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# Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand

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## Abstract

Deep-sea sharks approach neutral buoyancy by means of a large liver that contains large amounts of low-density lipids, primarily squalene and diacyl glyceryl ether (DAGE). As an animal increases in size and matures sexually, many biochemical changes take place within the animal. It was hypothesized that maintenance of neutral buoyancy in deep-sea sharks involves fine-scale changes in the chemical composition of the liver oil as individual sharks grow and develop. To test this hypothesis, the lipid composition of liver oil for individuals of different size and sex of deep-sea sharks from the Chatham Rise, New Zealand was compared. The composition of liver oil varied within and among species. Several species contained large amounts of squalene and DAGE, whereas only traces of these lipids were present in other species. The amounts of squalene and DAGE in liver oil were inversely related, and squalene content tended to decrease as sharks increased in size. Species with high squalene levels (> 80%) in liver oil were not abundant on the Chatham Rise, although levels of DAGE (a lipid of increasing commercial interest) were elevated in many species. Maintenance of neutral buoyancy in deep-sea sharks appears to involve changes in the composition of low-density liver lipids as the sharks increase in size and mature. © 2000 Elsevier Science Inc. All rights reserved.

**Keywords:** Deep-sea sharks; Diacyl glyceryl ether; Liver oil; Squalene; Buoyancy

## 1. Introduction

Deep-sea sharks approach neutral buoyancy, presumably to conserve energy in a nutrient poor environment (Bone et al., 1969; Corner et al., 1969). It has long been recognized that the liver is a hydrostatic organ in sharks (Hickling et al., 1930), and in deep-sea sharks, buoyancy is achieved by means of a large liver, which contains large quantities of low-density oil (Bone et al.,

1988). The liver oil of many deep-sea sharks contains several uncommon, low-density lipid classes, chiefly squalene and diacyl glyceryl ether (DAGE) (Deprez et al., 1990; Batista and Nunes, 1992). Squalene (density  $0.86 \text{ g ml}^{-1}$ ) and DAGE (density  $0.89 \text{ g ml}^{-1}$ ) provide substantially more lift per unit volume than triacylglycerol (TAG) (density  $0.92 \text{ g ml}^{-1}$ ), which is the common lipid storage form in most animals (Heilbron et al., 1926; Lewis, 1970).

Squalene is used commercially in cosmetics, pharmaceuticals, sunscreens, dyes, and lubricants (Buranudeen et al., 1986; Gopakumar et al., 1986). DAGE may aid in reduction of the severity of certain types of cancer, promote formation of

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blood cells, and provide protection against radiation injury (Hallgren et al., 1983). Possible anti-tumor properties of both squalene and DAGE have also been investigated (Ando et al., 1972; Ikekawa et al., 1986). The potential for commercial utilization of shark liver oil as a source of squalene and DAGE, and the possibility of targeting deep-sea sharks in commercial fisheries has recently been discussed (Summers, 1987; Davenport et al., 1989; Summers et al., 1992). In some of the deep-water fisheries of New Zealand and Australia, sharks form as much as 50% of the by-catch, but most of the sharks are discarded and the liver oil is unused (Deprez et al., 1990; Wetherbee, 1996). This is, in part, because little information is available upon which to base selective retention of sharks of a particular species, size, or sex that would yield the greatest quantities of liver oil, squalene or DAGE. Therefore, a rigorous analysis of the composition of liver oil from a variety of sharks would provide information that would contribute to improved utilization of this resource.

As most fish grow, changes occur in their biochemistry, physiology and ecology (Aleev et al., 1963). In deep-sea sharks, such changes may influence their overall buoyancy, which may in turn be reflected in the composition of their liver lipids. Previous workers have suggested that composition of liver oil of individual sharks might vary with species, age, sex, season, feeding success, growth rate, diet, location, depth, and reproductive status (Hickling et al., 1930; Stansby et al., 1967; Hayashi et al., 1981; Bakes et al., 1995; Hernandez-Perez et al., 1997). However, in most studies, only a few individuals have been sampled, or liver oil from multiple specimens was combined, thereby precluding examination of variability in composition of oil between individual sharks (Deprez et al., 1990; Batista and Nunes, 1992; Bakes et al., 1995; Magnussen et al., 1995). The purpose of our study was to compare the lipid composition of the liver oil of different species of deep-sea shark, and a range of individuals within species.

