

## COMMENTARY

# Does the physiology of chondrichthyan fishes constrain their distribution in the deep sea?

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## ABSTRACT

The deep sea is the largest ecosystem on Earth but organisms living there must contend with high pressure, low temperature, darkness and scarce food. Chondrichthyan fishes (sharks and their relatives) are important consumers in most marine ecosystems but are uncommon deeper than 3000 m and exceedingly rare, or quite possibly absent, from the vast abyss (depths >4000 m). By contrast, teleost (bony) fishes are commonly found to depths of ~8400 m. Why chondrichthyans are scarce at abyssal depths is a major biogeographical puzzle. Here, after outlining the depth-related physiological trends among chondrichthyans, we discuss several existing and new hypotheses that implicate unique physiological and biochemical characteristics of chondrichthyans as potential constraints on their depth distribution. We highlight three major, and not mutually exclusive, working hypotheses: (1) the urea-based osmoregulatory strategy of chondrichthyans might conflict with the interactive effects of low temperature and high pressure on protein and membrane function at great depth; (2) the reliance on lipid accumulation for buoyancy in chondrichthyans has a unique energetic cost, which might increasingly limit growth and reproductive output as food availability decreases with depth; (3) their osmoregulatory strategy may make chondrichthyans unusually nitrogen limited, a potential liability in the food-poor abyss. These hypotheses acting in concert could help to explain the scarcity of chondrichthyans at great depths: the mechanisms of the first hypothesis may place an absolute, pressure-related depth limit on physiological function, while the mechanisms of the second and third hypotheses may limit depth distribution by constraining performance in the oligotrophic abyss, in ways that preclude the establishment of viable populations or lead to competitive exclusion by teleosts.

**KEY WORDS:** Elasmobranch, Shark, Skate, Ray, Chimaera, Biogeography, Geographic range limit, Metabolism, Osmoregulation, Buoyancy, Energetics, Hydrostatic pressure, Nitrogen limitation

## Introduction

Sharks and their relatives (class Chondrichthyes) are an ancient, ecologically important group of fishes with a broad distribution across marine and freshwater habitats worldwide (Compagno, 1990). A conspicuous exception to this pervasiveness, however, is the deep sea – the largest habitat on Earth – yet one that poses significant environmental challenges for animal life (Box 1). Although chondrichthyans contribute significantly to the biomass in the

shallower regions of the deep sea (200–1500 m), they are uncommon deeper than 3000 m and are exceedingly rare, or perhaps absent, in the vast abyssal zone (>4000 m) (Priede et al., 2006). By contrast, teleosts have successfully colonized the deep sea to at least 8370 m (Priede et al., 2006; Jamieson and Yancey, 2012). While the logistical challenges of surveying the deep-sea biota (Merrett and Haedrich, 1997) preclude a definitive statement that chondrichthyans are absent at abyssal depths, decades of concerted efforts have failed to detect any reliable chondrichthyan presence in abyssal waters (Priede et al., 2006; Jamieson, 2015). Interestingly, the available data also clearly demonstrate that chondrichthyan species diversity declines with depth more steeply than for the other major classes of fishes, including teleosts (Priede et al., 2006; Priede and Froese, 2013). The factors limiting the depth distribution of chondrichthyans remain unresolved and their apparent absence from the abyss continues to be a curious biogeographical puzzle, the solution to which is particularly important because of their ecological significance and sensitivity to fisheries (Kyne and Simpfendorfer, 2010).

Here, we explore hypotheses that implicate physiological attributes of chondrichthyans as potential constraints on abyssal colonization. Physiological characteristics and capacities are thought to be important determinants of biogeographic patterns among animals, including those in the deep sea (Hochachka and Somero, 2002; Somero, 1998). Our approach involves comparisons of key physiological attributes between deep-sea chondrichthyans and deep-sea teleosts (Fig. 1; Box 2). In particular, we draw comparisons with the macrourids (Fig. 1D) and other large teleosts that successfully inhabit the abyss while sharing many lifestyle and niche characteristics with chondrichthyans, such as slow growth, large size and tertiary trophic level consumption ranging from carrion to megafauna to benthic macrofauna (Gartner et al., 1997; Kyne and Simpfendorfer, 2010; Priede et al., 2006). The scarcity of chondrichthyans in an environment where ecologically similar teleosts thrive (>3000 m), combined with the co-existence of these competing groups in the shallower deep sea (200–1500 m), imply that biological features unique to chondrichthyans could be constraints upon their competitiveness and adaptive potential in the abyss.

## Chondrichthyans successfully inhabit shallower regions of the deep sea

The prevalence of chondrichthyans along continental slopes indicates successful adaptation to shallower deep-sea zones, thus emphasizing their rarity below 3000 m. How has the challenging deep-sea environment shaped key features of chondrichthyan physiology?

## Locomotion, metabolism and buoyancy

Locomotory costs, buoyancy compensation and metabolic rate are intimately linked in fishes (Alexander, 1990; Pelster, 1997). Fishes maintain position by a combination of hydrodynamic lift

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**Glossary**

|                       |  |
|-----------------------|--|
| Abyssal zone or abyss | the region of the ocean deeper than 4000 m   |
| Aphotic zone          | the dark region of the ocean where little to no sunlight penetrates (deeper than 200–1000 m depending on local conditions)                                   |
| Bathyal zone          | the region of the ocean at depth of 1000–4000 m  |
| Buoyancy              | an upward force exerted by water that counteracts the weight of an underwater object   |
| Chondrichthyan        | a member of the Chondrichthyes, a vertebrate class including the subclasses Elasmobranchii (sharks, skates and rays) and Holocephali (chimaeras)             |
| Demersal              | living on or near the seafloor   |
| Elasmobranch          | a member of the subclass Elasmobranchii: the sharks, skates, and rays  |
| Hepatosomatic index   | a measure of relative liver size, specifically the proportion of total body mass that is attributable to the liver (i.e. liver mass/total body mass)         |
| Hydrodynamic lift     | buoyancy imparted by water flowing over an animal's surface (e.g. the body or fins)  |
| Hydrostatic lift      | buoyancy imparted by compounds lighter (less dense) than water (e.g. most lipids, all gases)   |
| Macrofauna            | small animal life, typically living on the seafloor (e.g. polychaete worms, amphipod crustaceans)  |
| Macrourid             | a member of the teleost family Macrouridae, commonly named grenadiers or rat-tail fishes, which are among the most abundant deep-sea fishes                  |
| Megafauna             | large, often highly mobile, animal life (e.g. decapod crustaceans, cephalopods, fishes, also carrion)  |
| Oligotrophy           | an environmental condition of low nutrient and/or food availability  |
| Photic zone           | the sunlit region of the ocean, from the surface to the depth at which light intensity is 1% of the surface (up to 200–1000 m depending on local conditions) |
| Teleost               | a member of the actinopterygian infraclass Teleostei, commonly named the bony fishes   |
| Ureotelism            | mode of excretion where urea is the predominant molecule excreted to maintain nitrogen balance   |

and hydrostatic lift, derived from muscle-powered body movement and the accumulation of low-density molecules (e.g. gases in the teleostean swim bladder, lipids), respectively. Hydrostatic lift is more economical than hydrodynamic lift at slower swimming speeds (Alexander, 1990), so the hydrostatic strategy is preferred among fishes with a slower pace of life

(e.g. lower activity levels, routine metabolic rates), such as most deep-sea species.

Measurement of oxygen consumption rate, a key proxy of metabolic rate, is difficult and rare for deep-sea fishes. Consequently, the activities of key enzymes of energy metabolism, especially in locomotory muscle, have been valuable for assessing metabolic capacities among deep-sea fishes because these enzyme activities typically correlate with metabolic rate in teleosts (Drazen and Seibel, 2007). Muscle enzyme activities decline with depth among teleost fishes (see below), which, combined with limited measurements of oxygen consumption rate, imply declines in metabolic rate with depth. Although complicated by factors such as feeding mode, locomotory mode and size scaling, the decline appears largely to occur over the transition from the photic to aphotic zones (0–1000 m). This has led to the visual interaction hypothesis (VIH), which posits that the depth-related decline in metabolic rate results from relaxed selection for locomotory capacity to capture prey or evade predators in an increasingly dark environment (Childress, 1995; Drazen and Seibel, 2007; Seibel and Drazen, 2007; Drazen et al., 2015). In the aphotic zone, where the transition to darkness is complete but oligotrophy continues to increase with depth, it has been argued also that a low basal metabolic rate, combined with associated low capacities for resource utilization (e.g. growth) and reduced locomotory activity, might be beneficial for persistence under the food-poor conditions (Cohen, 1977; Collins et al., 1999, 2005; Drazen, 2002; Bailey et al., 2003, 2005; Priede et al., 2003; Drazen and Yeh, 2012).

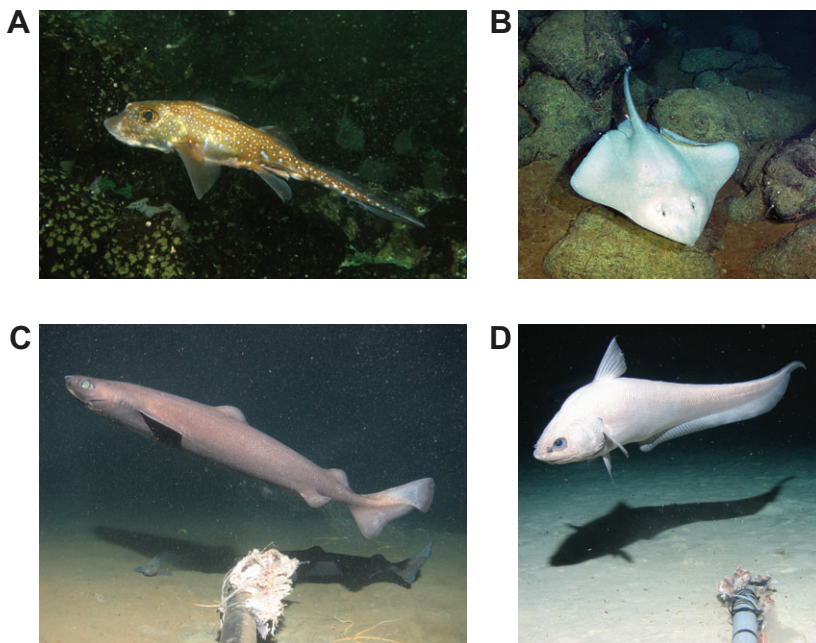
**Muscle enzymes as a proxy for metabolic rate**

In general, locomotory muscle in fishes is spatially separated into the aerobic ‘red’ muscle, used for slow sustained swimming, and the anaerobic ‘white’ muscle, recruited for burst swimming. In white muscle of teleosts, the activities of enzymes related to glycolytic capacity decrease in correlation with the depth-related decline in whole-animal oxygen consumption rate (Drazen and Seibel, 2007). Glycolytic enzyme activities also decrease with depth in white muscle of chondrichthyans, implying similarly low metabolic rates as deep-sea teleosts (Condon et al., 2012; Drazen and Seibel, 2007; Treberg et al., 2003). By contrast, the aerobic enzyme activities in both types of muscles decline with depth in teleosts but not in chondrichthyans (Condon et al., 2012; Dickson et al., 1993; Drazen et al., 2013; Drazen and Seibel, 2007; Speers-Roesch et al., 2006; Treberg et al., 2003). Other red muscle aerobic/oxidative properties are comparable between deep-sea and shallow demersal chondrichthyans (Bernal et al., 2003; Condon

**Box 1. Environmental challenges of the deep sea**

The deep sea can be broadly defined as depths >200 m, incorporating the midwater (200–1000 m), bathyal (1000–4000 m), abyssal (>4000 m) and hadal (trenches >6000 m) zones. The deep sea is characterized by five major environmental characteristics that pose challenges to the persistence of animal life (Herring, 2002).

| Challenges   | Environmental characteristics  |
|--|--|
| Can complicate sensing of food, predators and mates                                      | <ul style="list-style-type: none"> <li>⎧ Vast (the deep sea deeper than 1000 m comprises ~1 billion km<sup>3</sup>, or 88% of the world's ocean).</li> <li>⎧ Dark (the photic zone typically ends at 200 m but some light may penetrate to 1000 m).</li> </ul>   |
| Can interfere with physiological function and limit physiological or ecological capacity | <ul style="list-style-type: none"> <li>⎧ Cold (4–20°C at 200–1000 m depending on latitude and thermocline depth; typically 1–4°C below 1000 m).</li> <li>⎧ High hydrostatic pressure (increases at 1 atm per 10 m).</li> <li>⎧ Oligotrophic (nutrient availability generally declines markedly with depth).</li> </ul> |



**Fig. 1. Representative species belonging to three chondrichthyan groups common in the deep sea and a teleost group common in the deep sea.** (A) A chimaeroid, the spotted ratfish (*Hydrolagus colliei*). Northeast Pacific Ocean (Barkley Sound, Canada), 30 m. Species depth range: 0–971 m. Photo: Ben Speers-Roesch. (B) A rajoid, the deep-sea skate (*Bathyraja abyssicola*). Northeast Pacific Ocean (Endeavour Ridge), 2157 m. Species depth range: 362–2906 m. Photo: Ocean Networks Canada. (C) A squaloid shark, the Portuguese dogfish (*Centroscymnus coelolepis*). Southeast Atlantic Ocean (attracted to a baited lander offshore of Angola), 1598 m. Species depth range: 128–3675 m. Photo: Alan Jamieson, Oceanlab, University of Aberdeen. (D) A macrourid teleost, the abyssal grenadier (*Coryphaenoides armatus*). Northeast Atlantic Ocean (attracted to a baited lander on the Porcupine Abyssal Plain), 4800 m. Species depth range: 282–5180 m. Photo: Alan Jamieson, Oceanlab, University of Aberdeen. Photos are not to scale.

et al., 2012; Dickson et al., 1993; Kryvi et al., 1981; Treberg et al., 2003). Deep-sea chondrichthyans and teleosts swim at similar, slow speeds ( $\sim 0.1\text{--}0.3\text{ m s}^{-1}$ ) (Bagley et al., 1994; Carey and Clark, 1995; Watanabe et al., 2012), so the retention in deep-sea chondrichthyans of greater metabolic capacity in red muscle and aerobic capacity in white muscle suggests a greater reliance upon sustained swimming compared with deep-sea teleosts.

**Buoyancy through hydrostatic lift: lipid accumulation in liver**

Many deep-sea fishes have evolved strategies to achieve neutral buoyancy via hydrostatic lift, including watery tissues, low-density fluid-filled spaces, reduced skeletal systems, swim bladder enhancements (in some teleost groups, e.g. macrourids) to maintain inflation at high hydrostatic pressure and/or sequestration of low-density compounds (e.g. lipids) (Herring, 2002; Pelster, 1997).

Chondrichthyans lack a swim bladder but typically have a large, lipid-rich liver that serves as a hydrostatic buoyancy organ and as a lipid fuel depot (Table 1). The low molal density of chondrichthyan osmolytes provides additional hydrostatic lift (Withers et al., 1994). The accumulation of lower-density trimethylamine N-oxide (TMAO) in the place of urea in deep-sea chondrichthyans (see below) might enhance their buoyancy, but the benefit is small (<10%) relative to liver lift (Tables S1, S2). Compared with shallow species where hydrodynamic and hydrostatic lifts combine to confer buoyancy, it has been commonly accepted that deep-sea chondrichthyans have enlarged livers with higher lipid contents dominated by the low-density squalene and diacylglyceryl ether (DAGE), allowing them to approach neutral buoyancy by hydrostatic lift alone (Bone and Roberts, 1969; Corner et al., 1969; Nevenzel, 1989; Pethybridge et al., 2010; Wetherbee and Nichols, 2000). However, the patterns of lipid accumulation among deep-sea chondrichthyans are more complex and there is no available empirical evidence that benthic deep-sea species, such as skates, are neutrally buoyant.

**Depth-related trends in liver size and lipid accumulation**

Among chondrichthyans, the liver mass relative to whole body mass (hepatosomatic index, HSI) and liver lipid content both increase with median depth of occurrence (MDO) until approximately 1000 m, beyond which there is a plateau and possibly a decrease at the deepest depths (Fig. 2A,C). The increase with depth occurs within all chondrichthyan orders with a wide interspecies depth range, but squaloid sharks drive the depth-related maximum because the trends are linear among carcharhinoid sharks and batoids (Fig. 2B,D). The HSI and liver lipid contents of the deepest batoids and carcharhinoids do not exceed the upper limit seen in squaloids, suggesting a universal

**Box 2. Summary of comparative physiological and biochemical patterns in chondrichthyans and teleosts with increasing depth**

Trends in deep-sea versus shallow species

|                                | Chondrichthyans | Teleosts       |
|--------------------------------|-----------------|----------------|
| Whole animal                   |                 |                |
| Metabolic rate                 | ↓ <sup>a</sup>  | ↓              |
| Growth rate                    | ↓               | ↓              |
| Muscle function and metabolism |                 |                |
| Protein content                | ↔               | ↓ <sup>b</sup> |
| WM metabolic capacity          |                 |                |
| Glycolytic                     | ↓               | ↓ <sup>b</sup> |
| Aerobic                        | ↔               | ↓ <sup>b</sup> |
| RM metabolic capacity          | ↔               | ↓              |
| Liver                          |                 |                |
| HSI (% body mass)              | ↑               | ↔ <sup>c</sup> |
| Liver lipid (% of organ mass)  | ↑               | ↔ <sup>c</sup> |
| Protein stabilization          |                 |                |
| Extrinsic (organic osmolytes)  | ↑               | ↑              |
| Intrinsic (protein structure)  | ?               | ↑              |

<sup>a</sup>Presumed based on white muscle enzyme activities. <sup>b</sup>Exceptions occur with more active species. <sup>c</sup>May increase with depth in some taxa such as Gadiformes (i.e. macrourids); ?, pattern unknown (no data). See text for relevant citations. WM, white muscle; RM, red muscle; HSI, hepatosomatic index.

maximum for HSI and lipid accumulation in deep-sea chondrichthyans. This maximum simply might reflect the attainment of neutral buoyancy, with further increases in HSI or liver lipid content being unnecessary. Indeed, chondrichthyans approach neutral buoyancy when HSI reaches 15–20% and liver lipid content is 50–80%, at least in species with abundant squalene or DAGE (Bone and Roberts, 1969). These thresholds match the values of the depth-related apexes of HSI and liver lipid content among chondrichthyans (Fig. 2A,B). Extrahepatic density reductions might also occur in some groups (e.g. chimaeroids) (Corner et al., 1969). Unlike chondrichthyans, general depth-related trends for HSI or lipid content are absent in teleosts (Drazen, 2007). Certain common abyssal teleosts, however, have large, lipid-rich livers, but these primarily serve as a fuel depot (e.g. *Coryphaenoides armatus* and *Bathysaurus ferox*: HSI adult means, ~9–16%; liver lipid content, ~45% and ~70%, respectively; Drazen, 2007; Marshall and Merrett, 1977; Sulak et al., 1985).

