

**Investigation of the chemical  
composition and nutritional value of  
smoothhound shark (*Mustelus  
mustelus*) meat**

by

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## SUMMARY

The aim of this study was to determine the proximate composition of five individual body sites of the *Mustelus mustelus* shark in order to evaluate the cross carcass variation of the individual proximate components (moisture, protein, lipid, ash) of the meat. This variation was determined in order to find a representative sample of the edible part of the shark (fillet and body flap). Secondly, this sample representing the entire shark fillet was used to investigate the endogenous factors (gender, size and life cycle stage) and their effects on the individual proximate components and other meat components (amino acids, fatty acids, minerals, histamine and mercury contents). Finally, all this data was combined to describe the average chemical composition and nutritional value of *M. mustelus* meat.

None of the proximate components showed any variation between the different fillet positions. This indicated that the fillet is homogenous and samples for chemical analyses can be taken anywhere on the fillet as representative of the entire fillet.

It was found that all three main effects (gender, size and life cycle stage) did not have major influences on most of the components of the chemical composition of *M. mustelus* meat analysed. Higher fatty acid levels (SFA, MUFA and PUFA) were observed in large females than in large males as well as in non-pregnant large females compared to pregnant large females. According to statistical analysis, large males had higher total mercury levels than large females. The only component affected by size variation was the fatty acids, showing a trend to decrease in quantity before maturity was reached. Variation due to life cycle stages was mostly evident in the fatty acid component with some small effects on two mineral components, aluminium and copper, which had slightly higher levels in pregnant large females than in non-pregnant large females.

*M. mustelus* meat has an average proximate composition of 75% moisture, 23% protein, 1.6% lipids and 1.4% ash (weight per wet weight). The protein is, however, an over-estimation of the true protein value as the meat contains significant amounts of non-protein nitrogen (NPN) in the form of urea which contributes to the N concentration. *M. mustelus* meat is a good source of some essential amino acids, especially lysine and threonine (78% of the daily requirements for an adult in a 100g portion), but low in minerals. The meat has a healthy lipid content with a good ratio (>0.45) of PUFA:SFA (0.83) as well as a healthy (<4) n-6:n-3 fatty acid ratio of 0.39. The histamine content was very low or not detectable but some samples contained total mercury values above the maximum safe limit.

Although further research is needed for some meat components, these results are a valuable contribution to the new South African Food Composition Tables being compiled.

## OPSOMMING

Die doel van hierdie studie was om die proksimale samestelling van die vleis vanaf vyf afsonderlike posisies op die liggaam van die *Mustelus mustelus* haai te bepaal. Sodoende is die variasie, met betrekking tot die verskillende proksimale komponente (vog, proteïen, lipiede en as), in terme van die totale karkas, bepaal. Die proksimale variasie is bepaal om vas te stel hoe 'n verteenwoordigende monster van die totale karkas geneem kan word. Gevolglik is hierdie verteenwoordigende monster gebruik om die effek van geslag, grootte en die verskillende fases van die lewens-siklus op die afsonderlike proksimale komponente asook ander vleis komponente (aminosure, vetsure, minerale, histamien en kwik inhoud) te ondersoek. Laastens is al hierdie inligting gebruik om die algemene samestelling en voedingswaarde van *M. mustelus* vleis te bespreek.

Geen van die proksimale komponente het enige variasie getoon tussen afsonderlike liggaamsposisies nie. Hierdie resultaat dui daarop dat die vleis van 'n *M. mustelus* haai homogeen is regoor die karkas en dat 'n vleis monster vanaf enige posisie op die karkas geneem kan word as 'n verteenwoordigende monster.

Daar is gevind dat geslag, grootte en fase van die lewens-siklus geen merkwaardige invloed het op die vleis se samestelling nie. Hoër vetsuur konsentrasies (versadigde, mono-onversadigde en poli-onversadigde vetsure) is gevind in groot vroulike haaie en nie-dragtige vroulike haaie as in groot manlike haaie en dragtige vroulike haaie onderskeidelik. Statisties, het groot manlike haaie hoër vlakke van totale kwik as groot vroulike haaie. Die enigste vleis komponent wat beïnvloed is deur die grootte van die haai, is die vetsure, wat verminder het voor volwassenheid bereik is en dan weer vermeerder soos die haai groter word. Variasie as gevolg van die verskillende fases van die lewens-siklus is meestal gevind in die vetsuursamestelling, en die minimale het ook gevarieer ten opsigte van die elemente aluminium en boor wat effense hoër vlakke getoon het in dragtige haaie as in nie-dragtige haaie.

*M. mustelus* vleis het 'n gemiddelde proksimale samestelling van 75% vog, 23% proteïen, 1.6% lipiede en 1.4% as (nat massa). Die proteïen waarde is 'n oorskatting van die ware proteïen waarde as gevolg van hoë nie-proteïen stikstof in die vorm van ureum wat bydra tot die totale stikstof inhoud. *M. mustelus* vleis blyk 'n goeie bron van sommige essensiële aminosure soos lisien en treonien (78% van die daaglikse aanbevole dosis), maar laag in mineraal inhoud. Die vleis het 'n gesonde vet inhoud met 'n goeie ( $>0.045$ ) poli-onversadigde:versadigde vetsuur verhouding (0.83) asook 'n gesonde ( $<4$ ) omega 6 tot omega 3 vetsuur verhouding van 0.39. Die histamien inhoud van die vleis was baie laag of onder die meetbare limiet, maar sekere monsters het 'n totale kwik inhoud getoon wat bo die maksimum veilige limiet is.

Hoewel verdere navorsing ten opsigte van sekere van die vleis komponente vereis word, lewer hierdie resultate 'n waardevolle bydrae tot die nuwe Suid-Afrikaanse voedsel samestellings tabelle wat tans opgestel word.

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## Chapter 1

### INTRODUCTION

The first directed shark fishery in South Africa was reportedly initiated in the early 1930's (Kroese *et al.*, 1995). Although the shark industry in South Africa has since fluctuated, the demand for shark products has shown a steady increase since the early 1990's (Stuttaford, 1995). The smoothhound (*Mustelus mustelus*) shark, which is one of the shark species commonly caught off the Southern African coastline, has only been targeted as a food source since the late 1980's in this country (Smale & Compagno, 1997). Over the last decade, smoothhound catches off South Africa have steadily increased from 4 tons in 2001 to 85 tons in 2009 (Anonymous, 2011). This is also one of the main shark species that is exported to Australia (Fouche, 2011), where there is a large market for shark meat for use in 'fish and chips' and other minced fish products (Preston, 1984).

Even though *M. mustelus* meat is commonly consumed in many parts of the world, no information currently exists in terms of the chemical composition and nutritional value of this shark species. The newly published South African labelling legislation (R.146/2010) (DoH, 2010), as well as the South African Consumer Protection Act (R.467/2009) (DTI, 2009), aim to ensure that local consumers have access to honest, accurate information on foodstuff labels, which is not misleading in any way, and which will empower them to make informed purchasing decisions (Van der Riet, 2011).

According to the regulations promulgated under R.146/2010 (DoH, 2010), nutritional labelling is mandatory on those products for which nutrient-related health claims are made, but not on those where no such nutrient-related claims are made. Nonetheless, there is still an urgent need for the publication of comprehensive nutritional data for raw food products, particularly for when this information is included voluntarily on product labels and for incorporation into local and international food composition tables created for dietetic planning purposes.

From the limited information that is available on the nutritional composition of shark meat, it can be gathered that this is generally a healthy food source. Geiger and Borgstrom (1962) reported that shark protein is a good source of essential amino acids and can serve as a cheap food substitute to fulfil several amino acid deficiencies in protein-poor diets. Fish meat is known to be rich in omega-3 fatty acids (n-3 FA), with particularly marine species having a favourable omega-6 to omega-3 fatty acid ratio (<4) (Økland *et al.*, 2005), as well as a healthy ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (>0.45) (Huss, 1988). An increased intake of omega-3 polyunsaturated fatty acids has been recommended for the treatment and prevention of coronary heart disease (Simopoulos, 1991). Nevertheless, the western diet has a great deficiency

in omega-3 fatty acids (Simopoulos, 1991; Justi *et al.*, 2003). The inclusion of larger proportions of fish or shark meat into such diets could thus increase the intake of these important fatty acids and potentially decrease the risks for heart disease.

Along with these probable health benefits of shark meat, there are also a number of potential adverse effects associated with its consumption. The latter may include the formation of histamine, a biogenic amine formed from the free amino acid histidine, which can accumulate as a result of post-harvest bacterial contamination and time-temperature abuse of the meat. High levels of histamine in fish have been identified as the causative agent in histamine poisoning (also called scombroid or scrombotoxin poisoning) in humans (Ababouch *et al.*, 2004).

A further hazard associated with the consumption of shark meat is the potential for high levels of heavy metals to accumulate in their tissues, with mercury being the heavy metal of greatest concern. Since sharks are long-lived species and feed at a high trophic level in the marine food web, they are prone to the storage of high mercury levels in their muscles and organs due to the processes of bioaccumulation and biomagnification (Ababouch *et al.*, 2004). The consumption of high levels of mercury, especially in its organic methylmercury (MeHg) form, can lead to mercury poisoning in humans (Ruelas-Inzunza & Paez-Osuna, 2005).

Factors affecting the nutritional composition and the safety of shark meat are manifold and include genetic variation, individual variation, anatomical differences, physiological factors, gender differences, seasonal changes and environmental factors (Jacquot, 1961). In order to accurately determine the proximate composition of *M. mustelus* meat, it is first necessary to identify that sample that should be taken for such analyses that is the most representative of the entire edible portion of the fish, as well as the entire population of the species. A representative sample will also be one that takes all of the aforementioned possible factors of variation into account.

The first aim of this study was to determine the proximate composition of *M. mustelus* sharks at five different body sites so as to evaluate the cross-carcass variation existing within the meat for the individual proximate components (moisture, protein, lipid and ash). This variation was determined in order to identify the most representative sample of the edible part of the shark (fillet and body flap) that could be used for future chemical analyses. The second aim was to use the sample deemed to be most representative of *M. mustelus* meat to investigate the endogenous factors (gender, size and life cycle stage) and their effects on the individual proximate components and other meat components (amino acids, fatty acids, minerals, histamine and mercury content). The final aim was to utilise the data obtained from the two aforementioned study components to describe the average chemical composition and nutritional value of *M. mustelus* meat, which would prove extremely valuable for incorporation into the new South African Food Composition Tables being compiled by the Medical Research Council (MRC).

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## Chapter 2

### LITERATURE REVIEW

#### Background

Shark fishing is practiced worldwide and forms a significant part of the fishing industry in many countries. Sharks belong to the class chondrichthyes (cartilaginous fishes), subclass elasmobranchii and superorder selachimorpha (sharks) (Nelson, 2006). Globally, reported landings of chondrichthyan fishes exceeded 700 000 tons per annum in 1998, the majority of which was fairly evenly divided between sharks and batoid elasmobranchs (rays and skates) (Walker, 1998). Of the total recorded catch at this time, chondrichthyans and sharks provided approximately 1% and 0.5% of the world's fisheries products, respectively (Walker, 1998).

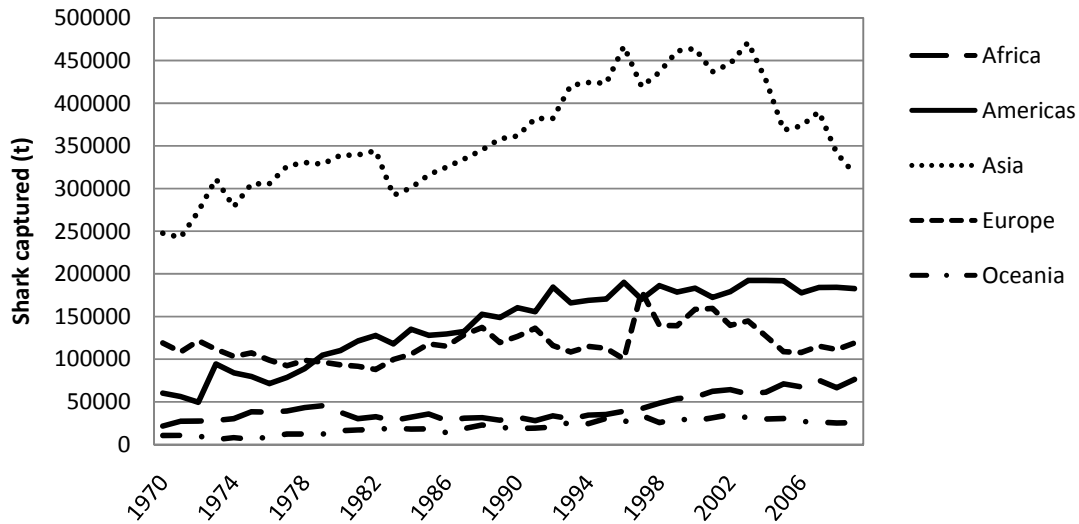
The countries with the highest shark catches in the world are ranked in the Top 20 list (Table 2.1) (Lack & Sant, 2011). The term 'shark' in this list refers to all chondrichthyan species (sharks, skates, rays and chimaeras). Catches by these 20 countries represent nearly 80% of the world's shark catches (Lack & Sant, 2011). The information in Table 2.1 is only based on shark catch data reported to the United Nations Food and Agriculture Organisation (FAO) and therefore does not likely truly represent all shark catches worldwide. According to this information, Indonesia is the top shark-catching country in the world at present, representing about 13% of the total shark catches worldwide (Lack & Sant, 2011). Most sharks caught are taken as by-catch by fisheries targeting other species and, as a result, most of this by-catch is reported as unidentified shark or not reported at all, providing very little accurate information on the shark catch industry (Walker 1998). A growing number of sharks caught incidentally in some fisheries are being landed for human consumption, but many are still being discarded at sea, with only their fins being kept (Sonu and Region 1998). The major shark groups caught are requiem sharks (family Carcharhinidae) and dogfish, followed by smoothhounds (*Mustelus* spp.) (Vannuccini, 1999).

An indication of the total annual shark catches per continent is provided in Figure 2.1 (Anonymous, 2011). From observation of this figure, it is evident that Asia is the leading continent in terms of shark catches. For many years, Japan was the world's largest harvesters and consumers of elasmobranchs, but the Japanese share of the world shark catch decreased during the late 1900s. To fulfil the demand for sharks, Japan increased shark imports from \$600 000 worth in 1976 to \$18 million in 1997 (Sonu & Region, 1998). The decrease in Japanese shark catches is portrayed in Figure 2.2 and is compared with the increases in catches in the rest of the world during the late 1900s.

**Table 2.1** Top 20 countries by % of global reported shark catch (adapted from (Lack & Sant, 2011))

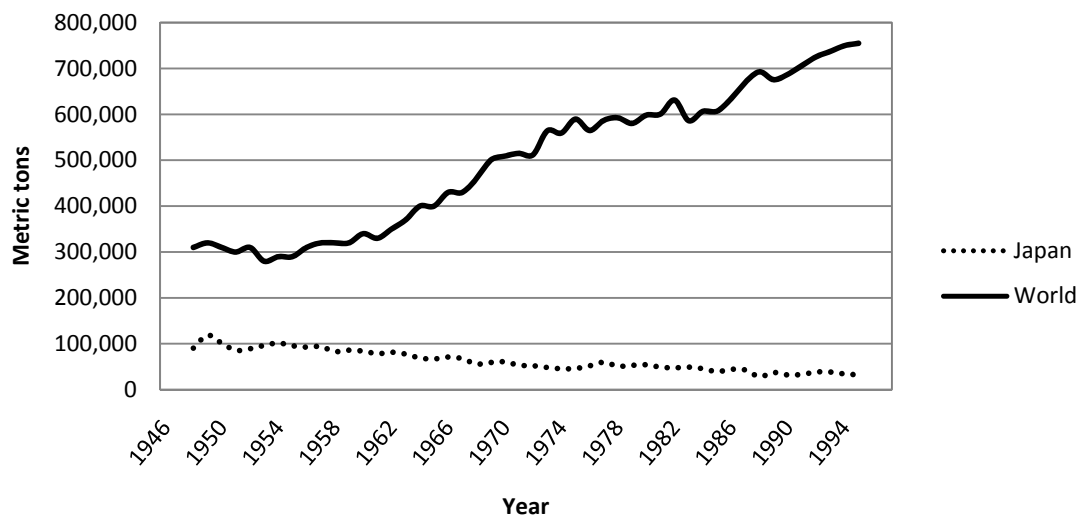
Top 20 Countries	% of global reported shark catch
Indonesia	13.0
India	9.0
Spain	7.3
Taiwan	5.8
Argentina	4.3
Mexico	4.1
Pakistan	3.9
United States	3.7
Japan	3.0
Malaysia	2.9
Thailand	2.8
France	2.6
Brazil	2.4
Sri Lanka	2.4
New Zealand	2.2
Portugal	1.9
Nigeria	1.7
Iran	1.7
U.K.	1.6
South Korea	1.4

### Annual shark catch per continent



**Figure 2.1** Annual shark catch (tons) per continent from 1970 to 2009 (adapted from Anon., 2011)

### Japanese vs global annual elasmobranch catches



**Figure 2.2** World and Japanes annual catches of elasmobranch (sharks, rays and skates) from 1948 to 1995 (metric tons) (adapted from (Sonu & Region, 1998)

## **Shark industry in sub-equatorial Africa**

There has been very little long-term data monitoring of chondrichthyan catches and fishing efforts in sub-equatorial Africa (Fowler, 2005). That long-term data that is available for this area has been recorded by the Natal Sharks Board in South Africa. In sub-equatorial Africa, increasing demands for chondrichthyan products locally and internationally (Clarke *et al.*, 2005) have motivated changes in local fisheries efforts. Such changes include the landing, drying, stock-piling and movement of large quantities of shark fins through major South African cities, such as Cape Town (Fowler, 2005). Kenya and Tanzania have substantial shark meat markets, with imports to Kenya from its neighbouring countries (Fowler, 2005). Kenya and South Africa act as African transshipment points for dried fins (Fowler, 2005).

Probable and possible major fisheries for cartilaginous fishes in the sub-equatorial Africa region include longline and drift gillnet by-catch of large oceanic sharks, semi-oceanic sharks and batoids (rays and skates) as part of the international high seas fisheries for scombroids (important marine food and game fishes found in all tropical and temperate seas). The bottom-trawl by-catch of sharks, batoids and chimaeras, which form part of the hake fisheries off South Africa and Namibia, also contribute to the catch. Chondrichthyans currently make up 11.6% of the total catch by weight of this inshore trawl catch (Attwood *et al.*, 2011). The sole fishery off South Africa and the prawn fishery off the KwaZulu-Natal coast of South Africa and Mozambique also land cartilaginous fish as by-catch (Fowler, 2005).

South Africa is not listed under the Top 20 shark fishing countries in the world (Lack & Sant, 2011), but it is, after Tanzania, the country with the most reported shark landings in sub-equatorial Africa (Fowler, 2005). Those landings reported by the FAO (Table 2.2) are significantly underestimated because these do not include the large chondrichthyan by-catch of demersal trawl fisheries that is largely discarded in this region (Fowler, 2005).

## **Shark industry in South Africa**

In a TRAFFIC Network Report, Rose (1996) listed South Africa as the only African country reporting a directed shark fishery on an industrial scale. Recently, however, due to concerns about high pelagic shark catches, these fisheries were phased out and incorporated into the tuna and swordfish longline fishery with a 2 000 ton limit on by-catch (DAFF, 2011). Fowler (2005) also reported that South Africa is the only country in sub-equatorial Africa reporting substantial yields (>1 000 tons in aggregate over 1985 - 2000) in terms of shark production and trade. South Africa produced 95 – 454 tons per annum of frozen shark meat and 52 – 66 tons per annum of shark fin from 1998 to 2000 (Fowler, 2005).



**Table 2.2** Elasmobranch landings (metric tonnes) by country within the Subequatorial African region as reported to FAO (2002) (adapted from (Fowler, 2005))

Country	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Angola	500	35	703	889	603	970	400	106	1 126	1 399	750
Comoros	-	-	58	58	-	-	-	-	-	-	-
Dem. Rep. of the Congo	748	580	596	597	445	380	315	250	185	120	45
Rep. of Cote d'Ivoire	255	297	379	335	256	258	288	501	407	265	762
Gabon	-	-	-	<0.5	5	55	1 439	799	2 023	1 535	800
Kenya	279	261	173	152	166	176	191	140	134	131	115
Madagascar	-	-	-	-	-	-	-	-	-	-	-
Mauritius	19	19	20	18	19	17	19	60	11	11	27
Mozambique	-	-	-	-	-	165	21	-	-	-	-
Namibia	2	76	24	1	96	247	332	438	278	608	1 548
Reunion	-	-	-	36	33	37	46	89	111	81	138
Seychelles	82	66	93	82	117	116	84	61	103	68	150
South Africa	2 513	2 476	2 620	2 933	2 209	1 833	1 719	2 174	2 075	1 801	1 665
Tanzania	3 865	4 381	4 500	3 473	3 863	4 510	5 600	5 000	4 675	4 875	5 000
Total	8 263	8 211	9 168	8 574	7 812	8 764	10 454	9 618	11 128	10 894	11 000

## History

Although sharks have long been utilised for their fins, skins, meat and for the production of fertilisers and oils, the first reported directed shark fishery in South Africa was initiated in the early 1930s off Durban, catching 8 609 elasmobranchs of which 6 681 were sharks (Kroese *et al.*, 1995). The shark industry was greatly stimulated during the Second World War by the increasing demand for vitamin A due to the disruption of cod fishing activities in the North Seas (Kroese *et al.*, 1995). Research conducted in South Africa found that shark livers contain oil suitable for vitamin A production and a shark fishery was initiated in around 1941 (Van Zyl, 1992). At this time, catches of up to 1 500 sharks were made per trip, each trip lasting about a week (Van Zyl, 1992). The artificial synthesis of vitamin A led the market for shark liver to collapse in 1950 (Lees, 1969; Van Zyl, 1992). Vitamin A production from shark liver continued in decreasing quantities until 1975, although sharks continued to be caught as by-catch and were exported as dried and/or salted meat to central Africa and as frozen carcasses to Europe, the Far East and Australia (Kroese *et al.*, 1995). Between 1968 and 1972, the demand for dried fish from Africa decreased drastically, presumably due to unacceptability of products from South Africa following decolonisation in these consumer countries. Consequently, most of the catch was sold frozen to Europe, the Far East and Australia (Kroese *et al.*, 1995). Shark catches, however, still continued and were reported as 144 832 landings in 1973 (Kroese *et al.*, 1995). The discovery of high mercury levels in shark meat in Australia (Walker, 1976) led to the severe restriction on international marketing of shark products (Kroese *et al.*, 1995). As a result, the demand for shark meat fluctuated between 1975 and 1990. Sharks processed for consumption were limited to smaller, younger sharks to avoid the risk of high mercury levels accumulated in larger, older sharks. Since then, the demand for shark meat and fins has shown a steady increase with approximately 18 tons of fins being exported from South Africa in 1993, of which 14.6 tons was destined to Hong Kong and 3.3 tons to Japan (Stuttaford, 1995).

## Shark catches

The total annual shark catches in South Africa is estimated at 3 500 tons with a significant increase in numbers over the past decade (Table 2.3). These were reported to be 0.36% of global shark catches in 2009 (Anonymous, 2011) and contributed approximately 0.3% (by mass) of South Africa's total commercial landings between 1979 and 1991 (MCM, 2010).

As most sharks caught are taken as by-catch, it has been difficult to record exact numbers of sharks caught and landed. Fisheries that have an impact on sharks and elasmobranchs in South Africa include demersal fisheries, longline fisheries, commercial line fisheries and shore-operated net fisheries as discussed below.

### *Demersal fisheries*

Based on fishery-independent demersal trawl survey data, South Africa's commercial trawl by-catch was estimated to be 22 000 tons in 1986 (Compagno *et al.*, 1994). The annual by-catch in the KwaZulu-Natal prawn fishery has been estimated to be approximately 315 tons, with about 75% consisting of teleosts and 15% elasmobranchs (Fennessy, 1994b; Fennessy, 1994a). Most of the teleosts are discarded, except for some commercially important species (about 17% of catch) and all the elasmobranchs are discarded (Fennessy, 1994a; Fennessy, 1994b). In commercial trawl fisheries in South Africa most of the elasmobranch by-catch is discarded, except for St. Joseph (*Callorhinchus capensis*), soupfin (*Galeorhinus galeus*) and smoothhound (*Mustelus spp.*) sharks, which are preferentially retained (Attwood *et al.*, 2011). Shark landings in the trawl fishery represent less than 0.2% for the offshore fishery, but approximately 10% for the inshore fishery (Kroese *et al.*, 1995), which is responsible for the greatest catch of a number of demersal sharks and other cartilaginous fish species (MCM, 2010). The impact of demersal fisheries on the status of elasmobranch stocks is unknown, however, it is known that the majority of elasmobranchs caught in trawls are discarded at sea (Kroese *et al.*, 1995). More recent recordings show that shark catches from deep sea trawlers in South Africa in 1997, 1998, 1999 and 2000 were 8 tons, 5 tons, 4 tons and 4 tons, respectively (Warman, 2003; Warman, 2004).