## 2. Materials and methods

### 2.1. Specimens

Sharks were collected with bottom otter trawls

during surveys for smooth oreo *Pseudocyttus maculatus* conducted by the Ministry of Agriculture and Fisheries on the Chatham Rise, off the east coast of New Zealand. Fishing was conducted aboard the RV Tangaroa between 24 October and 9 November 1993 at depths ranging from 740 to 1441 m. The latitude, longitude, depth and time of day were recorded for each trawl. Each shark examined was identified to species, weighed, measured and reproductive status was determined (Wetherbee, 1996). Whole livers were removed, weighed and homogenized with a blender. A subsample of the homogenate was immediately frozen in liquid nitrogen. Upon return to port, the liver samples were transferred to a  $-80^{\circ}\text{C}$  freezer.

For comparative purposes liver samples were also taken from one sandbar shark (*Carcharhinus plumbeus*) and one bluntnose sixgill shark (*Hexanchus griseus*) both caught on longlines set in waters off Oahu, Hawaii, and one spiny dogfish (*Squalus acanthias*) caught in shallow water (10 m) on the Chatham Rise. A small piece of liver was removed from these sharks and stored at  $-80^{\circ}\text{C}$  until analyzed.

### 2.2. Lipid extraction and analysis

Each liver sample was weighed and the oil was extracted using ether in a Soxhlet apparatus for 4 h. Previous trials with the more usual Bligh and Dyer (1959) extraction procedure gave similar yields of neutral lipids but the extraction was considerably more time consuming. The lipid extracted was collected in a pre-weighed glass beaker, transferred to a 4 ml amber vial and stored at  $-80^{\circ}\text{C}$ . For lipid quantification, all samples were made up to a known concentration in chloroform and stored at  $-20^{\circ}\text{C}$  until analysis.

Each liver oil sample was analyzed in duplicate using an Iatroscan MK5 TLC-FID (Iatron Laboratories, Japan) to determine individual lipid classes. A 1 ml sample was applied to silica gel SIII chromarods using disposable micropipets. The chromarods were developed for 25 min in a non-polar solvent system of hexane-diethyl ether (96:4 v/v). After development, the rods were oven-dried at  $100^{\circ}\text{C}$  for 10 min and analyzed in the Iatroscan. The Iatroscan was calibrated with standards for various lipid classes (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid,

squalene and triolein). Areas of peaks corresponding to each lipid class in liver oil samples were quantified (in mg) using DAPA software (Kalamunda, Western Australia) on an IBM-compatible computer. Each lipid class was then expressed as a percentage of the entire amount of lipid in the liver sample. Iatrosan results are generally reproducible to  $\pm 3$ –5% using the MK5 system (Nichols, unpublished results).

### 3. Results

#### 3.1. Specimens

Thirteen species of sharks belonging to four families were sampled: Hexanchidae (one species—*Hexanchus griseus*, a relatively deep-water shark), Squalidae (eight species, seven deep-sea and one shallow water), Scyliorhinidae (three deep-sea species in the genus *Apristurus*, which were classified as species 'A', 'C' and 'E' according to Paulin et al. (1989)), and Carcharhinidae (one species — *Carcharhinus plumbeus*, a shallow water shark) (Table 1). For sharks in the family Squalidae, individuals of both sexes, various sizes, maturity levels, reproductive status, and depths of capture were examined. Three species included in this study (*Etmopterus granulosus*, *Deania calcea*, and

*Centroscymnus crepidater*) form a major part of the by-catch in deep-sea trawl fishing in New Zealand and Australian waters (King and Clark, 1987; Deprez et al., 1990; Wetherbee, 2000).

#### 3.2. Lipid composition

##### 3.2.1. Deep-sea species

Liver oil of the single sixgill (*H. griseus*) specimen contained mostly diacyl glyceryl ether (DAGE), followed by triacylglycerol (TAG) and traces of squalene and wax ester (Table 2). By contrast, liver oil of the deep-sea squalids contained mostly squalene, DAGE and TAG, along with traces of wax ester, sterols, phospholipids and free fatty acids (Table 2). Squalene and DAGE accounted for approximately 90% of the liver oil of most of the deep-sea squalids, and the inverse relationship between squalene and DAGE was nearly linear (Fig. 1). Squalene content was high in the oil of deep-sea squalids with the exception of the Plunket shark (*Scymnodon plunketi*). The highest mean squalene content occurred in the kitefin shark (*Dalatias licha*), (nearly 80% of all of the oil), and squalene averaged between 54 and 70% for the other squalids. Mean DAGE content ranged from 11 to 35% and TAG from 2 to 22% for deep-sea squalids other than