Depth-related trends in the major lipid classes found in chondrichthyan livers show limited influence of species-specific MDO within orders, but highlight phylogenetic influences that, in certain cases, might relate to selection for neutral buoyancy during historical deep-sea adaptation of higher taxa (family or order; Fig. 3). For example, substantial hepatic accumulation of squalene is almost exclusive to members of five speciose deep-sea squaloid families, which all evolved from the ancestral family Squalidae (Klug and Kriwet, 2010), a shallow group lacking squalene accumulation (Fig. 3B; Table S3). Although squalene is optimal for buoyancy (Table 1), its accumulation is not required for deep-sea existence in chondrichthyans given its sporadic occurrence in other deep-sea orders (Fig. 3, Table S3).

Among certain deep-sea chondrichthyan orders lacking squalene, namely chimaeroids and hexanchoids (and a few squaloid species), DAGE accumulates to high levels (>40%) instead, possibly to confer neutral buoyancy (Fig. 3, Table S3). In chondrichthyan orders with lower DAGE levels, including most squaloids and carcharhinoids, DAGE might serve primarily in fine-scale buoyancy adjustment (Malins and Barone, 1970). Aside from the prevalence of squalene in deep-sea squaloids, squalene and DAGE contents are both poorly related to MDO, possibly as a result of phylogenetic influences or because in the accumulating species these lipids are uniformly retained up to but not exceeding the level required for neutral buoyancy.

Accumulation of squalene or DAGE strictly for buoyancy in deep-sea chondrichthyans may come at the expense of fuel storage (Fig. 3A,B). For example, in contrast to squalene and bulk DAGE accumulators, the TAG-rich livers of shallow-water squalids and virtually all carcharhinoids have low DAGE and no squalene

(Table S3; Fig. 3B). Indeed, squalene and TAG contents are inversely related among squaloids (Fig. S1).

Emerging from these data is a scenario illustrating how phylogeny, deep-sea adaptation and the multi-functional roles of lipids have led to several strategies of liver lipid accumulation among chondrichthyans. Shallow-water chondrichthyans (MDO<500 m), whose higher metabolic capacities allow them to rely more on hydrodynamic lift for buoyancy, do not greatly accumulate DAGE or squalene for buoyancy, allowing them to sequester more TAG as an energy store. Deep-sea carcharhinoids retain the TAG-rich lipid strategy of their shallow relatives despite penetrating to considerable depths. Conversely, buoyancy-related lipids dominate in deep-sea squaloid families and the largely deep-living chimaeroids and hexanchoids, with the trade-off of less lipid accumulation as metabolic substrate (e.g. TAG, DAGE). Given that chimaeroids and squaloids occur much deeper than carcharhinoids (see Fig. 2), selection for a dedicated and more efficient method of achieving neutral buoyancy (i.e. bulk use of low density squalene and/or DAGE) during deep-sea adaptation in chondrichthyans may have outweighed the benefits of storing lipid fuel in an oligotrophic environment. Unfortunately, the lipid accumulation strategy is unknown for deep-sea skates, which rival squaloids as the deepest chondrichthyans. In comparison, deep-sea teleosts such as macrourids store liver lipids as metabolic fuel rather than for buoyancy, so they have TAG-rich livers with negligible DAGE or squalene (Drazen, 2007; Drazen et al., 2009).

#### Osmoregulation

Marine chondrichthyans are ion-regulating osmoconformers. Urea is the primary osmolyte accumulated in shallow marine chondrichthyans, followed by trimethylamine oxide (TMAO) and glycine-betaine, methylamines that probably counteract the perturbation of macromolecular structure and function by high urea levels (Yancey, 2005). Hydrostatic pressure can also destabilize proteins (Yancey, 2005). Among teleosts, which have low urea levels, tissue levels of stabilizing methylamines rise with increasing depth (to ~380 mmol kg<sup>-1</sup> at 7000 m) (Yancey et al., 2014), possibly to counteract increasing pressure (Samerotte et al., 2007). A similar depth-related increase of muscle TMAO, and to a lesser extent glycine-betaine, occurs among chondrichthyans but only up to a MDO of ~1000–1500 m, after which a plateau at ~300 mmol kg<sup>-1</sup> appears to occur (Fig. 4A). Interestingly, urea content in muscle declines linearly with MDO among chondrichthyans (Fig. 4B). This reciprocal accumulation of stabilizing osmolytes with decreasing levels of urea is consistent with a strategy of recruiting the stabilizing effects of trimethylamines to balance the destabilizing effects of pressure and urea on proteins (Laxson et al., 2011).

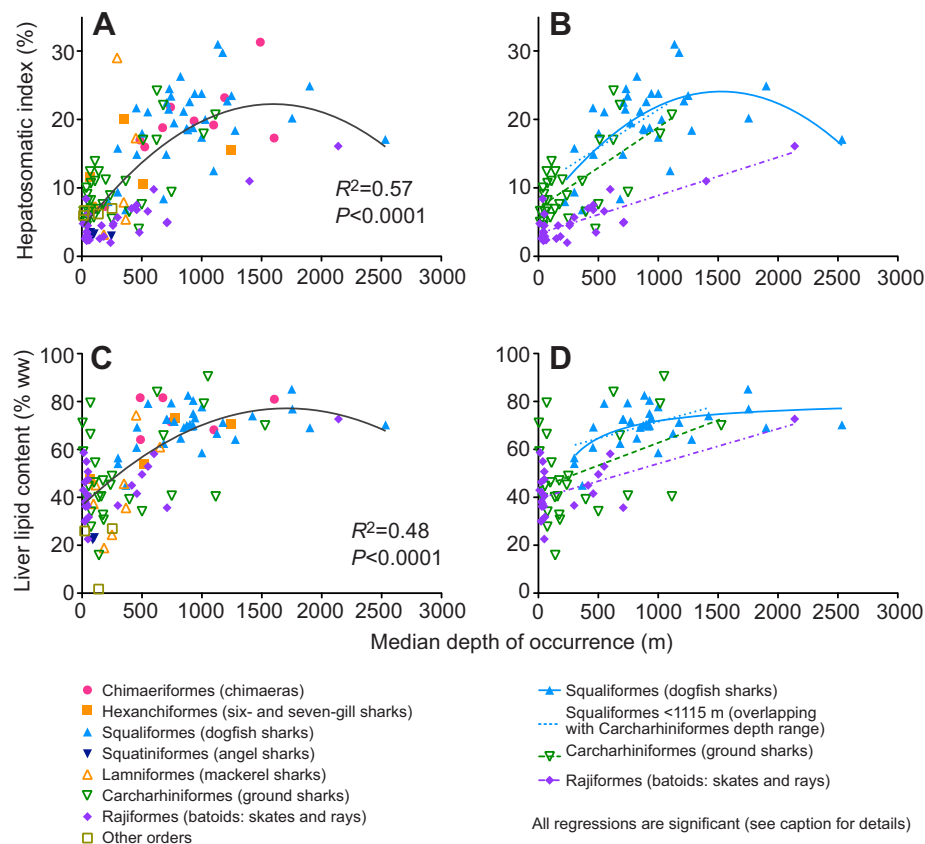
**Table 1. Major lipid classes accumulated in livers of chondrichthyans and their functional roles**

| Lipid                           | Density <sup>a</sup><br>(g l <sup>-1</sup> ) | Lift <sup>a</sup> (g per ml<br>lipid) | Functional role <sup>b</sup>   |
|---------------------------------|--|---------------------------------------|--|
| Triacylglycerols (TAG)          | 0.93   | +0.095                                | Primarily a metabolic fuel (released as free fatty acids or converted and released as ketone bodies) |
| Diacylglycerol ethers<br>(DAGE) | 0.89   | +0.135                                | Buoyancy control as well as metabolic fuel (but used at lower rates than TAG)                        |
| Squalene                        | 0.86   | +0.165                                | Primarily buoyancy; a relatively metabolically inert intermediate of cholesterol synthesis           |

The lipids accumulated are lighter than water and energetically rich so can serve roles in buoyancy or as metabolic fuel, respectively.

<sup>a</sup>Values for density (at 1 atm, 20°C) and lift (versus seawater of density 1.025 g ml<sup>-1</sup>) are taken from Phleger (1998).

<sup>b</sup>See Ballantyne (1997); Nevenzel (1989); Speers-Roesch and Treberg (2010).



**Fig. 2. Hepatosomatic index (HSI, % of body mass) and liver lipid content (% of liver wet mass) as a function of median depth of occurrence among chondrichthyans.** HSI and liver lipid in chondrichthyan fishes (A,C) or within each of the three most speciose and successful deep-sea chondrichthyan orders (B,D). Data points represent individual species. Median depth of occurrence was used to approximate the depths at which species are most commonly found. Our analysis includes species with MDO overlapping or approaching the deepest MDO known for chondrichthyan fishes, which are for species in the most successful chondrichthyan groups in the deep sea: carcharhinoids (~1500 m, *Apristurus* spp.), chimaeroids (1925 m, *Hydrolagus pallidus*), squaloids (2500 m, *Etmopterus princeps*) and batoids (2400 m, *Rajella bigelowi*; 2700 m, *Bathyraja pallida*) (Kyne and Simpfendorfer, 2010). Akaike information criterion (AICc; GraphPad Prism v5.0b) was used to determine the best fit of five biologically plausible non-linear regression models that commonly describe depth-related biological phenomena: linear, log-log, exponential, hyperbolic or quadratic polynomial. Regression equations (GraphPad) for A and C are:  $y=4.084+0.02275x-0.000007117x^2$ ;  $y=36.51+0.04756x-0.00001391x^2$ . Regression equations,  $R^2$  and  $P$  values: (B) Squaliformes:  $y=6.146+0.02352x-0.000007714x^2$ ,  $R^2=0.38$ ,  $P<0.001$ ; Squaliformes <1115 m:  $y=0.01114x+10.28$ ,  $R^2=0.26$ ,  $P<0.01$ ; Carcharhiniformes:  $y=0.004492x+15.39$ ,  $R^2=0.44$ ,  $P<0.0001$ ; Rajiformes:  $y=0.005586x+3.309$ ,  $R^2=0.69$ ,  $P<0.0001$ . (D) Squaliformes:  $y=80.95x/(125.8+x)$ ,  $R^2=0.36$ ,  $P<0.01$ ; Squaliformes <1115 m:  $y=0.01387x+57.65$ ,  $R^2=0.20$ ,  $P<0.05$ ; Carcharhiniformes:  $y=0.0189x+43.84$ ,  $R^2=0.16$ ,  $P<0.05$ ; Rajiformes:  $y=0.01457x+39.48$ ,  $R^2=0.36$ ,  $P<0.005$ . Raw data and references are provided in Table S3. Compiled literature values for HSI and liver lipid composition have been deposited in Excel format at <https://dx.doi.org/10.6084/m9.figshare.1614828>.

### Life history

As K-selected organisms, chondrichthyans grow slowly and invest in few, well-developed young. Deep-sea chondrichthyans have even slower growth rates, later maturation, lower fecundity and longer reproductive cycles compared with shallow species, a pattern similar to that seen in teleosts (Drzen and Haedrich, 2012; Kyne and Simpfendorfer, 2010; Rigby and Simpfendorfer, 2015). All three chondrichthyan reproductive modes (egg-laying, aplacental live birth, placental live birth) occur among deep-sea taxa (Rigby and Simpfendorfer, 2015). Deep-sea teleosts also feature diverse reproductive modes, including live birth (Merrett, 1994). However, the broadcast spawning and planktonic larval stages of many deep-sea teleosts, including macrourids, are distinct from the internally fertilizing, directly developing chondrichthyans (Priede et al., 2006).

### Why are chondrichthyans rare in the abyss?

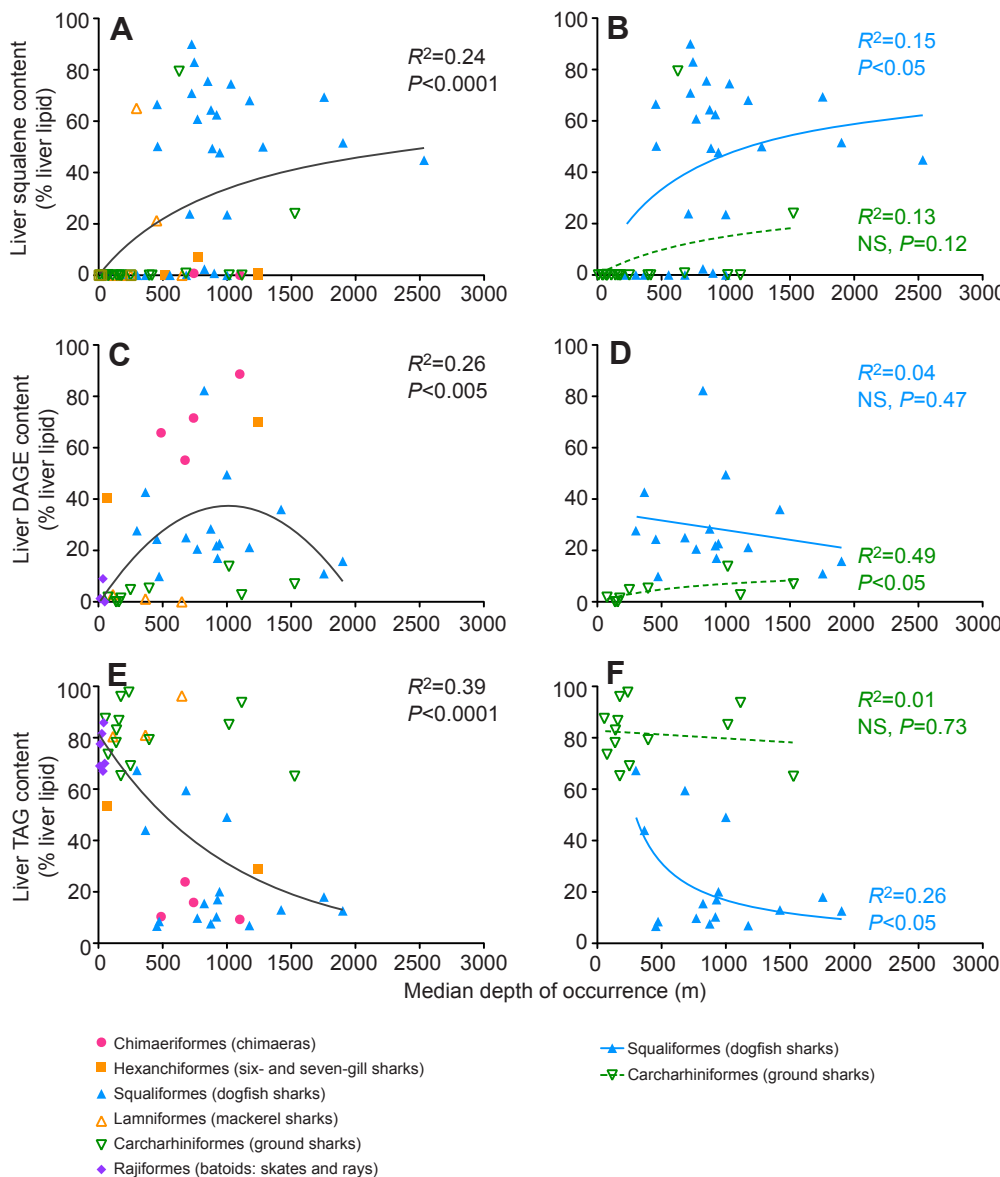
Has the physiology of chondrichthyans constrained their ability to tolerate, compete in, or adapt to the deep-sea environment? Priede et al. (2006) pointed out that, rather than a single explanation,

multiple factors might combine to limit chondrichthyan depth distribution. Below, we consider multiple hypotheses involving potential incompatibilities between chondrichthyan physiology and the environmental challenges of the deep sea (Box 1). Priede et al. (2006) convincingly discounted darkness and cold temperature as limitations, so we ignore these.

### High hydrostatic pressure

#### Constraints imposed by interaction with the chondrichthyan solute system

Recently, the capacity for organic osmolyte accumulation has been hypothesized to limit depth distribution in chondrichthyans, as well as teleosts (Laxson et al., 2011; Yancey et al., 2014). Among chondrichthyans, Laxson et al. (2011) showed that the decline in muscle urea content with depth of capture intercepts zero urea at approximately 4700 m, which is close to the maximum recorded depths of capture for chondrichthyans (~4000 m). Our regression, using MDO, intercepts zero urea near 6000 m, well beyond known chondrichthyan depth limits (Fig. 4B). Regardless, as depth passes 5000 m, urea theoretically



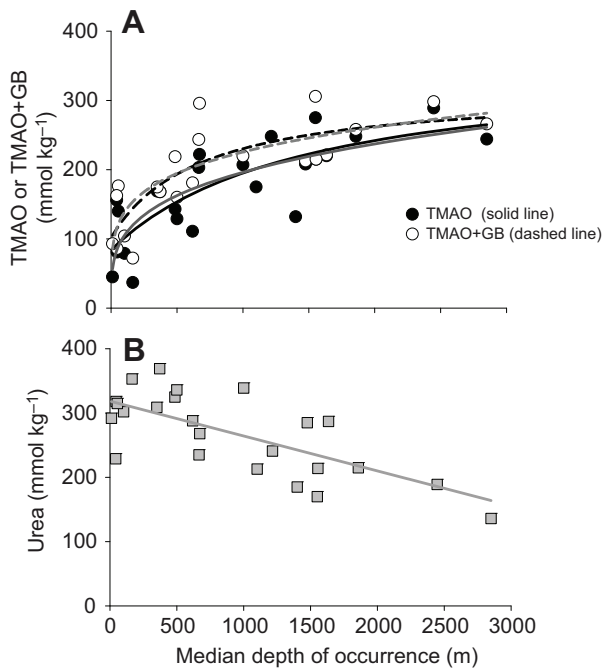
**Fig. 3. Liver triacylglycerol, diacylglycerol ether and squalene contents as a function of median depth of occurrence among chondrichthyans.** TAG, DAGE and squalene levels in chondrichthyan fishes (A,C,E) and within each of the two most speciose and successful deep-sea chondrichthyan orders (B,D,F). Data points represent individual species. See Fig. 2 for further details on AICc analyses. NS, not significant ( $P>0.05$ ). Regression equations (significant regressions only) are: (A)  $y=71.63x/(1128+x)$ ; (B) Squaliformes:  $y=78.98x/(684.5+x)$ ; (C)  $y=-0.7724+0.07536x-0.0000372x^2$ ; (D) Carcharhiniformes:  $y=13.01x/(853.2+x)$ ; (E)  $y=81.64e^{(-0.0009646x)}$ ; (F) Squaliformes:  $y=8180x^{-0.8958}$ . Raw data and references are provided in Table S2. Compiled literature values for liver lipid composition have been deposited in Excel format at <https://dx.doi.org/10.6084/m9.figshare.1614828>.

would have to be replaced entirely by stabilizing solutes in order to maintain osmoconformation. Yet, for unknown reasons, stabilizing solute accumulation does not appear to exceed  $\sim 300 \text{ mmol kg}^{-1}$  in chondrichthyans examined to date (Fig. 4A). Thus, the abyssal rarity of chondrichthyans may be related either to an inability to attain very low urea levels or to an inability to replace urea with other solutes as depth increases (Laxson et al., 2011). It remains unclear why deep-sea chondrichthyans could not eliminate urea and osmoconform solely with stabilizing solutes (e.g. TMAO). In fact, the deepest-sampled teleosts ( $\sim 7000 \text{ m}$ ) can accumulate higher muscle TMAO ( $\sim 380 \text{ mmol kg}^{-1}$ ), making their muscle nearly isosmotic with seawater; extrapolation of the interspecies depth trend for teleosts predicts complete isosmosis at approximately  $8000\text{--}8500 \text{ m}$ , coinciding remarkably well with the maximum depth at which teleost fish have been found (Yancey et al., 2014). Furthermore, although urea-requiring enzymes occur in marine elasmobranchs (Yancey and Somero, 1978), urea accumulation is not a prerequisite for chondrichthyans given the evolution of non-ureotelic freshwater stingrays. Perhaps non-ureotelic

osmoconformation could evolve in marine chondrichthyans inhabiting great depths, but with the insurmountable trade-off of a permanently abyssal existence that might be incompatible with other aspects of chondrichthyan biology.

