Sexual and Seasonal Dimorphisms in the Dermal, Dental and Ampullary Structures of the Lesser-Spotted Catshark, *Scyliorhinus canicula*

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ABSTRACT

Sexual dimorphisms in head morphology, integument and dentition of some elasmobranch species have been established. These dimorphisms are reportedly linked to reproductive behaviour, whereby male biting during copulation results in a dimorphism in head dimensions and dentition and, as a result, differences in skin thickness. The findings for *Scyliorhinus canicula* from the Solent support the findings of other authors, whereby adult males were found to possess longer, narrower mouths and a longer head than adult females. Juvenile male catsharks were found to possess a longer mouth than females. No head, mouth or jaw dimorphisms for hatchling catsharks were found. Adult male catsharks were found to possess unicuspid teeth, with large central cusps, in contrast to the pentacuspid form of female and immature catsharks. A sexual dimorphism was found in the tooth row numbers for hatchling and adult catsharks, with hatchling males possessing a greater number of tooth rows than hatchling females on the lower jaw and adult males possessing a greater number of tooth rows than adult females in the upper jaw.

Seasonal comparisons were made to ascertain whether morphological changes occurred that could indicate a mating season for the Solent population of *S. canicula*. Adult head length, mouth length and mouth width were found to be significantly different. Adult males sampled in all seasons possessed a longer mouth than females sampled in all seasons, whilst the lower jaw length was significantly greater for adult males in all seasons compared to adult females. Juvenile female catsharks were found to possess a thicker epidermis than juvenile male catsharks in all seasons of the year, whilst adult females possessed a thicker epidermal layer than adult males, findings not previously reported in this species. Adult females were found to possess a thicker dermal layer in all seasons compared to adult male catsharks. Adult females also possessed wider and longer dermal denticles on the pectoral fins than adult males. Hatchling catsharks had a greater dermal

denticle density on both fins indicating the possession of smaller dermal denticles than hatchling females. A sexual dimorphism was found in the Ampullae of Lorenzini with male catsharks possessing a greater number of alveoli than adult females, possibly both an ecological and reproductive adaptation. The seasonal and sexual dimorphisms found in this study do not directly indicate a specific mating season for this species in the Solent.

Contents

Whilst registered as a candidate for the degree of Doctor of Philosophy, I have not registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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LIST OF ABBREVIATIONS

A	Alveoli
AC	Ampullary Canal
AoL	Ampullae of Lorenzini
AoLP	Ampullae of Lorenzini Pore
BD	Cusp Base Diameter
C	Cusp
CL	Clasper length
CLSM	Confocal Laser Scanning Microscopy
CLT	Cusplet
CR	Crown
CS	Central Stage
CU	Cuboidal Cells
DF	Dorsal Fin
DS	Dorsal Skin
GSI	Gonosomatic index
GW	Gonad weight
HC	Hillock Shaped Cells
JD	Jaw depth
JDI	Jaw Diameter
JL	Jaw Length
JW	Jaw Width
LL	Lateral Line
MD	Mid Cusp Diameter
MoL	Mouth Length
MSD	Mouth to Snout Distance

MoW	Mouth Width
MW	Medial Walls
R	Root
RC	Receptor Cells
RL	Root Lobe
S	Symphysis
SC	Supportive Cells
SE	Sensory Epithelium
SEM	Scanning Electron Microscopy
ST	Sympyseal Teeth
TD	Cusp Tip Diameter
TH	Tooth Height
THL	Total Head Length
THW	Total Head Width
TL	Total body length
TW	Tooth Width
WT	Total body weight

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Chapter 1 – General Introduction

Chondrichthyan, or cartilaginous, fishes are numerous with over 1200 species recorded worldwide (Compagno, 2001). The class chondrichthyes comprises the rabbit fishes, skates, rays and sharks, with a majority of these forming the subclass Elasmobranchii (Compagno *et al.*, 2005). According to Compagno *et al.* (2005) and Fowler *et al.* (2005) there are currently reported to be approximately 440 accepted species of shark, although it is believed that not all have been described. It is evident that in recent years increasing numbers of shark species have been identified. Clark (1981) reported that there were 350 accepted species of shark worldwide, whereas Gilbert (1981) reported that worldwide there were between 300 and 350 species of shark recorded. Clark (1981) noted that less than a decade before this there were only 250 accepted species recorded.

It is their long ancestral lineage that has driven many scientists to examine the reasons why sharks have been so successful and have managed to survive, largely unchanged, for millions of years. It is reported that elasmobranch species have inhabited the world's oceans for more than 450 million years, and the first fossil record of sharks is three times as old as that of the dinosaurs (Maisey, 1990). Compagno *et al*. (2005) stated that many extant shark species maintain the anatomical features seen in extinct species that lived over 150 million years ago and that the body form remains largely unchanged. It is this morphology that is believed to have made elasmobranch species so successful throughout evolutionary history.

Gilbert (1981) suggested that one of the main reasons sharks have shown this remarkable survival rate is due largely to their reproductive capabilities. He puts this down to the fact that, in all species, semen is introduced into the female, fertilising the eggs internally, an act uncommon in a majority of fish species. In contrast to this, many authors reported that

in recent times the reproductive strategies of elasmobranchs to be potentially detrimental to their long-term survival and put this largely down to over-exploitation by humans. It was suggested by Holden (1974) and Holden (1977) that the life history strategies of elasmobranchs may make them susceptible to over-exploitation and could impede the recovery of depleted populations. Compagno *et al*. (2005) noted that elasmobranchs have life histories characterised by low fecundity, slow growth and late maturity. Pratt and Casey (1990) suggested that this suite of life history characteristics resulted in low reproductive potential and low capacity for population increase. The reason that sharks show these life history characteristics is due to their evolutionary position as a top predator, with few natural enemies.

It is very clear from the literature, however, that observations of mating in any species of shark are rare. Due to the very nature of the marine environment, the mating behaviours of few elasmobranch species have been observed. Many of those that have been observed are chance encounters, largely involving stingrays (Dasyatidae) and Skates (Rajidae) (Nordell, 1994; Kajiura and Tricas, 1996).

It also appears that the mating behaviours of elasmobranch species are poorly understood. Klimley (1980) recognised that a paucity of information existed on the mating behaviour of sharks. Demski (1990) noted that there had been virtually no observations of mating in pelagic species of shark. Gilbert (1981) also remarked that relatively few people had witnessed the mating activities of any shark species. However, he cited works by Dempster and Herald (1961) who described copulation in the horn shark, *Heterodontus franscisci*, Clark (1963) who witnessed courtship behaviour in the lemon shark, *Negaprion brevirostris*, and Schensky (1914) who observed and photographed copulation in the catshark, *Scyliorhinus canicula*. Tricas and Le Feuvre (1985) reported on the mating of the white tip shark, *Triaenodon obesus*, whilst Pratt and Carrier (1995) produced numerous

photographs of the mating of wild nurse sharks off the Florida coast. More recently the first observations of mating in the bamboo shark, *Hemiscyllium freycineti,* were noted (Cornish, 2005). It is noted by Demski (1990) that a majority of observations of courtship and copulation have only recently been observed in elasmobranchs and usually in small, near-shore, species. Despite these recorded observations, Johnson and Nelson (1978) noted that most mating behaviours had only been witnessed in aquaria, a situation that has changed little in over thirty years. The problems associated with studying marine elasmobranch species that are often nomadic and regularly inhabit inaccessible and murky environments has also been recognised (Gilbert, 1981).

To overcome the problems imposed by their environment, sharks are widely reported to use a large array of extremely acute senses. These are used for both hunting and, possibly, the location of conspecifics in order to mate. From the literature it is clear that in many shark species, all of the senses play a part in mate and prey detection, depending on the distance to the target. For example, the auditory sense is known to be the longest-range sense in many species of shark. Sharks are able to detect sound from several kilometres away and will swim towards the noise to investigate (Fowler *et al.,* 2002).

Other senses studied are those of sight, electromagnetic detection and olfaction. The presence of electrical receptors, known as Ampullae of Lorenzini (AoL) is well documented (Sand, 1938; Murray, 1957; Kalmijn, 1971). These detectors are thought to enable sharks to detect the electrical impulses given off by live prey as low as five billionths of a volt per centimetre (Tricas and Sisneros, 2004). It is also suggested that the electro-sense is important in some species during courtship and reproduction in allowing the detection of conspecifics (Sisneros and Tricas, 2002a). Research into the olfactory organs of *S. canicula* showed that the olfactory organs of *S. canicula* were sexually

dimorphic and that female catsharks exposed to homogenised testes exhibited behavioural changes, which could lead to olfactory-mediated mate location (Llewellyn, 2008).

The majority of literature that is available indicates that once conspecific detection has taken place and mating has occurred, females often bear bite wounds (Stevens, 1974; McCourt and Kerstitch, 1980; Nordell, 1994; Pratt and Carrier, 1995; Kajiura *et al.,* 2000). According to Kajiura *et al.* (2000) elasmobranch fishes exhibit a series of complex courtship and mating behaviours in which males inflict significant bite wounds on the body of females. In almost all cases of observed mating behaviours, males were observed to bite the pectoral fins or marginal discs of the females (McCourt and Kerstitch, 1980; Nordell, 1994; Pratt and Carrier, 1995; Kajiura and Tricas, 1996). This behaviour was clearly demonstrated by Pratt and Carrier (1995) who witnessed and photographed the mating of wild nurse sharks and captured footage of the males biting the pectoral fin of the females (Fig. 1.1)

Figure 1.1. A male nurse shark biting the pectoral fin of a female nurse shark during copulation (© Jeffery Carrier, 1995).

Several studies have focused upon the fact that in some species, a sexual dimorphism exists in the structure of the teeth to facilitate the males in obtaining a firm grip on the female during mating. Kajiura *et al.* (2000) believed that dental sexual dimorphism enhanced grip efficiency by the males during reproduction and it was suggested that in most species biting served to provide leverage for clasper insertion. The evidence of a periodic shift in male dentition, coupled with wounds left by this biting behaviour, has been used as an indication of the reproductive seasonality of many species (Kajiura *et al.,* 2000).

In response to this it is believed that females showed an adaptive response to male biting in the form of skin thickening (Nordell, 1994; Pratt, 1979). Brunnschweiler and Pratt (2008) noted that in free swimming zebra sharks, *Stegostoma fasciatum*, male – male interactions were witnessed, whereby the males were seen to bite each other on the pectoral fins in much the same way that males were seen to bite females. However, they disregarded a same sex hypothesis and found that the male – male behaviours were possibly agonistic in nature and no evidence of skin thickening in males has been suggested. It is therefore possible that if biting behaviour occurs in other species of elasmobranch, as noted in the literature, that the skin of female *S. canicula* could develop to be thicker to protect against biting from males.

1.1 Mouth, Jaw and Tooth Morphology

According to Ellis and Shackley (1995) morphological and dental features are useful for the taxonomy of elasmobranch fish. It is widely reported that sharks and rays continually replace their teeth throughout their life, a process known as polyphyodonty (Moss, 1972; Kajiura and Tricas, 1996). Moss (1972) indicated that tooth replacement is characteristic of elasmobranch fish and noted that tooth replacement in sharks is a mechanism by which

broken or worn teeth are replaced. Moss (1972) also stated that tooth replacement is related to body growth in sharks, a process that may equip larger sharks to cope with different prey as they grow.

It has been clearly demonstrated, however, that tooth replacement may not take place solely for feeding. Despite the hypothesis of Fedducia and Slaughter (1974) that tooth dimorphism is an adaptive feeding strategy; many authors believed that sexually dimorphic dentition is a reproductive adaptation. Nordell (1994) noted the existence of sexual dimorphism in the dentition of *Urolophus halleri*, and recognised that this may be of importance in reproductive behaviour.

Kajiura *et al.* (2000) note that elasmobranch courtship involves a series of complex behaviours, many of which involve the use of the mouth by males. In numerous instances during courtship males have been observed to bite the pectoral fins or marginal discs of females (McCourt and Kerstitch, 1980; Nordell, 1994; Pratt and Carrier, 1995; Kajiura and Tricas, 1996). Kajiura *et al.* (2000) believed that the larger teeth in males enhanced grip efficiency of the males during reproduction and they stated that in most species biting served to provide leverage for clasper insertion. They suggested that the evidence of a periodic shift in male dentition, coupled with wounds left by this biting behaviour, can be used as an indication of the reproductive seasonality of many species.

Studies carried out by Kajiura and Tricas (1996) and Kajiura *et al.* (2000) found that in the Atlantic stingray, *Dasyatis sabina*, male dentition showed a periodic shift from the female molariform to a recurved cuspidate form during the mating season. They stated that the reasons for this transformation from the cuspidate teeth to the molariform were due to the fact that molariform teeth were relatively inefficient for grasping. It appeared that the cuspidate form, which provided sharp dentition, provided males with an enhanced grip.

Males of some other species were also observed to have longer, more pointed teeth than females (Bigelow and Schroeder, 1953). McCourt and Kerstitch (1980) examined the teeth of museum specimens of *Urolophus concentricus* and found that the teeth of large males were markedly more pointed and recurved than those of females. A sexual dimorphism in the dentition of *S. canicula* was identified by Ellis and Shackley (1995). They reported that the males showed longer teeth than females. Springer (1979) noted that male Scyliorhinids often had longer teeth than females, and in one species, *Apristurus riveri,* male teeth are twice as long as the females.

This sexual dimorphism in the structure of the teeth as an adaptation to feeding was discounted by Lyle (1983). The research showed that the stomach contents of male and female catsharks that were examined displayed no differences in prey selection between the genders. *S. canicula* were found to feed mainly on small benthic invertebrates (crustaceans, gastropods, cephalopods, worms) (Compagno *et al*., 2005) and although Lyle (1983) found that composition of diet altered gradually with size, no significant sexual difference in the diet of *S. canicula* in Isle of Man waters occurred.

Kajiura and Tricas (1996) found that in the Atlantic stingray, *Dasyatis sabina*, even though males and females posses a very different dentition for part of the year, their diet consisted of the same prey items all year round. They noted that it was not clear whether the change to a cuspidate dentition had any influence on the ability of male stingrays to feed. The same phenomenon was found in the stingray, *Urobatis concentricus*, whereby despite the presence of sexually dimorphic teeth, the gut contents of males and females showed no difference (McEachran, 1977).

Several observations of mating in *S. canicula* have been made (Bolau, 1881, Schensky, 1914, Houziaux and Voss, 1997, Domi *et al*., 2000). During these observations it was noted that the male wrapped itself tightly around the female (Figure 1.2)

Figure 1.2. Mating in *S. canicula* showing the male wrapped tightly around the female (Photograph reproduced with permission of the estate of DPWilson - © DPWilson Ltd).

Despite these observations of mating no mention had previously been made as to whether biting took place during copulation in *S. canicula*. Stevens (1974) cited work by other authors who stated that many benthic species of shark showed torn and scarred pectoral fin margins during the mating season, in much the same way as pelagic species of sharks. Castro *et al.* (1988) noted that precopulatory behaviour and copulation in Scyliorhinids may involve the male biting the fins and body of the female. Biting as precopulatory behaviour in *S. canicula* was confirmed by Domi *et al.* (2000) whereby the male was seen to grasp the female with its mouth in the area posterior to the pectoral fin (Figure 1.3).

Figure 1.3. An adult male catshark biting a female catshark prior to copulation. (Image supplied courtesy of Domi *et al*., 2000).

Observations by Nordell (1994) demonstrated that not all biting by males resulted in copulation. He noted that male round stingrays frequently bit females during the mating season, but that most male biting did not result in copulation. Nordell (1994) also stated that where biting did not lead to reproduction, males bit the posterior (or occasionally the medial) portion of the females' disc. It appeared that when this was the case, the females often freed themselves from the males' grip. Studies by Kajiura and Tricas (1996) showed that female sharks and rays often appeared reluctant to mate and would flee from courting males. It was suggested by Kajiura and Tricas (1996) that the act of males biting females during copulation may have elicited females to cooperate and therefore reproduce.

It has been noted by several authors that in addition to dental sexual dimorphism in many species of sharks, there is also a distinct dimorphism of the jaws. Brough (1937) and Arthur (1950) both noted that the structure of the lower jaw in *S. canicula* changed with sexual maturity and that these changes were more pronounced in the mating season. It was

also apparent that in immature specimens no sexual dimorphism of the jaw was evident. Gosztonyi (1973) noted the same characteristics in *Halaelurus bivius*, whereby males possessed a U-shaped mouth compared to the V-shaped mouth of females. The shape of the jaw has been widely noted as being a sexually dimorphic in a range of shark species (Soto, 2001). It was suggested that this relates to the biting activities of males during copulation, whereby the longer, narrower mouth provides a greater overbite and allows the males to gain enhanced grip on the body and fins of females.

In some elasmobranch species a clear seasonal dimorphism was found to exist in the structure of the teeth between males and females (Kajiura and Tricas, 1996). Ellis and Shackley (1995) determined that the lesser-spotted catshark showed a sexual dimorphism in both the jaw dimensions and the length and form of the adult teeth. However, they did not determine the existence of any seasonal dimorphism with regard to tooth structure. Reports of the reproductive season of *S. canicula* vary from region to region. It is not yet clear, in any study, if the sexually dimorphic dentition described in *S. canicula* occurs at the onset of puberty and remains fixed or alters in adults depending on the season for reproductive purposes.

1.2 Skin Structure

The skin is the largest and outermost of the organ systems that make up the vertebrate body (Kemp, 1999). It is comprised of two layers, an outer layer of stratified epithelium, the epidermis, and an underlying layer of connective tissue that makes up the dermis. Fish skin is characterised by scales and in sharks this is no exception. Kemp (1999) stated that in sharks it is apparent that the skin is covered with numerous scales. Elasmobranch scales are characteristically flat, non-overlapping and are known as placoid scales. The scales of

sharks are formed by individual tooth-like appendages that are embedded in the skin and are aptly known as dermal denticles (Kemp, 1999).

Despite a relatively large amount of information on fish skin, much of it focuses on the skin of teleost fish. The literature that does exist regarding elasmobranch skin has largely focused around the presence of bite wounds that are mainly present on the skin of female elasmobranchs. The presence of mating scars has been observed in many species of elasmobranch (Pratt, 1979; Stevens, 1974; Kajiura and Tricas, 1996; Kajiura *et al.,* 2000). Nordell (1994) suggested that in response to male biting, it could be expected that the skin of mature females might be thicker than males in areas where males bite them. Stevens (1974) observed that many of the reports of bite wounds included damage to the pectoral fins of most shark species. In most cases the fins were either torn, or showed scarring where biting had taken place. Stevens (1974) also noted that in many of the reproductive observations in sharks, the males were shown to grasp the pectoral fins with their mouths prior to insertion of the claspers. Studies on the skin thickness of the blue shark, *Prionace glauca* (Pratt, 1979) and in the Atlantic stingray, *Dasyatis sabina* (Kajiura *et al.,* 2000) showed that in both species the pectoral fin dermis and disc margin of females was fifty percent thicker than that of males.

The study by Kajiura *et al.* (2000) found that in *Dasyatis sabina* the dermis of females showed a sexual dimorphism throughout both the mating and non-mating seasons. Pratt (1979) found that the difference in skin thickness of female blue sharks was not localised to a specific area, such as the pectoral fin, but was uniformly thicker over most of the body. Pratt (1979) added that in order to accommodate the aggressive mating behaviour shown by male blue sharks during the mating season, the skin of the females is thicker than the male's teeth are long. He concluded that although sharks often have puncture wounds to the epidermis, only occasionally do the teeth penetrate to the dermis and musculature.

Despite the evidence of this increased skin thickness, Kajiura *et al.* (2002) indicated that the temporal relationships between dental and dermal sexual dimorphisms were unknown for any species.

A study by Southall and Sims (2003) reported on the use of skin in feeding. The research focused on the structure of elasmobranch placoid scales, or dermal denticles. Southall and Sims (2003) found that *S. canicula* use their dermal denticles to anchor prey items during feeding. Although they noted that the behaviours were found to be conducted mainly by juveniles, adults were also occasionally witnessed to anchor food to the seabed with the dermal denticles.

There is no research currently concerned with the presence of a sexual, or seasonal, dimorphism in the skin of *S. canicula*. It is not clear if the skin thickness in female catsharks will thicken in response to male biting, or whether there will be a difference in the density and distribution of the dermal denticles, which could provide protection from biting during copulation. Another possibility is that, in accordance with the sightings of mating that have been recorded, males may have larger denticles in the pelvic region in order to anchor the females during copulation.

1.3 Ampullae of Lorenzini

Despite the long lineage of research on electroreception of aquatic organisms it is only relatively recently that the major function of the Ampullae of Lorenzini (AoL) has been fully understood. According to Hueter *et al.* (2004) all elasmobranch fishes possess an ampullary system. Collin and Whitehead (2004) stated that electroreception is an ancient sensory modality, having evolved more than 500 million years ago, and has been lost and subsequently re-evolved a number of times. The presence of the electroreceptive organs in

elasmobranchs was first noted in the 17th century. According to Fishelson and Baranes (1998) the study of the electroreceptive organs in elasmobranchs dates back to their initial description by Malpighi and is followed by the exact description by Lorenzini after whom the electroreceptors, or AoL, are named. Despite having been fully described by Lorenzini in 1679 (Fishelson and Baranes, 1998) it was not until the mid part of the 20th century that the major function of the ampullary organs of elasmobranchs began to be understood (von der Emde, 1997). Tricas (2001) noted that the early anatomists who described the gross anatomical features of the ampullary organs of elasmobranchs (Lorenzini, 1678; Ewart and Mitchell, 1891) were unaware of its ecological function. According to Waltman (1966) when Lorenzini first described the ampullary canals that now bear his name he thought the ampullae were glands and that the long canals served to distribute their gelatinous secretion over the surface of the fish. Raschi (1986) stated that a variety of functions had been ascribed to the AoL. Initially they were thought to be secretory, providing the normally thick external coating of mucus characteristic of Rajoids. This misconception was mainly due to the presence of a conductive mucopolysaccharide gel contained within the long canals of the AoL. This gel is released when pressure is applied to pores on the head of shark and ray species. Today the AoL are known to be organs of sense and not secretion although the debate over the function of the AoL gave rise to a range of theories on the use of these organs.

It appears that the structure of the AoL is common to most elasmobranch species. Sisneros and Tricas (2002a) described the structure of the AoL to be comprised of a small chamber (the ampulla) which leads to subdermal canal terminating in a single pore located on the surface of the skin. They went on to add that the wall of the ampulla is composed of a single layer sensory epithelium that contains hundreds of sensory receptor cells. The lumen of the ampullary chamber is filled with mucopolysaccharide jelly that forms the electrical core.

The AoL are located around the head region of sharks and the disc margins in rays and are visible as small pores (Figure 1.4)

Figure 1.4. The AoL on the head of a shark. (http://www.bio.davidson.edu).

It was discovered by Kalmijn (1971) that electroreception and the AoL are involved in prey detection. Blonder and Alevizon (1988) stated that nearly all living animals in seawater emit direct current (DC) electrical fields, which are the result of electrical potentials between body fluids and the water and between different parts of the body. According to Zakon (1988) sensory systems that operate in an aquatic environment face different environmental constraints than their terrestrial counterparts in the detection of stimuli. He added that the differing properties of the aquatic environment to those of air, means that the transmission of sound, light and chemical stimuli have imposed habitat specific differences in the structure and function of many sensory receptor organs. It is well documented that electroreception is restricted to the aquatic environment (von der

Emde, 1997; Zakon, 1988). This is because air behaves as an insulator and not a conductor and is why electroreception is restricted to water (Zakon, 1988).

The fact that all living organisms emit an electrical current is what allows elasmobranchs to detect prey, even when the prey are obscured from view (Kalmijn, 1971). Kalmijn (1971) carried out experiments on *S. canicula*, using plaice as a prey item. It was found that when plaice were buried under the sand they were detected by *S. canicula* from a distance of approximately 15cm. However, in order to eliminate the possibility that the sharks were able to see the plaice, Kalmijn (1971) positioned the plaice in an agar chamber that allowed the electrical impulses given off by the plaice to pass through, but no visual or chemical stimuli. It appeared that when the catsharks passed the agar chamber they demonstrated the same feeding response through well-aimed turnings toward their prey.

For a number of elasmobranch species vision is limited by the environment in which they live. Many, including *S. canicula*, often inhabit dark, murky waters and vision plays a limited role in many behaviours. For those species that do rely less on visual cues it is believed that another sense is used for mate location or prey detection. It is thought that not only is electroreception used for prey detection, but also plays a role in bringing males and females together during the mating season. Recent claims by Sisneros and Tricas (2002a) suggested that the electrosense of elasmobranchs is important during courtship and reproduction and not used solely as prey detection as previously stated by Kalmijn (1971). Sisneros and Tricas (2002b) carried out a study on the electrogenic ray, *Urobatis halleri*. Their research concluded that both male and female stingrays use their electrosense to detect and locate conspecifics during the mating season. The research carried out by Sisneros and Tricas (2002b) suggested that mate location occured in much the same way as prey detection did in *S. canicula,* as previously noted by Kalmijn (1971). They discovered that male rays were able to locate females that were buried in the sand and out of view.

The conclusions drawn by Sisneros and Tricas (2002b) suggested that the ampullary electrosense in the natural behaviour of sharks and rays can be classified into four major categories; detection of prey, mates, predators and competitors. Sisneros and Tricas (2002b) added another dimension to the use of electroreception stating that is also appeared that females formed large aggregations during the mating season, using their electrosense to locate conspecifics.

In light of recent studies by Sisneros and Tricas (2002b) several questions need addressing. One of the main questions that needs to be answered is whether the AoL in *S. canicula,* as well as other species, will show a sexual dimorphism in terms of the numbers of sensory and sustentacular cells in each ampullary organ. Despite the findings from Sisneros and Tricas (2002b) there is no evidence in the literature to suggest that the structure of the AoL in any species is sexually dimorphic in structure.

1.4 Reproductive Seasonality

The subject of secondary sexual dimorphisms in shark species has been well documented and heavily disputed. Dodd (1983) noted that the only striking secondary sexual characters of the males are the so-called claspers. However, Mellinger (1986) listed a number of other characters which showed sexual dimorphism in at least some male elasmobranchs, including smaller size at maturity, earlier onset of sexual maturity, shorter life span, modified teeth, stronger jaws, placoid spines on the wings of some skates (which are claw-like and retractile) greater activity and increased aggressiveness. Capapé, *et al.* (2008) noted that liver size is sexually dimorphic in chondrichthyan species, adding that a larger liver may allow females to maximize the production of yolk. They stated that sexually dimorphic livers have been identified in a range of elasmobranch species, including the

lesser guitarfish, *Rhinobatos annulatus,* the lesser-spotted catshark, *S. canicula*, the smallnose fanskate, *Sympterygia bonapartii,* and the thornback ray, *Raja clavata.*

The fact that sexual dimorphisms exist in many elasmobranch species is well proven. How the presence of sexual dimorphisms can help to determine reproductive cycles has been explored to a much lesser extent. Wourms (1997) distinguished 3 types of reproductive cycle in elasmobranchs: well defined annual or biennal cycles (e.g. *Squalus acanthias*) partially defined cycles with one of two peaks of activity (e.g. *Raja erinacea*) and reproduction throughout the year (e.g. *S. canicula*). Kimber *et al.* (2009) stated that it appeared that in some species the reproductive cycle drives sexual dimorphisms. Kajiura *et al.* (2000) noted that in the Atlantic stingray there is a shift in the dental structure of males during the year. They believed that this change in tooth shape coincided with the mating season and served as an indicator of when mating takes place. However, the mating season for any elasmobranch species is very difficult to determine due to their wide ranging habitats and the environment in which they live.

One aspect of shark behaviour that may assist in providing some evidence of the reproductive season is sexual segregation. Sexual segregation has been noted in many elasmobranch species (Wetherbee *et al*., 1997). They noted that in the grey reef shark males tended to occur at greater depths. Bullis (1967) noted that sexual segregation has been observed in the blue shark, *Prionace glauca*, white-tip shark, *Carcharhinus longimanus*, sandbar shark, *Carcharhinus plumbeus* (as *C. milberti* and *Eulamia milberti*), and the marbled catshark, *Galeus arae*. Compagno (1984) stated that juvenile *S. canicula* were found to be distributed in shallower water than adults, and that adults often occurred in unisexual schools. Rodriguez-Cabello *et al.* (2004) found that the distribution of *S. canicula* in the Cantabrian Sea is continuous along the continental shelf, although they may

aggregate by sex or size. Juveniles were found mostly at depths around 200m, while adults had a wider depth distribution, 50-450m (Rodriguez-Cabello *et al.,* 2004).

In a different study, Rodriguez-Cabello *et al.* (2007) noted that mature *S. canicula* females were found at depths ranging from 100m to 400m, with a greater proportion of individuals being larger in the deeper strata. Sexual segregation by depth has also been observed in *S. canicula* by Sims *et al.* (2001). Males in a tidal sea lough showed low activity during the day in deep water (12-24m) followed by more rapid movements into shallow areas (<4m) at night (Sims *et al*., 2001). Females showed a different behavioural strategy, refuging in shallow water (0.5-1.5m) in the day and were nocturnally active primarily in deep water (Sims *et al*., 2001). Springer (1967) suggested that a depth distribution of this nature might occur to avoid intraspecific predation. D'Onghia *et al.* (1995) did not concur with these findings and found that both sexes of juvenile and adult *S. canicula* in the north Aegean Sea were found together at depths greater than 200m. D'Onghia *et al.* (1995) suggested that Springer (1967) based his findings on observations of pelagic sharks and that pelagic sharks show a very different life history to demersal species.

The lesser-spotted catshark is an oviparous species that has been shown to exhibit a long breeding cycle, with females having a protracted egg-laying period and the ability to store sperm for long periods (Metten, 1939). Breeding can be differentiated from mating as breeding encompasses the egg laying season, whereas mating involves only the act of copulation. There have been conflicting views with regard to the exact timings of both breeding and mating in this species. Wourms (1997) suggested that *S. canicula* has no defined mating season, whereas Dobson and Dodd (1977) examined the testis of catsharks and stated that *S. canicula* undergoes an annual cycle of reproductive activity. Craik (1978) agreed with this theory of an extended breeding cycle. It was noted by Craik (1978) that vitellogenesis occurred throughout the year and that breeding in female *S. canicula* is

cyclical with the period of the cycle being unusually long. Ford (1921) noted that upon examination of the population of *S. canicula* from around the coast of Plymouth, South Devon (UK) the breeding season was protracted and eggs were laid throughout the year. However, Harris (1952) who studied *S. canicula* in Ilfracombe, North Devon (UK) found that the Ilfracombe population of *S. canicula* showed a rather more defined breeding season than the Plymouth population. Harris (1952) reported that breeding starts in November and continues until July, and from July to December only a third of females examined were carrying egg cases. However, it could be argued that in Ford's (1921) study the percentage of females carrying egg cases was considerably lower in September and October. Ford (1921) and Harris (1952) both report the highest occurrences of egg cases were in winter and spring. These findings were consistent with those of Sumpter and Dodd (1979) who concluded that the female lesser-spotted catshark has an extended breeding season, although the peak frequency of egg laying occurs in the winter and the spring.

Henderson and Casey (2001) studied a population of *S. canicula* from the west coast of Ireland and found that a similar pattern occurred, with females carrying egg cases throughout the year, indicating a protracted breeding season. It was also noted that peak egg production was in the spring (May) and minimal in October (Henderson and Casey, 2001). Henderson and Casey (2001) noted that male and female gonadal cycles were not in synchrony and concurred that sperm storage in females occurs. Ellis and Shackley (1997) found that the egg-laying season in *S. canicula* from the Bristol Channel lasted 10 months peaking in June and July, with the gonosomatic index greatest in May. Earlier studies, such as those carried out by Metten (1939) support this and report that *S. canicula* is sexually active throughout the year, although slightly more prolific during spring.

Geographical segregation in reproductive parameters has been documented in many elasmobranch species (Parsons, 1992; Taniuchi *et al*., 1993) and may be an indicator of a

more specific mating period in elasmobranch species. It is reported that sexual segregation occurs in catsharks in the Solent (UK) with findings similar to those of other researchers. Local fishers reported catches of either male or female catsharks at any one location throughout much of the year. It appeared that catches of both male and female catsharks at the same locations in the Solent occur during the spring and early summer months. Lyle (1983) found a similar pattern whereby males predominated in catches throughout the entire study except the winter months. Lyle (1983) added that since females in excess of 60cm were found to be mostly mature, it could be concluded that the adult females were only resident on the studied ground for a short period of time. The spring catches of both male and female catsharks at the same location in the Solent also coincides with the crossing, or flexion, of claspers (Fig. 1.5) and running milt in some male specimens caught at this time. This is an indication that mating may be taking place throughout this period. Flexion of the claspers is a good indication of mating behaviour as it is used to fill the siphon sacs prior to copulation (Gilbert and Heath, 1972).

Figure 1.5. Crossed claspers in *S. canicula* (Photograph courtesy of L. Llewellyn).

The reproductive cycle of male *S. canicula* has been little studied in comparison to that of the female. Garnier *et al.* (1999) studied the seasonal variations in sex steroids and male sexual characteristics in *S. canicula*. Previous studies showed that in another species of elasmobranch, *S. acanthias,* there is an annual cycle of 3ß-hydroxysteroid dehydrogenase activity correlated with changes in spermatogenesis stages (Simpson and Wardle 1967). Garnier *et al.* (1999) found that various aspects of reproductive function in male *S. canicula* appeared to be influenced by season, with sea temperature possibly being the major determinant in this respect. By using radioimmunoassays to measure the concentrations of reproductive hormones in the blood plasma, and recording the weights and sizes of the testes and sperm reserves, Garnier *et al.* (1999) found that testicular and epididymal weights, sperm reserves and clasper length varied throughout the year. They also discovered that testosterone was the principal steroid present, and most steroids except progesterone had an annual peak in February.

An additional consideration with regard to determination of a specific mating season in elasmobranchs, as previously mentioned, is the ability of females to store spermatozoa. It is a well established fact that in most shark species the female can store sperm in a specialised region of the anterior oviduct for many months (Reebs, 2003; Wourms, 1977). This ability to store sperm for extended periods means that female catsharks have a protracted egg laying period and have been known to lay eggs for 11 months of the year (Ford, 1921). Metten (1939) first described sperm storage in *S. canicula* and found isolated spermatozoa throughout the tubules of the shell-secreting zone, the nidamental gland. In freshly dissected females the spermatozoa found in this region were active. The storage of spermatozoa was also noted by Prasad (1945) in an additional five elasmobranch species. Clark (1922) referred to sperm storage as receptaculum seminis, the ability of an elasmobranch to self fertilise eggs from sperm reserves. The evidence to support this view was described by Clark (1922) who found that female blonde rays, *Raja brachyura*, kept

alone for 5-6 weeks laid 30 egg-cases all of which were fertile. Pratt (1993) describes 3 types of sperm storage in elasmobranchs (1) non-storage/immediate insemination for sharks such as porbeagle, *Lamna nasus* (2) short-term storage/delayed insemination found in sharks where ovulation is prolonged over weeks or months such as the whale shark, *Rhizoprionodon terraenovae* (3) long-term storage/repeated insemination, a characteristic of nomadic sharks such as the blue shark, *Prionace glauca.* This level of sperm storage evolved to allow free-roaming migrations and sexual segregation in shark species, and increases the chances of successful insemination (Pratt 1993). Sperm storage provides flexibility as it uncouples mating activities. This ensures that females can self-inseminate when each individual is physiologically prepared and bears mature ovarian eggs, healed mating wounds and greater energy reserves (Pratt 1993). However, this process makes it difficult to ascertain when the mating season occurs and therefore restricts the ability to observe courtship and copulation in sharks.

Despite all of the reproductive seasonality data relating to various populations of *S. canicula* it remains unclear when the precise mating season occurs for the population found in the Solent. Kajiura and Tricas (1996) and Kajiura *et al.* (2000) found a seasonal dimorphism in the dentition of the Atlantic stingray, *D. Sabina*, and attributed this to the mating season. However, as far as the author is aware there have been no other reports of this occurring in any other elasmobranch species, including *S. canicula*. Similarly, there is also a lack of data on the skin thickness of *S. canicula* in relation to reproduction and it appears that no literature exists on the thickness of skin of either male or female catsharks. The only literature relating to the skin of this species examines the dermal denticles as a tool for prey capture (Southall and Sims, 2003). The question remains unanswered as to how catsharks in the Solent achieve mate location.

It appears that single sex aggregations could form, as have been found in other populations, meaning that conspecific location could be important for this species. Therefore, examining whether there are sexual or seasonal dimorphisms in dental structures in males, a thickening of the skin and change in denticle structures in females and a sexual dimorphism in the structure of the AoL throughout the year could aid in confirming whether there is a specific mating period for the population of *S. canicula* in the Solent. As far as the author is aware, there is no literature available on the seasonal dimorphisms of tooth, skin, head and jaw dimensions and no literature on the sexual dimorphisms of the AoL for the lesser-spotted catshark.

1.5 Aims

Continuing on from previous studies that have focussed on secondary sexual dimorphisms in the head, jaws, teeth, skin and AoL of elasmobranchs the current study has the following overall aims:

1. To investigate the morphology and structure of the head, mouth, jaws and dentition of *S. canicula* and to compare the head morphology and dentition between sexes to determine whether a seasonal and sexual dimorphism occurs in any structural aspect.

2. To investigate the gross morphology of the skin of *S. canicula.* This will involve comparing the dermal and epidermal layers and the structure and morphology of the dermal denticles in hatchling, juvenile and adult catsharks and to compare the skin structure between sexes to determine whether a seasonal and sexual dimorphism occurs.

3) To investigate the structure of the AoL of *S. canicula.* This will involve comparing the alveolar epithelia and alveolar number adult catsharks and to compare the AoL between sexes to determine whether a sexual dimorphism occurs in any structural aspect.

The aims will be addressed by conducting generalised gross dissection and sampling procedures as outlined in Chapter 2. Chapter 3 investigates the morphometrics of the head, mouth and jaws, comparing the measurements of aspects of the gross morphology of hatchling, juvenile and adult male and female catsharks. Statistical analyses will be conducted to test for sexual and seasonal differences.

Chapter 4 examines the structure of the teeth of hatchling, juvenile and adult catsharks. Light Microscopy is utilised to make detailed measurements of the dentition of male and female catsharks and the measurements are compared statistically for any sexual or seasonal differences.

Chapter 5 investigates the structure of the skin of hatchling, juvenile and adult catsharks, examining the dermal and epidermal structures as well as the morphology for the dermal denticles. Scanning Electron Microscopy (SEM) is utilised to investigate the surface structure of the dermal denticles of males and females and to make qualitative comparisons.

Chapter 6 examines the structure of the AoL of adult *S. canicula*. Histology and light microscopy, as well as SEM and confocal laser microscopy will be used to determine the structure of the epithelium and alveoli of the AoL. Statistical tests will determine if any sexual differences occur.

The available literature relating to each topic is reviewed in detail in the introduction to each chapter. Gaps in our current knowledge are highlighted and the significance of the research conducted in each chapter is discussed. Chapter 6 provides a general discussion and overview of the results of the study, their significance and outlines areas for further research and investigation.

Chapter 2 - General Materials and Methods

A range of general materials and methods were employed in order to address the aims of the study. These ranged from collection of specimens, to maintenance and sampling of individuals. This chapter details the general materials and methods used for the research. Information is also provided on analysis of data that led to the classification of individuals into size classes and the categorisation of seasons based on monthly water temperatures.

2.1 Experimental Specimens

Between October 2002 and December 2007 specimens of *Scyliorhinus canicula* were captured in the eastern Solent off the coast of Southsea, Hampshire within a 0.5 km radius from Dean Tail (Figure 2.1) with the use of a long line or gill net from local fisherpeople. Samples were not obtained consistently throughout the year due to inclement weather and the seasonality of the marine fisheries industry. Frozen samples were avoided as they were sourced from unknown geographic locations and seasons and were gutted prior to freezing.

Figure 2.1. Collection site (Dean Tail) of *S. canicula* from the eastern Solent. (Maps adapted from Admiralty Leisure Chart Folio SC5600).

The specimens were maintained in a 1250 litre aerated holding tank at the University of Portsmouth's Langstone Harbour Marine Laboratories at Eastney (Figure 2.2).

Figure 2.2. Holding tank at the University of Portsmouth's Langstone Harbour Marine Laboratories at Eastney (Photograph courtesy of C. Waring)

Sharks were held at the facility for a period of between 1 week and 1 month. Those specimens maintained for a maximum of one month were selected for behavioural experiments and were captured at the beginning of a specific season. This method ensured that no catshark was captured during one season and sampled during another. All remaining individuals were sampled during the month, and therefore season, in which they were captured. Catches were designed to ensure that monthly and seasonal overlap was not encountered. The seasonal allocations were based on the date of sacrifice.

There was a constant flow of sand-filtered seawater (salinity, 34) pumped from Langstone Harbour, entering the tanks throughout the duration of captivity at a flow rate of approximately 8 l/min. Specimens were fed daily on a 1% maintenance diet which

consisted of squid and chopped fish. Daily checks were carried out to monitor the condition of the sharks and to collect seawater temperatures (°C) from the holding tank. Average monthly water temperatures ranged from 6.8 to 23.9°C throughout the year (Figure 2.3). In order to ascertain whether there were any intra-sexual and inter-sexual seasonal dimorphisms present in *S. canicula* the specimens were divided into seasons, depending on which month they were sampled. In order to differentiate the seasons, seawater temperature data were used.

These data were collected from Langstone Harbour using a Hanna Instruments HI140 data logger placed permanently 1 meter below the surface. Using these data the seasons were determined by grouping data together when the water showed a steady increase or decrease in temperature. The data were obtained between September 2002 and September 2004 (Figure 2.3) and is consistent with seasonal segregations noted by Lyle (1983).

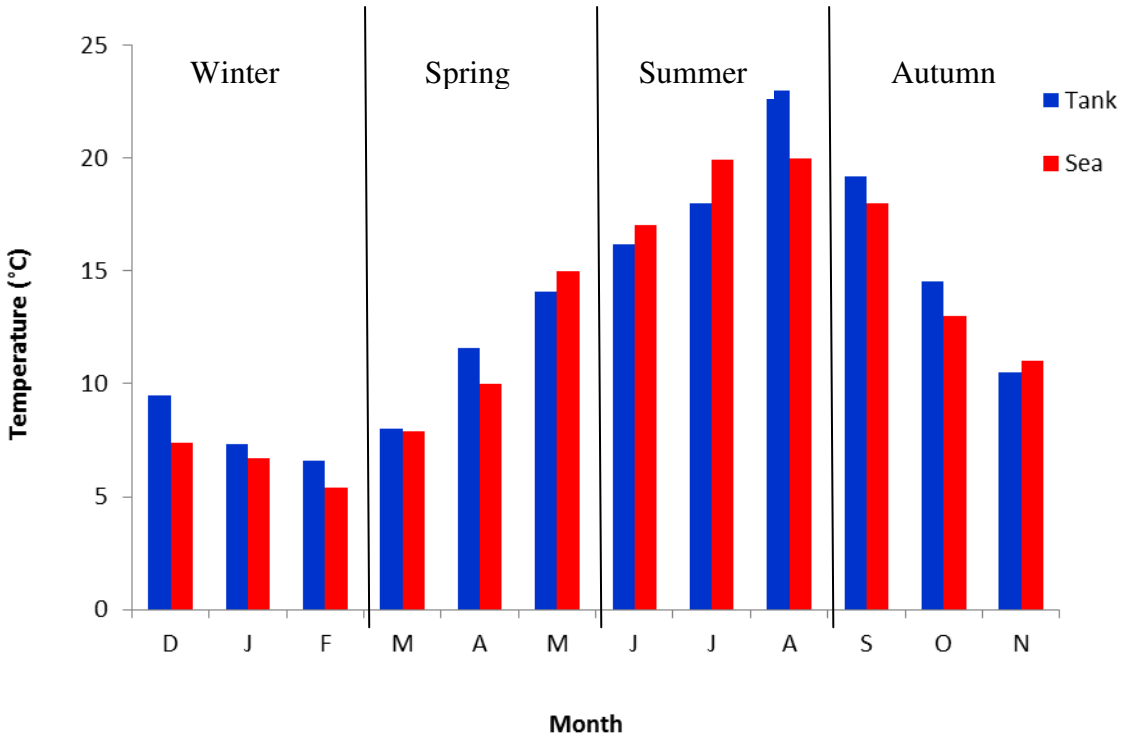

Figure 2.3. Average monthly seawater temperatures in the holding tanks over a three year period from 2003-2006 and in Langstone Harbour over a two year period from 2002-2004.

During captivity many of the female catsharks produced eggs, which were placed into external tanks. Again, the tanks were fed a constant flow of seawater from Langstone Harbour with a flow rate of approximately 8 l/min and maintained at temperatures between 6.8 and 23.9°C. Both juvenile and adult catsharks were killed by a sharp blow to the head followed by the destruction of the brain. Hatchlings were killed using an overdose of anaesthetic (0.5 ml l^{-1} 2-phenoxyethanol) followed by destruction of the brain.

2.2 Specimen Processing

Once sacrificed, a range of measurements were recorded. The adults were measured from the snout to the extremity of the upper caudal lobe to establish the total length (T_L) (mm). Weight (W_T) (g) was measured using a top-pan balance and the sex of each individual was recorded. For a range of males, the internal length of the right clasper was measured using Mitutoyo electronic callipers accurate to two decimal places (Figure 2.4).

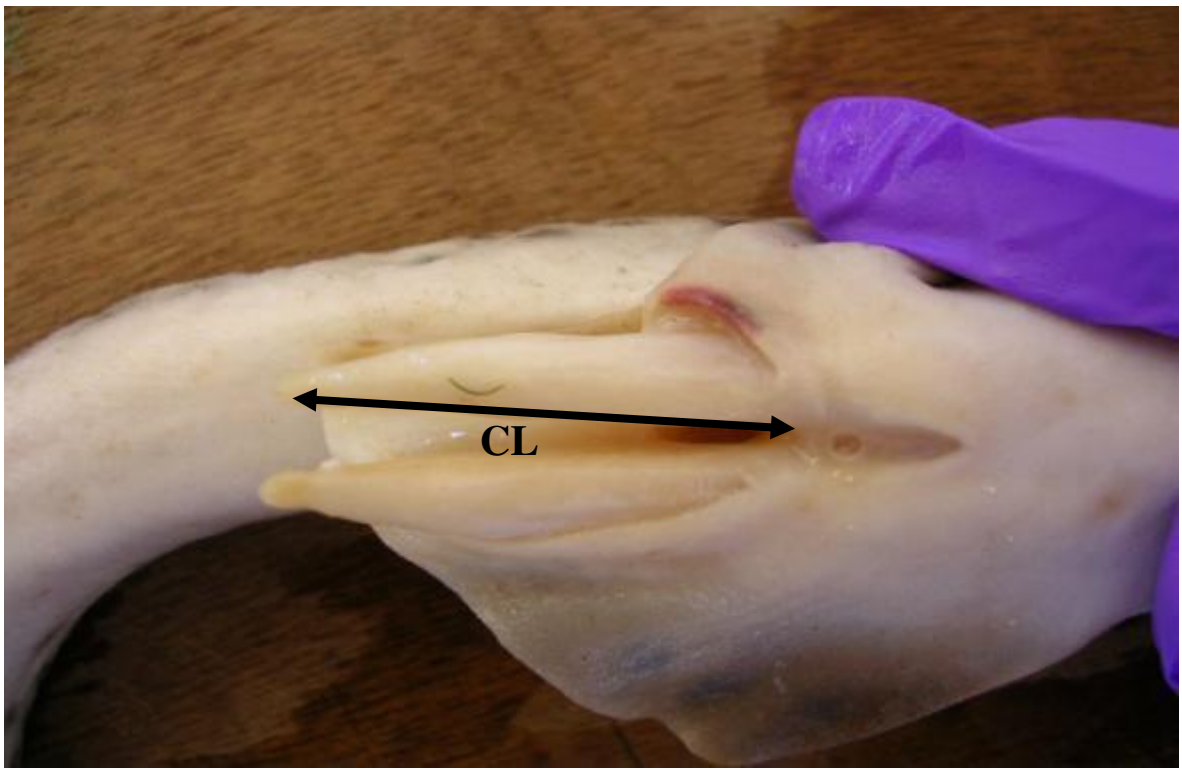

Figure 2.4. Internal length of the right clasper (CL) Photograph courtesy of C. Waring.

The specimens were dissected by entering through the vent and cutting along the ventral surface between the pelvic fins, terminating at the anterior end of the pectoral fins. The gonads of both sexes were examined for maturity status as described by Ivory *et al*., (2004). Notes were taken on any findings within the gonads (i.e. the presence of eggs or running milt). In males, mature specimens were determined by the presence of rigid claspers that were the same length as, or slightly longer than, the pectoral fins and when the testes were enlarged and the vas deferens extremely coiled. For a range of females the diameter of the right nidamental gland was also measured using Mitutoyo calipers. Mature females were found to possess large white nidamental glands and thick oviducts. The heads were removed and stored in unbuffered 10% formalin in seawater for later sampling of the jaws, teeth and AoL. A skin sample, approximately 1cm^2 was removed from an area just below the dorsal fin and above the lateral line on the left hand side of the body. The left and right pectoral fins were removed and place in unbuffered 10% formalin in seawater for later processing.

2.3 Hatchling Morphometrics

The complete hatchling catsharks were placed into unbuffered 10% formalin in seawater. A section of skin was taken from the hatchling catsharks. As with the adults, this was taken from an area below the dorsal fin and above the lateral line on the left hand side of the body and placed into a solution of unbuffered 10% formalin in seawater. Due to the smaller dimensions of the hatchlings the sections of skin removed were reduced in size to 0.5 mm^2. Both left and right pectoral fins were also removed and stored in unbuffered 10% formalin in seawater. Due to the size of the fins no disc was removed and the whole fin was used for analysis

2.4 Data Analysis

Previously, many researchers have used percentages to remove the effects of body size on the results. According to Packard and Boardman (1999) this approach can cause major discrepancies with the data and can provide wholly unreliable data. They stated that implementing the use of ratios assumes a linear relationship and yields less reliable results. In order to determine whether there was a seasonal sexual dimorphism in the morphology and physiology of *S. canicula,* a general linear model (GLM) was used. The use of an ANCOVA was employed in order to examine the effects of body size on the data from this study. Significance was accepted at $P<0.05$.

2.5 Results

2.5.1 Hatchling Samples

A total of 37 hatchling catsharks were sampled, comprising 23 males and 14 females. This gives a ratio of 1.64:1 in favour of males. Due to the small numbers obtained it was not possible to analyse any seasonal differences.

2.5.2 Juvenile and Adult Samples

A total of 220 lesser-spotted catsharks, comprising 75 males and 145 females were sampled, giving a ratio of approximately 2:1 in favour of females. This is in contrast to the hatchling catsharks that were used in this study, whereby males dominated. Although 220 specimens were sampled, not all specimens contributed to every parameter measured. This was due to the evolution of the research, whereby tissue samples were taken in later specimens that were not taken at the beginning of the study (e.g. inclusion of fin data).

The relationship between nidamental gland width and total body length revealed a significant non-linear relationship (P<0.05) although there were no seasonal differences. Nidamental glands were wider at a body length of 550mm and above (Figure 2.5) which can be correlated with the onset of maturity. Using the clasper length data and examining the relationship between total body length and clasper length, the males were classified into mature and immature individuals. The clasper length data revealed a significant relationship with body length indicating a positive correlation between clasper length and the onset of maturity. The relationship was not linear, but did suggest the greatest increase in clasper length was at a body length of 525mm (Figure 2.6). GLM analyses showed that season had a significant effect on clasper length (P<0.05) with males sampled in spring possessing longer claspers than those sampled during the remaining seasons.

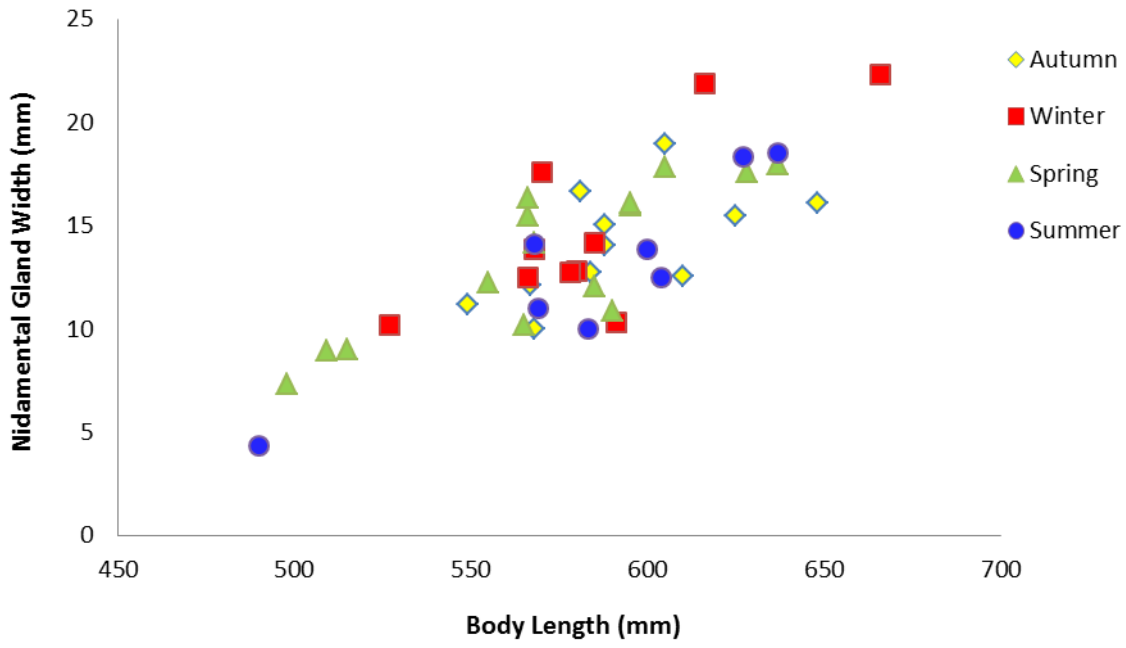

Figure 2.5. Scatterplot showing the relationship between nidamental gland width and total body length for each season in female *S. canicula*. (n= (A, 11) (W, 10) (Sp, 15) (Su, 8))

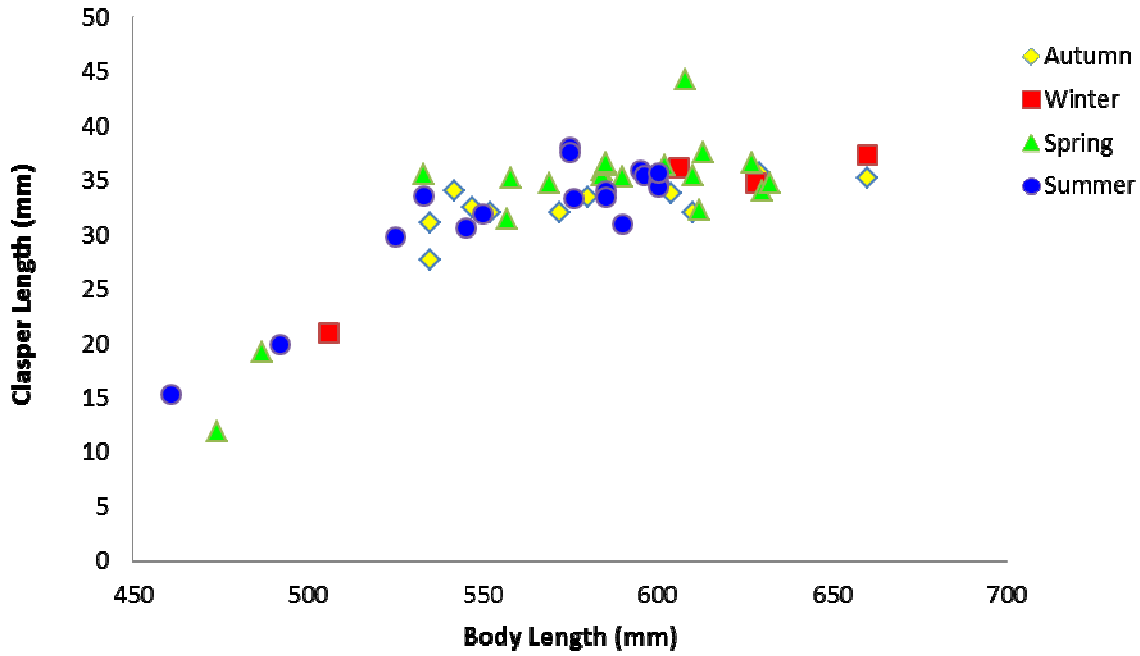

Figure 2.6. Scatterplot showing the relationship between clapser length and body length for each season in male *S. canicula*. (n= (A, 11) (W, 4) (Sp, 19) (Su, 17)). (*P*<0.05).

The number of mature male *S. canicula* that were sampled throughout the year as well as those that were producing milt can be seen in Figure 2.7.

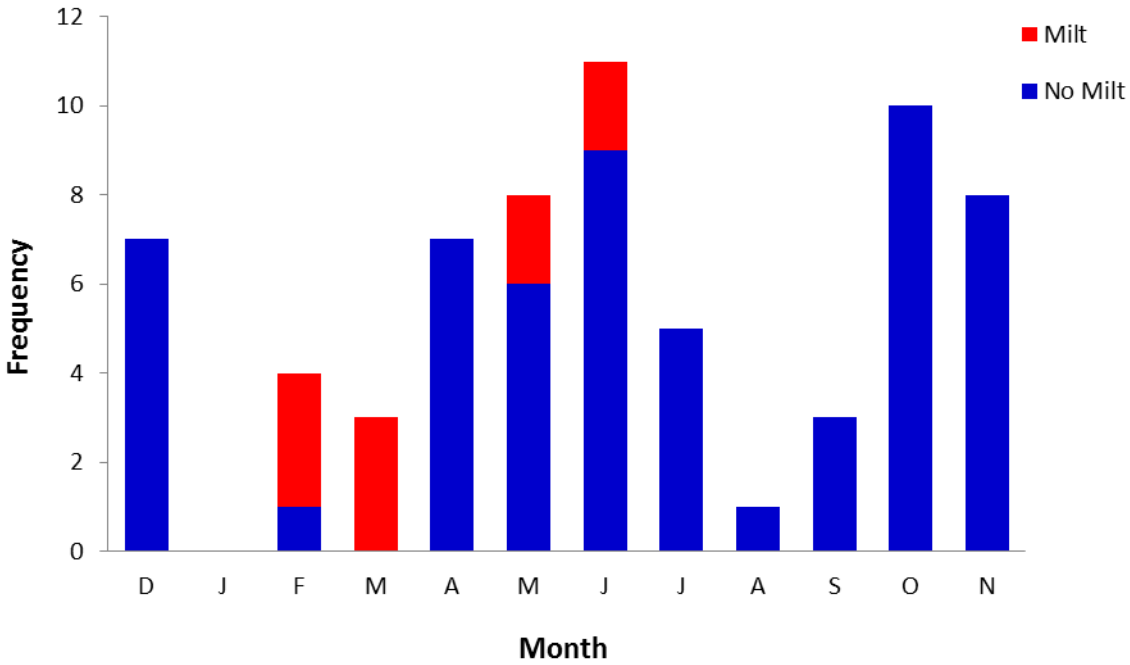

Figure 2.7. Total number of mature male catsharks sampled in each month of the year showing the proportion expressing milt when sampled.

Ivory *et al.* (2004) reviewed the growth and reproduction in *S. canicula*. The study contained a review of previously published data showing the length at 50% maturity of populations of *S. canicula* from various geographical locations (Table 2.1).

Table 2.1. A summary of the length (cm) at 50% maturity for *Scyliorhinus canicula* from various geographical locations (After Ivory *et al*., 2004).

Author	Sampling Area	Male	Female
Jennings *et al.* (1999)	Atlantic (North Sea)	58.0	58.0
Henderson and Casey (2001)	Atlantic (Ireland)	57.5	58.1
Ellis and Shackley (1997)	Bristol Channel	52.0	55.0
Ford (1921)	English Channel	57.0–60.0	57.0–60.0
Leloup and Olivereau (1951)	English Channel	52.0–60.0	52.0–60.0
Fauré-Frémiet (1942)	Atlantic (France)	52.0–60.0	52.0–60.0
Rodríguez-Cabello *et al.* (1998)	Atlantic (Spain)	–	54.2
Capapé *et al.* (1991)	Mediterranean (France)	44.0	41.0–47.0
Leloup and Olivereau (1951)	Mediterranean	37.0–44.0	37.0–44.0
Capapé (1977)	Mediterranean (Tunisia)	40.0	40.0–45.0

It can be seen from Table 2.1 that there is a large variation in the lengths at 50% maturity for *S. canicula* from European waters. These range from 37 cm (Leloup and Olivereau, 1951) for males, to 58 cm for both males and females (Jennings *et al.,* 1999). It appears that those individuals sampled from the warmer Mediterranean sites mature at a shorter length that those from around the colder waters of the UK.

Based on both the data collected for the current study and those from previous studies on length at maturity, the specimens of *S. canicula* used for this research were categorised into two size classes (Table 2.2). In previous studies that categorised samples into class sizes in order to analyse morphometrics of catsharks (Ellis and Shackley, 1995 and 1997) the samples were treated as five separate class sizes. For this study, however, it was not

feasible to do this due to the limited number of specimens found at some of the smaller body lengths. As well as size classes, samples were divided into seasonal groups depending on when they were sampled (Table 2.2).

Table 2.2. The number and length range of juvenile and adult male and female *S. canicula* sampled for each month and season.

Season	Months	Males				Females			
		< 525 mm		≥ 525 mm		< 550 mm		≥ 550 mm	
		n	Length Range (mm)	n	Length Range (mm)	n	Length Range (mm)	n	Length Range (mm)
Winter	December	2	506-522	7	550-626	2	527-547	16	552-666
	January								
	February	1	474	4	540-608	4	486-542	10	558-628
Spring	March			3	533-590	7	460-532	14	566-607
	April	1	487	7	585-632			5	575-595
	May	1	461	6	557-623			14	550-633
Summer	June	2	492-515	9	525-660	4	490-547	9	558-637
	July	1	420	5	545-600	2	490-540	11	550-648
	August			1	585			5	568-627
Autumn	September			3	535-660			6	565-600
	October	1	410-448	10	542-630	8	448-540	14	550-630
	November	2	510-520	8	535-767	3	403-549	10	550-632

Figure 2.8 shows length frequency histograms for the male and female, immature and mature catsharks used in this study. It can be seen that a majority of catsharks used for this research ranged in size from 525 – 650 mm in length. It is also clear from Figure 2.8 that fewer sharks were caught during the winter months when fishing effort was at its lowest.

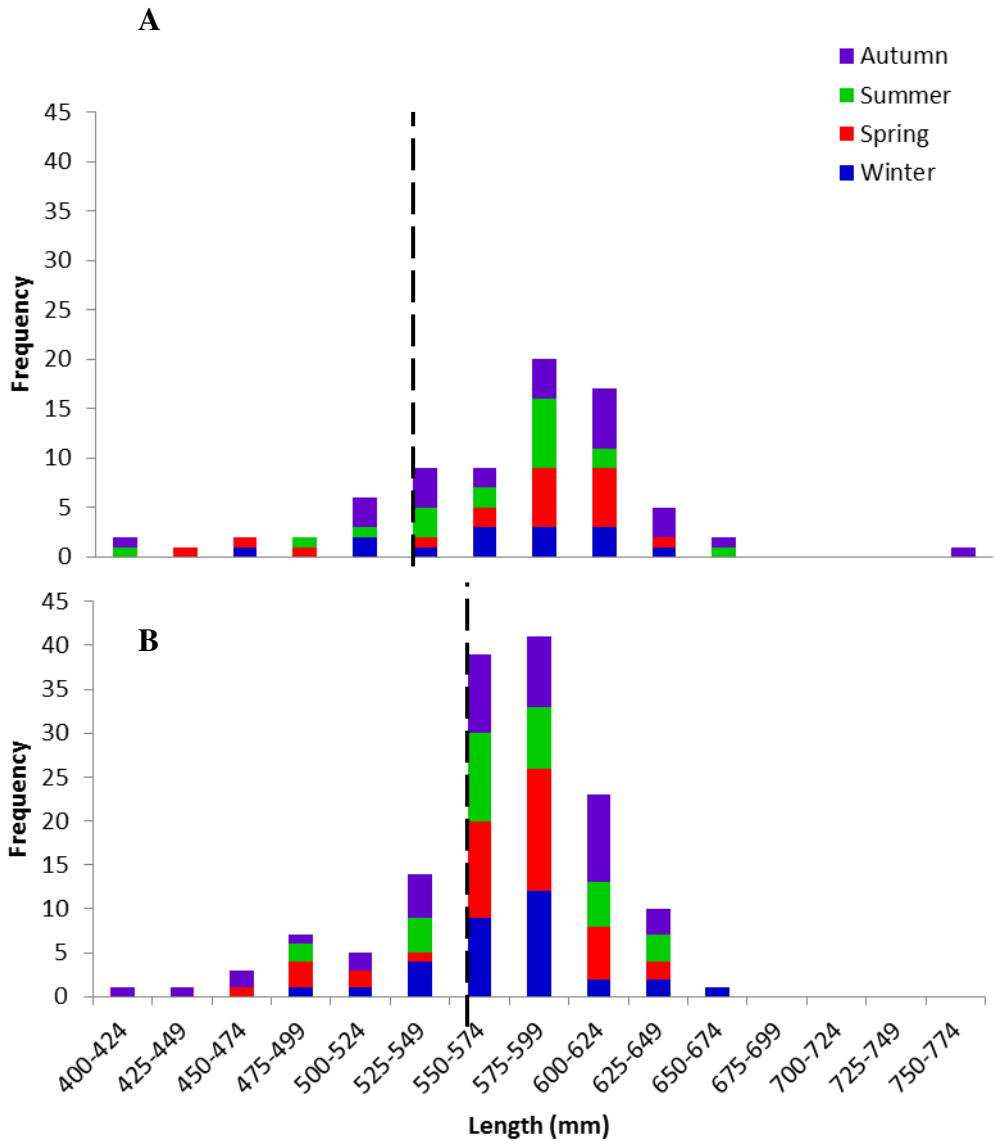

Figure 2.8. Total number of male (A) and female (B) immature and mature catsharks by size range showing numbers of each size range sampled in different seasons of the year. Dashed line indicates length split for mature and immature sharks.

The months were divided into seasons using the temperature readings taken from the Hanna Instruments HI140 data logger that was permanently placed in Langstone Harbour on a floating raft, 1 meter below the surface. The seawater that was pumped into the holding tanks at the Langstone Harbour Marine Laboratories in Eastney was on average 2°C warmer than the temperatures recorded in Langstone Harbour for each month of the study. However, despite this slightly higher water temperature the monthly temperature pattern in the holding tanks was consistent with that in the harbour. The slight increase in

water temperature could have occurred due to two factors. The first being that the water was held in settlement tanks after being pumped from the harbour and before being pumped into the holding tanks, meaning that it could have been warmed slightly. Secondly, the water entering the holding tanks would have been heated slightly by the internal temperature of the room that the holding tanks were housed in.

There was an increase in water temperature from March to August (spring to summer) and a decline from September to February (autumn and winter). The lowest recorded water temperatures in the holding tanks were taken in February, whilst in the harbour the month with the lowest recorded temperatures was January. The difference in temperature between the sea and the tank may have had an effect on the reproductive cycle, as temperature has been found to affect aspects of seasonal reproduction in male *S. canicula.* Garnier *et al.* (1999) stated that various aspects of the reproductive function of *S. canicula* appear to be influenced by season, the sea temperature being, most probably, a major determinant in this respect. The sea temperatures recorded from the harbour may not have mimicked the natural environment of *S canicula* as they were taken from the centre of the harbour at a depth of 1m. The harbour would have had provided different thermal properties to the water and may not have precisely replicated the habitat of *S. canicula*. However, it did allow for a seasonal classification of water temperatures, which are in line with other literature.

Females dominated during every month of sampling, except for April, and as previously stated the total number of female catsharks sampled was almost double that of males with the highest numbers of females sampled during March and October. Despite the increased incidence of males during the month of April, the ratio of females sampled during every season was always higher than that of males.

The number of males sampled was lowest during late summer to early autumn and highest in late summer and mid autumn. Ford (1921) reported similar observations in a population of *S. canicula* off Plymouth. He reported that females were found in highest numbers in the winter and spring, whilst during May and June there was an increase of male specimens. Harris (1952) found a slightly different variation in distribution from a population off Ilfracombe, whereby females were found in high numbers from September to January, whilst males were found in higher numbers from February.

It is difficult to draw any solid conclusions from this study in terms of the impacts of seasonal changes and how they can affect the sex ratio of *S. canicula* in the Solent. As the specimens were collected and supplied by local fishers precise information was not available on catch effort, exact geographic location of catches or methods employed to collect samples and whether the samples were caught consistently, using the same methods. This is also true for the published data on the topic of seasonal changes in the sex ratios of *S. canicula*. The literature does not clarify the catch effort that was employed or say which collection methods or sampling protocols were used. As with this study, which failed to collect samples in January, studies by Ford (1921) and Harris (1952) show that there were months where there was a failure to collect samples. Ford (1921) did not obtain samples during March and April, whilst Harris failed to obtain samples in August. Neither author comments on the fishing effort, although it was noted that, as with this study, the specimens were collected and provided by local fishers. The inconsistency with collecting samples seems to be a recurring problem as was noted by Henderson and Casey (2001). They conducted a study off the west coast of Ireland and stated that because of adverse weather conditions, and also seasonal changes in the type of fishing gear employed by vessels, sample material was available for only eight months of the study.

The 2:1 female/male ratio of catsharks found in this study could lead to the consideration that sexual segregation occurs within this population. It has been widely surmised that elasmobranchs segregate by sex, size and depth, although without sampling catches direct from commercial fisheries throughout the year, this assumption is hard to prove for *S. canicula* in the Solent.

Many authors describe sexual segregation in a number of elasmobranch species (Springer, 1967; Klimley, 1987; Sims *et al.,* 2001; Bass *et al.*, 1975). The assumption that sexual segregation occurs in elasmobranchs has been suggested through the disparity of landed catches and the fact that males and females have been caught in different areas. Research by Springer (1967) suggested that populations of sharks might be divided into social units of both sexes, and that mature females will segregate, as will mature males, forming unisex groups. According to Sims *et al.* (2001) intraspecific competition and alternative seasonal habitats may play a role in sexual segregation as well as reproductive choices associated with pre-or post-mating strategies. Other types of segregation have also been found to occur, such as depth segregation in the marbled catshark, *Galeus area* (Bullis, 1967). Similar findings have been reported in *S. canicula* whereby juveniles were found in shallower water than adults and that adults often occurred in unisexual schools (Compagno, 1984, Compagno *et al*., 2005). For this study no information was gathered on geographical locations or catch depths, although observations by local fishers suggested that catches of *S. canicula* are unisexual apart from during the spring when males and females are regularly caught in the same areas (Llewellyn, L, pers. comm.).

The majority of specimens sampled for this study were adult sharks (≥ 525mm, males and ≥ 550mm females) which could suggest that *S. canicula* in the Solent segregate by size and possibly depth. Despite previous findings by Compagno (1984) and Compagno *et al*. (2005) research carried out Rodriguez-Cabello *et al.* (2004) found that populations from

the Cantabrian Sea segregated by size, with juveniles occurring at similar depths and with a much narrower depth distribution than adults. However, size selectivity could have also occurred, leading to larger numbers of adult specimens. Research by Ellis *et al*. (2005) noted that in the Celtic Sea, despite juvenile specimens of *S. canicula* being caught often, they did not usually appear in beam trawls, although maturing and mature individuals did. It is possible that juveniles occur on substrates that are too rocky to fish using this method, making it size selective. The same selectivity could be true of the specimens caught for this study as they were captured using longlining or gillnets. These methods are size selective and could potentially target larger individuals. This would possibly eliminate catches of smaller individuals and explain the lack of smaller juvenile and hatchling catsharks.

In male catsharks the mean clasper length was greatest in fish sampled during the spring months, with the mean length greatest in May (36.8mm). December shows the greatest clasper length overall, however no conclusions can be drawn from this as the n value for December is only one. Catsharks sampled in February possessed the smallest clasper length, with the shortest recorded length being 30.57mm. Research by Garnier *et al.* (1999) suggested that there are annual variations in clasper size of *S. canicula*. Their study, however, showed that the catsharks sampled during March had the greatest clasper length. The study by Garnier *et al.* (1999) also found that greater clasper length coincided with peak sperm reserves, which were found to be greatest between the spring months of March to May, with the highest reserves being found in March. This information led to the assumption that sexual activity for *S. canicula* was at its peak during this time. It was also noted by Garnier *et al.* (1999) that the increase in sperm reserves correlated closely with an increase in the weight of the testis. The present research was not designed to be a detailed study into reproductive organs of *S. canicula* and neither sperm reserves nor the morphometrics of the testis were measured.

Although the present study uses total length and gonad morphometrics to determine sexual maturity in catsharks, it is possible that age may be used as a way of measuring sexual maturity in certain elasmobranch species. Several authors have employed a variety of techniques in an attempt to determine the age of elasmobranch fish. Ivory *et al.* (2004) describe a number of these techniques, which include tooth replacement rates, eye lens weight, and the enumeration of growth increments on dorsal spines and caudal thorns. However, the vertebral centra have proven the most useful and accurate structures in elasmobranchs for age determination. In certain species, such as the gray smoothound, *Mustelus californicus* and the Brown Smoothound, *Mustelus henlei* (Yudin and Cailliet, 1990) the centra have been found to contain concentric rings similar to those found on teleost scales and otoliths (Cailliet *et al*., 1986). Additional work would focus on a combination of length-frequency and age determination. In summary based on the data presented in the current and previous studies, the full data set was broken down into 2 size class sizes for further analysis:

Males - Size class 1 < 525mm total body length (immature/Juvenile)

Males - Size class 2 ≥ 525mm total body length (mature)

Females - Size class 1 < 550mm total body length (immature/Juvenile)

Females - Size class 2 ≥ 550mm total body length (mature)

Chapter 3 – Head, Mouth and Jaws

3.1 Introduction

3.1.1 Elasmobranch Head

Many studies have focused on the head, mouth and jaws of elasmobranch species. The fact that a sexual dimorphism exists in these anatomical structures in some species is well documented (Brough, 1937; Arthur, 1950; Gosztonyi, 1973; Ellis and Shackley, 1995; Filiz and Taskavak, 2006). Much of the work that has focused on the head of elasmobranch species has been concerned with the differences in shape and is restricted to a few species. Miller (1995) examined rostral development in the sawfish, *Pristis perotteti*, focusing on the embryonic development of the rostrum. The various species of hammerhead shark have also been widely studied with a view to understanding the development and function of the cephalofoil (Nakaya, 1995; Kajiura, 2001; Kajiura *et al*., 2005). A great deal of research carried out on the heads of sharks has focused on the musculature, especially in respect to jaw control (Moss, 1977; Frazzetta, 1994, Motta and Wilga, 1995; Wilga, 2002; Motta, 2004).

However, some observations of sharks, including those on *S. canicula*, showed that some secondary sexual dimorphisms existed in the heads of elasmobranch species. Brough (1937) noted that the heads of mature male catsharks were narrower than those of mature female catsharks, whilst Jardas (1979) discovered that in a population of *S. canicula* from the Adriatic Sea males possessed longer heads than females. Filiz and Taskavak (2006) found this to also be true of a population off the coast of Turkey. They measured a variety of head morphometrics and found measurements such as snout-spiracle distances to be significantly dimorphic. Bas (1964) carried out morphometric studies on *S. canicula* from the Mediterranean and found that they exhibited negative allometric growth of the head.

Ellis and Shackley (1995) also recorded significant sexual differences in the girth of the head and pre-oral, pre-branchial and head lengths were also recorded. Frazzetta (1994) examined the structure of the head skeleton from elasmobranch species, and stated that the head consists of three entities; the chondrocranuim, hyoid arch and jaws (Figure 3.1). The chondocranium, or cartilaginous braincase, is mounted on a slightly flexible vertebral column. The chondocranium is made up of several key components; the rostrum, nasal capsules, orbits and otic capsules. The chondrocranium not only protects the brain, but also supports the hyoid arch and jaws. According to Motta and Wilga (2001) the hyoid arch in elasmobranchs is composed of only three elements: a hyomandibula, ceratohyal and basihyal (Figure 3.1).

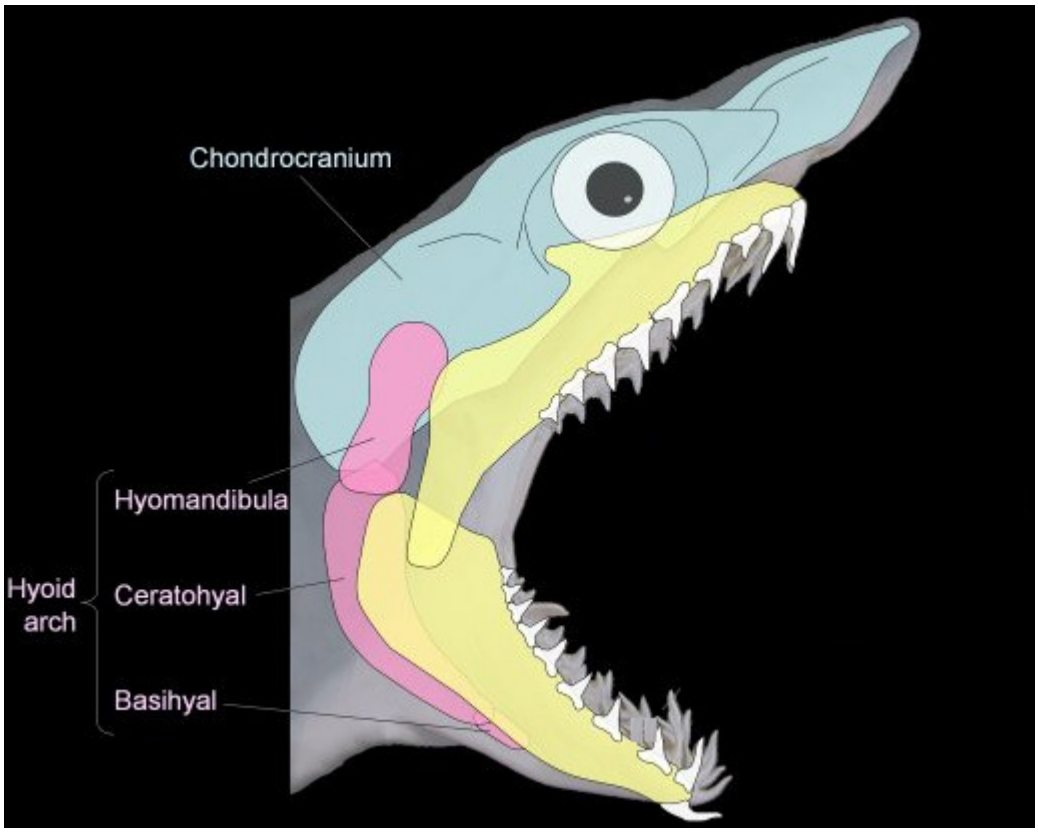

Figure 3.1. Diagram of the head of a shark showing the component parts. (Adapted from http://www.chalk.discoveringfossils.co.uk).

3.1.2 Elasmobranch Jaws

The role of the hyoid arch in many gnathostomes is to support the jaws. Part of the hyoid arch extends from the corner of the jaw to the otic region of the skull. This upper part of the hyoid arch forms the hyomandibula. The purpose of the hyomandibula is to secure the rear of the jaws against the skull, permitting a strong bite and at the same time allowing lateral flexibility.

Motta and Wilga (2001) suggested that from an evolutionary and functional standpoint chondrichthyan fishes represent a basal group of jawed fishes that share a common ancestor with bony fishes. Motta *et al.* (1997) note that the Chondrichthyes diverged from a common ancestor with the Teleostomi prior to the Devonian period and have retained the same major skeletal features for over 400 million years. However, jaw development in elasmobranch species has encountered a transition from an amphistylic jaw suspension, as seen in early elasmobranchs, to a hyostylic jaw suspension encountered in modern elasmobranchs (Motta, 2004). In amphistyly the upper jaw is braced against the cranium and also supported by the hyomandibula. Hyostyly represents a type of jaw suspension in which the upper jaw is attached to the cranium anteriorly only by means of ligaments and posteriorly by the hyomandibula. It is this ligament attachment that creates the flexibility of the feeding apparatus witnessed in modern day sharks. According to Motta and Wilga (2001) modern day sharks showing hyostyly can be characterised by having a sub-terminal mouth that opens ventrally, shorter jaws, more protrusible palatoquadrate cartilage with a smaller otic process and a dentition better suited for sawing and shearing compared to ancestral sharks.

Many authors have compared the feeding structures of modern day elasmobranchs to bony fishes. According to Motta *et al.* (1997) there have been fewer anatomical studies on

elasmobranch feeding structures when compared to studies on teleost fishes and even fewer data on natural feeding behaviour of sharks. Motta and Wilga (2001) stated that sharks retain a relatively simple feeding apparatus composed of a fused chondrocranium, jaws consisting of a palatoquadrate (upper jaw) and Meckels (lower jaw) cartilage and a hyoid arch. Motta (2004) noted that compared to the teleost skull, which has approximately 63 bones, the feeding apparatus of a shark is composed of just 10 cartilaginous elements. Further to this, elasmobranchs lack pharyngeal jaws and the ability to further process food by this secondary set of decoupled jaws, unlike bony fish (Motta, 2004). Frazzetta (1994) noted that despite this simple structuring of elasmobranch feeding apparatus, sharks utilise a wide variety of prey capture modes. These include suction, ram, bite, bite and gouge and filter feeding. Motta (2004) agreed with this, stating that the most remarkable thing about the elasmobranch feeding mechanism is its functional diversity despite its morphological simplicity.

Motta *et al.* (1997) stated that the elasmobranch mechanism of feeding, in the form of jaw protrusion, is very different from that of teleosts owing to a different anatomy. Motta and Wilga (1995) noted that during biting many sharks grasp the prey in the jaws and vigorously shake the body or head from side to side to cut through the prey with the saw like action of the teeth, which is characteristic of many shark species and is unlike feeding in most teleosts.

Another feature of many elasmobranch jaws is the presence of a flexible symphysis (Figure 3.2). Dean *et al.* (2005) stated that the majority of elasmobranch species possess flexible symphyses. According to Gerry *et al.* (2008) a highly mobile jaw symphysis is characteristic of many other vertebrates that process prey unilaterally. Scapino (1981) stated that the symphysis may contain a readily flexible joint that permits a moderate amount of independent movement of the two halves of the jaw, or hemimandibles, in the

upper or lower jaws of elasmobranchs. It appears that this provides greater efficiency for prey processing, allowing one side of the jaw to bite whilst the other can process the prey item (Gerry *et al*., 2008).

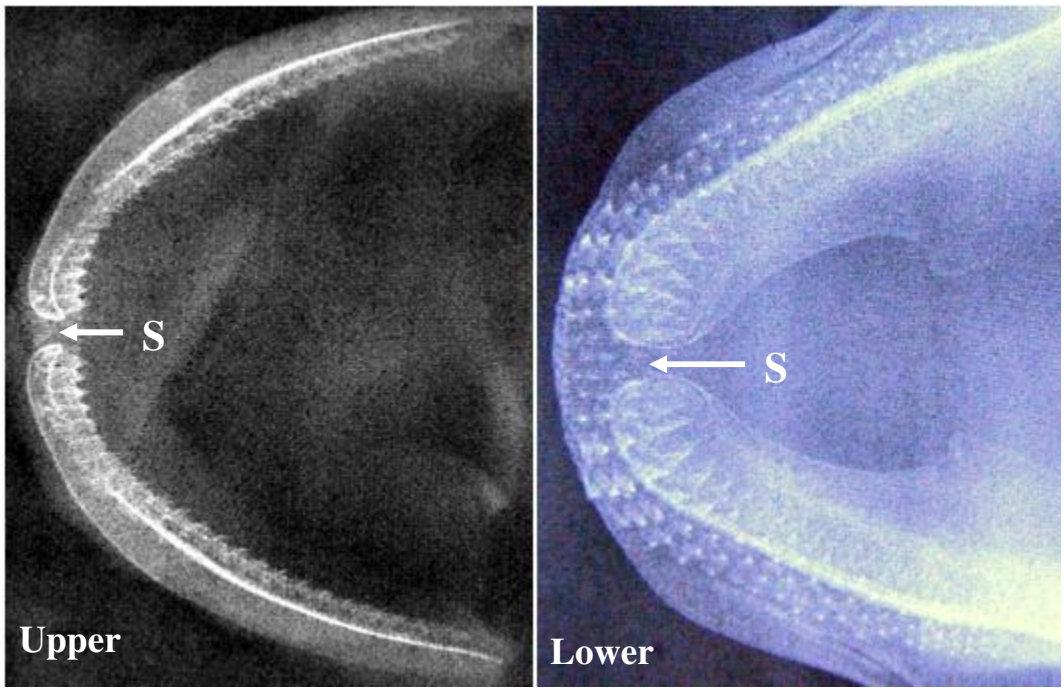

Figure 3.2. Radiographic image of the upper and lower jaws of an adult male catshark showing the symphysis (S) (Photographed by the author).

Wilga (2002) observed that due to their dorsoventrally depressed morphology, little skates, *Raja erinacea,* have a euhyostylic jaw suspension whereby the mandibular arch is suspended only by the hyomandibula and lacks anterior ligaments or articulations with the cranium. As with most elasmobranch species, in *R. erinacea* the two sides of the jaws are effectively separated into two functional halves and can work almost independently of each other. This combination of a flexible symphysis and jaw separation into two halves allows the skate to grasp the prey item in the corner of the jaws whilst biting repeatedly using only the adductors on that half of the jaw (Wilga, 2002). This appears to have the same effect as chewing in mammals, and which has been shown to be energetically efficient because muscles only fatigue on one side of the head (Ross *et al*., 2007).

Gerry *et al.* (2008) suggested that despite the presence of a flexible symphysis in many elasmobranchs they do not all function equally. They noted that although catsharks are able to use a high degree of asynchrony when feeding, this feature is unlikely to be due to an elevated level of symphyseal flexibility in the jaws, as has been suggested for skates. It appears that catsharks have a triangular symphysis that is wider posteriorly than anteriorly. It seems that this joint forms a tight connection between the two halves of the jaw anteriorly. However, the looser connection at the posterior end provides some flexibility, although the joint does not have the same degree of movement as in little skates (Gerry *et al*., 2008).

Due to the degree of flexibility found at the symphysis, Gerry *et al.* (2008) suggested that this joint may require some stability because prey is often positioned at the symphysis prior to head-shaking and held at the centre of the jaws while pieces are torn from it. They go on to add that in smoothhounds, *Mustelus* spp., the symphysis is rectangular and expands laterally to widen the distance between the tips of the Meckel's cartilage as the jaw opens. Although this type of symphysis is flexible, Gerry *et al.* (2008) suggested that smaller, symphyseal teeth overlay the symphysis and outer margins of the jaw and provide structural support, stiffening the jaws in response to applied force. Ellis and Shackley (1995) noted that in the lower jaw of *S. canicula* there were small median teeth in the symphyseal area separating the large anterior teeth at either side (Figure 3.3).