Table 1  
Description of specimens examined in this study

Species	Total length (cm)	Males (N)	Females (N)	Water depth (m)
Family Hexanchidae				
<i>Hexanchus griseus</i>	273.1	1	0	375
Family Squalidae				
<i>Centrophorus squamosus</i>	101.0–127.0	3	5	790–1112
<i>Centroscymnus crepidater</i>	35.6–95.5	9	19	759–1286
<i>Centroscymnus owstoni</i>	42.4–102	1	2	966–1115
<i>Dalatias licha</i>	50.5–142.9	0	5	791–976
<i>Deania calcea</i>	66.7–112.5	0	28	759–1114
<i>Etmopterus granulosus</i>	22.6–78.8	36	48	907–1441
<i>Scymnodon plunketi</i>	87.5–120.6	4	1	820–1056
<i>Squalus acanthias</i>	84.1	0	1	10
Family Scyliorhinidae				
<i>Apristurus</i> 'A'	65.3–70.8	1	1	966–1056
<i>Apristurus</i> 'C'	40.0–82.2	6	10	857–1150
<i>Apristurus</i> 'E'	40.8–86.0	4	8	1013–1441
Family Carcharhinidae				
<i>Carcharhinus plumbeus</i>	172	0	1	60

Table 2  
Lipid composition of liver oil of sharks examined<sup>a</sup>

Species	n	%Oil	Squalene	DAGE	TAG	WE	Other	DAGE:TAG
Family Hexanchidae								
<i>Hexanchus griseus</i>	1	82	1	70	29	<1	<1	2.5:1
Family Squalidae								
<i>Centrophorus squamosus</i>	8	83 ± 3	70 ± 10	11 ± 6	18 ± 5	<1	<1	0.6:1
<i>Centroscymnus crepidater</i>	28	78 ± 7	68 ± 11	22 ± 8	10 ± 8	<1	<1	2.1:1
<i>Centroscymnus owstoni</i>	3	65 ± 5	54 ± 13	23 ± 8	22 ± 7	<1	<1	1.0:1
<i>Dalatias licha</i>	5	79 ± 9	79 ± 13	18 ± 13	2 ± 2	<1	<1	10.0:1
<i>Deania calcea</i>	28	81 ± 6	67 ± 8	24 ± 6	9 ± 5	<1	<1	2.6:1
<i>Etmopterus granulosus</i>	84	72 ± 7	55 ± 12	35 ± 11	9 ± 7	<1	<1	3.9:1
<i>Scymnodon plunketi</i>	5	78 ± 1	<1	89 ± 6	10 ± 6	<1	<1	9.2:1
<i>Squalus acanthias</i>	1	52	0	12	87	0	<1	0.1:1
Family Scyliorhinidae								
<i>Apristurus 'A'</i>	2	43 ± 16	<1	<1	99 ± 0	<1	<1	
<i>Apristurus 'C'</i>	16	82 ± 12	<1	13 ± 26	86 ± 26	<1	<1	0.2:1
<i>Apristurus 'E'</i>	12	70 ± 21	24 ± 13	7 ± 6	65 ± 15	<1	<1	0.1:1
Family Carcharhinidae								
<i>Carcharhinus plumbeus</i>	1	18	0	0	83	1.2	16 <sup>b</sup>	

<sup>a</sup> % Oil, weight of oil as % weight of liver. Values for each lipid class are mean (± S.D.) percent of total liver lipid. WE, wax ester; other, free fatty acid (FFA); phospholipid (PL); sterol (ST); DAGE:TAG; ratio of diacyl glyceryl ether (DAGE) and triacylglycerol (TAG).

<sup>b</sup> FFA = 5, PL = 11, ST < 1.

*S. plunketi* (Table 2). Oil of only one species (*Centrophorus squamosus*) contained more TAG than DAGE, and the ratio of DAGE to TAG ranged from 0.6:1 to 10:1 for the deep-sea squalids (Table 2). Oil of *S. plunketi* differed considerably from that of the other deep-sea squalids, and contained very little squalene (< 1%), but a large amount (89%) of DAGE. Wax ester, phospholipid, free fatty acid and sterol were all present in very small quantities (< 1%) in liver oil of all species of deep-sea sharks examined. There was no indication of elevated wax ester content in species that regularly had orange roughly (*Hoplostethus atlanticus* — a teleost rich in wax esters) in their stomachs.

