphology between each of these species is the presence of a serrated cutting edge. This feature is not exclusive to individuals with this diet as *Prionace glauca* also possesses a serrated cutting edge but consumes a very different diet[33] (Appendix 8.1.1.10, Figure 1.4D).

Due to the known variation in diet between individuals of an extant elasmobranch species, and the lack of correlation between tooth morphology and dietary preference, it is unwise to use tooth morphology as a dietary discriminator in extinct elasmobranchs.

Bite marks preserved on fossilised mineralised tissues have been used to interpret diet of extinct species [35, 36], see Figure 1.2. Bite marks can be compared to the teeth of predatory species known to be residing in the environment at the time of attack.

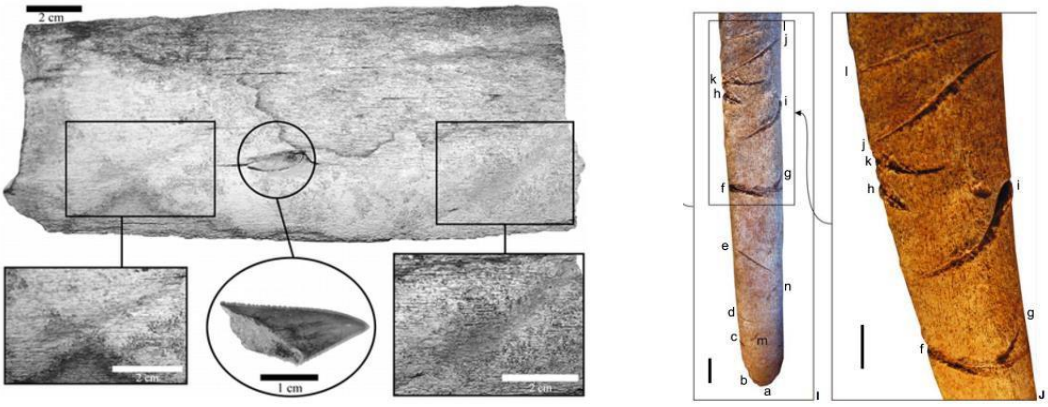


Figure 1.2: Examples of bite marks derived from existing literature [35, 36], inflicted by elasmobranchs, on the bones of marine mammals. Image on left: Mysticete mandible with white shark (*Carcharodon sp*.) tooth (MUSM 1470). The tooth is figured at centre. Boxes on the left and right show tooth scrapes[35]. Image on right: *Isurus hastalis* bite marks on the 6th right rib of the *Astadelphis gastaldii* skeleton (MGPT PU13884). Scale bars represent 100 mm for the complete ribs and 50 mm for the details[36].

From this, a snap shot view into the past can be achieved[34]. However, much like observation studies and stomach contents analyses, these insights do not provide a full description of diet for a species. The rarity of such finds prevents the generation of a comprehensive dietary analysis for any species. It is not possible to tell from bite marks whether they were the result of predation or scavenging, or indeed whether the prey item was a regular contributor to the diet of the species.

1.2.1.5 Implications of using traditional methods for dietary analysis in elasmobranchs

If conservation measures of modern elasmobranchs are to be successful, it is essential that diet is quantified and understood for each species. Given the successful nature of elasmobranchs in the fossil record it is also important to understand how elasmobranchs have shifted their diet in periods of biotic crisis in the past. It is likely that modern elasmobranchs might adopt similar dietary shifts. If this is understood, then conservation measures can encapsulate current dietary preferences as well as those likely in the future. With present methods of dietary analysis, this information is not forthcoming. For many species the use of stomach contents analyses, observation and isotopic analyses are impractical, and thus diet is unknown. It is necessary therefore, to develop alternative methods for dietary discrimination in elasmobranchs to help fill this knowledge gap.

1.2.2 Alternative methods of dietary discrimination

Analysis of tooth wear is becoming a popular non-lethal tool in the discrimination of diet for a wide variety of animal groups [37,-57]. Analysis of tooth wear can be conducted on a variety of scales, providing differing levels of separation both within and between species [37, 50, 57]. This is the first study to test tooth wear techniques on elasmobranchs, proving that these techniques can be used successfully to determine diet in this group.

1.2.2.1 Mesowear as a tool for dietary discrimination

Mesowear is the analysis of dental damage caused by the different physical properties of food items. Since the first study in 2000[37], mesowear studies have grown to encompass dietary niche analysis in almost all broad groupings of extant ungulates (hoofed mammals) as well as several extinct lineages[37-44]. They have also provided insight into environments our hominin ancestors inhabited[38]. The method classifies the gross wear to the cusps of a homologous tooth within the jaw of several individuals of a species. Percentage occurrence of each classification provides insight into the diet of the species. Classification of the cusp falls into two broad categories; cusp relief (classified as high or low) and cusp shape in the buccal view (classified as sharp, rounded or blunt). Mesowear in ungulates has proven to be a very powerful tool in the determination of diet and environment, both for extinct and extant species.

More recently Purnell and Jones[45] have adapted the classic mesowear methodology to investigate meso-style wear on conodont elements. Wear was classified in two regions on the blade and the platform of the conodont elements. On the blade, wear was classified as breakage, spalling, rounding and polishing. On the platform, wear was classified as polishing to the highs, polishing to the lows, and blunting.

This later methodology has the ability to be adapted and applied to elasmobranchs as it does not rely on the analysis of a homologous facet, or asingular tooth morphology. This method can encompass potential biases in tooth shape, position within the jaw and sample size[45]. Additionally, tooth wear accumulates over longer time frames generating a more accurate picture of individual diet, and avoiding the snap shot problem of stomach contents analyses[46]. As all individuals record tooth wear, sample sizes required for accurate dietary discrimination are smaller. The techniques can also be applied to dried museum specimens and fossil material. Meso-style wear analysis could provide a more

efficient approach to understanding dietary niches than stomach contents analysis.

1.2.2.2 Microwear as a tool for dietary discrimination

Microwear analyses investigate the small scale wear patterns produced on tooth surfaces as a result of feeding activities. Wear of this type is observed with the use of a high powered microscope, such as a SEM or IFM. Microwear analyses try to quantify wear features observed on a tooth surface, such as pits and scratches. The proportions and abundance of such features can then inform dietary preferences and partitioning within and between species (Figure 1.3 displays some sample images from microwear studies).

2D Microwear has been used to distinguish diets in a wide variety of organisms, including human ancestors[46-54], dinosaurs[55-56] and moles[57].

All 2D microwear studies share the same fundamental flaws. Depending on contrast and resolution settings, different levels of microwear may be apparent within the SEM image[52,58]. Secondly there is a large observer bias recorded within microwear studies. Purnell et al.[58] demonstrate that whilst observer effects are nonsignificant

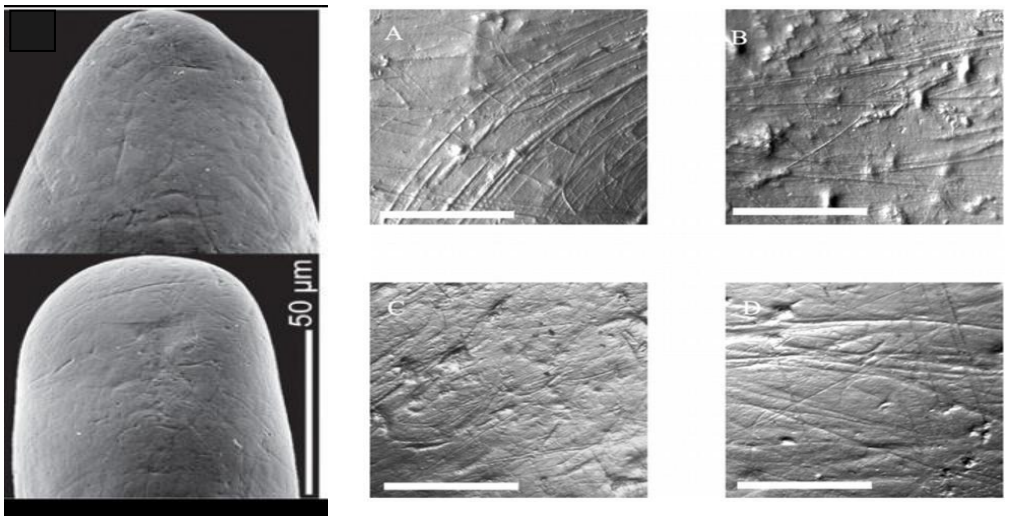


Figure 1.3 Tooth microwear images taken using SEM, sourced from existing literature [59, 100]. Left: surface wear to three spine stickleback fish (*Gasterosteus* aculeatus)[59]. Centre and right: Digitized photographs of phase I facet 3 in different taxa; scale bars = 300 µm. (A) *Gorilla gorilla gorilla*.(B) *Pongo pygmaeus*. (C) *Papio hamadryas hamadryas*. (D) *Ouranopithecus macedoniensi*[60].

between images investigated by a single individual, they become very significant when numerous operators investigate the same image. This indicates that only data collected by one operator is comparable, and thus not very practical.

1.2.2.3 Microtextural analysis as a tool for dietary discrimination

3D microtextural analyses are an improvement and advancement of 2D microwear techniques. 3D Microtextural analysis solves the problems of image generation and observer error, as it uses focus variation microscopy to generate 3D data point clouds of the tooth surface (Figure 1.4 displays example images from a 3D microtextural study [61]). These can then be utilised to measure surface roughness, which has been shown to be superior to counting pits and scratches from SEM

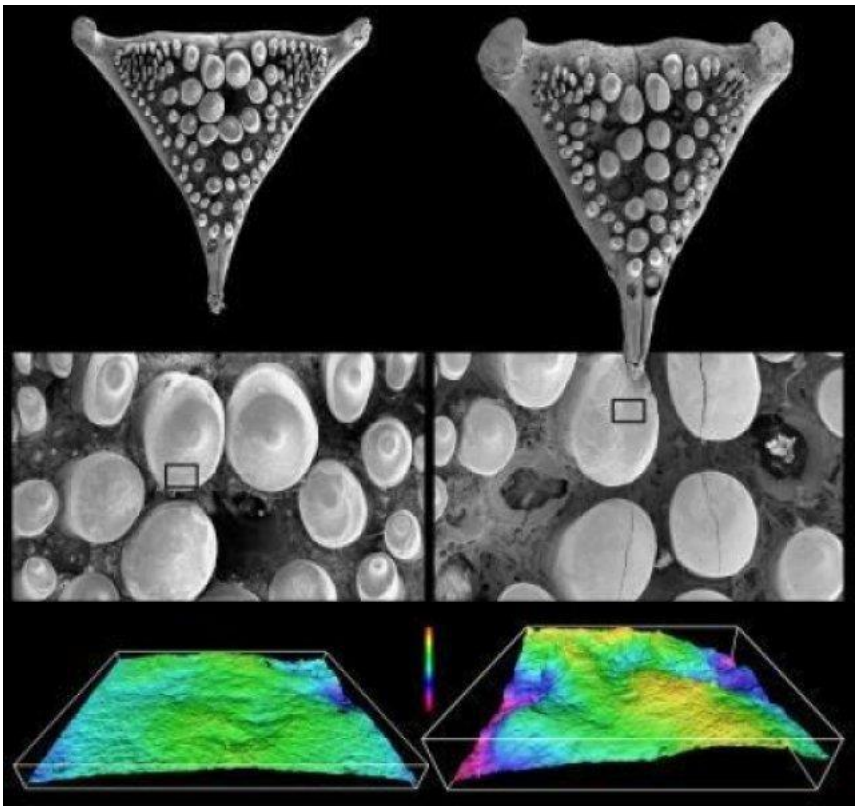


Figure 1.4: Image obtained from published literature[61]. The “throat teeth” of the cichlid fish *Astatorechromis allaudi*. The coloured images at the bottom show contoured surfaces (140µm across) showing the different roughness between cichlids that eat hard food (right) and those that do not (left)[61].

images. Due to standardised protocols in 3D microtextural analysis it is possible to compare results between different teeth of the same species, between different species and different animal groups, even when each data set is obtained through alternative operators[52, 58]. As with mesowear analyses, wear textures accumulate over longer periods of time, generating a more complete image of diet of the individual, and avoiding the snap shot problem of stomach contents analyses. Smaller sample sizes are thus required to generate an accurate view of the individuals and species diet. In contrast to mesowear techniques, the level of separation observed, both between and within species, provides a finer scale image of dietary preference. Thus, geographical and ontogenetic differences in diet can easily be detected.

Although untested in elasmobranchs, 3D microtextural analysis has been documented successfully in other fish genera, both extant and extinct[61-62]. Like meso-style analysis of elasmobranch teeth, 3D microtextural analysis has the potential to determine the dietary preferences of elasmobranchs in a more efficient, non-lethal manner to traditional dietary discriminating techniques; providing more complete overview of diet and dietary shifts both in extinct and extant species.

1.2.2.4 Potential downfalls and considerations

As with all studies there are drawbacks to different methodologies as it is not always possible to apply the methods or constrain all variables appropriately. The same is true of wear analyses. There are four issues relating to the methodologies and the application of the methodologies to elasmobranchs that need to be considered. For each however, careful testing and sampling strategies can help to minimise/ eliminate these issues.

- Many elasmobranchs are migratory, and thus are likely to have a varying diet at different points of their life cycle and stages of migration[63]. In order to understand the diet of the species it is

important to generate an inclusive sampling strategy that includes individuals of both genders, all ontogenetic stages and of varying geographical locations.

- Tooth position can potentially influence the level of food which comes into contact with the teeth[61, 64-65], and thus has the potential to skew data within both selachimorphs and extinct elasmobranchs. If investigating an entire jaw of an individual this can be overcome through careful sampling. In extant species the same tooth location in the jaw can be analysed, e.g. anterior only teeth. Much of the fossil record, however, is isolated teeth. Often there is no way to tell where in the jaw a tooth originated. This can result in a distortion of dietary preference displayed by a species. Although most teeth in the fossil record will have undergone a full wear cycle, and will preserve a good dietary signal.
- Tooth replacement is well documented in elasmobranchs. Length of time within the mouth creates biases towards the levels of wear obtained on the tooth. Elasmobranchs shed their teeth, but at different rates[66-68] and not all extinct species were capable of tooth replacement. This makes comparison between species problematic, as differential wear levels can accumulate for the same dietary preferences.
- Finally, taphonomic processes acting upon teeth, post-shedding, have the potential to alter meso-style wear and 3D microtextural analyses. Such processes have been shown to obliterate wear dietary signals detected by 2D microwear techniques[69-72].

Using careful sampling strategies it is possible to understand the impact of each of these issues in extant elasmobranchs, and provide assumptions and margins of error that can be applied to data collected from fossilised teeth.

1.2.3 Summary

3D Microtextural analysis and meso-style wear analysis ultimately measure two very different aspects of tooth wear, and are thus influenced by separate factors and biases. Meso-style wear is biased towards gross tooth

morphology. For example, a serrated tooth is more likely to exhibit wear in the mid-section of the tooth, than a non-serrated or molariform tooth, simply because it has features residing in this location. Different tooth morphologies are also liable to enhance the probability of meso-style wear in certain regions; a curved tooth is less likely to exhibit wear upon the inside edge as it is more “protected”.

3D Microtextural analysis provides a solution to these biases as it focuses on a small, micro-meter scale region of the tooth. However 3D microtextural analysis does have certain issues, which are not affected by meso-style wear analysis. 3D microtextural analysis is more inclined to be influenced by taphonomic processes, post-shedding, than meso-style wear. Whilst sediment abrasion may affect certain aspects of meso-style wear, not all features will be affected. On the other hand sediments have been shown to obliterate microwear[68-72], and thus are likely to impact upon 3D microtextural analysis as well.

Investigation into both techniques needs to be conducted in order to determine the best approach for both extinct and extant elasmobranchs. It is highly probable that both methods are necessary to obtain an accurate measure of diet.

2.0 Meso-style wear as a tool for dietary discrimination in elasmobranchs

Elasmobranchs are a globally successful marine vertebrate group, yet in recent years large population declines have been observed. This has knock on effects to lower trophic levels, impacting a wide range of species and human activities. Attempts to conserve and rebuild these declining population, relies on good ecological knowledge of species. Unfortunately, for most elasmobranchs information as simple as dietary preference is unknown. This reflects the difficulties faced when using current methods of dietary analysis. Here I show that methods, adapted from Purnell and Jones[45], can be applied to elasmobranchs to provide a new tool for dietary discrimination. Meso-style wear provides a measure of gross tooth wear across the upper jaw of each individual. All individuals analysed present a dietary signal, including dried museum specimens. As such, fewer individuals are required to provide insight into diet. This is especially relevant in the conservation of rare and endangered species. In addition to this, as wear accumulates over longer time frames, meso-style wear avoids the “snapshot bias” of stomach contents analyses. Variation in wear pattern reflects differences in dietary preference. Dietary differences have been documented both between and within species, showing that the method is capable of determining ontogenetic and geographical differences.

2.1 Introduction

Elasmobranchs are arguably one of the most successful marine vertebrate groups. During their 400 million year evolution they have been abundant and influential in the ecosystems they have inhabited. In recent years however, large declines in large elasmobranch populations have been recorded[6,73]. Some species, including *Carcharhinus leucas* (Müller & Henle, 1839), *Carcharhinus obscurus* (Lesueur, 1818) and *Sphyrna zygaena* (Linnaeus, 1758), have declined by up to 99% in the past 35 years[5]. Many species are now identified as endangered and many more are too rare to

categorise. Despite this, elasmobranchs still have a global distribution, inhabiting nearly all marine habitats and ecological niches. Their roles in marine ecosystems are acknowledged as the impacts of declining populations are being felt[15, 74]. Conservation measures, such as marine reserves, are being increasingly suggested to help conserve and rebuild many elasmobranch populations[74-76].

To do this effectively we need to know a species ecology, including dietary habits. The reality is that very little is known about a majority of elasmobranch species. Information relating to dietary preferences, in particular, is a mystery for most [77-79]. The current method of dietary analysis adopted by scientists studying elasmobranchs is stomach contents analysis. Whilst the method has been used to successfully determine diet in many elasmobranchs[10-18], it does have limitations[79]. Primarily, stomach contents analysis only provides a snapshot view into the dietary preferences of the individual. It does not provide a comprehensive view of the individual's diet[19]. There is also scientific evidence that hungry sharks are more likely to be caught, and thus the number of sharks caught with empty stomachs is high[25, 80-81]. This means that for stomach contents analyses to be comprehensive, a large number of sharks need to be culled[82]. Exacerbating this problem is the ability of elasmobranchs to empty their stomachs when distressed[212]. For stomach contents analyses to be reliable and in order to produce an accurate dietary picture, a large number of individuals need to be captured over a long timeframe. As a result, this method is not suitable for rarely caught and endangered species.

Recently, isotopic analyses have been used to gain insight into a species diet. Stable isotopes, particularly $\delta^{13}C$ and $\delta^{15}N$, can be used to estimate foraging location and trophic position within a food web[25]. The method avoids the problem of snapshot views and can be non-lethal, but it does not record actual diet[25, 83-84]. This makes comparison of individuals and populations difficult unless the isotopic composition of food items, in their respective tropic web, has been characterised. In addition to this, multiple dietary combinations can yield the same $\delta^{13}C$ and $\delta^{15}N$ values[25-27], making it necessary to combine isotopic and stomach contents analyses to generate an accurate dietary measure.

Meso-style wear provides an additional, potentially non-lethal, method for dietary discrimination in elasmobranchs. Mesowear is a method widely applied to terrestrial mammals to detect dietary differences between individuals and populations[85-86]. The method has never been applied to elasmobranchs. We present here the first evidence that meso-style wear varies with diet in modern elasmobranchs.

Meso-style wear methods are an adaptation of the mesowear methods used on terrestrial mammals, where analysis of gross wear (removal/ change in volume/shape of the tooth) is conducted on the occlusal surfaces of the m2 molar in the upper jaw[37]. Although the entire surface of a tooth is affected by wear, mesowear analysis focuses on the buccal cutting edge of the enamel surface where the ectoloph meets the occlusal plane. Wear to this region is defined by two variables; cusp relief (high or low) and cusp shape (sharp, rounded or blunt)[37, 85]. Mesowear methods stem from the assumption that rougher, harder and tougher foods will produce a greater level of wear to the tooth surface than a softer food. This method is not applicable to elasmobranchs. Tooth variation combined with the high rates of tooth replacement; require alteration of the mesowear methods to take these factors into account. The proposed meso-style wear method, is still a scoring method that measures the level of wear observed on the tooth surface using a standard binocular microscope, but is conducted across the entire upper jaw.

Meso-style wear methods have several advantages over traditional methods of dietary analysis. Wear levels accumulate over long periods of time thus avoiding the snap-shot issues associated with stomach contents analyses[37]. The methods are capable of detecting subtle dietary differences between individuals and populations, even with small sample sizes[85]. The method can also be used to assess the diet of extinct species where traditional methods are not practical[85]. In contrast to isotopic analyses, meso-style wear methods also provide an indication of broad prey preference (teleost, elasmobranch, cephalopod etc.) rather than the relative trophic level.

The focus of this study is to test the hypotheses that:

1) Meso-style tooth wear varies with and records diet in elasmobranchs.
2) Meso-style wear reflects ontogenetic shifts in diet. Elasmobranchs are known to display ontogenetic dietary shifts. Ontogenetic stage and diet of an individual can be inferred from literature, using its recorded total body length (Appendices 8.1.1.1-8.1.1.10)

Meso-style wear is a non-destructive approach that collects data from tooth surfaces. As all individuals record a dietary signal, and dried museum specimens and fossils can be utilised, smaller sample sizes are required in comparison to traditional techniques. As a result, the method can be applied to a wide range of situations, including conservation of endangered elasmobranch species and palaeontological investigations into extinct elasmobranchs. If combined with approaches that capture dietary information on differing timescales, it provides the possibility of a strong multiproxy approach to dietary analysis in elasmobranchs.

2.2 Materials and methods

2.2.1 Materials and sampling strategy

To provide baseline data, only species that have a well constrained and studied diet were used. Specimens ranged in age and size, and diet was thus recorded on an individual basis rather than by species. Ontogenetic stage and total body length were used to determine the diet of each individual based on existing literature (Appendices 8.1.1.1- 8.1.1.10). Where ontogenetic stage or length data were unavailable, the individuals' diet was recorded as unknown. Specimens used in this study are recorded in Table 2.1. All were dry specimens that had been cleaned and stored. The entire upper jaw on the labial side was documented, minus the commissural teeth, where possible. Entire jaws were analysed to reduce the impact of newly erupted teeth, which preserved no dietary data, affecting the meso-style wear signals.

To test the hypothesis that meso-style wear can detect ontogenetic shifts in diet, *Carcharias taurus, Carcharhinus plumbeus, Galeocerdo cuvier* and *Carcharodon carcharias* were individually investigated in greater detail. All data was used to test the hypothesis that meso-style wear varied with diet in elasmobranchs.

Specimens were obtained from a range of collections including, the American Museum of Natural History, Florida Museum of Natural History, David Ward's private collections (Orpington, UK) and Gordon Hubbell's private collections (Gainesville, Florida).

Ecological information for each species studied can be found in Appendices 8.1.1.1- 8.1.1.10, and a detailed breakdown of specimens studies can be found in Table 8.3.

2.2.2 Meso-style wear data acquisition

A binocular microscope, equipped with a X30 objective, was used to assess the level of damage to the labial surface of the teeth within the upper jaw of each individual. 36 variables of meso-style wear were recorded on a presence/absence basis. These variables were divided into four broad groups of wear type; breaks, spalls, rounding and general signs of wear (Table 2.2). The location of each wear type was also documented. Each tooth was divided into five broad regions, which could subsequently be combined. These categories were (Figure 2.1):

- Tooth tip- the uppermost third of the tooth. Tooth tip can be combined with either proximal or distal regions to provide a more exact wear location.
- Tooth middle- The mid-third of the tooth. Again the category can be combined with either proximal or distal regions.
- Tooth base- The lowermost third of the tooth. Again combining with either proximal or distal regions can provide a more accurate wear location.
- Proximal- The half of the tooth closest to the jaw symphysis.
- Distal- The half of the tooth furthest from the jaw symphysis.

Table 2.1: Summary of samples used within this study. Diet for each individual is in accordance documented diet from published studies (Appendices 8.1.1.1- 8.1.1.10).

Species	Specimen Number	Diet	Storage Location	Capture information	Individual information
Carcharhinus leucas	79914 SD	Teleost and elasmobranch	AMNH	Belize, 21/08/1985	2.18m male
	89085 SD	Teleost and elasmobranch	AMNH	Virginia 09/06/1987	2.09m male
	89166 SD	Teleost and elasmobranch	AMNH	Florida 11/07/1987	2.0m male
	10200 SD	unknown	AMNH	California, 1996	Unknown
Carcharhinus plumbeus	89068 SD	Teleost, cephalopod, elasmobranch	AMNH	Virginia 09/06/1987	1.98m female
	89131 SD	Teleost, cephalopod, elasmobranch	AMNH	New York 27/06/1987	2.02m female
	89254 SD	Teleost, cephalopod, elasmobranch	AMNH	Hawaii 18/11/1987	1.8m female
	89128 SD	Teleost, cephalopod, elasmobranch	AMNH	New York 27/06/1987	1.78m female
Carcharhinus brevipinna	89155 SD	Teleost, cephalopod	AMNH	Florida 08/07/1987	2.27m female
	88174 SD	Unknown	AMNH	Madagascar 15/07/1988	Unknown
	88175 SD	Unknown	AMNH	Madagascar 15/07/1988	Unknown
	88	Unknown	Ward	Gambia	Unknown
	63	Unknown	Ward	Philippines	Unknown
	274	Unknown	Ward	Philippines	Unknown
Carcharhinus melanopterus	79986 SD	Teleost	AMNH	Philippines 19/05/1987	0.86m male
	79982 SD	Teleost	AMNH	Philippines 19/05/1987	0.65m male
	79979 SD	Teleost	AMNH	Philippines 19/05/1987	0.77m female
Prionace glauca	89126 SD	Teleost, cephalopod	AMNH	New Jersey 19/04/1987	2.37m male
	89229 SD	Teleost, cephalopod	AMNH	Hawaii 02/10/1987	2.49m male
	89132 SD	Teleost, cephalopod	AMNH	New York 27/06/1987	2.16m female
	42154 SD	Unknown	AMNH	New Jersey 14/06/1980	Unknown

Carcharhinus falciformis	89053 SD	Teleost, cephalopod	AMNH	Philippines 19/05/1987	2.28m female
	89064 SD	Unknown	AMNH	Philippines 19/05/1987	Unknown
	89060 SD	Unknown	AMNH	Philippines 19/05/1987	Unknown
	89051 SD	Unknown	AMNH	Philippines 19/05/1987	Unknown
Galeocerdo cuvier	89252 SD	Teleost, cephalopod	AMNH	Hawaii 18/11/1987	1.9m male
	89251 SD	Teleost, cephalopod	AMNH	Hawaii 18/11/1987	1.84m female
	79968 SD	Teleost, cephalopod, marine mammal	AMNH	Mexico 18/07/1986	3.03m male
	59765 SD	Teleost, cephalopod	AMNH	Florida 04/05/1991	2.52m female
	62	Unknown	Ward	Hong Kong 1974	Unknown
	61	Unknown	Ward	Sydney, Australia	Unknown
	60	Unknown	Ward	Sydney Australia	Unknown
Carcharodon carcharias	20105018.011	Teleost, elasmobranch	FLMNH	Unknown	2.37m male
	29905026.02	Teleost, elasmobranch	FLMNH	Florida 05/02/1999	2.27m male
	F11582	Marine Mammal	Hubbell	Unknown	5.18m
	H8993	Marine Mammal	Hubbell	West Australia 04/06/1993	5.18m male
	F83083	Marine Mammal	Hubbell	Unknown	3.96m male
	F26808	Marine mammal	Hubbell	Unknown	3.96m male
	M91683	Marine Mammal	Hubbell	Unknown	5.94m female
Isurus oxyrinchus	10302002.04	Teleost, cehpalopod	FLMNH	Unknown	2.64m
	34	Unknown	Ward	Seychelles	Unknown
	190	Unknown	Ward	Philippines	Unknown
Carcharias taurus	19705007.02	Teleost, elasmobranch	FLMNH	Florida	2.4m
	UF 47900	Teleost, elasmobranch	FLMNH	Florida 03/1981	1.9m
		Teleost, elasmobranch	FLMNH	North Carolina 17/04/1975	2.78m
	27	Unknown	Ward	West Australia	Unknown
	25	Unknown	Ward	Florida	Unknown

It is a combination of the wear categories and locality regions which give rise to the 36 variables that were recorded in this study. Table 2.3 provides a summary of the variables studied.

Wear was recorded for each variable on a presence/ absence basis in a table as displayed in Table 8.2. Presence of a meso-style variable scored a 1, absence scored 0. Each tooth in the upper jaw was analysed, with the exception of the commissural teeth. This enabled a percentage occurrence score to be generated for each variable, for each individual's upper jaw. See equation below.

$$\frac{\textit{Number of teeth in the upper jaw displaying wear defined by a set variable}}{\textit{Number of teeth examined in the upper jaw of the individual}} \; X\,100 \;=\; \textit{Percentage occurrence of a parameter within an individual}$$

2.2.3 Statistical analysis

Meso-style wear data were normally distributed (Shiparo-Wilks tests) allowing for parametric testing in the rest of the analyses. Hypotheses were explored using analysis of variance (ANOVA), pairwise testing (Tukey HSD), correlations (Spearman's rank) and principal components analyses (PCA). Where unequal variance was detected (Bartlett and Levene tests) a Welch ANOVA was used.

Two sets of analyses were conducted to test the hypothesis that meso-style wear varies with diet in elasmobranchs. Initially ANOVA and Tukey HSD tests were used to test for differences in meso-style wear between differing dietary groups (teleost, teleost/elasmobranch, teleost/cephalopod, marine mammal). Parameters found to be significant were then used to conduct a PCA analysis of the data.

The second hypothesis, investigating whether meso-style wear can reflected the ontogenetic dietary shifts known to exist in *C. carcharias, C. plumbeus, G. cuvier* and *C. taurus*, was tested using rank correlations (Spearman's Rank). It was assumed that size is an accurate measure for

ontogenetic stage, and thus an increasing size can be used as a proxy for increased ontogenetic level. Based on literature (Appendices 8.1.1.5- 8.1.1.8) it was also assumed that dietary specialisation is linked to ontogenetic stage. Each individual within a species was ranked by total body length. These were then correlated with PC1 and PC2 values, which were based upon the parameters known to separate diet through ANOVA testing.

To ensure that meso-style wear was separating dietary groupings and not species, further ANOVA, Tukey HSD and PCA were conducted.

All statistical tests were carried out using JMP, version 12 (SAS Institute, Cary, NC, USA).

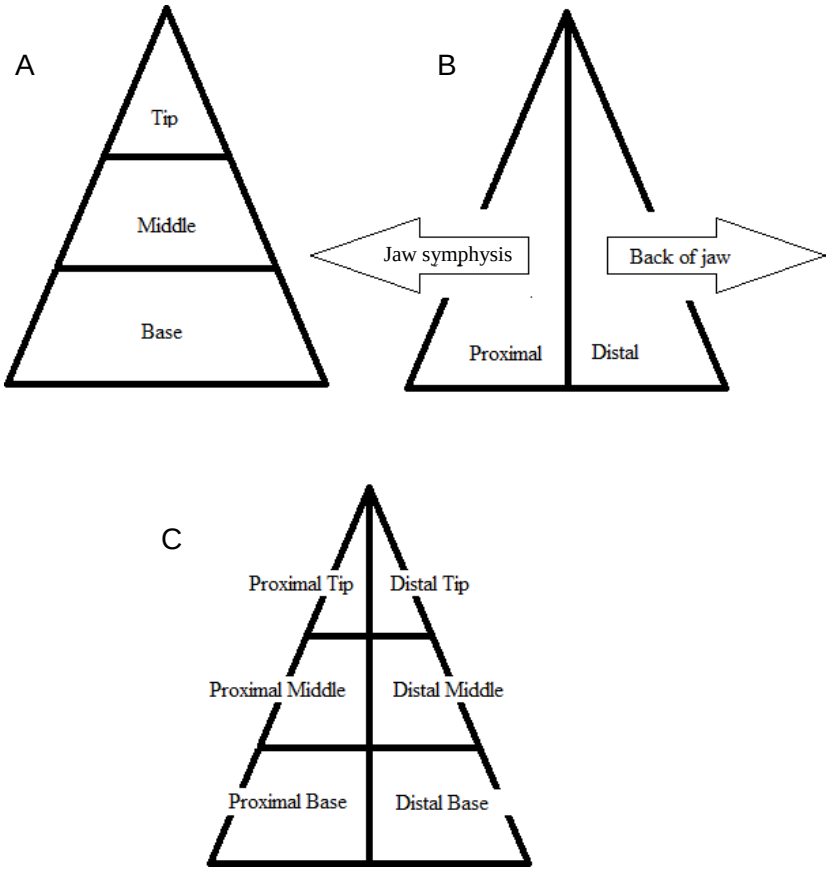


Figure 2.1: Diagrammatic representation of the tooth regions analysed.

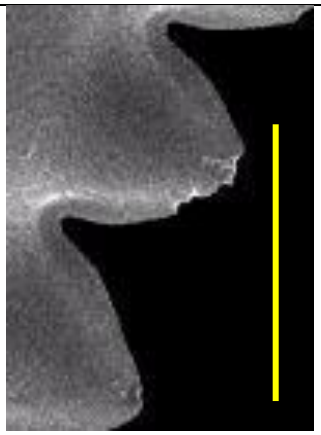
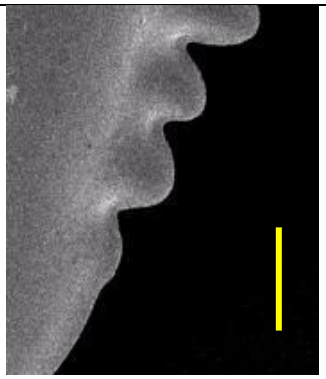
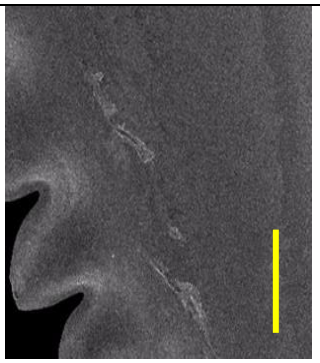
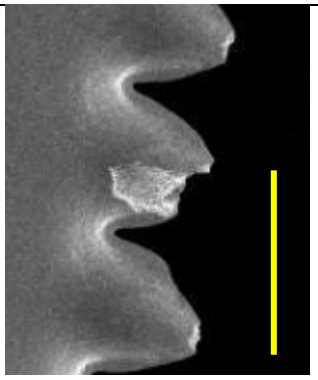
A) indicates the separation of the tooth into 3 sections tip, middle and base, B) indicates the separation of the tooth into the proximal and distal halves, C) displays how these regions come together to create the different wear regions on each tooth.

Table 2.2: Breakdown of the parameters analysed within this study.

Each variable is a combination of a wear type and location category. For example, Variable 4 documents the presence/ absence of breaks that occur on the upper third of the tooth on the proximal side.

Variable Number	Wear Type	Location
1	Breaks	Distal Tip (BDT)
2		Distal Middle (BDM)
3		Distal Base (BDB)
4		Proximal Tip (BPT)
5		Proximal Middle (BPM)
6		Proximal Base (BPB)
7		Tip (BT)
8		Middle (BM)
9		Base(BB)
10	Rounding	Distal Tip (RDT)
11		Distal Middle (RDM)
12		Distal Base (RDB)
13		Proximal Tip (RPT)
14		Proximal Middle (RPM)
15		Proximal Base (RPB)
16		Tip (RT)
17		Middle (RM)
18		Base (RB)
19	Spalls	Distal Tip (SDT)
20		Distal Middle (SDM)
21		Distal Base (SDB)
22		Proximal Tip (SPT)
23		Proximal Middle (SPM)
24		Proximal Base (SPB)
25		Tip (ST)
26		Middle (SM)
27		Base (SB)
28	Other signs of wear	Distal Tip (WDT)
29		Distal Middle (WDM)
30		Distal Base (WDB)
31		Proximal Tip (WPT)
32		Proximal Middle (WPM)
33		Proximal Base (WPB)
34		Tip (WT)
35		Middle (WM)
36		Base (WB)

Table 2.3: Categorization and examples of each wear type analysed on the tooth during the course of this study. All images are from a *Carcharodon carcharias* t ooth. Scale bar in each image represents 1mm.

Wear Type	Description	
Breaks	An area of tooth that is missing, through any means other than having been worn away through use. Typically a break will have a clean sharp edge, however some rounding can occur after the breakage.	
Rounding	The gradual abrasion of the tooth wearing away edges to leave a blunted/ rounded edge in its place.	
Other signs of wear	General term given to the occurrence of any type of wear to the tooth surface and edges that has not been described in this table. Typically this takes the form of scuffs and scratches to the tooth surface.	
Spalls	Peeling of the uppermost enameloid layers. Spalls nearly always coincide with breaks, but breaks are not synonymous with spalls.	

2.3 Results

Results of the ANOVA demonstrate that meso-style wear can be used to determine diet in elasmobranchs. 31 of the 36 variables differ significantly between the dietary groups (Table 2.4), with the greatest number of pairwise differences (Table 2.5) between teleost/ elasmobranch eating individuals and those consuming marine mammals. Pairwise differences can be found between all dietary groupings, except between teleost/elasmobranch and teleost diets, where there is overlap between the smallest individuals that consume a high proportion of teleosts and a small proportion of elasmobranchs.

PCA based on the 31 parameters that differ between diets reveals a clear pattern (Figure 2.2). PC axis 1 is influenced most by the parameters; breaks and spalls to the distal middle, the tooth tip and the tooth middle (Table 8.3) PC1 captures 55.4% of the variance and is strongly correlated with an increased consumption of elasmobranch in the diet (Rs= 0.811414 , p= 0.0469). PC axis 2 is influenced most by the parameters; rounding to the proximal tip, middle and base, to the distal middle and to the tooth middle and base (Table 8.3). PC2 also captures a dietary signal, capturing 22.4% of the variance. PC2 is most strongly correlated with increased consumption of cephalopods in the diet (Rs= 0.445692, p= 0.0177) (Figure 2.2). PC2 also trends with an increased consumption of marine mammals, although this trend is not significant (Rs= 0.709209, P= 0.0735), and the consumption of benthic elasmobranchs (Rs= 0.999382, p= 0.0158) (Figure 2.2). These latter correlations need to be treated with caution as they are based on very small sample sizes.

ANOVA analyses also reveal significant differences between species. All parameters displayed significant differences (Table 2.6). Not all parameters however, displayed Tukey HSD separation (Table 2.7). In many instances, no Tukey separation was found between two species that were ecologically distinct, for example *Carcharias taurus* and *Carcharodon carcharias* or *Carcharhinus brevipinna* and *Galeocerdo cuvier*. PCA analysis using all parameters most effectively separated individuals by diet, not species (Figure 8.11, Table 8.4).

Table 2.4: Results of ANOVA/Welch ANOVA comparing different dietary groups.

Text in bold indicates a significant result. 'w' indicates a Welch ANOVA was used. 31 of the 36 variables display dietary separation.

Variable			F	d.f.	p
Breaks	proximal	tip	**4.0826**	**3, 27**	**0.0163**
		middle	**3.9477**	**3, 27**	**0.0186**
		base	**5.4563**^{w}	**3, 10.049**	**0.0174**
	distal	tip	**3.3963**	**3, 27**	**0.0321**
		middle	**5.4071** ^{w}	**3, 10.049**	**0.0179**
		base	**9.2884** ^{w}	**3, 11.205**	**0.0023**
		tip	**5.1842**	**3, 27**	**0.0059**
		middle	**5.515**	**3, 27**	**0.0044**
		base	**6.4486**	**3, 27**	**0.002**
Rounding	proximal	tip	**47.3149** ^{w}	**3, 11.567**	**<0.0001**
		middle	**35.9217** ^{w}	**3, 12.374**	**<0.0001**
		base	**8.6339**	**3, 27**	**0.0004**
	distal	tip	**10.6978** ^{w}	**3, 11.108**	**0.0013**
		middle	**8.4272** ^{w}	**3, 11.946**	**0.0028**
		base	**9.0805** ^{w}	**3, 14.073**	**0.0013**
		tip	3.6776 ^{w}	3, 9.4624	0.0536
		middle	**37.1616** ^{w}	**3, 12.376**	**<0.0001**
		base	**14.8278** ^{w}	**3, 14.057**	**0.0001**
Other signs of wear	proximal	tip	**4.2489** ^{w}	**3, 8.3005**	**0.0434**
		middle	**13.3015** ^{w}	**3, 9.1383**	**0.0011**
		base	**5.0572**	**3, 27**	**0.0066**
	distal	tip	0.7275	3, 27	0.5445
		middle	2.7794	3, 27	0.0603
		base	**7.9507** ^{w}	**3, 12.082**	**0.0034**
		tip	3.3808 ^{w}	3, 8.2395	0.0729
		middle	**9.7735** ^{w}	**3, 9.1602**	**0.0033**
		base	**6.3565**	**3, 27**	**0.0021**
Spalling	proximal	tip	**4.0211**	**3, 27**	**0.0173**
		middle	**4.3229**	**3, 27**	**0.013**
		base	**6.3297** ^{w}	**3, 10.335**	**0.0105**
	distal	tip	3.3542 ^{w}	3, 10.385	0.0617
		middle	**10.3161** ^{w}	**3, 11.206**	**0.0015**
		base	**8.7707** ^{w}	**3, 11.152**	**0.0029**
		tip	**5.9417**	**3, 27**	**0.003**
		middle	**12.6672** ^{w}	**3, 10.842**	**0.0007**
		base	**8.6015** ^{w}	**3, 10.971**	**0.0032**

Table 2.5: Results of Tukey HSD testing.

Text on the upper right of the table indicates the parameters which display significant ($p<0.05$) separation between the dietary groups. Text in the lower left of the table indicates the total number of parameters which display significant separation between dietary groups.

	Teleost	**Teleost/ Cephalopod**	**Teleost/ Elasmobranch**	**Marine Mammal**
Teleost		WM, WPM, RB, RM, RT, RDM, RDT, RPT, RPM, RPB		SDB,
Teleost/ Cephalopod	10		BPB, BDP, BB, RPT, RPM, RM, SPB, SDB, SB	ST, WB, WM, WT, WDM, WPT, WPM, WPB, RM, RB, RDM, RPM, RPB, BT,
Teleost/ Elasmobranch	0	9		BPT, BPM, BPB, BDM, BDB, BT, BM, BB, WPM, WPB, WDB, WT, WM, WB, SPT, SPM, SPB, SDM, SDB, ST, SM, SB
Marine Mammal	1	14	22	

Projection of individuals where diet is not known onto the PCA based on dietary separation, reveals no individual with a surprising dietary prediction (Figure 8.12, Table 8.7).

Testing of ontogenetic shifts in diet revealed strong trends and correlations within each of the species tested (Figure 2.3). Ranked size, reflecting ontogenetic shifts, of *C. carcharias* individuals displayed a strong correlation with PC2 scores ($Rs= 0.6637$, $P= 0.0256$) and a lack of correlation with PC1 scores. This is in keeping with the ontogenetic dietary shifts in this species from a teleost/elasmobranch diet to a diet dominated by marine mammals. PC2 values increase with ontogeny for this species.

Table 2.6: ANOVA/ Welch ANOVA results testing for significant differences in variable score between species.

Bold text indicates a significant result, 'w' indicates a Welch ANOVA was used. All parameters displayed significant separation between species.

