Chapter 2.

Biochemical analyses of liver oils of selected sharks & chimaeras of the Indian EEZ
Chapter 2. Biochemical analyses of liver oils of selected sharks & chimaeras of the Indian EEZ

2.1 INTRODUCTION
Sharks, chimaeras and other elasmobranch resources are of great commercial importance the world over, apart from being a significant link in the marine ecology. The annual average landings of sharks, skates and rays during 1996-2006 were 60,866 t of which sharks constituted 60.1% (36,592 t) (CMFRI statistics, 1996-2006). Though a harvest potential of 1,85,000 t of elasmobranchs has been indicated for the Indian EEZ, they have not been fully exploited. Sixty-five species of shark have been sighted in Indian waters and over 20 of these, of the Carcharhinidae and Sphyrnidae families, contribute to the fishery. Tamil Nadu, Gujarat, Maharashtra, Kerala, Karnataka and Andra Pradesh supply around 85% of the shark landings in India. Despite the commercial importance, no serious attempts have so far been made at any targeted exploration of this valuable resource.

There are several types of gear that take sharks as incidental catch; the most important among them being trawl nets and gill nets (FAO, 1999). There is no detailed information on the landings of sharks by gear type but data available on shark production by mechanized boats at major fishing centres show that trawl nets account for 60% of the shark landings and gill nets account for 38%. Purse seine in Cochin and Mangalore and hook-and-line in Cochin and Bombay take a very small fraction of the catch.

Most of the sharks and chimaeras are usually obtained as by-catches during trawling operations from the open seas and find limited applications in the fisheries sector except for a few, being used for their fins or body oils. The sharks selected in our study Apristurus indicus, Centrophorus scalpratus, Centroselachus crepidater and the chimaeras Neoharriotta raleighana and Harriotta pinnata are presumably taken by bottom trawlers off the Indian coasts and find limited commercial applications. Therefore collection of such potential specimens for further research including molecular studies is essential. Information on the by-catches of sharks and chimaeras from the Indian EEZ (and other chondrichthyans) is urgently required and the conservation status of these species should be reassessed without delay when such information is obtained. Though many species constitute the shark fishery along Tamil Nadu and Kerala coast only a few comprise the regular fishery and the
species dominance in the fishery differs from gear to gear depending on the area, depth and mode of operation of the gear concerned.

Interest in the potential health benefits of lipids from deep sea fishes have emerged since the 1950s. Previous studies have reported benefits of cod liver oil on diabetes (Stene 2001), hypercholesterolemia (Brox et al., 2001), and arthritis (Gruenwald et al., 2002). An early finding was that the Eskimo of northern populations had a low incidence of heart disease despite high fat intake. It was found that the deepwater fish the Eskimo consumed are abundant with n-3 long-chain fatty acids (Calder, 2006). These early observations led to an increase in research examining the beneficial and/or preventative effects of lipid bioactives on numerous debilitating and common conditions, including cardiovascular disease (CVD), rheumatoid arthritis and asthma, among others.

Shark liver oil is typically obtained from sharks that are caught as a by-product of deep-sea fishing, making a valuable remedy from a natural resource that would have otherwise gone waste. The liver oils from deep sea sharks and chimaeras, which typically inhabit the cold, non-polluted waters of the sea, have been found to possess life-enhancing compounds including alkylglycerol (or alkoxyglycerol, AKG, Glycerol Ether Lipid), squalene, and natural trace elements in addition to fat soluble vitamins and long chain n3 polyunsaturated fatty acids. Alkylglycerols contained in shark liver oils may have anti-cancer properties. The antilipidemic hydrocarbon squalene, the antioxidant vitamin E and the membrane stabilizing n3 PUFAs contained in these oils help strengthen and regenerate the immune system while benefiting many other functions and organs of the body. Therefore it is rightfully promoted as a complementary or alternative form of treatment for cancer and other inflammatory diseases.

Numerous bio-analytical techniques are available today to assess and estimate the bioactive components present in fish oils. The thin layer chromatography clubbed with the flame ionisation detector (TLC-FID) is used to separate fish oils to their classes since it can separate them based upon their wide polarity due to various alkyl chains. Moreover, the TLC-FID method can simultaneously determine the hydrocarbon content, the triacyl glycerols, monoalkyl glycerols, fat vitamins, free fatty acids etc. contained in the fish oils. The different classes of fatty acids present in the fish oils can be determined using a Gas
Chromatograph with a flame ionisation detector (GC-FID). Better resolutions and accurate quantifications of the lipid components may be further achieved by the use of a high performance liquid chromatograph clubbed with an evaporative light scattering detector (HPLC-ELSD). This method specially yields in improved separation performance for neutral lipid classes which are highly variable and abundant in marine oils.

Giving all these aspects a thought and with an intention of understanding the lipid components of sharks and chimaeras obtained from our deep sea expeditions along the Indian EEZ, the major objectives of the study were framed as below.

1. To identify sharks and chimaeras with potential lipid bioactives, from the Indian EEZ which were abundantly caught as bycatches during trawling operations along the south west and south east coasts of the Indian EEZ.

2. To calculate and compare the hepatosomatic indices of the selected sharks & chimaeras of the Indian EEZ.

3. To estimate the major components of the liver oils of these sharks and chimaeras that contributes to their pharmacological behavior.

4. To conduct biochemical characterisation of liver oils of *Apristurus indicus*, *Centrophorus scaloratus*, *Centroselachus crepidater*, *Neoharriotta raleighana* and *Harriotta pinnata* obtained during cruises onboard the FORV Sagar Sampada using TLC-FID, GC-FID and HPLC-ELSD.
2.2 REVIEW OF LITERATURE

2.2.1 Elasmobranch resources of the Indian EEZ

The annual production of elasmobranchs in India is around 70,000 tonnes, over 4% of total marine fish landings (Dholakia et al., 2004). Sharks account for between 60 and 70% of this. Tamil Nadu, Gujarat, Maharashtra, Kerala, Karnataka and Andhra Pradesh supply around 85% of the shark landings in India. Sixty-five species of shark have been sighted in Indian waters and over 20 of these, of the Carcharinidae and Sphyrnidae families, contribute to the fishery. Sharks are of great commercial importance the world over, apart from being a significant link in the marine ecology. In India the present annual shark production is around 45,500 tonnes, obtained as a by-catch from a variety of gears. Despite the commercial importance, no serious attempts have so far been made at any targeted exploitation of this valuable resource. Information on the composition of the species of shark landings is very scarce apart from the gross catch statistics.

There are several types of gear that take sharks as incidental catch; the most important among them are trawl net and gill net (FAO 2000). There is no detailed information on the landings of sharks by gear type but data available on shark production by mechanized boats at major fishing centres show that trawl nets account for 60% of the shark landings and gill nets account for 38%. Purse seine in Cochin and Mangalore and hook-and-line in Cochin and Bombay take a very small fraction of the catch. The official sources also indicate that the fishery is limited to the 50–70m depth zone where the sharks are fished as a bycatch by many multispecies gears like trawls and the drift gillnets which are used all along the Indian coast. The shark fishery takes a number of smaller species, e.g. spade-nose shark (Scoliodon laticaudus), milkdog shark (Rhizoprionodon acutus), R. oligolinx, Carcharinus sorrah, C. dussumieri, C. brevipinna and C. macloti. Apart from these, juveniles of large species, such as C. melanopterus, C. limbatus, C. plumbeus, Sphyrna zygaena and a few others also are caught. The occurrence of many of these species is highly seasonal and their landings vary from centre to centre during the year.

In the past there was no organised shark fishery in the country and the sharks were caught incidentally and formed only a bycatch of the gears then used. During the 1950s and 1960s the shark fishery was more or less neglected and the resource
was not adequately studied for the reason that the shark flesh was less preferred as an edible meat owing to its pungent odour caused by the presence of trimethylamine (Anthoni et al., 1990). In those days in the absence of any demand for shark meat, all fishermen did was to remove the fins and throw the maimed sharks back into the sea. However, in later years shark meat gained popularity, both in domestic and international market, in part because of the increase in the demand for seafood in general. The high value fetched by the fins, liver oil, cartilage and skin also boosted the demand for shark that encouraged many to undertake shark fishing. This trend accelerated with the entry of sophisticated fishing trawlers into the fisheries and increasing export demand for shark products. Sharks emerged as a valuable catch and consequently fishermen went after sharks equipped with different gears exclusively to exploit sharks. As fishing for sharks gained momentum in recent years India emerged as a major shark producing country. Much of the trade is, however, still restricted to the west coast of India (Varma 2002).

Biological information and size composition of the various species of sharks exploited is scanty except for a limited number of studies. This is mostly due to the highly seasonal and erratic occurrences of most of the species in the bycatches taken by the various gears. Further, the high cost of sampling makes it difficult to carry out detailed studies on their biology.

2.2.2 Status of Chondrichthyan fishes in India

Despite the widespread recognition of the vulnerability of deepwater chondrichthyan fishes (sharks, batoids and holocephalans) to overfishing and their potential inability to recover from depletion, there is a lack of a concise overview of the present status of knowledge concerning the biodiversity and life history of this group. Deepwater chondrichthyans have been defined as those sharks, rays and holocephalans whose distribution is predominantly at, are restricted to, or spend the majority of their lifecycle at, depths below 200m (Dholakia, A.D., 2004). This depth is generally recognised as the continental and insular shelf edge, and therefore, deepwater species are those occurring on or over the continental and insular slopes and beyond, including the abyssal plains and oceanic seamounts. Of the global chondrichthyan fauna (1193 species), 581 species are considered to be deepwater (48.7% of the global total). The deepwater fauna is divided between 278 sharks
(55.8% of global), 257 batoids (39.8% of global) and 46 holocephalans (93.9% of global).

The bulk of the deepwater shark fauna is attributable to the squaloid dogfishes (Order Squaliformes) and scyliorhinid catsharks (Order Carcharhiniformes, Family Scyliorhinidae), together comprising 84.5% of deepsea sharks. Three families of skates (Arhynchobatidae, Rajidae and Anacanthobatidae) dominate the deepwater batoid fauna, together comprising 89.9% of deepsea batoids.

The total number of known species is ever increasing as exploratory and taxonomic work ensues. Undescribed taxa, those new or recently identified species yet to be formally treated by science, represent over one fifth (21%) of all known deepwater chondrichthyans and the systematics and inter-relationships of several groups of deepsea chondrichthyans remains unresolved. This high proportion not only highlights the overall lack of knowledge of the deepsea fauna at even the most basic (i.e. taxonomic) level, but also that the deepsea chondrichthyan fauna is far from fully documented.

Chondrichthyans are generally considered to be K-selected species, displaying conservative life history parameters such as relatively slow growth, late age at maturity, low fecundity and low natural mortality, resulting in limited reproductive output. These characteristics place them at risk of overexploitation and population depletion, with an inability to recover from reduced population levels once depleted. An understanding of the biological parameters of a species is important to accurately assess its productivity and thus make inferences concerning its vulnerability to fisheries.

For the vast majority of deepwater chondrichthyans, details of their life history characteristics are lacking and many groups remain very poorly-known. However, reported world landings of chondrichthyan fishes are a gross underestimate. They do not include the vast quantities caught as bycatch which are almost entirely undocumented and totally unregulated. Bycatch alone may represent 50% of the actual world cartilaginous fish catch (Bonfil 1994).

2.2.3 The Catch Statistics

There have traditionally been important fisheries for elasmobranchs in India with a relatively steady growth up to the mid 70's, followed by a period of stability during
most of the 80's, then a tremendous increase in catches in 1987 resulting in India becoming one of the top three elasmobranch producers in the last ten years. Indian production of sharks and rays represents 8.78% of the world elasmobranch catches! Still, because of large inland yields, elasmobranch resources comprised only 1.72% of total national catches in 1987–1991. Catch results are not given by species or families in the statistics and the composition of catches is only known by FAO areas. Approximately equal amounts (about 26,000 t/yr) were obtained from both FAO areas for the period 1977–1991. Catches from the west coast were slightly larger than those of the east coast during 1977–1991.

During 1983–1985 sharks comprised 55% of the elasmobranch catch of the country (Dholakia, A.D., 2004.). The main fishing areas in order of importance were Gujarat, Maharashtra, Kerala, Andhra Pradesh, Karnataka and Tamil Nadu and important fishing grounds for sharks are reported for Ashikode, Kerala Province (Anon. 1983). Sharks catches are incidental to other fisheries in India and are mainly taken with longlines, which vary in design by region, and are also as by catch of trawlers using disco nets off Ratnagiri (Maharashtra), with bottom set gillnets in Porto Novo (Tamil Nadu) and by shrimp trawlers of Kerala (Kulkorni and Sharangdher 1990). Rays are caught with bottom set gillnets in Gujarat, northwest India and Cudalore and are abundant on the outer shelf and slope off Kerala and Karnataka (Devadoss 1978; Kunjipalu and Kuttappan 1978; Sudarsan et al. 1988). Devadoss (1984) indicates that batoids comprise 10% of by catches in Calicut; 90% of the by catch comes from trawlers, 8% from gillnets and 2% from hook and lines. Both sharks and rays are abundant in Lakshadweep and form important by catches in trawl fisheries in Krishnapatnam (Swaminath et al. 1985; James 1988).