Liver oil of deep-sea catsharks (*Apristurus* spp.) contained primarily TAG, but the composition varied between species. Species of *Apristurus* were distinguishable from each other based on the lipid composition of their liver oils (Fig. 2). Oil of two individuals of species 'A' was almost entirely TAG (99%). Oil of species 'C' was also primarily TAG (85%), but also contained DAGE (13%), whereas that of species 'E' contained DAGE (7%) and squalene (24%) in addition to TAG (65%) (Table 2). Liver oil from two individuals identified as species 'C' contained much higher levels of

DAGE and much lower levels of TAG in comparison to other specimens classified as species 'C' (Fig. 3).

### 3.2.2. Shallow-water species

Oil composition of the shallow water sharks differed from that of the deep-sea squalids. Oil of the spiny dogfish (*S. acanthias*) contained mostly TAG (87%), some DAGE (12%), but no measurable squalene. The sandbar shark (*C. plumbeus*) also contained primarily TAG (83%), but was the only species that contained a relatively high proportion of free fatty acids and phospholipids (Table 2).

### 3.3. Intraspecific comparisons

Considerable variation was observed in the composition of liver oil within species of the deep-sea squalids. The factor most strongly associated with this variation appeared to be the size of the shark. Shark size and squalene content tended to be inversely related, whereas DAGE content tended to increase as shark size increased (Fig. 4). These changes in lipid composition were gradual over the size range of the species, and there were no significant differences in squalene,

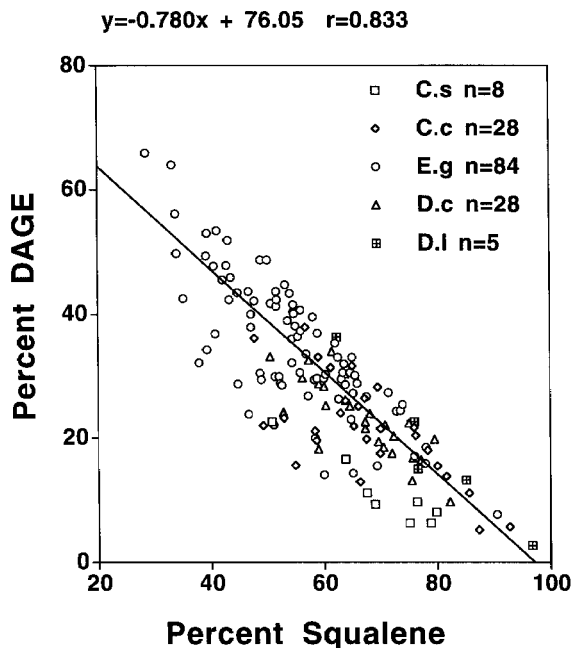


Fig. 1. Percentage of diacyl glyceryl ether (DAGE) versus percentage squalene in liver oil of deep-sea sharks belonging to the family Squalidae. C.s, *Centrophorus squamosus*; C.c, *Centroscymnus crepidater*; E.g, *Etmopterus granulosus*; D.c, *Deania calcea*; D.l, *Dalatias licha*. Each data point represents an individual shark.

DAGE, or TAG content between mature and immature sharks for any of the species investigated (*t*-test,  $P > 0.05$ ). Correlation analysis also showed that there was no significant relationship

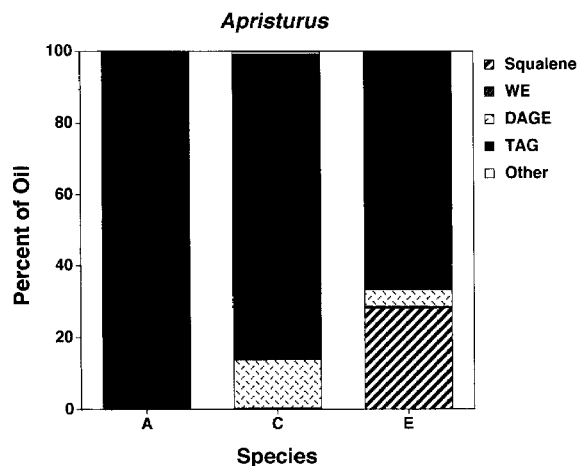


Fig. 2. Lipid composition of liver oil for three species of deep-sea sharks belonging to the genus *Apristurus*. WE, wax ester; DAGE, diacyl glyceryl ether; TAG, triacylglycerol; other, free fatty acids, sterols and phospholipids.