Variable			F	d.f.	p
Breaks	proximal	tip	**3.4263^{w}**	**9, 15.802**	**0.0157**
		middle	**4.6877 ^{w}**	**9, 15.802**	**0.0037**
		base	**3.2505 ^{w}**	**9, 15.875**	**0.0195**
	distal	tip	**4.8047 ^{w}**	**9, 16.145**	**0.0031**
		middle	**4.7284 ^{w}**	**9, 16.01**	**0.0034**
		base	**5.4911 ^{w}**	**9, 16.485**	**0.0014**
		tip	**3.764 ^{w}**	**9, 16.351**	**0.0098**
		middle	**12.255 ^{w}**	**9, 16.286**	**<0.0001**
		base	**4.7501 ^{w}**	**9, 16.349**	**0.0032**
Rounding	proximal	tip	**8.1666 ^{w}**	**9, 16.142**	**0.0002**
		middle	**10.5817**	**9, 44**	**<0.0001**
		base	**19.9463 ^{w}**	**9, 16.169**	**<0.0001**
	distal	tip	**12.7547 ^{w}**	**9, 16.294**	**<0.0001**
		middle	**5.4348**	**9, 44**	**<0.0001**
		base	**26.6128 ^{w}**	**9, 16.119**	**<0.0001**
		tip	**13.4808 ^{w}**	**9, 16.043**	**<0.0001**
		middle	**10.9664**	**9, 44**	**<0.0001**
		base	**17.7007 ^{w}**	**9, 16.184**	**<0.0001**
Other signs of wear	proximal	tip	**6.8308 ^{w}**	**9, 16.376**	**0.0004**
		middle	**13.3921 ^{w}**	**9, 16.253**	**<0.0001**
		base	**9.888 ^{w}**	**9, 16.144**	**<0.0001**
	distal	tip	**3.0569**	**9, 44**	**0.0063**
		middle	**4.1745**	**9, 44**	**0.0006**
		base	**22.3443 ^{w}**	**9, 15.533**	**<0.0001**
		tip	**6.8466**	**9, 44**	**<0.0001**
		middle	**7.2596**	**9, 44**	**<0.0001**
		base	**11.4951 ^{w}**	**9, 15.524**	**<0.0001**
Spalling	proximal	tip	**2.652 ^{w}**	**9, 15.057**	**0.0457**
		middle	**3.4748 ^{w}**	**9, 15.25**	**0.0158**
		base	**2.9265 ^{w}**	**9, 15.812**	**0.0299**
	distal	tip	**5.7393 ^{w}**	**9, 15.415**	**0.0014**
		middle	**3.1728 ^{w}**	**9, 15.37**	**0.0226**
		base	**5.3359 ^{w}**	**9, 14.932**	**0.0023**
		tip	**2.3312**	**9, 44**	**0.0303**
		middle	**9.9191 ^{w}**	**9, 15.762**	**<0.0001**
		base	**5.1214 ^{w}**	**9, 15.914**	**0.0023**

Table 2.7: Results of Tukey HSD testing for significant differences between species. Table displays parameters that differ between species, e.g. RPM, RPB, RDB and RM differ significantly between *C. brevipinna* and *C.carcharias*.

	C. brevipinna	***C. carcharias***	***C. falciformis***	***C. leucas***	***C. melanopterus***	***C. plumbeus***	***C. taurus***	***G. cuvier***	***I. oxyrinchus***	***P. glauca***
C. brevipinna		RPM, RPB, RDB, RM		BM, SDT, SM		BM, SDM, SM	BDB, RPM, RDM, RM, WT		BPT, BDT, BDM, BDB, BM, RPM, RPB, RDM, RDB, RM, RB, WPM, WDM, WT, WM	
C. carcharias	4		RPM, RPB, RDM, RB, WPM, WDM, WDB, WM	RPB		RPM, RM, WPM, WM		RPM, RPB, RM, RB, WPM, WM	BDT, BDM	RPM, RM
C. falciformis	0	8		RPM, RDM, RDB, RM, RB	RPM, RDM, RT, RM		RPT, RPM, RDT, RDM, RT, RM, WPT, WPM, WDM, WT, WM		BDT, RPT, RPM, RPB, RDT, RDM, RDB, RM, RB, WPT, WPM, WPB, WDT, WDM, WDB, WT, WM, WB	RDB, WDB
C. leucas	3	1	5					RPM, RM	WT, WM	
C. melanopterus	0	0	4	0		ST	RPM	RPM, RT, RM, WPM		RPM, RM
C. plumbeus	3	4	0	0	1		RDM, RM, WPT, WPM, WM		RPM, RDM, RM, WPT, WPM, WDM, WT, WM, WB	
C. taurus	5	0	11	0	1	5		RPT, RPM, RT, RM, WPT, WPM, WT, WM	BDT	RPM, RT, RM, WPT, WPM
G. cuvier	0	6	0	2	4	0	8		BDT, RPT, RPM, RPB, RM, RB, WPT, WPM, WPB, WT, WM, WB	
I. oxyrinchus	15	2	18	2	0	9	1	12		BDT, RPT, RPM, RM, WPT, WPM, WT, WM
P. glauca	0	2	2	0	2	0	5	0	8	

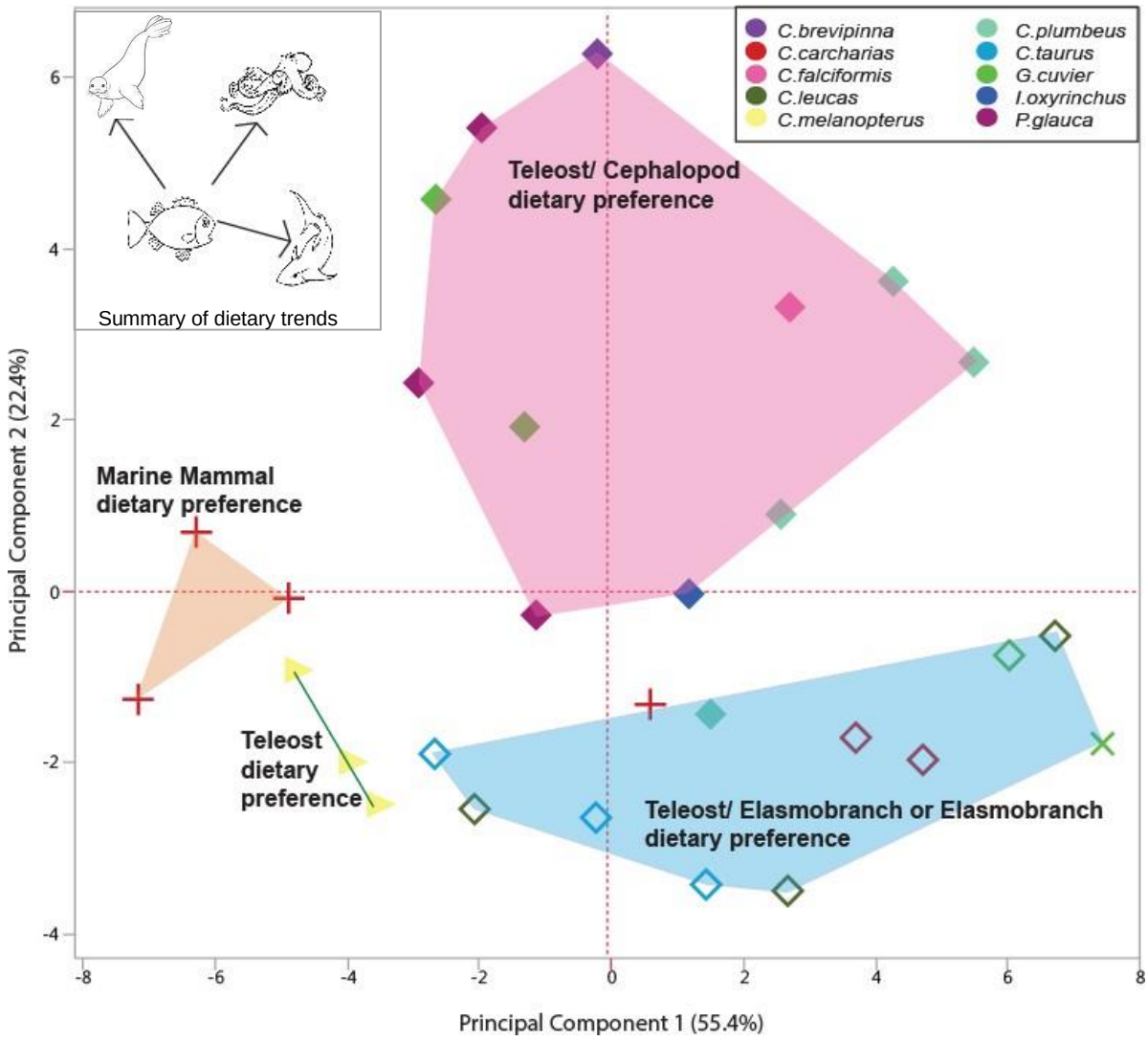


Figure 2.2: Principal component plot, based on the 31 parameters that significantly separate diet, displays dietary separation both within and between species.

An increase in PC1 values indicates an increase in the proportion of elasmobranch in the diet. An increase in PC2 values indicates an increase in the proportion of marine mammal in the diet. An increase in both PC1 and PC2 scores indicates an increase in the proportion of cephalopod in the diet. Representation of these can be seen in the upper left corner of the graph. There are two outliers on the graph, which fall within the teleost/elasmobranch dietary preference convex hull. In both instances, a diet including teleost/elasmobranch is possible for the individual represented.The symbols on the plot relate to the dietary preferences of that species. ◆= Teleost/ Cephalopod diet, ▲= Teleost diet, ◊= Teleost/ Elasmobranch diet, X= Elasmobranch diet, += Marine Mammal/ Teleost diet.

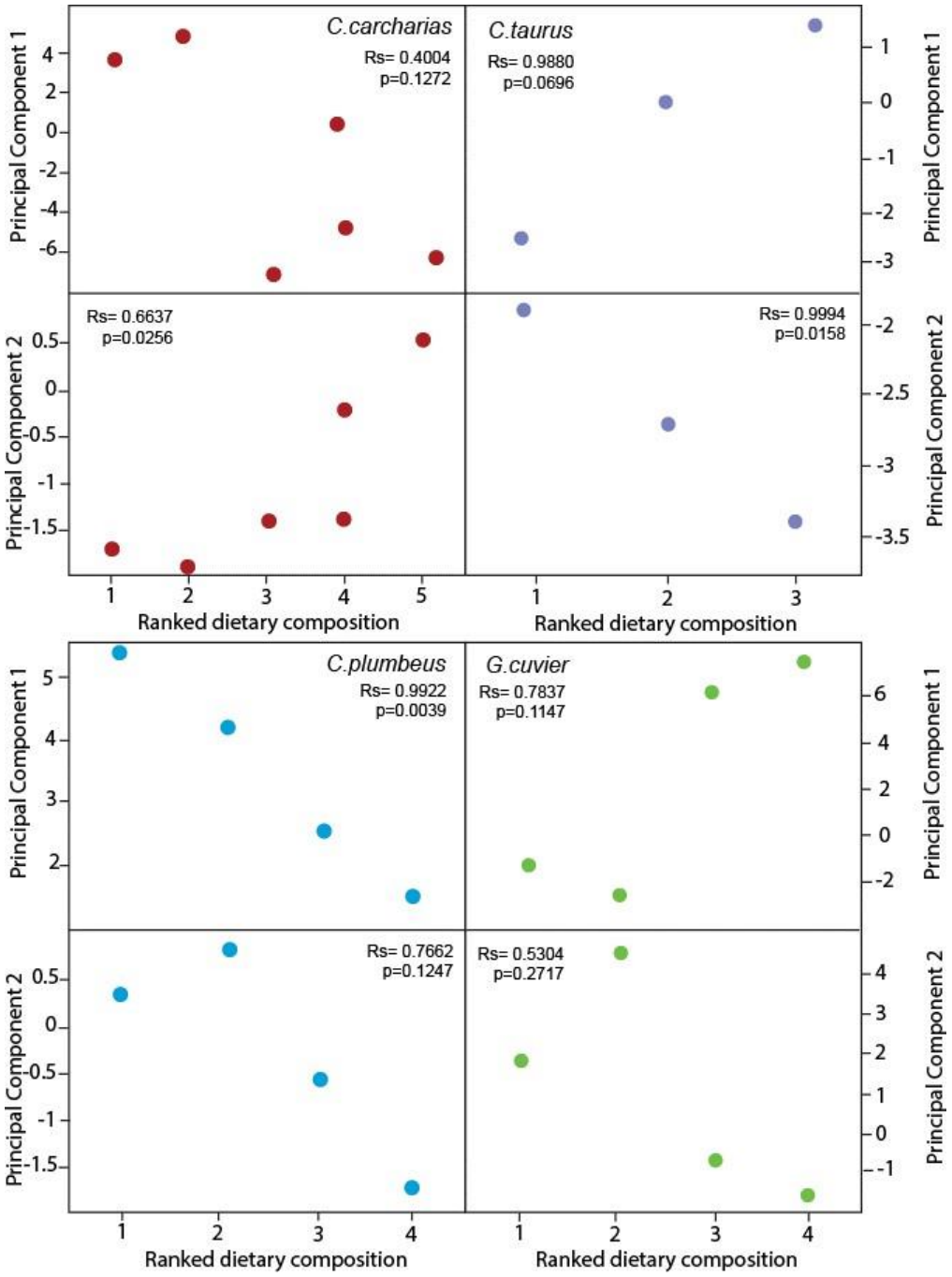


Figure 2.3: Biplots displaying the approximate linear relationships between Principal components values and ranked dietary composition based on ontogenetic stage, for four different elasmobranch species (each a different colour). Ranked dietary composition was calculated through comparison of proportions of teleost and "other" prey in diet. A high rank indicates a small proportion of teleost and a large proportion of an alternative food. Each species displayed has well documented dietary shifts as a result of ontogeny. *C.carcharias* sees an increase in marine mammal with age, *C. taurus* sees an increase in elasmobranch with age, *C. plumbeus* sees an increase in cephalopod with age and *G. cuvier* sees an increase in elasmobranch and occasional marine mammal with age. Correlation of ranked dietary composition by each PC score indicates the strength of the relationship between dietary trends and PC axes. For example PC2 displays a significant correlation with ranked dietary component for *C.carcharias*, but no relationship with PC1. This indicates that increased marine mammal consumption is tracked by increasing PC2 scores, but not PC1.

Analysis of ranked size for *C. plumbeus* reveals strong correlations with PC1 values (Rs= 0.9933, p= 0.0039). This reflects a dietary shift from cephalopod/teleost dominated to one that includes increasingly more elasmobranch items. PC1 values are seen to decrease with ontogenetic level in this species.

Ranked dietary data analysis for *C. taurus* revealed strong trends with PC1 values, however these were not significant (Rs= 0.9880, P= 0.0696). This lack of significance is likely due to small sample sizes. Ranked dietary data for *C. taurus* is also correlated with PC2 values (Rs= 0.9994, P=0.0158). Decreasing PC2 values combined with the strong increasing trends of PC1 values indicates the increased consumption of benthic elasmobranchs within individual's diets with age. This is in keeping with ontogenetic dietary shifts in this species.

Testing of ontogenetic shifts in *G. cuvier* reveals that neither PC axes produce a significant correlation with dietary change in this species. Both axes do display strong trends however, and further samples may produce a significant result. For this species, PC1 values tend to increase with ontogenetic stage, and PC2 values decrease. This reflects the shift in diet from teleost/ cephalopod dominated to one that includes elasmobranchs and a wider variety of prey items. Indeed with this species, as they reach adulthood and increased sizes, the dietary spectrum increases. This would produce a wider variety of meso-style wear values.

2.4 Discussion

The results from this study demonstrate that meso-style wear patterns exhibit a relationship with diet in elasmobranchs. This is the first time that mesowear techniques have been applied to the dietary analysis in elasmobranchs. As such it provides evidence for a new tool in the study of diet in this group.

The strong correlations displayed between PC1, PC2 and diet indicate that meso-style wear patterns are reflecting dietary differences, both within and

between species. A change in the proportion of elasmobranch in the diet tracks along PC axis 1. An increase in PC1 values corresponds to an increase in the consumption of elasmobranch in the diet, both benthic and pelagic. There is also a trend of decreasing PC2 values with the increased consumption of benthic elasmobranchs. An increase in the consumption of marine mammals and cephalopods tracks along PC axis 2. Higher PC2 values equate to an increase of marine mammal in the diet. An increase in PC2 values in combination with an increase in PC1 values, indicate an increased consumption of cephalopod. Low PC1 and PC2 values, indicates a diet dominated by teleosts.

Our analyses indicate that meso-style wear of individuals, for which we have no dietary data, are closely comparable to other individuals of the same species (Figure 8.12, Table 8.7). For example we interpret *Carcharhinus falciformis* individuals to be consuming a diet that ranges from cephalopod dominated with a small input of teleost to a diet of cephalopod and elasmobranch with a small teleost input.

Although ANOVA analyses revealed significant differences between species, this separation does not consistently track with Tukey HSD testing, or indeed is reflected in the Principal Components Analysis. Species with similar diets plot together within the Principal Component axes, creating a spread that reflects dietary change. Some species that are statistically similar track in similar ways, and inhabit the same dietary morphospace. For example *Carcharhinus falciformis* and *Galeocerdo cuvier* are statistically similar, however *G. cuvier* occupies a wider dietary range, in which *C. falciformis* sits. This dietary range is consistent with the dietary ranges of both species. Both species track along a Mid PC1/ High PC2 value to High PC1/ low PC2 value line. Other species, shown to be statistically similar, track differently within the PC axes. For example Tukey HSD testing revealed no separation between *G. cuvier* and *Carcharhinus plumbeus*, yet when analyses with principal components were conducted, species plot trends are quite different. *G. cuvier* displays an increase in PC1 scores and a decrease in PC2 scores with an increase in body size. Yet *C. plumbeus* trends from high PC1/PC2 values to mid PC1/ low PC2 values with an increase in body size. Both species are tracking ontogenetic dietary shifts,

which display some overlap thus accounting for Tukey HSD similarity.

Rank correlations and principal components analyses reveal that meso-style wear is capable of detecting and recording ontogenetic shifts in diet. For all species a range in diet is recorded both in principal components and variable values. The way these values present themselves in principal components axes also trend in a way that tracks diet on an individual species basis. This accounts for overlap between species and the spectrum of results observed.

A large level of variation between teeth within a single jaw was noted, and can be attributed to differential eruption rates. However, as all jaws experience differential eruption rates, this variation is thus a constant between all individuals. By taking an average of the whole jaw the potential issues associated with tooth replacement and unequal levels of wear have been resolved. It is unclear at present whether the techniques can be applied to shed and fossil teeth, which have undergone a full wear cycle. Further work is needed to ascertain the methods comparability with these teeth.

2.5 Conclusions

This is the first time that meso-style wear has been applied to elasmobranchs. Principal components axes reflect proportional changes in prey items within elasmobranch diet. This meso-style wear study provides evidence of a new method for dietary analysis in elasmobranchs. As all captured individuals preserve a dietary signal, fewer individuals are required to determine the diet of a species. Dried specimens from museums and collections can also be used. Due to differential tooth replacement and uneven wear it is necessary to analyse the entire upper jaw of an individual to determine diet. Whilst this does generate some noise in the dataset, it avoids accidental sampling of eruption extremes. The wear patterns build up over a period of time, thus avoiding the snapshot biases of stomach contents analysis. The method could be used as part of a multi-proxy approach to dietary analysis that captures information at different timescales.

3.0 Microtextural analysis as a tool for dietary discrimination in elasmobranchs

As abundant and widespread apex predators, elasmobranchs play influential roles in food-web dynamics of marine communities. This has obvious implications for fisheries management and marine conservation, yet elasmobranch diet is relatively understudied; for the majority of species little or no quantitative dietary data exist. This reflects the difficulties of direct observation of feeding and stomach contents analysis in wild elasmobranchs. Here, by quantifying the 3D surface textures that develop on tooth surfaces as a consequence of feeding, we show that tooth microwear varies with diet in elasmobranchs, providing a new tool for dietary analysis. The technique can be applied to small samples and animals with no stomach contents, and thus offers a way to reduce the impact on wild elasmobranch populations of analysing their dietary ecology, especially relevant in conservation of endangered species. Furthermore, because it accumulates over longer periods of time, analysis of microwear texture overcomes the 'snapshot bias' of stomach contents analysis. It has the potential to be a powerful tool for dietary analysis in extant and extinct elasmobranchs.

3.1 Introduction

As abundant and widespread apex predators, elasmobranchs play influential roles in food-web dynamics of marine communities[77]. This has obvious implications for fisheries management and marine conservation, yet elasmobranch diet is relatively understudied. For the majority of species little or no quantitative dietary data exist (e.g. refs 77-79). Systematic direct observation of wild feeding in large marine predators is difficult, but the difficulty and expense of stomach contents analysis is also a factor in this deficiency of dietary data. Problems relating to stomach contents analysis are a consequence of the large numbers of samples required for robust analysis (e.g. ref 87) and the difficulties in obtaining this data for many elasmobranchs[79]. Furthermore, stomach contents provide only a 'snapshot' view of diet over the

few hours prior to capture[19], and are subject to other inherent biases including: elevated counts of prey with “hard parts” in relation to those without; elevated capture rates of actively foraging (‘hungry’) individuals; and distress causing elasmobranchs to invert their stomachs before they can be analysed[25, 79, 80-81]. Because large sample sizes are required, stomach contents analysis based on lethal sampling can also be problematic for species with threatened conservation status[82].

More recently, additional methods have been employed to examine diet in elasmobranchs. Stable isotopes, particularly analyses of $\partial^{13}C$ or $\partial^{15}N$, can be used to estimate an organism’s foraging location and trophic position relative to that of others in the same food web. The approach avoids many of the pitfalls of stomach contents analysis, as it provides time integrated dietary information based on assimilated biomass[25] and can also be non-lethal and minimally invasive. It is not, however, without methodological limitations[83, 84]. Stable isotope analysis provides only a measure of the relative trophic position of a species within a specific trophic web, rather than actual diet. This makes comparison of individuals or populations from geographically distant areas difficult, unless the isotopic composition of food items in their respective trophic webs has been characterised. In addition to this, multiple dietary combinations can result in the same $\partial^{13}C$ and $\partial^{15}N$ values[25-27]. Genetic tools to identify specific prey species from stomach contents have also been developed[88-89].

3D texture analysis of tooth microwear represents an additional, potentially powerful tool for dietary discrimination and investigation of dietary ecology in elasmobranchs. Although the method is widely applied to terrestrial mammals[90-92], and is starting to be applied to teleost fishes[61,93], it has not previously been applied to elasmobranchs. This study presents the first evidence that 3D textures of tooth microwear vary with diet in sharks.

The approach is based on quantification of the 3D surface textures that develop as tooth surfaces wear as a consequence of feeding. Textural analysis of tooth microwear has several advantages over other approaches: it provides direct evidence of tooth use that is independent of functional analyses based on morphology of the jaws and teeth; the dietary signal accumulates over longer timescales than stomach contents, avoiding the ‘snapshot’ problem[19, 61]; it can

detect subtle dietary differences between individuals and populations, even when sample sizes are small[61, 94-95]. The method is also highly applicable to fossils and to specimens that are not amenable to stomach contents analysis, and in contrast to stable isotope analysis it provides evidence of the nature of food rather than the relative trophic level at which an organism is feeding.

This analysis is based on the Sand Tiger shark, *Carcharias taurus*. This species is an ideal model to investigate the use of tooth microwear texture analysis for dietary discrimination in elasmobranchs. It has worldwide distribution, and because it survives well in captivity, it is a relatively common species in aquaria (and thus teeth from individuals with controlled diets are available). Once relatively common, *C. taurus* is now one of the most threatened elasmobranchs in the world[96], and efforts to understand its ecology, linked to conservation priorities, mean that it is one of the few elasmobranch species where detailed dietary analysis of wild populations has been conducted. Wild *C. taurus* are known to consume mainly teleosts and elasmobranchs, with the proportions of each varying with geographical differences in prey distribution and with *C. taurus* ontogeny. Compared to small individuals (> *ca* 2 m) larger individuals consume more elasmobranchs (particularly benthic species), with elasmobranchs making up a greater proportion of their diet (in terms of % number, % frequency and % mass); the range of dietary items is also greater in larger individuals[96-98].

The focus of this study is to test the hypothesis that tooth microwear textures vary with diet in *C. taurus*. Unlike terrestrial mammals, the polyphyodont dentition of elasmobranchs makes it impossible to sample in multiple individuals the same location (i.e. on a homologous wear facet, on a homologous cusp of a homologous tooth). Consequently, we also tested the subsidiary hypotheses that non-dietary variation in microwear texture between samples from different parts of a tooth is greater than variation between individuals with different diets.

Because this approach is non-destructive, acquires data from teeth, and requires only a small number of samples, it has a wide range of potential applications, including conservation of endangered elasmobranch species and palaeontological investigation of extinct elasmobranchs. In combination with

other approaches that capture dietary information on different timescales[46], it provides independent data that could be used as part of a multiproxy approach to dietary analysis in elasmobranchs.

3.2 Materials and methods

3.2.1 Materials and sampling strategy

To provide baseline data from individuals known to consume only fish, six shed anterior teeth of captive *C. taurus* were obtained by divers from tanks at Sea Life London Aquarium (specimens 1a-1f; Table 3.1). These teeth are derived from four individuals that were approximately 270 cm in length (same ontogenetic stage), and were fed on a controlled diet of whole 'white fish' and occasional tropical *Caranx sp.* The specific individual from whom each tooth originated is unknown. Analysis of tooth loss rate indicates that a single tooth is in use for 80-90 days before being shed[99]; all aquarium teeth sampled were shed at the end of a replacement cycle.

Teeth from wild individuals were sampled in jaws of *C. taurus* captured in the western Atlantic, off the east coast of the USA (Specimens 2-4; Table 3.1). Individuals ranged in length from 190 cm to 278 cm and thus include both smaller and larger elasmobranchs, the latter known to consume a greater proportion of elasmobranchs (in terms of % number, % frequency and % mass) and a greater diversity of prey[96-98]. Six teeth anterior teeth were sampled from each individual.

An additional specimen captured in the Western Pacific and landed in the Philippines was also analysed (specimen 5). No dietary data are available for this specimen, or this population, but microwear data were used to test the hypothesis that textures in wild sharks vary as consequence of size, rather than diet.

The data used to test the hypothesis that non-dietary variation in microtextures between samples from different parts of a tooth is greater than variation between individuals with different diets, were obtained from an anterior tooth from specimen 2. Eight samples were obtained from positions ranging

across the labial surface of the tooth. For all other tests, data were collected from the central part of the labial surface, approximately 5 mm below the apex of the tooth.

The data used to test the hypothesis that microwear texture varies with diet were sampled from the 6 aquarium teeth (known, from their morphology, to be from anterior locations in the jaw), and 6 teeth per individual from the wild western Atlantic specimens (2-4). These teeth were selected at random from among the six anterior most teeth of the outer tooth row of the upper jaw and the equivalent teeth on the lower jaw. The same sampling strategy was applied to Specimen 5.

3.2.2 Surface texture data acquisition

Data were acquired from high fidelity surface replicas of teeth prepared using President Jet medium body polysiloxane dental moulding compound, and EpoTek 320 LV black epoxy. Both were mixed and applied following the manufacturer's instructions. Analysis of accuracy and precision of moulding compounds indicates that replicas made this way compare favourably with the most accurate and precise moulding compounds, with very small absolute differences in parameter values between replica and original[100].

High-resolution 3D surface data were captured following standard lab protocols[68,93,100], using an Alicona Infinite Focus microscope G4b (IFM; Alicona GmbH, Graz, Austria; software version 2.1.2), equipped with a x100 objective to give a field of view of 146 x 111 µm. The Alicona Infinite Focus microscope G4b has a CCD of 1624 x 1232 pixels. In theory, for a field of view of 146 µm, this equates to a lateral sampling distance of 0.09 µm, but the limits imposed by the wavelength of white light mean that lateral optical resolution is between 0.35–0.4 µm. For all samples, vertical and lateral resolutions were set at 20 nm and 440 nm respectively. Exposure settings were manually adjusted to maximize data quality (between 7.18 and 6.5 ms); contrast was set at 2.0. All data collection points were from the labial surface of each tooth, near the tooth tip. Point clouds were edited manually to delete measurement errors (e.g. single point data spikes) and extraneous dirt and dust particles from the surface. After

editing, point clouds were exported as '.sur' files and imported into SurfStand (software version 5.0; restore bad data option selected).

Table 3.1: Specimens of *Carcharias taurus* from whom tooth texture data were obtained.

Dietary preferences for specimens 2-4 were obtained from published analyses[96-98]. Specimen size indicates the total length of each individual, from snout to tail tip. Specimens 1a-1f represent individual teeth analysed from captive individuals. Specimens 2-5 are individual sharks in which six teeth were analysed from each.

Repository number	**Specimen number (this analysis)**	**Locality**	**Date of Capture**	**Specimen Size**	**Diet**
LEIUG 123402	Specimen 1a	Sea Life London Aquarium		~270 cm	Fish only
LEIUG 123403	Specimen 1b				
LEIUG 123404	Specimen 1c				
LEIUG 123405	Specimen 1d				
LEIUG 123406	Specimen 1e				
LEIUG 123406	Specimen 1f				
Florida Museum of Natural History UF47900	Specimen 2	Tanzler Waters Reef, Florida	03/1981	~190cm	Fish, relatively small proportion of elasmobranch
Florida Museum of Natural History 19705007.17	Specimen 3	Florida 29°06.77N 80°49.73W	01/081997	240 cm measured at capture	Fish, intermediate proportion of elasmobranch
Florida Museum of Natural History Un-catalogued	Specimen 4	North Carolina 35°09N 75°47W	17/04/1975	278 cm measured at capture	Fish, relatively larger proportion of elasmobranch
Private collection (Gordon Hubbell) GH-CT-P-12	Specimen 5	Cebu City, Philippines		~335 cm size estimated at capture	Unknown

Surfaces were then treated by levelling the surface and removing gross tooth form with a second order polynomial function, and applying a spline filter, with a nesting index of
0.025 mm. The resulting scale limited roughness surface was then used for calculation of ISO 25178-2 standard parameters[101], quantifying tooth surface texture (Table 8.2).

3.2.3 Statistical analysis

The texture data are normally distributed (Shapiro-Wilks tests) so parametric tests were used except for analysis of relationships between texture and non-normal data (dietary rank and length data for elasmobranchs). Hypotheses were explored using analysis of variance (ANOVA), correlations (Spearman's Rank), pairwise testing (Tukey HSD), and principal components analysis (on correlations; PCA). Where homogeneity of variance tests (Bartlett and Levene tests) revealed evidence of unequal variances, Welch ANOVA was used.

Two sets of analyses were performed to test the hypothesis that texture varies with diet. The first were ANOVA and pairwise tests of the sample of teeth from aquarium elasmobranchs fed a fish-only diet, and the wild, western Atlantic specimens (three samples, six teeth in each). Because the nature of sampling in the aquarium and wild datasets differs (in the aquarium, teeth represent random sampling of anterior teeth from multiple individuals, whereas each of the wild samples is random but from within an individual) a second set of tests was conducted to simply compare the six aquarium teeth with subsets of six randomly sampled teeth from the wild, Western Atlantic specimens. This simulated, for the wild specimens, the random sampling of shed teeth from the aquarium. This process was repeated 10 times (Table 8.9). PCA was based on the parameters found to differ in the ANOVA of the aquarium and three Western Atlantic sharks. The diet of specimen 5 was evaluated by projecting samples into this PCA.

All statistical tests were carried out using JMP, version 12 (SAS Institute, Cary, NC, USA).

3.3 Results

The results of ANOVA demonstrate that non-dietary variation in microwear texture between samples from different parts of a tooth is less than variation between individuals with different diets (Tables 3.2- 4). Comparison of within tooth samples (2a) with data sampled from multiple teeth in specimens (1-4) reveals that 2a does not differ in any parameters from specimen 2; it differs in two parameters from specimen 3, and differs from specimen 4, which consumed a greater proportion of elasmobranchs, in 8 parameters.

The same ANOVA allows us to reject the null hypothesis that microwear does not vary with diet in *C. taurus*. Nine of 23 parameters differ significantly between specimens (Table 3.2), with the greatest number of pairwise differences (Tables 3.3 and 3.4) between sample 1 (teeth from aquarium elasmobranchs fed fish only) and sample 4 (western Atlantic elasmobranch larger than the *ca* 2 m threshold for increased consumption of elasmobranchs). The number of pairwise differences between specimens increases with dietary difference (Table 3.4). For all microtexture parameters, values increase with consumption of elasmobranchs, except Sds, Str, Ssk, which decrease, and Smr2, Ssc, Sdr, which fluctuate. ANOVA based only on specimens with different diets (i.e. excluding the within tooth samples, 2a) yields a very similar result (Tables 8.6- 8.8; nine parameters exhibit significant differences). Subsampling to compare six random teeth from the wild specimens with the aquarium sample found significant differences in every sub-sampling ANOVA (Table 8.9): nine parameters were significant within more than 50% of sub-sample sets.

PCA based on the 14 parameters that differ between elasmobranchs with different diets (i.e. excluding the within tooth samples, 2a) reveals a clear pattern (Figure 3.1, Table 8.9). PC 1 captures 83.6 % of the variance and is strongly correlated with diet (R_S = 0.41252; P =0.0007); the mean and range of PC 1 values increases as diet includes more elasmobranchs and a greater diversity of prey (increasing from sample 1 - aquarium-fed fish-only diet - to sample 4 - highest proportion of elasmobranchs). PC 2 is also correlated with the proportion of elasmobranch in the diet, but to a lesser degree (R_s = -0.257156; P = 0.0114). Neither PC 1 nor PC 2 is correlated with body length

(PC 1: R_s = 0.05144, P = 0.2866; PC 2: R_s = 0.00004, P = 0.9758).

Projecting sample 5 into the PCA based only on samples 1 - 4 (18 parameter analysis) reveals that its microwear textures are comparable to those of samples 1 and 2. ANOVA of PC 1 scores reveals significant differences (F = 5.2296, d.f. 4, 25, *P* = 0.0034) between samples, and pairwise testing (Tukey HSD) indicates that sample 5 does not differ from samples 1, 2 and 3, and that samples 1, and 5 both differ from sample 4.

Table 3.2: Results of ANOVA comparing samples from multiple different individuals with different diets (wild and captive), and samples from multiple sites within a tooth. 'w' indicates Welch ANOVA; significant differences (P < 0.05) in bold.

Parameter	F	*p*	df
Sq	2.7789w	0.0781	4, 11.558
Ssk	2.0122w	0.1596	4, 11.457
Sku	**5.5796w**	**0.0117**	4, 10.413
Sp	1.6849	0.1825	4, 27
Sv	1.8052w	0.1926	4, 12.021
Sz	1.1382w	0.3873	4, 11.301
Sds	**18.8940**	**0.0001**	4, 27
Str	**3.5685w**	**0.0393**	4, 11.806
Sdq	**7.1046w**	**0.0037**	4, 11.855
Ssc	**3.8799w**	**0.0289**	4, 12.433
Sdr	**7.3744w**	**0.0030**	4, 12.069
Vmp	2.0855w	0.1505	4, 11.143
Vmc	2.5595w	0.0918	4, 12.24
Vvc	2.4292w	0.1042	4, 12.147
Vvv	3.1651w	0.0569	4, 11.334
Spk	1.9043w	0.1802	4, 10.958
Sk	**2.4630w**	**0.1001**	4, 12.334
Svk	3.2954w	0.0527	4, 10.96
Smr1	1.7856	0.1609	4, 27
Smr2	**4.5516w**	**0.0185**	4, 11.841
S5z	1.2853w	0.3350	4, 10.774
Sa	2.6142w	0.0888	4, 11.902

Table 3.3: Pairwise differences (Tukey HSD) between samples from multiple different individuals with different diets (wild, specimens 2 -4) and captive (specimen 1), and samples from multiple sites within a tooth (samples 2a). Parameters which display a significant difference between samples are included in the table below. For an explanation of what each parameter represents see page x.

4	differs from	1, 2, 2a	Sds
4	differs from	1, 2a	Sq, Sal, Vmp, Vvv, Spk, Svk
4	differs from	1	Sv, Sz, Vmc, Vvc, Sk, S5z, Sa
3	differs from	1, 2a	Sds, Sal
2	differs from	1	Sds

Table 3.4: Pairwise differences (Tukey HSD) between samples from multiple different individuals with different diets (wild specimens 2-4 and captive (specimen 1), and samples from multiple sites within a tooth (samples 2a).

	Sample 1	**Sample 2a**	**Specimen 2**	**Specimen 3**	**Specimen 4**
Sample 1			Sds	Sds, Sal	Sq, Sv, Sz, Sds, Sal, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, S5z, Sa
Sample 2a	0			Sds, Sal	Sq, Sds, Sal, Vmp, Vvv, Spk, Svk, Smr2
Specimen 2	1	0			Sds
Specimen 3	2	2	0		
Specimen 4	14	8	1	0	

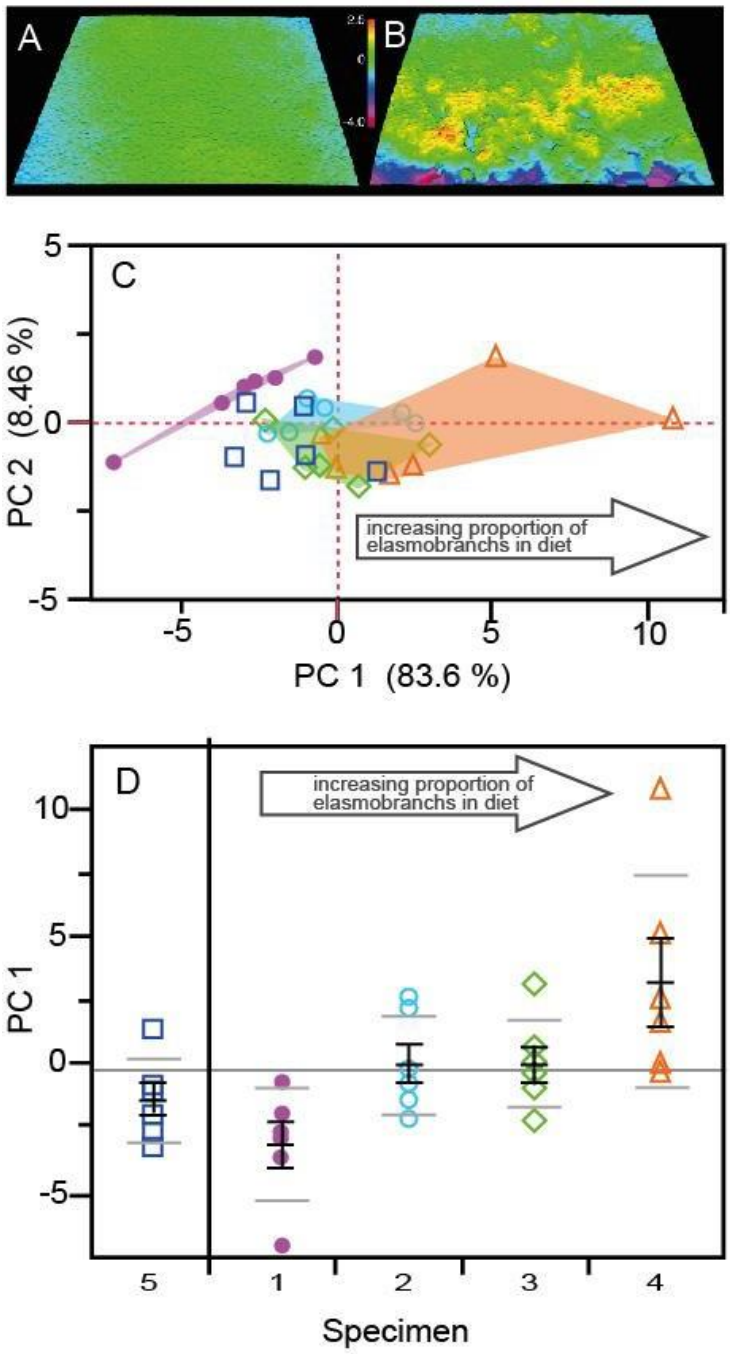


Figure 3.1: Tooth microwear textures of *Carcharias taurus*, and principal components analysis of International Organization for Standardization (ISO) texture parameters.

A, B. Digital elevation models showing levelled surface data (146 µm x 110 µm), one (A) fed fish-only diet (Aquarium specimen 1d: LEIUG 123404), one (B) consuming elasmobranchs (Wild specimen 4:CT001). Samples were taken from the labial surface of each tooth, near the tooth tip.

C. PCA of 14 ISO parameters that exhibit significant differences between elasmobranchs with different diets. PC1 is strongly correlated with diet, with higher scores linked to higher proportions of elasmobranch prey in the diet, and a greater range of prey types. Figure is based on data from specimens 1-4.

D. PCA scores by specimen, showing mean and standard error (black bars) and 95% confidence intervals on means; Horizontal line across plot in grand mean. Both means and variances of PC1 values are correlated with diet (Rs=1). Specimen 5, with no known diet, displays PC1 scores most similar to those of individuals with a teleost dominated diet.

3.4 Discussion

The results of this study demonstrate that tooth microwear textures vary with diet in *C. taurus*, and show that the approach can provide an additional, potentially powerful tool for dietary discrimination in elasmobranchs. The strong correlations between PC 1 and diet, and the absence of correlations with body size, indicate that microwear textures are tracking the transition to different diets with greater size, rather than the increase in size *per se*. The increase in the mean and range of the PC 1 values (which capture similar patterns in a number of different ISO texture parameters) reflects not only the increase in the proportion of elasmobranchs in the diet, but also the increase in consumption of benthic elasmobranchs[96-98], which may have an associated increase in the amount of sediment consumed with prey. The increase in variance of texture values with diet may also reflect increased diversity of prey types[96-98]. Alternatively, the greater variance might partly reflect the greater difference between maximum texture development in a tooth near the end of its functional life and minimum texture in a recently erupted tooth. Either way, the higher values for texture in specimens consuming more elasmobranchs indicates that texture tracks diet, but more work will be required to separate these additional factors.

These analyses indicate that the tooth microwear textures of Specimen 5, for which we have no dietary data (Table 3.1), are closely comparable to those of samples 1,2 and 3, in terms of both values and variances (Figure 3.1). On this basis we interpret specimen 5 to have had a diet dominated by fish. The large size of this specimen (at ca. 335 cm, larger than any other specimens analysed) lends further support to the hypothesis that microwear texture is tracking diet, and not size. Our dietary predictions regarding *C. taurus* from this area could be tested using traditional stomach contents analyses, but this is outside the scope of the present study.

Our results also suggest that for microwear texture analysis of the diet of individual elasmobranchs, sampling multiple teeth per individual will provide more reliable results than single teeth. Sub-sampling of the wild teeth, which found multiple significant differences between elasmobranchs that consume

only fish and those that eat mixed diets in all comparisons, supports this conclusion. Analyses based on single isolated teeth rather than those from jaws, which might be a more common situation in analyses of fossil teeth, have the potential to detect differences between populations and species with different diets, but will be less sensitive than analyses based on multiple teeth per individual. To a certain extent, this will be offset in collections of isolated fossil teeth because the differences in the accumulation of wear between well-used and recently erupted teeth will be diminished as the vast majority of fossil teeth are those that are shed at the end of a tooth's functional cycle. Due to the rate of tooth replacement in elasmobranchs, the number of teeth shed by an individual in its lifetime, outnumber the number of teeth in the individuals jaw at time of death by several orders of magnitude.

In some ways our results are perhaps surprising. Elasmobranchs are well known for the rate at which they replace their teeth, yet this analysis indicates that anterior teeth are retained long enough for dietarily informative microwear textures to develop. Furthermore, recent analysis indicates that *C. taurus* consume prey mostly in one piece[96], implying less interaction of teeth with prey than would be the case in animals that process their food before swallowing. For elasmobranchs that bite their prey, I predict that the relationship between diet and microwear texture will be stronger than that reported here.

Drawing wider comparisons with analyses in other groups of vertebrates of the relationship between diet and 3D microwear texture based on ISO parameters, the number of parameters that differ between samples of *C. taurus* is larger than most previous studies, probably due to greater differences in material properties of food between the samples compared. Purnell and Darras[93] found that Sdq, Sdr, Vmc, Vvv, Sk and Sa discriminated best between the specialist durophagous and more opportunist durophagous fish in their study (based on ANOVA and PCA), with these parameters also differing between populations of the opportunist durophage *Archosargus probatocephalus* with different proportions of hard prey in their diets. Of these parameters, Vmc, Vvv, Sk and Sa produce pairwise differences between *C.*

taurus samples (between 1 and 4). These parameters capture aspects of the volume and height of surfaces (Table 8.2). All increase in value as the proportion of elasmobranchs in the diet increases, the same as the pattern of increase with durophagy seen in *Archosargus probatocephalus* and *Anarhichas lupus*[93]. Vmc, Vvv, and Sk were also found to increase with the amount of hard-shelled prey in the diet of cichlids[61].

Other studies, although focused on terrestrial rather than aquatic vertebrates, have found similar patterns. Vmc, Vvc, Vvv, and Sa increase with more abrasive diets in grazing ungulate mammals[102]; Vmc, Vvv and Sk increase with increasingly 'hard' prey in insectivorous bats[94]. Unlike other studies, the latter found Sa to decrease with harder diets[94].