### *Longline fisheries*

*Demersal* – Limited data is available on elasmobranch longline catches in South Africa during the 80's, but landings of approximately 73 tons of sharks per annum (1987 - 1990), mostly soupfin sharks, were reported by Dudley & Compagno (1994). At the end of 1990, shark-directed demersal longlining was prohibited (Japp, 1993) and specific permits were required for shark-directed longlining in South African waters. By 1996, 31 permits issued between 1991 and 1994 were in use (Kroese *et al.*, 1995). An increase in international demand for fresh shark meat in the early 1990s motivated the targeting of certain shark species, primarily soupfin shark (*G. galeus*) and houndshark (*Mustellus spp.*) found in shallower inshore waters (Kroese *et al.*, 1995). From 1998, permits were again reduced due to poor fishery performance, with only six permits remaining since 2008 (MCM, 2010; DAFF, 2011).

The average catch per unit effort (CPUE) during 1992 to 1994 was 1 017 kg (dressed weight)/1 000 hooks for mako shark (*Isurus oxyrinus*) and 787 kg (dressed weight)/1 000 hooks for soupfin shark (*G. galeus*) (Kroese *et al.*, 1995). Since then these sharks, amongst others, have been increasingly exploited, as seen for soupfin shark and three other shark species in Table 2.3. From this data it is clear that smoothhound sharks are the most commonly caught shark in the South African demersal longline fisheries over the past few years.

*Pelagic* – The domestic pelagic longline fishery originally only targeted tuna and swordfish, with sharks as a by-catch. The foreign pelagic tuna-directed fisheries consists mainly of Japanese and Chinese vessels, which target offshore oceanic species such as mako sharks (*I. oxyrinus*), blue sharks (*Prionace glauca*), silky sharks (*Carcharhinus falciformes*) oceanic whitetip sharks (*Carcharhinus longimanus*), thresher shark (*Alopias vulpinus*, *A. pelagicus* and *A. superciliosus*), scalloped hammerhead (*Sphyrna lewini*) and porbeagle sharks (*Lamna nasus*) (MCM, 2010; DAFF, 2011).

**Table 2.3** Shark catches for demersal longline fisheries (kg dressed weight) (1992 – 2011)

Year	Southern sharks	Smoothhound sharks	Requiem sharks	Cow sharks	Total
1992	13955	-	-	-	13955
1993	5497	-	-	-	5497
1994	47946	79	-	30	48055
1995	43476	1141	-	433	45050
1996	47582	35	3	30	47650
1997	2015	20	-	-	2035
1998	18540	7068	862	243	26713
1999	77129	27111	6675	1118	112033
2000	53546	53263	22290	2607	131706
2001	17865	4723	1771	3171	27530
2002	8230	1503	1870	870	12473
2003	5497	-	1700	-	7197
2004	9922	5210	3007	180	18319
2005	2306	-	3103	1250	6659
2006	7992	21594	20327	46	49958
2007	9806	41579	31328	250	82963
2008	34025	64108	30098	2003	130234
2009	40496	56447	61586	1014	159543
2010	119703	121273	57398	1850	300224
2011	36995	75577	20429	25	133026

The total catches of pelagic sharks have increased sharply since 2003, from 394 to 537 tons in 2008, due to high market values and export markets to Europe and Asia (MCM, 2010). In 2010 the pelagic shark fishery landed shortfin mako (515 tons), blue sharks (198 tons), bronze whalers (25 tons) and skates (9 tons) and the large pelagic longline fishery landed shortfin mako (66 tons) and

blue sharks (100 tons) (DAFF, 2011). Aiming to decrease pelagic shark catches, this shark directed fishery was terminated and incorporated into the tuna- and swordfish-directed longline fishery in 2011 by issuing large pelagic rights to shark fishers, with a by-catch limit of 2 000 tons (DAFF, 2011).

#### *Commercial line fisheries*

This is the oldest fishery to have fished specifically for sharks and has been responsible for the biggest shark landings. Shark targeting has fluctuated greatly due to market demand. Even though an increased demand for shark products since 1991 has caused an upswing in the targeting of sharks and an increased numbers of shark landings, few commercial handline fishermen target sharks, but rather these fishermen target line fish for the more lucrative fresh fish market (Kroese *et al.*, 1995).

Sharks are targeted by line-fishermen mainly when teleost catches are low, or they are caught as a by-catch when targeting teleost species (Kroese *et al.*, 1995). The main shark species targeted by line-fishermen are soupfin sharks (*G. galeus*) and houndsharks (*Mustellus spp.*). Directed shark fishing is mainly concentrated on the southwest coast, with other fisheries operating primarily inshore off the south and east coast of South Africa. Other chondrichthyans being domestically caught include dusky sharks (*Carcharhinus obscurus*), copper sharks (*C. brachyurus*), spotted gully sharks (*Triakus megalopterus*), thresher sharks (family Alopiidae), cow sharks (family Hexanchidae), dogfish (*Squalus spp.*), catsharks (*Poroderma spp.*) and rays (family Dasyatidae). Of these, only a few are usually landed and the rest are discarded. Even though these sharks are discarded at sea, most sharks brought on deck are killed to simplify hook removal and then discarded, meaning that many more sharks are killed at sea than those that are being landed (Kroese *et al.*, 1995). Major shark catches in 2010 were reported as soupfin (89 tons), houndsharks (25 tons), Carcharhinid sharks (64 tons), blue sharks (13 tons) and skates (59 tons) (DAFF, 2011).

#### *Shore-Operated Net Fisheries*

A shark-directed commercial net fishery targeting St. Joseph shark (*C. capensis*) was established in the 1980s with an original catch of approximately 650 tons of St. Joseph shark per annum (DAFF, 2011). Commercial nets used traditionally include surface drift-nets, set-nets anchored at the bottom and beach-seine nets (Kroese *et al.*, 1995). The only nets targeting sharks in South Africa are in a legal bottom-set drift-net fishery for St. Josephs, an experimental beach seine fishery for sandshark (*Rhinobatus annulatus*), and an illegal gill-net fishery in the Langebaan estuary targeting houndsharks (*Mustelus spp.*) (Kroese *et al.*, 1995). Elasmobranch by-catch in beach seine nets targeting southern mullet (*Liza richardsoni*) in the False Bay area represent about 1.4% of the total catch (larger percentage by weight) (Lamberth *et al.*, 1994). Of the elasmobranch catch, skates and rays constitute almost 70% and sharks 30%, of which 15.9% are St. Joseph and 12.6% are smoothhound (*M. mustelus*) sharks (Kroese *et al.*, 1995).

Kroese et al. (1995) reported that approximately 5 tons (dressed weight) of houndshark are caught per month in an illegal houndshark fishery in the Langebaan estuary. These sharks are caught in shallow waters, which is probably a breeding and nursery area for smoothhound sharks (Kroese *et al.*, 1995).

Annual shark catches by beach seine and drift nets in South Africa in 1998, 1999 and 2000 were recorded as 14 tons, 100 tons and 100 tons, respectively (Warman, 2003; Warman, 2004).

#### *Total shark catches in South Africa*

Annual catches of different species of sharks, rays and chimaeras recorded or calculated by the FAO are depicted in Table 2.4 (Anonymous, 2011). From this data it is calculated that smoothhound sharks make up about 3% of the total catch of sharks, rays and chimaeras per year in South Africa. Table 2.5 provides information on the total catches, landings and values of shark per fishery per year in South Africa. Although some data are absent, it can be seen that the landed price as well as the landed value of shark has increased over these three years (1998 - 2000).

#### Shark processing

Fowler *et al.* (2005) reported that South Africa and Senegal were the only countries reporting substantial production of more than 1 000 tons of shark products in aggregate over the period of 1985 to 2000. South Africa produced 95 – 454 tons per annum of frozen shark meat from 1998 to 2000 (Fowler *et al.* 2005).

In South Africa, areas of inshore and demersal shark landings include Port Elizabeth, Mossel Bay, Vlees Bay, False Bay, Hout Bay, Gans Bay and Struis Bay. Large pelagic longliners land in Cape Town and Richards Bay. South Africa has four shark processing facilities (Fig. 2.3), of which three include Fishermen Fresh situated in Port Elizabeth, Eastern Cape (1), Selecta situated in Mitchell's Plain, Western Cape (2) and Xolile situated in Strand, Western Cape (3). Figure 2.3 shows the location of these three facilities as well as their areas of operation.

Small spotted gully sharks and two smoothhound species, *M. mustelus* and *M. palumbes*, are all processed and sold under the name gummy sharks. Bronze whaler sharks (*Carcharhinus brachyurus*), dusky sharks (*Carcharhinus obscurus*) and blacktip sharks (*Carcharhinus limbatus*) are all processed under the name bronzies. Blue shark and mako shark make up a small percentage of the sharks being processed (Da Silva 2007).

Shark meat is classified as “good”, “bad” or “big” by the industry (Da Silva 2007). “Good” sharks are those with high value flesh (smoothhound sharks, bronze whalers and soupfin shark) or good meat quality. The term “bad shark” describes sharks of lower value flesh (the larger spotted gully shark, hammerhead sharks and blue sharks) or inferior meat quality. “Big” sharks refer to sharks of large sizes that are not fit for human consumption due to the potential for high amounts of mercury to be present in their flesh.

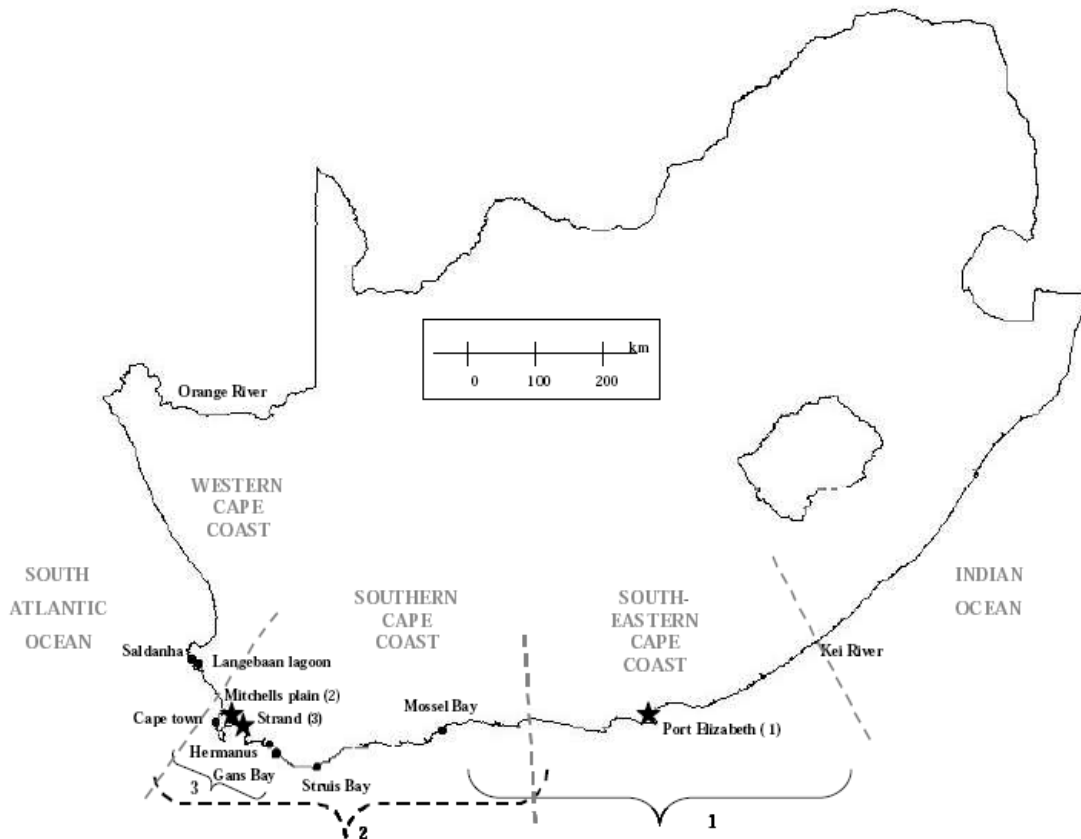
**Table 2.4** Catches of sharks, rays and chimaeras (tons) in South Africa (Anonymous, 2011)

Land Area	Species	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
South Africa	Sharks, rays, chimaeras										
	Blue shark	-	83	94	265	169	212	117	199	140	257
	Broadnose sevengill shark	0	0	0	0	0	0	0	4	12	12
	Cape elephantfish (St. Joseph)	380	405	422	524	559	645	749	702	585	623
	Copper shark	0	1	0	0	0	1	2	20	29	64
	Rays, stingrays, mantas nei*	1009	1152	1300	1507	1653	0	1220	1021	0	1
	Sharks, rays, skates, etc. nei*	410	167	357	209	435	1680	419	293	864	807
	Sharptooth houndshark	0	0	0	0	0	0	0	6	0	2
	Shortfin mako	-	79	31	147	659	689	453	548	48	491
	Smoothhound	-	4	2	1	24	81	81	90	76	85
	Thresher	0	2	-	-	-	4	1	3	5	2
	Tope shark	-	16	19	26	219	163	204	297	290	257
Total	Sharks, rays, chimaeras	1800	1909	2226	2679	3718	3475	3246	3183	2049	2601

\* Not elsewhere included

**Table 2.5** South African shark catches, landings and values per fishery per year (1998-2000) (Warman, 2003; Warman, 2004)

Fishery	Year	Nominal catch (t)	Landed mass (t)	Landed price (R/t)	Landed value (R'000)
SA Inshore	1998	214	89	479	43
	1999	117	49	507	25
	2000	117	49	3 000	147
SA line fish	1998	300	-	-	-
	1999	323	-	6 000	1 711
	2000	312	-	6 000	1 872
SA Misc nets	1998	6	9	3 100	19
	1999	100	100	3 000	300
	2000	100	100	3 240	324



**Figure 2.3** Map of South Africa showing distribution of shark fisheries and processing plants with their areas of operation marked with corresponding numbers (Da Silva, 2007) (1) = fishermen fresh, (2) = Selecta and (3) = Xoliile



Low meat quality refers to meat that is flaky or is slightly translucent in colour. This quality is usually determined by the initial handling and processing of the sharks. Larger sharks should not be picked up by their tails as this can cause the meat to ‘tear’ and become flaky (a phenomenon very similar to “gaping” found in traditional marine and fresh water fish).

For “good” quality shark meat, the sharks should be bled, beheaded and eviscerated immediately after capture, or within two to three days if refrigerated (Vannuccini, 1999). Bleeding must be performed immediately after capture by cutting the shark behind the head or just in front of the tail, letting it bleed for about 2 minutes or until most of the blood has drained from the carcass. The size of sharks classified as “big” is different for different species because some sharks grow faster than others and will therefore only reach potentially toxic levels of mercury at a larger size than other species. There is currently, however, a lack of information or guidelines on species-specific sizes relating to which sharks are classified as “big”.

#### Shark handling in a typical South African shark processing facility

The following information was obtained from a shark processing facility, Xolile, situated in Strand, Western Cape. Xolile is one of the four shark processing and export plants in South Africa. An average of 7 – 8 tons of shark is processed at this processing plant per month. All of the sharks are caught by hand line, being supplied by fishermen along the South Coast of South Africa, from Port Elizabeth to Cape Town (Fouche, 2011).

The sharks are euthanized and bled on the boat immediately after capture. Bleeding involves cutting the sharks behind the head, in line with the last gill slit and at the precaudal pit, perpendicular to the length of the shark. A cut is made along the ventral side from the anus (pelvic fins) to the anal fin in order to prevent the collection of blood in this region. The sharks are gutted and their heads are removed immediately after landing, after which they are transported to the processing facility without ice or cooling. When the sharks are received at the processing unit (0 – 3 days after death, depending on where the sharks have been caught), they are immediately packed in ice until further processing, which occurs immediately or a few hours after receiving. At the processing plant, the sharks are filleted, skinned and the fillets are packed into boxes. The packaged fillets are placed in a blast freezer overnight, following which they are labelled per species and are stored in a freezer until distribution.

Sharks destined for the export market include St. Josephs, bronze whaler and smoothhound sharks, which are purchased from fishermen at approximately R24 per kg (at the time that this information was obtained). These sharks are mainly exported to Australia, which has one of the biggest markets for shark meat. Sharks with meat of a lower quality, such as the blue shark, are purchased at approximately R5 per kg, processed and distributed to local restaurants or retailers as shark meat. Shark meat is sold in some local fish shops as ‘fish fillet’.

Random spot checks are performed regularly to determine the total mercury and cadmium contents of the shark meat, and the entire batch is discarded if mercury levels above  $1 \text{ mg}\cdot\text{kg}^{-1}$  (1 ppm) are detected in the meat. Mercury serves as an indicator of the heavy metal content of shark meat, as this metal is generally the first to reach the upper legal limit if the heavy metal content is high (Fouche, 2011).

### Exports

By 2005, the only countries in the sub-equatorial African region reporting exports exceeding 100 tons per annum of frozen shark were South Africa and Angola, with South Africa also playing a major role in shark fin exports to China. The FAO reported annual shark fin exports from South Africa at 52 - 66 tons whereas Hong Kong customs records showed imports from South Africa of approximately 195 tons in 2000 (Fowler, 2005). South African records show that the quantities of dogfish and other sharks exported as frozen fish in 2001 and 2002 amounted to 406 tons with a value of about 6 million Rand, and for 2002 and 2003 amounted to 445 tons with a value of 9.6 million Rand (Warman, 2003; Warman, 2004). Exports of shark fins in 2001 and 2002 were recorded at 49 tons with a value of 10 million Rand, while for 2002 and 2003 these were recorded at 14 tons with a value of one million Rand (Warman, 2003; Warman, 2004).

As is evident from Table 2.6, shark meat is also being imported into South Africa from a number of countries. It is, however, unclear what this shark meat is currently being used for. South Africa is also part of the shark fin trade, as can be seen in Table 2.7, with exports mainly to Eastern countries. The income for these products is extremely high as shark fins are one of the world's most expensive seafood products.

### **Shark products**

Most parts of sharks have been used in some way in the past, including the flesh, skin, liver, cartilage, teeth and fins. In some countries, the consumption of intestines, stomach, heart and skin is also common. Other products commonly made from sharks include fish meal, fertiliser, as well as liver oil, which is high in Vitamin A. Shark meat is consumed salted, dried or smoked in many communities (Walker, 1998). Dried and salted shark meat is popular as this processing method provides a convenient form in which to transport the product in areas where shelf-life would otherwise be limited (Vannuccini 1999).

Shark fin soup has been regarded as a delicacy in China for more than 2 000 years (Walker, 1998) with a value of up to R5 000 per kg (Hareide *et al.*, 2007). In some fisheries, only the meat is retained and the rest is discarded, while in other fisheries only the fins, liver or skin are retained. Few fisheries utilise all parts of the animals (Walker, 1998).

In many Pacific-Island countries, such as Australia, shark meat is commonly consumed as 'fish and chips' (Preston, 1984). Shark meat is often sold under names such as 'flake', 'grayfish',

'white boneless fillets', 'ocean fillets' or 'sokomoro' to disguise its true identity (Walker, 1998; Atkins, 2010).

**Table 2.6** South African annual imports and exports of shark meat (Warman, 2004)

Country	Year	Dogfish and other sharks			
		Import		Export	
		Mass (kg)	Rand	Mass (kg)	Rand
Australia	2002	-	-	79 741	3 226 105
	2003	-	-	97 307	6 530 638
Brazil	2002	-	-	-	-
	2003	-	-	49 614	214 711
Ecuador	2002	-	-	-	-
	2003	-	-	25 000	94 780
Germany	2002	-	-	11 714	41 233
	2003	-	-	23 500	141 000
Hong Kong	2002	-	-	-	-
	2003	-	-	500	93 230
Greece	2002	-	-	82 058	685 102
	2003	-	-	-	-
Italy	2002	-	-	92 903	1 341 395
	2003	-	-	190 516	2 378 611
Japan	2002	837 901	1 962 967	-	-
	2003	677 115	1 514 989	-	-
Malaysia	2002	-	-	-	-
	2003	-	-	9 872	59 232
Mauritius	2002	-	-	1 925	6 776
	2003	-	-	-	-
Mozambique	2002	-	-	8 018	15 630
	2003	-	-	-	-
Panama	2002	3 367	11 853	-	-
	2003	-	-	-	-
Portugal	2002	23 432	82 481	-	-
	2003	-	-	-	-
Seychelles	2002	-	-	-	-
	2003	20 042	49 967	-	-
Singapore	2002	-	-	13 823	82 938
	2003	-	-	-	-
Spain	2002	1 244	4 379	5 594	37 769
	2003	3 359	14 044	-	-
St Vincent & Grenadines	2002	30 981	109 053	-	-
	2003	26 386	78 376	-	-
Taiwan, Prov of China	2002	-	-	-	-
	2003	84 673	310 048	-	-
Thailand	2002	-	-	-	-
	2003	-	-	7	34
Tunisia	2002	-	-	1 408	4 956
	2003	-	-	-	-
UK	2002	-	-	-	-
	2003	-	-	4 000	22 057
Uruguay	2002	-	-	106 335	540 808
	2003	-	-	45 094	147 104

**Table 2.7** South African annual imports and exports of shark fins (2002, 2003) (Warman, 2004)

Country	Year	Shark fins			
		Import		Export	
		Mass (kg)	Rand	Mass (kg)	Rand
Hong Kong	2002	-	-	35 839	3 000 602
	2003	-	-	9 717	1 182 164
Japan	2002	-	-	-	-
	2003	1 784	161 472	4 320	872
St Vincent & Grenadines	2002	-	-	-	-
	2003	694	55 520	-	-
Taiwan, Prov of China	2002	9 570	671 050	-	-
	2003	9 201	920 100	-	-
UK	2002	-	-	-	-
	2003	153	306	-	-

Historically, shark meat and liver oil have been the main products being traded commercially and consumed locally throughout Eastern Africa and some Indian Ocean islands.

In Kenya, Tanzania and Seychelles, artisanal fishing involved sharks mainly in the production of dried/salted shark meat and the use of liver oil for maintenance of traditional vessels (Fowler, 2005). Being both nutritious and inexpensive, shark meat has served as a staple food for human consumption in the sub-equatorial African region (Fowler, 2005).

In Japan, shark meat is utilised raw, broiled, reconstituted after being dried, and in fish cakes. The fins are used for shark-fin soup, mainly in Chinese restaurants. The hides are processed into leather. Shark liver is also utilised for its oil, and the meat is made into fishmeal (Sonu & Region, 1998).

## Sustainability

Sharks are known as animals that are long-lived, slow growing, late maturing and producing few offspring. Overall, sharks have a low productivity that tends to be lower than that of other invertebrate groups of teleosts (Walker, 1998). Although this makes sharks vulnerable to over-fishing, a larger problem is, however, the lack of management of shark catches. The management of shark fishing has proven problematic due to a lack of co-ordinated research relating to the biology and stock assessment of commercially valuable sharks. Accurate stock assessment is made difficult by the large amount of illegal fishing and discards because sharks are largely taken as by-catch. The quantity of demersal sharks caught as by-catch in inshore trawl fisheries is higher than sharks caught by the directed demersal shark longline fishery (MCM, 2010). Greater efforts into the management of shark fishing are, however, currently being initiated (MCM, 2010).

Some shark species are much more vulnerable to overexploitation since they have lower productivity than others. Soupfin shark (*G. galeus*), sandbar/brown shark (*Carcharhinus plumbeus*), great white (*Carcharodon carcharias*) and some dogfish are some of the species with low productivity, whereas the gummy shark (*Mustelus antarcticus*) and other *Mustelus* species, Atlantic sharpnose shark (*Rizoprionodon terranovae*), bonnethead/shovelhead shark (*Sphyrna tiburo*) and blue shark (*Prionace glauca*) are species with a higher productivity (Walker, 1998).

The Southern African Sustainable Seafood Initiative (SASSI) was initiated in 2004 under the banner of the World Wildlife Foundation (WWF), with the aim of creating awareness about marine conservation impacts among participants of the fishing industry and consumers, as well as to promote compliance by the fishing industry with the prevailing South African fisheries regulations (Marine Living Resources Act, Act No. 18 of 1998) (SASSI, 2010). SASSI has established a detailed database and consumer seafood lists indicating the sustainability status of specific seafood species by classifying them under a green, orange or red list, based on abundance, conservation and legal status criteria. The green list includes fish species that come from healthy stocks and that can sustain current fishing pressure, while the orange list includes species that have worrying population trends, poor stock status or where the fishing method used for their capture has negative environmental impacts. The SASSI red list includes those species that are specially-protected, deemed for recreational fishing only, as well as those that are illegal to sell in South Africa (Anonymous, 2010). Shark species that are currently listed under the SASSI orange list include soupfin shark and houndshark (*Mustelus spp.*) caught by linefishing, while soupfin shark caught by inshore demersal trawlers are included under the SASSI red list. Nonetheless, it is often difficult in South Africa to obtain information at the point of sale relating to the fishing method used for capture (Cawthorn *et al.*, 2011), which limits the feasibility of this list to some extent when it comes to making the most sustainable seafood choices.