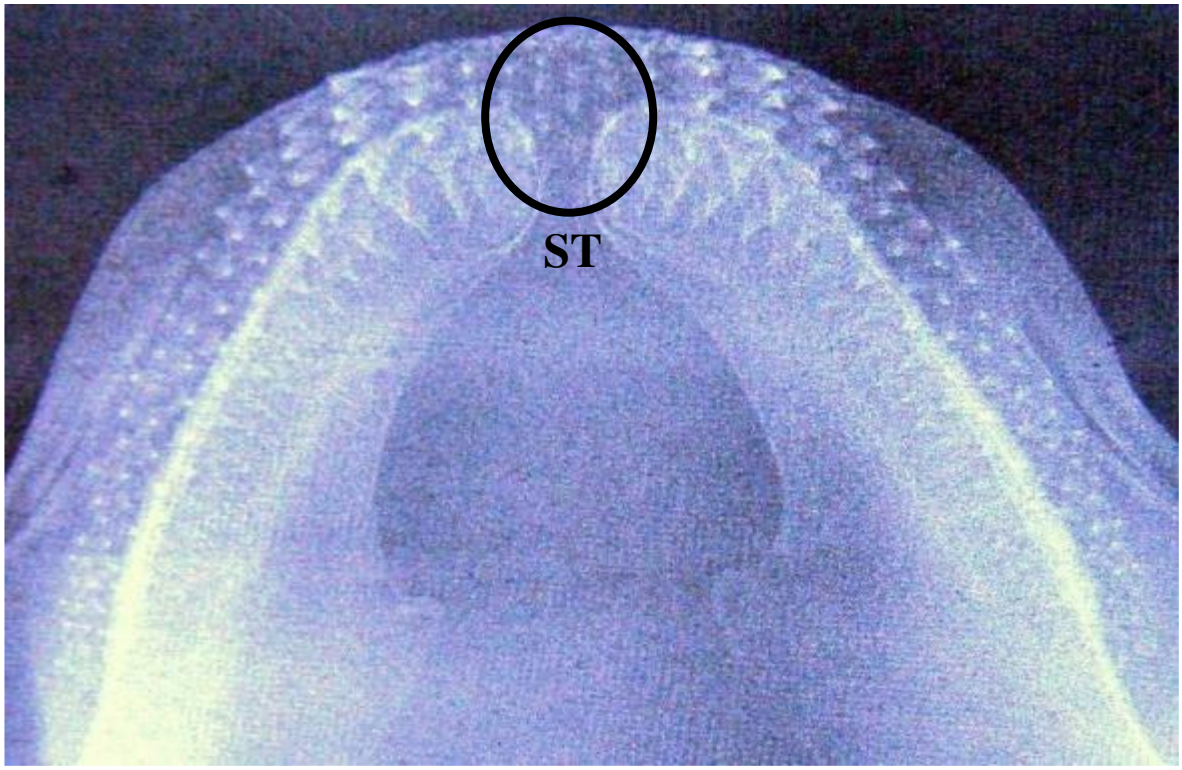

Figure 3.3. Radiographic image of the lower jaw of an adult male catshark showing the smaller symphyseal teeth (ST) (Photographed by the Author).

Wu (1994) examined the jaws of bamboo sharks. It was noted that the caudal-most ends of the Meckel's cartilage that compose the symphysis compress medially during feeding. This caused the medial facing surfaces of the symphyseal portions of the jaw halves to contact, possibly reinforcing the articulation. Gerry *et al.* (2008) noted that the symphysis in the bamboo shark is similar in shape to that of catsharks in that it was flexible enough to allow movement during feeding, but was not as flexible as the symphysis in either smooth-hounds or *R. erinacea*.

Fahle and Thomason (2008) investigated the flexibility of the jaws of newborn and adult *S. canicula.* They found that the jaws of newborn animals were significantly more viscoelastic than those of adults. They concluded that as a result, newborn lesser-spotted catshark might be unable to consume hard prey items, unlike adults. It appeared that there may be some possible advantages associated with the greater viscoelasticity of the jaws of newborn catsharks. Lesser-spotted catshark egg cases are c. 21–29 mm in width and

newborns eclode with a head girth of 22–28 mm. It is therefore possible that flexible jaws may allow greater cranial flexibility and aid eclosion from the egg case (Fahle and Thomason, 2008). They go on to add that numerous ontogenetic changes occur in the cranium of newborn *S. canicula*, with jaw muscles exhibiting positive, negative and isometric growth during ontogeny. They suggested that more malleable jaws may allow these structural changes to occur whilst minimizing functional consequences.

3.1.3 Mouth Morphometrics

Mouth morphometrics have also been well researched and are found to show a high degree of sexual dimorphism amongst many species of elasmobranch. The reasons for these sexual dimorphisms have been widely discussed and disputed. Fedducia and Slaughter (1974) suggested that sexual dimorphism in the feeding apparatus of skates is an adaptation to niche utilisation, whereby males and females have differing habitats and feeding habits. McEachran (1975) disputed this and noted that in the four species of skate studied; *R. erinacea, R. ocellata, R, senta* and *R. radiate*; not only was the tooth shape sexually dimorphic, but the shape of the jaw of males became more sinuous in mature individuals. Kajiura *et al.* (2005) studied bonnethead sharks and stated that although the shape of the mouth was not photographed or quantified, the cartilaginous jaw elements could also change concomitantly with the onset of sexual maturity in males. Soto (2001) examined specimens of mature *Schroederichthys* spp., including the lizard catshark, *Schroederichthys saurisqualus,* the slender catshark, *Schroederichthys tenius,* the narrowtail catshark, *Schroederichthys maculates,* the narrowmouth catshark, *Schroederichthys bivius,* and the redspotted catshark, *Schroederichthys chilensis.* Soto (2001) found that to varying degrees the mouths of the *Schroederichthys* spp. studied showed a secondary sexual dimorphism, whereby males possessed a longer mouth than females (Figure 3.4).

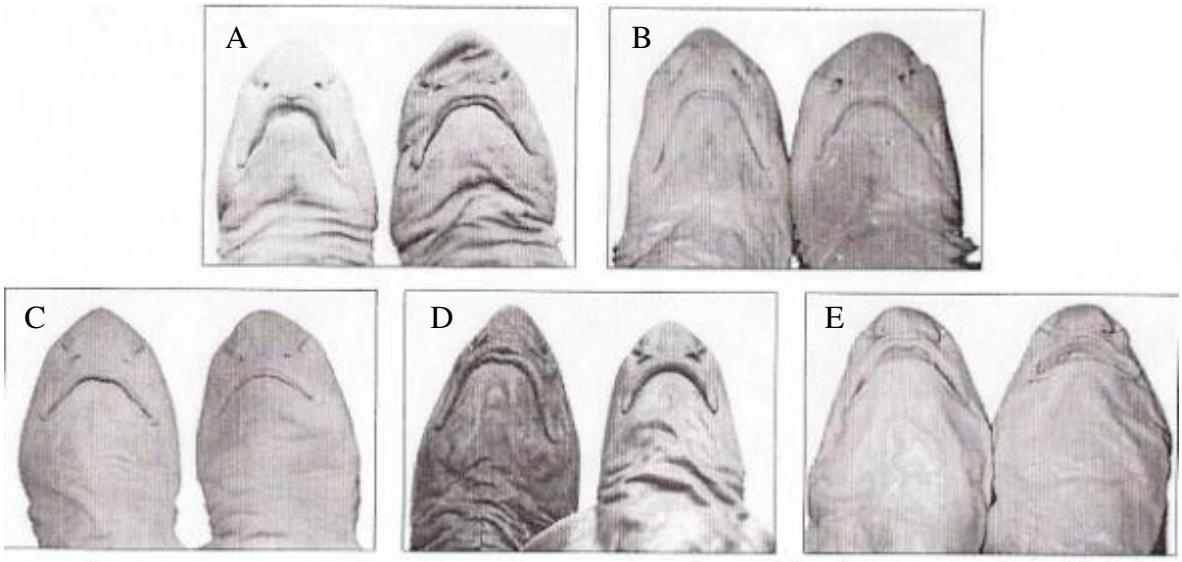

Figure 3.4. Secondary sexual dimorphism in the mouth of mature *Schroederichthys* spp. A) *S. saurisqualus* (male and female) B) *S. tenius* (male and female) C) *S. maculates* (male and female) D) *S. bivius* (male and female) E) *S. chilensis* (male and female). Adapted from Soto (2001).

Some species of Scyliorhinid catsharks are noted for their secondary sexual dimorphisms of the mouth. Mouth morphology, is characterised by a U-shaped and much longer mouth in males, but is V-shaped in females (Gosztonyi, 1973). Evidence of a sexual dimorphism in mouth morphology of *S. canicula* is well documented. In adult *S. canicula* Brough (1937) classified the mouth and jaws together and noted that the mouth was narrower and that the intermandibular separation of the jaw was less in male *S. canicula*. Brough (1937) stated that the changes in the lower jaw structure correlated to sexual maturity and the sexual dimorphic characters were more pronounced in the mating season. The presence of a sexual dimorphism in the mouth was not noted in sexually immature specimens (Brough, 1937). Arthur (1950) also noted sexual dimorphism in the mouth of *S. canicula*. The research found that the mouth length/width ratio of *S. canicula* was strongly sexually dimorphic. It was reported by Brough (1937) that this sexual dimorphism occurs relatively suddenly at the onset of maturity.

In keeping with the findings of Brough (1937) and Arthur (1950) Ellis and Shackley (1995) found that male *S. canicula* possess a longer and narrower mouth than females

resulting in a pronounced sexual dimorphism with respect to the mouth length/width ratio. Erdogan *et al.* (2004) studied a population of *S. canicula* and also note that males possessed a longer narrower mouth and that there was a clear sexual dimorphism in the length/width ratio of the mouths of catsharks. Filiz and Taskavak (2006) also studied the mouth dimensions in a Turkish population of *S. canicula*. They found that the length/width ratio of the mouth was sexually dimorphic, with males having a narrower, longer mouth than females. Possible explanations as to why mouth dimensions change in male *S. canicula* during maturation and the fact that males have bigger teeth include differential feeding habits and adaptations for reproductive behaviour (Ellis and Shackley, 1995).

It is possible that head, mouth and jaw morphometrics may change due to reproductive adaptations. If the population of *S. canicula* in the Solent have a distinct mating season the shape and size of the head, mouth and jaws may change to coincide with reproduction. This may be especially true if there is a seasonal shift in tooth morphology in males. As far as the author is aware the effects of seasonality on head, mouth and jaw morphometrics has not been studied before in *S. canicula*.

Therefore, the aims of this study are:

1. To determine if there is any sexual or seasonal dimorphism in the head of hatchling, juvenile and adult *S. canicula*.

2. To determine if there is any sexual or seasonal dimorphism in the mouth of hatchling, juvenile and adult *S. canicula*.

3. To determine if there is any sexual or seasonal dimorphism in the jaw structure of hatchling, juvenile and adult *S. canicula*.

3.2 Materials and Methods

3.2.1 Head and Jaws

For the head and jaw measurements the adult catsharks were categorised into size classes based on sexual maturity. The size classes used are:

Males - Size class 1 < 525mm total body length (immature)

Males - Size class 2 ≥ 525mm total body length (mature)

Females - Size class 1 < 550mm total body length (immature)

Females - Size class 2 ≥ 550mm total body length (mature)

The numbers of individuals sampled for the adult head, mouth and jaw parameters differed due to some early samples having a limited number of head and mouth morphometrics measured. In earlier samples only the lower jaws were extracted. In some instances the upper or lower jaws were damaged during removal and were deemed unusable for jaw morphometrics measurements. Some parameters, such as jaw depth, were later additions and were taken as the study evolved. The n-values for all analyses are noted.

3.2.2 Head and Mouth Measurements

Once the specimens had been sacrificed, head and mouth morphometrics measurements were taken. These included pre-branchial length, measured from the tip of the snout to the first gill, head width, pre-oral length, mouth width and mouth length (Figures 3.5 and 3.6). After the data were collected the head was removed and placed in a solution of unbuffered 10% formalin in seawater for later removal of the jaws and Ampullae of Lorenzini.

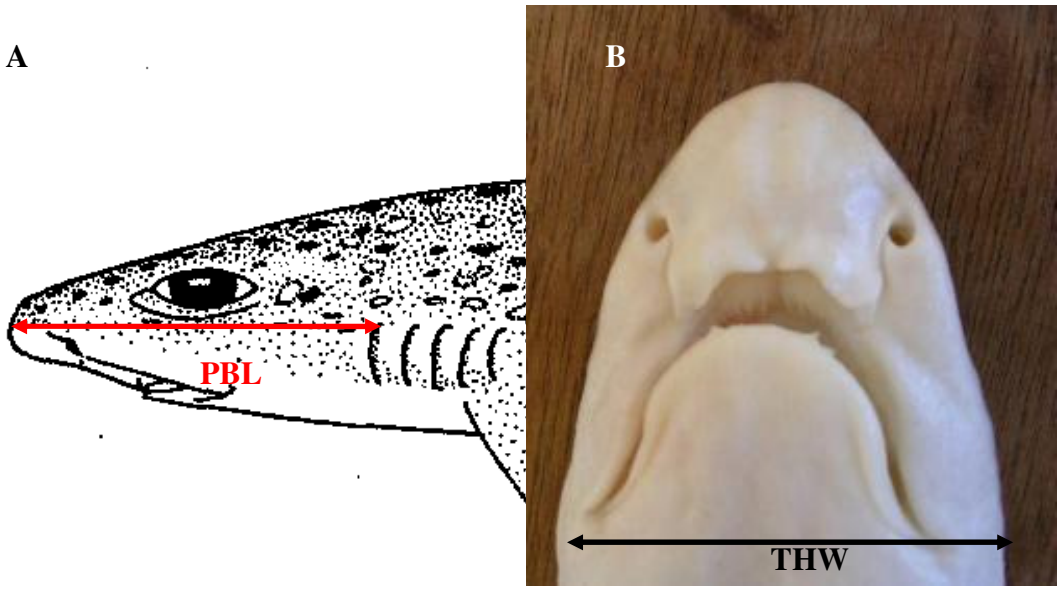

Figure 3.5. Measurements for pre-branchial length (PBL) (A) and total head width (THW) (B). Image A Adapted from Compagno (1984). Image B courtesy of P. Whiting (2002).

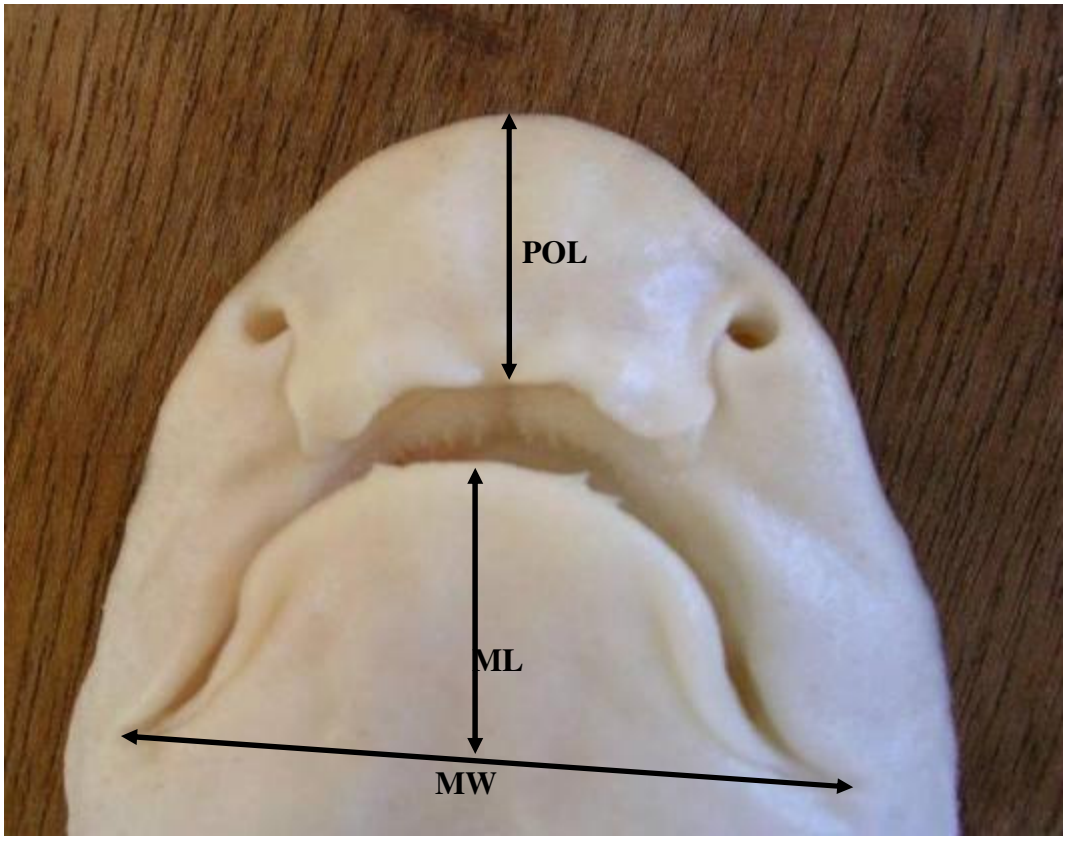

Figure 3.6. Measurements for pre-oral length (POL) mouth length (ML) and mouth width (MW). Photograph courtesy of P. Whiting (2002).

3.2.3 Jaw Preparation

The heads of the sharks were removed from the formalin and washed in running tap water for 45 minutes. The jaws were removed from the heads of the sharks by cutting between the skin and the Meckel's cartilage and palatoquadrate with a fine scalpel blade. Any remaining skin was cut away and the connective tissue was scraped away using the fine scalpel blade. The jaws were placed in four 45-minute washes of distilled water to remove any trace of formalin from the tissue. They were then submerged in a solution of 6% hydrogen peroxide solution for 24 hours in order to soften the connective tissue that was not removed initially with the use of the scalpel. After the 24 hour period in the H_2O_2 the jaws were washed in distilled water for 45 minutes and the softened connective tissue surrounding the cartilaginous jaw was removed with the use of a fine scalpel. The jaws were then left to dry for 24 hours. The jaws were air-dried in a fume hood. During the

drying process they maintained their shape and dried uniformly with no distortion or flexion (Figure 3.7).

3.2.4 Statistical Analyses

When the jaws had dried they were measured and the dimensions for jaw length (JL) jaw width (JW) and Jaw depth (JD) were recorded (Figure 3.7) and the numbers of tooth rows were counted.

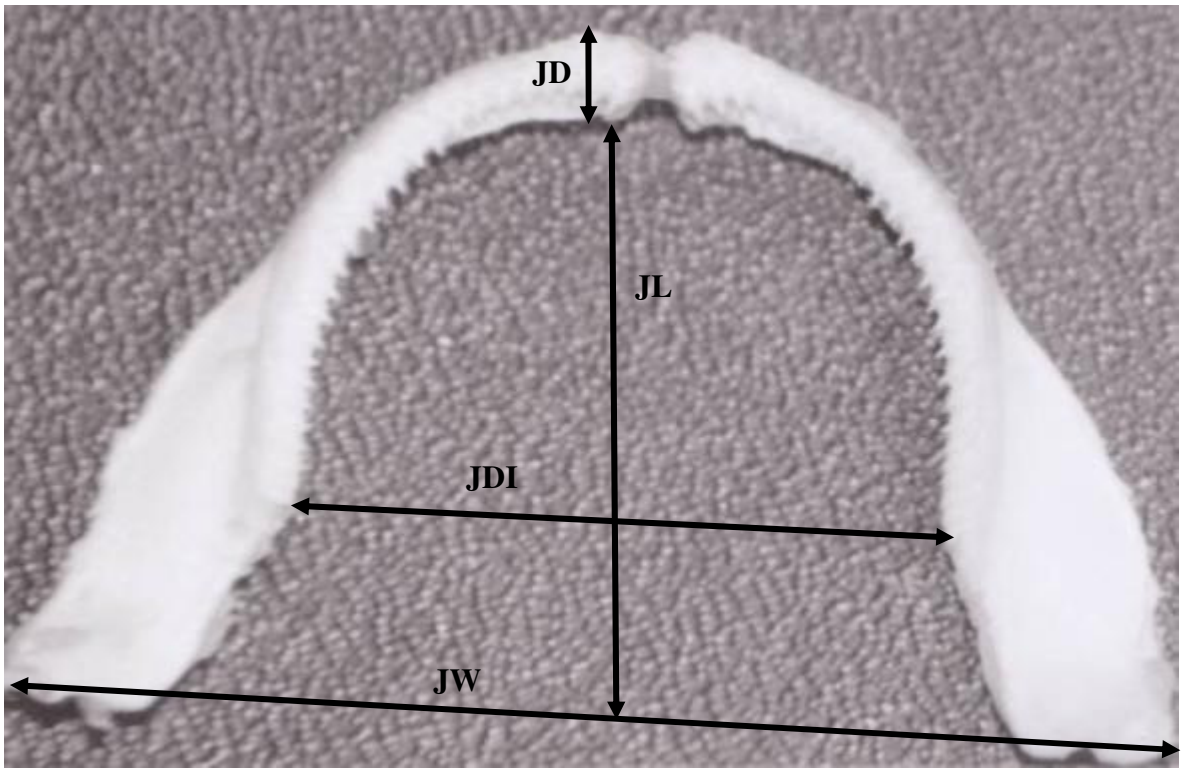

Figure 3.7. Excised upper jaw of an adult female catshark showing jaw depth (JD) jaw length (JL) Jaw Diameter (JDI) and jaw width (JW) (Photographed by the author).

Prior to employing parametric statistical tests, Kolmogorov-Smirnov Normality Tests were carried out to determine whether the data were normally distributed (Dytham, 2003). If necessary log 10 transformations were conducted. Significance was accepted when $p<0.05$. A range of analyses were performed on the head, mouth and jaws of hatchling, juvenile

and adult *S. canicula* in order to determine whether any seasonal or sexual dimorphism exists in the head morphometrics of the lesser-spotted catshark. An ANCOVA, with body length as a covariate, was performed on the head, mouth and jaw data of adult lesser-spotted catsharks in order to ascertain whether there were any sexually dimorphic differences in head and jaw structure. For hatchling catsharks only jaw length and width were recorded due to difficulties in excising and processing the jaws from these specimens.

Due to low numbers of both hatchling (n = M (23) F (14)) and juvenile (head, n = M (7) F (9) jaw, n= M (6) F (17)) catsharks sampled it was not possible to carry out any seasonal comparisons. A GLM was performed to determine whether there were any seasonal dimorphisms in the morphometrics of the head, mouth and jaws of the mature specimens. A Grubbs test for outliers was performed on the data (Grubbs, 1969) as per Attrill *et al*. (2007) in order to ascertain the presence of any outliers. The test revealed that no outliers were present in any of the data sets. Where body length was found to be significant a scatterplot was produced and an ANOVA was performed to ascertain whether there was a significant correlation. If body length and gender were found to be significant a scatter plot was created showing the male and female data. The regression lines were analysed to see if there was a significant difference between the male and female slopes.

Canonical discriminant analysis was carried on the adult and juvenile jaws to determine any correlation between the upper and lower jaw dimensions of adult and juvenile male and female catsharks. The morphological parameters (Jaw Depth, Jaw Length, Jaw Diameter and Jaw Width) were combined and two sets of analyses were performed, one for juvenile males and females and one for adult males and females. The two factors that contributed most strongly to any dimorphisms are displayed as functions in the axes.

3.3 Results

3.3.1 Head and Mouth Data

3.3.1.1 Hatchling Head Morphometrics

The results for the ANCOVA for hatchling head morphometrics can be seen in Table 3.1. There was no significant difference in the pre-branchial length between male and female hatchling *S. canicula* (ANCOVA, F=1.19; d.f.=1; *P*=0.293). Body length had no effect on the pre-branchial length of hatchling *S. canicula* (ANCOVA, F=3.06; d.f.=1; *P*=0.101). There were similar findings for the head width, with no significant differences found between male and female hatchling catsharks (ANCOVA, F=0.62; d.f.=1; *P*=0.436). However, body length had an effect on the head width of hatchling catsharks (ANCOVA, F=31.18; d.f.=1; *P*<0.001). It can be seen from Figure 3.8 that the head width of hatchling *S. canicula* increased as body length increased. The statistical analysis revealed no significant differences between the pre-oral length of hatchling catsharks (ANCOVA, F=1.39; d.f.=1; *P*=0.247). Body length was found to have an effect on the pre-oral length in hatchling catsharks, with larger individuals possessing a greater distance between the mouth and the tip of the snout (ANCOVA, F=19.85; d.f.=1; *P*<0.001) (Figure 3.9). There were no significant differences found between the mouth width or mouth length of male and female hatchling *S. canicula* (ANCOVA, F=0.01; d.f.=1; *P*= 0.928; ANCOVA, F=1.16; d.f.=1; *P*=0.286). Body length had no effect on the mouth length of hatchling catsharks (ANCOVA, F=2.70; d.f.=1; *P*=0.653). Body length did, however, have an effect on the mouth width of hatchling *S. canicula*, with larger individuals possessing a wider mouth (ANCOVA, F=11.37; d.f.=1; *P*=0.001) (Figure 3.10).

Table 3.1. Head and mouth data for male and female hatchling *S. canicula* showing means ± standard errors, range and *P*-values (n= M (23) F (14)).

Feature (mm)	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Pre-branchial length	13.89 ± 0.62 (11.34 – 16.46)	13.26 ± 0.36 (11.70 - 14.99)	**0.293**	**0.101**
Head Width	11.45 ± 0.14 (10.59 - 12.37)	11.43 ± 0.15 (9.95 - 12.84)	**< 0.001**	**0.436**
Pre-oral length	5.89 ± 0.14 (4.47 – 6.39)	6.14 ± 0.12 (5.28 - 7.28)	**< 0.001**	**0.247**
Mouth Length	3.50 ± 0.10 (2.78 - 3.60)	3.52 ± 0.40 (2.51 - 3.99)	**0.653**	**0.928**
Mouth Width	7.10 ± 0.14 (6.68 – 7.81)	6.97 ± 0.14 (5.39 - 8.56)	**0.001**	**0.286**

Figures 3.8 - 3.10 show a graphical representation of the head width, pre-oral length and mouth width against body length for hatchling catsharks. There was a significant correlation between body length and head width (ANOVA, F= 26.6; d.f = 1; $P<0.001$) pre-oral length (ANOVA, F= 14.93; d.f = 1; $P= 0.002$) and mouth width (ANOVA, F= 10.90; d.f = 1; $P<0.001$) for hatchling catsharks.

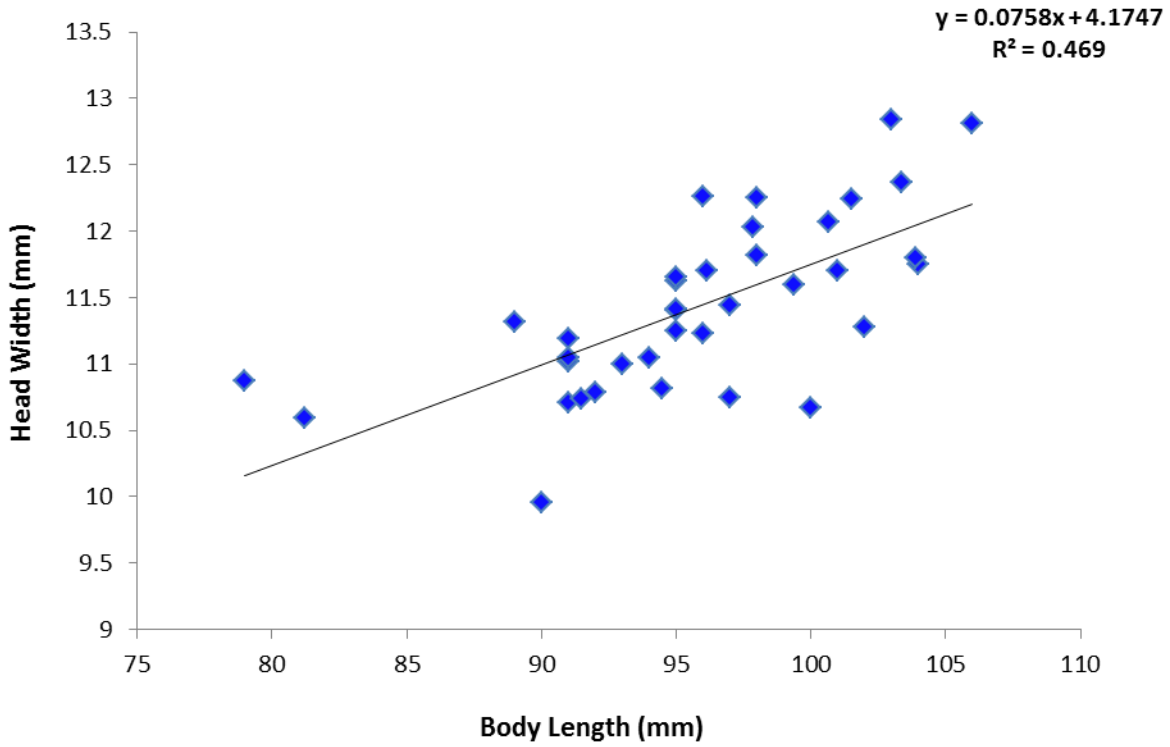

Figure 3.8. Scatterplot with regression for head width against body length for hatchling male and female *S. canicula* (n= 37) (*P*<0.001).

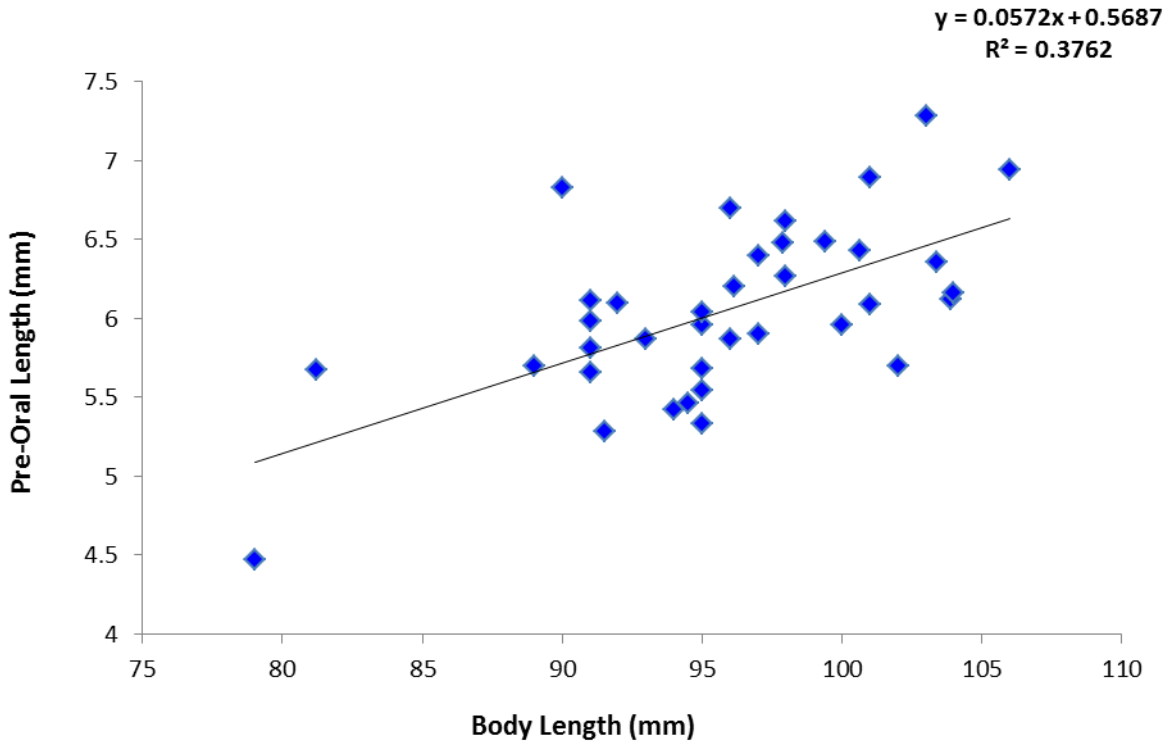

Figure 3.9. Scatterplot with regression for pre-oral length against body length for hatchling male and female *S. canicula* (n= 37) (*P*=0.002).

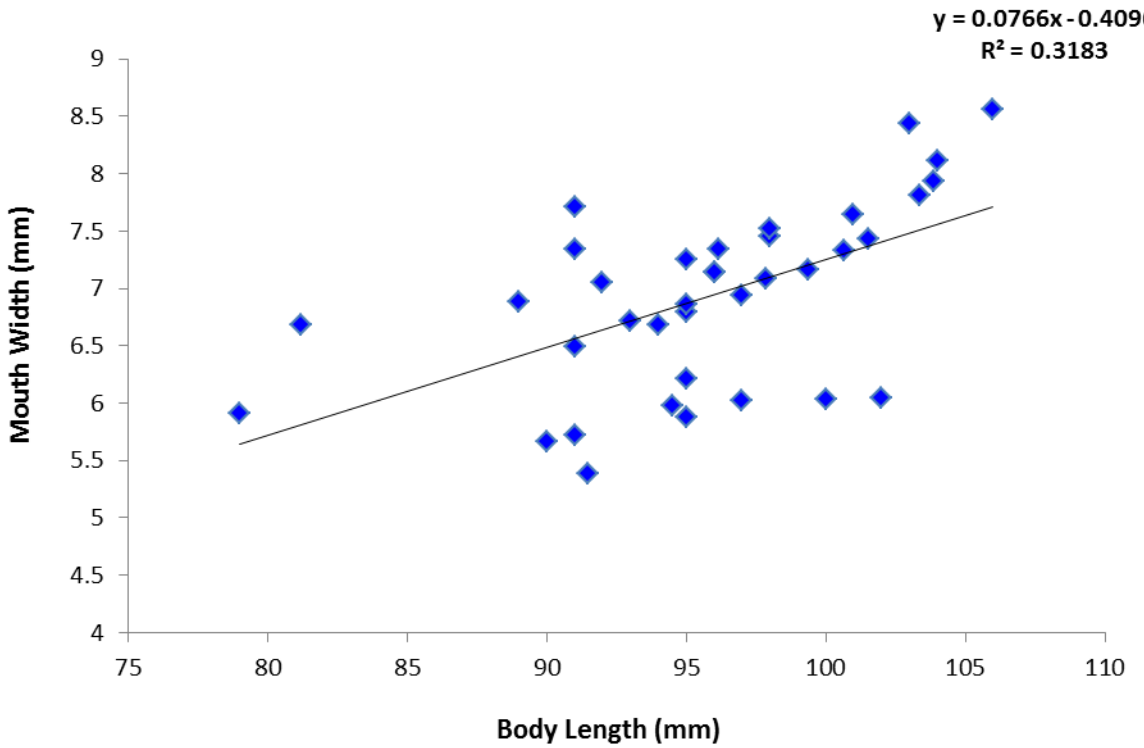

Figure 3.10. Scatterplot with regression for mouth width against body length for hatchling male and female *S. canicula* (n= 37) (*P*< 0.001).

3.3.1.2 Juvenile Head and Mouth Morphometrics

Table 3.2 shows the results from the ANCOVA for the head and mouth morphometrics for juvenile male and female catsharks The results of the ANCOVA show that neither body length, nor gender had a significant effect on the pre-branchial length of male and female catsharks (ANCOVA, F=0.69; d.f.=1; *P*=0.422; ANCOVA, F=0.55; d.f.=1; *P*=0.473). There was no significant difference in the head width of male and female juvenile catsharks (ANCOVA, F=0.69; d.f.=1; *P*=0.422). Body length was found to have an effect on the head width of juvenile catsharks, with larger individuals possessing a wider head than smaller individuals (ANCOVA, F=11.19; d.f.=1; *P*=0.005) (Figure 3.11). There were no significant differences found with respect to the pre-oral length for either gender or body length (ANCOVA, F=0.40; d.f.=1; *P*=0.539; ANCOVA, F=1.71; d.f.=1; *P*=0.214). There was a significant difference in the mouth length of juvenile catsharks. Males were found to possess significantly longer mouths than females (ANCOVA, F=6.03; d.f.=1;

P=0.029). Body length was also found to have a significant effect on the mouth length of juvenile *S. canicula*. It can be seen from Figure 3.12 that as the length of the catshark increases so does the mouth width (ANCOVA, F=6.26; d.f.=1; *P*=0.027). There was no significant difference found in the mouth width of juvenile *S. canicula*, with both males and females possessing an almost identical mouth width (ANCOVA, F=0.52; d.f.=1; *P*=0.484). Body length was found to have a significant effect on the mouth width of juvenile catsharks, with larger specimens having wider mouths than smaller ones (ANCOVA, F=13.31; d.f.=1; *P*=0.003) (Figure 3.13).

Table 3.2. Head and mouth data for male and female juvenile *S. canicula* showing means ± standard errors and *P*-Values (n= M (7) F (9)).

Feature (mm)	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length (*P*-Value)	Gender (*P*-Value)
Pre-branchial length	77.84 ± 3.65 (61.95 – 90.90)	71.64 ± 4.88 (53.65 – 88.55)	**0.422**	**0.473**
Head Width	49.00 ± 1.42 (44.45 – 56.03)	48.93 ± 1.69 (43.28 – 54.24)	**0.005**	**0.422**
Pre-oral length	20.83 ± 2.21 (16.95 – 38.22)	21.52 ± 1.36 (18.47 – 29.42)	**0.214**	**0.539**
Mouth Length	16.88 ± 0.99 (13.54 – 21.99)	17.92 ± 1.10 (13.83 – 22.10)	**< 0.001**	**0.015**
Mouth Width	35.58 ± 0.97 (30.86 – 39.83)	35.16 ± 1.25 (30.84 – 39.27)	**0.003**	**0.484**

Graphical representations of the head width, mouth length and mouth width against body length for juvenile catsharks can be seen in Figures 3.11 - 3.13. The correlation between body length and head width (ANOVA, F= 13.65; d.f = 1; *P*= 0.005) mouth length (Male = ANOVA, F= 8.41; d.f = 1; *P*= 0.003; Female = ANOVA, F= 23.48; d.f = 1; *P*= 0.002) and mouth width (ANOVA, F= 13.39; d.f = 1; *P*= 0.003) were found to be significant (Figures 3.11 – 3.13). Mouth length for male and female juvenile catsharks was found to increase with body length (Figure 3.12). However, there was no significant differences between the

regression lines of male and female juvenile catsharks (P= 0.666). Figure 3.13 shows that as body length increased in juvenile catsharks so did mouth width.

Further plots were made for head width, mouth length and mouth width with the data for the juvenile and adult catsharks combined (Appendix 1). The combined data showed a similar pattern with mouth length and mouth width, in general, increasing with body length.

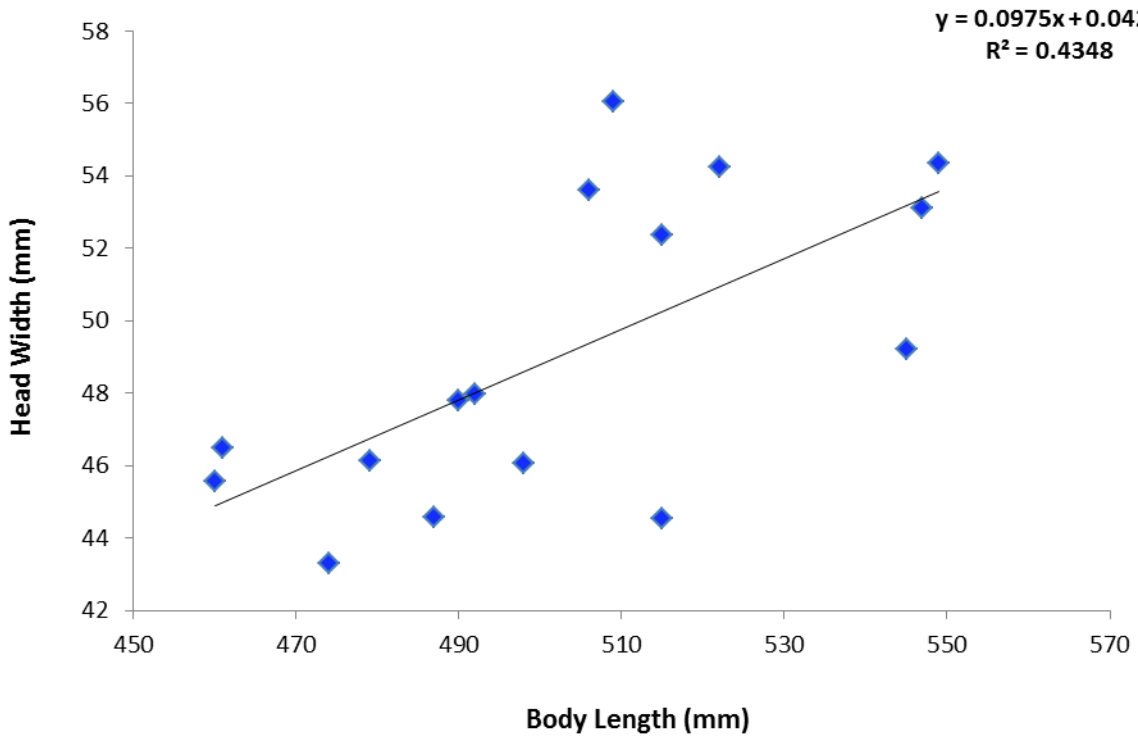

Figure 3.11. Scatterplot with regression for head width against body length for juvenile male and female *S. canicula* (n= 16) (*P*= 0.005).

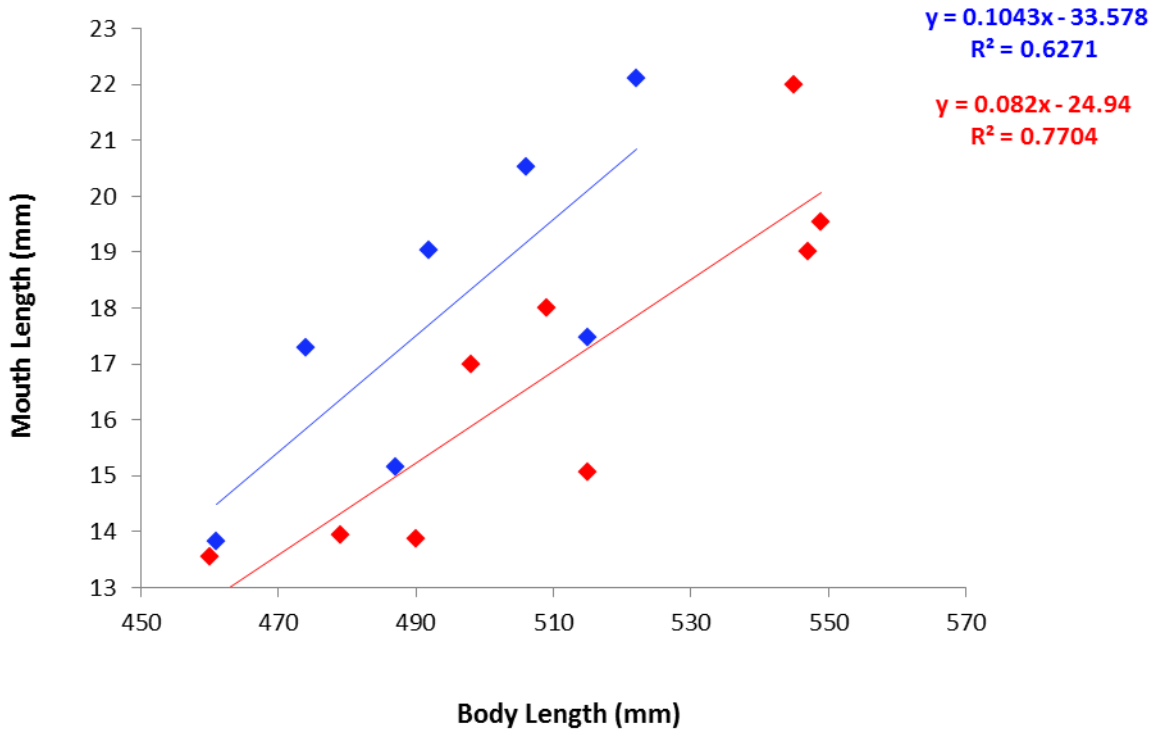

Figure 3.12. Scatterplot with regression for mouth length against body length for juvenile male and female *S. canicula*. (Male = Blue, Female = Red) (n= M (7) F (9)) (*P*= 0.666).

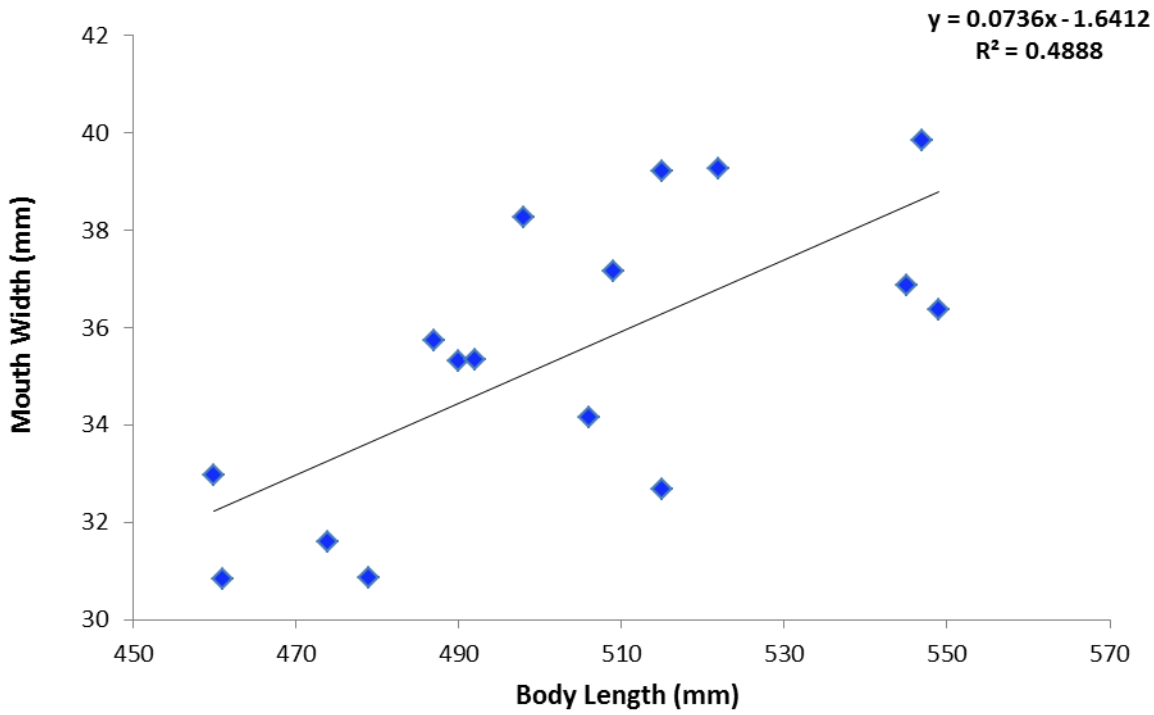

Figure 3.13. Scatterplot with regression for mouth width against body length for juvenile male and female *S. canicula* (n=16) (*P*= 0.003).

3.3.2 Adult Head and Mouth Morphometrics

The means ± standard errors and ranges for the head and mouth morphometrics of adult *S. canicula* can be seen in Appendix 2.

3.3.2.1 Pre-Branchial Length

Figure 3.14 shows a graphical representation of the pre-branchial length of adult male and female catsharks for all four seasons and for gender. In general, adult male catsharks had a greater pre-branchial length than adult female catsharks sampled in all corresponding seasons except winter. However, the results of the GLM showed that not all of these differences were significantly different.

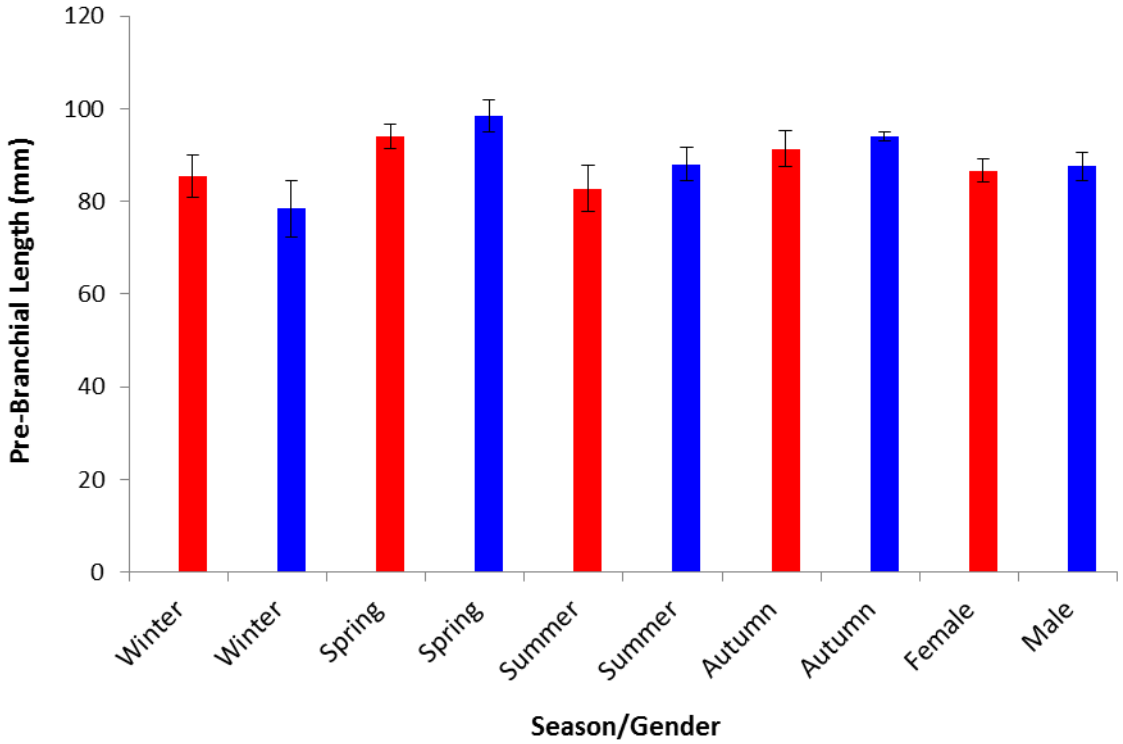

Figure 3.14. Gender and seasonal comparison of pre-branchial length for adult male and female catsharks showing means and ± standard errors (n= Female (45) (W, 16) (Sp, 8) (Su, 16) (A, 5) Male (26) (W, 10) (Sp, 7) (Su, 7) (A, 2)) (Male = Blue, Female = Red).

It can be seen from Table 3.3 that body length, gender and season within gender had no significant effect on the pre-branchial length of adult male and female catsharks. Season had a significant effect on the pre-branchial length of adult male and female catsharks, with catsharks sampled in spring having a greater pre-branchial length than catsharks sampled in winter and summer.

Table 3.3. Results from the GLM analyses for pre-branchial length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	624.6	953.5	953.5	3.91	**0.052**
Gender	1	67.3	166.7	166.7	0.68	**0.411**
Season	3	2052.9	2353.6	784.5	3.22	**0.029**
Season*Gender	3	798.2	798.2	266.1	1.09	**0.359**

Figure 3.15 shows a graphical representation of the pre-branchial length and body length adult male and female catsharks for each of the seasons. It can be seen that overall, those adult catsharks sampled in spring had a larger pre-branchial length than those adult catsharks sampled in all other seasons.

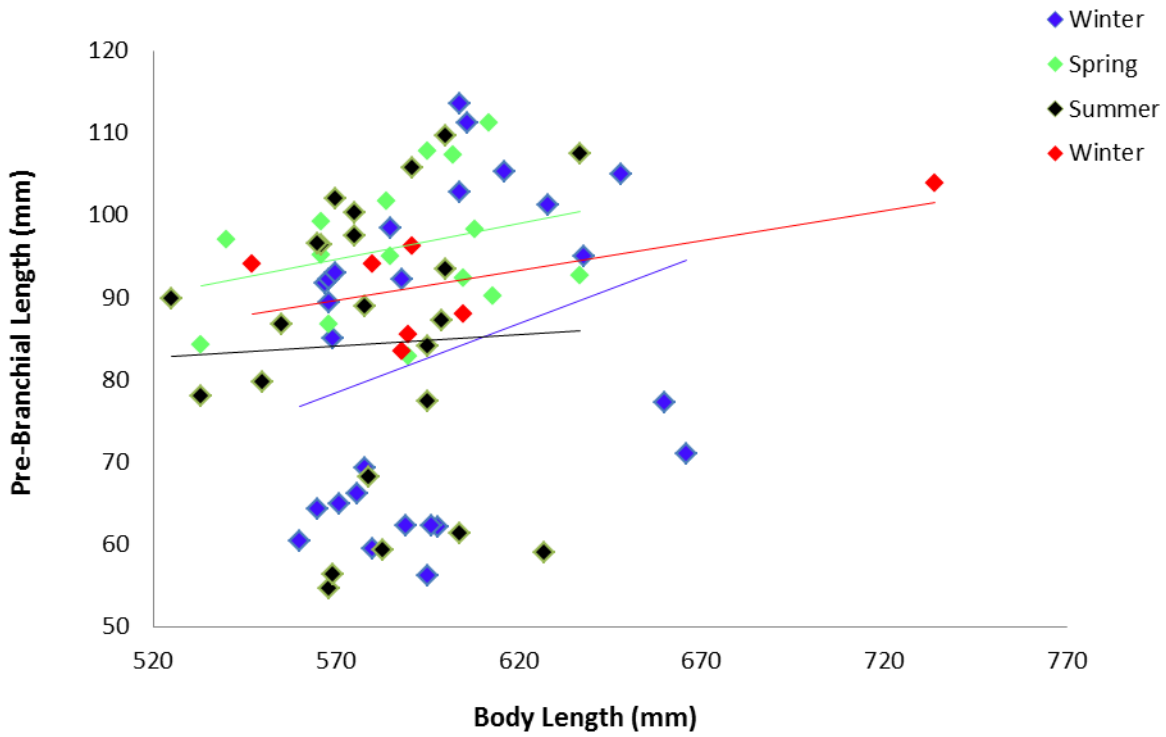

Figure 3.15. Scatterplot with regression for pre-branchial length against body length for adult catsharks for all four seasons (n= (W, 26) (Sp, 15) (Su, 23) (A, 7)).

3.3.2.2 Head Width

Figure 3.16 shows a graphical representation of the head width of adult male and female catsharks for all four seasons. The head width of adult male and female catsharks were similar within each season, except for autumn, whereby females generally had a wider head than males. However, the results of the GLM showed that there were no significant differences between the head width of male and female adult catsharks within any season.

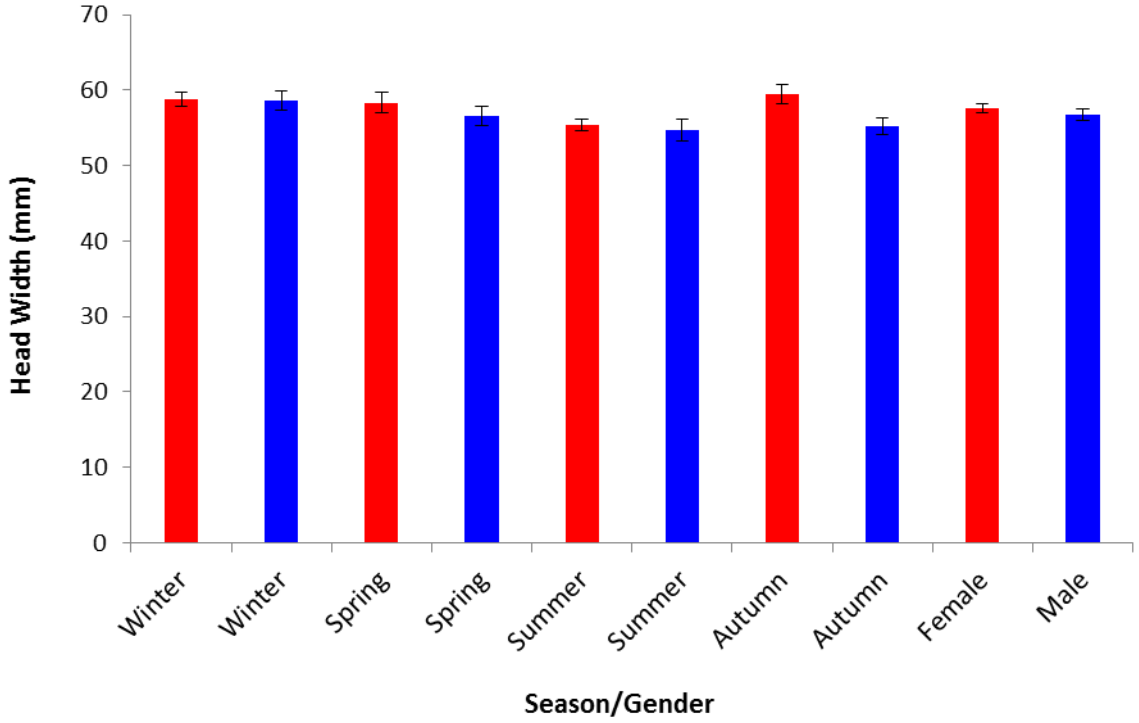

Figure 3.16. Gender and seasonal comparison of head width for adult male and female catsharks showing means and ± standard errors (n= Female (45) (W, 16) (Sp, 8) (Su, 16) (A, 5) Male (26) (W, 10) (Sp, 7) (Su, 7) (A,2)) (Male = Blue, Female = Red).

It can be seen from Table 3.4 that gender, season and season within gender had no significant effect on the head width of adult male and female catsharks. Body length had a significant effect on the head width of adult male and female catsharks.

Table 3.4. Results from the GLM analyses for head width of adult male and female *S. canicula.*

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	192.90	104.77	104.77	9.55	**0.003**
Gender	1	1.01	8.26	8.26	0.75	**0.389**
Season	3	105.06	83.96	27.99	2.55	**0.064**
Season*Gender	3	9.55	9.55	3.18	0.29	**0.832**

Figure 3.17 shows a graphical representation of the body length and head width of adult male and female catsharks. It can be seen that as body length increases so does the head width of adult male and female *S. canicula*. There was a significant correlation between body length and head width (ANOVA, F= 17.54; d.f = 1; *P*= <0.001).

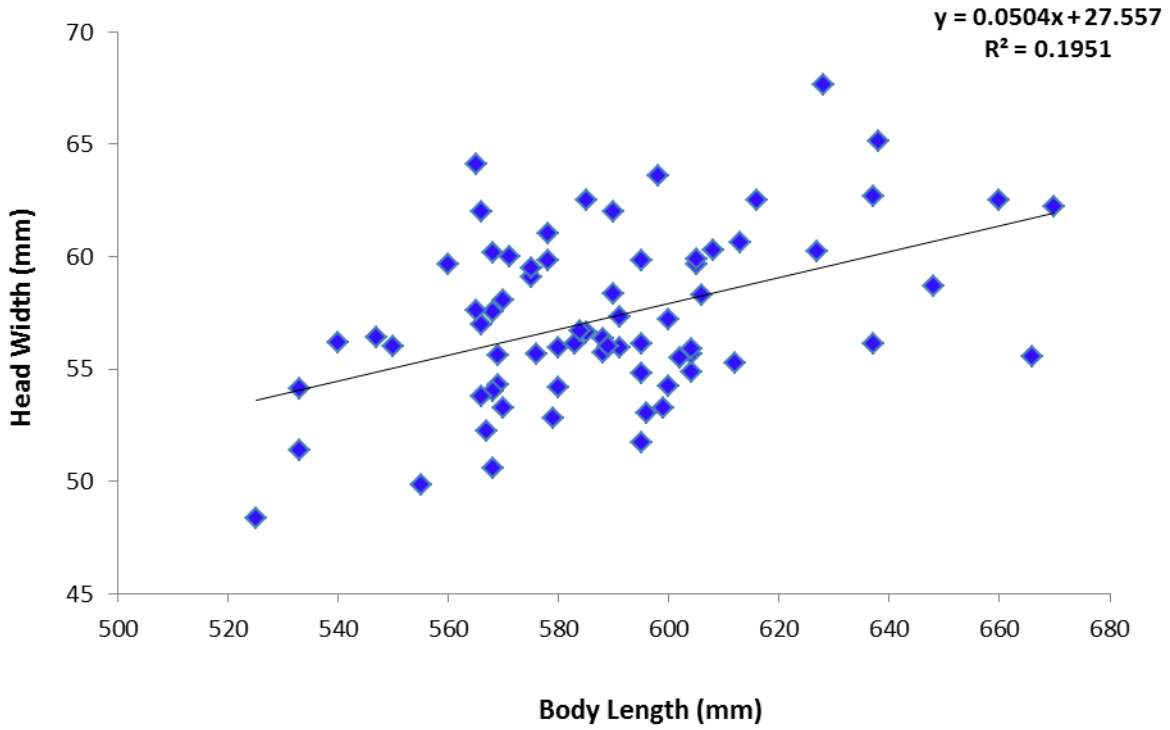

Figure 3.17. Scatterplot with regression showing head width against body length for adult male and female *S. canicula* (n= 71) (*P*= <0.001).

3.3.2.3 Pre-Oral Length

Figure 3.18 shows a graphical representation of the pre-oral length of adult male and female catsharks for all four seasons. Adult male and female catsharks sampled in winter had a similar pre-oral length, as did male and female adult catsharks sampled in spring. Female catsharks sampled in summer generally had a greater pre-oral length than males sampled in summer. However, the statistical analyses showed that there were no significant differences in the pre-oral length between the genders.

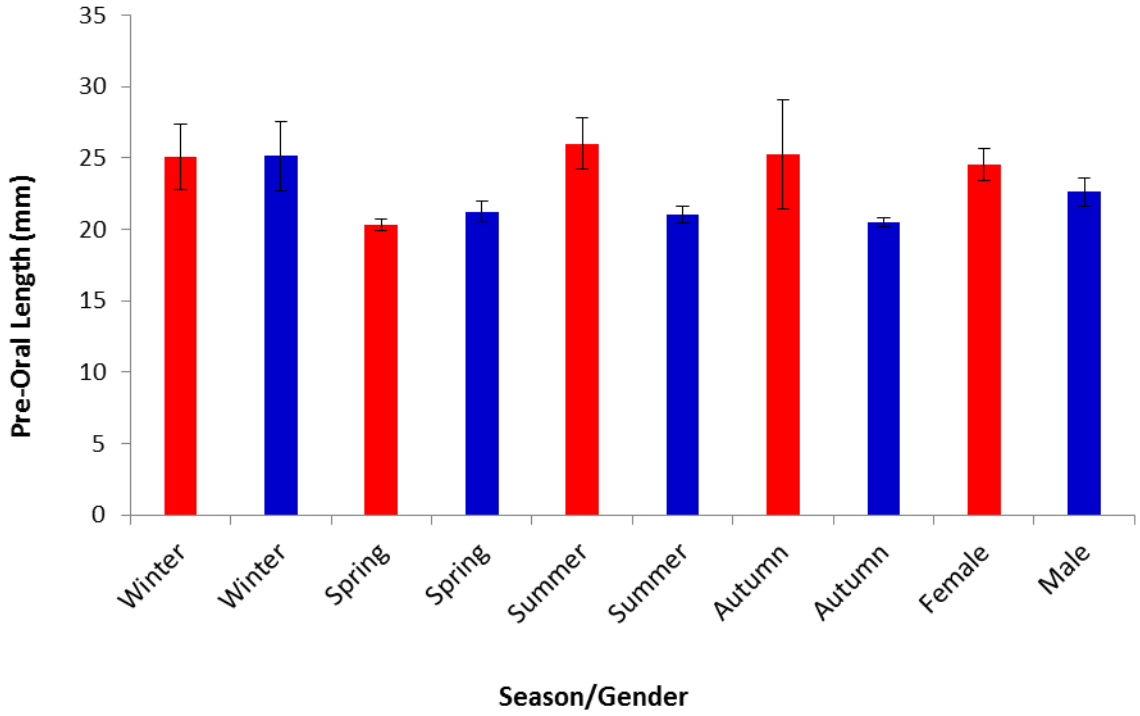

Figure 3.18. Gender and seasonal comparison of pre-oral length for adult male and female catsharks showing means and ± standard errors (n= Female (45) (W, 16) (Sp, 8) (Su, 16) (A, 5) Male (26) (W, 10) (Sp, 7) (Su, 7) (A, 2)) (Male = Blue, Female = Red).

It can be seen from Table 3.5 that body length, gender, season and season within gender had no significant effect on the pre-oral length of adult male and female catsharks.

Table 3.5. Results from the GLM analyses for pre-oral length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	207.39	129.30	129.30	2.89	**0.094**
Gender	1	31.50	17.72	17.72	0.40	**0.532**
Season	3	175.82	154.23	51.41	1.15	**0.337**
Season*Gender	3	64.95	64.95	21.65	0.48	**0.695**

3.3.2.4 Mouth Length

A graphical representation of the mouth length of adult male and female catsharks for all four seasons can be seen in Figure 3.19. Males generally had a longer mouth than females in all seasons sampled compared to females, although the statistical analyses revealed that this was not significant for all seasons.

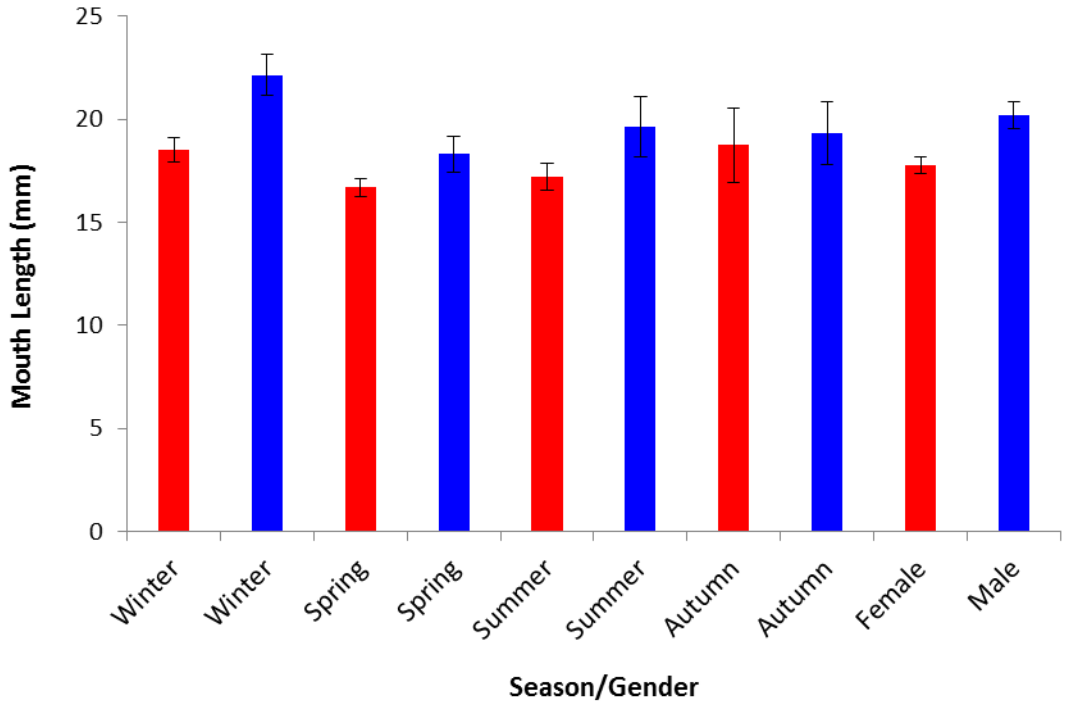

Figure 3.19. Gender and seasonal comparison of mouth length for adult male and female catsharks showing means and ± standard errors (n= Female (45) (W, 16) (Sp, 8) (Su, 16) (A, 5) Male (26) (W, 10) (Sp, 7) (Su, 7) (A, 2)) (Male = Blue, Female = Red).

It can be seen from Table 3.6 that body length and season within gender had no significant effect on the mouth length of adult male and female catsharks. Both gender and season had an effect on mouth length in adult male and female catsharks. Males generally had longer mouths than females. Catsharks sampled in winter were found to possess a greater mouth length than catsharks sampled in spring and summer.

Table 3.6. Results from the GLM analyses for mouth length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	4.902	3.01	3.01	0.39	**0.535**
Gender	1	110.04	53.45	53.45	6.92	**0.011**
Season	3	69.10	75.3	25.1	3.25	**0.028**
Season*Gender	3	13.06	13.06	4.35	0.56	**0.641**

3.3.2.5 Mouth Width

Figure 3.20 shows a graphical representation of the mouth width of adult male and female catsharks for all four seasons. It can be seen that adult female catsharks generally had wider mouths than adult male catsharks.

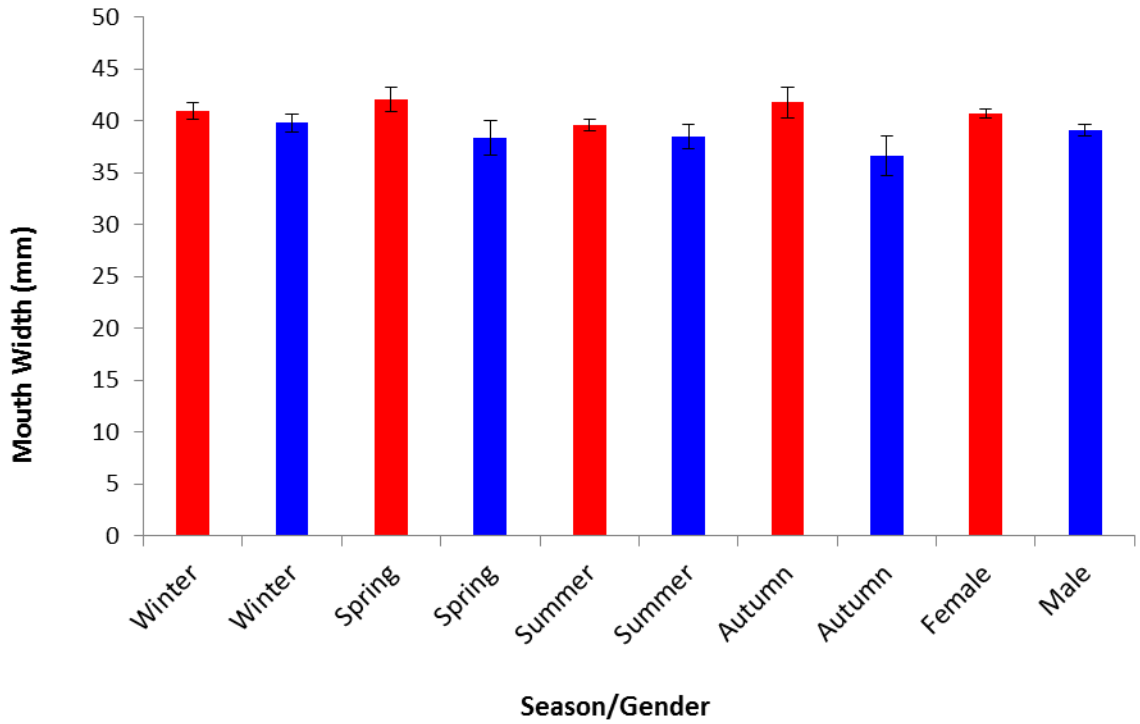

Figure 3.20. Gender and seasonal comparison of mouth width for adult male and female catsharks showing means and ± standard errors (n= Female (45) (W, 16) (Sp, 8) (Su, 16) (A, 5) Male (26) (W, 10) (Sp, 7) (Su, 7) (A, 2)) (Male = Blue, Female = Red).

It can be seen from Table 3.7 that season and season within gender had no effect on the mouth width of adult male and female catsharks. Both body length and gender had a significant effect on the mouth width in adult male and female catsharks. Adult females were generally found to possess a greater mouth width than adult males.

Table 3.7. Results from the GLM analyses for mouth width of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	113.67	70.91	70.91	8.34	**0.005**
Gender	1	41.41	51.99	51.99	6.12	**0.016**
Season	3	18.37	16.17	5.39	0.63	**0.596**
Season*Gender	3	21.85	21.85	7.28	0.86	**0.468**

Figure 3.21 shows a graphical representation of the body length and mouth width of adult male and female catsharks. It can be seen that as body length increases so does the mouth width of adult male and female *S. canicula*. The regression lines are similar and the increase in mouth width with body length is linear, with both male and female adult catsharks showing the same rate of mouth width increase in relation to body length increase. There was no significant difference found between the regression lines of adult male and female catsharks in relation to mouth width and body length (P= 0.636).

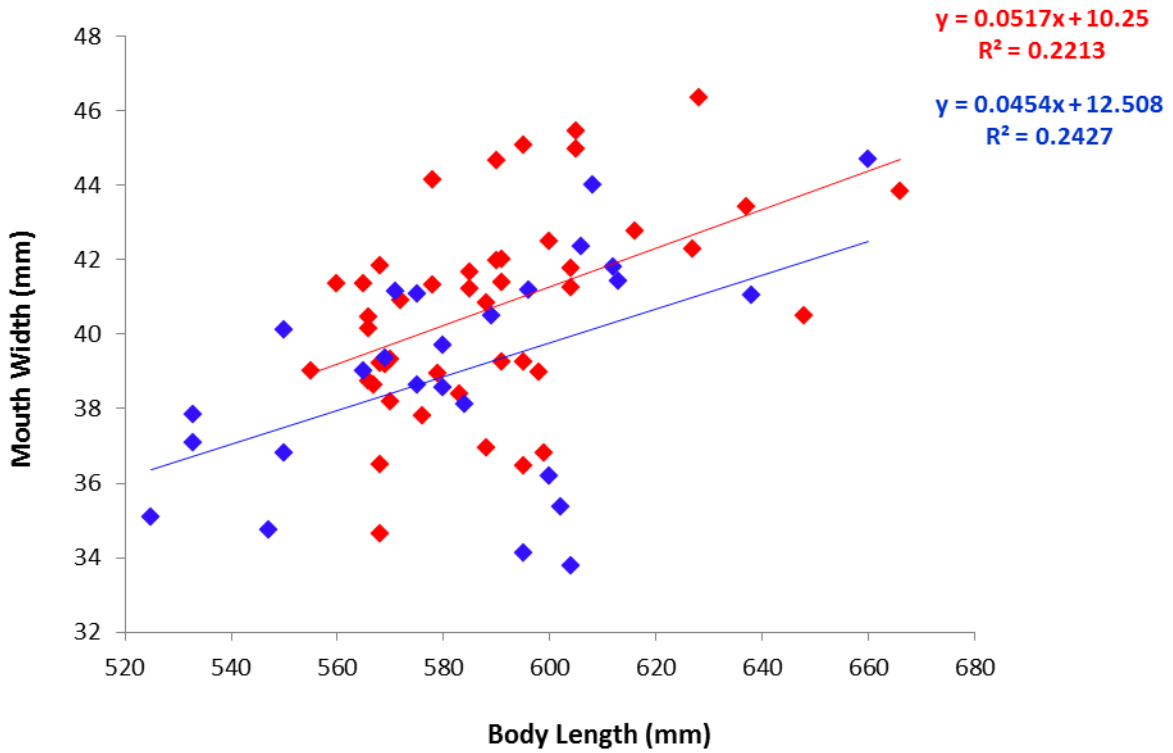

Figure 3.21. Scatterplot with regression for mouth width against body length for adult male and female *S. canicula* (Male = Blue, Female = Red) (n= M (26) F (45)) (*P*= 0.636).

3.3.3 Jaw Data

3.3.3.1 Hatchling Jaw Data

The results of the hatchling upper and lower jaw data can be seen in Table 3.8. In general male hatchling catsharks had a greater jaw width and length, although the statistical analyses showed that only the upper jaw width was significantly different. Male hatchling catsharks possessed a significantly wider upper jaw than female hatchling catsharks (ANCOVA, F=5.92; d.f.=1; *P*=0.035). Body length had no effect on the upper jaw width (ANCOVA, F=2.33; d.f.=1; *P*=0.158). Neither body length, nor gender had a significant effect on the upper jaw length (ANCOVA, F=2.34; d.f.=1; *P*=0.160; ANCOVA, F=0.02; d.f.=1; *P*=0.895) lower jaw length (ANCOVA, F=0.75; d.f.=1; *P*=0.407; ANCOVA, F=1.27; d.f.=1; *P*=0.286) and lower jaw width (ANCOVA, F=0.11; d.f.=1; *P*=0.750; ANCOVA, F=0.94; d.f.=1; *P*=0.358) of hatchling *S. canicula*.

Table 3.8 Upper and lower jaw data for male and female hatchling *S. canicula* showing means ± standard errors, range and *P*-values (n= M (23) F (14)).

Feature (mm)	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length (*P*-Value)	Gender (*P*-Value)
Upper Jaw Length	3.42 ± 0.07 (3.33 – 3.53)	3.42 ± 0.10 (2.96– 3.82)	**0.160**	**0.895**
Upper Jaw Width	5.6 ± 0.29 (4.74 – 6.16)	6.30 ± 0.10 (5.88 – 6.69)	**0.158**	**0.035**
Lower Jaw Length	1.87 ± 0.15 (1.46 – 2.11)	2.11 ± 0.09 (1.77 – 2.57)	**0.407**	**0.286**
Lower Jaw Width	6.2 ± 0.43 (4.90 – 6.74)	6.88 ± 0.16 (6.02 – 7.81)	**0.750**	**0.358**

3.3.4 Juvenile Jaw Data

3.3.4.1 Upper Jaw

The data for the upper jaw morphometrics can be seen in Table 3.9. Generally juvenile female catsharks possessed larger jaws than juvenile male catsharks. However, the results of the ANCOVA revealed that the differences between the length (ANCOVA, F=2.10; d.f.=1; *P*=0.163) width (ANCOVA, F=0.92; d.f.=1; *P*=0.350) diameter (ANCOVA, F=0.20; d.f.=1; *P*=0.661) and depth (ANCOVA, F=0.01; d.f.=1; *P*=0.929) of the upper jaws of male and female juvenile catsharks were not statistically significant. Body length had no significant effect on the upper jaw length (ANCOVA, F=0.92; d.f.=1; *P*=0.349) or upper jaw depth (ANCOVA, F=1.26; d.f.=1; *P*=0.282) of juvenile *S. canicula*. Body length was found to have a significant effect on the jaw width (ANCOVA, F=7.16; d.f.=1; *P*=0.015) and jaw diameter (ANCOVA, F=19.85; d.f.=1; *P*=0.001).

Table 3.9. Table 3.2 Upper jaw data for male and female juvenile *S. canicula* showing means ± standard errors and *P*-values (n= M (6) F (17)).

Feature (mm)	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length (*P*-Value)	Gender (*P*-Value)
Jaw Length	17.79 ± 0.72 (10.76 – 22.22)	15.1 ± 1.41 (10.16 – 20.39)	**0.163**	**0.349**
Jaw Width	32.31 ± 0.84 (24.58 – 38.50)	29.03 ± 0.51 (27.10 – 30.41)	**0.015**	**0.350**
Jaw Diameter	26.21 ± 0.75 (21.81 – 30.65)	24 ± 1.16 (22.48 – 27.28)	**0.001**	**0.661**
Jaw Depth	3.35 ± 0.13 (2.74 – 4.18)	3.17 ± 0.10 (3.03 – 3.48)	**0.282**	**0.929**

It can be seen from Figures 3.22 and 3.23 that as the body length of the juvenile catsharks increased so did the upper jaw width and upper jaw diameter. There was a significant correlation between body length and upper jaw width (ANOVA, F= 12.77; d.f = 1; *P*= 0.002) and upper jaw diameter (ANOVA, F= 57.27; d.f = 1; *P*= <0.001).

The combined data for the juvenile and adult upper jaw width and juvenile and adult upper jaw diameter were plotted to ascertain if jaw width and diameter increased with length to the same degree in adult catsharks as they did in juveniles (Appendix 1).

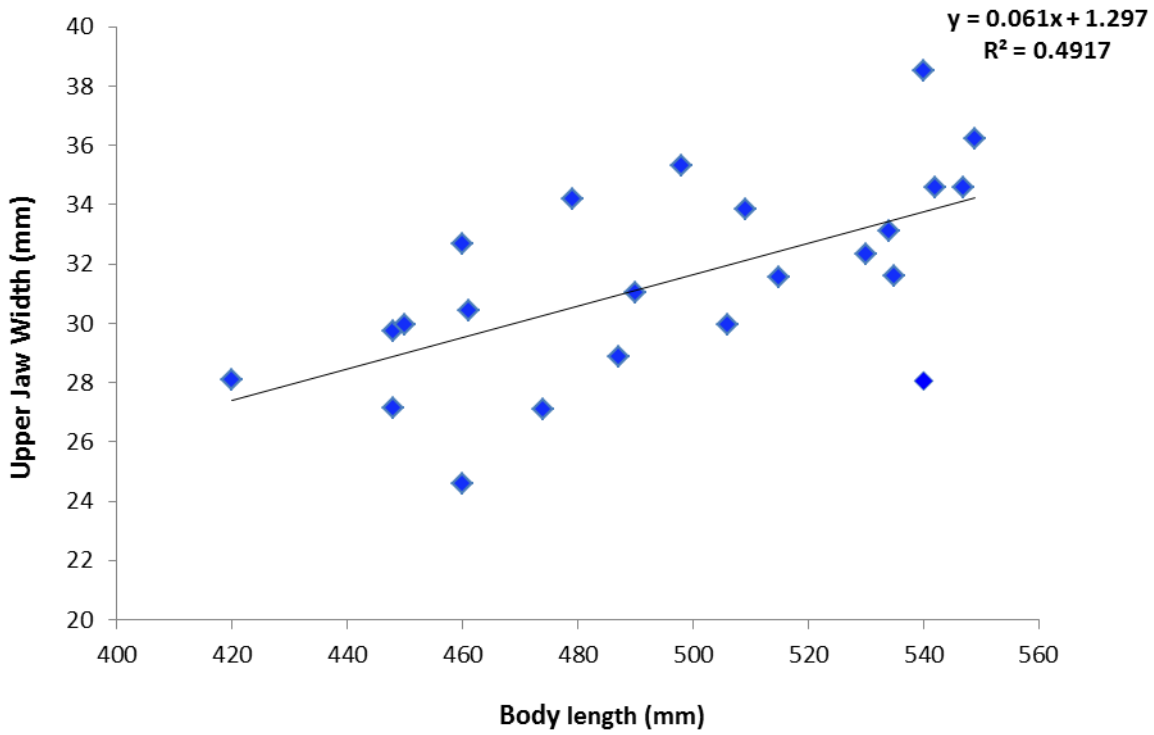

Figure 3.22. Scatterplot with regression showing upper jaw width against body length for juvenile male and female *S. canicula* (n= 23) (*P*= 0.002).

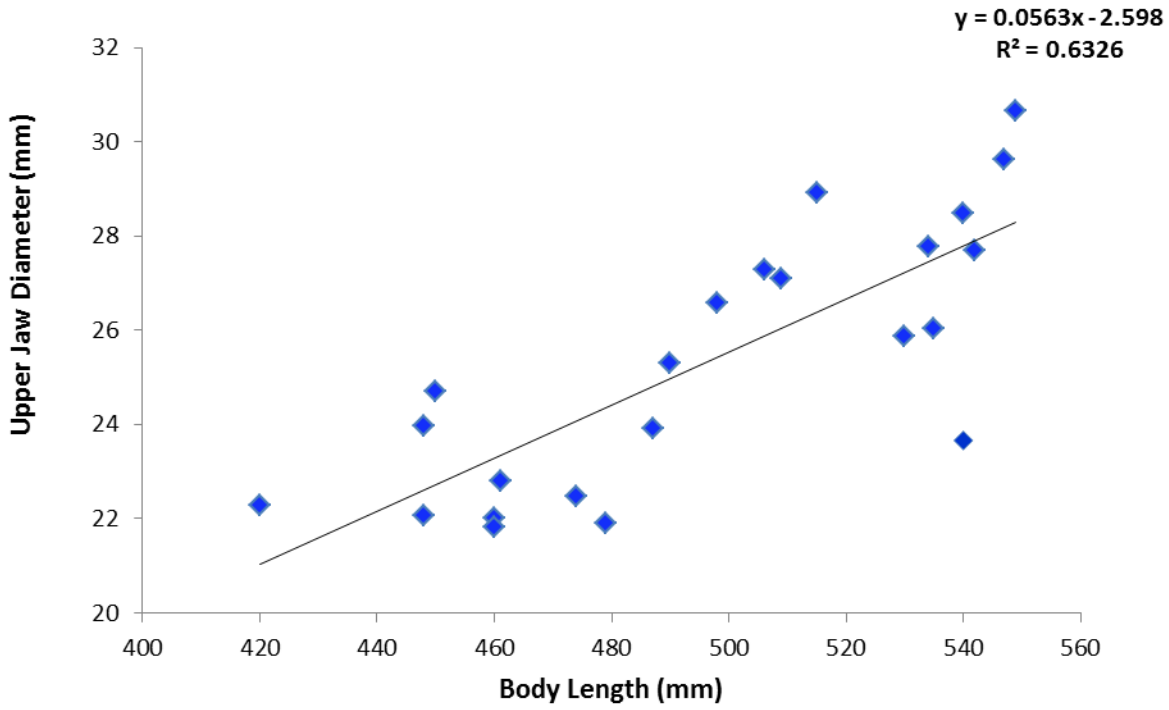

Figure 3.23. Scatterplot with regression showing upper jaw diameter against body length for juvenile male and female *S. canicula* (n= 23) (*P*= <0.001).

3.3.4.2 Lower Jaw

Table 3.10 shows the lower jaw morphometrics data for juvenile male and female *S. canicula*. There is a similar pattern shown in the lower jaw morphometrics with juvenile female catsharks possessing relatively larger lower jaws than juvenile male catsharks. However, there were no significant differences found in the lower jaw length (ANCOVA, F=0.04; d.f.=1; *P*=0.847) lower jaw width (ANCOVA, F=0.48; d.f.=1; *P*=0.497) and jaw diameter (ANCOVA, F=0.12; d.f.=1; *P*=0.735). There was a significant difference found in the lower jaw depth between juvenile male and female catsharks, with female catsharks possessing a greater lower jaw depth than male catsharks (ANCOVA, F=4.73; d.f.=1; *P*=0.036). Body length did not have a significant effect on lower jaw width of juvenile catsharks (ANCOVA, F=0.69; d.f.=1; *P*=0.417). Body length did have a significant effect on the lower jaw length (ANCOVA, F=13.11; d.f.=1; *P*=0.002) lower jaw diameter (ANCOVA, F=12.92; d.f.=1; *P*=0.003) and lower jaw depth (ANCOVA, F=5.35; d.f.=1; *P*=0.047) of juvenile catsharks (Figures 3.24 - 3.26).

Table 3.10. Lower jaw data for male and female juvenile *S. canicula* showing means ± standard errors, range and *P*-values (n= M (6) F (18)).

Feature (mm)	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length (*P*-Value)	Gender (*P*-Value)
Jaw Length	17.80 ± 0.67 (12.81 – 23.05)	15.68 ± 0.79 (13.05 – 18.09)	**0.002**	**0.847**
Jaw Width	31.24 ± 1.27 (25.14 – 41.59)	28.32 ± 1.55 (23.37 – 32.37)	**0.417**	**0.497**
Jaw Diameter	22.92 ± 0.43 (20.8 – 25.76)	21.77 ± 0.79 (20.26 – 23.30)	**0.003**	**0.735**
Jaw Depth	4.45 ± 0.16 (3.67 – 5.41)	4.98 ± 0.56 (4.20 – 6.62)	**0.047**	**0.036**

A graphical representation of the body length and lower jaw length, diameter and depth of juvenile catsharks can be seen in Figures 3.24 - 3.26. The graphs show that as body length increases the length, diameter and depth of the lower jaw of juvenile male and female *S.*

canicula increases. There was a significant correlation between body length and lower jaw length (ANOVA, F=18.65; d.f.=1; *P*= <0.001) lower jaw diameter (ANOVA, F=22.86; d.f.=1; *P*<0.05) and lower jaw depth (Males = ANOVA, F=76.07; d.f.=1; *P*= 0.009; Females = ANOVA, F=6.90; d.f.=1; *P*= 0.018) in juvenile catsharks.

The data for the juvenile and adult lower jaw diameter and juvenile and adult lower jaw depth were plotted to ascertain any ontogenic relationships. There was a clear linear relationship between the juvenile and adult samples (Appendix 1).

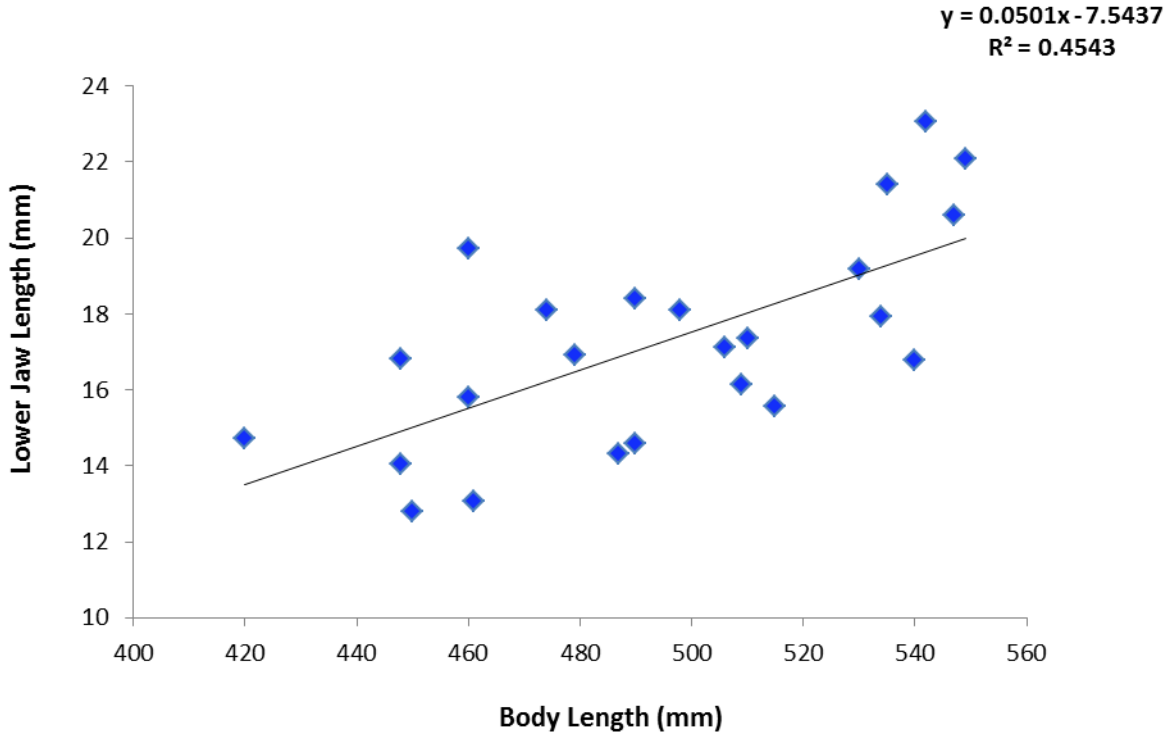

Figure 3.24. Scatterplot with regression showing lower jaw length against body length for juvenile male and female *S. canicula* (n= 24) (*P*= <0.001).