3.5 Conclusions

Our study of *C. taurus* and comparisons with previous work demonstrate that analysis of tooth microwear textures can provide an additional, potentially powerful tool for dietary discrimination in elasmobranchs. Dental microwear texture analysis provides significant results even on small samples and animals with no stomach contents, offering a potential means to reduce the impact on wild elasmobranch populations when analysing their dietary ecology. This is especially relevant in conservation of endangered elasmobranch species. Because it accumulates over longer periods of time analysis of microwear texture also overcomes the 'snapshot bias' of stomach contents analysis, and in combination with other approaches that capture dietary information on different timescales[46], it could be used as part of a multi-proxy approach to dietary analysis in elasmobranchs. In an evolutionary context, dental microwear texture analysis applied to extinct elasmobranchs has the potential to provide a robust new approach to testing hypotheses of trophic ecology, niche segregation and escalation through a fossil record of teeth and jaws spanning 400 million years.

4.0 The impact of sediment abrasion on tooth microtextures

Taphonomic processes have the potential to affect 3D tooth microtextures and distort the dietary signals preserved within them. A limited number of studies have investigated the impact of sediment abrasion to 2D tooth microwear signals. These studies found that, whilst sediment abrasion did not create false dietary signals, dietary signals were obliterated. Until now it was not known how 3D microtextural signals might be impacted by sediment abrasion.

This study shows the impact of sediment abrasion to 3D tooth microtextures, under a number of experimental conditions. These analyses indicate that like previous studies, sediment abrasion does not generate false dietary signals. Sediments are capable however, of distorting existing tooth microtextures and altering preserved dietary signals. Sediment impacts are most noticeable when specimens have been subjected to calcarenitic sediments, or specimens are compared which have been subjected to different sediment types. Our analyses indicate that in the future, studies using 3D tooth microtextural techniques on fossil teeth need to take care when samples originate from carbonate dominant sediments, or individually from very different sediment types.

4.1 Introduction

3D tooth microtextural analysis is becoming a popular tool for investigating dietary ecology among both extinct and extant species. The method has already demonstrated accurate dietary discrimination among groups of terrestrial mammals[21, 53, 90-91, 103] and fish[61, 93]. Yet to date there is no understanding of how taphonomic sedimentary processes impact upon 3D tooth microtextures, potentially distorting preserved dietary signals and our dietary interpretations.

A limited number of studies have previously investigated the impacts of taphonomic processes, such as post mortem transport, sediment movement and systematic reworking, on 2D tooth microwear signals. The most recent and robust of these[71] tumbled a limited number of fossilised human teeth under a range of sedimentary conditions in a commercial tumbler. Their qualitative

analyses found that 2D microwear signals were obliterated rather than replaced, causing the previously preserved dietary signal to be lost. Importantly they did not find evidence that a false dietary signal had been created. These findings were in keeping with earlier studies which employed similar methodologies and also acknowledged that 2D microwear signals were obliterated[69-70, 72]. What King et al[71] failed to find however, were the high levels of sedimentary damage to the tooth surfaces reported by these earlier studies.

3D tooth microtextural analyses are a development and an improvement on traditional 2D microwear analyses[53]. These texture analyses are based on the quantification of the sub-micron tooth surface textures that develop as a consequence of feeding activity. Textural methods have several advantages over other traditional dietary analyses. They provide direct evidence of tooth use that is independent of functional analyses based on morphology of the jaws and teeth. A microwear textural signal accumulates over longer time scales than stomach contents, avoiding the 'snapshot' problem[19, 61]. Microwear textural analyses can also detect subtle dietary differences between individuals and populations, even when sample sizes are small[61, 94-95]. In addition, microwear textural analyses are also highly applicable to specimens that are not amenable to stomach contents analysis and fossils. The method is not however without fault as extraneous variables, such as taphonomic sedimentary processes, potentially impact upon the preserved tooth texture[107]. This is particularly likely when applying to fossil specimens, which have lain in a sedimentary environment for many years.

Taphonomic sedimentary processes have already been shown to obliterate traditional 2D microwear signals[69-72]. It is likely therefore, that the same taphonomic sedimentary processes will also distort the tooth microtextural signals. This study presents the first quantitative analyses of the impacts of taphonomic sedimentary processes on the dietary signals preserved within tooth microtextures.

This analysis is a quantitative replication and advancement of the work conducted by King et al[71]. The use of human teeth proved impractical and instead elasmobranch teeth were used in our analysis. Elasmobranch teeth

are widely abundant, and although are coated in enameloid instead of enamel, have similar material properties to enamel teeth[104]. The findings from this study are thus applicable to all teeth that have a hard enamel-like covering. The increased number of samples per experimental testing condition, and the increased number of variables tested have also allowed for a greater understanding and application of the impacts of taphonomic processes upon tooth surface microtextures.

The focus of this study is to test the overarching hypothesis that sediment abrasion alters tooth microtextures. In keeping with previous studies, this was tested by investigating the following hypotheses:

- Sediment abrasion does not create false dietary signals on teeth that had never been used for food processing.
- Sediment abrasion removes or modifies tooth surface textures that were known to preserve dietary signal.

In order to fully investigate the above hypotheses, and to advance our understanding of the impacts of sedimentary abrasion on tooth microtextures, a number of variables were considered. Each of these variables represents a condition that is likely to be encountered by those using 3D tooth microtextures as a tool for dietary analysis on fossil teeth. The study of these variables, lead to the examination of the following subsidiary hypotheses:

- Different sediment types will have different effects on tooth microwear textures when being used as an abrasive. This simulates the comparison on teeth found in different sedimentary deposits.
- Sediment abrasion will affect teeth with differing previous wear levels in different ways. This simulates the comparison of shed and unshed teeth, found in both the same and different deposits.
- Sedimentary abrasion will affect different sized teeth differently. This simulates the comparison of teeth of differing sizes, found in both the same and different deposits.

- Sedimentary abrasion will affect different tooth morphologies differently. This simulates the comparison of teeth of different species, found in both the same and different deposits.
- Sediment abrasion will affect fossilised and fresh teeth in different ways. This simulates the comparison of teeth that have been reworked.

4.2. Materials and methods

4.2.1 Materials and sampling strategy

A number of experiments were conducted under differing experimental conditions to fully test the above hypotheses. The impacts of sediment type, tooth size and shape, fossilisation and previous wear levels were all investigated as possible variables that impact upon tooth microwear textures in abrasive environments. Table 4.1 provides a breakdown of the variables making up each experimental condition. Explanations of materials used to investigate each hypothesis can be found below.

Table 4.1: A breakdown of the 10 experimental conditions used to investigate the impacts of taphonomic processes on the preservation of tooth microwear textures. Conditions altered by sediment type, tooth morphology, tooth size, amount of previous tooth use and wear, and whether teeth are fossilised or not. For example experimental condition A ran with the following variables: siliciclastic sand, cuspate, non-serrated, unfossilised teeth that were erupted with a cusp height >2cm.

Experimental condition	Sediment type	Morphology	Worn/ Unworn	Cusp height	Fossilised/Non-Fossilised	N=
A	Siliciclastic	Cuspate non-serrated	Worn	>2cm	Non-fossilised	6
B				<1cm	Non-fossilised	6
C				>2cm	Fossilised	6
D		Cuspate serrated	Unworn	<1cm	Non-fossilised	6
E			Worn	<1cm	Non-fossilised	6
F	Calcarenitic	Cuspate non-serrated	Worn	>2cm	Non-fossilised	6
G				<1cm	Non-fossilised	6
H				>2cm	Fossilised	6
I		Cuspate serrated	Unworn	<1cm	Non-fossilised	6
J			Worn	<1cm	Non-fossilised	6

The hypothesis that sediment abrasion produces different textures on tooth surfaces when the abrading sediments have different compositions was investigated using two differing sediments: calcarenitic (carbonate rich) dominant sand and siliciclastic dominant sand. Calcarenitic sand originated from Porthmeor Beach, Cornwall, at the low tide line. This sediment has a calcium carbonate content of 77.14 % ± 0.61%[105] Siliclastic sand originated from Walton-on- the-Naze. Sand was collected from the beach just below the Red Crag Cliffs. The sand has a similar composition to the cliffs which have been described as marine, shelly quartz rich sands[106]. A coarse grain size (500-1000μm) was obtained for both sediment types by sieving sediments with a 1000 μm mesh sieve followed by a 500 μm mesh sieve. Approximately 500 g of sediment was used in each tumbling barrel for each experiment. See Table 4.1 for a summary of specimens and experimental conditions studied.

The hypothesis that sediment abrasion creates different textures on erupted and un-erupted teeth (taken to be worn and unworn, respectively) was studied using *Hemipristis elongatus* teeth. Teeth originated from a single specimen, donated by D. Ward (Orpington, UK). All teeth used had an approximate cusp height of 1cm, and were extracted from two different rows within the jaws. Worn teeth, preserving dietary tooth textures, were extracted from the front functional row within the jaw. Un-worn teeth, that have never been used for food processing, and thus preserve no previous wear signal, were removed from the first non- functional and un-erupted tooth row within the jaw. Teeth were extracted from the jaw, after the jaws had been soaking in distilled water for 24 hours. This softened the cartilage and allowed for the separation of teeth from jaw using tweezers. During the process, care was taken not to touch the enameloid surface with the tweezers, and thereby create a false wear signal. A total of 12 worn and 12 unworn teeth were used within four experimental conditions outlined in Table 4.1. These were experimental conditions D, E, I and J.

The hypothesis that sediment abrasion produces different textures on teeth of different sizes was investigated using *Carcharias taurus* teeth. Teeth were collected by divers from Deep Sea World, Edinburgh, from the bottom of their shark tank. Two tooth sizes were used in this study, those with a cusp

height in excess of 2cm and those with a cusp height below 1cm. A total of 24 teeth were analysed, 12 from each size category. All teeth had been shed and thus it has been assumed that these have undergone a full wear cycle and thus preserve a dietary signal (Chapter 3). Analysis of tooth loss rate indicates that a single tooth is in use in an individual's mouth for 80-90 days before being shed[99]. The experimental conditions investigating this hypothesis were A, B, F and G (Table 4.1).

The hypothesis that sediment abrasion creates different textures on the surface of teeth with different morphologies was investigated by comparing the cuspate, serrated teeth of *H. elongatus* with the cuspate, non-serrated teeth of *C. taurus* when all other variables were kept constant. All teeth had a cusp height that did not exceed 1cm, and only teeth tumbled in the same sediment were compared. 24 teeth were studied in total, 12 from each species. The experimental conditions investigating this hypothesis were B, E, G and J (Table 4.1).

The hypothesis that fossilised and fresh teeth respond differently to sediment abrasion was conducted by comparing fossil *Carcharias* sp. teeth to *C. taurus* teeth from Edinburgh to test for the impact of fossilisation upon the effects of sediment abrasion. Fossil teeth originated from deposits in Morocco that date to 54 MA. They are morphologically similar to *C. taurus* teeth, both displaying a long, narrow, slightly recurved main cusp and a single pair of lateral cusplets. All teeth used had a cusp height that was in excess of 2 cm. 24 teeth were compared in total, 12 from each grouping. The experimental conditions investigating this hypothesis were A, C, F, and H (Table 4.1).

Sediment grain size (500-1000 µm), tumbling speed (2400 revolutions per hour), time (up to 120 hours) equating to a distance of approximately 180km were kept constant for all experiments (Appendix 8.4.1).

Six tumbling barrels, replicating the same conditions, were run simultaneously for each experimental condition. Each barrel contained approximately 500g of sediment and a single tooth. The barrel was filled with tap water. Prior to the start of each experiment teeth were moulded and cast to

provide a reference against which to compare the effects of continued sediment abrasion on tooth microtextures. Barrels were tumbled over a period of 120 hours, with barrels being stopped and teeth removed, moulded and cast every 24 hours (~36 km of transportation).

4.2.2 Surface texture data acquisition

Data were acquired at each timing interval from high precision surface replicas of teeth, prepared using President Jet medium body polysiloxane dental moulding compound, and EpoTek 320 LV black epoxy. Both were mixed and applied following the manufacturer's instructions. Analysis of accuracy and precision of moulding compounds indicates that replicas produced this way display very small absolute differences in parameter values between replica and original[100].

High-resolution 3D surface data were captured following standard lab protocols[61, 93, 100], using an Alicona Infinite Focus microscope G4b (IFM; Alicona GmbH, Graz, Austria; software version 2.1.2), and a x100 objective to give a field of view of 146 x 111 µm. All data was collected from the labial surface of the tooth near the tooth tip. For all samples, vertical and lateral resolution were set at 20 nm and 440 nm respectively. The Alicona Infinite Focus microscope G4b has a CCD of 1624 x 1232 pixels, but limits imposed by the wavelength of white light results in a lateral optical resolution of 0.35–0.4 µm. Exposure settings were manually adjusted between 7.18 and 6.5 ms, to maximize data quality; contrast was set at 2.0. Point clouds were manually edited to delete measurement errors (e.g. single point data spikes) and extraneous dirt and dust particles from the surface. Point clouds were exported as '.sur' files into SurfStand (software version 5.0; restore bad data option selected). Surfaces were treated by levelling the surface and removing gross tooth form with a 2nd order polynomial function, and applying a spline filter (with a nesting index of 0.025 mm). The resulting scale limited roughness surface was used for calculation of ISO 25178-2 standard parameters[101], quantifying tooth surface texture. More details of materials and techniques can be found in the

Supplementary Material, including short definitions of ISO parameters (Table 8.2).

4.2.3 Statistical analysis

The texture data are normally distributed (Shapiro-Wilks tests) so parametric tests were used. Hypotheses were explored using analysis of variance (ANOVA) and pairwise testing (Tukey HSD). Where homogeneity of variance tests (Bartlett and Levene) revealed evidence of unequal variances, a Welch ANOVA was used.

All experimental conditions were initially analysed using ANOVA and pairwise Tukey HSD testing of each timing period to investigate the changes in the 3D microwear textural signal over time. For example A_0 was compared to each of A_{24}, A_{48}, A_{72} A_{96} and A_{120} in turn. These were followed by analyses of individual variables. Different experimental conditions were paired and compared using ANOVA and pairwise Tukey tests. Each pairing of conditions was set so that only a single variable varied. For each pairing only the samples from the same time period was compared. For example A_0 was compared to F_0, A_{24} to F_{24} etc. Table 4.2 provides a summary of the experimental pairings that were analysed to investigate the impacts of individual variables.

All statistical tests were carried out using JMP, versions 10 and 12 (SAS Institute, Cary, NC, USA).

Table 4.2: A summary of the experimental pairings used to test the impacts of different variables upon the abrasion of tooth microwear textures. Comparisons between experimental conditions were conducted using ANOVA and pairwise Tukey HSD tests at each timing interval between 0-120h. Only teeth from the same timing interval were compared.

Hypothesis	Experimental condition
Different sediment types produce different abrasive textures on tooth surfaces	A to F; B to G; C to H; D to I; E to J
Sediment abrasion creates different textures on teeth of different sizes	A to B; F to G
Sediment abrasion creates different textures on teeth with different morphologies	B to E; G to J
Sediment abrasion creates different textures on teeth with different previous levels of wear	D to E; I to J
Sediment abrasion creates different textures on teeth that have been fossilised to those that have not	A to C; F to H

4.3 Results

4.3.1 Does sediment abrasion significantly alter tooth microtextural signals through time?

ANOVA analyses, investigating the impact of sediment abrasion on tooth surfaces over time, revealed few significant differences (Tables 4.3 - 11). ANOVA analyses failed to differentiate between parameters measured at each timing interval, during experiments B, D and I. A single parameter was found to produce a significant difference during timing intervals for experiments A, E, F and J. The parameter for each varied, being Sds, Sk, S5z and Smr1, respectively. Ssc and Smr1 separated timing intervals during Experiment H. Sds, Ssc and Smr1 separated timing intervals in Experiment C. Sds, Ssc and Smr1 were the only parameters showing significant differences for more than one experimental condition.

In contrast to the lack of significant separation in other experimental conditions, Experiment G recorded ANOVA separation within 17 parameters (Table 4.12). 13 of these parameters are known dietary discriminators in elasmobranchs (Chapter 3). Further investigation using Tukey HSD analyses revealed that the values for parameters at timing interval D (72h) were greatly different from the rest of the experiment, and responsible for the separation

observed in ANOVA testing. Tukey testing separated timing interval D, from all other timing intervals (Table 4.12). No other timing intervals were shown to be significantly different from one another. When timing interval D data was removed and Experiment G data reanalysed using ANOVA and Tukey HSD testing, no parameter produced a significant result (Table 4.13).

Tukey HSD testing also identified few instances of separation between timing intervals during other experiments (Tables 4.3 - 11). 12 parameters throughout all experiments produced separation. Of these only Ssk and Smr1 separated initial surfaces from surfaces subjected to 120 hours of sediment abrasion. Only Smr1, during experiment H, produced maintained separation once detected. The other 11 parameters displayed intermittent variation through time.

Table 4.3: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment A (large, cuspate and unserrated teeth tumbled in siliciclastic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	P	Tukey HSD separation
Sq	1.5706 ^{w}	5, 8.2272	0.2690	a/d
Ssk	2.5018	5, 23	0.0599	a/f
Sku	1.4146 ^{w}	5, 9.0350	0.3061	
Sp	0.2447 ^{w}	5, 7.6815	0.9307	
Sv	0.3123 ^{w}	5, 8.0542	0.8923	
Sz	0.5351	5, 23	0.7476	
Sds	**3.6016**	**5, 23**	**0.0150**	e/d
Str	0.8045	5, 23	0.5581	
Sdq	1.4750	5, 23	0.2364	
Ssc	2.4557	5, 23	0.0636	
Sdr	1.6200	5, 23	0.1946	
Vmp	0.3984 ^{w}	5, 8.6960	0.8380	
Vmc	1.7705 ^{w}	5, 8.2906	0.2220	a/d d/f
Vvc	1.1786 ^{w}	5, 8.2670	0.3955	a/d, d/f
Vvv	3.3682 ^{w}	5, 8.5126	0.0580	a/d
Spk	1.9888	5, 23	0.1184	
Sk	1.8758 ^{w}	5, 8.2346	0.2019	a/d d/f
Svk	2.5671	5, 23	0.0550	a/d
Smr1	1.1100	5, 23	0.3824	
Smr2	0.8343	5, 23	0.5388	
S5z	1.2723 ^{w}	5, 7.6851	0.3650	
Sa	1.6750 ^{w}	5, 8.2815	0.2430	a/d d/f

Table 4.4: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment B (small, cuspate, unserrated teeth tumbled in siliciclastic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	1.5806 [w]	5, 11.698	0.2403	
Ssk	1.3687 [w]	5, 11.797	0.3036	
Sku	1.0983 [w]	5, 12.679	0.4081	
Sp	1.9221 [w]	5, 12.410	0.1618	
Sv	1.7012	5, 28	0.1671	
Sz	1.3047	5, 28	0.2903	
Sds	0.7402 [w]	5, 12.289	0.6076	
Str	0.2971	5, 28	0.9104	
Sdq	1.4087 [w]	5, 12.608	0.2864	
Ssc	0.4891	5, 28	0.7815	
Sdr	1.3602 [w]	5, 15.532	0.3030	
Vmp	1.3949 [w]	5, 12.660	0.2907	
Vmc	1.3654 [w]	5, 12.161	0.3030	
Vvc	1.3075 [w]	5, 12.410	0.3231	
Vvv	1.2847 [w]	5, 11.490	0.3336	
Spk	1.7062 [w]	5, 12.329	0.2059	
Sk	1.3815 [w]	5, 12.214	0.2972	
Svk	1.4631 [w]	5, 11.792	0.2732	
Smr1	1.7354	5, 28	0.1592	
Smr2	0.9356	5, 28	0.4732	
S5z	1.0084	5, 28	0.4312	
Sa	1.5416 [w]	5, 11.888	0.2498	

Table 4.5: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment C (large, cuspate, unserrated, fossil teeth tumbled in siliciclastic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	0.7743 [w]	5, 12.532	0.5857	
Ssk	1.6453 [w]	5, 13.614	0.2141	
Sku	2.3865 [w]	5, 13.395	0.0941	
Sp	2.0586 [w]	5, 11.172	0.1468	
Sv	0.8077 [w]	5, 13.045	0.5642	
Sz	0.7856 [w]	5, 12.796	0.5783	
Sds	**7.0344** [w]	**5, 12.979**	**0.0022**	
Str	1.4279	5, 30	0.2429	
Sdq	0.9868 [w]	5, 11.987	0.4650	
Ssc	**3.3854** [w]	**5, 11.882**	**0.0392**	
Sdr	1.2814	5, 12.270	0.3327	
Vmp	2.0764 [w]	5, 11.932	0.1395	a/d
Vmc	0.5419	5, 30	0.7430	
Vvc	0.8730	5, 30	0.5108	
Vvv	1.1944 [w]	5, 12.365	0.3669	
Spk	1.3665 [w]	5, 12.662	0.3003	
Sk	0.2634	5, 30	0.9295	
Svk	0.6711 [w]	5, 13.532	0.6522	
Smr1	**3.2289**	**5, 30**	**0.0189**	a/d a/e
Smr2	0.8693 [w]	5, 11.730	0.5299	
S5z	0.8767 [w]	5, 12.454	0.5241	
Sa	0.6885 [w]	5, 12.393	0.6412	

Table 4.6: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment D (Small, cuspate, serrated, unworn teeth tumbled in siliciclastic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	0.4673	5, 30	0.7975	
Ssk	0.8141	5, 30	0.5491	
Sku	2.3688 ^{w}	5, 12.134	0.1019	
Sp	0.6817	5, 30	0.6408	
Sv	0.7707 ^{w}	5, 13.533	0.5868	
Sz	0.6425	5, 30	0.6691	
Sds	1.0001	5, 30	0.4358	
Str	1.7687	5, 30	0.1519	
Sdq	0.2927	5, 30	0.9129	
Ssc	0.5130	5, 30	0.7641	
Sdr	0.3004	5, 30	0.9084	
Vmp	0.8369 ^{w}	5, 12.652	0.5471	
Vmc	0.8070	5, 30	0.5544	
Vvc	0.8294 ^{w}	5, 12.395	0.5520	
Vvv	0.6049	5, 30	0.6967	
Spk	0.3764	5, 30	0.8605	
Sk	0.9071	5, 30	0.4904	
Svk	0.6832	5, 30	0.6400	
Smr1	1.1259	5, 30	0.3696	
Smr2	1.7330	5, 30	0.1598	
S5z	0.7037	5, 30	0.6254	
Sa	0.6193	5, 30	0.6862	

Table 4.7: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment E (Small, cuspate, serrated, worn teeth tumbled in siliciclastic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	2.0215 ^{w}	5, 12.451	0.1450	
Ssk	0.6079	5, 30	0.6944	
Sku	1.2809 ^{w}	5, 12.806	0.3307	
Sp	1.6555 ^{w}	5, 13.629	0.2116	
Sv	0.8937	5, 30	0.4978	
Sz	1.5610	5, 30	0.2012	
Sds	1.1749	5, 30	0.3445	
Str	0.9586	5, 30	0.4585	
Sdq	2.1869	5, 30	0.0821	
Ssc	1.1775	5, 30	0.3433	
Sdr	1.9305	5, 30	0.1186	
Vmp	1.6508 ^{w}	5, 13.066	0.2153	
Vmc	2.9395 ^{w}	5, 13.270	0.0534	
Vvc	1.6747 ^{w}	5, 13.323	0.2083	
Vvv	1.9689 ^{w}	5, 12.436	0.1536	
Spk	1.8223 ^{w}	5, 13.010	0.1775	
Sk	**3.1910** ^{w}	**5, 13.401**	**0.0413**	
Svk	1.7242 ^{w}	5, 12.657	0.2001	
Smr1	0.8852	5, 30	0.5031	
Smr2	0.6252	5, 30	0.6817	
S5z	1.3561	5, 30	0.2685	
Sa	2.4043 ^{w}	5, 12.781	0.0951	

Table 4.8: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment F (large, cuspate, unserrated teeth tumbled in calcarenitic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	1.7004 [w]	5, 13.198	0.2028	
Ssk	1.0597	5, 30	0.4020	
Sku	1.6167	5, 30	0.1859	
Sp	2.0424 [w]	5, 13.648	0.1360	b/c b/f b/e
Sv	0.9803 [w]	5, 13.477	0.4646	
Sz	1.8767 [w]	5, 13.602	0.1642	
Sds	2.0417	5, 30	0.1011	
Str	1.3820	5, 30	0.2590	
Sdq	0.7531 [w]	5, 13.746	0.5978	
Ssc	0.7066	5, 30	0.6230	
Sdr	0.9254 [w]	5, 13.721	0.4940	
Vmp	1.2230 [w]	5, 13.706	0.3502	
Vmc	1.2879 [w]	5, 13.312	0.3260	
Vvc	1.4961 [w]	5, 13.498	0.2552	b/f, b/c
Vvv	1.8680 [w]	5, 13.659	0.1656	
Spk	1.5126 [w]	5, 13.736	0.2493	b/f b/c
Sk	1.2783 [w]	5, 13.250	0.3299	
Svk	2.0768 [w]	5, 13.570	0.1312	
Smr1	1.2492 [w]	5, 13.609	0.3399	
Smr2	1.1030	5, 30	0.3795	
S5z	**3.1319** [w]	**5, 13.538**	**0.0433**	b/f b/c
Sa	1.3976 [w]	5, 13.622	0.2857	

Table 4.9: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment H (large, cuspate, unserrated, fossil teeth tumbled in calcarenitic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	2.2141 [w]	5, 13.545	0.1128	
Ssk	1.0081 [w]	5, 13.912	0.4490	
Sku	0.9253 [w]	5, 13.913	0.4936	
Sp	1.2794 [w]	5, 13.573	0.3283	
Sv	1.5216 [w]	5, 13.982	0.2457	
Sz	1.1871 [w]	5, 14.102	0.3639	
Sds	2.7454 [w]	5, 12.605	0.0677	a/b a/d a/e a/f
Str	0.8744 [w]	5, 13.993	0.5227	
Sdq	2.2573 [w]	5, 12.784	0.1111	
Ssc	**3.3751** [w]	**5, 13.552**	**0.0342**	
Sdr	1.9687 [w]	5, 12.852	0.1515	
Vmp	2.5340 [w]	5, 13.230	0.0811	a/e
Vmc	2.2184 [w]	5, 13.593	0.1120	
Vvc	2.4869 [w]	5, 13.415	0.0845	
Vvv	2.0485 [w]	5, 13.336	0.1365	
Spk	2.5739 [w]	5, 13.179	0.0780	a/e
Sk	2.1056 [w]	5, 13.607	0.1269	
Svk	1.3921 [w]	5, 13.352	0.2887	
Smr1	**6.3063** [w]	**5, 12.863**	**0.0036**	a/b a/c a/d a/e a/f
Smr2	0.4564	5, 30	0.8054	
S5z	0.8173 [w]	5, 13.927	0.5572	
Sa	2.4867 [w]	5, 13.527	0.0840	

Table 4.10: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment I (small, cuspate, serrated, unworn teeth tumbled in calcarenitic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	0.6729	5, 29	0.6473	
Ssk	0.5275	5, 29	0.7536	
Sku	0.7507	5, 29	0.5923	
Sp	1.3402	5, 29	0.2755	
Sv	1.2058	5, 29	0.3312	
Sz	1.3352	5, 29	0.2774	
Sds	1.4282	5, 29	0.2438	
Str	1.0268	5, 29	0.4204	
Sdq	1.1079	5, 29	0.3778	
Ssc	1.0839 ^{w}	5, 12.668	0.4149	
Sdr	1.0477	5, 29	0.4090	
Vmp	0.5173	5, 29	0.7610	
Vmc	0.7353	5, 29	0.6030	
Vvc	0.6442	5, 29	0.6679	
Vvv	0.6502	5, 29	0.6636	
Spk	0.4594	5, 29	0.8031	
Sk	0.9750 ^{w}	5, 12.864	0.4689	
Svk	0.5801	5, 29	0.8408	
Smr1	1.3607 ^{w}	5, 13.172	0.3001	
Smr2	1.5185 ^{w}	5, 13.009	0.2508	
S5z	1.0805 ^{w}	5, 12.940	0.4157	
Sa	0.7277 ^{w}	5, 12.547	0.6154	

Table 4.11: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment J (small, cuspate, serrated, worn teeth tumbled in calcarenitic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	0.9850 ^{w}	5, 12.943	0.4634	
Ssk	1.5864 ^{w}	5, 12.735	0.2333	
Sku	1.9694 ^{w}	5, 13.180	0.1498	
Sp	0.8918	5, 30	0.4990	
Sv	0.4710	5, 30	0.7948	
Sz	0.7655	5, 30	0.5819	
Sds	0.5628	5, 30	0.7276	
Str	2.0740	5, 30	0.0965	
Sdq	1.2485	5, 30	0.3116	
Ssc	2.0517 ^{w}	5, 13.506	0.1352	
Sdr	1.4130	5, 30	0.2480	
Vmp	1.6821 ^{w}	5, 12.092	0.2127	
Vmc	1.0398 ^{w}	5, 12.917	0.4355	
Vvc	0.6482 ^{w}	5, 12.660	0.6681	
Vvv	1.2273 ^{w}	5, 13.584	0.3488	
Spk	1.5795 ^{w}	5, 12.215	0.2378	
Sk	1.0859 ^{w}	5, 12.919	0.4132	
Svk	1.5719 ^{w}	5, 13.807	0.2323	
Smr1	**3.6862**	**5, 30**	**0.0103**	a/e
Smr2	0.4185	5, 30	0.8321	
S5z	1.8723	5, 30	0.1290	
Sa	0.8816 ^{w}	5, 12.925	0.5204	

Table 4.12: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment G (small, cuspate, unserrated teeth tumbled in calcarenitic sediment). Text in bold indicates a significant result, 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	**14.8980**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Ssk	1.2190	5, 29	0.3253	
Sku	0.0915 [w]	5, 13.317	0.9922	
Sp	**14.0090**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Sv	**11.0647**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Sz	**15.5759**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Sds	**4.7836** [w]	**5, 11.844**	**0.0126**	d/b
Str	1.1434	5, 29	0.3603	
Sdq	**4.0233**	**5, 29**	**0.0068**	d/b d/c d/e d/f
Ssc	**3.8862**	**5, 29**	**0.0081**	d/c d/f
Sdr	**4.2577**	**5, 29**	**0.0050**	d/a d/b d/c d/e d/f
Vmp	**17.6564**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Vmc	**4.0257** [w]	**5, 12.861**	**0.0202**	d/a d/b d/c d/e d/f
Vvc	**10.6529**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Vvv	**7.5584** [w]	**5, 12.996**	**0.0016**	d/a d/b d/c d/e d/f
Spk	**16.9153**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Sk	**3.4712** [w]	**5, 12.919**	**0.0329**	d/a d/b d/c d/e d/f
Svk	**19.5965**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Smr1	0.4986 [w]	5, 13.208	0.7721	
Smr2	1.6965	5, 29	0.1670	
S5z	**15.4043**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f
Sa	**12.9366**	**5, 29**	**<0.0001**	d/a d/b d/c d/e d/f

Table 4.13: Results of ANOVA/ Welch ANOVA and Tukey HSD testing of tooth microtextural surfaces through time under Experiment G (small, cuspate, unserrated teeth tumbled in calcarenitic sediment), minus timing interval 'D' (72 hours). 'w' indicates the use of a Welch ANOVA.

Parameter	F	d.f	p	Tukey HSD Differences
Sq	0.5767	4, 25	0.6821	
Ssk	1.1668	4, 25	0.3492	
Sku	0.1173 [w]	4, 12.110	0.9738	
Sp	1.5092	4, 25	0.2298	
Sv	0.8095	4, 25	0.5309	
Sz	1.1030	4, 25	0.3770	
Sds	0.7094 [w]	4, 10.687	0.5558	
Str	0.8647	4, 25	0.4986	
Sdq	0.5657	4, 25	0.6898	
Ssc	1.2017	4, 25	0.3347	
Sdr	0.5170	4, 25	0.7239	
Vmp	2.1753	4, 25	0.1011	
Vmc	0.5475	4, 25	0.7025	
Vvc	0.5781	4, 25	0.6812	
Vvv	0.3784	4, 25	0.8219	
Spk	1.2728	4, 25	0.3070	
Sk	0.5550	4, 25	0.6972	
Svk	0.2240	4, 25	0.9224	
Smr1	0.5210 [w]	4, 12.099	0.7222	
Smr2	0.1769	4, 25	0.9482	
S5z	0.4829	4, 25	0.7480	
Sa	0.5506	4, 25	0.7003	

4.3.2 Does sediment abrasion create false dietary signals on previously unworn tooth surfaces?

ANOVA analyses of experimental conditions D and I through time, revealed no instance of significant parameter change as a result of sediment abrasion to the tooth surfaces. Tukey HSD testing also failed to identify any parameter that significantly differed at any timing interval, for either experiment.

4.3.3 Does sediment abrasion remove or modify dietary signals defined by tooth surface microtextures?

This was analysed via a number of subsidiary hypotheses that investigated the effects of tooth size, tooth morphology, previous tooth wear levels and sediment type on the impacts of sediment abrasion to tooth surfaces. The findings of each of these are described below.

4.3.3.1 Impact of different starting wear levels

Erupted and unerupted (worn and unworn) teeth were tumbled separately in sediment and their resulting microtextural surfaces compared. Experimental conditions D and E were compared, at all timing intervals, to investigate the impacts on siliciclastic sediment abrasion. Experimental conditions I and J were compared, likewise, to investigate the impact on calcarenitic sediment abrasion.

Siliciclastic sediments produce differential levels of wear to worn and unworn teeth that fluctuate throughout the duration of the experiment. Four parameters differ between worn and unworn teeth at the commencement of the experiment (D and E at 0 hours). Three of these are known dietary discriminators in elasmobranchs, Vmp, Spk and S5z (Chapter 3). A different nine parameters display separation between the two tooth groups during the remainder of the experiments (Table 4.14). Between one and eight parameters display significant separation between the two groups of teeth, at each timing interval. In all but one

instance, the parameters displaying separation are different from those initially separating the two groups. During the remainder of the experiment five known dietary defining parameters displayed significant differences; Sk, Svk, Sv, Sz, S5z (Chapter 3).

Calcarenitic sediments produce differential levels of wear on worn and unworn teeth. Ten parameters differ between worn and unworn tooth surfaces during the experiment (Table 4.15). Six parameters initially differed between the two tooth groups, Sds, Str, Sdq, Ssc, Sdr and Smr1. Of these only Sds is a known dietary separator in elasmobranchs (Chapter 3). After 24 hours eight parameters separated the two groups (I and J at 24 hours). Of these, four parameters differed from the original separating parameters (I and J at 0 hours). During the experiment the number of parameters differing between worn and unworn teeth varied from four to nine and on occasions included the dietary discrimination parameters Sv, Sz, Sds and S5z (Chapter 3).

4.3.3.2 Impact of tooth size

Large teeth (cusp height >2cm) and small teeth (cusp height <1cm) were tumbled separately in sediment and their resulting microtextural surfaces compared. Experimental conditions A and B were compared at each timing interval to investigate the impact of tooth size on siliciclastic sediment abrasion. Experimental F and G were compared, likewise, to investigate the impacts of tooth size on calcarenitic sediment abrasion

ANOVA analyses comparing the impacts of siliciclastic sediment abrasion on teeth of different sizes found nine parameters to significantly differ between the two groups throughout the duration of the experiment (Table 4.16). Of these, four were different from the parameters that originally differed between the microtextural surfaces of the two tooth sizes (A and B at 0 hours). Of the parameters known to separate diet in

elasmobranchs (Chapter 3), Sds, Sq, Vmp, Vmc, Vvc, Vvv Spk, Sk, Svk and Sa remained constant and were unaffected by sediment abrasion, regardless of tooth size. Sv, Sz and S5z, the level of differentiation varied throughout the experiment. The greatest difference in surface textures to the two tooth groups (A and B at 120 hours) was found after 120 hours of sediment abrasion, with nine parameters differing.

ANOVA analyses of different sized teeth subjected to calcarenitic sediment abrasion yielded very different results. Fifteen parameters produced significant differences throughout the course of the experiment (Table 4.17). Of these, six were different from the parameters that initially differentiated the surfaces of the two tooth sizes (F and G at 0 hours). At least four differences from the parameters initially differing between the two groups were noted at each timing interval. Seven of the parameters that displayed differences in this experiment are also known dietary discriminators in elasmobranchs (Chapter 3). Sq, Sds, Vvv, Svk and Sa initially displayed significant differences between the two tooth sizes. After 24 hours only Sds still produced a significant difference, indicating a removal of dietary defining microtextures. The parameters separating the two groups varied greatly between time periods, with more than half of the original parameters varying in outcome at each timing interval.

4.3.3.3 Impact of tooth morphology

Small, cuspate, unserrated teeth were compared to small, cuspate, serrated teeth to test the impact of tooth morphology on the effects of sediment abrasion. Experimental conditions B and E were compared at each timing interval to investigate the impacts of tooth morphology on siliciclastic sediment abrasion. Experimental conditions G and J were compared, likewise, to investigate the impacts of tooth morphology on calcarenitic sediment abrasion.

Siliciclastic sediments were found to affect teeth of different morphologies in the same way throughout a majority of the experiment

significant differences found. At 120 hours ANOVA analyses revealed tooth surfaces of the two tooth morphologies (B and E at 120 hours) were characterised as significantly different by five parameters. Of these three are known dietary discriminators in elasmobranchs, Sv, Sz, S5z (Chapter 3).

Calcarenitic sediments were found to generate differential wear to the tooth surfaces of different tooth morphologies much more rapidly (Table 4.19). At 48 hours three parameters generated significant differences between the tooth surfaces of the two groups (G and J at 48 hours). At 72 hours 19 parameters displayed significant differences, 11 of these parameters are known dietary discriminators (Chapter 3). After 72 hours little difference was once again found between the two tooth morphologies. In total 20 parameters were found to produce significant differences during the experiment. During experiment G, results produced at the 72 hour timing interval appear to be anomalous (as seen in Tables 4.12 and 4.13). If this timing interval is removed from further analysis, then ANOVA analyses only reveal four occasions of significant differentiation between the two tooth morphologies. None of these parameters are known dietary discriminators.

4.3.3.4 Impact of different sediments

To test for the impact of different sediment composition, each tooth type was tumbled under both sedimentary conditions (siliciclastic and calcarenitic).

Prior to sediment abrasion, 14 parameters differed between the tooth surfaces of two sets of large *C. taurus* teeth, to be tumbled in differing sediments (experimental condition A compared to F). After 24 hours ANOVA analyses found only one differing parameter, which was different to the initial differing parameters (Table 4.20). By 120 hours

there is no discernible difference between the surfaces of teeth tumbled in siliciclastic and calcarenitic sediments (Table 4.20).

In contrast, effects of sediment type on small *C. taurus* teeth (experimental condition B and G) generated a greater number of significant differences throughout the experiment (Table 4.21). Twenty parameters displayed significant differences during the experiment. The greatest number of parameter differences was observed after 72 hours and 120 hours. Of the parameters displaying differences, 13 are known dietary discriminators in elasmobranchs (Chapter 3). If the potential anomalous results displayed at 72 hours in experiment G (Tables 4.12 and 4.13) are removed from this analysis, the impacts of sediment type are not fully noticed until 120 hours. At 120 hours nine parameters differed between the two groups of *C. taurus* teeth. Four of these parameters are dietary discriminators, and six parameters are different from the parameters that differed between the two groups before commencement of study.

Siliciclastic and calcarenitic sediments quickly abrade unworn tooth surfaces in different ways (experimental conditions D and I). After 24 hours nine parameters were found to differ between tooth surfaces subjected to the different sediments. Of these parameters only two, Sds and Ssc, also differed between the two groups of teeth initially. After 24 hours there is a reduction in the number of parameters differing between tooth surfaces subjected to different sediment types (Table 4.22). Of the parameters that produce a significant result throughout the experiment, only Sds is a known dietary discriminator (Chapter 3). Sds significantly differs between the teeth tumbled under the two conditions at all time periods during this experiment. Thus sediment type does not affect any known dietary parameter during these experiments.

A greater number of differences are observed between previously worn teeth tumbled in different sediment types (experimental conditions E and J). Fourteen parameters were found to differ between the teeth tumbled in siliciclastic sediments from those tumbled in calcarenitic

sediments (Table 4.23) throughout the duration of the experiments. Of these parameters seven are known dietary discriminators in elasmobranchs (Chapter 3). Although there are a greater number of differences observed, the parameters detecting the differences are not constant through time. In general more than 50% of the original differing parameters vary at each timing interval. Sds, a dietary discriminating parameter, remains constant throughout the experiment, and thus is unaffected by abrasion from different sediment types. The other six discriminating parameters fluctuate in the level of their significance.

4.3.3.5 Impact of fossilisation

Fresh and fossil teeth, tumbled in each sediment type, were compared at each timing interval to understand the impact of fossilisation on sediment abrasion. Experimental conditions A and C were compared at each timing interval to understand the effects on siliciclastic sediment abrasion. Experimental conditions F and H were compared, to understand the effects on calcarenitic sediment abrasion.

When tumbled with siliciclastic sediments, ANOVA analyses revealed little in the way of significant differences between fresh and fossil teeth (Table 4.24). Smr1, a non-dietary defining parameter, initially differed between the tooth surfaces of fresh and fossil teeth. After 24 hours only Sds generated a significant difference. Throughout the rest of the experiment only one or two parameters displayed differences between fresh and fossil teeth at each timing interval. In addition to Smr1 and Sds, Vvv, Sk and Sp also displayed differences during the experiment. Of these Sds, Vvv and Sk are known dietary discriminators (Chapter 3).

With calcarenitic sediments the differences between fresh and fossil teeth were more pronounced. ANOVA analyses found nine parameters to significantly differ between the two groups (F and H) during the experiment (Table 4.25). Between one and four parameters

differed between fresh and fossil teeth during each timing interval. These parameters varied throughout the experiment, and were inconsistent in their differentiation. Often more than half of the original differing parameters had altered at each timing interval. Of the parameters found to produce significant differences during the experiment, three are known dietary discriminators in elasmobranchs (Chapter 3).

Experimental conditions H and C were compared at each timing interval, to investigate how difference sediment compositions affect fossilised tooth microtextural surfaces. Siliciclastic and calcarenitic sediments create a greater number of significant differences the longer fossil teeth are exposed to their effects (Table 4.26). No significant differences were detected between the two sets of fossil teeth prior to the commencement of sediment abrasion. By 120 hours teeth tumbled in siliciclastic and calcarenitic sediments displayed five parameter differences. Of these Sv, Sz are known dietary discriminators (Chapter 3). The parameters that displayed differences prior to 120 hours varied and did not overlap with those differing between the two groups after 120 hours.