**Constraints imposed by interactions of membranes with solute system and pressure**

Increasing hydrostatic pressure and decreasing temperature cause phospholipid bilayer membranes to become less fluid, which can perturb function of the membrane and membrane-bound proteins. In response, deep-sea animals have membranes with a composition of phospholipid fatty acids that increases inherent fluidity (e.g. by removing saturated fatty acids), thus combating pressure- and temperature-induced stiffening (Cossins and Macdonald, 1989; Somero, 1992). The impacts of ureotelism on membranes might constrain the potential for such deep-sea membrane adaptation in chondrichthyans: they have highly saturated membranes that could have lower intrinsic fluidity than those of teleosts, possibly as an adaptation to urea, which makes membranes more fluid (Barton et al., 1999; Glemet and Ballantyne, 1996). Furthermore, the depth-



**Fig. 4. Relationships between muscle trimethylamines or urea and median depth of occurrence (MDO) among chondrichthyan fishes.** (A) Muscle trimethylamine and (B) urea contents. Data points represent individual species. There is no clear intraspecific pattern of increasing muscle trimethylamine content with increasing depth of capture in chondrichthyans (Laxson et al., 2011), thus for species captured from multiple depths we have combined all solute values into a single mean. The power relationship [grey lines where TMAO is  $y=30x^{0.27}$  ( $R^2=0.65$ ,  $P<0.001$ ) or TMAO+GB is  $y=52x^{0.21}$  ( $R^2=0.66$ ,  $P<0.001$ ), respectively] is favoured by AICc, but prevents calculation of an asymptotic maximum content; as such we also include the results for a hyperbola [black lines where TMAO is  $y=84+(274x)/(1463+x)$  or TMAO+GB is  $y=101+(214x)/(668+x)$ , respectively], which mirror the modelled power relationships and have comparable  $R^2$  values. The urea line is best fit by the linear relationship of  $y=319-0.054x$  ( $R^2=0.5$ ,  $P<0.001$ ). TMAO, trimethylamine oxide; GB, glycine-betaine. Data are from Kelly and Yancey, 1999; Laxson et al., 2011; Robertson, 1989; Treberg et al., 2006; Treberg and Driedzic, 2007. For the unknown *Bathyraja* species from Kelly and Yancey (1999), we used depth of capture rather than MDO. Also included are the average values for two *Somniosus microcephalus* (urea: 226, 221 mmol kg<sup>-1</sup>; TMAO: 202, 149 mmol kg<sup>-1</sup>; median depth of occurrence 1100 m; J.R.T. and W. R. Driedzic, unpublished results) and values for *Amblyraja hyperborea* ( $N=9$ , urea: 185±8 mmol kg<sup>-1</sup>; TMAO: 132±8 mmol kg<sup>-1</sup>; median depth of occurrence 1400 m; C. Brandt, W. G. Anderson, J. D. Reist and J.R.T., unpublished results).

related decline of urea content in chondrichthyans might further exacerbate the stiffening effects of high pressure and low temperature. Thus, we propose that interactions between the membranes and solute system of chondrichthyans may limit depth distribution because the magnitude of fluidizing membrane adaptation required to counteract the combined effects of depth-related changes in urea, pressure and temperature eventually may become unattainable as depth increases. Unfortunately, comparable data on deep-sea chondrichthyan membranes are scant, warranting the study of membranes from diverse chondrichthyans across multiple depths.

#### Oligotrophy

Nutritional energy input (e.g. dissolved organic carbon levels) and animal biomass (prey availability) decline markedly with depth (Guo et al., 1995; Wei et al., 2010). The metabolically sluggish

lifestyle typical of deep-sea fishes is therefore advantageous, combining low basal metabolism, low activity, slow growth and, at least in some species, high food conversion efficiency (Koslow, 1996, 1997). Below, we assess the general hypothesis that chondrichthyans are excluded from the oligotrophic abyss because they have distinct metabolic and nutritional demands compared with teleosts (Musick and Cotton, 2015; Priede et al., 2006).

#### Metabolic and locomotory constraints

Contrary to this general hypothesis, enzymatic indices of white muscle glycolytic capacity suggest similar depth-related declines in metabolism between chondrichthyans and teleosts (Condon et al., 2012). However, the retention of aerobic capacity in muscles of chondrichthyans, but not teleosts, suggests a heavier reliance on costly endurance swimming, which could be inappropriate for an abyssal existence. Similarly, the abyssal exclusion of certain bathyal teleosts has been attributed to their higher activity levels and metabolic rates compared with abyssal macrourids such as *C. armatus* (Collins et al., 2005). Because of scarce data, this notion remains speculative, warranting further study.

#### Constraints imposed by the energetics of lipid-based buoyancy

Despite depth-related decreases in food supply, even the deepest-living chondrichthyans maintain an enlarged, lipid-rich liver, probably to improve buoyancy and locomotory efficiency (Fig. 2). Priede et al. (2006) argued that the depth distribution of chondrichthyans is limited primarily because this enhanced liver lipid accumulation is energetically unsustainable in the oligotrophic abyss, with the accumulated lipid costing 100–1000 times more energy than inflating a swimbladder to achieve the equivalent buoyancy in abyssal teleosts. Here, we deconstruct Priede et al.'s (2006) proposal in order to identify potential underlying mechanisms.

#### Buoyancy costs: lipid-based buoyancy as a growth penalty

Priede et al.'s (2006) comparison of the energy invested in buoyancy organs ignored the partitioning of lipid accumulation cost in chondrichthyan energy budgets and the cost of large, fatty livers found in certain abyssal teleosts. Unlike the swimbladder, lipid-based buoyancy is not a basal (i.e. constantly required for life) energy expenditure, but rather accrues cost only when the animal is growing. During growth, the additional body mass must be buoyed by synthesis or sequestration of new lipid, which will decrease growth efficiency under a given food intake compared with teleosts, where the equivalent buoyancy is achieved, at a lower cost, by swimbladder inflation (Box 3). Thus, refining Priede and colleagues' proposal, we suggest that the penalty of lipid-based buoyancy in deep-sea chondrichthyans is, simply, lower growth efficiency and later maturation compared with deep-sea teleosts. This penalty is best illustrated by the whole-body energy content (i.e. kJ invested per kg of fish) of deep-sea chondrichthyans, which, primarily because of their larger and fattier livers, is approximately 1.4- to 1.6-fold greater than for deep-sea macrourids (Bulman et al., 2002; Crabtree, 1995; J.R.T. and B.S.-R., unpublished calculations). In other words, given comparable food intake and the similar conversion efficiencies of teleosts and chondrichthyans (Wetherbee and Cortés, 2004), the chondrichthyans require 40–60% more digestible energy to equal the macrourid's growth. Thus, the actual consequence of lipid accumulation in deep-sea chondrichthyans should be a roughly 30–40% lower growth rate than abyssal teleosts. However, growth constants ( $k$ ) are similar between deep-sea chondrichthyans and teleosts, suggesting comparable growth rates

### Box 3. Where do energetic costs of hydrostatic buoyancy fit in energy budgets?

The simplest expression for an animal's energy metabolism is:

$$Energy_{in} \text{ (food energy)} = Energy_{out} \text{ (energy use and loss)}$$

This equation can be expanded:

$$Energy_{in} = \text{Food processing} + \text{Homeostasis} + \text{Growth} + \text{Reproduction}$$

$$Energy_{in} = \text{Digestion} + \text{Feces} + \text{Excretion} + \text{Metabolism} + \text{Activity} + \text{Growth} + \text{Reproduction}$$

Processes in red are obligatory costs, or losses (i.e. fecal or excretory loss)



Processes in green only occur when energy intake exceeds the obligatory costs and losses

Thus, lipid accumulation is the portioning of dietary carbon (energy) away from reproduction or growth as lean tissues mass, whereas swimbladder inflation is a constant basal cost regardless of feeding status. However, while swimbladder costs are relatively small, lipid accumulation for buoyancy requires a substantial diversion of energy, incurring a potential growth or reproduction penalty compared with swimbladder buoyancy (see text for further details).

(Drazen and Haedrich, 2012; Kyne and Simpfendorfer, 2010). The growth penalty of lipid accumulation therefore may be compensated by a greater caloric intake, as observed in one available study on the daily rations of deep-sea chondrichthyans and co-existing teleosts (Madurell and Cartes, 2005). If so, poor food availability in the abyss and resource competition with teleosts, combined with the aforementioned growth penalty, could make chondrichthyans more susceptible to competitive exclusion by more efficiently growing abyssal teleosts. Under this mechanistic proposal for how lipid accumulation in deep-sea chondrichthyans might constrain abyssal colonization, the barrier is set ultimately by biotic factors, acting upon a proximate growth penalty imposed by lipid accumulation.

#### Lipid-based buoyancy as a constraint on starvation tolerance

The dual role of liver lipids in buoyancy and energy storage in chondrichthyans may place additional constraints upon abyssal colonization. Musick and Cotton (2015) suggested that mobilization of lipid fuels (TAG, DAGE) to sustain fasts between infrequent meals in the abyss could compromise neutral buoyancy, leading to unsustainable locomotory costs. However, because of associated mobilization of body protein mass, HSI and liver lipid content of sharks are maintained during prolonged starvation (Baldrige, 1972; Kajimura et al., 2008), suggesting unperturbed buoyancy. Thus, the actual disadvantage in these species might be the loss of body protein, which could hinder performance compared with teleosts, where the swimbladder easily buffers any buoyancy loss resulting from lipid mobilization. Among abyssal teleosts lacking swimbladders (e.g. snailfishes), the reliance upon alternative density reduction strategies (e.g. buoyant fluid spaces) (Pelster, 1997) could buffer such buoyancy loss.

Accumulation of relatively inert squalene, and its trade-off against TAG levels (Fig. 3), could limit starvation endurance in deep-sea squaloids (Musick and Cotton, 2015). However, it is unclear whether a starvation (or any other) penalty of squalene accumulation constrains depth limits because squalene-accumulating squaloid sharks are the deepest chondrichthyans (along with skates, in which squalene presence remains

unexplored). In fact, the high lift of abundant squalene may lessen the impact upon buoyancy of mobilization of available TAG.

#### Reproductive constraints

Deep-sea chondrichthyans have notably low fecundity and long reproductive cycles. The growth penalty of lipid-based buoyancy proposed above could impact maturation and reproductive potential, constraining reproductive viability of abyssal populations. Indeed, the intrinsic rebound potential of deep-sea chondrichthyan populations decreases with depth but ceases to decline below a maximum depth of occurrence of ~1500 m, suggesting a lower viable limit on life history parameters (Simpfendorfer and Kyne, 2009). Furthermore, the egg yolk, which is the only nutrient supply for the developing embryo in egg-layers and most aplacental live-bearers, requires a substantial provision of hepatic lipid and protein (Koob and Callard, 1999; Pethybridge et al., 2011). Consequently, reproductively linked cycles in liver size and lipid content occur in females of many chondrichthyans, including deep-sea species (Clarke et al., 2001; Jakobsdóttir, 2001; Lucifora et al., 2002; Pethybridge et al., 2010; Rossouw, 1987). These cycles of energy investment by female chondrichthyans may be unsustainable in the abyss, or could perturb buoyancy. Although the lipids allocated from liver to yolk can make the eggs neutrally buoyant in aplacental live-bearers (Corner et al., 1969; Pethybridge et al., 2011), thus maintaining the mother's overall buoyancy, this contribution to buoyancy may decrease as the embryo depletes yolk and grows. If so, maternal buoyancy compensation might be required, for example by acquiring new liver lipid or relying more on hydrodynamic lift. All of these energetic challenges of reproduction are inapplicable to deep-sea teleosts such as macrourids, where the swimbladder obviates the growth penalty of lipid-based buoyancy and frees liver lipid for reproductive investment. Additionally, the planktonic larval development of macrourids and most other abyssal teleosts allows direct exploitation of productive surface waters (Priede and Froese, 2013).

#### Constraints imposed by dietary nitrogen limitation

Ureotelism may make chondrichthyans uniquely nitrogen limited (Wood, 2001), which could create distinct nutritional challenges for deep-sea species. Unlike teleosts, chondrichthyans seem unable to modulate nitrogen losses (specifically, urea efflux from the gills), irrespective of dietary nitrogen intake (Treberg and Driedzic, 2006; Wood et al., 2007). Indeed, urea nitrogen losses remain unchanged even during starvation, suggesting that losses represent an unavoidable consequence of ureotelism, rather than excretion per se as in teleosts. When a teleost is fasted, whole-body nitrogen losses remain low until the body lipid and glycogen stores are depleted and they shift to protein catabolism (Black and Love, 1986), at which point whole-body nitrogen losses are expected to increase. By contrast, post-prandial nitrogen losses might already be minimized in chondrichthyans. If so, unlike teleosts, elasmobranchs are physiologically poised to maximize retention of dietary nitrogen, which is consistent with urea-retention mechanisms identified in their gills and kidneys (Ballantyne and Robinson, 2011). Unavoidable urea/nitrogen losses leading to persistent nitrogen demand for urea synthesis may also explain the preferential catabolism of body protein, rather than liver lipid, during starvation in sharks (Baldrige, 1972; Kajimura et al., 2008). Thus, the low, stochastic and intermittent abyssal food supply may cause a regular, problematic demand for body protein catabolism for urea synthesis in chondrichthyans, to accommodate persistent losses at the gills. Note, diffusional losses are driven by the concentration



difference between organism and environment, with the latter being  $\sim 0$ . Thus, even considering the lower urea contents in deep-sea chondrichthyans, urea losses at the gills could remain significant enough to lead to a nitrogen limitation not found in teleosts, which might cause a reliance on body protein catabolism during food shortages that constrains performance and growth.

### Summary and conclusions

The interaction of multiple physiological and ecological factors probably explains why chondrichthyans are rare in the abyss. Three leading hypotheses have emerged, all of which implicate major roles for physiological mechanisms and none of which are mutually exclusive. First, the chondrichthyan osmoregulatory system may limit abyssal penetration as a result of the inability to balance stabilizing and destabilizing influences of pressure and osmolytes on proteins, and/or through the interactive effects of temperature, pressure and urea on membranes. Second, the reliance on lipid-based buoyancy has a unique energetic cost that could increasingly limit growth rate and reproductive output as oligotrophy intensifies with depth. Third, chondrichthyans may be unusually nutrient limited in the oligotrophic abyss because of their nitrogen-intensive osmoregulatory strategy combined with inherent, unavoidable nitrogen losses at their gills.

The first hypothesis, which involves an abiotic factor (hydrostatic pressure), proposes an intrinsic and absolute physiological limit on abyssal penetration because it implies that, if chondrichthyans descend beyond a certain depth, the required balance of urea:TMAO may be unattainable and/or acute, insurmountable physiological dysfunction might occur. The second and third hypotheses, by contrast, propose that the physiological attributes involved do not pose absolute limits on depth range, but rather confer limits on growth and reproduction in the abyss that prevent the establishment of viable populations or ultimately allow teleosts to outcompete and exclude chondrichthyan species. Under this scenario, the abyssal rarity is influenced by physiology but ultimately determined by biotic ecological factors, such that chondrichthyans could not spend long periods or complete life cycles (i.e. colonize) at abyssal depths but could make shorter, temporary forays (i.e. penetrate beyond population range limits). To resolve whether absolute (pressure-related) or relative (biotic) depth limits are involved, crucial albeit challenging studies are required on chondrichthyan, teleost and prey abundances along steep bathymetric gradients (e.g. canyon areas) (King et al., 2008; Jones et al., 2003), long-term observations of large food falls at abyssal depths (Kemp et al., 2006), telemetry (Rodríguez-Cabello and Sánchez, 2014) and the effects of hydrostatic pressure on chondrichthyans (still unstudied). Regional variability in the depth-related decline in food availability (Wei et al., 2010; Rex and Etter, 2010) is a particularly valuable, but unexploited, tool to test whether oligotrophy constrains chondrichthyan depth distribution, in which case the depth limit should vary regionally depending on prey biomass at depth. This prediction is testable provided detailed regional data on depth gradients in abundance and biomass of chondrichthyans and their prey are available. Interestingly, one localized bathymetric study showed that suitable prey can be abundant below depths where chondrichthyans disappear (Jones et al., 2003), possibly hinting that oligotrophy alone does not set a depth limit. Still, seasonal lows in seafloor biomass (Rex and Etter, 2010) could prevent year-round abyssal persistence of chondrichthyans. Additionally, energetic constraints on chondrichthyans remain a possible explanation for their steeper decline in species diversity compared with the less energetically demanding, and thus more competitive, teleosts.