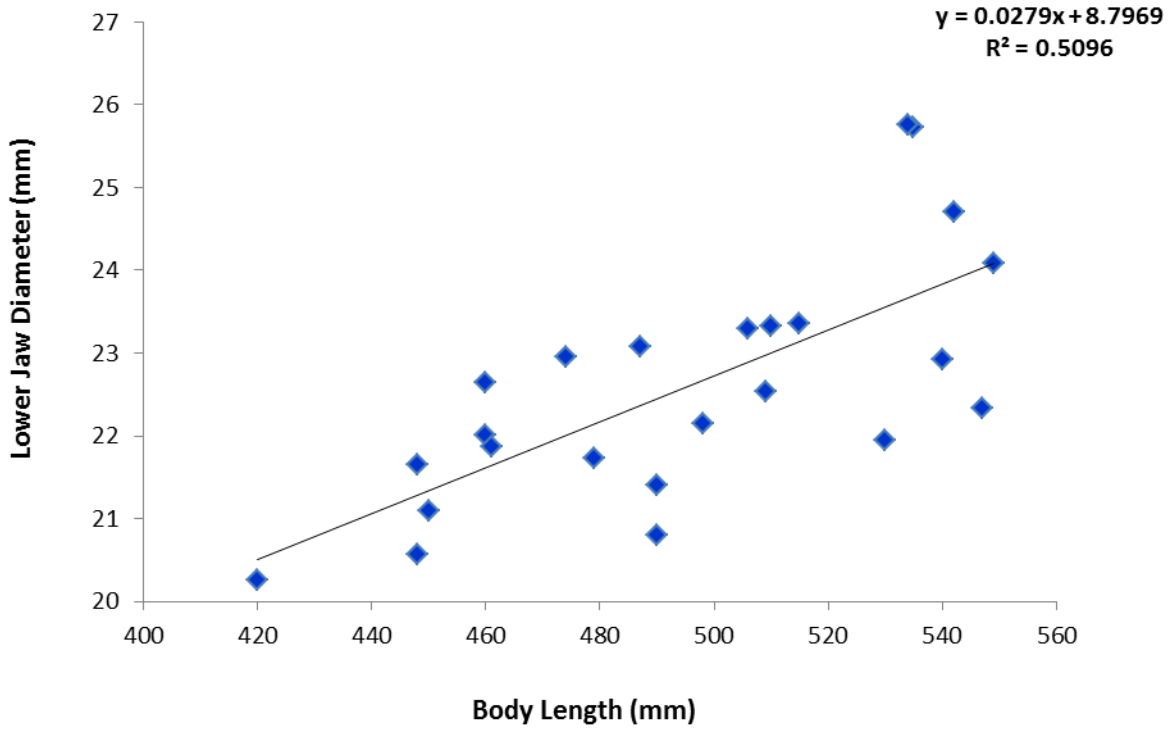

Figure 3.25. Scatterplot with regression showing lower jaw diameter against body length for juvenile male and female *S. canicula* (n= 24) (*P*< 0.009).

It can be seen from Figure 3.25 that as the length of the sharks increases the differentiation in jaw depth between males and females begins to become more pronounced with males developing a greater jaw depth than females with increased body length. However, a comparison of the regression lines for juvenile males and females revealed that there were no significant differences in the correlation between juvenile male and female catsharks with regard to lower jaw depth and body length (*P*= 0.316).

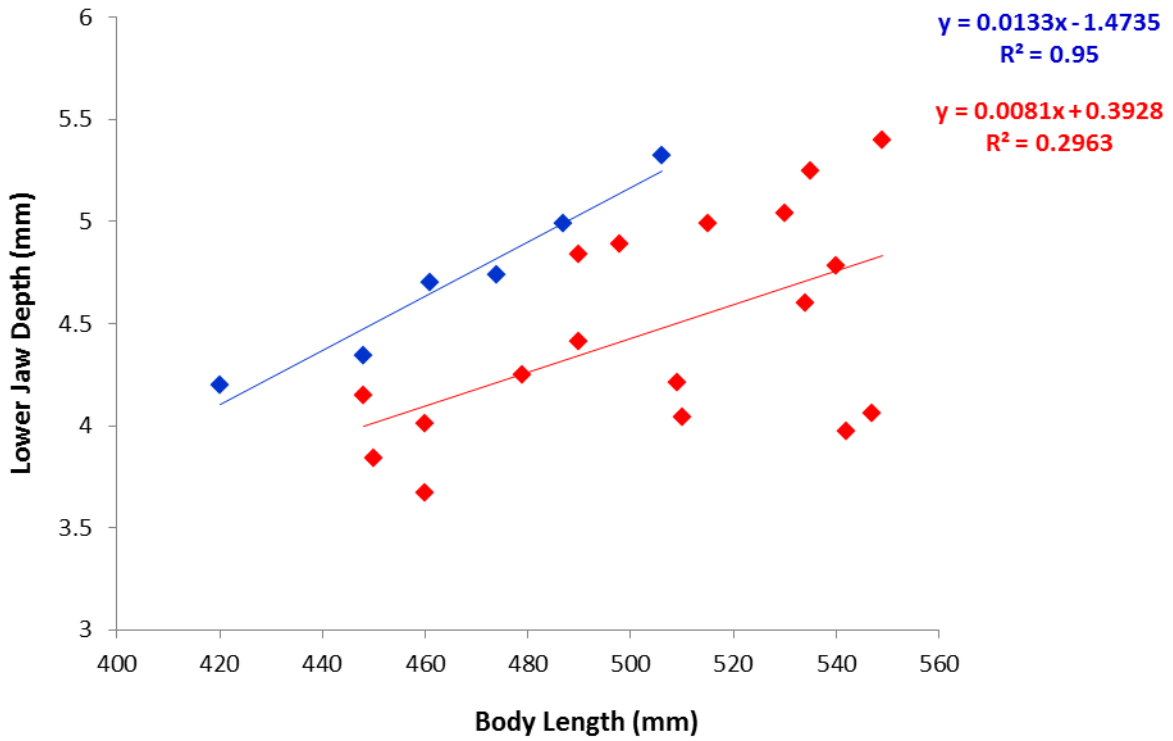

Figure 3.26. Scatterplot with regression showing lower jaw depth against body length for juvenile male and female *S. canicula* (Male = Blue, Female = Red) (n=M (6) F (18)) (*P*= 0.316).

3.3.5 Adult Jaw Data

The means ± standard errors and ranges for the jaw morphometrics of adult *S. canicula* can be seen in Appendix 2.

3.3.5.1 Upper Jaw Length

Figure 3.27 shows a graphical representation of the upper jaw length of adult male and female catsharks for all four seasons. The results show that adult male catsharks sampled in every season possess a greater jaw length than adult female catsharks sampled throughout the year. Jaw length did not differ significantly between adult males sampled throughout the year. Adult females sampled throughout the year also possessed a similar jaw size, except in autumn, when females had a longer upper jaw than adult females

sampled in all other seasons. However, the results of the GLM show that there were no significant intra-gender dimorphisms present in upper jaw length in adult male and female *S. canicula*.

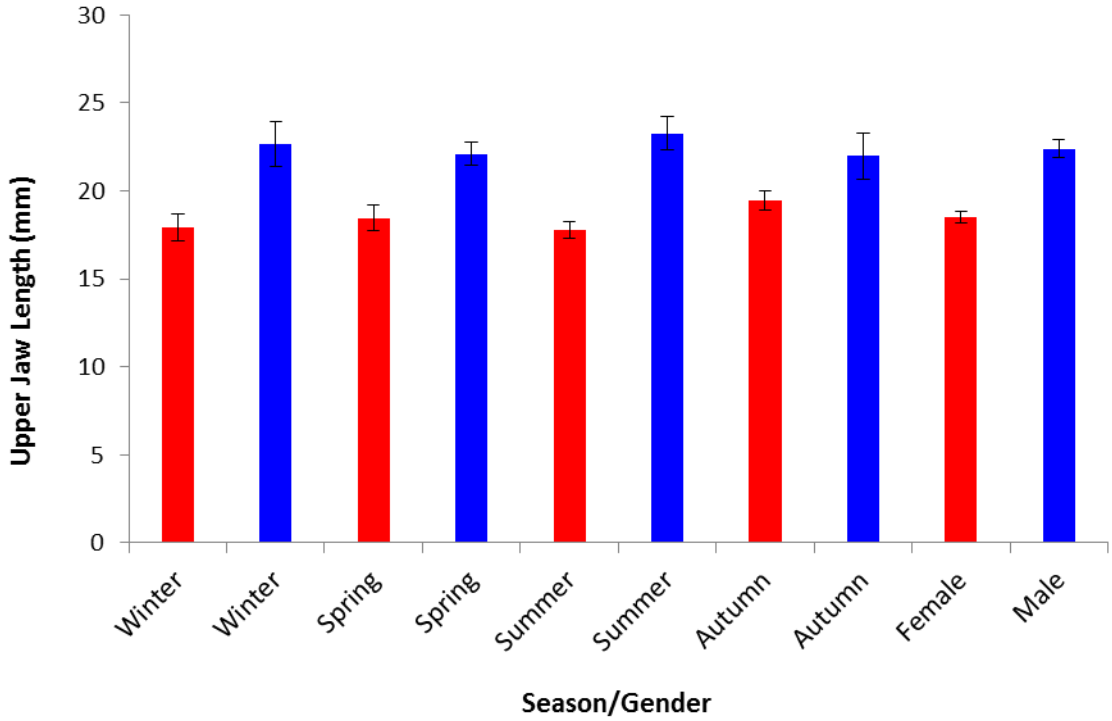

Figure 3.27. Gender and seasonal comparison of upper jaw length for adult male and female catsharks showing means and ± standard errors (n= Female (47) (W, 9) (Sp, 11) (Su, 11) (A, 16) Male (35) (W, 4) (Sp, 14) (Su, 8) (A, 9)) (Male = Blue, Female = Red).

Table 3.11 shows that body length, season and season within gender had no effect on the upper jaw length of adult male and female catsharks. Gender had a significant effect on the upper jaw length of adult male and female catsharks, with males having a greater upper jaw length than females.

Table 3.11. Results from the GLM analyses for upper jaw length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	24.38	16.62	16.62	3.40	**0.069**
Gender	1	338.56	322.86	322.86	65.98	**<0.001**
Season	3	17.277	12.295	4.098	0.84	**0.478**
Season*Gender	3	10.325	10.325	3.442	0.70	**0.553**

3.3.5.2 Upper Jaw Width

Figure 3.28 shows a graphical representation of the upper jaw width of adult male and female catsharks for all four seasons. The graph shows that in general females sampled within each season had marginally wider upper jaws than males sampled within the same season, except in autumn. However, the statistical analyses show that there were no specific gender differences in the jaw width of adult male and female catsharks.

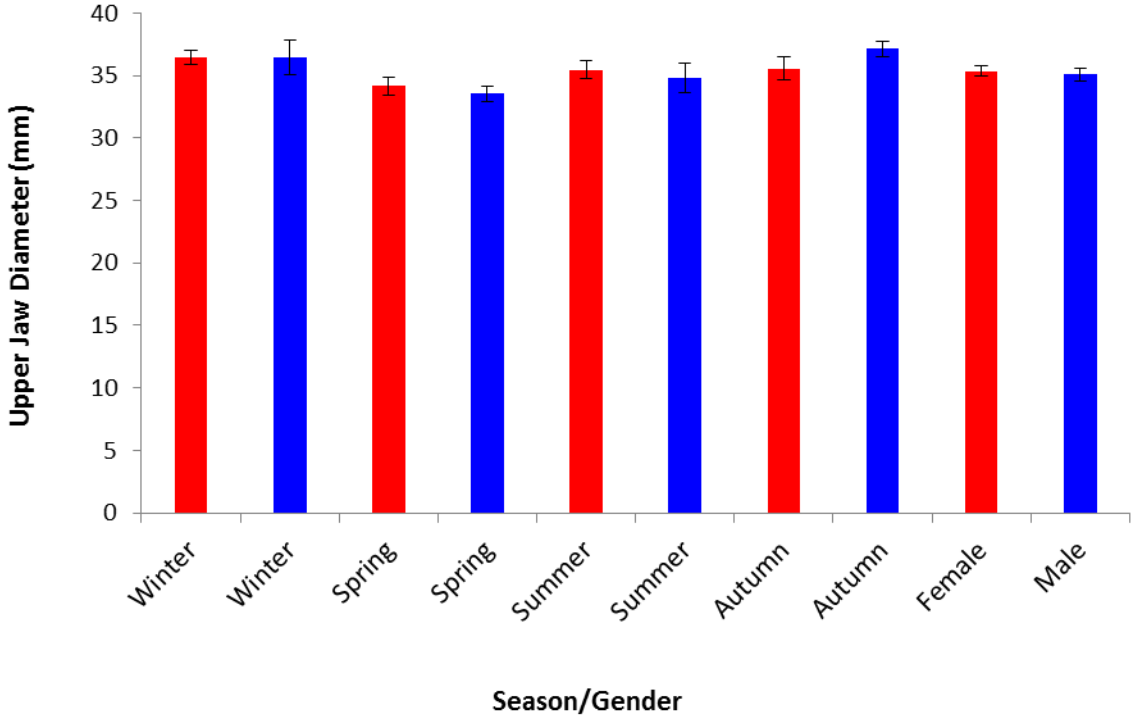

Figure 3.28. Gender and seasonal comparison of upper jaw width for adult male and female catsharks showing means and ± standard errors (n= Female (47) (W, 9) (Sp, 11) (Su, 11) (A, 16) Male (35) (W, 4) (Sp, 14) (Su, 8) (A, 9)) (Male = Blue, Female = Red).

It can be seen from Table 3.12 that gender and season within gender had no effect on the upper jaw width of adult male and female catsharks. Body length and season had a significant effect on the upper jaw width of adult male and female catsharks, with males and females in winter having a greater jaw width than males and females in spring. Adult catsharks sampled in autumn and winter had a greater upper jaw width than adult catsharks sampled in spring.

Table 3.12. Results from the GLM analyses for upper jaw width of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	33.02	31.68	31.68	5.79	**0.019**
Gender	1	0.23	0.393	0.393	0.07	**0.790**
Season	3	70.24	71.18	71.18	4.33	**0.007**
Season*Gender	3	35.75	35.75	11.92	2.18	**0.100**

Figure 3.29 shows a graphical representation of the body length and upper jaw width of adult male and female catsharks. There was a significant correlation between body length and upper jaw width of adult catsharks (ANOVA, F=5.28; d.f.=1; *P*= 0.024). It can be seen that as body length increases so does the upper jaw width of adult *S. canicula*.

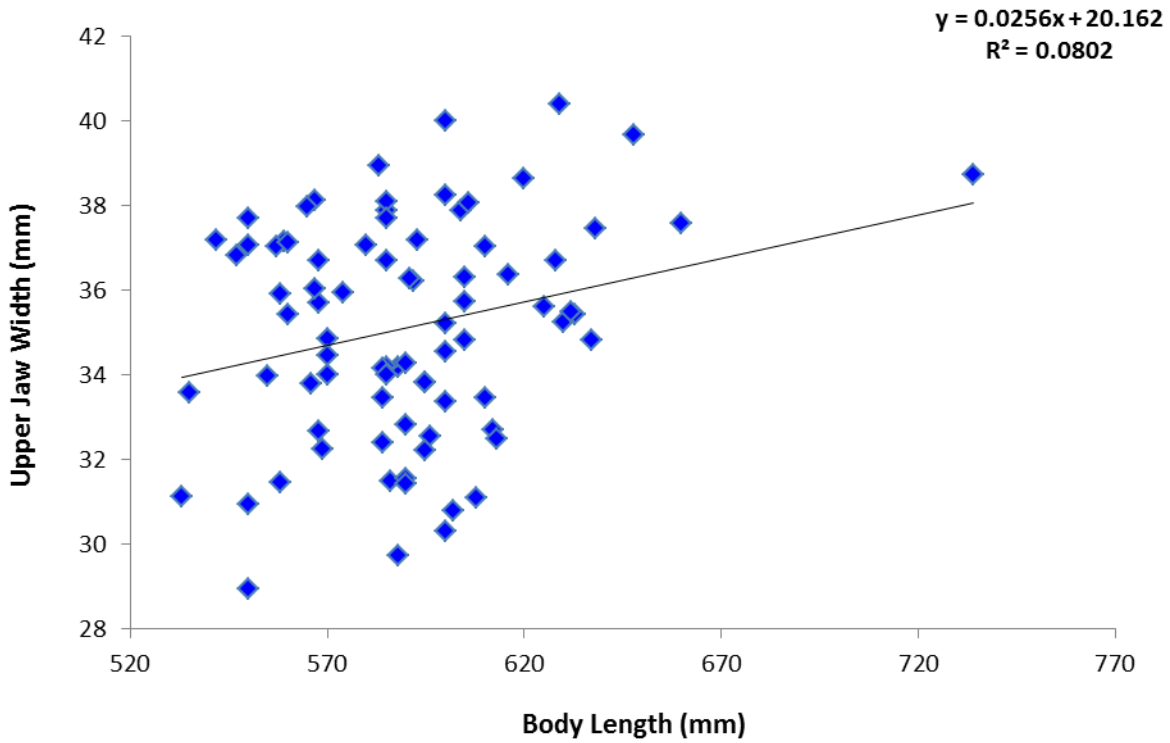

Figure 3.29 Scatterplot with regression showing upper jaw width against body length for adult male and female *S. canicula* (n= 82) (*P*= 0.024).

3.3.5.3 Upper Jaw Diameter

The means and standard errors for the upper jaw diameter in adult male and female catsharks can be seen in Figure 3.30. It can be seen from the graph that in all seasons except winter that males generally have a greater jaw diameter than females. However, the results of the GLM show that there are no significant differences in the jaw diameter between male and females throughout the year.

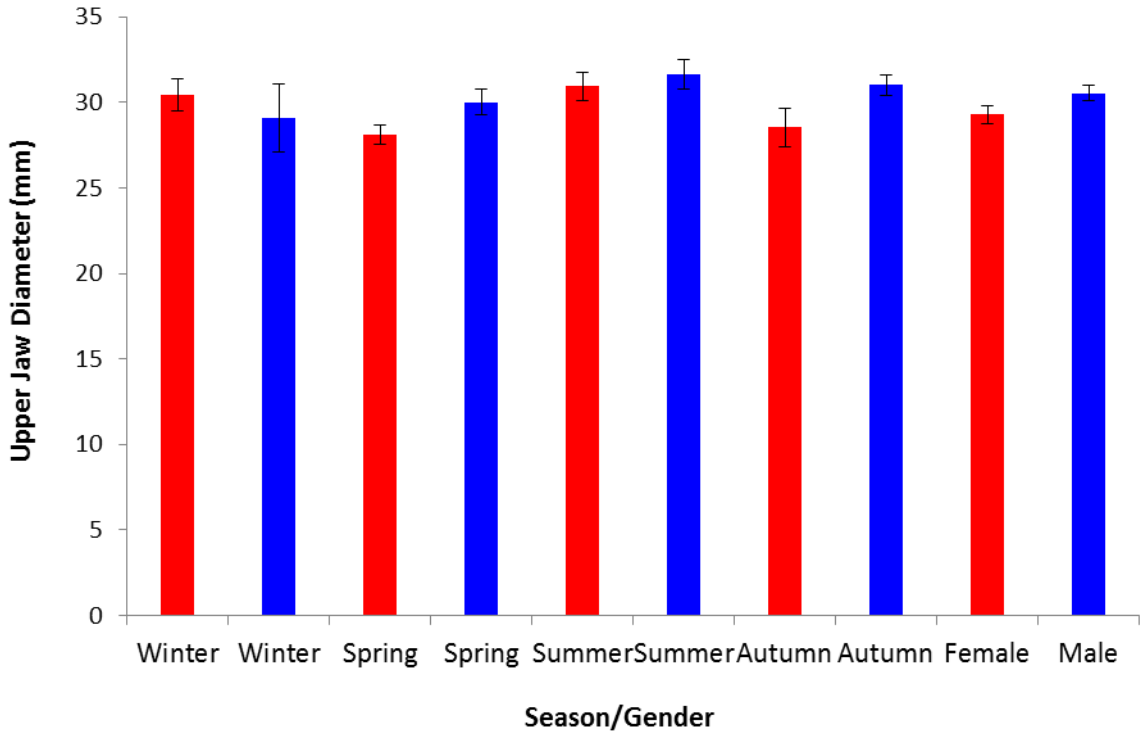

Figure 3.30. Gender and seasonal comparison of upper jaw diameter for adult male and female catsharks showing means and ± standard errors (n= Female (41) (W, 9) (Sp, 9) (Su, 7) (A, 16) Male (28) (W, 4) (Sp, 9) (Su, 6) (A, 9)) (Male = Blue, Female = Red).

Table 3.13 shows that gender, season and season within gender had no effect on the upper jaw diameter of adult male and female catsharks. Body length had a significant effect on the upper jaw diameter of both adult male and female catsharks.

Table 3.13. Results from the ANCOVA analyses for the upper jaw diameter of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	27.158	37.818	37.818	7.13	**0.010**
Gender	1	9.748	3.716	3.716	0.70	**0.406**
Season	3	44.806	48.662	16.221	3.06	**0.095**
Season*Gender	3	24.615	24.615	8.205	1.55	**0.211**

Figure 3.31 shows a graphical representation of the body length and upper jaw diameter of adult male and female catsharks. The correlation between body length and upper jaw diameter was found to be significant for adult catsharks (ANOVA, F=4.58; d.f.=1; *P*= 0.035). It can be seen that as body length increases so does the upper jaw diameter of adult *S. canicula*.

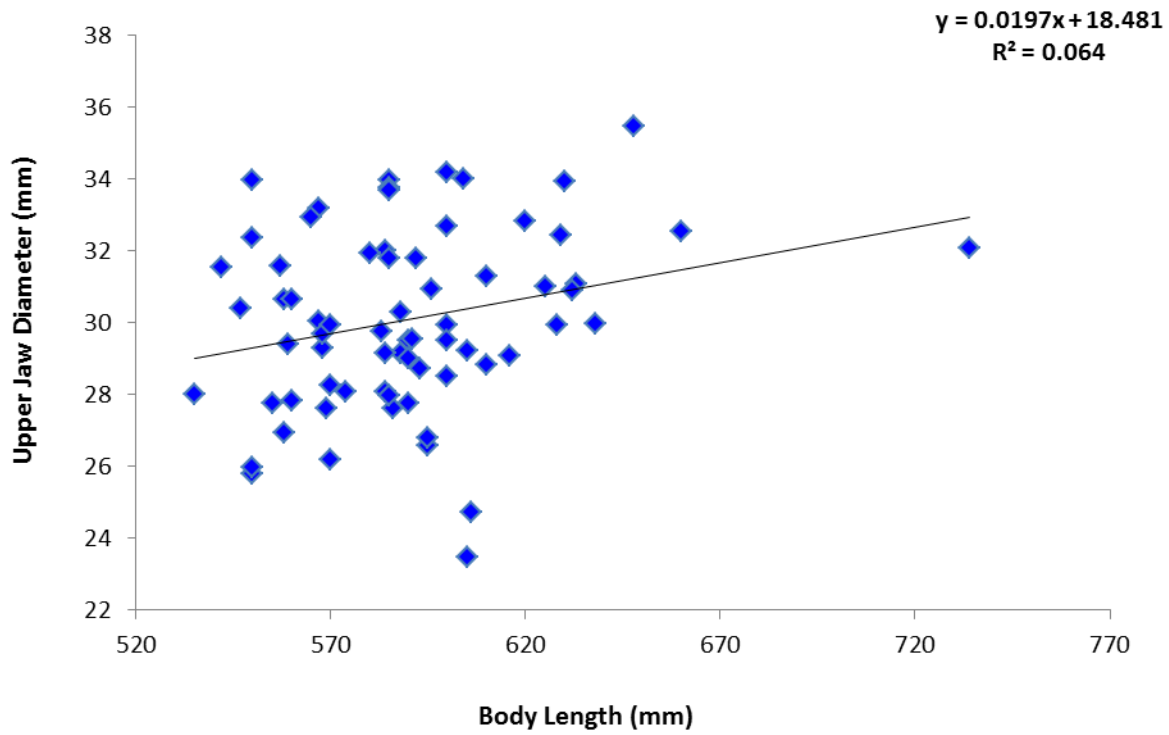

Figure 3.31. Scatterplot with regression showing upper jaw diameter against body length for adult male and female *S. canicula* (n= 69) (*P*= 0.035).

3.3.5.4 Upper Jaw Depth

Figure 3.32 shows the means and standard errors for the upper jaw depth in male and female adult catsharks. The results show the upper jaw depth in adult male catsharks is greater than in adult female catsharks during every season of the year.

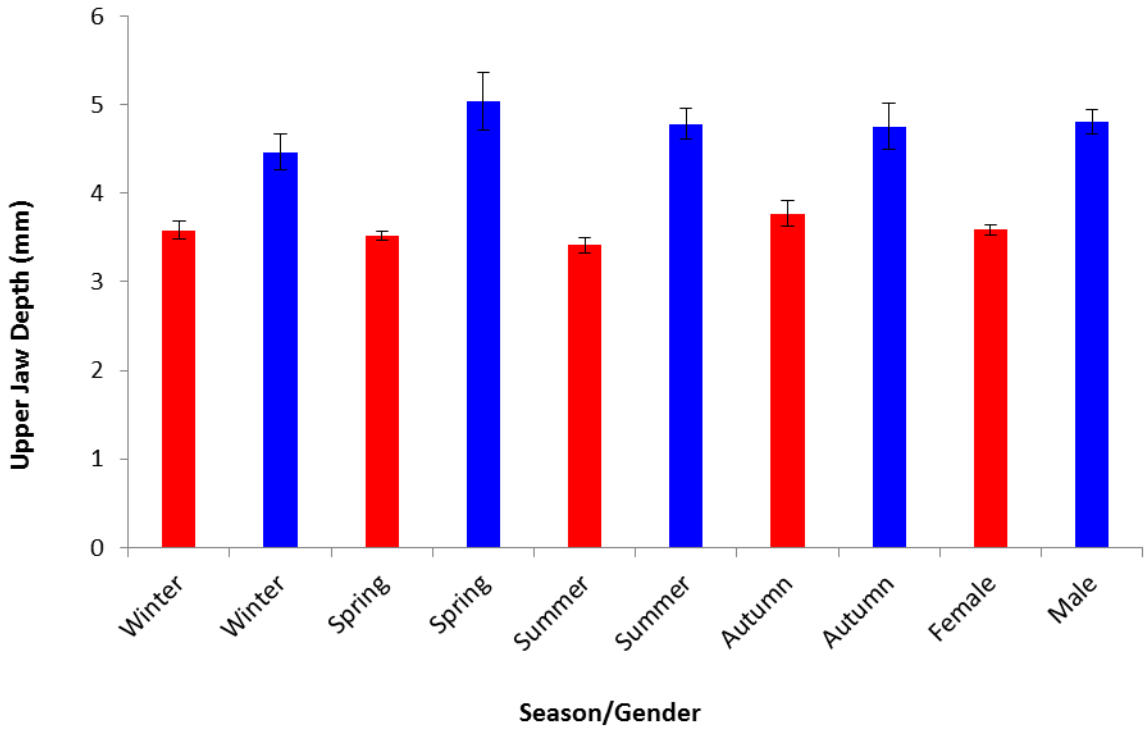

Figure 3.32. Gender and seasonal comparison of upper jaw depth for adult male and female catsharks showing means and ± standard errors (n= Female (35) (W, 9) (Sp, 9) (Su, 7) (A, 10) Male (28) (W, 4) (Sp, 9) (Su, 6) (A, 9)) (Male = Blue, Female = Red).

Table 3.14 shows that season and season within gender had no effect on the upper jaw depth of adult male and female catsharks. Body length and gender had a significant effect on the upper jaw depth of adult male and female catsharks. Males sampled in all seasons had significantly deeper jaws than females sampled in all season.

Table 3.14. Results from the ANCOVA analyses for the upper jaw depth of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	3.13	1.89	1.89	6.76	**0.012**
Gender	1	21.65	18.07	18.07	64.72	**<0.001**
Season	3	0.70	0.95	0.32	1.14	**0.342**
Season*Gender	3	1.27	1.27	0.42	1.51	**0.221**

Figure 3.33 shows a graphical representation of the body length and upper jaw depth of adult male and female catsharks. The correlation between body length and upper jaw depth were not significantly different for adult male (ANOVA, F=1.91; d.f.=1; *P*= 0.180) and adult female (ANOVA, F=0.89; d.f.=1; *P*= 0.351). The regression between the upper jaw depth of adult male and female catsharks was not significant (*P*= 0.698).

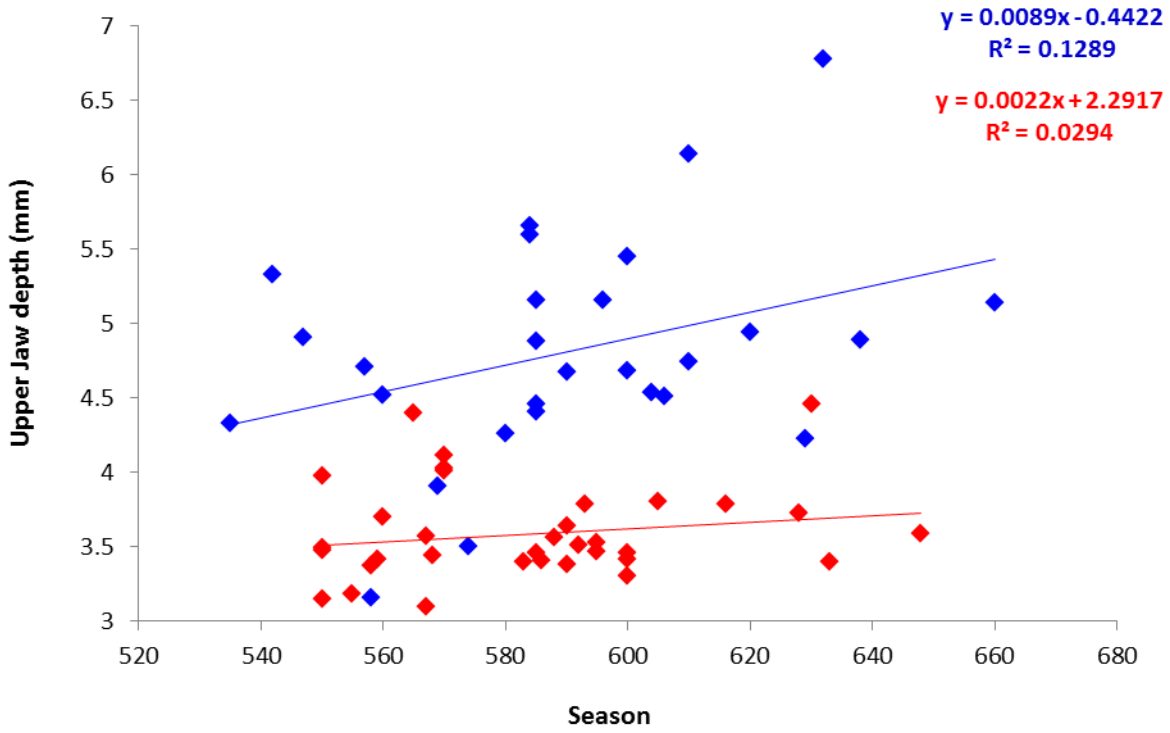

Figure 3.33. Scatterplot with regression showing upper jaw depth against body length for adult male and female *S. canicula* (Male = Blue, Female = Red) (n=M (28) F (35)) (*P*= 0.698).

3.3.5.5 Lower Jaw Length

Figure 3.34 shows a graphical representation of the lower jaw length of adult male and female catsharks for all four seasons. Lower jaw length in adult females was less than lower jaw length in adult males throughout the year.

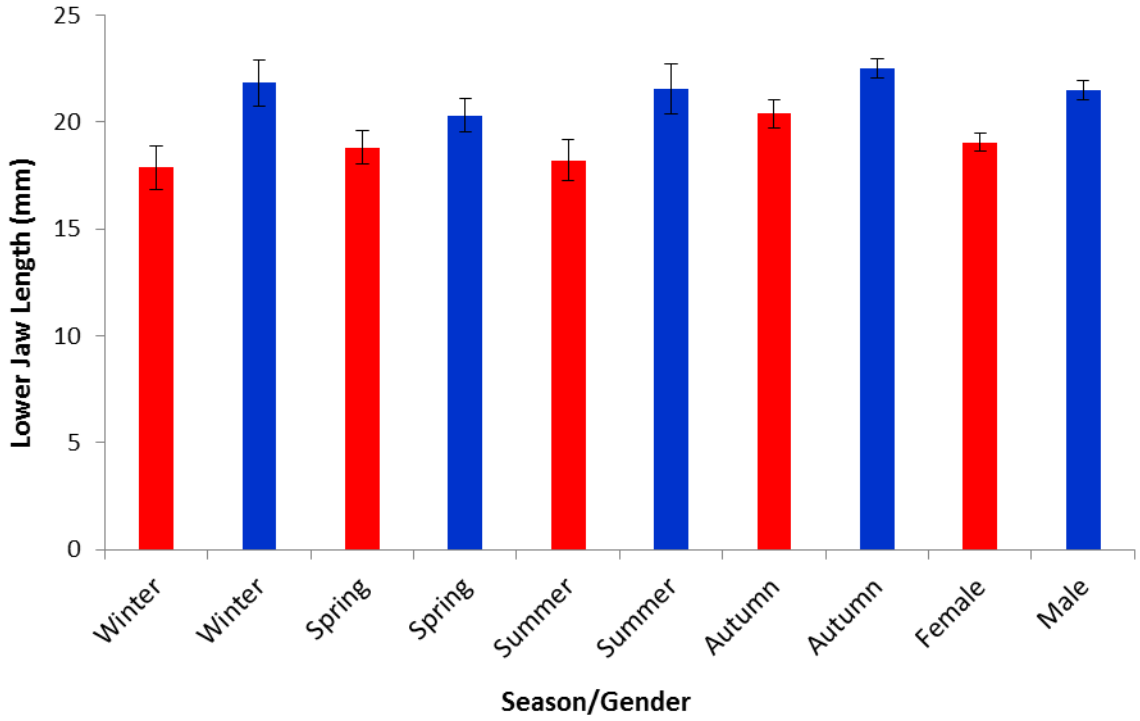

Figure 3.34. Gender and seasonal comparison of lower jaw length for adult male and female catsharks showing means and ± standard errors (n= Female (49) (W, 9) (Sp, 11) (Su, 12) (A, 17) Male (38) (W, 4) (Sp, 13) (Su, 8) (A, 13)) (Male = Blue, Female = Red).

Table 3.15 shows that body length, season and season within gender had no effect on the lower jaw length of adult male and female catsharks. Gender had a significant effect on the lower jaw length of adult male and female catsharks, with males having a greater lower jaw length than females.

Table 3.15. Results from the GLM analyses for lower jaw length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	22.57	9.42	9.42	1.26	**0.264**
Gender	1	120.43	128.28	128.28	17.22	**<0.001**
Season	3	64.35	54.43	18.14	2.44	**0.071**
Season*Gender	3	14.60	14.60	4.87	0.65	**0.583**

3.3.5.6 Lower Jaw width

Figure 3.35 shows a graphical representation of the lower jaw width of adult male and female catsharks for all four seasons. The data shows that within each season, except summer, the lower jaw width of adult male and female catsharks were very similar in size, with very little difference evident throughout the seasons.

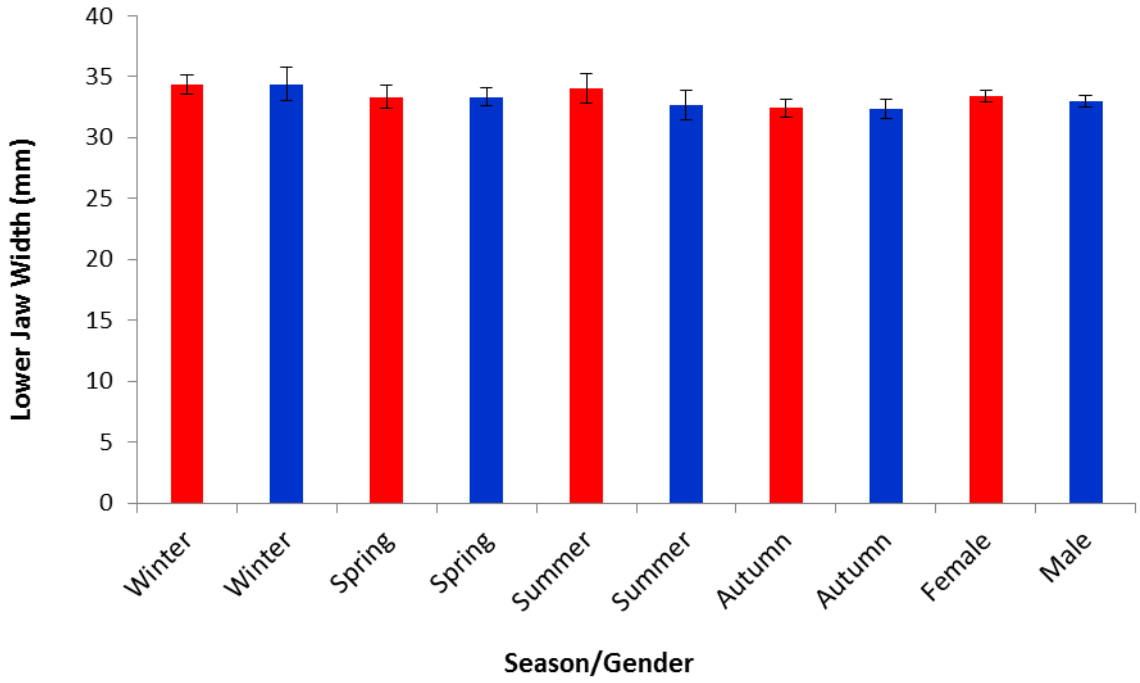

Figure 3.35. Gender and seasonal comparison of lower jaw width for adult male and female catsharks showing means and ± standard errors (n= Female (49) (W, 9) (Sp, 11) (Su, 12) (A, 17) Male (38) (W, 4) (Sp, 13) (Su, 8) (A, 13)) (Male = Blue, Female = Red).

Table 3.16 shows that gender, season and season within gender had no effect on the lower jaw width of adult male and female catsharks. Body length had a significant effect on the lower jaw width of adult male and female catsharks.

Table 3.16. Results from the GLM analyses for lower jaw width of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	94.52	105.70	105.70	12.78	**0.001**
Gender	1	7.05	6.32	6.32	0.76	**0.385**
Season	3	44.67	39.40	13.13	1.59	**0.199**
Season*Gender	3	8.80	8.80	2.93	0.35	**0.786**

Figure 3.36 shows a graphical representation of the body length and lower jaw width of adult male and female catsharks. There was a significant correlation between body length and lower jaw width (ANOVA, F=11.82; d.f.=1; $P< 0.001$). It can be seen that as body length increases so does the lower jaw width of adult *S. canicula*.

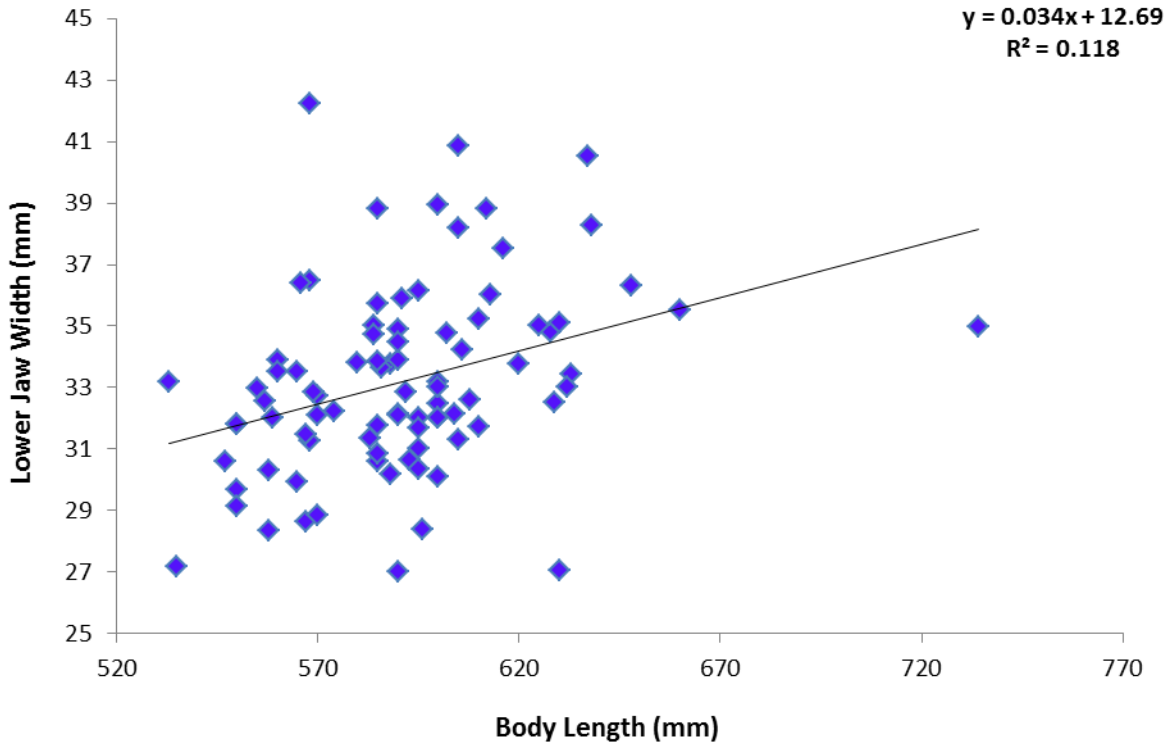

Figure 3.36. Scatterplot with regression showing lower jaw width against body width for adult male and female *S. canicula* (n= 87) (*P*< 0.001).

3.3.5.7 Lower Jaw Diameter

Figure 3.37 shows the means and standard errors for the lower jaw diameter in male and female adult catsharks. It can be seen that females in winter possessed the largest lower jaw diameter of all groups sampled, although the results of the GLM revealed that there were no significant differences in the lower jaw diameter of adult male and female *S. canicula*.

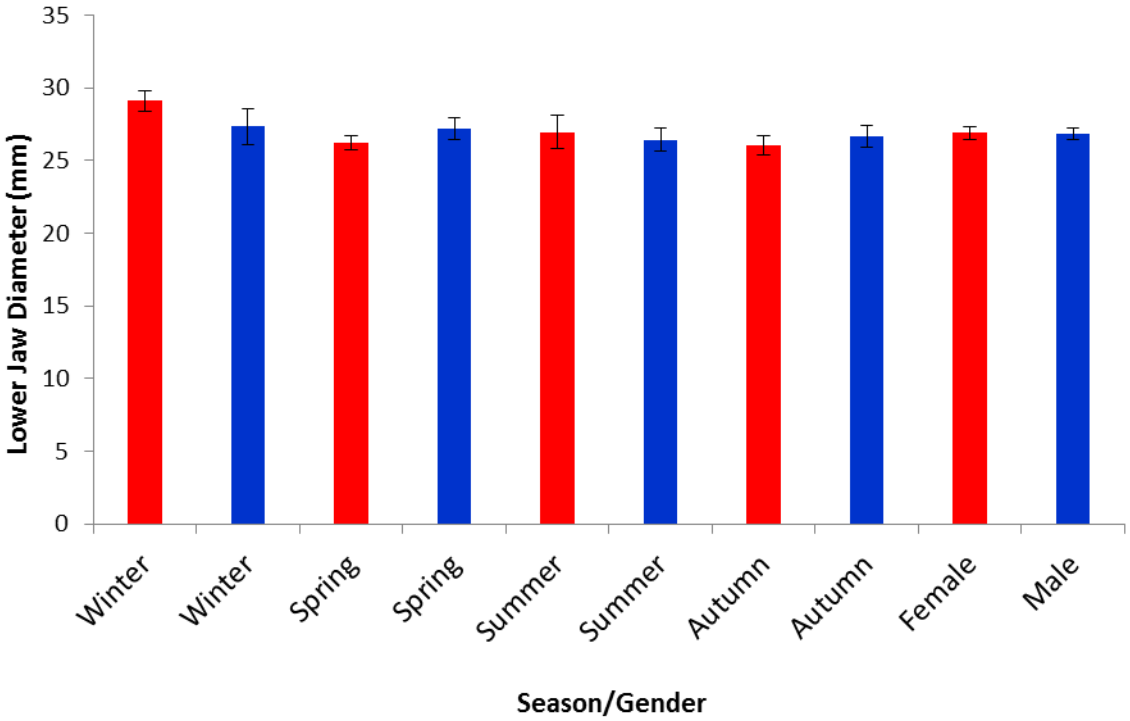

Figure 3.37. Gender and seasonal comparison of lower jaw diameter for adult male and female catsharks showing means and ± standard errors (n= Female (42) (W, 9) (Sp, 9) (Su, 8) (A, 16) Male (32) (W, 4) (Sp, 9) (Su, 6) (A, 13)) (Male = Blue, Female = Red).

Table 3.17 shows that gender, season and season within gender had no effect on the lower jaw diameter of adult male and female catsharks. Body length had a significant effect on the lower jaw diameter of adult male and female catsharks.

Table 3.17. Results from the ANCOVA analyses for the lower jaw diameter of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	32.84	33.93	33.93	6.01	**0.017**
Gender	1	0.42	1.74	1.74	0.31	**0.581**
Season	3	44.23	26.04	8.68	1.54	**0.213**
Season*Gender	3	20.03	20.03	6.68	1.18	**0.323**

Figure 3.38 shows a graphical representation of the body length and lower jaw diameter of adult male and female catsharks. The was a significant correlation between lower jaw diameter and body length (ANOVA, F=5.53; d.f.=1; *P*= 0.021). It can be seen that as body length increases so does the lower jaw diameter of adult *S. canicula*.

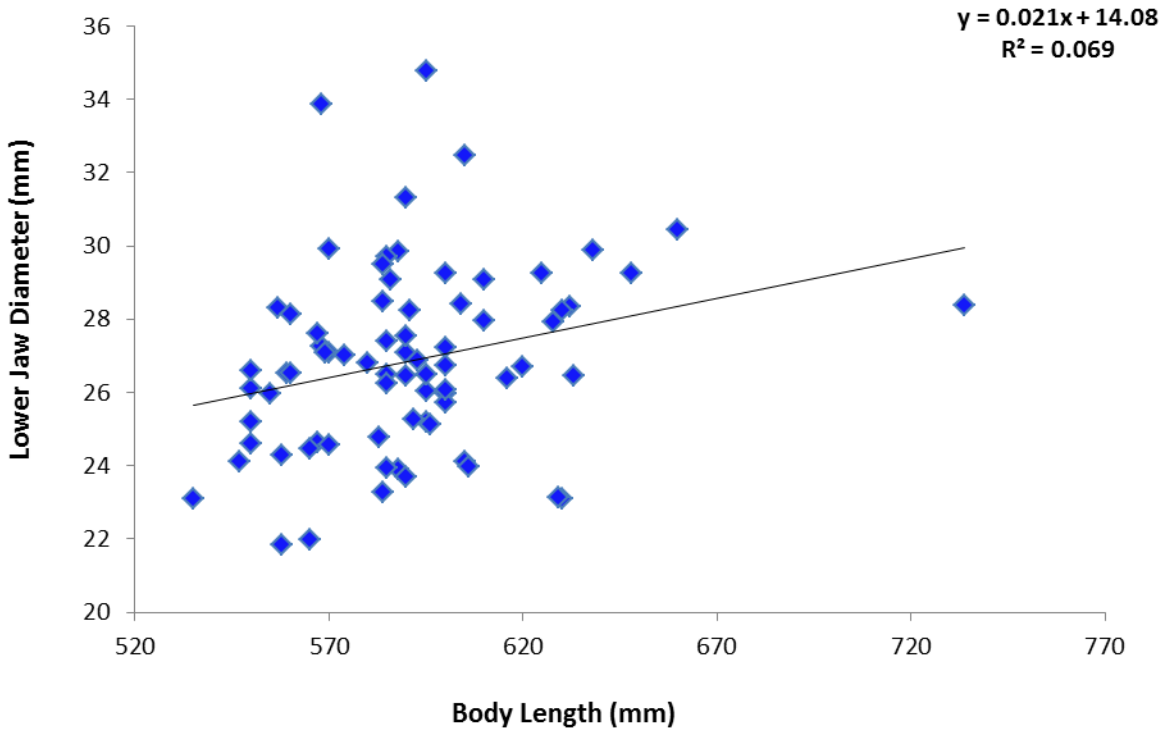

Figure 3.38. Scatterplot with regression showing lower jaw diameter against body length for adult male and female *S. canicula* (n= 75) (*P*= 0.021).

3.3.5.8 Lower Jaw Depth

Figure 3.39 shows the means and standard errors for the lower jaw depth in male and female adult catsharks. It is clear from the data that adult males have a deeper lower jaw than adult females, with adult male catsharks in winter and spring having a greater jaw depth than all other groups sampled.

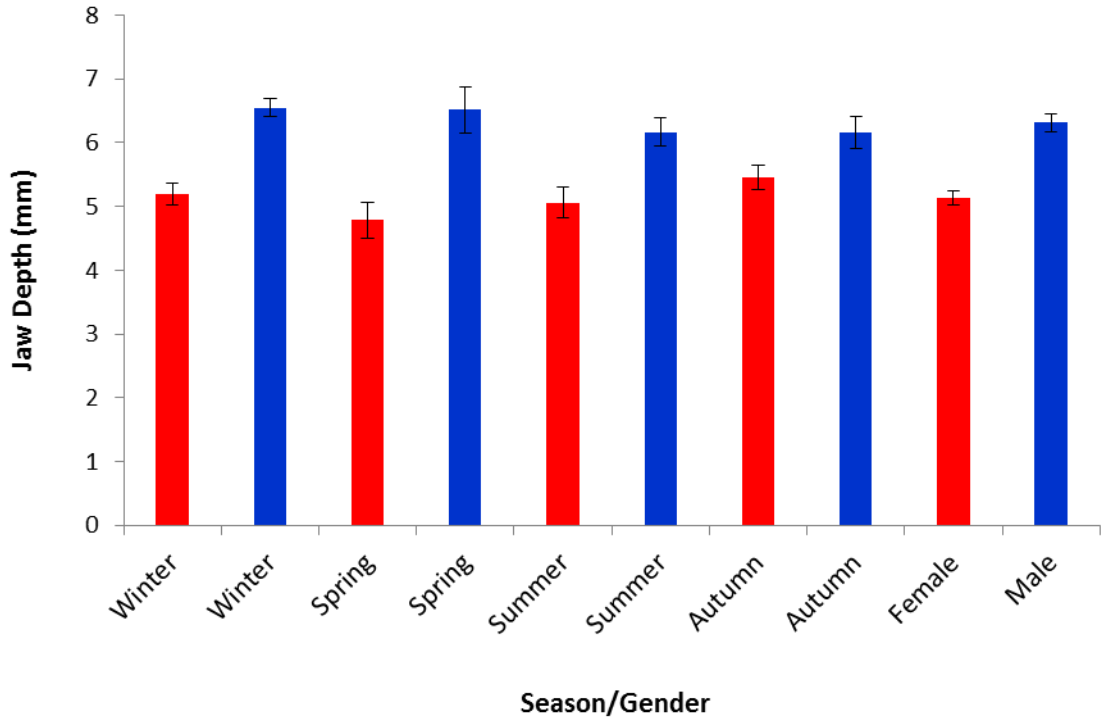

Figure 3.39. Gender and seasonal comparison of lower jaw depth for adult male and female catsharks showing means and ± standard errors (n= Female (42) (W, 9) (Sp, 9) (Su, 8) (A, 16) Male (32) (W, 4) (Sp, 9) (Su, 6) (A, 13)) (Male = Blue, Female = Red).

Table 3.18 shows that body length, season and season within gender had no effect on the lower jaw depth of adult male and female catsharks. Gender had a significant effect on the lower jaw depth of adult male and female catsharks. Males had significantly deeper lower jaws than females.

Table 3.18. Results from the ANCOVA analyses for the lower jaw depth of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	3.83	1.67	1.67	2.94	**0.092**
Gender	1	20.91	19.83	19.83	34.78	**<0.001**
Season	3	0.28	0.39	0.13	0.23	**0.877**
Season*Gender	3	3.36	3.36	1.12	1.97	**0.129**

3.3.6 Discriminant Analysis

Canonical discriminant analysis was carried out on the jaws of both adult and juvenile *S. canicula*. The pooled data of Jaw Width, Jaw Length, Jaw Diameter and Jaw Depth for males and females were analysed separately to ascertain if there were any differences in the structure of the upper and lower jaws between adults and juveniles. Figure 3.40 shows the results for the adult and juvenile male and female upper and lower jaw analysis. The canonical discriminant analysis showed that significant differences were observed between functions 1 and 5 (Wilks-Lambda, $P< 0.001$) functions 2–5 (Wilks-Lambda, $P< 0.001$) functions 3–5 (Wilks-Lambda, $P< 0.001$) but not functions 4-5 (Wilks-Lambda, $P> 0.05$) nor function 5 (Wilks-Lambda, $P> 0.05$). The first function accounts for 57.5% of the total variation with Jaw Depth showing the strongest correlation with this discriminating function. It can be seen from Figure 3.39 that there are clear gender distinctions between the upper and lower jaw dimensions of all adult and juvenile female catsharks with the upper and lower jaw morphology of each gender in each size class being distinctly different. It can also be seen from Figure 3.40 that there is a clear distinction between the upper and lower jaws in both male and female and adult and juvenile catsharks.

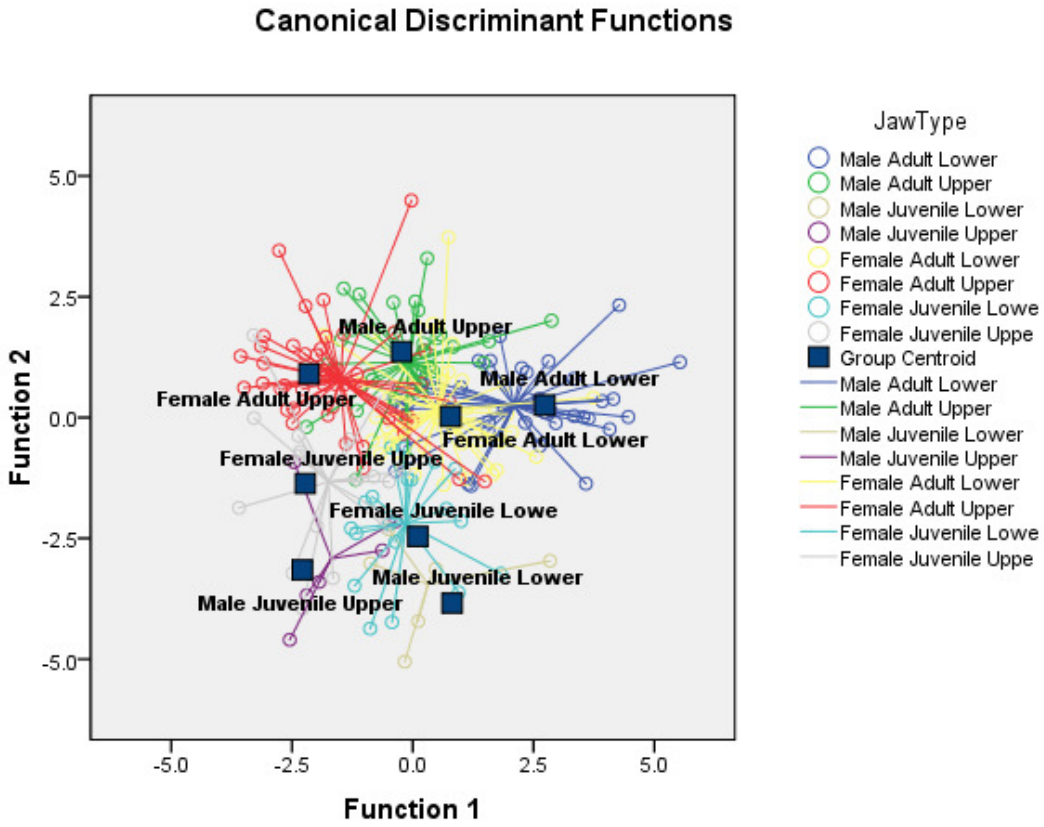

Fig. 3.40. Discriminant analysis of morphometric characters of the upper and lower jaws of adult and juvenile male and female *S. canicula*.

3.4 Discussion

Some literature exists on the head and mouths of elasmobranch species (Brough, 1937; Arthur, 1950; Gosztonyi, 1973; Ellis and Shackley, 1995; Kajiura *et al*., 1995; Nakaya, 1995; Filiz and Taskavak, 2006, Kajiura, 2001). Kajiura *et al.* (2005) found that the shape of the cephalofoil of bonnethead sharks showed a secondary sexual dimorphism. They stated that the females possess a broadly rounded anterior margin to the cephalofoil whereas the male cephalofoil is characterised by a distinct bulge along the anterior margin (Figure 3.41).

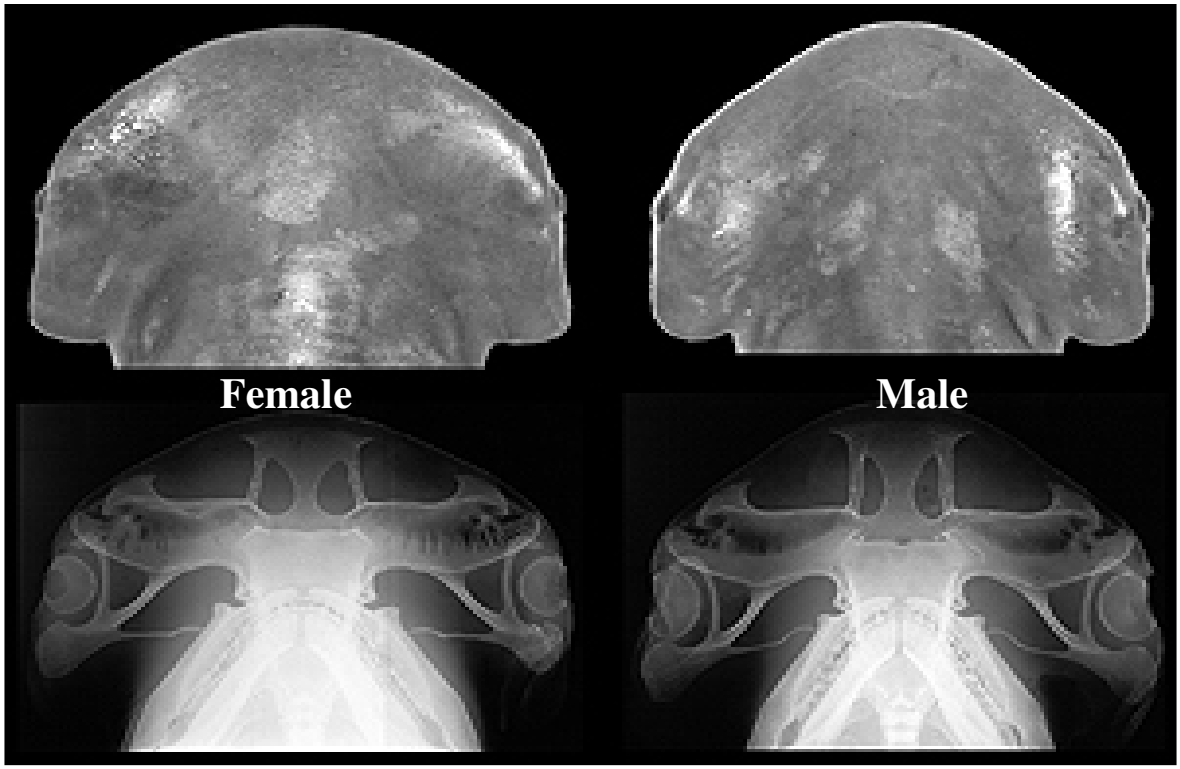

Figure 3.41. The cephalofoil of male and female bonnethead sharks (Adapted from Kajiura *et al*., 2005).

Much of the research focusing on head morphometrics relates directly to the function and structure of the jaw (Ellis and Shackley, 1995; Frazzetta, 1994; Wu, 1994; Motta and Wilga, 1995; Motta *et al*., 1997; Motta and Wilga, 2001; Motta, 2004; Fahle and Thomason, 2008; Gerry *et al*., 2008).

Several authors have found that the shape and size of the head, mouth and jaws of several elasmobranch species are sexually dimorphic (Brough, 1937; Bas, 1964; Jardas, 1979; Wu, 2008; Ellis and Shackley, 1995; Filiz and Taskavak, 2006). To date little, if any, literature exists on the seasonal dimorphisms of the head, mouth and jaws in elasmobranch species. As far as the author is aware there is no research investigating seasonal dimorphisms of these morphometrics parameters for the lesser-spotted catshark, *S. canicula*.

3.4.1 Head and Mouth Data

3.4.1.1 Hatchling Head and Mouth Morphometrics

The results of the study revealed that the heads of hatchling *S. canicula* were not sexually dimorphic. Body length affected the width and pre-oral length, but not the length of the heads of hatchling catsharks, showing that the larger individuals possessed larger mouth widths and pre-oral lengths. There was also no sexual dimorphism found in the mouths of hatchling *S. canicula.* These findings concur with those of Brough (1937) who noted that male specimens of *S. canicula* possessed longer mouths than those of females. Brough (1937) added that this increase in mouth length was only apparent in mature individuals and that the change in mouth shape occurred quickly at the onset of maturity. Other research focused on the head morphometrics of hatchling catsharks. Fahle and Thomason (2008) made some note of the head girth of hatchling *S. canicula*, noting that the girth ranged from 22–28 mm. However, they didn't investigate the possibility of any sexual dimorphism and therefore it is not clear whether the lack of any sexual dimorphism in the heads of hatchling *S. canicula* is common amongst all populations.

3.4.1.2 Juvenile Head and Mouth Morphometrics

No sexual dimorphism was found in the heads of juvenile male and female catsharks. Body length had no effect on the pre-branchial length or pre-oral length, although it did affect the head width, with larger catsharks showing a greater head width. The results suggest that mouth length of juvenile catsharks were sexually dimorphic, with juvenile male catsharks, having longer mouths than juvenile female catsharks. The width of the mouth of juvenile catsharks was not found to be sexually dimorphic. These initial findings appeared to contradict those of Brough (1937) who noted that the mouth was narrower in male *S.*

canicula, but only became so with the onset of maturity. It is possible that different populations experience the onset of maturation at different rates, due to the environmental conditions in which they live. If this is the case, the population of *S. canicula* from the Solent may begin to develop secondary sexual dimorphisms before they reach full sexual maturity. However, it is apparent that the small sample sizes used for this study could be affecting the data.

Many shark species have been shown to sexually segregate (Yano and Tanaka, 1988; Economakis and Lobel, 1998) and this has been reported for *S. canicula*. Compagno (1984) reported that juvenile *S. canicula* were distributed in shallower water than adults. In the Cantabrian Sea, Rodriguez-Cabello *et al.* (2004) found that the distribution of *S. canicula* is continuous along the continental shelf although they may aggregate by sex or size. Juveniles were found mostly at depths around 200m, while adults had a wider depth distribution, 50-450 m. Work by Lyle (1983) showed that despite these reported size and gender segregations that there were no differences in the prey types consumed by *S. canicula*. A range of sizes of catshark were examined, the smallest of which was 29cm. This indicated that there were no gender differences in feeding behaviours of juvenile lesser-spotted catsharks. Henderson and Dunne (1999) also examined the stomach contents of 144 specimens of *S. canicula* from Galway Bay. They note the similar findings to Lyle (1983) stating that the food consumed by catsharks ranging in size from 35cm to 75cm did not differ between the genders. If there is a sexual dimorphism in the mouth of juvenile catsharks from the Solent, as indicated, it is possibly due to reproduction later in life as suggested by Ellis and Shackley (1995) and not for differences in prey preferences.

3.4.1.3 Adult Head and Mouth Morphometrics

The head width and pre-oral length for adult *S. canicula* showed no sexual or seasonal dimorphisms. The head width was affected by body length, with larger individuals possessing a wider head. The pre-branchial length of adult *S. canicula* was found to be seasonally dimorphic. However, the small number of specimens caught during the autumn months could have affected the results. Adult catsharks sampled in spring had longer heads than adult catsharks sampled in winter and summer. It has been noted by fishermen in the Solent that catches of *S. canicula* in April and May consist of both males and females, but at all other times of the year only males or females are caught. This supports the theory of Sims (2005) that male and female catsharks segregate sexually. Compagno (1984) found that adult *S. canicula* often occurred in unisexual schools, whilst research by Rodriguez-Cabello *et al.* (2004) found that *S. canicula* in the Cantabrian Sea may aggregate by sex or size. During sampling it was also noted that males demonstrated red, crossed claspers that were running milt in some individuals sampled during the spring and early summer months (pers. obs.). The differences in head morphology found in this study could be due to the fact that *S. canicula* demonstrates a specific mating season as suggested by Harris (1952) and not a protracted one as noted by Ford (1921) and Wourms (1977).

There was a distinct sexual dimorphism found in both the mouth length and mouth width for adult *S. canicula*. Body length had no effect on mouth length, but did have an effect on mouth width, with larger individuals possessing wider mouths. Gender had an effect on the mouth length of adult *S. canicula*, with males having longer mouths than females. Adult female catsharks were found to possess wider mouths than adult male catsharks. There was a seasonal difference found in the mouth length of adult catsharks, with specimens sampled in winter possessing a longer mouth than adults sampled in all other seasons. Again, the small sample sizes of some groups could have affected these data. However,

these findings agree with those found in the literature (Brough, 1937; Arthur, 1950; Gosztonyi, 1973; Ellis and Shackley, 1995; Filiz and Taskavak, 2006). The reasons for these dimorphisms is suggested to be related to reproduction (Ellis and Shackley, 1995) whereby the males possess longer, narrower mouths in order to grasp the pectoral fins of the females more firmly prior to clasper insertion. The presence of a seasonal dimorphism in mouth length may indicate a mating period, although anecdotal evidence suggested that the mating season for this species in the Solent is later in the year.

3.4.2 Jaw Morphometrics

3.4.2.1 Hatchling Upper and Lower Jaw Morphometrics

The jaws of hatchling catsharks showed very little in the way of sexual dimorphism. The upper jaw length and the lower jaw length and width were not sexually dimorphic. The upper jaw width was sexually dimorphic with hatchling male catsharks possessing a wider jaw than hatchling female catsharks. This is contrary to the findings of Brough (1937) who found that the jaws of male catsharks were narrower than those of female catsharks. The samples examined by Brough (1937) were adults and could have differed in morphology from hatchling catsharks. The head measurements of hatchling *S. canicula* showed no sexual dimorphism, although the results for the mouth dimensions show that male hatchling catsharks possess a narrower mouth than female hatchling catsharks.

3.4.2.2 Juvenile Upper and Lower Jaw Morphometrics

The jaws of juvenile catsharks showed a similar pattern to those of hatchling catsharks, whereby there was very little sexual dimorphism present. Body length and gender had no effect on the upper jaw length and jaw depth, or the lower jaw width. Body length did have

an effect on the upper jaw width and jaw diameter and the lower jaw length and jaw diameter, with the larger specimens possessing increased jaw dimensions. The initial data suggests that the jaw depth of juvenile *S. canicula* is sexually dimorphic, with juvenile males possessing a greater jaw depth than juvenile females, although a greater sample size would have confirmed these findings. It is possible that if the jaw depth is sexually dimorphic that this is due to the fact that the juvenile male catsharks are possibly nearing maturity and the jaw is developing to accommodate the onset of the development of larger, unicuspid teeth associated with sexual maturity. According to Moss (1977) the teeth of *S. canicula* were found to be small, multi-cusped teeth that are well suited for grasping, rather than shearing. Moss (1972) found that in *Mustelus canis* many individuals possessed sheared or smashed teeth in the lower jaw dentition. This possibly indicated that the lower jaw is used for grasping prey, or possibly mates, and more pressure is placed on this part of the jaw. As it is well documented that the males of many elasmobranch species, including *S. canicula*, bite during copulation it is feasible that males will develop a deeper jaw to accommodate the larger teeth used for grasping females prior to clasper insertion.

3.4.2.3 Adult Upper Jaw Morphometrics

There were a number of sexual and seasonal dimorphisms found in the upper jaw of adult *S. canicula*. Body length had effects on all parameters of the upper jaw that were measured. It was evident that the larger the individual the larger the dimensions of the jaw. The jaw length in adult catsharks was found to be sexually dimorphic, with males having a greater jaw length than females. Jaw width was also found to be seasonally dimorphic with catsharks sampled in winter possessing a wider jaw than catsharks sampled in spring and summer.

The data strongly agrees with the findings of other authors who found that the jaws of male catsharks were narrower and longer than those of female catsharks. Brough (1937) found that the intermandibular separation (Jaw diameter) of the jaw of male *S. canicula* was less than that of females. The pattern of jaw morphometrics in adult catsharks closely follows the dimensions of the head with males possessing a longer narrower head, and therefore jaws, while females possess a shorter wider head, hence shorter wider jaws.

Jaw depth was also found to be sexually dimorphic. Male catsharks sampled in all seasons were found to have a greater jaw depth than females sampled in all seasons. This increased jaw depth is present possibly to accommodate not only larger teeth, but increased tooth rows. In many elasmobranch species, including *S. canicula*, upper jaw protrusion is evident. Protrusion is an integral part of feeding behaviour in most sharks and likely serves numerous functions (Wilga and Motta, 2001). Protrusion of the upper jaw is also believed to facilitate the cutting action of the teeth and allow deep gouging bites to be made into oversized prey (Moss 1977; Tricas & McCosker 1984; Wilga and Motta, 2001). According to Moss (1972) upper jaw protrusion may enable the shark to grasp items from the substrate with more precision and this is especially developed in benthic species such as *S. canicula*. Frazzetta & Prange (1987) stated that in addition, nearly simultaneous protrusion of the upper jaw while the lower jaw is elevating may also provide the shark with a better grasp of struggling or elusive prey. This may also be the case when the sharks are reproducing. Males must ensure a firm grip on the female, either on the pectoral fin or the area behind the head. Not unlike the capture of prey, during copulation female sharks have been witnessed to struggle (Pratt and Carrier, 2001) forcing the males to maintain a firm grasp. In order to achieve this male sharks may possess larger teeth and in return require the jaw depth to be greater in order to accommodate the increased tooth size and possibly more tooth rows.

3.4.2.4 Adult Lower Jaw Morphometrics

Sexual dimorphisms were found to exist in the lower jaws of adult catsharks. Body length had an effect on both jaw width and jaw diameter, but there were no sexual dimorphisms found for these parameters. The length of the lower jaws was found to be sexually dimorphic, with males possessing longer lower jaws than females. Jaw depth followed the same pattern, with males possessing a deeper jaw than females. The sexual dimorphism is more defined in the lower jaw and possibly relates directly back to the use of the lower jaw for anchoring prey, or mates, due to the grasping nature of the teeth (Moss, 1977). However, the

Brough (1937) noted that the changes in the lower jaw structure correlate to sexual maturity and the sexual dimorphic characters are more pronounced in the mating season. However, there was no indication of a seasonal dimorphism in the lower jaws of adult *S. canicula* from the present study despite the occurrence of a seasonal dimorphism in pre-branchial length of this species. The lack of a seasonal dimorphism could be down to the small sample size for the seasonal groups. However, it seems that the increase in jaw depth of the lower jaw coincides with the possible increase of tooth size in adult catsharks.

3.4.3 Discriminant Analysis

The results of canonical discriminant analysis showed that there was a clear distinction between the structure of the upper and lower jaws of juvenile and adult male and female *S. canicula*, possibly related to growth rates between adults and juveniles. This difference is highlighted more in the female jaw structures with a clearer separation of the upper and lower jaws of juvenile and adult female catsharks. The reason for the greater difference between the female adult and juvenile catshark jaws is unclear, especially if adult female

teeth do not develop as much as the adult male teeth. It was also noted that there was greater overlap in the jaw dimensions between adult and juvenile male catsharks than in adult and juvenile female catsharks. The smaller numbers of juvenile female catsharks sampled could be a reason for this overlap.

In conclusion, the data obtained for this study revealed that the population of *S. canicula* from the Solent are, to varying degrees, sexually dimorphic in terms of head, mouth and jaw dimensions. Contrary to other research, juvenile catsharks from the Solent potentially showed a sexual dimorphism in the mouth. The findings also reveal that adult male *S. canicula* from the Solent have longer, narrower mouths than female *S. canicula* much in keeping with previously published data. There is also a potential seasonal dimorphism in relation to head morphometrics, findings that have not been previously reported. However, in order to reveal the true extent of these sexual and seasonal dimorphisms, more research, with larger sample sizes, needs to be carried out to determine if the Solent population shows a seasonal dimorphism in relation to a distinct mating season.

The next chapter will investigate the structure and function of the dentition of elasmobranchs and in particular *S. canicula*. The chapter will involve structural measurements and morphometric investigations to determine if any further sexual dimorphisms exist with regards to the dentition of *S. canicula.*

Chapter 4 – Teeth

4.1. Introduction

4.1.1 Tooth Development

Tooth development in elasmobranch species has been well documented and carries certain similarities to that of other Gnathostomata, or jaw-bearing vertebrates. According to Reif (1984) shark dentitions are complex and undergo developmental processes throughout the ontogeny of an individual. Like dentitions of all Gnathostomata, dentitions of sharks are formed by a dental lamina, or a band of epithelial tissue (Reif, 1984).

James (1953) stated that the development of teeth in all animals depends upon the formation of a dental epithelial structure known as an enamel organ. The enamel organ is formed from a band of ectodermal cells growing from the epithelium, or dental lamina, of the embryonic jaws into the underlying mesenchyme. The anatomical form and function of this organ is well recognised and documented (James, 1953). During development the enamel organ becomes bell-shaped and the hollow of the bell is lined with a single layer of columnar cells known as the internal dental epithelium (James, 1953). These cells are responsible for the formation of dentine. As dentine is the first calcified tooth tissue to be formed, the shape of the crown is determined by the internal dental epithelial layer (James, 1953). The epithelial ingrowth from the dental lamina, which covers the dental papilla, furnishes a mould for the shape of a developing tooth, and forms the dental enamel. Enamel may be added to the dentine later, and is seen upon the external surface of the exposed part of the completed tooth. However, Grady (1970) disputed the presence of tooth enamel in sharks and indicated that the highly mineralised outer layer of tissue found on the tips of shark teeth has been the subject of controversy for nearly a hundred years.

According to Grady (1970) the highly mineralised outer cap of tissue on shark teeth is not enamel but a form of modified dentine.

The epithelial ingrowth described by James (1953) is an ectodermal fold which develops during embryogenesis. In sharks, teeth are formed in the anterior interface between the ectodermal fold and the surrounding mesoderm. In the same way as the fold deepens during embryogenesis new teeth are added at the basal end of the dental lamina. Tooth germs are constantly transported upwards in the fold throughout ontogeny. It should therefore be assumed that the cell clusters which differentiate into tooth germs are derived from the basal part of the dental lamina (Reif, 1984). Near the end of embryogenesis the deepening of the fold ends and the teeth are transported into position by a conveyor belt system situated between the jaw cartilage and the dental lamina (Reif, 1984).

4.1.2 Tooth Replacement

The replacement of teeth in elasmobranch species has been well documented. As far back as the late 18th century an attempt was made to prove that the teeth of sharks are perpetually renewed. Andre (1784) stated that the teeth of elasmobranch were continuously replaced and that the anterior teeth appeared to have been replaced up to twelve times. Ifft & Zinn (1948) concurred with this theory, stating that shark teeth are continually replaced as the animal grows. Moss (1972) noted that a characteristic feature of elasmobranchs is the apparently continual replacement of upper and lower jaw teeth throughout their lifetimes. The jaws of sharks are characterised by having several rows or sets of teeth in succession, a type of dentition called polyphyodont Luer *et al*. (1990).

However, despite all of the evidence, not all authors support the theory of tooth replacement in sharks. Cawston (1938) found little evidence of tooth loss in sharks and

suggested that sharks didn't lose teeth under natural conditions, but only lost teeth during capture or from contact with a metal object or the spines of stingrays. The report went on to add that the dentition of sharks is a complete entity and there is no constant replacement of lost teeth. Cawston (1938) also pointed out that sharks teeth are not found in aquaria where they would often occur if they were constantly being shed under natural conditions. This observation was not agreed upon by Breder (1942) who found that a large number of teeth from sand tiger sharks were found to be littering the floor of the aquarium's shark exhibit. Cawston (1938) also noted that as bony fish replace their teeth at the site where one has been lost there is no good reason for supposing that sharks do not do the same. By direct observation, Breder (1942) observed specific teeth as they moved forward in the sharks jaw and were eventually shed. One theory presented by Breder (1942) in response to the claims made by Cawston (1938) regarding the absence of shed teeth was that the teeth could have also been lost to the digestive tracts of the sharks by ingestion.

The methods by which elasmobranch species replace teeth are not seen in any other living animal group. The continuous replacement of teeth throughout their lifetimes is a well known phenomenon in modern sharks (Williams, 2001). The movement of the developing and formed teeth of elasmobranchs has long been recognised, an alteration in position that does not occur to the same degree in other animals (James, 1953). There are some differences that make tooth succession in elasmobranchs unique. In other animals where tooth succession occurs the teeth are usually ankylosed to the underlying bone. In elasmobranchs the mode of attachment of the teeth by the fibrous tissues, or Sharpey's fibres, is quite distinctive and characteristic (James, 1953).

According to Wetherbee *et al.* (1997) the fully formed teeth erupt from the gum at the outer jaw margin, are fully functional for a short time and are shed as the next tooth in the series takes its place. Luer *et al.* (1990) found that the order in which teeth are shed from

the outer jaw does not always follow a consistent pattern. The methods by which elasmobranchs shed their teeth differ from species to species. A comparison of the number of semi-erect replacement teeth to the number of functional teeth in 13 species of shark carried out by Strasbourg (1963) indicated that tooth replacement rates are variable between species. Overstrom (1991) noted that some species shed nearly all of their teeth, either individually or in entire sets of upper or lower dentition, whereas others shed only a few periodically. Strasburg (1963) observed that the cookiecutter shark, *Isistius brasiliensis,* shed its relatively large triangular teeth as a set and not individually. However, Castro (1983) noted that continuous tooth replacement is common to all elasmobranchs that had been studied. Reif (1984) supported this, stating that in any given jaw a large number of replacement teeth are present in addition to the functioning teeth. Reif (1984) also noted that shark dentitions are always organised into tooth families, i.e. a functional tooth and its successors. This observation was also made by Andre (1784) who classified sharks teeth into two groups, passive and active. Active teeth being described as the anterior teeth that were standing with their point upwards, whilst the passive teeth were described as those that were lying one upon the other, like tiles upon a house.

The rate at which teeth are replaced varies both within and between species and can be influenced by age, diet, seasonal changes and water temperature (Motta, 2004). Luer *et al.* (1990) found that the rates of tooth replacement in the nurse shark, *Ginglymostoma cirratum,* did not decrease with increasing size as the animal aged, but varied during each year depending on water temperature.

Markel and Laubier (1969) measured the time taken for a tooth to move from one position to the next in the lesser-spotted catshark. They concluded that the replacement rates for *S. canicula* can take a maximum of 12 weeks, whereas the tooth replacement rate in immature specimens of the dusky smoothound, *Mustelus canis,* was determined to be

approximately one tooth row every 10-12 days. Many authors have measured the tooth replacement rates in other species. Replacement rates varied from 8-10 days for the lemon shark, *Negaprion brevirostris* (Moss, 1967) 9-12 days for the leopard shark, *Triakis semifasciata* (Reif, 1978a) 9-28 days for the nurse shark, *Ginglymostoma cirratum,* in summer and 51-70 in winter (Reif, 1978a, Luer *et al*., 1990) and approximately 28 days for *Heterodontus* (Reif, 1976).

Many authors have studied the tooth replacement rates of a range of elasmobranch species and it appears that different life stages of individuals dictates the rate at which teeth are replaced. It was discovered that even before birth sharks start shedding teeth. The uterus of a pregnant great white shark and the stomach of its 1.2 meter unborn pup contained teeth that had been shed by the unborn shark (Anonymous, 1996).

Tooth shedding in elasmobranch species appears to perform two functions. The first being to increase tooth size as an individual grows. Luer *et al.* (1990) suggested that tooth shedding accounts for the continual presence of a complete dentition, containing teeth which are at a size relative to growth. According to Moss (1972) tooth replacement is therefore related to body growth in sharks. It is further suggested that as an individual grows the food preferences alter and as a result the teeth need to increase in size to accommodate the changes in diet (Lyle, 1983). Cawston (1938) noted that sharks teeth increase in size with the age of the fish. Research carried out by Luer *et al.* (1990) found that the size of the functional teeth increased as the total length of the animal increased. Wetherbee *et al.* (1997) also found that, in general, the replacement teeth need to be larger than the functional ones in order to accommodate growth while maintaining tooth spacing. If tooth size does increase with body length then the fastest rate of replacement should be the juvenile stages (Wass, 1973; Luer *et al*., 1990; Williams 2001). Wetherbee *et al.* (1997) concurred with this and went on to add that as growth rate is much faster in

juveniles, replacement rates should be greatest in pups. Wass (1973) showed this to be the case in sandbar sharks, *Carcharhinus plumbeus,* noting that tooth retention time increases from 18 days in young to 36 days in mature animals.

The second function of tooth shedding is to replace broken or damaged teeth. Tooth replacement in sharks quite obviously serves to renew worn or broken teeth; a function which may be of crucial importance to these predators (Moss, 1972). Wetherbee *et al.* (1997) suggested that rapid and well coordinated tooth replacement is absolutely essential in order to maintain a sharp dental battery for adequate feeding in marine apex predators. A study by Moss (1972) on *M. canis* found broken teeth in the functional series in 50% of the specimens examined. Examination of the lower jaw dentition showed that approximately 50% of the samples possessed several gouged, sheared or smashed teeth well within the exposed replacement series. Food preferences can also determine the tooth replacement rates in certain species. Rapid tooth replacement in these crustacean-eating specialists is a necessary adaptation to ensure the maintenance of an adequate dentition (Moss, 1972).

4.1.3 Tooth Row Counts

Tooth row counts have been used to characterise sharks and rays. The use of tooth row counts results in the production of a dental formula and is usually stated as the number of rows of teeth on each half of the upper and lower jaw and the area of the sympheses (Ellis and Shackley, 1995). The use of tooth row counts to characterise shark species can be a useful tool as they are easily accessible, but can prove to be unreliable as they can be inaccurate when small teeth at the sides of the jaw have to be counted (Bass, 1973). Other problems may also arise with the growth of individuals, whereby the tooth rows change as the fish grows (Bass, 1973). Sexual differences can also affect the number of tooth rows (Bass, 1973) and this has been shown in the teeth of *S canicula*, whereby the females had

significantly more rows of teeth in both the upper and lower jaw (Ellis and Shackley, 1995).

4.1.4 Tooth Morphology and Feeding

The dentition and feeding of elasmobranch species has been widely studied (Fedducia and Slaughter, 1974; Robinson and Motta, 2002). It is clear from the literature that there is a large range of inter-species variation in terms of tooth design in elasmobranchs. Much of the research carried out has been largely focused on the prey and habitat preferences of a range of shark species (Fedducia and Slaughter, 1974). It is apparent that tooth design is dictated by the life habits of a particular species. Goto (2001) noted that high cusps, sharp cutting edges and serrated margins of the teeth in many sharks can be considered as the adaptation for carnivorous habit (Figure 4.1).

Figure 4.1. Tooth from an adult great white shark, *Carcharadon carcharias*, demonstrating the high cusp and sharp cutting edge (Photographed by the author).

According to Motta (2004) modern extant sharks (and batoids) display a diversity of forms that are often ascribed functional roles (e.g. seizing/grasping, tearing, cutting, crushing and grinding). Motta (2004) provided a summary of the functions of various tooth forms. It

appears that teeth used for seizing prey prior to swallowing are generally small, with multiple rows of lateral cusps. These teeth are generally found in species such as the nurse shark, *Ginglymostoma cirratum* (Figure 4.2) which are generally thought of as being benthic. Motta (2004) suggested that teeth suited to seizing and tearing are found in species such as the shortfin mako, *Isurus oxyrinchus*. This species has long, pointed teeth with smooth, narrow cusps anteriorly and triangular cutting teeth posteriorly (Figure 4.2). James (1953) noted that this type of tooth design is found in pelagic sharks that have developed sharp pointed teeth for seizing prey.

According to Frazzetta (1988) slender, smooth-edged teeth can readily pierce prey, but are of less use in slicing it. Smooth bladed teeth can pierce prey with less resistance and are less prone to binding (becoming immobilized) in the prey tissue. Some sharks are equipped with cutting teeth, such as the tiger shark, *Galeocerdo cuvier*, in which many of the teeth are serrated (Figure 4.2). This tooth design aids in cutting through durable tissues, such as turtle shells (Witzell, 1987). Serrated teeth can make greater use of the available biting forces, and they have a greater cutting effect than do smooth-edged teeth (Frazzetta, 1988) stated that. The serrations vary from one species to another in coarseness and in distribution along tooth edges (Frazzetta, 1988).

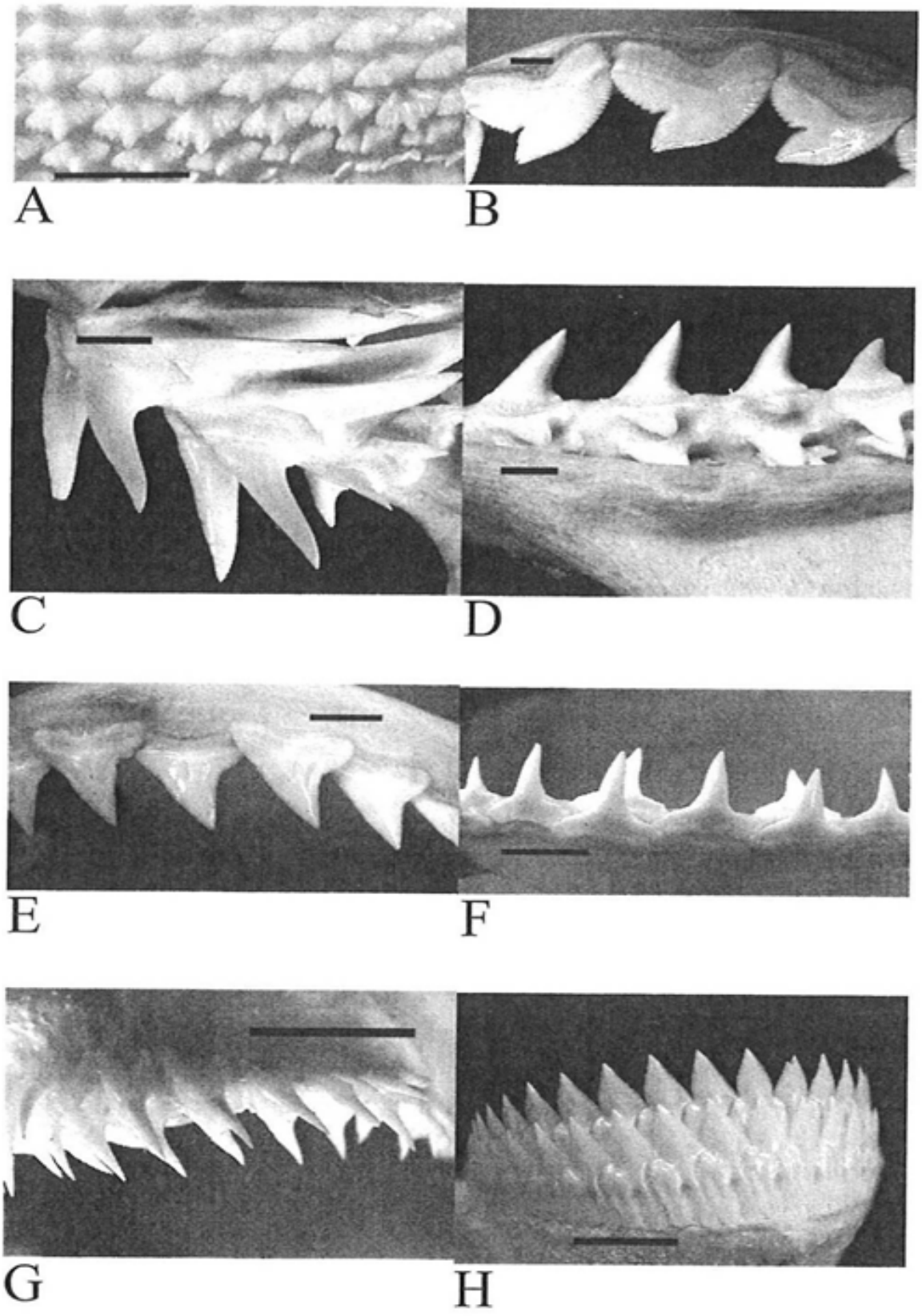

Figure 4.2. Tooth shapes of a range of modern elasmobranch species (A) nurse shark, *Ginglymostoma cirratum* (B) tiger shark, *Galeocerdo cuvier* (C&D) shortfin mako, *Isurus oxyrinchus* (E&F) sandbar shark, *Carcharinus plumbeus* (G&H) kitefin shark, *Dalatias licha* (adapted from Motta, 2004).