Table 4.14: ANOVA/Welch ANOVA analyses comparing the impacts of previous tooth wear on the effects of siliciclastic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
Parameter	**F**	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	3.5608	1, 5.6731	0.1109	0.6217	1, 10	0.4487	2.8013	1, 10	0.1251	0.6762	1, 9	0.4301	0.5046	1, 9	0.4937	4.1537	1, 5.1194	0.0958
Ssk	1.4156	1, 5.4996	0.2829	**8.5972**	**1, 10**	**0.0150**	**6.504**	**1, 10**	**0.0288**	5.9382	1, 5.1893	0.0571	0.7489	1, 9	0.4071	0.2962	1, 5.4748	0.6077
Sku	3.9757	1, 5.092	0.1017	1.5118	1, 5.1325	0.2722	**7.2168**	**1, 5.8703**	**0.0370**	4.1407	1, 5.5731	0.0917	0.0273	1, 9	0.8720	0.5351	1, 5.9104	0.4924
Sp	3.7677	1, 6.24	0.0984	0.1337	1, 10	0.7222	3.401	1, 5.5812	0.1184	1.7479	1, 9	0.2156	1.0671	1, 9	0.3259	2.379	1, 5.4032	0.1793
Sv	4.5902	1, 5.8406	0.0771	0.8005	1, 10	0.3920	**6.0278**	**1, 10**	**0.0340**	1.4957	1, 6.1754	0.2659	0.0021	1, 9	0.9640	0.8077	1, 10	0.3899
Sz	4.9353	1, 6.0184	0.0675	0.4949	1, 10	0.4978	**6.2559**	**1, 10**	**0.0314**	1.9009	1, 9	0.1980	0.2248	1, 9	0.6456	1.6209	1, 6.0663	0.2496
Sds	0.9855	1, 10	0.3443	0.0526	1, 10	0.8232	0.311	1, 10	0.5893	0.0028	1, 9	0.9592	0.7812	1, 9	0.3998	3.2495	1, 10	0.1016
Str	1.1811	1, 10	0.3026	0.1639	1, 10	0.6941	0.007	1, 10	0.9350	1.4405	1, 9	0.2607	**9.6848**	**1, 9**	**0.0125**	0.5453	1, 10	0.4772
Sdq	2.4513	1, 5.5226	0.1727	0.8774	1, 10	0.3710	**10.4263**	**1, 6.1858**	**0.0172**	2.89	1, 4.2406	0.1603	1.3417	1, 9	0.2766	5.6234	1, 5.5466	0.0588
Ssc	2.3125	1, 6.072	0.1786	1.2607	1, 10	0.2878	4.0041	1, 5.0972	0.1007	4.789	1, 4.4869	0.0852	0.4238	1, 9	0.5313	2.5563	1, 6.2113	0.1593
Sdr	2.1713	1, 5.573	0.1947	0.8883	1, 10	0.3682	**7.271**	**1, 10**	**0.0224**	2.8516	1, 4.1672	0.1637	1.3387	1, 9	0.2771	**6.1658**	**1, 6.115**	**0.0469**
Vmp	**6.8625**	**1, 5.9563**	**0.0399**	2.1904	1, 10	0.1697	0.5027	1, 10	0.4945	0.142	1, 9	0.7151	0.5635	1, 9	0.4720	1.4541	1, 5.0625	0.2812
Vmc	2.093	1, 10	0.1192	0.4708	1, 6.2427	0.5173	1.4556	1, 10	0.2554	0.0947	1, 9	0.7653	0.3206	1, 9	0.5851	6.1988	1, 5.4995	0.0508
Vvc	4.8423	1, 10	0.0524	0.8091	1, 6.2148	0.4019	0.3933	1, 6.1181	0.5533	0.0024	1, 9	0.9624	0.1822	1, 9	0.6795	3.4357	1, 5.2723	0.1200
Vvv	2.3329	1, 5.45	0.1824	0.3154	1, 10	0.5868	4.95	1, 10	0.0503	3.1228	1, 9	0.1110	0.7349	1, 9	0.4135	4.3755	1, 5.3465	0.0871
Spk	**6.1075**	**1, 6.0474**	**0.0481**	1.3715	1, 10	0.2687	0.9767	1, 10	0.3463	0.28	1, 9	0.6095	0.1894	1, 9	0.6737	1.9774	1, 5.086	0.2177
Sk	2.4375	1, 10	0.1495	0.4281	1, 6.2681	0.5362	1.4162	1, 6.3483	0.2766	0.0044	1, 9	0.9483	0.245	1, 9	0.6325	**9.4679**	**1, 6.2428**	**0.0207**
Svk	3.0608	1, 5.4489	0.1358	0.0336	1, 10	0.8583	**5.5661**	**1, 10**	**0.0400**	2.8038	1, 9	0.1284	0.4993	1, 9	0.4977	3.2916	1, 5.5152	0.1239
Smr1	**8.8526**	**1, 10**	**0.0139**	2.7626	1, 10	0.1275	1.7596	1, 10	0.2142	0.0989	1, 9	0.7712	0.0006	1, 9	0.9804	0.0002	1, 10	0.9900
Smr2	1.1859	1, 10	0.3017	0.0392	1, 10	0.8471	3.0676	1, 10	0.1104	4.8586	1, 9	0.0550	0.8224	1, 4.7463	0.4081	1.4536	1, 10	0.2257
S5z	**5.5724**	**1, 10**	**0.0399**	0.6371	1, 10	0.4433	**7.3909**	**1, 10**	**0.0216**	2.6856	1, 9	0.1357	0.4068	1, 9	0.5395	1.5279	1, 6.0375	0.2623
Sa	3.4765	1, 6.1056	0.1107	0.6611	1, 10	0.4351	1.6112	1, 10	0.2331	0.4228	1, 9	0.5318	0.3737	1, 9	0.5561	4.6316	1, 5.2214	0.0817

Table 4.15: ANOVA/Welch ANOVA analyses comparing the impacts of previous tooth wear on the effects of calcarenitic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	1.6547	1, 5.0506	0.2541	0.8095	1, 10	0.3894	0.3783	1, 5.1435	0.5647	0.0458	1, 5.07	0.8389	0.0716	1, 4.6266	0.8006	0.1813	1, 5.7752	0.6856
Ssk	0.7289	1, 5.0398	0.4319	4.8795	1, 6.2951	0.0672	3.73	1, 5.2844	0.1082	1.9761	1, 5.1092	0.2176	3.0722	1, 8	0.1177	2.7181	1, 10	0.1302
Sku	1.6633	1, 5.0037	0.2536	6.4633	1, 5.0089	0.0517	2.1325	1, 5.0059	0.2040	5.0271	1, 5.0107	0.0749	5.0272	1, 4.0949	0.0869	0.2181	1, 10	0.6505
Sp	0.8328	1, 5.0505	0.4029	4.93	1, 5.256	0.0745	1.3079	1, 5.0709	0.3039	2.1082	1, 5.2331	0.2037	**7.5905**	**1, 4.1964**	**0.0485**	0.0246	1, 5.639	0.8809
Sv	0.3188	1, 5.3466	0.5952	**17.645**	**1, 6.1252**	**0.0054**	2.7185	1, 5.0166	0.1599	**6.6595**	**1, 5.0759**	**0.0487**	**9.8628**	**1, 4.1109**	**0.0335**	1.0159	1, 5.4173	0.3564
Sz	0.5163	1, 5.2055	0.5034	**14.265**	**1, 5.507**	**0.0108**	2.2225	1, 5.0197	0.1960	4.2838	1, 5.1217	0.0919	**9.8267**	**1, 4.0541**	**0.0344**	0.4354	1, 5.4264	0.5363
Sds	**212.42**	**1, 5.1051**	**0.0001**	**216.47**	**1, 5.1932**	**0.0001**	**469.77**	**1, 6.0922**	**0.0001**	**100.03**	**1, 5.0837**	**0.0002**	**137.79**	**1, 4.3709**	**0.0002**	**50.877**	**1, 5.1497**	**0.0007**
Str	**10.497**	**1, 11**	**0.0079**	**36.340**	**1, 10**	**0.0001**	4.2607	1, 10	0.0659	2.8143	1, 10	0.1244	**6.1398**	**1, 8**	**0.0382**	**4.1331**	**1, 5.9423**	**0.0888**
Sdq	**13.166**	**1, 5.1447**	**0.0144**	**186.60**	**1, 10**	**0.0001**	**22.357**	**1, 5.0951**	**0.005**	**32.281**	**1, 5.1743**	**0.0021**	**47.367**	**1, 4.4372**	**0.0016**	**16.327**	**1, 5.5227**	**0.0081**
Ssc	**419.08**	**1, 5.2333**	**0.0001**	**2998.2**	**1, 10**	**0.0001**	**607.57**	**1, 5.3253**	**0.0001**	**94.865**	**1, 5.0295**	**0.0002**	**1107.6**	**1, 8**	**0.0001**	**17.084**	**1, 5.0563**	**0.0088**
Sdr	**16.348**	**1, 5.0414**	**0.0097**	**220.96**	**1, 5.8554**	**0.0001**	**22.671**	**1, 5.0171**	**0.005**	**45.199**	**1, 5.0475**	**0.0011**	**47.448**	**1, 4.1105**	**0.0021**	**19.552**	**1, 5.1018**	**0.0066**
Vmp	1.7287	1, 5.0785	0.2448	0.0034	1, 10	0.9549	0.101	1, 5.3524	0.7626	0.0066	1, 5.5356	0.9381	0.1753	1, 8	0.6864	0.0025	1, 5.9083	0.9621
Vmc	2.7722	1, 5.0425	0.1563	3.6401	1, 10	0.0855	0.1868	1, 5.2118	0.6829	0.6815	1, 5.0334	0.4464	0.1544	1, 8	0.7047	0.2289	1, 6.28	0.6485
Vvc	2.2973	1, 5.0472	0.1895	2.5846	1, 10	0.1390	0.226	1, 5.2321	0.6537	0.6148	1, 5.0752	0.4680	0.4188	1, 8	0.5356	0.351	1, 6.3191	0.5741
Vvv	1.3323	1, 5.0583	0.3000	0.2449	1, 10	0.6314	0.3809	1, 5.0848	0.5637	0.0172	1, 5.0765	0.9008	0.3635	1, 4.1076	0.5783	0.1449	1, 6.0605	0.7164
Spk	1.0297	1, 5.0897	0.3560	0.2096	1, 10	0.6568	0.2202	1, 5.2598	0.6577	0.0037	1, 5.4591	0.9539	0.2107	1, 8	0.6585	0.1502	1, 6.2258	0.7112
Sk	2.6832	1, 5.0455	0.1618	4.2515	1, 10	0.0662	0.1985	1, 5.2245	0.6738	0.754	1, 5.0243	0.4247	0.2928	1, 8	0.6032	0.0515	1, 6.5493	0.8273
Svk	0.4083	1, 5.1008	0.5504	1.3129	1, 6.2344	0.2939	0.7565	1, 5.0437	0.4239	0.3016	1, 5.0801	0.6062	1.6222	1, 4.0448	0.271	0.4237	1, 6.295	0.5382
Smr1	**11.786**	**1, 5.6309**	**0.0154**	3.5133	1, 10	0.0904	0.0757	1, 10	0.7889	0.01	1, 10	0.9223	0.1779	1, 8	0.6843	0.9959	1, 5.0321	0.3638
Smr2	5.6605	1, 5.4092	0.0594	0.9163	1, 10	0.3610	0.0355	1, 10	0.8543	1.0951	1, 5.0048	0.3432	1.8657	1, 8	0.2091	0.9322	1, 5.0097	0.3786
S5z	0.2702	1, 5.107	0.6250	**16.787**	**1, 5.6195**	**0.0073**	2.659	1, 5.0121	0.1638	4.02	1, 5.0366	0.1009	**8.2873**	**1, 4.0561**	**0.0443**	1.4015	1, 6.0651	0.2808
Sa	2.2987	1, 5.0465	0.1894	2.0464	1, 10	0.1519	0.2328	1, 5.1807	0.6492	0.4555	1, 5.0549	0.5294	0.0358	1, 8	0.8547	0.0226	1, 6.3432	0.8851

Table 4.16: ANOVA/Welch ANOVA analyses comparing the impacts of tooth size on the effects of siliciclastic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	d.f	p	F	d.f	P	F	d.f	p	F	d.f	p	F	d.f	p	F	d.f	p
Sq	0.0828	1, 9	0.78	0.8335	1, 9	0.3851	0.0494	1, 7	0.8304	0.9405	1, 7	0.3645	0.1831	1, 9	0.6788	1.4194	1, 5.291	0.2842
Ssk	4.2512	1, 9	0.0693	0.7	1, 9	0.4244	0.2921	1, 7	0.6056	0.3495	1, 7	0.573	0.2402	1, 9	0.6358	0.2395	1, 10	0.6351
Sku	0.6153	1, 9	0.4529	0.6095	1, 4.271	0.476	0.8419	1, 7	0.3894	0.3512	1, 7	0.5721	1.047	1, 9	0.3329	3.3402	1, 10	0.0976
Sp	0.2281	1, 4.770	0.617	0.1094	1, 9	0.7484	1.24	1, 5.524	0.3116	0.0664	1, 7	0.804	0.9819	1, 9	0.3476	**9.0051**	**1, 5.4859**	**0.0268**
Sv	0.9631	1, 9	0.3521	0.378	1, 4.7537	0.5669	0.3429	1, 7	0.5766	0.0189	1, 7	0.8944	1.3462	1, 9	0.2758	**14.339**	**1, 59.429**	**0.0093**
Sz	0.4169	1, 9	0.5346	0.0936	1, 9	0.7825	0.4467	1, 7	0.5253	0.0443	1, 7	0.7393	1.1889	1, 9	0.3039	**13.490**	**1, 5.8108**	**0.011**
Sds	**448.90**	**1, 9**	**0.0001**	**62.193**	**1, 4.4074**	**0.0009**	**11.631**	**1, 7**	**0.0113**	**19.428**	**1, 7**	**0.0031**	**47.163**	**1, 9**	**0.0001**	**29.085**	**1, 5.218**	**0.0026**
Str	1.0496	1, 9	0.3323	0.0528	1, 9	0.8234	**8.413**	**1, 7**	**0.023**	0.9475	1, 7	0.3628	2.7258	1, 9	0.1331	**5.6685**	**1, 10**	**0.0386**
Sdq	**105.51**	**1, 9**	**0.0001**	**20.529**	**1, 9**	**0.0014**	1.9587	1, 7	0.2044	4.6137	1, 7	0.0688	**12.211**	**1, 9**	**0.0068**	**19.982**	**1, 5.4471**	**0.0012**
Ssc	**2948.7**	**1, 9**	**0.0001**	**658.85**	**1, 9**	**0.0001**	**185.71**	**1, 7**	**0.0001**	**670.33**	**1, 7**	**0.0001**	**517.77**	**1, 9**	**0.0001**	**931.35**	**1, 10**	**0.0001**
Sdr	**85.493**	**1, 4.710**	**0.0001**	**32.345**	**1, 9**	**0.0003**	1.7372	1, 7	0.229	**9.1451**	**1, 5.3817**	**0.0267**	**12.694**	**1, 9**	**0.0061**	**13.562**	**1, 5.0651**	**0.0042**
Vmp	2.2931	1, 6.196	0.1792	1.8126	1, 9	0.2111	0.0321	1, 7	0.8629	0.5675	1, 7	0.4758	0.2398	1, 9	0.6361	0.7691	1, 5.642	0.4163
Vmc	0.0115	1, 9	0.917	0.5900	1, 9	0.4621	0.0088	1, 7	0.9279	1.1942	1, 7	0.3107	0.039	1, 9	0.8479	1.0422	1, 5.3087	0.3516
Vvc	0.1333	1, 9	0.7235	0.9208	1, 9	0.3623	0.0745	1, 7	0.7927	1.0167	1, 7	0.3469	0.0366	1, 9	0.8526	0.7073	1, 5.4054	0.436
Vvv	0.3794	1, 9	0.5532	0.8456	1, 9	0.3818	0.1234	1, 7	0.7357	0.9669	1, 7	0.3582	0.3009	1, 9	0.5966	0.8596	1, 5.4269	0.3932
Spk	2.0140	1, 9	0.1895	1.5293	1, 9	0.2476	0.002	1, 7	0.9756	0.3907	1, 7	0.5517	0.2389	1, 9	0.6367	2.1682	1, 5.8036	0.1929
Sk	0.0631	1, 9	0.8072	0.5263	1, 9	0.4866	0.0351	1, 7	0.8567	1.3076	1, 7	0.2904	0.0273	1, 9	0.8724	1.6187	1, 5.4204	0.2551
Svk	0.5028	1, 9	0.4962	0.3657	1, 9	0.5603	0.1573	1, 7	0.7035	0.6681	1, 7	0.4406	0.5104	1, 9	0.4931	1.531	1, 6.0688	0.2617
Smr1	2.0375	1, 9	0.1872	2.6257	1, 9	0.1396	0.0297	1, 7	0.8682	0.0312	1, 7	0.8647	0.0072	1, 9	0.9343	4.0797	1, 5.6228	0.0931
Smr2	0.4859	1, 9	0.5034	0.1298	1, 9	0.7269	0.0608	1, 7	0.8124	0.355	1, 7	0.5701	0.2806	1, 9	0.6092	0.9576	1, 10	0.3509
S5z	**6.5323**	**1, 9**	**0.0309**	0.0348	1, 9	0.8562	0.5761	1, 7	0.4726	0.0314	1, 7	0.8644	1.433	1, 9	0.2619	**14.435**	**1, 5.2682**	**0.0115**
Sa	0.0085	1, 9	0.9287	0.7991	1, 9	0.3947	0.0024	1, 7	0.9626	1.0929	1, 7	0.3306	0.0912	1, 9	0.7695	1.4555	1, 5.2817	0.2789

Table 4.17: ANOVA/Welch ANOVA analyses comparing the impacts of tooth size on the effects of calcarenitic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	d.f	p	F	d.f	P	F	d.f	p	F	d.f	p	F	d.f	p	F	d.f	p
Sq	**10.50**	**1, 10**	**0.009**	1.439	1, 10	0.258	0.0287	1, 10	0.869	0.322	1, 10	0.5829	0.421	1, 10	0.531	1.6171	1, 5.627	0.2535
Ssk	1.936	1, 6.200	0.194	0.0825	1, 10	0.780	1.4768	1, 6.157	0.2688	**8.5789**	**1, 10**	**0.0151**	0.7104	1, 10	0.419	0.366	1, 10	0.5587
Sku	3.740	1, 10	0.082	0.4302	1, 5.828	0.537	1.1263	1, .352	0.3341	0.1102	1, 6.1943	0.7508	2.595	1, 6.080	0.1622	0.8159	1, 5.2904	0.5046
Sp	0.054	1, 10	0.821	0.0581	1, 10	0.814	**5.6963**	**1, 10**	**0.0382**	1.0668	1, 10	0.326	**7.9375**	**1, 5.346**	**0.0346**	4.8108	1, 10	0.053
Sv	0.207	1, 10	0.659	0.2635	1, 10	0.619	1.3498	1, 5.352	0.2945	4.0753	1, 10	0.0711	2.496	1, 10	0.1464	2.8303	1, 10	0.1234
Sz	0.152	1, 10	0.705	0.041	1, 10	0.844	3.2074	1, 5.935	0.124	2.4341	1, 10	0.1498	4.8076	1, 10	0.0531	**5.0143**	**1, 10**	**0.0491**
Sds	**71.63**	**1, 5.805**	**0.001**	**60.034**	**1, 5.501**	**0.001**	**75.012**	**1, 5.404**	**0.0002**	**229.22**	**1, 6.248**	**0.0001**	**69.573**	**1, 6.042**	**0.0002**	**106.99**	**1, 5.766**	**0.0001**
Str	0.766	1, 6.297	0.414	0.5123	1, 10	0.495	0.0009	1, 10	0.9761	1.9244	1, 6.106	0.2139	**9.1251**	**1, 5.449**	**0.0264**	4.5287	1, 10	0.0592
Sdq	**65.39**	1, 10	**0.001**	**6.8849**	**1, 10**	**0.025**	**56.488**	**1, 10**	**0.0001**	**124.25**	**1, 10**	**0.0001**	**35.536**	**1, 10**	**0.0001**	**60.022**	**1, 10**	**0.0001**
Ssc	**1269**	1, 10	**0.001**	**373.50**	**1, 5.592**	**0.001**	**1050.2**	**1, 5.131**	**0.0001**	**2832.8**	**1, 10**	**0.0001**	**808.84**	**1, 5.773**	**0.0001**	**1049.1**	**1 ,5.642**	**0.0001**
Sdr	**61.86**	1, 10	**0.001**	**21.557**	**1, 10**	**0.001**	**70.629**	**1, 10**	**0.0001**	**247.56**	**1, 10**	**0.0001**	**55.513**	**1, 10**	**0.0001**	**85.398**	**1, 10**	**0.0001**
Vmp	3.223	1, 10	0.103	0.4114	1, 10	0.536	0.4762	1, 10	0.5059	0.0642	1, 10	0.8052	0.0149	1, 10	0.9051	0.0101	1, 10	0.9219
Vmc	3.593	1, 10	0.087	2.0885	1, 10	0.179	0.0011	1, 10	0.9738	0.233	1, 10	0.6397	0.6953	1, 10	0.4238	1.527	1, 5.777	0.2644
Vvc	4.075	1, 10	0.071	1.9486	1, 10	0.193	0.1827	1, 10	0.6781	0.0008	1, 10	0.9779	0.4156	1, 10	0.5336	0.5093	1, 6.005	0.5022
Vvv	**8.401**	**1, 10**	**0.0159**	0.9848	1, 10	0.3444	0.6649	1, 10	0.4338	2.7295	1, 10	0.1295	0.6995	1, 10	0.4225	**7.1172**	**1, 6.250**	**0.0357**
Spk	2.527	1, 10	0.143	0.938	1, 10	0.3556	0.6639	1, 10	0.4342	0.1077	1, 10	0.7495	0.2189	1, 10	0.6499	0.106	1, 10	0.7517
Sk	3.116	1, 10	0.108	1.9286	1, 10	0.1951	0.0168	1, 10	0.8993	0.0055	1, 10	0.9422	0.5371	1, 10	0.4805	1.5255	1, 6.020	0.2628
Svk	**6.108**	**1, 10**	**0.033**	0.3765	1, 10	0.5532	0.0262	1, 10	0.8747	0.7001	1, 10	0.4223	0.1093	1, 10	0.7478	2.394	1, 10	0.1528
Smr1	0.253	1, 10	0.6259	0.2152	1, 10	0.6256	**5.7175**	**1, 10**	**0.0379**	0.4111	1, 10	0.5358	0.7085	1, 10	0.4196	2.4979	1, 5.521	0.1693
Smr2	0.124	1, 10	0.2906	0.516	1, 10	0.4892	**13.427**	**1, 10**	**0.0044**	**9.932**	**1, 10**	**0.0103**	2.697	1, 5.382	0.1573	3.006	1, 10	0.1136
S5z	0.022	1, 5.698	0.8875	0.028	1, 10	0.8707	2.9733	1, 5.928	0.136	2.082	1, 10	0.1797	**5.584**	**1, 10**	**0.0398**	**6.668**	**1, 6.120**	**0.0409**
Sa	**6.259**	**1, 10**	**0.0315**	1.665	1, 10	0.226	0.0134	1, 10	0.9105	0.425	1, 10	0.529	0.524	1, 10	0.4859	1.643	1, 5.654	0.25

Table 4.18: ANOVA/Welch ANOVA analyses comparing the impacts of tooth morphology on the effects of siliciclastic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA

	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
Parameter	**F**	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	2.2	1, 9	0.1722	1.6058	1, 9	0.2369	0.0607	1, 10	0.8104	0.1456	1, 5.8244	0.7163	0.0524	1, 10	0.8235	2.739	1, 5.027	0.1585
Ssk	0.0237	1, 9	0.881	2.4564	1, 9	0.1515	0.954	1, 5.7856	0.3678	0.9752	1, 10	0.3467	0.1123	1, 10	0.7444	1.837	1, 10	0.2051
Sku	5.058	1, 9	0.0511	0.0002	1, 9	0.9894	1.8337	1, 5.656	0.2273	0.9044	1, 10	0.364	0.4298	1, 10	0.5269	0.0004	1, 6.3345	0.9837
Sp	0.0067	1, 9	0.9365	3.0485	1, 9	0.1148	1.0056	1, 5.8485	0.3556	0.0002	1, 10	0.9881	0.8882	1, 10	0.3682	**6.1691**	**1, 5.9253**	**0.0481**
Sv	0.0587	1, 9	0.814	0.4293	1, 9	0.5287	0.6957	1, 10	0.4237	0.8324	1, 10	0.3731	0.4799	1, 10	0.5042	**6.9144**	**1, 10**	**0.0252**
Sz	0.0356	1, 9	0.8545	1.0656	1, 9	0.3289	0.8596	1, 6.145	0.3888	0.1453	1, 10	0.711	0.6738	1, 10	0.4309	**7.7385**	**1, 10**	**0.0194**
Sds	0.2026	1, 9	0.6632	0.5712	1, 9	0.4691	0.4223	1, 10	0.5305	0.0103	1, 6.0957	0.9222	0.0002	1, 10	0.9902	4.6496	1, 5.787	0.0761
Str	0.5333	1, 9	0.4838	0.5203	1, 9	0.489	0.2946	1, 10	0.5991	1.9144	1, 10	0.1966	1.9441	1, 6.227	0.2109	0.3665	1, 10	0.5584
Sdq	1.6735	1, 9	0.228	0.3269	1, 9	0.5815	0.1116	1, 5.2406	0.7513	0.1647	1, 5.4262	0.7004	0.044	1, 10	0.8381	3.7466	1, 5.1943	0.1085
Ssc	1.1918	1, 9	0.3033	0.0968	1, 9	0.7628	0.539	1, 5.0531	0.4955	0.431	1, 10	0.5263	0.3751	1, 5.847	0.5539	3.2642	1, 6.0959	0.1201
Sdr	1.517	1, 9	0.2493	0.2656	1, 9	0.6187	0.1092	1, 5.2939	0.7538	0.2926	1, 5.2371	0.6107	0.0673	1, 10	0.8006	3.2653	1, 5.1955	0.1284
Vmp	0.8102	1, 9	0.3915	4.8909	1, 5.6241	0.072	0.0225	1, 10	0.8841	0.0761	1, 10	0.7883	0.0485	1, 10	0.8301	1.7558	1, 5.1241	0.2412
Vmc	3.5088	1, 9	0.0938	0.7728	1, 9	0.4022	0.1231	1, 10	0.733	0.2246	1, 5.1486	0.655	0.182	1, 10	0.6787	1.6388	1, 5.0393	0.2563
Vvc	4.1061	1, 9	0.0734	1.6013	1, 9	0.2375	0.3594	1, 10	0.5622	0.2171	1, 5.2925	0.6598	0.2381	1, 10	0.6361	1.2936	1, 5.0667	0.3063
Vvv	0.8805	1, 9	0.3726	1.2492	1, 9	0.2927	0.4014	1, 10	0.5406	0.2024	1, 6.0241	0.6686	0.008	1, 10	0.9306	2.424	1, 5.0193	0.18
Spk	0.6591	1, 9	0.4378	5.0093	1, 6.1444	0.0655	0.0095	1, 10	0.9243	0.0831	1, 10	0.7791	0.2374	1, 10	0.6366	3.5885	1, 5.181	0.1147
Sk	3.6913	1, 9	0.0869	0.5868	1, 9	0.4633	0.2175	1, 10	0.651	0.2134	1, 5.1107	0.6631	0.1936	1, 10	0.6693	2.3921	1, 5.0675	0.1818
Svk	0.386	1, 9	0.5498	1.2852	1, 9	0.2862	0.6007	1, 6.1067	0.4673	0.0134	1, 10	0.9103	0.0128	1, 10	0.9123	3.8002	1, 5.0537	0.1081
Smr1	0.0028	1, 9	0.9593	**9.9236**	**1, 9**	**0.0117**	0.3888	1, 10	0.5471	0.115	1, 10	0.7416	0.581	1, 10	0.4635	2.7302	1, 5.4534	0.1545
Smr2	0.513	1, 9	0.492	0.0886	1, 9	0.7727	1.3521	1, 10	0.2822	0.4494	1, 10	0.5178	1.0099	1, 6.1952	0.3526	**5.8108**	**1, 10**	**0.0366**
S5z	0.0598	1, 9	0.8123	1.4995	1, 9	0.2518	1.0325	1, 5.8663	0.3496	0.0297	1, 10	0.8666	0.1409	1, 10	0.7153	**6.0222**	**1, 10**	**0.0439**
Sa	3.0631	1, 9	0.114	1.2774	1, 9	0.2876	0.0096	1, 10	0.924	0.1872	1, 5.3844	0.6821	0.1112	1, 10	0.7456	2.5441	1, 5.0331	0.1712

Table 4.19: ANOVA/Welch ANOVA analyses comparing the impacts of tooth morphology on the effects of calcarenitic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA

	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
Parameter	**F**	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	1.5459	1, 5.2566	0.2663	1.8125	1, 10	0.2079	2.0205	1, 10	0.1856	**38.2034**	**1, 4.1919**	**0.003**	2.8904	1, 9	0.1233	0.1842	1, 10	0.6769
Ssk	3.3619	1, 6.2753	0.1143	1.5507	1, 10	0.2414	0.0175	1, 10	0.8974	**9.5847**	**1, 9**	**0.0128**	0.0984	1, 9	0.7608	2.0983	1, 10	0.1781
Sku	1.2587	1, 11	0.2858	0.2339	1, 5.2175	0.6483	0.0268	1, 10	0.8732	2.2504	1, 9	0.1678	0.3248	1, 9	0.5817	0.6948	1, 10	0.4240
Sp	4.1177	1, 5.4845	0.0932	2.4820	1, 10	0.1462	**5.5553**	**1, 10**	**0.0402**	**46.5672**	**1, 9**	**<0.0001**	1.2944	1, 9	0.2846	0.888	1, 10	0.3682
Sv	0.0073	1, 11	0.9334	1.0581	1, 10	0.3279	0.2684	1, 6.0074	0.6229	**33.5772**	**1, 4.3335**	**0.0034**	2.578	1, 9	0.1428	0.3006	1, 10	0.5955
Sz	0.9384	1, 11	0.3535	2.0191	1, 10	0.1858	1.8544	1, 6.306	0.2199	**68.7099**	**1, 9**	**<0.0001**	3.4981	1, 9	0.0942	0.6181	1, 10	0.4500
Sds	1.0205	1, 11	0.3341	2.3969	1, 10	0.1526	2.973	1, 5.2005	0.1430	4.8147	1, 9	0.0559	0.1078	1, 9	0.7502	2.0396	1, 10	0.1837
Str	3.0491	1, 11	0.1086	0.0326	1, 10	0.8604	0.3006	1, 10	0.5956	4.1476	1, 9	0.0722	1.9466	1, 9	0.1964	1.1931	1, 10	0.3003
Sal	2.1538	1, 11	0.1702	0.1508	1, 5.362	0.7127	3.3471	1, 10	0.0973	**14.1247**	**1, 9**	**0.0045**	0.001	1, 9	0.9756	0.2941	1, 10	0.5995
Sdq	2.1411	1, 5.8963	0.1946	3.6207	1, 10	0.0862	**6.2433**	**1, 10**	**0.0315**	**26.803**	**1, 4.5485**	**0.0046**	3.4326	1, 9	0.0969	0.1327	1, 10	0.7232
Ssc	3.118	1, 5.851	0.1291	3.5047	1, 10	0.0907	4.8838	1, 10	0.0516	**32.444**	**1, 4.5769**	**0.0031**	0.8162	1, 9	0.3898	0.0504	1, 10	0.8269
Sdr	2.0422	1, 5.7943	0.2046	3.3112	1, 10	0.0988	**6.1045**	**1, 6.2823**	**0.0466**	**20.2197**	**1, 4.2252**	**0.0096**	3.4154	1, 9	0.0976	0.0991	1, 10	0.7593
Vmp	4.4425	1, 5.4259	0.0845	0.6935	1, 10	0.4244	0.8251	1, 10	0.3851	**71.3551**	**1, 9**	**<0.0001**	0.6741	1, 9	0.4328	1.7361	1, 10	0.2170
Vmc	1.297	1, 5.1687	0.3048	1.8300	1, 10	0.2059	2.1813	1, 10	0.1750	**25.6589**	**1, 4.0583**	**0.0069**	3.8554	1, 9	0.0812	0.0056	1, 10	0.9417
Vvc	1.7584	1, 5.1958	0.2401	1.4398	1, 10	0.2578	1.9844	1, 10	0.1893	**26.3698**	**1, 4.138**	**0.0062**	1.9799	1, 9	0.1930	0.0828	1, 10	0.7794
Vvv	0.7208	1, 5.4904	0.4313	2.6239	1, 10	0.1363	1.7283	1, 10	0.2180	**53.6582**	**1, 4.255**	**0.0015**	4.332	1, 9	0.0671	0.0204	1, 10	0.8893
Spk	4.487	1, 5.4627	0.0830	0.1779	1, 10	0.6821	1.4581	1, 10	0.2550	**73.9337**	**1, 9**	**<0.0001**	0.683	1, 9	0.4299	1.1724	1, 10	0.3043
Sk	1.3373	1, 5.167	0.2982	1.9035	1, 10	0.1978	2.4729	1, 10	0.1469	**22.3191**	**1, 4.0402**	**0.0089**	3.7878	1, 9	0.0835	0.0002	1, 10	0.9901
Svk	0.473	1, 6.0635	0.5170	2.5816	1, 10	0.1392	1.4547	1, 10	0.2555	**68.107**	**1, 4.3153**	**0.0008**	4.5387	1, 9	0.0602	0.0213	1, 10	0.8867
Smr1	1.6852	1, 5.7357	0.2440	0.1954	1, 5.4685	0.6754	0.0416	1, 10	0.8426	0.6141	1, 9	0.4534	2.2441	1, 9	0.1683	**9.2373**	**1, 10**	**0.0125**
Smr2	2.3996	1, 11	0.1496	0.1785	1, 10	0.6816	1.1182	1, 10	0.3152	**7.9068**	**1, 9**	**0.0203**	0.0346	1, 9	0.8566	0.0588	1, 10	0.8133
S5z	1.59	1, 5.8474	0.2553	2.4551	1, 10	0.1482	2.4757	1, 5.5838	0.1703	**98.6805**	**1, 4.3881**	**0.0004**	4.3098	1, 9	0.0677	0.7364	1, 10	0.4109
Sa	1.4392	1, 5.2145	0.2819	1.7017	1, 10	0.2213	2.0869	1, 10	0.1806	**31.3788**	**1, 4.1119**	**0.0046**	0.1087	1, 9	0.1087	0.0542	1, 10	0.8206

Table 4.20: ANOVA/Welch ANOVA analyses comparing the impacts of different sediment types upon the microtextural surfaces of large C. taurus teeth. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
Parameter	**F**	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	**17.3464**	**1, 10**	**0.0019**	3.1248	1, 10	0.1076	0.0115	1, 10	0.9174	3.4405	1, 10	0.106	1.3233	1, 10	0.2796	0.4359	1, 10	0.5240
Ssk	**7.2848**	**1, 10**	**0.0224**	0.2746	1, 10	0.6116	0.2062	1, 10	0.6635	4.4387	1, 10	0.0731	1.2778	1, 10	0.2875	0.0284	1, 10	0.8695
Sku	0.8249	1, 10	0.3851	1.8301	1, 10	0.2059	2.4206	1, 10	0.1637	4.2495	1, 10	0.0782	0.0135	1, 10	0.9100	0.2258	1, 10	0.6449
Sp	0.0040	1, 10	0.9511	1.9092	1, 10	0.1917	**7.0149**	**1, 10**	**0.0330**	0.6237	1, 10	0.4556	0.9159	1, 10	0.3636	1.5733	1, 10	0.2382
Sv	**4.0006**	**1, 10**	**0.0734**	2.2398	1, 10	0.1654	0.9936	1, 10	0.3521	0.1753	1, 10	0.6880	0.2767	1, 10	0.6116	0.0004	1, 10	0.9850
Sz	2.8617	1, 10	0.1216	2.6476	1, 10	0.1348	2.2495	1, 10	0.1773	0.3726	1, 10	0.5609	0.0100	1, 10	0.9225	0.3012	1, 10	0.5951
Sds	**15.6649**	**1, 10**	**0.0027**	0.7025	1, 10	0.4215	0.7774	1, 10	0.4072	**16.7121**	**1, 10**	**0.0046**	0.7884	1, 10	0.3977	1.3023	1, 10	0.2804
Str	0.4424	1, 10	0.5210	0.0124	1, 10	0.9137	5.2051	1, 10	0.0565	0.0128	1, 10	0.9131	0.0885	1, 10	0.7729	0.1613	1, 10	0.6964
Sdq	**12.4532**	**1, 10**	**0.0055**	2.3894	1, 10	0.1532	0.5476	1, 10	0.4834	2.9972	1, 10	0.127	0.1738	1, 10	0.6866	0.2095	1, 10	0.6569
Ssc	**17.1684**	**1, 10**	**0.0020**	1.6870	1, 10	0.2231	0.0049	1, 10	0.9462	0.0344	1, 10	0.8582	0.2014	1, 10	0.6642	0.7378	1, 10	0.4105
Sdr	**12.7534**	**1, 10**	**0.0051**	2.9072	1, 10	0.1190	0.4146	1, 10	0.5402	3.5185	1, 10	0.1028	0.0123	1, 10	0.9143	0.6684	1, 10	0.4326
Vmp	0.0302	1, 10	0.8654	2.1791	1, 10	0.1707	0.7186	1, 10	0.4246	3.2610	1, 10	0.1139	0.5992	1, 10	0.4588	1.2226	1, 10	0.2947
Vmc	**12.3935**	**1, 10**	**0.0055**	2.8037	1, 10	0.1250	0.0658	1, 10	0.8050	4.3383	1, 10	0.0758	1.4330	1, 10	0.2619	0.0213	1, 10	0.8868
Vvc	**7.2217**	**1, 10**	**0.0228**	3.2909	1, 10	0.0997	0.0071	1, 10	0.9351	4.8222	1, 10	0.0641	1.7604	1, 10	0.2173	0.0785	1, 10	0.7851
Vvv	**24.3228**	**1, 10**	**0.0006**	3.1710	1, 10	0.1053	0.2250	1, 10	0.6497	1.6212	1, 10	0.2436	1.2125	1, 10	0.2994	1.1847	1, 10	0.3019
Spk	0.0524	1, 10	0.8325	3.1172	1, 10	0.1079	1.2039	1, 10	0.3089	3.2335	1, 10	0.1152	0.4989	1, 10	0.4978	1.3555	1, 10	0.2715
Sk	**13.1789**	**1, 10**	**0.0046**	2.2459	1, 10	0.1649	0.0259	1, 10	0.8767	4.5107	1, 10	0.0713	1.3453	1, 10	0.2759	0.0580	1, 10	0.8146
Svk	**18.5458**	**1, 10**	**0.0015**	3.2639	1, 10	0.1010	0.1591	1, 10	0.7018	0.9596	1, 10	0.3599	0.9801	1, 10	0.3480	1.4704	1, 10	0.2532
Smr1	1.2743	1, 10	0.2853	**6.6329**	**1, 10**	**0.0306**	3.0865	1, 10	0.1224	0.2140	1, 10	0.6577	0.0908	1, 10	0.7700	3.7392	1, 10	0.0819
Smr2	1.2208	1, 10	0.2951	0.5913	1, 10	0.4597	2.4269	1, 10	0.1632	2.9520	1, 10	0.1295	1.5484	1, 10	0.2448	0.0088	1, 10	0.9270
S5z	**21.6599**	**1, 10**	**0.0009**	3.5197	1, 10	0.0901	1.3509	1, 10	0.2832	0.6874	1, 10	0.4344	0.3238	1, 10	0.5833	0.1509	1, 10	0.7058
Sa	**114.3494**	**1, 10**	**0.0036**	2.8791	1, 10	0.1206	0.0080	1, 10	0.9311	3.9107	1, 10	0.0885	1.3534	1, 10	0.2746	0.0758	1, 10	0.7887

Table 4.21: ANOVA/Welch ANOVA analyses comparing the impacts of different sediment types upon the microtextural surfaces of small C. taurus teeth. Bold text indicates a significant result, a decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	d.f	p	F	d.f	P	F	d.f	p	F	d.f	p	F	d.f	p	F	d.f	p
Sq	3.4524	1, 5.1821	0.1202	0.9275	1, 9	0.3607	0.0047	1, 9	0.9467	**16.763**	**1, 9**	**0.0027**	0.7274	1, 9	0.4137	1.1977	1, 9	0.2994
Ssk	0.8108	1, 9	0.3913	0.0012	1, 9	0.9729	1.3020	1, 5.3263	0.3025	**5.4969**	**1, 9**	**0.0437**	1.0211	1, 9	0.3361	0.4885	1, 9	0.5006
Sku	4.1534	1, 9	0.072	0.0393	1, 9	0.8478	2.8080	1, 5.0389	0.1542	0.0848	1, 9	0.7775	0.8738	1, 6.019	0.3859	2.2650	1, 9	0.1632
Sp	0.7310	1, 9	0.4147	0.0284	1, 9	0.87	0.5261	1, 56797	0.497	1.8652	1, 6.017	0.2209	0.298	1, 6.259	0.604	**10.150**	**1, 9**	**0.0097**
Sv	0.2087	1, 9	0.6586	0.3431	1, 9	0.5724	1.9468	1, 5.5749	0.216	**6.3451**	**1, 9**	**0.0328**	0.4166	1, 6.268	0.5332	**15.999**	**1, 9**	**0.0025**
Sz	0.0198	1, 9	0.8912	0.1412	1, 9	0.7158	1.4043	1, 5.6064	0.2838	3.6873	1, 9	0.087	0.3748	1, 5.868	0.5541	**15.304**	**1, 9**	**0.0031**
Sds	**402.57**	**1, 9**	**0.0001**	**68.056**	**1, 9**	**0.0001**	**36.074**	**1, 5.0102**	**0.0018**	**43.742**	**1, 5.166**	**0.001**	**73.983**	**1, 9**	**0.0001**	**30.886**	**1, 5.0271**	**0.0025**
Str	0.4397	1, 9	0.5239	2.6170	1, 9	0.1402	5.8114	1, 6.2899	0.0506	0.0957	1, 9	0.764	4.2485	1, 9	0.0663	**11.158**	**1, 9**	**0.0075**
Sdq	4.4491	1, 9	0.0641	**21.399**	**1, 9**	**0.0012**	5.0776	1, 58935	0.0659	0.7942	1, 9	0.396	**16.644**	**1, 5.499**	**0.0078**	**18.825**	**1, 5.7961**	**0.0053**
Ssc	**336.23**	**1, 5.3795**	**0.0001**	**474.45**	**1, 9**	**0.0001**	**421.07**	1, 9	**0.0001**	**338.86**	**1, 9**	**0.0001**	**740.07**	**1, 9**	**0.0001**	**1152.1**	**1, 9**	**0.0001**
Sdr	**15.071**	**1, 9**	**0.0037**	**28.386**	**1, 9**	**0.0005**	4.0092	1, 5.2215	0.0992	2.8298	1, 9	0.1268	**16.524**	**1, 5.155**	**0.0091**	**13.075**	**1, 5.1192**	**0.0147**
Vmp	4.8795	1, 5.6304	0.0722	0.2787	1, 9	0.6103	0.0370	1, 9	0.8514	**9.1928**	**1, 9**	**0.0142**	0.552	1, 9	0.4746	0.7555	1, 9	0.4051
Vmc	3.3984	1, 5.2303	0.122	0.8205	1, 9	0.3886	0.6634	1, 9	0.4343	**15.975**	**1, 9**	**0.0031**	1.9661	1, 9	0.1911	0.5773	1, 9	0.4649
Vvc	3.7701	1, 5.2852	0.1067	0.9391	1, 9	0.3578	0.8318	1, 9	0.3832	**13.646**	**1, 9**	**0.005**	1.5828	1, 9	0.2369	0.5312	1, 9	0.4828
Vvv	2.5768	1, 5.3154	0.1659	0.9130	1, 9	0.3643	0.1333	1, 5.8751	0.7278	**22.590**	**1, 9**	**0.001**	0.0447	1, 9	0.8369	0.9801	1, 9	0.3455
Spk	4.5622	1, 5.6287	0.0796	0.8456	1, 9	0.3818	0.0001	1, 9	0.992	**7.6268**	**1, 9**	**0.0221**	0.352	1, 9	0.5662	1.7906	1, 9	0.2105
Sk	3.4222	1, 5.2564	0.1207	0.7407	1, 9	0.4118	1.0042	1, 9	0.3399	**14.888**	**1, 9**	**0.0039**	2.0697	1, 9	0.1808	0.8452	1, 9	0.3795
Svk	1.4713	1, 5.51	0.2746	0.2619	1, 9	0.6212	0.5343	1, 5.8034	0.4933	**21.106**	**1, 9**	**0.0013**	0.0509	1, 5.784	0.8293	2.1164	1, 9	0.1764
Smr1	0.2577	1, 9	0.6253	1.1689	1, 5.2107	0.3271	0.7480	1, 9	0.4074	1.1334	1, 9	0.3148	0.1493	1, 9	0.7073	0.2330	1, 9	0.6397
Smr2	0.0362	1, 9	0.8533	0.0088	1, 9	0.9272	0.2832	1, 9	0.6062	**7.3458**	**1, 9**	**0.0240**	0.256	1, 9	0.6238	2.7930	1, 9	0.1256
S5z	0.0077	1, 9	0.9322	0.0811	1, 9	0.7822	1.4844	1, 5.7557	0.2707	**5.5742**	**1, 9**	**0.0426**	0.6537	1, 5.577	0.4519	**12.440**	**1, 9**	**0.0055**
Sa	3.4000	1, 5.1973	0.1181	0.9080	1, 9	0.3655	0.1844	1, 9	0.6767	**16.584**	**1, 9**	**0.0028**	1.2546	1, 9	0.2889	1.0383	1, 9	0.3322