Dahlgren (1992) noted that directed fisheries for sharks are developing on a seasonal basis on the east coast of India. About 500 vessels, both sail - powered and motorized, fish for sharks with bottom or drift longlines of the coasts of Orissa Andhra Pradesh and Tamil Nadu. Bottom longlines are usually set in waters 80–150m deep and occasionally as deep as 500m for bull sharks and tiger sharks. The longlines have up to 400 hooks and the meat is usually salted on board during the trip. In Orissa alone, about 200 boats are engaged in drift longlining on a seasonal basis (December - March). The most common species caught by drift longlines are silky sharks and scalloped hammerhead sharks.
Catch composition data are not readily available but the multispecies nature of these fisheries is evident from the literature. Appukuttan and Nair (1988) reported that more than 20 species of sharks (mainly carcharhinids and sphyrids) are commonly caught. Their data for Pamban and Kilakkarai show that Rhizoprionodon acutus, R. oligolinx, Carcharhinus limbatus, C. sorrah, C. hemiodon, Sphyrna lewini and Eusphyra blochii are the most important species. Other species caught are C. melanopterus and Scoliodon laticaudus (Devadoss 1988). Important batoids are: Dicerobatis eregoodoo, Rhynchobatus djiddensis, Rhinobatus granulatus, Himantura uarnak, H. bleekeri, Dasyatis sephen, D.jenkinsii, Aetobatus narinari, A. flagellum, Aetomylus nichoffi and Mobula diabolus (Devadoss 1978, 1983; Kunjipalu and Kuttappan 1978).

Local assessments of the state of the fisheries for elasmobranchs exist (Krishnamoorthi et al. 1986, Devadoss et al. 1988, Sudarsan et al. 1988), but no overall studies exist (Appukuttan and Nair 1988). Devadoss (1978) reports that ray resources off Calicut were apparently overfished by 1980 while according to Reuben et al. (1988) shark and ray resources of Northeast India were still underexploited in 1985. Devadoss et al. (1988) did local assessments using Schaefer’s model and made suggestions for effort changes for the different areas. The present situation needs careful monitoring. There appears to be a high level of catches of elasmobranchs in India (peak of 73 500t in 1988) and it is unlikely that such large yields are sustainable over a long periods. The collapse of the neighbouring Pakistani elasmobranch fisheries in 1983 could indicate future catch reductions for the Indian elasmobranch fisheries.

2.2.4 The fisheries
The annual average landings of sharks, skates and rays during 1996-2006 were 60,866 t of which sharks constituted 60.1% (36,592 t) (CMFRI statistics, 1996-2006). Though a harvest potential of 1,85,000 t of elasmobranchs has been indicated for the Indian EEZ, they are not fully exploited. Catches in the exploratory surveys by the Government of India tuna longliners indicate that the pelagic sharks constitute 42% in the Arabian Sea, 36% in the Bay of Bengal, 43% in the Andaman Sea and 31% in equatorial areas. In Kerala the sharks, chimaeras, skates and rays formed an average catch of 8000t/yr accounting for nearly 2% of the total landings of Kerala.
They formed 13% of the total elasmobranch catches at the all India level. In 1975, landings were 10000t, which is the highest so far recorded in Kerala. The minimum was 4900t in 1987. During 1994–97 the shark landings in Kerala varied from 1647t in 1997 to 3781t in 1994 with an average of 2600.3t which formed 0.5% of the total marine fish production by Kerala.

2.2.5 Species composition

About 70 species of sharks occur in Indian seas of which about 22 species have only limited occurrence and value; around 12 are moderately abundant though not frequently caught and only six are major species (Table 2) in the fishery. The most common and abundantly fished shark is *Scoliodon laticaudus* followed by *Rhizoprionodon acutus*. Among the requiem sharks, *Carcharhinus sorrah*, *C. limbatus* and *C. melanopterus* and the hammerhead shark *Sphyrna lewini* are common and reach a maximum length of one to 2.5m. Other sharks which occur moderately in the catches are the grey sharks, *C. macloti*, *C. hemiodon*, *C. dussumieri*, *C. Sealei*, *Loxodon macrorhinus* and *Rhizoprionodon oligolinx*. These sharks grow up to a metre in length. The snaggletooth shark (*Hemipristis elongatus*), mako shark (*Isurus oxyrinchus*), and the tiger shark (*Galeocerdo cuvier*) are larger sharks and grow to more than 2m. The hammerhead sharks, *Sphyrna mokarran* and *Eusphyra blochii* grow up to 2m also occur in the catch.

About 68% of the sharks landed are along the west coast. *Scoliodon laticaudus* is the dominant species in the catch (83.3%) along the Gujarat and Maharashtra coasts followed by *Carcharhinus* spp. (13%), *Rhizoprionodon* spp. (2%) and the tiger shark along with other minor groups constitute the rest of the catch. On the southwest coast (Kerala, Karnataka and Goa) the grey sharks (*Carcharhinus* spp.) and the dogfishes (*Centrophorus* spp.) form 56.1% of the major catch of 56.1% followed by the hammerhead sharks (*Sphyrna lewini*, *S. mokarran*) - 26.5%, *S. laticaudus* - 3% and other *Carcharhinus* shark and hammerhead sharks form 14.4%. On the east coast (Chennai region) the major grey sharks contribute 59.4% of the shark fishery followed by the hammerheads (*Sphyrna lewini* and *Eusphyra blochii*) - 23%, *Rhizoprionodon* spp. - 15%. The other sharks which include the tiger shark *Galeocerdo cuvieri*, *Isurus oxyrinchus*, *C. Sealei*, *Hemipristis elongatus*, *Isurus oxyrinchus*, *C. Sealei*, *Hemipristis elongatus*, *Sphyrna mokarran* and *Eusphyra blochii* grow up to 2m also occur in the catch.
Chiloscyllium griseum comprise 2.5% of the value (Devadoss 1988). The chimaeras contribute almost 18% of the total fish catch along the south west coast.

In Tamil Nadu and Kerala as many as 30 to 40 species of sharks belonging to 15 genera occur along the Tamil Nadu and Kerala coasts. Five species of skates and 15 species of rays belonging to three and seven genera respectively also occur in the fishery. However, only a few species constitute the commercial fishery.

The following species of sharks have been observed in the shark fishery along the Tamil Nadu and Kerala coast: Alopias vulpinus, Apristurus indicus, Stegostoma fasciatum, S. obesus, Chiloscyllium indicus, C. griseum, Rhincodon typus, Galeocerdo cuvieri, Scoliodon laticaudus, S. walbeehmii, Rhizoprionodon oligolinx, R. acutus, Eulamia elioti, Sphyra blochii, S. zygaena, S. tudes, S. lewini, Carcharhinus sorrah, C. dussumieri, C. gangeticus, C. limbatus, C. longimanus, C. melanopterus, C. brevipinna, Centrophorus moliuccensis, C. scalpratus, C. crepidater, Echinorhinus brucus, Hemipristis elongatus, Loxodon macrorhinus. Scoliodon laticaudus, Rhizoprionodon acutus, Carcharhinus sorrah, C. limbatus, C. melanopterus, Sphyra blochii, S. zygaena, S. tudes, Laxodon macrorhinus and Stegostoma fasciatum. Among the skates, Pristis microdon, P. cuspidatus, Rhinobatus granulatus, djiddensis, R. obtusus, R. armalus, and Raja mamillidens are recorded in the fishery. Rays recorded in the fishery are Dasyatis bleekeri, D. kuhlii, D. zugei, D. uarnak, D. sephen, D. imbricata, Aetobatus flagellum, A. narinari, Rhinoptera javanica, Manta birostris, Narcine indica, Tygon zuge and Gymnura poecilura. Apart from sharks, rays and skates the chimaeras (Neoharriotta raleighana, Harriotta pinnatta) are also an important resource among the marine fishes caught from the Arabian Sea and the Indian Ocean.

2.2.6 Distribution of the fishery
Shark fishery is multispecies and no species is dominant throughout the entire coast of India. Neither a single species, nor a group of species, dominates in the different states. Scoliodon dominates the fishery in the Gujarat and Bombay regions and Grey sharks and hammerhead sharks dominate the catch in Kerala and Karnataka states.

The whale shark (Rhincodon typus) has become the target fishery using harpoons at Veraval on the Gujarat coast at some other places. Carcharhinus spp. are targeted and are hunted for their liver and fins.
Though many species constitute the shark fishery along Tamil Nadu and Kerala coast only a few comprise the regular fishery and the species dominance in the fishery differs from gear to gear depending on the area, depth and mode of operation of the gear concerned (FAO, 1999). Mostly the drift gillnet with larger mesh size (140-200mm) and the hook and line gear, especially that with larger hooks, exploit the shark resource more effectively than the other gears. The large mesh gillnets and hook and line units land bigger sharks such as the *Carcharhinus sorrah*, *C. melanoptera*, *Laxodon macrorhinus*, *Sphyraena tudes*, *S. blochii*, *Galeocerdo cuvieri*, etc. The trawl net and the gillnets with smaller mesh size (40-90mm) land smaller sized sharks such as *Scoliodon laticaudus*, *Chiloscyllium indicus*, etc. Along the southeastern coast of Tamil Nadu *C. sorrah*, *L. macrorhinus* constitute the major part of the shark landings and along the northeastern coast *Rhizoprionodon acutus* and *S. acutus* form the major portion of the landings. Studies on the biology and stock assessment of these species reveal that *C. sorrah* is exposed to higher fishing pressure and the females are more severely fished than the males along the Gulf of Mannar. Similarly *R. acutus* is exposed to higher fishing pressure (Krishnamoorthy and Jagdish 1986) along the Madras coast.

A closer look at the pattern of catches shows that pelagic sharks are more abundant on the West Coast. A different pattern emerges on the east coast where even though sharks contributed 32% to the total sharks caught the share of the batoid fishes taken constituted 53% of the total elasmobranch catch in India (Devadoss et al. 1997). Sudarsan et al. (1988) identified the existence of potentially rich grounds for pelagic sharks off the Gulf of Mannar, (on the East Coast in the state of Tamil Nadu). The incidence of non-conventional species of shark like the bramble shark as shown by their catch by deep sea water trawlers operating off Tuticorin in the Gulf of Mannar is an encouraging feature. The present fishing area (which is mainly exploited by trawlers) falls within a narrow coastal zone up to the 70m isobath and the knowledge of sharks in this area is based on the catches within this zone. The projected potential Indian yield for sharks and rays is about 0.18 million tonnes of which the share for sharks is 0.12 million tonnes (Sudarsan et al. 1988). At the present level of fishing a large gap exists between the projected potential yield and the actual catch in the offshore waters. It has been also reported that the total potential of the EEZ of India is estimated at 4,470,000t. Of these about 2,260,000t or
50.6% lies within the 50m isobath, around 38% lies within 200m and 11% beyond 200m.

Generally speaking, exploitation of elasmobranchs at present along the Indian coast fishing is unbalanced. Some regions are excessively exploited and some are totally unexploited. Kerala comes under the former category with a high level of exploitation. There is scope for expanding the commercial exploitation of sharks (Chen and Yuan, 2006), but this needs to be done carefully as discussed below.

Availability of food is largely the limiting factor for any fishery including sharks. On the west coast along Kerala and Karnataka states the sharks fishery is at its peak when mackerel and oil sardines appear in shoals. That the pelagic sharks hunt the fast moving pelagic fishes is evident by the exclusive presence of mackerel and oil sardine in their stomachs during this period. The distribution of S. laticaudus in large concentrations along Gujarat and Bombay coasts is also due to the availability of preferred food. Demersal sharks are abundant in this region and forage on bottom living fauna. The juveniles also inhabit the bottom and are caught by bottom trawls. The large adults, with greater mobility, come to the surface where they are taken by driftgill net gear. The large Scoliodons prefer pelagic fishes while the juveniles feed on the bottom fauna such as crabs, squilla, small prawn, etc. (Chen and Yuan, 2006).