Due to the declines in linefish species caught off the South African coastline, demersal sharks such as smoothhound shark (*Mustelus mustelus*) have been increasingly exploited both as a target and as by-catch. Houndsharks are mainly caught by traditional linefishing, as well as being targeted by recreational line-fishermen and spear-fishermen. In terms of recreational fishing, a bag limit for houndsharks of 10 per day has been set. There is no minimum size limit and also no management measures in place for *M. mustelus*. As stated by the WWF, *M. mustelus* is likely to be less vulnerable to fishery pressure than other *Mustelus* species, but the absence of specific management measures could threaten the sustainability of this fishery (Anonymous, 2010).

According to Walker (1998), it is possible to harvest sharks sustainably. The challenge, however, is to limit the harvest rates to avoid further depletion of stocks. This can be done by implementing fishery management plans. Nonetheless, by 1998, of the 26 countries reporting annual shark catches greater than 10 000 tons, only South Africa, Australia, New Zealand and the United States had shark fishery management plans in place (Walker, 1998).

## Commercial species

Shark species that are targeted in South Africa by both commercial linefishing and demersal longline fisheries include smoothhound sharks (*M. mustelus*, *M. palumbes*), soupfin shark (*G. galeus*), bronze whaler shark (*C. brachyurus*), dusky shark (*C. obscurus*), hammerhead species (*Sphyrna spp.*), gully sharks (*Triakis megalopterus*), cow sharks (*Notorhynchus cepedianus*) and St. Josephs (*C. capensis*) (Da Silva, 2007).

### Mustelus spp.

There are over 20 shark species that are classified within the genus *Mustelus*, order Carcharhiniformes and family Triakidae. These sharks are bottom-dwelling, mostly found on the shelves and uppermost slopes of temperate and tropical continental seas (Smale & Compagno, 1997) and are also often abundant in closed bays with soft bottoms (Compagno, 1984b).

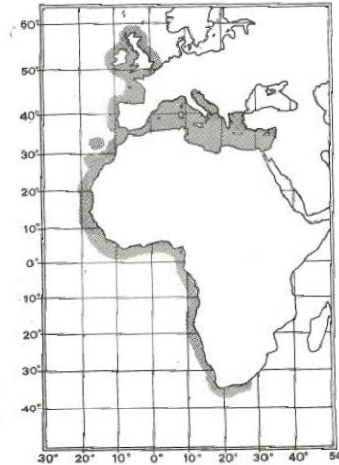
Sharks of the *Mustelus* genus are usually slender houndsharks with long parabolic subangular snouts, dorsolateral eyes, angular mouths, teeth formed into a pavement with cusps usually obsolete or absent and the second dorsal fin nearly as large as the first (Compagno, 1984b).

### Mustelus mustelus

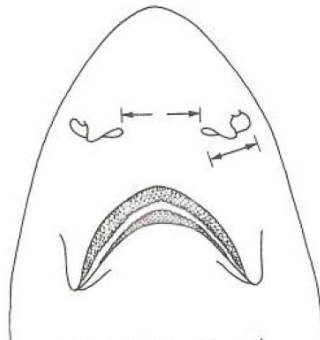
*Mustelus mustelus*, also known as a smoothhound shark, occurs off the coast of Southern Africa from Namibia to KwaZulu-Natal, as well as in the Mediterranean (Fig. 2.4) (Compagno, 1984b). These sharks have a short head (Fig. 2.5) and rounded snout, broad internarial space, large eyes, teeth with low, bluntly rounded cusps arranged in multiserial rows adapted for preying on crustaceans and other invertebrates. Their diet mainly includes crabs, shrimp, prawn, lobster, cephalopods, bony fish and offal. As the sharks grow, there appears to be a shift in the preferences of their diets from crustaceans and polychaetes to cephalopods and other fish, as well as in terms of the depth and location of their prey (Smale & Compagno, 1997). Smoothhounds are fairly slender with flattened ventral surfaces on the head and body as an apparent adaptation to benthic feeding (feeding on the surface of bottom sediments) (Fig. 2.6). Their colour is uniform grey or grey-brown on the top part of their body and light on their ventral surface, with some specimens having dark spots (Compagno, 1984b; Heemstra & Heemstra, 2004).

Males mature at 950 to 1 300 mm (6 - 9 years) and females slightly later, at 1 250 to 1 400 mm (12-15 years). The female sharks can reach a size of 1 700 mm total length (TL) (Smale & Compagno, 1997; Heemstra & Heemstra, 2004; Da Silva, 2007). Maturity can be determined by evaluating the clasper length which lengthens rapidly in males of 950 – 1 050 mm TL and calcification of the claspers which usually occurs at about 1 000 mm TL. A clear sign of maturity in males can be determined by observing the *vas deferens*, which changes upon maturity from being

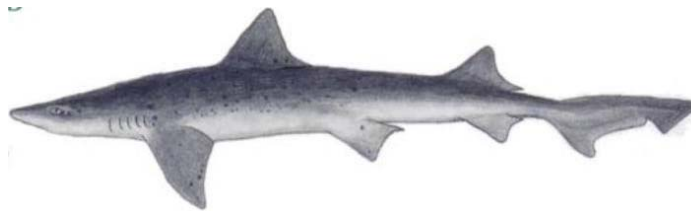
straight to becoming tightly coiled (Smale & Compagno, 1997). In females, maturity is characterised by an enlargement of the nidamental gland and widening of the uterus, as well as the ovarian eggs increasing in size and becoming more yolky and yellow in colour once they have exceed a diameter of about 5 mm (Smale & Compagno, 1997).



**Figure 2.4** Occurrence of *M. mustelus* (Compagno 1984)



**Figure 2.5** Underside of *M. mustelus* head (Compagno, 1984)



**Figure 2.6** Smoothhound shark *Mustelus mustelus*, 1.4 m mature female (Heemstra & Heemstra, 2004)

Mating occurs at the beginning of the year and the females have a gestation period of 9 to 11 months. The average litter size is 11 to 12 pups, but this can range from 2 to 23 pups per litter (Smale & Compagno, 1997). The sharks have viviparous development (the embryos develop inside the uterus), leading to live birth. The size of new-born pups ranges from 35 to 42 cm.

### **Commercial use**

Smoothhounds are commonly caught off the Southern African coast by commercial trawlers, linefishing boats and shore-based anglers (Smale & Compagno, 1997). In the past, these sharks have not been used for human consumption in this area, even though they are fished commercially and are considered as food fish in many other parts of the world. However, this started to change in the late 1980s, when smoothhounds began being targeted in the Western Cape of South Africa, particularly when numbers of prime teleost species were low. Today, areas of intensive smoothhound fishing include Struis Bay, Saldanha Bay and St Helena Bay (Smale & Compagno, 1997). The flesh of these sharks is dried and utilised locally or exported to other parts of the world such as Australia, Europe and Africa, while the fins of large sharks are exported to the East (Smale, 1997).

### **Nutritional composition of shark meat**

As early as 1918, it was recognised that fish represents a food of high digestibility and nutritive value for the purpose of human nutrition (Geiger & Borgstrom, 1962). Numerous further studies have confirmed that fish meat has an excellent amino acid composition and is a good source of nutrients and easily digestible proteins (YÁÑEZ *et al.*, 1976). Since the bodies of fish are supported by water, they tend to have less connective tissue than terrestrial animals, resulting in a desirable tender texture (Økland *et al.*, 2005).

#### Variation of proximate composition

Fish meat comprises several components, such as moisture, protein, lipids, vitamins and minerals, all of which contribute to the overall composition of the meat. These components may differ in quantity and nature according to their function and availability (Huss, 1988). The meat composition may therefore vary between individuals and different species, differing with seasons, gender, size, life cycle stage and anatomical position.

Proteins in fish muscle tissue can be divided into the following three groups (Huss, 1988):



1. Structural proteins (actin, myosin, tropomyosin and actomyosin), which constitute 70 - 80% of the total protein content (compared with 40% in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength ( $\geq 0.5$  M).
2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which constitute 25 - 30% of the total protein and are soluble in neutral salt solutions of low ionic strength ( $< 0.15$  M).
3. Connective tissue proteins (collagen), which constitute approximately 3% of the protein in teleostii and about 10% in elasmobranchii (compared with 17% in mammals).

While in some fish the protein can be evenly distributed across the muscular tissue, large pelagic species, such as tuna, are recognised to have distinct tissue groups within the muscular tissue (Balshaw *et al.*, 2008). The muscular tissue can be classified as 'white' or 'red' muscle. Red muscle is used for continuous swimming or 'cruising' and is usually high in myoglobin, hence its red colour (Bone, 1979). The white muscle is used for short bursts of swimming and therefore has limited myoglobin, resulting in the white colour. The positioning of the red muscle tissue depends on the swimming action of the fish, but the amount of red muscle usually increases towards the tail end. In most fish species, the white muscle tissue constitutes the major part of the muscle tissue, with red muscle never constituting more than 25% and in most cases less than 10% (Bone, 1979).

Unlike certain shark or fish species (such as shortfin mako, tuna and mackerel) that have continuous swimming motion, the smoothhound shows limited continuous movement. The latter sharks are found on sandy bottoms, where they are in search of benthic prey. Only occasionally would these sharks rise well above the bottom of the sea bed and swim faster (Smale & Compagno, 1997). The meat of smoothhound sharks is therefore composed mainly of white muscle, with smaller amounts of red muscle. As in many cartilaginous fish, the red muscle is situated as a thin subcutaneous sheet near the lateral line (Donley & Shadwick, 2003). Whereas stiff-bodied fish display lateral displacement restricted mainly to the caudal (tail) region when swimming, smoothhounds have highly undulatory movement (lateral displacement over much of the body) (Donley & Shadwick, 2003). This movement is caused by the activation of the layer of red muscle. The activation of these muscles causes the local bending of the body and the sequential wave of muscle contraction along the body, providing the forward movement of the shark (Donley & Shadwick, 2003). This specific distribution of red and white muscles as related to the shark's movement can therefore result in significant variation in the proximate composition of the meat across the body of the shark, since these muscle types differ in composition. Red muscle is usually more nutritious and has a higher polyunsaturated lipid content than white muscle (Love, 1988).

A component which causes substantial variation within the body of a fish is the lipid component. As soon as the lipid content exceeds 1% in a body region, that region can be classified as a fat depot (Huss, 1988). These fat depots are mostly located in the subcutaneous tissue, the

belly flap, the collagenous tissue between the muscle fibres and in the head section (Huss, 1988). The fat content is known to show large variation within species and individuals (Jacquot, 1961).

Certain fish species have also been found to exhibit an increase in oil/lipid content as their size increases (Huss, 1988), with sardines showing apparent fluctuations in the lipid content after maturity has been reached (Jacquot, 1961). In most fish, the maturation process is accompanied by a decrease in lipid reserves in the muscle due to the transportation of lipids to the gonads. Early stages of maturation are usually associated with an increase in body weight, with a corresponding weight loss as the gonads grow (Love, 1980). Gender also has a significant effect on the lipid content in many fish species. Female fish species generally require higher fat reserves, which are used during the development of their gonads during sexual maturation and embryonic development during gestation (Love, 1980).

An example of this variation is evident when considering the fat contents of salmon and herring, which can range from 0.35 to 14% and from 2 to 22%, respectively (Jacquot, 1961). For halibut, the fat content ranges from 0.5 to 9.6%, whereas the protein content stays constant at about 18% (Jacquot, 1961). From these observations it is clear that the protein and lipid contents are independent of each other. The lipid content is rather correlated to moisture content of the fish tissue, with an increase in the lipid content being associated with a corresponding decrease in the moisture content (Jacquot, 1961).

With regards to anatomical variation, the lipid content is the main component of variation. In certain fish, such as albacore, the lipid content in the ventral region is significantly higher than that in the dorsal and the anterior regions of the fish. This finding was confirmed in a study conducted by Suwandi (1995) on the chemical composition of dogfish, in which it was found that the lipid content of the belly flap is much higher than that of the fillet. Suwandi (1995) also reported that the fillet of the dogfish has a higher protein content than the belly flap.

It is claimed that female fish contain more protein than male fish. This is true for fish such as salmon, but it has also been found that the opposite is true for fish such as cod and immature Australian sea mullet (Jowett & Davies, 1938). Some authors have, however, reported no significant differences between genders with regards to the meat composition (Jacquot, 1961). Nonetheless, some species (e.g. horse mackerel) show significant differences in terms of protein content between males and females, although the magnitude of these differences depends on the season (Jacquot, 1961). The variation in meat composition can also be seasonal, due to changes in the diet and the stage of sexual development. The meat composition of fish, particularly the lipid levels, is in most cases closely linked to their diet composition. Many fish feeding on diets such as plankton, which subject to changes in abundance and composition, show variations in their flesh with changes in season (Jacquot, 1961).

The stages of sexual development can influence both the composition of the meat as well as the meat quality, which includes appearance and texture. Sardines, for example, have a relatively constant lipid content before maturity, after which the lipid content increases and begins

to fluctuate (Jacquot, 1961). In certain fish, the protein content of the females fluctuates during the sexual cycle. Frequently, the flesh attains its maximum fat content and best meat quality prior to spawning, whereas during spawning the fish lose weight and have a poorer meat quality at the end of this period (Jacquot, 1961). The latter observation may, however, also be associated with a lower dietary intake during the spawning period, making it difficult to distinguish between the influences of sexual stage and feeding (Jacquot, 1961). Nevertheless, this seasonal variation is not true for all species. Some species of Cape hake (*Merluccius* spp.) maintain a practically stable nitrogen content throughout the year (Jacquot, 1961).

It has been observed that the oil (lipid) content of some fish species varies with size, with larger fish having a higher oil content than smaller fish as cited in Huss (1988).

#### General proximate composition of sharks

All fish meat, including shark, consists mainly of water, crude protein and lipids. Sharks have been described as having an average proximate composition of 77.2% water, 19% protein, 2.5% lipid and 1.3% ash (Jacquot, 1961). The different proximal components will be discussed in more detail for fish in general, but more specifically for elasmobranchs and sharks, where literature could be sourced (it is worth noting that most of the data sourced is dated).

#### *Protein*

The protein content of sharks as cited by Geiger and Borgstrom (1962) forms approximately 22% of the flesh. As mentioned earlier, the protein in fish can be divided into three groups: structural proteins (70 - 80% of total protein content), sarcoplasmic proteins (25 - 30% of total protein content) and connective tissue proteins which constitutes a larger fraction in elasmobranchs (10%) than in teleost fishes (3%) (Huss, 1988).

The amino acid composition of fish varies between species as well as within species, between fish of different ages and sizes. When comparing shark and skate protein with casein, studies have shown that the fish proteins are very rich in lysine, arginine, alanine, glutamic acid, threonine and cysteine and contain higher levels of arginine, isoleucine and methionine than that found in casein (Geiger & Borgstrom, 1962). Casein is, however, superior to the fish protein in terms of the phenylalanine, tyrosine, proline, threonine and tryptophan contents (Geiger & Borgstrom, 1962) and elasmobranchs appear to lack albumin (Irisawa & Irisawa, 1954). Shark and skate proteins are therefore considered to be comparable, if not superior to casein, with regards to the amino acid composition. Consequently, shark protein can be considered as a good source of essential amino acids and can serve as a cheap substitute to fulfil several amino acid deficiencies in protein-poor diets (Geiger & Borgstrom, 1962).

Fish muscle also contains nitrogen-containing compounds of non-protein nature. In elasmobranchs, this non-protein nitrogen (NPN) can make up approximately 34 - 38% of the total

nitrogen (Geiger & Borgstrom, 1962). In sharks, the largest fraction of this NPN consists of urea, which occurs not only in the liver (as in land animals), but all over the body. Urea is formed in a process where arginine (released by autolysis of the tissue proteins) is converted to urea and ornithine with the catalytic action of arginase (a hydrolytic enzyme). Unlike teleost fish species, elasmobranches are ureotelic organisms, meaning that they produce urea as their main nitrogenous excretory product (Baldwin, 1960). Additionally, in elasmobranches, arginase is present in large concentrations throughout the body rather than in the liver only as in other ureotelic organisms (Baldwin, 1960). Urea also acts as a major osmolyte in marine elasmobranches and is retained in the body fluids in large quantities (Hazon *et al.*, 2003). Elasmobranch gill epithelia is particularly impermeable to urea, but the large surface area combined with a significant concentration gradient allows for diffusional loss of urea through the gills (Hazon *et al.*, 2003). The rate of loss of urea is, however, almost equivalent to the rate of urea synthesis, causing the concentration of urea in the body to remain almost constant (Hazon *et al.*, 2003) and much higher than in other animals. Although urea is non-toxic, it is converted to ammonia by bacteria and can result in a strong ammonia taste and odour if the shark meat is not handled correctly (Vannuccini, 1999). Sharks should be bled immediately after capture and should be dressed and iced as soon as possible in order to prevent the urea in the blood from contaminating the meat (Vannuccini, 1999).

Trimethylamine oxide (TMAO) is another NPN compound occurring in large quantities (more than 2.5% dry weight) in the meat of elasmobranches and which can reach 26% of the total nitrogen in certain sharks (Jacquot, 1961). Urea and TMAO are compounds which assist sharks to maintain their osmotic balance (Hazon *et al.*, 2003).

Table 2.8 lists some of the NPN components contained in shark meat compared to that found in cod fish. In terms of free amino acids, shark and cod have a similar composition, but the levels of TMAO and urea are markedly higher in sharks than in cod.

**Table 2.8** Differences in muscle extractives between cod and shark species (Huss, 1988)

	Cod	Shark spp.
	mg·100 g <sup>-1</sup> wet weight	
Total free amino acids	75	100
Arginine	<10	<10
Glycine	20	20
Glutamic acid	<10	<10
Histidine	<1.0	<1.0
Proline	<1.0	<1.0
TMAO	350	500 – 1 000
Urea	0	2 000

### *Lipids*

Fish, and more specifically sharks, are known to have lipids of high nutritional value. Fish meat is rich in omega-3 fatty acids (n-3 FA), with deep sea fish having a particularly good ratio of n-6 to n-3 FAs (Økland *et al.*, 2005). The recommended maximum for the n-6:n-3 fatty acid ratio is 4 as specified by the Department of Health of the United Kingdom (UK) (Justi *et al.*, 2003). Fish lipids are highly unsaturated, with up to five or six double bonds (Huss, 1988). Okland *et al.* (2005) found that polyunsaturated fatty acids (PUFAs) form a significant part (48 to 63%) of the total fatty acids in elasmobranchs, and most cartilaginous fish have lower levels of free fatty acids as well as lower levels of cholesterol than bony fish (Økland *et al.*, 2005). The lipids of cartilaginous fish such as sharks may have diacyl alkyl glyceryl ethers or hydrocarbon squalene as significant components of their fat (Huss, 1988). Jacquot (1961) classified shark as a semi-fatty fish with an average lipid content of 2.5%.

Fish oils, in general, contain about 15 - 40% (of total fatty acids weight) saturated fatty acids, with the main saturated fatty acid being palmitic acid (C16:0) with myristic acid (14:0) and stearic acid (18:0) occurring in small amounts (Tsuchiya, 1961). The fatty acid, *n*-tetracosanoic acid (C24:0), has also been detected in shark liver oils (Jacquot, 1961). An unsaturated fatty acid widely occurring in most fish oils and other marine animal oils is oleic acid (18:1n9). Eicosanoic acids have additionally been found specifically in several elasmobranch fishes and in the liver oil of the sharks. Other trienoic acids found to be present in some shark species include eicosatrienoic acid (20:3n3) and docosatrienoic acid (22:3) (Jacquot, 1961). Docosatetraenoic acid (22:4) has been noted to occur in the liver oil of sharks and appears to occur widely in the oil derived from numerous marine species (Tsuchiya, 1961).

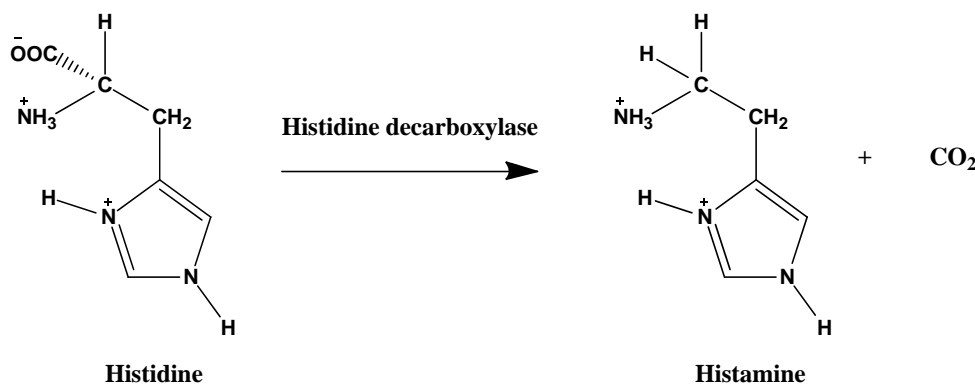
### **Safety of shark meat**

Although shark meat is considered to have a favourable nutritional value, there are, nevertheless, certain substances that may be present in shark meat which can have adverse effects on human health.

#### Biological safety

Histamine poisoning is an intoxication resulting from the ingestion of food containing unusually high levels of histamine (Taylor, 1986). It is often referred to as scombrototoxin or scombroid fish poisoning since it has historically been associated with the consumption of spoiled scombroid fish, such as tuna, skipjack or mackerel. Nonetheless, non-scombroid fish, such as marlin and many other fish species, have more recently also been found to be a causative agent in this illness (Taylor, 1986).

Histamine is a biogenic amine produced in foods by the decarboxylation of the corresponding free amino acid, histidine. This decarboxylation reaction is catalysed by bacterial amino acid decarboxylases (Ababouch & Gram, 2004). Free histidine is a naturally occurring amino acid in fish muscle and is generally found in large amounts in the muscle of fatty, red-meat active and migratory fish species (Ababouch & Gram, 2004). This free amino acid serves as a substrate for bacterial histidine decarboxylase with the subsequent formation of histamine (Fig. 2.7).



**Figure 2.7** Reaction equation for the formation of histamine (Ababouch & Gram, 2004)

The formation of high amounts of histamine in fish tissue is generally associated with time and temperature abuse of the harvested fish. Due to the poor handling and treatment of sharks post-harvest, shark meat is expected to be susceptible to bacterial contamination, with the subsequent potential for histamine formation. As sharks have a high content of urea, they should be cut and bled immediately after capture. The meat is therefore rapidly exposed to environmental conditions, making it vulnerable to bacterial contamination. If the carcasses are not cooled immediately after bleeding, as is the case on most fishing boats, optimal conditions are created for histamine to form if there is a sufficient concentration of histidine in the muscle to serve as a substrate.

In general, fish should be placed on ice, in cooled seawater or in brine at a temperature of 4.4 °C or lower for 12 hours after death, or at 10 °C or lower for 9 hours after death. Fish exposed to water or air exceeding 28.3 °C must be put on ice, in cooled seawater or brine at a temperature of 4.4 °C or lower for 6 hours after death (Smajlovi *et al.*, 1999). Such treatments should prevent rapid development of the bacterial histidine decarboxylase enzyme. This prevention is very important since heating inactivates the bacteria and their enzymes, but neither heating nor freezing can eliminate histamine once it has formed (Ababouch & Gram, 2004).

Histamine is more toxic when consumed in fish than when in its pure form and even small amounts in fish can be more toxic than larger amounts of pure histamine. Guidelines have been

set by the FAO for the maximum levels of histamine in fish (Ababouch & Gram, 2004). Fish with levels lower than  $5 \text{ mg} \cdot 100 \text{ g}^{-1}$  are considered to be safe for consumption, fish with levels between 5 and  $20 \text{ mg} \cdot 100 \text{ g}^{-1}$  are possibly toxic, fish with levels between 20 and  $100 \text{ mg} \cdot 100 \text{ g}^{-1}$  are probably toxic and levels above  $100 \text{ mg} \cdot 100 \text{ g}^{-1}$  are toxic and unsafe for human consumption (Ababouch & Gram, 2004). The FDA has established a  $50 \text{ mg} \cdot \text{kg}^{-1}$  (ppm) upper limit for histamine in seafood (Ababouch & Gram, 2004).

Although information on histamine levels in shark meat is currently limited, Huss (1988) reported that the histidine content in shark meat is relatively low ( $<1.0 \text{ mg} \cdot 100 \text{ g}^{-1}$  wet weight) and Amano and Bito (1951) reported that high levels of histamine do not appear to be formed in shark meat. However, thorough research has not yet been done on this topic.

### Chemical safety

Most sharks are large, long-lived fish and are at the upper level of the marine food chain. As such, they are highly susceptible to the bioaccumulation in their flesh of heavy metals, such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As). Mercury is considered to be the most significant heavy metal contaminant in shark meat. The consumption of high levels of mercury can lead to mercury poisoning, which mainly affects the central nervous system of the human body (Ruelas-Inzunza & Paez-Osuna, 2005).