Mustelus species are found to possess crushing teeth that are described as being low, with cutting edges with bluntly rounded apices (Bigelow and Schroeder, 1948) (Figure (4.3). James (1953) described the crushing teeth of bottom feeding species as being pavement-like plates used for crushing hard cased animals, such as molluscs.

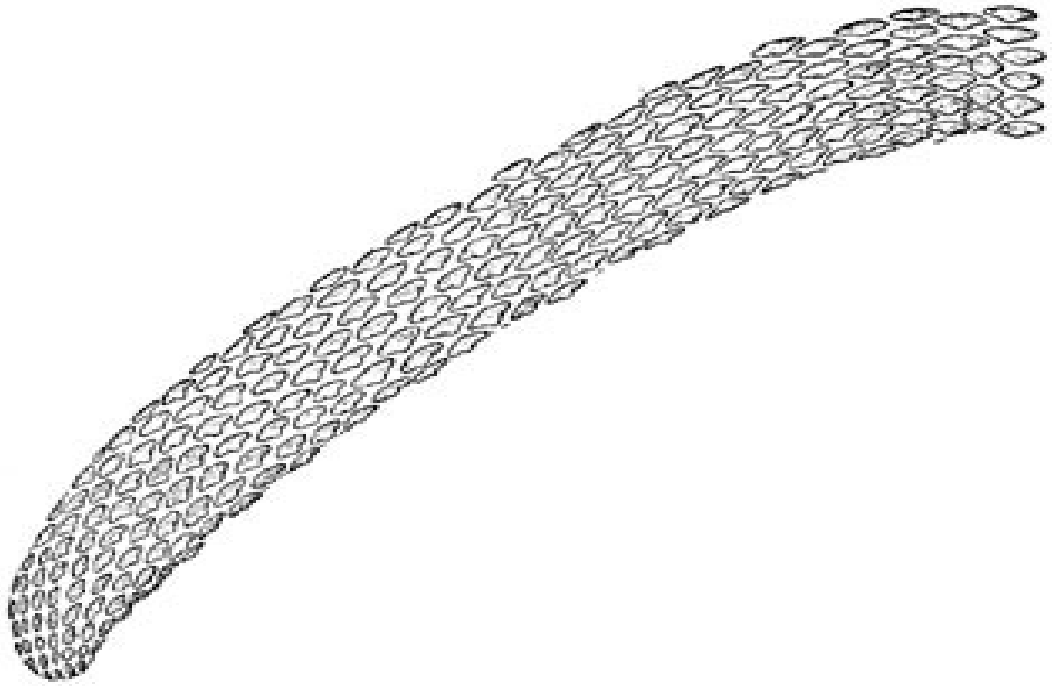

Figure 4.3. The upper jaw teeth of *Mustelus canis* (Bigelow and Schroeder, 1948).

According to Moss (1977) the tooth morphology of the lesser-spotted catshark, *S. canicula*, suggested that their small, sharp, cuspoid teeth are used primarily for prey grasping rather than shearing. Herman *et al.* (1990) described the teeth of *S. canicula* as having a rather broad based, but elongated principle cusp. The root shows two root lobes that are relatively long and narrow (Figure 4.4).

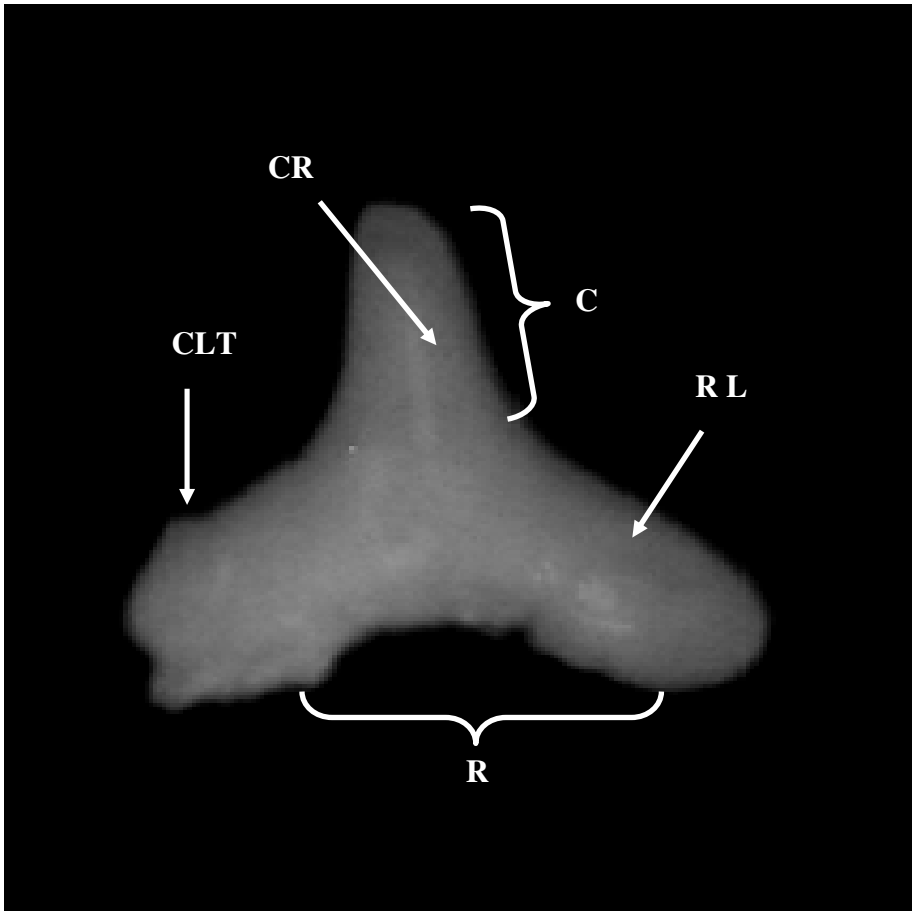

Figure 4.4. Anatomy of a sharks tooth, showing the crown (CR) cusp (C) root lobe (RL) root (R) and cusplette (CLT) (Photographed by the author).

Many authors have noted the presence of a sexual dimorphism in the teeth of many shark species, including that of *S. canicula* and this will be discussed in detail later. However, Fedducia and Slaughter (1974) suggested that sexual dimorphism in tooth shape may relate to differences in foraging habits between the sexes. This suggestion, regarding differences in feeding behaviour, was also noted by Arthur (1950). Lyle (1983) noted that *S. canicula* feeds opportunistically on a wide range of macrobenthic fauna with hermit crabs, cockles and whelks being the dominant prey. It was noted by Lyle (1983) that the composition of the diet altered gradually with catshark size, whereby the reliance on small crustaceans declined and consumption of hermit crabs, molluscs, cephalopods and teleosts increased with growth. However, there was no evidence that the genders differed in their dietary preferences.

Eales (1949) examined the stomach contents of 450 specimens of *S. canicula* and noted that the records showed a remarkable uniformity, although there was no mention of differences in feeding activity by males and females. Eales (1949) concluded that the diet of the lesser-spotted catshark consists of whelks, shrimps, hermit crabs, cuttlefish and small fish of various species. Henderson and Dunne (1999) supported this and found the stomach contents of a population of *S. canicula* in Irish waters containing fifteen different prey items, including crustaceans, polychaetes and echinoderms. It was concluded by Lyle (1983) that *S. canicula* is a general, opportunistic feeder on benthic and pelagic animals, scooping some up from the bottom and catching others, such as herring, while swimming.

Rodriguez-Cabello (2007) examined the stomach contents of 2234 specimens of *S. canicula* and discovered that diet composition did not vary between males and females, but did vary with increasing body length. Individuals in the same size class did not have differing diets. This has also been found to be the case in other species. McEachran (1975) examined over 1600 stomachs of four elasmobranch species with sexually dimorphic teeth (*R. erinacea, R. ocellata, R, senta* and *R. radiata*) and found no significant difference between the food consumed between the sexes for either young or mature specimens. It was also noted by McEachran (1975) that many of the organisms were found whole inside the stomachs of specimens with wear-induced dimorphisms. This indicated that the food items were not ground or crushed prior to ingestion, further supporting the theory that the existence of sexually dimorphic teeth did not necessarily result in intra-specific differential prey selection. McCourt and Kerstitch (1980) studied the stomach contents of the stingray *Urobatis concentricus* and found that food habits between genders of this species showed no differences.

4.1.5 Dental Sexual Dimorphism

An understanding of the role of sexual dimorphisms in elasmobranchs has evolved greatly in the past century. Hussakof and Bryant (1918) stated that comparisons between male and female sharks of one species are sometimes made, but it is very seldom that actual measurements are given that would allow of a detailed comparison in bodily proportions between the two sexes. It is now widely recognised that sexual dimorphisms in elasmobranchs are a major feature of their morphology. Bass (1973) suggested that the fact that sexual dimorphism occurs is well established and that in many shark species the female generally attains a greater total length than the male.

As previously discussed the act of the male biting the fins and body of the female during copulation is a widely recognised behaviour in elasmobranchs. The reports of males using their teeth to manoeuvre the female into a mating position and to hold the female during copulation have been recorded in many shark and ray species, including the blue shark, *Prionace glauca* (Stevens, 1974) the stingray, *Urobatis concentricus* (McCourt and Kerstitch, 1980) and the round stingray, *Urobatis halleri* (Nordell, 1994). Castro *et al.* (1988) noted that both precopulatory and copulatory behaviour in scyliorhinids may involve the male biting the fins and body of the female. Observations by Domi *et al.* (2000) confirmed that male *S. canicula* do bite the fins and body of females during copulation (Figure 4.5).

Figure 4.5. An adult male catshark biting the body behind the right pectoral fin of a female catshark prior to copulation (Image supplied courtesy of Domi *et al*., 2000).

The existence of a dental sexual dimorphism in many elasmobranch species is well recognised. McCourt and Kerstitch (1980) stated that there are numerous instances of sexual dimorphism in dentition among skates and rays. Taniuchi and Shimizu (1993) concurred and noted that dental sexual dimorphism was observed in the stingray, *Dasyatis akajei,* whereby adult males were found to possess teeth with a pointed cusp and adult females possessed flattened teeth. Sexual dimorphism has also been reported frequently in many shark species, including scyliorhinid sharks, whereby some species of catsharks are noted for their secondary sexual dimorphisms in tooth morphology (Gosztonyi, 1973). Springer (1979) reported that male scyliorhinids often have longer teeth and in one species the teeth were twice as long in males than in females of a similar size.

It appears that in most cases, male elasmobranchs possess larger, more pointed teeth than those of females. In the stingray, *Urobatis concentricus*, males possess a pointed cusp on each tooth, whilst females have virtually flat teeth with irregular surfaces (McCourt and Kerstitch 1980). This differing tooth morphology has been noted in several species, including the stingray, *Dasyatis akajei* (Taniuchi and Shimizu, 1993). In the

narrowmouthed catshark, *S. bivius*, Gosztonyi (1973) noted that in adults, male teeth are much longer than those of females and they are unicuspid with smooth and bulbous bases. Arthur (1950) found that the teeth of male *S. canicula* are larger than those of females, while Ellis and Shackley (1995) noted that the anterior teeth are significantly larger in male fish. According to McEachran (1975) in some ray species the teeth of both males and females were rounded to a bluntly conical shape prior to maturity. After maturity it appears that males develop teeth with sharp conical cusps.

Heterodonty, whereby an animal possess more than one type of tooth morphology is common in sharks. A primary type of heterodonty occurring in sharks is when the upper teeth are quite different from the lower teeth (Applegate, 1967). Applegate (1967) added that heterodonty in sharks involves a number of distinct variations. It is evident that there is a general increase in tooth height, which coincides with a similar increase in the total length of the shark. The changes in tooth structure due to maturity have been reported in various species of shark and ray. McCourt and Kerstitch (1980) found no differences in the dentition in juvenile stage, *U. concentricus*, but heterodonty was clearly observed in larger, mature specimens. In the narrowmouthed catshark, Gosztonyi (1973) observed that mouth ontogeny and tooth characteristics showed a succession of dentition during the fishes lifetime. It appeared that foetal dentition consisted of tricuspid teeth, while juvenile dentition was pentacuspid. Gosztonyi (1973) noted that the adult dentition depended strongly on the gender. Kerr (1955) noted considerable minor heterodonty in *S canicula.* Ellis and Shackley (1995) described the same findings, whereby the gradual replacement of tricuspid to unicuspid dentition occurs as the male fish grows larger. Ellis and Shackley (1995) found that in both the upper and lower jaws of male *S. canicula* the anterior teeth are large (1-2mm in height) with one small cusp on either side of the large, prominent

central cusp. The posterior teeth were much smaller, with a much less prominent central cusp and one to two lateral cusps.

With these clear sexual dimorphisms in the dentition of many elasmobranch species and the fact that diets have been found to be similar for males and females, many researchers suggested that dental dimorphisms are related to reproduction. The fact that McEachran (1977) found no differentiation in stomach contents suggested that sexually dimorphic dentition is an adaptation to sexual reproductive behaviour (Ellis and Shackley, 1995). This is backed up by the findings of McEachran (1975) who suggested that a more plausible interpretation of dental sexual dimorphism is of more importance in reproductive behaviour than in differential niche utilisation. McCourt and Kerstitch (1980) suggested that heterodonty is closely related to the mating behaviour of male stingrays. Ellis and Shackley (1995) gave a possible explanation as to why males have bigger teeth as being an adaptation for reproductive behaviour and proposed that males possess more pointed teeth to grasp the pectoral fins of the female during copulation.

Kajiura and Tricas (1996) suggested that although more subtle dental dimorphisms are known in sharks the possibly of periodic changes remains to be demonstrated. It is clearly demonstrated in the Atlantic stingray, *Dasyatis sabina,* that there are seasonal differences in the tooth morphology of males and females. Kajiura and Tricas (1996) found that female *Dasyatis sabina,* over a consecutive 24 month period showed stable molariform morphology. However, their research showed that males exhibited a periodic shift in dentition from a female-like molariform to a recurved cuspidate form during the reproductive season. It appeared that the grip tenacity of the male dentition was greater for the cuspidate form that occurred during the mating season than for the molariform dentition that occurred during the non-mating season. Kajiura and Tricas (1996) predicted that

periodic dental dimorphism would be most prominent in species with mating and courtship behaviours that require vigorous grasping by the male for successful copulation.

It is possible, therefore, that if the Solent population of *S. canicula* have a distinct mating season that there will be a change in the shape and size of the teeth of the male catsharks to coincide with reproduction. As far as the author is aware the effects of seasonality on tooth structures has not previously been studied in *S. canicula*.

Therefore, the aims of this study are:

1. To determine if there is any sexual or seasonal dimorphism in the dentition of hatchling and juvenile *S. canicula*.

2. To determine if there is any sexual dimorphism in the dentition of mature *S. canicula*.

3. To determine if there is any seasonal dimorphism in the dentition of mature *S. canicula*.

4.2 Materials and Methods

For the tooth morphometrics the adult catsharks were categorised into size classes based on sexual maturity. The size classes used are:

Males - Size class 1 < 525mm total body length (immature/Juvenile)

Males - Size class 2 ≥ 525mm total body length (mature)

Females - Size class 1 < 550mm total body length (immature/Juvenile)

Females - Size class 2 ≥ 550mm total body length (mature)

The numbers of individual adult catsharks sampled for the upper and lower jaw tooth dimensions differed. In earlier samples used for this study only the lower jaws were extracted. The n-values for all analyses are reported.

4.2.1 Tooth Row Counts

Hatchling tooth row counts were taken *in situ*, as the jaws were too small to excise. The mouths of the hatchling catsharks were examined under a Leica GZ6 stereomicroscope and any extraneous tissue was cut away with a fine scalpel to reveal the unexposed tooth rows. Adult and juvenile tooth rows were counted from the jaws immediately after removal from the head of the sharks. The extraneous tissue was removed with a fine scalpel to reveal the unexposed tooth rows. The rows were counted at the front section of the jaw (Figure 4.6)

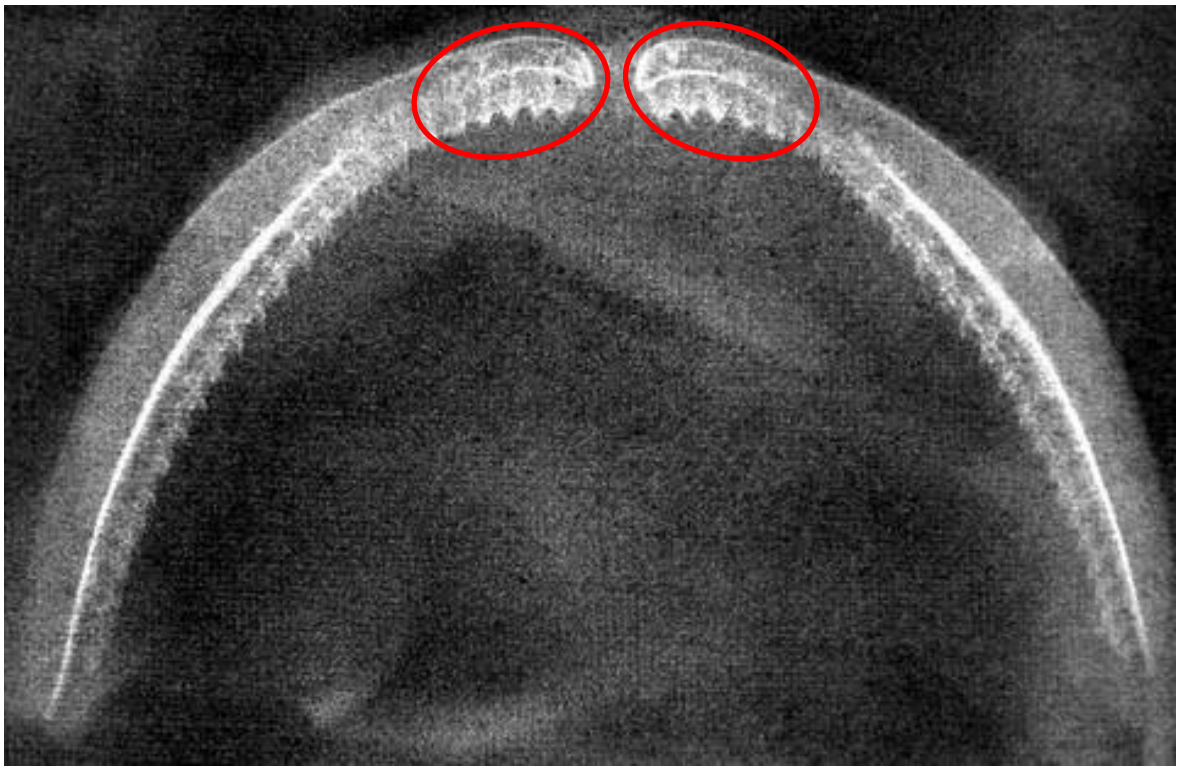

Figure 4.6. Radiography image of the upper jaw of a female lesser-spotted catshark. Red circles indicate the location of the tooth row counts (Photographed by the author).

4.2.2 Dental Formula

The dental formula of adult male and female specimens was ascertained after the jaws had been excised from the head. A Leica Zoom 2000 stereomicroscope was used to count the number of teeth on each side of the mouth and those in the symphyses (Figure 4.7). The value of the upper and lower jaws were combined to calculate the dental formula.

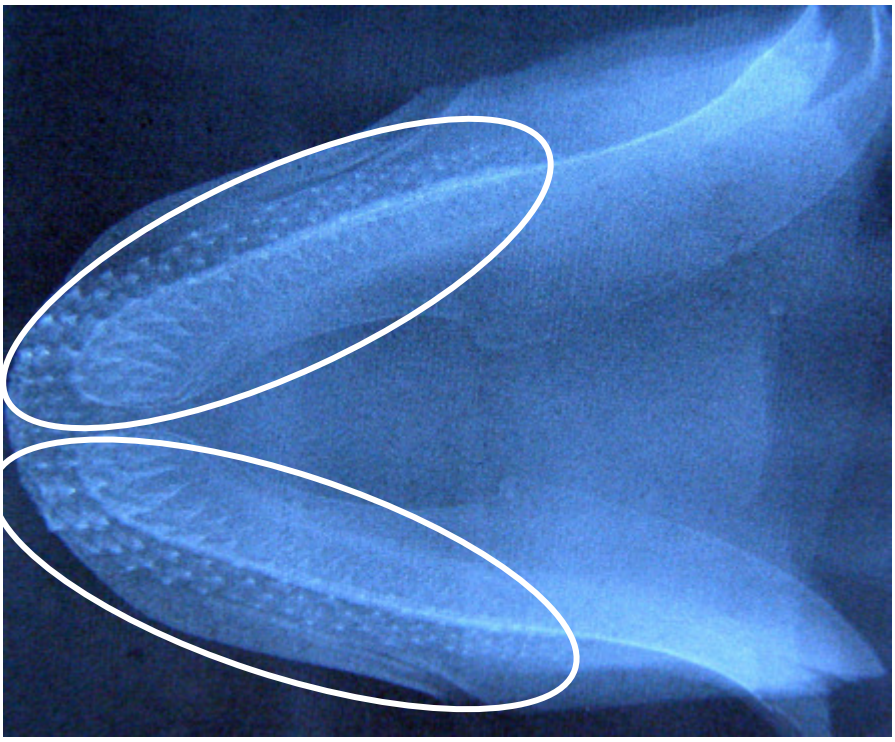

Figure 4.7. The lower jaw of an adult male catshark showing the areas where tooth row counts were taken (Photographed by the author).

4.2.3 Radiography

Six sets of shark jaws were imaged using commercial radiography techniques in order to examine the benefits of using such a system to accurately record tooth row and dental formula details.

The excised shark jaws were removed from the formalin and put through 6 x 45 minute washes of running tap water. The jaws were immersed in distilled water held in plastic containers and transported to the Queen Alexandra Hospital, Cosham, Hampshire. Prior to imaging the jaws were towel dried. Images were captured using a Siemens dental machine (no serial number) at 60 kilovoltage (peak) 7mA. Exposure time depended on sample size and ranged from 0.16 - 0.25 seconds. Images were processed using dental occlusal film (Agfa dentals M2) to produce a radiographic image (Figure. 4.8).

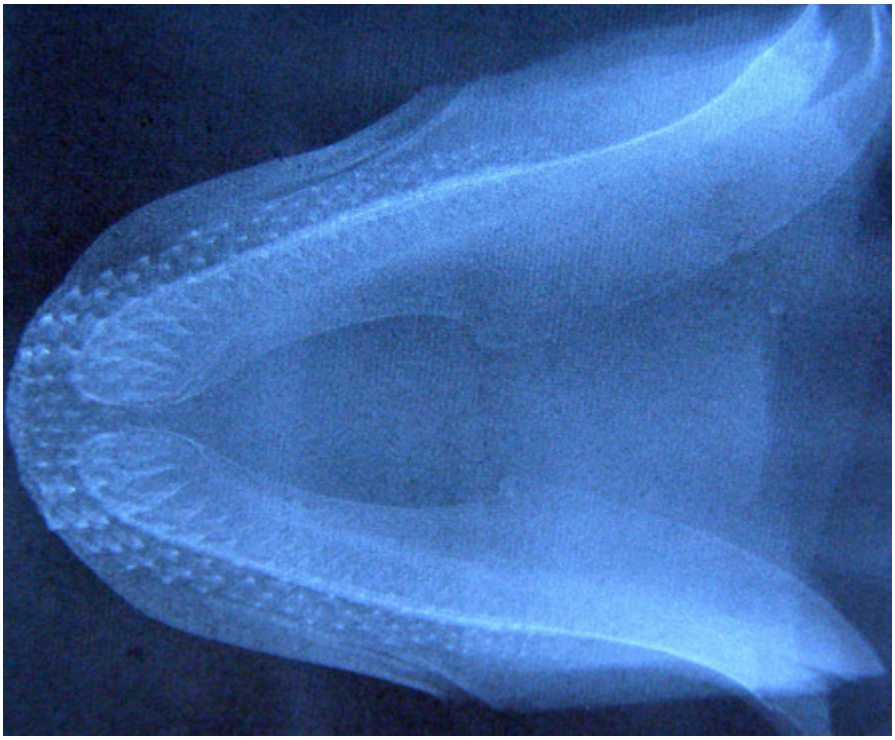

Figure 4.8. Radiographic image of the lower jaw of a male catshark (Photographed by the author).

4.2.4 Tooth Morphometrics

Due to the small size of the hatchling teeth it was not possible to remove them from the jaws and no individual tooth data were recorded for hatchling catsharks. Both adult and juvenile teeth were extracted from the upper and lower jaws once the jaws had been air dried for 24 hours. The jaws were examined under a Leica GZ6 stereomicroscope and a

fine scalpel and pair of size 5 tweezers were used to ease the teeth out from the jaw. Any excess Sharpey's fibres were removed with the fine scalpel blade in order that the tooth could be laid as flat as possible before being imaged.

Five teeth were extracted from the upper jaw and five from the lower of each specimen as per Nordell (1994). The teeth were extracted from the front portion of the jaw and were only taken from the third to sixth rows as these were the functional teeth (Figure 4.9). This was to ensure that the newest teeth were extracted and not those that had been subjected to wear or breakages. Teeth from the back of the jaw were not measured as they would be unlikely to be used in the mating process due to their position far back in the mouth.

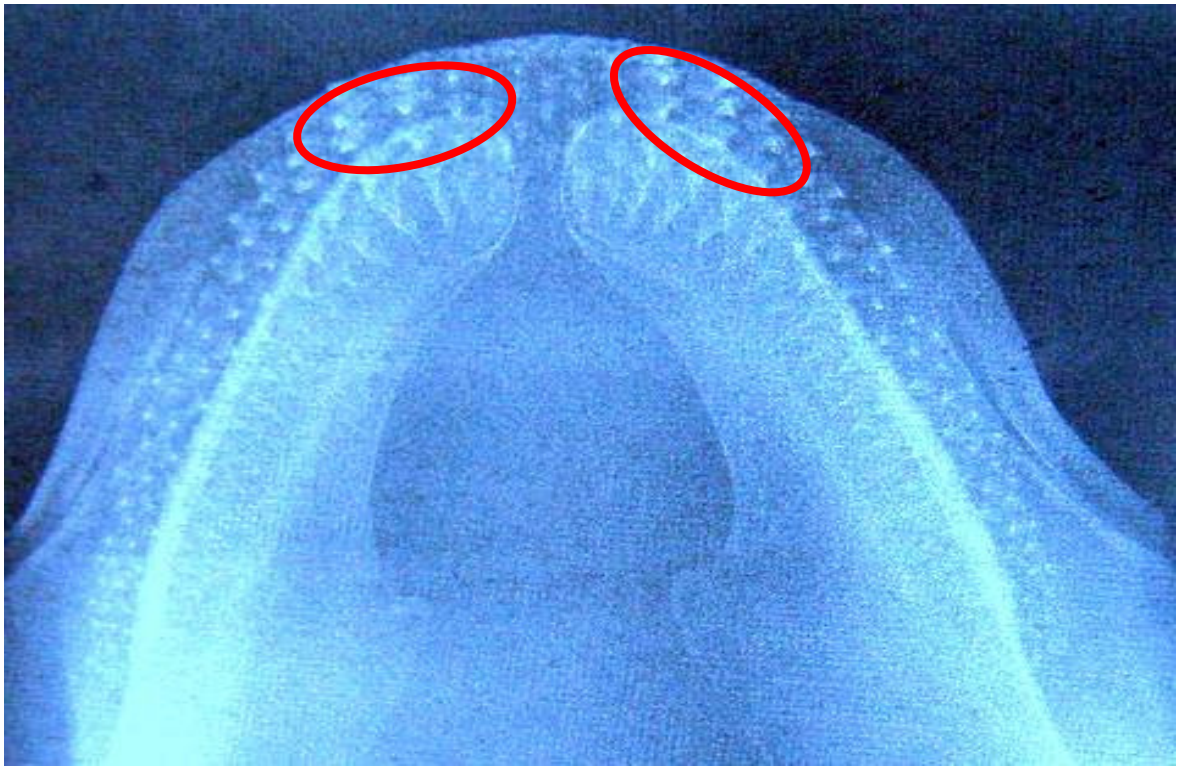

Figure 4.9. Lower jaw of an adult male lesser-spotted catshark. Circles indicate position of tooth extraction showing tooth rows 3-6 (Photographed by the author).

Once the teeth had been removed they were photographed using a Leica GZ6 stereomicroscope and imaged with the use of a JVC Digital Camera KY-F1030U. The

images were then stored and an image analysis package (UTHSCSA image tool) was used to measure each individual tooth. Six measurements from each tooth were taken.

The number of cusps visible on each tooth was counted (Figure 4.10). Damaged cusps that were visible and where the tip was missing were counted. Any completely missing cusp was excluded from the cusp count.

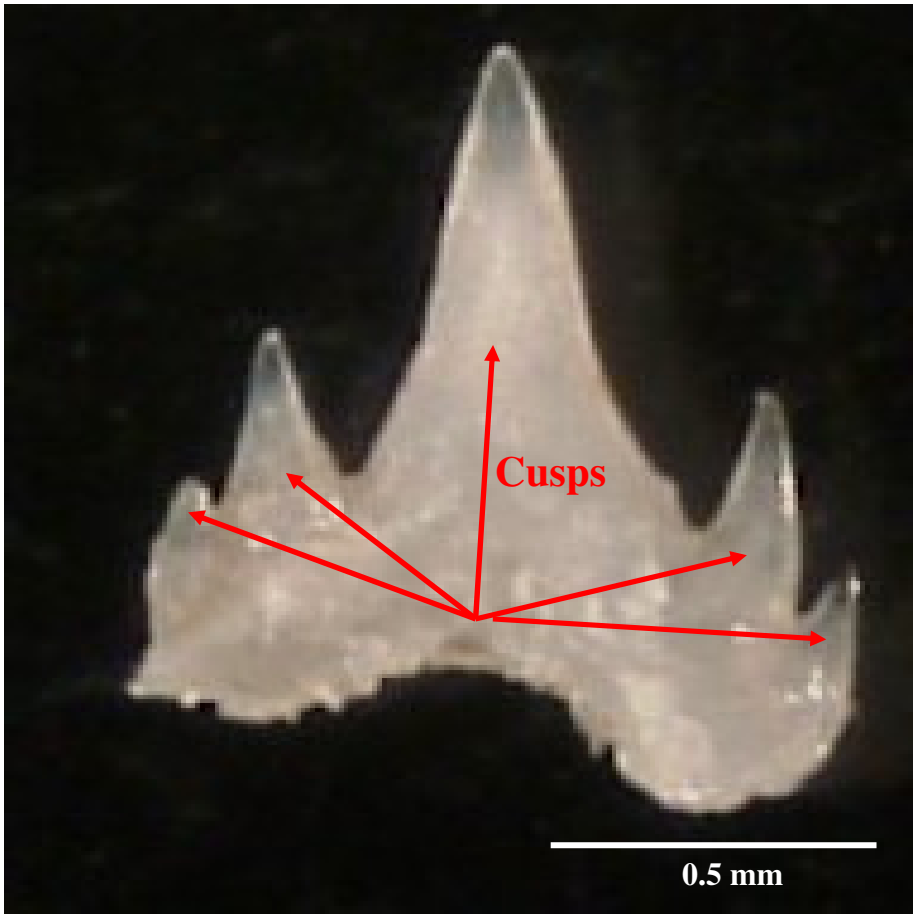

Figure 4.10. Showing tooth cusps on a pentacuspid tooth extracted from a female specimen of *S. canicula* (Photographed by the author).

The width (TW) from the extreme edges of the root of the tooth and the tooth slope height (TSH) from cusp to the far point of the root lobe was measured for each tooth (Figure 4.11).

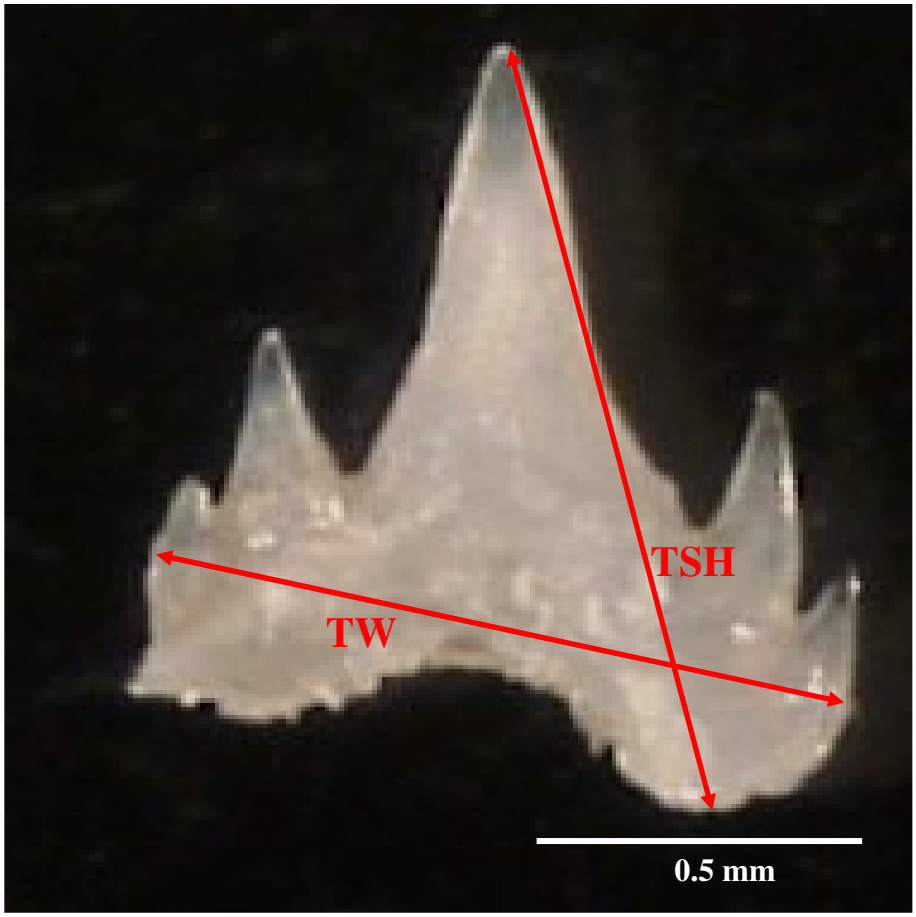

Figure 4.11. The area of measurement for tooth slope height (TSH) and tooth width (TW) on a pentacuspid tooth extracted from a female specimen of *S. canicula* (Photographed by the author).

Several measurements of the tooth cusps were taken from each tooth (Figure 4.12). The diameter of the base of the central cusp (BD) was taken for both males and females. The diameter of the mid section of the central cusp (MD) was also taken. The mid section of the cusp was ascertained by measuring cusp length (CL) and determining the median point of the cusp. The diameter of the tip of the central cusp (TD) was also measured (Figure 4.12). Care was taken when extracting teeth to avoid broken or visibly worn teeth in order to provide consistent measurements for the tip diameter.

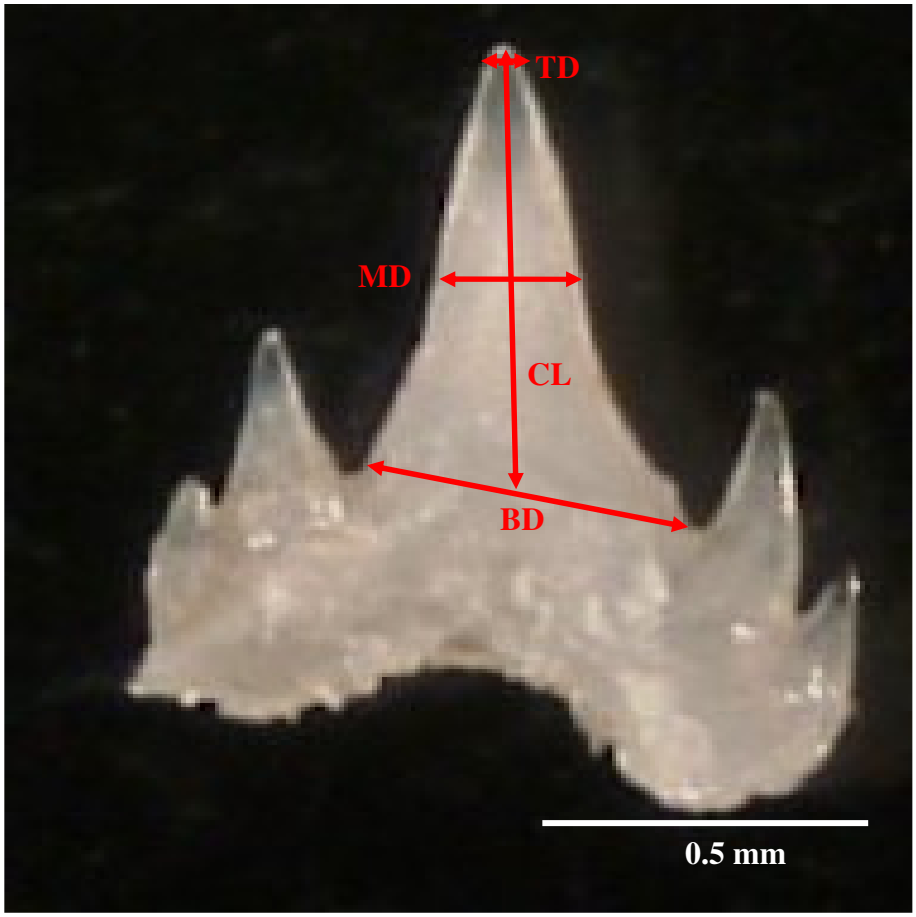

Figure 4.12. The area of measurement for cusp base diameter (BD) Mid cusp diameter (MD) and cusp tip diameter (TD) and cusp length (CL) on a pentacuspid tooth extracted from a female specimen of *S. canicula* (Photographed by the author).

4.2.5. Statistical Analyses

A range of analyses were performed on the teeth of hatchling, juvenile and adult *S. canicula* in order to determine whether any seasonal or sexual dimorphism exists in the dental structure of the lesser-spotted catshark. Prior to employing parametric statistical tests, Kolmogorov-Smirnov Normality Tests were carried out to determine whether the data were normally distributed (Dytham, 2003). If necessary log 10 transformations were conducted. Significance was accepted when $P<0.05$.

The tooth measurements were grouped according to season for both mature adult male and female catsharks and a GLM was used to determine any seasonal differences within each gender. Due to the low numbers of both hatchling and juvenile catsharks sampled it was not possible to carry out any seasonal comparisons. In order to determine the presence of outliers. A Grubbs test for outliers was performed on the data (Grubbs, 1969) as per Attrill *et al*. (2007) in order to ascertain the presence of any outliers. The test revealed that no outliers were present in any of the data. If body length had a significant effect on any parameter a scatterplot was produced. The regression line was analysed using an analysis of covariance (ANCOVA) to see if there was a significant correlation between body length and a particular measurement. If both gender and body length was found to be significant the male and female regression slopes were compared to determine any correlation between the morphology of the teeth and body length between the genders.

Canonical discriminant analysis was carried on the adult and juvenile upper and lower jaw tooth data to determine whether any correlation between the upper and lower jaw tooth dimensions of adult and juvenile male and female *S. canicula* existed. The tooth morphometrics (TH, TW, BD, MD, TD) were combined and two sets of analyses were performed, one for juvenile males and females and one for adult males and females. The results show the two factors that contributed most strongly to any dimorphisms. These are represented as functions in the axis.

4.3 Results

4.3.1 Hatchling Data

For the hatchling catsharks only tooth row numbers were counted as the teeth were too small to successfully remove and measure.

4.3.1.1 Tooth Rows - Upper and Lower Jaw

The results of the ANCOVA for the tooth row counts for male and female hatchling catsharks can be seen in table 4.1. There was no significant difference in the tooth row counts for male and female hatchling catsharks for the upper jaw (ANCOVA, F=1.39; d.f.=1; *P*=0.256) and body length was found to have no effect (ANCOVA, F=2.35; d.f.=1; *P*=0.144). Body length had no effect on the number of tooth rows in the lower jaw of male and female hatchling catsharks (ANCOVA, F=1.35; d.f.=1; *P*=0.268). There was an inter-gender difference in the number of tooth rows for the lower jaw of hatchling catsharks. Male hatchling catsharks were found to possess a significantly greater number of tooth rows in the lower jaw than female hatchling catsharks (ANCOVA, F=7.35; d.f.=1; *P*=0.018).

Table 4.1. Tooth row data for male and female hatchling *S. canicula* showing means ± standard errors and *P*-Values and range (n= M (23) F (14)).

Feature	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Tooth Row Number Upper Jaw	4.6 ± 0.48 (3-7)	5.8 ± 0.61 (3-8)	**0.144**	**0.256**
Tooth Row Number Lower Jaw	5.0 ± 0.37 (4-6)	6.2 ± 0.32 (5-8)	**0.268**	**0.018**

4.3.2 Juvenile Tooth Data

4.3.2.1 Upper Jaw

The ANCOVA results for the upper jaw tooth morphometric data and tooth row counts for male and female juvenile catsharks can be seen in Table 4.2. The results show that neither

gender (ANCOVA, F=0.73; d.f.=1; *P*=0.405) nor body length (ANCOVA, F=3.08; d.f.=1; *P*=0.096) had a significant effect on the height of the teeth for the upper jaw in juvenile catsharks. Gender was found to have no significant effect on the tooth width of the upper jaw in juvenile catsharks (ANCOVA, F=3.41; d.f.=1; *P*=0.081). However, body length was found to have a significant effect on the tooth width of juvenile catsharks (ANCOVA, F=5.46; d.f.=1; *P*=0.031). The results of the ANCOVA for the cusp base diameter of the upper jaw teeth of juvenile *S. canicula* showed that neither body length (ANCOVA, F=1.59; d.f.=1; *P*=0.223) or gender (ANCOVA, F=1.85; d.f.=1; *P*=0.190) had a significant effect. Body length had no significant effect on the mid cusp diameter of the upper jaw teeth in juvenile catsharks (ANCOVA, F=1.14; d.f.=1; *P*=0.300). However, gender was found to have a significant effect on the mid cusp diameter of the upper jaw teeth of juvenile catsharks (ANCOVA, F=5.59; d.f.=1; *P*=0.030). The mid cusp diameter of the upper jaw teeth in juvenile male *S. canicula* was found to be greater than in juvenile female *S. canicula*. The cusp tip diameter in the upper jaw teeth of juvenile male and female catsharks was not found to be significantly different for either body length (ANCOVA, F=0.48; d.f.=1; *P*=0.498) or gender (ANCOVA, F=3.92; d.f.=1; *P*=0.063). Tooth cusp number of the upper jaw of juvenile male and female catsharks was not found to be significantly different. Neither body length (ANCOVA, F=0.60; d.f.=1; *P*=0.449) nor gender (ANCOVA, F=2.62; d.f.=1; *P*=0.123) had an effect on upper jaw tooth cusp numbers in juvenile catsharks. There were no significant differences found in the upper jaw tooth row numbers with neither body length (ANCOVA, F=1.21; d.f.=1; *P*=0.283) or gender (ANCOVA, F=0.44; d.f.=1; *P*=0.513) having an effect on the number of rows of teeth in the upper jaws of juvenile *S. canicula*.

Table 4.2. Tooth morphometrics for the upper jaw of juvenile male and female *S. canicula* showing means ± standard errors and *P*-Values and range (n= M (5) F (15)).

Feature	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length (*P*-Value)	Gender (*P*-Value)
Upper Jaw Tooth slope height (mm)	1.03 ± 0.04 (0.8 – 1.43)	1.03 ± 0.11 (0.67 – 1.30)	**0.096**	**0.405**
Upper Jaw Tooth Width (mm)	0.84 ± 0.02 (0.64 – 0.97)	0.87 ± 0.04 (0.77 – 0.96)	**0.031**	**0.081**
Upper Jaw Cusp Base Diameter (mm)	0.39 ± 0.02 (0.23 – 0.57)	0.43 ± 0.06 (0.27 – 0.63)	**0.223**	**0.190**
Upper Jaw Mid Cusp Diameter (mm)	0.21 ± 0.01 (0.17 – 0.29)	0.25 ± 0.03 (0.17 – 0.34)	**0.300**	**0.030**
Upper Jaw Cusp Tip Diameter (mm)	0.08 ± 0.01 (0.06 – 0.10)	0.10 ± 0.02 (0.5 – 0.14)	**0.498**	**0.063**
Upper Jaw Tooth Cusp Number	3.94 ± 0.17 (2 – 5)	3.4 ± 0.4 (3 - 5)	**0.449**	**0.123**
Upper Jaw Tooth Row Number	6.0 ± 0.2 (4 - 7)	5.5 ± 0.2 (5 - 6)	**0.513**	**0.283**

It can be seen from Figure 4.13 that as body length increases so does upper tooth width. There was no significant correlation between the body length and upper jaw tooth width of juvenile catshark (ANOVA, F= 3.70; d.f = 1; *P*=0.070). Further plots were made for upper jaw tooth width, combining the data for the juvenile and adult catsharks (Appendix 2). The combined data showed a similar pattern with tooth width and cusp base diameter, in general, increasing with body length.

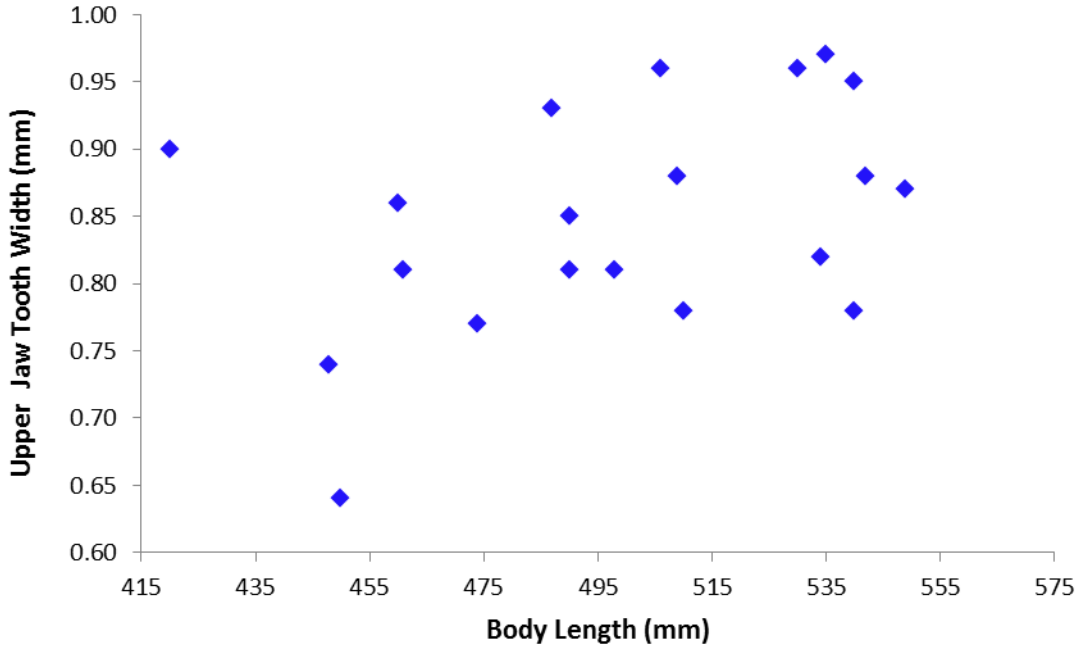

Figure 4.13. Scatterplot showing upper jaw tooth width against body length for juvenile *S. canicula* (n= M (5), F (15)).

4.3.2.3 Lower Jaw

The ANCOVA results for the lower jaw tooth morphometrics and tooth row counts for juvenile male and female catsharks can be seen in Table 4.3. Tooth slope height in the lower jaw of juvenile *S. canicula* was not found to be dimorphic, with no significant differences with regard to body length (ANCOVA, F=2.48; d.f.=1; *P*=0.133) or gender (ANCOVA, F=2.84; d.f.=1; *P*=0.109). Although juvenile male catsharks generally had wider teeth in the lower jaw they were not found to be sexually dimorphic. There was no significant difference found in the width of the teeth in the lower jaw of juvenile *S. canicula* (ANCOVA, F=3.39; d.f.=1; *P*=0.082). With respect to lower jaw width in juvenile catsharks body length was found to have a significant effect (ANCOVA, F=7.57; d.f.=1; *P*=0.013). The same pattern was observed in the diameter of the cusp base, whereby gender had no significant effect on the cusp base diameter of the lower jaw of juvenile catsharks (ANCOVA, F=2.79; d.f.=1; *P*=0.113) but body length did (ANCOVA,

F=11.24; d.f.=1; *P*=0.004). There were no significant differences found in the mid cusp diameter of the lower jaws of juvenile male and female *S. canicula*. Neither body length (ANCOVA, F=2.12; d.f.=1; *P*=0.163) nor gender (ANCOVA, F=1.99; d.f.=1; *P*=0.175) had a significant effect on the mid cusp diameter of juvenile catsharks. Body length was also found to have no significant effect on the cusp tip diameter of juvenile catsharks (ANCOVA, F=1.34; d.f.=1; *P*=0.263). However, gender was found to have a significant effect on the cusp tip diameter in the lower jaws of juvenile catsharks, with juvenile male *S. canicula* possessing a significantly wider cusp tip than juvenile female *S. canicula* (ANCOVA, F=7.35; d.f.=1; *P*=0.015). There were no significant differences in the number of tooth cusps present on the lower jaw teeth of juvenile male and female catsharks. Body length (ANCOVA, F=0.40; d.f.=1; *P*=0.534) and gender (ANCOVA, F=0.59; d.f.=1; *P*=0.451) had no effect on the cusp numbers in the lower jaw teeth of juvenile catsharks. Neither body length (ANCOVA, F=0.57; d.f.=1; *P*=0.460) nor gender (ANCOVA, F=0.21; d.f.=1; *P*=0.653) had a significant effect on the number of tooth rows in the lower jaw of juvenile catsharks.

Table 4.3. Tooth morphometrics for the lower jaw of juvenile male and female *S. canicula* showing means ± standard errors and *P*-Values (n= M (5) F (15)).

Feature	Female $\bar{x}$ ± SE (Range)	Male $\bar{x}$ ± SE (Range)	Body Length (*P*-Value)	Gender (*P*-Value)
Lower Jaw Tooth slope height (mm)	1.13 ± 0.03 (0.85 – 1.38)	1.21 ± 0.08 (0.93 – 1.45)	**0.133**	**0.109**
Lower Jaw Tooth Width (mm)	1.02 ± 0.04 (0.76 – 1.25)	1.06 ± 0.06 (0.84 – 1.22)	**0.013**	**0.082**
Lower Jaw Cusp Base Diameter (mm)	0.45 ± 0.02 (0.32 – 0.63)	0.45 ± 0.04 (0.33 – 0.63)	**0.004**	**0.113**
Lower Jaw Mid Cusp Diameter (mm)	0.23 ± 0.01 (0.19 – 0.34)	0.25 ± 0.02 (0.18 – 0.34)	**0.163**	**0.175**
Lower Jaw Cusp Tip Diameter (mm)	0.08 ± 0.01 (0.05 – 0.10)	0.11 ± 0.01 (0.11 – 0.80)	**0.263**	**0.015**
Lower Jaw Tooth Cusp Number	4.25 ± 0.23 (2 - 5)	4 ± 0.32 (3 - 5)	**0.534**	**0.451**
Lower Jaw Tooth Row Number	5.6 ± 0.2 (4 - 8)	6 ± 0.1 (5 - 6)	**0.460**	**0.653**

It can be seen from Figures 4.14 and 4.15 that as body length of juvenile catsharks increases the tooth width (ANOVA, F= 7.83; d.f = 1; *P*=0.012) and the diameter of the cusp base (ANOVA, F= 7.48; d.f = 1; *P*=0.014) also increases. There was a significant correlation between body length and the width and cusp base diameter of the lower jaw teeth of juvenile *S. canicula*. Further plots were made for lower jaw tooth width and cusp base diameter with the data for the juvenile and adult catsharks combined (Appendix 2). The combined data showed a similar pattern with tooth width and cusp base diameter, in general, increasing with body length.

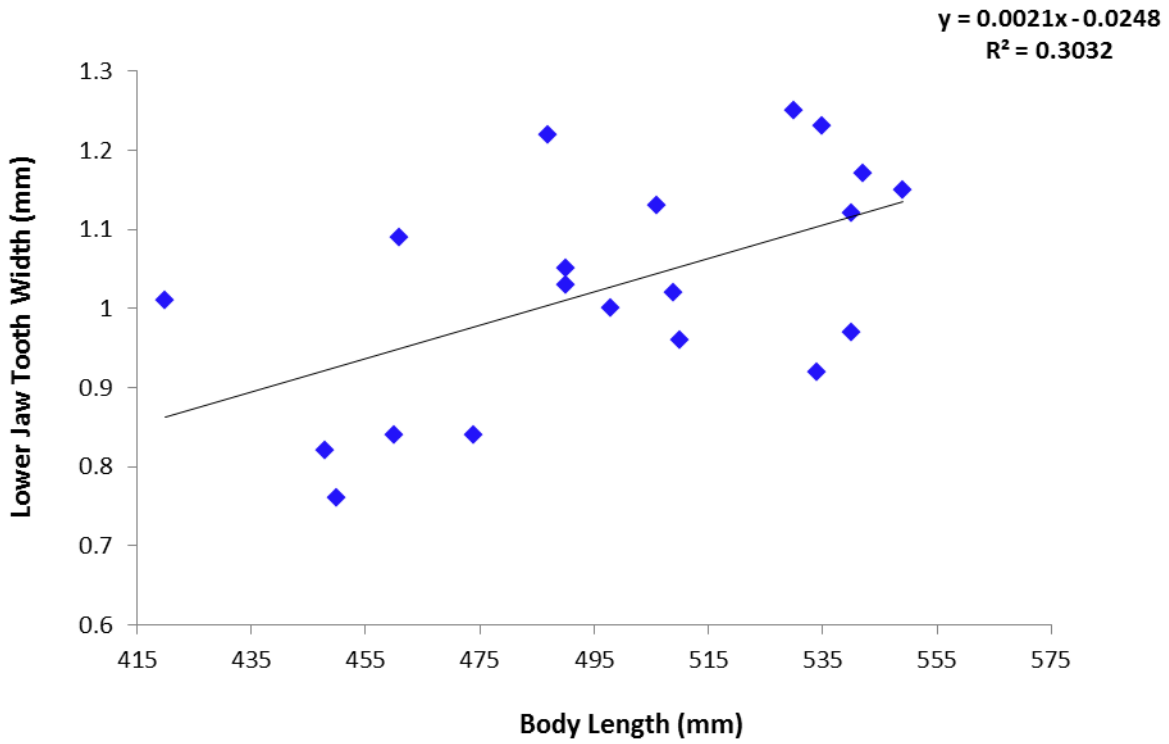

Figure 4.14. Scatterplot with regression showing lower jaw tooth width against body length for juvenile *S. canicula*. (n= M (5), F (15)) (*P*=0.012).

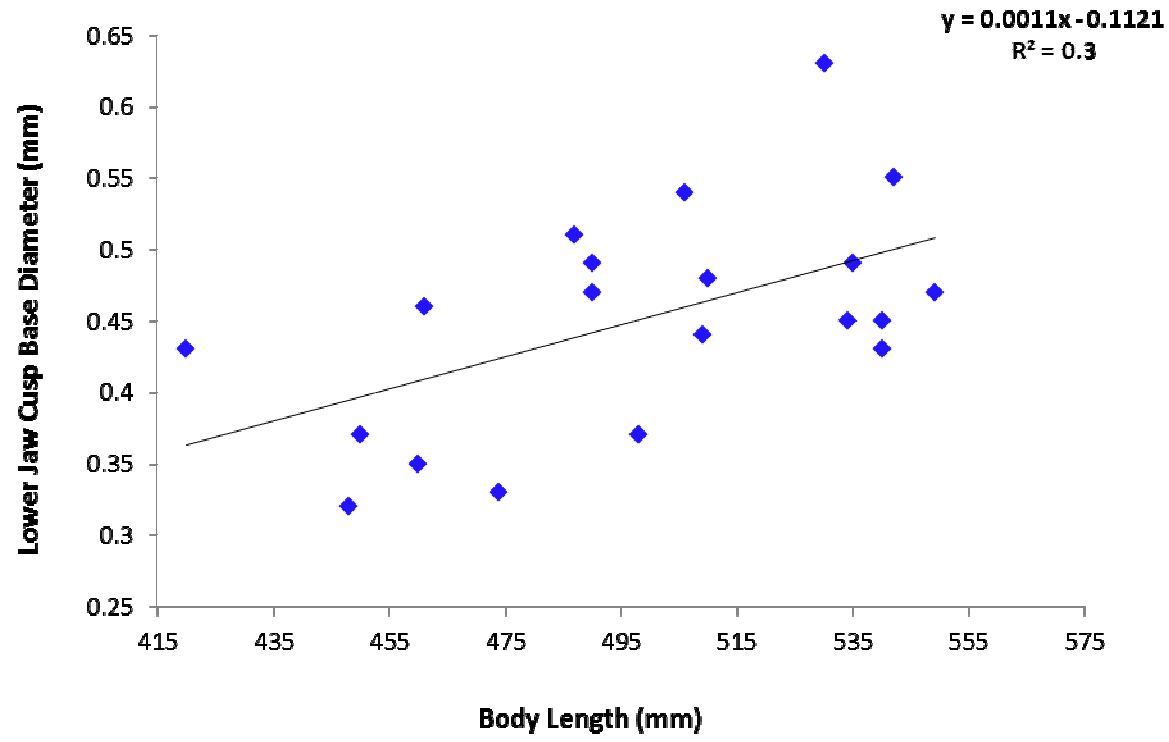

Figure 4.15. Scatterplot with regression showing lower jaw tooth cusp base diameter against body length for juvenile *S. canicula*. (n= M(5), F(15)) (*P*=0.014).

4.3.3 Adult Tooth Measurements - Upper Jaw

The means ± standard errors and ranges for the upper jaw tooth morphometrics of adult *S. canicula* can be seen in Appendix 3.

4.3.3.1 Tooth Slope Height

The results of the descriptive statistics are dispalyed in Figure 4.16. The graphical representation shows that adult male catsharks had a greater tooth slope height than adult female catsharks thoughout the year.

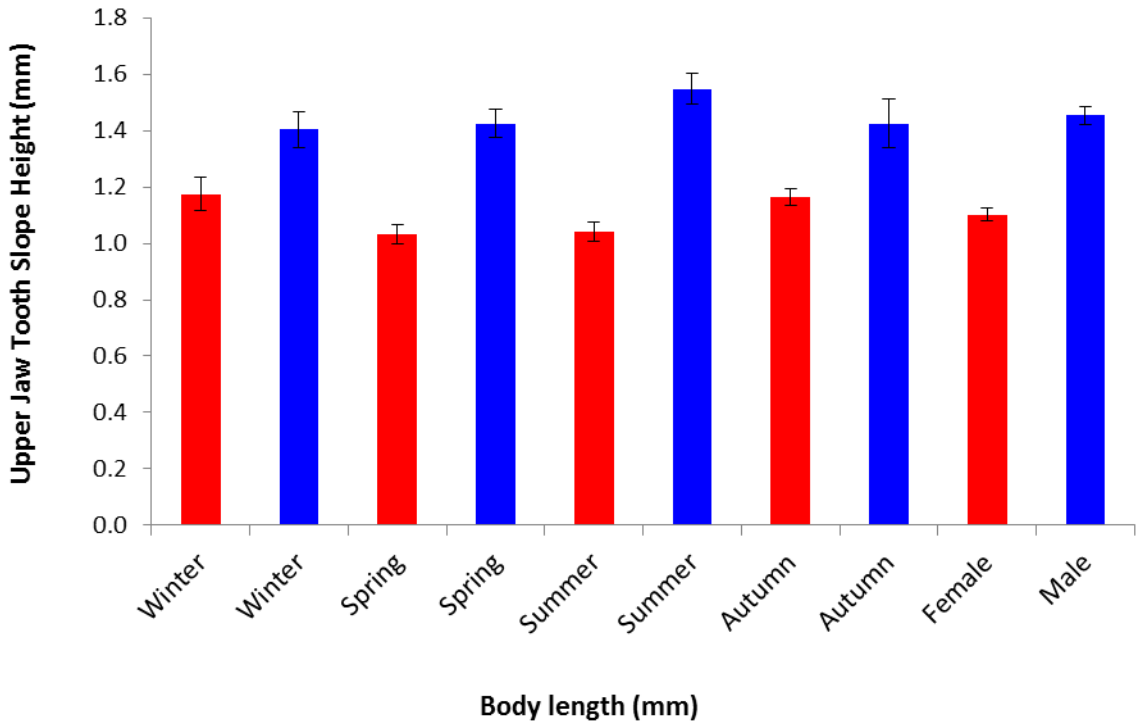

Figure 4.16. Gender and seasonal comparison of upper jaw tooth slope height for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

It can be seen from Table 4.4 that season had no intra-gender effect on the upper jaw tooth slope height of adult male and female catsharks. Body length, gender and gender within

season were found to have a significant effect, with adult male catsharks sampled in spring and summer possessing teeth with a greater height than adult females sampled in winter, spring, summer and autumn. Adult male catsharks sampled in winter and autumn had teeth with a greater height than females sampled in spring and summer.

Table 4.4. Results from the ANCOVA analyses for the upper jaw tooth slope height of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.59389	0.33528	0.33528	18.20	**<0.001**
Gender	1	2.06886	1.65250	1.65250	89.68	**<0.001**
Season	3	0.08636	0.06933	0.02311	1.25	**0.297**
Season*Gender	3	0.26165	0.26165	0.08722	4.73	**0.005**

Figure 4.17 shows a graphical representation of the upper jaw tooth slope height of individual male and female adult catsharks. It can be seen that as body length increases so does upper jaw tooth slope height. It is also clear from the regression analyses that there is a clear gender separation with adult female catsharks possessing shorter teeth than adult male catsharks. It can also be seen that the tooth slope height of both males and females increases with body length. The regression lines show that this increase in tooth slope height relative to body length follows a similar pattern in both adult male and female catsharks. There was no significant difference between the regression lines of adult male and female catsharks (P= 0.845).

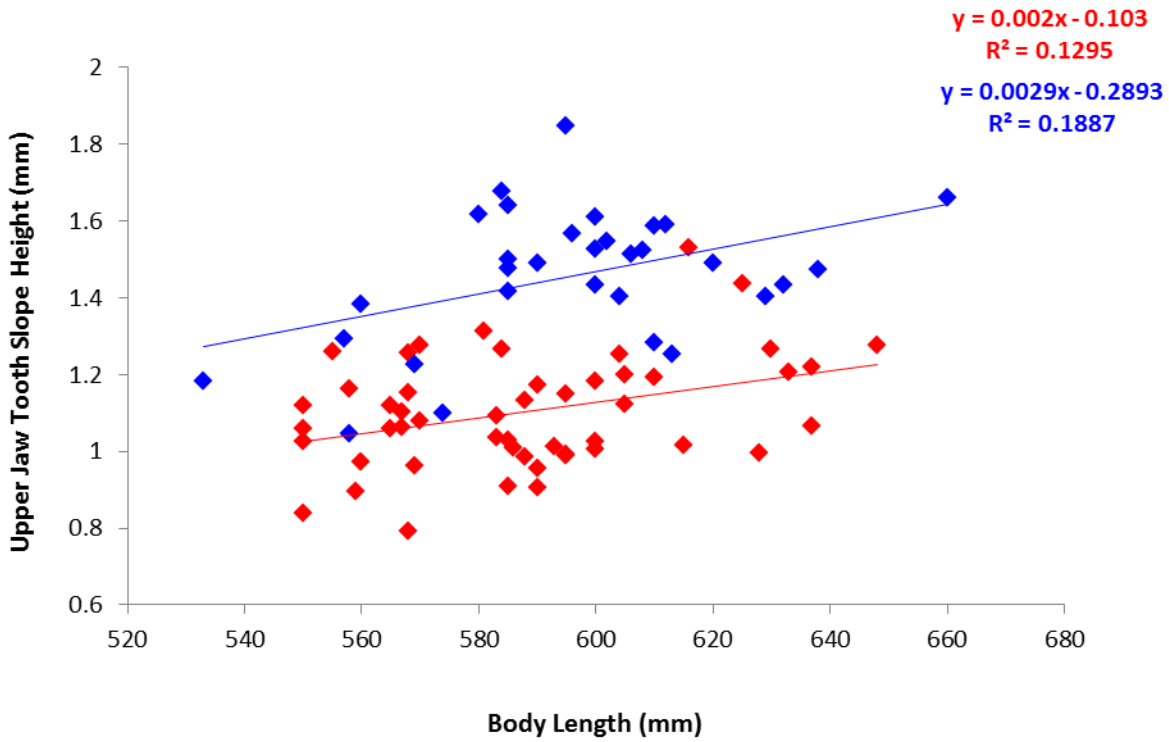

Figure 4.17. Scatterplot with regression for upper jaw tooth slope height against body length for adult male and female *S. canicula* (n= M (31), F (50)) (Male = Blue, Female = Red) (*P*= 0.845).

Table 4.5 shows that although there was a significant different for tooth slope height for gender within season these differences could not be identified (*P*>0.05).

Table 4.5. *P*-values for seasonal comparison of upper jaw tooth slope height for adult male and female catsharks.

Season	Winter	Spring	Summer	Autumn	
Winter		0.995	0.379	1.000	Male
Spring	0.291		0.475	0.990	
Summer	0.675	0.999		0.247	
Autumn	1.000	0.109	0.429		
	Female				

Table 4.6 shows that there were significant differences in the upper jaw tooth slope height of adult male and female catsharks in spring and summer, with males in these two seasons having a greater tooth slope height in the upper jaw than females sampled in spring and summer. There were no significant differences in the upper jaw tooth slope height between adult males and females sampled in autumn and adult males and females sampled in winter.

Table 4.6. *P*-values for seasonal comparison of upper jaw tooth slope height of adult male and female catsharks.

		Male			
		Winter	Spring	Summer	Autumn
Female	Winter	0.236	0.001	<0.001	0.117
	Spring	0.001	<0.001	<0.001	<0.001
	Summer	0.007	<0.001	<0.001	0.001
	Autumn	0.193	<0.001	<0.001	0.079

4.3.3.2 Tooth Width

The data for the upper jaw tooth width in adult male and female catsharks shows a similar pattern to the upper jaw tooth slope height (Figure 4.18). Throughout the year adult males possess wider teeth in the upper jaw than adult females.

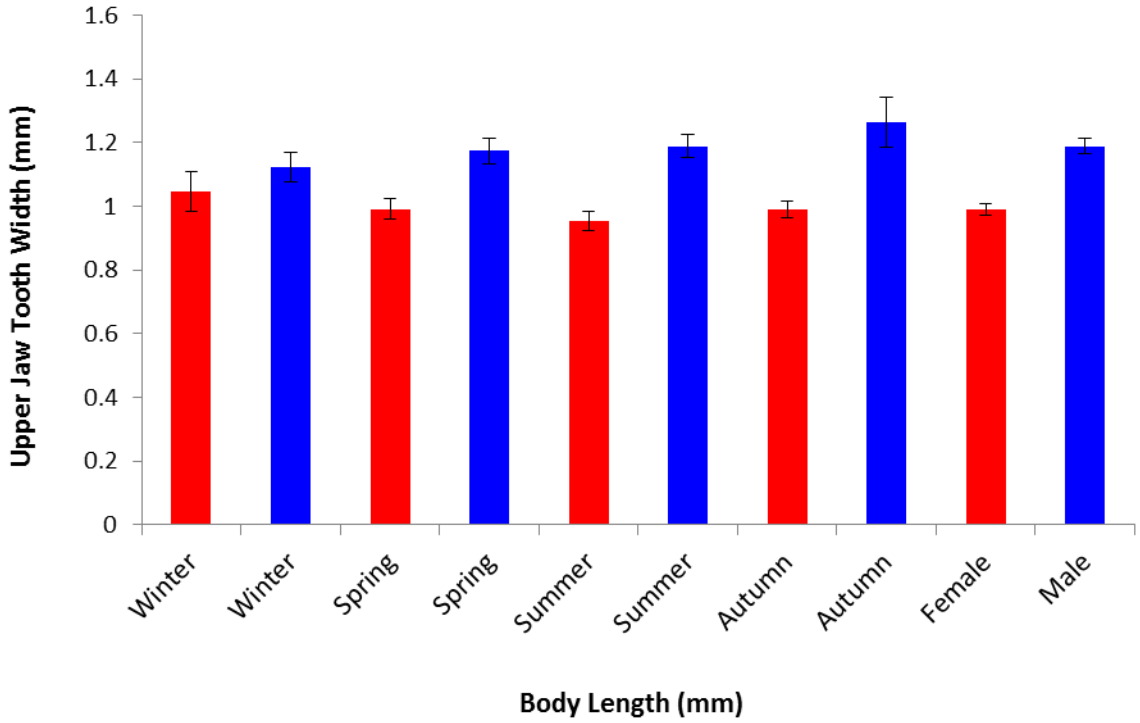

Figure 4.18. Gender and seasonal comparison of upper jaw tooth width for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

It can be seen from Table 4.7 that season and gender within season had no significant effect on the upper jaw tooth width of adult male and female catsharks. Body length and gender were found to have a significant effect, with adult male catsharks possessing wider teeth than adult female catsharks.

Table 4.7. Results from the ANCOVA analyses for the upper jaw tooth width of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.36211	0.19704	0.19704	13.06	**0.001**
Gender	1	0.61497	0.47701	0.47701	31.63	**<0.001**
Season	3	0.00566	0.01327	0.00442	0.29	**0.830**
Season*Gender	3	0.06013	0.06013	0.02004	1.33	**0.272**

Figure 4.19 shows a graphical representation of the upper jaw tooth width of male and female adult catsharks. It can be seen that as body length increases so does upper jaw tooth width. There is a clear gender split, with adult males possessing a greater tooth width in the upper jaw than adult females. It can also be seen that the tooth width of both males and females increases with body length. There was no significant difference between the regression lines of adult male and female catsharks (*P*= 0.289).

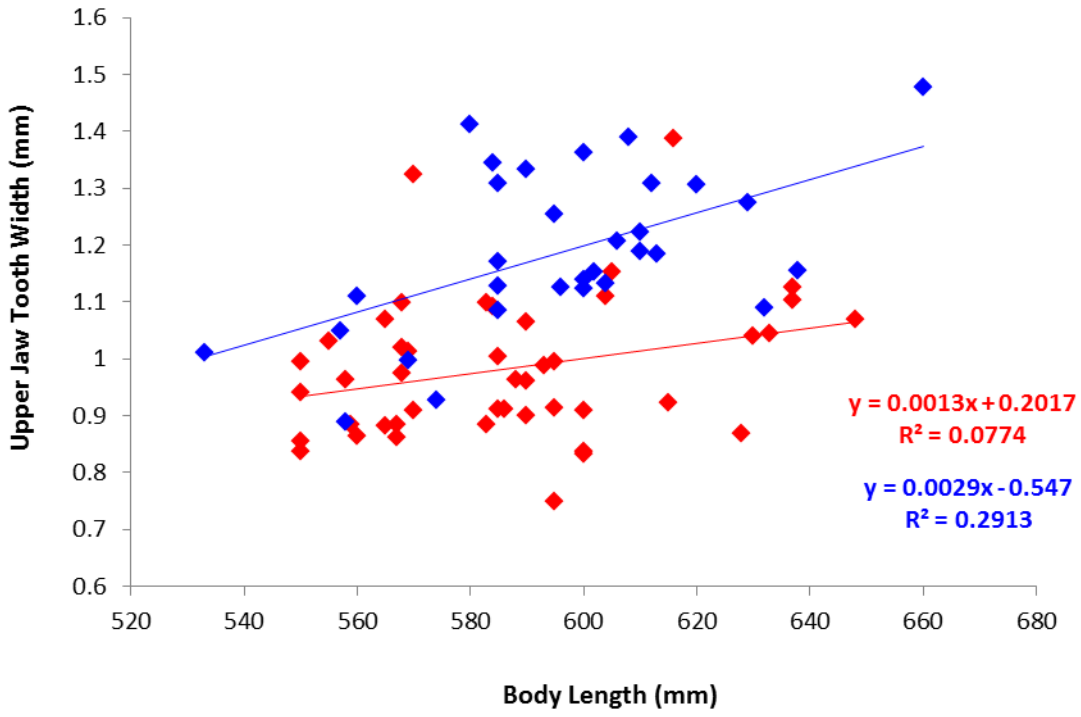

Figure 4.19. Scatterplot with regression for upper jaw tooth width against body length for adult male and female *S. canicula* (n= M (31), F (50)) (Male = Blue, Female = Red) (*P*= 0.289).

4.3.3.3 Tooth Cusp Base

Figure 4.20 shows a graphical representation of the means and standard errors for the upper jaw tooth slope height in adult male and female catsharks for all seasons. The cusp base diameter of adult male catsharks was shown to be greater than that of adult female catsharks throughout the year.

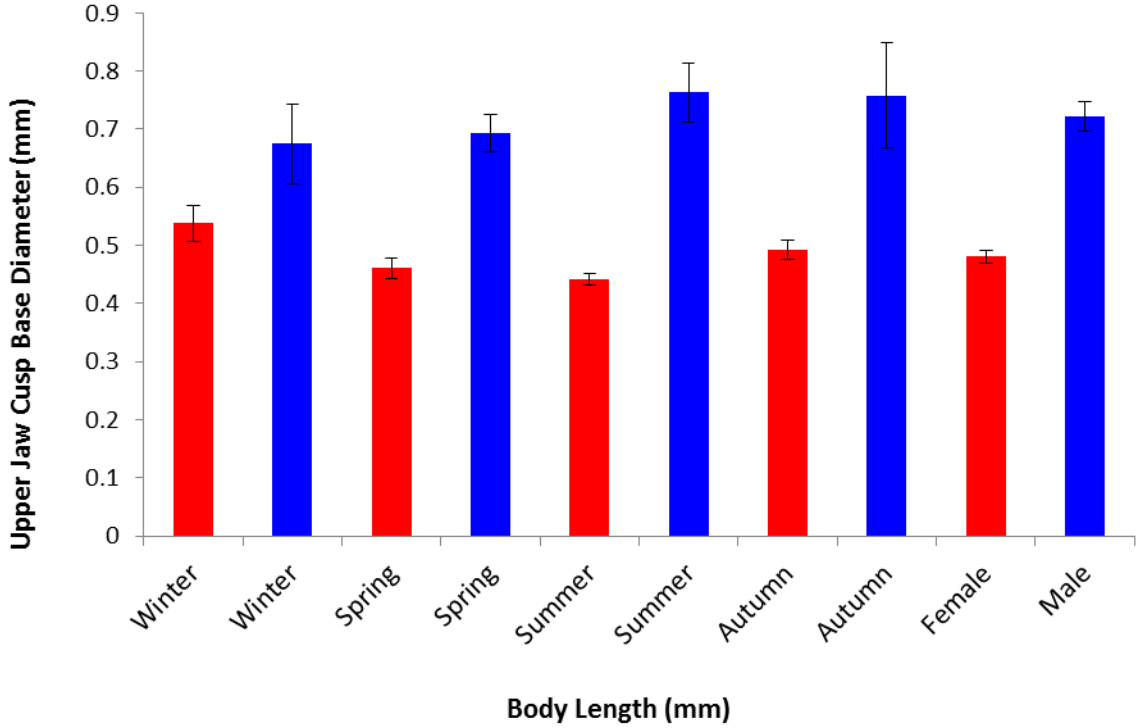

Figure 4.20. Gender and seasonal comparison of upper jaw tooth cusp base diameter for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

It can be seen from Table 4.8 that season and gender within season had no effect on the upper jaw tooth cusp base diameter of adult male and female catsharks. Body length and gender were found to have a significant effect, with adult male catsharks possessing wider cusp base than adult females.

Table 4.8. Results from the ANCOVA analyses for the upper jaw tooth cusp base diameter of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.19315	0.07075	0.07075	6.92	**0.010**
Gender	1	1.00461	0.83106	0.83106	81.26	**<0.001**
Season	3	0.02132	0.01677	0.00559	0.55	**0.652**
Season*Gender	3	0.06285	0.06285	0.02095	2.05	**0.115**

Figure 4.21 shows a graphical representation of the upper jaw tooth cusp base diameter of male and female adult catsharks. It can be seen that as body length increases so does the cusp base of the upper jaw teeth. There is a clear gender division, with adult female catsharks possessing a larger cusp base diameter in the upper jaw teeth than adult female catsharks. It can also be seen from Figure 4.21 that as the body length of the catsharks increases the difference in cusp base diameter becomes more pronounced. Comparison of the male and female regression lines showed that there was no significant difference between the increase of the upper jaw tooth cusp base diameter for adult catsharks (*P*= 0.226)

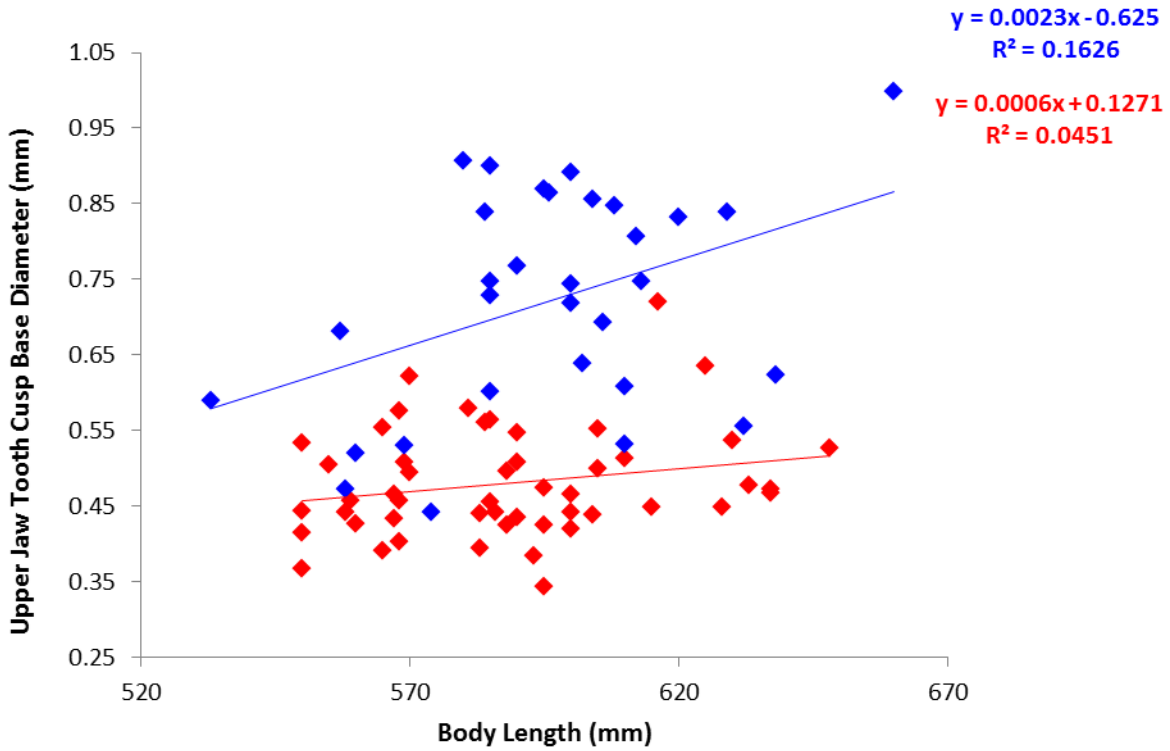

Figure 4.21. Scatterplot with regression upper jaw tooth cusp base diameter against body length for adult *S. canicula* (n= M (31), F (50)) (Male = Blue, Female = Red) (*P*= 0.226).

4.3.3.4 Tooth Cusp Mid Diameter

The results for the upper jaw mid tooth cusp diameter in adult catsharks can be seen in Figure 4.22. The mid cusp diameter in the upper jaw teeth was found to be greater in adult male catsharks than in adult female catsharks throughout the year.

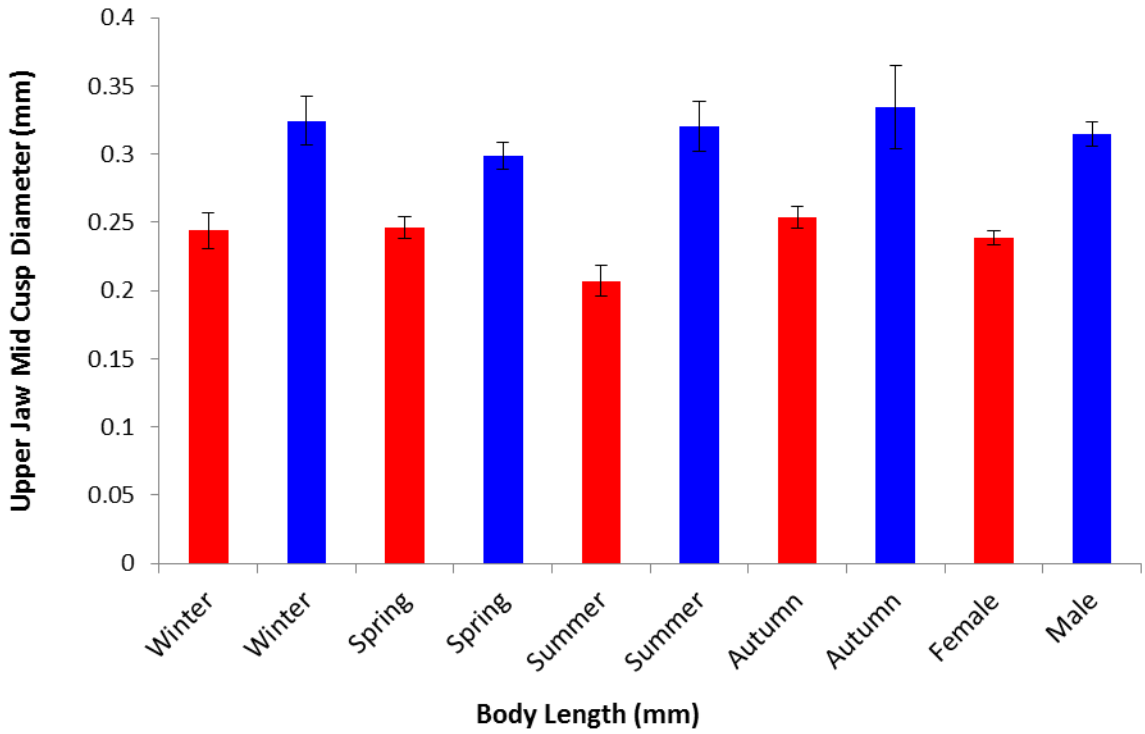

Figure 4.22. Gender and easonal comparison of upper jaw tooth mid cusp diameter for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

Table 4.9 shows that season and gender within season had no significant effect on the upper jaw tooth mid cusp diameter of adult male and female catsharks. Body length and gender were found to have significant effects, with adult male catsharks possessing wider mid cusp diameter than adult females.

Table 4.9. Results from the ANCOVA analyses for the upper jaw tooth mid cusp diameter of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.030121	0.010805	0.010805	7.16	**0.009**
Gender	1	0.095517	0.095550	0.095550	63.34	**<0.001**
Season	3	0.008763	0.005577	0.001859	1.23	**0.304**
Season*Gender	3	0.008863	0.008863	0.002954	1.96	**0.128**

Figure 4.23 shows a graphical representation of the upper jaw tooth mid cusp diameter of male and female adult catsharks. It can be seen that as body length increases so does the mid cusp diameter of the upper jaw teeth. It can also be seen from Figure 4.23 that there is a clear differentiation between the genders, with adult females generally having a smaller mid cusp diameter in the upper jaw teeth than adult males. The results of the ANOVA showed that there was no significant difference between the increase of the upper jaw mid cusp diameter against body length for adult catsharks (P= 0.061).

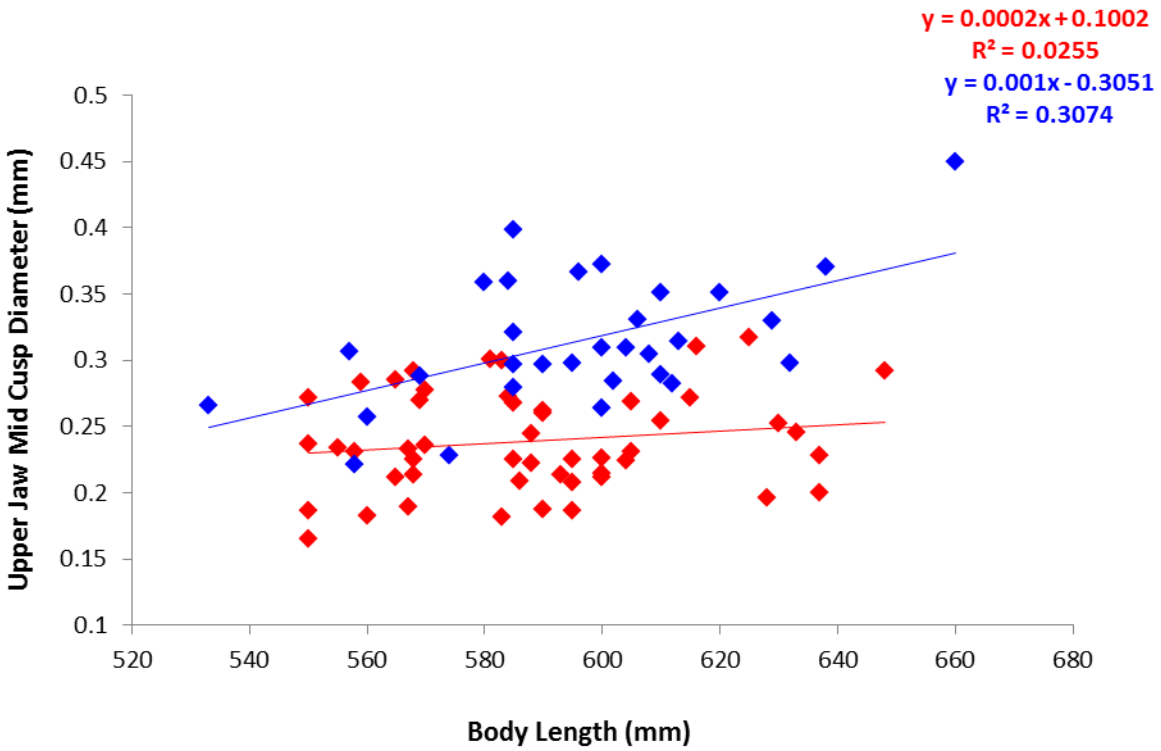

Figure 4.23. Scatterplot with regression for upper jaw tooth mid cusp diameter against body length for adult male and female *S. canicula* (n= M (31), F (50)) (Male = Blue, Female = Red) (*P*= 0.061).

4.3.3.5 Tooth Cusp Tip Diameter

Figure 4.24 shows a graphical representation of the means and standard errors for the upper jaw tooth cusp tip diameter in adult male and female catsharks for all seasons. The findings for the upper jaw cusp tip diameter follow the same pattern for the other upper jaw tooth dimensions, whereby adult males possess a greater cusp tip diameter than adult females throughout the year.