Kerala: This state's coastline of 590km is almost one-tenth of Indian's total coastline. Sharks are landed in 222 landing centres along the Kerala coast. Important fisheries ports are Cochin, Sakhthikulangara, Munambam, Azheekal, Ponnani, Beypore, Vizhinjam, Quilandi and Azhikkode. The major gears which catch sharks along the Kerala coast are hook and line, drift/set gillnets and trawls. In hook and line fisheries, elasmobranchs form 22% of the catch. Other associated species are catfish (31%), carangids (24%), tunnies and mackerels (5%), and seerfish (2%). The hook and line fishery is prevalent mostly in Trivandrum and Quilon districts and to some extent in Kozhikode. In drift gillnetting elasmobranchs constituted 26% of the catch; the other resource groups include tunnies (25%), catfish (18%), seerfish (14%) and Pomfrets (3%).
2.2.7 Distribution of landings

The distribution of elasmobranchs in both the Arabian Sea and the Bay of Bengal is not uniform. Gujarat state contributes a little over 50% of the total catch from the west coast and Maharashtra and Gujarat share 81% of the shark catches on the west coast and 55% of all Indian shark catch. The east coast contributed 11000t constituting 32% in the total sharks caught. Tamil Nadu and Andhra Pradesh together account for 78% of the total sharks fished along the east coast and 25% of the total sharks caught in Indian coastal waters. Even though sharks appear to be distributed all along the coast, there are places where particular species or groups of sharks are present in large numbers. As stated earlier, S. laticaudus and R. acutus are found in large sharks, which are highly pelagic, are more prevalent along Kerala, Karnataka and Tamil Nadu coasts. The pelagic sharks, C. limbatus, C. sorrah and C. melanopterus start appearing in the fishery along Tamil Nadu coast from April until September coinciding with the mackerel and sardine fishery (Sudarshan et al., 1988).

2.2.8 Species selected for the study

The elasmobranch resources Centrophorus scalpratus, Centroselachus crepidater, Apristurus indicus, Neoharriotta raleighana and Harriotta pinnata were obtained during cruises onboard the FORV Sagar Sampada from along the south west and south east coasts of the Indian EEZ. None of these species have been recorded in the “vulnerable” and “near threatened” species as categorized within the IUCN red list.

1) **Centrophorus scalpratus** McCulloch, 1915

**Kingdom:** Animalia  
**Phylum:** Chordata  
**Class:** Chondrichthyes  
**Order:** Squaliformes  
**Family:** Centrophoridae  
**Red list category and Criteria:** Data deficient  
**Synonyms:** Centrophorus moluccensis Bleeker, 1860; Atractophorus armatus Gilchrist, 1922.  
**FAO Names:** Smallfin gulper shark; Endeavour dogfish  
**Marine fishing areas:**
Indian Ocean – western; Indian Ocean – eastern; Pacific – northwest; Pacific – southwest; Pacific – western central

Field Marks:
No anal fin, two dorsal fins with large spines, bladelike unicuspitate teeth in upper and lower jaws, with lowers much larger than uppers, a moderately long snout, moderate-sized first dorsal fin and very small second dorsal, blocklike sessile-crowned, wide-spaced, cuspidate lateral denticles, and rear tips of pectoral fins narrowly angular and greatly elongated.

Diagnostic Features:
Snout moderately long and parabolic, preoral snout greater than mouth to pectoral origins; upper anterolateral teeth with semierect or oblique cusps. First dorsal fin fairly high and short; second dorsal very small, half height of first dorsal or less, with base less than 1/2 to nearly 3/5 length of first dorsal base, and spine origin well behind rear tips of pelvic fins; distance from first dorsal insertion to origin of second dorsal spine greater than distance from tip of snout to pectoral insertions in adults; free rear tips of pectoral fins formed into narrow, angular and greatly elongated lobes that reach well beyond the level of first dorsal spine, inner margins equal or longer than distance from second dorsal spine to caudal origin; caudal fin with a deeply notched post ventral margin in adults. Lateral trunk denticles not overlapping each other, block like, with crowns sessile on bases and no pedicels, crowns broad, squared or vertically rhomboidal in adults, with a strong main cusp and no lateral cusps on their posterior edges.

Geographical Distribution:
Western Indian Ocean: South Africa, southern Mozambique. Western Pacific: Japan (Okinawa), Indonesia (Ambon), Australia (Victoria), New Hebrides, New Caledonia.

Habitat and Biology:
A common deepwater dogfish of the outer continental shelves and upper slopes, thrives on or near the bottom at depths from 128 to 823 m. Ovoviviparous, number of young two per litter. Full term fetuses were found in summer off South Africa. Feeds primarily on bony fish, including lanternfish, bramids, carangids, worm-eels, bonito, hairtails, oilfishes, as well as other dogfish sharks, squid, octopi, shrimp, and even tunicates.
Size: Attains maximum at about 100 cm; males maturing between 69 and 73 cm and reaching 86 cm; females maturing above 89 cm and reaching up to 98-100 cm; size at birth is about 31 to 37 cm.

Interest to Fisheries: Presumably taken by bottom trawlers off South Africa, India and Australia. Potentially important for its abundance off the coasts of South Africa and southern Mozambique. The shark finds limited commercial applications to the fisheries sector and is mainly used as fish meal for its meat, fins and liver oil.

2) Centrophorus crepidater/Centroscymnus crepidater Barbosa du Bocage & de Brito Capello, 1864

Kingdom: Animalia
Phylum: Chordata
Class: Chondrichthyes
Order: Squaliformes
Family: Somniosidae
Synonyms: Centroselachus crepidater, Barbosa du Bocage & de Brito Capello, 1864, Centrophorus jonsonnii Saemundsson, 1922.
FAO Names: Long nose velvet dogfish, Golden dogfish.
Red list category and Criteria: Least Concern

Geographical distribution
A fairly common but poorly studied species with a wide but patchy distribution. Occurs in the eastern Atlantic (Iceland to southern Africa), Indian Ocean (Aldabra Islands and India), eastern Pacific (northern Chile) and the western Pacific, from New Zealand and southern Australia, on or near the bottom of continental and insular shelves in depths of 270-1,300 m. Locally from Sydney (New South Wales) to Perth (Western Australia), including Tasmania and the southern seamounts. Native to Angola; Australia (New South Wales, South Australia, Tasmania, Victoria, Western Australia); Benin; Cameroon; Chile; Congo; Côte d’Ivoire; Equatorial Guinea; France; Gabon; Gambia; Ghana; Guinea; Guinea-Bissau; Iceland; India; Ireland; Liberia; Mauritania; Morocco; Namibia; New Zealand; Nigeria; Portugal; Senegal; Seychelles (Aldabra); Sierra Leone; South Africa; Spain (Canary Is.); Togo; United Kingdom; Western Sahara

Marine fishing areas
Atlantic – northeast; Atlantic – southeast; Atlantic – eastern central; Indian Ocean – western; Indian Ocean – eastern; Pacific – southwest

Habitat and ecology
Demersal on the slope in depths of 270 to 1,300 m; off Australia most common in 780 to 1,100 m. Feeds mainly on fish and cephalopods. In the Rockall Trough in the northeastern Atlantic, the diet was dominated by squid and micronektonic fish including myctophids. This species would appear to feed clear of the seabed on benthopelagic organisms (Mauchline and Gordon 1983). The lack of a seasonal pattern to reproduction, with females breeding throughout the year, means that the gestation period is currently unknown. Litter sizes average six with a range from 3 to 9. Annual fecundity is unknown. The productivity of this species appears to be low, with age at maturity in Australia of 15 years at 64 cm (males) and 22 years at 82 cm (females), and longevity of around 60 years (S. Irvine, pers. comm.). The maximum size of specimens in Australia is 105 cm (Daley et al. 2002).

Size
The male attains a maximum size of 130 cm with a reported age of 54 years.

Field marks
Snout greatly elongated, preoral length about equal to distance from mouth to pectoral fin origins. Upper labial furrows greatly elongated, their lengths greater than distance between their anterior ends.

Diagnostic features
Dorsal spines (total): 2; Anal spines: 0. Black or blackish brown in color, dorsal fins with very small fin spines, very long snout, greatly elongated labial furrows that nearly encircle mouth, lanceolate upper teeth and bladelike lower teeth with moderately long, oblique cusps, fairly slender body that does not taper abruptly from pectoral region, moderately large lateral trunk denticles with partly smooth, oval, cuspidate crowns in adults and subadults.

Interest to fisheries
Mainly a bycatch species taken by trawl and hook, although with some limited targeting for its flesh and oil. Catches in Australia have been increasing in the last few years with relaxation of mercury laws and fishers looking for non-quota species in the South East Trawl Fishery. Biomass surveys extending over 10 years in New Zealand show an increasing trend, but may be confounded by the use of different...
vessels. The productivity of this species appears to be low, with age at maturity in Australia of 15 years (males) and 22 years (females), and longevity of around 60 years, thus further increases in catches should be viewed with concern. However, the species is currently still abundant and a Near Threatened assessment cannot be justified at this time, although the situation should be monitored carefully.

3) **Apristurus indicus**\(^{(a,b)}\) Brauer, 1906

**Kingdom:** Animalia  
**Phylum:** Chordata  
**Class:** Chondrichthyes  
**Order:** Carcharhiniformes  
**Family:** Scyliorhinidae  
**Synonyms:** *Scyliorhinus indicus*  
**FAO Names:** smallbelly catshark  
**Red list category and Criteria:** Not evaluated  
**Marine fishing areas:** Western Indian Ocean, Gulf of Aden, Oman, Southeast Atlantic Ocean  
**Size:** 34 – 45cm TL male/unsexed  
**Depths:** 700 – 1800 m  
**Biology:** found on continental slopes, Oviparous  
**Interest to fisheries:** probably caught with bottom trawls, Not evaluated

4) **Neoharriotta raleighana**\(^{(a,b)}\) Goode & Bean, 1895

**Kingdom:** Animalia  
**Phylum:** Chordata  
**Class:** Chondrichthyes  
**Order:** Chimaeriformes  
**Family:** Rhinochimaeridae  
**Synonyms:** *Anteliochimaera chaetirhamphus*, Tanaka 1909; *Harriotta curtissjamesi* Townsend & Nichols 1925; *Harriotta opisthoptera* Deng, Xiong & Zhan 1983.  
**FAO Names:** Bentnose Rabbitfish, Long-nosed Chimaera, Narrow-nose Chimaera  
**Red list category and Criteria:** Least Concern
Marine fishing areas: The fish is native to the oceans of the Atlantic—eastern central; Atlantic—northeast; Atlantic—northwest; Atlantic—southeast; Atlantic—southwest; Indian Ocean—eastern; Pacific—southwest; Pacific—northwest; Pacific—eastern central; Pacific—southeast

Diagnostic features
Dorsal spines (total): 1; Anal spines: 0; Anal soft rays: 0. A longnose chimaera with a rather long, narrow, depressed snout, a small eye situated above or behind the mouth, a rather long first dorsal fin and spine, knobby tooth plates, and caudal fin lanceolate with no tubercles on upper edge but with a long terminal filament. Dark brown or blackish in color. No separate anal fin. Claspers are rod-like, rather slender, unbranched, with tip somewhat swollen. Jugular and oral canals arising separately from orbital, with a short interspace; angular (maxillary) canal joining suborbital about 2/7 of distance from front level of eye toward tip of snout.

Geographical distribution
Range appears to be widespread and worldwide (although not widely recorded in the Indian Ocean at present), with the largest numbers recorded from the western Pacific and northern Atlantic. Nothing is known of population structure, although molecular evidence may support regional populations. Other species of chimaeroids appear to have wide ranges (e.g., R. atlantica and R. pacifica), but H. raleighana is the only chimaeroid that may be global in its distribution.

Habitat & biology
Found on the continental slope and ocean floor. Appears to feed mainly on shellfish and crustaceans. Maximum length 120 cm without tail filament
Size: 120 cm OT male/unsexed; (Ref. 26346); 102.5 cm TL (female)

Environment
Bathydemersal; marine; depth range 200-2600 m.

Interest to fisheries
This species appears to be the only chimaeroid with a widespread, global distribution. Occurs in deep waters of the continental slopes in depths of 380 to 2,600 m in both the Atlantic and Pacific Oceans. Also occurs in the Indian Ocean (off southern Australia). They seem to be somewhat common in the Northern Atlantic, Northwest Pacific and Southwest Pacific, however, very little is known about the biology of this species. They are oviparous but nothing is known of spawning and
reproduction and very few juveniles have been collected. As with many other chimaeroids adults and juveniles may occupy different habitats. Known to be captured in deepwater research trawls and as bycatch in deepwater commercial trawls. Data from the South Tasman Rise Trawl Fishery (south of Tasmania, Australia) indicates that this species is a negligible component of bycatch. Increased deepwater trawl fisheries could pose a potential threat to habitats and populations in the future. At present this species appears to be widespread geographically and bathymetrically and relatively abundant with no immediate threats to the population and is thus classified as least concern. However, bycatch data from other fisheries and the monitoring of expanding deepwater fisheries are required.