The mercury levels in fish tend to increase with an increase in trophic level from plankton-feeding fish to larger predatory species, due to a process known as biomagnification (Ababouch & Gram, 2004). Another reason for the high levels of mercury in large fish species, such as sharks, is the accumulation of mercury in the body of an individual over its life span (Ababouch & Gram, 2004). The on-going accumulation is explained by the fact that organic mercury (methylmercury), which constitutes about 70 - 93% of total Hg in muscle, is a stable form of mercury in the meat since it binds strongly to thiol groups of proteins, the content of which increases with age (Storelli *et al.*, 2002; Järup, 2003). Mercury is mostly released into the environment in its inorganic form by pollution, but is then converted by bacteria in the sediment into its organic form, methylmercury (MeHg), which is the more toxic forms to humans.

The maximum allowable level of mercury in fish and fish products according to the regulations set in the United States, European Union and South Africa is 1 ppm or  $1 \text{ mg} \cdot \text{kg}^{-1}$  wet weight (FDA (US Food and Drug Administration), 1998; EC (European Commission), 2001b; DoH, 2004). As larger sharks contain higher levels of mercury due to bioaccumulation, there is a limit on the size of sharks used for human consumption. Some shark species have lower mercury content at a larger size than others and can therefore be used for consumption at large sizes. Soupfin sharks, for example, are sold from 1.5 – 12 kg, but sharks over 12 kg contain dangerous levels of mercury. On the other hand, smoothhound sharks above 12 kg are still used for consumption, but these sharks are, however, sold at a lower price due to their fillets being of a lower quality (Da



Silva, 2007). Storelli *et al.* (2002) studied mercury levels of several sharks in the Mediterranean Sea and reported that hammer head sharks had the highest mean mercury levels (18.29 mg·kg<sup>-1</sup> muscle) among the evaluated species. Gulper sharks (*Centrophorus granulosus*), longnose spurdog (*Squalus blainville*) and kitefin (*Dalatias licha*) sharks were also found to have high mean levels of mercury (9.66, 4.53 and 4.38 mg·kg<sup>-1</sup> muscle, respectively) whereas velvet belly (*Etmopterus spinax*) and smoothhound (*M. mustelus*) sharks had the lowest levels of mercury (0.63 and 0.31 mg·kg<sup>-1</sup> muscle, respectively).

Walker (1976) evaluated the effects of species, sex, length and locality on the mercury content of two sharks, the school shark (*Galeorhinus australis*) and gummy sharks (*Mustelus antarcticus*), and showed that there was significant variation in the mercury content of these sharks. School sharks had significantly higher mercury levels than gummy sharks and the mercury content increased exponentially with increasing shark length. This variation between species could be expected to be due to the varying diets of the different species, with prey from higher trophic levels having higher mercury levels than those from lower trophic levels. The exponential increase with size can be anticipated to be due to bioaccumulation of mercury in the shark flesh. Medium-sized and large males had significantly higher mercury levels than the females of the corresponding sizes and species. The latter observation could be explained by the different growth rates of the genders, with females having a higher growth rate than males. There are therefore many factors that can cause variation of mercury contents in shark meat.

In 2007, two rapid alert notices were issued in South Africa by the National Regulator for Compulsory Specifications (NRCS) for levels of heavy metals exceeding maximum legal limits for frozen fish, one of which was for mercury in frozen shark meat (Dreyer, 2010). The high levels of mercury reported by the NRCS for frozen shark products on the local market has led to an increase in monitoring action and as a result, shark processing factories are obliged to test every batch for mercury before it may be released for sale on the market (Da Silva, 2010; Fouche, 2011). The Council for Scientific and Industrial Research (CSIR) also recently published a report which stated that 'mercury poisoning could result from daily intake of some of our local fish' (Basson, 2009). As mercury is associated with the protein component in the meat, variation in the protein content across the carcass would result in some variation in the mercury content. There are, however, no guidelines as to where in the body samples should be sourced for mercury analyses. Thorough investigation into the variation of mercury content in shark muscle is therefore needed in order to set up guidelines for sampling, as well as into which sharks (species, size and gender) should be safe for human consumption.



## Conclusion

Even though the shark industry forms a substantial part of South Africa's fishing industry, and shark meat is consumed locally as well as worldwide, there is a lack of research on the meat composition of sharks caught in and exported from South Africa. From literature gathered on fish and shark meat in general, it appears that shark meat is a good source of essential amino acids and comprises a healthy lipid composition in terms of human nutrition. Shark meat is, therefore, a cheap source of high food value. Shark meat may, however, contain substances of concern, such as mercury and histamine which may accumulate and form in the flesh of the sharks and can have adverse effects on human health when consumed.

Thorough research on the chemical composition of *Mustelus mustelus* meat, which is one of South Africa's main commercial species, is therefore of great importance as consumers are increasingly wanting to become more aware of the nutritional value of the food they eat and the affects it will have on their health.

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## Chapter 3

### **CROSS CARCASS VARIATION IN THE PROXIMATE COMPOSITION OF *MUSTELUS MUSTELUS* MEAT**

#### **Summary**

The aim of this study was to determine the anatomical variation of the proximate components (moisture, protein, lipids and ash) in *Mustelus mustelus* meat. No significant differences between five different fillet sites ( $n = 23$ ) were detected for all the proximate components. An average proximate composition of 75.31% moisture, 23.08% protein, 1.58% lipid and 1.36% ash can therefore be considered as representative of the entire edible fillet. The protein content was negatively correlated with the moisture content (Pearson's correlation co-efficient: -0.88). The lipid content displayed the highest variation between individual sharks and fillet sites (CV = 61). From these results, *Mustelus mustelus* was confirmed to have a uniform meat composition across the carcass and sampling for further chemical analyses can be performed at any position on the fillet.

#### **Introduction**

Fish meat consists of several components that all contribute to its overall chemical composition. These components, which include moisture, protein, lipids and minerals, can differ in nature and quantity according to their function and availability (Love, 1980; Huss, 1988). Factors that play a role in the meat composition can be both endogenous (genetic) and exogenous (related to diet and the environment) (Shearer, 1994). The meat composition may also vary at the different anatomical positions of the body since these have different functions and therefore different chemical compositions (Love, 1980). The aforementioned differences can largely be attributed to the fact that fish have two basic muscle types, namely red muscle tissue and white muscle tissue. The red muscle is used for slow, continuous movements, whereas the white muscle is used for rapid, sudden movements (Love, 1980). Thus, fish species that exhibit a high level of activity will have a greater proportion of red muscle tissue than those that are fairly sedentary (Love, 1988).

*Mustelus mustelus* sharks belong to the order Carcharhiniformes and the family Triakidae (Compagno, 1984a). They are most commonly found on the shelves and uppermost slopes of temperate and tropical continental seas (Smale & Compagno, 1997). These sharks are benthic feeders, found on sandy bottoms, mostly swimming not more than 50 mm off the bottom in pursuit of prey, and moving by lateral undulation (Smale & Compagno, 1997). They therefore have a layer of sub-cutaneous red muscle situated near the lateral line which is active in the lateral bending of the body (Donley & Shadwick, 2003). White and red muscle differs in composition as they have different functions. The red muscle usually contains higher levels of polyunsaturated lipids and is



more nutritious than the white muscle (Love, 1988). It can therefore be expected that the composition of the meat will vary across the carcass as some locations in the body of the shark will have larger proportions of red muscle (fillet) than others (belly flap).

The lipid content contributes significantly to the variation in meat composition across the carcass of fish. If the lipid content exceeds 1% (weight per wet sample of meat) at any specific area in the fish body, that area is classified as a fat depot (Huss, 1988). These fat depots are usually situated in the head section, the belly flap, the sub-cutaneous tissue and the collagenous tissue between the muscle fibres (Huss, 1988). The lipid content of the meat is known to be inversely related to the moisture content. Thus, as the levels of lipids in the meat increase, it can be anticipated that there will be a corresponding decrease in the moisture levels (Huss, 1988). Particularly in fish with large fat depots, variations in the proximate composition (moisture, protein and lipids) can consequently be expected across the body of the fish.

*Mustelus mustelus* is one of the commercial shark species in South Africa which is consumed locally as well as exported globally. To date, however, no information has been published relating to the chemical composition and nutritive value of its meat. Consequently, it is currently considered to be a low-value product and a large quantity of the shark meat is discarded and wasted at sea by fishermen targeting species of a higher value.

For the purpose of determining the overall chemical composition of *M. mustelus* meat, it is imperative that the sample analysed is representative of the entire shark. However, in order to determine which portion of the meat is the most representative for such analyses, it is necessary to first determine the degree of variation in the chemical composition across the carcass of the shark. For shark fillets used commercially, the sample used for chemical composition determinations should be taken in the most economical manner possible, causing little damage to the fillet but still being representative of the entire fillet.

The aim of this study was to determine the proximate composition of *M. mustelus* sharks at different body sites so as to evaluate the cross-carcass variation existing within the meat for the individual proximate components (moisture, protein, lipid and ash). This variation was determined in order to identify the most representative sample of the edible part of the shark (fillet and body flap) for future proximate analysis applications.

## **Materials and methods**

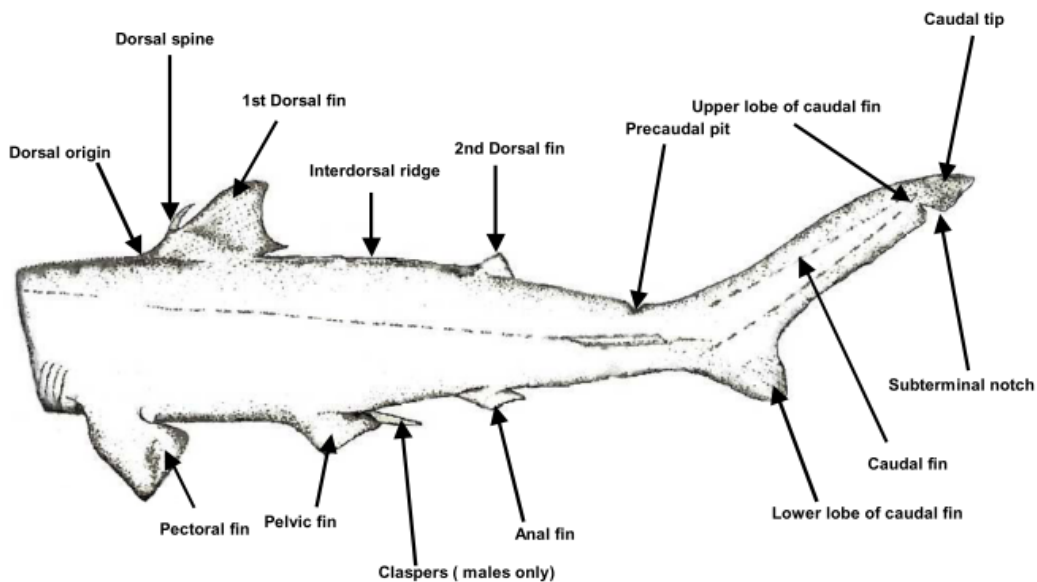
### Sampling

#### *Harvesting*

The sharks were caught from a small fishing boat in the Langebaan lagoon, Western Cape, South Africa (ethics clearance number: 2009V17CA) with the use of fishing rods. Sampling was conducted over four fishing trips from the end of September 2010 to the middle of December 2010,

with an average catch rate of 16 sharks per trip. The aim was to obtain an equal distribution of sharks over all different sizes and both genders, thus not all caught sharks were euthanized and landed, but some were released after determining gender and/or size.

The sharks were euthanized by a sharp blow on the head. Thereafter the sharks were bled by making a cut on the ventral side of the precaudal pit (the area or notch found at the narrowest part of the base where the caudal (tail) fin begins) (Fig. 3.1), and holding it vertically with the tail hanging downwards for about 2 minutes or until most of the blood had drained. The dead sharks were then kept in cool sea water until the vessel returned to shore (maximum 3 hours). The sharks were loaded into containers of crushed ice and transported directly to the Department of Agriculture, Forestry and Fisheries (DAFF) laboratory in Cape Town, where they were either kept on ice to be dissected the following day or frozen for later dissection.



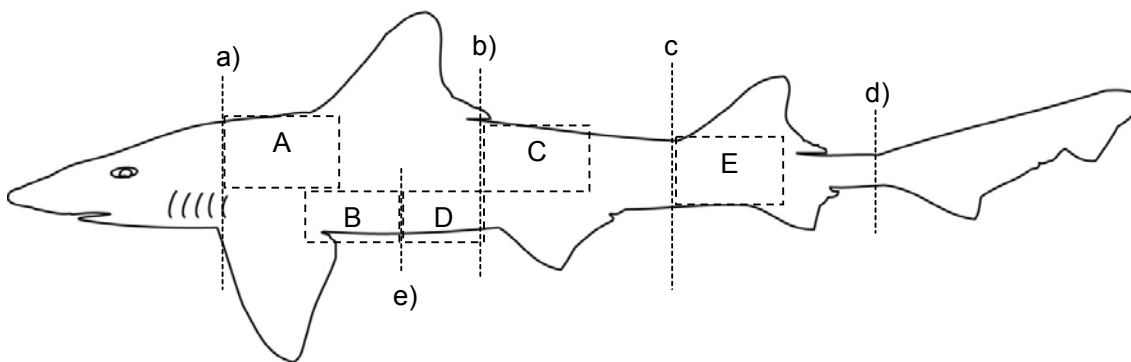
**Figure 8** Anatomy of a generalised shark (Da Silva, 2007)

### *Dissection*

The sharks were weighed to record the live mass and measured to record total length (TL) before being gutted. The heads were cut off behind the last gill slit (a) (Fig. 3.2) and the tails were removed at the precaudal pit (d) (Fig. 3.2) where the cut was made for bleeding the shark, with direct vertical cuts perpendicularly to the length of the fish. The fins were removed prior to the sharks being filleted. After filleting, the belly flaps were cut from the fillets at the position where the stomach cavity ends. The fillets with their skin on, excluding the belly flap, were weighed before cutting the fillet into three samples and the belly flap into two samples for a total of five samples per shark carcass. For large sharks, only the right fillet was used, but for smaller sharks both the left and right fillets were used for samples in order to supply sufficient meat per sample. The fillets



were divided into three samples by cutting the fillets after the first dorsal fin (b) (Fig. 3.2) and before the second dorsal fin (c) (Fig. 3.2). The belly flaps were divided into an anterior and a posterior sample by cutting them in the middle of the belly flaps, at the position that is in line with the tip of the first dorsal fin (e) (Fig. 3.2). The reason for taking these samples at the specified anatomical positions rather than at specific distances on the fillet was due to the large variation seen in the fillet sizes of the different sized sharks. This method of sampling provides anatomical consistency between samples of different sharks. The five specified samples were labelled A, B, C, D and E as shown in figure 3.2. Samples were vacuum packed in polyethylene bags and transported back to Stellenbosch University where they were frozen at  $-18^{\circ}\text{C}$  until further processing.



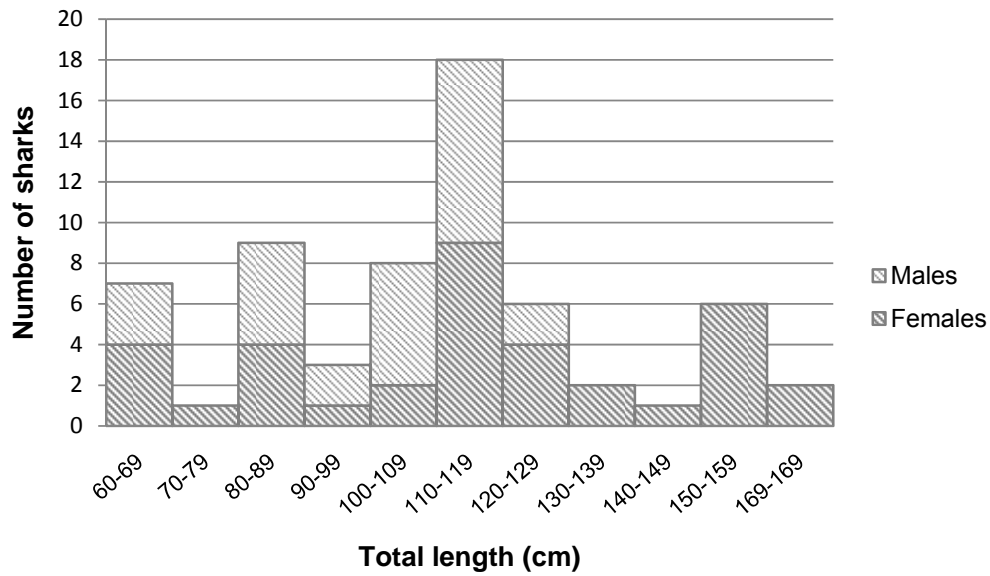
**Figure 9** Diagram of a smoothhound (*M. mustelus*) shark showing the positions at which the carcass was cut (a, b, c, d and e) and the positions of the 5 different sites and the corresponding sample codes (A, B, C, D and E)

#### *Sample demographics*

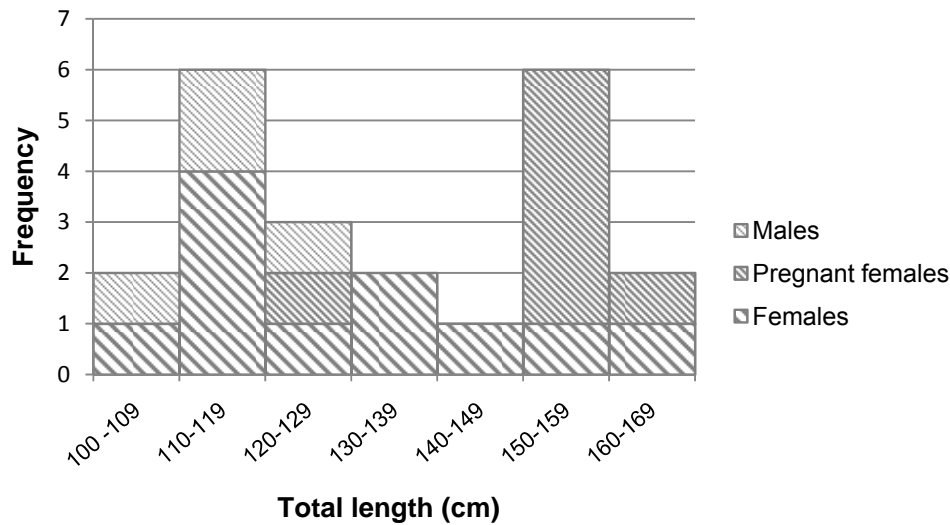
A total of 64 sharks from both genders across the entire size range (57 – 165 cm) (Fig. 3.3) were caught and processed. The entire sample set consisted of 27 males and 37 females of which 7 were pregnant. Two of the sharks were too small to take sufficient samples from, therefore only 62 sharks were used for chemical analyses.

#### Sub-sampling

From the total sample set, 23 sharks were selected for cross-carcass variation analyses. These 23 sharks were all from the top section of the size range (103 – 165 cm) in order to have large enough meat samples for all five fillet sites. The sub-sample included both males ( $n = 4$ ) and females ( $n = 19$ ), of which seven were pregnant, in order to obtain a sub-sample which was representative of the population (Fig. 3.4).



**Figure 10** Distribution by length and gender of the total sample group of smoothhound (*M. mustelus*) sharks (n = 64); shown as a categorisation of continuous data.



**Figure 11** Distribution by length, gender and life cycle stage of smoothhound (*M. mustelus*) sharks (n = 23) used for the investigation of cross-carcass variation, shown as a categorisation of continuous data.

## Proximate Analysis

### *Sample preparation*

The frozen vacuum packed fillet samples were removed from the freezer 24 hours prior to processing and these were thawed at 4°C. The skins were removed from the thawed samples and the meat samples were homogenised, vacuum packed and frozen at -18°C until chemical analyses. The frozen, homogenised samples were removed from the freezer and thawed at 4°C 24 hours before the chemical analyses.

### *Moisture content*

The moisture contents (% wet weight) of 2.5 g homogenised meat samples were determined for all samples in duplicate by drying for 24 hours at 100°C as described in the official method of the Association of Official Analytical Chemists (AOAC, 2002b).

### *Total protein content*

The total crude protein (% wet weight) of the defatted, dried and ground meat samples was analysed in duplicate by means of the Dumas combustion method 992.15 (AOAC, 2002a). The samples (0.1 g) were encapsulated in a Leco™ foil sheet and analysed in a Leco Nitrogen/Protein Analyser (FP – 528, Leco Corporation). The Leco analyser was calibrated with ethylene-diamine-tetra-acetic acid (EDTA) before each batch of samples were analysed. A calibration sample of known protein content was run after every 10 samples in order to ensure the accuracy and recovery rate of the method. The results were obtained as percentage nitrogen (N), which was then converted to total crude protein (%) by multiplying the nitrogen value with a conversion factor of 6.25. This was then converted to percentage protein per gram of meat sample by using the following formula:

$$\% \text{ protein} = \% \text{ crude protein} \times (100 - \% \text{ moisture} - \% \text{ fat}) / 100$$

### *Total lipid content*

The total lipid content (% wet weight) of 5 g homogenised meat samples were determined in duplicate using the chloroform/methanol extraction gravimetric method described by Lee *et al.* (1996). A chloroform/methanol solution concentration of 1:2 (v/v) was used since the samples were expected to contain less than 5% fat (Lee, 1996).

### *Ash content*

The ash content (% wet weight) of the moisture free samples were determined in duplicate using the official AOAC method 942.05 by ashing for 6 hours at 500°C (AOAC 2002).

### Statistical analysis of data

As the main objective was to evaluate the cross-carcass variation of the proximate composition of *M. mustelus* meat, a representative sub-sample of the population, containing both genders as well as pregnant females, was included. Therefore, the only main effect tested was anatomical position. The General Linear Models (GLM) procedure of SAS 9.1 was used for the statistical analysis of the data using a one-way analysis of variance (ANOVA) (SAS, 2006). The model below was fitted for the main effect (carcass position):

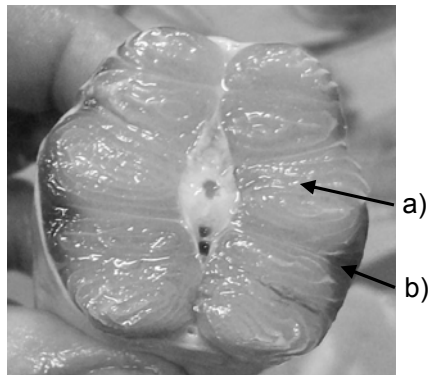
$$y_{ij} = \mu + \alpha_i + \varepsilon_j$$

where  $Y_{ij}$  is the  $j^{\text{th}}$  observation of the  $i^{\text{th}}$  treatment (carcass position),  $\mu$  is the common mean,  $\alpha_i$  is the effect of carcass position, and  $\varepsilon_{ij}$  is the residual effect of carcass position.

The least square means (LS Means) were calculated and used to express the average values of the different groups (positions) and standard error to describe the variances of the means. Correlations between proximate components were evaluated by calculating the Pearson's correlation co-efficient and the p-value in order to reject or accept the null hypothesis, which stated that there is no correlation between individual proximate components.

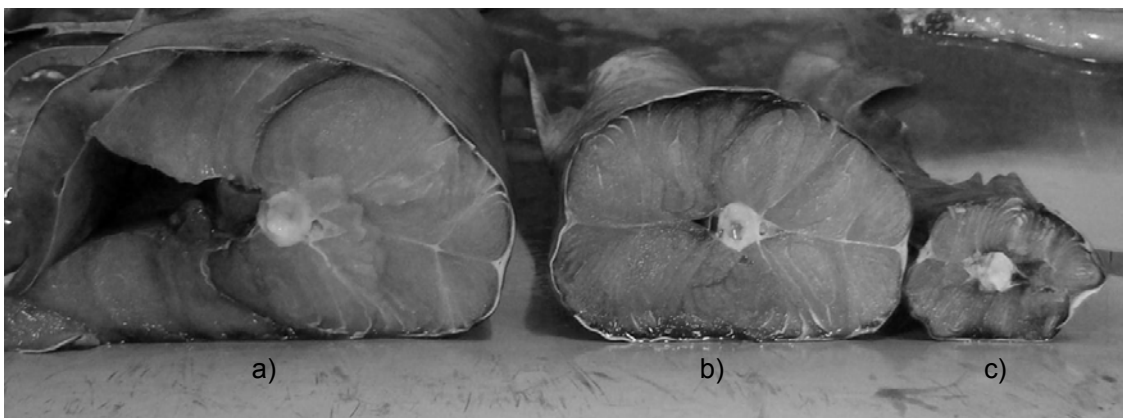
### **Results**

*M. mustelus* meat consists predominantly of white muscle, with some red muscle situated as a thin sub-cutaneous layer on the lateral sides of the fish (Fig. 3.5).