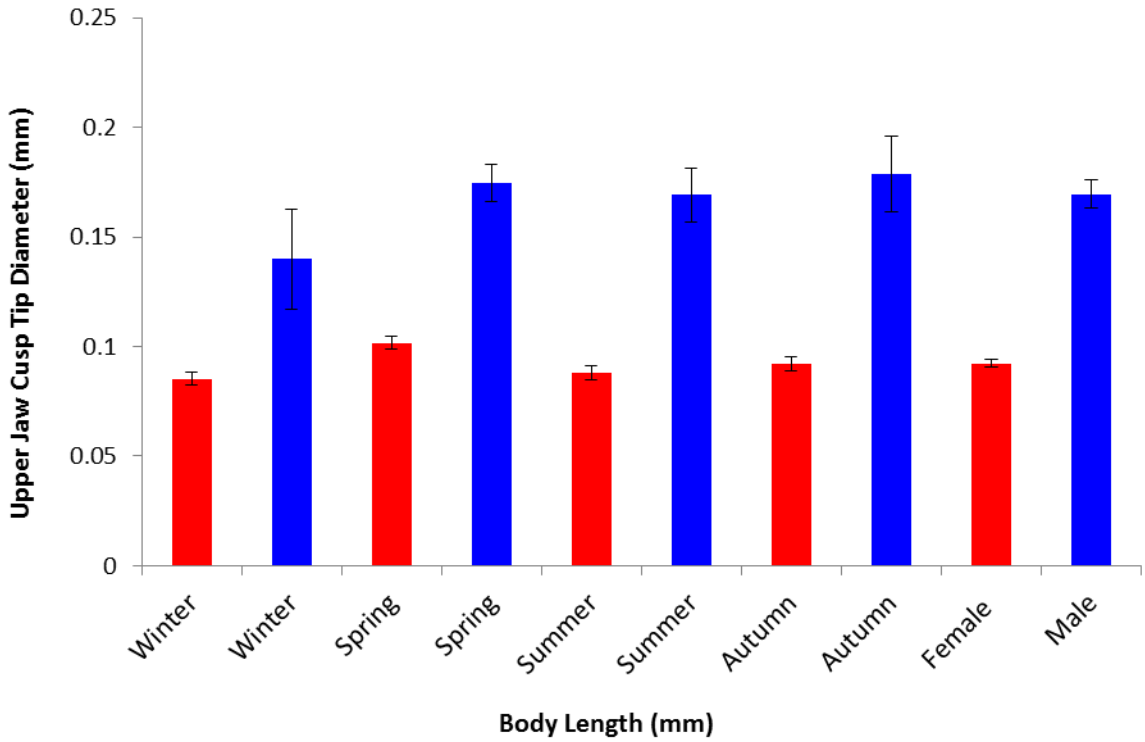

Figure 4.24. Gender and seasonal comparison of upper jaw tooth cusp tip diameter for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

Table 4.10 shows that gender within season had no effect on the upper jaw tooth cusp tip diameter of adult male and female catsharks. Body length, gender and season were found to have significant effects, with adult male catsharks possessing wider mid cusp diameter than adult females. Females sampled in spring were also found to have a larger tip diameter compared to females sampled in winter and summer.

Table 4.10. Results from the ANCOVA analyses for the upper jaw tooth cusp tip diameter of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.010308	0.002950	0.002950	5.54	**0.021**
Gender	1	0.106282	0.082139	0.082139	154.22	**<0.001**
Season	3	0.004734	0.006022	0.002007	3.77	**0.014**
Season*Gender	3	0.001698	0.001698	0.000566	1.06	**0.370**

It can be seen from Figure 4.25 that as the body length of adult catsharks increases so does the cusp tip diameter of the upper jaw teeth. The results of the ANOVA showed that there was no significant difference between the regression lines for adult male and female catsharks in terms of upper jaw tooth cusp diameter increase against body length for male and female *S. canicula* (*P*= 0.320).

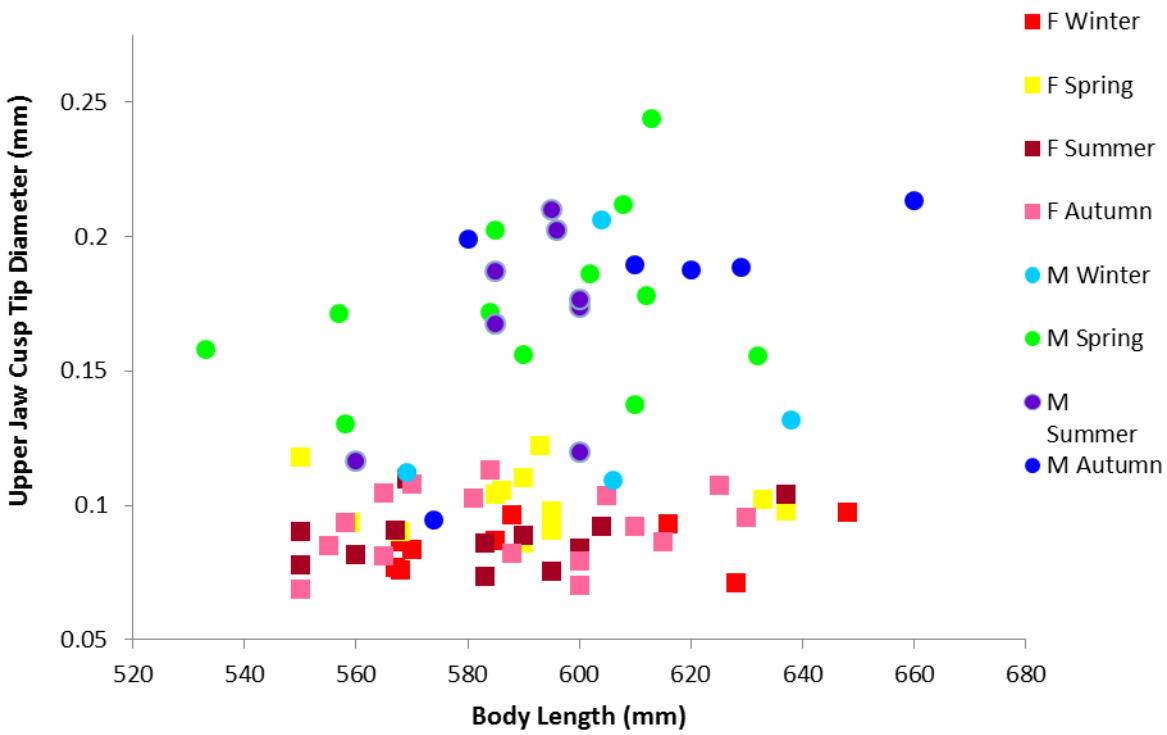

Figure 4.25. Seasonal scatterplot for upper jaw tooth cusp tip diameter against body length for adult male and female *S. canicula*. (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (*P*= 0.320).

4.3.3.6 Tooth Cusp Number

Figure 4.26 shows a graphical representation of the means and standard errors for the upper jaw tooth cusp number in adult male and female catsharks for all seasons. It is clear that the number of cusps in adult female catsharks is greater than in adult male catsharks and that this pattern occurs throughout the year.

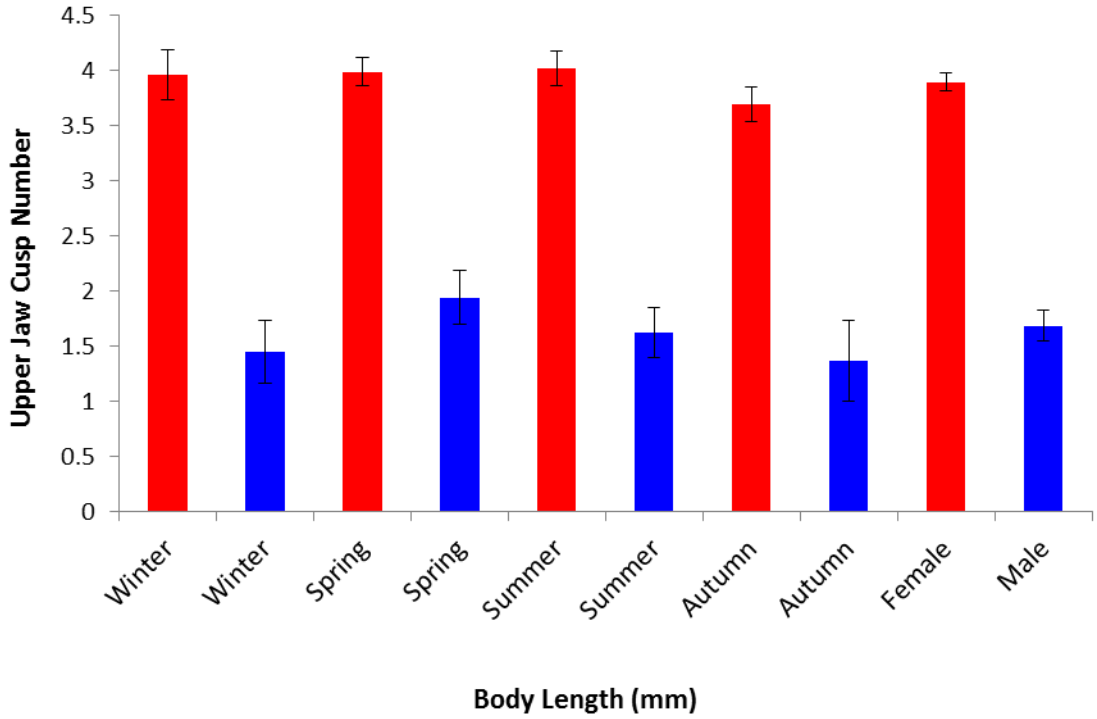

Figure 4.26. Gender and seasonal comparison of upper jaw tooth cusp number for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

Table 4.11 shows that body length, season and gender within season had no effect on the upper jaw tooth cusp number of adult male and female catsharks. Gender was found to have a significant effect, with adult female catsharks possessing more tooth cusps than adult males.

Table 4.11. Results from the ANCOVA analyses for the upper jaw tooth cusp number of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	2.744	0.000	0.000	0.00	**0.980**
Gender	1	90.611	86.882	86.882	192.94	**<0.001**
Season	3	2.087	2.026	0.675	1.50	**0.222**
Season*Gender	3	0.557	0.557	0.186	0.41	**0.744**

4.3.3.7 Tooth Row Number

It can be seen from Figure 4.27 that throughout the year adult male catsharks possess a greater number of tooth rows in the upper jaw than adult female catsharks.

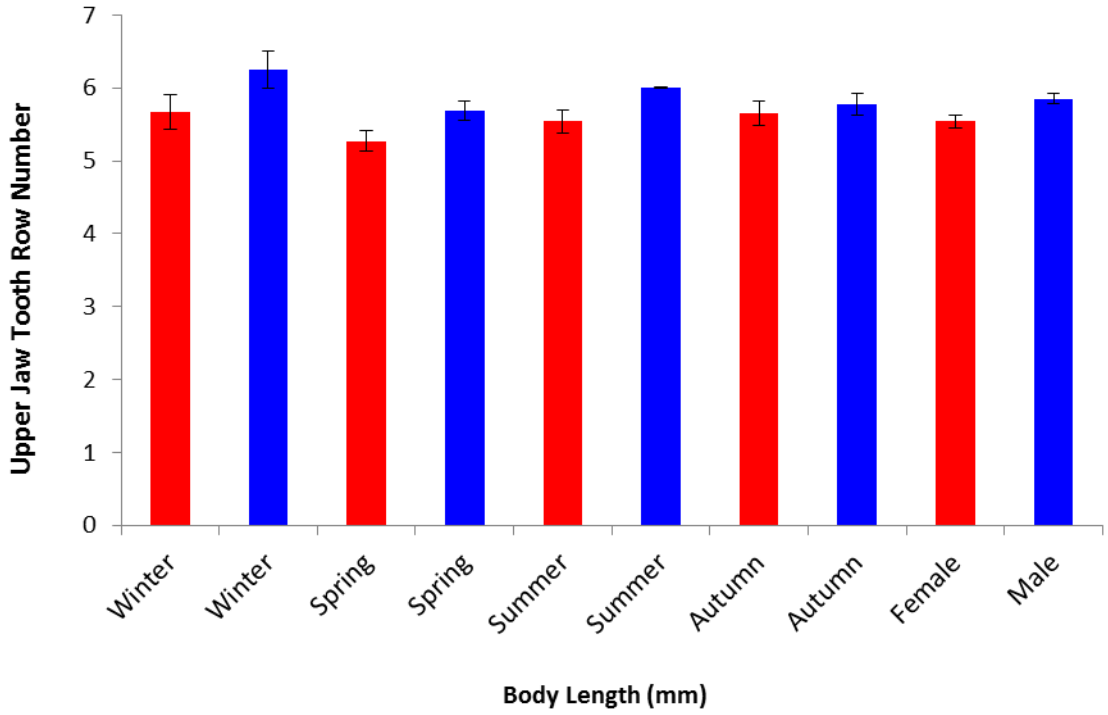

Figure 4.27. Gender and seasonal comparison of upper jaw tooth row number for adult catsharks showing means and ± standard errors (n= Female (50) (W, 9) (Sp, 13) (Su, 12) (A, 16) Male (31) (W, 4) (Sp, 13) (Su, 8) (A, 6)) (Female = Red, Male = Blue).

Table 4.12 shows that body length, season and gender within season had no effect on the upper jaw tooth row number of adult male and female catsharks. Gender was found to have a significant effect, with adult male catsharks possessing more tooth rows than adult females.

Table 4.12. Results from the ANCOVA analyses for the upper jaw tooth row number of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.3007	0.1426	0.1426	0.48	**0.491**
Gender	1	1.8781	2.6614	2.6614	8.94	**0.004**
Season	3	1.7474	1.9002	0.6334	2.13	**0.104**
Season*Gender	3	0.4598	0.4598	0.1533	0.52	**0.673**

4.3.4 Adult Tooth Data – Lower Jaw

The means ± standard errors and ranges for the lower jaw tooth morphometrics of adult *S. canicula* can be seen in Appendix 3.

4.3.4.1 Tooth Slope Height

The results for the adult tooth slope height in the lower jaw can be seen in Figure 4.28 It is clear from the data that throughout the year adult males posses teeth with a greater height than adult females.

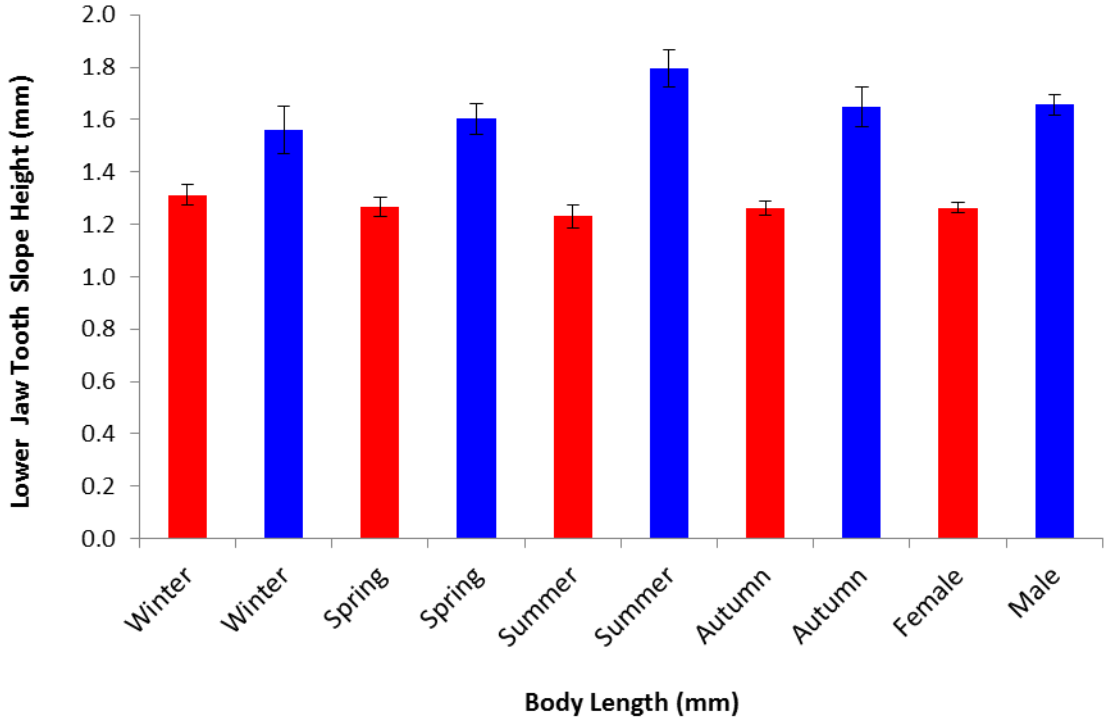

Figure 4.28. Gender and seasonal comparison of lower jaw tooth slope height for adult catsharks showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

It can be seen from Table 4.13 that season had no effect on the lower jaw tooth slope height of adult male and female catsharks. Body length, gender and gender within season were found to have a significant effect, with adult male catsharks possessing teeth with a greater height than adult females sampled in all seasons.

Table 4.13. Results from the ANCOVA analyses for the lower jaw tooth slope height of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.62460	0.23099	0.23099	9.11	**0.003**
Gender	1	2.71144	2.27911	2.27911	89.88	**<0.001**
Season	3	0.08413	0.11660	0.03887	1.53	**0.213**
Season*Gender	3	0.25015	0.25015	0.08338	3.29	**0.025**

Figure 4.29 shows a graphical representation of the lower jaw tooth slope height of individual male and female adult catsharks. It can be seen that as body length increases so does lower jaw tooth slope height. However, the results of the ANOVA show that there is no significant difference in the regression lines for adult male and female catsharks with regard to the increase in lower jaw tooth slope height and body length (*P*= 0.484).

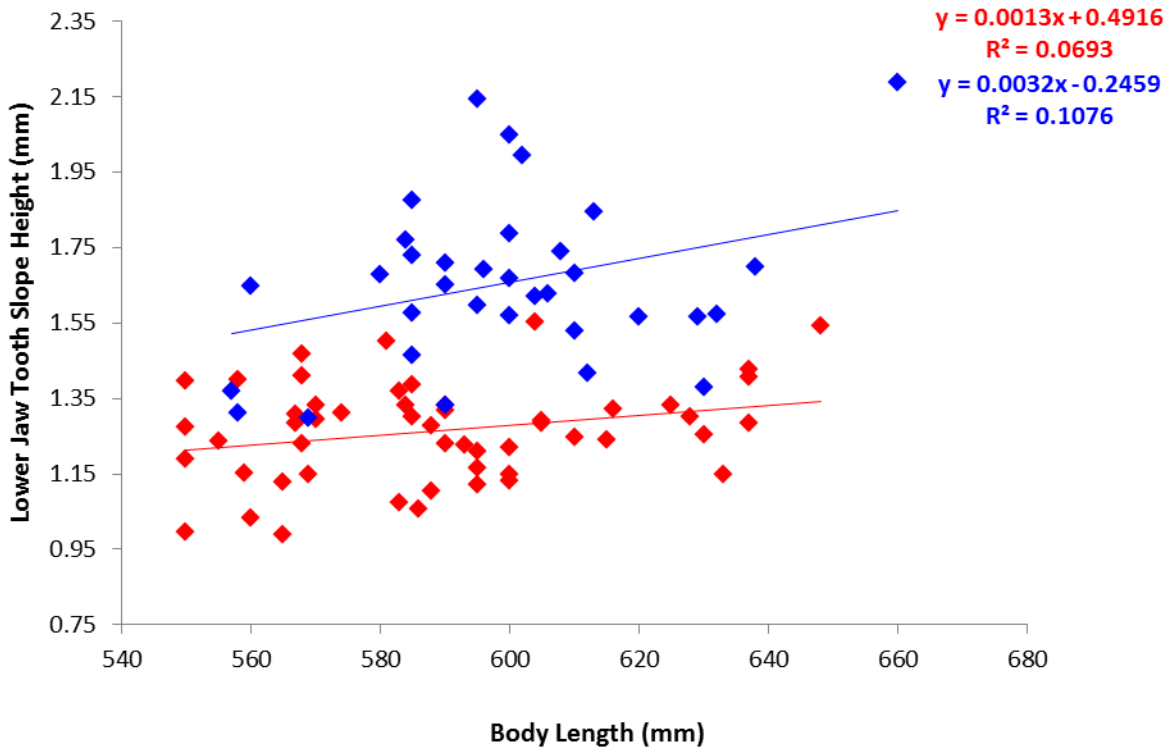

Figure 4.29. Scatterplot with regression upper jaw tooth slope height against body length for adult male and female *S. canicula* (n= M (34), F (52)) (Male = Blue, Female = Red) (*P*= 0.484).

Table 4.14 shows that there was a significant difference in the lower jaw tooth slope height of adult male and female catsharks sampled in summer compared with those sampled in winter. Adult male catsharks sampled in summer had a greater tooth slope height than those sampled in winter. There were no seasonal differences between female catsharks.

Table 4.14. *P*-values for seasonal comparison of lower jaw tooth slope height for adult male and female catsharks.

Season	Winter	Spring	Summer	Autumn	
Winter		0.972	0.040	0.964	Male
Spring	0.999		0.105	1.000	
Summer	0.978	0.999		0.183	
Autumn	0.999	1.000	1.000		
	Female				

Table 4.15 shows that there are significant differences in the lower jaw tooth slope height of adult male and female catsharks in all seasons except winter. The teeth of adult males are larger than those of females in all seasons, except winter.

Table 4.15. *P*-values for seasonal comparison of lower jaw tooth slope height for adult male and female catsharks.

		Male			
		Winter	Spring	Summer	Autumn
Female	Winter	0.324	0.003	<0.001	0.004
	Spring	0.085	<0.001	<0.001	<0.001
	Summer	0.039	<0.001	<0.001	<0.001
	Autumn	0.075	<0.001	<0.001	<0.001

4.3.4.2 Tooth Width

Figure 4.30 shows a graphical representation of the means and standard errors for the lower jaw tooth slope height in adult male and female catsharks for all seasons. As with

lower jaw tooth slope height, the lower jaw tooth width of adult male *S. canicula* is greater that that of adult females catsharks throughout the year.

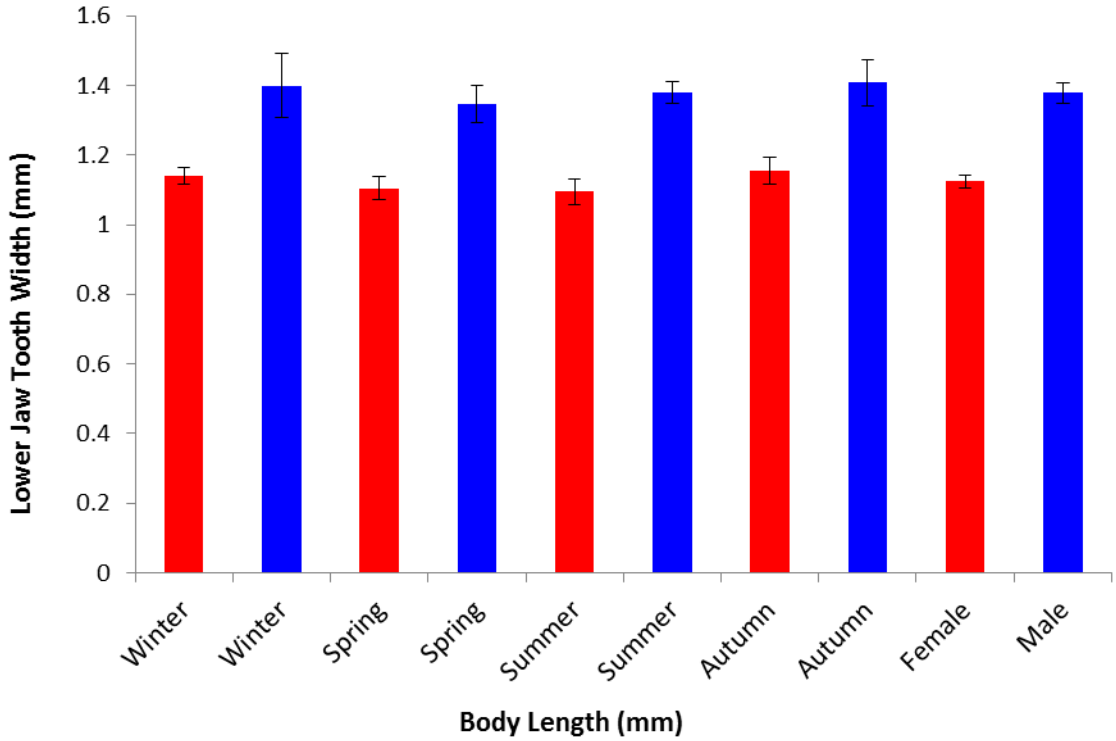

Figure 4.30. Gender and seasonal comparison of lower jaw tooth width for adult catsharks showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

Table 4.16 shows that season and gender within season had no effect on the lower jaw tooth width of adult male and female catsharks. Body length and gender were found to have a significant effect, with adult male catsharks possessing wider teeth than adult females.

Table 4.16. Results from the ANCOVA analyses for the lower jaw tooth width of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.48085	0.23616	0.23616	11.18	**0.001**
Gender	1	0.11835	1.01087	1.01087	47.87	**<0.001**
Season	3	0.02654	0.02261	0.00754	0.36	**0.784**
Season*Gender	3	0.00975	0.00975	0.00325	0.15	**0.927**

Figure 4.31 shows a graphical representation of the lower jaw tooth width of male and female adult catsharks. It can be seen that as body length increases so does the width of the lower jaw teeth. At shorter body lengths there is less distinction between the genders with regards to width of the lower jaw teeth. As the body length increases there is a gradual separation between the genders, with adult male catsharks possessing increasingly wider teeth on the lower jaw than adult female catsharks. The comparison of the male and females regression lines showed that there was no significant difference between the genders in the increase in lower jaw tooth width with body length for adult catsharks (*P*= 0.087).

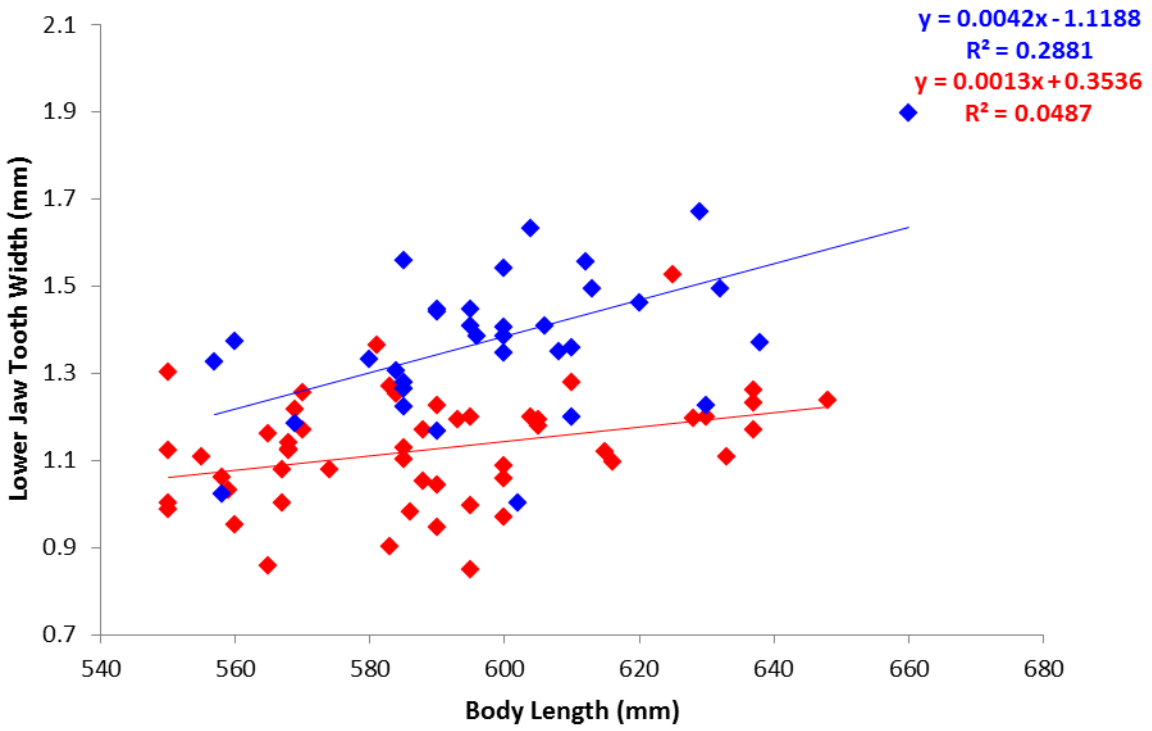

Figure 4.31. Scatterplot with regression lower jaw tooth width against body length for adult male and female *S. canicula* (n= M (34), F (52)) (Male = Blue, Female = Red) (*P*= 0.087).

4.3.4.3 Tooth Cusp Base Diameter

The results of the statistical analyses for the cusp base diameter of the lower jaw teeth from adult male and female *S. canicula* can be seen in Figure 4.32. The data shows that adult male *S. canicula* possess a greater cusp base diameter in the lower jaw teeth throughout the year compared to adult female catsharks.

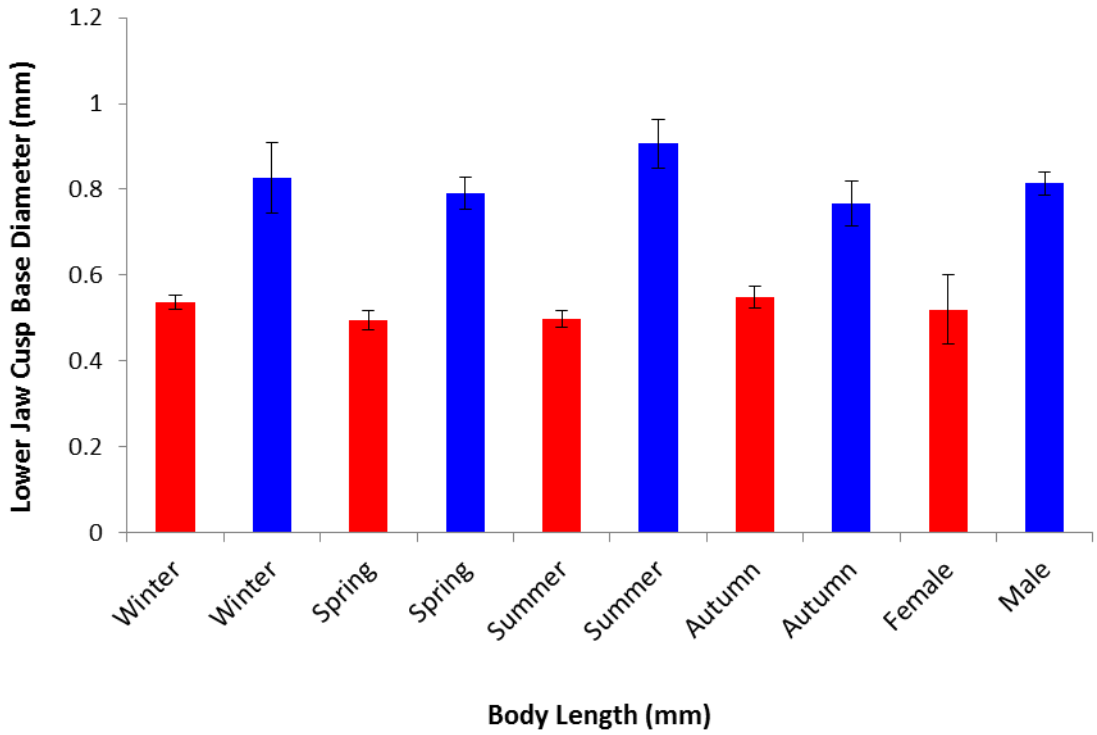

Figure 4.32. Gender and seasonal comparison of lower jaw tooth cusp base diameter for adult catsharks, showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

It can be seen from Table 4.17 that season had no effect on the lower jaw tooth cusp base diameter of adult male and female catsharks. Body length, gender and gender within season were found to have a significant effect, with adult male catsharks sampled in all seasons possessing a wider tooth cusp base diameter than adult females in all seasons.

Table 4.17. Results from the ANCOVA analyses for the lower jaw tooth cusp base diameter of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.23223	0.09067	0.09067	6.98	**0.010**
Gender	1	1.63606	1.50195	1.50195	115.58	**<0.001**
Season	3	0.03445	0.05514	0.01838	1.41	**0.245**
Season*Gender	3	0.11765	0.11765	0.03922	3.02	**0.035**

Figure 4.33 shows a graphical representation of the lower jaw tooth cusp base diameter of male and female adult catsharks. It can be seen that as body length increases so does the cusp base diameter of the lower jaw teeth. However, no significant difference was found between the two regression lines for lower tooth cusp base diameter for adult male and female *S. canicula* (*P*= 0.905).

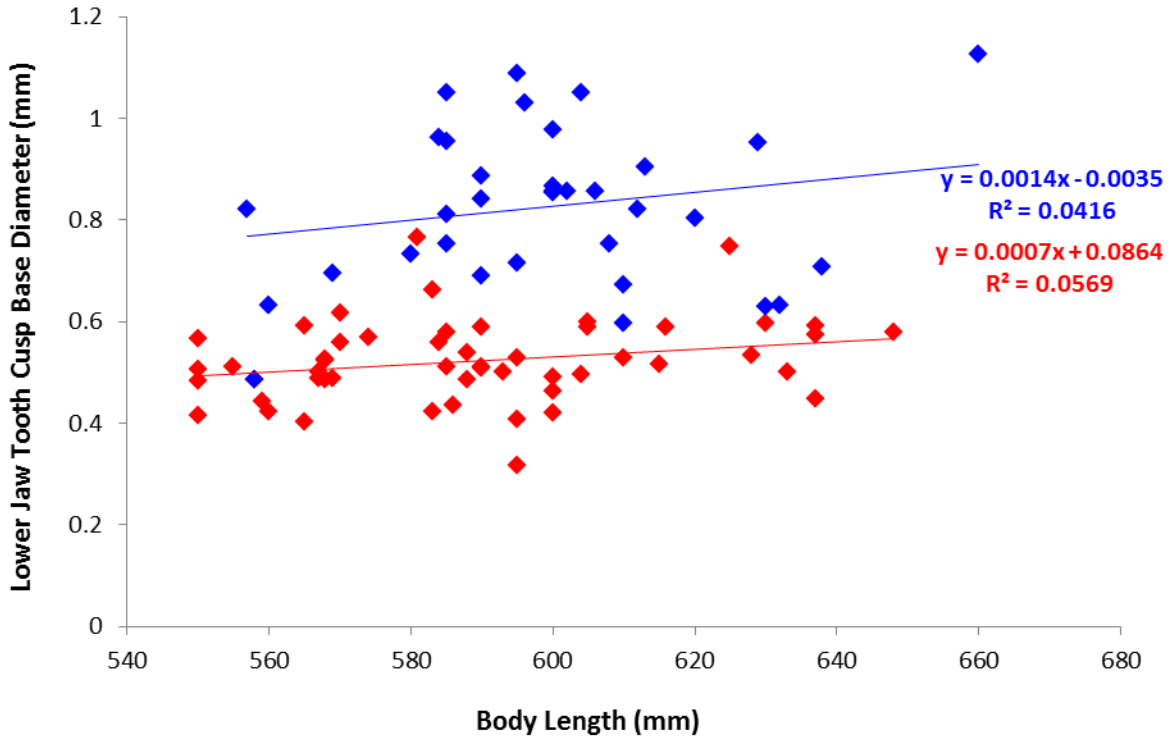

Figure 4.33. Scatterplot with regression lower jaw tooth cusp base diameter against body length for adult male and female *S. canicula* (n= M (34), F (52)) (Male = Blue, Female = Red) (*P*= 0.905).

Table 4.18 shows that there were no significant seasonal differences in the tooth cusp base diameter of the lower jaws of adult male and female catsharks (P>0.05).

Table 4.18. *P*-values for seasonal comparison of lower jaw tooth cusp base diameter for adult male catsharks.

Season	Winter	Spring	Summer	Autumn	
Winter		1.000	0.856	0.979	Male
Spring	0.993		0.330	0.977	
Summer	1.000	1.000		0.065	
Autumn	1.000	0.850	0.971		
	Female				

Table 4.19 shows that there are significant differences in the lower jaw tooth cusp base diameter of adult male and female catsharks in all seasons. The cusp base diameter of the lower jaw teeth of adult males is larger than those of females in all seasons.

Table 4.19. *P*-values for seasonal comparison of lower jaw tooth cusp base diameter for adult male and female catsharks.

Female	Male				
		Winter	Spring	Summer	Autumn
	Winter	0.003	<0.001	<0.001	0.002
	Spring	<0.001	<0.001	<0.001	<0.001
	Summer	0.001	<0.001	<0.001	<0.001
	Autumn	0.004	<0.001	<0.001	0.002

4.3.4.4 Tooth Cusp Mid Diameter

Figure 4.34 shows a graphical representation of the means and standard errors for the lower jaw tooth mid cusp diameter in adult male and female catsharks for all seasons. Throughout the year adult male catsharks possess a greater mid cusp diameter than adult female catsharks.

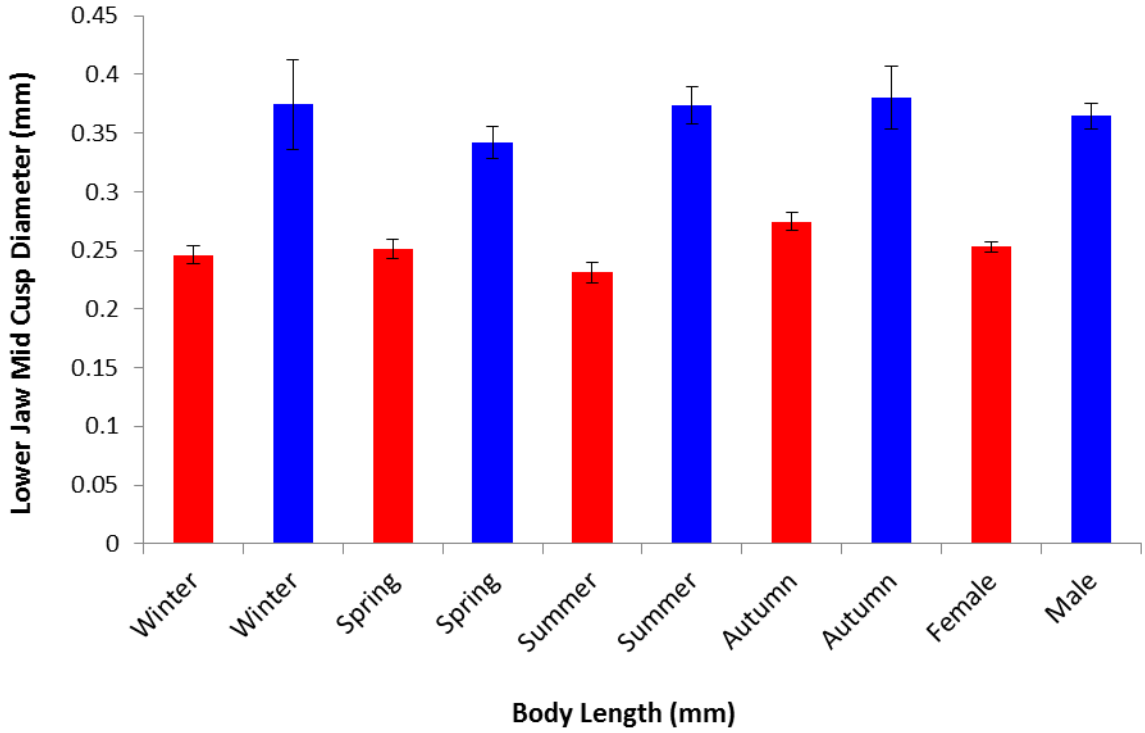

Figure 4.34. Gender and seasonal comparison of lower jaw tooth mid cusp diameter for adult catsharks showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

It can be seen from Table 4.20 that season and gender within season had no effect on the lower jaw tooth cusp base diameter of adult male and female catsharks. Body length and gender were found to have a significant effect, with adult male catsharks sampled in all seasons possessing a wider mid cusp diameter on lower jaw teeth than adult females in all seasons.

Table 4.20. Results from the ANCOVA analyses for the lower jaw tooth mid cusp diameter of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.064702	0.024815	0.024815	12.36	**0.001**
Gender	1	0.225121	0.216046	0.216046	107.64	**<0.001**
Season	3	0.010910	0.008766	0.002922	1.46	**0.233**
Season*Gender	3	0.007892	0.007892	0.002631	1.31	**0.277**

Figure 4.35 shows a graphical representation of the lower jaw tooth mid cusp diameter of male and female adult catsharks. It can be seen that as body length increases so does the cusp base diameter of the lower jaw teeth. As with the lower jaw cusp base diameter the regression slopes show that this increase in mid cusp base diameter relative to body length diversifies between adult males and females with body length. As adult male catsharks grow they develop a greater cusp base diameter relative to body length compared to adult female catsharks. The results of the ANOVA showed that there was a significant difference between the regression lines of male and female catsharks in relation to lower jaw mid cusp diameter. Adult male catsharks showed a greater mid cusp diameter than adult females with an increase in body length (P= 0.042).

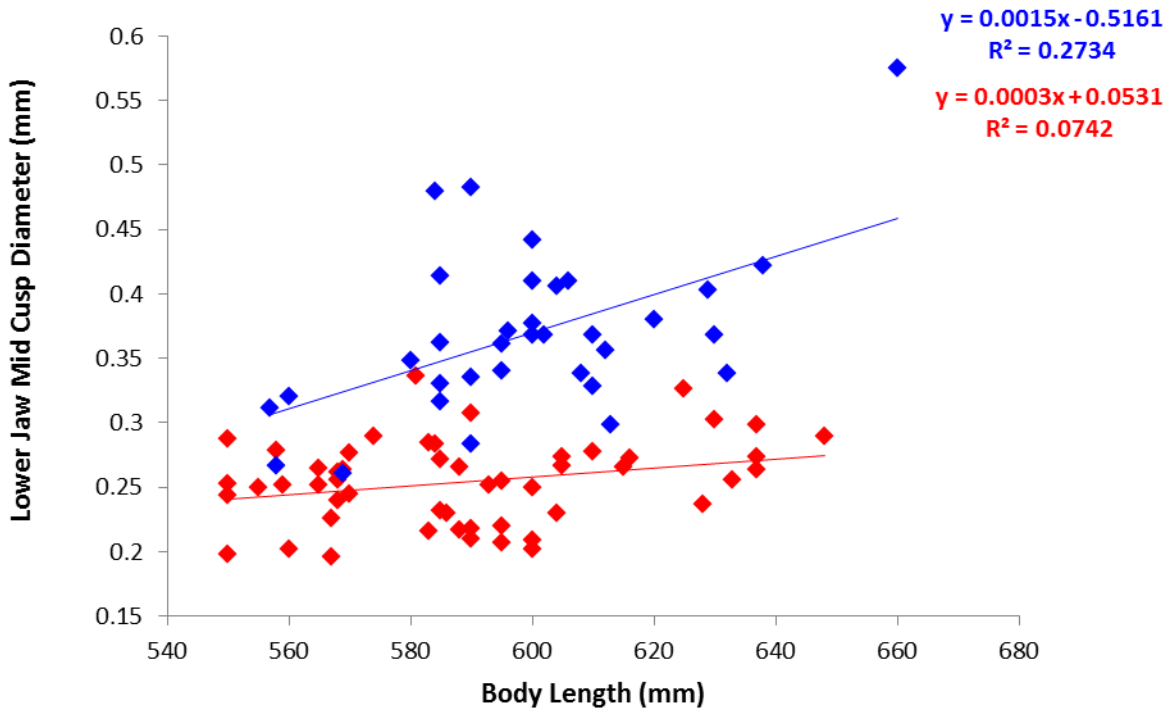

Figure 4.35. Scatterplot with regression lower jaw tooth mid cusp base against body length for adult male and female *S. canicula* (n= M (34), F (52)) (Male = Blue, Female = Red) (*P*= 0.042).

4.3.4.5 Tooth Cusp Tip Diameter

The results for the lower jaw cusp tip diameter are shown in Figure 4.36 Throughout the year adult male catsharks possess a wider cusp tip on the lower jaw teeth than adult females catsharks.

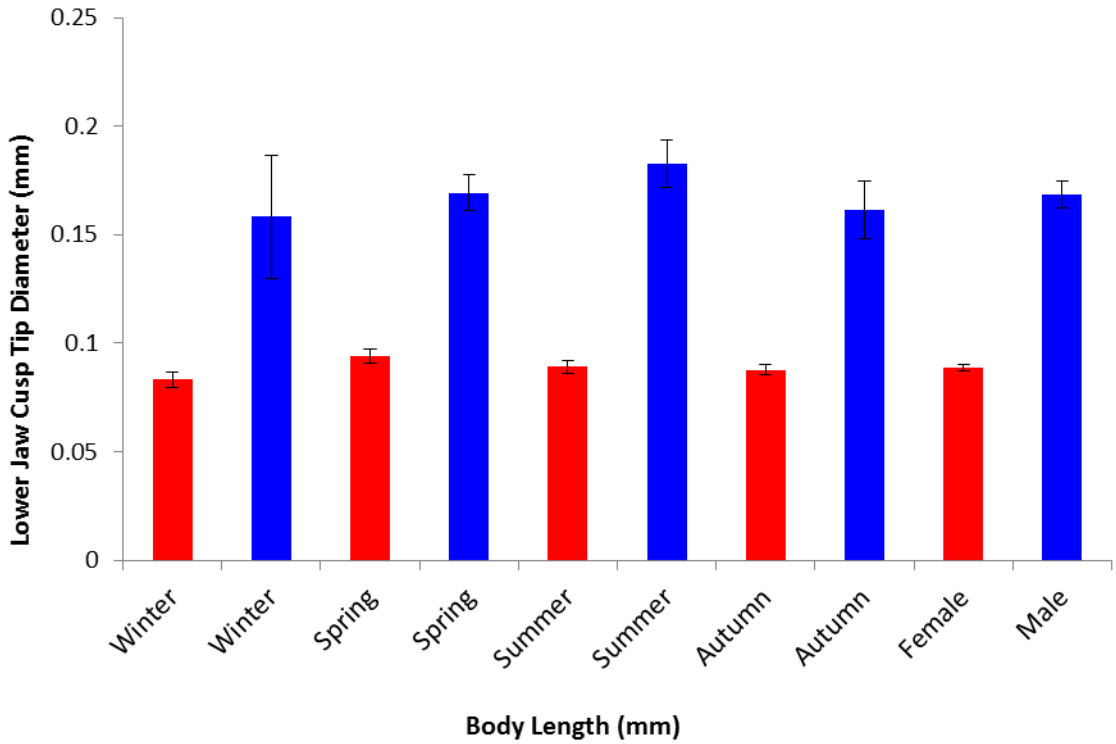

Figure 4.36. Gender and seasonal comparison of lower jaw tooth cusp tip diameter for adult catsharks showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

It can be seen from Table 4.21 that body length, season and gender within season had no significant effect on the lower jaw tooth cusp base diameter of adult male and female catsharks. Gender was found to have a significant effect, with adult male catsharks sampled in all seasons possessing a wider cusp tip diameter on lower jaw teeth than adult females sampled in all seasons.

Table 4.21. Results from the ANCOVA analyses for the lower jaw tooth cusp tip diameter of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.006686	0.000958	0.000958	1.44	**0.234**
Gender	1	0.127107	0.108235	0.108235	162.46	**<0.001**
Season	3	0.002308	0.002852	0.000951	1.43	**0.241**
Season*Gender	3	0.001414	0.001414	0.000471	0.71	**0.551**

4.3.4.6 Cusp Number

Figure 4.37 shows a graphical representation of the means and standard errors for the lower jaw tooth cusp number in adult male and female catsharks for all seasons. It can be seen from Figure 4.38 that the pattern of cusp numbers of the lower jaw teeth mirrors that of the upper jaw teeth, whereby adult females have more cusps than adult males throughout the year.

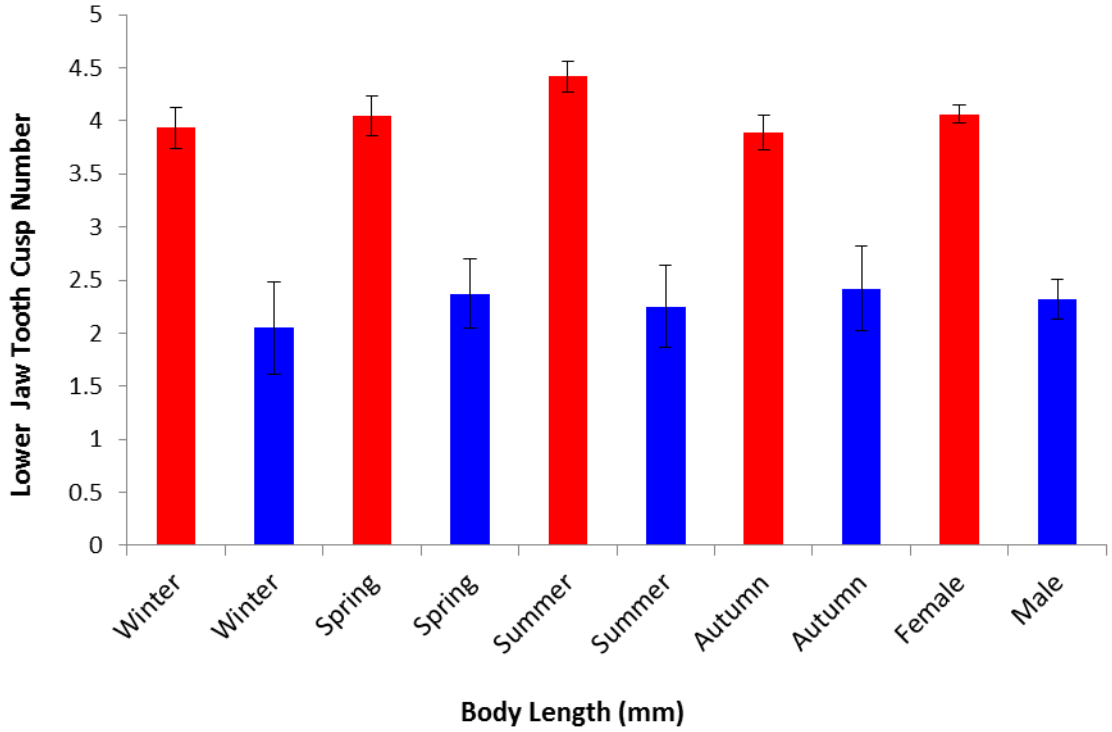

Figure 4.37. Gender and seasonal comparison of lower jaw tooth cusp number for adult catsharks showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

It can be seen from Table 4.22 that body length, season and gender within season had no significant effect on the lower jaw tooth cusp number of adult male and female catsharks. Gender was found to have a significant effect, with adult female catsharks possessing more tooth cusps on the lower jaw teeth than adult males.

Table 4.22. Results from the ANCOVA analyses for the lower jaw tooth cusp number of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	2.9664	0.0889	0.0889	0.11	**0.740**
Gender	1	60.6964	56.5177	56.5177	70.66	**<0.001**
Season	3	1.0716	0.7595	0.2532	0.32	**0.813**
Season*Gender	3	1.4693	1.4693	0.4898	0.61	**0.609**

4.3.4.7 Tooth Row Numbers

It can be seen from Figure 4.38 that adult male catsharks possess a greater number of tooth rows in the lower jaw than adult female catsharks.

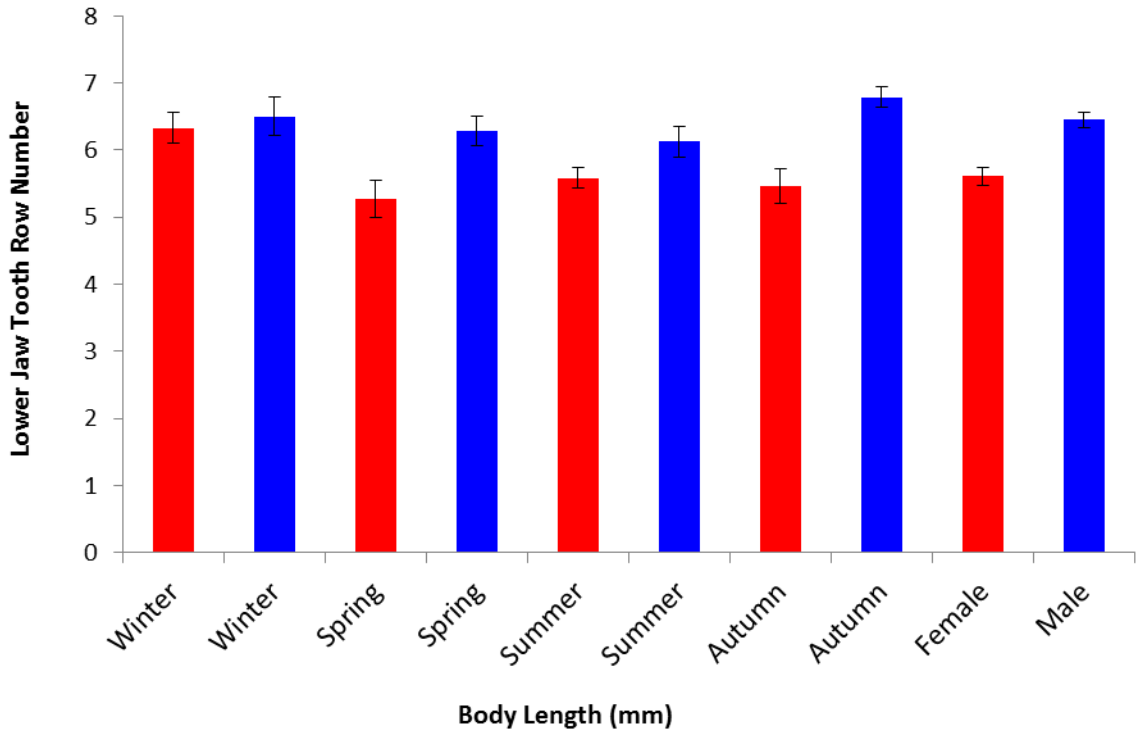

Figure 4.38. Gender and seasonal comparison of lower jaw tooth row number for adult catsharks showing means and ± standard errors (n= Female (52) (W, 9) (Sp, 13) (Su, 13) (A, 17) Male (34) (W, 4) (Sp, 12) (Su, 8) (A, 10)) (Female = Red, Male = Blue).

It can be seen from Table 4.23 that body length, season and gender within season had no significant effect on the lower jaw tooth row number of adult male and female catsharks. Gender was found to have a significant effect, with adult male catsharks possessing more tooth rows on the lower jaw than adult females.

Table 4.23. Results from the ANCOVA analyses for the lower jaw tooth row number of adult male and female catsharks.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	1.6694	0.9406	0.9406	1.52	**0.221**
Gender	1	14.9801	10.1360	10.1360	16.39	**<0.001**
Season	3	5.1019	3.5303	1.1768	1.90	**0.136**
Season*Gender	3	3.7550	3.7550	1.2517	2.02	**0.117**

4.3.5 Discriminant Analysis

Canonical discriminant analysis was carried out on the teeth of both adult and juvenile *S. canicula*. The data for Tooth Slope Height, Tooth Width, Cusp Base Diameter, Mid Cusp Diameter and Cusp Tip Diameter were pooled for adult and juveniles and male and female specimens. Adults and juveniles were analysed separately to ascertain if there were any differences in the structure of the upper and lower jaw teeth of adult and juvenile *S. canicula*. Figure 4.39 shows the results for the adult and juvenile male analysis. The canonical discriminant analysis showed significant differences between functions 1 and 3 (Wilks-Lambda, $P< 0.001$) and between function 2–3 (Wilks-Lambda, $P< 0.001$) but not function 3 (Wilks-Lambda, $P> 0.05$). The first function accounts for 59.3% of the total variation with tooth width showing the strongest correlation with this discriminating function. It can be seen from Figure 4.39 that there are clear differences between the upper and lower jaw tooth dimensions of adult and juvenile male catsharks with some overlap on both the upper and lower jaw teeth for male and female catsharks. This overlap could account the fact that some of the juveniles sampled would have been on the cusp of adulthood and may be expressing adult teeth.

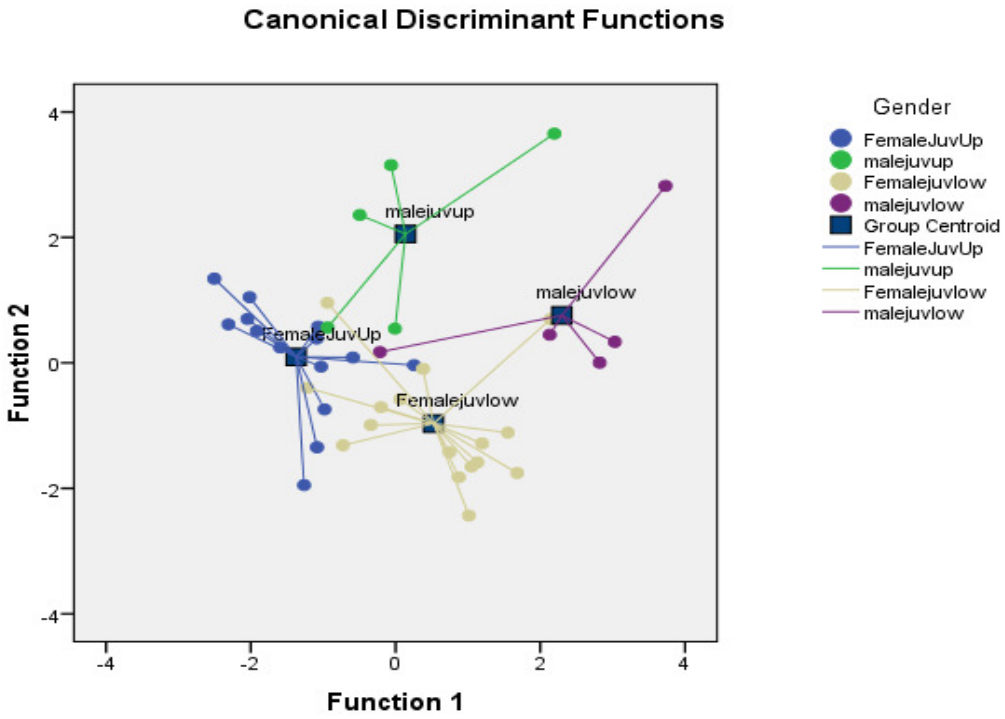

Figure 4.39. Disciminant analysis of upper and lower jaw tooth structure for juvenile male and female *S. canicula*.

Figure 4.40 shows the results for the adult male and female analysis. The canonical discriminant analysis showed that significant differences were observed between functions 1 and 3 (Wilks-Lambda, $P< 0.001$) and between function 2–3 (Wilks-Lambda, $P< 0.001$) but not function 3 (Wilks-Lambda, $P> 0.05$). The first function accounts for 83.8% of the total variation with tooth width showing the strongest correlation with this discriminating function. It can be seen from Figure 4.40 that there are clear distinctions between the upper and lower jaw tooth dimensions of adult and juvenile female catsharks. There is some overlap between male and female teeth with adult males falling into the adult female groups. This again could be due to the fact that some of the individuals sampled were on the cusp of adulthood and were expressing adult dental form.

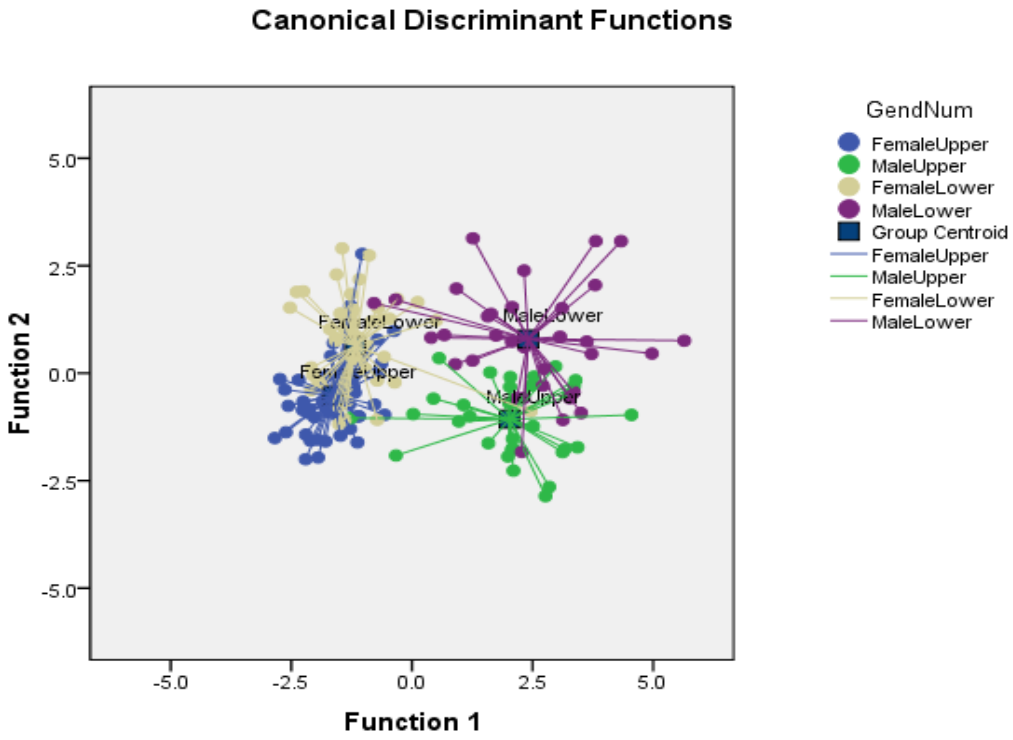

Figure 4.40. Disciminant analysis of upper and lower jaw tooth structure for adult male and female *S. canicula*.

4.3.6 Dental Formula

The dental formula for adult catsharks was taken as a direct comparison against studies of other populations of *S. canicula.* Within these studies the sexes were combined to give an overall representation of the dental formula of *S. canicula*. Previously published data can be seen in Table 4.24.

Table 4.24. Previously published dental formula data for *S. canicula*.

Author	Dental Formula
Springer (1979)	43-48 / 37-45
Compagno (1988)	46 / 40
Ellis and Shackley (1995)	41-60 / 41- 60

The dental formula for the current study has been calculated and can be seen in table 4.25 The data dispayed shows the tooth counts of both the upper and lower jaws and then the combined dental formula of adult male and female catsharks. The dental formula are then combined, inline with data gathered by Springer (1979) Compagno (1988) and Ellis and Shackley (1995).

Table 4.25. Dental formula for upper and lower jaws of adult male and female catsharks (n= F (6) M (6)).

	Male	Female
Upper Jaw	(21 - 25) + (0 - 1) + (21 - 25)	(23 - 28) + (0 - 2) + (24 - 29)
Lower Jaw	(20 - 23) + (0 - 1) + (19 - 23)	(19 - 27) + (0 - 2) + (18 - 29)
Dental Formula	41-50 / 41-50	43-55 / 43-58
Combined Dental Formula	41-55 / 41-58	

4.4 Discussion

There is a plethora of literature available on the dentition of a range of elasmobranch species, including work on the development and use of teeth. A great deal of the literature has focused on the development (James, 1953; Reif, 1984) tooth replacement (Ifft & Zinn, 1948; Moss, 1972; Luer *et al*., 1990) tooth morphology (Fedducia and Slaughter, 1974; Goto, 2001) and feeding (Lyle, 1983; McEachran, 1975) of elasmobranch species and some research has been carried out on the seasonal sexual dimorphisms in certain species (McCourt and Kerstitch, 1980; Kajiura and Tricas, 1996). Dental sexual dimorphisms have been noted in *S. canicula* (Ellis and Shackley, 1995) but as far as the author is aware no research currently reporting on seasonal sexual dimorphisms in the dental structures exists for the lesser-spotted catshark.

4.4.1 Hatchling Catsharks

The analysis carried out on the hatchling catsharks was limited to tooth row counts as removal of whole teeth was extremely difficult due to their small size. The analyses carried out on the tooth row counts showed that a sexual dimorphism exists in respect to the lower jaw, whereby hatchling male catsharks had a greater number of tooth rows as compared to female hatchling catsharks. Body length had no effect on the number of tooth rows for hatchling catsharks. The reason for this dimorphism in the numbers of tooth rows on the bottom jaw could be due to the feeding habits of hatchling catsharks. According to Southall and Sims (2003) the teeth of hatchling catsharks are small relative to their body size, and tooth morphology suggested they are used primarily for prey grasping rather than shearing. The teeth of the bottom jaw appear to be used for grasping prey and it is possible that these teeth are more prone to damage than the upper jaw teeth and the lower jaw therefore possesses an increased number of tooth rows. The fact that a sexual dimorphism exists is possibly due to fact that male hatchling catsharks are born with the dimorphism in preparation for mating as they mature. It is feasible that some of the secondary sexual characteristics, including tooth row numbers, occur at birth and do not develop as the individuals reach sexual maturity.

4.4.2 Juvenile Catsharks

There were a number of sexual dimorphisms present in the dentition of both the upper and lower jaws of juvenile catsharks. In the upper jaw of juvenile catsharks the width of the teeth were found to increase in males. Body length had an effect on the width of the teeth with males possessing wider teeth than females of a similar length. The mid cusp diameter was also found to be significantly different, with males having a larger diameter in the mid section of the central cusp than females.

The same findings were evident in the lower jaw, whereby the tooth width, cusp base diameter and cusp tip diameter were all larger in males when compared to similar sized females. This increase in tooth size in relation to body length was noted by Luer *et al.* (1990) who stated that in the nurse shark, *Ginglymostoma cirratum*, the size of the functional teeth increase as the individuals increase in length. As previously mentioned, if tooth size increases with body length the fastest rate of replacement would occur at the juvenile stages (Wass, 1973; Luer *et al*., 1990; Williams 2001). It is possible that the reported changes in tooth cusp diameter and tooth width in the adult stages of male catsharks is taking place much earlier than previously stated, although given the small sample size this cannot be fully determined within this study.

There is no evidence for a difference in feeding habits of juvenile catsharks (Lyle, 1993; Kabasakal, 2001) and the initial data could be an indication that the evolution of wider teeth in juvenile male *S. canicula* is an adaptation to future mating. Rodríguez-Cabello *et al.* (2004) found that juvenile and adult *S. canicula* behaved differently in terms of segregation. They found that juveniles were mostly found in the southern corner of the Bay of Biscay at depths around 200 m, while adults had a wider depth distribution of between 50–450 m. However, it was suggested that the juveniles had a similar habitat and feeding preference, further supporting the fact that the changes in tooth morphology could be driven by reproduction, even in the juvenile stages of development. As mentioned in a previous chapter, head and jaw morphology changes as catsharks mature (Brough, 1937; Arthur, 1950; McEachran, 1975; Ellis and Shackley, 1995). It is therefore possible that tooth morphology changes to accommodate the changes that may take place in jaw shape during the juvenile stages of development.

Ellis and Shackley (1995) noted that the teeth of juvenile male and female catsharks closely resembled the teeth of adult female catsharks, showing the same pentacuspid

design. The data found in this study supports this as there were no significant differences in the numbers of cusps present on the teeth of juvenile male and female catsharks. It was also seen that tooth slope height was not significantly different between male and female juvenile catsharks.

However, the differences in the width and central cusp diameters suggested that male catsharks at the juvenile stage of development have differing tooth morphology than in other elasmobranch species. Many authors report similar dentitions between juvenile males and females of many sharks, skates and rays. In two ray species reported in the literature, *U. concentricus* (McCourt and Kerstitch, 1980) and *D. akajei* (Taniuchi and Shimizu, 1993) no differences were found in the dentition at the juvenile stage. The results of canonical discriminant analysis showed that the juvenile tooth form of male and female catsharks are not as distinct as they are in adult specimens, with a great deal of overlap in the morphological structures observed in the teeth of male and female juvenile catsharks.

There were no significant differences found in the number of tooth rows of juvenile male and female lesser-spotted catsharks. This is in contrast to the hatchling data which showed that hatchling male catsharks had a greater number of tooth rows than hatchling females in the upper jaw. These data could be hindered by two factors. Firstly, the difficulty in accurately counting the tooth rows of hatchling catsharks due to their small size. Secondly, the small samples size used for the juvenile data could have meant that the samples used were not representative and could have skewed the data.

4.4.3 Adult Catsharks

It is clear from the data that there were a range of seasonal and sexual dimorphisms in the tooth structure of adult catsharks. The tooth morphology of both the upper and lower jaws

of adult *S. canicula* was found to be sexually, and in some cases, seasonally dimorphic. Ellis and Shackley (1995) found that in adult *S. canicula* the tooth morphology changed dramatically, with males moving from the female pentacuspid form to the unicuspid form as they matured. Lyle (1983) also noted that there were seasonal patterns in the consumption of certain prey, overlying the changes in diet with size of *S. canicula,* although this does not account for the sexual dimorphisms found in various populations of *S. canicula*. The changes from pentacuspid to unicuspid teeth in adult male *S. canicula* can be noted in both the upper and lower jaw teeth of adult male lesser-spotted catsharks. This distinction can be seen more clearly in the results of the canonical discriminant analysis, whereby the teeth of adult male and female catsharks are clearly separated. The analysis did, however, show a degree of overlap whereby several adult male samples were found to fall within the grouping of the adult females. For this study the adult and juvenile sharks have been classified according to clasper length and nidamental gland width. It is highly possible that there is no definite cut off point for maturity and that the smaller individuals sampled were on the cusp of adulthood and were classified as adults, despite showing the possession of the female pentacuspid dentition. The discriminant analysis did, however, show a strong distinction between the tooth morphometrics of both adult male and female catsharks, showing that the morphology of adult male teeth in both the upper and lower jaws was very different to that of adult female catsharks.

4.4.4 Dental Formula

The dental formula of the upper and lower jaws of adult male and female catsharks was taken and compared to the published data. Ellis and Shackley (1995) stated that the dental formula of *S. canicula* from the Bristol Channel was 41-60 / 41- 60. Work by Springer (1979) and Campagno (1980) gave the dental formula of *S. canicula* as 43-48/37-45 and 46/40 respectively. The data obtained for this study closely matches that of the population

of *S. canicula* in the Bristol Channel as published by Ellis and Shackley (1995). The number of teeth in the jaws of adult male and female catsharks differed significantly with adult females possessing more teeth when compared to adult males. This could be driven by the fact that the teeth of adult female catsharks are significantly smaller than those adult males.

4.4.5 Adult Upper Jaw Tooth Dimensions

The data for the upper jaw of adult *S. canicula* showed males to possess larger teeth overall. When comparing all parameters, except cusp number, the teeth of adult males were found to be significantly larger than those of adult females.

Body length was found to have a significant effect on all parameters measured, except cusp number and tooth row number. It is expected that as body length increases so does tooth size (Wass, 1973; Luer *et al*., 1990; Williams 2001). Gender was also found to have a significant influence on tooth morphology, with gender differences being found for all parameters measured, whereby males had greater tooth dimensions, but fewer cusps.

Tooth slope height was found to be significantly different in terms of body length, gender and season within gender. However, the statistics do not differentiate the seasonal differences when comparing males and females sampled within the various seasons. It is clear from the data that males possess teeth with greater height than females and it is evident that the presence of a large central cusp signifies a different usage of the teeth between adult male and female *S. canicula*. It can be seen that adult males possessed a greater tooth slope height compared to females except in those male fish that were sampled during the winter and autumn months compared to females that were sampled during the winter and autumn months. During the spring and summer months adult males had a

greater tooth slope height than adult females. This ties in with anecdotal evidence from local commercial fishers who noted that male and female specimens were only caught within the same fishing grounds for the late spring and early summer months. It appeared that the rest of the year the catches of *S. canicula* consisted of either males or females. According to Lyle (1983) seasonal changes in the composition of the diet were observed. Feeding intensity was greatest during summer, related in part to increased prey availability and was least in autumn. However, Lyle (1983), Kabasakal (2001) and Rodriguez-Cabello *et al.* (2007) found there to be no differences in the diet composition of male and female *S. canicula*. With some other elasmobranch species being found to exhibit the same feeding behaviours, despite sexually dimorphic teeth (McEachran, 1975) it can be concluded that the increase in tooth slope height could be attributed so some function other than feeding.

Tooth width and the cusp diameters that were measured were all significantly affected by body length and gender. Males were found to have wider teeth than females and the central cusp dimensions were all found to be larger in males than in females. The difference in the number of cusps on the upper jaw teeth of adult male and female catsharks supported the findings of Ellis and Shackley (1995) with males possessing fewer cusps than females. Males were found to possess one large central cusp, with two small cusplettes, as opposed to females that possessed pentacuspid teeth. The fact that males possess a unicuspid tooth form and show no differences in prey selection (Lyle, 1983) lends weight to the notion that adult male *S. canicula* move from a pentacuspid to unicuspid tooth form for reproductive purposes. Similar changes have been noted in *D. akajei* (Taniuchi and Shimizu, 1993) and *D. sabina* (Kajiura and Tricas, 1996), whereby males show a differing dental morphology to females, moving from molariform to cusped dentition for reproductive purposes.

Adult male catsharks were also found to possess significantly more tooth rows in the upper jaw than adult female catsharks in all seasons except autumn. It is documented that for

most species only a few teeth are replaced at a time, although some sharks have different replacement rates for upper and lower jaws (Moss 1967). With no clear distinction between feeding habits or diet between adult male and female catsharks it is plausible that reproduction is driving males to possess extra teeth rows. As males have been shown to use their mouths during copulation the extra rows may limit the impact of excessive tooth loss, especially during the mating season.

4.4.6 Adult Lower Jaw Tooth Dimensions

The lower jaw data showed a similar pattern to that of the upper jaws, whereby the teeth of adult male catsharks were significantly larger than those of adult female catsharks. Body length had an impact on tooth size, with the larger individuals possessing larger teeth. Gender also had a big impact, with males showing an increased tooth size in all parameters measured except for cusp number. Females possessed more cusp numbers than males, with males showing the unicuspid tooth form and females showing the pentacuspid tooth form.

Tooth slope height was found to be significantly different in all seasons except winter, with adult males possessing teeth with a greater slope height than adult females. There was an intra-gender dimorphism for adult male catsharks, with males sampled in summer having significantly larger slope height than males sampled in all other seasons of the year. It is therefore possible that these changes in male tooth structure are an indication of mating activity. This increase in tooth length could indicate a potential mating season for this species. It is possible that the teeth of adult male catsharks do change to some degree, showing a periodic shift as described by Kajiura and Tricas (1996) for male *D. sabina.* It is possible that this shift occurs in male *S. canicula*, although to a lesser degree than in stingrays.

The data for the lower jaw tooth width and cusp diameter parameters shows a very clear sexual dimorphism, with adult males possessing larger teeth than adult females. There were clearer sexual dimorphisms in the structure of the teeth on the lower jaw than those of the upper jaw. These changes in upper and lower jaw teeth have been seen in many elasmobranch species. According to Motta (2004) many squaloid sharks have a multicuspid grasping upper dentition and blade-like lower cutting teeth. In contrast to this Frazzetta (1988) noted that the slender, smooth-edged, teeth used to readily pierce prey are typical of the lower jaw dentition in many sharks and that many sharks possess upper teeth with serrations along the edges and they have a greater cutting effect than do smooth-edged teeth. This isn't the case with *S. canicula*, where both the upper and lower teeth are smooth edged. It does seem plausible however that the clear dimorphism in the lower jaw teeth is due to their grasping function. The data also demonstrated that the lower jaw teeth have more cusps than the upper jaw teeth and this appears indicative of teeth used for grasping (Frazzetta, 1998). It appeared that the benthic nature of *S. canicula* and the feeding habits exhibited by bottom feeding elasmobranchs has driven the development of grasping teeth. This seems to be the case in the lower jaw teeth of *S. canicula*, whereby the teeth of adult male catsharks are larger and possess more cusps than the upper jaw teeth. This is a possible indication that the grasping design is well suited to holding a female in position prior to mating.

In conclusion, the data obtained in this study concurs with previous findings that adult male and female catsharks have differing tooth morphology, with adult males possessing larger teeth than females and showing a unicupsid dentition as opposed to the pentacuspid form found in females and juveniles of both sexes. It is also apparent that this change becomes more prevalent after maturation. In terms of seasonal dimorphisms the answer is still unclear, with very few seasonal changes having been determined in hatchling, juvenile or adult catsharks. Again, this could be due to small samples sizes in some cases. The one

factor that could lead to a possible determination of a mating season for the Solent population of *S. canicula* is the significant increase in tooth slope height of the lower jaw 'grasping' teeth during the summer for adult male catsharks. With more research and greater numbers of specimens from different populations it may be possible to determine a specific mating season for this species using the changes in the tooth and jaw dimensions.

The next chapter will investigate the structure and function of the skin of elasmobranchs and in particular *S. canicula*. The chapter will involve structural measurements and morphometric investigations to determine if any further sexual dimorphisms exist with regards to the skin of *S. canicula*.

Chapter 5 – Skin

5.1 Introduction

5.1.1 Fish Skin

In vertebrates the skin functions as the outer protective barrier that separates the animal from its environment (Kemp, 1999). Aside from protection from the external environment, Moss (1972) recognised that vertebrate skin has a wide range of functions, including detection of sensations, secretion, water balance, thermal regulation and many others. According to Naresh *et al.* (1997) fish skin, including shark skin, is similar to all other vertebrate skin and is built upon the same architectural pattern with an outer epidermis followed by dermis and flesh (Figure 5.1).

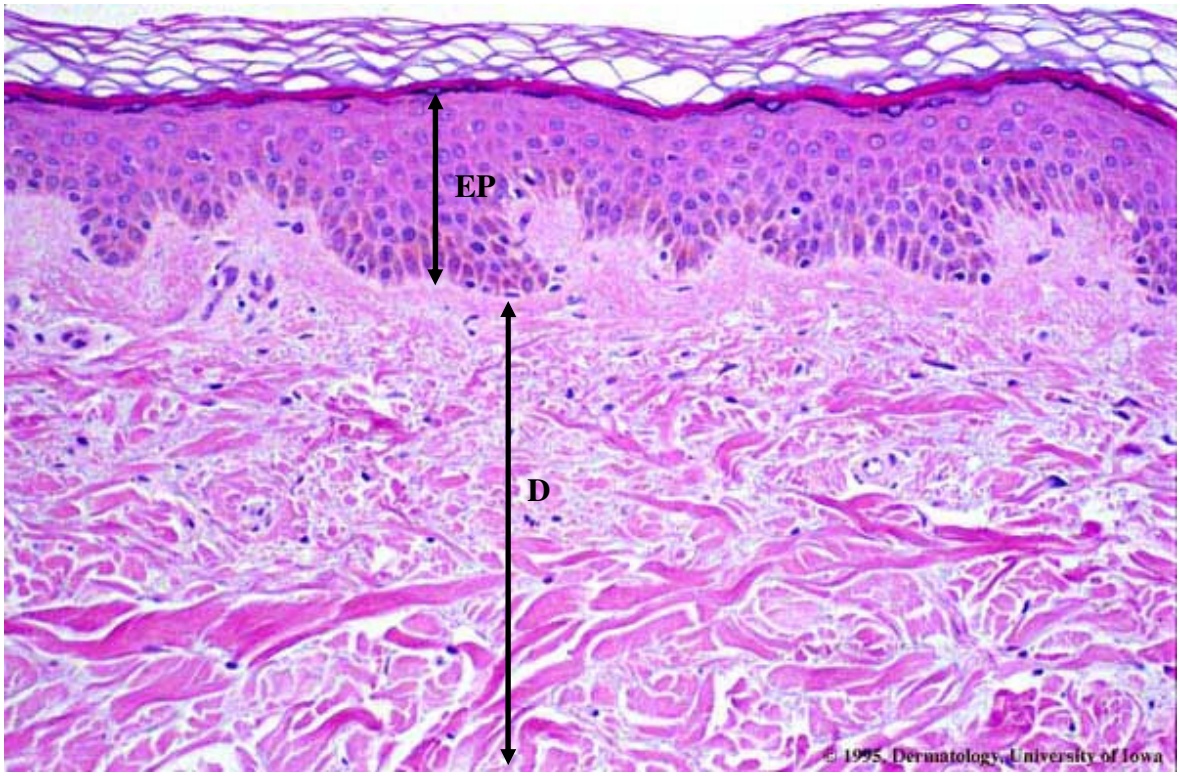

Figure 5.1. The epidermis (EP) and dermis (D) of vertebrate (human) skin. (http://slohs.slcusd.org/).

The outer layer of skin, or epidermis, in sharks is covered by a layer of scales, the structure and function of which will be discussed in more detail later. There is little information available on the epidermal layer of shark skin, except in relation to the protection from the external environment mentioned previously. However, there is more information available on the dermal layer, although this is still relatively little compared to other elements of shark skin that have been researched.

The dermis is defined as the connective tissue layer immediately subjacent to the epidermis, together with which it forms the skin (Moss, 1972). Moss (1972) also stated that the dermis proper is a uniquely vertebrate structure. Moss (1972) added that it is within elasmobranchs that true elastic fibres are found for the first time in conjunction with the division of the dermis into a superficial layer of looser construction and a deeper more compact layer. Lewis and Piez (1964) noted the elasticity of elasmobranch skin and found

that the skin collagen from the spurdog, *Squalus acanthias*, is closely related to the collagens of higher vertebrates in its structure and function.

5.1.2 Shark Dermis and Epidermis

There is a large amount of literature on the structure and function of shark dermal and epidermal structures, much of it focused around the presence of bite wounds that are largely present on the skin of female elasmobranchs. The presence of mating scars has been observed in many species of elasmobranch (Pratt, 1979; Stevens, 1974; Kajiura and Tricas, 1996; Kajiura *et al.,* 2000).

Nordell (1994) suggested that in response to male biting, it could be expected that the skin of mature females might be thicker than males in areas where males bite them. Stevens (1974) observed that many reports of bite wounds showed damage to the pectoral fins of most shark species. In most cases the fins were either torn, or showed scarring where biting had taken place. Stevens (1974) also noted that in many of the reproductive observations in sharks, the males were shown to grasp the pectoral fins with their mouths prior to insertion of the claspers. Observations by Domi *et al.* (2000) who recorded mating behaviour in *S. canicula,* supported the theory that males bite the females on the pectoral fins prior to copulation. Studies on the skin thickness of the blue shark, *Prionace glauca* (Pratt, 1979) and in the Atlantic stingray, *Dasyatis sabina* (Kajiura *et al.,* 2000) showed that in both species the pectoral fin dermis of females was fifty percent thicker than that of males.

The study by Kajiura *et al.* (2000) found that in *D. sabina* the dermis of females showed a sexual dimorphism throughout both the mating and non-mating seasons. Pratt (1979) found that the difference in skin thickness of female blue sharks was not localised to a specific area, such as the pectoral fins, but was uniformly thicker over most of the body. Pratt

(1979) added that in order to accommodate the aggressive mating behaviour shown by male blue sharks during the mating season, the skin of the females was thicker than the males teeth were long. He concluded that although sharks often had puncture wounds to the epidermis, only occasionally did the teeth penetrate to the dermis and musculature. Despite the evidence of this increased skin thickness, Kajiura *et al.* (2002) indicated that the temporal relationships between dental and dermal sexual dimorphisms were unknown for any species.

5.1.3 Fish Scales

One of the key characteristics of fish skin is the fact that it is covered by scales. In general fish skin incorporates a multitude of scales. This is especially true of the skin of sharks, which is covered in a large number of small, modified teeth. These scales are responsible for its considerable roughness (Sudo *et al*., 2002). Agassiz (1833-44) classified modern fish into three groups according to their scale types, namely ganoid, cycloid and ctenoid scales. This group has now been extended, with the placoid scales from elasmobranch fishes having been added. Examples of the different scale types are given in Figure 5.2.

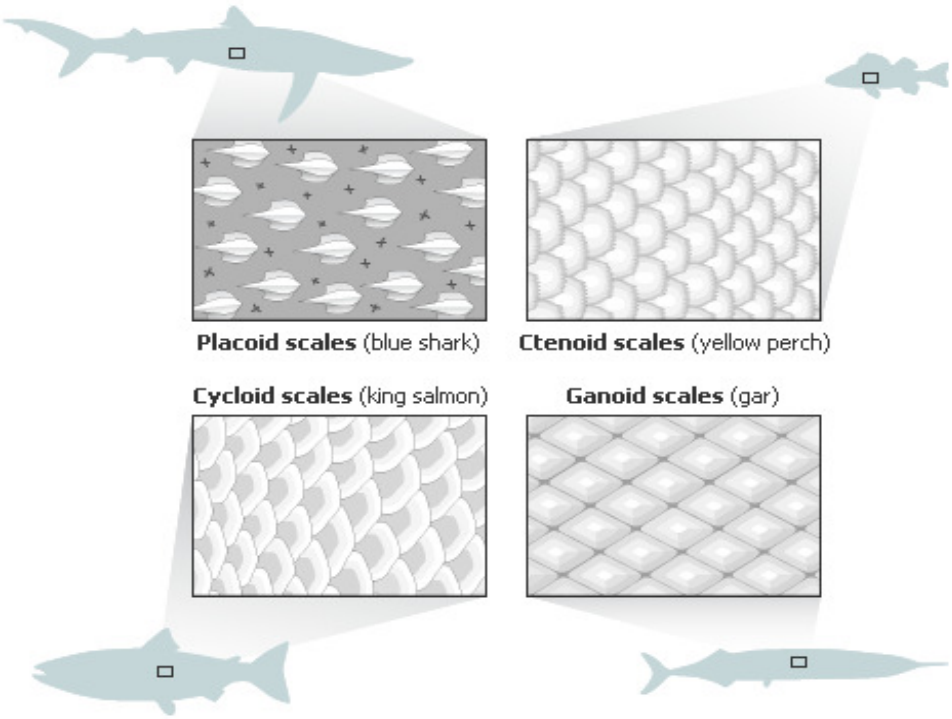

Figure 5.2. Different types of fish scales categorised by shape.
http://images.encarta.msn.com/xrefmedia/aencmed/targets/illus/ilt/T013949A.gif

The complete squamation of the shark covers the whole integument, including the fins, claspers (males) nictating membrane (where present) oral cavity, gill bars and the inside of the gill slits (Reif, 1985). The importance of this coverage of scales on elasmobranch fishes has generated a great deal of literature. Hence, the majority of literature that is available on the structure and function of elasmobranch skin focuses mainly on the scales and is of an evolutionary nature, examining the divergence of fish scales from prehistoric samples.

5.1.4 Elasmobranch Scales – Evolution and Form

There is a diverse terminology associated with shark scales and one which needs further explanation. Shark scales are commonly referred to as placoid scales, the term referring to the plate-like structure of the scales themselves. The scales of sharks are formed by individual tooth-like appendages that are embedded in the skin (Kemp, 1999). Deynat and

Seret (1996) described the dermal armature of the chondrichthyan fishes as consisting of numerous dermo-epidermic structures called dermal denticles. Dermal denticles are so called due their close structure and resemblance to the teeth (dermal = skin, denticle = teeth) which will be discussed later. Ørvig (1967) first proposed the term ododonte to describe skeletons that shared their development and structural properties with teeth. Schaeffer (1977) described a simple way to distinguish the differences between odontodes and teeth. He stated that teeth are regarded as dental units which are situated on the biting margins or biting faces of the jaws and are used in the catching, crushing etc of food. Odontodes are dermal units which occupy positions anywhere else on the entire dermal skeleton (Shaeffer, 1977). Kemp (1999) noted that abruptly around the margins of a shark's mouth and the mantle of the head that scales end and teeth begin. Whatever terminology is used, ododontes, placoid scales, or dermal denticles, the one commonality they possess is that they are characteristic of the skin of elasmobranchs. From herein elasmobranch scales will be referred to as dermal denticles.

The development and origins of shark skin is something that has been well studied and is widely reported. The dermal skeleton develops from a single modifiable morphogenetic field (Schaeffer, 1977). Morphogenetic fields are groups of cells that are able to respond to discrete, localized biochemical signals leading to the development of specific morphological structures or organs.

Kemp (1999) described the development of dermal denticles from an evolutionary standpoint. He stated that dermal denticles develop as a result of the inductive interaction between the dermal papillae of neural crest-derived mesenchyme and overlying cells of the epidermal stratum germinativum, a relationship that Moss (1972) named the epidermal coparticipation hypothesis. Dentine and pulp are produced in the dermal papilla. Epidermal ameloblasts contribute matrix for the outer enameloid cap of the denticle mesenchyme.

The base of the denticle differentiates as the bony base embeds in the stratum vasculare, or the outer layer of the dermis. In evolutionary terms, Kemp (1999) described teeth as being modified denticles and suggested that it was necessary to go back in time to the Palaeozoic era for information about the origin of vertebrate denticles and how they were adapted as the basis for tooth evolution. However, it is far beyond the scope of this study to research the evolutionary pathway of dermal denticles.