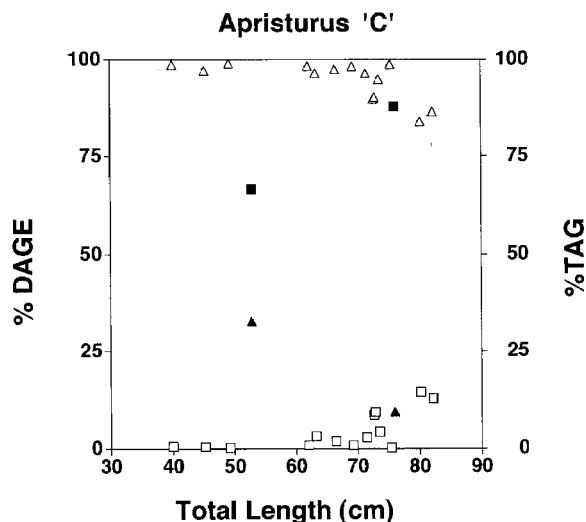


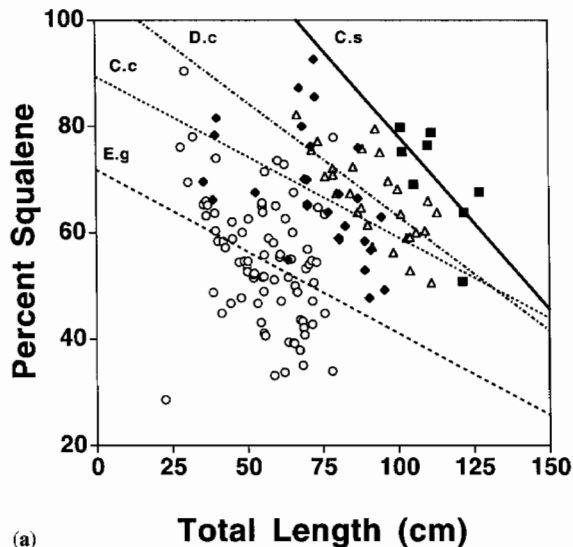
Fig. 3. Percentage of diacyl glyceryl ether (%DAGE; squares) and triacylglycerol (%TAG; triangles) in liver oil of various size individuals of *Apristurus* species 'C'.

between composition of liver oil and depth of capture, or reproductive development (size of ova and weight of ovary and testes) of sharks ( $P > 0.05$ ). However, for the two species with the largest sample sizes, a *t*-test revealed that males had significantly more squalene in their liver oil than females (*E. granulosus*,  $P = 0.006$ ,  $df = 81$ ; *C. crepidater*,  $P = 0.001$ ,  $df = 14$ ). Examination of both mature and immature sharks indicated that the difference between oil composition of the sexes was attributable to differences between the sexes for mature sharks. Mature males of both *E. granulosus* (*t*-test,  $P = 0.026$ ,  $df = 37$ ) and *C. crepidater* ( $P = 0.016$ ,  $df = 7$ ) had more squalene than mature females, whereas squalene content of immature males and immature females did not differ significantly for either *E. granulosus* (*t*-test,  $P = 0.16$ ,  $df = 30$ ) or *C. crepidater* ( $P = 0.49$ ,  $df = 4$ ).

#### 4. Discussion

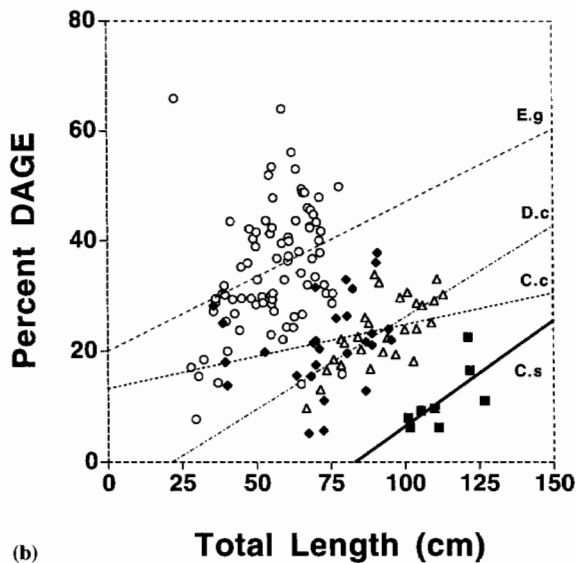
This study compared the liver lipids of an ecologically and evolutionarily diverse array of species. The sixgill shark (*H. griseus*) is a comparatively primitive species, whereas sharks in the genera *Apristurus* and *Carcharhinus* are evolutionarily advanced (Compagno, 1977). Both shallow-water (*S. acanthias*) and deep-sea species in the family Squalidae, and deep-sea (*Apristurus*) and shallow-water (*C. plumbeus*) species in the