Table 4.22: ANOVA/Welch ANOVA analyses comparing the impacts of different sediment types upon the microtextural surfaces of small unworn H. elongatus teeth. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	d.f	p	F	d.f	p	F	d.f	P	F	d.f	p	F	d.f	p	F	d.f	p
Sq	3.5254	1, 10	0.0899	4.7012	1, 10	0.0553	0	1, 5.2488	1	0.4632	1, 5.4853	0.5238	0.2925	1, 8	0.6017	0.0322	1, 10	0.8613
Ssk	0.0984	1, 10	0.7603	0.0597	1, 10	0.812	0.2238	1, 10	0.6463	0.6123	1, 9	0.4521	2.1947	1, 4.4518	0.2055	0.0094	1, 10	0.9245
Sku	0.1807	1, 10	0.6798	0.5406	1, 10	0.4791	0.0000	1, 10	0.9995	0.1402	1, 9	0.7159	4.2191	1, 4.0982	0.1075	0.9142	1, 5.5578	0.3787
Sp	0.5759	1, 10	0.4654	0.1334	1, 10	0.7229	0.0002	1, 10	0.988	0.289	1, 9	0.6026	4.7699	1, 4.4953	0.0868	0.8105	1, 10	0.3891
Sv	1.0359	1, 10	0.3328	1.1586	1, 10	0.307	0.0765	1, 6.3079	0.7912	0.0164	1, 9	0.9006	4.8798	1, 4.3327	0.0865	0.0001	1, 10	0.991
Sz	0.9604	1, 10	0.3502	0.6719	1, 10	0.4315	0.0265	1, 6.3659	0.8758	0.1161	1, 9	0.7403	5.3035	1, 4.4375	0.0762	0.1834	1, 10	0.6776
Sds	**34.188**	**1, 10**	**0.0002**	**28.441**	**1, 10**	**0.0003**	**83.474**	**1, 10**	**0.0001**	**13.450**	**1, 9**	**0.0052**	**21.477**	**1, 8**	**0.0017**	**8.7985**	**1, 5.4191**	**0.0284**
Str	0.0396	1, 5.777	0.849	**12.165**	**1, 10**	**0.0058**	0.0024	1, 10	0.9616	0.0238	1, 5.3224	0.8832	**7.4932**	**1, 8**	**0.0256**	0.2639	1, 6.1317	0.6254
Sdq	0.2526	1, 10	0.6261	**9.8146**	**1, 10**	**0.0106**	3.5827	1, 5.8006	0.1089	0.5311	1, 9	0.4753	**8.1477**	**1, 8**	**0.0213**	0.4437	1, 10	0.5204
Ssc	**66.909**	**1, 10**	**0.0001**	**55.633**	**1, 10**	**0.0001**	**126.38**	**1, 10**	**0.0001**	**14.923**	**1, 5.3754**	**0.0103**	**276.92**	**1, 8**	**0.0001**	1.3171	1, 5.0349	0.3027
Sdr	0.5838	1, 10	0.4625	**8.6743**	**1, 10**	**0.0147**	4.1956	1, 6.1767	0.0851	1.1710	1, 9	0.3076	**7.8953**	**1, 8**	**0.0228**	1.8492	1, 10	0.2037
Vmp	**5.3974**	**1, 10**	**0.0425**	0.3019	1, 10	0.5948	0.013	1, 5.3924	0.9135	0.1785	1, 5.330	0.6892	1.3076	1, 8	0.2859	0.0388	1, 10	0.8477
Vmc	3.8714	1, 5.6313	0.0998	**13.059**	**1, 10**	**0.0047**	0.0031	1, 5.0745	0.9574	1.0943	1, 5.1148	0.3424	1.5078	1, 8	0.2544	0.5536	1, 10	0.474
Vvc	4.1287	1, 5.6503	0.0914	**7.8767**	**1, 10**	**0.0186**	0.0003	1, 5.0755	0.9878	1.0009	1, 5.1157	0.362	1.886	1, 8	0.2069	0.9983	1, 10	0.3413
Vvv	2.6293	1, 10	0.1360	2.6532	1, 10	0.1344	0.0003	1, 5.3428	0.987	0.8176	1, 9	0.3894	0.0428	1, 8	0.8413	0.2096	1, 5.5797	0.6644
Spk	4.8347	1, 10	0.0525	0.1732	1, 10	0.6861	0.0049	1, 5.532	0.9469	0.4705	1, 6.202	0.5176	0.1199	1, 8	0.738	0.4536	1, 10	0.5159
Sk	3.7196	1, 5.6862	0.1047	**13.086**	**1, 10**	**0.0047**	0.0037	1, 5.0608	0.9537	1.0861	1, 5.0953	0.3442	1.9623	1, 8	0.1988	0.2688	1, 6.027	0.6226
Svk	2.0199	1, 10	0.1857	0.1317	1, 10	0.7242	0.0041	1, 5.5097	0.9511	0.7007	1, 9	0.4242	0.1582	1, 8	0.7012	0.2708	1, 5.9354	0.6216
Smr1	0.4106	1, 10	0.5361	**6.4342**	**1, 10**	**0.0295**	0.0903	1, 10	0.7708	0.2660	1, 9	0.6185	0.0129	1, 8	0.9124	1.2839	1, 5.4209	0.3048
Smr2	3.5707	1, 10	0.0881	0.6252	1, 10	0.4477	0.0209	1, 10	0.8888	0.9317	1, 5.0167	0.3786	0.9123	1, 8	0.3675	1.0929	1, 5.0056	0.3437
S5z	1.3555	1, 10	0.2713	0.5644	1, 10	0.4698	0.0597	1, 6.1942	0.8149	0.2635	1, 9	0.6202	3.1067	1, 8	0.116	0.1765	1, 10	0.6833
Sa	3.8192	1, 5.9716	0.0987	8.4225	1, 10	0.0158	0.0007	1, 5.1308	0.9802	1.012	1, 5.2339	0.3586	0.9656	1, 8	0.3545	0.1606	1, 10	0.697

Table 4.23: ANOVA/Welch ANOVA analyses comparing the impacts of different sediment types upon the microtextural surfaces of small worn H. elongatus teeth. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	d.f	p	F	d.f	P	F	d.f	p	F	d.f	p	F	d.f	p	F	d.f	p
Sq	0.3564	1, 10	0.5626	2.7674	1, 10	0.1272	0.8167	1, 10	0.3874	2.841	1, 10	0.1228	0.4379	1, 10	0.5247	4.7052	1, 5.2702	0.0794
Ssk	1.0972	1, 10	0.3173	0.011	1, 10	0.9186	0.0793	1, 10	0.7839	0.0735	1, 10	0.7918	0.0992	1, 10	0.76	1.2551	1, 10	0.2888
Sku	0.5705	1, 10	0.4659	1.5624	1, 5.5955	0.2611	1.0607	1, 5.3058	0.3477	2.4878	1, 5.144	0.1739	0.999	1, 10	0.3435	0.0614	1, 10	0.8093
Sp	1.8752	1, 7.4392	0.2218	**8.4196**	**1, 6.1657**	**0.0264**	1.8441	1, 10	0.2043	2.1468	1, 5.8488	0.1945	4.7226	1, 10	0.0578	0.1272	1, 10	0.7288
Sv	0.5502	1, 10	0.4738	4.8364	1, 10	0.0525	1.8522	1, 5.2684	0.2289	**12.105**	**1, 5.5239**	**0.015**	5.1754	1, 5.9761	0.0634	2.3627	1, 10	0.1553
Sz	1.0775	1, 10	0.3215	**6.788**	**1, 6.107**	**0.0397**	2.8874	1, 5.6937	0.1428	**6.2649**	**1, 5.663**	**0.0487**	5.9371	1, 5.4139	0.0551	1.5868	1, 10	0.2364
Sds	**107.52**	**1, 5.2689**	**0.0001**	**66.266**	**1, 5.153**	**0.0004**	**94.194**	**1, 6.0092**	**0.0001**	**316.72**	**1, 5.9477**	**0.0001**	**78.049**	**1, 6.1525**	**0.0001**	**131.32**	**1, 10**	**0.0001**
Str	**18.211**	**1, 10**	**0.0013**	**11.812**	**1, 10**	**0.0064**	**8.6407**	**1, 10**	**0.0148**	**9.3815**	**1, 10**	**0.012**	3.897	1, 4.7369	0.1085	3.7881	1, 10	0.0802
Sdq	**88.886**	**1, 10**	**0.0001**	**112.62**	**1, 9.3122**	**0.0001**	**171.11**	**1, 10**	**0.0001**	**257.47**	**1, 10**	**0.0001**	**67.548**	**1, 10**	**0.0001**	**78.875**	**1, 10**	**0.0001**
Ssc	**2544.3**	**1, 10**	**0.0001**	**2340.4**	**1, 8.1803**	**0.0001**	**4365.7**	**1, 5.4546**	**0.0001**	**5061.5**	**1, 10**	**0.0001**	**2280.2**	**1, 10**	**0.0001**	**1382.5**	**1, 5.7565**	**0.0001**
Sdr	**75.222**	**1, 6.0534**	**0.0001**	**102.83**	**1, 6.033**	**0.0001**	**133.96**	**1, 5.9732**	**0.0001**	**375.80**	**1, 10**	**0.0001**	**67.484**	**1, 10**	**0.0001**	**116.81**	**1, 10**	**0.0001**
Vmp	0.4215	1, 10	0.5295	3.2614	1, 10	0.1011	0.4793	1, 10	0.5045	1.3473	1, 10	0.2727	0.0626	1, 10	0.8081	**6.5041**	**1, 5.4584**	**0.0473**
Vmc	0.7599	1, 10	0.402	2.0125	1, 10	0.1864	0.7722	1, 10	0.4005	**6.1701**	**1, 10**	**0.0323**	0.5168	1, 10	0.4905	3.2094	1, 5.5302	0.1276
Vvc	0.0601	1, 10	0.8109	2.3328	1, 10	0.1577	0.962	1, 10	0.3498	3.1041	1, 10	0.1086	0.2733	1, 10	0.6137	2.7759	1, 5.8023	0.1484
Vvv	1.1179	1, 10	0.313	2.4824	1, 10	0.1462	0.2529	1, 10	0.2529	2.015	1, 10	0.1862	0.8919	1, 10	0.3696	3.116	1, 5.188	0.1357
Spk	0.6915	1, 10	0.4233	3.7881	1, 10	0.0802	0.6033	1, 10	0.4553	1.415	1, 10	0.2617	0.4621	1, 10	0.5138	4.6257	1, 5.563	0.0786
Sk	0.5464	1, 10	0.4753	1.8579	1, 10	0.2028	0.8972	1, 10	0.3659	**8.3184**	**1, 10**	**0.0163**	0.5317	1, 10	0.4844	3.2867	1, 5.8196	0.1213
Svk	0.1367	1, 10	0.6998	3.2998	1, 10	0.0993	0.8012	1, 10	0.3918	3.9929	1, 6.3125	0.0903	3.117	1, 5.8794	0.1289	1.3439	1, 5.3772	0.2952
Smr1	**12.636**	**1, 10**	**0.0045**	1.2574	1, 10	0.2884	1.1637	1, 10	0.306	0.2718	1, 10	0.6135	0.4158	1, 10	0.5351	0.3976	1, 5.5741	0.5533
Smr2	**7.9218**	**1, 10**	**0.0168**	0.0006	1, 10	0.9814	4.1335	1, 10	0.0694	0.3805	1, 10	0.5511	0.0003	1, 4.5422	0.9867	1.0832	1, 10	0.3225
S5z	1.8162	1, 10	0.2049	**9.7453**	**1, 6.2119**	**0.0196**	4.6139	1, 10	0.0573	**7.425**	**1, 5.2339**	**0.0396**	**15.856**	**1, 10**	**0.0032**	0.9809	1, 10	0.3453
Sa	0.5193	1, 10	0.4862	2.3061	1, 10	0.1598	0.7645	1, 10	0.4024	3.8645	1, 10	0.0777	0.4405	1, 10	0.5235	3.9158	1, 5.361	0.1009

Table 4.24: ANOVA/Welch ANOVA analyses comparing the impacts of fossilisation state on the effects of siliciclastic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
Parameter	**F**	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	3.3029	1, 5.0466	0.1283	0.8829	1, 10	0.3695	1.1385	1, 7	0.3214	1.3749	1, 2.12	0.3518	1.8997	1, 7	0.2106	3.8367	1, 6.8374	0.0962
Ssk	0.0030	1, 10	0.9572	2.3439	1, 5.5574	0.1805	0.2142	1, 7	0.6576	2.8243	1, 8	0.1314	0.4233	1, 7	0.5361	0.5181	1, 11	0.4867
Sku	1.9610	1, 10	0.1917	0.8720	1, 5.0626	0.3928	0.0402	1, 7	0.8467	0.9510	1, 6.259	0.3656	0.6752	1, 7	0.4383	0.0350	1, 11	0.8550
Sp	2.8212	1, 5.0371	0.1534	0.3113	1, 10	0.5891	0.0081	1, 7	0.9309	3.0210	1, 8	0.1204	1.1108	1, 7	0.3269	**11.995**	**1, 11**	**0.0053**
Sv	3.0371	1, 5.4041	0.1374	0.0380	1, 10	0.8494	0.1736	1, 7	0.6894	0.0000	1, 8	0.9953	1.4907	1, 7	0.2616	2.2431	1, 11	0.1623
Sz	2.9990	1, 5.1319	0.1424	0.0497	1, 10	0.8281	0.0743	1, 7	0.7930	0.5462	1, 8	0.4810	1.7743	1, 7	0.2246	4.0774	1, 11	0.0685
Sds	4.0189	1, 10	0.0728	**13.599**	**1, 10**	**0.0042**	0.9883	1, 7	0.3533	**13.110**	**1, 8**	**0.0068**	2.3966	1, 7	0.1655	1.5863	1, 11	0.2339
Str	0.1981	1, 10	0.6658	1.5974	1, 10	0.2349	0.2954	1, 7	0.6036	1.5687	1, 8	0.2458	0.0032	1, 7	0.9563	0.0077	1, 11	0.9317
Sdq	3.0951	1, 5.1875	0.1367	0.6764	1, 10	0.4300	1.1020	1, 7	0.3287	3.1135	1, 8	0.1157	0.1078	1, 7	0.7523	1.6197	1, 11	0.2294
Ssc	4.7548	1, 5.837	0.0733	3.9792	1, 10	0.0740	1.0065	1, 7	0.3491	0.5162	1, 8	0.4929	0.1635	1, 7	0.6980	0.1347	1, 11	0.7205
Sdr	2.2103	1, 5.063	0.1965	1.2671	1, 10	0.2866	1.6227	1, 7	0.2434	0.2779	1, 8	0.1340	0.0221	1, 7	0.8859	1.7859	1, 11	0.2084
Vmp	4.5683	1, 5.1637	0.0839	0.5784	1, 10	0.4645	1.2217	1, 5.5323	0.3148	3.2247	1, 2.1465	0.2057	0.0497	1, 7	0.8300	2.0765	1, 6.8375	0.1938
Vmc	3.7755	1, 5.4673	0.1047	0.0077	1, 10	0.9316	0.4873	1, 7	0.5077	2.8059	1, 2.2328	0.2228	0.5162	1, 7	0.4958	0.8187	1, 11	0.3850
Vvc	4.7116	1, 5.4158	0.0779	0.1379	1, 10	0.7181	0.4663	1, 7	0.5167	3.1217	1, 2.2396	0.2057	0.4058	1, 7	0.5444	1.3415	1, 11	0.2713
Vvv	3.7755	1, 5.0524	0.1090	0.0311	1, 10	0.8636	2.1975	1, 5.2771	0.1953	**6.6481**	**1, 8**	**0.0327**	0.2351	1, 7	0.6426	0.4699	1, 7.3638	0.5140
Spk	3.0872	1, 5.0508	0.1387	1.4927	1, 10	0.2498	1.4467	1, 5.4985	0.2783	3.5287	1, 8	0.0971	0.9184	1, 7	0.3698	4.3168	1, 6.5437	0.0791
Sk	5.5537	1, 5.4979	0.0604	0.2242	1, 10	0.6460	0.9732	1, 7	0.2294	1.3366	1, 2.1226	0.3612	2.3460	1, 7	0.1695	**7.1831**	**1, 11**	**0.0214**
Svk	2.5309	1, 5.0132	0.1734	0.3471	1, 10	0.5688	1.1863	1, 7	0.1900	1.0711	1, 8	0.3310	1.4639	1, 7	0.2656	1.5518	1, 6.9273	0.2534
Smr1	**9.9136**	**1, 10**	**0.0104**	2.1616	1, 5.4271	0.1943	0.1678	1, 7	0.6157	0.6172	1, 8	0.4547	0.4238	1, 7	0.5338	0.2825	1, 11	0.6056
Smr2	4.7662	1, 5.4811	0.0760	0.1721	1, 10	0.6870	1.3780	1, 7	0.2788	2.1517	1, 8	0.1572	0.0026	1, 7	0.9606	0.5721	1, 11	0.4653
S5z	3.6917	1, 5.0455	0.1122	0.0117	1, 10	0.9159	0.0140	1, 7	0.9093	1.4097	1. 2.2337	0.3460	0.4822	1, 7	0.5098	2.9653	1, 11	0.1130
Sa	3.9154	1, 5.0918	0.1037	0.6322	1, 10	0.6322	1.0741	1, 7	0.3345	1.3929	1. 2.1741	0.3590	2.1163	1, 7	0.1891	5.3421	1, 7.6111	0.0512

Table 4.25: ANOVA/Welch ANOVA analyses comparing the impacts of fossilisation state on the effects of calcarenitic sediment abrasion to tooth surface microtextures through time. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA

	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
Parameter	**F**	**d.f**	**p**	**F**	**d.f**	**P**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**	**F**	**d.f**	**p**
Sq	1.4903	1, 5.0089	0.2765	**4.1329**	**1, 10**	**0.0695**	1.213	1, 5.1822	0.2965	0.0881	1, 10	0.7726	3.4377	1, 5.3512	0.1191	0.1435	1, 10	0.7127
Ssk	2.8942	1, 10	0.1197	0.1482	1, 10	0.7083	0.1508	1, 10	0.7059	0.0026	1, 10	0.9601	1.0336	1, 11	0.3118	0.5646	1, 10	0.4697
Sku	1.4451	1, 10	0.2570	0.0695	1, 10	0.7974	0.4792	1, 10	0.5046	0.3963	1, 10	0.5431	1.6669	1, 6.3288	0.2418	0.2427	1, 10	0.6329
Sp	1.1506	1, 5.6049	0.3086	2.8421	1, 10	0.1227	2.8912	1, 10	0.1199	0.0561	1, 10	0.8175	0.8198	1, 11	0.3846	0.0012	1, 10	0.9727
Sv	0.0181	1, 5.4943	0.8979	2.5287	1, 5.5221	0.1429	3.804	1, 5.8961	0.0999	0.5434	1, 10	0.4780	0.1251	1, 11	0.7303	1.9591	1, 10	0.1919
Sz	0.2632	1, 5.3735	0.6283	3.5932	1, 10	0.0873	4.2674	1, 5.8589	0.0855	0.2496	1, 10	0.6282	0.2798	1, 11	0.6073	0.6346	1, 10	0.4442
Sds	**32.7344**	**1, 10**	**0.0002**	1.201	1, 10	0.2988	**9.6853**	**1, 10**	**0.0110**	**6.5943**	**1, 10**	**0.0280**	6.3452	1, 5.2305	0.0511	**15.1682**	**1, 10**	**0.0030**
Str	2.0893	1, 10	0.1789	1.2622	1, 10	0.2875	**6.7531**	**1, 10**	**0.0266**	0.0564	1, 10	0.8170	1.8158	1, 11	0.2049	1.6313	1, 10	0.2304
Sdq	0.407	1, 5.0918	0.5511	5.5985	1, 6.0137	0.0557	0.2662	1, 5.9503	0.6245	0.0068	1, 10	0.9358	**11.8182**	**1, 5.5492**	**0.0157**	1.0798	1, 10	0.3232
Ssc	0.0674	1, 5.1722	0.8051	**5.8758**	**1, 10**	**0.0358**	3.2939	1, 10	0.0996	0.3239	1, 10	0.5818	**15.7814**	**1, 5.6376**	**0.0083**	4.9321	1, 10	0.0506
Sdr	0.5125	1, 5.0413	0.5059	**6.8704**	**1, 6.30305**	**0.0393**	0.0199	1, 10	0.8907	0.0711	1, 10	0.7952	**9.6292**	**1, 5.3707**	**0.0243**	2.5543	1, 10	0.1411
Vmp	4.8119	1, 5.153	0.0781	1.9651	1, 6.3386	0.208	1.2666	1, 5.2596	0.3091	0.0663	1, 10	0.8020	3.6648	1, 6.0163	0.1039	0.593	1, 5.6191	0.4591
Vmc	1.2449	1, 5.032	0.315	4.7272	1, 5.6936	0.0751	1.1836	1, 5.1826	0.3246	0.6412	1, 10	0.4419	3.8311	1, 5.2533	0.1049	0.005	1, 10	0.9448
Vvc	1.7624	1, 5.0463	0.2412	4.7761	1, 5.6495	0.0743	1.1995	1, 5.1768	0.3217	0.4553	1, 10	0.5151	**6.6097**	**1, 5.4774**	**0.0459**	0.0082	1, 10	0.9295
Vvv	1.2254	1, 5.0161	0.3186	2.6207	1, 5.4288	0.1618	1.0915	1, 5.1331	0.3428	0.0203	1, 10	0.8894	2.0946	1, 5.1817	0.2055	0.4699	1, 5.4553	0.5211
Spk	5.4173	1, 5.1798	0.0656	3.0634	1, 6.32896	0.1284	1.3346	1, 5.245	0.2979	0.056	1, 10	0.8177	3.7705	1, 5.9049	0.1010	0.5187	1, 5.874	0.4990
Sk	1.1112	1, 5.0481	0.3396	4.7135	1, 5.9413	0.0734	1.1969	1, 5.2469	0.3216	0.8543	1, 10	0.3771	4.2299	1, 5.3569	0.0911	0.0263	1, 10	0.8745
Svk	1.0582	1, 5.0382	0.3505	2.4277	1, 5.5658	0.1740	1.4827	1, 5.1804	0.2759	0.0028	1, 10	0.9591	1.5954	1, 5.2657	0.2596	0.3396	1, 5.503	0.5831
Smr1	**5.9859**	**1, 10**	**0.0345**	3.7731	1, 10	0.0808	1.4596	1, 10	0.2548	0.1683	1, 10	0.6903	1.8128	1, 5.2165	0.2337	0.287	1, 10	0.6039
Smr2	3.8953	1, 10	0.0767	2.0521	1, 10	0.1825	0.0464	1, 10	0.8337	0.3434	1, 10	0.5709	0.0008	1, 6.3381	0.9772	4.3485	1, 10	0.0636
S5z	0.6494	1, 5.0577	0.4565	5.061	1, 6.0428	0.0652	2.6281	1, 5.5894	0.1597	0.6728	1, 10	0.4312	1.1507	1, 11	0.3064	0.01	1, 10	0.9223
Sa	1.4127	1, 5.0196	0.2878	42879	1, 5.4595	0.0884	1.181	1, 5.1876	0.3251	0.2179	1, 10	0.6507	3.756	1, 5.2631	0.1075	0.0744	1, 10	0.7906

Table 4.26: ANOVA/Welch ANOVA analyses comparing the impacts of different sediment types upon the microtextural surfaces of fossil Carcharias sp. teeth. Bold text indicates a significant result, decimalised d.f value indicates the use of a Welch ANOVA.

Parameter	0 hours			24 hours			48 hours			72 hours			96 hours			120 hours		
	F	d.f	p	F	d.f	P	F	d.f	P	F	d.f	p	F	d.f	p	F	d.f	p
Sq	0.0003	1, 10	0.9855	1.3983	1, 5.4783	0.2858	0.0538	1, 10	0.8213	0.0004	1, 10	0.9841	4.9176	1, 3.4554	0.1015	2.1837	1, 10	0.1675
Ssk	0.2091	1, 10	0.6572	1.844	1, 10	0.2043	0.2958	1, 5.6415	0.6073	0.1995	1, 10	0.6638	2.4946	1, 6.6527	0.1605	0.0307	1, 10	0.8641
Sku	2.8181	1, 10	0.1241	0.5294	1, 5.2393	0.4981	0.9521	1, 5.7471	0.3684	0.0279	1, 10	0.8704	2.1724	1, 6.0817	0.1903	0.5558	1, 7.2316	0.4795
Sp	0.3751	1, 10	0.5539	0.8816	1, 10	0.3699	0.1731	1, 10	0.6862	0.3291	1, 10	0.5777	3.956	1, 3.377	0.1305	**10.786**	**1, 5.7561**	**0.0178**
Sv	0.7325	1, 10	0.4121	0.1131	1, 6.203	0.7477	0.6824	1, 10	0.429	1.1456	1, 7.2638	0.3187	1.3509	1, 10	0.275	**5.8369**	**1, 10**	**0.0343**
Sz	0.5778	1, 10	0.4647	0.1558	1, 10	0.7014	0.4673	1, 10	0.5098	0.3791	1, 10	0.5506	5.4226	1, 10	0.0448	**10.080**	**1, 10**	**0.0088**
Sds	1.8987	1, 10	0.1983	**5.5833**	**1, 10**	**0.0398**	0.8072	1, 10	0.3901	0.2897	1, 10	0.1168	4.784	1, 3.3359	0.1074	0.1524	1, 7.84	0.7076
Str	1.5775	1, 10	0.2377	0.0317	1, 10	0.8623	1.0219	1, 10	0.3359	3.3355	1, 10	0.095	0.6848	1, 10	0.4293	2.4196	1, 10	0.1481
Sal	1.4497	1, 10	0.2563	0.0853	1, 6.3445	0.7796	0.9639	1, 10	0.3494	0.0238	1, 10	0.8802	1.9492	1, 10	0.1962	0.4016	1, 10	0.5392
Sdq	0.0108	1, 10	0.9192	0.0626	1, 10	0.8075	0.6627	1, 10	0.4346	0.1771	1, 10	0.682	9.1528	1, 3.2678	0.0506	3.5959	1, 10	0.0845
Ssc	0.0419	1, 5.9641	0.8447	1.2418	1, 10	0.2912	0.1913	1, 10	0.6711	0.9314	1, 10	0.3552	**17.474**	**1, 3.452**	**0.0189**	2.8184	1, 10	0.1213
Sdr	0.0055	1, 10	0.9425	0.0088	1, 10	0.927	0.5454	1, 10	0.4772	0.0443	1, 10	0.8372	7.579	1, 3.1633	0.0665	**6.6618**	**1, 10**	**0.0255**
Vmp	0.3562	1, 10	0.5639	0.4462	1, 10	0.5193	0.0235	1, 10	0.8811	1.9178	1, 10	0.1935	1.0001	1, 3.3536	0.3839	0.5075	1, 10	0.491
Vmc	0.8184	1, 5.5202	0.4034	0.1646	1, 5.5535	0.7001	0.3577	1, 10	0.5631	2.5988	1, 10	0.1352	1.8528	1, 3.1912	0.2616	0.2602	1, 10	0.6201
Vvc	0.5471	1, 6.3283	0.486	0.4664	1, 5.682	0.5215	0.1833	1, 10	0.6776	2.4434	1, 10	0.1463	2.2652	1, 3.208	0.2236	1.0487	1, 10	0.3278
Vvv	0.2631	1, 10	0.6191	0.0037	1, 5.2876	0.9536	0.0192	1, 10	0.8926	**4.9578**	**1, 10**	**0.0474**	0.8638	1, 3.4465	0.4131	0.1222	1, 10	0.7333
Spk	0.7074	1, 6.2809	0.4312	1.4253	1, 10	0.2601	0.0021	1, 10	0.9642	0.0381	1, 10	0.8488	**7.2647**	**1, 10**	**0.0246**	2.118	1, 10	0.1735
Sk	0.5696	1, 5.6184	0.4809	1.166	1, 6.0819	0.3212	0.3086	1, 10	0.5908	0.1053	1, 10	0.7517	5.2929	1, 3.2591	0.0979	4.0257	1, 10	0.07
Svk	0.0086	1, 10	0.928	0.0851	1, 5.4991	0.7812	0.0014	1, 10	0.9705	0.0464	1, 10	0.8333	**5.6362**	**1, 10**	**0.0416**	1.3708	1, 10	0.2664
Smr1	1.3045	1, 10	0.28	1.2244	1, 6.3592	0.3086	0.5909	1, 10	0.4598	0.0019	1, 10	0.9657	1.2387	1, 3.1479	0.3434	3.2768	1, 10	0.0976
Smr2	0.0137	1, 10	0.9092	1.9968	1, 10	0.188	0.0375	1, 10	0.8504	0.6406	1, 6.0646	0.4109	4.0825	1, 10	0.0741	**7.7964**	**1, 10**	**0.0175**
S5z	0.443	1, 10	0.5208	0.0319	1, 10	0.8618	0.2552	1, 10	0.6244	0.0384	1, 10	0.8483	1.8532	1, 10	0.2065	2.8411	1, 10	0.12
Sa	0.0913	1, 10	0.7687	1.3138	1, 5.6179	0.2982	0.1185	1, 10	0.7378	0.0156	1, 10	0.903	5.4099	1, 3.308	0.0944	2.4568	1, 10	0.1453

4.4 Discussion

The results from this study show that the levels of sediment abrasion and composition of sediments within which teeth are found need to be considered before microtextural analyses are conducted. Whilst the effects of sediment abrasion under a single experimental condition are minimal, there are subtle changes in parameter score. When comparing two different experimental conditions these subtle differences often compound to produce significant differences, altering the microtextural signal.

Two main hypotheses were addressed during this study, which included the analysis of several variables. The first of these, “Does sediment abrasion create a false dietary signal?” was investigated through the analysis of sediment abrasion on previously un-erupted and unworn teeth. Analyses revealed that in both instances variance in parameter score, and parameter scores themselves, fluctuated subtly over the time frame of the experiment, but failed to generate significant differences. The implication of this is that sediment abrasion, regardless of sediment type, does not generate a false dietary signal on tooth surfaces. This is in keeping with other studies which found that false 2D dietary microwear signals were not produced by sediments[71]. Our analyses indicate that whilst sediment abrasion to unworn surfaces can generate some variance on the tooth microtextural surface, it is insufficient to generate a false signal that could impact upon the dietary separation between individuals and species in future study.

The second hypothesis addressed is “Does sediment abrasion remove or modify dietary signals preserved in tooth surface microtextures?” This was investigated through a number of variables (level of previous wear, tooth size and tooth morphology) to enable a fuller understanding of how sediment abrasion impacts upon tooth microtextures. Impacts of sediment abrasion were found to vary with different sediment types. When comparing different teeth tumbled under the same sedimentary conditions, calcarenitic sediments had a greater effect on tooth surface microtextures, and preserved dietary signal, than siliciclastic sediments. Throughout all experiments, calcarenitic sediments

produced larger and more variable levels of differentiation between parameters than siliciclastic sediments.

With siliciclastic sediments, previous tooth wear levels, tooth size and tooth morphology appear to have little effect on how sediments abrade tooth surface microtextures. Only after 120 hours were the differential impacts of sediment abrasion on teeth of different sizes and morphologies noticed. Siliciclastic sediment abrasion on teeth, with noted previous wear, produced fluctuating levels of significant variation within parameters. Parameters displaying significant differences were often different from those generating initial separation between samples tumbled in different sediments before abrasion. Investigation of the parameters, known to separate diet in elasmobranchs, reveal that only Sv, Sz and S5z detect separation, as a result of siliciclastic sediment abrasion, on more than one occasion.

With calcarenitic sediments, significant differences between the sediment abraded surfaces of teeth with differing levels of previous wear, different sizes and morphologies were more noticeable. Significant differences were typically sporadic in their parameter definition, and included a greater number of parameter separations during each time frame, than under siliciclastic conditions with siliciclastic sediments, With calcarenitic sediments, tooth morphology did not greatly affect the impacts of sediment abrasion, with sporadic parameter differentiation displayed by a few parameters in a couple of the timing intervals. Tooth size in contrast appeared to influence sediment abrasion to a much greater degree. Between four and eight parameters separated the two tooth sizes at different points during the experiment. Of these, seven are known dietary discriminators in elasmobranchs. Calcarenitic sediment abrasion, on teeth with differential starting wear levels, found that at least 50% of the parameters separating the two groups varied from those initially producing separation. Three of the parameters are known dietary discriminators (Chapter 3). Throughout all calcarenitic experiments, known dietary defining parameters most likely to reveal separation were Sq, Sv, Sds, Vvv, Sz and S5z.

The implications of these analyses are that if teeth are sourced from

siliciclastic sediments then all teeth can be compared unless there are signs of heavy surface abrasion. This implies that siliciclastic sediments are not replacing or removing dietary microtextural signals to the extent that the dietary separation is lost. These findings are in contrast to previous work which found that sediments obliterate the 2D microwear signal on tooth surfaces[71]. If teeth are sourced from calcarenitic sediments then greater care needs to be taken. Our findings indicate that calcarenitic sediments are capable of removing dietary defining microtextures from tooth surfaces. A greater number of samples are thus required to reduce the impacts of separation due to differential sediment wear, particularly if teeth of different sizes are being analysed. These findings reflect those of previous studies investigating sediment abrasion on 2D Microwear signals where dietary defining tooth surfaces were obliterated and replaced[71].

Comparing samples between different sedimentary sources is also problematic. Abrasion from different sediment types was found to increase the number of significant differences between the surfaces of similar groups of teeth. This was in all cases except for large teeth and unworn tooth surfaces where, after initial separation, convergence was noted. Our analyses indicate that different sediment types create differential wear rates on teeth with previous surface wear of comparable size and morphology. In general, similar teeth tumbled in different sediments produce different wear textures and a greater number of significant differences. Worn and small teeth (<1cm) are most rapidly affected, producing a greater number of significant differences after a shorter period of tumbling. Teeth larger than 2cm produce a different signal. Whilst there were high levels of initial separation in tooth surface microtextures of the two groups of teeth, this separation diminished through time. This implies that on large extant teeth different sediments remove and alter the original dietary signal, producing a convergent microtextural signal.

Results from this study suggest that different sediment types are regularly removing and replacing the dietary preserved microtextural signal in different ways, making it difficult to compare samples from very different sedimentary sources. As a result, any separation that is noted between teeth from different sedimentary sources could be a result of taphonomic processes

rather than dietary differences. Future work using samples that fall in this category need to treat their findings with care.

Under the second hypothesis, fossilised teeth were also investigated in order to understand the impacts of reworking on tooth microtextures. Siliciclastic sediments produced little change to fossilised tooth surfaces through time. Three parameters displayed significant separation, with one displaying additional Tukey HSD separation between the original surfaces and those that had been abraded. Siliciclastic sediments also produced sporadic separation, between extant and fossil teeth, through time. The dietary defining parameters Sds, Vvv and Sk occasionally displayed significant separation. Calcarenitic sediment abrasion produced significant changes to two parameters defining tooth microtextures. Tukey HSD separation also identified Sds and Smr1 as being particularly susceptible to abrasion changes. Calcarenitic sediment abrasion also resulted in sporadic separation between extant and fossil teeth. Up to four parameters displayed significant separation at each timing interval. Fossil teeth appear to be fairly resilient to the impacts of sediment abrasion but are more susceptible than extant teeth. Fossils that have been reworked still preserve accurate dietary defining microtextures so long as the reworking has not been extensive.

Tooth surface microtextures appear to respond in much the same way to sediment abrasion as 2D microwear signals do. This study finds that, like those before it, sediments do not create false dietary signals, but they do remove tooth microtextural surfaces. Unlike previous studies however, microtextural surfaces appear to be replaced, and dietary signals altered. This study has also found that sediment composition differences can have a significant influence on the impacts of sediment abrasion with regards to removal and replacement of surfaces and dietary signals. Like King et al[71] these experiments have also failed to replicate the level of abrasion described by earlier taphonomic studies[69-70, 72] or indeed the levels of abrasion often observed on fossilised teeth[107]. After 120 hours of sediment abrasion, teeth in this study were only just beginning to display visible signs of surface wear particularly those tumbled in calcarenitic sediments. As such, teeth which do not display a scuffed and dull

surface (the first signs of visible surface abrasion found within this study) can be used in a microtextural dietary study with some confidence. Longer tumbling time frames, particularly with fossilised teeth are thus needed to ascertain levels and timings of abrasion required to replicate the levels of abrasion described in previous studies, and reflect the polished and pitted surfaces displayed by many fossilised teeth.

4.5 Conclusions

This is the first study to investigate the impacts of sediment abrasion on tooth surface microtextures. Our results show that the levels of sediment abrasion and composition of sediments, within which teeth are found, need to be considered before microtextural analyses are conducted.

Below is a summary of our key findings with regards to the impacts of sediment abrasion on tooth microtextures:

- Our analyses indicate that whilst sediment abrasion to unworn surfaces can generate some variance on the tooth microtextural surface, it is insufficient to generate a false signal that could impact upon the dietary separation between individuals and species in future study.
- Results from this study indicate that if teeth are sourced from siliciclastic sediments then all teeth can be compared unless there are signs of heavy surface abrasion. It should be noted, however, that more work needs to be done in order to confirm this. Siliciclastic sediments are not replacing or removing dietary microtextural signals to the extent that the dietary separation is lost.
- Results from this study indicate that calcarenitic sediments are capable of removing dietary defining microtextures from tooth surfaces. A greater number of samples are thus required to reduce the impacts of separation due to differential sediment wear, particularly if teeth of different sizes are being analysed. Again further work is needed to ascertain why calcarenitic sediments in this study were having this impact.
- Different sediment types create differential wear rates on teeth, with

previous surface wear, of comparable size and morphology. Different sediment types are regularly removing and replacing the dietary preserved microtextural signal in different ways, making it difficult to compare samples from very different sedimentary sources.

- Fossil teeth appear to be fairly resilient to the impacts of sediment abrasion but are more susceptible than fresh teeth. Fossils that have been reworked can still preserve accurate dietary defining microtextures so long as the reworking has not been extensive, and no surface abrasion is visible to the naked eye.
- Tooth surface microtextures appear to respond in much the same way to sediment abrasion as 2D microwear signals do. This study finds that, like those before it, sediments do not create false dietary signals, but they do remove and replace tooth microtextural surfaces.
- Like King et al[71] these experiments have also failed to replicate the level of abrasion described by earlier taphonomic studies[69-70, 72] or indeed the levels of abrasion often observed on fossilised teeth. After 120h of sediment abrasion, teeth in this study were only just beginning to display visible signs of surface wear.

5.0 Microtextural analysis as a tool for dietary discrimination in the fossil record- Did *Carcharodon carcharias* cause the extinction of *Carcharocles megalodon?*

5.3 Million years ago numbers of the giant shark, *Carcharocles megalodon,* began to decline and the species disappeared from many of its traditional habitats. There are three leading hypotheses which provide possible explanations for this decline. In this study the hypothesis that states that the extinction of *Cs. megalodon* was caused by dietary competition with the modern Great White Shark, *Carcharodon carcharias,* will be investigated. Until now, evidence of dietary competition between these species has been ambiguous, and relies on the extremely rare bite marks preserved on marine mammalian bones within the fossil record. This evidence does not, however, provide a comprehensive measure of either species diets. Here we show, for the first time, that *Cs. megalodon* and *Cn. carcharias* were in direct dietary competition.

3D microtextures have revealed that the diet of adult *Cn. carcharias* has complete overlap with that of juvenile and sub-adult *Cs. megalodon*. Tooth microtextures also reveal that *Cs. megalodon* diet did not change in reaction to the evolution of *Cn. carcharias* and the competition it created. This indicates that although the evolution of *Cn. carcharias* created an additional selection pressure, it was not enough to independently cause the demise of *Cs. megalodon*. This is the first time that microtextural techniques have been applied to the study of diet in fossil elasmobranchs. We anticipate this study to be the first of many to use microtextural techniques to investigate the diet of fossil elasmobranch species.

5.1 Introduction

Carcharocles megalodon is an extinct species of elasmobranch that inhabited global waters from 15.9-2.6 million years ago[108]. Dubbed the ‘Megatooth shark’, this giant predator grew to at least 16 metres in length and is the largest

elasmobranch species known to have existed[108-110]. *Cs. megalodon* fossils have been found globally and indicate a preference for sub-tropical to temperate habitat[28, 109, 111]. Individuals typically lived in offshore waters, but entered shallow coastal regions to give birth. These shallow coastal areas acted as nurseries for juvenile *Cs. megalodon*, providing a plentiful supply of food and a reduced risk of predation[28, 111]. Due to ontogenetic habitat differences, it is thought that adult and juvenile *Cs. megalodon* consumed different diets[111]. Bite marks on bones indicate that adult *Cs. megalodon* ate cetaceans[34, 112], whales[28, 112], pinnipeds[110], sirenians, porpoises and turtles[28]. Juveniles would have targeted smaller prey and consumed a higher proportion of teleosts[111].

At the beginning of the Pliocene, 5.3 million years ago, *Cs. megalodon* numbers started to decline[108], with fossil evidence becoming scarce towards the Pliocene/Pleistocene boundary. The lack of fossils approaching the Pliocene-Pleistocene boundary makes pinpointing their extinction difficult. Recent calculations, however, have provided an extinction date of *Cs. megalodon* at 2.6 million years ago[108]. There are three leading hypotheses for the extinction of *Cs. megalodon*. One such hypothesis is that a change in global climate, cooling nursery waters and the resulting in the mass movement of marine mammals into waters where *Cs. megalodon* was poorly adapted[113], caused *Cs. megalodon*'s extinction. The other two hypotheses are linked to dietary competition with newly evolved macropredators; the Killer Whale, *Orcinus orcus*, and the Great White shark, *Carcharodon carcharias*. It is the competition with the latter of these two species, *Cn. carcharias*, which is the focus of this study.

The Great White shark, *Cn. carcharias*, had evolved by the Miocene-Pliocene transition, 5.3 million years ago. As with all species exact emergence dates are unknown. Skeletal remains found in Peru however, dating to 6.5 million years ago[114], have been identified as an intermediate species between *Cn. carcharias* and its ancestor *Isurus hastalis* (Figure 5.1). This indicates an emergence date of *Cn. carcharias* after this time. *Carcharodon hubbelli* displays a dental morphology that encompasses features of both *Cn. carcharias* and *I.hastalis*[115].

Early *Cn. carcharias* were on average larger than individuals found today, but smaller than adult *Cs. megalodon*[213]. Bite marks on bones indicate that early *Cn. carcharias* consumed dolphins and small whales[112-116] .This would have made *Cn. carcharias* a direct dietary competitor to *Cs. megalodon*. Despite its smaller size *Cn. carcharias* had potential evolutionary advantage over *Cs. megalodon,* for as global temperatures cooled *Cn. carcharias* was able to adapt more efficiently than *Cs. megalodon*. *Cn. carcharias* is capable of elevating its body temperature above that of the surrounding oceanic environment[136]. This allows the species to inhabit cooler waters, and thus track endothermic prey. The lack of fossil evidence outside of warm waters would suggest that *Cs. megalodon* was incapable of elevating its body temperature.

In order to investigate the idea that the evolution of *Cn. carcharias* lead to the extinction of *Cs. megalodon* through dietary competition, one has to fully understand the diet of both species at the time. Determining the diet of extinct species is problematic. The traditional methods used for determining diet in extant elasmobranch species cannot be applied to fossil specimens: stomach contents rarely fossilise and observation studies are obviously unsuitable. Tooth morphology is often used as a dietary indicator in other extinct animal groups[137-138]. In elasmobranchs however, individuals of the same species with identical tooth morphology can have differing diets[97-98, 139]. More so, several extant species consume similar diets but have very different dental morphologies. For example *Cn. carcharias, Galeocerdo cuvier* and *Carcharhinus leucas* have different dental morphologies but consume very similar diets, particularly the largest individuals[12-13, 32,140-144] The only morphological feature linking these three dentitions is coarsely serrated cutting edges. This feature is not universal to species consuming this particular diet. *Prionace glauca* which specialises on a teleost/cephalopod diet[145], also possesses coarse serrations. This makes dental morphology an unreliable indicator of diet in extinct elasmobranch species. Alternative methods that have been applied to extant elasmobranchs are isotopic analyses and analyses of tooth surface microtextures. Isotopic analyses are unsuitable for this investigation as they only provide a measure of trophic level, not prey preference[26, 83, 146-147].