5.1.5 Dermal Denticle Design

There are many descriptive accounts of the structure of dermal denticles. Reif (1978a) described the dermal denticles of recent sharks as consisting of a crown, neck region and base (Figure 5.3) with the denticles having a simple pulp cavity and pores for blood vessels.

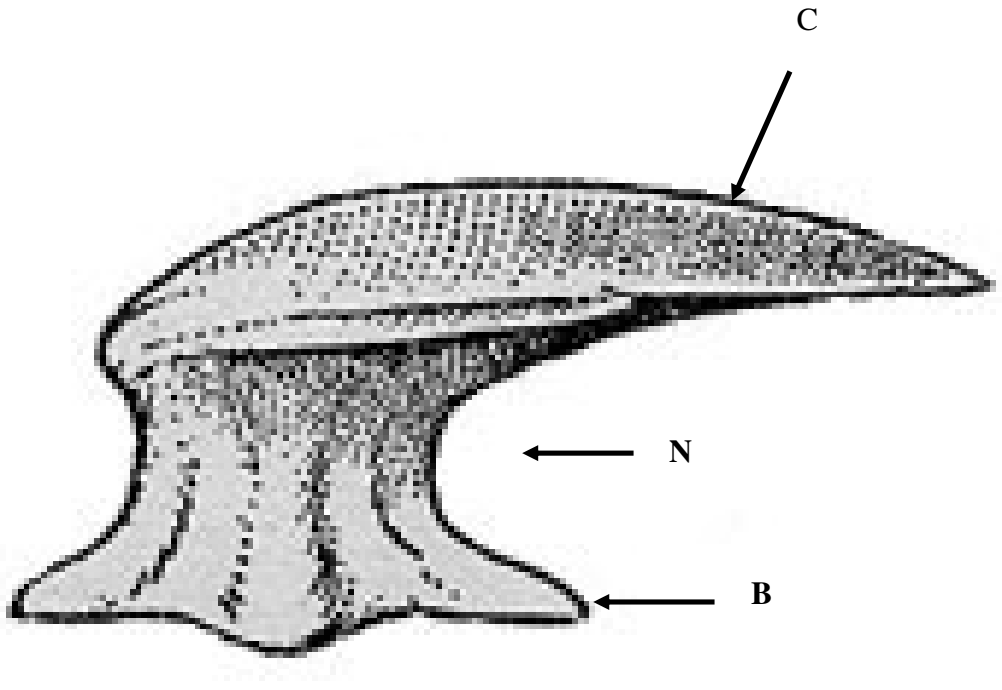

Figure 5.3. Dermal denticle of a shark showing the crown (C) neck (N) and base (B).http://www.flmnh.ufl.edu/fish/Gallery/Descript/SnoseSgillshark/denticles.JPG

Applegate (1967) described dermal denticles as typically consisting of a basal plate embedded into the dermis, with a pedicel that rises from the base and forms a neck connecting with the exposed outer portion, or crown. Ørvig (1967) gave a more in depth description stating that there are several characteristics of dermal denticles, which included the following:

- Formation within a single undivided dental papilla bounded by an epithelial dental organ.
- Formation superficially in the dermis and not from a subepithelial dental lamina.
- Replacement from below, but may be replaced laterally or may remain and form odonto-complexes by lateral and superimpositional growth.

The placoid denticles are formed and anchored in the dermis (Reif, 1985). The dermal denticles are embedded firmly in the dermal layer of the skin and protrude past the epidermal layer forming a protective covering of the epidermis (Ørvig, 1967).

5.1.6 Utilisation of Dermal Denticles

The presence of hardened dermal denticles embedded on the surface of elasmobranch skin has been recognised for as long as sharks have been a utilizable resource (Raschi and Tabit, 1992). The earliest Greek artisans found the integument of sharks useful for the denticles on dried skin (shagreen) which was used for the fine sanding of wood (McCormick *et al*., 1963). Robinson (1971) reported that Japanese sword craftsmen used dermal denticle studded shark skin as a covering for the tsuka (hilt) and saya (scabbard cover). In modern times the structure of shark skin has been utilised further. Ball (1999) described studies in shark skin that led to the development of drag-reducing coatings. It

appeared that the ribbed texture of the denticles of a shark provided hydrodynamic efficiency relative to a smooth surface because of the way that the corrugations affect the viscous boundary layer of the water. This design has been applied within the aeronautical industry and the findings showed that a film with the same texture with ribs parallel to the flow helped reduce the drag of an aircraft by up to 8.5%, representing a fuel saving of 1.5% (Ball, 1999). More recently the structure of shark skin was used to assist athletes to achieve greater swimming speeds. The concept of using specifically designed swimming suits, modelled on shark skin, to achieve drag reduction by controlling the near-wall turbulence and skin-friction forces received much attention (Polidori *et al.,* 2006). Work carried out by Bechert *et al.* (2000) in idealized laboratory experimental conditions, found up to a 7.3% decrease in turbulent shear stress when compared to a smooth reference plate. Fluid dynamic experiments showed that small riblet surfaces induced drag reductions of up to about 10% compared to smooth surfaces (Koeltzsch *et al.,* 2002; Bechert *et al.,* 2000). However, in real pool conditions Toussaint *et al.* (2002) showed that a statistically non-significant 2% reduction in drag was found when wearing fast-skin suits compared to conventional ones. Shark skin was also recently utilised as a food source. Collagen is the major fraction of connective tissue in skin and has been used in the food, pharmaceutical and photographic industries. Commonly, the main sources for collagen production are pig skin, cattle skin and bone. Kittiphattanabawon *et al*. (2010) observed that the outbreak of bovine spongiform encephalopathy (BSE) resulted in justified anxiety amongst users of cattle collagen. Kittiphattanabawon *et al*. (2010) pointed out that due to its thickness, shark skin can be used as an excellent source of collagen and is being increasingly utilised as a resource in place of bovine collagen.

5.1.7 Function of Dermal Denticles

According to Raschi and Tabit (1992) the function of dermal denticles can be considered along three perspectives. The first of these focuses on the historical context, whereby the skin of sharks has been modified over millions of years to carry out a range of functions, These functions include protection from predators and ectoparasites, reduction of mechanical abrasion, accommodation of bioluminescent and sensory organs and reduction of frictional drag. The second factor suggested that the lifestyle of sharks has meant that the dermal denticles have evolved to occur on specific areas of the body in order to cope with the demands of the habitat in which individual species live. Lastly, Raschi and Tabit (1992) suggested that the denticles are designed to increase water flow dynamics and assist in energy efficiency whilst swimming. Each of these perspectives will be discussed in more detail.

Fish skin has a wide range of protective adaptations which enable them to occupy habitats ranging from rocky substrata to turbulent waters (Hawkes, 1974). Shark denticles clearly perform a wide variety of functions, presumably in response to numerous selective pressures (Raschi and Tabit, 1992). Hawkes (1974) stated that because of its watery environment, fish skin is subjected to at least two types of stress. These were described as osmotic pressure gradients between the cells and the water and physical forces, not only from the water itself, but from other environmental hazards, for example rocks. The fact that dermal denticles are adapted to cope with various stresses associated with lifestyles of certain shark species is widely accepted.

Many authors have commented on the fact that the dermal denticles are modified depending on where they are located on the body. According to Reif (1985) dermal denticle morphology varies in the different regions of the sharks' integument and from

growth stage to growth stage. In elasmobranchs dermal denticles may, to a varying degree, undergo modifications in external morphology when they are adapted to serve specific functions in the organism (Ørvig, 1977; Reif, 1985). Dermal denticles often acquire a special shape and/or size when they have definite tasks to perform, such as defence (Ørvig, 1977; Raschi and Tabit, 1992).

The dermal denticles in some species can also indicate maturity. According to Reif (1985) the denticles of the embryos were comparatively widely spaced in all species that were studied. Deynat and Seret (1996) noted that dermal denticles presented both important variations in their morphology, size and arrangement due to their localisation on the body and indicated the degree of maturity of individuals. In the starspotted smoothound, *Mustelus manazo*, Sudo *et al.* (2002) found that the dermal denticles were smaller on the head region than on the tail. Reif (1978c) noted that during embryogenesis a differentiated dermal skeleton is formed, which is made of non-growing denticles. They differed in size and shape and from body region to body region, a term described by Reif (1978c) as lateral differentiation. Despite the occurrence of lateral differentiation, Reif (1985) noted that on a small skin sample of any shark and any growth stage the squamation is comparatively uniform in shape. Although there is variation in denticle size and shape this variation is only found to a limited extent (Reif, 1985). The denticles are repeatedly replaced during postnatal ontogeny and the total number of denticles also increases. The new dermal denticles always differ from the old ones (ontogenetic differentiation). As a rule, lateral differentiation in the dermal skeleton of sharks is much stronger in adults than in young sharks (Reif, 1978c). Reif (1985) noted that the dermal denticles were formed in a single morphogenetic step and they did not grow, but were replaced with new ones. Once the denticles were calcified they did not grow (Reif, 1985).

Raschi and Tabit (1992) noted that denticles differed around the mouth and fins and they suggested that this adaptation possibly helped to cope with the demands of differing water flow over the body of sharks. The theory of dermal denticle design as a hydrodynamic function has been fully examined and is widely accepted. Reif (1978a) found that fast swimming sharks have a type of denticle that differed considerably from all other sharks and that it was likely that this type of denticle reduced drag and facilitated high speed swimming. Reif (1978a) also noted that the dermal denticles grew so that the grooves usually developed in a posterior position. This was to facilitate the flow of water over the denticles and channel it down the length of the body. Sudo *et al.* (2002) suggested that the complicated structure of smoothound skin leads to the theory of channelled microflow. They believed that the water is channelled through the grooves in the dermal denticles and is prevented from moving away from the body. The smoother flow of water that this causes reduced the drag that would be caused by more turbulent waters passing over the skin in the absence of grooves. Subsequent theoretical and experimental studies showed that longitudinal grooves with V-shaped ridges and U-shaped valleys (Walsh and Weinstein, 1978; Bechert *et al*., 1985) can reduce frictional drag by up to 8%. Neumann and Dinkelacker (1989) increased this figure to 13% by refinement of the valley topography. Figure 5.4 clearly shows the structure of the dermal denticles in sharks, indicating the ridges and valleys on the crown of the dermal denticle of a shark.

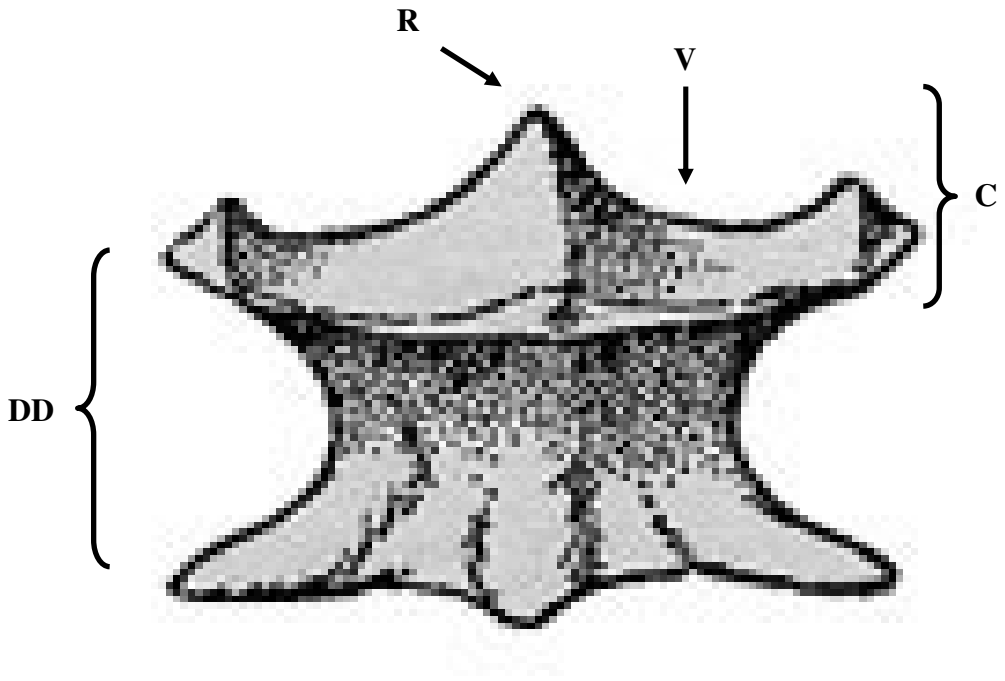

Figure 5.4. The valleys (V) and ridges (R) of the dermal denticles (DD) on the crown (C) of a shark. http://www.flmnh.ufl.edu

Other uses for dermal denticles have been suggested by other authors. Ford (1921) found that hatchling catsharks emerged with numerous, well-formed dermal denticles. Grover (1974) noted that the juvenile swell shark, *Cephaloscyllium ventriosum,* possessed two rows of enlarged denticles on the dorsal side, running down to the caudal area. He suggested that in the case of oviparous sharks the denticles facilitated the exit from the egg case. The formation of dermal denticles in hatchling sharks has been documented for some time. The same formation of dermal denticles was found to be present in *Heterodontus portusjacksoni*, *Heterodontus galeatus* (Johanson *et al*., 2007) and *S. canicula* (Ballard *et al.,* 1993). The newly hatched catshark had two rows of special placoid denticles along its back and that these and the four rows of special caudal denticles all appear before hatching (Ballard *et al.,* 1993). All of these denticle rows are lost during ontogeny and replaced by more randomly developing scattered dermal denticles (Johanson *et al.,* 2008).

A study by Southall and Sims (2003) reported on the use of skin in feeding. The research focused on the structure of elasmobranch dermal denticles. Southall and Sims (2003) noted that *S. canicula* had been shown to use their dermal denticles to anchor prey items during feeding. Although they noted that the behaviours were found to be conducted mainly by hatchlings and juveniles, adults were also occasionally witnessed to anchor food to the seabed using dermal denticles

Another area of study that has interested researchers is the fact that sharks are able to replace their dermal denticles. Reif (1985) noted that it was unclear whether denticles are shed during embryogenesis and that denticle replacement was believed to start around birth (or hatching). Dermal denticle replacement occurs as body length increases, or when sharks suffered an injury (Reif, 1978a). Denticle size increase was found to show a strong negative allometry relative to body size, hence an adult shark had many more denticles than a young shark (Reif, 1985). The dermal denticles, like the teeth of sharks do not grow, but are regularly replaced (Markel and Laubier, 1969; Reif, 1978a). Dermal denticles are shed by resorption of the anchoring fibres and that the bony base is not affected by this resorption (Reif, 1985). The replacement and insertion of new denticles continues throughout ontogeny, although the functional life of dermal denticles has never been measured successfully (Reif, 1985).

Reif (1978c) examined the ability of sharks to recover their dermal denticles after skin damage, similar to that from an attack by another shark, or by a heavy abrasion. He recorded that most scar tissue was covered with dermal denticles after 4 months. However, he also found that in the area where the skin had regenerated the dermal denticles showed a high degree of variability. The dermal skeleton in the scar area differed from the original skeleton in five ways (size, variability, shape, arrangement and orientation of dermal denticles). The dermal denticles that grew back were much bigger (30-50% larger) and

much more complex than the dermal denticles that were present in areas that had not been damaged. Reif (1978c) also noted that the dermal denticles that grew in the area that had been damaged were more randomly laid out. They were no longer arranged in diagonal rows and were not so perfectly aligned in a posterior direction. In essence the research showed that the dermal denticles were replaced when the skin suffered a trauma, such as an abrasion, but the original pattern of the dermal skeleton was not regenerated.

Considering the previous research that has been carried out on the skin of sharks and the fact that reproduction involves the males biting females it is possible that male and female lesser-spotted catsharks show a sexual dimorphism in both the thickness of the epidermal and dermal layers of the skin as well as in the dermal denticles. It is clear from previous reports that during copulation males bite the pectoral fins of females in order to secure the female prior to inserting their claspers (Stevens, 1974; Domi *et al.,* 2000). It is also clear that sharks can shed their dermal denticles in much the same way that they can shed their teeth (Markel and Laubier, 1968; Reif, 1978b).

It is possible that both the epidermal and dermal skin layers in female *S. canicula* will be thicker than those of males and that the skin layers in females may show a seasonality in their thickness as an adaptation to the reproductive pressures of male biting during mating. The dermal denticles of *S. canicula* may also play a role in mating, whereby the females may have larger, longer, more densely packed dermal denticles than males, in order to provide some degree of protection from biting from the males during copulation. It is also possible that as sharks are able to shed their dermal denticles that there will also be a change in the shape and size of the denticles of the female catsharks to coincide with the mating season. As far as the author is aware the effects of seasonality on skin structures has not been studied before in *S. canicula*.

Therefore, the aims of this study are:

1) To determine if there are any sexual and seasonal dimorphisms in the dermal and epidermal skin layers of hatchling, juvenile and adult *S. canicula*.

2) To determine if there are any sexual and seasonal dimorphisms in the dermal denticles of hatchling, juvenile and adult *S. canicula*.

3) To determine if there is any symmetry or lateralisation in the pectoral fin dermal denticles of hatchling, juvenile and adult *S. canicula.*

5.2 Materials and Methods

For the skin morphometrics the adult catsharks were categorised into size classes based on sexual maturity. The size classes used are:

Males - Size class 1 < 525mm total body length (immature/juvenile)

Males - Size class 2 ≥ 525mm total body length (mature)

Females - Size class 1 < 550mm total body length (immature/juvenile)

Females - Size class 2 ≥ 550mm total body length (mature)

5.2.1 Dermal and Epidermal Preparation

A sample of skin approximately 1cm^2 (Reif, 1985) was cut from below the dorsal fin and above the lateral line on the left side of the body of each specimen (Figure 5.5) and stored in unbuffered 10% formalin in seawater. This area was chosen as Stevens (1974) and Pratt

(1979) noted that in female blue sharks the skin thickness in this region was greater than in males of the same species.

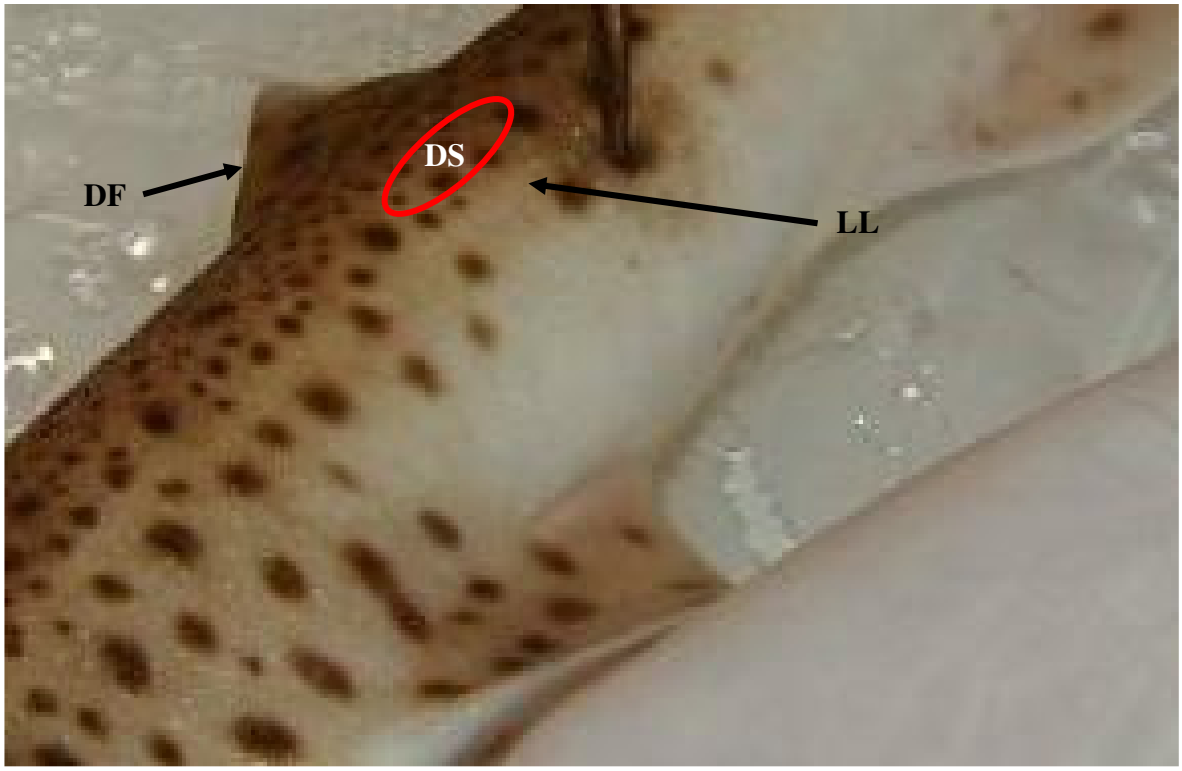

Figure 5.5. Dissection of dorsal skin (DS) from between the dorsal fin (DF) and the lateral line (LL).

Both adult and juvenile skin samples were removed from the formalin and placed in distilled water for four 45-minute washes. They were then prepared for sectioning. Due to the skin being heavily calcified, a method adapted from Naresh *et al.* (1997) was employed. This involved the use of a cryostat (Bright Instruments, model OTF) with a freezing microtome (Bright Instruments, model 5040) and a tungsten carbide knife (Bright Instruments, model B1009DR). The skin sections were placed onto a cryostat chuck and covered in cryo-m-bed (Bright Instruments). The tissue and chuck were placed in a chuck holder and the chuck was then submerged in liquid nitrogen. Once the cryo-m-bed turned opaque the skin sample was also submerged. After approximately 15 seconds the chuck and sample were removed from the liquid nitrogen and placed inside the cryostat chamber

to warm to –22°C. Once at –22°C the sample was sectioned on the microtome at a thickness of 12 μm and mounted onto Poly- L- Lysine glass microscope slides.

Once sectioned the samples were put through a rapid haematoxylin and eosin (H&E) staining procedure. The slides were immersed in haematoxylin for 2 minutes, washed with acid alcohol for 3 seconds, placed in 5% Eosin for 1 minute, washed in water for 30 seconds and then dehydrated. The dehydration process consisted of a five second wash in 90% ethanol, followed by two five second washes in 100% ethanol. Finally the slides were placed in xylene and cover slips applied and mounted with DPX. The slides were photographed using a Leitz Dialux 22EB optical microscope at x40 magnification and a JVC TK-C1381 colour video camera. Measurements were taken using UTHSCSA imaging tool.

Two measurements were carried out with regard to skin thickness, the dermal and epidermal thickness. Figure 5.6 shows a cross section through the skin of a female sampled during the summer (June) measuring 595mm and weighing 625g. The dermis and epidermis can be clearly seen, as can a cross section through the dermal denticles and the skeletal muscle.

A total of 15 measurements of both the epidermis and dermis were taken from each specimen in order to give an average thickness of both skin layers. Five skin sections were used for each specimen and 3 measurements of each section were taken for the dermal and epidermal thicknesses. This was due to the presence of large numbers of dermal denticles embedded in the dermis. Areas of the dermis where the dermal denticles were present were avoided and sections were only measured where the dermal layer was lying adjacent to the epidermis (Figure 5.6).

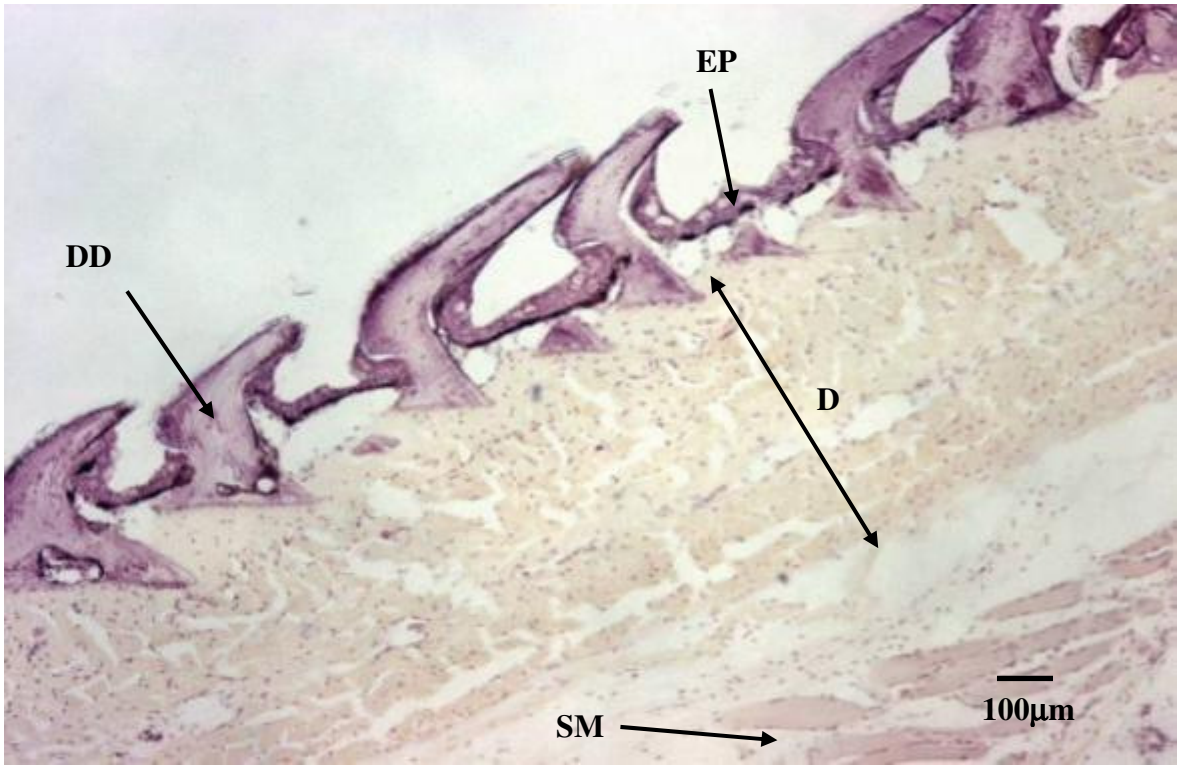

Figure 5.6. A cross section of skin from a female catshark showing the dermal denticles (DD) epidermis (EP) dermis (D) and skeletal muscle (SM).

A mean skin thickness for the dermis and epidermis was ascertained for each specimen and a General Linear Model (GLM) was then performed to compare the effects of season, gender and season and gender on skin thickness. This was done to determine the existence of a seasonal sexual dimorphism in the dermal and epidermal thicknesses of *S. canicula*. An Analysis of Covariance (ANCOVA) was also performed, with body length as a covariate, in order to determine the effect of body length on skin thicknesses.

5.2.2 Dermal Denticle Dimensions

The adult pectoral fins were removed from both male and female sharks and stored in unbuffered 10% formalin in seawater. The formalin was removed by passing the left and right fins through four 45-minute washes of distilled water. A 7mm cork borer was then used to take a section of skin 2cm from the posterior edge of each fin. In order to standardise the area from which the skin was taken a measurement was made of the

maximum length of the fin and then a measurement 2cm from posterior edge of the fin was taken. The area where the two axes crossed was the area from which the skin sample was taken (Figure. 5.7). This was done in order to take into account the bite radius of the male catsharks.

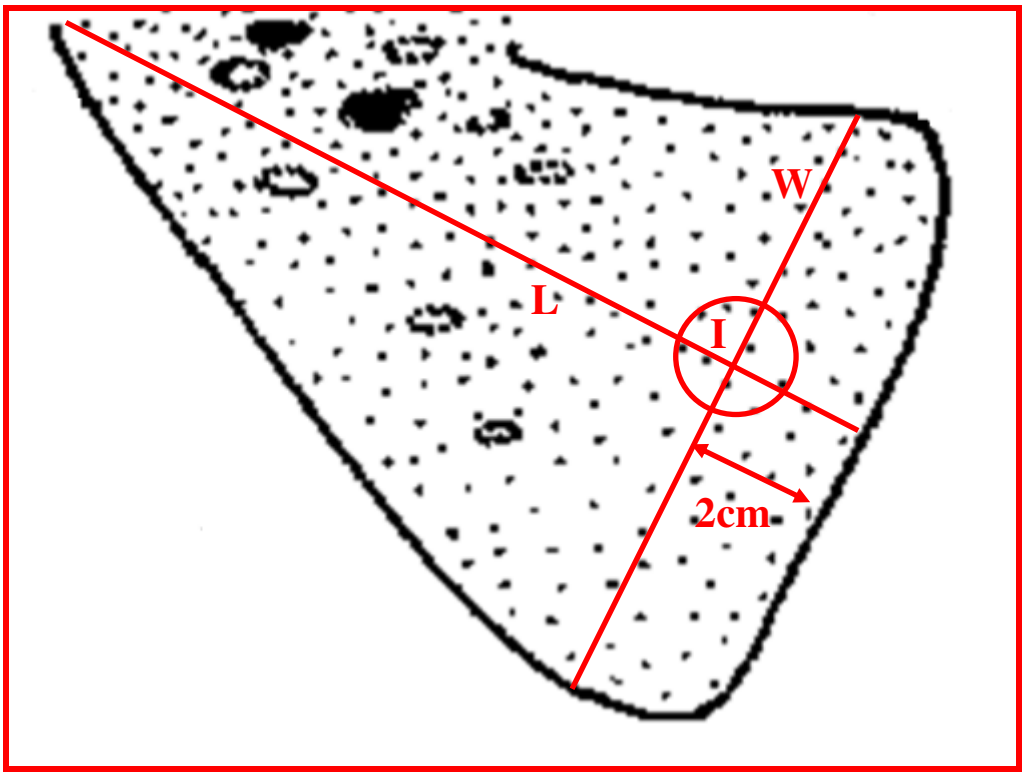

Figure 5.7. Schematic of a pectoral fin from *S. canicula* showing the length (L), width (W) and intercept (I) from the area the skin sample was taken. Adapted from Compagno (1984).

The skin discs were placed on filter paper to remove any excess water and then photographed using a Wild M5 dissecting microscope at x24 magnification and an analogue Panasonic F15 camera. Since Reif (1985) noted that on a small skin sample of any shark there is limited variation in denticle size (Figure 5.9) measurements of the length and width of five dermal denticles from each fin were taken. Power analysis was carried out on the dermal denticle measurements (Figure 5.9) This produced a power of 0.83 for a sample size of 5 with differences of 85µm per sample. Results were accepted above 0.80,

which indicated that the measurement of 5 dermal denticles per fin was adequate. The density of the dermal denticles in an area of 1 mm^2 of skin were also measured.

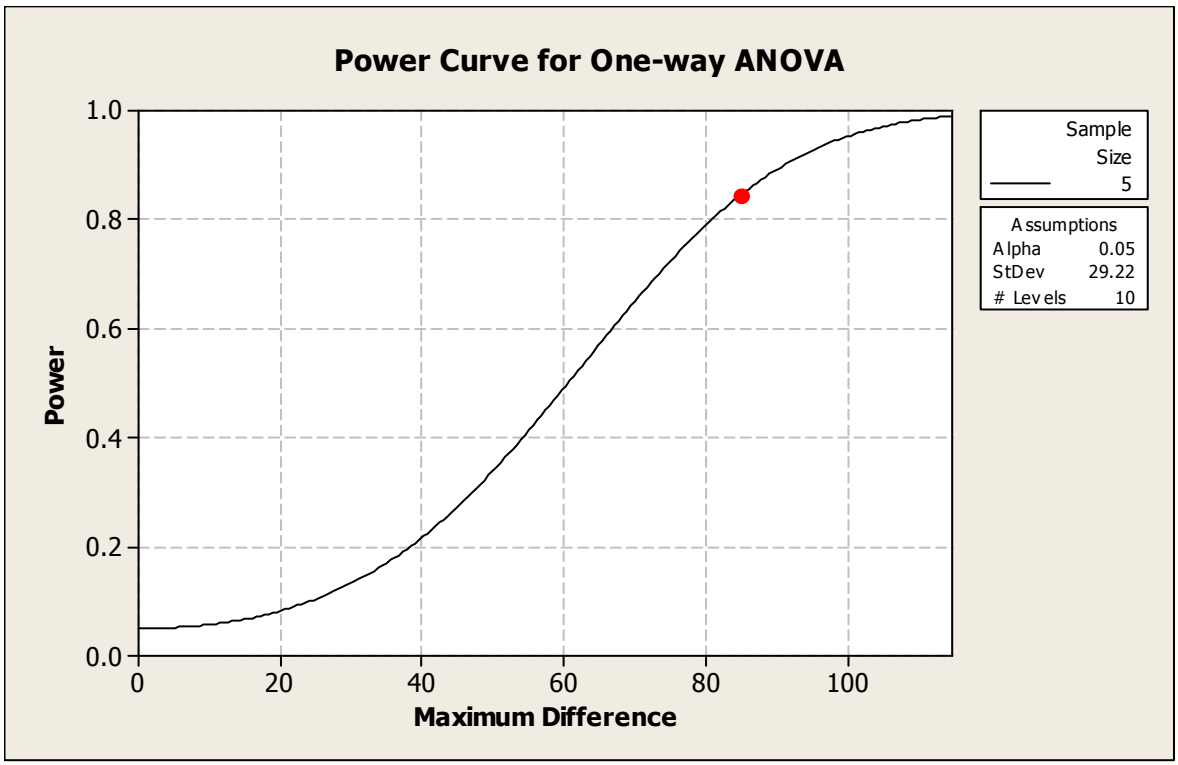

Figure 5.8. Power analysis on the dermal denticle measurements of adult *S. canicula*.

A Leica QWin Image Analysis package was used to record these measurements. Any broken or abraded dermal denticles were not measured. Methods for counting the number of dermal denticles were adapted from those used to count cells with a haemocytometer. Dermal denticles positioned along the right hand and bottom boundary lines were counted. Dermal denticles that were positioned on the left hand and top boundary lines were excluded. Figure 5.9 shows an image of the denticles from the fin of a male catshark sampled during the winter (February) with a length of 569 mm and weighing 475g.

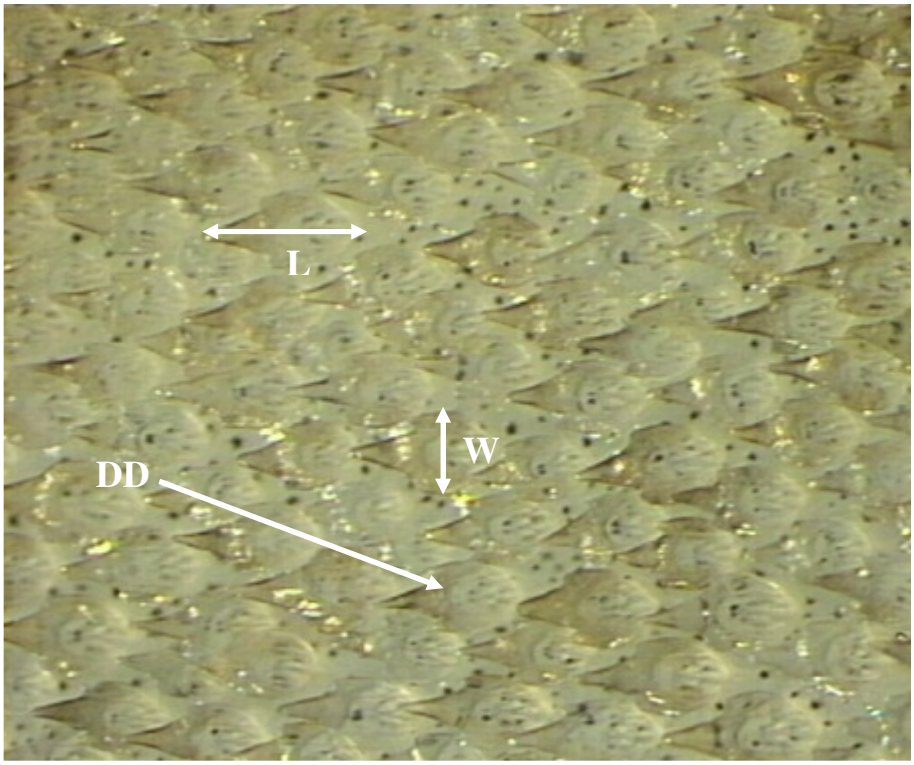

Figure 5.9. The skin of a male catshark showing the dermal denticles (DD) and the measurements that were taken (width (W) and Length (L)).

The hatchling catsharks were removed from the formalin and the pectoral fins were removed. The extracted fin was then washed in four 45-minute washes of distilled water. The fin was dried using filter paper and images were captured using a Wild M5 dissecting microscope at x24 magnification and an analogue Panasonic F15 camera. A Leica Qwin image analysis package was used. Due to their size, the entire fins were used and the total number of denticles per 1mm^2 were counted (Figure 5.10).

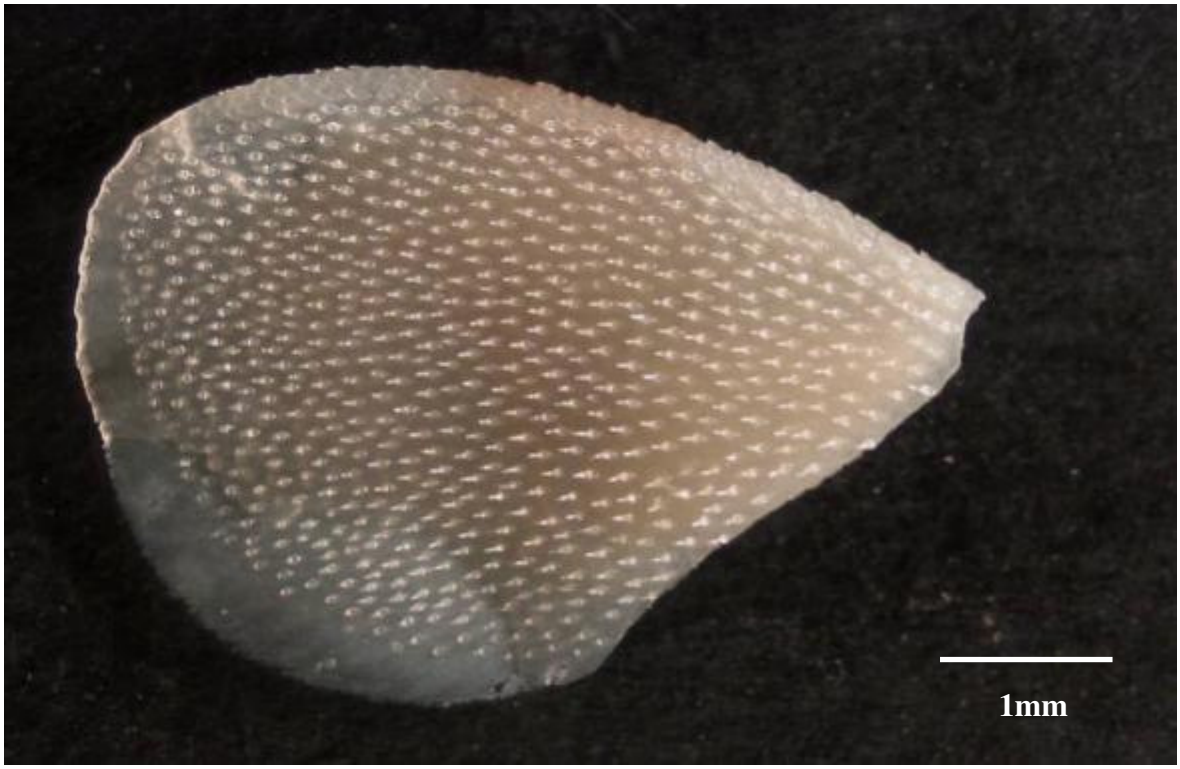

Figure 5.10. An excised left fin of a hatchling female catshark.

As with the juvenile and adult specimens the length and width of five randomly selected denticles were also measured with the use of UTHSCSA imaging tool.

A canonical discriminant analysis was carried on the adult dermal denticles to determine any seasonal correlation between adult catsharks. The morphological parameters (denticle length, denticle width and denticle density) were combined to ascertain whether there was any separation between the seasons in terms of these parameters for adult males and females.

5.2.3 SEM

Scanning Electron Microscopy (SEM) was performed on the dermal denticles of the catsharks. A method, adapted from Dingerkaus and Kostler (1986) was used to remove mucous and debris from the denticles prior to preparation, whereby samples were

ultrasonicated for 15 mins and air dried before being prepared for SEM. After drying samples were immersed in 4% gluteraldehyde in a 0.2M sodium cacodylate seawater fixative solution (pH 7.4) for one hour (Cragg and Nott, 1977). The skin samples were then osmicated in 4% osmium tetroxide (OsO_4) in 0.1M Sodium Phosphate Buffer (pH 7.4) with a volume sufficient to cover the samples. The samples were then left for 60 minutes or until they turned black.

Samples were rinsed in buffer wash at least twice more following post-fixation to remove any remaining osmium before being further dehydrated. The samples were taken through a dehydration series consisting of 30 minute washes of 50%, 60%, 70%, 80%, 90% and 100% ethanol solutions. The samples were then placed in a 50/50 mix of 100% ethanol and acetone, followed by a 30-minute wash in 100% acetone.

5.2.4 Mounting

Once dehydrated, samples were transferred onto aluminium stubs. Samples were affixed to the stub by use of sticky carbon tabs which served both to attach specimens and provided good conductivity for SEM imaging. The samples were then DC-sputter coated with a gold/palladium mix for 2 ½ minutes. Samples were then observed in JEOL JSM-65C SEM at 15KV x44 magnification.

5.3 Results

A range of analyses were carried out on the skin of hatchling, juvenile and adult lesser-spotted catsharks to determine whether any sexual dimorphism existed in the structure of the skin. Paired t-tests were used to determine any intra-gender dimorphisms in the dermal, epidermal and denticle structures comparing data from the left and right pectoral fins. An

ANCOVA was used to determine any inter-gender differences in the epidermis, dermis and dermal denticles of male and female specimens. A Grubbs test for outliers was performed on the data (Grubbs, 1969) as per Attrill *et al*. (2007) in order to ascertain the presence of any outliers. The test revealed that no outliers were present in any of the data. A GLM was used to determine whether there were any seasonal differences. The dermis and epidermis of adult ($F \geq 550$mm, $M \geq 525$mm) samples were analysed to discover whether there were any seasonal variations in the skin dimensions between the genders. For the dermal denticles adult samples were analysed to discover whether there were any seasonal variations in the denticle dimensions between the genders. This seasonal comparison was not carried out for the juvenile and hatchling catsharks due to the low numbers of individuals sampled.

Both the dermal and epidermal layers were analysed to determine the thickness of the different layers of skin in order to ascertain if there was a sexual dimorphism in the skin of hatchling, juvenile and adult male and female catsharks (Figure 5.11).

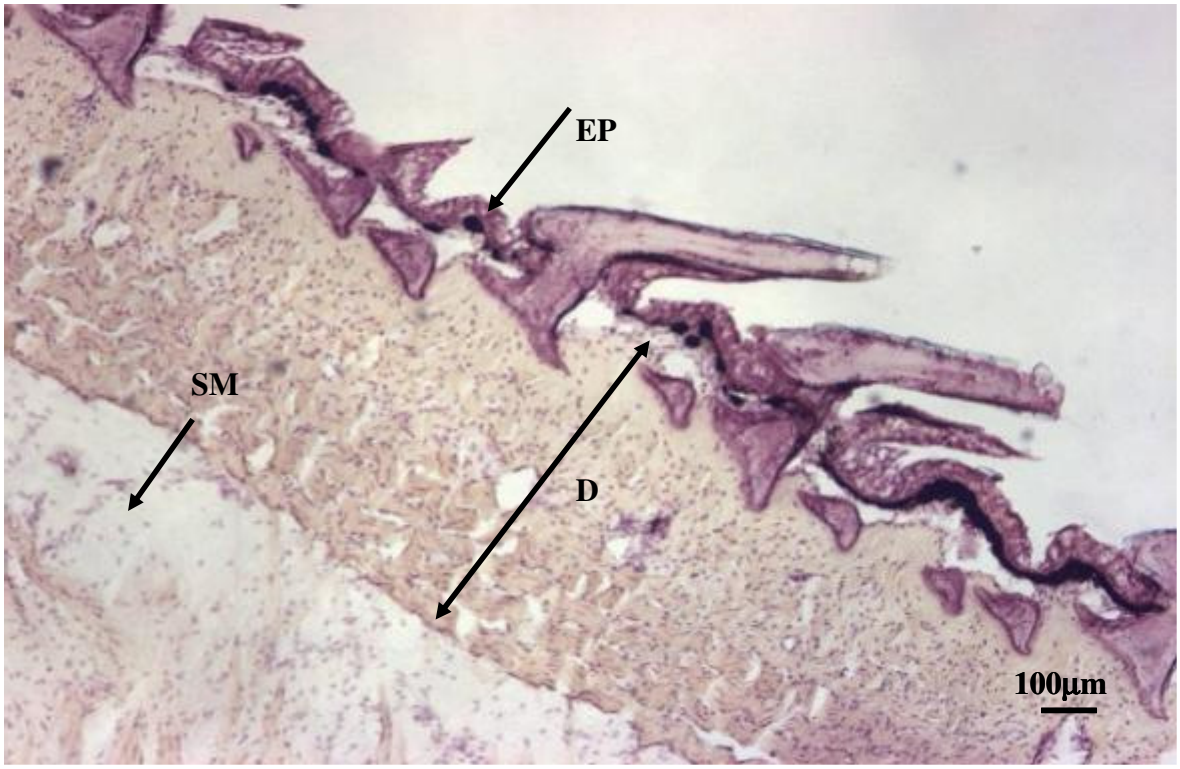

Figure 5.11. The epidermis (EP) dermis (D) and skeletal muscle (SM) of the skin of *S. canicula*.

5.3.1 Hatchling Dermal and Epidermal Results

Comparisons of the epidermis and dermis of male and female catsharks were made and the results for the ANCOVA can be seen in Table 5.1.

Body length had no significant effect on the epidermal thickness of hatchling catsharks (ANCOVA, F=3.01; d.f.=1; *P*=0.086). Gender was found to have a significant effect on the epidermal thickness of hatchling catsharks (ANCOVA, F=6.06; d.f.=1; *P*=0.015) with hatchling females possessing a thicker epidermal layer than hatchling males. Neither body length nor gender had a significant effect on the dermal thickness of hatchling catsharks (ANCOVA, F=2.84; d.f.=1; *P*=0.095; ANCOVA, F=0.043; d.f.=1; *P*=0.515).

Table 5.1. Results from the ANCOVA for the epidermis and dermis of hatchling male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F (14) M (23)).

Feature (µm)	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Epidermal Thickness (Range)	46.13 ± 1.44 (15.41-74.21)	41.26 ± 1.44 (24.94-80.64)	**0.086**	**0.015**
Dermal Thickness (Range)	133.52 ± 4.05 (91.31-203.14)	137.98 ± 3.27 (74.63-28.52)	**0.095**	**0.515**

5.3.2 Juvenile Epidermal and Dermal Results

5.3.2.1 Juvenile Epidermal Thickness

Figure 5.12 shows a graphical representation of the epidermal thickness of juvenile male and female catsharks. Juvenile female catsharks were found to possess a greater epidermal thickness than juvenile male catsharks.

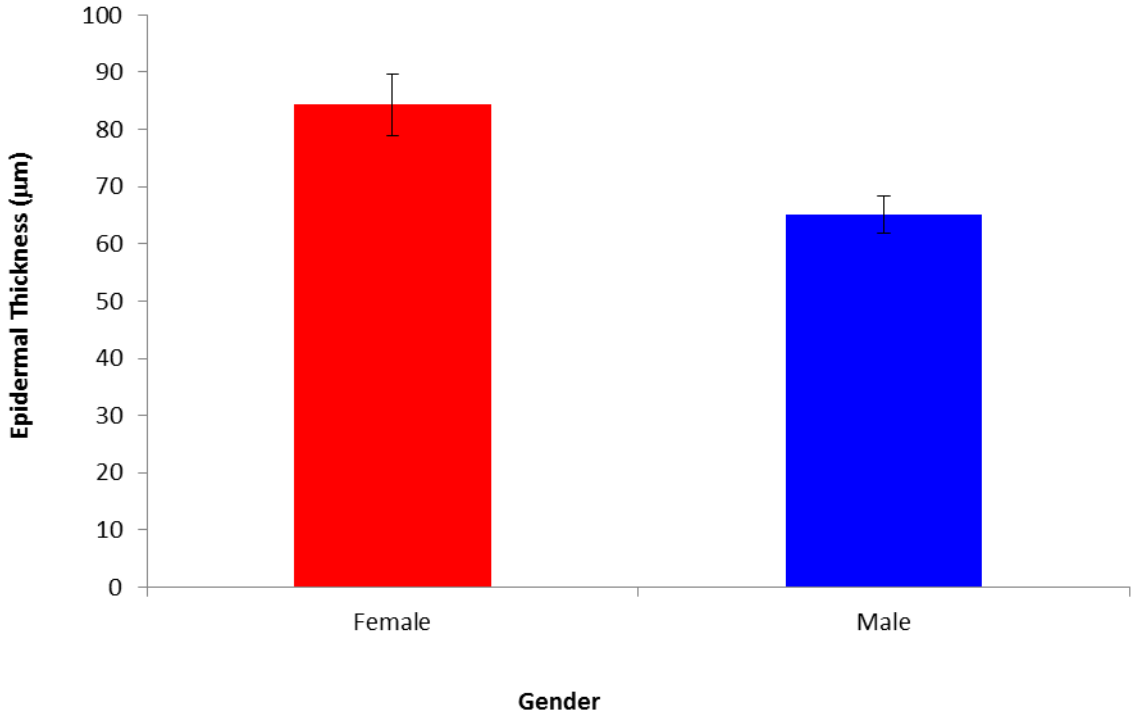

Figure 5.12. Gender comparison of epidermal thickness for Juvenile catsharks showing means and ± standard errors (n= Female (19), Male (10).

Table 5.2 shows the output from the GLM for the epidermal thickness of male and female juvenile catsharks. It can be seen from Table 5.2 that body length did not affect the epidermal thickness in juvenile catsharks. Gender had a significant effect on the epidermal thickness in juvenile catsharks, with juvenile females possessing a significantly thicker epidermal layer than juvenile males.

Table 5.2. Results from the GLM analyses for the epidermis of juvenile male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	128.2	28.3	28.3	0.07	**0.795**
Gender	1	3519.6	1854.4	1854.4	4.57	**0.046**

5.3.2.2 Juvenile Dermal Thickness

Figure 5.13 shows a graphical representation of the dermal thickness of juvenile male and female catsharks for all four seasons. There was no significant difference in the dermal thickness of male and juvenile catsharks.

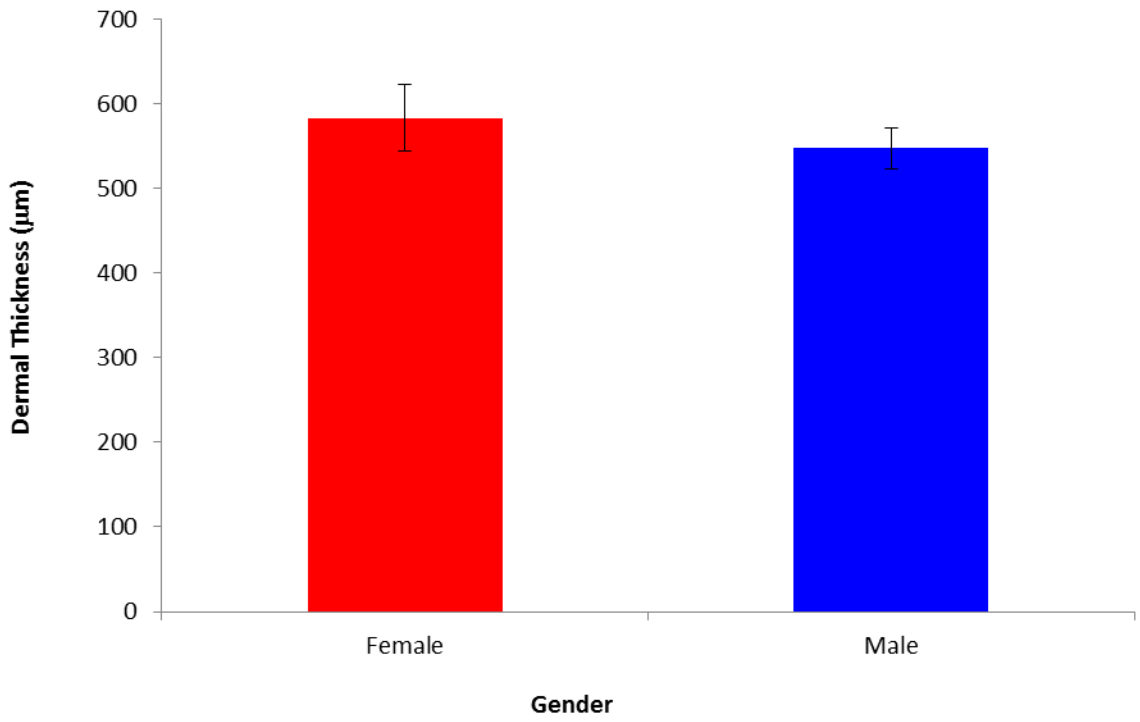

Figure 5.13. Gender comparison of dermal thickness for Juvenile catsharks showing means and ± standard errors (n= Female (19), Male (10).

The results from the GLM for dermal thickness in male and female juvenile catsharks can be seen in Table 5.3. Body length did not affect the dermal thickness in juvenile catsharks. It can also be seen that gender had no effect on the dermal thickness in juvenile catsharks.

Table 5.3. Results from the GLM analyses for the dermis of juvenile male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	1317895	10627	10627	1.00	**0.327**
Gender	1	24331	82	82	0.01	**0.931**

5.3.3 Adult Epidermal and Dermal Results

The means ± standard errors and ranges for the epidermal and dermal morphometrics of adult *S. canicula* can be seen in Appendix 3.

5.3.3.1 Adult Epidermal Thickness

Figure 5.14 shows a graphical representation of the epidermal thickness of adult male and female catsharks for all four seasons. In general adult female catsharks had a thicker epidermis than adult male catsharks.

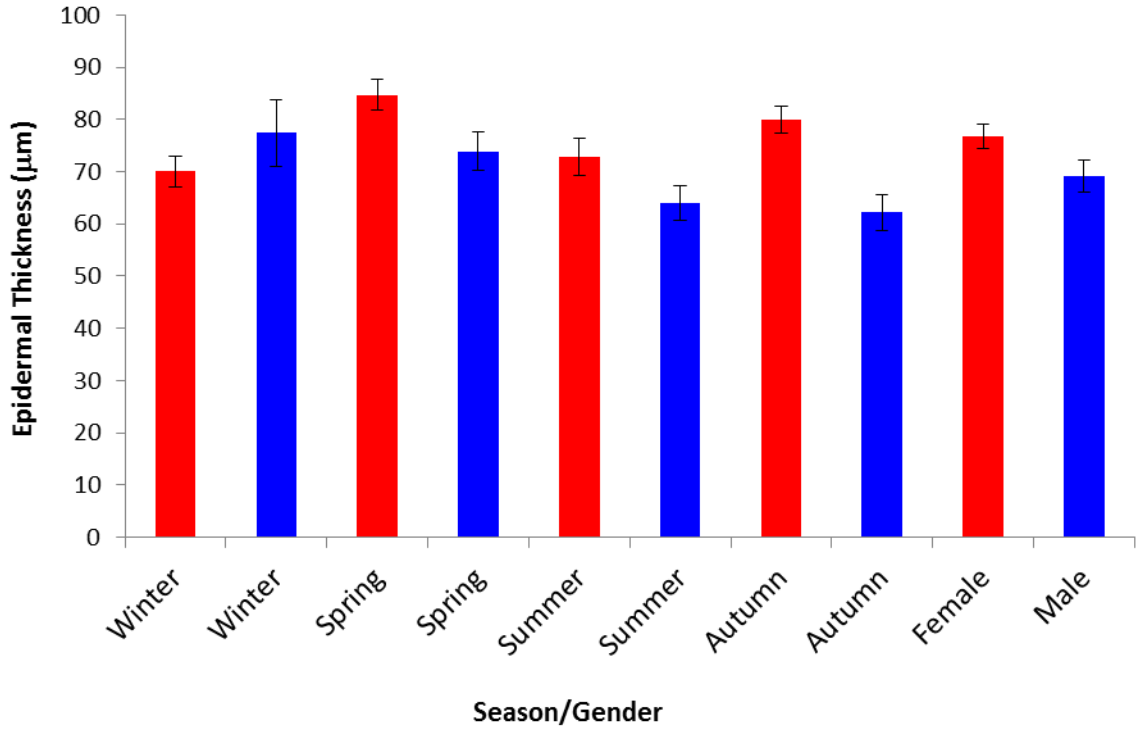

Figure 5.14. Gender and seasonal comparison of epidermal thickness for adult catsharks showing means and ± standard errors (n= Female (62) (W, 15) (Sp, 13) (Su, 15) (A, 19) Male (43) (W, 8) (Sp, 13) (Su, 13) (A, 9)) (Female = Red, Male = Blue).

Table 5.4 shows the results from the GLM analyses for the epidermis of adult male and female *S. canicula*. The results show that gender had a significant effect on the epidermal skin thickness of male and female *S. canicula* with females possessing a thicker epidermis than males. Body length, season, and gender within season had no effect on the epidermal thickness of adult male and female *S. canicula.*

Table 5.4. Results from the GLM analyses for the epidermis of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	802.2	664.3	664.3	1.98	**0.162**
Gender	1	1572.3	1481.6	1481.6	4.42	**0.038**
Season	3	1512.8	1503.1	501.0	1.50	**0.221**
Season*Gender	3	1817.6	1817.6	605.9	1.81	**0.151**

5.3.3.2 Adult Dermal Thickness

Figure 5.15 shows a graphical representation of the dermal thickness of adult male and female catsharks for all four seasons. It can be seen that in general adult female catsharks possessed a greater dermal thickness than adult male catsharks.

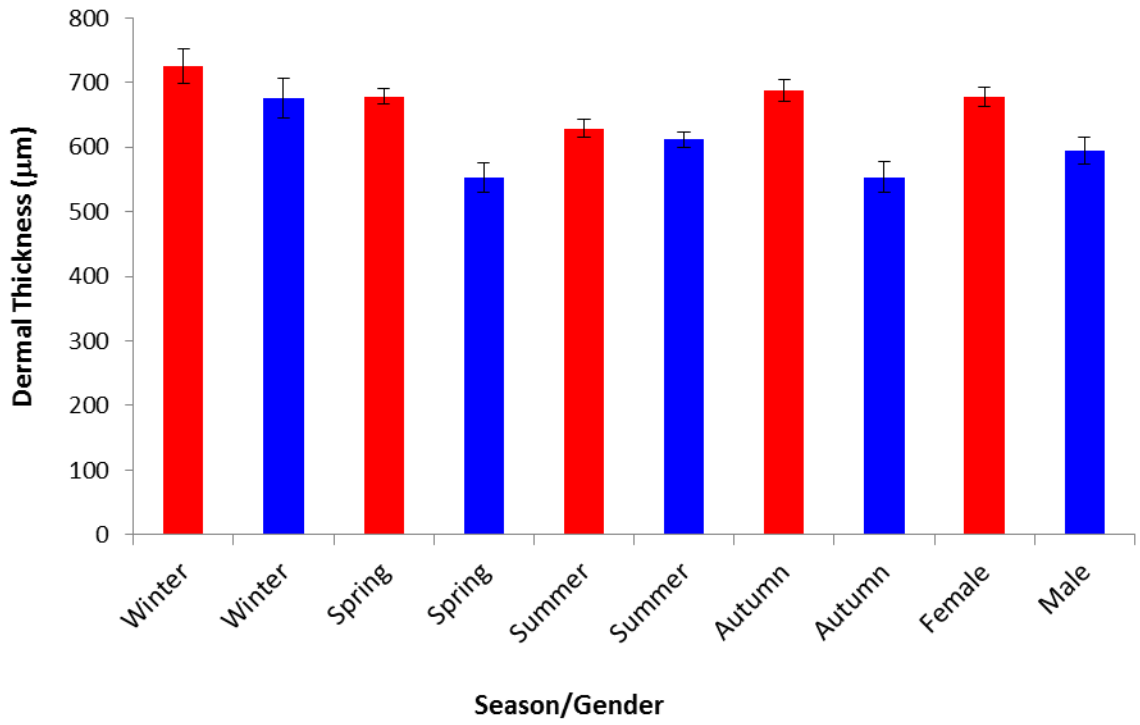

Figure 5.15. Gender and seasonal comparison of dermal thickness for adult catsharks showing means and ± standard errors (n= Female (62) (W, 15) (Sp, 13) (Su, 15) (A, 19) Male (43) (W, 8) (Sp, 13) (Su, 13) (A, 9)) (Female = Red, Male = Blue).

Table 5.5 shows the results from the GLM analyses for the dermis of adult male and female *S. canicula*. It can be seen from Table 5.5 that body length, season and gender within season had no effect on the dermal thickness of adult male and female catsharks. Gender was found to have a significant effect, with adult females possessing a thicker dermis compared to adult males.

Table 5.5. Results from the GLM analyses for the dermis of adult male and female *S. canicula.*

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	61281	53130	53130	3.58	**0.062**
Gender	1	181021	165273	165273	11.13	**0.001**
Season	3	52007	70907	23636	1.59	**0.197**
Season*Gender	3	83108	83108	27703	1.86	**0.141**

Figure 5.16 shows skin sections from male and female catsharks. Juvenile and adult female catsharks possessed thicker dermal and epidermal layers than sub-adult and mature male catsharks sampled in the same season. Male and female juvenile catsharks were not found to possess a significantly different dermal layer. However, juvenile females possessed a significantly thicker epidermis compared to males.

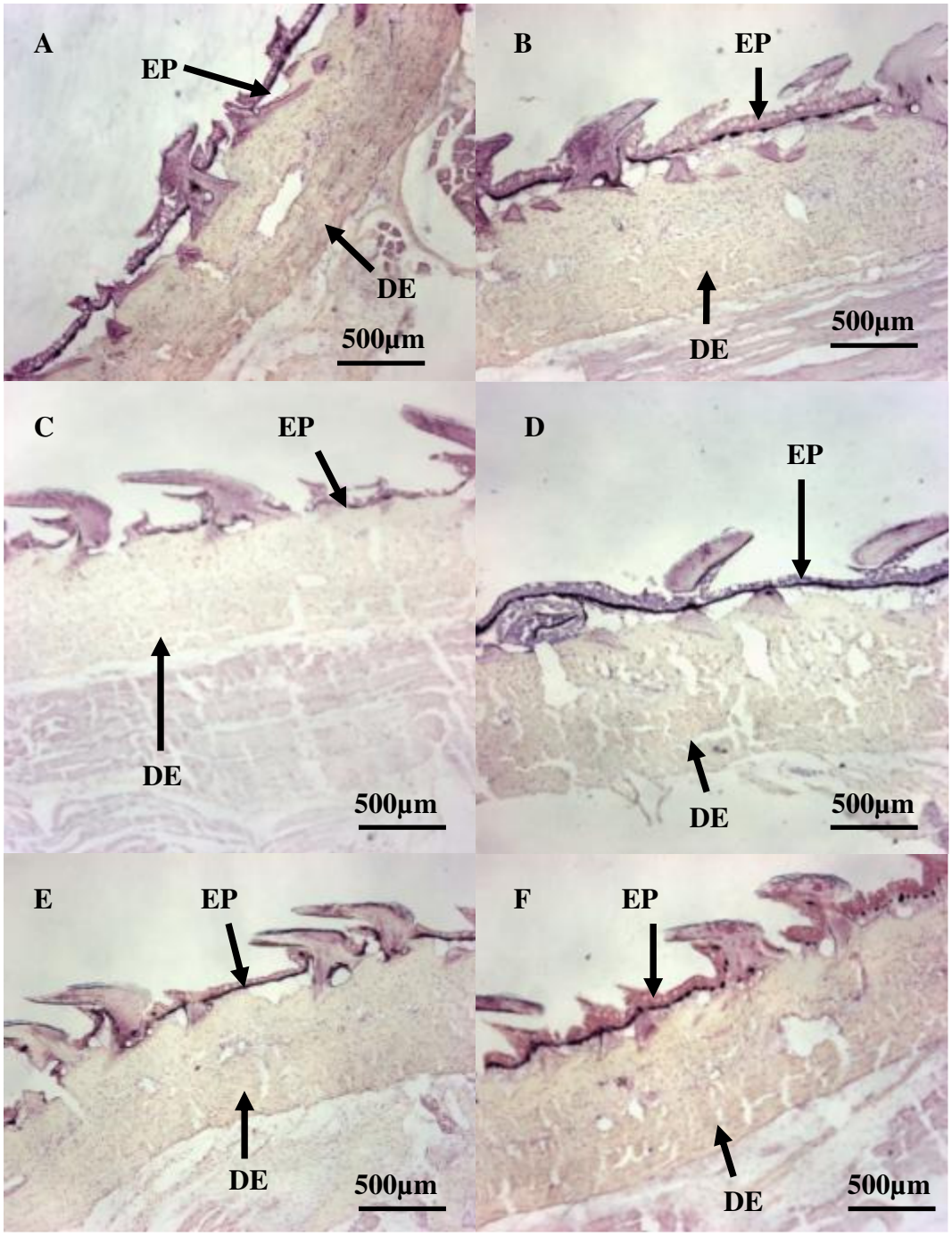

Figure 5.16 Section of skin showing dermis (DE) and epidermis (EP) from (A) juvenile male (506mm TL) and (B) juvenile female (509mm TL) *S. canicula* sampled in winter, (C) sub-adult male (535mm TL) and (D) sub-adult female (545mm TL) *S. canicula* sampled in autumn and (E) mature male (600mm TL) and (F) mature female (600mm TL) sampled in summer.

5.3.4 Dermal Denticle Morphometrics

5.3.4.1 Hatchling Right Pectoral Fin

The results for the ANCOVA for dermal denticle morphometrics for the right pectoral fin of hatchling catsharks can be seen in Table 5.6. There were no significant effects on the denticle length for body length (ANCOVA, F=0.18; d.f.=1; *P*=0.674) or gender (ANCOVA, F=0.01; d.f.=1; *P*=0.917) on the right pectoral fin of hatchling catsharks. Neither body length nor gender had a significant effect on the denticle width of the right pectoral fin of hatchling catsharks (ANCOVA, F=0.05; d.f.=1; *P*=0.829; ANCOVA, F=3.30; d.f.=1; *P*=<0.078). Body length had no significant effect on the dermal denticle density of the right pectoral fin in hatchling catsharks (ANCOVA, F=0.56; d.f.=1; *P*=0.458). There was a significant difference in the dermal denticle density of hatchling male and female catsharks, with hatchling male catsharks possessing a significantly higher dermal denticle density on the right pectoral fin that hatchling female catsharks (ANCOVA, F=6.00; d.f.=1; *P*<0.020).

Table 5.6. Results from the ANCOVA for the denticle width, denticle length and denticle density on the right pectoral fin of hatchling male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F (14) M (23)).

Feature	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Denticle Length (µm) (Range)	281 ± 5 (205.8-316.1)	281 ± 8 (106.4-392.3)	**0.674**	**0.917**
Denticle Width (µm) (Range)	113 ± 2.5 (72.4-178.8)	104 ±1.2 (78.5-140.3)	**0.829**	**0.078**
Density (mm^2) (Range)	56.6± 3 (41-79)	65.7 ± 2.5 (46-85)	**0.458**	**0.020**

5.3.4.2 Hatchling Left Pectoral Fin

The results for the ANCOVA for dermal denticle morphometrics for the left pectoral fin of hatchling catsharks can be seen in Table 5.7. Neither body length nor gender had a significant effect on the denticle length of the left pectoral fin of hatchling catsharks (ANCOVA, F=0.20; d.f.=1; *P*=0.655; ANCOVA, F=1.97; d.f.=1; *P*=0.170). There was no significant effect on the denticle width for body length (ANCOVA, F=1.80; d.f.=1; *P*=0.188). Gender did have a significant effect on the width of the dermal denticles of the left fin of hatchling catsharks (ANCOVA, F=5.78; d.f.=1; *P*=0.022) with hatchling female catsharks possessing wider dermal denticles than hatchling male catsharks. Body length had no significant effect on the denticle density on the left pectoral fin of hatchling catsharks (ANCOVA, F=0.95; d.f.=1; *P*=0.338). There was a significant difference in the dermal denticle density of the left pectoral fin of hatchling male and female catsharks, with hatchling male catsharks possessing a significantly higher dermal denticle density on the left pectoral fin than hatchling female catsharks (ANCOVA, F=9.97; d.f.=1; *P*=0.003).

Table 5.7. Results from the ANCOVA for the denticle width, denticle length and denticle density on the left pectoral fin of hatchling male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F (14) M (23)).

Feature	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Denticle Length (µm) (Range)	276 ± 5.8 (189.6-375)	293 ± 6.7 (199.9-402.2)	**0.655**	**0.170**
Denticle Width (µm) (Range)	116 ± 2.7 (66.1-197.1)	108 ± 1.3 (79.4-139.7)	**0.188**	**0.022**
Density (per mm^2) (Range)	56.3 ± 2.8 (39-79)	66.74 ± 2 (48-88)	**0.338**	**0.003**

5.3.4.3 Hatchling Combined Pectoral Fin Data

The results for the ANCOVA for the combined data for the dermal denticle morphometrics of the left and right pectoral fins of hatchling catshark are shown in Table 5.8. Neither body length nor gender had a significant effect on the denticle length of the pectoral fins of hatchling catsharks (ANCOVA, F=0.24; d.f.=1; *P*=0.629; ANCOVA, F=0.69; d.f.=1; *P*=0.413). There was no significant effect on the denticle width for body length (ANCOVA, F=0.34; d.f.=1; *P*=0.561). Gender had a significant effect on the combined width of the dermal denticles of the left and right fins of hatchling catsharks (ANCOVA, F=5.87; d.f.=1; *P*=0.021). Hatchling female catsharks were found to possess wider dermal denticles on the pectoral fins that hatchling male catsharks. Body length had no significant effect on the denticle density on the pectoral fins of hatchling catsharks (ANCOVA, F=0.84; d.f.=1; *P*=0.365). There was a significant difference in the dermal denticle density of the pectoral fins of hatchling male and female catsharks. Hatchling male catsharks were found to possess a significantly higher dermal denticle density on the pectoral fins than hatchling female catsharks (ANCOVA, F=8.94; d.f.=1; *P*=0.005).

Table 5.8. Results from the ANCOVA for the combined denticle length, denticle width and denticle density of the left and right pectoral fins of hatchling male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F (14) M (23)).

Feature	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Denticle Length (µm) (Range)	278.6 ± 10.5 (206.3-340)	287.8 ± 6.4 (236-373.1)	**0.629**	**0.413**
Denticle Width (µm) (Range)	114.5 ± 4.7 (90.1-161.1)	105 ± 1.2 (95.0-116.5)	**0.561**	**0.021**
Density (mm^2) (Range)	56.5 ± 2.5 (42-77)	66.4 ± 2.1 (49-85)	**0.365**	**0.005**

5.3.4.4 Hatchling Intra-Gender Pectoral Fin Lateralisation

Table 5.9 shows the intra-gender pectoral fin lateralisation comparisons of the denticle dimensions and densities for the right and left pectoral fins of female hatchling catsharks. It can be seen from Table 5.8 that there were no significant differences in the length, width or density of the denticles on the left and right pectoral fins of female hatchling catsharks.

Table 5.9. Intra-gender pectoral fin lateralisation for female hatchling *S. canicula*, showing the means, standard errors, range and *P*-Values (n=14).

Gender	Feature	($\bar{x}$ ± SE) (Right fin)	($\bar{x}$ ± SE) (Left fin)	*P*-Value
Female	Denticle Length (µm) (Range)	281 ± 5.4 (205.8-316.1)	276 ± 5.8 (189.6-374)	**0.775**
Female	Denticle Width (µm) (Range)	113 ± 2.5 (72.4-178.8)	116 ± 2.7 (66.1-197.1)	**0.791**
Female	Density (per mm^2) (Range)	56.6 ± 2.7 (41-79)	56.4 ± 2.8 (39-79)	**0.956**

Table 5.10 shows the intra-gender pectoral fin lateralisation comparisons of the denticle dimensions and numbers for the right and left pectoral fins of male hatchling catsharks. There were no significant differences in the dermal denticle length, width or density on the pectoral fins of hatchling male catsharks (Table 5.10).

Table 5.10. Intra-gender pectoral fin lateralisation for male hatchling *S. canicula*, showing the means, standard errors, range and *P*-Values (n= 23).

Gender	Feature	($\bar{x}$ ± SE) (Right fin)	($\bar{x}$ ± SE) (Left fin)	*P*-Value
Male	Denticle Length (µm) (Range)	281 ± 7.8 (106.4-392.4)	293 ± 6.7 (199.9-402.2)	**0.245**
Male	Denticle Width (µm) (Range)	104 ±1.21 (78.5-140.3)	108 ± 1.3 (79.4-139.7)	**0.526**
Male	Density (per mm^2) (Range)	65.7 ± 2.5 (46-85)	66.7 ± 2 (48-88)	**0.758**

5.3.5 Juvenile Dermal Denticle Measurements

5.3.5.1 Right Pectoral Fin Measurements

The results for the dermal denticle morphometrics on the right pectoral fin of juvenile males and female catsharks can be seen in Table 5.11. Neither body length nor gender had a significant effect on the dermal denticle length of juvenile catsharks (ANCOVA, F=2.95; d.f.=1; *P*=0.146 ANCOVA, F=0.10; d.f.=1; *P*=0.762). Dermal denticle width was not found to be significantly different on the right pectoral fin for either body length or gender for juvenile catsharks (ANCOVA, F=1.73; d.f.=1; *P*=0.245; ANCOVA, F=0.06; d.f.=1; *P*=0.810). There were no significant differences for dermal denticle density on the right pectoral fin on juvenile catsharks. Neither body length nor gender had an effect on dermal denticle density of juvenile catsharks (ANCOVA, F=2.44; d.f.=1; *P*=0.179; ANCOVA, F=0.24; d.f.=1; *P*=0.648).

Table 5.11. Results from the ANCOVA for the denticle length, denticle width and denticle density on the right pectoral fin of juvenile male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F(15) M(13)).

Feature	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Denticle Length (µm)	411.9 ± 33.4 (348.4-461.6)	391.5 ± 17.9 (330.6-431.3)	**0.146**	**0.762**
Denticle Width (µm)	267.6 ± 26.1 (228.5-317.1)	255.3 ± 11.4 (230.4-296.5)	**0.245**	**0.810**
Density (mm^2)	55.7 ± 0.7 (55-57)	57.8 ± 2.1 (50-62)	**0.179**	**0.648**

5.3.5.2 Left Pectoral Fin Measurements

The results for the dermal denticle morphometrics on the left pectoral fin of juvenile males and female catsharks can be seen in Table 5.12. Neither body length nor gender had a significant effect on the dermal denticle length of juvenile catsharks (ANCOVA, F=4.43; d.f.=1; *P*=0.089 ANCOVA, F=1.55; d.f.=1; *P*=0.268). Dermal denticle width was not found to be significantly different on the left pectoral fin for either body length or gender for juvenile catsharks (ANCOVA, F=2.17; d.f.=1; *P*=0.201; ANCOVA, F=0.66; d.f.=1; *P*=0.454). There were no significant differences in dermal denticle density on the left pectoral fin on juvenile catsharks. Neither body length nor gender had an effect on dermal denticle density of juvenile catsharks (ANCOVA, F=0.07; d.f.=1; *P*=0.799; ANCOVA, F=3.33; d.f.=1; *P*=0.128).

Table 5.12. Results from the ANCOVA for the denticle length, denticle width and denticle density on the left pectoral fin of juvenile male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F(15) M(13)).

Feature	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Denticle Length (µm) (Range)	415.4 ± 32.3 (352.3-458.6)	369.3 ± 18.1 (320.2-421.3)	**0.089**	**0.268**
Denticle Width (µm) (Range)	286.3 ± 29.1 (239.7-339.7)	261.25 ± 8.52 (230.9-282.7)	**0.201**	**0.454**
Density (mm^2) (Range)	51 ± 1.2 (49-53)	56.6 ± 2 (53-64)	**0.799**	**0.128**

5.3.5.3 Combined Pectoral Fin Measurements

The results for the ANCOVA for the combined data for the dermal denticle morphometrics of the left and right pectoral fins of juvenile catshark are shown in Table 5.13. Neither body length nor gender had a significant effect on the denticle length of the pectoral fins of

juvenile catsharks (ANCOVA, F=3.77; d.f.=1; *P*=0.110; ANCOVA, F=0.60; d.f.=1; *P*=0.473). There was no significant effect on the combined dermal denticle width for body length (ANCOVA, F=2.09; d.f.=1; *P*=0.208) or gender (ANCOVA, F=0.30; d.f.=1; *P*=0.609). Neither body length or gender had a significant effect on the denticle density on the pectoral fins of juvenile catsharks (ANCOVA, F=0.41; d.f.=1; *P*=0.552; ANCOVA, F=1.88; d.f.=1; *P*=0.229).

Table 5.13. Results from the ANCOVA for the combined denticle length, denticle width and denticle density on the left and right pectoral fins of juvenile male and female *S. canicula* showing means and ± standard errors, range and *P*-Values (n= F(15) M(13)).

Feature	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Body Length ANCOVA (*P*-Value)	Gender ANCOVA (*P*-Value)
Denticle Length (µm) (Range)	413.7 ± 32.8 (350.4-460.1)	380.4 ± 17.6 (325.4-426.2)	**0.110**	**0.473**
Denticle Width (µm) (Range)	277 ± 27.6 (234.1-328.4)	258.3 ± 9.2 (235.9-289.6)	**0.208**	**0.609**
Density (mm^2) (Range)	53 ± 0.7 (52-54)	57 ± 1.8 (56-62)	**0.552**	**0.229**

5.3.5.4 Intra-Gender Pectoral Fin Lateralisation

Table 5.14 shows the intra-gender pectoral fin lateralisation comparisons of the denticle dimensions and density for the right and left pectoral fins of juvenile female catsharks. It can be seen from Table 5.14 that there were no significant differences in the length or width of the dermal denticles on the left pectoral fins of juvenile female catsharks compared to those on the right. There was a significant difference between the density of dermal denticles on the right and left pectoral fins of juvenile female catsharks, with the right fins having higher densities of denticles than the left fins.

Table 5.14. Intra-gender pectoral fin lateralisation for female juvenile *S. canicula*, showing the means, standard errors, range and *P*-Values (n=15).

Gender	Feature	($\bar{x}$ ± SE) (Right fin)	($\bar{x}$ ± SE) (Left fin)	*P*-Value
Female	Denticle Length (µm) (Range)	411.9 ± 33.4 (348.4-461.6)	415.4 ± 32.3 (352.3-458.6)	**0.945**
Female	Denticle Width (µm) (Range)	267.6 ± 26.1 (228.5-317.1)	286.3 ± 29.1 (239.7-339.7)	**0.665**
Female	Density (mm^2) (Range)	55.7 ± 0.7 (55-57)	51 ± 1.2 (49-53)	**0.039**

Table 5.15 shows the intra-gender pectoral fin lateralisation comparisons of the denticle dimensions and densities for the right and left pectoral fins of male juvenile catsharks. It can be seen from Table 5.13 that there were no significant differences in the length, width or densities of the dermal denticles on the left and right pectoral fins of juvenile male catsharks.

Table 5.15. Intra-gender pectoral fin lateralisation for male juvenile *S. canicula*, showing the means, standard errors, range and *P*-Values (n=13).

Gender	Feature	($\bar{x}$ ± SE) (Right fin)	($\bar{x}$ ± SE) (Left fin)	*P*-Value
Male	Denticle Length (µm) (Range)	391.5 ± 17.9 (330.6-431.3)	369.3 ± 18.1 (320.2-421.3)	**0.412**
Male	Denticle Width (µm) (Range)	255.3 ± 11.4 (230.4-296.5)	261.25 ± 8.52 (230.9-282.7)	**0.688**
Male	Density (per mm^2) (Range)	57.8 ± 2.1 (50-62)	56.6 ± 2.0 (53-64)	**0.696**

5.3.6 Adult Dermal Denticle Measurements

There were no adult male or female specimens available during the autumn months for denticle measurements. A GLM was carried out to determine the gender and seasonality, with an ANCOVA using body length as a covariate, to ascertain any seasonal and sexual dimorphisms in the length, width and densities of dermal denticles on the left and right pectoral fins of adult specimens. Paired t-tests were used to identify any intra-gender differences between the right and left pectoral fins in males and the right and left pectoral fins in females. The means ± standard errors and ranges for the dermal denticle morphometrics of adult *S. canicula* can be seen in Appendix 3.

5.3.7 Adult Pectoral Fin Denticle Length

5.3.7.1 Right Pectoral Fin Denticle Length

Figure 5.17 shows a graphical representation of the length of the dermal denticles on the right pectoral fin of adult male and female catsharks for all three seasons. The denticle length on the right pectoral fin was found to be generally greater in adult female catsharks than in adult male catsharks, although the results of the ANCOVA showed no significant differences between genders.

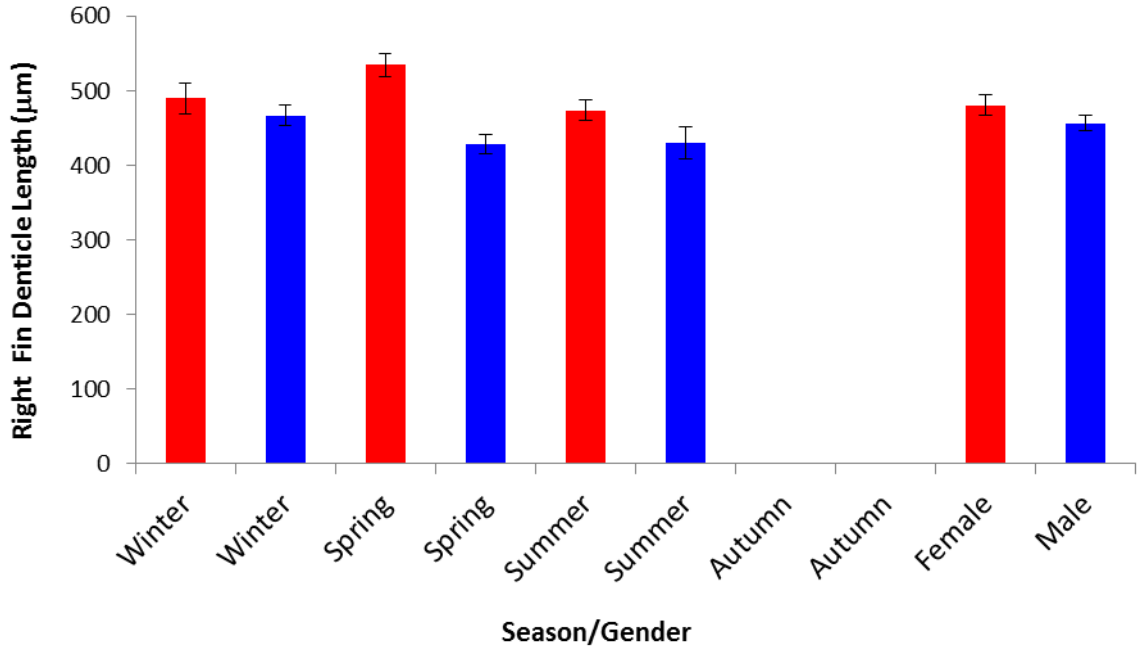

Figure 5.17. Gender and seasonal comparison of right pectoral fin denticle length for adult catsharks showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

Table 5.16 shows the output of the GLM for the right pectoral fin denticle length of adult male and female catsharks. It can be seen from Table 5.16 that body length, gender and gender within season had no effect on denticle length in the right pectoral fins of adult male and female adult catsharks. It can be seen from Table 5.16 that season had an effect on denticle length with catsharks sampled in spring having longer denticles on the right pectoral fins than catsharks sampled during winter, spring and summer.

Table 5.16. Results from the GLM analyses for the right pectoral fin denticle length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	19925	5684	5684	2.46	**0.125**
Gender	1	3263	5489	5489	2.38	**0.131**
Season	3	29262	26985	13492	5.84	**0.006**
Season*Gender	3	7813	7813	3907	1.69	**0.198**

5.3.7.2 Left Pectoral Fin Denticle Length

Figure 5.18 shows a graphical representation of the length of the dermal denticles on the left pectoral fin of adult male and female catsharks for all three seasons. There were no seasonal or gender differences in the dermal denticle length of adult *S. canicula*.

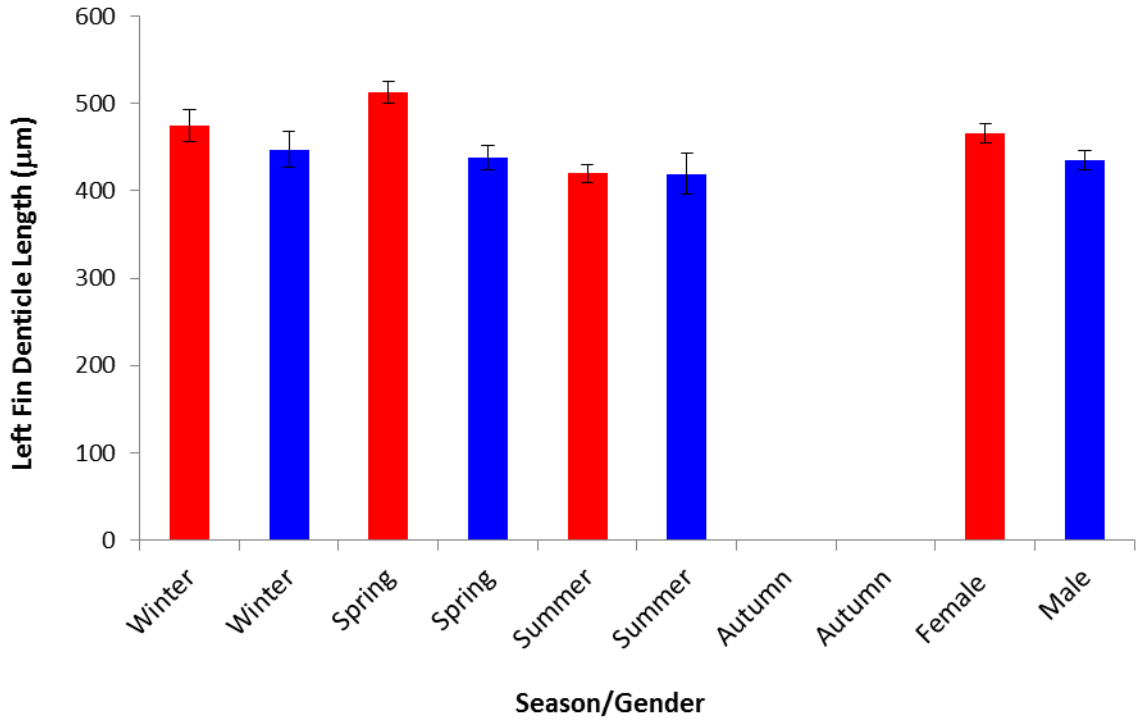

Figure 5.18. Gender and seasonal comparison of left pectoral fin denticle length for adult catsharks showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.17 that body length had an effect on the denticle length of the left pectoral fins. Gender, season and gender within season had no effect on the length of the dermal denticles on the left pectoral fin of adult male and female *S. canicula*.

Table 5.17. Results from the GLM analyses for the left pectoral fin denticle length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	31320	16010	16010	7.52	**0.009**
Gender	1	5432	7542	7542	3.54	**0.067**
Season	2	11792	10089	5045	2.37	**0.107**
Season*Gender	2	11830	11830	5915	2.78	**0.074**

Figure 5.19 shows a graphical representation of the body length and left pectoral fin denticle length of individual adult male and female catsharks. It can be seen that as body length increased so did denticle length on the left pectoral fin.