Further study of fishes from the Mediterranean deep sea, which has unusually warm waters ( $\sim 13^{\circ}\text{C}$  versus  $2\text{--}4^{\circ}\text{C}$  elsewhere) that could increase metabolic costs and thus accentuate metabolic constraints, would improve our understanding of the potential energetic determinants of chondrichthyan or teleost depth distribution.

By combining large-scale field experiments and mechanistic physiological approaches, it will be possible to evaluate the hypotheses raised here to explain the vexing scarcity of chondrichthyans in the abyss. Although deep-sea research is challenging and expensive, these studies will also better position us to forecast how anthropogenic impacts on the deep sea, including expected changes in food supply and abiotic conditions resulting from climate change (Smith et al., 2008), might alter the distribution, physiology and ecological role of deep-sea chondrichthyan fishes.

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### Competing interests

The authors declare no competing or financial interests.

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### Supplementary information

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### References

- Alexander, R. M. (1990). Size, speed and buoyancy adaptations in aquatic animals. *Am. Zool.* **30**, 189–196.
- Bagley, P. M., Smith, A. and Priede, I. G. (1994). Tracking movements of deep demersal fishes in the Porcupine Seabight, north-east Atlantic Ocean. *J. Mar. Biol. Assoc. UK* **74**, 473–480.
- Bailey, D. M., Bagley, P. M., Jamieson, A. J., Collins, M. A. and Priede, I. G. (2003). In situ investigation of burst swimming and muscle performance in the deep-sea fish *Antimora rostrata*. *J. Exp. Mar. Biol. Ecol.* **285–286**, 295–311.
- Bailey, D. M., Genard, B., Collins, M. A., Rees, J.-F., Unsworth, S. K., Battle, E. J. V., Bagley, P. M., Jamieson, A. J. and Priede, I. G. (2005). High swimming and metabolic activity in the deep-sea eel *Synaphobranchus kaupii* revealed by integrated in situ and in vitro measurements. *Physiol. Biochem. Zool.* **78**, 335–346.
- Baldrige, H. D., Jr. (1972). Accumulation and function of liver oil in Florida sharks. *Copeia* **1972**, 306–325.
- Ballantyne, J. S. (1997). Jaws: the inside story. The metabolism of elasmobranch fishes. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **118**, 703–742.
- Ballantyne, J. S. and Robinson, J. W. (2011). Chondrichthyes: physiology of sharks, skates, and rays. In *Encyclopedia of Fish Physiology* (ed. A. P. Farrell), pp. 1807–1818. San Diego: Academic Press.
- Barton, K. N., Buhr, M. M. and Ballantyne, J. S. (1999). Effects of urea and trimethylamine N-oxide on fluidity of liposomes and membranes of an elasmobranch. *Am. J. Physiol.* **276**, R397–R406.
- Bernal, D., Sepulveda, C., Mathieu-Costello, O. and Graham, J. B. (2003). Comparative studies of high performance swimming in sharks I. Red muscle morphometrics, vascularization and ultrastructure. *J. Exp. Biol.* **206**, 2831–2843.

- Black, D. and Love, R. M.** (1986). The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. *J. Comp. Physiol. B* **156**, 469–479.
- Bone, Q. and Roberts, B. L.** (1969). The density of elasmobranchs. *J. Mar. Biol. Assoc. UK* **49**, 913–937.
- Bulman, C. M., He, X. and Koslow, J. A.** (2002). Trophic ecology of the mid-slope demersal fish community off southern Tasmania, Australia. *Mar. Freshwater Res.* **53**, 59–72.
- Carey, F. G. and Clark, E.** (1995). Depth telemetry from the sixgill shark, *Hexanchus griseus*, at Bermuda. *Environ. Biol. Fishes* **42**, 7–14.
- Childress, J. J.** (1995). Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends Ecol. Evol.* **10**, 30–36.
- Clarke, M. W., Connolly, P. L. and Bracken, J. J.** (2001). Aspects of reproduction of the deep water sharks *Centroscymnus coelolepis* and *Centrophorus squamosus* from west of Ireland and Scotland. *J. Mar. Biol. Assoc. UK* **81**, 1019–1029.
- Cohen, D. M.** (1977). Swimming performance of the gadoid fish *Antimora rostrata* at 2400 meters. *Deep Sea Res.* **24**, 275–277.
- Collins, M. A., Priede, I. G. and Bagley, P. M.** (1999). In situ comparison of activity in two deep-sea scavenging fishes occupying different depth zones. *Proc. R. Soc. B Biol. Sci.* **266**, 2011–2016.
- Collins, M. A., Bailey, D. M., Ruxton, G. D. and Priede, I. G.** (2005). Trends in body size across an environmental gradient: a differential response in scavenging and non-scavenging demersal deep-sea fish. *Proc. R. Soc. B Biol. Sci.* **272**, 2051–2057.
- Compagno, L. J. V.** (1990). Alternative life-history styles of cartilaginous fishes in time and space. *Environ. Biol. Fishes* **28**, 33–75.
- Condon, N. E., Friedman, J. R. and Drazen, J. C.** (2012). Metabolic enzyme activities in shallow- and deep-water chondrichthyans: implications for metabolic and locomotor capacity. *Mar. Biol.* **159**, 1713–1731.
- Corner, E. D. S., Denton, E. J. and Forster, G. R.** (1969). On the buoyancy of some deep-sea sharks. *Proc. R. Soc. B Biol. Sci.* **171**, 415–429.
- Cossins, A. R. and Macdonald, A. G.** (1989). The adaptation of biological membranes to temperature and pressure: fish from the deep and cold. *J. Bioenerg. Biomembr.* **21**, 115–135.
- Crabtree, R. E.** (1995). Chemical composition and energy content of deep-sea demersal fishes from tropical and temperate regions of the western North Atlantic. *Bull. Mar. Sci.* **56**, 434–449.
- Dickson, K. A., Gregorio, M. O., Gruber, S. J., Loeffler, K. L., Tran, M. and Terrell, C.** (1993). Biochemical indices of aerobic and anaerobic capacity in muscle tissues of California elasmobranch fishes differing in typical activity level. *Mar. Biol.* **117**, 185–193.
- Drazen, J.** (2002). Energy budgets and feeding rates of *Coryphaenoides acrolepis* and *C. armatus*. *Mar. Biol.* **140**, 677–686.
- Drazen, J. C.** (2007). Depth related trends in proximate composition of demersal fishes in the eastern North Pacific. *Deep Sea Res. I Oceanogr. Res. Papers* **54**, 203–219.
- Drazen, J. C. and Haedrich, R. L.** (2012). A continuum of life histories in deep-sea demersal fishes. *Deep Sea Res. I Oceanogr. Res. Papers* **61**, 34–42.
- Drazen, J. C. and Seibel, B. A.** (2007). Depth-related trends in metabolism of benthic and benthopelagic deep-sea fishes. *Limnol. Oceanogr.* **52**, 2306–2316.
- Drazen, J. C. and Yeh, J.** (2012). Respiration of four species of deep-sea demersal fishes measured in situ in the eastern North Pacific. *Deep Sea Res. I Oceanogr. Res. Papers* **60**, 1–6.
- Drazen, J. C., Phleger, C. F., Guest, M. A. and Nichols, P. D.** (2009). Lipid composition and diet inferences of abyssal macrourids in the eastern North Pacific. *Mar. Ecol. Prog. Ser.* **387**, 1–14.
- Drazen, J. C., Dugan, B. and Friedman, J. R.** (2013). Red muscle proportions and enzyme activities in deep-sea demersal fishes. *J. Fish. Biol.* **83**, 1592–1612.
- Drazen, J. C., Friedman, J. R., Condon, N. E., Aus, E. J., Geringer, M. E., Keller, A. A. and Clarke, M. E.** (2015). Enzyme activities of demersal fishes from the shelf to the abyssal plain. *Deep Sea Res. I Oceanogr. Res. Papers* **100**, 117–126.
- Gartner, J. V., Crabtree Roy, E., Jr and Sulak, K. J.** (1997). Feeding at depth. In *Deep-Sea Fishes, Fish Physiology*, Vol. 16 (ed. D. J. Randall and A. P. Farrell), pp. 115–182. San Diego: Academic Press.
- Glemet, H. C. and Ballantyne, J. S.** (1996). Comparison of liver mitochondrial membranes from an agnathan (*Myxine glutinosa*), an elasmobranch (*Raja erinacea*) and a teleost fish (*Pleuronectes americanus*). *Mar. Biol.* **124**, 509–518.
- Guo, L., Santschi, P. H. and Warnken, K. W.** (1995). Dynamics of dissolved organic carbon (DOC) in oceanic environments. *Limnol. Oceanogr.* **40**, 1392–1403.
- Herring, P.** (2002). *The Biology of the Deep Ocean*. Oxford: Oxford University Press.
- Hochachka, P. W. and Somero, G. N.** (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford: Oxford University Press.
- Jakobsdóttir, K. B.** (2001). Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii* (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. *Fish. Res.* **51**, 247–265.
- Jamieson, A. J.** (2015). *The Hadal Zone: Life in the Deepest Oceans*. Cambridge: Cambridge University Press.
- Jamieson, A. J. and Yancey, P. H.** (2012). On the validity of the Trieste flatfish: dispelling the myth. *Biol. Bull.* **222**, 171–175.
- Jones, E. G., Tselepidis, A., Bagley, P. M., Collins, M. A. and Priede, I. G.** (2003). Bathymetric distribution of some benthic and benthopelagic species attracted to baited cameras and traps in the deep eastern Mediterranean. *Mar. Ecol. Prog. Ser.* **251**, 75–86.
- Kajimura, M., Walsh, P. J. and Wood, C. M.** (2008). The spiny dogfish *Squalus acanthias* L. maintains osmolyte balance during long-term starvation. *J. Fish Biol.* **72**, 656–670.
- Kelly, R. H. and Yancey, P. H.** (1999). High contents of trimethylamine oxide correlating with depth in deep-sea teleost fishes, skates, and decapod crustaceans. *Biol. Bull.* **196**, 18–25.
- Kemp, K. M., Jamieson, A. J., Bagley, P. M., McGrath, H., Bailey, D. M., Collins, M. A. and Priede, I. G.** (2006). Consumption of large bathyal food fall, a six month study in the NE Atlantic. *Mar. Ecol. Prog. Ser.* **310**, 65–76.
- King, N. J., Jamieson, A. J., Bagley, P. M. and Priede, I. G.** (2008). Deep-sea scavenging demersal fish fauna of the Nazaré Canyon system, Iberian coast, north-east Atlantic Ocean. *J. Fish Biol.* **72**, 1804–1814.
- Klug, S. and Kriwet, J.** (2010). Timing of deep-sea adaptation in dogfish sharks: insights from a supertree of extinct and extant taxa. *Zool. Scripta* **39**, 331–342.
- Koob, T. J. and Callard, I. P.** (1999). Reproductive endocrinology of female elasmobranchs: lessons from the little skate (*Raja erinacea*) and spiny dogfish (*Squalus acanthias*). *J. Exp. Zool.* **284**, 557–574.
- Koslow, J. A.** (1996). Energetic and life-history patterns of deep-sea benthic, benthopelagic and seamount-associated fish. *J. Fish Biol.* **49**, 54–74.
- Koslow, J. A.** (1997). Seamounts and the ecology of deep-sea fisheries: the firm-bodied fishes that feed around seamounts are biologically distinct from their deepwater neighbors - and may be especially vulnerable to overfishing. *Am. Sci.* **85**, 168–176.
- Kryvi, H., Flatmark, T., Flatmark, T. and Totland, G. K.** (1981). The myoglobin content in red, intermediate and white fibres of the swimming muscle in three species of shark: a comparative study using high-performance liquid chromatography. *J. Fish Biol.* **18**, 331–338.
- Kyne, P. M. and Simpfendorfer, C. A.** (2010). Deepwater chondrichthyans. In *Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation* (ed. J. C. Carrier, J. A. Musick and M. R. Heithaus), pp. 37–113. Boca Raton: CRC Press.
- Laxson, C. J., Condon, N. E., Drazen, J. C. and Yancey, P. H.** (2011). Decreasing urea:trimethylamine N-oxide ratios with depth in chondrichthyes: a physiological depth limit? *Physiol. Biochem. Zool.* **84**, 494–505.
- Lucifora, L. O., Menni, R. C. and Escalante, A. H.** (2002). Reproductive ecology and abundance of the sand tiger shark, *Carcharias taurus*, from the southwestern Atlantic. *ICES J. Mar. Sci.* **59**, 553–561.
- Madurell, T. and Cartes, J. E.** (2005). Trophodynamics of a deep-sea demersal fish assemblage from the bathyal eastern Ionian Sea (Mediterranean Sea). *Deep Sea Res. I Oceanogr. Res. Papers* **52**, 2049–2064.
- Malins, D. C. and Barone, A.** (1970). Glycerol ether metabolism: regulation of buoyancy in dogfish *Squalus acanthias*. *Science* **167**, 79–80.
- Marshall, N. B. and Merrett, N. R.** (1977). The existence of a benthopelagic fauna in the deep-sea. *Deep Sea Res.* **24**, 483–497.
- Merrett, N. R.** (1994). Reproduction in the North Atlantic oceanic ichthyofauna and the relationship between fecundity and species' sizes. *Environ. Biol. Fishes* **41**, 207–245.
- Merrett, N. B. and Haedrich, R. L.** (1997). *Deep-sea Demersal Fish and Fisheries*. London: Chapman & Hall.
- Musick, J. A. and Cotton, C. F.** (2015). Bathymetric limits of chondrichthyans in the deep sea: a re-evaluation. *Deep Sea Res. II Top. Stud. Oceanogr.* **115**, 73–80.
- Nevenzel, J. C.** (1989). Biogenic hydrocarbons of marine organisms. In *Marine Biogenic Lipids, Fats, and Oils*, Vol. 1 (ed. R. G. Ackman), pp. 3–72. Boca Raton: CRC Press.
- Pelster, B.** (1997). Buoyancy at depth. In *Deep-Sea Fishes, Fish Physiology*, Vol. 16 (ed. D. J. Randall and A. P. Farrell), pp. 195–237. San Diego: Academic Press.
- Pethybridge, H., Daley, R., Virtue, P. and Nichols, P.** (2010). Lipid composition and partitioning of deepwater chondrichthyans: inferences of feeding ecology and distribution. *Mar. Biol.* **157**, 1367–1384.
- Pethybridge, H., Daley, R., Virtue, P. and Nichols, P. D.** (2011). Lipid (energy) reserves, utilisation and provisioning during oocyte maturation and early embryonic development of deepwater chondrichthyans. *Mar. Biol.* **158**, 2741–2754.
- Phleger, C. F.** (1998). Buoyancy in marine fishes: direct and indirect role of lipids. *Am. Zool.* **38**, 321–330.
- Priede, I. G. and Froese, R.** (2013). Colonization of the deep sea by fishes. *J. Fish Biol.* **83**, 1528–1550.
- Priede, I. G., Deary, A. R., Bailey, D. M. and Smith, K. L.** (2003). Low activity and seasonal change in population size structure of grenadiers in the oligotrophic abyssal central North Pacific Ocean. *J. Fish Biol.* **63**, 187–196.
- Priede, I. G., Froese, R., Bailey, D. M., Bergstad, O. A., Collins, M. A., Dyb, J. E., Henriques, C., Jones, E. G. and King, N.** (2006). The absence of sharks from abyssal regions of the world's oceans. *Proc. R. Soc. B Biol. Sci.* **273**, 1435–1441.