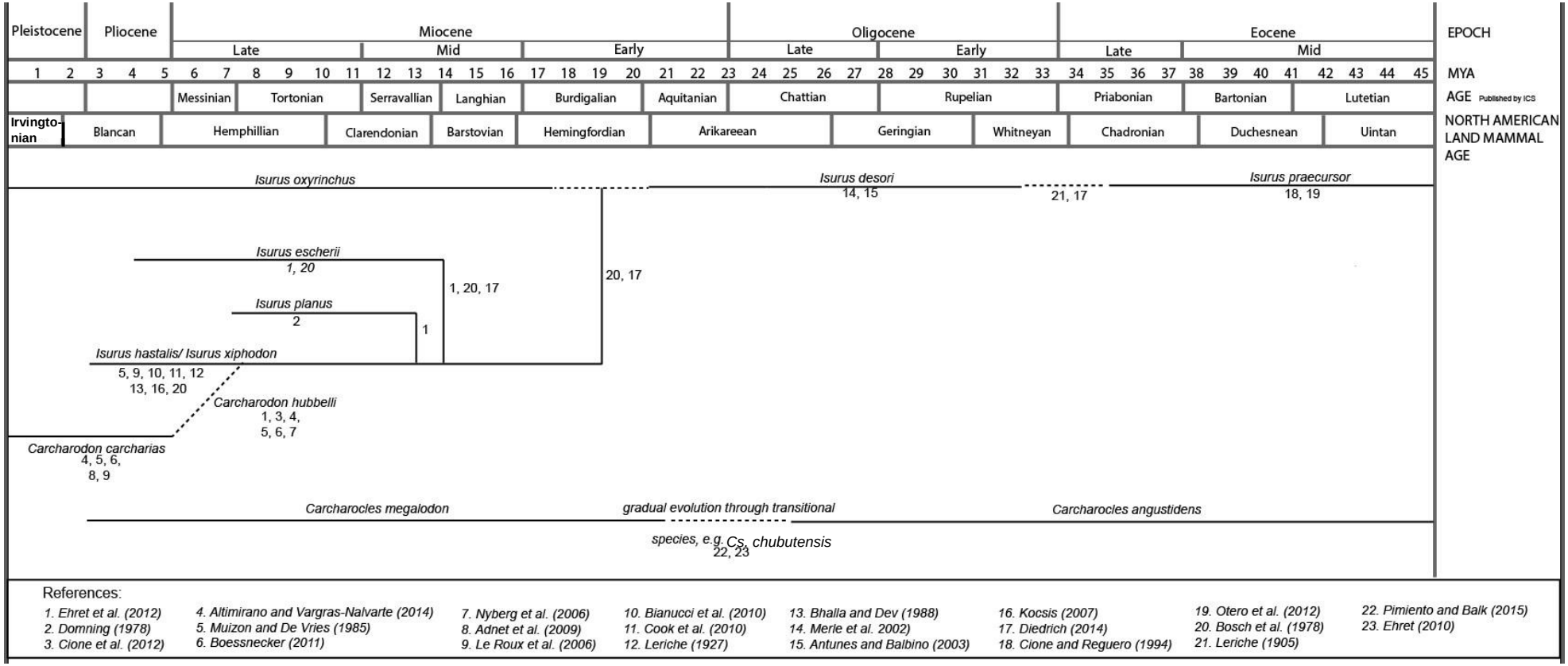


Figure 5.1: Stylised evolutionary pathways of *Carcharocles megalodon* and *Carcharodon carcharias*. The numbers relate to literature supporting evolutionary pathways and species occurrences. Full references can be found in the reference list: 113-114, 117-135.

Dietary preference needs to be identified for both species in order to determine dietary competition between *Cn. carcharias* and *Cs. megalodon*.

3D microtextural analysis has been shown to be an effective dietary discriminator in extant elasmobranch species (Chapter 2) and other aquatic vertebrates[61,93], however the method has yet to be applied to extinct elasmobranchs. As such this paper investigates not only the question of *Cn. carcharias* and *Cs. megalodon* dietary competition, but also acts as a proof of concept study for the application of microtextural analyses in the study of diet in extinct elasmobranchs. This study demonstrates that 3D microtextural analyses can be used on fossil teeth, and thus can be used to test the following hypotheses:

1) For there to be dietary competition between *Cs. megalodon* and *Cn. carcharias* they must have consumed the same diet. Tooth surface microtextures are known to differ between taxa and individuals with different diets (Chapter 3). A hypothesis of dietary competition can therefore be rejected if microtextural analyses provide evidence that their diets differ.

2) When resource availability is limited, one organism will have negative effects upon another by controlling access to, or by consuming, this resource[148]. This results in resource partitioning, where one species modifies its dietary niche in order to survive. Resource partitioning can be successful, where both species survive, or unsuccessful, where one species becomes extinct[1, 149]. If dietary competition between *Cn. carcharias* and *Cs. megalodon* was the cause of *Cs. megalodon's* extinction there should be a deviation in microtextural score between the two species, indicating resource partitioning between the two species. A hypothesis of dietary competition can be rejected if there is no niche partitioning when a resource is limited. A hypothesis of competition leading to the extinction of *Cs. megalodon* can also be rejected if there is no resource partitioning between *Cs. megalodon* and *Cn. carcharias*.

5.2 Materials and methods

5.2.1 Materials and sampling strategy

All samples were collected from Florida, USA. Thirteen sites were located and used if both *Carcharocles megalodon* and *Carcharodon carcharias* teeth were found within the same horizon of the same deposit. This was to ensure that both species were utilising the same habitat at the same time. For localities pre- dating the emergence of *C. carcharias,* sites were selected based on the co- occurrence of *Cs. megalodon* and *Isurus hastalis*, the ancestor of *Cn. carcharias*. This was to ensure that the locality was capable of supporting several large species of elasmobranch at the same time, enabling comparability between sites through time. Sites ranged in age from 18.9 MA to 2.6 MA, covering the full timeline of *Cn. carcharias/ Cs. megalodon* cohabitation as well as the existence of *Cs. megalodon* prior to the emergence of *Cn. carcharias.* Table 5.1 provides a summary of the samples used, indicating where they were found and where they are currently housed.

Individual teeth of *Cs. megalodon* ranged in size from 45mm cusp height to 119mm cusp height, representing a range of ontogenetic stages for this species. *Cn. carcharias* cusp heights ranged from 40mm to 55mm. It is assumed that the size of *Cn. carcharias* teeth in comparison to body size is comparable between modern and Pliocene individuals. It is also assumed that size at maturation is also comparable. On this basis all individuals investigated within this study were adult. *I.hastalis* cusps ranged in height from 34mm to 45mm. Using *Cn. carcharias* as a model this indicates a sub-adult to adult sample

All samples used within this study were fossilised. Some samples had undergone surface texture alteration, due to sediment abrasion[107] (Chapter 4), preparation, diagenesis or a combination of the three. As a result large numbers of samples were excluded from the study as they did not record an accurate dietary signal. This is a feature of any fossil dietary study using 3D microtextural techniques. Teeth which displayed a dull, scuffed surface and irregular pitting textures were removed from analysis, as were those which had polished roots

and overly rounded edges. Wear as a result of feeding accumulates in regular patterns in specific regions on a tooth, any tooth displaying irregular wear in unusual locations were thus eliminated[107]. Sample sizes used within this study are a reflection of this exclusion, with some study sites only producing dietary data for one species, despite multiple species being present.

Table 5.1: Summary of samples used for this study.

The table includes information on deposit name, ID and age and species identification and name.

Date of Deposit	**Deposit locality**	**Species**	**Specimen ID Number**	**Specimen locality**
5.0- 4.0 MA Blancan z.1, Pliocene	DU006	*Carcharodon carcharias, Isurus hastalis*	UF223349 UF223343 UF223342	Atlantic Beach, Duval, Florida
5.0-4.5 MA Hemphillian z.4, Early Pliocene	PO001	*Isurus hastalis, Carcharocles megalodon*	UF 17862 UF 5365 UF 5352 UF 5337 UF 5374	Bone Valley Formation, Palmetto Mine, Polk, Florida
5.0-4.5 MA Hemphillian z.4, Early Pliocene	PO010 Hh	*Carcharocles megalodon*	UF16025 UF 15165 UF 16017 UF 15166 UF 16020	Bone Valley Formation, Phosphoria Mine, Polk, Florida
5.0-4.5 MA Hemphillian z.4, Early Pliocene	PO016	*Carcharocles megalodon*	UF 16975 UF 16975	Bone Valley Formation, Nichols Mine, Polk, Florida
5.0-4.5 MA Hemphillian z.4, Early Pliocene	PO018	*Isurus hastalis, Carcharocles megalodon*	UF 217145 UF 14444 UF 14443	Bone Valley Formation, Kingsford Mine, Polk, Florida
5.7-2.6 MA Hemphilian z.4- Blancan z.1, Latest Miocene-Pliocene	NA004	*Carcharodon carcharias, Carcharocles megalodon*	UF111787 UF111788 UF111786	Coosawhatchie Formation, North Fernandina Beach, Nassau, Florida
7.5-6.8 MA Hemphillian z.2, Latest Miocene	SA004	*Isurus hastalis, Carcharocles megalodon*	UF 61872 UF 61867 UF 61868	Bone Valley Formation, Lockwood Meadows, Sarasota, Florida
9.0-5.3 MA Hemphilian, Late Miocene	DU002	*Carcharodon carcharias, Isurus hastalis, Carcharocles*	UF234883 UF234837 UF234884 UF234843	St John's River, Duval, Florida

		megalodon	UF109888 UF104563 UF109842 UF109722 UF109658 UF105222 UF104958	
9.0- 9.5 MA Clarendonian z.3, mid-Miocene	AL001	*Isurus hastalis, Carcharocles megalodon*	UF231341 UF231256	Alachua formation, Love Bone Bed, Alachua, Florida
10.0- 12.0 MA Clarendonian z. 2, Early-Miocene	HA002	*Carcharocles megalodon*	UF120085	Statenville Formation, Hawthorn Group, Suwannee River Mine, Hamilton, Florida
10.0- 12.0 MA Clarendonian z.2, Mid-Miocene	HA003	*Isurus hastalis, Carcharocles megalodon*	UF232616 UF232627 UF232629	Statenville Formation, Swift Creek Mine, Hamilton, Florida
16.0- 12.0 MA Barstovian z.2-Clarendonian z.2, Mid-Miocene	HR046	*Isurus hastalis, Carcharocles megalodon*	UF130161 UF130159	Bone Valley Formation, Hickey Branch Site, Hardee, Florida
18.9 - 12.5 MA Hemingfordian and Barstovian, Early-Mid Miocene	SA024	*Isurus hastalis, Carcharocles megalodon*	UF 231961 UF 231991 UF 231985 UF 231982 UF 231983 UF 231989 UF 231988 UF 231871 UF 231990 UF 231992	Arcadia Formation, Dean's Trucking Pit, Sarasota, Florida

5.2.1.1 Localities

All samples originated from the locations described below. All sites contained *Cs. megalodon* teeth and *I.hastalis* and/or *Cn. carcharias* teeth. Each locality is also summarised in Figure 5.2. As all locations are within North America, and are catalogued at North American institutes, North American Land Mammal ages have been used rather than the standard stratigraphy of the Neogene as outlined by the International Commission on Stratigraphy. Figure 8.13 provides

a comparison of each stratigraphy timeline for reference. All localities have been reconstructed as near shore marine environments, based on the fossil assemblages recovered from each.

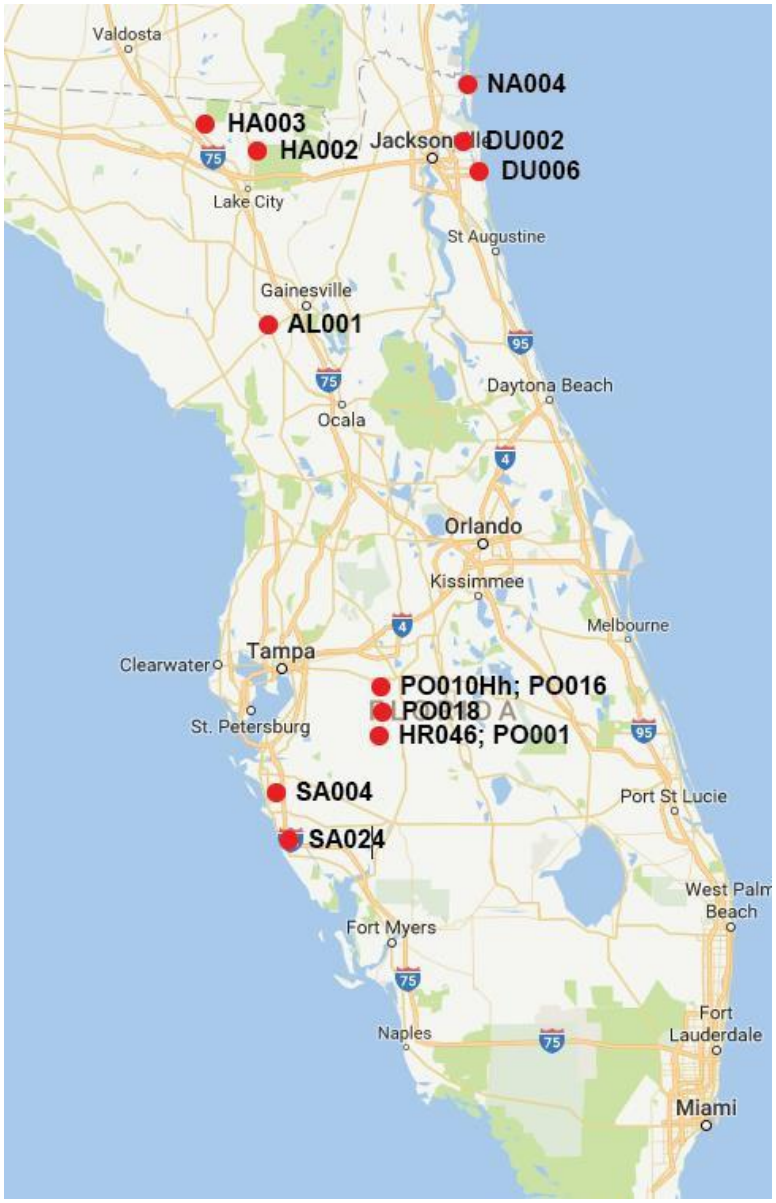


Figure 5.2: The approximate locations of each fossil site, using a base map from google maps.

AL001- Alachua Formation, Love Bone Bed, Alachua, Florida

Location: 29.55°N, 82.52°W[150]

Age: 9.5-9.0 million years old- Latest Clarendonian[150] (Figure 8.13)

Description: The deposit has been interpreted as a fluvial influenced deposit with coarse, cross-bedded phosphatic sands. It contains gravel that fines

upwards to orange clays that infill a channel cut into the Late Eocene Crystal River Formation. Several environments are represented at the site including estuarine, swamp, woodland and savannah. As such, at the time of deposition this locality would have been near shore marine, with a strong fluvial input. The bones of the terrestrial vertebrates are highly rounded as a result of fluvial transport to point of deposition[151]. Elasmobranch teeth are predominately isolated.

Species collected: *Cs. megalodon, I.hastalis*

Species analysed: *Cs. megalodon, I.hastalis*

DU002- St John's River Dredge, Duval, Florida

Location: 30.39°N, 81.53°W[150]

Age: 9-5 million years old – Hemphillian, late Miocene[150] (Figure 8.13)

Description: Little geological information is recorded for this locality. Specimens are found as a result of a dredge of the St. John's River in Duval County, Florida. As such accurate information on stratigraphy and exact age estimates are not possible. Based upon the species found however, the dredged section of river yields a combination of fossils that indicate a late Miocene age for the region[150].

Species Collected: *Cs. megalodon, I.hastalis, Cn. carcharias*

Species analysed: *Cs. megalodon, I.hastalis, Cn. carcharias*

DU006- Atlantic Beach, Duval, Florida

Location: 30.34°N 81.39°W[150]

Age: 4.8-4.5 million years old- Blancan earliest Pliocene[150] (Figure 8.13)

Description: As with the St. Johns River Dredge site, Duval County, Florida

there is little documentation on this locality. Fossils are found as a result of a dredge, as such it is unclear on the stratigraphy surrounding the fossils found. Based on the combination of species found, it has been possible to date the region to the early Pliocene[150].

Species collected: *Cs. megalodon, I.hastalis, and Cn. carcharias*

Species analysed: *I.hastalis, Cn. carcharias*

HA002, HA003- Statenville Formation, Hawthorn Group, Hamilton, Florida

Location: HA002 Suwannee River Mine 30.46°N, 82.75°W; HA003 Swift Creek Mine 30.43°N, 82.90°W[150]

Age: 12-10 million years old- mid- Clarendonian[150] (Figure 8.13)

Description: The Statenville Formation occurs at or near the surface at several localities within Hamilton County, Florida on the North East side of the Ocala Platform. The formation comprises interbedded clays, sands and dolostones. Phosphatic grains are very common in the formation and both HA002 and HA003 are mined for phosphate. The sands are light to olive grey, fine to coarse grained, phosphatic and interspersed with gravels and fossils. The clays are yellow to olive grey and vary in levels of phosphate and sand. The dolostones are typically thin beds that are yellow grey- light orange. They can be sandy, clayey or phosphatic and are inter-dispersed with mollusc moulds and casts[152].

Species collected: *Cs. megalodon, I. hastalis*

Species analysed: At HA002 *Cs. megalodon*. At HA003 *Cs. megalodon, I.hastalis*

HR046- Bone Valley Formation, Hickey Branch Site, Hardee, Florida (C.F. Industries)

Location: 27.64°N, 81.93°W[150]

Age: 14.5-10 million years old late Barstovian to mid Clarendonian[150] (Figure 8.13)

Description: This locality is part of the Hawthorn Group. It is a clastic unit of sand sized and larger phosphatic grains within a matrix of clay, silt and quartz sand. The lithology at the site is highly variable with sediments sizes ranging from silts and clays to coarse sands. The units are poorly consolidated and range in colour from white, light brown, yellow to olive greys and blue green. Elasmobranch teeth are one of the most abundant fossils from this group[153]. Based on the fossils found at the site it has been dated to mid-Miocene in age[153].

Species collected: *Cs. megalodon, I. hastalis*

Species analysed: *Cs. megalodon, I. hastalis*

NA004- Coosawhatchie Formation, North Fernandina Beach, Nassau, Florida

Location: 30.69°N, 81.43°W[150]

Age: 5.8- 4.5 million years old- latest Hemphillian to earliest Blancan[150] (Figure 8.13)

Description: The Coosawhatchie Formation consists of varying units of quartz sands, dolostones and clay. The quartz sand deposits are dolomitic, clayey and phosphatic in nature. They are fine to medium sub-angular sized grains that can be poor to moderately well sorted. The dolostone units are quartz-sandy, clayey and phosphatic. They are finely crystalline and infrequently contain moulds and fossils[152]. Clay units can be quartz dominant, sandy, silty, dolomitic or phosphatic in nature. They are more common in the base of the formation and are often dominated by smectite[154]. The formation as a whole dips to the north east at around 0.8mkm^{-1}. The Charlton Member sits near the top of the formation. It is characterised by interbedded carbonates and clays, which are less sandy in nature compared to the rest of the formation. This member is

highly fossiliferous and it is likely this is where the teeth used in this study originated.

Species collected: *Cs. megalodon, Cn. carcharias*

Species analysed: *Cs. megalodon, Cn. carcharias*

PO001, PO010Hh, PO016, PO018- Palmetto Fauna, Bone Valley Formation, Polk, Florida

Location:PO001- Palmetto mine 27.71°N, -81.93°W; PO010Hh- Phosphoria mine 27.83°N, 81.93°W; PO016 Nichols Mine 27.86°N, 82.01°W; PO018- Kingsford Mine 27.80°N, 82.03°W[150]

Age: 5.0-4.5 million years old[150, 156-157] (Figure 8.13)

Description: The deposits originate from the central Florida Phosphate district. All fossils are found as a by-product of phosphate mining in the area. The Bone Valley Formation is between 7.5 and 15 metres thick and overlies the Arcadia Formation. The boundary between the two formations is unconformable and characterised by the presence of coarse phosphatic rubbles and clays. The Bone Valley Formation is overlain by Pleistocene sands. The depositional environment of the Bone Valley sedimentary deposits has been interpreted as near shore marine with a range of energy environments. The most common fossils are those of isolated elasmobranch teeth. These range in preservation quality from pristine to highly worn and eroded[152]. The marine fossils are comparable to those found within the Pliocene age Yorktown Formation, Lee Creek Mine, North Carolina[155-157].

Species collected: *Cs. megalodon, I.hastalis, Cn. carcharias*

Species analysed: At PO001 *Cs. megalodon I.hastalis*; At PO001h *Cs. megalodon*; At PO016 *Cs. megalodon*; At PO018 *Cs. megalodon, I.hastalis*

SA004- Bone Valley Formation, Lockwood Meadows, Sarasota, Florida

Location: 27.36°N, 82.51°W[150]

Age: 7.5-6.8 million years old mid- Hemphillian[150] (Figure 8.13)

Description: Located within the city of Sarasota this deposit is an extension of the Upper Bone Valley Formation located within Polk County, Florida (See PO001, PO010Hh, PO016, PO018). In Sarasota the deposit is described as a buff, fine grained sand that contains dark grey, poorly sorted phosphatic pebbles and well-rounded, light rose metaquartzite pebbles[158]. Based on the mammal remains found at the locality, it is estimated to have been deposited in the early Hemphillian[159]. This deposit precedes, therefore, the Bone Valley Formation deposits of Polk County, Florida.

Species collected: *Cs. megalodon, I.hastalis*

Species analysed: *Cs. megalodon, I.hastalis*

SA024- Arcadia Formation, Dean's Trucking Pit, Sarasota, Florida

Location: 27.16°N, 82.39°W[150]

Age: 19-12.5 million years old- Hemingfordian to Barstovian[150] (Figure 8.13)

Description: The Arcadia Formation is predominately a carbonate unit with a variable siliciclastic component. The formation is composed primarily of interbedded sandy/clayey limestones and phosphatic dolostones. These vary in colour from light olive grey to light brown. They are typically micro to finely crystalline. Clays in the formation are yellow grey to light olive grey, moderately hard as well as having varying sandy, silty, phosphatic and dolomitic elements. Sands in the formation are yellow grey, vary from very fine to medium grained and vary in the levels of clay, dolomite and phosphate[152].

Species collected: *Cs. megalodon, I.hastalis*

Species analysed: *Cs. megalodon, I.hastalis*

5.2.2 Surface texture data acquisition

Microtextural data were collected from high fidelity casts of original teeth. Each tooth replica was produced using President Jet medium body polysiloxane dental moulding compound and black epoxy resin (Epotek-120 LV), mixed to manufacturer's guidelines. This method has been shown to produce no significant difference between the original tooth surface and that of the cast with regards to accuracy and precision[100].

High-resolution 3D surface data were captured following standard lab protocols[68, 93, 100], using an Alicona Infinite Focus microscope G4b (IFM; Alicona GmbH, Graz, Austria; software version 2.1.2), equipped with a x100 objective to give a field of view of 146 x 111 µm. The Alicona Infinite Focus microscope G4b has a CCD of 1624 x 1232 pixels. In theory, for a field of view of 146 µm, this equates to a lateral sampling distance of 0.09 µm, but the limits imposed by the wavelength of white light mean that lateral optical resolution is between 0.35–0.4 µm. For all samples, vertical and lateral resolutions were set at 20 nm and 440 nm respectively. Exposure settings were manually adjusted to maximize data quality (between 7.18 and 6.5 ms); contrast was set at 2.0. Point clouds were edited manually to delete measurement errors (e.g. single point data spikes) and extraneous dirt and dust particles from the surface. After editing, point clouds were exported as '.sur' files and imported into SurfStand (software version 5.0; restore bad data option selected). Surfaces were then treated by levelling the surface and removing gross tooth form with a second order polynomial function, and applying a spline filter, with a nesting index of 0.025 mm. The resulting scale limited roughness surface was then used for calculation of ISO 25178-2 standard parameters[101], quantifying tooth surface texture (Table 8.2).

5.2.3 Statistical analysis

The microwear texture data was normally distributed (Shiparo-Wilks test), thus no data transformations were performed and subsequent testing was parametric.

Hypotheses were tested using Analysis of Variance (ANOVA), pairwise testing (Tukey HSD), rank correlation (Spearman's Rank) and principal components analyses (PCA). Where unequal variance was detected in datasets (Bartlett and Levene tests) a Welch ANOVA was utilised in the place of a standard ANOVA.

In order to test whether *Cs. megalodon* was in dietary competition with *Cn. carcharias* and/or *I.hastalis,* comparison of tooth microtextural surfaces were investigated using ANOVA at specific time slices; late Miocene, mid-Miocene and Miocene/Pliocene boundary. In addition to this, principal components and pairwise comparisons were used to further investigate the level of dietary competition between the species.

In order to test the hypothesis that dietary competition caused the extinction of *Cs. megalodon*, variation in parameters and principal component 1 (PC1) scores through time was investigated for this species using ANOVA, pairwise testing and rank correlation. ANOVA, pairwise testing and rank correlation were also conducted on *I.hastalis* and *Cn. carcharias* individuals through time.

All statistical tests were carried out using JMP (versions 11 and 12, SAS institute, CANY, NC, USA).

5.3 Results

Analysis of the variance in microtextures between samples from different parts of the same tooth and microtextures between different teeth, from the same geological deposit, reveal no significant differences (Table 8.13). This is in accordance with other microtextural studies on elasmobranchs (Chapter 3).

ANOVA/Welch statistical tests reveal that there are minimal significant differences between *Cs. megalodon, I.hastalis* and *Cn. carcharias* during the mid-Miocene (16MA-11MA), late Miocene (11MA- 7MA) and at the Miocene/Pliocene boundary (7MA- 4MA) (Table 5.2). Analysis of the texture

parameters using Tukey HSD testing, revealed no separation between any species from any of the above time frames.

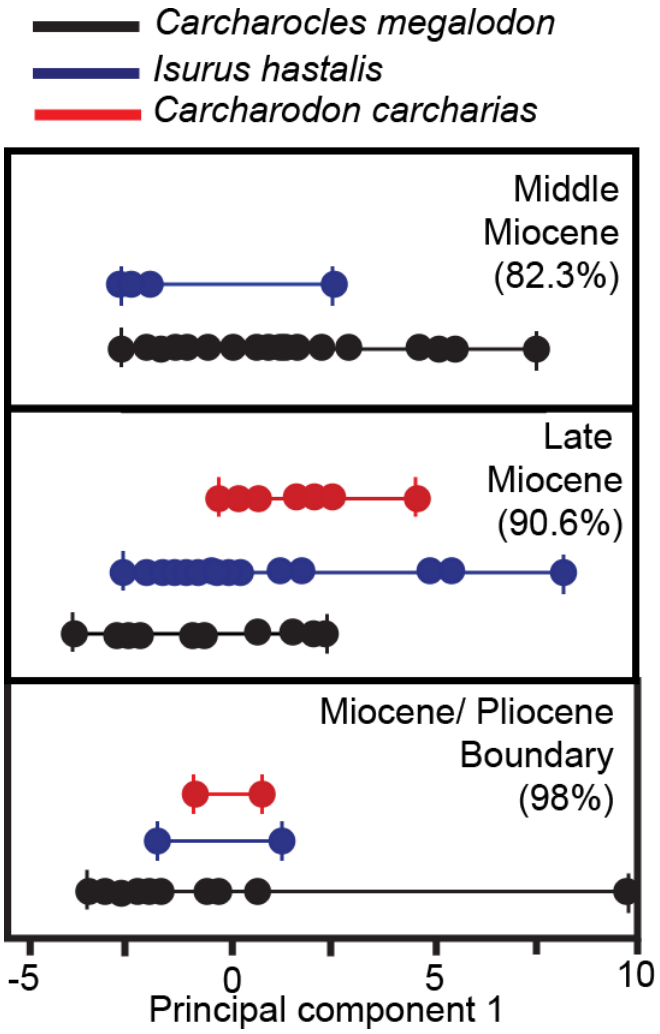


Figure 5.3: Graphical representation of principal components plots for three key time frames. The number in brackets beneath the time period indicates the amount of variance captured by principal component axis one in each instance. Mid-Miocene captured data between 16MA and 11MA , the late Miocene 11MA-7MA, the Miocene/ Pliocene boundary- 7MA-4MA.

Further analysis using principal components on the eight parameters (Sq, Sa, Spk, Vmp, Vvv, Sk, Svk, s5z,) that weighted most heavily in the generation of PC1 scores once again revealed no separation between the species. Between 82.9 % and 98% of the variation was accounted for by principal components axis 1 (Figure 5.2, Table 8.12). During all three time frames *Cs. megalodon*, *I.hastalis* and *Cn. carcharias* encompassed the same space bound by PC axes 1 and 2.

Table 5.2: Results of ANOVA comparing the microwear textures of 46 *Cs. megalodon*, 26 *I.hastalis* and 10 *Cn. carcharias* across three different time frames. 'w' indicates Welch ANOVA; significant differences (P>0.05) in bold. Tukey HSD testing revealed no significant pairwise differences.

	mid Miocene			**late Miocene**			**Miocene/Pliocene**		
	p	**d.f.**	**F**	**P**	**d.f.**	**f**	**p**	**d.f.**	**f**
Sq	0.1492	1, 25	2.2144	0.3898	2, 31	0.9713	0.9976	2, 15	0.0024
Ssk	**0.0259^{w}**	**1, 18.870**	**5.8499**	0.4735	2, 31	0.7659	0.0643	2, 15	3.3140
Sku	**0.0004^{w}**	**1, 22.515**	**16.8968**	0.1329^{w}	2, 18.316	2.2581	0.2082	2, 15	1.7457
Sp	0.1221	1, 25	2.5607	0.9334	2, 31	0.0691	0.7411	2, 15	0.3057
Sv	0.0471	1, 25	4.3623	0.9318	2, 31	0.0708	0.8950	2, 15	0.1118
Sz	0.0601	1, 25	3.8775	0.9991	2, 31	0.0009	0.8647	2, 15	0.1468
Sds	0.9920^{w}	1, 4.3967	0.0001	0.0687^{w}	2, 17.267	3.1406	0.7236	2, 15	0.3306
Str	0.3646	1, 25	0.8526	0.3332	2, 31	1.1391	0.3455^{w}	2, 2.4319	1.6983
Sdq	0.1048	1, 25	2.8323	0.4397	2, 31	0.8438	0.6207	2, 15	0.4923
Ssc	0.3952	1, 25	0.7483	0.5895	2, 31	0.5376	0.5343	2, 15	0.6537
Sdr	0.1304	1, 25	2.4460	0.5094	2, 31	0.6895	0.6679	2, 15	0.4147
Vmp	0.4929	1, 25	0.4843	0.3533	2, 31	1.0761	0.9880	2, 15	0.0120
Vmc	0.8866	1, 25	0.0280	**0.0276^{w}**	**2, 19.623**	**4.3324**	0.9842	2, 15	0.0159
Vvc	0.9711	1, 25	0.0013	**0.0274^{w}**	**2, 19.660**	**4.3410**	0.9945	2, 15	0.0055
Vvv	0.4293	1, 25	0.6454	0.3441	2, 31	1.1043	0.9849	2, 15	0.0153
Spk	0.1732	1, 25	1.9654	0.6055	2, 31	0.5099	0.9808	2, 15	0.0194
Sk	0.3346	1, 25	0.9679	0.1231	2, 31	1.6259	0.9917	2, 15	0.0084
Svk	0.0537	1, 25	4.0984	0.8023	2, 31	0.2218	0.9045	2, 15	0.1011
Smr1	0.5167	1, 25	0.4326	0.6896^{w}	2, 14.232	0.3816	0.8290	2, 15	0.1899
Smr2	0.3184	1, 25	1.0365	0.1364	2, 31	2.1258	0.4610	2, 15	0.8158
S5z	0.0702	1, 25	3.5785	0.9702	2, 31	0.0303	0.9437	2, 15	0.0581
Sa	0.2149	1, 25	1.6195	0.2799	2, 31	1.3270	0.9965	2, 15	0.0036

ANOVA of tooth microtextures for *Cs. megalodon* through time revealed only two parameters that differ, Sku and Smr2 (Table 5.3); of these Tukey HSD testing was only able to significantly separate the time points 13.8 MA and 5.115 MA for the parameter Sku (Table 5.4). For all other parameters no change was observed from 16.8 to 5.115 MA, and parameter trends display very little variation (Table 5.3). Rank correlation analyses found Sku to significantly increase through time. PC1 scores and all other parameters were uncorrelated with time (Table 5.3). ANOVA analyses also failed to find any differences in principal component 1 (PC1) scores through time (based on the parameters Sq, Sa, Spk, Vmp, Vvv, Sk, Svk and S5z) (Table 5.3, Figure 5.3). In summary texture parameters recorded the same surface textures for *Cs. megalodon* living before and after the evolution of *Cn. carcharias*.

ANOVA of *I.hastalis* tooth microtextures revealed no significant differences through time. ANOVA also revealed no significant differences in PC1 scores through time (Tables 5.5 and 5.6, Figure 5.3). Parameter trends are recorded in Table 5.5. Parameter trends appear to typically display an increase in score towards the late Miocene, then a decrease in score thereafter. The exceptions to this rule are Ssk, Sku, Sv, Sz, Svk which display no score change, Sds, Str, Sal, Ssc and Smr1 which fluctuate and finally Smr2 which decreases to the late Miocene then increases thereafter. No parameter displayed a significant correlation with time.

In contrast to analyses of *Cs. megalodon* and *I.hastalis*, ANOVA analyses on *Cn. carcharias* revealed nine significant differences in parameter scores through time (Table 5.7). Tukey HSD tests also separated the time frames 7.8 MA from 4.25 MA for the parameters Sq, Vmc, Vvv and Sa (Table 5.8). Rank correlations on parameter scores against time revealed that Sq, Vmc, Vvc, Sk and Sa all displayed a significant decline in parameter score towards the present day. A majority of parameters displayed a decreasing score towards the present day (Table 5.7). The exceptions to this were Ssk, Sku Str and Sal which varied and Sds, Smr1 and Smr2 which increased. Tukey HSD testing revealed that PC1 scores also showed a significant difference in microtextures between 7.8MA and 4.25MA (Table 5.7, Figure 5.3).

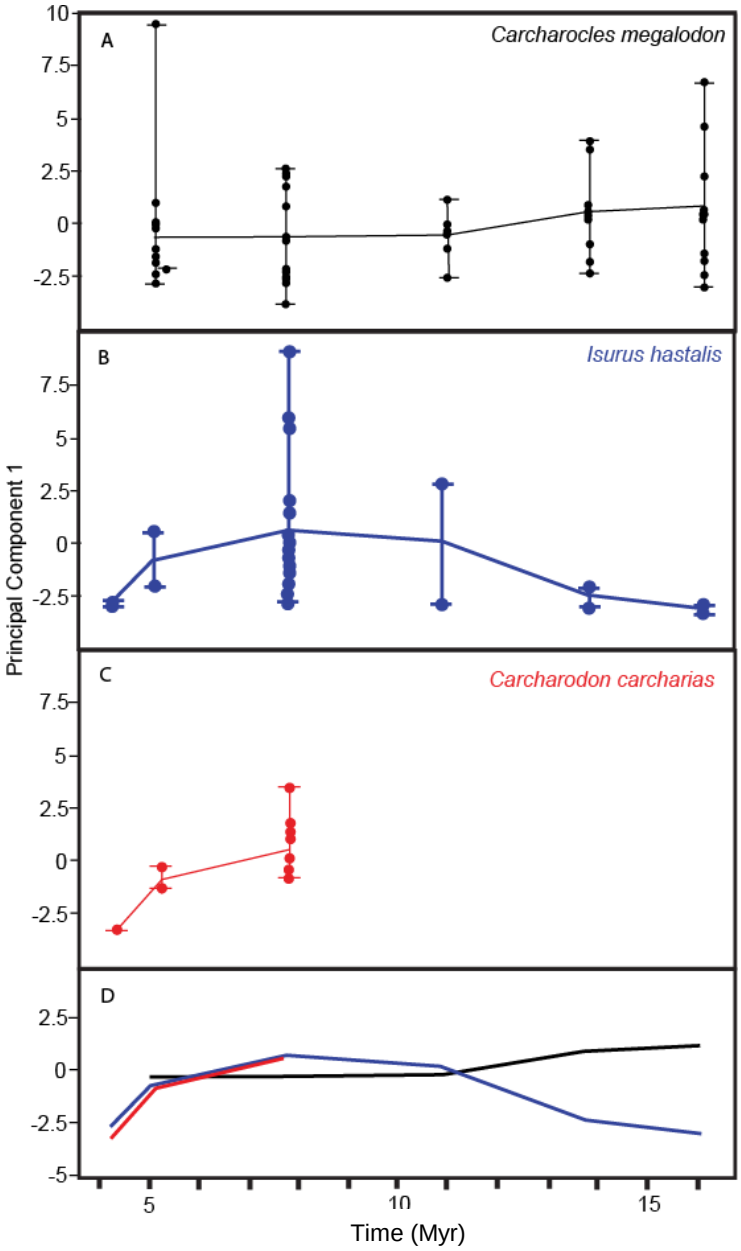


Figure 5.4: A-C- A series of bi-plots of principal component 1 scores through time for each species. D- summary plot of mean principal component scores through time for each species. Whiskers represent data ranges for each timing interval. A change in PC1 score (indicating dietary preference) can be observed for *Cn. carcharias* and *I. hastalis* from 7.5 MA, with a sharp change in score from 5MA. *Cs. megalodon* PC1 scores remain relatively constant through time.

Table 5.3: Results of ANOVA comparing 46 *Cs. megalodon* samples through time. /\ indicates an increase and then decrease in parameter score from 16.135 to 5.115 MA, \/ indicates a decrease followed by an increase in parameter score from 16.135 to 1.115 MA, ^^ indicates a fluctuation in score through time and – indicates no change in score through time. [W] denotes the use of a Welch ANOVA and significant results (P<0.05) are in bold.

	F	P	d.f.	Trend towards modern day
Sq	0.8106	0.5491	5, 40	-- Rs=0.0469 , p=0.3557
Ssk	1.0401[W]	0.4201	5, 14.524	^^ Rs=0.0419 , p=0.3980
Sku	**4.5089** [W]	**0.0157**	**5, 13.561**	**/\ Rs=0.1846 , p=0.0124**
Sp	0.6982	0.6280	5, 40	-- Rs=0.0595 , p=0.2672
Sv	1.3087	0.2798	5, 40	-- Rs=0.1052 , p=0.0917
Sz	1.0270	0.4149	5, 40	-- Rs=0.0907 , p=0.1295
Sds	1.1847	0.3338	5, 40	-- Rs=0.0001 , p=0.9988
Str	1.5412	0.1990	5, 40	\/ Rs=0.07560 , p=0.1845
Sal	1.3863	0.2500	5, 40	-- Rs=0.0974 , p=0.1104
Sdq	1.3962	0.2464	5, 40	-- Rs=0.0332 , p=0.4834
Ssc	1.3818	0.2517	5, 40	-- Rs=0.0252 , p=0.5781
Sdr	1.4437	0.2299	5, 40	-- Rs=0.0288 , p=0.5336
Vmp	0.0747	0.9957	5, 40	-- Rs=0.0037 , p=0.9229
Vmc	0.6655	0.6517	5, 40	-- Rs=0.0662 , p=0.2294
Vvc	0.4454	0.8140	5, 40	-- Rs=0.0441 , p=0.3792
Vvv	0.1801	0.9685	5, 40	-- Rs=0.0018 , p=0.9613
Spk	0.8505	0.5225	5, 40	↓ Rs=0.0706 , p=0.2071
Sk	1.0362	0.4098	5, 40	-- Rs=0.0245 , p=0.5873
Svk	1.0269	0.4150	5, 40	↓ Rs=0.0898 , p=0.1324
Smr1	1.0608	0.3963	5, 40	-- Rs=0.1024 , p=0.0980
Smr2	**2.5044**	**0.0460**	**5, 40**	^^ Rs=0.0944 , p=0.1186
S5z	0.9665	0.4498	5, 40	-- Rs=0.0851 , p=0.1479
Sa	1.6857 [W]	0.1945	5, 19.123	-- Rs=0.0329 , p=0.4871
PC1	0.5403	0.7445	5, 40	-- Rs=0.0274 , p=0.5500

Table 5.4: Pairwise differences (Tukey HSD) between *Cs. megalodon* samples from multiple time periods (16.135 MA, 13.8 MA, 10.965 MA, 7.8 MA, 5.33 MA, 5.115 MA) each with multiple individuals. Lower left side of matrix tallies differences, upper right shows the parameters that differ.

	5.115 MA	5.33 MA	7.8 MA	10.965 MA	13.8 MA	16.135 MA
5.115 MA					Sku	
5.33 MA	0					
7.8 MA	0	0				
10.965 MA	0	0	0			
13.8 MA	1	0	0	0		
16.135 MA	0	0	0	0	0	

Table 5.5: Results of ANOVA comparing 26 *I.hastalis* samples through time. /\ indicates an increase and then decrease in parameter score from 16.135 to 4.25 MA, \/ indicates a decrease followed by an increase in parameter score from 16.135 to 4.25 MA, ^^ indicates a fluctuation in score over time and – indicates no change in score over time.

	F	P	d.f.	Trend towards modern day
Sq	0.7736	0.5800	5, 20	/\ Rs=0.1372 , p=0. 3451
Ssk	0.3132	0.8992	5, 20	-- Rs=0.0651 , p=0.6793
Sku	0.3540	0.8854	5, 20	-- Rs=0.0753 , p=0.6237
Sp	0.6969	0.6320	5, 20	/\ Rs=0.0776 , p=0.6114
Sv	0.5809	0.7142	5, 20	-- Rs=0.10009 , p=0.4950
Sz	0.6459	0.6677	5, 20	-- Rs=0.0944 , p=0.5260
Sds	2.0175	0.1198	5, 20	^^ Rs=0.0548 , p=0.7369
Str	0.6623	0.6562	5, 20	^^ Rs=0.0861 , p=0.5674
Sal	1.0013	0.4423	5, 20	^^ Rs=0.1287 , p=0.3770
Sdq	0.9280	0.4837	5, 20	/\ Rs=0.1542 , p=0.2880
Ssc	1.9691	0.1275	5, 20	^^ Rs=0.0923 , p=0.5362
Sdr	0.7580	0.5903	5, 20	/\ Rs=0.1289 , p=0.3762
Vmp	0.8527	0.5291	5, 20	/\ Rs=0.1276 , p=0.3810
Vmc	1.0119	0.4366	5, 20	/\ Rs=0.1382 , p=0.3416
Vvc	1.0378	0.4228	5, 20	/\ Rs=0.1440 , p=0.3212
Vvv	1.0293	0.4273	5, 20	/\ Rs=0.1627 , p=0.2623
Spk	0.7099	0.6244	5, 20	/\ Rs=0.1165 , p=0.4260
Sk	0.7251	0.6126	5, 20	/\ Rs=0.1338 , p=0.3574
Svk	0.8710	0.5178	5, 20	-- Rs=0.1601 , p=0.2697
Smr1	0.7699	0.5825	5, 20	^^ Rs=0.1010 , p=0.4949
Smr2	0.2528	0.9334	5, 20	\/ Rs=0.0408 , p=0.8165
S5z	0.6473	0.6668	5, 20	/\ Rs=0.1039 , p=0.4814
Sa	0.7248	0.6128	5, 20	/\ Rs=0.1299 , p=0.3721
PC1	0.7919	0.5679	5, 20	/\ Rs=0.0790 , p=0.6042

Table 5.6: Pairwise differences (Tukey HSD) between *I.hastalis* samples from multiple time periods (16.135 MA, 13.8 MA, 10.965 MA, 7.8 MA, 5.115 MA, 4.25 MA) each with multiple individuals. Lower left side of matrix tallies differences, upper right shows the parameters that differ.

	4.25 MA	**5.115 MA**	**7.8 MA**	**10.965 MA**	**13.8 MA**	**16.135 MA**
4.25 MA						
5.115 MA	0					
7.8 MA	0	0				
10.965 MA	0	0	0			
13.8 MA	0	0	0	0		
16.135 MA	0	0	0	0	0	

Table 5.7: Results of ANOVA comparing 10 *Cn. carcharias* samples through time. w indicates Welch ANOVA; significant differences (P<0.05) in bold. A ↓ indicates a decrease in parameter score from 7.8 to 4.25 MA, an ↑ indicates and increase in parameter score from 7.8 to 4.25MA and ^^ indicates a fluctuation in parameter score between 7.8 and 4.25MA.