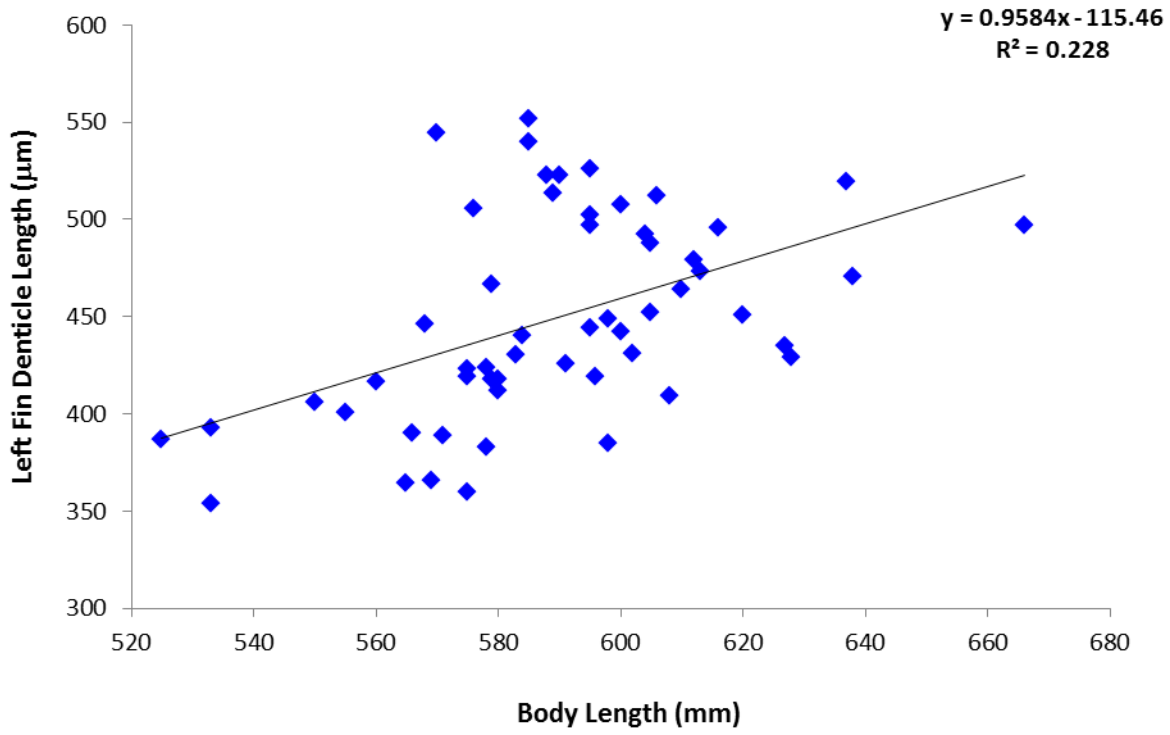

Figure 5.19. Scatterplot with regression showing left pectoral fin denticle length against body length for adult male and female *S. canicula* (n= 55) (*P* <0.001).

5.3.7.3 Combined Pectoral Fin Denticle Length

Figure 5.20 shows a graphical representation of the combined length of the dermal denticles from the left and right pectoral fins of adult male and female catsharks for all three seasons. The denticle length on the pectoral fins of adult female catsharks was found to be generally greater than in adult male catsharks, although the results of the ANCOVA showed no significant differences between genders.

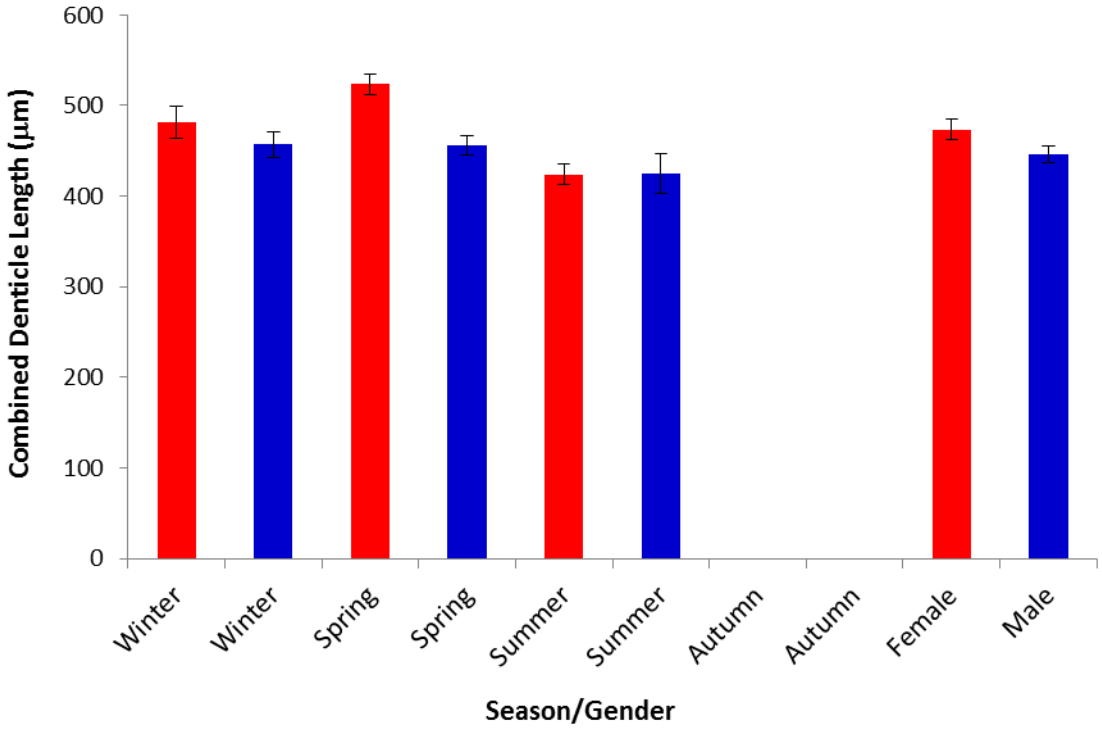

Figure 5.20. Gender and seasonal comparison of combined pectoral fin denticle length for adult catsharks showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.18 that body length had an effect on the combined denticle length for adult male and female catsharks. Gender and gender within season had no effect on the length of the dermal denticles on the left pectoral fin of adult male and female *S. canicula*. Season did have an effect on the combined dermal denticle length of adult catsharks with adult catsharks sampled in summer having significantly shorter dermal denticles than adult catsharks sampled in both winter and spring.

Table 5.18. Results from the GLM analyses for the combined pectoral fin denticle length of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	25302	10193	10193	5.70	**0.022**
Gender	1	4279	6475	6475	3.62	**0.065**
Season	2	19537	17511	8756	4.89	**0.013**
Season*Gender	2	9718	9718	4859	2.72	**0.079**

Figure 5.21 shows a graphical representation of the seasonal body length and combined denticle length on the pectoral fins of individual adult male and female catsharks. It can be seen that as body length increases so does denticle length on the pectoral fins. Adult catsharks sampled in winter showed a difference in rate of increase of denticle length with body length compared to adults sampled in spring and summer.

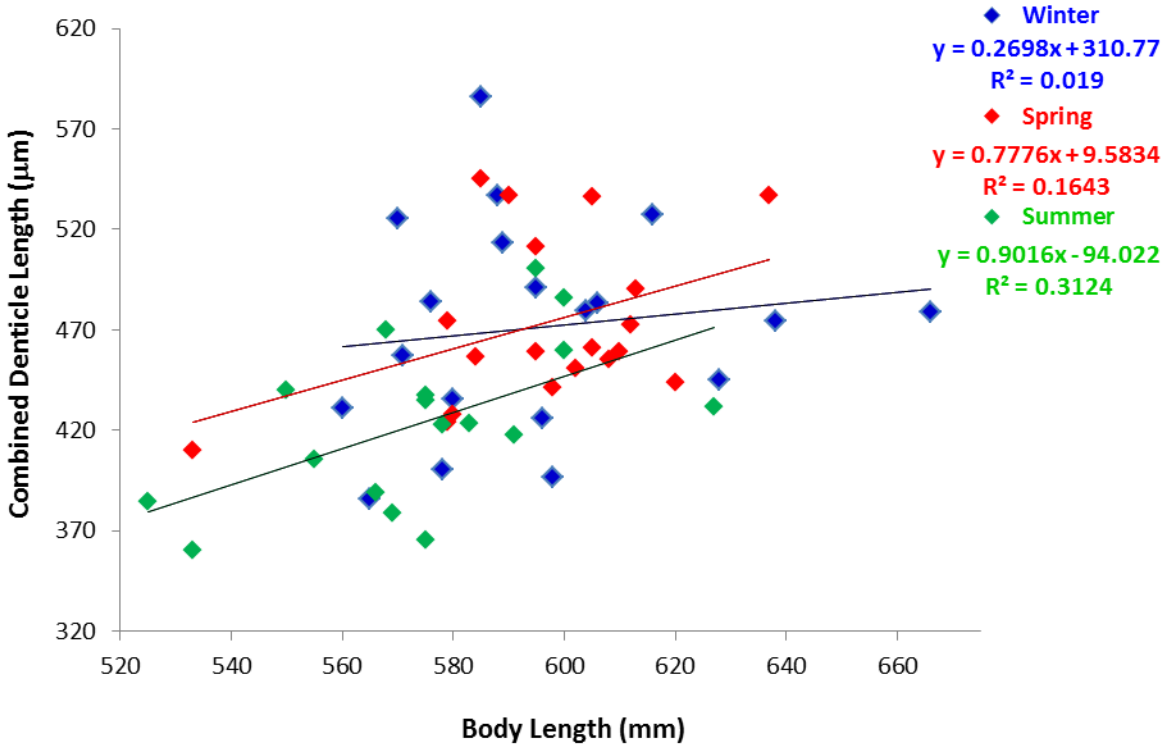

Figure 5.21. Scatterplot with regression showing the seasonal comparison for the combined pectoral fin denticle length against body length for adult male and female *S. canicula* (n= 55 (W, 19) (Sp, 19) (Su, 17) (*P*=0.013).

5.3.8 Adult Pectoral Fin Denticle Width

5.3.8.1 Right Pectoral Fin Denticle Width

Figure 5.22 shows a graphical representation of the seasonal data for dermal denticle width on the right pectoral fin of adult male and female catsharks. There were no seasonal or gender differences in the denticle width of the right pectoral fin of adult catsharks.

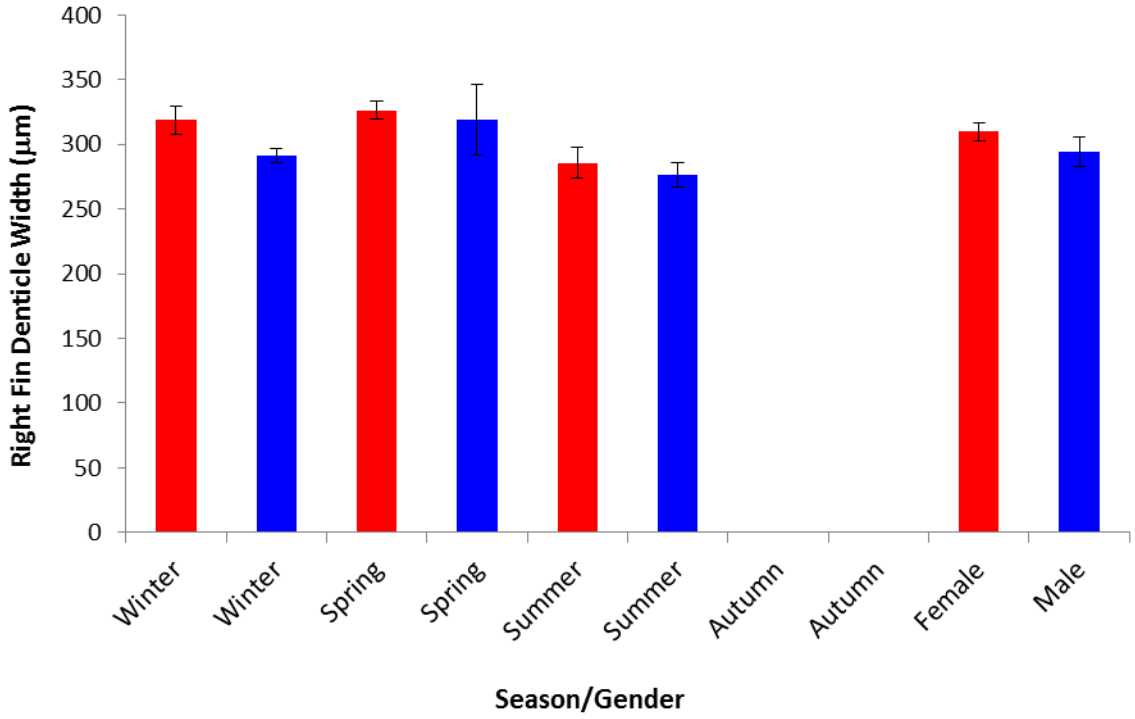

Figure 5.22. Gender and seasonal comparison of right pectoral fin denticle width for adult catsharks showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.19 that body length, gender, season and gender within season had no effect on the denticle width of the right pectoral fin of male and female catsharks

Table 5.19. Results from the GLM analyses for the right pectoral fin denticle width of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	5443	1321	1321	0.76	**0.389**
Gender	1	1576	1607	1607	0.92	**0.343**
Season	2	8828	8333	4167	2.39	**0.105**
Season*Gender	2	1241	1241	621	0.36	**0.702**

5.3.8.2 Left Pectoral Fin Denticle Width

Figure 5.23 shows a graphical representation of the width of the dermal denticles on the left pectoral fin of adult male and female catsharks for all three seasons. The left fin denticle width was found to be significantly different for adult male and female catsharks.

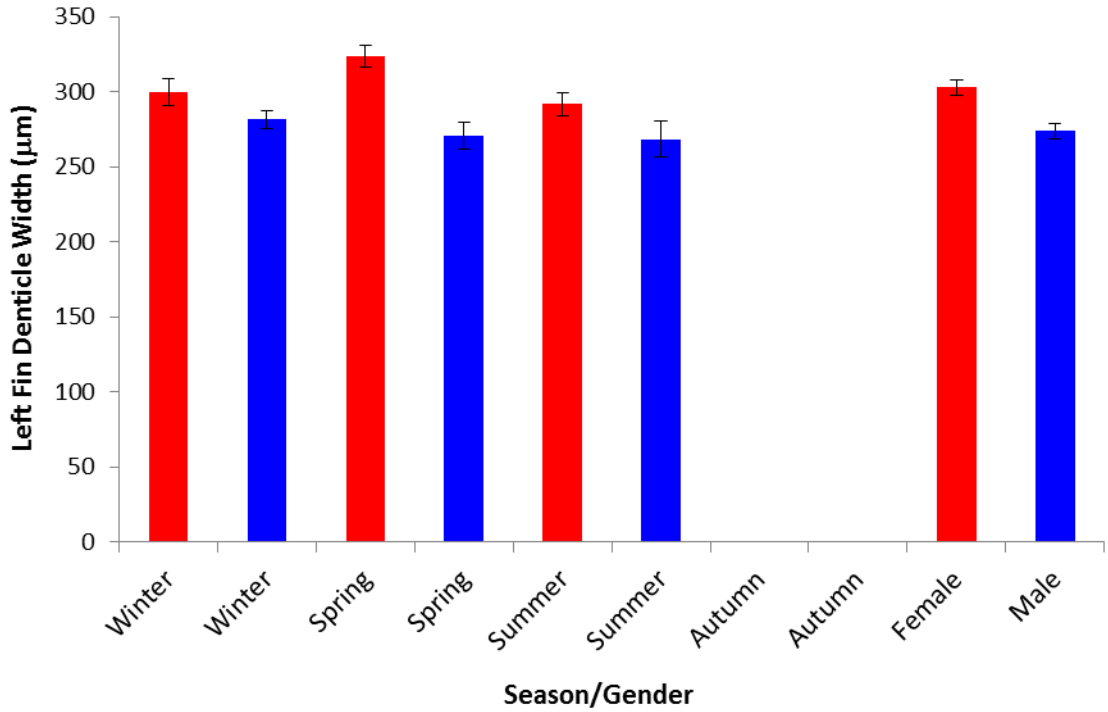

Figure 5.23. Gender and seasonal comparison of left pectoral fin denticle width for adult catsharks showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.20 that body length, season and gender within season had no effect on the denticle width of the left pectoral fin of adult male and female catsharks. Gender was found to have a significant effect, with adult female catsharks possessing wider dermal denticles on the left pectoral fins than adult male catsharks.

Table 5.20. Results from the GLM analyses for the left pectoral fin denticle width of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	2718.3	1217.9	1217.9	2.19	**0.147**
Gender	1	6353.1	7522.0	7522.0	13.54	**0.001**
Season	2	1514.3	1086.0	543.0	0.98	**0.386**
Season*Gender	2	1715.1	1715.1	857.6	1.54	**0.227**

5.3.8.3 Combined Pectoral Fin Denticle Width

Figure 5.24 shows a graphical representation of the seasonal data for combined left and right pectoral fin dermal denticle widths of adult male and female catsharks. There was a significant difference in the dermal denticle widths between adult male and female catsharks.

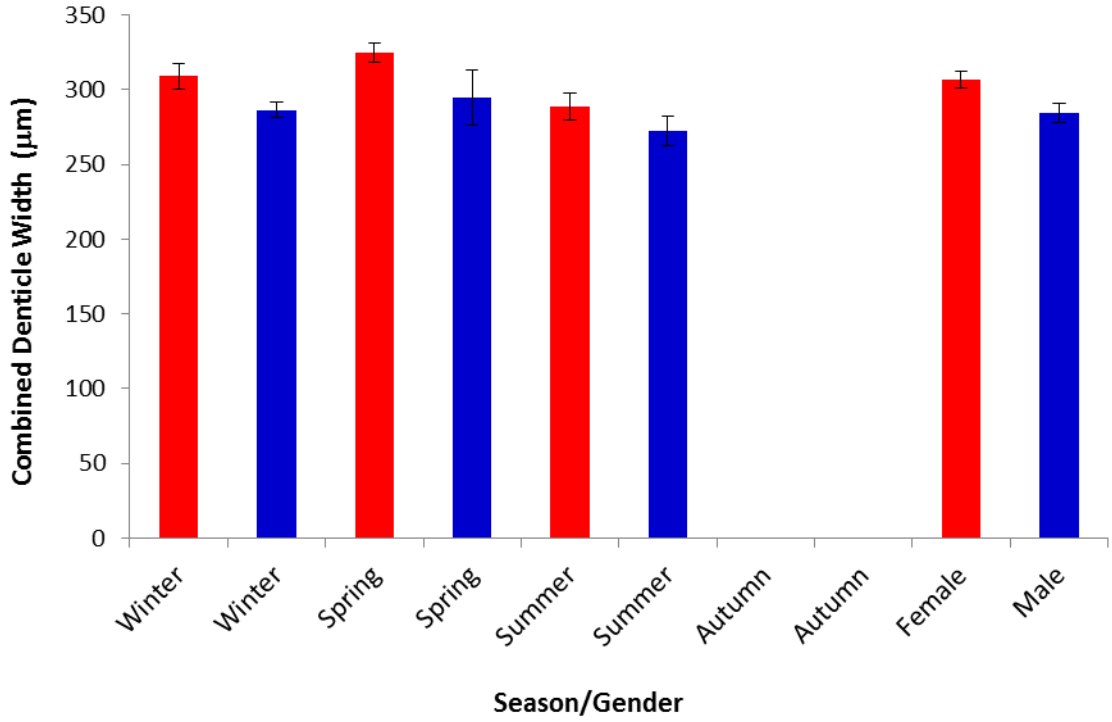

Figure 5.24. Gender and seasonal comparison of the combined pectoral fin denticle width for adult catsharks showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.21 that body length, season and gender within season had no effect on the combined denticle width for adult male and female catsharks. Gender was found to have a significant effect on the combined dermal denticle width of adult catsharks with adult female catsharks having significantly wider dermal denticles than adult male catsharks.

Table 5.21. Results from the GLM analyses for the combined pectoral fin denticle width of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	5767.4	1938.7	1938.7	2.73	**0.106**
Gender	1	3874.9	4400.2	4400.2	6.20	**0.017**
Season	2	3711.6	3483.7	1741.9	2.45	**0.099**
Season*Gender	2	543.3	543.3	271.7	0.38	**0.684**

5.3.9 Adult Pectoral Fin Denticle Densities

5.3.9.1 Right Pectoral Fin Denticle Density

Figure 5.25 shows a graphical representation of the densities of the dermal denticles on the right pectoral fin of adult male and female catsharks. Both seasonal differences and gender within season were found to have an effect on the denticle density on the right pectoral fin of adult *S. canicula*.

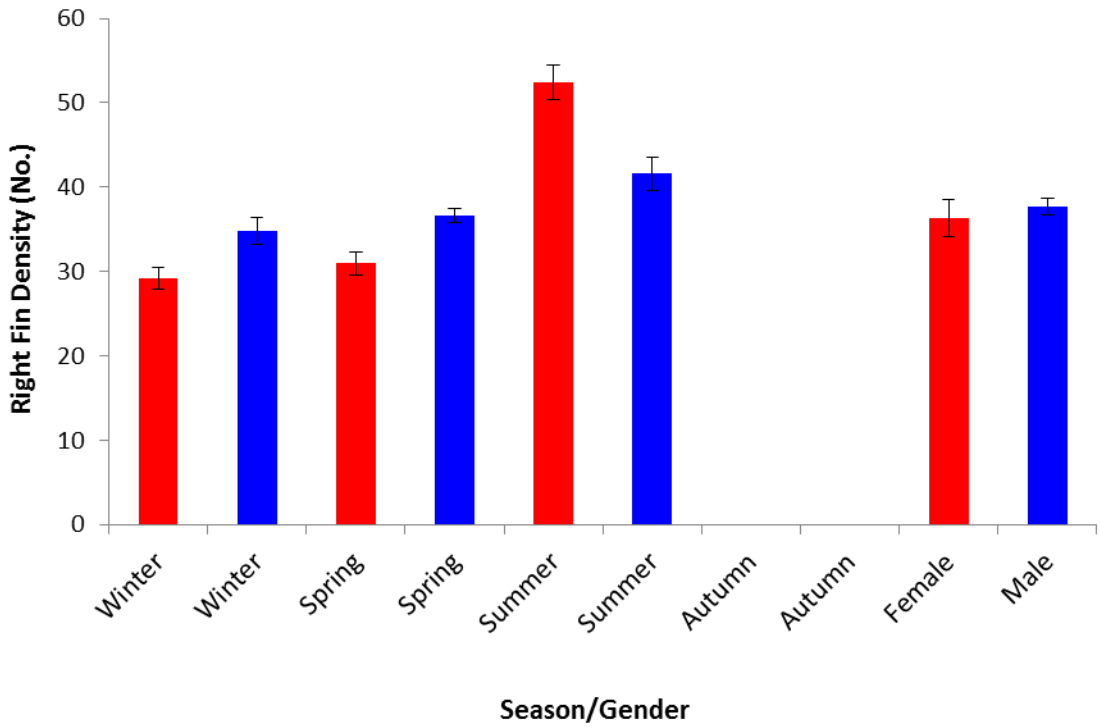

Figure 5.25. Gender and seasonal comparison of right pectoral fin denticle density per mm^2 for adult catsharks, showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.22 that neither body length nor gender had an effect on the dermal denticle density of the right pectoral fin of male and female catsharks. Season and gender within season had a significant effect.

Table 5.22. Results from the GLM analyses for the right pectoral fin denticle density of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	1027.45	45.63	45.63	2.20	**0.144**
Gender	1	66.01	2.47	2.47	0.12	**0.731**
Season	2	973.45	1157.36	578.68	27.94	**<0.001**
Season*Gender	2	774.76	774.76	387.38	18.71	**<0.001**

The following tables show the intra-gender comparisons of season and gender from the GLM for the dermal denticle density in the right pectoral fin of male and female adult *S. canicula*.

Table 5.23 shows that there were no significant differences in the densities of dermal denticles on the right pectoral fins of adult male catsharks ($P>0.05$). There was a significant difference in the density of dermal denticles on the right pectoral fins of adult female catsharks (Table 5.23). Adult female catsharks sampled in summer had a higher dermal denticle density on the right pectoral fin than females sampled during winter and spring ($P<0.05$).

Table 5.23. *P*-values for intra-gender seasonal comparison of right pectoral fin denticle numbers per mm^2 for adult male and female catsharks (ND = No Data).

Season	Winter	Spring	Summer	Autumn	
Winter		0.976	0.254	ND	**Male**
Spring	0.979		0.590	ND	
Summer	<0.001	<0.001		ND	
Autumn	ND	ND	ND		
	Female				

Table 5.24 shows that there were significant differences in the densities of dermal denticles on the right pectoral fins of adult male and female catsharks (P<0.05) with adult females sampled in winter possessing a lower density of dermal denticles on the right pectoral fin than adult males in spring and summer. Adult female catsharks sampled in spring also possessed lower densities of dermal denticles on the right pectoral fin than adult male catsharks in summer. Adult Female catsharks sampled in summer possessed a higher density of dermal denticles on the right pectoral fins than males in winter, spring and summer.

Table 5.24. *P*-values for seasonal comparison of right pectoral fin denticle numbers per mm^2 for adult male and female catsharks (ND = No Data).

Female		**Male**			
		Winter	Spring	Summer	Autumn
	Winter	0.072	0.011	<0.001	ND
	Spring	0.324	0.074	0.001	ND
	Summer	<0.001	<0.001	<0.001	ND
	Autumn	ND	ND	ND	ND

5.3.9.2 Left Pectoral Fin Denticle Density

Figure 5.26 shows a graphical representation of the densities of the dermal denticles on the left pectoral fin of adult male and female catsharks for all three seasons. There were both significant seasonal and sexual dimorphisms found in the denticle density on the left pectoral fins of adult male and female catsharks.

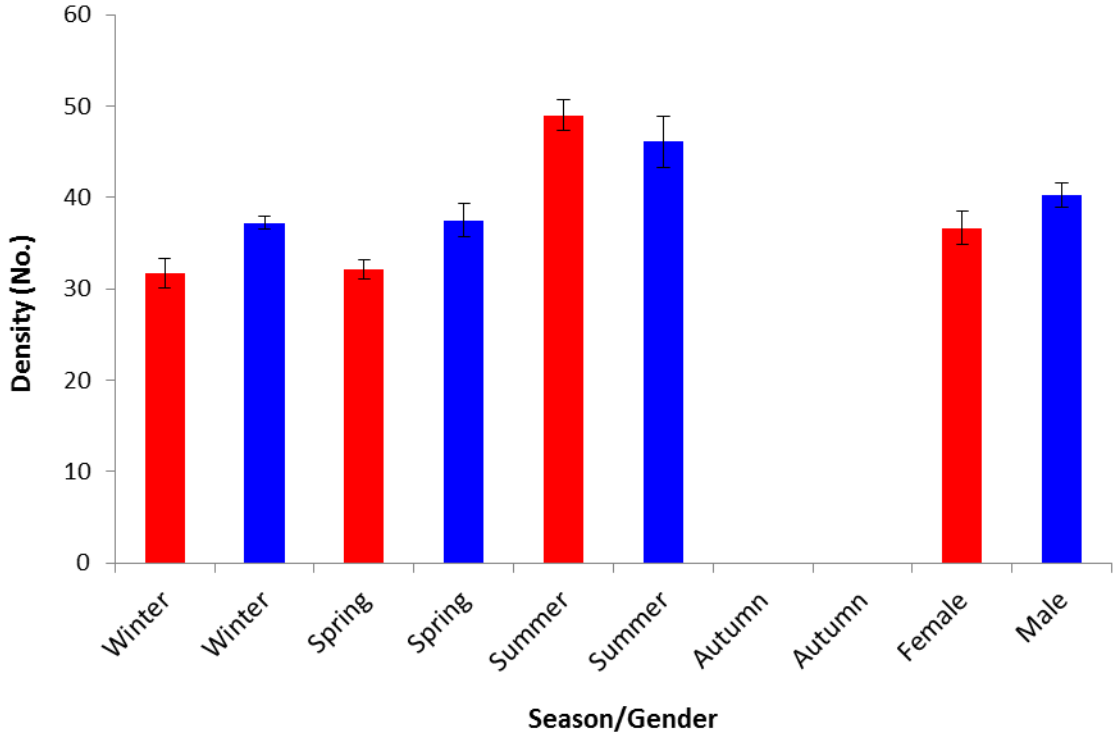

Figure 5.26. Gender and seasonal comparison of left pectoral fin denticle density per mm^2 for adult catsharks, showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

It can be seen from Table 5.25 that neither body length nor gender had an effect on the dermal denticle density of the left pectoral fin of adult catsharks. Season and gender within season had a significant effect, with adult male and females in summer possessing a higher density of dermal denticles than adult male and females sampled in winter and spring. Adult male catsharks sampled in winter and spring had a greater dermal denticle density on the left pectoral fin that adult females sampled in winter and spring.

Table 5.25. Results from the GLM analyses for the left pectoral fin denticle density of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	670.25	4.48	4.48	0.15	**0.700**
Gender	1	254.66	101.08	101.08	3.39	**0.072**
Season	2	1041.52	1130.36	565.18	18.95	**<0.001**
Season*Gender	2	200.89	200.89	100.45	3.37	**0.043**

The following tables show the differences between gender and season for the left fin dermal denticle density for adult male and female *S. canicula*. There were significant intra-gender differences between the dermal denticle density on the left pectoral fins of male catsharks ($P<0.05$). Males sampled in summer had a greater denticle density than males sampled in winter and spring (Table 5.26). There were significant intra-gender differences between the dermal denticle densities of the left pectoral fins of female catsharks ($P<0.05$). Females sampled in summer had a greater denticle density than females sampled in winter and spring (Table 5.26).

Table 5.26. *P*-values for intra-gender seasonal comparison of left pectoral fin denticle density per mm^2 for adult male and female catsharks (ND = No Data).

Season	Winter	Spring	Summer	Autumn	
Winter		1.000	0.046	ND	Male
Spring	1.000		0.041	ND	
Summer	<0.001	<0.001		ND	
Autumn	ND	ND	ND		
	Female				

There were significant differences between the dermal denticle densities of the left pectoral fins of male and female catsharks (P<0.05). Females sampled in summer had a greater denticle density than males sampled in winter and spring. Males sampled in summer had a greater denticle density than females sampled in winter and spring (Table 5.27).

Table 5.27. *P*-values for seasonal comparison of left pectoral fin denticle density per mm^2 for adult male and female catsharks (ND = No Data).

		Male			
		Winter	Spring	Summer	Autumn
Female	Winter	0.241	0.202	<0.001	ND
	Spring	0.323	0.268	<0.001	ND
	Summer	0.010	0.008	0.903	ND
	Autumn	ND	ND	ND	ND

5.3.9.3 Combined Pectoral Fin Denticle Density

Figure 5.27 shows a graphical representation of the combined densities of the dermal denticles on the right and left pectoral fins of adult male and female catsharks for all three seasons. There were both significant seasonal and sexual dimorphisms found in the combined denticle density on the pectoral fins of adult male and female catsharks.

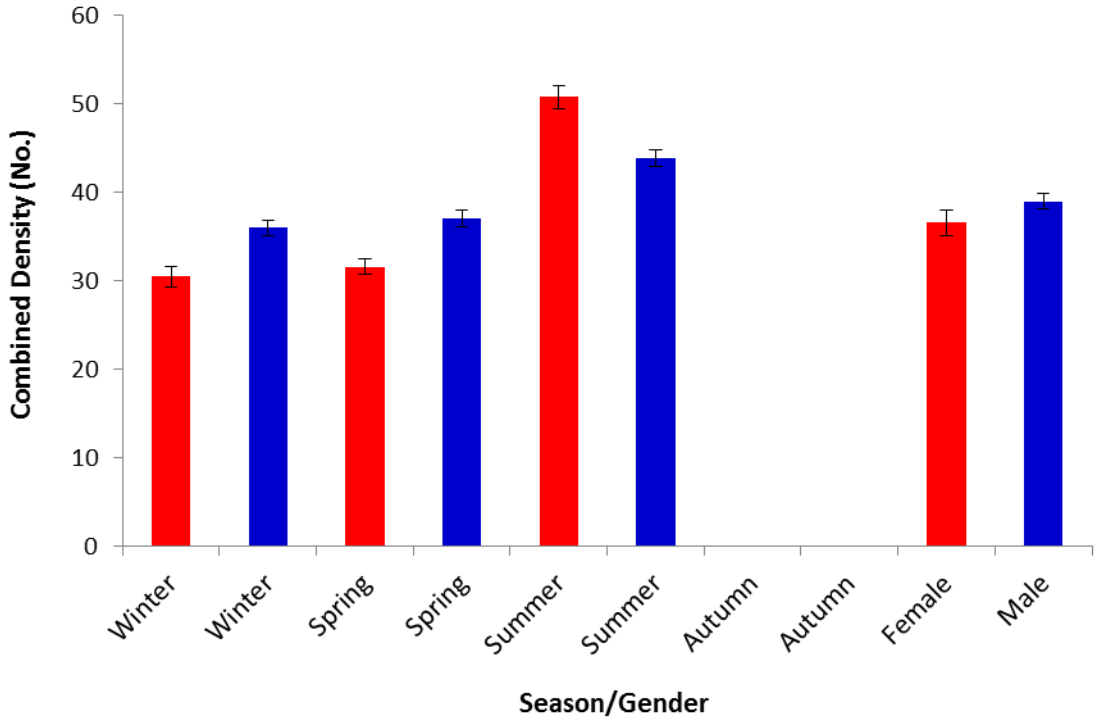

Figure 5.27. Gender and seasonal comparison of combined pectoral fin denticle density per mm^2 for adult catsharks, showing means and ± standard errors (n= Female (25) (W, 9) (Sp, 9) (Su, 7) Male (30) (W, 10) (Sp, 10) (Su, 10)) (Female = Red, Male = Blue).

Body length and gender had no effect on the combined denticle density for adult male and female catsharks (Table 5.28). Both season and gender within season were found to have a significant effect on the combined dermal denticle density of adult catsharks with adult catsharks sampled in summer having a higher density of dermal denticles than adult catsharks sampled in all other seasons. Adult male catsharks were also found to have a greater dermal denticle density than adult female catsharks in all seasons sampled except for summer (Table 5.28).

Table 5.28. Results from the GLM analyses for the combined denticle density of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	839.35	19.68	19.68	1.09	**0.301**
Gender	1	144.99	33.79	33.79	1.87	**0.177**
Season	2	1003.40	1140.31	570.16	31.62	**<0.001**
Season*Gender	2	441.15	441.15	220.57	12.23	**<0.001**

The intra-gender pectoral fin dermal denticle densities of adult male and female catsharks were found to be significantly different ($P<0.05$). Males sampled in summer had a greater denticle density than males sampled in winter (Table 5.29). Female catsharks sampled in summer had a greater dermal denticle density than female catsharks sampled in winter and spring (Table 5.29).

Table 5.29. *P*-values for intra-gender seasonal comparison of combined pectoral fin denticle density per mm^2 for adult male and female catsharks (ND = No Data).

Season	Winter	Spring	Summer	Autumn	
Winter		0.998	0.035	ND	Male
Spring	0.997		0.070	ND	
Summer	<0.001	<0.001		ND	
Autumn	ND	ND	ND		
	Female				

There were significant differences between the dermal denticle densities of the pectoral fins of male and female catsharks ($P<0.05$) (Table 5.30). Adult male catsharks sampled in

spring and summer had a greater dermal denticle density than adult female catsharks sampled in winter. Adult male catsharks sampled in summer had a greater dermal denticle density than females sampled in spring. Female catsharks sampled in summer had a greater denticle density than males sampled in winter, spring and summer (Table 5.30).

Table 5.30. *P*-values for seasonal comparison of combined pectoral fin denticle density per mm^2 for adult male and female catsharks (ND = No Data).

		Male			
		Winter	Spring	Summer	Autumn
Female	Winter	0.052	0.016	<0.001	ND
	Spring	0.165	0.586	<0.001	ND
	Summer	<0.001	<0.001	0.030	ND
	Autumn	ND	ND	ND	ND

Images of dermal denticle samples from the left fins of juvenile, sub-adult and mature specimens of *S. canicula* can be seen in Figure 5.28. There were no differences in the dermal denticle morphometrics of juvenile catsharks. Adult females were found to have wider and longer dermal denticles than adult male catsharks, whereas adult male catsharks were found to possess a greater dermal denticle density than adult female catsharks (Figure 5.28).

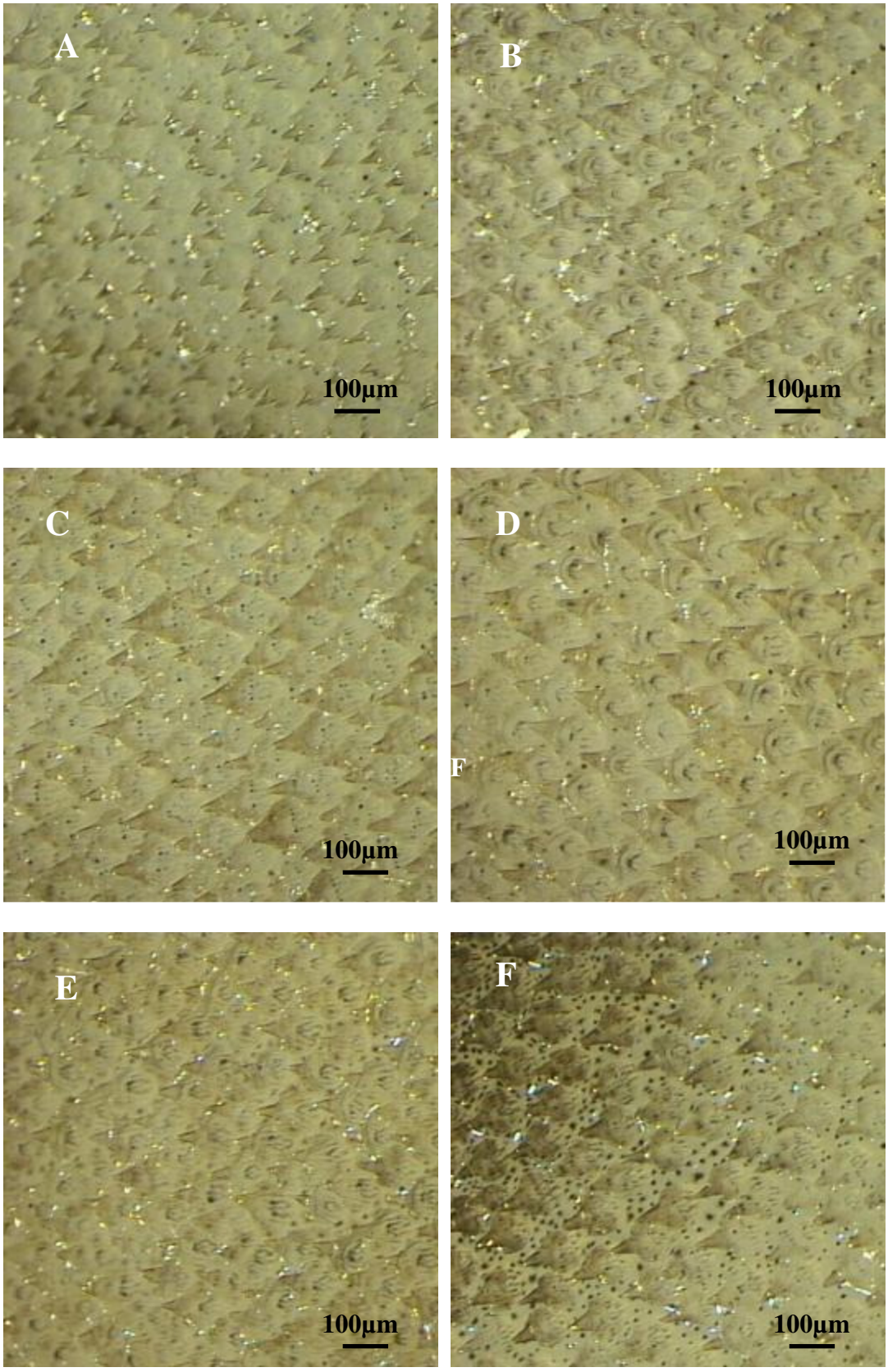

Figure 5.28 Left pectoral fin skin samples from (A) juvenile male (474mm TL) and (B) juvenile female (479mm TL) *S. canicula* sampled in winter, (C) presumed mature male (569mm TL) and (D) presumed mature female (566mm TL) *S. canicula* sampled in spring and (E) mature male (628mm TL) and (F) mature female (638mm TL) sampled in winter.

5.3.10 Adult Intra-Gender Pectoral Fin Lateralisation

Table 5.31 shows the intra-gender pectoral fin lateralisation comparisons of the denticle dimensions and density for the right and left pectoral fins of adult male catsharks. It can be seen from Table 5.31 that the denticles on the right pectoral fins of male catsharks were both significantly longer and wider than those on the left. The dermal denticles on the right pectoral fins were also found to be wider than those on the left pectoral fins of male adult catsharks. There were no significant differences between the densities of dermal denticles of the right and left pectoral fins of male adult catsharks.

Table 5.31. Intra-gender pectoral fin lateralisation for adult male *S. canicula*, showing the means, standard errors, range and *P*-Values (n= 30).

Gender	Feature	($\bar{x}$ ± SE) (Right fin)	($\bar{x}$ ± SE) (Left fin)	*P*-Value
Male	Denticle Length (µm) (Range)	441 ± 4.9 (366.4-525.7)	422 ± 5.2 (353.9-513.6)	**0.008**
Male	Denticle Width (µm) (Range)	293 ± 5.4 (241-387.5)	276 ± 4.1 (225.9-310.5)	**0.011**
Male	Density (per mm^2) (Range)	39.5±1.4 (28-64)	41±1.5 (30-61)	**0.228**

Table 5.32 shows the intra-gender pectoral fin lateralisation comparisons of the denticle dimensions and densities for the right and left pectoral fins of adult female catsharks. It can be seen from Table 5.32 that there were no significant differences in the length, width or densities of dermal denticles on the right or left pectoral fins of adult female *S. canicula*.

Table 5.32. Intra-gender pectoral fin lateralisation for adult female *S. canicula*, showing the means, standard errors, range and *P*-Values (n= 25).

Gender	Feature	($\bar{x}$ ± SE) (Right fin)	($\bar{x}$ ± SE) (Left fin)	*P*-Value
Female	Denticle Length (µm) (Range)	480 ± 13 (387.7-631.8)	466 ± 11 (365.6-551.6)	**0.157**
Female	Denticle Width (µm) (Range)	309 ± 7.0 (243.7-387.5)	302 ± 5.4 (250.2-345.5)	**0.392**
Female	Density (per mm^2) (Range)	36.4 ± 2.2 (23-57)	36.7 ± 1.8 (28-57)	**0.910**

5.3.11 Discriminant Analysis

Canonical discriminant analysis was carried out on the dermal denticles of adult *S. canicula*. The pooled data of dermal denticle width, dermal denticle length and dermal denticle densities for adult males and females were analysed separately to ascertain if there were any differences in the structure of the dermal denticles between adult male and female catsharks. Figure 5.29 shows the results for the adult male and female analysis. The canonical discriminant analysis showed significant differences between functions 1 and 3 (Wilks-Lambda, $P< 0.001$) but not between function 2–3 (Wilks-Lambda, $P> 0.05$) and not function 3 (Wilks-Lambda, $P> 0.05$). The first function accounts for 88.3% of the total variation with females sampled in winter showing the strongest correlation with this discriminating function. It can be seen from Figure 5.29 that there is a clear difference between females sampled in winter and spring and females sampled in summer. The same can be seen with adult male catsharks, with males sampled in winter and spring being distinctly different compared to the males sampled in summer.

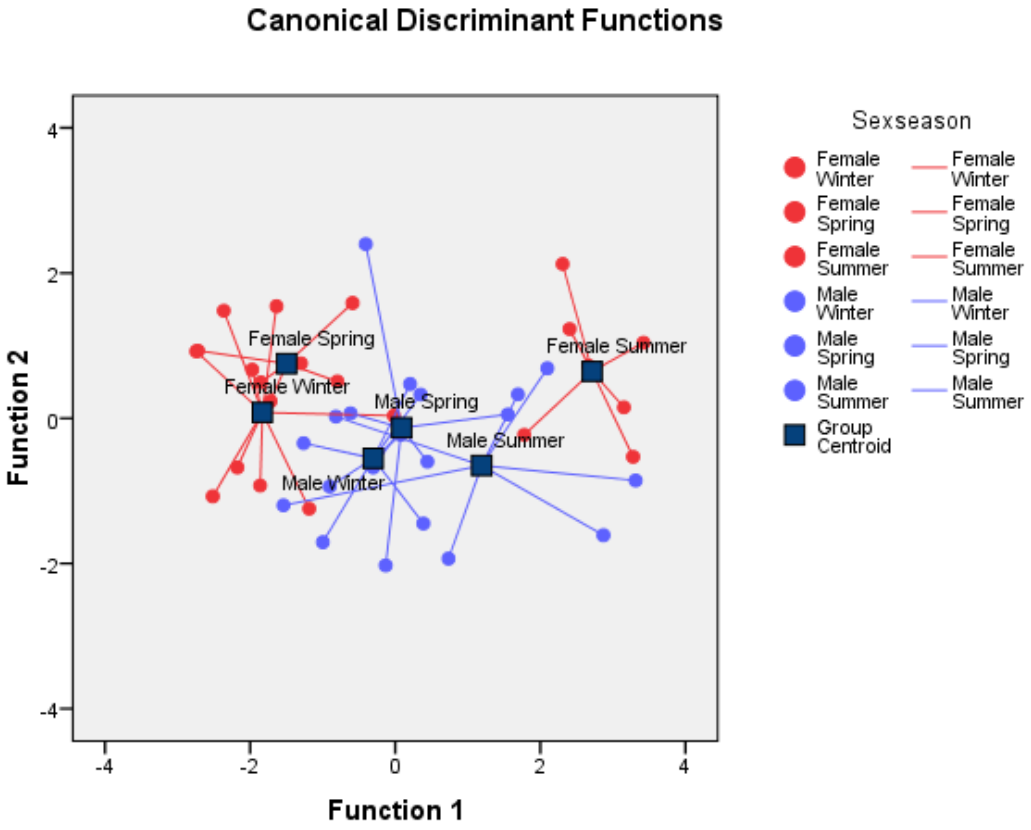

Figure 5.29. Discriminant analysis for the dermal denticles for adult *S. canicula.*

5.3.12 SEM

The dermal denticles were examined using SEM to determine whether the number of ridges and valleys differed between adult male and female catsharks.

Figure 5.30 Shows an SEM image of the dermal denticles of an adult male catshark

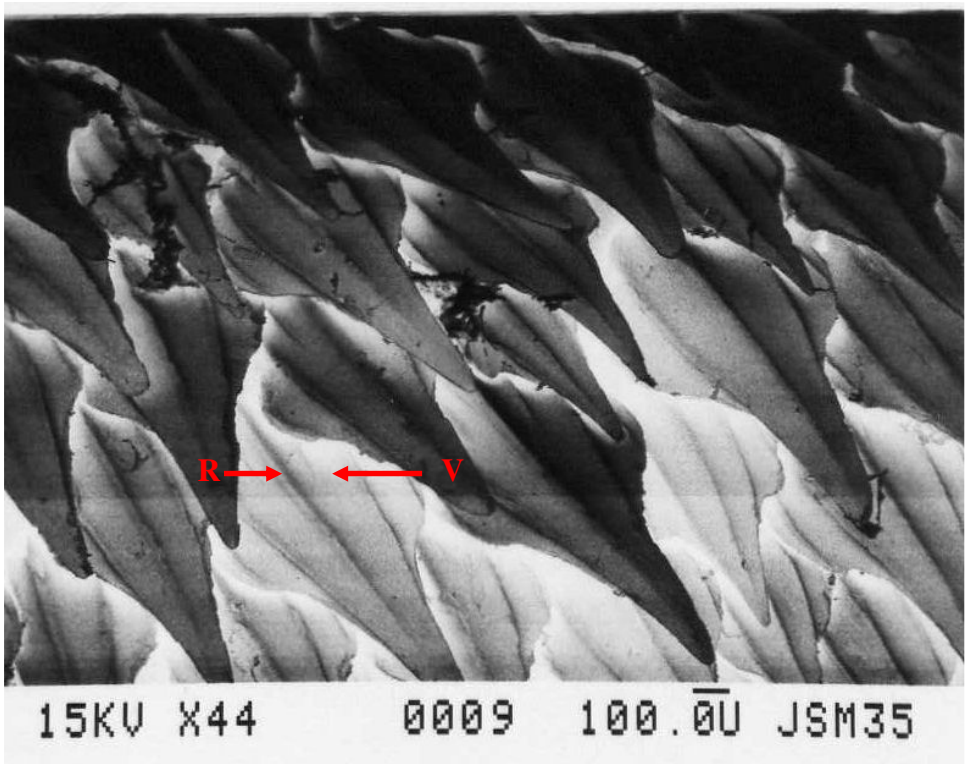

Figure 5.30. SEM of the dermal denticles of an adult male catshark showing a ridge (R) and valley (V).

Table 5.33 shows the numbers of ridges and valleys present on the dermal denticles of adult male and female *S. canicula*.

Table 5.33. The mean number of ridges and valleys on the dermal denticles of adult male and female *S. canicula* (n= F (6) M (6)).

	Male	Female
Ridges	**5 ± 0**	**5 ± 0**
Valleys	**4 ± 0**	**4 ± 0**

There was no sexual dimorphism found with regards to the numbers of ridges and valleys found on the dermal denticles of adult male and female dermal denticles. In every instance both male and female samples possessed 5 ridges and 4 valleys.

5.4 Discussion

There is a large amount of literature available on the skin of elasmobranch species, including work on the epidermal and dermal layers. As far as the author is aware there is no research investigating seasonal dimorphisms of the skin layers of the lesser-spotted catshark, *S. canicula*. Much of the literature that has focused on the dermal denticles is largely focused on the origins and evolution of these denticles (Ørvig, 1977; Schaeffer, 1977; Miyake *et al*., 1999; Reif, 2002; Sire and Huyesseune, 2003) or is a descriptive review on the characteristics of the dermal denticles (Nelson, 1970; Schofield and Burgess, 1997; Deynat, 1998; Yano *et al*., 1997; Azevedo *et al*., 2003; Baranes, 2003). Several authors have found that the shape, size and arrangement of the dermal denticles vary depending on where they are located on the body of individuals (Reif, 1978a; Reif, 1985; Raschi and Tabit, 1992; Deynat and Seret, 1996). To date little, if any, literature exists on the sexual or seasonal dimorphisms of the dermal denticles in elasmobranch species.

5.4.1 Hatchling Catsharks

The results of this study revealed that sexual dimorphisms exist in several elements of the integument of hatchling *S. canicula*. The results of the ANCOVA found that sexual dimorphisms were present in the epidermis of hatchling *S. canicula* with females possessing a thicker epidermis than males. There was no significant difference in the hatchling catsharks with regards to the thickness of the dermis.

Significant differences were also found with regard to the size and densities of dermal denticles on the fins of hatchling catsharks. This demonstrated that *S. canicula* potentially hatch with sexual dimorphisms that are not brought about by puberty. The fact that hatchling *S. canicula* emerge with numerous, well formed dermal denticles was

highlighted by Ford (1921). However, at this life stage individuals would not necessarily be primed for reproductive purposes and this has prompted several authors to investigate alternative uses for the denticles. A study by Southall and Sims (2003) investigated the use of dermal denticles in juvenile catsharks as a tool for feeding. They deduced that juvenile catsharks, aged between 3–7-months, possessed numerous, well defined dermal denticles in the lateral region. It was suggested by Southall and Sims (2003) that juvenile catsharks use the denticles to anchor prey to the seabed in order to secure the food prior to consumption. Grover (1974) suggested that in another elasmobranch species, the swell shark, *Cephaloscyllium ventriosum*, two rows of larger dermal denticles formed on developing embryos, which were referred to as enlarged juvenile denticles. According to Grover (1974) these denticles eventually disappeared as the hatchling grew. Ford (1921) also found a similar arrangement of denticles in hatchling *S. canicula*. It appeared that these denticles were used for emergence as these larger denticles did not appear on juveniles from live bearing sharks. It is believed that the presence of the large denticles allow the embryos of oviparous shark species easier movement within the egg cases in order for them to be able to hatch more easily (Grover, 1974; Southall and Sims, 2002). Apart from the presence of claspers in males, Grover (1974) found no other sexual dimorphisms in hatchling *C. ventriosum.*

The results from this study indicated that there are sexual dimorphisms in the denticle width and density of hatchling catsharks. Hatchling male *S. canicula* were found to have a higher density of denticles than females on the right fins, left fins and when the left and right fin denticle measurements were combined. Hatchling female catsharks possessed wider dermal denticles than male catsharks on the left fin and when the dermal denticle measurements for the right and left fins were combined. The fact that hatchling male catsharks were found to have a greater dermal denticle density on both fins than females indicated that the dermal denticles of males were smaller than those of females. It is

unclear whether the dimorphisms seen in the dermal denticles of hatchling catsharks is a preparatory state for reproductive purposes. Reif (1985) noted that the dermal denticles in many shark species become more numerous and do not necessarily increase in size. The reasons for this apparent sexual dimorphism in the dermal denticles of hatchling catsharks are unclear, as the same was not found for juvenile catsharks. However, the small samples sizes used for the juvenile study could have meant that any sexual dimorphisms were not detected.

There was no intra-gender dimorphism found in the dermal denticle morphometrics of hatchling catsharks.

5.4.2 Juvenile Catsharks

As with the hatchling catsharks sampled, the epidermis of juvenile catsharks was found to be sexually dimorphic with female catsharks possessing a thicker epidermal layer than male catsharks. This is consistent with findings from other researchers (Pratt, 1979; Kajiura *et al*., 2000) who found that skin thickness differed markedly in two species of elasmobranch. However, in both cases the research focused on adults and didn't consider whether skin thickness varied in juveniles. The initial indication from this study suggested that the skin of juvenile *S. canicula* could be sexually dimorphic. These findings, along with those in hatchling catsharks, suggest that the Solent population of *S. canicula* are born with and maintain a sexually dimorphic epidermal layer through to adulthood.

There were no sexual or seasonal dimorphisms present in the dermis of juvenile catsharks sampled in this study. Again, this conforms to findings in hatchling samples and may be due to the fact that the individuals sampled were not sexually mature and therefore do not require the level of protection as adults who are involved in reproductive processes.

There were no sexual dimorphisms found in the dermal denticles of the pectoral fins of juvenile *S. canicula*, contrary to findings in hatchling catsharks. One reason for these findings could be the fact that sample size of juvenile specimens used in this study was low and any interpretations made need to consider this fact. It is widely reported, however, that dermal denticles are shed throughout ontogeny (Markel and Laubier, 1969; Reif, 1978a; Reif, 1978c; Reif, 1985) and this could lend weight to the theory that the dermal denticles could play a vital role in the biting during copulation that is widely reported. As the juvenile catsharks are presumably sexually inactive it would appear that any protection from biting would be unnecessary. It remains unclear why there were sexual dimorphisms found in the dermal denticle morphometrics of hatchling catsharks but not juveniles, although again the small sample size could have affected the juvenile results.

There was very little in the way of intra-gender dimorphisms in the dermal denticles of male and female juvenile catsharks, with females having a higher density of denticles on the right pectoral fins than on the left. It is unclear why this may be the case, but as previously mentioned in many cases lateralisation does occur. The different behavioural strategies described by Southall and Sims (2003) could go some way to support these data, whereby females were found to inhabit rock crevices more often than males. This increased number of dermal denticles could indicate protection from abrasion from the rocky substrate. Catsharks appear to hatch with many sexually dimorphic characteristics in the integument and these dimorphisms disappear in juveniles and re-appear in adults. However, the reasons for this are unclear, but it is possible that the hatching and mating processes could be driving these changes.

5.4.3 Adult Catsharks

It is apparent from the data that there are a number of both sexual and seasonal dimorphisms in the integument of adult catsharks. In terms of the epidermal and dermal thicknesses it can be clearly seen that in all cases where a significant difference was found females possessed thicker skin layers than males. It could be seen from Figure 5.13 that the thickest epidermal layers occurred in females sampled in spring and only during the winter months was the male epidermis found to be thicker than females. With respect to the dermis, females were found to possess a thicker dermal layer than males during every season that they were sampled. These data compliment previous findings from other authors, such as Pratt (1974) and Kajiura *et al.* (2000) who found differences in the epidermal layers of blue shark, *Prionace glauca,* and the Atlantic stingray, *Dasyatis sabina*, respectively. In both cases females possessed a thicker epidermal layer than males. However, their research didn't consider any seasonal dimorphisms.

It is possible that some form of desquamation occurs in the skin of elasmobranchs, although no reference for this phenomenon has been found. It is known that elasmobranch fishes do replace their dermal denticles (Reif, 1985). This could help to support the theory that the epidermal thickness changes seasonally to coincide with the mating season in *S. canicula* as epithelial cells may be removed along with the dermal denticles. Reif (1985) stated that once the dermal denticles have calcified they cease to grow, but are shed after a certain time and replaced with new, larger denticles. Markel and Laubier (1969) stated that dermal denticles are shed, although this occurs less regularly than for the teeth. They didn't however state the rate at which the denticles were replaced. Kapoor and Khanna (2004) suggested that denticles were replaced when the collagen fibres that secure them retract and the denticle is lost, which could potentially remove epithelial cells along with them.

The dermal denticle data showed that there were significant differences in the size, shape and density of dermal denticles from the pectoral fins of adult *S. canicula*. In terms of length of dermal denticles, females sampled during spring had longer dermal denticles on the right fin than males sampled during summer. It could also be seen that adult females sampled in spring possessed the longest dermal denticles on the right pectoral fin than adult female specimens sampled in any other season. It is feasible that in much the same way fish show lateralisation in behaviour (Cantalupo *et al*., 1995; Bisazza *et al*., 1997) they could also show a lateralisation in the way they reproduce. If this is the case then a preference for adult male catsharks biting the right fins of adult female catsharks during copulation could result in the sexual dimorphism of the dermal denticles found here. Whitney *et al.* (2004) noted that in the whitetip reef shark the clasper used by each male (left or right) corresponded to the female pectoral fin that was grasped. For example, a male would use the left clasper when the female's left pectoral fin had been grasped. However, due to the low numbers of sightings of shark mating behaviours (Gilbert, 1981; Tricas and Le Feurve, 1985; Pratt and Carrier, 1985; Whitney *et al*., 2004; Domi *et al*., 2000; Cornish, 2005) it is difficult to ascertain whether sharks demonstrate a lateralisation in regard to clasper insertion and therefore pectoral fin biting.

There were no significant differences found in the width of the dermal denticles on the right fins of male or female catsharks, although females were found to possess wider denticles than males in all seasons except summer. On the left fin females were found to have wider denticles than males in all seasons. The combined data showed that in general females were found to possess both wider and longer denticles than males. In terms of dermal denticle densities on the fins there were differences between seasons and for gender within season. For the right pectoral fin adult males sampled in spring and summer were found to have longer dermal denticles than adult females sampled during winter and

spring. However, adult female catsharks sampled in summer had a higher density of dermal denticles than males and females sampled during all other seasons. For the left pectoral fin adult male catsharks had a higher density of dermal denticles than males and females in all seasons, except summer, when females had the highest density of dermal denticles than any group sampled. The surface structure of the dermal denticles was not found to be different between adult male and female catsharks, with both genders possessing five ridges and four valleys on each denticle examined.

For the intra-gender data significant differences were found in the width and length of the dermal denticles of adult male catsharks. The right fins of adult male catsharks possessed both longer and wider dermal denticles than the left fins. There were no significant differences found in the density of dermal denticles in adult males or any intra-gender parameter in adult females. It is not clear why the adult males showed this lateralisation in the right pectoral fin. It is possible that in the same way fish showed a bias to which direction they swam to avoid predation (Cantalupo *et al*., 1995; Bisazza *et al*., 1997) male catsharks favour a specific side for clasper insertion during copulation. This could mean that there is a dimorphism in the size of the denticles that are closer to the female during copulation in order to reduce the abrasive impacts on the males' skin when the male and female are coupled together.

Despite this apparent lateralisation in males, adult female catsharks were found to have larger dermal denticles than adult male catsharks. As would be expected, due to the larger dermal denticles on the pectoral fins of females, there were generally lower densities of denticles on the pectoral fins of females. It is possible that this difference in size is an adaptive response to male biting during copulation.

Raschi and Tabit (1992) suggested that the shape and size of dermal denticles vary depending on habitat preferences of different shark species. Reif (1974) found that in *Heterodontus* spp. the denticles change as habitat preference changes. In the case of *Heterodontus* spp. adult denticles grow larger to protect the skin from the rocky habitats that adults inhabit, as opposed to the muddy bottoms inhabited by juveniles. It is possible that is the case for *S. canicula*. It is well recognised that male and female *S. canicula* segregate by gender and work carried out by Sims *et al.* (2001) and Wearmouth and Sims (2008) showed that this is the case for the population inhabiting Lough Hyne in Ireland. Sims *et al.* (2001) showed that male and female *S. canicula* exhibited alternative behavioural strategies. Males were observed to be crepuscularly and nocturnally active, moving from deep (12–24 m) to shallower (<4 m) water to feed at dusk and during the night. Females refuged in shallow water (0.5–1.5 m) rock crevices and caves during daytime and were nocturnally active in deeper water only once every 2 or 3 days.

It could be this difference in behaviour and the very fact that females begin inhabiting different habitats that have caused the sexual dimorphisms in the denticles of *S. canicula*. According to Wearmouth and Sims (2008) comparatively little was known about its natural, free-ranging behaviour until relatively recently. However, this information relates to the population of *S. canicula* inhabiting Lough Hyne and is based on the behaviour of four individuals. Due to the variation in topography and exposure of other habitats within the habitable range of *S. canicula* it is difficult to surmise whether this behaviour is repeated within every population. As previously mentioned, local fishermen suggested that catches of *S. canicula* are segregated by gender, apart from during the spring when males and females are regularly caught in the same areas (Dr. Leanne Llewellyn, pers. comm.). It could be assumed that this difference in habitat is what is driving the sexual dimorphism in the dermal denticles seen here.

It was previously stated that many authors suggested that the dermal denticles of elasmobranchs have evolved as a protective mechanism. Raschi and Tabit (1992) noted that protection is one of the most widely proposed functions of dermal denticles. They noted that in many demersal species (that forage in rocky outcrops or coral reefs) individuals are often found with abrasions to the skin surface. It was also highlighted by Raschi and Tabit (1992) that many authors have suggested that dermal denticles were used as a defence mechanism against predators. However, Moss (1984) suggested that as most elasmobranchs are preyed upon mainly by larger elasmobranchs that dermal denticles may play only a minor role against predation.

Considering the results found in this study and the fact that there is clear evidence of a seasonal and sexual dimorphism in the dermal denticle size (whereby females possessed larger denticles but in lower densities in spring) may suggest that adult female *S. canicula* are using their dermal denticles as a protective measure against the biting action of males. The bite force applied to the pectoral fins of females during copulation would be far less than that used during a predatory attack. As previously discussed in chapter 3, Motta *et al.* (1997) and Motta and Wilga (1999) studied the bite forces of the nurse shark, *Ginglymostoma cirratum,* and the lemon shark, *Negaprion brevirostris*. They found that the forces created during a predatory attack were extremely powerful. Evidence from observed mating behaviours has shown that the bite of males on female pectoral fins during courtship and copulation serves the purpose of both initiating copulation and of gripping the female and holding her in position in readiness for the insertion of the clasper (West and Carter, 1990; Domi *et al*., 2000; Pratt and Carrier, 2001). It is unlikely that the bite forces applied during copulation mimic those during a predatory attack. It is possible, therefore, that the enlarged denticles could offer some form of protection during mating. This, combined with the increased thickness of the epidermis during spring could help in determining whether the Solent population of *S. canicula* has a specific mating season.

The results of the canonical discriminant analysis support the suggestion of a seasonal dimorphism in the dermal denticle density of *S. canicula*. The analysis showed that the dermal denticle densities of females in winter and spring were very closely matched, possibly indicating a change in denticle density in preparation for mating. The denticle densities of female catsharks in summer were very distinct when compared against the dermal denticle densities for adult female catsharks sampled in winter and spring. The same pattern can be seen in male catsharks with a clear distinction against males sampled in winter and spring and those sampled in summer. This again could lead to an indication of a specific mating season, especially if male catsharks are using their pectoral fins to anchor females to the seabed prior to copulation. However, with no autumn dermal denticle data available for either adult male or female catsharks it is not possible to make any definite conclusions as to whether there are any distinct seasonal differences.

In conclusion, the findings in this chapter show that not only does a sexual dimorphism exist in the skin (epidermis, dermis and dermal denticles) of hatchling, juvenile and adult catsharks, but that in some cases these dimorphisms are seasonal in nature. With more research with larger sample sizes and using various populations of *S. canicula* these data could help to determine whether the Solent population of *S. canicula* has a defined mating season.

Chapter 6 – Ampullae of Lorenzini

6.1 Introduction

6.1.1 Elasmobranch Senses

Elasmobranchs are widely reported to possess a large array of extremely acute senses, which are used for hunting, predator avoidance and possibly the location of conspecifics for mating purposes. Sharks are highly evolved and possess well-developed brains and associated sensory systems. The combination of these senses enables remarkable acuity of orientation (Bres, 1993). There is a distance hierarchy of senses within many shark species and these vary according to distance and environmental conditions. Olfaction, hearing and vision are believed to operate over greater distances, whereas mechanosensory and electrosensory systems are relatively short range (Montgomery and Walker, 2001).

Traditionally elasmobranchs were believed to possess poorly developed visual systems, mainly due to the assumption that the eyes had only rod-like retinae and were therefore specialised for dim light (scotopic) (Hart *et al*., 2004). Recent research, however, has now shown that elasmobranchs have great visual acuity with capabilities for both day and night vision. The shark eye has a structure close to that of the standard vertebrate eye, allowing a greater range of vision than previously thought. A study by Fouts and Nelson (1999) investigated vision in the Pacific angel shark, *Squatina californica*. The research revealed that visually-sensed prey movement was possibly the most important cue for eliciting daytime attacks on prey items, whereas night time attacks were possibly instigated through the sighting of turbulence-mediated bioluminescence.

The auditory sense in elasmobranchs is also well utilised, often in the location of prey items. According to Hodgson (1978) attraction of sharks by sound is commonly exploited

by indigenous peoples in the Southwestern Pacific. Many species of Pacific and Atlantic sharks have been found to be attracted to pulsed, low-frequency sounds (Nelson and Gruber, 1963; Nelson and Johnson, 1972). The first audiogram for any elasmobranch species was created by Kritzler and Wood (1961). This was carried out on the bull shark, *Carcharhinus leucas*. The results indicated that bull sharks detected acoustical frequencies between 100Hz to 1500Hz and showed an optimal sensitivity between 400 and 600 Hz. Work by Nelson and Gruber (1963) found that free-ranging sharks, such as lemon and bull sharks, were attracted to low frequency underwater sounds similar to the sound frequencies caused by the struggling actions of speared fish. It is these sounds that are re-created by fishers in their attempts to attract sharks to boats. Nelson and Gruber (1963) found that the sharks were most attracted to irregular, rapid and pulsing sounds with frequencies below 60Hz. They discovered that high frequency sounds, bands 400-600Hz, were far less of an attractant and continuous sound attracted no sharks. The findings indicated that elasmobranchs use their auditory sense widely in prey detection, but it is unclear whether this sense is utilised in the detection of conspecifics.

Olfaction has been shown to be a very important sense in many elasmobranch species. Historically, studies on the role of olfaction in elasmobranchs have focused on predation and prey location (Sheldon; 1909; Parker; 1914; Tester, 1963; Hodgeson and Mathewson, 1971; Silver, 1979; Johnson and Teeter, 1985; Zeiske *et al*., 1986). However, laboratory and field studies indicated that elasmobranchs may communicate with the use of reproductive (olfactory-mediated) pheromones (Johnson and Nelson, 1979; Klimley, 1980; Gordon, 1993; Houziaux and Voss, 1997). Possible olfaction-mediated pair formation in two species of carcharhinid sharks, the blackfin reef shark, *Carcharhinus melanopterus* and the reef white-tip shark, *Triaenodon obesus* was reported by Johnson and Nelson (1978). They reported close-following behaviour whereby the female swam with the tail raised up with the male orientating to the posterior part of the females body. Similar

behaviours were described by Klimley (1980) in the nurse shark, *Ginglymostoma cirratum*. Klimley (1980) described this behaviour as "parallel swimming" and these observations were made during acts of courtship and copulation. Other authors provide further evidence of the use of pheromones for olfactory-mediated cues in elasmobranchs when mating behaviours have been observed. These include mating in the captive sandtiger sharks, *Carcharias taurus* (Gordon, 1993) and captive *S. canicula* (Houziaux and Voss, 1997; Domi *et al*., 2000). Llewellyn (2008) found that certain aspects of the olfactory system of *S. canicula* were sexually dimorphic. The numbers and dimensions of the olfactory lamellae were found to be greater in mature male specimens compared to females. A sexual dimorphism was also found in the density of olfactory receptor cells, with males possessing greater densities than females, further evidence of olfaction being used in mating behaviours in *S. canicula*.

6.1.2 Electroreception – The Ampullae of Lorenzini

Despite being fairly common place amongst animals, the electric sense is not only one of the most recently discovered animal senses, but it is one of the last senses to be fully understood (von der Emde, 1998). According to Collin and Whitehead (2004) the electric sense is a complex and specialised sense found in a large range of aquatic vertebrates. Although it is believed that the electric sense emerged with the earliest vertebrates, its real purpose was discovered only a few decades ago (Heiligenberg, 1991). von der Emde (1998) stated that the anatomical features now known to be electroreceptor organs have been known for a long time, but their correct function was not recognised. This is evidenced by Murray (1961) who reported that the biological function of the Ampullae of Lorenzini (AoL) of elasmobranch fish remained uncertain at that point. Murray (1961) went on to note that considerable evidence existed concerning the different types of stimuli capable of eliciting responses in the sensory nerves of shark species, although there was

little hard evidence. Many theories have been put forward as to the purpose of electroreceptive organs in elasmobranch species. Sand (1938) noted that the AoL are sensitive to small changes in temperature and that a rise in temperature of 0.1°C could be detected. Murray (1957) suggested that the AoL are depth receptors, sensitive to hydrostatic pressure. Research by Murray (1957) noted that a response to mechanical stimuli does in fact occur, but only to those stimuli which increase or reduce the pressure within the ampullae themselves relative to the pressure outside.

Work carried out by Kalmijn (1971) and Kalmijn (1974) determined that the electroreceptive organs of elasmobranch species, the AoL, were able to detect minute electrical fields in the environment created from both animate and inanimate objects. Kalmijn (1971) noted that a suspicion of the electrosensitivity of elasmobranch fishes dates back to 1935. It appeared that Dijkgraaf (Unpublished) (Cited in Kalmijn, 1971) who at that time was working with *S. canicula*, showed that the catshark was sensitive to a rusty steel wire placed in seawater. It wasn't until the 1960s, however, that this theory was proven by Dijkgraaf and Kalmijn (1962) who repeated these experiments and described the sharks as showing escape reactions to the wire, despite having been blindfolded. The ability of sharks to use the AoL to detect electrical fields was reported by Collin and Whitehead (2004). They found that electroreceptors are primarily designed for the detection of a weak bioelectric field. Brown (2003) also reported that the AoL serve as acute electrosensors for sharks. Many studies have now focused on the thresholds of detection of the AoL. Studies by Bromm *et al.* (1976) on the sensitivity of the AoL to electrical current found that the lowest threshold current for a single ampulla was approximately 0.01nA at temperatures of between 13°C and 19°C. They noted that the threshold currents increased with lower (7°C) and higher (25°C) temperatures by a factor of approximately ten. Araneda and Bennett (1993) discovered that marine elasmobranchs are extraordinarily sensitive to voltage, responding reliably to gradients of <1μV/m(1).

von der Emde (1998) suggested that because electroreception needs a conductive medium it is always associated with aquatic organisms. He went on to add that many marine and freshwater fishes, with the important exception of most teleosts, are electroreceptive. The peripheral component of the elasmobranch electroreceptive system has been studied in over 150 species (Raschi *et al.,* 2001). Research suggested that the structure of the AoL is common to most elasmobranch species, where the initial structure of the AoL begins with pores visible on the surface of the skin which then open into canals that lead to the sensory ampullae (Wueringer, *et al*., 2009) (Figure 6.1).

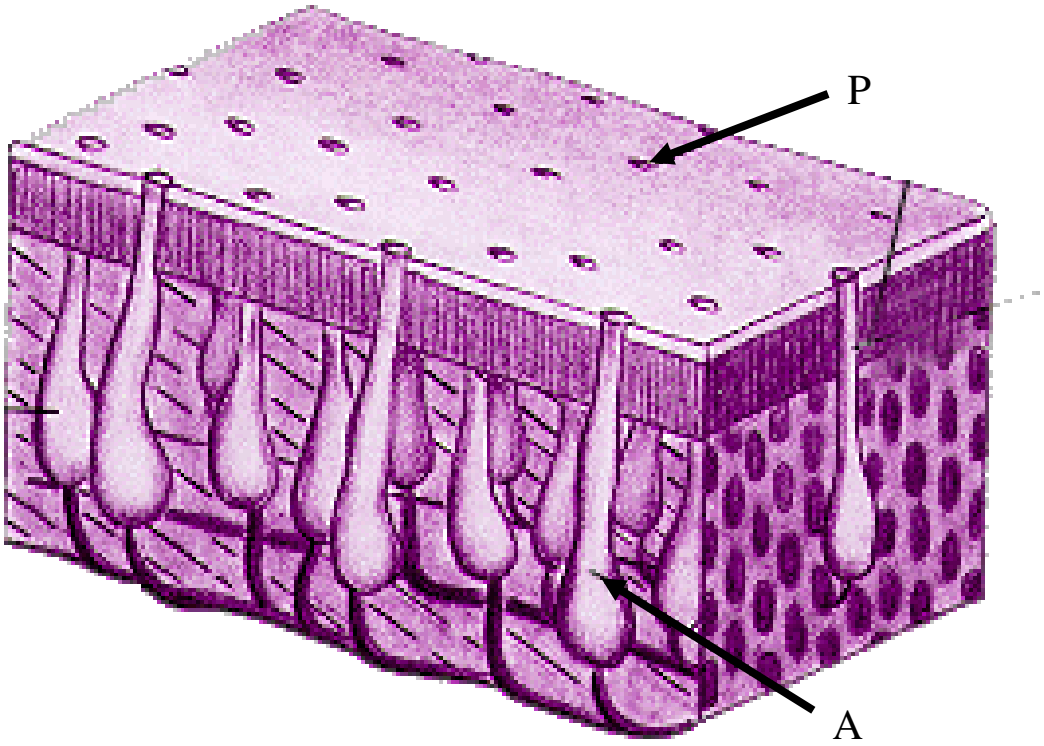

Figure 6.1. Schematic of the skin of a shark showing the AoL pore (P) and the ampulla (A) http://www.seaworld.org/infobooks/Sharks&Rays/images/ampullae.gif.

The make-up and structure of the AoL differs between species. The length of the ampullary canals ranges from 5 to 20 cm in marine elasmobranch species (Brown, 2002) and the number of alveoli varies between species (Wueringer *et al*., 2009). The pore pattern and distribution has also been found to vary considerably. Mello (2009) found that

the identification of hammerhead sharks using the patterns of AoL (together with the head shape) provided a means to correctly identify the species where only cephalofoils were available. It has been suggested that the lateral expansion of the head of hammerhead sharks affords a greater area for electroreceptor organs (the AoL) and therefore enhanced the electroreception capacity, enabling greater efficiency of prey detection and capture (Lim *et al.,* 2010).

6.1.3 Structure of the Ampullae of Lorenzini

Sisneros and Tricas (2002a) reported that the structure of the AoL in marine species comprised of an ampulla and a long, subdermal canal that projects to a single pore on the surface (Figure 6.2).

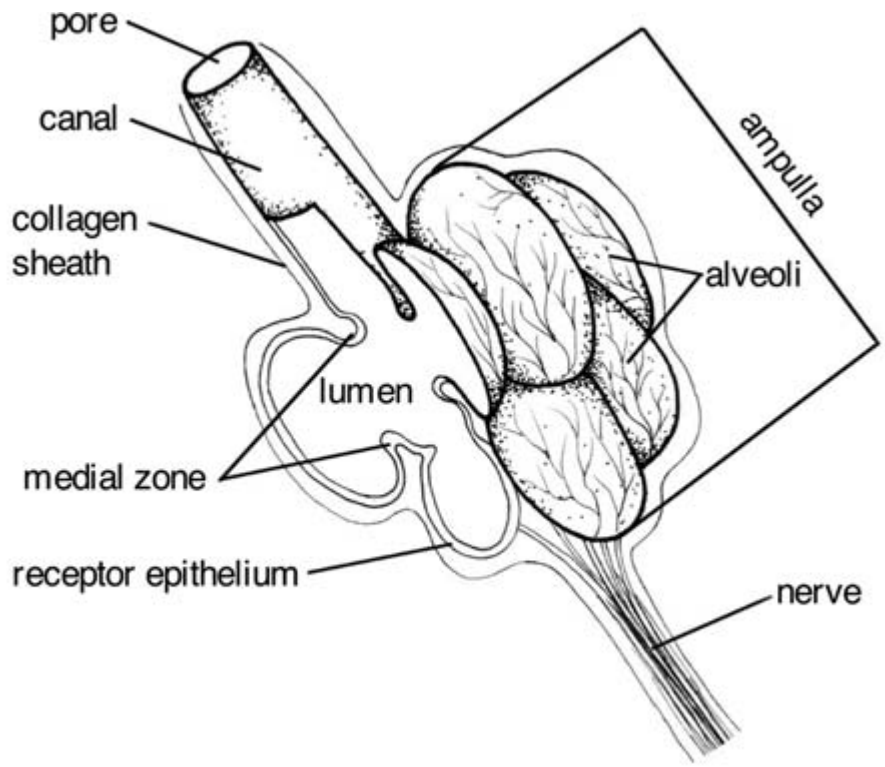

Figure 6.2. Schematic of a stylised Ampullae of Lorenzini (Adapted from Wueringer *et al*., 2009).

The lumen of the ampullary chamber is filled with mucopolysaccharide jelly that forms the electrical core and is a conductive material (Figure 6.3) (Sisneros and Tricas, 2002a). The ampullae are grouped into clusters by envelopes of connective tissue (Norris, 1929; Jorgensen, 2005). In marine elasmobranchs many individual ampullae are grouped into discrete, bilateral cephalic clusters from which project the subdermal canals that radiate in many different directions to terminate at individual skin pores on the head of sharks and rays (Hueter *et al*., 1994).

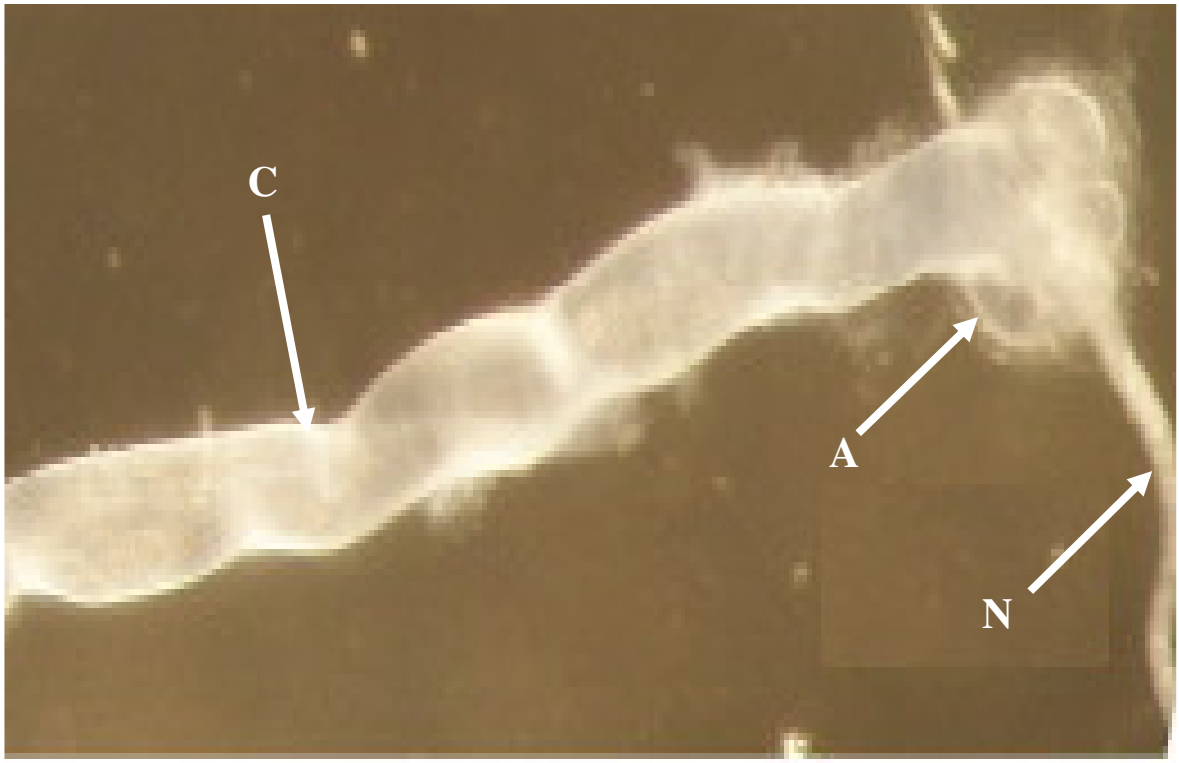

Figure 6.3. An excised AoL showing the gel filled canal (C) ampulla (A) and nerve (N) (Adapted from Fields *et al.* (2007).

The internal structure of the Aol and the associated cells have been well described. The wall of the ampulla is composed of a single layer of sensory epithelium that contains hundreds of sensory and receptor cells (Sisneros and Tricas, 2002a). Hueter *et al*. (2004) described each alveolus as containing hundreds of sensory hair cell receptors and support cells exposed to the internal lumen of the ampulla chamber. The canal wall is lined with large hillock-shaped cells that apparently secrete copious amounts of a high-potassium, mucopolysaccharide gel that fills the ampullary canal (Figure 6.4) (Whitehead, 2002). The canal consists of a double layer of connective tissue fibres and squamous epithelial cells (Figure 6.4) that are tightly joined together to form a highly electrical resistance between the outer and inner canal wall (Whitehead, 2002; Hueter *et al*., 2004).

The junction between the canal and the alveolar sacs the hillock cells terminates and the wall of the ampulla then consists of cuboidal epithelial cells (Figure 6.4) (Whitehead, 2002). These cells abut with the sensory epithelium of the alveoli, which comprises

numerous receptor and supportive cells (Whitehead, 2002) (Figure 6.4). The pear-shaped receptor cells possess a central nucleus and a single kinocilium extends into the ampullary lumen. Supportive cells produce an uneven interior surface to the alveolar sacs (Whitehead, 2002).

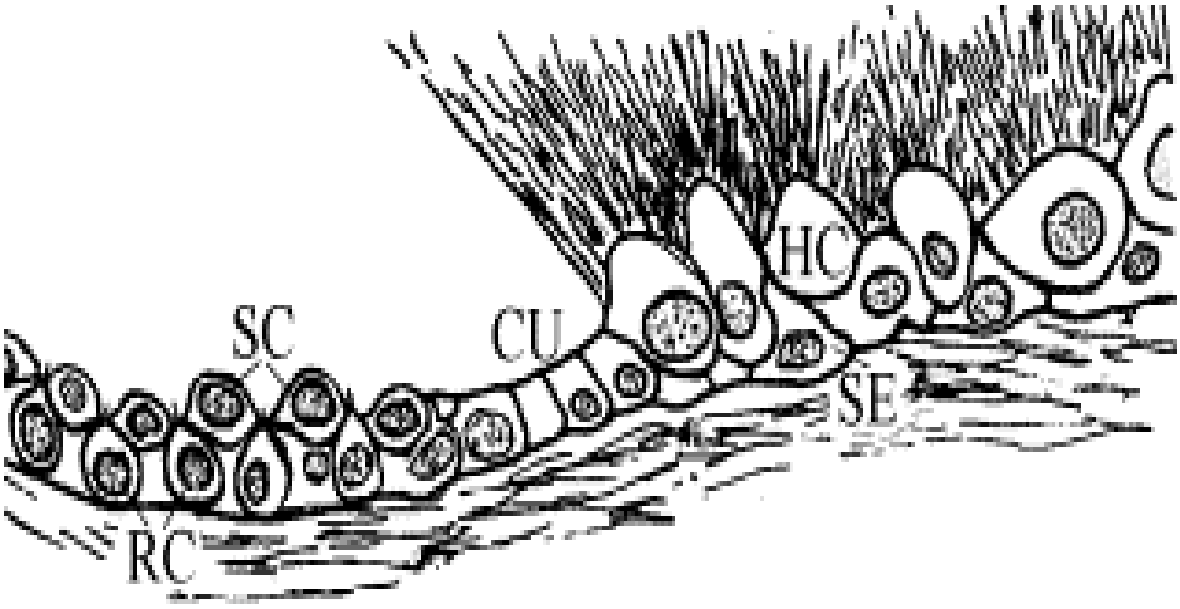

Figure 6.4. Longitudinal illustration of the cells of an AoL showing the hillock-shaped cells (HC) squamous epithelial cells (SE) cuboidal epithelial cells (CU) receptor (RC) and supportive cells (SC) (Adapted from Whitehead, 2002).

6.1.4 Function of the Ampullae of Lorenzini

The distribution and function of the AoL in *S. canicula,* has been investigated by Al-Zahaby *et al.* (1996) and they noted that the AoL are located around the head region of sharks and the disc margins in rays. Al-Zahaby *et al.* (1996) performed a histological study of the ampullae and found that there were a large number of mitochondrial, as well as sensory and sustentacular cells. von der Emde (1997) also found that the sensory cells and sustentacular cells of the AoL in many other elasmobranch species are occupied by large numbers of mitochondria and vesicles and are also covered by flattened epithelial cells. From such findings it became widely accepted that the AoL act as both thermo- and

electroreceptors, which is in stark contrast to earlier assumptions that the AoL functioned purely as mechanoreceptors (Parker, 1909) (Figure 6.5).

Figure 6.5. Ventral surface of the head of *S. canicula* showing AoL distribution. (http://seaexplorers.net76.net/SHARK%20LAB/Ampullae%20of%20Lorenzini.JPG).

Despite this detailed understanding of the structure of the AoL, the function is only recently being discovered. According to Raschi *et al*. (2001) little work has been carried out on the ecomorphological role played by the AoL. As previously stated, the ampullary organs of elasmobranch fishes are now known to be important in detecting bioelectric stimuli (Kalmijn, 1971).

Many authors suggested that the ampullary organs are also important in the ability of elasmobranchs to navigate using the earth's magnetic field (Kalmijn, 1971; Raschi, 2001; Kajiura, 2001). Further research has shown that far from having a single purpose the electroreceptors are, in fact, multifunctional.

It was discovered by Kalmijn (1971) that electroreception and the AoL function in a predatory role. Experiments were carried out on *S. canicula*, setting a number of plaice in differing environments. Kalmijn (1971) noted that when the plaice were buried under the sand they were detected by the catsharks from a distance of approximately 15cm. Plaice were then hidden in an agar chamber that allowed the electrical impulses, given off by the plaice, to pass through, but did not allow any other visual or chemical stimuli. It appeared that the catsharks, upon passing the agar chamber, showed the same clear feeding response through well-aimed turns toward their prey. The ability of *S. canicula* to detect prey hidden in the substrate was further investigated by Filer *et al.* (2008). They investigated the ability of *S. canicula* to detect electric fields under different types of substrate. It was found that detection rates decreased over pebbles and rocks compared with sand and the control (no substratum). Filer *et al.* (2008) then presented electrical fields beneath different depths of sand to examine the depth-limits of fish electroreception. They found that turn and bite rates were significantly lower at depths below 10 mm, with no bites towards electrodes made when the depth was greater than 30 mm.

The discovery that the AoL assist prey detection is supported by recent studies showing that sharks can detect the electrical impulses given off by live prey as low as five billionths of a volt per centimetre (Tricas and Sisneros, 2004). Clark (1981) claimed that the dusky smoothound, *Mustelus canis,* had the best electrical acuity of any animal and can detect an electrical field 25 million times weaker than that detected by any human being.

Fishelson and Baranes (1998) found a direct link between the AoL and prey detection is that the densities of the ampullary organs in some species of skate are directly proportional to the average density of prey. Kimber *et al.* (2009) noted that male and female catsharks demonstrated differing levels of response when exposed to electrode activity, with female catsharks showing a greater response to the electrode than males despite similar foraging

behaviours. It was also noted that in the presence of the opposite sex the foraging behaviour of both genders was reduced, indicating differing reproductive strategies aided by the AoL.