- C.s  $y = -0.654x + 143.670$   $r = 0.670$ ,  $p=0.019$
- ◆ C.c  $y = -0.304x + 89.459$   $r = 0.461$ ,  $p=0.019$
- E.g  $y = -0.307x + 71.801$   $r = 0.342$ ,  $p=0.001$
- △ D.c  $y = -0.431x + 106.179$   $r = 0.694$ ,  $p=0.001$



(a)

- C.s  $y = 0.383x - 31.793$   $r = 0.796$ ,  $p=0.018$
- ◆ C.c  $y = 0.116x + 13.286$   $r = 0.247$ ,  $p=0.206$
- E.g  $y = 0.269x + 19.973$   $r = 0.313$ ,  $p=0.004$
- △ D.c  $y = 0.333x - 7.141$   $r = 0.696$ ,  $p=0.001$



(b)

Fig. 4. Percentage of (a) squalene and (b) diacyl glyceryl ether (DAGE) in liver oil of individuals of various size for four species of deep-sea sharks belonging to the family Squalidae. See Fig. 1 for species abbreviations.

order Carcharhiniformes were represented in this study.

#### 4.1. Lipid composition

##### 4.1.1. Deep-sea sharks

The liver oil of the sixgill shark consisted primarily of DAGE (70%), but contained only a trace of squalene. This species is found over a range of depths similar to those inhabited by the squalid sharks examined in this study (Compagno et al., 1984) and our specimen was caught at 375 m. However, the sixgill may attain lengths of nearly 500 cm, whereas the squalid sharks in our study all have maximum sizes of less than 170 cm (Compagno et al., 1984). It is possible that such large deep-sea sharks rely less on squalene for reduction of density than do smaller squalid sharks. For example, liver oil of the Pacific sleeper shark *Somniosus pacificus* (maximum size 700 cm (Compagno et al., 1984) consisted of nearly 50% DAGE, but contained no squalene (Bakes et al., 1995). Since sharks lack a swim bladder, the organ of buoyancy in most teleost fishes, they rely on a combination of hydrostatic lift (provided by low-density matter such as lipid) and hydrodynamic lift (generated by fins and tail as they move through the water). One mechanism for providing additional buoyancy in large deep-water sharks may be an elevated lipid content in skeletal muscle. Muscle taken from a specimen of *S. pacificus* was nearly 25% lipid, compared to 2–3% lipid in muscle of the smaller deep-sea squalids examined in this study (Wetherbee, unpublished results). It would be of interest to examine the lipid content of non-liver-tissue such as skeletal muscle in other large, deep-water sharks.

For each species of deep-sea squalid examined in this study, squalene and DAGE combined to represent roughly 90% of the liver oil. All of these species except *S. plunketi* had substantial levels of both squalene and DAGE. With a squalene content of 1–80% and DAGE content of 11 to 89%, squalene and DAGE were inversely proportional in liver oil of the deep-sea squalids. These high levels of low-density lipids in liver oil presumably reflect their function of increasing buoyancy in these species, because compared to TAG, squalene and DAGE provide 80% and 14% more lift respectively, per unit volume (Corner et al., 1969; Malins and Baron, 1970). Squalene provides so much more lift than TAG that, even consider-

ing the energetic cost of synthesis, squalene is a more economical way of increasing buoyancy (Corner et al., 1969). Other studies have shown that squalene content influences the overall density of the liver oil, as well as the density of the whole liver (Higashi et al., 1953b,c; Batista and Nunes, 1992). Thus, the amount of squalene in liver oil has a direct bearing on the amount of hydrostatic lift provided by the liver.

All except one species of the deep-sea squalids contained more DAGE than TAG, and the DAGE to TAG ratio was high for several species. Because DAGE and TAG are not usually separated in the process of DAGE extraction and refinement for health aid products, those species with high DAGE to TAG ratios are most desirable for this industry. Given the life-history characteristics of most sharks it is doubtful that a fishery specifically targeting deep-sea sharks would be sustainable (Wetherbee, 1996), however, those species with particularly valuable liver oil could be utilized from the by-catch of the fisheries rather than discarded.