	F	P	d.f.	trend towards modern day
Sq	**5.4254**	**0.0378**	**2, 7**	↓ **Rs=0.7549, p= 0.0147**
Ssk	1.5553	0.2761	2, 7	^^ Rs=0.3287, p= 0.3025
Sku	0.8734	0.4586	2, 7	^^ Rs=0.2000, p= 0.5120
Sp	**4.9948**	**0.0449**	**2, 7**	↓ Rs=0.5753, p= 0.0766
Sv	3.3241	0.0966	2, 7	↓ Rs=0.5014, p= 0.1239
Sz	4.4001	0.0579	2, 7	↓ Rs=0.5611, p= 0.0846
Sds	2.4392	0.1571	2, 7	↑ Rs=0.4342, p= 0.1811
Str	0.7132^{w}	0.5504	2, 1.0285	^^ Rs=0.2962, p= 0.3486
Sal	0.1012	0.9050	2, 7	^^ Rs=0.1346, p= 0.6481
Sdq	2.8667	0.1232	2, 7	↓ Rs=0.4598, p= 0.1567
Ssc	0.8648	0.4617	2, 7	↓ Rs=0.2643, p= 0.3982
Sdr	2.5517	0.1471	2, 7	↓ Rs=0.4300, p= 0.1852
Vmp	**10.6181^{w}**	**0.0163**	**2, 6.2481**	↓ Rs=0.4526, p= 0.1641
Vmc	**5.1825**	**0.0416**	**2, 7**	↓ **Rs=0.7775, p= 0.0110**
Vvc	**6.1262**	**0.0290**	**2, 7**	↓ **Rs=0.7690, p= 0.0123**
Vvv	3.3271	0.0965	2, 7	↓ Rs=0.6186, p= 0.0555
Spk	**11.1088^{w}**	**0.0138**	**2, 6.5683**	↓ Rs=0.4786, p= 0.1418
Sk	4.5230	0.0548	2, 7	↓ **Rs=0.7412, p= 0.0173**
Svk	2.3423	0.1664	2, 7	↓ Rs=0.4915, p= 0.1315
Smr1	0.0891	0.9158	2, 7	^^ Rs=0.0338, p= 0.9021
Smr2	3.4195	0.0920	2, 7	↑ Rs=0.4728, p= 0.1465
S5z	3.0674	0.1105	2, 7	↓ Rs=0.4719, p= 0.1473
Sa	**5.5462**	**0.0360**	**2, 7**	↓ **Rs=0.7763, p= 0.0112**
PC1	**4.8167**	**0.0484**	**2, 7**	↓ Rs=0.5538, p= 0.0888

Table 5.8: Pairwise differences (Tukey HSD) between *Cn. carcharias* samples from multiple time periods with multiple individuals (7.8 MA, 5.33MA, 4.25MA). Lower left side of matrix tallies differences, upper right shows the parameters that differ.

	4.25 MA	**5.33 MA**	**7.8 MA**
4.25 MA			Sq, Vmc, Vvv, Sa
5.33 MA	0		
7.8 MA	4	0	

5.4 Discussion

As in other aquatic vertebrates, dietary differences are reflected in differences in tooth microtexture[61, 93] (Chapter 3). Analysis of microtextures in *Cs. megalodon* and *Cn. carcharias* failed to reject the null hypothesis that these taxa differ in their dietary preference. This in turn is consistent with the hypothesis that *Cn. carcharias* and *Cs. megalodon* were direct dietary competitors inhabiting the same environment. The lack of significant separation between the microtextures on *I.hastalis* and *Cs. megalodon* teeth indicate dietary overlap between these species also. The overlap in diet with *Cs. megalodon,* suggests the existence of a potential selection pressure prior to the evolution of *Cn. carcharias*.

Through time the microtextural signals from *Cn. carcharias* and *Cs. megalodon* did not differ significantly from one another. In addition, no separation was also found between these species and *I.hastalis*. The microtextural signals of *Cn. carcharias* sat within the range of signals produced by *Cs. megalodon* (Figure 5.3)*. Cs. megalodon* displays a greater variance in microtextural signal than that of *Cn. carcharias.* This is most likely due to ontogenetic shifts in diet of this species. All *Cn. carcharias* individuals investigated were adult and relatively similar in size. *Cs. megalodon* on the other hand displayed a range of ages, with teeth similar in size to those of *Cn. carcharias* to teeth that were over 10cm in height. Extremes of tooth size were not found within the same locality, suggesting segregated living in relation to ontogenetic stages. This is in keeping with existing literature which suggests *Cs. megalodon* had designated nursery areas[28, 66]. Segregated living based on ontogenetic stage is also observed in modern *Cn. carcharias*[160].

Competition between species, resulting in niche partitioning, only occurs if the resource being used by both is limited[148]. Thus no competition, and no niche partitioning, will occur if the resource is unlimited. During the Miocene, *Cs. megalodon, I.hastalis* and *Cn. carcharias* record the same tooth microtextures, and thus had the same dietary preferences. The lack of niche partitioning at this time suggests that food was in abundance, and other selection pressures were controlling population explosions.

A previously unlimited resource can become limited if environmental pressures change. This has recently been seen in the Northwest Atlantic with the collapse of fish stocks and the resulting niche partitioning in whales[149]. The Miocene/Pliocene boundary marks the beginning of a regional drop in temperature[208], due to the closure of the Isthmus of Panama[209]. At the same time, and possibly in response to climatic changes, there is a mass migration of large marine mammals away from tropical waters and into cooler ones[113]. This movement of prey would have resulted in a limitation of the dietary resource. Evidence of this is found within the microtextural signals on the teeth of *Cs. megalodon* and *Cn. carcharias*. *Cn. carcharias* records a sudden change in dietary preference away from that shared with *Cs. megalodon* (Figure 5.4), suggesting the occurrence of niche partitioning between the species. It was not possible to collect data for *Cs. megalodon* specimens dating to the Pliocene; as such statistical analysis of this potential partitioning has not been possible.

As environmental conditions in the Caribbean changed[209], and large marine mammals migrated to higher latitudes[210, 211], results from this study suggest that *Cs. megalodon,* unable to compete with *Cn. carcharias,* was unable to adapt effectively to the changing environmental pressures and thus became extinct.

With fossil evidence of consumption of large marine mammals by large *Cs. megalodon* documented[28, 34, 112], a high PC1 score, obtained by the largest *Cs. megalodon* samples, can be attributed to a large marine mammal focused diet. Assuming that fossil adult *Cn. carcharias* were consuming similar prey items to those consumed today, the principal components scores obtained by *Cn. carcharias* samples can be attributed to small to medium sized marine mammals. To be certain of diet, comparison of fossil textural scores to those from extant *Cn. carcharias* with a controlled mammal diet is ideally needed. This would create a base line reference point for a mammalian/fish diet.

This is the first study to successfully use 3D microtextural analyses to investigate diet and dietary competition in fossil elasmobranchs. As such it provides evidence for a new tool in the discrimination of dietary change in other

fossil elasmobranch species; opening the door for ecological, palaeontological and marine environmental studies.

In comparison to other 3D microtextural studies (e.g. 93) once again Vvv, Sk and Sa have been shown to be dietary discriminators. Although in this study the differences displayed through ANOVA analyses were not significant, these parameters did weight heavily in the generation PC1 scores. In addition to this the additional discriminatory parameters shown in modern elasmobranchs (Chapter 3) Sq, Spk, Vmp, Svk and S5z also generate separation between *Cs. megalodon* and *Cn. carcharias*. Although once again these to do not display significant separation, but do weight strongly in the generation of PC1 scores. It appears that the parameters utilised in this study generate good separation within elasmobranch species, both fossil and extant.

5.5 Conclusions

This is the first time that tooth microwear textures have been used to investigate diet and dietary competition in fossil elasmobranchs. The parameters creating the greatest separation between species are consistent with those creating dietary separation in other elasmobranch studies. The tooth microwear textures of *Cn. carcharias* and *Cs. megalodon* display no significant differences. This is consistent with a hypothesis of direct dietary competition between the two species. During the Pliocene *Cn. carcharias* alters it's dietary preference, generating resource partitioning of prey between *Cn. carcharias* and *Cs. megalodon.* There is no change in *Cs. megalodon* tooth surface microtextures at this time, suggesting a consistency in prey preference. The significant shift in the diet of *Cn. carcharias* suggests environmental pressures were present, limiting dietary resources and that whilst *Cn. carcharias* was adapting to meet these pressures *Cs. megalodon* was not.

6.0 Discussion

With elasmobranch populations in decline[5-6], and current methods of determining species diet often inappropriate, it is necessary to develop alternative techniques which require smaller sample sizes and provide a more complete overview of diet. The results of this thesis have demonstrated for the first time that tooth wear, both meso-style and microtextural, can be used as an alternative dietary discriminator in elasmobranchs. These techniques have several advantages over traditional techniques. Most importantly perhaps, as all specimens record a dietary signal, wear techniques require a smaller number of samples to generate the same dietary signal as stomach contents analyses. This is particularly important when studying rare and endangered species. In addition to this, microtextural analyses can be used to determine dietary interaction in the fossil record, helping us to understand how elasmobranchs responded to biotic crises in the past. Through understanding elasmobranch "coping" mechanisms and determining the diet of endangered species, more effecting conservation measures can be implemented to help counter declining populations.

6.1 Considerations for application

The application of wear analyses, traditionally conducted on mammals, to elasmobranchs require certain methodological modifications. This is largely necessary, due to tooth replacement mechanisms. Due to the rate of tooth replacement in elasmobranchs[99], it was important to establish whether dietary defining wear signals accumulate during the period a tooth is in use. For both methods, the separation between species and individuals, and between individuals suggest that a tooth is in use long enough to generate a dietary defining wear surface. That said it is not usually possible to distinguish, with the naked eye, between teeth that are newly erupted and those that have been used to process prey. As a result it is necessary to sample multiple teeth from an individual where possible. This has the result of reducing the impact of newly erupted teeth distorting the dietary signal for an individual. Whilst this generates "noise" in the data set it also ensures that an accurate representation of diet is

obtained. Sampling multiple teeth for each individual also reduces the impact of tooth position and individual prey processing preferences.

Obtaining wear patterns from multiple teeth in an extinct individual is more problematic. However, because the number of shed teeth outnumbers the number of teeth in the jaw upon death by orders of magnitude, the probability of analysing a newly erupted tooth that displays no dietary signal is very small. As such any singular tooth analysed has the potential to divulge the dietary preferences of the individual from which it originated

A further consideration is also required when applying wear techniques to fossil material of any animal group. The third question, addressed by this thesis, examined the taphonomic impacts of sediment abrasion to tooth microtextures. Results from this study show that the amount of sediment abrasion and the composition of sediments in which fossil teeth are found need to be considered before microtextural analyses are conducted. Different sediment types alter tooth microtextures in subtly different ways. If all teeth originate from the same deposit, or deposits with similar sedimentary compositions, comparisons between teeth yield accurate differences between individuals. This is assuming that exposure to sediment has not been extensive. Comparison of teeth originating from different sedimentary deposits, with different sedimentary compositions, need to be treated with care. Different sediments alter tooth microtextures in different ways. This can be sufficient to generate false separation, or false similarity between individuals.

This is the first study to investigate the impact of sediment abrasion on tooth microtextures. The findings are however comparable to those obtained by King et al[73] who investigated sedimentary effects on 2D microwear signals. This study, like King et al.[73], found that sediments removed existing tooth surfaces after a period of sedimentary exposure. Unlike King et al.[73], and other previous studies[71-72, 74], our results found that sediments often produced textures that replaced the tooth microtextural surface. It was only towards the end of the experiment that significant differences began to be generated. This study also failed to generate the level of abrasion, often observed on fossil teeth[107] or described in

early taphonomic studies on 2D microwear[71-72, 74]. The teeth in this study were only just beginning to display outward signs of sediment abrasion.

It is therefore suggested that teeth which do not display:

- wear in non-dietary processing locations[107]
- wear in an irregular manner[107]
- a dull or scuffed surface (first signs of sediment abrasion obtained in this study)

can be used with some confidence in microtextural analyses. However care should be taken when comparing teeth originating in calcarenitic dominant sediments, or teeth that originate from different deposits with different sedimentary compositions

6.2 Meso-style wear

The first question in this thesis sought to determine whether mesowear techniques could be adapted and applied to elasmobranchs to successfully determine dietary preferences. The results of this study demonstrate that meso-style wear patterns exhibit a relationship with diet in elasmobranchs. Strong correlations between PC1/PC2 scores and diet indicate that meso-style wear patterns are reflecting dietary differences, both within and between species. Using ontogenetic dietary shifts, in the species *C. carcharias, C. taurus, C. plumbeus* and *G. cuvier,* it has been possible to determine that:

1. an increase in PC1 score indicates an increase in the consumption of elasmobranchs
2. an increase in PC1 score combined with a decrease in PC2 score indicates an increased consumption of benthic elasmobranchs
3. an increase in PC2 score indicates an increase in the consumption of marine mammals
4. an increase in PC1 and PC2 scores indicates an increase in the consumption of cephalopods.

This is the first time that mesowear methods have been adapted and applied to elasmobranchs. Although these results confirm an association between food processing and tooth wear, direct comparison to other studies is difficult. Traditional mesowear analyses aim to score the level of wear of a facet on a homologous tooth position/s by examining cusp relief, caused by blunting and rounding[37, 85]. This is not possible in elasmobranchs due their constant tooth replacement. Purnell and Jones[45] adapted traditional mesowear methods, examining wear features across a conodont element. Whilst microscopic in size the scoring method used is comparable to those used in other mesowear analyses, and more applicable to elasmobranchs. The wear features, breakage, spalling and rounding used in this study, originated from Purnell and Jones[45]. Due to the the lack of occlusion between teeth, and the tooth replacement cycle in elasmobranchs, variable wear levels were noted throughout the jaw of an individual shark. This made analysis of a select location in the jaw impractical. It was thus deemed necessary to analyse wear across an entire jaw to average out the effects of tooth replacement and individual hunting strategy.

This method provides a broad overview of dietary preference for elasmobranchs. Although specific species are not revealed, knowing the species residing in a habitat range, and the dietary preference of the elasmobranch species, it is possible to put in place conservation measures which protect the main prey groups, and thus the elasmobranch species in question.

6.3 Microtextural analysis

With respect to the second research question, “can microtextural analyses be used to determine diet in elasmobranchs?”, our results show that tooth surface microtextures vary with diet in *C. taurus*. The strong correlations between PC1 values and diet, and the absence of correlation with body size, indicate that microtextures are tracking the ontogenetic transition of diet, rather than the increase in individual size *per se*. This indicates for the first time that microtextural analyses can provide an additional, potentially powerful tool for dietary discrimination in extant elasmobranchs.

The fourth question addressed in this thesis was the application of microtextural analyses to extinct elasmobranchs. This was specifically tested through the analysis of dietary competition between the macro-predators *Cs. megalodon* and *Cs. carcharias*. The results from this thesis further support the idea that *Cs. megalodon* and *Cn. carcharias* were living in the same habitats and consuming the same prey during late Miocene and Pliocene[162]. The results of this study failed to reject the null hypothesis that these taxa differ in their dietary preference. The occurrence of apparent nice partitioning at the start of the Pliocene indicates a limitation on the shared dietary resource[148], with *Cn. carcharias* opting to alter its foraging habits in light of new selection pressures. The methodological implications of these results suggest that insight into dietary competition and dietary preference in extinct elasmobranch species is possible via the application of microtextural analyses.

Despite the different mineral properties of extinct and extant shark's teeth, similar microtextural parameters displayed significant differences during experiments. These parameters are consistent with those of previous work analysing diet in aquatic organisms[93,68]. Studies investigating the diet of terrestrial vertebrates also found these parameters displayed separation between the different dietary groups[94,102]. Parameters relating to height and volume appear to play the largest role in the separation of diet within and between species (Table 8.3).

These studies suggest that microtextural analyses can be successfully used to obtain detailed dietary information for endangered, rare and extinct elasmobranch species.

6.4 Comparison to traditional techniques

Wear analyses pose three advantages over traditional techniques.

1. They require much smaller sample sizes to generate the same dietary information.
2. Dietary data accumulates over a longer time frame, providing a more accurate overview of an individual's dietary preferences.

3. The techniques can be applied to specimens that have no stomach contents. This includes individuals that are caught with empty stomachs, dried museum specimens and fossil material.

Wear analyses are thus particularly suited to the study of rare and endangered species, where culling large numbers of individuals is not possible, and to the study of extinct species.

Where possible the combination of wear analyses with stomach contents would provide a particularly strong measure of a species diet; with data captured at different time frames and specific prey species identified. Table 6.1 below highlights some of the advantages and disadvantages of each dietary defining method.

Table 6.1: Summary table of advantages and disadvantages for each dietary defining technique used on elasmobranchs.

	Stomach contents	Isotope	Observation/ morphology	Meso-style	Microtextural
Sample size	Large	Small	Large	Small	Small
Indication of specific prey	Yes	No	Yes	No	No
Indication of broad prey preference	Yes	No	Yes	Yes	Yes
Timescale	Short	Long	Short	Long	Long
Fossil?	No	No	No	Yes	Yes
Endangered species friendly?	No	Yes	Yes	Yes	Yes
Dried specimens	No	No	No/Yes	Yes	Yes

The findings of this thesis indicate that wear analyses may help us to understand the dietary ecology of many elasmobranch species. The study of jaws stored in museums and private collections would enable the analysis of diet for various elasmobranch species, without the need to capture any live individuals.

The application of wear techniques to fossil material also creates opportunities to study marine community strength, and elasmobranch dietary change during periods of biotic crises and in response to human impacts on the oceans.

6.5 further work

This thesis was designed to investigate the possibility of using wear analyses in the study of elasmobranch diet. As previously never tested, this work provides evidence of proof of concept. As such further work is required to answer further questions relating to the application of wear analyses to elasmobranch dietary study.

In particular analysis of individuals with known stomach contents data is required to calibrate both techniques. Such individuals are difficult to source, but essential if wear analyses are to be widely adopted in the study of elasmobranch diet. Further investigation into the taphonomic impacts of sediments to tooth microtextures, are also required to further advance our understanding of how techniques can be applied to any fossil material.

With respect to further advancement within specific chapters, see the notes below

- With regards to Chapter 2, data should be further tested by predicting the diet of individuals with stomach contents data. This would highlight the accuracy of meso-style wear at predicting diet.
- With regards to Chapter 3, an investigation into the diet of *C. taurus* residing in eastern waters would act as a test for the prediction of diet of the Philippines individual with no previously known diet.
- Further experimentation investigating the impacts of other sedimentary types, the impacts of stomach acid to tooth surfaces and subsequent sediment abrasion and the impacts of extended periods of sediment abrasion could also be studied to help advance our understanding of taphonomic processes and influences on tooth microtextures. The application of sedimentary abrasion via means other than a commercial tumbler, e.g a flume tank, may yield different results and should also be

investigated. Understanding of taphonomic processes will enable a more accurate investigation of extinct species diet.

- With regards to Chapter 5, projection of data from extant *Cn. carcharias* with known diet onto the principal components plots for fossil *Cn. carcharias, I.hastalis* and *Cs. megalodon* would provide markers with which to determine the diet of these fossil species. The comparison of data from individuals with known diet onto plots generated from fossil data can provide an indication of diet in extinct species. To do this, however, data is needed from individuals with known diets. A wide range of individuals with different diets would also help to strengthen our understanding and application of microtextural techniques.

7.0 Conclusion

This study set out to investigate the possibility of applying wear analyses (mesowear and microtextural) to the study of elasmobranch diet. With this came the consideration of several elasmobranch specific features, specifically the implications of tooth replacement.

This study has shown that mesowear techniques can be adapted, and successfully applied, to the study of elasmobranch diet. When broad wear features, similar to those of other studies[45], are applied to the entire upper jaw of elasmobranch individuals, a dietary signal is detected. This signal was capable of detecting broad prey preferences and ontogenetic shifts in individual species. This technique can be applied to live caught individuals that preserve no stomach contents to determine diet. The technique can also be used to study dried and museum specimens.

This research has also shown that microtextural analyses can be applied to, both extinct and extant, elasmobranchs to determine elements of dietary ecology. Microtextural analyses provide a measure of individual diet, and ontogenetic differences in preference. This technique can too, be applied to the study of specimens captured without stomach contents and dried museum specimens. In addition, the methods have been shown to reflect dietary ecology in extinct elasmobranch species.

The application of microtextural techniques to the study of fossil material has also been investigated through the consideration of taphonomic processes. This study has found that taphonomic processes have the potential to alter tooth microtextures to the extent that false separation or similarity is generated. Results also suggest that comparison of specimens originating from different sedimentary deposits need to be treated carefully, as microtextural signals may be falsely altered.

The results of this thesis have highlighted the continued strengths of wear analyses in the study of diet. The techniques have been applied here to elasmobranchs for the first time, and although the results are promising, further work is required to fully understand the full potential of these techniques.

8.0 Appendices

8.1- Appendices relating to all chapters

8.1.1- Species information

A series of sheets detailing the demographics, ecology, dentition and diet of each species discussed in this study

8.1.1.1- Carcharhinus brevipinna

Demographics

Demographic information for this species is often confused due to individual mis-identification as *Carcharhinus limbatus*[161]. *Carcharhinus brevipinna* is found worldwide in all tropical and warm temperate waters, except those of the Eastern Pacific. Individuals are found in both coastal and off-shore waters to a depth of 100 meters. *C.brevipinna*, however typically prefers waters that are less than 30 meters in depth[199]. Juveniles have been known to enter bays, but avoid brackish waters. Juveniles also prefer cooler waters than adults, leading to some habitat segregation between juveniles and adults.

Life Traits

C. brevipinna is capable of reaching 3 meters in length; however adults typically average closer to 2 meters. As with most species males mature at a smaller size and a younger age than females. 1.3m and 4-5 years vs 1.5-1.6m and 7-8 years respectively. Although individuals are mature by 8 years of age they do not typically reproduce until an age of 12 years. It is thought that the longevity for this species is between 15 and 20 years[164]. Individuals from South Africa mature later and at a greater size than other sub-populations of this species[162]. Neonates are born in shallow coastal nurseries[163]. Upon maturing, individuals move away from the coast, forming large shivers that are segregated by age and gender[2].

Dentition and Diet

C. brevipinna has a similar dentition in both its upper and lower jaws. The upper jaw supports 34, on average, long narrow single cuspate teeth that are finely serrated along both cutting edges. The lower jaw supports 32 teeth, on average, that are the same as the upper jaw except that they are unserrated,

Figure 8.1. This dentition does not lend itself to cutting and tearing, as a result prey are typically consumed whole. The diet of this species is based mainly around teleosts and cephalopods[165-166].

Figure 8.1: Upper left hand dentition of *Carcharhinus brevipinna*[207].

8.1.1.2 Carcharhinus falciformis

Demographics

C. falciformis has a worldwide distribution in tropical waters that exceed 23°C. They inhabit a pelagic habitat away from the continental shelf once matured. Juveniles are born and mature at the edge of the continental shelf. Despite inhabiting open waters *C. falciformis* typically spends its time less than 50m from the water surface, with occasional dives down as deep as 500m[170]. Although *C. falciformis* has a worldwide distribution there is limited genetic movement.

This has resulted in the formation of at least four genetically distinct sub-populations, based upon differing life traits. These sub-populations are in the; North-West Atlantic, Western and Central Pacific, Eastern Pacific and Indian Oceans[171].

Life Traits

C. falciformis commonly obtain lengths up to 3.3 meters. Growing and maturing quickly, males reach maturity between 6 and 10 years or 1.8- 2.4 meters. Females mature between the ages of 7 and 12 years or at a length between 1.8 and 2.6 meters. Pacific individuals mature earlier and at a smaller size in comparison to other *C. falciformis* populations; Indian Ocean individuals mature later and at a greater size. It is though that individuals of this species have a longevity exceeding 22 years[168-169, 172].

Dentition and Diet

Carcharhinus falciformis has 30 teeth, on average, in each of its upper and lower jaws. The dentition of the upper jaw is triangular, cuspate and highly

serrated with a notch along the posterior cutting edge of the tooth. Teeth in the upper jaw are erect at the anterior but become increasingly oblique as you travel along the jaw towards the posterior. The lower jaw dentition is uniform, only decreasing in cusp height from the anterior towards the posterior. Lower jaw teeth are narrow, erect and single cusped with smooth sharp cutting edges, see Figure 8.2. Typical prey for *C. falciformis* are teleosts and cephalopods[167].

Figure 8.2: Upper right hand dentition of *Carcharhinus falciformis*[207].

8.1.1.3 Carcharhinus leucas

Demographics

Carcharhinus leucas is common in tropical and subtropical waters worldwide. During summer months it will occasionally enter warm temperate waters. *C. leucas* is not restricted to marine settings and is frequently observed entering and inhabiting freshwater lakes and rivers172. The species is also capable of living in hypersaline conditions. Individuals of this species are typically found in waters no more than 30 meters deep. Due to the species ability to inhabit freshwater and its preference for coastal waters, individuals frequently come into contact with humans[173].

Life Traits

C. leucas can grow up to 3.4 meters in length and live to approximately 16 years[174], with females averaging a larger length than males. Males also mature at a smaller size than females, 1.57-2.26m and 1.80- 2.30m respectively. Upon reaching adulthood this species almost always remains solitary, returning to freshwater or estuarine lagoons to breed. Neonates and juveniles remain in these nursery areas for several years. This makes *C. leucas* vulnerable to anthropogenic caused change[175-176].

Dentition and Diet

C. leucas has a broad potential diet, although typically restrains itself to teleosts and elasmobranchs. This diet can include turtles, mammals, birds, crustaceans, echinoderms, teleosts and elasmobranchs[170]. High dietary diversity is only utilised by the largest individuals. Again with this species there is documented ontogenetic dietary change. Cliff and Dudley[177] recorded that in South Africa there is a shift in the dominant prey from teleosts to elasmobranchs as individuals grow. In Florida, juveniles are recorded eating small rays and catfish almost exclusively, with adults consuming a wider variety of other prey[175].

C. leucas has the ability to consume a wide range of prey due partly to its versatile dentition. *C. leucas* has differing dentition between its upper and lower jaws, like all carcharhinids. The lower jaw dentition is similar, but broader than typical carcharhiniform lower dentition. In the largest individuals this lower dentition can be weakly serrated. The upper jaw sports broad, triangular teeth that are serrated along both cutting edges. The serrations coarsen towards the tooth base. Moving from the anterior towards the posterior of the jaw the teeth begin to curve back towards the jaw commissure and decrease in size, see Figure 8.3.

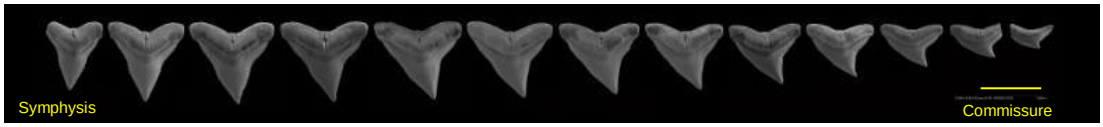


Figure 8.3: Upper left hand dentition of *Carcharhinus leucas*[207].

8.1.1.4 Carcharhinus melanopterus

Demographics

Carcharhinus melanopterus is very common in tropical and sub-tropical shallow, coastal reef waters of the Indian and Pacific Oceans, as well as the Mediterranean Sea. The species is absent from the Atlantic Ocean and the American Pacific coast. *C. melanopterus* is typically found in waters only a few meters deep. They have been known to enter brackish water very occasionally[170].

Life Traits

C. melanopterus is a medium sized elasmobranchs that grows to approximately 1.8 meters in length. Individuals mature between the lengths of 0.9 and 1.1 meters[170, 178]. The species is easily identified by the black tips to all of its fins, which are often observed above the water line. *C. melanopterus* typically reside in small habitat ranges of only a few kilometres, which they will remain in for many years rather than migrating long distances like many other species.

Dentition and Diet

The diet of *C. melanopterus* consists primarily of teleosts, although they will occasionally take crustaceans, cephalopods, molluscs[178] and sea snakes[179]. The upper jaw consists of 24 narrow cuspate teeth that are finely serrated along the cutting edges, coarsening towards the base. Teeth also become increasingly reclined towards the posterior, as you move towards the jaw commissure. The lower jaw dentition is similar to that of the upper dentition, but is only very finely serrated in the largest of individuals, with a majority of individuals having an unserrated lower dentition, see Figure 8.4. The male dentition is more reclined that that of the female.

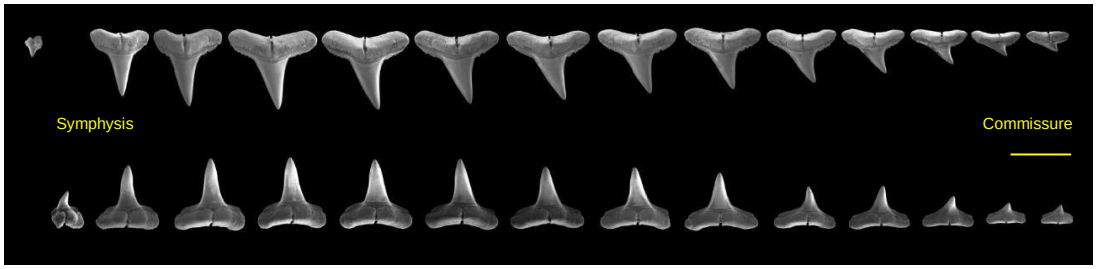


Figure 8.4: Upper and lower right hand dentition of *Carcharhinus melanopterus*[207].

8.1.1.5 Carcharhinus plumbeus

Demographics

Carcharhinus plumbeus has a global distribution within warm temperate and sub-tropical waters. They are also the most abundant large elasmobranch in the Western Atlantic. Ontogenetic habitat variation is documented for this species, with juveniles living in warm temperate waters and adults in sub-tropical waters. Regardless of water temperature this species is most commonly found in

shallow waters, between 20-65 meters in depth, which have sandy or muddy substrate floors[180].

Life Traits

C. plumbeus is a slow growing species, with evident sexual dimorphism. Females are capable of obtaining lengths of 2.5 meters, maturing at 16 years and at a length between 1.29 and 1.58 meters. Males on the other hand only grow to 1.8m, mature after 13 years and at a size between 1.23 and 1.56 meters. Geographic variation is also recorded with individuals in eastern waters maturing younger and obtaining a shorter final length than western water counterparts[181-182].

Dentition and Diet

C. plumbeus has classic carcharhiniform lower dentition; small, narrow single cuspate teeth. Its upper dentition identifies this species from other carcharhiniforms. In the upper jaw, *C. plumbeus* has broad triangular cuspate teeth that are finely serrated along both cutting edges. Serrations become coarser towards the base of the tooth. Teeth become increasingly more reclined towards the commissure of the jaw (Figure 8.5).

As with many elasmobranchs there is ontogenetic dietary variation, this is heightened in *C. plumbeus* by ontogenetic spatial variation. As individuals increase in size they move from a diet dominated by benthic invertebrates and teleosts to one that is more varied and includes teleosts, elasmobranchs and cephalopods. Benthic invertebrates play little part in the adult diet[183-184].

Figure 8.5: Upper left hand dentition of *Carcharhinus plumbeus*[207].

8.1.1.6 Carcharias taurus

Demographics

Carcharias taurus has a worldwide distribution. The species can be found in all subtropical and warm-temperate coastal waters, except those of western North and South America. Due to their wide distribution they are known by many

different common names globally, which can confuse the literature. These include: The sand tiger shark, grey nurse shark and spotted ragged-tooth shark. *C. taurus* is most commonly located in waters between 15 and 20 meters in depth, although they have been recorded at a depth of 200 meters[186-187].

Life Traits

This species is capable of obtaining lengths around 3.2 meters[97], with both sexes maturing around 2 meters in length. Goldman[188] identified that males mature between the ages of 6 and 7 years and at a slightly smaller size to females that mature between the age of 9 and 10 years. Longevity for *C. taurus* is unknown, but they live in captivity up to 16 years[185]. *C. taurus* is a highly adaptable species capable of living and hunting alone or in a shiver of up to 80 individuals.

Dentition and Diet

C. taurus has tall narrow cuspate teeth that are recurved. A single large cusplet is positioned either side of the main cusp, see Figure 8.6 . Crown height decreases and crown width increases as you move from the anterior to the posterior of the jaw. The species "toothy" appearance has given *C. taurus* an unjustified, reputation in many parts of the world as a "man-eater". In practice this placid elasmobranch consumes a diet of teleosts and elasmobranchs. Ontogenetic, but not geographic, variation is documented in this species. Juveniles consume a diet of predominately teleosts and the occasional small elasmobranch, as an individual increases in size prey diversity and prey size increases. There is also a documented decrease in the importance of teleosts in the diet, with a proportional increase in importance of elasmobranchs[96-98].The species of prey taken is heavily dependent on local prey abundances.

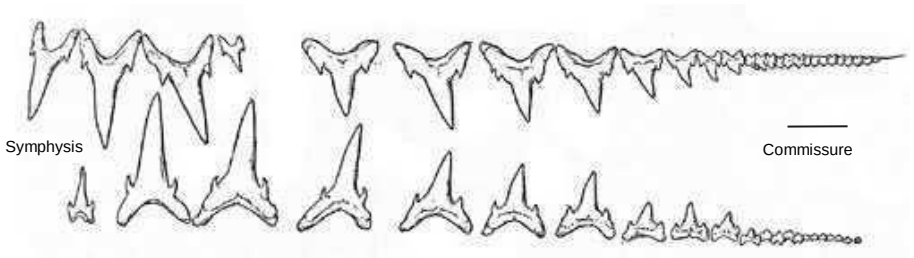


Figure 8.6: Upper and lower right hand dentition of *Carcharias taurus*[214]

8.1.1.7 *Carcharodon carcharias*

Demographics

C. carcharias can and does inhabit almost all coastal and open waters that have a sea surface temperature between 12°C and 24°C. They are found in higher numbers off the coast of Southern USA, South Africa, Japan, Oceania, Chile and the Mediterranean. They are common both at the surface and down to depths of 1200m[189].

Life Traits

Carcharodon carcharias is a large slow growing species, capable of obtaining lengths of 7m+. Males typically mature quicker than their female counterparts. Males considered mature once they reach a length between 3.5m and 4m, this typically takes 26 years. Females mature between lengths of 4m and 4.5m, around an age of 33 years[193-194].

Dentition and Diet

The dentition of *Carcharodon carcharias* is stereotypical of the commonly thought of “shark’s tooth”. They have a broad, triangular cuspate dentition that is coarsely serrated along both edges, see Figure 8.7. This strong dentition enables this species to consume a wide variety of large prey items. As with most elasmobranch species, ontogenetic dietary variation has been documented. Juveniles typically consume fish and elasmobranchs. Once an individual has obtained a length of approximately 3m they will start to consume marine mammals, by 4m in length their diet is almost exclusively that of marine mammals. The species preyed upon is dependent on prey abundance and individual preferences. A typical *C. carcharias* has a diet containing the following in varying proportions: fish, elasmobranchs, cetaceans, pinnipeds, turtles, otters
and birds. They have also been documented to scavenge from whale carcasses[141, 144, 190-191].

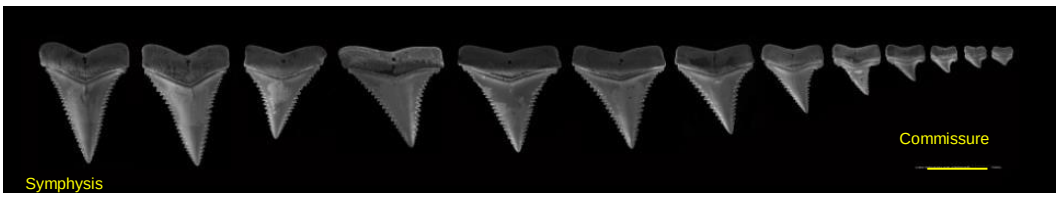


Figure 8.7: Upper left hand side dentition from a juvenile *Carcharodon carcharias*[207].

8.1.1.8 *Galeocerdo cuvier*

Demographics

G. cuvier has a worldwide distribution. They are found within tropical waters during the winter months, migrating into temperate waters during the summer, following warm currents. They are primarily classified as a deep water species, inhabiting the waters just off of coastal reefs. Although observed in shallow waters, 3m in depth, they are thought to reside at an average depth of 20m[197].

Life Traits

G. cuvier is one of the largest extant elasmobranchs, with a maximum length of 5m. With their camouflaged skin and slow swim speed they are able to stalk prey effectively, releasing a burst of speed to seal the kill. These elasmobranchs reach maturity between 2.5 and 3m for males and 3 and 3.5m for females[196].

Dentition and Diet

G. cuvier has one of the most iconic dentitions of any elasmobranch species, see Figure 8.8. Its dentition is designed to cut and tear flesh. The posterior edge of the tooth is coarsely serrated, not unlike that of many elasmobranch species. It is the distal edge that identified this species from others. The top half of the distal edge is serrated, like the proximal side, and is inclined back on itself. The lower half of the distal edge slopes back down to the root edge and displays a series of coarse cusplets. The effect of these two different halves of the distal edge creates a notch and a backwards facing barb-like tooth tip. This tooth morphology allows *G. cuvier* to have a varied and opportunistic diet. Although this species is an opportunistic feeder, feeding on the easiest and most abundant prey in an area, there are dietary trends emerging through quantitative stomach contents studies. As with most species there are ontogenetic and geographical differences in diet. Lowe et al (1996)[195] described these trends as follows, as individual size increases there is a correlated increase in prey diversity and prey size. In Hawaii this is observed as a decrease in the importance of teleosts and cephalopods and an increase in the abundance of elasmobranchs, marine mammals, turtles and miscellaneous anthropogenic objects as the elasmobranch reaches adulthood. Lowe et al (1996)[195] also noted a differentiation in hunting habits between adults and

juveniles, further accentuating ontogenetic dietary differences. They noted that juveniles hunt only at night as bottom feeders, in contrast adults will feed from the surface during the day and feed from the bottom, like the juveniles, during the night. Similar dietary splits are observed at various locations around the coasts of Australia[196].

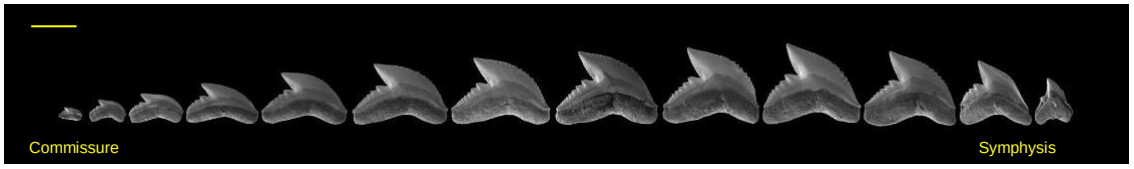


Figure 8.8: Lower right hand dentition from *Galeocerdo cuvier*[207].

8.1.1.9 Isurus oxyrinchus

Demographics

I.oxyrinchus is an offshore species found in temperate and tropical waters worldwide. Keeping its distance from shore, with only occasional forays into inlets and around islands, individuals are typically located within the top 150 meters of the water column. *Isurus oxyrinchus* is documented to migrate over long distances in search of prey and mates. During these migrations *I.oxyrinchus* rarely enters waters less than 16°C, despite being an endothermic species[199].

Life Traits

Capable of growing to 4m in length, females typically obtain a greater length than their male counterparts. *I.oxyrinchus* displays elevated growth rates over those of other lamnid sharks[199]. In 2006 a series of experiments redefined the longevity and maturation ages of this species. Females typically mature at 18 years of age and with a longevity of 32 years. Males mature after only 8 years and live to around 29 years of age. These ages are regardless of global location[200-201].

Dentition and Diet

Geographical variation in diet is documented for this species. Individuals from the Northern Atlantic and Australia consume cephalopods and large teleosts,

such as tuna and swordfish[81, 202]. Individuals from South Africa specialise in consuming other elasmobranchs[203]. In general the species consumes predominately large teleosts and cephalopods, but will occasionally take elasmobranchs, porpoises, dolphins, turtles and birds. An ontogenetic change in dentition allows larger individuals to take on these larger prey types. Adult *I.oxyrinchus* have wider and flatter interior teeth, which enable this change in diet. In general *I.oxyrinchus* has narrow, hooked cuspate teeth that are curved towards the centre of the mouth. Teeth become smaller and broader as you move from the anterior towards the posterior of the jaw. They also become more reclined towards the posterior as you move towards the jaw commissure. Teeth are unserrated but still have very sharp cutting edges, see Figure 8.9.

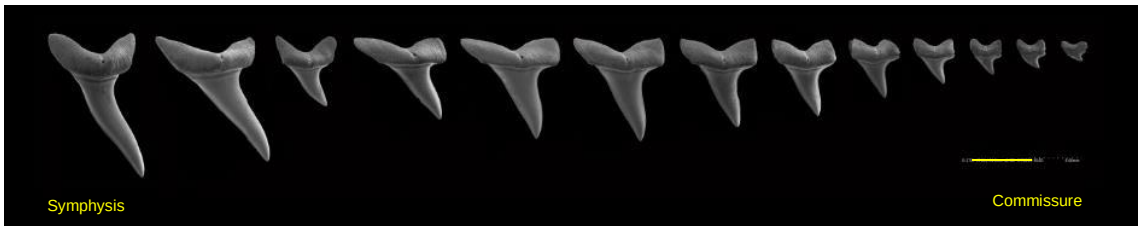


Figure 8.9: Upper left hand dentition of a juvenile *Isurus oxyrinchus*[207].

8.1.1.10 Prionace glauca

Demographics

Prionace glauca is an abundant pelagic and oceanic elasmobranch, inhabiting most waters between latitudes of 60°N and 50°S. It prefers waters with temperatures between 7°C and 16°C, but will tolerate warmer waters. Due to its temperature preferences it is found at differing depths globally. In tropical waters it is usually found in deeper waters, up to 350m in depth. In temperate oceans it is often located close to the surface and close to the shore. It has been documented in estuarine localities in temperate environments[170].

Life Traits

P.glauca commonly obtains lengths approaching 3.8m, and have a longevity of approximately 20 years. Individuals are classified as juvenile below the length of 1.7m and adult above 2.2 m. Between these sizes individuals are classified as sub-adult. *P.glauca* is a migratory species, documented to cover distances up to

10,000km. Their migration patterns are associated with breeding cycles and prey migrations. This species is found both individually or living in groups. When living in groups they are known to hunt co-operatively[206].

Dentition and Diet

Prionace glauca have coarsely serrated, recumbent, triangular, cuspate teeth. Teeth become more recumbent towards the posterior of the jaw, see Figure . There is documented geographical variation in the diet of this species, however this usually refers to differing proportions of key prey groups. A typical *P.glauca* diet consists of small pelagic fish and cephalopods, typically squid. They are also known to occasionally eat small benthic invertebrates and fish as well as small sharks. Markaida and Sosa-Nishizaki (2010)[204] recorded the predominant component of *P.glauca* in Mexico was cephalopods, with fish secondary. On the other hand in Australia it is documented that this species consumes mainly fish, with cephalopods as a secondary component[205]. There are no large ontogenetic dietary shifts in this species, only an increase in prey size with individual growth.

Figure 8.10: Upper left hand dentition of *Prionace glauca*[207].

8.1.2 Specimen numbers

Table 8.1: The table highlights the University of Leicester accession number for the mould and cast, original specimen number and location of original specimen.