5) *Harriotta pinnata* Schnakenbeck 1931

**Kingdom:** Animalia  
**Phylum:** Chordata  
**Class:** Chondrichthyes  
**Order:** Chimaeriformes  
**Family:** Rhinochimaeridae  
**FAO Names:** Siclefin chimaera  
**Synonyms:** *Neoharriotta pinnata* Schnakenbeck 1931

**Marine fishing areas:** the fish is native to Atlantic – southeast; Atlantic – eastern central; Indian ocean - eastern

**Diagnostic features:**
A longnose chimaera with a narrow, slightly flattened snout, and blunt-edged, ridged tooth plates; pectoral fins short and broad, anal fin large and curved, and caudal fin with no tubercles on upper edge but with a short terminal filament (Ref. 5578). Dark brown in color

**Geographical distribution**
The occurrence of the species is distributed across the Eastern Atlantic: Cape Blanc, Mauritania to Walvis Bay, Namibia (Ref. 4444). Western Indian Ocean: Arabian Sea

**Habitat & biology**
Very poorly known species. Occupies a relatively shallower shelf and slope habitat than other rhinochimaerids at depths of 200 to 470 m. Few adult specimens have
been collected, but males and females appear to reach sexual maturity at about 50 to 60 cm body length (BDL), as all specimens greater than 60 cm BDL are sexually mature. Oviparous, likely exhibiting similar reproductive patterns to other chimaeroids. Diet is unknown but probably consists primarily of a variety of benthic invertebrates.

**Size:** 130 cm TL male/unsexed

**Environment:** Bathydemersal; marine; depth range 150 - 700 m, usually 200 - 470 m

**Red list category and Criteria:** Data deficient

**Interest to fisheries**

*Neoharriotta pinnata* appears to be widespread in the Atlantic off the coast of Western Africa and in the Arabian Sea off the south west coast of India. Although widely reported, only a relatively small number of voucher specimens have been collected and the species does not appear to be common. Not known to be targeted in any commercial fishery or utilized in any way, however, its occurrence at depths ranging from 200 to 700 m puts this species within range of most deepwater trawling operations and it is likely collected as bycatch. Nothing is known of the biology of this species, particularly reproduction, population structure, habitat and ecology and it is recommended that further studies be conducted. In particular, collection of specimen data (locality, size, sex, reproductive state) from each capture would enhance our understanding of geographic range and basic population structure of this species. Collection of additional specimens for further research including molecular studies is also essential. Information on the bycatch of this species (and other chondrichthyans) is urgently required and the conservation status of this species should be reassessed without delay when such information is obtained.

Over the decade, the increasing use of fish oil for various ailments has promoted worldwide research and analyses on the bioactive potentials of marine lipids. Marine lipids and those extracted from livers of sharks inhabiting waters beyond 500 m depth and of the nutraceutical and pharmacological values they possess has been the topic of discussion in major lipid conventions and seminars. Scientists today are on the verge of discovery of major bioactives from shark liver oils that could serve as powerful anti-inflammatory and anti-tumour agents.
2.2.9 Shark liver oils – an overview

Shark liver oil is extracted from the livers of deep-water sharks which typically inhabit the cold, non-polluted waters of the sea. Raw shark liver oil that is minimally processed contains life-enhancing compounds like alkylglycerol (or Alkoxyglycerol, AKG, Glycerol Ether Lipid), squalene, and natural trace elements in addition to fat soluble vitamins A, D and E and long chain n3 poly-unsaturated fatty acids (Brunel et al., 2005). Alkylglycerols contained in shark liver oils may have anti-cancer properties. Shark liver oil is typically obtained from sharks that are caught as a by-product of deep-sea fishing, making a valuable remedy from a natural resource that would have otherwise gone waste. This oil is known to help strengthen and regenerate the immune system while benefiting many other functions and organs of the body. Therefore it is rightfully promoted as a complementary or alternative form of treatment for cancer and other inflammatory diseases.

Shark liver oil is widely used alongside conventional cancer treatment in northern Europe and is sold as a dietary supplement in the United States. Available scientific evidence does not support claims that shark liver oil supplements are effective against cancer in humans (Calder 2006). Recent research has focused on certain components of shark liver oil (alkylglycerols, squalamine, and squalene). Early laboratory studies suggest that they may have anti-tumor effects in animals, but their effects in humans are not yet known. Clinical trials are currently under way.

2.2.10 Biochemical constituents of shark liver oils

a) Fat vitamins

Shark liver oil is promoted as a dietary supplement as it contains fat vitamins A, D and E used to boost the immune system, fight off infections, heal wounds, treat cancer and lessen the side effects of conventional cancer treatment. Shark liver oils are rich in antioxidants like vitamin E (Devaraj and Jialal 2000) which reduce inflammation by decreasing C-reactive protein levels and by blocking the activity of TNF-α (tumour necrosis factor-alpha) series 2-prostaglandins (PGE-2) and cyclooxygenases (James et al. 2003). Antioxidants are well known to alleviate the inflammatory processes mediated by allergic substances. They also curb inflammation by quenching hazardous molecules called free radicals, which stimulate inflammation (Vittala and Newhouse 2004).
In the pharmaceutical industry, vitamins are used in supplement preparations such as tablets or capsules. Vitamins are also used in the cosmetics industry in skin care, hair care and oral hygiene products. Vitamins have been added to skin care products to boost the skin's antioxidant or anti-inflammatory response. They also function as immune system strengtheners, clarifiers or wrinkle reducers.

The major fat soluble vitamins present in fish oils are vitamin A and vitamin E.

b) Vitamin A

A fat-soluble vitamin occurs in two principal forms in nature: retinol and certain carotenoids. Retinol is found only in animal sources, in foods such as fish, meat, eggs and full-fat milk. In plant foods, vitamin A can be obtained from a family of substances called carotenoids that are found in brightly coloured fruit and vegetables, and leafy green vegetables. The best known form of carotenoid is β-carotene (pro-vitamin A).

c) Vitamin E

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet (Schneider 2005). The four tocopherol homologues (d-a-, d-b-, d-g-, and d-d-) have a saturated 16-carbon phytol side chain, whereas the tocotrienols homologues (d-a-, d-b-, d-g-, and d-d-) have three double bonds on the side chain. There is also a widely available synthetic form, dl-a-tocopherol, prepared by coupling trimethylhydroquinone with isophytol. Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalised into the aromatic ring structure thereby increasing its stability (Bello et al., 2005).

The major biologic role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (Ibrahim et al., 1999). Although vitamin E is primarily located in cell and
organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules. This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (Hsu et al., 2001).

There are many signs of vitamin E deficiency in animals most of which are related to damage to cell membranes and leakage of cell contents to external fluids. Disorders provoked, for example, by traces of peroxidized PUFAs in the diets of animals with low vitamin E status are cardiac or skeletal myopathies, neuropathies, and liver necrosis (Suzuki et al., 1999). Muscle and neurological problems are also a consequence of human vitamin E deficiency (Bieri et al., 1984). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis. Several animal models (Cho and Choi, 1994) suggest that increasing intakes of vitamin E inhibit the progression of vascular disease by preventing the oxidation of LDL. Evidence suggests that oxidized lipoprotein is a key event in the development of the atheromatous plaque which may ultimately occlude the blood vessel (Suzuki et al., 1999).

It is suggested that when the main PUFA in the diet is linoleic acid, a d-a-tocopherol-PUFA ratio of 0.4 (expressed as mg tocopherol per g PUFA) is adequate for adult humans, and the ratio has been recommended in the United Kingdom for infant formulas (Li et al., 1999). Use of this ratio to calculate the vitamin E requirements of men and women with energy intakes of 2550 and 1940 kcal/day containing PUFA at 6 percent of the energy intake (approximately 17 and 13 g, respectively) (Horwitt MK, 2001) produced values of 7 and 5 mg/day of a-TEs, respectively. In both the United States and the United Kingdom, median intakes of a-TE are in excess of these amounts and the a-tocopherol-PUFA ratio is approximately 0.6, which is well above the 0.4 ratio which would be considered adequate. The Nutrition Working Group of the International Life Sciences Institute Europe has suggested an intake of 12 mg a-tocopherol for a daily intake of 14 g PUFAs to compensate for the high consumption of soya oil in certain countries where over 50 percent of vitamin E intake is accounted for by the less biologically active g form.

d) Alkylglycerols
Alkylglycerols were discovered by two Japanese scientists in 1922 (Tsujimoto M, Toyama, 1922). They are naturally occurring esters, which are chemicals formed by the combination of a fatty acid and an alcohol molecule. The most common alcohols in these compounds are batyl alcohol, chimyl alcohol, and selachyl alcohol. Hallgren and Larsson studied the occurrence of alkylglycerols in humans, cattle and sharks. When they occur in nature, the alkylglycerols are found esterified with fatty acids. In animals and humans the alkylglycerol-esters are found in red blood cells, the spleen, liver and especially, the bone marrow. They are also involved in the production of white blood cells in the bone marrow. They appear to be as essential to white blood cell production as iron is to red blood cell production. Alkylglycerols also occur naturally in mother’s milk. There are ten times more alkylglycerols in human milk than in cow’s milk. But the most abundant source of alkylglycerols is the liver oil of certain sharks (Pelton and Overholser).

Early research with leukemia patients showed that taking alkylglycerols during the course of radiation therapy may reduce and even prevent leukopenia and thrombocytopenia. Another study demonstrated that women who took alkylglycerols prophylactically starting eight days before the beginning of radiation therapy had lower mortality rates and greater survival than women who consumed them during the course of radiation therapy. Comparison with controls showed that the prophylactic administration of alkylglycerols before starting radiation also slowed tumor growth.

Radiation therapy can produce a wide range of tissue damage and injury. Several studies have been published showing that the number of radiation-induced injuries is substantially lower in patients treated with alkylglycerols. (Ko et al. 2002; Pedrono et al. 2004; Arita et al. 2005) The more advanced tumors (stages IIB-IV) regressed toward less advanced stages. Alkylglycerols have also been shown to cause a regression of tumor growth in studies on mice in a laboratory environment. (Deniau et al., 2009)

It has been suggested that these alkylglycerols fight cancer by killing tumor cells indirectly. Proponents claim they activate the immune system in two ways: by stimulating immune system cells called macrophages, which consume invading germs and damaged cells; and by inhibiting protein kinase C, which is a key regulator of cell growth. Proponents also claim that alkylglycerols reduce the side
effects of chemotherapy and radiation treatment, supposedly because of their ability to protect cell membranes. Because of their supposed immune-boosting effects, alkylglycerols are also claimed to help against colds, flu, chronic infections, asthma, psoriasis, arthritis and AIDS. Since macrophages are also important in wound healing, alkylglycerols are said to have healing effects. These claims have not been studied in controlled clinical trials. Shark liver oils comprise mainly of 1-O-alkylglycerols which constitute about 10-30% of the unsaponifiable matter of the oils (Hallgren and Larsson 1962). These alkylglycerols or AKGs are indeed responsible for reducing pain or inflammation in the body (Pedrono et al. 2004). The exact mechanism by which they function has not been fully understood but it has been proposed that they work by either inhibiting the synthesis, release or action of inflammatory mediators, namely histamine, serotonin and prostaglandins that might be involved in inflammation. It has been reported that naturally occurring AKGs have potent biological activities on various cells or systems (Devaraj and Jialal 2000).

e) Squalene

Squalene is a remarkable nutrient produced in our body and is also found in nature. Chemically, squalene is a polyunsaturated aliphatic hydrocarbon of low density, which can produce oxygen by combining with water. It belongs to a class of antioxidants called isoprenoids. An isoprenoid is a cell-friendly molecule that neutralizes the harmful effects of excessive free radicals in the body. Squalene is a pure isoprenoid wherein it is not attached to any other molecule. It has six isoprene units, which provides stability in its function as an antioxidant (Kohno et al., 1995; Ko et al., 2002). The stability of the isoprenoid molecule determines its effectiveness in combating cell damaging free radicals. The more stable the isoprenoid, the more free radicals are neutralized, and the more cells are protected. Scientific research and clinical trials have shown that squalene is safe as a dietary supplement in food and in capsules and no untoward incidents have been reported in the use of squalene. Japanese people have been using squalene for centuries and have attributed their strength and health to this substance.