The relative proportions of squalene, DAGE and TAG in liver oil of each species in the family Squalidae in our study were similar to the lipid composition reported for the same species in other studies (Heilbron et al., 1926; Higashi et al., 1954b; Summers, 1987; Deprez et al., 1990; Batista and Nunes, 1992; Bakes et al., 1995; Borch-Jensen et al., 1995). However, lipid composition within each species was more variable in our study than in previous studies and for most species the maximum percentages of squalene and DAGE in an individual exceeded those previously reported. These differences may be a result of the larger number of specimens examined in our study than in previous studies. The highest values of squalene as a percentage of liver oil in an individual shark in our study were 93% for *C. crepidater*, 90% for *E. granulosus*, 85% for *D. licha*, 82% for *D. calcea*, and 80% for *C. squamosus*, which exceed the highest values found in previous studies (Tsujimoto et al., 1916; Heilbron et al., 1926; Heller et al., 1957; Hayashi et al., 1981; Deprez et al., 1990; Batista and Nunes, 1992; Bakes et al., 1995; Borch-Jensen et al., 1995). Similarly, the highest values we observed for DAGE as a percentage of the liver oil (97% for *S. plunketi*, 66% for *E. granulosus*, 36% for *D. licha*, 34% for *D. calcea*, and 32% for *Centroscyrmnus owstoni*) were all higher

than those reported in previous studies (Hayashi et al., 1981; Deprez et al., 1990; Bakes et al., 1995; Borch-Jensen et al., 1995).

The lipid composition of *S. plunketi* is of particular interest because it differs so markedly from other closely related species. Phylogenetically *S. plunketi* is closely related to sharks in the genus *Centroscyrmnus*, and until recently it was considered *Centroscyrmnus plunketi* (Compagno et al., 1984; Yano et al., 1984). However, the liver oil of *S. plunketi* contained much less squalene and much more DAGE than that of the *Centroscyrmnus* species. This classification appears to be valid on a biochemical basis as well because species in the genus *Scymnodon* apparently share the characteristic of containing low levels of squalene in their liver oil. Batista and Nunes (1992) found that liver oil of *Scymnodon ringens* contained less than 1% squalene, and Higashi et al. (1954c) found that squalene accounted for as little as 1% of the liver oil for some individuals of *Scymnodon squamulosus* (although *S. squamulosus* has been grouped in the genus *Zameus* by some workers (see Taniuchi and Garrick, 1986; Wetherbee and Crow, 1996). Deprez et al. (1990) reported a squalene content of 1% for liver oil of an individual identified as *Centrophorus squamosus*, but squalene content ranged from 50 to 80% in our specimens of *C. squamosus*, and between 60 and 80% in other studies (Heilbron et al., 1926; Summers, 1987; Batista and Nunes, 1992; Borch-Jensen et al., 1995; Hernandez-Perez et al., 1997). It is likely that this individual identified as *C. squamosus* by Deprez et al. (1990) was actually *S. plunketi*, which is similar in appearance.

Species in the genus *Apristurus* differ from deep-sea squalids in that they do not store vast amounts of low-density lipids in their liver oil. Although the liver oil of *Apristurus* 'E' contained DAGE and squalene (as high as 46% in one individual), combined levels of these lipids were considerably lower than the 90% levels typical of the deep-sea squalids in this study. Squalene is generally associated with sharks in the family Squalidae, however, squalene has been found in liver oil of species belonging to the families Chlamydoselachidae, Odontaspidae, Pseudocarchariidae, Cetorhinidae, Carcharhinidae (Tsujimoto et al., 1920; Abe et al., 1968, 1969; Sargent

et al., 1973) and now Scyliorhinidae (in *Apristurus* 'E').

The *Apristurus* spp. collected in our study are difficult to distinguish from each other without close visual inspection, however, these species differed considerably in the composition of their liver oils, and species 'E' can easily be separated from the others on this basis. The *Apristurus* spp. examined also differed in the morphology of their livers. Species 'E' had a two-lobed, gray-brown liver, typical of many species of shark, whereas species 'A' and 'C' both had essentially one-lobed livers that were more yellow-green. The liver lipid of our two specimens of species 'A' was almost entirely TAG-typical of shallow water sharks. Specimens of species 'A' also had smaller livers and proportionately less oil in their livers than species 'C' or 'E'. The liver oils contained moderate concentrations of DAGE in species 'C' and 'E' and considerable concentrations of squalene in some specimens of species 'E'. Based on liver characteristics species 'A' would be expected to be less buoyant than species 'C' and 'E'. These differences may be related to depth inhabited by each species because preliminary data indicate that species 'E' is found at greater depths than species 'A' and 'C'.

Two individuals in our study identified as *Apristurus* species 'C' had elevated DAGE content in liver oil compared to other sharks placed in this group. These differences may indicate variation within the species, or that these two individuals were not species 'C'. Both specimens showed a rosette pattern of ampullae of Lorenzini on the dorsal surface of the snout, which was not noted for the other specimens and suggests that these two were of a separate species. *Apristurus* were separated following the key of Paulin et al. (1989), but the classification scheme for these undescribed species may need further refinement. Further biochemical studies may aid in separation of these closely related species. For example, Higashi et al. (1954a) separated specimens grouped as *Centrophorus* spp. into two species based on two distinct patterns of liver oil composition.