**Figure 12** Cross section of a (*M. mustelus*) shark cut near the precaudal pit, showing the predominance of white muscle (a) with some sub-cutaneous dark muscle (b)

The proportion of red meat to white meat appeared to increase moving from the head towards the tail of the shark carcass (Fig. 3.6), however, this was not quantified.



**Figure 13** Cross sections of the smoothhound (*M. mustelus*) shark a) behind the head and before the pectoral fin, b) between the two dorsal fins and c) after the second dorsal fin.

Results from the one-way ANOVA for the proximate composition of the fillets showed that there were no significant differences ( $P > 0.05$ ) between any of the fillet sites. This was true for all four proximate components (Table 3.1). There was, therefore, no variation in the basic proximate composition of the shark fillets between the anterior (sample A), middle (sample C) and posterior (sample E) sections (moving from the head section towards the tail end). The belly flap (samples B and D) also had a similar proximate composition to the rest of the shark fillet (samples A, C and E).

As there was no significant anatomical variation in the proximate composition, a mean value can be given for the overall proximate composition of *M. mustelus* meat as expressed in Table 3.1. The variation of the values within the individual proximate components are given by the coefficients of variation (CV) of 1.96 for moisture, 6.49 for protein, 42.61 for lipids and 20.80 for ash contents.

Results for the statistical analysis of the correlation between the proximate components of moisture, protein, lipids and ash, showed that there was a significant negative correlation between the protein and moisture contents, with a Pearson's correlation coefficient of -0.88. Fat was positively correlated with ash (Pearson's correlation co-efficient of 0.20) and was negatively correlated with the moisture content (Pearson's correlation coefficient of -0.33), but with a very weak correlation. There were no significant correlations between any of the other proximate components.

## Discussion

Some fish species, especially those of larger sizes (e.g. tuna), vary in their proximate composition with anatomical location (Balshaw *et al.*, 2008). Similarly, dogfish are reported to also show significant variation between anatomical locations with regards to the moisture, protein and lipid

**Table 9** Mean values (g·100 g<sup>-1</sup> meat) for proximate components of meat samples (23 samples in duplicate per fillet site) at different body locations of smoothhound (*M. mustelus*) shark with the overall means, the ranges of individual values and coefficients of variation for each component. Values given as LS Means ± standard error (SE) (n = 23)

Proximate component	A (n = 23)	B (n = 23)	C (n = 23)	D (n = 23)	E (n = 23)	Mean	Coefficient of variation	Range
	g·100 g <sup>-1</sup> meat							
Moisture	74.99 ± 0.31	75.96 ± 0.31	74.93 ± 0.32	75.52 ± 0.29	75.13 ± 0.31	75.31	1.96	71.38 - 79.35
Protein	23.02 ± 0.38	22.42 ± 0.28	23.48 ± 0.31	22.96 ± 0.29	23.51 ± 0.29	23.08	6.49	18.05 - 27.71
Lipids	1.63 ± 0.09	1.62 ± 0.10	1.49 ± 0.09	1.46 ± 0.09	1.45 ± 0.08	1.53	28.91	0.70 - 2.73
Ash	1.39 ± 0.07	1.39 ± 0.09	1.38 ± 0.06	1.34 ± 0.03	1.28 ± 0.04	1.36	20.80	0.32 - 2.73

A = Anterior fillet site

B = Anterior belly flap site

C = Mid-fillet site

D = Posterior belly flap site

E = Posterior fillet site

Mean = overall mean of means

Range = difference from smallest to largest individual values for all five sites

contents (Suwandi, 1995). The variation has been found to exist mainly between the belly flap and the fillet, as well as at the anterior, middle and posterior parts of the fillet. This is mainly due to the distribution of red and white muscle, as well as the distribution of lipids in the fat depots at different locations in the carcass (Jacquot, 1961). These fat depots are mostly located in the sub-cutaneous tissue, the belly flap, the collagenous tissue between the muscle fibres and the head section (Huss, 1988). The red meat usually has a higher lipid content than the white meat (Love, 1988). These differences, particularly in the muscle fibre types, are linked to the swimming activity of the fish (Donley & Shadwick, 2003). The anatomical parts of the shark that are more active during movement and swimming require more energy than the rest of the body. This energy is supplied by the red muscle which contains more myoglobin and has a higher anabolic rate than the white muscle (Jacquot, 1961). The amount of red muscle will therefore be more abundant in fish that are active, strong swimmers, such as predator species.

Contrary to what was expected in larger fish species such as shark, the data from this study indicated that *M. mustelus* sharks have no significant variation in terms of the proximate composition between different body locations. Even though *M. mustelus* sharks are large fish, they are benthic feeders, feeding mostly on crustaceans (crabs, shrimps and prawn) (Smale & Compagno, 1997). Little movement is therefore required by these sharks for feeding as their prey is predominantly found on the surface of the bottom sediment. Since this feeding behaviour does not require continuous, strong swimming motion, this would explain why the meat of these sharks is composed mainly of white muscle, as this muscle type is used for short, fast bursts of swimming. There is, therefore, uniformity in the muscle type of the shark across the carcass.

Variation could, however, still occur within the white muscle at different body locations, due to a greater fat deposition in some sections compared to others, but this was not found to be the case for *M. mustelus* in this study. According to Huss (1988), when the fat content in fish exceeds 1% in a body region, it can be classified as a fat depot. Even though the fat content of *M. mustelus* meat was slightly higher than 1%, it was still very low, which might explain why these fat depots at specific body positions cannot be identified by observing the proximate data in Table 3.1.

The lipid component had the highest co-efficient of variation (CV = 42.61), which was expected since it is known that the lipid fraction is usually the component in meat which shows the greatest variation (Huss, 1988). Even though the mean lipid values of the individual fillet sites differed from one another, these differences were not statistically significant since the variation in the lipid component was too large.

The moisture content was anticipated to vary in correspondence with the lipid content, as these two components have been reported to be negatively correlated (Jacquot, 1961; Huss, 1988). For *M. mustelus* however, this did not seem to be the case, as the moisture component had the lowest CV (1.96) and the correlation between moisture and lipids was not significant (Pearson's correlation coefficient of -0.33). Protein and moisture were the only components that showed a significant negative correlation (Pearson's correlation coefficient of -0.88).

In total, the moisture, protein, lipid and ash percentages of the individual fillet sites, as well as the overall mean values of each proximate component, were in excess of 100% of the total meat sample weight. This could have been due to the high levels of non-protein nitrogen (NPN) in shark meat, which exists in the form of urea and ammonia (Geiger & Borgstrom, 1962). It is thus suggested that the NPN fraction be analysed, quantified and subtracted from the total N fraction, obtained from the Leco analysis, in order to calculate the true protein value. This will be investigated and discussed in Chapter 5.

## Conclusion

Samples taken for chemical and proximate analyses of shark meat should be expected to be representative of the entire edible fillet. Nonetheless, a thorough knowledge on the cross-carcass variation is required in terms of these parameters in order to ensure that the sample being analysed is, in fact, a representative sample. To date, no data have been published on the chemical and proximate composition of *M. mustelus* meat, nor is information available on the compositional variation across the carcass of these sharks. In this study, no significant variation was found in terms of the proximate composition between the different locations sampled from the body of the *M. mustelus* shark. As a result, it can be recommended that samples from any of the investigated sites could be considered as representative of the entire fillet. With regards to commercial use, the sample site causing the least damage to the fillet and which is the simplest to obtain would be sample A. This sample is close to the head and sampling at this site could be performed from the end of the fillet, leaving the main part of the fillet intact. Sample A was therefore used for all further analyses conducted in this study.

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## Chapter 4

### THE EFFECTS OF GENDER, SIZE AND LIFE CYCLE STAGE ON THE CHEMICAL COMPOSITION OF *MUSTELUS MUSTELUS* MEAT

#### Summary

The aim of this study was to determine the effects of gender, size and life cycle stage on the chemical components (proximate, amino acid, fatty acid and mineral compositions, as well as mercury and histamine contents) of *Mustelus mustelus* meat. The proximate components (moisture, protein, lipids and ash), the amino acids and most of the evaluated minerals were not affected by any of the three aforementioned variables. The fatty acid content was higher in females compared to males, as well as in non-pregnant females compared to pregnant females. Some fatty acids decreased in quantity in medium sized sharks (before maturity was reached). Pregnant females had higher levels of aluminium (Al) and copper (Cu) than non-pregnant females and mercury levels were higher in large males than in large females. These results suggest that only some of the chemical components of *M. mustelus* meat are found to have small variations within the species and, therefore, the entire set of results gives a good indication of the average composition of *M. mustelus* meat.

#### Introduction

Fish meat consists of several components, such as moisture, protein, lipids, vitamins and minerals, all of which contribute to the overall meat composition. These components can differ in nature and quantity according to their function and availability (Love, 1980; Huss, 1988). Meat composition is affected by both exogenous and endogenous factors (Shearer, 1994). Exogenous factors that affect meat composition include the diet of the animal (composition, frequency) and the environment in which it is found (salinity, temperature). On the other hand, endogenous factors that affect meat composition include gender, size, life cycle stage and body position (Shearer, 1994).

Conflicting reports have emerged in the scientific literature relating to whether the protein content of fish differs with gender. Some authors have claimed that some female fish have higher protein contents than male fish, while the opposite has been found for other fish species (Jacquot, 1961). In addition, the protein content of certain fish species has been found not to differ significantly between genders (Jowett & Davies, 1938). Therefore, the protein variations between genders appear to be dependent on specific fish species.

The composition and quality of meat can also be influenced by the stage of sexual development of the fish. In some fish species, such as sardines, fluctuations in the lipid contents

begin to become apparent after maturity has been reached (Jacquot, 1961). The sexual cycles of female fish often cause variation in the protein content and the best meat quality is usually obtained just before spawning. After spawning, some fish species lose weight, which could be partly due to a decrease in their dietary intake during this period (Jacquot, 1961). Certain fish species have also been found to exhibit an increase in oil/lipid content as their size increases (Huss, 1988). In most fish, the maturation process is accompanied by a decrease in lipid reserves in the muscle due to the transportation of lipids to the gonads. Early stages of maturation are usually associated with an increase in body weight, with a corresponding weight loss as the gonads grow (Love, 1980). The gonads of female fish are larger than those of males, with the former requiring more energy during maturation. Males show a less severe depletion of body reserves during maturation and use both glycogen and lipid stores as energy sources at this time. Female fish, on the other hand, utilise only lipid stores as an energy source during maturation, thus requiring the accumulation of larger lipid reserves prior to this stage (Love, 1980). Fish embryos use these lipid reserves that have been transported to the ovaries during maturation as they develop, which leads to a decrease or depletion of the lipid stores in the muscle of the female fish during the gestation period (Love, 1980). It can therefore be expected that the lipid and fatty acid components will not show a linear increase from small to larger sharks, but will rather show some degree of fluctuation due to the aforementioned factors.

Many studies have shown that the meat composition of fish is directly related to the composition of their diet (Jacquot, 1961; Huss, 1988). *Mustelus mustelus* sharks tend to show a shift in the main constituents of their diets as the sharks increase in size (age), changing from a diet primarily comprising crustaceans and polychaetes, to one comprising predominantly cephalopods (Smale & Compagno, 1997). This modification in the diet of the sharks can be expected to reflect in variations in the meat composition, which should become apparent when comparing the composition of small, medium and large sharks.

As sharks are at a high trophic level in the marine food web, they are known to accumulate chemical contaminants, such as the heavy metal mercury, through the food chain (Ababouch & Gram, 2004). Another reason for the possibly high levels of mercury associated with shark meat is due to the fact that these fish are long-lived and mercury is not excreted from the body. Rather, mercury is known to bind to the protein components in the animal's flesh, causing the bioaccumulation of mercury in the meat during the life span of the shark (Ababouch & Gram, 2004). Consequently, weight limits are generally set for sharks that are destined for human consumption (Fouche, 2011). These limits should, however, be species specific, since different shark species appear to reach the recommended maximum limit for mercury content at different ages and sizes. Preliminary research appears to indicate that *M. mustelus* sharks accumulate lower levels of mercury compared to other shark species (Storelli *et al.*, 2002).

Fish tissue containing high levels of histamine can cause a severe form of illness in humans, commonly referred to as histamine- or scombroid-poisoning (Ababouch & Gram, 2004).

The formation of elevated levels of histamine in fish meat *post mortem* is associated with the growth of certain spoilage bacteria, including members of the genera *Vibrio*, *Photobacterium*, *Klebsiella* and *Morganella*, together with time-temperature abuse of the harvested fish (Ababouch & Gram, 2004). The levels of histamine formed in fish are, however, highly dependent on the presence and concentration in the flesh of the amino acid histidine, which serves as a substrate in the decarboxylation reaction brought about by bacterial histidine decarboxylase enzymes, with the subsequent production of histamine (Ababouch & Gram, 2004). Thus, the manner in which individual sharks are handled post-harvest will potentially influence the levels of histamine formed in the meat, as will the variation in the histidine content between sharks of different genders, sizes and life cycle stages.

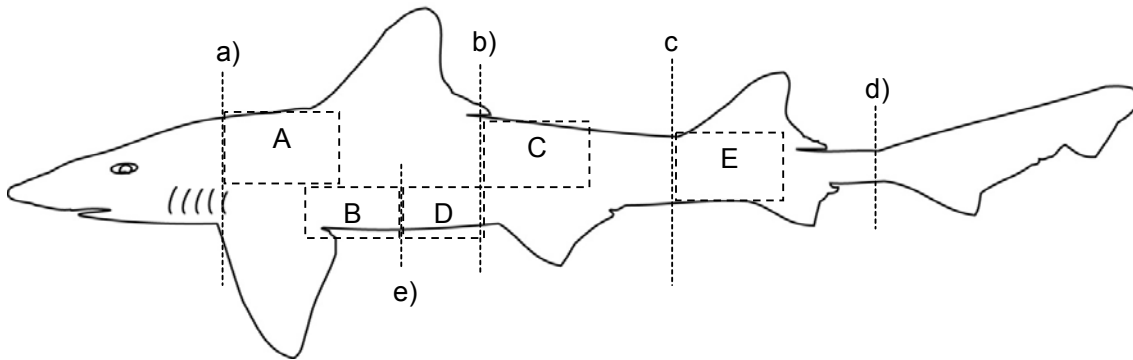
There are, therefore, a number of variables that can affect the overall chemical composition of fish meat. Nonetheless, little information currently exists on the overall chemical composition and the effects of gender, size and life cycle stage on the individual chemical components of *M. mustelus* meat. To determine the overall chemical composition of the meat, all the aforementioned factors of variation need to be investigated and taken into account to describe a chemical composition which is representative of the entire species. The variation in the mercury and histamine contents in *M. mustelus* meat certainly remains an area that requires further investigation and clarification if the true risks to human health associated with the consumption of this shark species are to be elucidated.

In the present research, the endogenous factors (gender, size and life cycle stage) were investigated. Based on the results of Chapter 3, it was assumed samples from any position of the fillet can be considered representative of the entire edible part of the body. The samples used for the present research were taken at the anterior end of the fillet, just behind the head. Exogenous factors such as diet and environment were assumed not to influence carcass composition as the sharks were all caught in the same area over a short period of time. The effects of the factors of variation were investigated for the overall proximate components, the amino acid, fatty acid and mineral compositions, as well as the mercury and histamine contents.

## **Materials and methods**

### Sampling

Fish capture, tissue dissection and sample preparation was performed as described in Chapter 3. Samples at site A (anterior end of the fillet) (Fig. 4.1) were used as representative of the entire body, since these samples can be taken with minimal damage to the fillet.



**Figure 14** Diagram of a smoothhound (*M. mustelus*) shark showing the positions at which the carcass was cut (a, b, c, d and e) and the positions of the 5 different sites and the corresponding sample codes (A, B, C, D and E)

### Sub-sampling

For the analysis of variation in the main proximate components, one sample per shark (in duplicate) was taken from 62 sharks at the same body location. For the remainder of the chemical analyses (amino acids, fatty acids, mineral, mercury and histamine), a sub-sample of 30 sharks was selected from the total sample group. This group of 30 sharks consisted of five different categories of six sharks each (Table 4.1). The five categories included three size categories of non-pregnant female sharks (small female, medium female and large female), one category of large pregnant sharks and one category of large male sharks. The large size categories for both male and female *M. mustelus* sharks are the size categories in which these sharks mature (1 250 – 1 400 mm total length in females and 950 – 1 300 mm in males (Smale & Compagno, 1997; Heemstra & Heemstra, 2004)). These sample groups were selected in order to be able to compare individual sample groups with each other and cancel out the rest of the variables.

**Table 10** Subsample (n = 30) distribution of sharks divided into six different categories according to total length

	Small (400 - 850 mm)	Medium (850 - 1 250 mm)	Large (>1 250 mm)
Female non-pregnant	6	6	6
Female pregnant	-	-	6
	Small (400 - 700 mm)	Medium (700 - 1 000 mm)	Large (>1 000 mm)
Male	-	-	6

## Analytical methods

### *Proximate composition*

The proximate analyses (moisture, protein, lipids and ash) were conducted as described in Chapter 3.

### *Fatty acids*

After thawing, a 2 g sample was extracted with a chloroform:methanol (2:1; v/v) solution according to the method of Folch *et al.* (1957). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (WiggenHauser Homogeniser, D-500 fitted with a standard shaft 1; speed setting D) was used to homogenise the sample with the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) to quantify the individual fatty acids. Of the extracted lipids, 250  $\mu\text{L}$  was transmethylated for 2 h at 70 °C with 2 mL of a methanol/sulphuric acid (19:1; v/v) solution as transmethylating agent. After cooling to room temperature, the resulting fatty acid methyl esters (FAMES) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen. Fifty  $\mu\text{L}$  hexane was added to the dried sample of which 1  $\mu\text{L}$  was injected.

The FAME were analysed using a Thermo Finnigan Focus gas-chromatograph (Thermo Electron S.p.A, Strada Rivoltana, 20090 Rodana, Milan, Italy) equipped with a flame ionisation detector, using a 60 m BPX70 capillary column with an internal diameter of 0.25 mm and 0.25  $\mu\text{m}$  film (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia) with a run time of approximately 45 minutes. The temperature programme was linear at 7 °C·min<sup>-1</sup> with the temperature settings as follows: initial temperature of 60 °C (5 min) and the final temperature at 160 °C, an injector temperature of 220 °C and a detector temperature of 260 °C. The gas flow rate of the hydrogen carrier gas was 30 ml·min<sup>-1</sup>. The FAME of the samples were identified by comparing the values with the retention times of a standard FAME mixture (Supelco™ 37 Component FAME mix, 10 mg·min<sup>-1</sup> in CH<sub>2</sub>Cl<sub>2</sub>, Cat no. 47885-U. Supelco™, North Harrison Rd, Bellefonte, PA 16823-0048, USA). Values were recorded as mg·g<sup>-1</sup> meat sample.

### *Amino acids*

For the analysis of the amino acid constituents, dried and defatted protein samples were first hydrolysed in a glass hydrolysis tube, with 0.1 gram protein samples and 6 mL 6N hydrochloric acid (HCl) and 15% phenol, sealed in a vacuum using nitrogen gas. The samples were hydrolysed in an oven at 110 °C for 24 hours, following which the hydrolysed samples were stored at -20 °C in Eppendorf tubes until further analysis.

For the preparation of the amino acids for injection on a Dionex high performance liquid chromatography (HPLC) unit, 1 mL samples were filtered through a 33 mm Millex-HV 0.45  $\mu\text{m}$  filter

into a second Eppendorf tube. The following was added to 10  $\mu\text{L}$  of sample in an Erlenmeyer flask: 4 mL distilled water, 800  $\mu\text{L}$  Borate buffer, 10  $\mu\text{L}$  NorValine. The sample solutions (1 mL) were then injected on the Dionex Summit HPLC with RF2000 Fluorescence detector and a Nova-Pak C18 4  $\mu\text{m}$ , 3.9 x 150 mm column using Chromeleon 6.80 software. The results were read as amount of moles per mL sample and converted to  $\text{g}\cdot 100\text{ g}^{-1}$  meat sample.

### *Minerals*

Mineral content was determined on 0.5 g dried and defatted, finely ground meat samples. The samples were ashed at 460 – 480  $^{\circ}\text{C}$  for 6 hours. After cooling, 5 mL of 6M HCl was added and the samples were placed in an oven at 50  $^{\circ}\text{C}$  for 30 minutes. Subsequently, 35 mL distilled water was added and the solution was filtered and made up to a final volume of 50 mL with distilled water (ALASA, 2007). Elements were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Element concentrations were calculated using iTEVA Analyst software. Argon gas flow rate was 2 - 5  $\text{ml}\cdot\text{min}^{-1}$  and instrument settings were as follows: camera temperature: -27  $^{\circ}\text{C}$ ; generator temperature: 24  $^{\circ}\text{C}$ ; optics temperature: 38  $^{\circ}\text{C}$ ; RF power: 1150 W; pump rate: 50 rpm; aux. gas flow: 0.5  $\text{L}\cdot\text{min}^{-1}$ ; nebuliser: 0.7  $\text{L}\cdot\text{min}^{-1}$ ; coolant gas: 12  $\text{L}\cdot\text{min}^{-1}$  and normal purge gas flow. Wavelengths for the elements were as follows: Al (167.079 nm), B (249.773 nm), Ca (317.933 nm), Cu (324.754 nm), Fe (259.940 nm), K (766.490 nm), Mg (285.213 nm), Mn (257.610 nm), Na (589.592 nm), P (177.495 nm) and Zn (213.856 nm). After the samples, standards with a high, medium and low range were analysed for quality control. Results were given as percentage or  $\text{mg}\cdot\text{kg}^{-1}$ .

### *Mercury*

The total mercury contents of the meat samples were analysed by a modified version of the cold vapour atomic absorption spectroscopy method as described by Iskandar *et al.* (1972). For sample preparation, 1 g homogenised sample was digested in a flask containing a cold finger with sulphuric acid and nitric acid for 2 – 6 hours at temperatures not exceeding 60 $^{\circ}\text{C}$  in order to prevent losses of volatile mercury. This was followed by an oxidising step with potassium permanganate as oxidising agent. This solution was then treated with 10% hydroxyl amine which destroys the potassium permanganate, resulting in a clear solution which is then analysed in an atomic absorption spectrophotometer unit at a wavelength of 251.6 nm. This method has a limit of detection of 0.01  $\text{mg}\cdot\text{kg}^{-1}$  (ppm) total mercury.

### *Histamine*

The RIDASCREEN® Histamine competitive enzyme-linked immunosorbant assay (ELISA) kit (Art. No. R1601, supplied by AEC Amersham, Cape Town, South Africa) was used for the extraction, derivatisation and quantification of histamine in all shark meat samples, in accordance with the instructions of the kit manufacturer. This ELISA kit has a limit of detection of  $<2.5 \text{ mg}\cdot\text{kg}^{-1}$  (ppm) histamine and a range of quantification of  $2.5 - 250.0 \text{ mg}\cdot\text{kg}^{-1}$  (ppm) histamine in fresh and frozen fish products. The antibodies utilised in the kit are reported to exhibit 100% specificity to histamine, with no cross reaction with other amino acids or amines. Assays were performed in duplicate on all samples. The standards supplied in the test kit were utilised during the assay performance and the two control samples included were also employed to verify the accuracy of the generated results. Quantification of the histamine levels in the samples was performed using RIDA®SOFT Win software, with results being expressed as  $\text{mg}\cdot\text{kg}^{-1}$  (ppm) histamine.

### Statistical analysis of data

All statistical analyses were performed using the General Linear Models (GLM) procedure of SAS 9.1 (SAS Institute, 2006). The model below was fitted for the main effects (gender, size and life cycle stage):

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijkl}$$

where  $Y_{ijkl}$  is the  $l$ th observation of the  $k$ th treatment (gender, size and life cycle stage),  $\mu$  is the common mean,  $\alpha_i$  is the effect of gender,  $\beta_j$  is the effect of size,  $\delta_k$  is the effect of life cycle stage and  $\varepsilon_{ijk}$  is the residual effect of gender, size and life cycle stage.

The data were analysed using SAS 9.1 by means of a one way or two way analysis of variance (ANOVA). The least square means (LSMeans) were calculated and used to express the average values of the different groups and standard error to describe the variances of these means.

## **Results**

### Proximate composition

Gender was found to have no significant ( $P > 0.05$ ) influence in terms of the four proximate components (moisture, protein, lipids and ash) measured in *M. mustelus* shark meat samples (Table 4.2). Similarly, there were no significant differences ( $P > 0.05$ ) in the proximate composition data of the three different sized groups of sharks (Table 4.3) or between the mean values of the proximate components of pregnant and non-pregnant sharks (Table 4.4).