Squalene is usually bound to sterol-carrier proteins in hepatocytes (Scallen et al., 1971). In 1926, squalene was first proposed as a precursor of cholesterol (Channon, 1926; Helibron et al., 1926). Squalene is synthesized from acetate and is
metabolized to cholesterol in liver (Langdon and Bloch, 1953; Tchen and Bloch, 1957; Srikantaiah et al., 1976). The endogenous synthesis of squalene begins with the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA). The initial reduction of HMG CoA (a niacin-dependent reaction) results in the formation of mevalonate. The enzyme involved in this reduction, HMG CoA reductase, has been the target of a class of cholesterol-lowering drugs. Mevalonate is then phosphorylated in three stages (via magnesium-dependent enzymes) and finally decarboxylated to form delta 3-isopentenyl diphosphate, the donor molecule for all polyprenyl compounds. Successive additions of prenyl groups result in the formation of the 15-carbon farnesyl diphosphate (Langdon and Bloch, 1953; Tchen and Bloch, 1957; Srikantaiah et al., 1976). Two molecules of farnesyl diphosphate are then enzymatically joined and reduced (niacin dependent) resulting in the formation of squalene (Goodman and Popjak, 1960; Bloch, 1965; Radisky and Poulter, 2000). After its biosynthesis, squalene can be transported to other areas of the body for incorporation into tissues or it can be further metabolized, resulting in the eventual formation of cholesterol (Tchen and Bloch, 1957; Bloch, 1965; Srikantaiah et al., 1976) and its steroid metabolites.

In addition to the contribution to cholesterol biosynthesis, a portion of the hepatic squalene pool is secreted via bile. In fact, the squalene levels are much higher in bile than in plasma, and in subjects on squalene-free diets, squalene appears also in feces (Liu et al., 1976). In rats, approximately 31% and 34% of absorbed \(^{3}H\)-labeled squalene were excreted as fecal neutral steroids and bile acids, respectively (Tilvis and Miettinen, 1983b). In humans, squalene feeding elevated fecal excretion of neutral steroids and bile acids by 31% and 20%, respectively (Strandberg et al., 1990).

Squalene helps to clean, purify, and detoxify the blood from toxins, facilitating circulation. It cleanses the gastrointestinal tract and kidneys, causing better bowel movement and urination. Many diseases are cured if the blood is purified, by supplementing squalene (Gregory and Kelly, 1999). Experimental evidence suggests that squalene can act as a “sink” for highly lipophilic xenobiotic assisting with their elimination from the body. Since it is a nonpolar substance, it appears to have the highest affinity for unionized drugs. Squalene can be used as an alternative to paraffin to enhance the elimination of \([14C]\) hexachlorobenzene (HCB) and
organochlorine xenobiotic (Richter et al., 1982). Dietary treatment with squalene is as effective as paraffin in markedly enhancing fecal excretion of HCB. The amount of HCB excreted with feces is about three times higher and the half-life of HCB elimination from the body is markedly lower (mean 34-38 days as compared to 110 days for controls) in squalene-treated animals (Richter and Schafer, 1982a).

Sharks inhabiting waters beyond 600m depth are believed to possess reasonably high content of this hydrocarbon (Ko et al. 2002), the antioxidant with potent pharmaceutical values. Its role as an antilipidemic agent and membrane stabilizer has been reported (Qureshi et al. 1996). Because some early studies have shown that squalamine can slow the growth of tumor blood vessels, proponents claim it may help to treat cancer, either alone or combined with chemotherapy. It is also being studied for use against macular degeneration, an eye condition that results in loss of vision. Squalene has been promoted as having cell-protecting abilities, which may reduce the side effects of chemotherapy.

f) n-3 PUFA
Fish oil is a rich source of long-chain n-3 PUFA, which has been shown to reduce symptoms in rheumatoid arthritis (RA) and other inflammatory disorders (Belluzzi et al.,1996), to increase the interval between relapses in Crohn's disease, and to reduce progression to renal failure in IgA nephropathy. Dietary n-3 PUFAs have also been shown to reduce cardiovascular risk factors and events, especially sudden cardiac death (Watkins et al.,2007). Reduction in the latter has been shown to correlate with erythrocyte EPA (20:5n-3) levels. Cleland, L.G. et al. (2003) showed that fish oil supplementation exerted anti-inflammatory effects (partly) by displacing arachidonic acid (AA) from the pool of highly unsaturated fatty acids in the sn-2 position of membrane phospholipids, from which it is released by phospholipase A2 to provide substrate for eicosanoid-forming enzymes (Boudrault et al.,2009). EPA acts as an alternate substrate and inhibitor of AA metabolism.

The proposed mechanisms for health benefits of n-3 fatty acids appear to be related to the incorporation of the fatty acids into membrane phospholipids. This results in increasing the production of series 3 eicosanoids, prostaglandin I3, thromboxane A3, and series 5 leukotriene B5 via the cyclooxygenase and lipoxygenase pathways. Eicosanoids, produced by both n-6 and n-3 fatty acids, are involved in the regulation
of inflammation, platelet aggregation, and vasoconstriction/dilation. Both EPA and n-6 arachidonic acid (ARA) (C20:4) compete for the common cyclooxygenase and lipoxygenase enzymes; thus the n-6:n-3 fatty acid ratio seems to be a determining factor for the outcome of the enzymatic pathways. Compared to EPA, ARA produces more potent inflammatory and pro-aggregatory eicosanoids. This is particularly important when considering the abundance of n-6 fatty acids and the scarcity of n-3 fatty acids in our diets (Boudrault et al., 2009).

Depending on the commercial preparation, shark liver oil may also be rich in omega-3 fatty acids. Shark liver oils contain high proportions of γ-linolenic acid (Zurier et al. 1996), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (James et al. 2003). It has been shown that these long chain n-3 PUFAs (James et al. 2003) lower the incidence of inflammatory diseases such as asthma and arthritis (Shahidi and Senanayake 2006). These dietary fatty acids are known to reduce the levels of arachidonic acid metabolites and lower the formation of proinflammatory compounds, like prostaglandins and leukotrienes, by blocking their activity (Olivera 2004). Early studies reviewed by Stamp et al. (2005) and Calder (2006) attributed the anti-inflammatory effects of fish oils to competition with arachidonic acid for production of inflammatory eicosanoids. Anti-inflammatory effects of EPA and DHA have been studied by several workers (Arita et al. 2005; Lukiw et al. 2005; Hudert et al. 2006). EPA and DHA contained in fish oils provide nutrients needed to build anti-inflammatory prostaglandin series 1 and 3 (Simopoulos 1991). An immunomodulatory effect of these latter fatty acids is suggested by epidemiological studies which show that populations such as Greenland Eskimos, who consume large quantities of marine mammal and fish oils which are rich in eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids, have a very low incidence of inflammatory and autoimmune disorders (4). Furthermore, a number of clinical studies reported that fish oil supplementation has some beneficial effects in rheumatoid arthritis, psoriasis, lupus, and inflammatory bowel disease and prolongs the survival of grafts (Calder, 1999). The potential clinical use of oils rich in n-6 or n-3 PUFAs has given rise to a number of investigations of the effects of fatty acids and dietary oils upon immune cell functions.

Parks et al. 1992, showed that fish oil feeding reduced plasma and liver cholesterol levels in African green monkeys fed on a high fat diet. The reduced level
of cholesterol absorption in the blood was attributed to the increased content of n-3 fatty acids in the diet. The mixed micelles in the intestine that contain n-3 fatty acids may not solubilize the dietary cholesterol as efficiently as for absorption. Studies in human beings have shown that oleinate reduces total plasma cholesterol and LDL cholesterol concentrations with no effect on HDL cholesterol (Mattson and Grundy 1985).

g) Sterols
Cholesterol is the major sterol found in the lipids of the deep sea sharks. It is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma of all animals (Leah 2009). It is an essential structural component of mammalian cell membranes, where it is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile acids, steroid hormones, and several fat-soluble vitamins. Cholesterol is the principal sterol synthesized by animals, but small quantities are synthesized in other eukaryotes, such as plants and fungi.

h) Regulation of cholesterol synthesis
Biosynthesis of cholesterol is directly regulated by the cholesterol levels present, though the homeostatic mechanisms involved are only partly understood. A higher intake from food leads to a net decrease in endogenous production, whereas lower intake from food has the opposite effect. The main regulatory mechanism is the sensing of intracellular cholesterol in the endoplasmic reticulum by the protein SREBP (sterol regulatory element-binding protein 1 and 2) (Espenshade 2007). In the presence of cholesterol, SREBP is bound to two other proteins: SCAP (SREBP-cleavage-activating protein) and Insig1. When cholesterol levels fall, Insig-1 dissociates from the SREBP-SCAP complex, allowing the complex to migrate to the Golgi apparatus, where SREBP is cleaved by S1P and S2P (site-1 and -2 protease), two enzymes that are activated by SCAP when cholesterol levels are low. The cleaved SREBP then migrates to the nucleus and acts as a transcription factor to bind to the SRE (sterol regulatory element), which stimulates the transcription of many genes. Among these are the LDL receptor and HMG-CoA reductase. The former scavenges circulating LDL from the bloodstream, whereas HMG-CoA
reductase leads to an increase of endogenous production of cholesterol (Brown and Goldstein, 1997). A large part of this signaling pathway was clarified by Dr. Michael S. Brown and Dr. Joseph L. Goldstein in the 1970s.

Cholesterol synthesis can be turned off when cholesterol levels are high, as well. HMG CoA reductase contains both a cytosolic domain (responsible for its catalytic function) and a membrane domain. The membrane domain functions to sense signals for its degradation. Increasing concentrations of cholesterol (and other sterols) cause a change in this domain's oligomerization state, which makes it more susceptible to destruction by the proteosome. This enzyme's activity can also be reduced by phosphorylation by an AMP-activated protein kinase. Because this kinase is activated by AMP, which is produced when ATP is hydrolyzed, it follows that cholesterol synthesis is halted when ATP levels are low.

Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins, complex spherical particles which have an exterior composed of amphiphilic proteins and lipids whose outward-facing surfaces are water-soluble and inward-facing surfaces are lipid-soluble; triglycerides and cholesterol esters are carried internally. Phospholipids and cholesterol, being amphipathic, are transported in the surface monolayer of the lipoprotein particle.

In addition to providing a soluble means for transporting cholesterol through the blood, lipoproteins have cell-targeting signals that direct the lipids they carry to certain tissues. For this reason, there are several types of lipoproteins within blood called, in order of increasing density, chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The more cholesterol and less protein a lipoprotein has the less dense it is. The cholesterol within all the various lipoproteins is identical, although some cholesterol is carried as the "free" alcohol and some is carried as fatty acyl esters referred to as cholesterol esters. However, the different lipoproteins contain apolipoproteins, which serve as ligands for specific receptors on cell membranes. In this way, the lipoprotein particles are molecular addresses that determine the start- and endpoints for cholesterol transport.

Synthesis of the LDL receptor is regulated by SREBP, the same regulatory protein as was used to control synthesis of cholesterol de novo in response to cholesterol presence in the cell. When the cell has abundant cholesterol, LDL
receptor synthesis is blocked so that new cholesterol in the form of LDL molecules cannot be taken up. On the converse, more LDL receptors are made when the cell is deficient in cholesterol. When this system is deregulated, many LDL molecules appear in the blood without receptors on the peripheral tissues. These LDL molecules are oxidized and taken up by macrophages, which become engorged and form foam cells. These cells often become trapped in the walls of blood vessels and contribute to atherosclerotic plaque formation. These plaques are the main causes of heart attacks, strokes, and other serious medical problems, leading to the association of so-called LDL cholesterol (actually a lipoprotein) with "bad" cholesterol (Tymoczko et al., 2002). Also, HDL particles are thought to transport cholesterol back to the liver for excretion or to other tissues that use cholesterol to synthesize hormones in a process known as reverse cholesterol transport (RCT) (Lewis and Rader, 2005) Having large numbers of large HDL particles correlates with better health outcomes (Gordon et al., 1989). In contrast, having small numbers of large HDL particles is independently associated with atheromatous disease progression within the arteries.

2.2.11 Cholesterol levels in deep sea sharks
The cholesterol levels in salmon sharks have been reported to range from 1.6% in females to 3.4% in males (Jayasinghe et al., 2003). Bordier et al., 1996 reported the cholesterol levels in liver oils of the deep sea shark Centrophorus squamosus to be 4.5% of the total lipids.

2.2.12 The hepatosomatic index of fishery resources
Sharks have no swim bladder and their large livers saturated with oil maintain their buoyancy in water. Deep sea sharks such as gulper shark (Centrophorus granulosus), smallfin gulper shark (Centrophorus scalpratus), basking shark and tope shark are the major species targeted for this purpose, as they contain a higher yield of oil. Batista and Nunes (1992) report that the size and weight of a shark's liver varies by species and season. The weight of the liver of some shark species constitutes almost one fifth of its weight. Weight tends to increase with size as the larger the shark, the greater the relative weight of the liver. The ratio of liver weight to total body weight of some species is as follows:
Kitefin (Dalatias licha) 19.2%
Tiger (Galeocerdo cuvieri) 17.5%
Salmon (Lamna ditropis) 12.0%
Thresher (Alopias pelagicus) 10.0%
Soupfin (Galeorhinus japonicus) 2.9%

Jayasinghe et al., 2003 reported the hepatosomatic indices (HSI) of salmon sharks to be 6.8% in males and 7.3-8.0% in females. However the average HSI for the black dogfish or squalid shark Centroscyllium fabricii was 20% for both males and females. The hepatosomatic indices of holocephalian fishes was examined by Oguri (1977). Compared to those in teleostean fishes, the index was remarkably high in the fishes studied nearly 16.6% for Hydroichthys coliei. He even recorded the high amounts of fat droplets in the liver tissues.