- Rex, M. A. and Etter, R. J.** (2010). *Deep-Sea Biodiversity: Pattern and Scale*. Cambridge, MA: Harvard University Press.
- Rigby, C. and Simpfendorfer, C. A.** (2015). Patterns in life history traits of deep-water chondrichthyans. *Deep-Sea Res. II Top. Stud. Oceanogr.* **115**, 30-40.
- Robertson, J. D.** (1989). Osmotic constituents of the blood plasma and parietal muscle of *Scyliorhinus canicula* (L.). *Comp. Biochem. Physiol. A Physiol.* **93**, 799-805.
- Rodríguez-Cabello, C. and Sánchez, F.** (2014). Is *Centrophorus squamosus* a highly migratory deep-water shark? *Deep Sea Res. I Oceanogr. Res. Papers* **92**, 1-10.
- Rossouw, G. J.** (1987). Function of the liver and hepatic lipids of the lesser sand shark, *Rhinobatos annulatus* (Müller & Henle). *Comp. Biochem. Physiol. B Comp. Biochem.* **86**, 785-790.
- Samerotte, A. L., Drazen, J. C., Brand, G. L., Seibel, B. A. and Yancey, P. H.** (2007). Correlation of trimethylamine oxide and habitat depth within and among species of teleost fish: an analysis of causation. *Physiol. Biochem. Zool.* **80**, 197-208.
- Seibel, B. A. and Drazen, J. C.** (2007). The rate of metabolism in marine animals: environmental constraints, ecological demands and energetic opportunities. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 2061-2078.
- Simpfendorfer, C. A. and Kyne, P. M.** (2009). Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environ. Conserv.* **36**, 97-103.
- Smith, C. R., De Leo, F. C., Bernardino, A. F., Sweetman, A. K. and Arbuzo, P. M.** (2008). Abyssal food limitation, ecosystem structure and climate change. *Trends Ecol. Evol.* **23**, 518-528.
- Somero, G. N.** (1992). Adaptations to high hydrostatic pressure. *Ann. Rev. Physiol.* **54**, 557-577.
- Somero, G. N.** (1998). Adaptation to cold and depth: contrasts between polar and deep-sea animals. In *Cold Ocean Physiology*, Vol. 66 (ed. H. O. Portner and R. C. Playle), pp. 33-57. Soc. Exper. Biol. Sem. Ser. Cambridge, UK: Cambridge University Press.
- Speers-Roesch, B. and Treberg, J. R.** (2010). The unusual energy metabolism of elasmobranch fishes. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **155**, 417-434.
- Speers-Roesch, B., Robinson, J. W. and Ballantyne, J. S.** (2006). Metabolic organization of the spotted ratfish, *Hydrolagus collieri* (Holocephali: Chimaeriformes): insight into the evolution of energy metabolism in the chondrichthyan fishes. *J. Exp. Zool. A Comp. Exp. Biol.* **305A**, 631-644.
- Sulak, K. J., Wenner, C. A., Sedberry, G. R. and Guelpen, L. V.** (1985). The life history and systematics of deep-sea lizard fishes, genus *Bathysaurus* (Synodontidae). *Can. J. Zool.* **63**, 623-642.
- Treberg, J. R. and Driedzic, W. R.** (2006). Maintenance and accumulation of trimethylamine oxide by winter skate (*Leucoraja ocellata*): reliance on low whole animal losses rather than synthesis. *Am. J. Physiol.* **291**, R1790-R1798.
- Treberg, J. R. and Driedzic, W. R.** (2007). The accumulation and synthesis of betaine in winter skate (*Leucoraja ocellata*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **147**, 475-483.
- Treberg, J. R., Martin, R. A. and Driedzic, W. R.** (2003). Muscle enzyme activities in a deep-sea squaloid shark, *Centroscyllium fabricii*, compared with its shallow-living relative, *Squalus acanthias*. *J. Exp. Zool.* **300A**, 133-139.
- Treberg, J. R., Speers-Roesch, B., Piermarini, P. M., Ip, Y. K., Ballantyne, J. S. and Driedzic, W. R.** (2006). The accumulation of methylamine counteracting solutes in elasmobranchs with differing levels of urea: a comparison of marine and freshwater species. *J. Exp. Biol.* **209**, 860-870.
- Watanabe, Y. Y., Lydersen, C., Fisk, A. T. and Kovacs, K. M.** (2012). The slowest fish: swim speed and tail-beat frequency of Greenland sharks. *J. Exp. Mar. Biol. Ecol.* **426-427**, 5-11.
- Wei, C.-L., Rowe, G. T., Escobar-Briones, E., Boetius, A., Soltwedel, T., Caley, M. J., Soliman, Y., Huettmann, F., Qu, F. Yu, Z. et al.** (2010). Global patterns and predictions of seafloor biomass using random forests. *PLoS ONE* **5**, e15323.
- Wetherbee, B. M. and Cortés, E.** (2004). Food consumption and feeding habits. In *Biology of Sharks and their Relatives* (ed. J. C. Carrier, J. A. Musick and M. R. Heithaus), pp. 225-246. Boca Raton: CRC Press.
- Wetherbee, B. M. and Nichols, P. D.** (2000). Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **125**, 511-521.
- Withers, P., Heffer, G. and Pang, T. S.** (1994). Role of urea and methylamines in buoyancy of elasmobranchs. *J. Exp. Biol.* **188**, 175-189.
- Wood, C. M.** (2001). Influence of feeding, exercise, and temperature on nitrogen metabolism and excretion. In *Nitrogen Excretion, Fish Physiology*, Vol. 20 (ed. P. Wright and P. M. Anderson), pp. 201-238. San Diego: Academic Press.
- Wood, C. M., Bucking, C., Fitzpatrick, J. and Nadella, S.** (2007). The alkaline tide goes out and the nitrogen stays in after feeding in the dogfish shark, *Squalus acanthias*. *Resp. Physiol. Neurobiol.* **159**, 163-170.
- Yancey, P. H.** (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* **208**, 2819-2830.
- Yancey, P. H. and Somero, G. N.** (1978). Urea-requiring lactate dehydrogenases of marine elasmobranch fishes. *J. Comp. Physiol. B* **125**, 135-141.
- Yancey, P. H., Geringer, M. E., Drazen, J. C., Rowden, A. A. and Jamieson, A.** (2014). Marine fish may be biochemically constrained from inhabiting the deepest ocean depths. *Proc. Nat. Acad. Sci. USA* **111**, 4461-4465.

**Table S1. Contribution to hydrostatic lift from liver lipids in squaloid sharks.**

|  | Shallow-water squaloid | Deep-sea squaloid |
|--|------------------------|-------------------|
| HSI (%)                                      | 10                     | 25                |
| Liver lipid (% wet mass)                     | 60                     | 80                |
| Liver lipid (g · kg of shark <sup>-1</sup> ) | 60                     | 200               |
| Liver lipid density (g ml <sup>-1</sup> )    | 0.91 <sup>a</sup>      | 0.87 <sup>c</sup> |
| <b>Lift per kg of shark (g)<sup>b</sup></b>  | <b>7.58</b>            | <b>35.6</b>       |

<sup>a</sup> Assumes 50% TAG, 50% DAGE with a net density based on densities given in Table 1 (main text).

<sup>b</sup> Lift = [liver lipid/liver lipid density] \* [seawater density – lipid density], where seawater density is 1.025 g ml<sup>-1</sup>.

<sup>c</sup> (Corner et al. 1969)

Determining sources of hydrostatic lift in shallow and deep-sea squaloid (dogfish) sharks requires evaluating lift due to *i) liver lipids* and *ii) osmolytes*. For liver lipids we estimated an average HSI and % lipid content from the data in Table S3 and S4. We assumed the bulk of the liver lipid is comprised of TAG, DAGE and hydrocarbon (squalene). Lipid density for a shallow squaloid shark assumes a composition of 50% TAG and 50% DAGE using the density values from Table 1 in the main text. Deep-sea squaloid shark lipid density of 0.87 g ml<sup>-1</sup> is from (Corner et al. 1969). Influences of temperature and pressure are ignored for simplicity.

For osmolytes, we assumed deep-sea squaloid sharks have comparable levels of urea and TMAO as the deepest sampled skates. The contributions from minor osmolytes (e.g. betaine, sarcosine, taurine) are ignored. Because of the effects of solutes on solution density, the contribution of urea and TMAO can be calculated using the partial molal volume approach outlined by Withers and colleagues (Withers et al 1994a; Withers et al. 1994b).

**Table S2. Contribution to hydrostatic lift from osmolytes in squaloid sharks**

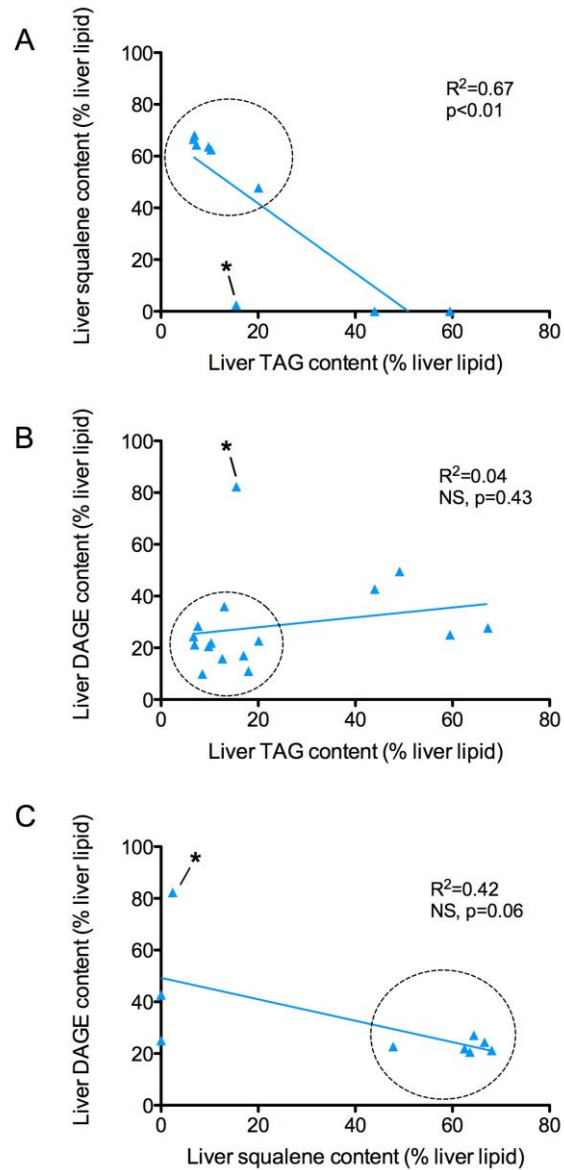
|   | Shallow-water squaloid | Deep-sea squaloid |
|---|------------------------|-------------------|
| Water volume<br>(kg water · kg wet mass <sup>-1</sup> ) | 0.72 <sup>d</sup>      | 0.58 <sup>e</sup> |
| TMAO (mmol l <sup>-1</sup> )                            | 84                     | 370               |
| Urea (mmol l <sup>-1</sup> )                            | 365                    | 170               |
| <b>Lift per kg of shark (g)<sup>f</sup></b>             | <b>4.37</b>            | <b>6.59</b>       |

<sup>d</sup>Thorson 1962)

<sup>e</sup>No data available as far as we know; however, for simplicity the wet to dry mass ratio is assumed to be the same as shallow-water sharks with the exception of the space occupied by liver lipid. Liver lipid would be part of the Thorson (1962) estimate, therefore we assumed the additional liver lipid (140 g kg<sup>-1</sup> of deep-sea Squaliform based on Table S1) displaces tissue with a content of 0.72 l water kg wet mass<sup>-1</sup> (Thorson 1962).

<sup>f</sup>Determined using equations and values given in (Withers et al. 1994a, Withers et al. 1994b). Note urea and TMAO concentrations are estimates for muscle based on Fig. 3 (main text), adjusted for an assumed 80% water content of the muscle. Although TMAO is preferentially accumulated in the intracellular fluid (ICF) over the extracellular fluid (ECF), for simplicity we assumed [TMAO] is equal between the ICF and ECF, which will overestimate the contribution of TMAO to lift.

Combining the results from Tables S1 and S2 we find that the shallow-water squaloid shark gains a combined lift of 7.58 (lipid) + 4.37 (TMAO+urea) = 11.95 g of lift per kg of shark. The deep-sea squaloid shark has 35.6 (lipid) + 6.59 (TMAO+urea) = 42.19 g of lift per kg. Therefore, the deep-sea shark has 30.24 g more lift, of which less than 10% (6.59-4.37 = 2.22 g) comes from the contribution of the altered osmolyte composition between shallow and deep-sea species.



**Fig. S1. Relationships between % composition of lipid classes (squalene; diacylglycerol ether, DAGE; and triacylglycerols, TAG) in livers of squaloid (dogfish) sharks.** Squalene and TAG contents are inversely related (A), whereas a relationship is lacking between TAG and DAGE contents (B) and a weak inverse trend exists for squalene vs. DAGE contents (C). Data points represent mean values for individual species. The dashed circle encompasses squalene-accumulating species; the data point indicated by a line with an asterisk represents *Proscymnodon plunketi* (family Somniosidae), which, unlike most deep-sea squaloids, accumulates low levels of squalene but high levels of DAGE. Linear regression equations (GraphPad Prism v5.0b) are: (A)  $y = -1.348x + 68.68$ ; (B)  $y = 0.1904x + 24.15$ ; (C)  $y = -0.4143x + 49.27$ . NS, not significant ( $p > 0.05$ ). Raw data and references are provided in Supplementary Tables.

**Table S3. Hepatosomatic index (HSI, = liver mass/body mass) of chondrichthyan fishes.** Species means were used to plot Fig. 1A,B. Minimum and maximum depths were obtained from Kyne and Simpfendorfer (2010), White and Somerville (2010), Ebert et al. (2013), Last and Stevens (2009), or Froese and Pauly (2014). Sample sizes are noted where n<3. References appended.

| Order                                   | Family                                     | Species  | Min depth (m) | Max depth (m) | Median depth (m) | HSI (%) | HSI species mean (%) | Reference and notes  |
|---|--|--|---------------|---------------|------------------|---------|----------------------|--|
| Chimaeriformes (Chimaeras)              |  |  |               |               |                  |         |                      |  |
|   | Rhinochimaeridae (Longnose chimaeras)      |  |               |               |                  |         |                      |  |
|   |  | <i>Harriotta raleighana</i>                              | 380           | 2600          | 1490             | 31.3    | 31.3                 | González et al., 2007  |
|   |  | <i>Rhinochimaera pacifica</i>                            | 191           | 1290          | 741              | 21.9    | 21.8                 | Pethybridge et al., 2010   |
|   |  |  |               |               |                  | 21.6    |                      | Hayashi et al., 1983. n=2.                                       |
|   | Callorhynchidae (Plownose chimaeras)       |  |               |               |                  |         |                      |  |
|   |  | <i>Callorhynchus capensis</i>                            | 10            | 374           | 192              | 7.3     | 7.3                  | Nibam, 2011. Average of males and females estimated from figure. |
|   | Chimaeridae (Shortnose chimaeras)          |  |               |               |                  |         |                      |  |
|   |  | <i>Chimaera fulva</i>                                    | 780           | 1095          | 938              | 19.8    | 19.8                 | Pethybridge et al., 2010. n=1.                                   |
|   |  | <i>Chimaera lignaria</i>                                 | 400           | 1800          | 1100             | 19.2    | 19.2                 | Pethybridge et al., 2010   |
|   |  | <i>Chimaera monstrosa</i>                                | 50            | 1000          | 525              | 16.0    | 16.0                 | Oguri, 1978  |
|   |  | <i>Hydrolagus affinis</i>                                | 300           | 2909          | 1605             | 17.3    | 17.3                 | Corner et al., 1969. n=1.  |
|   |  | <i>Hydrolagus barbouri</i>                               | 250           | 1100          | 675              | 18.8    | 18.8                 | Hayashi et al., 1983. n=2.                                       |
|   |  | <i>Hydrolagus colliei</i>                                | 0             | 971           | 486              | 17.0    | 17.0                 | Oguri, 1978  |
|   |  | <i>Hydrolagus mirabilis</i>                              | 450           | 1933          | 1192             | 23.2    | 23.2                 | González et al., 2007  |
| Hexanchiformes (Cow and frilled sharks) |  |  |               |               |                  |         |                      |  |
|   | Hexanchidae (Sixgill and sevengill sharks) |  |               |               |                  |         |                      |  |
|   |  | <i>Heptranchias perlo</i>                                | 27            | 1000          | 514              | 7.0     | 10.6                 | van Vleet et al., 1984. n=1.                                     |
|   |  |  |               |               |                  | 14.1    |                      | Tsujimoto, 1920. n=1.  |
|   |  | <i>Hexanchus griseus</i>                                 | 0             | 2490          | 1245             | 15.5    | 15.5                 | Studies cited in Bone and Roberts, 1969.                         |
|   |  | <i>Hexanchus vitulus</i> (= <i>Hexanchus nakamurai</i> ) | 90            | 621           | 356              | 20.0    | 20.0                 | van Vleet et al., 1984. n=1.                                     |
|   |  | <i>Notorynchus cepedianus</i>                            | 0             | 136           | 68               | 11.6    | 11.6                 | Pethybridge et al., 2010. n=1.                                   |
| Squaliformes (Dogfish sharks)           |  |  |               |               |                  |         |                      |  |
|   | Echinorhinidae (Bramble sharks)            |  |               |               |                  |         |                      |  |
|   |  | <i>Echinorhinus brucus</i>                               | 18            | 900           | 459              | 14.9    | 14.9                 | Bone and Roberts, 1969   |
|   | Squalidae (Dogfish sharks)                 |  |               |               |                  |         |                      |  |

|  |     |      |      |                              |      |  |
|--|-----|------|------|------------------------------|------|--|
| <i>Squalus acanthias</i>                             | 0   | 600  | 300  | 8.7<br>9.7<br>9.2<br>10.1    | 9.4  | Pethybridge et al., 2010<br>Malins and Barone, 1970<br>Bone and Roberts, 1969<br>Schmidt-Nielsen et al., 1934  |
| <i>Squalus chloroculus</i><br>(= <i>mitsukurii</i> ) | 4   | 1360 | 682  | 8.4                          | 8.4  | Pethybridge et al., 2010. Used min depth for <i>S. mitsukurii</i> and max depth for <i>S. chloroculus</i>  |
| <i>Squalus cubensis</i>                              | 60  | 380  | 220  | 8.0                          | 8.0  | van Vleet et al., 1984. n=3 mixed sexes.   |
| <i>Squalus megalops</i>                              | 0   | 732  | 366  | 6.8                          | 6.8  | Pethybridge et al., 2010   |
| Centrophoridae (Gulper sharks)                       |     |      |      |                              |      |  |
| <i>Centrophorus</i> sp.                              | 50  | 1400 | 725  | 21.5                         | 21.5 | Peyronel et al., 1984 (probably <i>Centrophorus uyato</i> )  |
| <i>Centrophorus</i> sp.                              |     |      | 731  | 24.5                         | 24.5 | Higashi et al., 1953c, cited in Corner et al. 1969. MDO is average of other <i>Centrophorus</i> .  |
| <i>Centrophorus acus</i>                             | 150 | 950  | 550  | 21.1                         | 21.1 | Tsujimoto, 1920. Female, n=1.  |
| <i>Centrophorus atromarginatus</i>                   | 150 | 450  | 300  | 15.8                         | 15.8 | Tsujimoto, 1920. Male, n=1.  |
| <i>Centrophorus granulosus</i>                       | 50  | 1440 | 745  | 23.8<br>23.0                 | 23.4 | Batista and Nunes, 1992. n=3.<br>van Vleet et al., 1984 n=1. Female.   |
| <i>Centrophorus lusitanicus</i>                      | 300 | 1400 | 850  | 21.2                         | 21.2 | Hernandez-Perez et al., 1997   |
| <i>Centrophorus squamosus</i>                        | 230 | 3280 | 1755 | 21.2<br>21.4<br>19.2<br>19.0 | 20.2 | Batista and Nunes, 1992. n=3.<br>Corner et al., 1969. n=2.<br>Clarke et al., 2001. Female, multiple maturity stages.<br>Hernandez-Perez et al., 1997 |
| <i>Centrophorus zeehaani</i>                         | 208 | 701  | 455  | 21.7                         | 21.7 | Pethybridge et al., 2010   |
| <i>Deania</i> spp.                                   | 70  | 1785 | 928  | 18.8                         | 18.8 | Higashi et al., 1953b, cited in Corner et al., 1969. Min and max depths are average of those of known species of <i>Deania</i> spp.                  |
| <i>Deania calcea</i>                                 | 70  | 1470 | 770  | 20.5<br>18.7<br>19.3         | 19.5 | Corner et al., 1969. n=3.<br>Batista and Nunes, 1992<br>Pethybridge et al., 2010   |
| <i>Deania hystricosum</i>                            | 470 | 1300 | 885  | 18.5                         | 18.5 | Hernandez-Perez et al., 1997   |
| <i>Deania profundorum</i>                            | 275 | 1785 | 1030 | 20.0                         | 20.0 | Hernandez-Perez et al., 1997   |