Kajiura and Fitzgerald (2009) noted that despite a great deal of research being carried out on the function of the AoL the majority of research on elasmobranch electroreception has focused on how it is employed in prey detection. However, Sisneros and Tricas (2002a) suggested an alternative use for electroreception, stating that the electrosense of elasmobranch species is important during courtship and reproduction. They noted that in non-electrogenic stingrays the electric sense was used during reproduction and courtship for conspecifics detection and localisation. Their study, however, focused on the electrogenic ray, *Urobatis halleri*. The study concluded that the electrosense of electrogenic rays is used for communication during social and reproductive interactions and that both male and female stingrays used electrosense to detect and locate conspecifics during the mating season. Sisneros and Tricas (2002a) discovered that male and female *U. halleri* used their electric sense in different ways. It appeared that male stingrays used their electric sense to detect and locate conspecific females, presumably for the purposes of reproduction. In contrast to this, females used electroreception to either locate and join other non-buried receptive females to attract a mate, or joined buried less-receptive females for refuge. The conclusions drawn by Sisneros and Tricas (2002a) showed that the ampullary electrosense in the natural behaviour of sharks and rays can be classified into four major categories. They list these as being the detection of prey, mates, predators and competitors.

It is possible that if elasmobranch species are using electroreception for mate location as suggested by Sisneros and Tricas (2002a) then the AoL of *S. canicula*, a non-electrogenic elasmobranch, could be sexually dimorphic. If male *S. canicula* are actively using their

electric sense to detect females for reproductive purposes then it is feasible that the structure of the AoL in male catsharks may vary from that of female catsharks. As far as the author is aware the presence of a sexual dimorphism in the structure of the AoL has not been studied before in any elasmobranch species

Therefore, the aims of this study are:

1. To determine if there is any sexual dimorphism in the epithelial thickness of the AoL of adult *S. canicula*.
2. To determine if there is any sexual dimorphism in the epithelial cell count of adult *S. canicula*.
3. To determine if there is any sexual dimorphism in the number of alveoli in the ampullae of adult *S. canicula*.

6.2 Materials and Methods

The head of each catshark was removed from the unbuffered 10% formalin in seawater and placed in 4 one-hour washes of distilled water. The tip of the snout was removed, anterior of the nares, to expose the ampullae (Figure 6.6). The ampullae were located from a region between the front of the cartilaginous olfactory capsule and the snout. Six ampullae were removed from the left hand side of each catshark and stored in unbuffered 10% formalin in seawater.

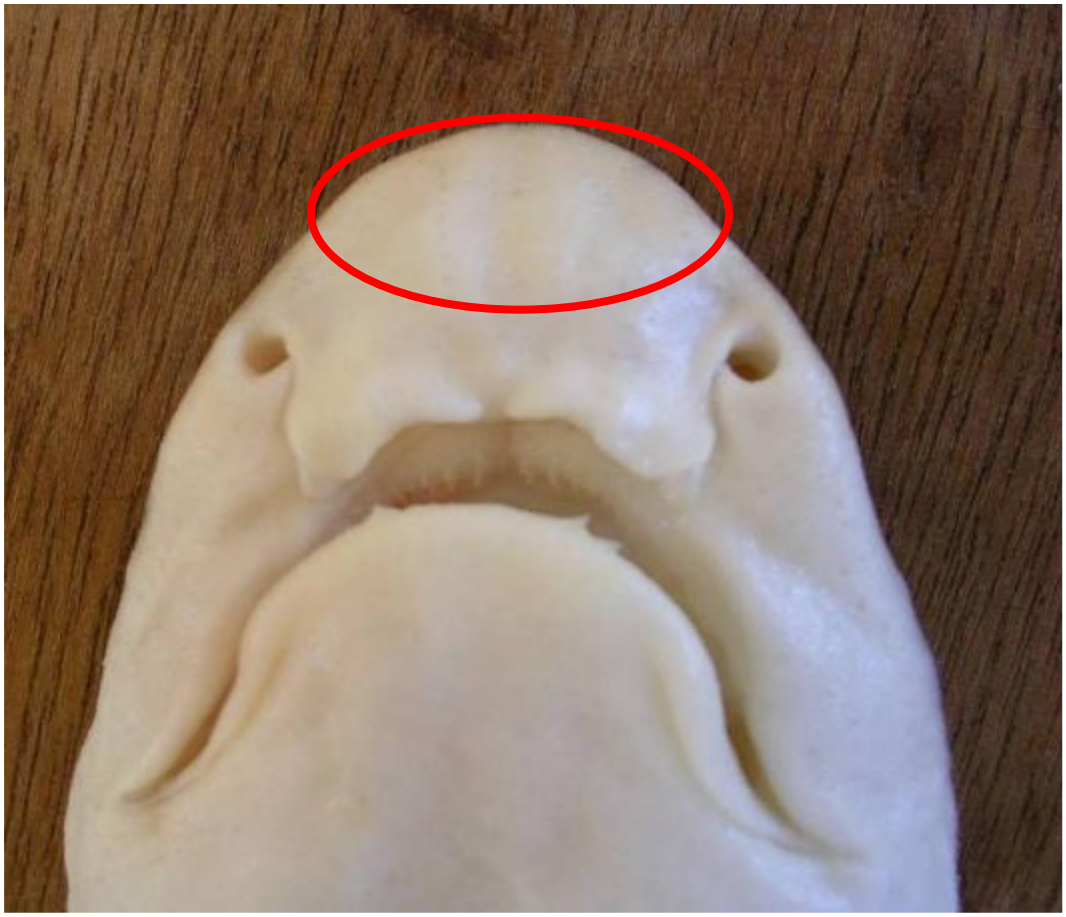

Figure 6.6. Showing the section of snout removed in order to expose the encapsulated ampullary organs (Photographed by author).

6.2.1 Histology

When the ampullae were ready for sampling they were removed from the formalin and were placed in two 45-minute washes of distilled water. Each ampulla was dipped in haematoxylin for 5 seconds to lightly stain the mucous layer, allowing easy location through the cryo-m-bed during sectioning. They were then mounted onto a cork disc (RA Lamb Medical Supplies, Eastbourne, UK).

A base layer of cryo-m-bed was first applied to the cork and was frozen to –51°C using freeze-it spray (RS components, Corby). The ampullae were placed on top of the base layer of frozen cryo-m-bed and another layer of cryo-m-bed was applied. The top layer was again frozen to –51°C using the freeze-it spray. The cork was then mounted onto a cryostat

chuck by applying a thin layer of cryo-m-bed to the outer edge of the chuck, placing the cork onto the chuck and spraying freeze-it spray around the edge of the chuck and cork.

The ampullae were sectioned with the use of a cryostat (Bright Instruments, model OTF) housing a freezing microtome (Bright Instruments, model 5040). The chuck was placed into the cryostat chamber and allowed to warm up to -22°C. The ampullae were cut either transversely (Figure 6.7) or longitudinally (Figure 6.8) to a thickness of 10μm and mounted onto Poly-L-Lysine microscope slides. The rapid H&E staining method was employed. For the ampullae, sections were exposed to the haematoxylin for one minute. The ampullae were washed in acid alcohol for 3 seconds and then stained with eosin for 30 seconds. The slides were rinsed in two washes of distilled water to remove any excess stain. They were then passed through 90% ethanol for 5 seconds and two washes of absolute ethanol for 5 seconds. They were finally placed into xylene and cover slips were applied with the use of DPX.

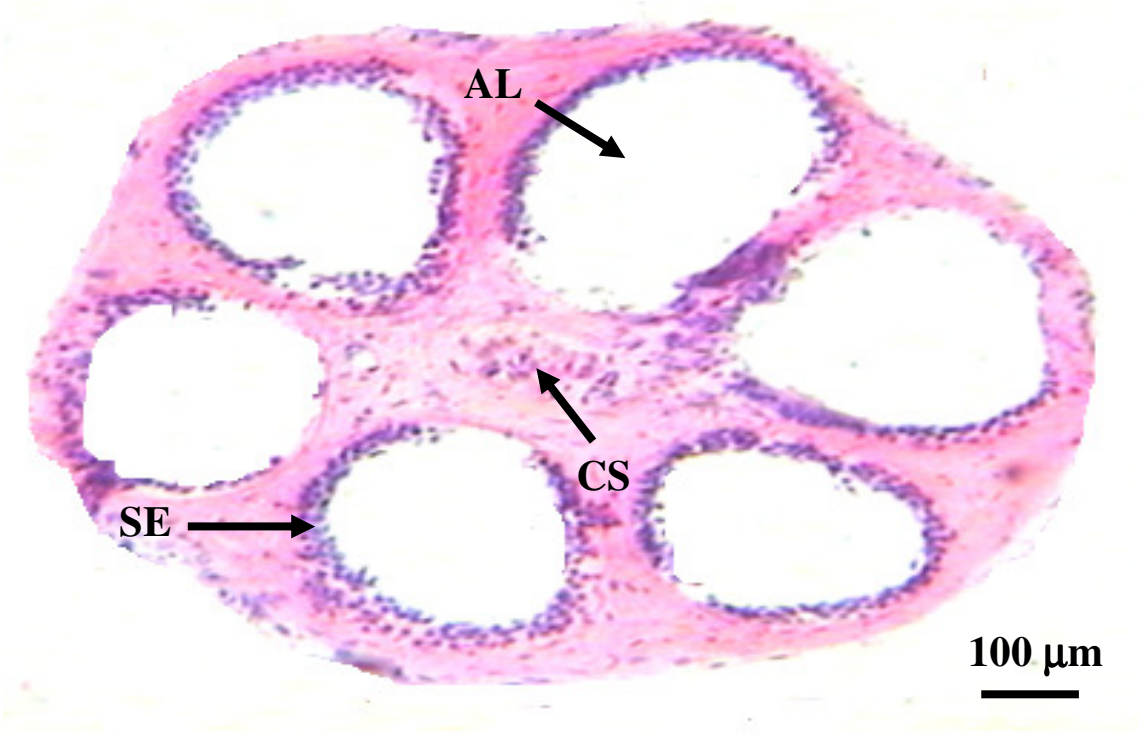

Figure 6.7. A transverse section of the AoL of an adult female catshark showing the sensory epithelium (SE) and the alveoli (AL) and central stage (CS) (Photographed by author).

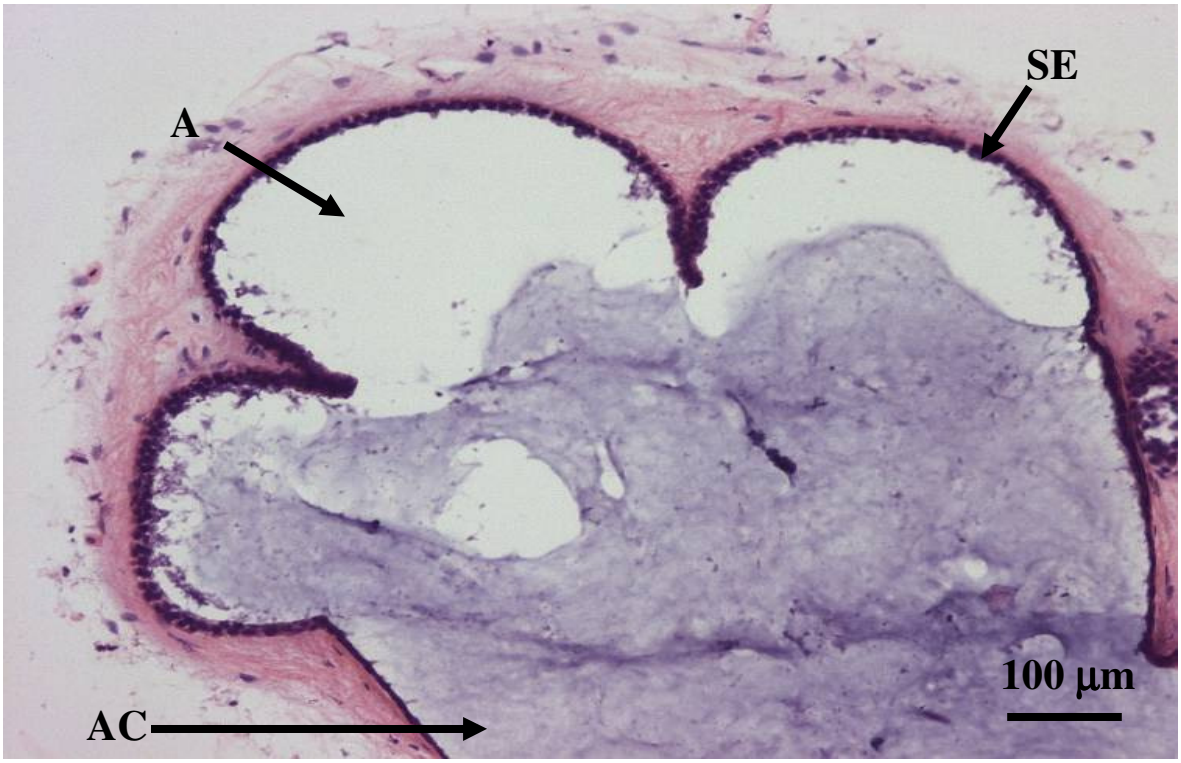

Figure 6.8. A longitudinal section of an ampulla showing the ampullary canal (AC) alveoli (A) and sensory epithelium (SE) from an AoL in a mature male catshark (Photographed by author).

The slides were photographed using a Leitz Dialux 22EB optical microscope at x40 magnification and a JVC TK-C1381 colour video camera. Measurements were taken using UTHSCSA imaging tool.

The epithelial thickness of each ampulla was recorded in three places around the bulb at the base of the ampullae. The number of sensory cells identified within an area of 100µm were counted and compared for males and females. An ANCOVA, with body length as a covariate, was performed on the data collected to determine the existence of a sexual dimorphism in the epithelial thickness, sensory cell density and alveoli numbers of *S. canicula*.

6.2.2 SEM

Scanning Electron Microscopy (SEM) was performed on the AoL of the catsharks. A method adapted from Dingerkaus and Kostler (1986) was used to remove mucous and debris from the denticles prior to preparation. Samples were ultrasonicated for 15 mins and air dried before being prepared for SEM. After drying, samples were fixed in 4% gluteraldehyde in a 0.2M sodium cacodylate seawater fixative solution (pH 7.4) for one hour (Cragg and Nott 1977). The skin samples were then osmicated in 4% osmium tetroxide (OsO_4) in 0.1M Sodium Phosphate Buffer (pH 7.4) with a volume sufficient to cover the samples. The samples were then left for 60 minutes or until they turned black.

Samples were rinsed in buffer wash at least twice more following post-fixation to remove any remaining osmium before being further dehydrated. The samples were taken through a dehydration series consisting of 30 minute washes of 50%, 60%, 70%, 80%, 90% and 100% ethanol solutions. The samples were then placed in a 50/50 mix of 100% ethanol and acetone, followed by a 30-minute wash in 100% acetone.

6.2.3 SEM Mounting

Once dehydrated, samples were transferred onto aluminium stubs. Samples were affixed to the stub by use of sticky carbon tabs which served both to attach specimens and provided good conductivity for SEM imaging. The samples were then DC-sputter coated with a gold/palladium mix for 2 ½ minutes. Samples were then observed in a JEOL JSM-65C SEM at 15KV x44 magnification.

6.2.4 Confocal Laser Scanning Microscopy

The AoL were placed into glass vials for fixation with 4% paraformaldehyde with 0.55% glutaraldehyde in 0.2M phosphate buffered saline solution (PBS). Fixed ampullae were placed on glass slides and excess fluid drawn off with strips of filter paper. Slides were then desiccated in the dark, overnight, before being mounted in glycerol and observed on a Carl Zeiss LSM 510 confocal laser scanning microscope with AxioCam HRc camera using a 488nm long pass barrier and 355-425 nm excitation.

6.3 Results

The AoL were analysed to determine whether there were any sexual dimorphisms in the epithelial thickness, the density of epithelial cells and the number of alveoli in each terminal bud. Due to the seasonal distribution of the individuals sampled and the complexity of accurately orientating, and therefore, cutting the ampullae it was not possible to perform a seasonal analysis of the structure of the AoL. An ANCOVA was used to determine any inter-gender differences. For the analysis of the AoL only adult specimens were used (i.e. males $\geq$ 525mm and females $\geq$ 550mm). A Grubbs test for outliers was performed on the data (Grubbs, 1969) as per Attrill *et al*. (2007) in order to

ascertain the presence of any outliers. The test revealed that no outliers were present in any of the data.

6.3.1 Epithelial Thickness

The means and standard errors for the epithelial thickness of the AoL in adult male and female catsharks (F, 13.26 ± 1.01µm (Range = 7.86 - 21.32µm)) (M, 10.71 ± 0.98µm (Range = 6.23 – 18.86)) (n= M (13) F (16)) showed that females generally had a thicker epithelial thickness than males. However, the statistical analyses showed that no significant differences existed between males and females.

The results from the ANCOVA for the epithelial thickness of the AoL in adult catsharks can be seen in Table 6.1. Neither body length nor gender had any significant effect on epithelial thickness of the AoL in adult catsharks.

Table 6.1. Results from the ANCOVA analyses for the epithelial thickness of the AoL of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	3.03	0.05	0.05	0.00	0.956
Gender	1	43.50	43.50	43.50	2.86	0.103

6.3.2 Epithelial Cell Density

The means and standard errors for the epithelial cell density in adult male and female catsharks were almost identical (F, 12.37 ± 0.38 per 100µm (Range = 9 – 16 per 100µm)) (M, 12.11 ± 0.25 per 100µm (Range 11-14 per 100µm)) (n= M (13) F (16)).

The results from the ANCOVA for the density of epithelial cells in the AoL of adult catsharks can be seen in Table 6.2. Neither body length nor gender had a significant effect on epithelial cell density of the AoL in adult catsharks.

Table 6.2. Results from the ANCOVA analyses for the epithelial cell density of the AoL of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.043	0.000	0.000	0.00	**0.993**
Gender	1	0.421	0.421	0.421	0.25	**0.624**

6.3.3 Alveoli Number

The means and standard errors for the number of alveoli in the ampulla of adult male and female catsharks (M, 7.2 ± 0.1 (Range 6-8)) (F, 6.1 ± 0.2 (Range 6-7)) (n= M (13) F (16)) showed that adult male catsharks were found to possess a greater number of alveoli in the ampulla than adult female catsharks.

The results from the ANCOVA for the number of alveoli in the ampulla of adult catsharks can be seen in Table 6.3. Body length had no effect on the number of alveoli in the ampulla of adult catsharks. Gender did have an effect on the number of alveoli in the ampulla of adult catsharks, with adult male catsharks possessing significantly more alveoli in the ampulla than adult female catsharks.

Table 6.3. Results from the ANCOVA analyses for the number of alveoli in the ampulla of adult male and female *S. canicula*.

	DF	Seq SS	Adj SS	Adj MS	F	***P*-Value**
Body Length	1	0.1447	0.0134	0.0134	0.03	0.856
Gender	1	1.7247	1.7247	1.7247	4.32	0.048

Adult male catsharks were found to have a significantly greater number of alveoli in the AoL than adult female catsharks. Adult male catsharks were found to have an average of 7 alveoli, whilst adult females were found to have an average of 6 (Figure 6.9).

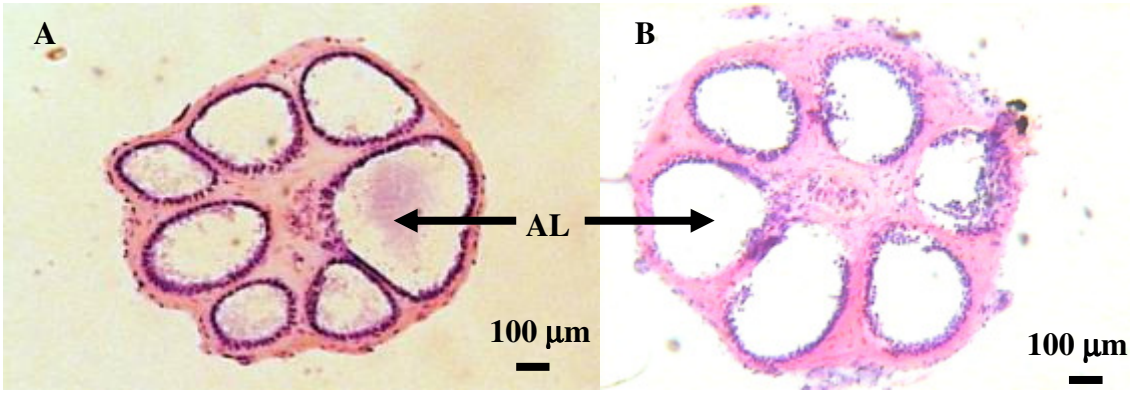

Figure 6.9. A transverse section of the AoL of an adult male catshark (A) and an adult female catshark (B) showing the alveoli (AL) (Photographed by author).

6.3.4 SEM

SEM was performed on the AoL of adult male and female *S. canicula* in order to determine the number of alveoli present in the ampullae. Figure 6.10 shows an SEM image of an AoL from an adult male catshark. It became clear, however, that the procedure wasn't suitable for the AoL due the collagen sheath that surrounds the ampulla making observation of the individual alveoli impossible.

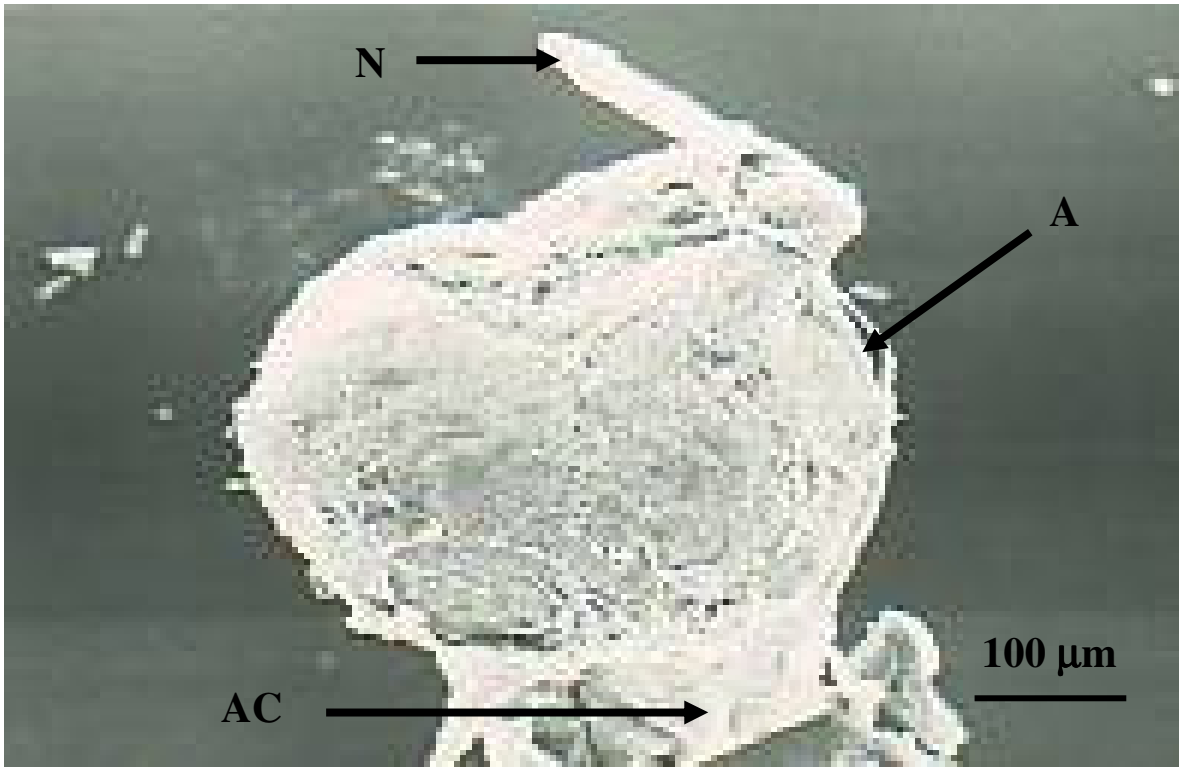

Figure 6.10. SEM image of an AoL from an adult male catshark showing the ampullary canal (AC) Ampulla (A) and the Nerve (N) (Photographed by author).

An attempt was made to dissect the tip of the ampulla as demonstrated by Whitehead (2002) (Figure 6.11). However, the ampullae used in this study were much smaller than those used by Whitehead (2002) from *C. leucas*. The cutting action crushed the ampulla and destroyed the structure of the alveoli, preventing the internal structure from being clearly viewed. An attempt was also made to remove the lower part of the ampullae just above the terminal point of the ampullary canal. This had a similar effect on the AoL causing it to be crushed when cut.

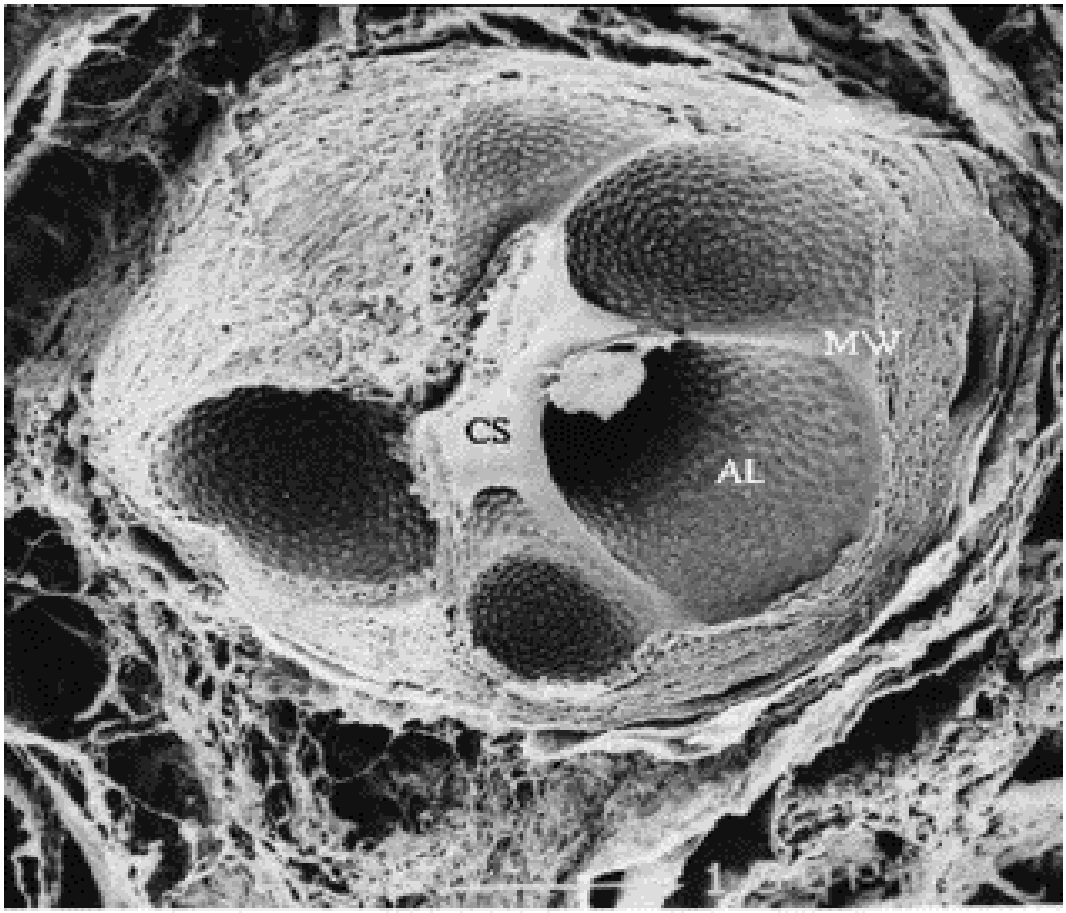

Figure 6.11. SEM image of a transversely cut AoL from the bull shark (*Carcharhinus leucas*) showing the alveoli (AL) central stage (CS) and the medial walls (MW). (Adapted from Whitehead, 2002).

6.3.5 Confocal Laser Scanning Microscopy

In response to limitations presented by the SEM, laser scanning confocal microscopy was performed on the AoL of adult male and female *S. canicula* in order to determine the number of alveoli present in the ampullae. Figure 6.12 shows an image from the confocal microscope of an AoL from an adult female catshark. This form of microscopy clearly showed the ampulla and the individual alveoli, but due to the focal range of the microscope it was not possible to gain a bird's eye view of the ampulla. It was therefore not possible to ascertain from this technique the number of alveoli present in each ampulla.

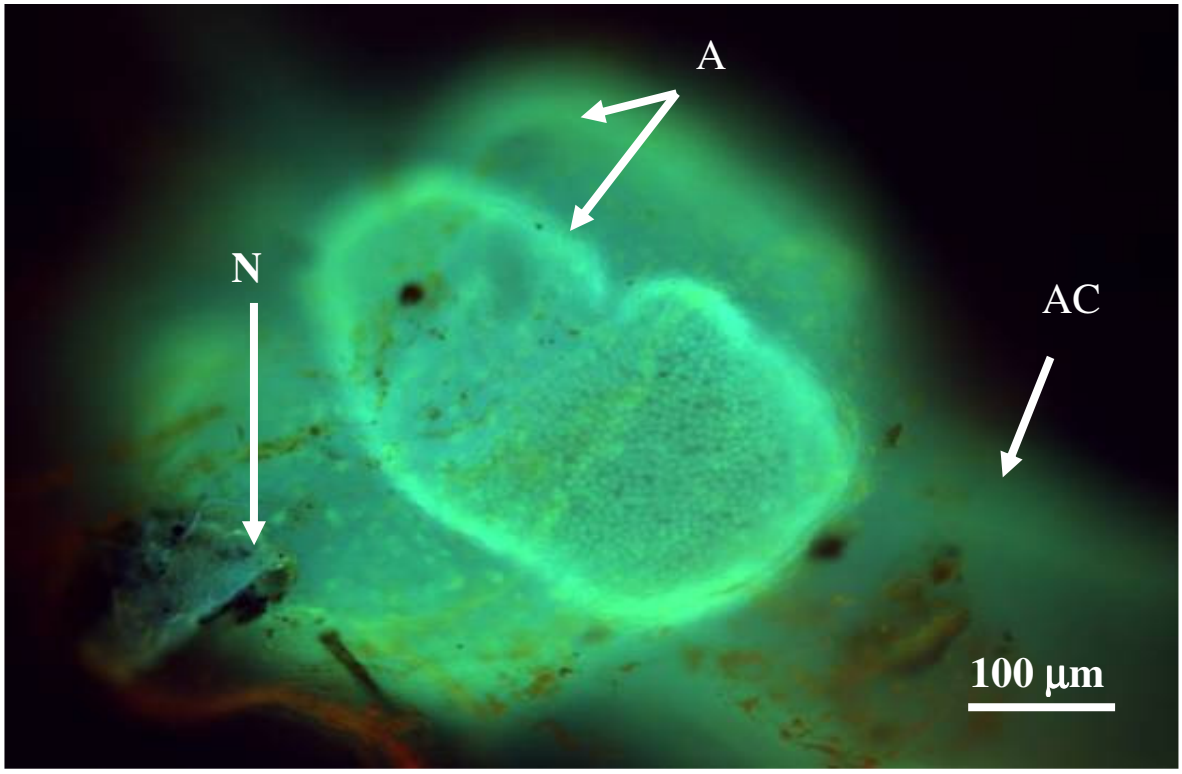

Figure 6.12. Confocal image of an AoL of a mature female catshark, showing the alveoli (A) ampullary canal (AC) and nerve (N) (Photographed by author).

6.4 Discussion

There is a large amount of literature available on the AoL of elasmobranch species. Much of the literature that has focused on the AoL is largely centered on the form and function of these organs. Several authors have found that the shape, size and arrangement of the AoL varied depending on species (Brown, 2002; Mello, 2009; Lim *et al*., 2010). To date little, if any, literature exists on the sexual dimorphisms of the AoL in elasmobranch species. As far as the author is aware there is no research investigating sexual dimorphisms of the AoL for the lesser-spotted catshark, *S. canicula,* or any other species of elasmobranch.

6.4.1 SEM

The results obtained for the SEM proved to be inconclusive. An attempt was made to obtain a bird's eye view of the ampulla and ascertain a count of the alveoli from the

images. The SEM images obtained were marred by the presence of a collagen sheath that surrounds the ampullary canal, ampulla and nerve. An attempt was made to remove the ampullary canal and expose the internal structure of the ampulla. However, the lumen of the canal was too small to reveal the greater extent of structure of the alveoli and when cut higher the structure of the AoL collapsed. An attempt was also made to replicate work by Whitehead (2002) and remove the tip of the ampullae. This resulted in destruction of the ampullary structure, further reducing the ability to view the individual alveoli. Whitehead (2002) carried out the work on *C. leucas*, a much larger species than *S. canicula*. The use of this much larger species would have made dissection of the ampullary structures much more feasible, and therefore ascertaining the alveoli number much easier.

6.4.2 Confocal Laser Scanning Microscopy

The use of confocal microscopy eliminated the problems associated with penetrating the collagen that sheathed the ampulla. However, this technique proved to be limiting due to the fact that the focal depth of the microscope was not great enough to allow a bird's eye view of the ampulla. The alveoli were clearly visible, but a quantitative measurement of the alveoli numbers could not be made. An attempt was made to rotate the ampullae to count the number of alveoli, but this proved inaccurate and resulted in an unreliable data set.

6.4.3 Epithelial Morphology

The results of this study revealed that there was no sexual dimorphism in the epithelial structure of the AoL of adult catsharks from the Solent. Neither epithelial thickness nor epithelial cell density were found to be significantly different.

The structure of the sensory epithelium of the AoL should be examined in relation to the sensory cells when considering the existence of a sexual dimorphism. Sisneros and Tricas (2002b) found that the wall of the ampullary canal is composed of two layers of flattened epithelial cells and is highly resistive. Hundreds of electroreceptor cells are grouped together at the base of a single epidermal pit with only the small (approximately 1%) apical portions of their membranes protruding into the lumen (von der Emde, 1998). It is the highly sensitive surface of these sensory receptors that protrude into the lumen of the ampulla that cause the highly efficient electrosense to exist (Hueter *et al*., 1994; von der Emde, 1998).

In light of the findings by Al-Zahaby *et al.* (1996) the fact that the ampullae posses a large number of mitochondrial, as well as sensory and sustentacular cells demonstrated that these cells are highly physiologically active and it is possible that an increase in the number of cells could demonstrate an increased ability to detect electrical impulses. This increased sensitivity to electrical fields would be especially beneficial in the detection of conspecifics for mating purposes, as proposed by Sisneros and Tricas (2002a). This would be especially true in an environment that is often murky and sometimes devoid of light. The fact that there was no sexual dimorphism found in the thickness or cell density of the epithelium of the AoL could indicate that adult male and female *S. canicula* are using electroreception equally in order to locate conspecifics.

6.4.4 Alveoli Number

It was apparent from the data that a sexual dimorphism exists in relation to the number of alveoli in the ampullae of adult *S. canicula*. Adult male catsharks were found to have a higher number of alveoli compared to adult female catsharks. Males possessed on average seven alveoli, whilst females were found to possess an average of six. Research on *C.*

leucas carried out by Whitehead (2002) revealed the presence of six alveoli in the ampullae. Wueringer *et al.* (2009) found that the number of alveoli varied between species and that in the eastern shovelnose ray, *Aptychotrema rostrata*, the ampulla contained an average of six alveolar bulbs. Raschi (1986) examined 40 different rajid species and found that the AoL contained between 8.3 to 20.5 alveoli per ampulla. This is in contrast to the alveoli of the bigeye hound shark, *Iago omanensis*, which was found to possess between seven to nine alveoli per ampulla (Fishelson and Baranes, 1998). Raschi (1986) suggested that the size of each ampullae and the number of alveoli associated with it are directly related to the depth occupied by a specific species. He proposed that shallow-water species have smaller ampullae with fewer alveoli than species that live below 1000m. This implies that deeper dwelling species may rely more on electric sense due to the reduced visual input and low light conditions associated with deeper water.

Despite there being no significant difference in the epithelial structures of the AoL in *S. canicula*, a sexual dimorphism in the alveoli number could indicate that the AoL do in fact play an important role in the location of mates in this species. With an increase in the number of alveoli there would be an increase in the surface area of the ampullae and therefore an increase in the number of receptor cells. This would potentially provide an increase in the electrosensory capacity of adult male *S. canicula*. These findings mimic those of Llewellyn (2008) who found that the density of receptor cells in the olfactory organ of *S. canicula* were sexually dimorphic. The findings revealed that adult male catsharks possessed a greater density of receptor cells in the olfactory organs than adult female catsharks. Llewellyn (2008) suggested that the increase in olfactory receptor cell densities in male catsharks could be linked to reproductive behaviour by males who use the increased number of receptor cells to detect female pheromones. The receptor cells of males may be sensitive to any pheromone compounds released by females and the increased densities may lead to the quicker, more localised, detection of females, by

enhancing the gradient searching capacity of males. This pattern could also be occurring in the AoL of *S. canicula*, whereby the increased density of receptor cells allows a more acute detection of females by male catsharks for mating.

However, the life habits of *S. canicula* should not be overlooked when considering the use and function of the senses, especially the AoL. Raschi (1986) found a direct link to the AoL and prey detection and stated that the densities of the ampullary organs in some species of skate are inversley proportional to the average density of prey. However, this does not take into account any different feeding strategies observed between male and female elasmobranchs and it has been made clear by the research of several authors that prey selection and feeding habits do not differ between male and female elasmobranchs (McEachran, 1977) or indeed catsharks (Lyle, 1983).

Research carried out by Sims *et al.* (2001) revealed that adult male and female *S. canicula* exhibited varying behavioural strategies. Their research showed that sexual segregation occured in *S. canicula*, with males inhabiting depths of between 12 and 20 m and females that refuged in rock crevices and caves at depths of between 0.5 and 1.5 m. This sexual segregation could also be an important factor in the development of the AoL as the electrosense has been closely linked with predator avoidance (Sisneros and Tricas, 2002b). If male and female catsharks are sexually segregating by depth then it is feasible that male catsharks would encounter a differing array of predators. Sims *et al.* (2001) noted that unlike males, female catsharks did not refuge under rocks and caves, but remained lying on the gravel substrate. Sims *et al.* (2001) also discounted the theory that sexual segregation exposed adult catsharks to predation, stating that adult catsharks had few predators in Lough Hyne. However, the population studied by Sims *et al.* (2001) is an isolated one and therefore predator avoidance could be a key part of the sexual dimorphism exhibited in the AoL of *S. canicula* from the Solent. Sims *et al.* (2001) concluded that sexual segregation in

S. canicula is possibly a result of reproductive behaviour, lending support to theory that the sexual dimorphism found in the AoL in adult catsharks from the Solent could be used for mate location by males. Despite not being electrogenic, it is possible that *S. canicula* can locate conspecifics from the electrical output specific to that species. It is clear from the work carried out Kalmijn (1971) that catsharks are able to determine that plaice are an item that they can prey upon. It is therefore feasible that catsharks are able to locate mates by distinguishing them from prey, using their AoL. This could be especially true if there were a distinct mating season, whereby females may produce a very distinctive electrical signature, which would be more easily picked up by the males.

Another theory that seems plausible is the fact that the species segregates and the increased activity of the AoL could function to serve in an ecological role. If males in the Solent are demonstrating similar depth segregations to those noted by Sims *et al.* (2003) and Rodriguez-Cabello *et al.* (2007) then male catsharks could possess a greater ability to detect prey in an environment where light intensity is less than it is closer to the surface. Whatever the reason for the presence of this sexual dimorphism in the AoL of the lesser-spotted catshark, it is clear that much more research is required in order to fully understand these findings.

In conclusion, the findings in this chapter shows that a sexual dimorphism doesn't exist in the epithelial structures of the AoL, but does exist in the number of alveoli present in each ampulla. It appears that the presence of the sexual dimorphism in the AoL could be directly related to reproductive behaviour and specifically conspecific location. With more research on various populations of *S. canicula* and other elasmobranch species the exact reason for the existence of a sexual dimorphism in the AoL could be established. It could also be possible that with greater numbers and the ability to segregate samples into seasons, that seasonal dimorphisms may also be determined in the AoL of *S. canicula*.

Chapter 7 General Discussion

7.1 Sexual Dimorphism

The fact that secondary sexual dimorphisms exist in a number of elasmobranch species is well established (Brough, 1937; Arthur, 1950; Bass, 1973; Gosztonyi, 1973; Fedducia and Slaughter, 1974; Stevens, 1974; McEachran, 1975; Taniuchi and Shimizu, 1993; Pratt, 1979; Wu, 1994; Ellis and Shackley, 1995; Kajiura and Tricas, 1996; Kajiura *et al.,* 2000; Soto, 2001; Kajiura *et al.,* 2002; Erdogan *et al*., 2004; Kajiura *et al*., 2005; Filiz and Taskavak, 2006;). These sexual dimorphisms have been noted in a range of anatomical and morphological features; including the head, mouth and jaws (Brough, 1937; Arthur, 1950; Gosztonyi, 1973; McEachran, 1975; Ellis and Shackley, 1995; Soto, 2001, Erdogan *et al*., 2004; Kajiura *et al.,* 2005) teeth (Gosztonyi, 1973; Ellis and Shackley, 1995) and skin (Stevens, 1974; Pratt, 1979; Taniuchi and Shimizu, 1993; Nordell, 1994; Kajiura *et al.,* 2000).

Much of the work carried out on these structures has centred on the development of adult specimens and many studies have noted limited investigations into the presence or absence of secondary sexual dimorphisms in juvenile or hatchling catsharks. The overall aims of the initial part of this study revealed sexual dimorphisms of the head, mouth, jaws and teeth of the Solent population of *S. canicula* (Appendix 4). These are similar findings to those in other populations. In keeping with previous studies, adult male catsharks were found to possess a longer, narrower head, mouth and upper jaw than female catsharks (Appendix 4). In this study the depth of the jaws was also investigated and it was found that adult male catsharks had a greater jaw depth than female catsharks (Appendix 4).

The teeth of adult catsharks were also found to be sexually dimorphic in both shape and size. The results revealed that in all aspects of tooth dimensions in both the upper and lower jaws, except for the number of cusps, adult males had significantly larger teeth than females (Appendix 4). This supports work by other authors and also provides an explanation for the presence of a sexual dimorphism in the jaw depth of both the upper and lower jaws of adult specimens. The discriminant analyses also showed that there were clear distinctions between the size of the upper and lower jaws in both male and female catsharks and between adult and juvenile catsharks. This concurs with previously reported research and these differences could be based on the differences in morphological structures of teeth of male and female catsharks.

The presence of larger teeth in adult males could be driving the need for a deeper jaw to accommodate the increased tooth size. Tooth row counts could also be contributing to the greater jaw depth as adult male catsharks were found to possess significantly more tooth rows than adult female catsharks in both the upper and lower jaws. The reasons for this increase in tooth rows appear to indicate an adaptation for mating, as stomach contents were not found to be significantly different between the genders (Lyle, 1983; Henderson and Dunne, 1999) and therefore feeding is not likely to be driving the sexual dimorphism. The fact that male catsharks have been witnessed to bite females during copulation (Domi, *et al*., 2000) could indicate that the teeth of male catsharks are under more pressure and are more prone to breakages and loss than females.

In terms of the presence of sexual dimorphisms in the head, mouth, jaws and teeth of hatchling and juvenile catsharks the results revealed that there were limited sexual dimorphisms present in these size classes. Juvenile male catsharks were found to possess longer mouths than juvenile female catsharks. Both hatchling and juvenile catsharks were found to be sexually dimorphic in terms of the jaws. Male hatchling catsharks were found

to possess a wider upper jaw than female hatchling catsharks. Juvenile male catsharks were found to possess a greater jaw depth than juvenile female catsharks (Appendix 4). The findings for the hatchling sharks is in contrast to the findings of Brough (1937) who found that different mouth morphology in *S. canicula* was only apparent in mature individuals and that the change in mouth shape occurs quickly at the onset of maturity. It is not clear why the hatchlings showed a sexual dimorphism in the jaw sizes, which was then lost in the juvenile samples. The small sample size used for the determination of the juvenile head, mouth and jaw morphometrics could have had an impact on the results. However, it is also possible that the Solent population of *S. canicula* does show a sexual dimorphism in the head, mouth and jaws at the juvenile stage and begin to develop sexual dimorphisms before fully reaching sexual maturity. For example, tooth size was found to be greater in juvenile male catsharks, which would account for the greater jaw depth in juvenile male catsharks.

There were sexual dimorphisms present in the teeth of hatchling and juvenile catsharks. Hatchling catsharks were found to be sexually dimorphic in terms of the lower jaw tooth rows, with hatchling male catsharks possessing more tooth rows than hatchling female catsharks (Appendix 4). As previously mentioned in chapter 4 the fact that a sexual dimorphism exists in the tooth rows of hatchling catsharks could indicate that catsharks are born sexually dimorphic with respect to certain features. Certain sexual dimorphisms were also found in the teeth of juvenile catsharks with mid cusp diameter on the upper jaw and cusp tip on the lower jaw teeth being sexually dimorphic. In all instances males were found to possess greater tooth dimensions than females (Appendix 4). The reasons for this appear to be developmental, whereby there is a gradual change from the pentacuspid form in juvenile males to the unicuspid form in adult males. The fact that *S. canicula* does not shed teeth as complete rows as is seen in some other species (Overstrom, 1991) could indicate that juvenile catsharks nearing maturity will possess teeth that are normally found in

individuals at varying stages of development. The discriminant analysis carried out on the teeth of adult and juvenile male and female catsharks showed that there were distinct differences between the upper and lower jaws of these sampled groups. This concurred with previous findings, whereby there are distinct differences between adult and juvenile teeth for both sexes.

Despite several investigations into the skin of other species; including the blue shark, *Prionace glauca* (Pratt, 1979) and the Atlantic stingray, *Dasyatis sabina* (Kajiura *et al.,* 2000) and observations by Domi *et al.* (2000) of male biting during reproduction in *S. canicula,* no literature exists on the presence or absence of a sexual dimorphism in the skin thickness of *S. canicula*. This is also true for the dermal denticles, whereby much literature exists on their form and function (Grover, 1974; Reif, 1978a; Raschi and Tabit, 1992; Southall and Sims, 2003; Johanson *et al*., 2007) although no investigation into the presence or absence of a sexual dimorphism currently exists.

A sexual dimorphism was found to exist in the epidermis in all size classes of *S. canicula* studied (Appendix 4). Only in adult specimens was there a sexual dimorphism found in the dermal layer (Appendix 4). This sexual dimorphism in the dermal and epidermal layers supports the findings for other species. It is possible that increased skin thickness is a response to male biting. Nordell (1994) suggested that it could be expected that the skin of mature females might be thicker than males in areas where males bite them during copulation. The fact that female hatchling catsharks were found to possess a thicker epidermal layer than male hatchling catsharks suggests that *S. canicula* is born sexually dimorphic in respect of skin thickness.

The study of the dermal denticles revealed that a sexual dimorphism exists in both morphology and density in hatchling, juvenile and adult catsharks (Appendix 4). The

morphology and density of the dermal denticles could be driven by both habitat preferences and reproductive behaviour, whereby larger or more densely distributed dermal denticles provide increased protection to the epidermal layer. Hatchling catsharks showed a range of sexual dimorphisms with regard to dermal denticle shape. On the right and left pectoral fins the denticles of males were found in higher densities than those of females. The left fin the dermal denticles were wider in females than in males. When both fin data were combined the density was greater in males than in females. It is possible that hatchling catsharks are born sexually dimorphic and that the dimorphisms observed in the dermal denticles are an adaptation for later life when mating occurs.

Juvenile catsharks showed no sexual dimorphisms in the size and density of dermal denticles. However, there was a lateralisation with juvenile female catsharks showing a greater density on the right fin than on the left fin (Appendix 4). The denticle width on the left fin of adult catsharks was found to be significantly different with adult females having wider dermal denticles than adult males (Appendix 4). The fin lateralisation also showed that males had longer and wider dermal denticles on the right fin as opposed to the left fin (Appendix 4). This could indicate that male catsharks show a preference for clasper insertion during mating, especially if they using their fins in any way to brace against the body of females during copulation.

The lateralisation studies showed that in all cases the right fin possessed dermal denticles that were significantly different to the left fin in hatchling, juvenile and adult catsharks. This would indicate a preference of use in the pectoral fins of the lesser-spotted catshark. It is possible that the sharks are using their pectoral fins to anchor their prey to the seabed, much the same way that Southall and Sims (2003) noted that hatchling and juvenile catsharks use their tails to anchor prey. If the pectoral fins are used for some aspect of mating then the lateralisation could indicate a preference for other reproductive behaviours,

such as clasper insertion. Some studies have been carried out on the pre-copulatory behaviours of sharks. Gordon (1993) witnessed pre-copulatory behaviours in captive sandtiger sharks and noted that a series of behaviours occurred prior to mating. It is feasible that these behaviours, if witnessed further could lead to males to potentially have a preferred mating strategy where they have a preferential side for approach and clasper insertion. More research is required to ascertain whether this theory holds true or not. The dermal denticle densities in adult catsharks could be a determinant of the mating season.

There have been a range of investigations into the structure and function of the AoL (Murray, 1961; Kalmijn, 1971; Kalmijn, 1974; Bromm *et al*., 1976; Heiligenberg, 1991; von der Emde, 1998; Collin and Whitehead, 2004; Wueringer, *et al*., 2009). Again, there is no mention of the possible existence of a sexual dimorphism in the structure of the AoL in the literature for any elasmobranch species. There was a clear sexual dimorphism in the structure of the alveoli in the AoL of *S. canicula*. Males were found to have an increased number of alveolar bulbs compared to female catsharks. The alveoli are lined with sensory epithelium which contains sensory cells. As previously discussed, it is possible that male and female catsharks inhabit different ecological niches, with males living deeper than females (Sims *et al*., 2001; Sims, 2005). This could account for the increase in ampullary alveoli. This increase electrosensory ability could also be utilised for mate location as reported by Sisneros and Tricas (2002a). It is possible that in the murky environment of the Solent that male *S. canicula* use their increased electrosensory capabilities to not only forage for food in deeper water, but to locate mates when their visual sense is impaired. However, as the use of the AoL for the location of conspecifics for *S. canicula* has not been recorded more research needs to be carried out to confirm whether electroreception is used for mate location in this species.

7.2 Seasonal Dimorphism

Despite the plethora of information that has been available on the sexual dimorphisms of elasmobranch species it was only relatively recently that the sexually dimorphic dentition of elasmobranchs was reported to be influenced by the reproductive season (Kajiura and Tricas, 1996). In some species of ray, seasonal dimorphisms have been found in the dentition, changing to coincide with the mating season (Kajiura and Tricas, 1996). A study by Capapé *et al*. (1990) investigated clasper length in two species of angel shark and found that there were no seasonal dimorphisms relating to this anatomical structure. It is evident from the literature that there is a paucity of information concerning seasonal dimorphisms for elasmobranch species. As far as the author knows there has been one investigation into the seasonal effects on any morphological structure in *S. canicula*. Garnier *et al*. (1999) found that clasper length varied throughout the year in this species. The seasonal dimorphisms were examined with a view of trying to accurately determine whether there is a distinct mating season for the Solent population of *S. canicula*. The main difficulty with doing this is that *S. canicula* have been found to show a protracted egg laying period (Henderson and Casey, 2001; Ford, 1921; Sumpter and Dodd, 1979). However evidence from studies examining peak periods of egg laying indicated that spring/summer seems the most likely reproductive season. Differences in the literature suggested that there are variations in the proposed mating season from populations around the UK and Europe. This is based on a number of factors including catches of male and female catsharks at the same time in the same place at certain times of year, crossing of claspers and running milt and the presence of sperm in the gonads at certain times of year (See chapter 2). This would seem feasible, as water temperature would vary around the coast depending on longitude and latitude, therefore driving reproduction to occur when the environmental conditions were right.

With regards to seasonal dimorphisms in the head, mouth and jaw of *S. canicula*, only adults showed any seasonal dimorphism (Appendix 4). Mouth length was found to be greater in winter than in all other seasons. The increased mouth length of adult catsharks has been reported previously and the presence of an increased mouth length in winter does not fit entirely with speculation from other studies that suggested spring or summer as the mating season. However, the categorisation of the seasons is potentially moveable as each season accounts for three months of the year. It is clear that seasons cannot be defined by distinct cut-off dates and that in light of climatic changes the seasons may overlap quite considerably. Upper jaw width was found to be greater in sharks sampled in spring than in all other seasons. The reasons for this increase in mouth width is not entirely clear, especially for males, as they have been found to generally possess a longer, narrower mouth than females. The coincidence of the wider jaw in spring could point towards a reproductive adaptation, as spring is believed to be the reproductive season for *S. canicula* in the Solent.

Elements of the skin were found to be seasonally dimorphic, with all of the seasonal dimorphisms relating to the dermal denticles of adult catsharks (Appendix 4). On the right pectoral fin the denticle length was greater in spring than in all other seasons. The denticle density was also found to be greater in summer than in spring and winter. Female catsharks were also found to possess greater dermal denticle densities on the right fins in winter and spring compared to males in winter and spring. On the left pectoral fins the dermal denticle density was found to be greater in summer compared to all other seasons (Appendix 4). Female denticle densities were found to be greater in summer as compared to male dermal denticle densities in summer (Appendix 4). This increase in denticle length and density during the spring and summer lends weight to the argument that for the Solent population of *S. canicula* the mating season occurs during spring and summer. As male *S. canicula* have been seen to bite females during reproduction it is possible that the densities of

dermal denticles would change throughout the year to coincide with the mating season. Reif (1978c) noted the ability of sharks to regenerate scales and from this evidence it is feasible that catsharks shed and re-grow their dermal denticles in much the same way that they shed their teeth. Again, further research would be needed to ascertain whether this is the case or not.

In the lower jaw the cusp tip diameter was greater in winter than in all other seasons, whilst for the lower jaw teeth cusp base diameter was greater for males in all seasons compared to females in all seasons (Appendix 4). There appears to be a limited effect of seasonality on the teeth of adult *S. canicula* unlike the finding of Kajiura and Tricas (1996) for the Atlantic stingray. The fact that the breeding season seems to be protracted, based on observed egg laying patterns (Henderson and Casey, 2001; Ellis and Shackley, 1997) could lead the teeth of *S. canicula* to be sexually dimorphic all year. It appears that in *S. canicula* the dentition does not indicate the timing of the mating season as it does in *D. sabina*.

In conclusion the results from this study show that the Solent population of *S. canicula* were found to be sexually dimorphic in respect of the head, mouth, jaws and teeth. These findings are in line with other research and support the findings of other authors. These sexual dimorphisms appear to be reproductive in nature and become more pronounced in adult catsharks. There are some sexual dimorphisms also present in hatchling and juvenile catsharks that have not been previously reported by other authors. The epidermis and dermis of *S. canicula* was also found to be sexually dimorphic, results not previously published before for this species. There were also some sexual dimorphisms of the dermal denticles of *S. canicula*, again believed to be reproductive in nature. This has not been reported previously for any species. There was some sexual dimorphism in the structure of the AoL, which could be linked to habitat selection or reproduction. Again, results not previously published for any species.

There were some seasonal dimorphisms present in *S. canicula*, although these were not entirely conclusive in resolving the issue of the determining whether there is a specific mating season for the Solent population of *S. canicula*. The seasonal dimorphisms for *S. canicula* were not as pronounced as in *D. sabina*, but showed an annual difference in dentition. The dermal denticle data showed a more pronounced seasonal dimorphism and could lead to some identification of a specific mating period for this species. In order to ascertain if this is the case more research needs to be carried out with a greater sample base. Further investigations are required to determine the effects of the seasons on the secondary sexual characters of *S. canicula* and whether these effects can lead to an indication of a specific mating season for this species.

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Appendix 1

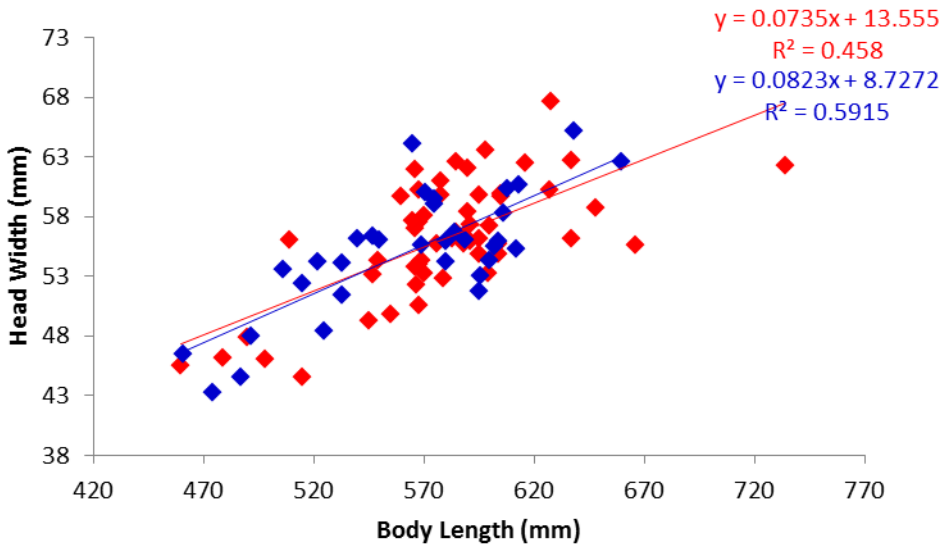

Figure A.1.1. Scatterplot with regression for head width in male and female juvenile and adult *S. canicula.* (n= (M, 33)(F, 54)) (*P*= 0.61) (Female = Red, Male = Blue).

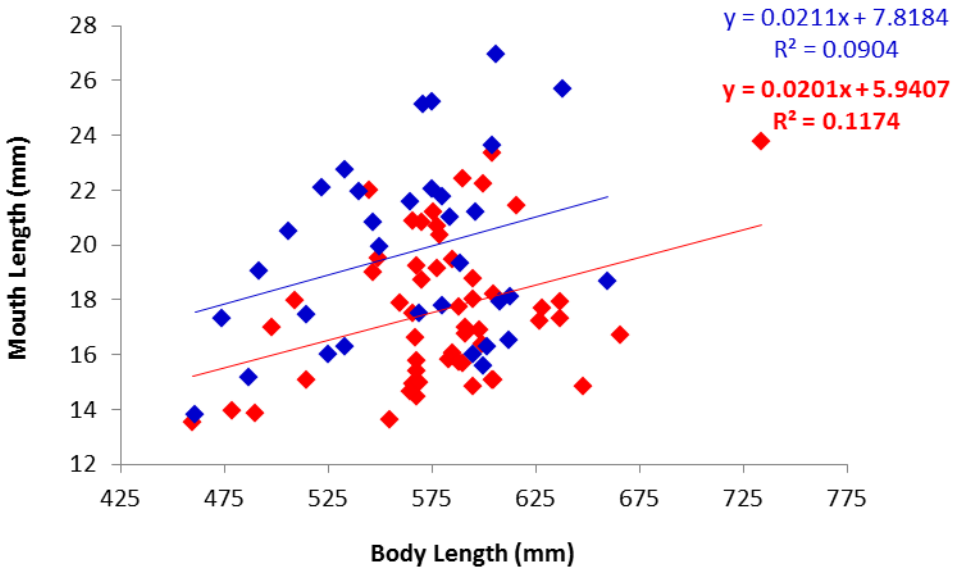

Figure A.1.2. Scatterplot with regression for mouth length in male and female juvenile and adult *S. canicula.* (n= (M, 33)(F, 54))(*P*= 0.67) (Female = Red, Male = Blue).

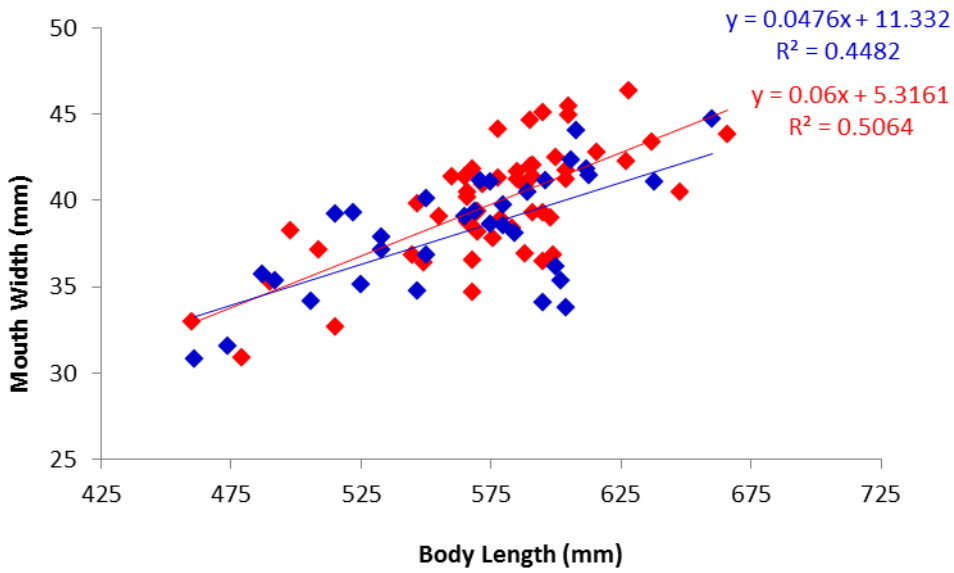

Figure A.1.3. Scatterplot with regression for mouth width in male and female juvenile and adult *S. canicula.* (n= (M, 33)(F, 54))(*P*= 0.40) (Female = Red, Male = Blue).

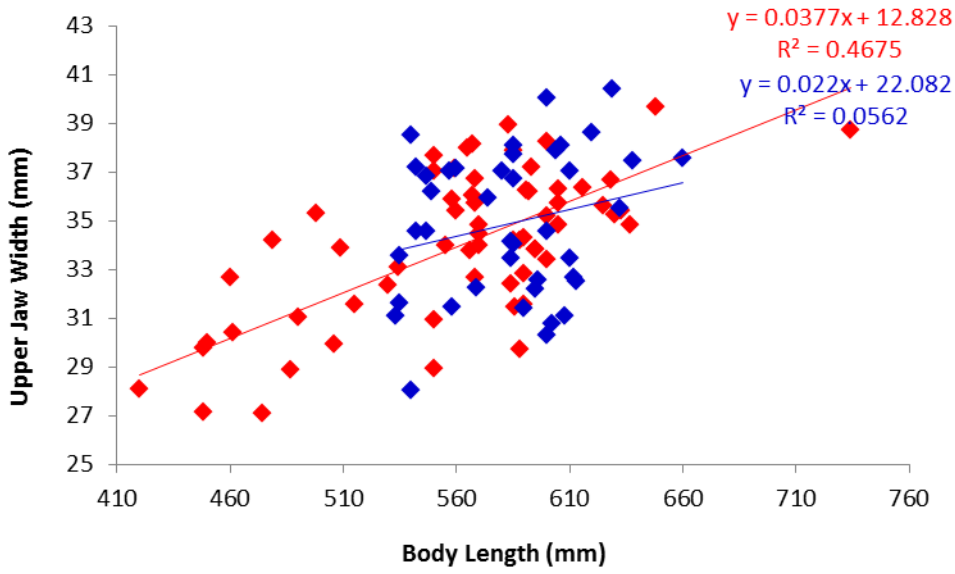

Figure A.1.4 Scatterplot with regression for upper jaw width in male and female juvenile and adult *S. canicula.* (n= (M, 41)(F, 64)) (*P*= 0.30) (Female = Red, Male = Blue).

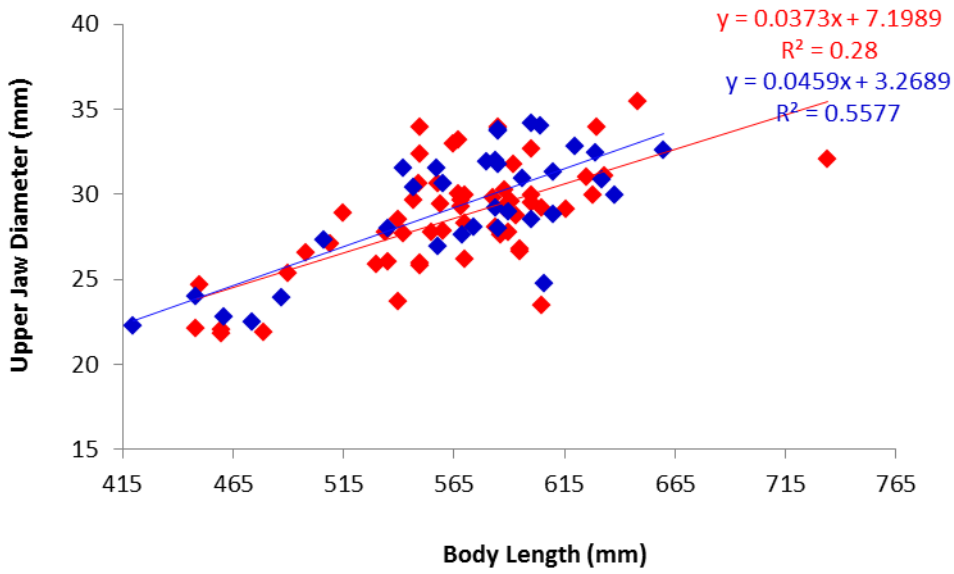

Figure A.1.5. Scatterplot with regression for upper jaw diameter in male and female juvenile and adult *S. canicula.* (n= (M, 34)(F, 58))(*P*= 0.84) (Female = Red, Male = Blue).

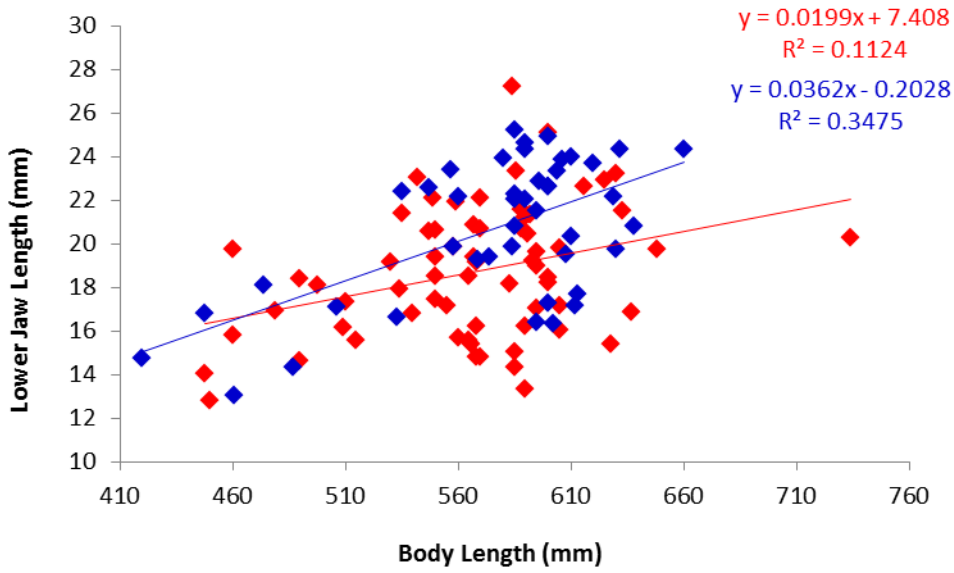

Figure A.1.6. Scatterplot with regression for lower jaw length in male and female juvenile and adult *S. canicula.* (n= (M, 44)(F, 67))(*P*= 0.20) (Female = Red, Male = Blue).

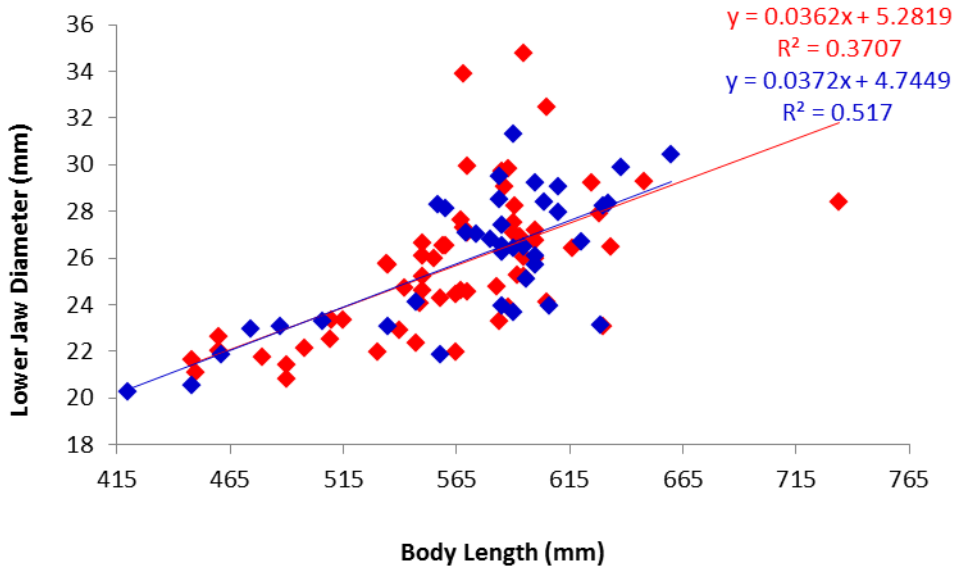

Figure A.1.7. Scatterplot with regression for lower jaw diameter in male and female juvenile and adult *S. canicula.* (n= (M, 38)(F, 61))(*P*= 0.91) (Female = Red, Male = Blue).

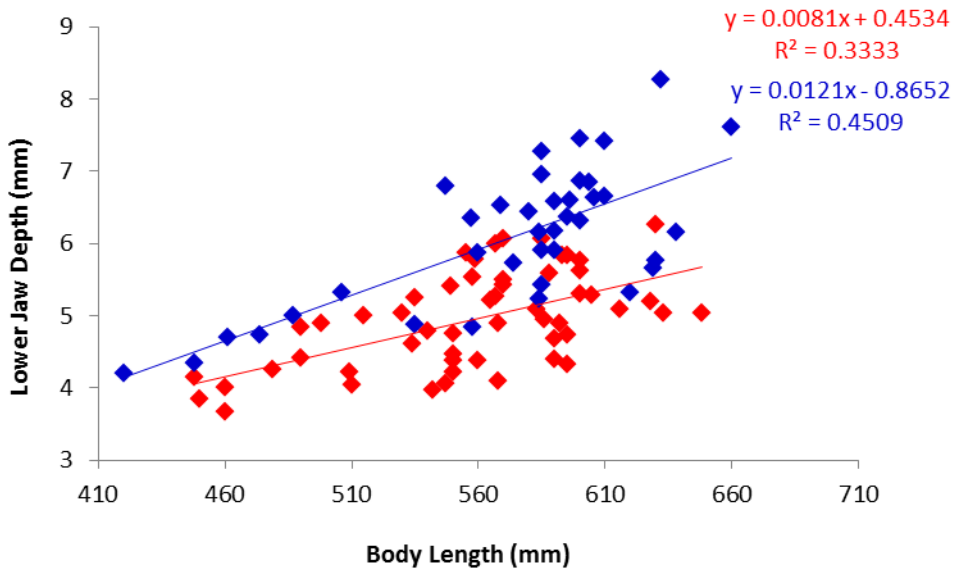

Figure A.1.8. Scatterplot with regression for lower jaw depth in male and female juvenile and adult *S. canicula.* (n= (M, 38)(F, 54))(*P* = 0.15) (Female = Red, Male = Blue).

Appendix 2

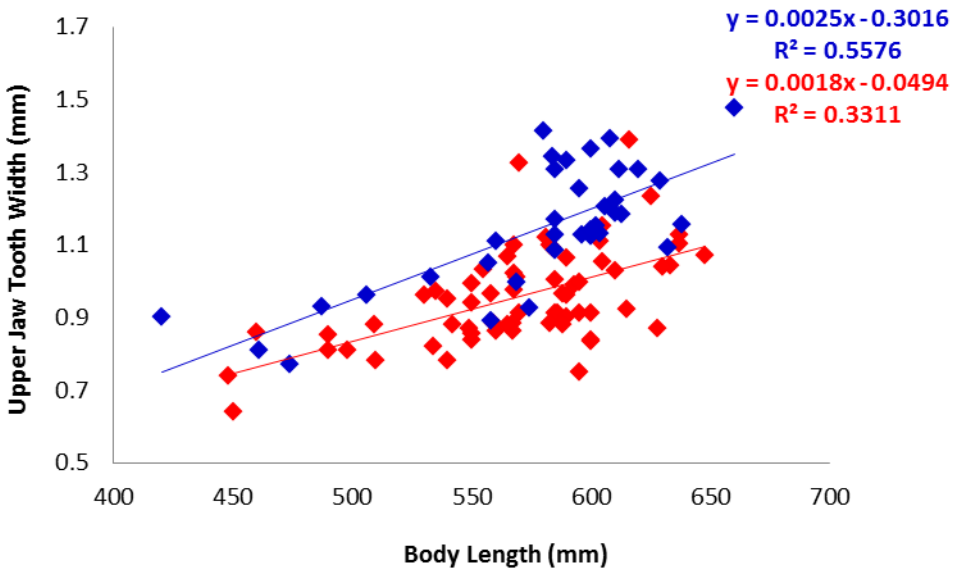

Figure A.2.1. Scatterplot with regression for upper jaw tooth width in male and female juvenile and adult *S. canicula.* (n= (F, 50)(M, 31))(*P*= 0.10) (Female = Red, Male = Blue).

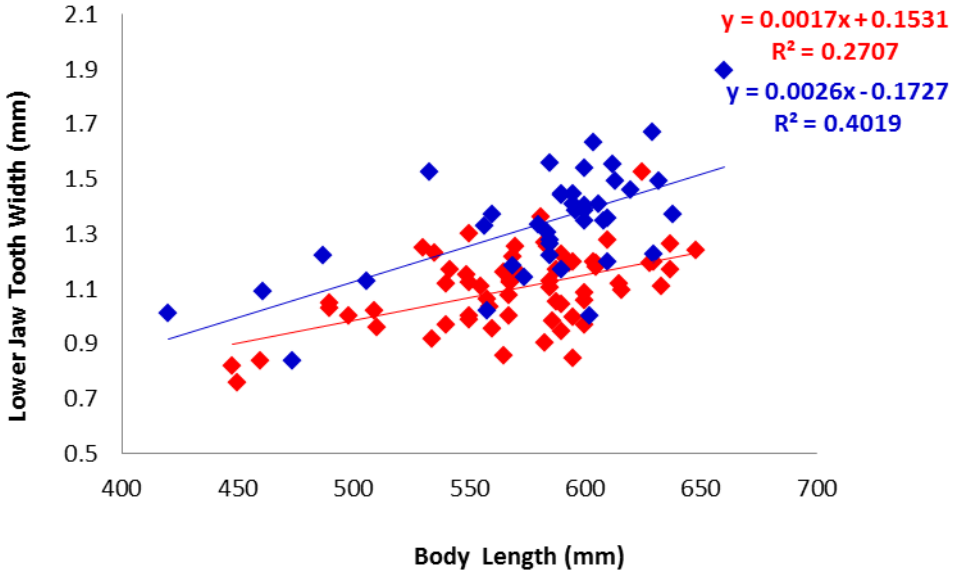

Figure A. 2.1. Scatterplot with regression for lower jaw tooth width in male and female juvenile and adult *S. canicula.* (n= (F, 52)(M, 34))(*P*= 0.08) (Female = Red, Male = Blue).

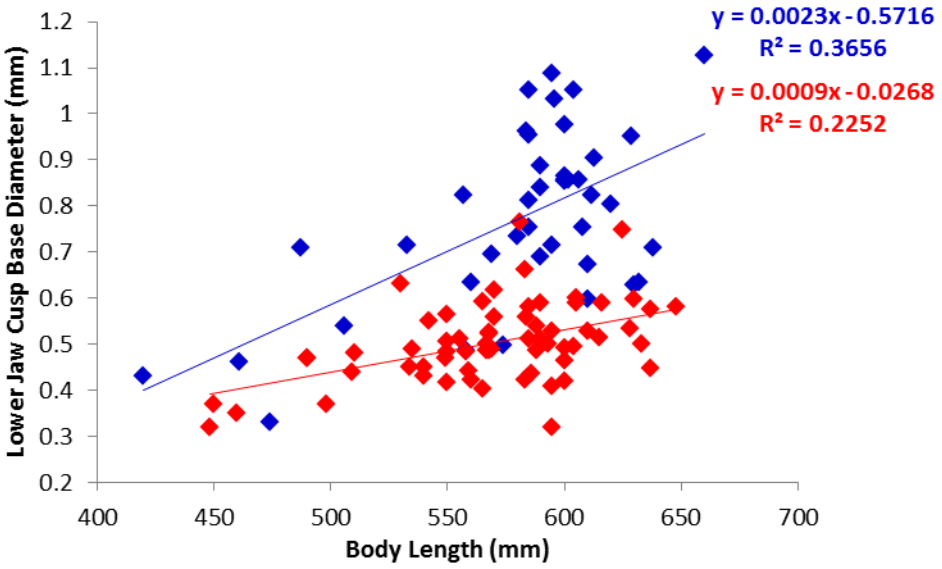

Figure A.2.3. Scatterplot with regression for lower jaw tooth cusp base diameter in male and female juvenile and adult *S. canicula.* (n= (F, 52)(M, 34))(*P*= 0.06) (Female = Red, Male = Blue).

Appendix 3

Table A.3.1. The means ± standard errors and ranges for the head, mouth and jaw morphometrics of adult *S. canicula.*

Head, Mouth and Jaw Data		
Feature (mm)	**Female $\bar{x}$ ± SE (Range)**	**Male $\bar{x}$ ± SE (Range)**
Pre-Branchial Length	86.64 ± 2.52 (54.60 - 113.50)	87.63 ± 3.06 (59.39 - 111.23)
Head Width	57.58 ± 0.55 (49.85 - 67.64)	56.77 ± 0.76 (48.35 - 65.13)
Pre-Oral Distance	24.55 ± 1.13 (17.87- 41.60)	22.61 ± 1.01 (18.56 - 46.67)
Mouth Length	17.76 ± 0.39 (13.64 - 23.79)	20.22 ± 0.66 (15.61 - 26.94)
Mouth Width	40.67 ± 0.41 (33.79 - 46.33)	39.08 ± 0.60 (34.66 - 44.68)
Jaw length (Upper)	18.53 ± 0.32 (13.84 - 23.97)	22.67 ± 0.39 (17.77 - 26.90)
Jaw Width (Upper)	35.15 ± 0.35 (28.93 - 39.67)	35.08 ± 0.49 (30.31 - 40.38)
Jaw Diameter (Upper)	29.27 ± 0.53 (19.16 - 35.47)	30.54 ± 0.45 (24.71 - 34.17)
Jaw Depth (Upper)	3.59 ± 0.06 (2.98 - 3.79)	4.81 ± 0.14 (3.16 - 6.78)
Jaw Length (Lower)	19.04 ± 0.42 (13.35 - 27.21)	21.48 ± 0.42 (16.33 - 25.24)
Jaw Width (Lower)	33.39 ± 0.46 (27.06 - 42.23)	32.98 ± 0.46 (27.03 - 38.93)
Jaw Diameter (Lower)	26.88 ± 0.41 (21.97 - 34.77)	26.83 ± 0.41 (21.86 - 31.32)
Jaw Depth (Lower)	5.13 ± 0.12 (3.04 - 6.26)	6.31 ± 0.15 (4.66 - 8.27)

Table A.3.2. The means ± standard errors and ranges for the tooth morphometrics of adult *S. canicula.*

Tooth Data		
Feature (µm) (Number)	**Female $\bar{x}$ ± SE (Range)**	**Male $\bar{x}$ ± SE (Range)**
Tooth Slope Height (Upper)	1.10 ± 0.02 (0.79 - 1.53)	1.45 ± 0.03 (1.05 - 1.85)
Tooth Width (Upper)	0.99 ± 0.02 (0.75 - 1.39)	1.19 ± 0.03 (0.89 - 1.48)
Cusp Base Diameter (Upper)	0.48 ± 0.01 (0.34 - 0.72)	0.72 ± 0.03 (0.44 - 0.10)
Mid Cusp Diameter (Upper)	0.24 ± 0.01 (0.17 - 0.32)	0.31 ± 0.01 (0.22 - 0.45)
Cusp Tip Diameter (Upper)	0.09 ± 0.01 (0.07 - 0.12	0.17 ± 0.01 (0.09 - 0.24)
Cusp Number (Upper)	3.89 ± 0.08 (2.4 - 5.0)	1.68 ± 0.14 (1.0 - 3.4)
Number of Rows (Upper)	5.54 ± 0.09 (5.0 -7.0)	5.85 ± 0.08 (5.0 -7.0)
Tooth Slope Height (Lower)	2.73 ± 0.15 (1.39 - 4.20)	2.71 ± 0.18 (1.28 - 4.12)
Tooth Width (Lower)	1.13 ± 0.02 (0.85 - 1.52)	1.38 ± 0.03 (1.00 - 1.90)
Cusp Base Diameter (Lower)	0.52 ± 0.01 (0.32 - 0.77)	0.81 ± 0.03 (0.49 - 1.13)
Mid Cusp Diameter (Lower)	0.25 ± 0.004 (0.20 - 0.34)	0.36 ± 0.01 (0.24 - 0.58)
Cusp Tip Diameter (Lower)	0.09 ± 0.01 (0.06 - 0.18)	0.17 ± 0.01 (0.08 - 0.23)
Cusp Number (Lower)	4.06 ± 0.09 (2.2 - 5.0)	2.32 ± 0.19 (1.0 - 4.6)
Number of Rows (Lower)	5.61 ± 0.13 (3.0 - 7.0)	6.44 ± 0.12 (4.0 - 8.0)

Table A.3.3. The means ± standard errors and ranges for the skin morphometrics of juvenile and adult *S. canicula.*

Skin Data		
Feature (µm)	**Female $\bar{x}$ ± SE (Range)**	**Male $\bar{x}$ ± SE (Range)**
Juvenile		
Epidermis	84.28 ± 5.27 (51.83 - 133.73)	65.07 ± 3.27 (50.09 -78.04)
Dermis	547.5 ± 24.4 (361.7 - 743.8)	582.7 ± 39.3 (414.6 - 771.2)
Adult		
Epidermis	69.09 ± 3.03 (38.24 136.14)	76.79 ± 2.29 (34.68 126.64)
Dermis	677.1 ± 14.9 (432.1 - 960.1)	594.3 ± 20.8 (270.9 - 944.5)
Denticle Length (Right Fin)	466.0 ± 11.3 (365.6 - 551.6)	435.1 ± 11.5 (353.9 - 513.6)
Denticle Length (Left Fin)	480.8 ± 13.1 (387.7 - 631.8)	456.63 ± 9.83 (366.45 - 525.66)
Denticle Width (Right Fin)	309.94 ± 6.99 (243.68 387.49)	294.4 ± 11.2 (241.0 501.5)
Denticle Width (Left Fin)	302.80 ± 5.36 (250.21 - 345.48)	273.94 ± 5.13 (225.85 - 310.48)
Denticle Density (Right Fin)	36.36 ± 2.21 (23 - 57)	37.67 ± 1.00 (28 – 49)
Denticle Density (Left Fin)	36.68 ± 1.76 (26 - 57)	40.27 ± 1.34 (30 - 58)

Table A.3.4. The means ± standard errors and ranges for the morphometrics of the Ampullae of Lorenzini of adult *S. canicula.*

Ampullae of Lorenzini Data		
Feature (µm) (Number)	**Female $\bar{x}$ ± SE (Range)**	**Male $\bar{x}$ ± SE (Range)**
Epithelial Cell Density	12.37 ± 0.38 (9.44 - 15.5)	12.11 ± 0.25 (10.56 - 13.75)
Epithelial Diameter	13.26 ± 1.01 (7.86 - 21.32)	10.713 ± 0.982 (6.23 - 18.86)
Alveolar Number	6.69 ± 0.18 (6 – 7)	7.21 ± 0.15 (6 – 8)

Appendix 4

Table A.4.1. Significant differences for the head, mouth and jaws of hatchling, juvenile and adult *S. canicula*.

Head, Mouth and Jaws		
Feature (mm)	**Significance**	***P*-Value**
Hatchling Head and Mouth Measurements		
Head Width	**Body Length**	**< 0.001**
Pre-oral length	**Body Length**	**< 0.001**
Mouth Width	**Body Length**	**0.001**
Juvenile Head and Mouth Measurements		
Head Width	**Body Length**	**0.005**
Mouth Length	**Body Length/ Gender**	**< 0.001 / 0.015**
Mouth Width	**Body Length**	**0.003**
Adult Head and Mouth Measurements		
Pre branchial length	**Season**	**0.029**
Head Width	**Body Length**	**0.003**
Mouth Length	**Gender/ Season**	**0.011/ 0.028**
Mouth Width	**Body Length/ Gender**	**0.005/ 0.016**
Hatchling (Upper Jaw)		
Jaw Width	**Body Length**	**0.035**
Juvenile (Upper Jaw)		
Jaw Width	**Body Length**	**0.015**
Jaw Diameter	**Body Length**	**0.001**
Juvenile (Lower Jaw)		
Jaw Length	**Body Length**	**0.002**
Jaw Diameter	**Body Length**	**0.003**
Jaw Depth	**Body Length/ Gender**	**0.047/ 0.036**
Adult (Upper Jaw)		
Jaw Length	**Gender**	**<0.001**
Jaw Width	**Body Length/ Season**	**0.019/ 0.007**
Jaw Diameter	**Body Length**	**0.010**
Jaw Depth	**Body Length/ Gender**	**0.012/ <0.001**
Adult (Lower Jaw)		
Jaw length	**Gender**	**<0.001**
Jaw Width	**Body Length**	**0.001**
Jaw Diameter	**Body Length**	**0.017**
Jaw Depth	**Gender**	**<0.001**

Table A.4.2. Significant differences for the teeth of hatchling, juvenile and adult *S. canicula*.

Teeth		
Feature (mm)	**Significance**	***P*-Value**
Hatchling (Lower Jaw)		
Tooth Row Number	**Gender**	**0.018**
Juvenile (Upper Jaw)		
Tooth Width	**Body Length**	**0.031**
Mid Cusp Diameter	**Gender**	**0.030**
Juvenile (Lower Jaw)		
Tooth Width	**Body Length**	**0.013**
Cusp Base Diameter	**Body Length**	**0.004**
Cusp Tip Diameter	**Gender**	**0.015**
Adult (Upper Jaw)		
Tooth Slope Height	**Body Length/ Gender/ Season*Gender**	**<0.001/ <0.001/ 0.005**
Tooth Width	**Body Length/Gender**	**0.001/<0.001**
Cusp Base Diameter	**Body Length/Gender**	**0.010/ <0.001**
Mid Cusp Diameter	**Body Length/Gender**	**0.009/ <0.001**
Cusp Tip Diameter	**Body Length/ Gender/ Season**	**0.021/ <0.001/ 0.014**
Tooth Cusp Number	**Gender**	**<0.001**
Tooth Row Number	**Gender**	**0.004**
Adult (Lower Jaw)		
Tooth Slope Height	**Body Length/ Gender/ Season*Gender**	**0.003/ <0.001/ 0.025**
Tooth Width	**Body Length/ Gender**	**0.001/ <0.001**
Cusp Base Diameter	**Body Length/ Gender/ Season*Gender**	**0.010/ <0.001/ 0.035**
Mid Cusp Diameter	**Body Length/Gender**	**0.001/ <0.001**
Cusp Tip Diameter	**Gender**	**<0.001**
Cusp Number	**Gender**	**<0.001**
Tooth Row Number	**Gender**	**<0.001**

Table A.4.3. Significant differences for the skin of hatchling, juvenile and adult *S. canicula*.

Skin		
Feature (µm)(mm^2)	**Significance**	***P*-Value**
Hatchling		
Epidermal Thickness	**Gender**	**0.015**
Juvenile		
Epidermal Thickness	**Gender**	**0.046**
Adult		
Epidermal Thickness	**Gender**	**0.038**
Dermal Thickness	**Gender**	**0.001**
Hatchling (Right Fin)		
Density	**Gender**	**0.020**
Hatchling (Left Fin)		
Denticle Width	**Gender**	**0.022**
Density	**Gender**	**0.003**
Hatchling Combined Fins		
Denticle Width	**Gender**	**0.021**
Density	**Gender**	**0.005**
Juvenile Intra-Gender (Female)		
Density	N/A	**0.039**
Adult (Right Fin)		
Denticle Length	**Season**	**0.006**
Denticle Density	**Season/ Season*Gender**	**<0.001/ <0.001**
Adult Intra-Gender (Male)		
Denticle Length	**N/A**	**0.008**
Denticle Width	**N/A**	**0.011**
Adult (Left Fin)		
Denticle Length	**Body Length**	**0.009**
Denticle Width	**Gender**	**0.001**
Denticle Density	**Season/ Season*Gender**	**<0.001/ 0.043**
Adult (Combined Fins)		
Denticle Length	**Body Length/ Season**	**0.022/ 0.013**
Denticle Width	**Gender**	**0.017**
Denticle Density	**Season/ Season*Gender**	**<0.001/ <0.001**

Table A.4.4. Significant differences for the Ampullae of Lorenzini of adult *S. canicula*.

Ampullae of Lorenzini		
Feature	**Significance**	***P*-Value**
Adult		
Alveoli Number	**Gender**	**0.048**