2.2.13 Chromatographic techniques for the determination of lipid classes
In recent years, enormous strides have been made in the development of methods for the analysis of lipids using high-performance liquid chromatography (HPLC). It is almost certainly true to say that this technique has replaced preparative gas chromatography entirely. In addition, it could be claimed that there is no type of lipid separation for which thin-layer chromatography (TLC) was once favoured that cannot now be done by HPLC (Christie, 2003). The latter offers great versatility in that it can be used in the adsorption, reversed-phase, ion exchange and silver ion modes. It operates at room temperature, so is particularly suited to molecules containing thermally labile functional groups. A host of bonded-phases, offering varying selectivities in specific analyses, are available commercially and many have yet to be properly explored in lipid applications.

With TLC in the adsorption mode (silica gel), the principle application in lipid analysis is for the separation of different lipid classes from animal and plant tissues. It is a relatively easy matter to resolve each of the main simple lipids from a tissue in one step, i.e. cholesterol esters, triglycerides, free fatty acids, cholesterol and diacylglycerols, using mobile phases consisting of a mixture of hexane and diethyl ether, with a little formic acid to ensure that the free acids migrate successfully. Complex lipids such as phospholipids and glycosphingolipids will remain at the origin, and they can then be quantified as if they were a single lipid class (Christie,
In the analysis of plasma lipids from clinical experiments, such separations often afford adequate information for diagnostic purposes, and there are many other circumstances where this type of separation is sufficient. It is perhaps one of the disadvantages of HPLC that there is no analogous method for the determination of complex lipids as a single entity. High-performance TLC makes use of silica gel of a very uniform and small particle size, permitting excellent separations with comparatively short elution times. For example, most of the important lipid classes in clinical samples can be achieved by one-dimensional TLC in a single chromatographic run.

TLC offers considerable versatility and precision in lipid analysis with relatively low capital costs.

2.2.14 The TLC-FID using Iatroscan® MK-6s

Analysis of the various lipid components in a system (crude oil, carbon products, marine oils and sediments, foods etc.) may be analysed using a Thin layer chromatograph clubbed with a flame ionisation detector, in a single run. This principle is made use of the Iatroscan MK-6s instrument. Here a specially designed Chromarod (a quartz rod coated with a thin layer of silica or alumina on which the sample is developed and separated), is advanced at a constant speed through the flame of the FID, the substances are ionized through energy obtained from the hydrogen flame. Effected by the electric field applied to the poles of the FID the ions generate electric current with an intensity proportional to the amount of each organic substance entering the flame. The analysis with IATROSCAN MK-6s is favorable in maintenance and price, because of the low consumption of solvents and the reusable Chromarods.

The separation is made with the TLC Method on so called Chromarods and the Detection with a Flame Ionization Detector (FID). With an additional FP-Detector it is possible to analyze sulphur and phosphorus, too (MK-6). The Analysis is made with different complementary accessories.

The spotting of the sample is made with a specially developed Application System (Autospotter or Full-Automatic-Sample Spotter) on the 10 Chromarods lying in the Rod Holder. The Chromarods are developed in a special Development Tank. After the separation of the substances the Holder is put in the IATROSCAN. The Chromarods are scanned through the Hydrogen flame. The collector, which is placed
above the flame is generating an analogue signal, which is evaluated with a PC and
the SES ChromStar Software.

Numerous workers have made use of the TLC-FID analytical technique for the
detection and quantification of various components. Cebolla et. al. (1995) , Karlsen
et al.,(1991) and Sol et al. (1985), have used this technique for the analysis of crude
hydrocarbons - saturates, aromatics, resins and asphaltenes (SARA) constituents in
crude oils and solvent extracts. Itoh et al 1985 separated the methanalysis products
of neutral sphingolipid and archaebacterial neutral glycolipid on Chromarods S-II
(silica gel) with a double developing system. The lipid constituents separated on the
rods were scanned automatically with a hydrogen flame ionization detector
(latroscan). The molar ratios of the constituents determined by this system were very
close to the theoretical values of the lipid. Reiffova et al., (2003) used the technique
for the separation of oligosaccharides. Chromatography was performed on
Chromarods S III with two different mobile phases, ethyl acetate-formic acid-water
and butanol-ethanol-water. Pretreatment of the biological samples was minimal.

2.2.15 The HPLC with an evaporative light scattering detector (HPLC-ELSD)²
Lipid analysts were initially slow to come to terms with the potential of high­
performance liquid chromatography (HPLC), largely because of the non-availability
of a sensitive universal detector. In contrast the flame-ionisation detector, commonly
used in gas chromatography (GC), is highly sensitive and exhibits a linear response
over a wide range of sample sizes. Transport-flame ionisation detectors for HPLC
have always looked promising, but have never been a commercial success. The
evaporative light scattering detector has the advantages of being simple yet versatile
in the commercial analysis of samples.

With this instrument, the solvent emerging from the end of the column is evaporated
in a stream of air in a heated chamber, the solute does not evaporate, but is
nebulized and passes in the form of minute droplets through a light beam, which is
reflected and refracted. The amount of scattered light is measured and bears a
relationship to the concentration of material that is eluting.

There are no special wavelength requirements for the light source, and in some
commercial instruments, it is simply a projector lamp. Such a detector can be
considered to be universal in its applicability, in that it will respond to any solute that
does not evaporate before passing through the light beam. The instrument gives excellent results under gradient elution conditions, and it is simple and rugged in use. The sensitivity is comparable to that of a refractive index detector, but the evaporative light-scattering detector is not affected by changes in the mobile phase or small variations in the room temperature or in the flow rate of the mobile phase, as is the former.

Once the instrument is warmed up and is running, there is little base-line drift during continuous operation even with abrupt changes in solvent composition. Most organic solvents, including acetone and chloroform, for example, can be used, and these can contain up to 20% water and small amounts of ionic species even. The minimum detection limit is dependent to a certain extent on the nature of the mobile phase, the nature of the sample, and the specific instrument, but it is certainly less than one microgram.

There has also been some debate about the efficacy of the evaporative light-scattering detector in quantification. However, Herslof and Kindmark (1996) obtained good reproducibility for the relative proportions of different molecular species in analyses of the triacylglycerols of soybean oil. When the technique is used in research with triacylglycerols differing widely in composition, the best approach to quantification consists in collecting fractions and adding an odd-chain fatty acid as an internal standard prior to transesterification and GC analysis, i.e. the technique long used with thin-layer chromatography. The fatty acid composition and the amount of each fraction are thereby obtained simultaneously.

Graeve and Janssen (2009) in their study on the separation of lipid classes of marine zooplanktons presented an improved HPLC method, which allowed better resolution and quantification of a broad range of lipid classes with special regard to neutral lipids. Marine zooplankton species often produce high amounts of exceptional lipids, especially at high latitudes, in order to cope with the harsh environmental conditions and strong seasonality in food supply. Major neutral lipid classes analysed in their study were wax esters, triacylglycerols, diacylglycerol ethers, free fatty alcohols and sterols. Neutral and polar lipids were separated and identified on a monolithic silica column (Chromolith® Performance-Si) using high performance liquid chromatography (HPLC) with an evaporative light scattering detector (ELSD). Their method resolved a broad spectrum of lipids, varying in
polarity from squalene to lysophosphatidylcholine in a single run. The total run time was 35 min including column re-equilibration. The calibration was made at levels of 0.1–60 µg lipid/injection, but a 10–15-fold greater amount could have been injected if single lipid classes had to be separated, e.g. for further determination of individual fatty acids. The method was applied to representative Arctic zooplankton species (copepods, pteropods, euphausiids and ctenophores) that were known to biosynthesize in particular neutral lipids like diacylglycerol ethers and free fatty alcohols.

Homan and Anderson (1998) achieved rapid separation and quantitation of combined neutral and polar lipid classes by the HPLC-ELSD method. In their study, substitution of acetone for 2-propanol in a portion of the solvent gradient program yielded consistent resolution of diacylglycerol and cholesterol without sacrificing baseline resolution of the remaining major lipid classes. Moreover, previously noted instabilities in triacylglycerol retention time are eliminated. The introduction of acetone also enabled a 20% reduction in flow-rate without an increase in total run time. As a further modification of the mobile phase composition, acetic acid and ethanolamine were substituted for the serine-ethylamine combination that was originally shown to improve column performance. The combination of acetic acid and ethanolamine yielded the same result but the increased volatility of these solutes over serine resulted in decreased baseline noise. Finally, 1,2-hexadecanediol was introduced as an internal standard that well suited for their method. The chromatographic performance obtained with the modifications was demonstrated in compositional analyses of lipid extracts from rat liver, heart, kidney and brain.
2.3 MATERIALS AND METHODS
All the elasmobranch resources *Apristurus indicus*, *Centrophorus scalpratus*, *Centroselachus crepidater*, *Neoharriotta raleighana* and *Harriotta pinnata* were caught during Cruises 250 and 252 on the Fisheries Oceanic Research Vessel (FORV) Sagar Sampada from beyond 600 m depth along the southwest and eastern coasts of India. Expo model trawl nets were used to catch these deep sea fishery resources. The liver was separated from these fishes, the total lengths and body weights of each fish were recorded; the corresponding liver weights were also noted down to calculate the ratio between the body and liver weights or the hepatosomatic index (HSI). The fish along with their respective livers were immediately frozen at -20°C onboard the vessel; brought to the laboratory for further analyses. The catch details of the five different elasmobranch resources, whose liver oils were analyzed for their bioactives, are shown in Table 2a. All chemicals and reagents used were obtained from Merck (Darmstadt, Germany). The chemical standards used for the calibration and standardisation were purchased from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA).

2.3.1 Extraction of liver oils
Accurately weighed liver of each fish was subjected to lipid extraction by the method of Folch et al. (1957). Briefly, minced liver was homogenized in a 2:1 (v/v) mixture of chloroform-methanol and filtered. 20% water was added to this mixture and the layers were allowed to separate overnight. The aqueous layer was discarded the following day and the total solvent extract was concentrated (i.e solvents were removed *in vacuo*) using rotary evaporation at 40°C. The oil was made up to a known volume in chloroform and stored in amber-coloured bottles under nitrogen at -20°C. A portion of the oil was saponified (Hallgren and Larsson 1962), in a mixture of 150% potassium hydroxide (w/v) and absolute ethanol for 2 h in a water bath at 75°C under an inert atmosphere of nitrogen. The resulting mixture was extracted with ether, water-washed, dried over anhydrous sodium sulphate and finally condensed to a known volume. A small portion of the ether layer was air dried to estimate the fraction of the non-saponifiable matter (NSM) present in the oils.

2.3.2 Analysis of lipid components using TLC-FID

81
Aliquots of the ether extract or the diluted crude liver oil were analyzed using an Iatroscan MK-6s (M/s. Mitsubishi Kagaku Iatron Inc. Tokyo, Japan) employing the TLC-FID method, to determine the abundances of individual lipid classes (hydrocarbons, alkoxyglycerols, triacylglycerols, fatty acids) (Bakes and Nichols 1995). Samples were applied in triplicates to silica gel SIII chromarods (5 µm particle size) using 1 µl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was chloroform-methanol-water-ammonia (47:23:4:0.25 v/v/v/v), a mobile phase separating polar lipids. A second non-polar solvent system of hexane-diethyl ether (60:15 v/v) was also used to resolve the non-polar lipid components. After development, the chromarods were oven dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The flame ionization detector (FID) was calibrated for each compound class (squalene, monopalmitoyl-rac-glycerol, di-oleoyl-rac-glycerol, cholesterol, oleic acid, tripalmitin, α-tocopherol, retinol, phosphatidylcholine). The peaks obtained via a Chromatocorder were quantified and tabulated. The standards were prepared in five different concentrations ranging from 0.1- 2 µg/ml and 1 ul of each standard was spotted on chromarods to determine the coefficient of linearity.

2.3.3 Analysis of lipid components using HPLC-ELSD

It is well established that separation in normal phase chromatography is based on the polar groups of the molecule regardless the non-polar side chain. This fact permits one to separate lipid classes regardless the number of carbon atoms and degree of saturation of the compound. However, the whole structure of the molecule contributes on the separation. Although the effect of alkyl groups to the retention is limited, it should be also taken into account. In addition, steric effects have also influence on the retention making possible the separation of cis and trans isomers. Hence, this type of chromatography is preferred when commercial mixtures of oils have to be analyzed because of the presence of complex mixture of chemical species.