**Table 11** Comparison of LSMeans  $\pm$  standard error of female and male smoothhound (*M. mustelus*) shark meat with regards to the proximate composition (n = 62)

Proximate component (g·100 g <sup>-1</sup> )	Female	Male	P-value
Moisture	74.93 $\pm$ 0.225	75.15 $\pm$ 0.300	0.565
Protein	23.46 $\pm$ 0.280	23.08 $\pm$ 0.374	0.416
Lipids	1.57 $\pm$ 0.144	1.51 $\pm$ 0.192	0.810
Ash	1.40 $\pm$ 0.065	1.45 $\pm$ 0.086	0.629

**Table 12** Comparison of the LSMeans  $\pm$  standard error between female smoothhound (*M. mustelus*) sharks of different sizes with regards to the proximate composition of the meat (n = 62)

Proximate component (g·100 g <sup>-1</sup> )	Large	Medium	Small
Moisture	75.49 $\pm$ 0.346	74.88 $\pm$ 0.472	75.79 $\pm$ 0.584
Protein	22.77 $\pm$ 0.494	23.72 $\pm$ 0.675	23.35 $\pm$ 0.834
Lipids	1.51 $\pm$ 0.285	1.68 $\pm$ 0.389	1.01 $\pm$ 0.481
Ash	1.41 $\pm$ 0.081	1.45 $\pm$ 0.110	1.52 $\pm$ 0.137

**Table 13** Comparison of LSMeans  $\pm$  standard error between pregnant and non-pregnant female smoothhound (*M. mustelus*) sharks of the same size category with regards to the proximate composition of their meat (n = 62)

Proximate component (g·100 g <sup>-1</sup> )	Pregnant females	Non-pregnant females	P-value
Moisture	75.93 $\pm$ 0.610	74.84 $\pm$ 0.254	0.124
Protein	23.08 $\pm$ 0.871	23.48 $\pm$ 0.363	0.690
Lipids	1.22 $\pm$ 0.502	1.58 $\pm$ 0.209	0.539
Ash	1.53 $\pm$ 0.143	1.39 $\pm$ 0.060	0.421

### Amino acid composition

No significant differences ( $P > 0.05$ ) were found between female and male *M. mustelus* sharks in terms of the 15 amino acids analysed in this study (Table 4.5). There were also no significant differences ( $P > 0.05$ ) in the quantities of individual amino acids between the three different size categories of female *M. mustelus* shark (Table 4.6). In addition, pregnancy had no significant influence ( $P > 0.05$ ) on the amino acid composition of the shark fillets from females that were in the same size category (Table 4.7).

**Table 14** Comparison of the LSMeans  $\pm$  standard error of the amino acid composition (n = 30) of female and male smoothhound (*M. mustelus*) sharks of the same size category

Amino acid (g·100 g <sup>-1</sup> )	Female	Male	P-value
Asparagine	1.82 $\pm$ 0.094	1.85 $\pm$ 0.094	0.845
Glutamine	2.86 $\pm$ 0.166	3.02 $\pm$ 0.166	0.520
Serine	0.73 $\pm$ 0.035	0.76 $\pm$ 0.035	0.564
Histidine	0.38 $\pm$ 0.036	0.29 $\pm$ 0.036	0.112
Glycine	0.71 $\pm$ 0.040	0.77 $\pm$ 0.040	0.340
Threonine	0.89 $\pm$ 0.057	0.77 $\pm$ 0.057	0.168
Arginine	0.83 $\pm$ 0.047	0.88 $\pm$ 0.047	0.494
Alanine	1.08 $\pm$ 0.055	1.18 $\pm$ 0.055	0.252
Tyrosine	0.69 $\pm$ 0.034	0.71 $\pm$ 0.034	0.692
Valine	0.88 $\pm$ 0.044	0.88 $\pm$ 0.044	0.976
Methionine	0.49 $\pm$ 0.031	0.51 $\pm$ 0.031	0.573
Phenylalanine	0.80 $\pm$ 0.040	0.82 $\pm$ 0.040	0.740
Isoleucine	0.90 $\pm$ 0.046	0.92 $\pm$ 0.046	0.764
Leucine	1.49 $\pm$ 0.069	1.55 $\pm$ 0.069	0.508
Lysine	1.66 $\pm$ 0.093	1.54 $\pm$ 0.093	0.409

**Table 15** Comparison of the LSMeans  $\pm$  standard error of the amino acid composition (n = 30) of female smoothhound (*M. mustelus*) sharks of different sizes

Amino acid (g·100 g <sup>-1</sup> )	Large	Medium	Small
Asparagine	1.82 $\pm$ 0.092	1.91 $\pm$ 0.159	1.86 $\pm$ 0.159
Glutamine	2.92 $\pm$ 0.156	3.15 $\pm$ 0.269	3.00 $\pm$ 0.269
Serine	0.74 $\pm$ 0.038	0.81 $\pm$ 0.066	0.76 $\pm$ 0.066
Histidine	0.38 $\pm$ 0.027	0.31 $\pm$ 0.047	0.41 $\pm$ 0.047
Glycine	0.72 $\pm$ 0.041	0.88 $\pm$ 0.071	0.82 $\pm$ 0.071
Threonine	0.86 $\pm$ 0.051	0.82 $\pm$ 0.089	0.90 $\pm$ 0.089
Arginine	0.86 $\pm$ 0.046	0.94 $\pm$ 0.080	0.90 $\pm$ 0.080
Alanine	1.09 $\pm$ 0.057	1.25 $\pm$ 0.098	1.15 $\pm$ 0.098
Tyrosine	0.71 $\pm$ 0.039	0.76 $\pm$ 0.068	0.72 $\pm$ 0.068
Valine	0.88 $\pm$ 0.046	0.93 $\pm$ 0.080	0.91 $\pm$ 0.080
Methionine	0.51 $\pm$ 0.028	0.55 $\pm$ 0.049	0.55 $\pm$ 0.049
Phenylalanine	0.81 $\pm$ 0.042	0.85 $\pm$ 0.073	0.83 $\pm$ 0.073
Isoleucine	0.90 $\pm$ 0.047	0.95 $\pm$ 0.081	0.93 $\pm$ 0.081
Leucine	1.52 $\pm$ 0.079	1.63 $\pm$ 0.136	1.55 $\pm$ 0.136
Lysine	1.70 $\pm$ 0.089	1.63 $\pm$ 0.155	1.71 $\pm$ 0.155

**Table 16** Comparison of the LSMeans  $\pm$  standard error of the amino acid composition (n = 30) of pregnant and non-pregnant female smoothhound (*M. mustelus*) sharks of the same size category

Amino acid (g·100 g <sup>-1</sup> )	Pregnant	Non-Pregnant	P-value
Asparagine	1.81 $\pm$ 0.091	1.82 $\pm$ 0.091	0.907
Glutamine	2.97 $\pm$ 0.171	2.86 $\pm$ 0.171	0.644
Serine	0.75 $\pm$ 0.039	0.73 $\pm$ 0.039	0.808
Histidine	0.37 $\pm$ 0.028	0.38 $\pm$ 0.028	0.767
Glycine	0.73 $\pm$ 0.040	0.71 $\pm$ 0.040	0.708
Threonine	0.82 $\pm$ 0.060	0.89 $\pm$ 0.060	0.458
Arginine	0.89 $\pm$ 0.050	0.83 $\pm$ 0.050	0.440
Alanine	1.09 $\pm$ 0.053	1.08 $\pm$ 0.053	0.858
Tyrosine	0.73 $\pm$ 0.039	0.69 $\pm$ 0.039	0.506
Valine	0.88 $\pm$ 0.045	0.88 $\pm$ 0.045	0.948
Methionine	0.53 $\pm$ 0.033	0.49 $\pm$ 0.033	0.365
Phenylalanine	0.82 $\pm$ 0.043	0.80 $\pm$ 0.043	0.783
Isoleucine	0.90 $\pm$ 0.047	0.90 $\pm$ 0.047	0.961
Leucine	1.55 $\pm$ 0.082	1.49 $\pm$ 0.082	0.604
Lysine	1.74 $\pm$ 0.119	1.66 $\pm$ 0.119	0.614

#### Fatty acid composition

The data in Table 4.8 indicate that female *M. mustelus* sharks had higher fatty acid levels than male sharks, even though the difference was only significant ( $P < 0.05$ ) in the case of certain individual fatty acids. The total saturated fatty acid (SFA) content was significantly higher ( $P < 0.05$ ) in females than in males, as was the total monounsaturated fatty acids (MUFAs) and the total polyunsaturated fatty acids (PUFAs). Both the ratio of PUFA to SFA and the ratio of omega-6 (n6) to omega-3 (n3) fatty acids did not show significant variation for any of the variables (gender, size and life cycle stage) (Tables 4.8, 4.9 and 4.10).

As the sharks increased in size, most of the individual fatty acids increased in quantity from small to large sharks, even though the majority of these differences were insignificant ( $P > 0.05$ ) (Table 4.9). There were, however, a greater number of fatty acids that differed significantly ( $P < 0.05$ ) in quantity between the small and medium sharks, as well as between the medium and large sharks. This can be explained by the trend visible from the data that the quantities of fatty acids decreased from small to medium sized sharks, and increased from medium to large sized sharks.

Non-pregnant female *M. mustelus* sharks had significantly higher ( $P < 0.05$ ) levels of total SFA, MUFAs and PUFAs than pregnant females of the same size category (Table 4.10). Most individual fatty acids were present at higher values in non-pregnant females than these were in pregnant females, although not all of these differences were statistically significant ( $P > 0.05$ ). Some of the individual fatty acids were not affected by any of the category variables. Palmitic acid

(16:0), heneicosanoic acid (21:0), palmitoleic acid (16:1n7), oleic acid (18:1n9), alpha linolenic acid (18:3n3) and docosapentaenoic acid (22:5n3) seemed to show more variation, as these fatty acids were significantly affected by gender, size and life cycle stage.

**Table 17** Comparison of the LSMeans  $\pm$  standard error of the fatty acids composition (n = 30) of female and male smoothhound (*M. mustelus*) shark meat

Fatty acid (mg·g <sup>-1</sup> meat sample)	Lipid names	Female	Male	P-value
Mysteric acid	14:0	0.06 $\pm$ 0.007	0.03 $\pm$ 0.007	0.010
Pentadecanoic acid	15:0	0.02 $\pm$ 0.002	0.02 $\pm$ 0.002	0.056
Palmitic acid	16:0	2.60 $\pm$ 0.188	1.61 $\pm$ 0.188	0.004
Stearic acid	18:0	1.28 $\pm$ 0.085	0.74 $\pm$ 0.085	0.001
Arachidic acid	20:0	0.02 $\pm$ 0.002	0.02 $\pm$ 0.002	0.303
Heneicosanoic acid	21:0	0.02 $\pm$ 0.002	0.01 $\pm$ 0.002	0.002
Behenic acid	22:0	0.09 $\pm$ 0.011	0.08 $\pm$ 0.011	0.502
Lignoceric acid	24:0	0.02 $\pm$ 0.002	0.01 $\pm$ 0.002	0.298
	Total SFA	4.11 $\pm$ 0.279	2.52 $\pm$ 0.279	0.002
Myristoleic acid	14:1	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.176
Pentadecenoic acid	15:1	0.02 $\pm$ 0.001	0.01 $\pm$ 0.001	0.049
Palmitoleic acid	16:1n7	0.18 $\pm$ 0.017	0.07 $\pm$ 0.017	0.001
Oleic acid	18:1n9	0.80 $\pm$ 0.039	0.39 $\pm$ 0.039	<.0001
Gadoleic acid	20:1n9	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.012
Erucic acid	22:1n9	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.069
Nervonic acid	24:1n9	0.05 $\pm$ 0.004	0.03 $\pm$ 0.004	0.007
	Total MUFA	1.08 $\pm$ 0.055	0.53 $\pm$ 0.055	<0.0001
Linoleic acid	18:2n6	0.11 $\pm$ 0.017	0.07 $\pm$ 0.017	0.113
Alpha linolenic acid	18:3n3	0.06 $\pm$ 0.003	0.02 $\pm$ 0.003	<.0001
Gamma linolenic acid	18:3n6	0.02 $\pm$ 0.003	0.01 $\pm$ 0.003	0.059
Eicosadienoic acid	20:2	0.04 $\pm$ 0.003	0.02 $\pm$ 0.003	0.019
Dihomo-gamma-linolenic acid	20:3n6	0.69 $\pm$ 0.088	0.66 $\pm$ 0.088	0.815
Eicosatrienoic acid	20:3n3	0.02 $\pm$ 0.003	0.01 $\pm$ 0.003	0.230
Arachidonic acid	20:4n6	0.03 $\pm$ 0.004	0.02 $\pm$ 0.004	0.015
Eicosapentaenoic acid	20:5n3	0.10 $\pm$ 0.017	0.10 $\pm$ 0.017	0.982
Docosadienoic acid	22:2	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.849
Docosapentaenoic acid	22:5n3	0.48 $\pm$ 0.057	0.26 $\pm$ 0.057	0.022
Docosahexaenoic acid	22:6n3	1.81 $\pm$ 0.185	1.15 $\pm$ 0.185	0.030
	Total PUFA	3.35 $\pm$ 0.262	2.32 $\pm$ 0.262	0.019
	PFA:SFA	0.82 $\pm$ 0.042	0.92 $\pm$ 0.042	0.119
	n6:n3	0.36 $\pm$ 0.059	0.52 $\pm$ 0.059	0.080

**Table 18** Comparison of the LSMeans  $\pm$  standard error of the fatty acid composition (n = 30) of smoothhound (*M. mustelus*) sharks of different sizes

Fatty acid (mg·g <sup>-1</sup> meat sample)	Large	Medium	Small	P-value L/M	P-value M/S	P-value L/S
14:0	0.05 $\pm$ 0.006	0.04 $\pm$ 0.010	0.04 $\pm$ 0.010	0.630	1	1
15:0	0.02 $\pm$ 0.002	0.02 $\pm$ 0.004	0.02 $\pm$ 0.004	1	1	1
16:0	2.14 $\pm$ 0.160	1.26 $\pm$ 0.278	1.29 $\pm$ 0.278	0.037	1	0.470
18:0	1.04 $\pm$ 0.082	0.63 $\pm$ 0.143	0.79 $\pm$ 0.143	0.064	0.994	0.447
20:0	0.02 $\pm$ 0.001	0.02 $\pm$ 0.002	0.02 $\pm$ 0.002	0.407	0.767	1
21:0	0.02 $\pm$ 0.002	0.01 $\pm$ 0.003	0.02 $\pm$ 0.003	0.039	0.048	1
22:0	0.07 $\pm$ 0.010	0.07 $\pm$ 0.018	0.05 $\pm$ 0.018	1	0.966	0.655
24:0	0.02 $\pm$ 0.001	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.058	0.884	0.475
Total SFA	3.38 $\pm$ 0.255	2.05 $\pm$ 0.442	2.24 $\pm$ 0.442	0.050	1	0.112
14:1	0.01 $\pm$ 0.001	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	1	1	1
15:1	0.02 $\pm$ 0.001	0.01 $\pm$ 0.003	0.02 $\pm$ 0.003	0.450	0.418	1
16:1n7	0.13 $\pm$ 0.014	0.03 $\pm$ 0.025	0.12 $\pm$ 0.025	0.012	0.019	1
18:1n9	0.64 $\pm$ 0.044	0.32 $\pm$ 0.077	0.49 $\pm$ 0.077	0.006	0.204	0.365
20:1n9	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.292	1	1
22:1n9	0.01 $\pm$ 0.001	0.01 $\pm$ 0.002	0.01 $\pm$ 0.001	0.760	0.705	1
24:1n9	0.04 $\pm$ 0.003	0.02 $\pm$ 0.006	0.02 $\pm$ 0.006	0.008	1	0.005
Total MUFA	0.86 $\pm$ 0.059	0.41 $\pm$ 0.102	0.68 $\pm$ 0.102	0.004	0.106	0.467
18:2n6	0.10 $\pm$ 0.014	0.07 $\pm$ 0.023	0.07 $\pm$ 0.023	0.947	1	1
18:3n3	0.04 $\pm$ 0.004	0.01 $\pm$ 0.006	0.03 $\pm$ 0.006	0.001	0.014	0.753
18:3n6	0.02 $\pm$ 0.003	0.02 $\pm$ 0.006	0.02 $\pm$ 0.006	1	1	1
20:2	0.03 $\pm$ 0.004	0.02 $\pm$ 0.006	0.03 $\pm$ 0.006	1	1	1
20:3n6	0.59 $\pm$ 0.068	0.36 $\pm$ 0.117	0.24 $\pm$ 0.117	0.291	1	0.051
20:3n3	0.01 $\pm$ 0.001	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.099	0.999	0.629
20:4n6	0.03 $\pm$ 0.003	0.02 $\pm$ 0.005	0.03 $\pm$ 0.005	0.855	0.725	1
20:5n3	0.08 $\pm$ 0.013	0.06 $\pm$ 0.023	0.02 $\pm$ 0.023	1	0.316	0.069
22:2	0.01 $\pm$ 0.002	0.01 $\pm$ 0.003	0.01 $\pm$ 0.003	1	0.719	1
22:5n3	0.36 $\pm$ 0.041	0.15 $\pm$ 0.071	0.38 $\pm$ 0.071	0.056	0.027	1
22:6n3	1.48 $\pm$ 0.136	0.89 $\pm$ 0.235	0.93 $\pm$ 0.235	0.121	1	0.167
Total PUFA	2.76 $\pm$ 0.233	1.62 $\pm$ 0.403	1.78 $\pm$ 0.403	0.071	1	0.144
PUFA:SFA	0.82 $\pm$ 0.036	0.77 $\pm$ 0.062	0.79 $\pm$ 0.062	All P>0.05		
n-6:n-3	0.39 $\pm$ 0.032	0.41 $\pm$ 0.055	0.29 $\pm$ 0.055	All P>0.05		

**Table 19** Comparison of the LSMeans  $\pm$  standard error of the fatty acid composition (n = 30) of pregnant and non-pregnant female smoothhound (*M. mustelus*) sharks

Fatty acid (mg·g <sup>-1</sup> meat sample)	Lipid names	pregnant	non-pregnant	P-value
Mysteric acid	14:0	0.04 $\pm$ 0.009	0.06 $\pm$ 0.009	0.171
Pentadecanoic acid	15:0	0.02 $\pm$ 0.003	0.02 $\pm$ 0.003	0.437
Palmitic acid	16:0	1.68 $\pm$ 0.211	2.60 $\pm$ 0.211	0.012
Stearic acid	18:0	0.81 $\pm$ 0.109	1.28 $\pm$ 0.109	0.013
Arachidic acid	20:0	0.02 $\pm$ 0.002	0.02 $\pm$ 0.002	0.947
Heneicosanoic acid	21:0	0.01 $\pm$ 0.002	0.02 $\pm$ 0.002	0.003
Behenic acid	22:0	0.06 $\pm$ 0.017	0.09 $\pm$ 0.017	0.240
Lignoceric acid	24:0	0.02 $\pm$ 0.001	0.02 $\pm$ 0.001	0.310
	Total SFA	2.66 $\pm$ 0.335	4.11 $\pm$ 0.335	0.012
Myristoleic acid	14:1	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.095
Pentadecenoic acid	15:1	0.01 $\pm$ 0.001	0.02 $\pm$ 0.001	0.328
Palmitoleic acid	16:1n7	0.08 $\pm$ 0.018	0.18 $\pm$ 0.018	0.003
Oleic acid	18:1n9	0.48 $\pm$ 0.051	0.80 $\pm$ 0.051	0.001
Gadoleic acid	20:1n9	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.136
Erucic acid	22:1n9	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.155
Nervonic acid	24:1n9	0.04 $\pm$ 0.005	0.05 $\pm$ 0.005	0.044
	Total MUFA	0.63 $\pm$ 0.068	1.08 $\pm$ 0.068	0.001
Linoleic acid	18:2n6	0.09 $\pm$ 0.023	0.11 $\pm$ 0.023	0.662
Alpha linolenic acid	18:3n3	0.03 $\pm$ 0.003	0.06 $\pm$ 0.003	0.0003
Gamma linolenic acid	18:3n6	0.02 $\pm$ 0.005	0.02 $\pm$ 0.005	0.969
Eicosadienoic acid	20:2	0.02 $\pm$ 0.005	0.04 $\pm$ 0.005	0.112
Dihomo-gamma-linolenic acid	20:3n6	0.50 $\pm$ 0.101	0.69 $\pm$ 0.101	0.221
Eicosatrienoic acid	20:3n3	0.01 $\pm$ 0.002	0.02 $\pm$ 0.002	0.099
Arachidonic acid	20:4n6	0.02 $\pm$ 0.004	0.03 $\pm$ 0.004	0.108
Eicosapentaenoic acid	20:5n3	0.07 $\pm$ 0.018	0.10 $\pm$ 0.018	0.247
Docosadienoic acid	22:2	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.867
Docosapentaenoic acid	22:5n3	0.23 $\pm$ 0.045	0.48 $\pm$ 0.045	0.003
Docosahexaenoic acid	22:6n3	1.16 $\pm$ 0.198	1.81 $\pm$ 0.198	0.044
	Total PUFA	2.17 $\pm$ 0.319	3.35 $\pm$ 0.319	0.025
	PUFA:SFA	0.81 $\pm$ 0.055	0.82 $\pm$ 0.055	0.905
	n6:n3	0.42 $\pm$ 0.053	0.36 $\pm$ 0.053	0.378

Mineral content

The results from the analyses of the mineral content of *M. mustelus* sharks showed that neither gender nor size had a significant effect ( $P > 0.05$ ) on the quantity of the eleven minerals analysed (Tables 4.11 and 4.12). For nine of the eleven minerals analysed, there were no significant differences ( $P > 0.05$ ) in their quantities between pregnant and non-pregnant *M. mustelus* females of the same size category (Table 4.13). Aluminium (Al) and Copper (Cu) were the only two minerals that showed statistically significant ( $P < 0.05$ ) differences in pregnant female sharks compared to non-pregnant female sharks, with higher values in the pregnant sharks.

**Table 20** Comparison of the LSMeans  $\pm$  standard error of the mineral content ( $n = 30$ ) of female and male smoothhound (*M. mustelus*) shark meat

Mineral (mg·100 g <sup>-1</sup> )	Female	Male	P-value
Phosphorus	199.42 $\pm$ 15.416	218.00 $\pm$ 15.416	0.414
Potassium	202.28 $\pm$ 18.471	259.10 $\pm$ 18.471	0.055
Calcium	16.08 $\pm$ 3.424	13.94 $\pm$ 3.424	0.667
Magnesium	32.88 $\pm$ 2.743	33.95 $\pm$ 2.743	0.788
Sodium	29.83 $\pm$ 3.923	24.99 $\pm$ 3.923	0.404
Iron	2.06 $\pm$ 0.806	0.85 $\pm$ 0.806	0.314
Copper	0.10 $\pm$ 0.013	0.10 $\pm$ 0.013	0.823
Zinc	0.50 $\pm$ 0.038	0.48 $\pm$ 0.038	0.785
Manganese	0.03 $\pm$ 0.004	0.02 $\pm$ 0.004	0.244
Boron	0.02 $\pm$ 0.001	0.02 $\pm$ 0.001	0.109
Aluminium	2.37 $\pm$ 0.805	2.66 $\pm$ 0.805	0.804

**Table 21** Comparison of the LSMeans  $\pm$  standard error of the mineral content ( $n = 30$ ) of female smoothhound (*M. mustelus*) sharks of different sizes

Mineral (mg·100 g <sup>-1</sup> )	Large	Medium	Small
Phosphorus	212.82 $\pm$ 9.531	218.18 $\pm$ 16.508	223.18 $\pm$ 16.508
Potassium	217.11 $\pm$ 12.656	245.34 $\pm$ 21.920	250.34 $\pm$ 21.920
Calcium	16.26 $\pm$ 2.011	14.47 $\pm$ 3.482	23.41 $\pm$ 3.482
Magnesium	35.38 $\pm$ 1.347	36.81 $\pm$ 2.333	36.81 $\pm$ 2.333
Sodium	30.38 $\pm$ 2.333	31.30 $\pm$ 4.040	31.54 $\pm$ 4.040
Iron	1.79 $\pm$ 0.409	0.51 $\pm$ 0.709	0.38 $\pm$ 0.709
Copper	0.15 $\pm$ 0.018	0.16 $\pm$ 0.032	0.15 $\pm$ 0.032
Zinc	0.57 $\pm$ 0.027	0.59 $\pm$ 0.048	0.56 $\pm$ 0.048
Manganese	0.03 $\pm$ 0.003	0.02 $\pm$ 0.004	0.02 $\pm$ 0.004
Boron	0.02 $\pm$ 0.002	0.02 $\pm$ 0.003	0.02 $\pm$ 0.003
Aluminium	4.69 $\pm$ 0.538	5.04 $\pm$ 0.932	4.38 $\pm$ 0.932

**Table 22** Comparison of LSMeans  $\pm$  standard error of the mineral content (n = 30) of pregnant and non-pregnant female smoothhound (*M. mustelus*) sharks

Mineral (mg·100 g <sup>-1</sup> )	Pregnant	Not Pregnant	P-value
Phosphorus	226.22 $\pm$ 17.85	199.42 $\pm$ 17.85	0.313
Potassium	231.94 $\pm$ 23.73	202.28 $\pm$ 23.73	0.398
Calcium	16.44 $\pm$ 3.40	16.08 $\pm$ 3.40	0.942
Magnesium	37.88 $\pm$ 2.46	32.88 $\pm$ 2.46	0.180
Sodium	30.94 $\pm$ 3.80	29.83 $\pm$ 3.80	0.840
Iron	1.53 $\pm$ 0.81	2.06 $\pm$ 0.81	0.650
Copper	0.21 $\pm$ 0.03	0.10 $\pm$ 0.03	0.039
Zinc	0.63 $\pm$ 0.05	0.50 $\pm$ 0.05	0.068
Manganese	0.031 $\pm$ 0.005	0.025 $\pm$ 0.005	0.411
Boron	0.021 $\pm$ 0.002	0.015 $\pm$ 0.002	0.056
Aluminium	7.01 $\pm$ 0.87	2.37 $\pm$ 0.87	0.004

### Mercury content

Results from the 30 *M. mustelus* meat samples analysed for total mercury showed that neither size nor life cycle stage had a significant effect ( $P > 0.05$ ) on the quantity of this heavy metal in the meat (Table 4.14). The difference in the mean mercury content of the six males compared to that of the six females was statistically significant, with higher mercury levels being found in the males than in the females (Table 4.14).