Original specimen number	Original location	UoL accession number of mould and cast
Chapter 3		123402/1-3
Specimen 1a	Sea Life, London	123403/1-3
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ExpB96a	Deep Sea World, Edinburgh	123453/1-2
ExpB120a	Deep Sea World, Edinburgh	123454/1-3
ExpB0b	Deep Sea World, Edinburgh	123455/1-2
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ExpB72b	Deep Sea World, Edinburgh	123458/1-2
ExpB96b	Deep Sea World, Edinburgh	123459/1-2
ExpB120b	Deep Sea World, Edinburgh	123460/1-3
ExpB0c	Deep Sea World, Edinburgh	123461/1-2
ExpB24c	Deep Sea World, Edinburgh	123462/1-2
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ExpB96c	Deep Sea World, Edinburgh	123465/1-2
ExpB120c	Deep Sea World, Edinburgh	123466/1-3
ExpB0d	Deep Sea World, Edinburgh	123467/1-2
ExpB24d	Deep Sea World, Edinburgh	123468/1-2
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ExpC72f	Morrocco, 54 MA, David Ward	123518/1-2
ExpC96f	Morrocco, 54 MA, David Ward	123519/1-2
ExpC120f	Morrocco, 54 MA, David Ward	123520/1-3
ExpD0a	David Ward	123521/1-2
ExpD24a	David Ward	123522/1-2
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ExpD0c	David Ward	123533/1-2
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ExpD72c	David Ward	123536/1-2
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ExpD24d	David Ward	123540/1-2
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ExpE0b	David Ward	123563/1-2
ExpE24b	David Ward	123564/1-2
ExpE48b	David Ward	123565/1-2
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ExpE96b	David Ward	123567/1-2
ExpE120b	David Ward	123568/1-3
ExpE0c	David Ward	123569/1-2
ExpE24c	David Ward	123570/1-2
ExpE48c	David Ward	123571/1-2
ExpE72c	David Ward	123572/1-2
ExpE96c	David Ward	123573/1-2
ExpE120c	David Ward	123574/1-3
ExpE0d	David Ward	123575/1-2
ExpE24d	David Ward	123576/1-2
ExpE48d	David Ward	123577/1-2
ExpE72d	David Ward	123578/1-2
ExpE96d	David Ward	123579/1-2
ExpE120d	David Ward	123580/1-3
ExpE0e	David Ward	123581/1-2
ExpE24e	David Ward	123582/1-2
ExpE48e	David Ward	123583/1-2
ExpE72e	David Ward	123584/1-2

ExpE96e	David Ward	123585/1-2
ExpE120e	David Ward	123586/1-3
ExpE0f	David Ward	123587/1-2
ExpE24f	David Ward	123588/1-2
ExpE48f	David Ward	123589/1-2
ExpE72f	David Ward	123590/1-2
ExpE96f	David Ward	123591/1-2
ExpE120f	David Ward	123592/1-3
ExpF0a	Deep Sea World, Edinburgh	123593/1-2
ExpF24a	Deep Sea World, Edinburgh	123594/1-2
ExpF48a	Deep Sea World, Edinburgh	123595/1-2
ExpF72a	Deep Sea World, Edinburgh	123596/1-2
ExpF96a	Deep Sea World, Edinburgh	123597/1-2
ExpF120a	Deep Sea World, Edinburgh	123598/1-3
ExpF0b	Deep Sea World, Edinburgh	123599/1-2
ExpF24b	Deep Sea World, Edinburgh	123600/1-2
ExpF48b	Deep Sea World, Edinburgh	123601/1-2
ExpF72b	Deep Sea World, Edinburgh	123602/1-2
ExpF96b	Deep Sea World, Edinburgh	123603/1-2
ExpF120b	Deep Sea World, Edinburgh	123604/1-3
ExpF0c	Deep Sea World, Edinburgh	123605/1-2
ExpF24c	Deep Sea World, Edinburgh	123606/1-2
ExpF48c	Deep Sea World, Edinburgh	123607/1-2
ExpF72c	Deep Sea World, Edinburgh	123608/1-2
ExpF96c	Deep Sea World, Edinburgh	123609/1-2
ExpF120c	Deep Sea World, Edinburgh	123610/1-3
ExpF0d	Deep Sea World, Edinburgh	123611/1-2
ExpF24d	Deep Sea World, Edinburgh	123612/1-2
ExpF48d	Deep Sea World, Edinburgh	123613/1-2
ExpF72d	Deep Sea World, Edinburgh	123614/1-2
ExpF96d	Deep Sea World, Edinburgh	123615/1-2
ExpF120d	Deep Sea World, Edinburgh	123616/1-3
ExpF0e	Deep Sea World, Edinburgh	123617/1-2
ExpF24e	Deep Sea World, Edinburgh	123618/1-2
ExpF48e	Deep Sea World, Edinburgh	123619/1-2
ExpF72e	Deep Sea World, Edinburgh	123620/1-2
ExpF96e	Deep Sea World, Edinburgh	123621/1-2
ExpF120e	Deep Sea World, Edinburgh	123622/1-3
ExpF0f	Deep Sea World, Edinburgh	123623/1-2
ExpF24f	Deep Sea World, Edinburgh	123624/1-2
ExpF48f	Deep Sea World, Edinburgh	123625/1-2
ExpF72f	Deep Sea World, Edinburgh	123626/1-2
ExpF96f	Deep Sea World, Edinburgh	123627/1-2
ExpF120f	Deep Sea World, Edinburgh	123628/1-3
ExpG0a	Deep Sea World, Edinburgh	123629/1-2
ExpG24a	Deep Sea World, Edinburgh	123630/1-2
ExpG48a	Deep Sea World, Edinburgh	123631/1-2
ExpG72a	Deep Sea World, Edinburgh	123632/1-2
ExpG96a	Deep Sea World, Edinburgh	123633/1-2

ExpG120a	Deep Sea World, Edinburgh	123634/1-3
ExpG0b	Deep Sea World, Edinburgh	123635/1-2
ExpG24b	Deep Sea World, Edinburgh	123636/1-2
ExpG48b	Deep Sea World, Edinburgh	123637/1-2
ExpG72b	Deep Sea World, Edinburgh	123638/1-2
ExpG96b	Deep Sea World, Edinburgh	123639/1-2
ExpG120b	Deep Sea World, Edinburgh	123640/1-3
ExpG0c	Deep Sea World, Edinburgh	123641/1-2
ExpG24c	Deep Sea World, Edinburgh	123642/1-2
ExpG48c	Deep Sea World, Edinburgh	123643/1-2
ExpGA72c	Deep Sea World, Edinburgh	123644/1-2
ExpG96c	Deep Sea World, Edinburgh	123645/1-2
ExpG120c	Deep Sea World, Edinburgh	123646/1-3
ExpG0d	Deep Sea World, Edinburgh	123647/1-2
ExpG24d	Deep Sea World, Edinburgh	123648/1-2
ExpG48d	Deep Sea World, Edinburgh	123649/1-2
ExpG72d	Deep Sea World, Edinburgh	123650/1-2
ExpG96d	Deep Sea World, Edinburgh	123651/1-2
ExpG120d	Deep Sea World, Edinburgh	123652/1-3
ExpG0e	Deep Sea World, Edinburgh	123653/1-2
ExpG24e	Deep Sea World, Edinburgh	123654/1-2
ExpG48e	Deep Sea World, Edinburgh	123655/1-2
ExpG72e	Deep Sea World, Edinburgh	123656/1-2
ExpG96e	Deep Sea World, Edinburgh	123657/1-2
ExpG120e	Deep Sea World, Edinburgh	123658/1-3
ExpG0f	Deep Sea World, Edinburgh	123659/1-2
ExpG24f	Deep Sea World, Edinburgh	123660/1-2
ExpG48f	Deep Sea World, Edinburgh	123661/1-2
ExpG72f	Deep Sea World, Edinburgh	123662/1-2
ExpG96f	Deep Sea World, Edinburgh	123663/1-2
ExpG120f	Deep Sea World, Edinburgh	123664/1-3
ExpH0a	Morrocco, 54 MA, David Ward	123665/1-2
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ExpH48a	Morrocco, 54 MA, David Ward	123667/1-2
ExpH72a	Morrocco, 54 MA, David Ward	123668/1-2
ExpH96a	Morrocco, 54 MA, David Ward	123669/1-2
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ExpH72b	Morrocco, 54 MA, David Ward	123674/1-2
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ExpH24c	Morrocco, 54 MA, David Ward	123678/1-2
ExpH48c	Morrocco, 54 MA, David Ward	123679/1-2
ExpH72c	Morrocco, 54 MA, David Ward	123680/1-2
ExpH96c	Morrocco, 54 MA, David Ward	123681/1-2
ExpH120c	Morrocco, 54 MA, David Ward	123682/1-3

ExpH0d	Morrocco, 54 MA, David Ward	123683/1-2
ExpH24d	Morrocco, 54 MA, David Ward	123684/1-2
ExpH48d	Morrocco, 54 MA, David Ward	123685/1-2
ExpH72d	Morrocco, 54 MA, David Ward	123686/1-2
ExpH96d	Morrocco, 54 MA, David Ward	123687/1-2
ExpH120d	Morrocco, 54 MA, David Ward	123688/1-3
ExpH0e	Morrocco, 54 MA, David Ward	123689/1-2
ExpH24e	Morrocco, 54 MA, David Ward	123690/1-2
ExpH48e	Morrocco, 54 MA, David Ward	123691/1-2
ExpH72e	Morrocco, 54 MA, David Ward	123692/1-2
ExpH96e	Morrocco, 54 MA, David Ward	123693/1-2
ExpH120e	Morrocco, 54 MA, David Ward	123694/1-3
ExpH0f	Morrocco, 54 MA, David Ward	123695/1-2
ExpH24f	Morrocco, 54 MA, David Ward	123696/1-2
ExpH48f	Morrocco, 54 MA, David Ward	123697/1-2
ExpH72f	Morrocco, 54 MA, David Ward	123698/1-2
ExpH96f	Morrocco, 54 MA, David Ward	123699/1-2
ExpH120f	Morrocco, 54 MA, David Ward	123700/1-3
ExpI0a	David Ward	123701/1-2
ExpI24a	David Ward	123702/1-2
ExpI48a	David Ward	123703/1-2
ExpI72a	David Ward	123704/1-2
ExpI96a	David Ward	123705/1-2
ExpI120a	David Ward	123706/1-3
ExpI0b	David Ward	123707/1-2
ExpI24b	David Ward	123708/1-2
ExpI48b	David Ward	123709/1-2
ExpI72b	David Ward	123710/1-2
ExpI96b	David Ward	123711/1-2
ExpI120b	David Ward	123712/1-3
ExpI0c	David Ward	123713/1-2
ExpI24c	David Ward	123714/1-2
ExpI48c	David Ward	123715/1-2
ExpI72c	David Ward	123716/1-2
ExpI96c	David Ward	123717/1-2
ExpI120c	David Ward	123718/1-3
ExpI0d	David Ward	123719/1-2
ExpI24d	David Ward	123720/1-2
ExpI48d	David Ward	123721/1-2
ExpI72d	David Ward	123722/1-2
ExpI96d	David Ward	123723/1-2
ExpI120d	David Ward	123724/1-3
ExpI0e	David Ward	123725/1-2
ExpI24e	David Ward	123726/1-2
ExpI48e	David Ward	123727/1-2
ExpI72e	David Ward	123728/1-2
ExpI96e	David Ward	123729/1-2
ExpI120e	David Ward	123730/1-3
ExpI0f	David Ward	123731/1-2

ExpI24f	David Ward	123732/1-2
ExpI48f	David Ward	123733/1-2
ExpI72f	David Ward	123734/1-2
ExpI96f	David Ward	123735/1-2
ExpI120f	David Ward	123736/1-3
ExpJ0a	David Ward	123737/1-2
ExpJ24a	David Ward	123738/1-2
ExpJ48a	David Ward	123739/1-2
ExpJ72a	David Ward	123740/1-2
ExpJ96a	David Ward	123741/1-2
ExpJ120a	David Ward	123742/1-3
ExpJ0b	David Ward	123743/1-2
ExpJ24b	David Ward	123744/1-2
ExpJ48b	David Ward	123745/1-2
ExpJ72b	David Ward	123746/1-2
ExpJ96b	David Ward	123747/1-2
ExpJ120b	David Ward	123748/1-3
ExpJ0c	David Ward	123749/1-2
ExpJ24c	David Ward	123750/1-2
ExpJ48c	David Ward	123751/1-2
ExpJ72c	David Ward	123752/1-2
ExpJ96c	David Ward	123753/1-2
ExpJ120c	David Ward	123754/1-3
ExpJ0d	David Ward	123755/1-2
ExpJ24d	David Ward	123756/1-2
ExpJ48d	David Ward	123757/1-2
ExpJ72d	David Ward	123758/1-2
ExpJ96d	David Ward	123759/1-2
ExpJ120d	David Ward	123760/1-3
ExpJ0e	David Ward	123761/1-2
ExpJ24e	David Ward	123762/1-2
ExpJ48e	David Ward	123763/1-2
ExpJ72e	David Ward	123764/1-2
ExpJ96e	David Ward	123765/1-2
ExpJ120e	David Ward	123766/1-3
ExpJ0f	David Ward	123767/1-2
ExpJ24f	David Ward	123768/1-2
ExpJ48f	David Ward	123769/1-2
ExpJ72f	David Ward	123770/1-2
ExpJ96f	David Ward	123771/1-2
ExpJ120f	David Ward	123772/1-3
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UF223349 UF223343 UF223342	University of Florida/ Florida Museum of Natural History, DU006	123773/1-2 123774/1-2 123775/1-2

UF 17862 UF 5365 UF 5352 UF 5337 UF 5374	University of Florida/ Florida Museum of Natural History, PO001	123776/1-2 123777/1-2 123778/1-2 123779/1-2 123780/1-2
UF16025 UF 15165 UF 16017 UF 15166 UF 16020	University of Florida/ Florida Museum of Natural History,PO010Hh	123781/1-2 123782/1-2 123783/1-2 123784/1-2 123785/1-2
UF 16975 UF 16975	University of Florida/ Florida Museum of Natural History, PO016	123786/1-2 123787/1-2
UF 217145 UF 14444 UF 14443	University of Florida/ Florida Museum of Natural History, PO018	123788/1-2 123789/1-2 123790/1-2
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UF130161 UF130159	University of Florida/ Florida Museum of Natural History, HR046	123814/1-2 123815/1-2
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8.2 Appendices relating to chapter 2

8.2.1 Example data gathering table

Table 8.2: Example data collection table used in Chapter 2. A separate sheet was used for each species. Each tooth was scored from the upper left hand side of the jaw to the upper right (looking at the jaw face on). Commissural teeth were not scored. Presence of a wear type in a defined location scored 1, absence scored 0. D refers to the distal side of the tooth, P to the proximal side, T equates to the tip, M to the middle and B to the base of the tooth.

Species	xxx	Specimen Number	xxx	Location	xxx
Catch data	xxx	Length	xxx	Age	xxx
Diet	xxx	Comments	xxx		

Tooth	Breaks									Rounding									Spalls									Other Signs of Wear								
	DT	DM	DB	PT	PM	PB	T	M	B	DT	DM	DB	PT	PM	PB	T	M	B	DT	DM	DB	PT	PM	PB	T	M	B	DT	DM	DB	PT	PM	PB	T	M	B
1	1	0	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1
2	1	0	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1

8.2.2 Parameter loadings for Figure 2.2

Table 8.3: Loading of parameters on PC axes 1 and 2 (Figure 2.2)

Variable			PC 1	PC2.
Breaks	proximal	tip	0.19046	-0.14295
		middle	0.19822	-0.12935
		base	0.18158	-0.18460
	distal	tip	0.19001	-0.11528
		middle	0.21041	-0.08747
		base	0.18708	-0.16131
		tip	0.20419	-0.06840
		middle	0.21368	-0.11340
		base	0.19705	-0.16500
Rounding	proximal	tip	0.10229	0.28617
		middle	0.12561	0.29736
		base	0.14593	0.25505
	distal	tip	0.08692	0.23953
		middle	0.12561	0.25845
		base	0.15889	0.18646
		middle	0.12706	0.29593
		base	0.15491	0.25046
Other signs of wear	proximal	tip	0.13894	0.21630
		middle	0.17630	0.20645
		base	0.18328	0.13926
	Distal	base	0.19030	0.05703
		middle	0.18518	0.18138
		base	0.19163	0.11721
Spalling	proximal	tip	0.19545	-0.13252
		middle	0.19794	-0.11490
		base	0.18578	-0.17673
	distal	middle	0.21490	-0.09607
		base	0.18833	-0.15860
		tip	0.20822	-0.04525
		middle	0.21488	-0.09975
		base	0.19843	-0.15921

8.2.3 PCA results of meso-style data separated by species

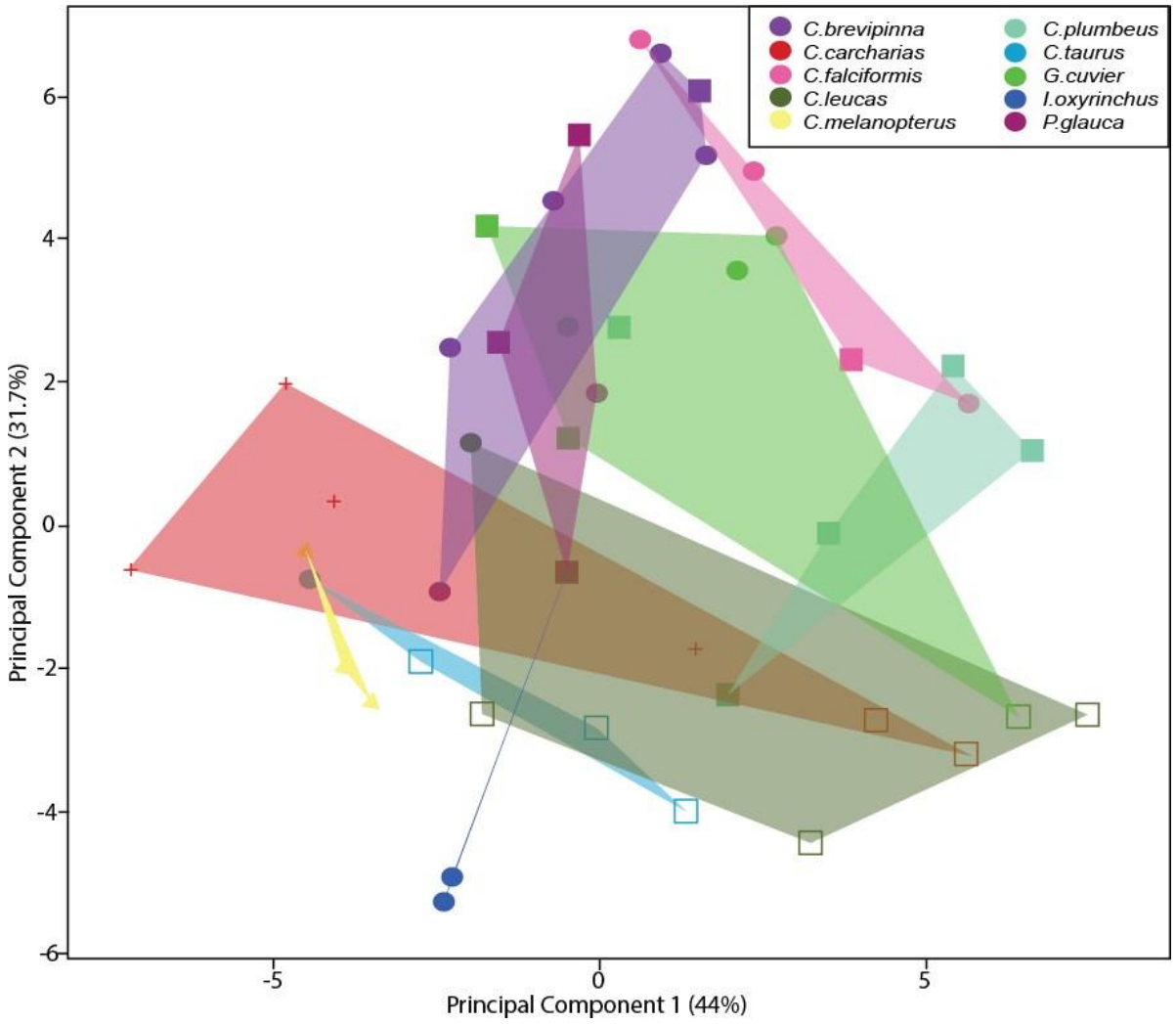

Figure 8.11: PCA plot displaying separation by species, based on the ISO parameters found to separate species through ANOVA testing.

No clear separation between species can be identified in this plot. Contrary to this dietary preferences are grouping on this plot with only a couple of outliers. Weighting of the individual ISO parameters onto PC axes 1 and 2 can be found in Table 8.3.The symbols on the plot relate to the dietary preferences of that species. ◆= Teleost/ Cephalopod diet, ▲= Teleost diet, □= Teleost/ Elasmobranch diet, += Marine Mammal/ Teleost diet, ●= unknown diet. Each species is denoted by the colour assigned in the key.

Table 8.4 : Weighting of parameters onto PC axes 1 and 2 (Figure 8.11)

Variable			PC1	PC2
Breaks	proximal	tip	0.17279	-0.15152
		middle	0.17895	-0.14348
		base	0.15805	-0.19475
	distal	tip	0.17698	-0.12640
		middle	0.19410	-0.10597
		base	0.16333	-0.17964
		tip	0.19000	-0.08467
		middle	0.19542	-0.12987
		base	0.17424	-0.17902
Rounding	proximal	tip	0.11247	0.26360
		middle	0.12942	0.25607
		base	0.14349	0.20583
	distal	tip	0.10063	0.22883
		middle	0.13236	0.22572
		base	0.15658	0.1468
		tip	0.14204	0.21177
		middle	0.13098	0.25512
		base	0.15233	0.20197
Other signs of wear	proximal	tip	0.14555	0.20005
		middle	0.17356	0.16817
		base	0.17294	0.09621
	distal	tip	0.13318	0.16014
		middle	0.17849	0.11758
		base	0.17798	0.02206
		tip	0.15960	0.15268
		middle	0.18175	0.14580
		base	0.18008	0.07587
Spalling	proximal	tip	0.17692	-0.14481
		middle	0.17837	-0.13201
		base	0.16220	-0.18852
	distal	tip	0.18661	-0.11503
		middle	0.19656	-0.11799
		base	0.16440	-0.17764
		tip	0.19430	-0.06553
		middle	0.19574	-0.12080
		base	0.17529	-0.17479

8.2.4 Prediction of dietary preference using meso-style wear

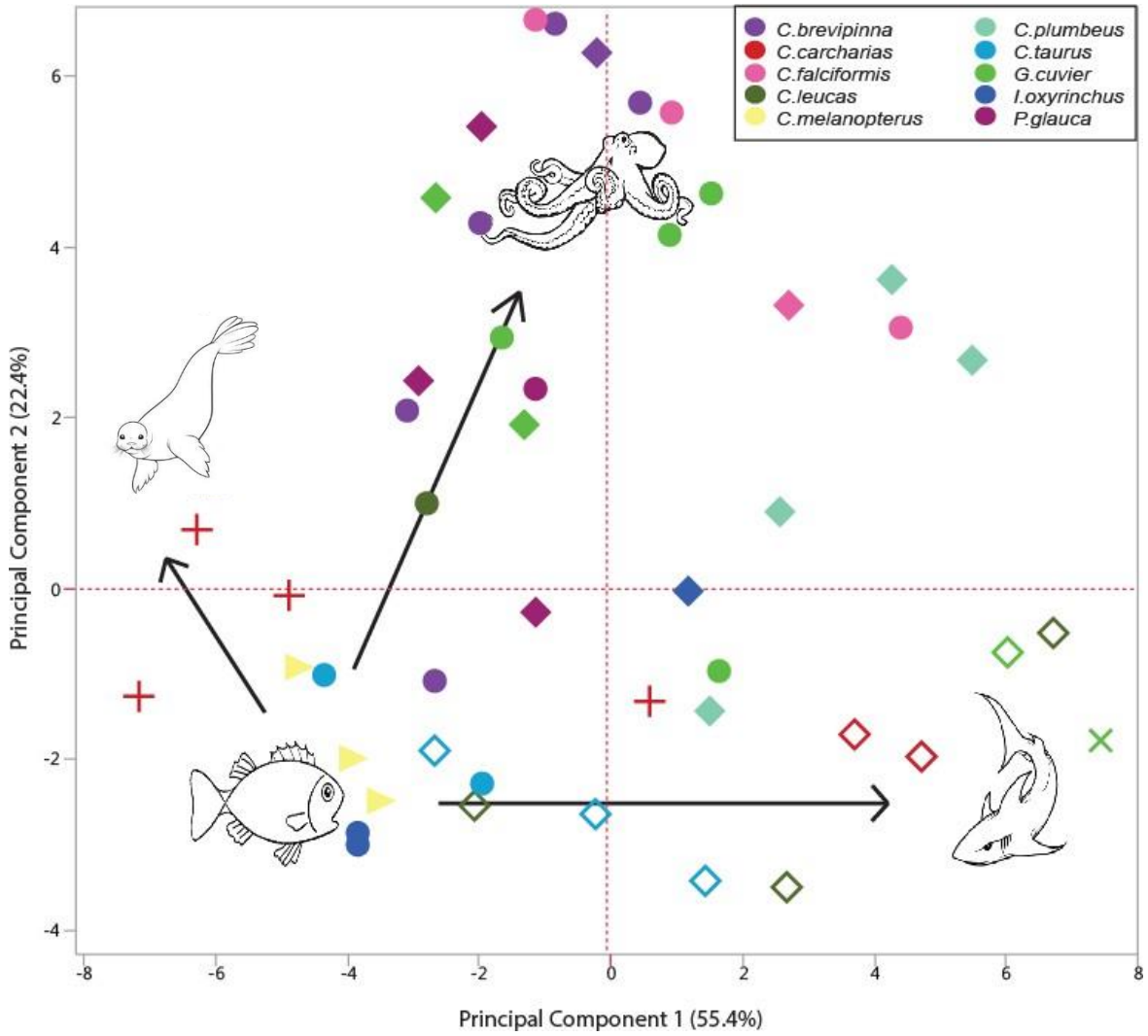

Figure 8.12: projection of individuals with unknown diet (filled circles) onto the PCA plot generated from the variables that were found to separate diet through ANOVA testing.

An increase in PC1 values indicates an increase in the proportion of elasmobranch in the diet. An increase in PC2 values indicates an increase in the proportion of marine mammal in the diet. An increase in both PC1 and PC2 scores indicates an increase in the proportion of cephalopod in the diet. From this plot the diet of individuals represented by a filled circle can be predicted. The symbols on the plot relate to the dietary preferences of that species. ◆= Teleost/ Cephalopod diet, ▲= Teleost diet, ◊= Teleost/ Elasmobranch diet, X= Elasmobranch diet, += Marine Mammal/ Teleost diet, ●= individual with no stomach contents data.

Table 8.7: Table displaying the predicted dietary preferences of individuals with unknown diet. Individuals with unknown data were projected into the dietary morphospace generated by the PCA based upon the parameters found to significantly separate diet based on ANOVA analyses.

Species	**Predicted diet**
C. falciformis	Range from high cephalopod, low teleost to high cephalopod, some elasmobranch, low teleost
G. cuvier	Range from cephalopod/ teleost to elasmobranch teleost
C. taurus	Low elasmobranch high teleost
P.glauca	Varying proportions of teleost and cephalopod
C.leucas	Combination of teleost, cephalopod and elasmobranch
I.oxyrinchus	Teleost
C.brevipinna	Varying proportions of cephalopod and teleost

8.3 Appendices relating to Chapter 3

8.3.1 ANOVA/Tukey HSD results for individuals with different diets detected through microtextures

Table 8.6: Results of ANOVA comparing samples from multiple individuals with different diets (wild and captive; specimens 1 - 4), w indicates Welch ANOVA; significant differences (P<0.05) in bold.

Parameter	F	*p*	df
Sq	**5.6677**	**0.0056**	3, 20
Ssk	1.1095	0.3686	3, 20
Sku	1.0342	0.3988	3, 20
Sp	1.7329	0.1925	3, 20
Sv	2.1293^{w}	0.1622	3, 9.6444
Sz	2.3187	0.1063	3, 20
Sds	**21.5572**	**0.0001**	3, 20
Str	1.6460	0.2106	3, 20
Sal	**6.8788**	**0.0023**	3, 20
Sdq	1.5501^{w}	0.2604	3, 10.279
Ssc	**3.8640^{w}**	**0.0445**	3, 10.144
Sdr	1.3992^{w}	0.2977	3, 10.374
Vmp	**3.5550**	**0.0328**	3, 20
Vmc	3.5150^{w}	0.0556	3, 10.296
Vvc	3.3413^{w}	0.0624	3, 10.356
Vvv	**5.3667**	**0.0071**	3, 20
Spk	**3.5394**	**0.0333**	3, 20
Sk	3.3593^{w}	0.0641	3, 9.8549
Svk	**4.5676**	**0.0136**	3, 20
Smr1	1.1868	0.3400	3, 20
Smr2	1.9279	0.1576	3, 20
S5z	2.6399	0.0775	3, 20
Sa	**5.7277**	**0.0054**	3, 20

Table 8.7: Pairwise differences, of parameters, (Tukey HSD) between samples from multiple individuals with different diets (wild; specimens 2 - 4) and aquarium sharks (sample 1; fish-only diet).

1	differs from	2,3,4	Sds
1	differs from	3, 4	Sal
1	differs from	4	Sq, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Sa
2	differs from	4	Sds

Table 8.9: Pairwise differences, of parameters, (Tukey HSD) between samples from multiple individuals with different diets (wild; specimens 2 - 4) and aquarium sharks (sample 1; fish-only diet). Lower left side of matrix tallies differences, upper right showing the parameters that differ.

	Sample 1	**Specimen 2**	**Specimen 3**	**Specimen 4**
Sample 1			Sds, Sal	Sq, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Sa, Sds, Sal
Specimen 2	0			Sds
Specimen 3	2	0		
Specimen 4	11	1	0	

8.3.2 ANOVA results for wild vs captive subsampling

Table 8.9: Results of ANOVA testing the hypothesis that microwear textures on six randomly subsampled wild teeth do not differ from those of the six teeth from aquarium specimens of C. taurus (w indicates Welch ANOVA; significant differences ($p<0.05$) in bold).

Parameter	*Sub-sample 1*			*Sub-sample 2*			*Sub-sample 3*			*Sub-sample 4*			*Sub-sample 5*		
	F	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**
Sq	**7.5273**	**0.0207**	1, 10	**8.2959**	**0.0164**	1, 10	**5.4979**	**0.0410**	1, 10	**5.0335**	**0.0487**	1, 10	6.1554w	0.0526	1, 5.349
Ssk	1.6821	0.2238	1, 10	0.9779	0.3460	1, 10	2.8132	0.1244	1, 10	1.0941	0.3202	1, 10	3.9191	0.0759	1, 10
Sku	0.6401	0.4423	1, 10	0.0000	0.9986	1, 10	0.3212[w]	0.5886	1, 6.996	0.1141	0.7425	1, 10	0.1230	0.7331	1, 10
Sp	2.6954	0.1317	1, 10	0.2650	0.6179	1, 10	1.4522	0.2559	1, 10	0.2569	0.6232	1, 10	0.1089w	0.7522	1, 6.301
Sv	**7.5794**	**0.0204**	1, 10	2.8608	0.1216	1, 10	**5.2689**	**0.0446**	1, 10	1.1881	0.3013	1, 10	**5.0533**	**0.0483**	1, 10
Sz	**5.2584**	**0.0448**	1, 10	1.4440	0.2572	1, 10	3.4150	0.0944	1, 10	0.7161	0.4172	1, 10	2.1109	0.1769	1, 10
Sds	**36.8229**	**0.0001**	1, 10	**31.0817[w]**	**0.0014**	1, 6.075	**19.2529[w]**	**0.0053**	1, 5.677	**67.6199**	**0.0001**	1, 10	**31.9545w**	**0.0012**	1, 6.192
Str	3.4110	0.0945	1, 10	4.8400	0.0524	1, 10	1.2809[w]	0.3011	1, 5.978	3.8536	0.0780	1, 10	2.4649w	0.1651	1, 6.303
Sal	**16.2000**	**0.0024**	1, 10	**8.1818**	**0.0169**	1, 10	**16.2000**	**0.0024**	1, 10	**18.8462**	**0.0015**	1, 10	**9.8000**	**0.0107**	1, 10
Sdq	1.0289	0.3343	1, 10	0.5989	0.4569	1, 10	0.4786	0.5048	1, 10	0.0095[w]	0.9260	1, 5.411	0.0027	0.9597	1, 10
Ssc	0.1146	0.7419	1, 10	0.2407	0.6343	1, 10	0.0125	0.9132	1, 10	1.7855	0.2111	1, 10	1.8029	0.2090	1, 10
Sdr	0.9052	0.3638	1, 10	0.5476	0.4763	1, 10	0.3122	0.5887	1, 10	0.1324[w]	0.7295	1, 5.515	0.0153	0.9041	1, 10
Vmp	4.9505	0.0503	1, 10	4.6831	0.0557	1, 10	2.8036	0.1250	1, 10	2.0230	0.1854	1, 10	1.7351	0.2171	1, 10
Vmc	**8.1668**	**0.0170**	1, 10	**8.9663**	**0.0135**	1, 10	**7.0975**	**0.0237**	1, 10	**5.0163**	**0.0490**	1, 10	5.4327w	0.0607	1, 5.714
Vvc	**8.1852**	**0.0169**	1, 10	**9.0758**	**0.0131**	1, 10	**6.4194**	**0.0297**	1, 10	4.7692	0.0539	1, 10	5.3855w	0.0619	1, 5.662
Vvv	**5.9782**	**0.0345**	1, 10	**5.6400**	**0.0390**	1, 10	3.8972[w]	0.0931	1, 6.358	4.1442	0.0691	1, 10	**6.1870**	**0.0321**	1, 10
Spk	4.7055	0.0552	1, 10	3.8691	0.0775	1, 10	2.6770	0.1329	1, 10	1.7714	0.2128	1, 10	1.4473	0.2567	1, 10
Sk	**8.3521**	**0.0161**	1, 10	**9.5260**	**0.0115**	1, 10	**7.8068**	**0.0190**	1, 10	5.0210[w]	0.0646	1, 6.236	5.3829w	0.0595	1, 5.997
Svk	**5.6671**	**0.0386**	1, 10	4.5509	0.0587	1, 10	3.7126[w]	0.1015	1, 6.097	3.2091	0.1035	1, 10	**6.0905**	**0.0332**	1, 10
Smr1	0.1399[w]	0.7189	1, 7.41	0.5570	0.4726	1, 10	0.1337	0.7222	1, 10	0.0336	0.8583	1, 10	0.0531	0.8224	1, 10
Smr2	0.0139	0.9086	1, 10	0.8460	0.3793	1, 10	0.0715	0.7946	1, 10	0.2684	0.6157	1, 10	0.3491	0.5677	1, 10
S5z	4.5638	0.0584	1, 10	1.7583	0.2143	1, 10	2.3585	0.1556	1, 10	0.7926	0.3942	1, 10	1.2556w	0.3058	1, 5.938
Sa	**7.9504**	**0.0182**	1, 10	**8.9876**	**0.0134**	1, 10	**6.2075**	**0.0319**	1, 10	**5.0876**	**0.0477**	1, 10	5.8589w	0.0569	1, 5.343

Parameter	*Sub-Sample 6*			*Sub-sample 7*			*Sub-sample 8*			*Sub-sample 9*			*Sub-sample 10*		
	F	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**
Sq	4.1013	0.0704	1, 10	**9.7333**	**0.0109**	1, 10	**5.5465**	**0.0403**	1, 10	**5.7745**	**0.0371**	1, 10	**6.5220**	**0.0287**	1, 10
Ssk	1.2554	0.2887	1, 10	2.4744	0.1468	1, 10	1.3809	0.2672	1, 10	0.9556	0.3514	1, 10	3.5344	0.0895	1, 10
Sku	0.0413[w]	0.8445	1, 7.393	0.6815	0.4283	1, 10	**1.2046[w]**	**0.3084**	1, 7.068	1.1937	0.3002	1, 10	0.9347[w]	0.3665	1, 6.864
Sp	0.5376	0.4803	1, 10	1.3588	0.2708	1, 10	0.9275	0.3582	1, 10	1.9887	0.1888	1, 10	1.4238	0.2603	1, 10
Sv	2.8624	0.1215	1, 10	**6.8390**	**0.0258**	1, 10	2.9620	0.1160	1, 10	2.7157	0.1304	1, 10	**4.9911**	**0.0495**	1, 10
Sz	1.6058	0.2338	1, 10	4.0081	0.0731	1, 10	1.9038	0.1977	1, 10	2.4713	0.1470	1, 10	3.1631	0.1057	1, 10
Sds	**31.476[w]**	**0.0013**	1, 6.075	**41.4737**	**0.0001**	1, 10	**43.255[w]**	**0.0005**	1, 6.230	**32.773[w]**	**0.0013**	1, 5.972	**48.3854**	**0.0001**	1, 10
Str	1.8284[w]	0.2260	1, 5.878	**11.0907**	**0.0076**	1, 10	3.1500[w]	0.1197	1, 6.913	3.1488	0.1064	1, 10	5.5360[w]	0.0567	1, 6.026
Sal	**6.4811[w]**	**0.0250**	1, 6.481	**8.1818**	**0.0169**	1, 10	**10.9459**	**0.0079**	1, 10	**16.2000**	**0.0024**	1, 10	**35.5882**	**0.0001**	1, 10
Sdq	0.3674	0.5579	1, 10	0.5585	0.4721	1, 10	0.2403	0.6346	1, 10	0.1601	0.6975	1, 10	0.1776	0.6823	1, 10
Ssc	0.1431	0.7131	1, 10	0.2297	0.6421	1, 10	0.5790	0.4643	1, 10	0.2064	0.6593	1, 10	0.3130	0.5882	1, 10
Sdr	0.2201	0.6490	1, 10	0.5243	0.4856	1, 10	0.1165	0.7399	1, 10	0.0420	0.8417	1, 10	0.0663	0.8020	1, 10
Vmp	2.0729	0.1805	1, 10	**5.2845**	**0.0443**	1, 10	3.5260[w]	0.1066	1, 6.374	4.2768	0.0655	1, 10	3.2598[w]	0.1184	1, 6.344
Vmc	**5.2433**	**0.0450**	1, 10	**8.8337**	**0.0140**	1, 10	**6.0967**	**0.0332**	1, 10	**5.9664**	**0.0347**	1, 10	**7.2790**	**0.0224**	1, 10
Vvc	**5.0918**	**0.0477**	1, 10	**8.6754**	**0.0146**	1, 10	**6.2178**	**0.0318**	1, 10	**6.3395**	**0.0305**	1, 10	**6.9224**	**0.0251**	1, 10
Vvv	2.8831[w]	0.1379	1, 6.319	**10.8943**	**0.0080**	1, 10	4.1697[w]	0.0847	1, 6.339	4.6215	0.0571	1, 10	**5.6270**	**0.0391**	1, 10
Spk	1.8879	0.1994	1, 10	4.7723	0.0538	1, 10	3.2071	0.1036	1, 10	4.1131	0.0700	1, 10	3.0526	0.112	1, 10
Sk	**6.1203**	**0.0329**	1, 10	**8.1077**	**0.0173**	1, 10	**6.6785**	**0.0272**	1, 10	**6.0844**	**0.0333**	1, 10	**8.0590**	**0.0176**	1, 10
Svk	2.5719[w]	0.1590	1, 6.110	**9.8839**	**0.0104**	1, 10	3.9119[w]	0.0947	1, 6.082	4.1845[w]	0.0857	1, 6.134	5.4832[w]	0.0553	1, 6.359
Smr1	0.1569	0.7003	1, 10	0.0604	0.8108	1, 10	0.3804	0.5512	1, 10	1.2794	0.2844	1, 10	0.0056	0.9418	1, 10
Smr2	0.3869	0.5479	1, 10	0.0234	0.8815	1, 10	0.0234	0.8815	1, 10	0.0281	0.8702	1, 10	0.0025	0.9610	1, 10
S5z	1.3639	0.2699	1, 10	3.6307	0.0858	1, 10	2.3907	0.1531	1, 10	2.6206	0.1366	1, 10	2.7503	0.1282	1, 10
Sa	4.7044	0.0553	1, 10	**9.4091[w]**	**0.0120**	1, 9.902	**5.8723**	**0.0359**	1, 10	**6.0129**	**0.0341**	1, 10	**6.8897**	**0.0254**	1, 10

8.3.3 Parameter Loadings for Figure 3.1

Table 8.10: Loadings of parameters onto PC axes 1 and 2 (Figure 3.1).

Parameter	PC 1	PC 2
Sq	0.29096	0.01654
Sv	0.26755	0.20103
Sz	0.27388	0.19996
Sds	-0.18000	0.65064
Sal	0.18642	-0.64283
Vmp	0.27865	0.05518
Vmc	0.27863	0.08711
Vvc	0.28216	0.06606
Vvv	0.28250	-0.07348
Spk	0.27945	0.06609
Sk	0.26944	0.10026
Svk	0.27849	-0.09528
S5z	0.27599	0.19059
Sa	0.28721	0.05050

8.4 Appendices relating to Chapter 4

8.4.1 Calculation of maximum distance travelled by a single tooth during an experiment.

Based on a barrel rotating 40 revolutions per minute, and having a circumference of 20cm each tooth will travel a maximum distance of 1.5 km in 1 hour

Time	0h	24h	48h	72h	96h	120h
Distance	0km	36km	72km	108km	144km	180km

Maximum distance travelled in 1 hour= $60\pi dr$

Where d= diameter of barrel

r=number of barrel rotations in 1 minute

8.5 Appendices relating to Chapter 5

8.5.1 Geological timescale

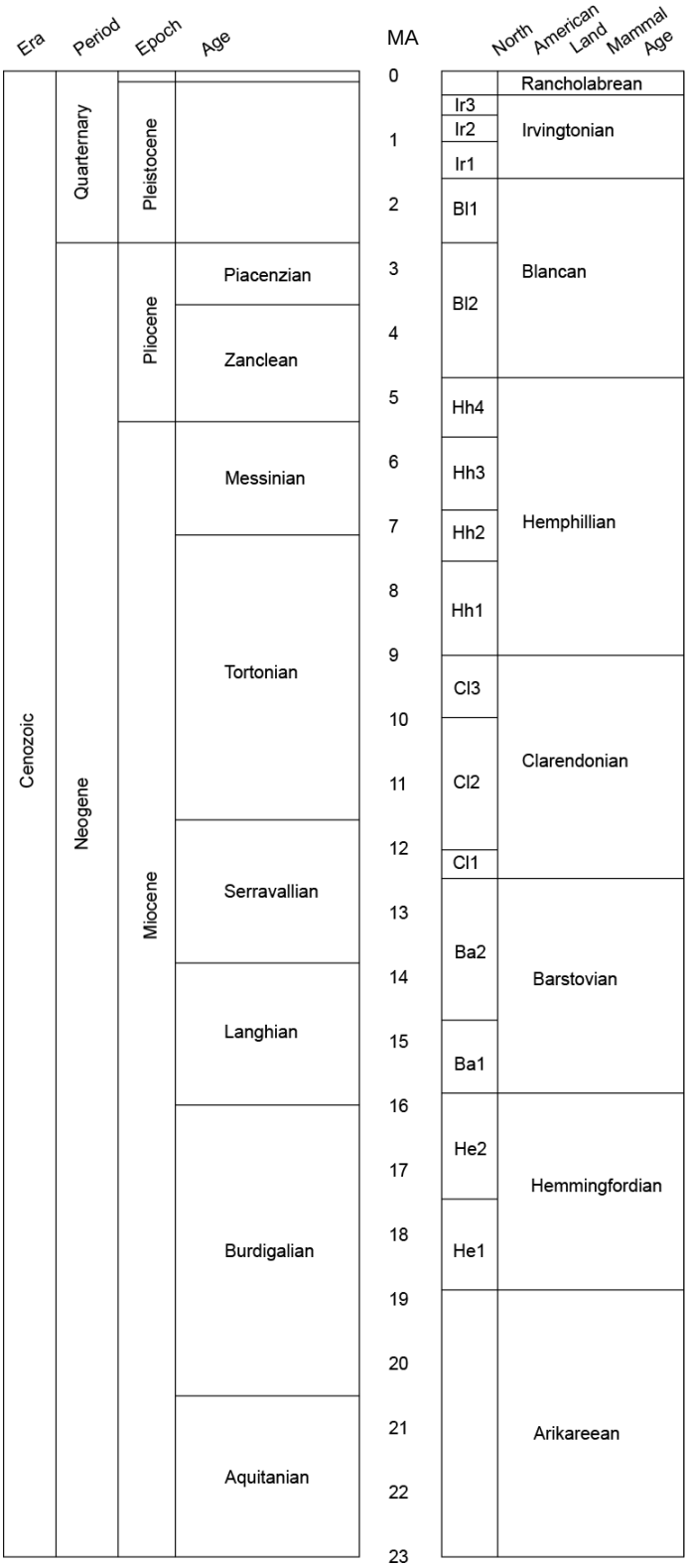

Figure 8.13: Geological timescale highlighting the correlation of the timing intervals of the ICS published timeline and the North American Land Mammal Ages.

8.5.2 ANOVA results comparing within and between individuals

Table 8.11: Results of ANOVA testing the differences between samples taken from within the tooth of a single individual to those of multiple individuals from the same fossil deposit. A decimalised d.f. value indicates the use of a welch ANOVA. Bold text indicates a significant difference.

Parameter	**F**	**d.f.**	**P**
Sq	1.5655	1, 14	0.2314
Ssk	0.1049	1, 14	0.7508
Sku	0.0453	1, 14	0.8345
Sp	0.5244	1, 14	0.4809
Sv	0.2200	1, 14	0.6462
Sz	0.3496	1, 14	0.5638
Sds	0.2020	1, 14	0.6600
Str	0.0034	1, 14	0.9545
Sal	0.0733	1, 14	0.3659
Sdq	2.5432	1, 6.51	0.1580
Ssc	2.6222	1, 6.66	0.1516
Sdr	2.6168	1, 6.35	0.1541
Vmp	0.0009	1, 14	0.9765
Vmc	0.8720	1, 6.58	0.3834
Vvc	0.7607	1, 7.29	0.4109
Vvv	0.1119	1, 14	0.7430
Spk	0.4091	1, 14	0.5328
Sk	3.9180	1, 6.50	0.0915
Svk	0.5856	1, 14	0.4568
Smr1	0.0845	1, 14	0.7756
Smr2	2.3595	1, 7.54	0.1654
S5z	0.2994	1, 14	0.5929
Sa	1.9895	1, 7.37	0.1992

8.5.3 Loading of parameters for Figure 5.2.

Table 1.14: Parameter loadings for Figure 5.2.

	Miocene/ Pliocene boundary	Late Miocene	Mid Miocene
Sq	0.35678	0.36974	0.38375
Vmp	0.35375	0.35130	0.32302
Vvv	0.35580	0.35496	0.30458
Spk	0.35305	0.35641	0.37324
Sk	0.34878	0.34847	0.34574
Svk	0.35162	0.34743	0.36858
S5z	0.35300	0.33455	0.34440
Sa	0.35557	0.36441	0.37725

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