#### 4.1.2. Shallow-water sharks

Liver oil of our specimen of the relatively shallow-water squalid, *S. acanthias* was dominated by TAG (87%), but also contained a modest amount of DAGE (12%). Other workers have reported levels of between 45 and 61% for TAG, and 11

and 45% for DAGE for this species (Kayama et al., 1971; Sargent et al., 1971; Hayashi et al., 1983). Kayama et al. (1969) also found traces of squalene in liver oil of *S. acanthias*, although we detected none. This species has a very wide geographical distribution, and though it is generally found in shallow water, it has been reported as deep as 900 m (Compagno et al., 1984). Therefore, it is not surprising that the liver oil of this squalid shark varies in composition among individuals, and contains low-density lipids.

The sandbar shark was the only species examined in which substantial proportions of free fatty acids and phospholipids were found in liver oil. In this shark, a relatively small fraction of the liver consisted of oil (18%, compared to 70–80% for the deep-sea species), so the actual quantity of these lipid classes is relatively small, but they contribute a large proportion of the total amount of lipid in the liver oil.

#### 4.2. Intraspecific comparisons

The finding that liver oil of mature male, deep-sea squalid sharks contained significantly more squalene than that of mature females is likely related to differences in reproductive characters between the sexes. Because squalene has a key role in sterol biosynthesis (Bone et al., 1988), it is possible that differences in squalene concentrations in liver oil between the sexes reflect differential regulation of steroid hormone levels between mature males and females. Mature females of the deep-sea squalids also carry large eggs (sometimes in excess of 5 cm diameter), with yolk that contains a fair amount of oil (20–40%), which has a fairly high squalene content (9–32%) (Higashi et al., 1953a, Wetherbee, unpublished results). These low-density, buoyant eggs are a source of hydrostatic lift not found in males or in immature females, and mature females may achieve neutral buoyancy even though lower levels of squalene are stored in liver oil. Higashi et al. (1954b) also found that the liver oil of male *C. owstoni* contained more squalene than the oil of females, but other studies have detected no differences in the lipid composition of males and females (Heller et al., 1957; Higashi et al., 1953c; Hayashi et al., 1983). In our study, no relationship was obvious between size of eggs (maximum ova diameter) and squalene content of liver oil of female sharks.

Comparison of the density of the liver-free body of mature females with that of male and immature sharks may reveal whether mature females derive more hydrostatic lift from non-liver tissue such as eggs.

The observed increase in DAGE and concomitant decrease in squalene with increased shark size, were rather gradual and continuous changes, not the kind of rapid alterations that might be associated with events such as the onset of maturity. It is unclear why lipid composition changes with increased size of shark, but these changes may be related to limits in the rate of synthesis of squalene or DAGE, or the role of squalene in biosynthesis of steroid hormones in mature sharks of both sexes (Bloch, 1965). Although squalene provides more lift per unit volume than DAGE, the synthesis of squalene requires more energy, and once formed squalene is less labile than DAGE (Corner et al., 1969). Unlike DAGE, squalene cannot be catabolized for metabolic energy, and the only known route of synthesis of squalene is in the pathway for synthesis of cholesterol and bile salts (Sargent et al., 1973).

Corner et al. (1969) hypothesized that fine-scale regulation of buoyancy would more likely depend on lipids other than squalene, and that selective metabolism of DAGE would provide a mechanism for delicate maintenance of neutral buoyancy in deep-sea sharks. The free fatty acids of DAGE and TAG are very similar and possibly interchangeable from one lipid class to the other (Hayashi et al., 1981). Malins and Baron (1970) described just such a rapid (50 h) accumulation of significant amounts of DAGE in *S. acanthias* when weights were attached to the fins of sharks held in tanks.

There appears to be strong selective pressure for neutral buoyancy in deep-sea sharks and the variation in lipid composition of liver oil for sharks of different species, sexes, and sizes may reflect fine-scale control of buoyancy of whole sharks, in that neutral buoyancy is attained despite changes in the biochemical make-up of an individual as it grows and matures. Research examining the relationship between composition of the liver oil and such factors as liver size, quantity of oil in the liver, and density of the individual shark would provide further insight into the role of the liver in the overall buoyancy of deep-sea sharks.

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