**Table 23** Comparison of the LSMeans  $\pm$  Standard error of the mercury content (n = 30) of the different smoothhound (*M. mustelus*) categories. Mercury values given as meat sample

Category	Variable	Total mercury (mg·kg <sup>-1</sup> )	P-value
Gender	Female	0.74 $\pm$ 0.196	0.048
	Male	1.37 $\pm$ 0.196	
Size	Large	1.00 $\pm$ 0.137	> 0.05
	medium	1.05 $\pm$ 0.237	
	small	0.59 $\pm$ 0.237	
Life cycle stage	non-pregnant	0.74 $\pm$ 0.248	> 0.05
	Pregnant	1.26 $\pm$ 0.248	

### Histamine

Of the 30 histamine samples analysed, 17 (ca. 57%) were found to contain histamine levels below the limit of detection of the ELISA kit utilised (<2.5 mg·kg<sup>-1</sup> (ppm)). The 13 samples which were found to contain quantifiable levels of histamine were not associated with specific shark categories with regards to gender, size or life cycle stage. The data could, therefore, not be used to compare



the different categories and determine the variation in histamine content due to gender, size and life cycle stage. The quantitative data obtained from the 13 samples will be discussed further in Chapter 5.

## Discussion

The effects of the different endogenous factors (main effects) investigated on the separate chemical components of *M. mustelus* meat are summarised in Table 4.15. There were no significant differences ( $P > 0.05$ ) in the four main proximate components correlated to changes in the three variables (gender, size and life cycle stage). It can therefore be assumed that the proximate composition of *M. mustelus* sharks does not undergo any significant changes during the growth, maturation and sexual cycles of the shark. As the diets of fish have a direct influence on their meat composition, this can be an indication that the *M. mustelus* sharks in the Langebaan lagoon area maintain a similar diet throughout their life span, rather than having a shift with maturity in their main diet components (from crustaceans and polychaetes to cephalopods), as is common for these sharks (Smale & Compagno, 1997). This proposition was confirmed by evaluating the stomach contents of these sharks (data not shown), which was not found to differ significantly between smaller and larger sharks.

The amino acid composition of the meat was not significantly affected by any of the variables (gender, size and life cycle stage), showing that the shark maintains a fairly constant amino acid composition throughout its life span and sexual cycles for both genders. It has been shown in prior studies that fish protein as a whole is relatively uniform, with insignificant variation between species (Matsuura *et al.*, 1955; Konosu *et al.*, 1956; Connell & Howgate, 1959). Jacquot (1961) reported, however, that there is undoubtedly variation in the amino acid composition of fish, but that this occurs mainly between species and is especially evident in the arginine and histidine contents. Some basic differences in the amino acid composition have also been found to exist between male and female fish, during reproductive cycles and between red and white muscle tissue (Sekinè, 1921; Matsuura *et al.*, 1955)

The fatty acid composition was clearly the one meat component which was found to be most affected by all the variables (gender, size and life cycle stage) and, as a result, had the most variation. This finding is in agreement with that data reported by Jacquot (1961) and Love (1980), who stated that the lipid component varies between species and individuals as it is largely effected by anatomical location, seasonal changes, gender and sexual cycles. The total amount of fatty acids (SFA, MUFA and PUFA) was found to be higher in females and non-pregnant females compared to males and pregnant females ( $n = 30$ ), respectively.

**Table 24** Summary of the three main effects (gender, size and life cycle stage) on individual meat components of the smoothhound (*M. mustelus*) shark

	Gender	Size	Life cycle stage
<b>Proximate components</b>			
Moisture	No effect	No effect	No effect
Total protein	No effect	No effect	No effect
Total lipids	No effect	No effect	No effect
Ash	No effect	No effect	No effect
<b>Amino acids</b>	No effect	No effect	No effect
<b>Fatty acids</b>			
Total SFA, MUFA & PUFA	The total SFA, MUFA and PUFA levels are all significantly higher in females than in males	Only the MUFA levels are significantly higher in large sharks compared to medium sized sharks	The total SFA, MUFA and PUFA levels are all significantly higher in non-pregnant females than in pregnant females
Individual fatty acids	All individual fatty acids have higher levels in females even though only some differences are statistically significant	Some fatty acids show no or little increase from small to large sharks, but with a significant decrease in quantity in medium size sharks	All individual fatty acids have equal or higher levels in non-pregnant females even though only some differences are statistically significant
<b>Minerals</b>	No effect	No effect	Only the levels of copper and aluminium are significantly higher in pregnant females than in non-pregnant females
<b>Mercury</b>	According to statistical analyses, male sharks have higher levels than females	No effect	No effect

For the total lipids (as determined by the method of Lee *et al.*, (1996)), however, no statistically significant differences were found between these different groups (n = 62). This can be explained by the fact that fatty acids only make up the triglyceride component (fat) of total lipids which include other lipid components, such as phospholipids, cholesterol, waxes and more. In cartilaginous fish such as sharks, a significant quantity of fat may consist of diacyl alkyl glyceryl ethers or of the hydrocarbon squalene (Huss, 1988). During the fat extraction process (Lee, 1996), more lipid components, other than fatty acids, are therefore extracted from the meat sample, which adds to

the final lipid weight. A change in the fatty acid quantity therefore does not necessarily cause a change in the total lipid quantity.

The total SFA, MUFA and PUFA, however, all had significantly higher levels in female sharks than in male sharks, as well as in non-pregnant sharks compared to pregnant sharks. As explained by Love (1980), it is expected that female fish will have higher lipid stores than male fish due to their requirement for lipids during maturation and embryo development. This use of lipids for the development of fish embryos and the subsequent decrease or depletion in body lipid stores also explain the lower fatty acid level in pregnant fish compared to non-pregnant fish of the same size category. However, where it was expected for the sharks to have an increase in the lipid/fatty acid levels before maturation (Love, 1980), especially as these samples were obtained three to four months before spawning, the fatty acids for *M. mustelus* shark evaluated in this study tended to decrease in quantity before maturation (1 250 - 1 400 mm in female *M. mustelus* sharks). Further research is therefore required on the biology of these sharks in order to explain these unexpected variations in fatty acids levels.

The eleven individual minerals analysed did not appear to be affected by either gender or size according to statistical analysis. Pregnant females had higher levels of copper and aluminium than non-pregnant females of the corresponding size category. Even though the differences in copper values were found to be statistically significant, these small differences may, in reality, not prove to be biologically significant. Pelgrom *et al.* (1994) reported that the accumulation of copper by juvenile Tilapia was higher for non-fed fish than for fed fish. This might explain the higher copper values in the pregnant *M. mustelus* sharks, as these sharks did not seem to be feeding close to the end of their gestation periods as was evident from the empty stomachs of these sharks upon capture (diet data not shown) and the sharks may thus have accumulated higher levels of copper from the surrounding seawater. No explanation has yet been suggested in literature to account for the higher aluminium values in pregnant *M. mustelus* sharks.

The results from this study indicated that the mercury content in *M. mustelus* sharks was not affected by either size or life cycle stage, even though the larger sharks were expected to have much higher mercury levels in the flesh than smaller sharks due to bioaccumulation (Ababouch & Gram, 2004). The group of large male sharks was found to contain a significantly ( $P < 0.05$ ) higher mean mercury value than the large female sharks. This observation may be due to the fact that male sharks have a lower growth rate than females and the average total length of the large males is less than that of the large females; the mercury content in the flesh of the male sharks is therefore more concentrated than in the larger female sharks. The sample groups, however, only consisted of six sharks each and further detailed analyses are therefore suggested to investigate mercury content variation in *M. mustelus* shark meat.

Even though the variation by size was only investigated for female sharks, a similar pattern can be expected for males. The gonads of female fish are larger than those of males, which can cause the depletion of body reserves during maturation to be more marked in females than in

males (Love, 1980). The variation in the fatty acids seen in the females sharks can therefore be expected to be similar in male sharks, but to a lesser extent.

## Conclusion

Compared to data reported for other fish and shark species, *M. mustelus* appears to represent a shark species that exhibits limited variation in terms of meat composition. The only meat component found to be significantly affected by all three factors (gender, size and life cycle stage) was the fatty acids. Even though the variation within the fatty acids was statistically significant, it was still relatively small and not necessarily biologically significant. The biological significance of the differences in the Al and Cu contents between pregnant and non-pregnant sharks with regards to human nutrition and safety will depend on the recommended daily dietary allowance (RDA) or the safe maximum limits for these elements, which will be discussed in Chapter 5. The difference in the total mercury content of large male and female sharks should be further investigated as this may be of major biological significance due to the fact that the mean value for large males exceeded the maximum safe limit of mercury in seafood (1 ppm), whereas the mean content in large females was below this limit.

From these results on the variation in the chemical composition of the meat within the *M. mustelus* species, it can be suggested that the composition of the meat is fairly consistent and an average meat composition can, therefore, be determined, taking into consideration the variation in some components.

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## Chapter 5

### THE INVESTIGATION OF THE CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF *MUSTELUS MUSTELUS* MEAT

#### Summary

The aim of this study was to determine the overall chemical composition and nutritional value of *Mustelus mustelus* meat. The results indicated that the meat of this species can be considered as a lean meat (1.6 g·100 g<sup>-1</sup> (wet weight) lipids), albeit that it contains considerable quantities of omega-3 polyunsaturated fatty acids. A 100 g portion of fillet would provide a large proportion (≥50%) of the RDA/EDI of most essential amino acids. The mineral content of the meat was found to be low, but the total mercury content exceeded the maximum safe limit in some meat samples. Histamine was only detected in some meat samples in very low quantities. *M. mustelus* meat can thus be regarded as healthy in terms of human nutrition, except for the possible hazard of high mercury levels in sharks, which should be further investigated.

#### Introduction

Fish meat has long been recognised as a very nutritious food source, particularly due to its high content of protein and essential amino acids (Geiger & Borgstrom, 1962; YÁÑEZ *et al.*, 1976). Jacquot (1961) described shark meat, in general, as having a proximate composition of 77.2% water, 19% meat protein, 2.5% lipid and 1.3% ash.

Shark protein has been found to be slightly superior when compared to casein (milk protein) as a standard reference with regards to the amino acid composition, being rich in lysine, arginine, alanine, glutamic acid, threonine and cysteine (Geiger & Borgstrom, 1962). Fish muscle also contains nitrogen-containing compounds of non-protein nature. This non-protein nitrogen exists mainly in the form of urea and trimethylamine oxide (TMAO) in shark meat and can make up a significant part of the total nitrogen content. These compounds play an important role in osmoregulation in the shark's body, but can negatively affect the meat quality and flavour if the carcass and meat is not handled correctly after catch (Vannuccini, 1999).

Shark meat is known to contain high levels of omega-3 fatty acids, comprising highly unsaturated fatty acids with up to five or six double bonds (Huss, 1988). Okland *et al.* (2005) found that polyunsaturated fatty acids (PUFAs) form a significant part (48-63%) of the total fatty acids in elasmobranchs and most cartilaginous fish have lower levels of free fatty acids, as well as lower levels of cholesterol, than bony fish (Økland *et al.*, 2005). Shark meat, therefore, appears to have a lipid composition of high nutritional value.

As sharks feed at a high trophic level in the marine food web, they are known to accumulate chemical contaminants through the food chain, such as the heavy metal mercury, in a

process called biomagnification (Ababouch & Gram, 2004). Another reason for the possibly high levels of mercury in sharks can be attributed to the fact that these fish are long-lived and mercury is not excreted. Rather, mercury binds to the protein component in the meat, causing the bioaccumulation of this heavy metal in the meat during the life span of the shark (Ababouch & Gram, 2004). The consumption of high levels of mercury can lead to mercury poisoning in humans. In many countries, including South Africa, the maximum limit for total mercury in seafood is therefore specified as  $1 \text{ mg}\cdot\text{kg}^{-1}$  (ppm) (Ababouch & Gram, 2004).

Histamine is a biogenic amine produced in foods by the decarboxylation of the corresponding free amino acid, histidine, in a process catalysed by bacterial amino acid decarboxylases (Ababouch & Gram, 2004). Histidine is a naturally occurring amino acid in fish muscle and is generally found in large amounts in the muscle of fatty, red-meat active and migratory fish species (Ababouch & Gram, 2004). The formation of histamine in seafood species is predominantly a result of time-temperature abuse. The consumption of high levels of histamine can lead to histamine poisoning (scombrotoxic poisoning) in humans and histamine levels therefore need to be monitored. Although there is currently limited information in the scientific literature pertaining to histamine levels in shark meat, Huss (1988) reported that the histidine content in shark meat is relatively low ( $<1.0 \text{ mg}\cdot 100 \text{ g}^{-1}$  wet weight) and a study by Amano and Bito (1951) suggested that histamine does not seem to be readily formed in shark meat. However, thorough research has not yet been done on this topic.

Even though *M. mustelus* meat is consumed commercially and is one of South Africa's major export shark species, there is currently no specific information on the chemical composition and nutritional value of this shark meat. The variation found in the chemical composition of shark meat is discussed in chapters 3 and 4. In the current chapter, data from the previous two chapters is combined to describe the average chemical composition and nutritional value of *M. mustelus* meat. This data will not only be beneficial for voluntary nutritional labelling of this food commodity, but it will also make a valuable contribution to the new South African Food Composition Tables being compiled by the Medical Research Council.

## **Materials and methods**

Sampling and proximate analyses were performed as described in chapter 3 and sub-sampling and analytical methods as described in chapter 4, with the exception of the urea analyses. The results from chapter 3 and chapter 4 were used for the description of the overall chemical composition of *M. mustelus* meat in the present chapter.

### Urea and ammonia analysis

The concentrations of urea and ammonia in 10 shark meat samples were measured using the R-Biopharm urea/ammonia enzymatic assay test kit (Cat. No.10 542 946 035, supplied by AEC



Amersham, Cape Town, South Africa). Sample preparation was performed in accordance with the instructions detailed in the kit insert for meat and meat products. This enzymatic method is based on the hydrolysis of urea to ammonia and carbon dioxide in the presence of the enzyme urease. Ammonia then reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase (GIDH) and reduced nicotinamideadenine dinucleotide (NADH), resulting in the oxidation of NADH. The consumption of NADH is measured at 340nm to determine the concentration of ammonia and urea present in the sample. The absorbance values ( $A_{340}$ ) were determined with an Aurius, 2000 Series spectrophotometer (Part No. 2021 00 01, Cecil instruments limited, Milton technical centre, Cambridge, CB4 6AZ, England), and these were used to calculate the concentration of urea and ammonia in the sample solution ( $\text{g}\cdot\text{L}^{-1}$  sample solution) and consequently the amount of urea and ammonia in the shark meat samples ( $\text{g}\cdot 100 \text{ g}^{-1}$  meat sample).

The urea values were thereafter used to calculate the amount of N present in the meat in the form of urea. This  $N_{\text{urea}}$  was subtracted from the  $N_{\text{total}}$  obtained from the LECO analyses of total protein in the meat sample, in order to obtain an estimated value for  $N_{\text{protein}}$ . This  $N_{\text{protein}}$  was then multiplied by the N:P conversion factor 6.25 to calculate the corrected protein value of *M. mustelus* meat.

## Results

The average values for the proximate composition of *M. mustelus* sharks are calculated from one sample analysed in duplicate per shark (total of 62 sharks) (Table 5.1). These values can be considered to be representative of the entire *M. mustelus* population in the Langebaan lagoon as it was found in chapters 3 and 4 that there were no significant differences with regards to the proximate components between different body locations within the shark or between sharks of different genders, sizes and life cycle stages.

**Table 25** The mean values ( $\text{g}\cdot 100 \text{ g}^{-1}$  meat) and the standard error for the overall proximate composition of smoothhound shark (*M. mustelus*) meat (n = 62)

Proximate component	Range	Mean $\pm$ Std Error	Coefficient of variation
Moisture	72.63 - 77.91	74.90 $\pm$ 0.17	1.71
Protein	18.05 - 27.60	23.41 $\pm$ 0.21	2.62
Lipids	0.68 - 7.08	1.59 $\pm$ 0.10	0.67
Ash	0.99 - 3.63	1.43 $\pm$ 0.05	0.13

The average amino acid composition (Table 5.2) calculated from one sample (in duplicate) per shark (sub-sample of 30 sharks) can also be considered to be representative of the *M. mustelus* population as no significant variations were found with regards to the gender, size and life cycle stage in the quantities of these individual fatty acids (Chapter 4). Glutamic acid was found to be the



most abundant amino acid ( $2.98 \pm 0.09 \text{ g}\cdot 100 \text{ g}^{-1}$  meat sample) with aspartic acid ( $1.85 \pm 0.05$ ), leucine ( $1.54 \pm 0.04$ ) and lysine ( $1.64 \pm 0.05$ ) in high concentrations and histidine ( $0.36 \pm 0.02$ ) and methionine ( $0.52 \pm 0.02$ ) in low concentrations.

**Table 26** The mean values ( $\text{g}\cdot 100 \text{ g}^{-1}$  meat) and the standard error for the overall amino acid composition of smoothhound shark (*M. mustelus*) meat (n = 30)

Amino acid	Range	Mean $\pm$ Std Error	Coefficient of variation
Asp	1.49 - 2.65	$1.854 \pm 0.053$	0.087
Glu	2.33 - 4.32	$2.976 \pm 0.090$	0.245
Ser	0.58 - 1.10	$0.761 \pm 0.022$	0.014
His	0.08 - 0.57	$0.356 \pm 0.018$	0.011
Gly	0.57 - 1.26	$0.777 \pm 0.026$	0.020
Thr	0.48 - 1.26	$0.856 \pm 0.032$	0.031
Arg	0.67 - 1.26	$0.877 \pm 0.027$	0.022
Ala	0.88 - 1.71	$1.149 \pm 0.034$	0.035
Tyr	0.52 - 1.04	$0.713 \pm 0.022$	0.015
Val	0.71 - 1.31	$0.899 \pm 0.027$	0.021
Met	0.40 - 0.80	$0.518 \pm 0.016$	0.008
Phe	0.65 - 1.20	$0.819 \pm 0.024$	0.018
Ile	0.74 - 1.34	$0.918 \pm 0.027$	0.022
Leu	1.19 - 2.23	$1.543 \pm 0.045$	0.060
Lys	1.12 - 2.40	$1.639 \pm 0.053$	0.085

The mean fatty acid values (Table 5.3) are representative of the entire *M. mustelus* shark population, including both genders, all sizes and both pregnant and non-pregnant females. Some fatty acids, however, were found to be present at higher levels in females than in males. These fatty acids included C14:0, C16:0, C18:0, C21:0, C16:1n7, C18:1n9, C20:1n9, C24:1n9, C18:3n3, C20:2, C20:4n6, C22:5n3, C22:6n3. Most of these fatty acids showing a difference with gender (C16:0, C18:0, C21:0, C16:1n7, C18:1n9, C24:1n9, C18:3n3, C22:5n3, C22:6n3) also exhibited higher levels in non-pregnant females than in pregnant females. With gender and life cycle stage variation, the total groups of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) as well as poly-unsaturated fatty acids (PUFA) were all present at higher levels in females and in non-pregnant females compared to males and pregnant females respectively. Fatty acids found to be present in significant amounts in *M. mustelus* meat included palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9), dihomo-gamma-linolenic acid (C20:3n6) and docosahexaenoic acid (C22:6n3). The total amount of PUFAs was only slightly lower than that of the total SFAs. The total

MUFAs made up only a small amount of the total fatty acids. The amount of n-3 PUFAs was higher than that of the n-6 PUFAs, giving a n-6:n-3 ratio of 0.39.

**Table 27** The mean values (g·100 g meat<sup>-1</sup> sample) and the standard error for the overall fatty acid composition of smoothhound shark (*M. mustelus*) meat (n = 30)

Fatty acid	Range	Mean ± Std Error	Coefficient of variation
14:0	0.02 - 0.09	0.045 ± 0.004	0.0004
15:0	0.01 - 0.04	0.021 ± 0.001	0.0001
16:0	0.68 - 3.44	1.872 ± 0.109	0.357
18:0	0.36 - 1.67	0.943 ± 0.056	0.096
20:0	0.01 - 0.03	0.018 ± 0.001	0.00003
21:0	0.01 - 0.03	0.015 ± 0.001	0.00004
22:0	0.02 - 0.15	0.075 ± 0.006	0.001
24:0	0.01 - 0.03	0.015 ± 0.001	0.00003
14:1	0.01 - 0.02	0.013 ± 0.001	0.00002
15:1	0.01 - 0.03	0.014 ± 0.001	0.00003
16:1n7	0.04 - 0.28	0.116 ± 0.012	0.004
18:1n9	0.26 - 0.97	0.562 ± 0.036	0.039
20:1n9	0.00 - 0.01	0.008 ± 0.000	0.000005
22:1n9	0.00 - 0.02	0.010 ± 0.001	0.00001
24:1n9	0.01 - 0.07	0.035 ± 0.002	0.0002
18:2n6	0.04 - 0.20	0.085 ± 0.008	0.002
18:3n3	0.01 - 0.07	0.035 ± 0.003	0.0003
18:3n6	0.01 - 0.05	0.020 ± 0.002	0.0001
20:2	0.01 - 0.06	0.030 ± 0.002	0.0001
20:3n6	0.08 - 1.03	0.527 ± 0.045	0.061
20:3n3	0.00 - 0.03	0.011 ± 0.001	0.00003
20:4n6	0.01 - 0.05	0.025 ± 0.002	0.0001
20:5n3	0.02 - 0.20	0.077 ± 0.009	0.002
22:2	0.00 - 0.03	0.011 ± 0.001	0.00003
22:5n3	0.08 - 0.69	0.351 ± 0.033	0.032
22:6n3	0.27 - 2.78	1.316 ± 0.087	0.226
Σ SFA	1.15 - 5.36	3.005 ± 0.172	0.884
Σ MUFA	0.38 - 1.30	0.758 ± 0.049	0.073
Σ PUFA	0.58 - 4.81	2.487 ± 0.148	0.661
PUFA:SFA	0.50 - 1.09	0.826 ± 0.022	0.015
n-6:n-3	0.18 - 0.64	0.385 ± 0.026	0.021

The mineral content of *M. mustelus* shark, as presented in Table 5.4, is representative for both genders, all sizes and pregnant as well as non-pregnant females, except for aluminium which has higher levels in pregnant females. The main minerals in *M. mustelus* meat are phosphorus,

potassium, magnesium, sodium and calcium in decreasing quantity, with trace amounts of iron, copper, zinc, manganese, boron and aluminium.