|                                  |     |      |      |      |      |   |
|----------------------------------|-----|------|------|------|------|---|
| Etmopteridae (Lanternsharks)     |     |      |      |      |      |   |
| <i>Centroscyllium fabricii</i>   | 180 | 2250 | 1215 | 20.0 | 22.7 | Jakobsdottir, 2001. Average of males and females.                             |
|                                  |     |      |      | 25.3 |      | González et al., 2007   |
| <i>Centroscyllium ritteri</i>    | 320 | 1100 | 710  | 19.6 | 22.5 | Kayama et al., 1969. n=1?   |
|                                  |     |      |      | 25.3 |      | Tsujimoto, 1920. Average of males and females.                                |
| <i>Etmopterus baxteri</i>        | 250 | 1500 | 875  | 18.7 | 18.7 | Pethybridge et al., 2010  |
| <i>Etmopterus hillianus</i>      | 311 | 695  | 503  | 18.0 | 18.0 | van Vleet et al., 1984. n=1. Female.  |
| <i>Etmopterus princeps</i>       | 567 | 4500 | 2534 | 19.5 | 17.1 | Corner et al., 1969. n=1. Jakobsdottir, 2001. Average of males and females.   |
|                                  |     |      |      |      | 11.8 | Hernandez-Perez et al., 1997  |
| <i>Etmopterus pusillus</i>       | 274 | 1998 | 1136 | 31.0 | 31.0 | Coelho and Erzini, 2007. Estimated from figure.                               |
| <i>Etmopterus spinax</i>         | 70  | 2490 | 1280 | 23.5 | 18.4 | Coelho and Erzini, 2008. Average of males and females. Estimated from figure. |
|                                  |     |      |      | 14.5 |      | Aranha et al., 2009. Average of males and females.                            |
|                                  |     |      |      | 17.3 |      | Schmidt-Nielsen et al., 1934  |
| Somniosidae (Sleeper sharks)     |     |      |      |      |      |   |
| <i>Centroscymnus coelolepis</i>  | 128 | 3675 | 1902 | 27.1 | 24.9 | Corner et al., 1969. n=2.   |
|                                  |     |      |      | 23.4 |      | Clarke et al., 2001. Females, average of multiple maturity stages.            |
|                                  |     |      |      | 29.4 |      | Batista and Nunes, 1992   |
|                                  |     |      |      | 21.7 |      | Pethybridge et al., 2010  |
|                                  |     |      |      | 23.1 |      | Hernandez-Perez et al., 1997  |
| <i>Centroscymnus owstoni</i>     | 426 | 1459 | 943  | 22.4 | 23.8 | Hernandez-Perez et al., 1997  |
|                                  |     |      |      | 22.8 |      | Higashi et al., 1953a, cited in Corner et al., 1969.                          |
|                                  |     |      |      | 20.8 |      | Pethybridge et al., 2010  |
|                                  |     |      |      | 29.1 |      | Tsujimoto, 1920. n=1.   |
| <i>Centroselachus crepidater</i> | 270 | 2080 | 1175 | 29.8 | 29.8 | Pethybridge et al., 2010  |
| <i>Proscymnodon plunketi</i>     | 219 | 1427 | 823  | 26.3 | 26.3 | Pethybridge et al., 2010  |
| <i>Scymnodon ringens</i>         | 200 | 1600 | 900  | 22.6 | 22.6 | Batista and Nunes, 1992. n=1.   |



|   |     |      |      |      |      |  |
|---|-----|------|------|------|------|--|
| <i>Somniosus longus</i>                   | 250 | 1160 | 705  | 14.9 | 14.9 | Higashi et al., 1954, cited in Corner et al., 1969. Published as <i>Heteroscymnus longus</i> .   |
| <i>Somniosus microcephalus</i>            | 0   | 2200 | 1100 | 12.5 | 12.5 | Studies cited in Bone and Roberts, 1969.   |
| <i>Somniosus pacificus</i>                | 0   | 2000 | 1000 | 17.4 | 17.4 | Higashi et al., 1954, cited in Corner et al., 1969.  |
| <i>Zameus squamulosus</i>                 | 550 | 1450 | 1000 | 23.8 | 23.8 | Tsujimoto 1920. Average of males and females. n=2.   |
| Dalatiidae (Kitefin sharks)               |     |      |      |      |      |  |
| <i>Dalatias licha</i>                     | 37  | 1800 | 1250 | 26.9 | 23.5 | Lewis, 1969  |
|   |     |      |      | 20.8 |      | Higashi et al., 1954, cited in Corner et al., 1969.  |
|   |     |      |      | 25.2 |      | Batista and Nunes, 1992  |
|   |     |      |      | 20.9 |      | Pethybridge et al., 2010   |
| Squatiniiformes (Angelsharks)             |     |      |      |      |      |  |
| Squatinae (Angelsharks)                   |     |      |      |      |      |  |
| <i>Squatina guggenheim</i>                | 35  | 115  | 75   | 3.5  | 3.5  | Colonello et al., 2007. Average of males and females.  |
| <i>Squatina africana</i>                  | 0   | 494  | 247  | 3.0  | 3.0  | Shelmerdine and Cliff, 2010. Average of males and females.                                       |
| <i>Squatina squatina</i>                  | 5   | 200  | 103  | 3.3  | 3.3  | Bone and Roberts, 1969   |
| <i>Squatina japonica</i>                  | 0   | 300  | 150  | 3.0  | 3.0  | Tsujimoto 1920 n=2. Male and female.   |
| Pristiophoriformes (Sawsharks)            |     |      |      |      |      |  |
| Pristiophoridae (Sawsharks)               |     |      |      |      |      |  |
| <i>Pristiophorus japonicus</i>            | 5   | 500  | 253  | 7    | 7    | Tsujimoto, 1920. n=1.  |
| Heterodontiformes (Bullhead sharks)       |     |      |      |      |      |  |
| Heterodontidae (Bullhead sharks)          |     |      |      |      |      |  |
| <i>Heterodontus japonicus</i>             | 6   | 37   | 22   | 6.7  | 6.7  | Tsujimoto, 1920. n=1.  |
| <i>Heterodontus portusjacksoni</i>        | 0   | 275  | 138  | 8.0  | 6.2  | Powter and Gladstone, 2008. Estimated from figure of values for males and females over one year. |
|   |     |      |      | 4.4  |      | Withers et al., 1994   |
| Orectolobiformes (Carpets sharks)         |     |      |      |      |      |  |
| Hemiscyllidae (Longtailed carpets sharks) |     |      |      |      |      |  |
| <i>Chiloscyllium plagiosum</i>            | 0   | 30   | 15   | 6.0  | 6.0  | Chen and Liu, 2006. Average of values for males and females over one year.                       |

|                                       |     |      |      |      |      |  |
|---------------------------------------|-----|------|------|------|------|--|
| Lamniformes (Mackerel sharks)         |     |      |      |      |      |  |
| Alopiidae (Thresher sharks)           |     |      |      |      |      |  |
| <i>Alopias superciliosus</i>          | 0   | 732  | 366  | 5.4  | 5.4  | Jayasinghe et al., 2003a. Average of males and females.                          |
| <i>Alopias vulpinus</i>               | 0   | 366  | 183  | 3.2  | 3.2  | Schmidt-Nielsen et al., 1934. n=1.   |
| Odontaspidae (Raggedtooth sharks)     |     |      |      |      |      |  |
| <i>Carcharias taurus</i>              | 0   | 191  | 96   | 5.0  | 7.3  | Lucifora et al., 2002. Average of males and females.                             |
|                                       |     |      |      | 9.6  |      | Davidson and Cliff, 2002   |
| Lamnidae (Mackerel sharks)            |     |      |      |      |      |  |
| <i>Lamna ditropis</i>                 | 0   | 225  | 113  | 7.3  | 7.3  | Jayasinghe et al., 2003b. Average of values for males and females over one year. |
| <i>Lamna nasus</i>                    | 0   | 700  | 350  | 6.3  | 7.9  | Bone and Roberts, 1969   |
|                                       |     |      |      | 9.4  |      | Schmidt-Nielsen et al., 1934. n=1.   |
| Cetorhinidae (Basking sharks)         |     |      |      |      |      |  |
| <i>Cetorhinus maximus</i>             | 0   | 904  | 452  | 15.9 | 17.3 | Bone and Roberts, 1969. n=2.   |
|                                       |     |      |      | 20.0 |      | Schmidt-Nielsen et al., 1934. n=1.   |
| Pseudocarchariidae (Crocodile sharks) |     |      |      |      |      |  |
| <i>Pseudocarcharias kamoharai</i>     | 0   | 590  | 295  | 29   | 29.0 | Oliveira et al., 2010. Females only. Estimated from figure.                      |
| Carcharhiniformes (Ground sharks)     |     |      |      |      |      |  |
| Scyliorhinidae (Catsharks)            |     |      |      |      |      |  |
| <i>Apristurus macrorhynchus</i>       | 220 | 1140 | 680  | 22.1 | 22.1 | Kayama et al., 1969. n=1?  |
| <i>Apristurus melanoasper</i>         | 512 | 1520 | 1016 | 17.9 | 17.9 | Pethybridge et al., 2010   |
| <i>Apristurus sinensis</i>            | 940 | 1290 | 1115 | 20.7 | 20.7 | Pethybridge et al., 2010   |
| <i>Cephaloscyllium laticeps</i>       | 0   | 220  | 110  | 11   | 11.0 | Awruch et al., 2008. Estimated from figure for males and females.                |
| <i>Cephaloscyllium umbratile</i>      | 20  | 200  | 110  | 13.9 | 13.9 | Tsujimoto, 1920. n=1.  |
| <i>Figaro boardmani</i>               | 150 | 640  | 395  | 6.5  | 6.5  | Pethybridge et al., 2010   |
| <i>Galeus arae</i>                    | 292 | 732  | 512  | 17.0 | 17.0 | van Vleet et al., 1984   |
| <i>antillensis</i>                    |     |      |      |      |      | n=1. Female  |
| <i>Galeus eastmani</i>                | 100 | 900  | 500  | 7.6  | 7.6  | Tsujimoto, 1920. Male, n=1. Depth IUCN Database.                                 |

|                                 |     |      |     |              |      |  |
|---------------------------------|-----|------|-----|--------------|------|--|
| <i>Galeus melanostomus</i>      | 55  | 1440 | 748 | 9.4          | 9.4  | Schmidt-Nielsen et al., 1934   |
| <i>Halaaelurus dawsoni</i>      | 50  | 1200 | 625 | 17.0         | 17.0 | Francis, 2006. Estimated from figure.  |
| <i>Holohalaelurus regani</i>    | 40  | 910  | 475 | 4.0          | 4.0  | Richardson et al., 2000. Average of males and females.   |
| <i>Parmaturus pilosus</i>       | 358 | 895  | 627 | 24.2         | 24.2 | Tsujimoto, 1920. Male, n=1.  |
| <i>Scyliorhinus canicula</i>    | 0   | 110  | 55  | 5.4<br>7.5   | 6.5  | Bone and Roberts, 1969<br>Capape et al., 2008. Average of males and females estimated from figure. |
| <i>Scyliorhinus hesperius</i>   | 274 | 457  | 366 | 11.0         | 11.0 | van Vleet et al., 1984. Female, n=1.   |
| <i>Scyliorhinus torazame</i>    | 0   | 100  | 50  | 8.1          | 8.1  | Tsujimoto 1920. Males and females.   |
| Triakidae (Houndsharks)         |     |      |     |              |      |  |
| <i>Galeorhinus galeus</i>       | 2   | 471  | 237 | 7.3<br>10.5  | 8.9  | Bone and Roberts, 1969<br>Schmidt-Nielsen et al., 1934   |
| <i>Mustelus asterias</i>        | 0   | 200  | 100 | 5.7          | 5.7  | Bone and Roberts, 1969   |
| <i>Mustelus norrisi</i>         | 0   | 84   | 42  | 5.1          | 5.1  | Baldrige, 1970. n=2.   |
| <i>Triakis scyllium</i>         | 0   | 150  | 75  | 5.9<br>7.8   | 6.9  | Tsujimoto, 1920. n=1.<br>Kayama et al., 1969. n=1?   |
| Carcharhinidae (Requiem sharks) |     |      |     |              |      |  |
| <i>Carcharhinus acronotus</i>   | 18  | 64   | 41  | 4.5          | 4.5  | Baldrige, 1970. n=2.   |
| <i>Carcharhinus brevipinna</i>  | 0   | 75   | 38  | 10.0         | 10.0 | Davidson and Cliff, 2002   |
| <i>Carcharhinus leucas</i>      | 0   | 152  | 76  | 10.2<br>11.2 | 10.7 | Baldrige, 1970<br>Davidson and Cliff, 2002   |
| <i>Carcharhinus limbatus</i>    | 0   | 30   | 15  | 6.6          | 6.6  | Davidson and Cliff, 2002   |
| <i>Carcharhinus longimanus</i>  | 0   | 152  | 76  | 8.2          | 8.2  | Jayasinghe et al., 2003a. Average of males and females.  |
| <i>Carcharhinus obscurus</i>    | 0   | 400  | 200 | 11.2         | 11.2 | Davidson and Cliff, 2002   |
| <i>Carcharhinus perezii</i>     | 0   | 30   | 15  | 5.0          | 5.0  | van Vleet et al., 1984. Male, n=1.   |
| <i>Carcharhinus plumbeus</i>    | 1   | 280  | 141 | 12.4         | 12.4 | Baldrige, 1970   |
| <i>Galeocerdo cuvier</i>        | 0   | 140  | 70  | 10.4<br>14.3 | 12.4 | Baldrige, 1970<br>Davidson and Cliff, 2002   |
| <i>Negaprion brevirostris</i>   | 0   | 92   | 46  | 9.1          | 9.1  | Baldrige, 1970   |
| <i>Prionace glauca</i>          | 0   | 350  | 175 | 8.4<br>6.7   | 7.6  | Bone and Roberts, 1969<br>Jayasinghe et al., 2003a. Average of males and                           |

|   |                                 |      |      |      |            |   |
|---|---------------------------------|------|------|------|------------|---|
|   |                                 |      |      |      |            | females.  |
|   | <i>Carcharhinus falciformis</i> | 0    | 500  | 250  | 5.1<br>5.9 | 5.5<br>Navarro-Garcia et al., 2000<br>Jayasinghe et al., 2003a.<br>Average of males and females.                    |
| Sphyrnidae                              |                                 |      |      |      |            |   |
|   | <i>Sphyrna lewini</i>           | 0    | 275  | 138  | 7.9<br>5.8 | 6.9<br>Davidson and Cliff, 2002<br>Jayasinghe et al., 2003a.<br>Average of males and females.                       |
| Rajiformes (Skates and rays or Batoids) |                                 |      |      |      |            |   |
| Rhinobatidae (Guitarfishes)             |                                 |      |      |      |            |   |
|   | <i>Rhinobatos annulatus</i>     | 0    | 73   | 37   | 3.0        | 3.0<br>Rossouw, 1987. Male and female. Estimated from figure of values over one year.                               |
|   | <i>Rhinobatos percellens</i>    | 0    | 110  | 55   | 2.4        | 2.4<br>Rocha and Gadig, 2013.<br>Average of male and female values over one year.                                   |
| Arhynchobatidae (Softnose skates)       |                                 |      |      |      |            |   |
|   | <i>Bathyraja albomaculata</i>   | 55   | 861  | 458  | 7.5        | 7.5<br>Ruocco et al., 2006.<br>Estimated from figure.   |
|   | <i>Bathyraja kincaidii</i>      | 55   | 1372 | 714  | 5.0        | 5.0<br>Perez-Brazen, 2014.<br>Estimated from values over one year for males and females.                            |
|   | <i>Bathyraja richardsoni</i>    | 1370 | 2909 | 2140 | 16.1       | 16.1<br>Templeman 1973.<br>Captured at ~2000 m.<br>Males and females.   |
| Rajidae (Hardnose skates)               |                                 |      |      |      |            |   |
|   | <i>Amblyraja hyperborea</i>     | 300  | 2500 | 1400 | 11.0       | 11.0<br>Treberg Atchison and Reist., unpublished.<br>Trawled from 500-1250m, Canadian region of Beaufort Sea.       |
|   | <i>Amblyraja radiata</i>        | 18   | 1400 | 709  | 6.0<br>3.9 | 4.9<br>Sulikowski et al., 2005.<br>Average of male and female values over one year.<br>Schmidt-Nielsen et al., 1934 |
|   | <i>Atlantoraja castelnaui</i>   | 10   | 100  | 55   | 2.9        | 2.9<br>Colonello et al., 2012.<br>Average of male and female values over one year.                                  |

|                                 |     |      |     |            |     |   |
|---------------------------------|-----|------|-----|------------|-----|---|
| <i>Atlantoraja cyclophora</i>   | 0   | 300  | 150 | 2.6        | 2.6 | Oddone and Velasco 2006. Average of male and female values over summer and winter. Depth from paper.    |
| <i>Dipturus batis</i>           | 100 | 1000 | 550 | 6.6        | 6.6 | Schmidt-Nielsen et al., 1934  |
| <i>Dipturus nidrosiensis</i>    | 200 | 1000 | 600 | 9.8        | 9.8 | Schmidt-Nielsen et al., 1934  |
| <i>Dipturus oxyrinchus</i>      | 15  | 900  | 458 | 6.8        | 6.8 | Schmidt-Nielsen et al., 1934  |
| <i>Leucoraja fullonica</i>      | 30  | 800  | 415 | 7.0        | 7   | Schmidt-Nielsen et al., 1934  |
| <i>Leucoraja ocellata</i>       | 0   | 90   | 45  | 3.5        | 3.5 | Treberg and Driedzic, 2007  |
| <i>Leucoraja naevus</i>         | 20  | 500  | 260 | 4.5        | 4.5 | Maia et al 2012. Average of male and female values over one year.                                       |
| <i>Malacoraja senta</i>         | 46  | 914  | 480 | 3.5        | 3.5 | Sulikowski et al., 2007. Average of male and female values over one year.                               |
| <i>Psammobatis bergi</i>        | 0   | 81   | 41  | 2.3        | 2.3 | San Martin et al., 2005. Average of male and female values over a year.                                 |
| <i>Raja clavata</i>             | 20  | 577  | 299 | 5.2<br>6.5 | 5.7 | Bone and Roberts, 1969. Saglam and Ak, 2012. Estimated from values over one year for males and females. |
|                                 |     |      |     | 5.4        |     | Tufan et al 2013. Average of males and females.   |
| <i>Raja miraletus</i>           | 17  | 462  | 240 | 2.0        | 2.0 | Kadri et al 2012. Estimated from values over one year for males and females.                            |
| <i>Raja montagui</i>            | 20  | 345  | 183 | 2.9        | 2.9 | Bone and Roberts 1969.  |
| <i>Rioraja agassizi</i>         | 0   | 130  | 65  | 2.4        | 2.4 | Colonello et al., 2009. Average of male and female values over one year.                                |
| <i>Zearaja chilensis</i>        | 28  | 500  | 264 | 4.8        | 4.8 | Bustamante et al., 2012. Females, estimated from figure.  |
| Dasyatidae (Whiptail stingrays) |     |      |     |            |     |   |
| <i>Dasyatis americana</i>       | 0   | 53   | 27  | 2.6        | 2.6 | Navarro-Garcia et al., 2009.  |
| <i>Dasyatis brevis</i>          | 1   | 70   | 36  | 8.4        | 8.4 | Navarro-Garcia et al., 2009.  |
| <i>Dasyatis chrysonota</i>      | 0   | 100  | 50  | 4.5        | 4.5 | Ebert and Cowley, 2009. Estimated from figure.  |
| Gymnuridae (Butterfly rays)     |     |      |     |            |     |   |

|                                     |    |     |     |     |     |   |
|-------------------------------------|----|-----|-----|-----|-----|---|
| <i>Gymnura marmorata</i>            | 0  | 100 | 50  | 6.2 | 6.2 | Navarro-Garcia et al., 2004.                        |
| Myliobatidae (Eagle and manta rays) |    |     |     |     |     |   |
| <i>Aetobatus narinari</i>           | 1  | 80  | 41  | 3.6 | 3.6 | Navarro-Garcia et al., 2009.                        |
| <i>Rhinoptera bonasus</i>           | 0  | 22  | 11  | 4.8 | 4.8 | Navarro-Garcia et al., 2009.                        |
| Urolophidae (Stingarees)            |    |     |     |     |     |   |
| <i>Urolophus bucculentus</i>        | 65 | 265 | 165 | 4.5 | 4.5 | Trinnie et al., 2012. Males. Estimated from figure. |