The quantification of the different lipid classes of squalene, cholesterol palmitate, α-tocopherol, tripalmitin and monopalmitoyl-rac-glycerol in the liver oils of the 5 elasmobranchs was further confirmed with the help of HPLC-ELSD technique. The
lipid separation was accomplished by normal phase HPLC, using quaternary gradient pumps (Merck Hitachi L-2700 Germany). 20μl volume of sample was injected into the column using a 20μl sample loop. The Chromatographic column was a LiChrosphere Si60 Nor Ph. The flow rate was 1.0ml/min and the column temperature was maintained at 35°C during all runs. An evaporative light scattering detector (Alltech 2000 ES USA) was used for the detection and nitrogen was used for the gas. The detector temperature was 45°C and the air pressure was 2.0 bar. The photomultiplier sensitivity was adjusted to the mean value of the total photomultiplier range (gain 7). The detector signal was recorded and integrated by a personal computer and a software program (GynkoSoft Chromatography Data System, version 4.22, Gynkotek, Munich, Germany).

The lipid classes were separated by three different solvent and gradient systems. A combination Gradient is shown in Table 2e. Isooctane with 0.5% of MTBE enables the separation of squalene, cholesterol as their corresponding esters, and mono-alkyl glycerols in a time of analysis of 50 min.

2.3.4 Analysis of fatty acids using GC-FID

Aliquots of the ether extract of the liver oil (three replicates) were methylated using BF3-methanol and the resulting fatty acid methyl esters (FAME) (Davidson and Cliff 2002) were injected into the Trace GC Ultra gas chromatograph (M/s. Thermo Electron Corporation, Milan, Italy) equipped with Perkin Elmer Elite 225, 50% cyanopropyl phenyl – 50% methyl capillary column (30 m × 0.25 mm i.d.), a flame ionization detector (FID) and a split/splitless injector. Nitrogen was the carrier gas. Briefly, the aliquots were injected in splitless mode at an oven temperature of 110°C. After 4 min the oven temperature was raised to 240°C at 2.7°C/min. Peaks were analyzed and quantified using Chromcard software, with the help of running authentic standards.

2.3.5 Statistical analysis

Data obtained from three replicates of the liver oil sample were subjected to descriptive statistics using SPSS 16.0 Software Package and the values were expressed as mean ± SD.
2.4 RESULTS

The sharks and chimaeras obtained for the study from the south west coasts of India namely Apristurus indicus, Centrophorus scalpratus, Centroselachus crepidater and the chimaeras Neoharriotta raleighana and Harriotta pinnata were taken by bottom trawlers off the Indian coasts (Plate II.1, Table 2a). These species formed the dominant catches among the elasmobranchs analysed during our expeditions but however they find limited commercial applications. Therefore the need to understand and utilise such potential specimens for research including molecular studies is essential.

The hepatosomatic indices of the elasmobranchs were studied and tabulated as in Table 2b. The body to liver weight ratio of all the species studied was approximately 20% as observed by several other workers. The weight of the liver of some shark species constitutes almost one fifth of its weight. Weight tends to increase with size as the larger the shark, the greater the relative weight of the liver.

The lipid composition of oils extracted from the liver of four species of deep sea sharks found in southern Indian waters was determined. The oils of NR, CS and CC recorded high NSM content of 80, 73 and 60 %, respectively (Table 2c). However, Al oil had the lowest content of NSM (25%) among the four oils examined. Alkylglycerols and hydrocarbons (HCs), predominantly the isoprenoid squalene, were the major components of the NSM. HC content varied significantly among the species analyzed. Oils of Al species recorded the lowest amount of squalene at 20.1 %, while that of CS was 67.4 %. Oils of CC and NR species contained 52 and 62 % squalene, respectively. AKGs comprising of both mono- and di-alkoxy-glycerols were present in all shark species at levels between 12.2 and 21.1 %. Polar lipids were either present in low abundance (<2%) or were not detected in the extracted oils.

Triacylglycerols composition ranged from 2-4% of total lipids in liver of the selected elasmobranchs. Cholesterol was observed at almost 5% levels in NR, CS and AI and at half its amount in CC. Significantly high amounts of monoacylglycerols (7% total lipid) were observed in the livers of the Neoharriotta sp. Vitamin E at 2% lipid levels of liver were observed in both the Neoharriotta sp. and the Centrophorus sp. Traces of Vitamin A were also recorded in the selected species. However the lipids of Apristurus sp recorded high levels of saponifiable matter (68% total lipids).
Table 2a. Details of shark species collected during cruises on the FORV Sagar Sampada

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Region</th>
<th>Lat N</th>
<th>Long E</th>
<th>Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Apristurus indicus (Al) Small-belly catfish</td>
<td>Azhikkal</td>
<td>12°04</td>
<td>74°16</td>
<td>735</td>
</tr>
<tr>
<td>2.</td>
<td>Centrophorus scalpratus (CS) Endeavour dogfish</td>
<td>Diglipur</td>
<td>13°21</td>
<td>93°07</td>
<td>695</td>
</tr>
<tr>
<td>3.</td>
<td>Centrosymnus crepidater (CC) Deep sea dogfish</td>
<td>Kasargode</td>
<td>12°25</td>
<td>74°07</td>
<td>740</td>
</tr>
<tr>
<td>4.</td>
<td>Neoharriotta raleighana (NR) Long-nosed ratfish</td>
<td>Alleppey</td>
<td>09°17</td>
<td>75°38</td>
<td>724</td>
</tr>
<tr>
<td>5.</td>
<td>Harriotta pinnata Sickle-finned chimaera</td>
<td>Alleppey</td>
<td>09°17</td>
<td>75°38</td>
<td>724</td>
</tr>
</tbody>
</table>

Table 2b. Fork lengths, body & liver weights and hepatosomatic indices of sharks and chimaeras of the Indian EEZ

<table>
<thead>
<tr>
<th>Sharks/chimaeras (N=3)</th>
<th>Total length (cm)</th>
<th>Body weights (kg)</th>
<th>Liver weights (kg)</th>
<th>Hepatosomatic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. scalpratus</td>
<td>160.6 ± 5.3</td>
<td>416.2 ± 2.3</td>
<td>83.1 ± 1.6</td>
<td>20.1</td>
</tr>
<tr>
<td>C. crepidater</td>
<td>127.5 ± 4.6</td>
<td>318.6 ± 1.1</td>
<td>72.2 ± 1.4</td>
<td>22.7</td>
</tr>
<tr>
<td>A. indicus</td>
<td>52.3 ± 4.7</td>
<td>99.2 ± 1.3</td>
<td>16.1 ± 1.2</td>
<td>16.3</td>
</tr>
<tr>
<td>N. raleighana</td>
<td>120.3 ± 2.7</td>
<td>312.4 ± 1.9</td>
<td>71.7 ± 1.4</td>
<td>23.1</td>
</tr>
<tr>
<td>H. pinnata</td>
<td>134.5 ± 6.1</td>
<td>281.4 ± 1.2</td>
<td>55.8 ± 1.5</td>
<td>19.8</td>
</tr>
</tbody>
</table>

Results are mean±SD for n=3 determinations.
### Table 2c: Characterisation of liver lipids of elasmobranch resources of the Indian EEZ

<table>
<thead>
<tr>
<th></th>
<th>Neoharriotta raleighana (NR)</th>
<th>Centrosymnus crepidater (CC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fat content (w/w)</td>
<td>69.28 ± 1.83</td>
<td>77.43 ± 2.85</td>
</tr>
<tr>
<td>Non-saponifiable matter</td>
<td>80.01 ± 2.03</td>
<td>60.7 ± 3.60</td>
</tr>
<tr>
<td>Hydrocarbon (squalene)</td>
<td>59.43 ± 2.52</td>
<td>52.71 ± 1.29</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>3.89 ± 3.07</td>
<td>2.01 ± 1.37</td>
</tr>
<tr>
<td>Diacylglycerol ether</td>
<td>3.46 ± 4.23</td>
<td>1.24 ± 1.94</td>
</tr>
<tr>
<td>Monoacylglycerol ether</td>
<td>7.61 ± 3.80</td>
<td>2.42 ± 1.20</td>
</tr>
<tr>
<td>Sterol (cholesterol)</td>
<td>5.73 ± 2.15</td>
<td>2.73 ± 1.05</td>
</tr>
<tr>
<td>Vitamin E (tocopherol)</td>
<td>2.55 ± 0.59</td>
<td>0.35 ± 0.32</td>
</tr>
<tr>
<td>Vitamin A (retinol)</td>
<td>0.96 ± 0.88</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>Saponifiable matter</td>
<td>22.86 ± 0.45</td>
<td>28.26 ± 2.45</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.85 ± 0.20</td>
<td>0.87 ± 0.16</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>21.18 ± 4.85</td>
<td>26.63 ± 2.35</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>0.83 ± 0.06</td>
<td>0.76 ± 0.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid composition of liver oil of</th>
<th>Neoharriotta pinnata, pinnata</th>
<th>Centrophorus scalpratus (CS)</th>
<th>Apristurus indicus (AI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fat content (w/w)</td>
<td>69.28 ± 3.33</td>
<td>79.46 ± 2.27</td>
<td>70.58 ± 1.87</td>
</tr>
<tr>
<td>Lipid composition (as % total lipid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-saponifiable matter</td>
<td>78.01 ± 3.43</td>
<td>73.6 ± 1.50</td>
<td>30.90 ± 0.70</td>
</tr>
<tr>
<td>Hydrocarbon (squalene)</td>
<td>52.43 ± 8.29</td>
<td>60.67 ± 0.84</td>
<td>18.10 ± 0.36</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>3.89 ± 3.07</td>
<td>2.65 ± 1.02</td>
<td>4.43 ± 1.37</td>
</tr>
<tr>
<td>Diacylglycerol ether</td>
<td>3.46 ± 4.23</td>
<td>1.46 ± 0.23</td>
<td>3.46 ± 4.23</td>
</tr>
<tr>
<td>Monoacylglycerol ether</td>
<td>10.61 ± 3.80</td>
<td>2.62 ± 1.53</td>
<td>0.84 ± 1.80</td>
</tr>
<tr>
<td>Sterol (cholesterol)</td>
<td>5.73 ± 2.15</td>
<td>5.73 ± 1.17</td>
<td>4.73 ± 1.24</td>
</tr>
<tr>
<td>Vitamin E (tocopherol)</td>
<td>2.55 ± 0.59</td>
<td>2.74 ± 0.15</td>
<td>0.55 ± 0.59</td>
</tr>
<tr>
<td>Vitamin A (retinol)</td>
<td>0.96 ± 0.88</td>
<td>0.34 ± 0.10</td>
<td>0.52 ± 0.31</td>
</tr>
<tr>
<td>Saponifiable matter</td>
<td>22.86 ± 0.45</td>
<td>24.26 ± 0.45</td>
<td>68.83 ± 0.46</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.85 ± 0.20</td>
<td>0.28 ± 0.11</td>
<td>1.94 ± 0.25</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>21.18 ± 4.85</td>
<td>23.09 ± 1.63</td>
<td>66.35 ± 2.05</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>0.83 ± 0.06</td>
<td>0.89 ± 0.23</td>
<td>0.54 ± 0.16</td>
</tr>
</tbody>
</table>