**Table 28** The mean values ( $\text{mg}\cdot 100\text{ g}^{-1}$  meat sample) and the standard error for the overall mineral composition of smoothhound shark (*M. mustelus*) meat (n = 30)

$\text{mg}\cdot 100\text{ g}^{-1}$ meat	Range	Mean $\pm$ Std Error	Coefficient of variation
Phosphorus	109.36 - 289.48	211.64 $\pm$ 5.64	954.51
Potassium	96.49 - 321.64	231.87 $\pm$ 7.75	1800.70
Calcium	8.58 - 38.60	16.80 $\pm$ 1.26	47.91
Magnesium	17.15 - 42.89	34.67 $\pm$ 0.87	22.54
Sodium	17.61 - 54.43	29.50 $\pm$ 1.33	53.14
Iron	0.35 - 7.71	1.17 $\pm$ 0.24	1.69
Copper	0.03 - 0.41	0.12 $\pm$ 0.01	0.005
Zinc	0.25 - 0.81	0.53 $\pm$ 0.02	0.010
Manganese	0.01 - 0.05	0.022 $\pm$ 0.002	0.0001
Boron	0.01 - 0.04	0.018 $\pm$ 0.001	0.00003
Aluminium	0.25 - 8.98	3.36 $\pm$ 0.46	6.35

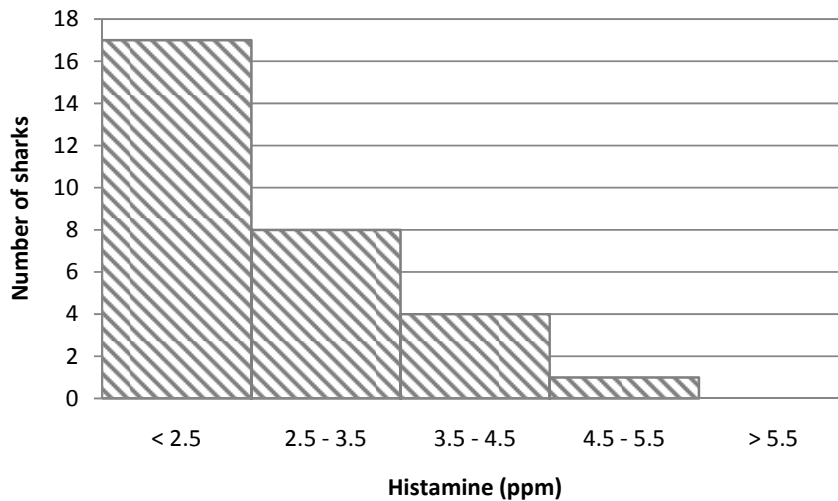
The mean value for the mercury content analysed from 30 sharks was  $0.90\text{ mg}\cdot\text{kg}^{-1}$ . Even though this mean value is below the legal limit according to EU and US regulation, some sharks were found to contain mercury levels far exceeding the maximum legal limit, whereas others had levels far below the limit. This mean value can therefore not be accepted to be representative of the entire *M. mustelus* population, as the mercury content varied significantly between individual sharks (Table 5.5).

Of the 30 histamine sample analysed, 17 of these samples (ca. 57%) were found to contain histamine levels which were below the limit of detection of the enzyme-linked immunosorbant Assay (ELISA) kit utilised ( $<2.5\text{ mg}\cdot\text{kg}^{-1}$  (ppm)) (Fig. 5.1). Of the 13 samples which were found to contain quantifiable levels of histamine, the maximum histamine level found among all analysed *M. mustelus* samples was  $4.5\text{ mg}\cdot\text{kg}^{-1}$  (ppm).

The amount of urea in the 10 meat samples analysed ranged between 1.0 and  $1.9\text{ g}\cdot 100\text{ g}^{-1}$  (wet weight). The corrected protein values were 2.9 to 5.5% lower than the total protein values calculated from the LECO analyses, giving a mean total protein value (n=10) of  $19.5\text{ g}\cdot 100\text{ g}^{-1}$  meat.

**Table 29** Total mercury content ( $\text{mg}\cdot\text{kg}^{-1}$ ) of 30 individual smoothhound (*M. mustelus*) shark samples

Sample nr	Category	Total Hg ( $\text{mg}\cdot\text{kg}^{-1}$ )
3	Female large	1.26
36	Female large	0.32
20	Female large	0.55
22	Female large	0.32
63	Female large	0.80
64	Female large	1.19
38	Female medium	0.54
43	Female medium	0.70
31	Female medium	1.27
48	Female medium	1.13
53	Female medium	0.50
54	Female medium	0.60
4	Female large pregnant	0.90
5	Female large pregnant	2.78
6	Female large pregnant	0.96
9	Female large pregnant	1.14
45	Female large pregnant	0.89
46	Female large pregnant	0.88
16	Female small	0.21
17	Female small	0.12
19	Female small	0.33
23	Female small	0.76
60	Female small	0.28
61	Female small	0.30
42	Male large	0.57
47	Male large	1.11
49	Male large	1.39
50	Male large	2.11
56	Male large	1.23
58	Male large	1.78



**Figure 15** Histogram showing the amount of histamine detected in 30 smoothhound shark (*M. mustelus*) meat samples

## Discussion

Comparing the proximate composition of *M. mustelus* meat to that of ten other shark species (including *Isurus oxyrinchus* (shortfin mako shark), *Lamna nasus* (porbeagle shark), *Scoliodon sorrakowah*, *Heterodontus francisci* (horn shark), *Carcharhinus brachyurus* (copper shark), *Carcharhinus longimanus* (white tipped shark), *Sphyrna* spp. (hammerhead shark), *Carcharhinus falciformis* (silky shark), *Galeocerdo cuvier* (tiger shark) and *Centrophorus squamosus* (leafscale gulper shark)) (Gordievskai<sup>a</sup> & Kizevetter, 1973; Chandrashekar & Deosthale, 1993; Vlieg *et al.*, 1993; Økland *et al.*, 2005), and general information on fish fillets (Huss, 1988) (Table 5.6), the average moisture ( $74.9 \pm 0.17 \text{ g} \cdot 100 \text{ g}^{-1}$ ) and ash ( $1.4 \pm 0.05 \text{ g} \cdot 100 \text{ g}^{-1}$ ) contents were found to fall within the range of the average values of ten other shark species (moisture: 74 - 82  $\text{g} \cdot 100 \text{ g}^{-1}$ , ash: 0.6 - 1.8  $\text{g} \cdot 100 \text{ g}^{-1}$ ) as well as the general values for fish fillet (moisture: 66 - 81  $\text{g} \cdot 100 \text{ g}^{-1}$ , ash: 1.2 - 1.5  $\text{g} \cdot 100 \text{ g}^{-1}$ ). The amount of total lipids ( $1.6 \pm 0.10 \text{ g} \cdot 100 \text{ g}^{-1}$ ) was, however, higher in *M. mustelus* meat than that which has been recorded for the ten other shark species (average 0.6  $\text{g} \cdot 100 \text{ g}^{-1}$ ). This could be related to the activity levels of these sharks, which is lower than some other species. *Mustelus mustelus* mainly rests on the bottom of the seabed (Smale & Compagno, 1997) and little energy is therefore burnt resulting in a higher muscle fat content.

The lipid component is known to be the proximate component with the most variation between individual sharks and different shark species (Huss, 1988), thus accounting for the large range seen in terms of lipid percentages in fish fillet (Table 5.6). Fish meat is generally grouped into different categories according to their fat content: high fat ( $>8 \text{ g} \cdot 100 \text{ g}^{-1}$ ), medium fat (4

- 8 g·100 g<sup>-1</sup>), low fat (2 - 4 g·100 g<sup>-1</sup>) and lean meat (<2 g·100 g<sup>-1</sup>) (Ackman, 1989). *Mustelus mustelus* meat has a relatively low lipid content and can be classified as lean fish, owing to its average lipid content of 1.6 ± 0.10 g·100 g<sup>-1</sup>.

The value for total protein in *M. mustelus* meat (23.41 ± 0.21 g·100 g<sup>-1</sup>) is higher than the values for nine other shark species (16 - 22 g·100 g<sup>-1</sup>) as well as for fish fillets in general (16 - 21 g·100 g<sup>-1</sup>). This high value may, however, be due to an error in the conversion factor (6.25) when converting the N value to percentage protein. This error can be due to the fact that elasmobranchs contain high levels of non-protein nitrogen in the form of urea/ammonia and trimethylamine oxide (TMAO) which plays a role in osmoregulation in the shark's body (Geiger & Borgstrom, 1962). This error in the protein value will be discussed later in this chapter.

**Table 30** Proximate composition (g·100 g<sup>-1</sup> wet weight) of smoothhound shark (*M. mustelus*) meat compared to the average of 10 other shark species and fish fillet in general

Proximate component (g·100 g <sup>-1</sup> )	Variation of fish fillet <sup>1</sup>	Range of 10 shark species <sup>2</sup>	Average of 10 shark species <sup>2</sup>	Range of <i>M. mustelus</i>	Average of <i>M. mustelus</i>
Moisture	66 – 81	74 – 82	77.9	72.63 - 77.91	74.9
Protein	16 – 21	16 – 22	19.4	18.05 - 27.60	23.4
Lipids	0.2 – 25	0.1 – 1.3	0.6	0.68 - 7.08	1.6
Ash	1.2 – 1.5	0.6 – 1.8	1.2	0.99 - 3.63	1.4

<sup>1</sup> (Huss 1988)

<sup>2</sup> Shortfin mako shark, porbeagle shark, *Scoliodon sorrakowah*, horn shark, copper shark, white tipped shark, hammerhead shark, silky shark, tiger shark and leafscale gulper shark (Gordievskaja and Kizevetter 1973, Chandrashekar and Deosthale 1993, Vlieg et al. 1993, Økland et al. 2005)

The mean amino acid concentrations (in g·100 g<sup>-1</sup> protein) of *M. mustelus* meat from the present study are compared in Table 5.7 to the average of the amino acid profiles of four food fishes (cod (*Gadus callarias*), haddock (*G. aeglefinus*), lemon sole (*Pleuronectes microcephalus*) and herring (*Clupea harengus*)) as described by Connel and Howgate (1959), as well as one other shark species (*Scoliodon sorrakowah*) as described by Chandrashekar and Deosthale (1993). The amino acid concentrations for *M. mustelus* are lower than that of the fish fillets and most of the amino acids have slightly lower concentrations than those found in the *Scoliodon sorrakowah* shark. The proportions of the amino acid concentrations are, however, mostly similar to that of fish fillets and *Scoliodon sorrakowah* shark, with the exceptions of histidine having the lowest concentration in *M. mustelus*, while methionine and serine have the lowest concentrations in fish fillets and *Scoliodon sorrakowah* shark, respectively.

Table 5.8 lists some of the essential amino acids and their daily requirements for human nutrition (FAO, 2007). A 100 g portion of *M. mustelus* fillet provides more than 50% of the daily requirements of threonine, isoleucine, leucine and lysine with threonine and lysine meeting the daily requirements with 78%, whereas about 50% of the daily requirement for valine, methionine,

phenylalanine and histidine are met by the same portion. Several fish species have high levels of lysine with a deficiency in methionine (Geiger & Borgstrom, 1962). The *M. mustelus* meat appears to be especially high in lysine (10% of the total amino acids), with a 100 g portion providing 78% of the daily requirement of a 70 kg adult (FAO, 2007).

**Table 31** The mean values (g·100 g<sup>-1</sup> protein) for the amino acid composition of smoothhound shark (*M. mustelus*) meat compared to one other shark species and the average of four food fishes

Amino acid	<i>M. mustelus</i>	Shark <sup>1</sup>	Fish <sup>2</sup>
Aspartic acid	8.04	8.4	11.25
Glutamic acid	12.92	13.9	16.89
Serine	3.29	2.4	5.79
Histidine	1.58	3.9	3.61
Glycine	3.27	3.9	5.09
Threonine	3.72	3.9	5.52
Arginine	3.78	5.4	6.96
Alanine	4.94	5.0	7.12
Tyrosine	3.15	3.1	4.12
Valine	3.93	5.0	5.83
Methionine	2.16	4.1	2.68
Phenylalanine	3.63	4.0	4.73
Isoleucine	3.89	5.1	5.03
Leucine	6.79	7.1	9.23
Lysine	7.24	9.3	10.59
Total	72.33		

<sup>1</sup> *Scoliodon sorrakowah* (Chandrashekar & Deosthale, 1993)

<sup>2</sup> Cod, haddock, lemon sole and herring (Connell & Howgate, 1959)

**Table 32** Daily amino acid requirements and the amount provided by a 100 g smoothhound shark (*M. mustelus*) fillet servings

Amino acid	Daily requirements of a 70 kg adult <sup>2</sup>	g·100 g <sup>-1</sup> <i>M. mustelus</i> meat	Percentage covered when consuming 100 g fillet
Threonine	1.1	0.86	78
Valine	1.8	0.90	50
Methionine	1.1	0.52	47
Phenylalanine	1.8	0.82	46
Isoleucine	1.4	0.92	66
Leucine	2.7	1.54	57
Lysine	2.1	1.64	78
Histidine	0.7	0.36	51

<sup>2</sup>(FAO, 2007)

The ratio of PUFA to SFA of *M. mustelus meat* ( $0.83 \pm 0.022$ ) is above the recommended minimum of 0.45 as specified by the Department of Health of the United Kingdom (UK) (Justi *et al.*, 2003; Ozogul *et al.*, 2007).

In terms of human nutrition, omega-3 fatty acids are essential for normal growth. Simopoulos (1991) suggested an optimal daily intake of 800 - 1100 mg of linolenic acid (18:3n3) and 300 - 400 mg of long-chain n-3 PUFAs. From the results of this study, a 100 g portion of *M. mustelus* fillet contains 174 mg of long-chain n-3 PUFAs in the form of eicosapentaenoic acid (20:5n3), docosapentaenoic acid (22:5n3) and docosahexaenoic acid (22:6n3) which is about half that of the suggested optimal daily intake.

Omega-6 fatty acids are important components of cell membranes, but, in excess, can present a risk for heart disease (Simopoulos, 1991). It is therefore essential to maintain the correct ratio of n-6 to n-3 fatty acids in the diet. The recommended maximum ratio as specified by the Department of Health of the UK (Justi *et al.*, 2003; Ozogul *et al.*, 2007) is 4. The n-6:n-3 ratio in *M. mustelus* meat ( $0.39 \pm 0.026$ ) is well below this maximum. This shark meat can therefore be considered as a healthy source of omega-3 fatty acids. Compared to the average fatty acid profile of nine marine fish determined in a Turkish study, the n-6:n-3 fatty acid ratio is within the ranges of these fish species (maximum 0.59, minimum 0.009) (Ozogul *et al.*, 2007).

Lean fish with a low SFA content is beneficial for the prevention of heart diseases, but so are fish with high levels of n-3 fatty acids (Økland *et al.*, 2005). It is therefore important to look not only at the total lipid content of fish meat, but also at the fatty acid composition with regards to the n-3 PUFAs. *Mustelus mustelus* meat can therefore be considered as a healthy lipid food source as it has a low total lipid content ( $1.6 \pm 0.10 \text{ g} \cdot 100 \text{ g}^{-1}$ ), of which a significant amount consists of n-3 PUFAs.

Nine of the 11 elements analysed in this study are considered to be essential in terms of human nutrition. These include Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn. The amounts of these minerals in 100 g of *M. mustelus* fillet are all far below the recommended daily dietary allowance (RDA) or estimated safe and adequate daily dietary intake (EDI) as determined by the National Research Council (NRC) of the United States (Table 5.9) (Teeny *et al.*, 1984). Compared to the mineral content of shortfin mako shark as described by Teeny *et al.* (1984), *M. mustelus* meat is higher in Ca ( $16.80 \pm 1.26 \text{ mg} \cdot 100 \text{ g}^{-1}$  meat) than shortfin mako shark ( $12 \text{ g} \cdot 100 \text{ g}^{-1}$  meat), but much lower in Cu, K, Mn and Na.

The aluminium content in *M. mustelus* meat ( $3.36 \pm 0.46 \text{ mg} \cdot 100 \text{ g}^{-1}$  meat) was similar to that found in other fish samples analysed by Muller *et al.* (1998), which ranged from 1.2 to 5.5  $\text{mg} \cdot 100 \text{ g}^{-1}$  meat sample. Boron levels in *M. mustelus* meat were found to be very low ( $0.02 \pm 0.001 \text{ mg} \cdot 100 \text{ g}^{-1}$  meat), results in agreement with previous studies (Saiki *et al.*, 1993). These results indicate that boron is not biomagnified in the aquatic food chain and does therefore not accumulate in fish. Although the safe daily intake of B had not been determined, an acceptable intake of  $13 \text{ mg} \cdot \text{day}^{-1}$  was recommended by Nielsen (1997). *Mustelus mustelus* meat can therefore



be considered to be safe for human consumption with regards to the mineral elements, since none of these appeared to exceed toxic limits.

**Table 33** RDA and EDI of essential minerals and that supplied by a 100 g serving of smoothhound shark (*M. mustelus*) fillet compared to shortfin mako shark fillet

Mineral element	Recommended intake (mg) <sup>1</sup>		mg·100 g <sup>-1</sup> <i>M. mustelus</i> fillet	% of RDA or EDI	mg·100 g <sup>-1</sup> shortfin mako fillet <sup>2</sup>
	RDA	EDI			
Ca	800	-	16.80	2.1	12
Cu	-	2.0 - 3.0	0.12	4.0 - 6.0	35
Fe	10	-	1.17	11.7	1.2
K	-	1875 – 5625	231.87	4.1 - 12.4	325
Mg	350	-	34.67	9.9	25
Mn	-	2.5 - 5.0	0.02	0.4 - 0.8	5
Na	-	1100 – 3300	29.50	0.9 - 2.7	104
P	800	-	211.64	26.5	220
Zn	15	-	0.53	3.5	0.4

RDA = Recommended Daily Dietary Allowance

EDI = Estimated Safe and Adequate Daily Dietary Intake

<sup>1</sup> Source: (Teeny *et al.*, 1984)

<sup>2</sup> Source: (Vlieg *et al.*, 1993)

There was significant variation in the mercury content between individual samples, with some samples having mercury levels far above (2.78 mg·kg<sup>-1</sup>) the maximum limit of 1 ppm (mg·kg<sup>-1</sup>) (FDA (US Food and Drug Administration), 1998; EC (European Commission), 2001a) and other samples safely below this limit (0.12 mg·kg<sup>-1</sup>). From the present data, no conclusion as to the safety of *M. mustelus* meat regarding total mercury content can therefore be drawn. The actual toxic mercury component in fish meat is the organic form of mercury (methylmercury), which is also the predominant form (63 – 90% of total mercury) (Storelli *et al.*, 2002). Further research is therefore needed to conclusively determine the toxicity of *M. mustelus* meat in relation to mercury.

Histamine levels were all well below the suggested maximum limit of 50 ppm histamine in seafood as published in a paper by the FAO (Ababouch & Gram, 2004), with most samples not even containing detectable (<2.5 ppm) levels of histamine. High levels of histamine were, however, not expected as the free amino acid, histidine, which acts as a substrate in histamine formation, appears in very low levels in *M. mustelus* meat (0.36 g·100 g<sup>-1</sup> meat) (Table 5.2). Histamine formation would therefore not likely present a significant cause of concern in *M. mustelus* meat, even if the cold chain is not maintained after catch.

Previous studies have shown the amount of urea in shark meat in general ranges from 1.0 to 2.1% (Simidu, 1961; Huss, 1988). The urea results obtained in the current study showed similar variation (1.0 to 1.9%). This variation could be due to the inconsistency in the bleeding process

after the sharks had been caught, as the meat is contaminated by the urea in the blood if the shark is not bled properly after capture (Vannuccini, 1999).

The corrected protein values, calculated by subtracting the  $N_{\text{urea}}$  from the  $N_{\text{total}}$ , ranging between 17.7 and 23.3% (average 19.5%) are closer to the protein values for shark and fish meat found in literature (Gordievskai<sup>a</sup> & Kizevetter, 1973; Huss, 1988; Chandrashekar & Deosthale, 1993; Vlieg *et al.*, 1993; Økland *et al.*, 2005). For a more accurate protein content calculation, other non-protein nitrogen fractions such as ammonia and TMAO should also be taken into account.

## Conclusion

The proximate composition of *M. mustelus* meat is similar to that of other shark species, but contains a slightly higher lipid content. It is, however, still classified as a lean meat since it has a lipid content below 2%. *Mustelus mustelus* meat can be regarded as a good source of essential amino acids and has a healthy lipid content with a good ratio (>0.45) of PUFA:SFA (0.83), as well as a healthy (<4) n-6:n-3 fatty acid ratio of 0.39. *Mustelus mustelus* meat has a low mineral content, with a 100 g portion of meat only providing a small percentage of the RDA/EDI. The meat appears to be safe with regards to histamine and the mineral elements analysed. Nonetheless, the high levels of mercury found in certain samples of *M. mustelus* meat are of concern, warranting further research into the mercury content of shark meat.

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## Chapter 6

### GENERAL DISCUSSION AND CONCLUSIONS

In recent years, consumer trends towards health and nutrition have led to an enormous increase in the consumption of seafood products (Gil, 2007). At the same time, consumers have begun to express the desire to obtain accurate information on the food they eat, especially as pertaining to the correspondence of the food contents with what is declared on the label. There is thus an urgent need to obtain comprehensive nutritional data for a large variety of food products, both for voluntary nutritional labelling and for incorporation into food composition tables for dietetic applications.

The smoothhound shark (*Mustelus mustelus*) is caught commercially in South Africa and is consumed both locally and internationally. Nonetheless, there is presently no information on the nutritional value or safety of this shark meat for human consumption. This study aimed to fill this research void by primarily determining the overall chemical composition and nutritional value of *M. mustelus* meat. In order to fulfil this aim, however, a number of objectives needed to be achieved.

Firstly, the proximate composition of five individual body sites of the *M. mustelus* shark was determined in order to evaluate the cross-carcass variation in the meat in terms of the individual proximate components (moisture, protein, lipid and ash). This variation was determined in order to identify a representative sample of the edible part of the shark (fillet and body flap) which could be used for subsequent chemical analyses. Secondly, the sample, found to be most representative of the entire shark fillet, was used to investigate the endogenous factors (gender, size and life cycle stage) and their effects on the individual proximate components and other meat components (amino acids, fatty acids, minerals, histamine and mercury contents). Finally, the data obtained from the two aforementioned objectives were combined to describe the average chemical composition and nutritional value of *M. mustelus* meat.

The results from this study showed that there was no significant variation in the proximate composition of the meat taken at different locations of the body of the *M. mustelus* shark. Any of the samples evaluated in this study could thus be considered as representative of the entire edible fillet of the shark meat. From a commercial and economical perspective, the sample site causing the least damage to the fillet and which is the simplest to obtain would be preferable to take for chemical analyses. In this study, this site was identified to be the sample close to the head (sample A), since this sample could be removed from the end of the fillet, leaving the main part of the fillet intact. Thus, sample A was used for all further analyses conducted in this study.

Subsequently, it was found that all three main effects (gender, size and life cycle stage) did not have any major influences on most of the chemical components analysed in *M. mustelus* meat. The only significant variations in the chemical composition seen in terms of gender were the higher

values obtained for fatty acids in females (corresponding to the data presented by Love (1980), as well as the higher levels of mercury in large male sharks (which had mercury levels exceeding the maximum level of 1 ppm for this heavy metal). The only chemical component affected by size variation was the level of fatty acids, which showed a trend to decrease in quantity before maturity was reached, contradicting the increase in quantity that is expected from the scientific literature (Love, 1980). Variation due to life cycle stages was mostly evident in the fatty acid component, with some small effects on two mineral components, aluminium and copper, which were present at slightly higher levels in non-pregnant large females. The biological significance of this finding is, however, unclear. In terms of the fatty acids, the pregnant females had lower levels than the non-pregnant females, which once again correlated with the data reported in this regard in the literature (Love, 1980). Therefore, compared to the data reported for many other fish and shark species (Jacquot, 1961), *M. mustelus* appears to be a shark species that exhibits limited variation in terms of meat composition.

From these results on the variation in the chemical composition of the meat within the *M. mustelus* species, an average meat composition was determined, taking note of the variation in certain components. The proximate composition of *M. mustelus* meat was found to be similar to that of other shark species, but was seen to contain a slightly higher lipid content. The meat of this species can, however, still be classified as a lean meat, since it had a lipid content below 2%. *Mustelus mustelus* meat appears to be a good source of essential amino acids and has a healthy lipid content with a good ratio (>0.45) of PUFA:SFA (0.83), as well as a healthy (<4) n-6:n-3 fatty acid ratio of 0.39. *Mustelus mustelus* meat was determined to have a low mineral content, with a 100 g portion of meat only providing a small percentage of the recommended daily dietary allowance (RDA) or the estimated safe and adequate daily dietary intake (EDI). The meat appears to be safe for consumption with regards to histamine and mineral contaminants (Aluminium, Boron) analysed. However, the mercury levels exceeding 1 ppm that were measured in certain shark species may warrant concern from a health perspective.

Overall, this study has, for the first time, described the chemical composition and nutritional value of *M. mustelus* meat. The generated data will prove invaluable for both voluntary nutritional labelling in this country, as well as for incorporation into the new South African Food Composition Tables being compiled by the Medical Research Council (MRC). In addition, new light has been shed on the health benefits of the consumption of this meat, as well as on certain safety concerns (mercury) for human consumption.

A number of avenues for further research have been identified from this study. Firstly, investigation into the non-protein nitrogen (NPN) components in *M. mustelus* shark meat is required, since these seem to be present in significant amounts, especially in the form of urea/ammonia and trimethylamine oxide (TMAO). From this study it is clear that these NPN compounds affect the total protein analyses, resulting in higher values than the true total protein values. Further research on the fatty acids composition linked with the biology of the shark should

be conducted in order to explain the trend for the fatty acid levels of *M. mustelus* to decrease prior to maturity, which is contrary to what has been for sharks found in the past. Lastly, a comprehensive evaluation of the mercury content found in this shark meat is imperative in order to accurately assess the health hazards posed by this heavy metal in *M. mustelus* shark meat and, if necessary, to create guidelines on suggested portion sizes that will be safe for consumption.

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