**Table S4. Lipid content and composition in liver of chondrichthyan fishes.** Species means were used to plot Fig. 1C,D and Fig. 2. Minimum and maximum depths were obtained from Kyne and Simpfendorfer (2010), White and Somerville (2010), Ebert et al. (2013), Last and Stevens (2009), or Froese and Pauly (2014). Sample sizes are noted where n<3. Abbreviations for lipid classes: TAG = triacylglycerol; DAGE = diacylglycerol ether; WE = wax ester; HC = hydrocarbon; NEFA = non-esterified fatty acids; ST= sterol; PL = phospholipid. NEFA, ST, and PL values are included in notes, when available. References appended.

| Order                                   | Family                                | Species                           | Min depth (m) | Max depth (m) | Median depth (m) | <i>Species mean for lipid class (% of total lipid)</i> |      |          |             | Reference and notes  |
|---|---------------------------------------|-----------------------------------|---------------|---------------|------------------|--|------|----------|-------------|--|
|   |                                       |                                   |               |               |                  | TAG  | DAGE | Squalene | Liver lipid |  |
| Chimaeriformes (Chimaeras)              |                                       |                                   |               |               |                  |  |      |          |             |  |
|   | Rhinochimaeridae (Longnose chimaeras) |                                   |               |               |                  |  |      |          |             |  |
|   |                                       | <i>Rhinochimaera pacifica</i>     | 191           | 1290          | 741              | 15.9   | 71.6 | 0.8      | 71.6        | Pethybridge et al., 2010<br>Hayashi et al., 1983. n=2.   |
|   | Chimaeridae (Shortnose chimaeras)     |                                   |               |               |                  |  |      |          |             |  |
|   |                                       | <i>Chimaera lignaria</i>          | 400           | 1800          | 1100             | 9.3  | 88.7 | 0.0      | 68.2        | Pethybridge et al., 2010. Other (%): 0.6 NEFA, 0.7 ST, 7.6 PL  |
|   |                                       | <i>Hydrolagus affinis</i>         | 300           | 2909          | 1605             |  |      |          | 81.0        | Morris et al., 1983. n=1   |
|   |                                       | <i>Hydrolagus barbouri</i>        | 250           | 1100          | 675              | 23.9   | 55.2 | -        | 81.6        | Hayashi et al., 1983. n=2  |
|   |                                       | <i>Hydrolagus colliei</i>         | 0             | 971           | 486              |  |      |          |             | Patent, 1970   |
|   |                                       | <i>Hydrolagus novaezealandiae</i> | 25            | 950           | 488              | 10.4   | 65.8 | -        | 64.1        | Hayashi and Takagi, 1980. n=1 female. Other (%): 10.5 fatty acids/sterols/hydrocarbons, 12.8 unknown, 0.5 PL |
| Hexanchiformes (Cow and frilled sharks) |                                       |                                   |               |               |                  |  |      |          |             |  |
|   | Chlamydoselachidae (Fried)            |                                   |               |               |                  |  |      |          |             |  |

|  |                                   |    |      |      |      |      |      |      |   |
|--|-----------------------------------|----|------|------|------|------|------|------|---|
| sharks)                                    |                                   |    |      |      |      |      |      |      |   |
|  | <i>Chlamydoselachus anguineus</i> | 50 | 1500 | 775  | -    | -    | 7.1  | 73   | Tsujimoto, 1920. Females, n=2<br>Shimma and Shimma, 1970. n=1   |
| Hexanchidae (Sixgill and sevengill sharks) |                                   |    |      |      |      |      |      |      |   |
|  | <i>Heptranchias perlo</i>         | 27 | 1000 | 514  | -    | -    | 0.0  | 54.1 | Tsujimoto, 1920. n=1.   |
|  | <i>Hexanchus griseus</i>          | 0  | 2490 | 1245 | 29.0 | 70.0 | 0.5  | 70.5 | Tsujimoto, 1920. Female, n=1<br>Average of range from studies cited in Bone and Roberts, 1969.<br>Hamm, 1950. n=1.<br><br>Wetherbee and Nichols, 2000. n=1  |
|  | <i>Notorynchus cepedianus</i>     | 0  | 136  | 68   | 53.6 | 40.4 | 0.0  | 47.6 | Pethybridge et al., 2010. n=1   |
| Squaliformes (Dogfish sharks)              |                                   |    |      |      |      |      |      |      |   |
| Echinorhinidae (Bramble sharks)            |                                   |    |      |      |      |      |      |      |   |
|  | <i>Echinorhinus brucus</i>        | 18 | 900  | 459  | -    | -    | 50.2 | 69.3 | Tsujimoto, 1922, cited in Nevenzel, 1989.<br><br>Karnovsky and Rapson, 1947, cited in Nevenzel, 1989<br>Bone and Roberts, 1969<br>Tsujimoto, 1921, cited in Nevenzel, 1989                        |
| Squalidae (Dogfish sharks)                 |                                   |    |      |      |      |      |      |      |   |
|  | <i>Squalus acanthias</i>          | 0  | 600  | 300  | 67.3 | 27.7 | 0.0  | 54.1 | Wetherbee and Nichols, 2000. n=1<br><br>Pethybridge et al., 2010. Other (%): 2.0 NEFA, 0.7 ST, 5.7 PL<br>Heller et al., 1957. Published as <i>Squalus fernandinus</i> .<br>Bone and Roberts, 1969 |



|                                |  |     |      |      |      |      |      |   |
|--------------------------------|--|-----|------|------|------|------|------|---|
|                                |  |     |      |      |      |      |      | Sargent et al., 1973. Other: All ST.  |
|                                |  |     |      |      |      |      |      | Malins et al., 1965. Other (%): 1.8 ST, 1.3 PL, 3.8 NEFA, 1.3 Unknown.  |
|                                |  |     |      |      |      |      |      | Schmidt-Nielsen et al., 1934<br>Malins and Barone, 1970<br>Pethybridge et al., 2010. Other (%): 0.7 NEFA, 0.8 ST, 10.8 PL               |
|                                | <i>Squalus chloroculus</i><br>(= <i>mitsukurii</i> ) | 4   | 1360 | 682  | 59.5 | 25.0 | 0.0  | 62.5  |
|                                | <i>Squalus cubensis</i>                              | 60  | 380  | 220  | -    | -    | 0.0  | -   |
|                                | <i>Squalus megalops</i>                              | 0   | 732  | 366  | 44.0 | 42.7 | 0.0  | 45.0  |
|                                |  |     |      |      |      |      |      | Heller et al., 1957<br>Pethybridge et al., 2010. Other (%): 0.9 NEFA, 1.2 ST, 10.5 PL   |
| Centrophoridae (Gulper sharks) |  |     |      |      |      |      |      |   |
|                                | <i>Centrophorus</i> spp.                             | 50  | 1400 | 725  | -    | -    | 70.9 | -   |
|                                |  |     |      |      |      |      |      | Peyronel et al., 1984. Probably <i>Centrophorus uyato</i> . Determined on liver oil extract.  |
|                                | <i>Centrophorus acus</i>                             | 150 | 950  | 550  | -    | -    | -    | 79.2  |
|                                | <i>Centrophorus atromarginatus</i>                   | 150 | 450  | 300  | -    | -    | -    | 56.5  |
|                                |  |     |      |      |      |      |      | Tsujimoto, 1920. Male, n=1.   |
|                                | <i>Centrophorus granulosus</i>                       | 50  | 1440 | 745  | -    | -    | 83.0 | 79.4  |
|                                |  |     |      |      |      |      |      | Tsujimoto, 1920. Females, n=2   |
|                                |  |     |      |      |      |      |      | Batista and Nunes, 1992. n=3<br>Andre and Canal, 1929, cited in Nevenzel, 1989.   |
|                                | <i>Centrophorus lusitanicus</i>                      | 300 | 1400 | 850  | -    | -    | 75.6 | 69.2  |
|                                |  |     |      |      |      |      |      | Hernandez-Perez et al., 1997  |
|                                | <i>Centrophorus moluccensis</i>                      | 125 | 820  | 473  | 8.5  | 9.9  |      |   |
|                                |  |     |      |      |      |      |      | Bakes and Nichols, 1995. Other (%): 0 PL, 0 NEFA, 0 unknown. Measured on commercial liver oil extract (composite sample of 1-6 livers). |
|                                | <i>Centrophorus squamosus</i>                        | 230 | 3280 | 1755 | 18.0 | 11.0 | 69.4 | 76.9  |
|                                |  |     |      |      |      |      |      | Wetherbee and Nichols, 2000.  |
|                                |  |     |      |      |      |      |      | Batista and Nunes, 1992<br>Hernandez-Perez et al., 1997   |

|                               |     |      |      |      |      |      |      |  |
|-------------------------------|-----|------|------|------|------|------|------|--|
| <i>Centrophorus uyato</i>     | 50  | 1400 | 725  | -    | -    | 90.0 | -    | Heller et al., 1957.   |
| <i>Centrophorus zeehaani</i>  | 208 | 701  | 455  | 6.7  | 24.4 | 66.6 | 60.9 | Pethybridge et al. 2010  |
| <i>Deania</i> sp.             | 70  | 1785 | 928  | -    | -    | -    | 75.0 | Morris et al., 1983. n=1. Min and max depths are averages of min to max for the known species of <i>Deania</i> .   |
| <i>Deania</i> sp.             | 70  | 1785 | 928  | 17.0 | 17.0 | -    | 70.0 | Sargent et al., 1973. n=1. Other: All ST. Min and max depths are averages of min to max for the known species of <i>Deania</i> .   |
| <i>Deania</i> sp.             | 70  | 1785 | 928  | -    | -    | -    | 80.5 | Higashi et al., 1953b, cited in Corner et al., 1969. Min and max depths are averages of min to max for the known species of <i>Deania</i> .  |
| <i>Deania calcea</i>          | 70  | 1470 | 770  | 9.8  | 20.6 | 60.8 | 71.7 | Corner et al. 1969 n=1<br>Wetherbee and Nichols, 2000.<br>Pethybridge et al., 2010<br>Tsujimoto, 1920. n=1<br>Batista and Nunes, 1992<br>Bakes and Nichols, 1995. Other (%): 0 PL, 0 NEFA, 0 unknown. Measured on commercial liver oil extract (composite sample of 1-6 livers). |
| <i>Deania hystricosum</i>     | 470 | 1300 | 885  | -    | -    | 49.4 | 82.6 | Hernandez-Perez et al., 1997   |
| <i>Deania profundorum</i>     | 275 | 1785 | 1030 | -    | -    | 74.5 | -    | Hernandez-Perez et al., 1997   |
| Etmopteridae (Lanternsharks)  |     |      |      |      |      |      |      |  |
| <i>Centroscyllium ritteri</i> | 320 | 1100 | 710  | -    | -    | 23.9 | 72.6 | Kayama et al., 1969. n=1<br><br>Tsujimoto, 1920. Average of males and females.   |

|                                 |     |      |      |      |      |      |      |   |
|---------------------------------|-----|------|------|------|------|------|------|---|
| <i>Etmopterus baxteri</i>       | 250 | 1500 | 875  | 7.6  | 28.4 | 64.4 | 70.1 | Wetherbee and Nichols 2000. Published as <i>E. granulosus</i> , but <i>E. granulosus</i> is now known only from S. America and is easily confused with <i>E. baxteri</i>  |
|                                 |     |      |      |      |      |      |      | Pethybridge et al. 2010<br>Bakes and Nichols 1995. Other (%): 0.4 PL, 4.2 NEFA, 1.1 unknown. Published as <i>E. granulosus</i> . Average of two samples measured on commercial liver oil extract (each a composite sample of 1-6 livers). |
| <i>Etmopterus princeps</i>      | 567 | 4500 | 2534 | -    | -    | 44.8 | 70.3 | Corner et al., 1969. n=1  |
| <i>Etmopterus spinax</i>        | 70  | 2490 | 1280 | -    | -    | 50.0 | 64.2 | Hernandez-Perez et al., 1997<br>Heilbron et al., 1926, cited in Nevenzel, 1989  |
| <i>Etmopterus virens</i>        | 196 | 915  | 556  | -    | -    | 0.0  | -    | Schmidt-Nielsen et al., 1934<br>Heller et al., 1957   |
| <i>Etmopterus unicolor</i>      | 750 | 1500 | 1125 | -    | -    | -    | 66.7 | Shimma and Shimma, 1966. n=2  |
| Somniosidae (Sleeper sharks)    |     |      |      |      |      |      |      |   |
| <i>Centroscymnus coelolepis</i> | 128 | 3675 | 1902 | 12.6 | 15.8 | 51.6 | 69.1 | Corner et al., 1969. n=2  |
|                                 |     |      |      |      |      |      |      | Pethybridge et al., 2010<br>Batista and Nunes, 1992.<br>Morris et al., 1983. n=1<br>Hernandez-Perez et al., 1997<br>Hernandez-Perez et al., 1997  |
| <i>Centroscymnus owstoni</i>    | 426 | 1459 | 943  | 20.1 | 22.7 | 47.8 | 73.2 | Pethybridge et al., 2010<br>Wetherbee and Nichols, 2000.<br>Tsujimoto, 1920. n=1<br>Higashi et al., 1953a, cited in Corner et al., 1969.  |

|                                  |     |      |      |      |      |      |      |   |
|----------------------------------|-----|------|------|------|------|------|------|---|
| <i>Centroscymnus sp.</i>         | 277 | 2567 | 1422 | 13.0 | 36.0 | -    | 74.0 | Sargent et al., 1973. Other: All ST. Depths are averages of the two other <i>Centroscymnus</i> species.   |
| <i>Centroselachus crepidater</i> | 270 | 2080 | 1175 | 6.9  | 21.2 | 68.1 | 71.3 | Wetherbee and Nichols, 2000.<br><br>Pethybridge et al., 2010<br>Bakes and Nichols, 1995. Other (%): 0.7 PL, 0 NEFA, 1.3 unknown. Measured on commercial liver oil extract (composite sample of 1-6 livers). |
| <i>Proscymnodon plunketi</i>     | 219 | 1427 | 823  | 15.5 | 82.3 | 2.4  | 64.7 | Wetherbee and Nichols, 2000.<br><br>Pethybridge et al., 2010<br>Bakes and Nichols, 1995. Other (%): 0 PL, 0 NEFA, 0 unknown. Measured on commercial liver oil extract (composite sample of 1-6 livers).     |
| <i>Scymnodon ringens</i>         | 200 | 1600 | 900  | -    | -    | 0.8  | 70.5 | Batista and Nunes, 1992. n=1, n=2 for squalene.   |
| <i>Somniosus longus</i>          | 250 | 1160 | 705  | -    | -    | -    | 72.6 | Higashi et al., 1954, cited in Corner et al., 1969. Published as <i>Heteroscymnus longus</i> .  |
| <i>Somniosus pacificus</i>       | 0   | 2000 | 1000 | 49.1 | 49.5 | 0.0  | 58.6 | Higashi et al., 1954, cited in Corner et al., 1969.<br>Tsujimoto 1920 n=1<br>Bakes and Nichols, 1995. Other (%): 1.4 PL. n=1  |
| <i>Zameus squamulosus</i>        | 550 | 1450 | 1000 | -    | -    | 23.6 | 77.8 | Tsujimoto 1920. Average of males and females. n=2.  |
| Dalatiidae (Kitefin sharks)      |     |      |      |      |      |      |      |   |
| <i>Dalatias licha</i>            | 37  | 1800 | 919  | 10.3 | 21.9 | 62.5 | 69.7 | Wetherbee and Nichols, 2000.<br>Pethybridge et al., 2010<br>Lewis, 1969   |