Results are mean % of three replicates of the liver oil ± S.D.
Table 2d. Total fatty acid composition of liver oils from deep-sea sharks collected from Indian waters*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>A. indicus</th>
<th>C. crepidater</th>
<th>C. sculpratus</th>
<th>N. raleghana</th>
<th>N. pinnata</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.65</td>
<td>1.17</td>
<td>0.21</td>
<td>1.72</td>
<td>0.72</td>
</tr>
<tr>
<td>14:1</td>
<td>0.82</td>
<td>0.16</td>
<td>1.30</td>
<td>0.30</td>
<td>0.28</td>
</tr>
<tr>
<td>15:0</td>
<td>1.00</td>
<td>1.00</td>
<td>0.06</td>
<td>1.98</td>
<td>0.78</td>
</tr>
<tr>
<td>15:1</td>
<td>0.70</td>
<td>0.11</td>
<td>0.54</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>16:0</td>
<td>14.15</td>
<td>12.43</td>
<td>10.82</td>
<td>12.36</td>
<td>11.36</td>
</tr>
<tr>
<td>16:1</td>
<td>2.89</td>
<td>4.00</td>
<td>3.34</td>
<td>4.71</td>
<td>4.85</td>
</tr>
<tr>
<td>17:0</td>
<td>0.15</td>
<td>0.35</td>
<td>0.16</td>
<td>0.30</td>
<td>0.22</td>
</tr>
<tr>
<td>17:1</td>
<td>2.93</td>
<td>4.98</td>
<td>3.82</td>
<td>3.10</td>
<td>2.85</td>
</tr>
<tr>
<td>18:0</td>
<td>9.79</td>
<td>5.65</td>
<td>4.17</td>
<td>3.60</td>
<td>4.60</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>16.34</td>
<td>24.11</td>
<td>33.43</td>
<td>27.68</td>
<td>29.68</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>1.39</td>
<td>ND</td>
<td>1.68</td>
<td>2.46</td>
<td>2.16</td>
</tr>
<tr>
<td>20:0</td>
<td>0.21</td>
<td>0.47</td>
<td>0.31</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>20:1</td>
<td>23.85</td>
<td>15.99</td>
<td>15.87</td>
<td>11.35</td>
<td>12.05</td>
</tr>
<tr>
<td>20:3(n-3)</td>
<td>7.15</td>
<td>5.82</td>
<td>8.75</td>
<td>5.20</td>
<td>5.11</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.62</td>
<td>0.88</td>
<td>1.87</td>
<td>4.53</td>
<td>5.03</td>
</tr>
<tr>
<td>22:0</td>
<td>0.32</td>
<td>0.37</td>
<td>0.28</td>
<td>0.67</td>
<td>1.87</td>
</tr>
<tr>
<td>22:1</td>
<td>10.17</td>
<td>14.01</td>
<td>7.46</td>
<td>6.22</td>
<td>6.92</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>3.88</td>
<td>4.08</td>
<td>3.10</td>
<td>10.03</td>
<td>9.11</td>
</tr>
<tr>
<td>24:1</td>
<td>1.50</td>
<td>2.42</td>
<td>1.93</td>
<td>2.40</td>
<td>2.93</td>
</tr>
<tr>
<td>Others</td>
<td>1.50</td>
<td>1.99</td>
<td>1.01</td>
<td>0.88</td>
<td>0.68</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Sum Saturates</td>
<td>26.26</td>
<td>21.43</td>
<td>18.01</td>
<td>21.03</td>
<td>19.03</td>
</tr>
<tr>
<td>Sum</td>
<td>59.21</td>
<td>65.79</td>
<td>68.59</td>
<td>49.96</td>
<td>57.87</td>
</tr>
<tr>
<td>monounsaturates</td>
<td>13.04</td>
<td>10.79</td>
<td>15.40</td>
<td>21.92</td>
<td>22.22</td>
</tr>
<tr>
<td>polyunsaturates</td>
<td>Total Fatty Acid</td>
<td>687.82</td>
<td>282.71</td>
<td>242.34</td>
<td>220.10</td>
</tr>
</tbody>
</table>

* All values are expressed as percentage of the total fatty acids unless otherwise stated.
GC results are subject to an error of ± 1%. ND = not detected.
Table 2e. Gradient mobile phase composition (%).*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Percent Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isooctane</td>
</tr>
<tr>
<td>0</td>
<td>99.5</td>
</tr>
<tr>
<td>3</td>
<td>99.5</td>
</tr>
<tr>
<td>3.01</td>
<td>98.5</td>
</tr>
<tr>
<td>20</td>
<td>89</td>
</tr>
<tr>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>30.01</td>
<td>71</td>
</tr>
<tr>
<td>50</td>
<td>71</td>
</tr>
<tr>
<td>50.01</td>
<td>75</td>
</tr>
<tr>
<td>60</td>
<td>75</td>
</tr>
<tr>
<td>70</td>
<td>99.5</td>
</tr>
</tbody>
</table>

*MTBE contained 0.01% (v/v) of formic acid.
compared to the others. The total free fatty acids were found to be at less than 1% levels in all the species evaluated.

The fatty acid (FA) and total fatty acid content of the four species of shark are given in Table 2d. The total fatty acids ranged from 22 to 28% of the total lipid in NR, CS and CC species whereas it was as high as 68% in the liver oils of AI species. The predominant fatty acids in all species were the monounsaturates (MUFA) ranging from 55 to 67% with 18:1(n-9), 20:1 and 22:1 being the major fatty acids. Polyunsaturated fatty acid (PUFA) levels varied from 10 to 22% of the total fatty acid content in oils.

2.5 DISCUSSION

The composition of liver oils from the elasmobranchs Apristurus indicus, Centrophorus scalpratus, Centroselachus crepidater and the chimaera Neoharriotta raleighana have not been previously documented. These sharks chimaeras are frequently caught in deep-sea fishery trawls along the southwest coast of India. The liver oil of these species was found to contain high levels of the HC squalene which is typical of liver oils from deep-water elasmobranchs inhabiting water depths between 600 and 1000 m (Bakes and Nichols 1995). Triacylglycerols, di- and monoacyl glyceryl ethers together formed 18% of the liver oils of N. pinnata. According to Deprez et al. (1990), the levels of these specific lipids have been found to vary from 18% in certain species of dogfish sharks (Centrophorus scalpratus) to as high as 90% in Plunket (Somniosus pacificus) and sleeper sharks (Centroscymnus plunketi). The role of specific lipids and hydrocarbons as buoyancy regulators in the liver of deep sea sharks has been documented (Malins and Barone 1970; Phleger and Grigor 1990) and it is apparent that different sharks regulate liver lipid composition to maintain buoyancy. The levels of squalene, triacylglycerols, di- and monoacyl glyceryl ethers and the unusually high amounts of tocopherol in the liver oils of chimaera could also be affected by the dietary intake of specific components and seasonal variations (Kayama et al. 1971; Hayashi and Takagi 1981). Phleger and Grigor (1990) showed that Hoplostethus atlanticus found at similar depths to these deep-sea chimaeras use lipid deposits to control buoyancy.
The total fatty acid content obtained from the liver oil of *N. pinnata* was 211 mg/g. Previous reports by Buranudeen and Richards-Rajadurai (1986) confirmed the variations in the total fatty acid content in certain *Centrophorus* species (possessing high squalene contents in their liver oils), to range from 95–600 mg/g. The fatty acids in the liver oils of *N. pinnata* were mainly the mono- and poly-unsaturated types. The role of EPA and DHA in lipid fluidity has been previously documented (Russell 1990) and the high levels of DHA in chimaeras and sharks may complement the levels of AKGs in them and play a role in maintaining their fluidity. AKGs are important in the treatment of haematopoiesis and radiation sickness (Devaraj and Jialal 2000; Pedrono *et al.* 2004).

It has also been proved that long chain *n*-3 PUFAs (James *et al.* 2003) lower the incidence of inflammatory diseases such as asthma and arthritis (Calder 2006). These dietary fatty acids are known to reduce the levels of arachidonic acid metabolites and lower the formation of proinflammatory compounds, like prostaglandins and leukotrienes, by blocking their activity (Olivera *et al.* 2004). Early studies reviewed by Stamp *et al.* (2005) and Calder (2006) attributed the anti-inflammatory effects of fish oils to competition with arachidonic acid for production of inflammatory eicosanoids. EPA and DHA contained in fish oils also help to increase levels of digestive enzymes in the body thereby providing nutrients needed to build anti-inflammatory prostaglandin series 1 and 3, which helps in weight loss (Simopoulos 1991).

In addition, the high levels of squalene (Ko *et al.* 2002) and tocopherol (Devaraj and Jialal 2000) in the liver lipids of *N. pinnata* help reduce inflammation by decreasing C-reactive protein levels by blocking the activity of TNF-α (tumour necrosis factor-alpha) series 2-prostaglandins (PGE-2) and cyclooxygenases (James *et al.* 2003). The role of squalene as an antilipidemic agent (Qureshi *et al.* 1996) and membrane stabilizer has been well documented (Sabeena *et al.* 2004).

All the instruments used in the study had been calibrated for the purpose of quantification of the standards. Most of the calibration curves reported in the literature for the Iatroscan follow the same basic form and are fitted by a power law equation (*FID response versus weight of lipid, y=ax^n*) for loads ranging from 1 to 10.
ug of standard lipid. Usually, FID response is linear, but the range of load used in TLC–FID is too large to obtain this linearity in one curve. Besides, the high sensitivity of the FID detector allows the quantification of low amounts of lipid (0.1 ug) with a low coefficient of variation (S.D.<6%, n=3) for each standard class. Thus, it was unnecessary to fit calibration curves in a larger range than 0.1 to 2 ug lipid. Thus, the power law equation was accurate enough for a satisfactory fitting ($r^2=0.9905$ to 0.9992, $n=3$) and easier to use in data processing.

Linear response curves have been reported for the detection of lipid classes by ELSD, although only for a narrow range of amount injected. Torres et al. (2005) prepared standard curves for non-polar lipids and observed that the relationship between light scattering and solute concentration was generally linear, but second order polynomial regression analysis gave the best fit. Figure 2.1 shows the calibration curves of the different lipid classes studied. The amount of sample injected was in the range between 1 and 50 ug injected. This broad range of concentrations studied permits one to simultaneously quantify minor constituents in fats and oil in concentrations as low as 1% (w/w) of the total and simultaneously with the rest of neutral lipid existing in the sample. Hence, this methodology not only separates the different lipid classes but also is able to estimate the relative proportion in which they are found in a broad range of concentrations.
Figure 2.1 Calibration curves of lipid components using TLC-FID

**Calibration plot for squalene**

Area vs. concentration (µg/ml)

**Calibration plot for triacylglycerols**

Area vs. concentration (µg/ml)

**Calibration plot for diacylglycerylether**

Area vs. concentration (µg/ml)
Figure 2.1 Calibration curves of lipid components using TLC-FID (contd.)

Calibration plot for monoacylglycerol ether

Calibration plot for cholesterol

Calibration plot for α-tocopherol
Figure 2.1 Calibration curves of lipid components using TLC-FID (contd.)

Calibration plot for retinol

Calibration plot for oleic acid

Calibration plot for phosphatidyl choline
2.6 SUMMARY AND CONCLUSION

Studies on the potential health benefits of lipids from deep sea fishes is a rapidly emerging area. Our study is only a preliminary initiative to assess the bioactive potentials of marine lipids using species otherwise considered by people as wastes.

The major findings of the study were as follows

1) *Apristurus indicus, Centrophorus scalpratus, Centroselachus crepidater, Neoharriotta raleighana* and *Harriotta pinnata* were the major elasmobranchs that dominated our catches during the cruises along the south west coasts of the Indian EEZ.

2) There was an approximately 20% ratio of body to liver weights of almost all the species analysed.

3) High levels of non-saponifiable matter was observed in the liver oils of the CS, CC and NR species whereas those of AI recorded high levels of the saponifiable matter in their liver oils.

The use of these liver oils in biomedical applications needs to be looked upon. The liver oils of deep sea sharks and chimaeras possess multitudes of bioactives whose immense pharmacological applications deserve strong scrutiny.
PLATE II.1 Elasmobranch resources from the Indian EEZ

*Centrophorus scalpratus*

*Centroselachus crepidater*

*Aprimurus indicus*

*Neoharriotta raleighana*

*Neoharriotta pinnata*
<table>
<thead>
<tr>
<th>Name: CHROMAT715 09:10 MAR. 24, 2006</th>
<th>Sample: 009</th>
<th>ATTN: 64</th>
<th>POSI: 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>INJECT: 0.07 Retinol oleic acid squalene a-tocopherol tripalmitin cholesterol di-oleoyl-rac-glycerol monopalmitoyl-rac-glycerol phosphatidylcholine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conc. Levels:**
- **Calc. Method:** 0 (Area%)
- **PA:** 1.00000
- **PB:** 1.00000

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT</th>
<th>AREA</th>
<th>MAR2</th>
<th>CONC</th>
<th>HEIGHT</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.176</td>
<td>0.212</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.27</td>
<td>0.000</td>
<td></td>
<td></td>
<td>0.374</td>
<td></td>
</tr>
</tbody>
</table>

**INJECT:**
- **Name:** CHROMAT715
- **08:19 MAR. 24, 2006 | CB1 ATTN: 64 SPEED: 10.0**
Plate II.3 Determination of lipid classes using HPLC-ELSD
2.7 REFERENCES


Bonfil R (1994) Trends and patterns in world and Asian elasmobranch fisheries in Wildlife Conservation Society, Bronx, USA


Buranudeen F, Richards-Rajadurai PN (1986) Squalene. *Infofish Marketing Digest* 1, 42-43


Dahlgren, T (1992) Shark long line catches, on India’s east coast. *Bay of Bengal’s news*, 48: 10-12.


Internet references
a) FAO(Aquaculture: Fisheries: species profile)
b) www.fishbase.com
c) www.ses-analysesysteme.de/latroscan
d) www.lipidlibrary.aocs.org