|                                     |                                    |    |      |      |   |   |     |      |   |
|-------------------------------------|------------------------------------|----|------|------|---|---|-----|------|---|
|                                     |                                    |    |      |      |   |   |     |      | Heller et al., 1957<br>Hayashi and Takagi, 1981. n=1<br>Batista and Nunes, 1992<br>Higashi et al., 1954<br>Morris et al., 1983. n=1 |
|                                     | <i>Isistius brasiliensis</i>       | 0  | 3500 | 1750 | - | - | -   | 85.2 |   |
| Squatiniiformes (Angelsharks)       |                                    |    |      |      |   |   |     |      |   |
| Squatinae (Angelsharks)             |                                    |    |      |      |   |   |     |      |   |
|                                     | <i>Squatina japonica</i>           |    |      |      | - | - | 0.0 | 22.3 | Tsujimoto 1920 n=2. Male and female.  |
|                                     |                                    | 20 | 158  | 89   |   |   |     |      |   |
|                                     | <i>Squatina squatina</i>           | 5  | 200  | 103  | - | - | -   | 23.2 | Bone and Roberts, 1969  |
| Pristiophoriformes (Sawsharks)      |                                    |    |      |      |   |   |     |      |   |
| Pristiophoridae (Sawsharks)         |                                    |    |      |      |   |   |     |      |   |
|                                     | <i>Pristiophorus japonicus</i>     | 5  | 500  | 253  | - | - | 0.0 | 27.0 | Tsujimoto, 1920. n=1  |
| Heterodontiformes (Bullhead sharks) |                                    |    |      |      |   |   |     |      |   |
| Heterodontidae (Bullhead sharks)    |                                    |    |      |      |   |   |     |      |   |
|                                     | <i>Heterodontus japonicus</i>      | 6  | 37   | 22   | - | - | 0.0 | 26.0 | Tsujimoto, 1920. n=1  |
|                                     | <i>Heterodontus portusjacksoni</i> | 0  | 275  | 138  | - | - | -   | 1.7  | Withers et al., 1994  |
| Orectolobiformes (Carpetsharks)     |                                    |    |      |      |   |   |     |      |   |
| Ginglymostomatidae (Nurse sharks)   |                                    |    |      |      |   |   |     |      |   |
|                                     | <i>Ginglymostoma cirratum</i>      | 0  | 12   | 6    | - | - | 0.0 | -    | Heller et al., 1957   |
| Lamniformes (Mackerel sharks)       |                                    |    |      |      |   |   |     |      |   |
| Alopiidae (Thresher sharks)         |                                    |    |      |      |   |   |     |      |   |
|                                     | <i>Alopias vulpinus</i>            | 0  | 366  | 183  | - | - | 0.0 | 18.9 | Schmidt-Nielsen et al., 1934. n=1<br><br>Tsujimoto, 1920. Male, n=1   |

|                                       |   |      |     |      |     |      |      |   |
|---------------------------------------|---|------|-----|------|-----|------|------|---|
| <i>Alopias superciliosus</i>          | 0 | 732  | 366 | 81.0 | 0.0 | -    | 35.6 | Jayasinghe et al., 2003a. Average of males and females. Other (%): 9.9 sterol esters, 0.7 NEFA, 1.5 ST, 0.7 1,2-diacylglycerol 0.6 PL   |
| Odontaspidae (Raggedtooth sharks)     |   |      |     |      |     |      |      |   |
| <i>Carcharias taurus</i>              | 0 | 191  | 96  | -    | -   | -    | 37.3 | Davidson and Cliff, 2011. Males and females over one year.  |
| Lamnidae (Mackerel sharks)            |   |      |     |      |     |      |      |   |
| <i>Carcharodon carcharias</i>         | 0 | 1300 | 650 | 96.3 | 0.0 | 0.0  | 61.0 | Pethybridge et al., 2014. Other (%): 1.9 PL, 0.4 ST, 0.1 NEFA.  |
| <i>Isurus oxyrinchus</i>              | 0 | 500  | 250 | -    | -   | 0.0  | 24.4 | Heller et al., 1957<br>Tsujimoto, 1920. Male, n=1   |
| <i>Lamna ditropis</i>                 | 0 | 225  | 113 | 80.4 | 2.8 | 0.0  | 45.2 | Jayasinghe et al., 2003b. Average of males and females over one year.   |
| <i>Lamna nasus</i>                    | 0 | 700  | 350 | -    | -   | -    | 45.7 | Tsujimoto 1920, n=1<br>Bone and Roberts, 1969<br>Schmidt-Nielsen et al., 1934   |
| Cetorhinidae (Basking sharks)         |   |      |     |      |     |      |      |   |
| <i>Cetorhinus maximus</i>             | 0 | 904  | 452 | -    | -   | 21.3 | 74.3 | Tsujimoto, 1920. Males. Determined on commercial liver oil samples.<br><br>Blumer, 1967. n=1, juvenile male.<br><br>Tsujimoto, 1920b, cited in Nevenzel, 1989.<br>Tsujimoto, 1935, cited in Nevenzel, 1989<br><br>Bone and Roberts, 1969. n=2<br>Schmidt-Nielsen and Eriksen, 1946, cited in Nevenzel, 1989 |
| Pseudocarchariidae (Crocodile sharks) |   |      |     |      |     |      |      |   |

|                                   |     |      |      |      |      |      |      |  |
|-----------------------------------|-----|------|------|------|------|------|------|--|
| <i>Pseudocarcharias kamoharai</i> | 0   | 590  | 295  | -    | -    | 65.0 | -    | Abe et al., 1969, cited in Oliveira et al., 2010                       |
| Carcharhiniformes (Ground sharks) |     |      |      |      |      |      |      |  |
| Scyliorhinidae (Catsharks)        |     |      |      |      |      |      |      |  |
| <i>Apristurus macrorhynchus</i>   | 220 | 1140 | 680  | -    | -    | 0.8  | 65.8 | Tsujimoto, 1920.   |
| <i>Apristurus melanoasper</i>     | 512 | 1520 | 1016 | 85.1 | 13.8 | 0.1  | 79.1 | Kayama et al., 1969. n=1<br>Wetherbee and Nichols, 2000.               |
| <i>Apristurus pinguis</i>         | 996 | 2057 | 1527 | 65.0 | 7.0  | 24.0 | 70.0 | Pethybridge et al., 2010<br>Wetherbee and Nichols, 2000.               |
| <i>Apristurus sinensis</i>        | 940 | 1290 | 1115 | 93.7 | 2.7  | 0.0  | 40.4 | Wetherbee and Nichols, 2000.<br>Pethybridge et al., 2010. Other (%): 0 |
| <i>Cephaloscyllium umbratile</i>  | 20  | 200  | 110  | -    | -    | 0.0  | 54.5 | NEFA, 1.2 ST, 5.1 PL<br>Tsujimoto, 1920. n=1                           |
| <i>Figaro boardmani</i>           | 150 | 640  | 395  | 79.2 | 5.3  | 0.0  | 39.2 | Pethybridge et al., 2010. Other (%): 0.9<br>NEFA, 1.5 ST, 11.6 PL      |
| <i>Galeus eastmani</i>            | 100 | 900  | 500  | -    | -    | 0.0  | 34.2 | Tsujimoto, 1920. Male, n=1.  |
| <i>Galeus melanostomus</i>        | 55  | 1440 | 748  | -    | -    | -    | 40.7 | Schmidt-Nielsen et al., 1934   |
| <i>Parmaturus pilosus</i>         | 358 | 895  | 627  | -    | -    | 79.4 | 84.0 | Tsujimoto, 1920. Male, n=1.  |
| <i>Scyliorhinus canicula</i>      | 0   | 110  | 55   | 87.5 | -    | -    | 44.7 | Bone and Roberts, 1969   |
| <i>Scyliorhinus retifer</i>       | 75  | 754  | 415  | -    | -    | 0.0  | -    | Craik, 1978<br>Heller et al., 1957                                     |
| <i>Scyliorhinus torazame</i>      | 0   | 320  | 160  | 86.7 | 0.0  | 0.0  | 40.4 | Tsujimoto, 1920. Mixed sexes.<br><br>Hayashi, 1983                     |
| Pseudotriakidae (False catsharks) |     |      |      |      |      |      |      |  |
| <i>Pseudotriakis microdon</i>     | 200 | 1900 | 1050 | -    | -    | -    | 90.5 | Morris et al., 1983. n=1   |
| Triakidae (Houndsharks)           |     |      |      |      |      |      |      |  |

|                                  |   |     |     |      |     |     |      |  |
|----------------------------------|---|-----|-----|------|-----|-----|------|--|
| <i>Mustelus antarcticus</i>      | 0 | 350 | 175 | 96.0 | -   | -   | 47.0 | Nichols et al., 1998. Females. Others are all PL; no NEFA or ST  |
| <i>Mustelus asterias</i>         | 0 | 200 | 100 | -    | -   | -   | 46.2 | Bone and Roberts, 1969   |
| <i>Mustelus manazo</i>           | 0 | 360 | 180 | -    | -   | 0.0 | -    | Tsujimoto, 1920. n=1.  |
| <i>Galeorhinus galeus</i>        | 2 | 471 | 237 | 97.7 | -   | -   | 45.2 | Nichols et al., 1998. Females. Others are all PL no NEFA or ST   |
| <i>Triakis scyllium</i>          | 0 | 150 | 75  | -    | -   | 0.2 | 27.7 | Bone and Roberts, 1969<br>Tsujimoto, 1920. n=1<br>Kayama et al., 1969. n=1   |
| Carcharhinidae (Requiem sharks)  |   |     |     |      |     |     |      |  |
| <i>Carcharhinus leucas</i>       | 0 | 152 | 76  | -    | -   | 0.0 | -    | Heller et al., 1957  |
| <i>Carcharhinus melanopterus</i> | 0 | 10  | 5   | -    | -   | -   | 71.1 | Hamm, 1950. Published as <i>Carcharias melanopterus</i> .  |
| <i>Carcharhinus sorrah</i>       | 0 | 140 | 70  | -    | -   | -   | 66.4 | Hamm, 1950. Published as <i>Carcharias spallanzani</i> .   |
| <i>Galeocerdo cuvier</i>         | 0 | 140 | 70  | -    | -   | 0.0 | 79.5 | Heller et al., 1957<br>Hamm, 1950  |
| <i>Prionace glauca</i>           | 0 | 350 | 175 | 65.2 | 1.4 | 0.0 | 32.7 | Bone and Roberts, 1969<br>Tsujimoto, 1920. n=1.<br>Sargent et al., 1973. Others: All ST.   |
| <i>Carcharhinus plumbeus</i>     | 1 | 280 | 141 | 83.0 | 0.0 | 0.0 | 15.9 | Jayasinghe et al., 2003a. Average of males and females. Other (%): 8.6 sterol esters, 1.4 NEFA, 1.6 ST, 1.1 1,2-diacylglycerol 0.8 PL<br>Wetherbee and Nichols, 2000. n=1. Other (%): 5 NEFA, 11 PL, ST <1 |
| <i>Carcharhinus falciformis</i>  | 0 | 500 | 250 | 69.1 | 4.7 | 0.0 | 48.9 | Tsujimoto, 1920. n=1.<br>Heller et al., 1957<br>Navarro-Garcia et al., 2000. Other: ST; no PL.   |



|   |                                |   |     |     |      |     |     |      |  |
|---|--------------------------------|---|-----|-----|------|-----|-----|------|--|
|   |                                |   |     |     |      |     |     |      | Heller et al., 1957<br>Hamm, 1950. Published as <i>Carcharias menisorrah</i> .<br>Jayasinghe et al., 2003a. Average of males and females. Other (%): 4 sterol esters, 4.4 NEFA, 1.5 sterols, 1.8 1,2-DAG, 1.5 PL |
|   | <i>Carcharhinus longimanus</i> | 0 | 152 | 76  | 73.5 | 1.8 | 0.0 | 34.0 | Heller et al., 1957<br><br>Jayasinghe et al 2003a. Average of males and females. Other: 7.9 sterol esters, 5.9 NEFA, 1.9 ST, 2.4 1,2-diacylglycerol, 1.9 PL  |
|   | <i>Negaprion brevirostris</i>  | 0 | 92  | 46  | -    | -   | 0.0 | -    | Heller et al., 1957  |
|   | <i>Rhizoprionodon lalandii</i> | 3 | 70  | 37  | -    | -   | -   | 39.7 | Hamm, 1950. Published as <i>Scoliodon intermedius</i> .  |
| Sphyrnidae                              |                                |   |     |     |      |     |     |      |  |
|   | <i>Sphyrna lewini</i>          | 0 | 275 | 138 | 78.1 | 0.0 | -   | 40.1 | Jayasinghe et al., 2003a. Average of males and females. Other (%): 6.5 sterol esters, 3.8 NEFA, 1.7 ST 1.2 1,2-diacylglycerol, 1.1 PL  |
|   | <i>Sphyrna tiburo</i>          | 0 | 80  | 40  | -    | -   | 0.0 | -    | Heller et al., 1957  |
|   | <i>Sphyrna zygaena</i>         | 0 | 20  | 10  | -    | -   | 0.0 | 59.1 | Heller et al., 1957<br>Tsujiimoto, 1920. n=1.<br>Hamm, 1950. n=2.  |
| Rajiformes (Skates and rays or Batoids) |                                |   |     |     |      |     |     |      |  |
| Rhinobatidae (Guitarfishes)             |                                |   |     |     |      |     |     |      |  |
|   | <i>Glaucostegus cemiculus</i>  | 0 | 100 | 50  | -    | -   | -   | 40.6 | El Kebir, 2003. Average of males and females.  |
|   | <i>Rhinobatos annulatus</i>    | 0 | 73  | 37  | -    | -   | -   | 55.0 | Rossouw, 1987. Male and female. Estimated from figure of values over one year.   |

|                                   |      |      |      |      |     |   |      |  |
|-----------------------------------|------|------|------|------|-----|---|------|--|
| <i>Rhinobatos glaucostigma</i>    | 0    | 112  | 56   | -    | -   | - | 31.8 | Navarro-Garcia et al., 2014.   |
| Arhynchobatidae (Softnose skates) |      |      |      |      |     |   |      |  |
| <i>Bathyraja richardsoni</i>      | 1370 | 2909 | 2140 | -    | -   | - | 72.7 | Templeman, 1973. Captured at ~2000 m. Average of males and females.  |
| Rajidae (Hardnose skates)         |      |      |      |      |     |   |      |  |
| <i>Amblyraja radiata</i>          | 18   | 1400 | 709  | -    | -   | - | 35.7 | Schmidt-Nielsen et al., 1934. n=2  |
| <i>Dipturus batis</i>             | 100  | 1000 | 550  | -    | -   | - | 52.9 | Schmidt-Nielsen et al., 1934. n=2  |
| <i>Dipturus nidrosiensis</i>      | 200  | 1000 | 600  | -    | -   | - | 58.2 | Schmidt-Nielsen et al., 1934   |
| <i>Dipturus oxyrinchus</i>        | 15   | 900  | 458  | -    | -   | - | 41.6 | Schmidt-Nielsen et al., 1934   |
| <i>Leucoraja fullonica</i>        | 30   | 800  | 415  | -    | -   | - | 45.0 | Schmidt-Nielsen et al., 1934   |
| <i>Raja clavata</i>               | 20   | 577  | 299  | -    | -   | - | 36.7 | Bone and Roberts, 1969<br>Tufan et al., 2013. Average of male and female values.   |
| <i>Raja rhina</i>                 | 0    | 1000 | 500  | -    | -   | - | 49.6 | Wu et al., 2011  |
| Dasyatidae (Whiptail stingrays)   |      |      |      |      |     |   |      |  |
| <i>Dasyatis americana</i>         | 0    | 53   | 27   | 81.6 | -   | - | 38.2 | Navarro-Garcia et al., 2009. Other (%): 5.2 PL, 1.5 diacylglycerol, 6 ST, 3.1 unknown.   |
| <i>Dasyatis brevis</i>            | 1    | 70   | 36   | 67.0 | 9.0 | - | 37.3 | Navarro-Garcia et al., 2004. Other (%): 3.2 PL, 13.1 ST, 7.7 unknown.  |
| <i>Dasyatis chrysonota</i>        | 0    | 100  | 50   | -    | -   | - | 47.5 | El Kebir, 2003. Average of males and females. Listed as <i>D. marmorata</i> but this may be subspecies of <i>D. chrysonota</i> . |
| <i>Dasyatis dipterura</i>         | 0    | 50   | 25   | -    | -   | - | 46.4 | Navarro-Garcia et al., 2014  |

|  |   |     |    |      |     |   |      |   |
|--|---|-----|----|------|-----|---|------|---|
| <i>Himantura bleekeri</i>                | 0 | 30  | 15 | 77.6 | 1.3 | - | 58.7 | Nechet et al., 2007. n=1. Other (%): 8.8 free fatty acids/fatty alcohols, 15.2 diacylglycerols, 7.3 glyceryl ethers/monoacylglycerols |
|  |   |     |    |      |     |   |      | Pal et al., 1998. n=1. HC composed of: 6.7% pristane, 3.5% squalene, remainder other HCs.   |
| <i>Himantura uarnak</i>                  | 0 | 45  | 23 | -    | -   | - | 30   | Hamm, 1950. n=2. Published as <i>Dasyatis uarnak</i> .  |
| Urotrygonidae (American round stingrays) |   |     |    |      |     |   |      |   |
| <i>Urobatis halleri</i>                  | 0 | 91  | 46 | -    | -   | - | 36.3 | Navarro-Garcia et al., 2014   |
| <i>Urotrygon chilensis</i>               | 0 | 60  | 30 | -    | -   | - | 36.2 | Navarro-Garcia et al., 2014   |
| Gymnuridae (Butterfly rays)              |   |     |    |      |     |   |      |   |
| <i>Gymnura marmorata</i>                 | 0 | 100 | 50 | 70.0 | 0.0 |   | 50.7 | Navarro-Garcia et al., 2004. Other (%): 4.9 PL, 12.5 ST, 12.6 unknown.  |
| Myliobatidae (Eagle and manta rays)      |   |     |    |      |     |   |      |   |
| <i>Aetobatus narinari</i>                | 1 | 80  | 41 | 85.9 | -   | - | 41.2 | Navarro-Garcia et al., 2009. Other (%): 1.7 PL, 1.9 diacylglycerol, 7 ST, 4 unknown.  |
| <i>Rhinoptera bonasus</i>                | 0 | 22  | 11 | 69.0 | -   | - | 43.0 | Navarro-Garcia et al., 2009. Other (%): 3.3 PL, 8.9 diacylglycerol, 12.9 ST, 9 unknown.   |
| <i>Rhinoptera marginata</i>              | 0 | 100 | 50 | -    | -   | - | 22.6 | El Kebir, 2003. Average of males and females.   |
| <i>Rhinoptera steindachneri</i>          | 0 | 65  | 33 | -    | -   | - | 30.7 | Navarro-Garcia et al., 2014   |

## References:

## References

- Aranha, A., Menezes, G., and Pinho, M. R.** (2009). Biological aspects of the velvet belly lantern shark, *Etmopterus spinax* (Linnaeus, 1758) off the Azores, North East Atlantic. *Mar. Biol. Res.* **5**, 257-267.
- Awruch, C. A., Pankhurst, N. W., Frusher, S. D., and Stevens, J. D.** (2008). Endocrine and morphological correlates of reproduction in the draughtboard shark *Cephaloscyllium laticeps* (Elasmobranchii: Scyliorhinidae). *J. Exp. Zool. A* **309**, 184-197.
- Bakes, M. J., and Nichols, P. D.** (1995). Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. *Comp. Biochem. Physiol. B* **110**, 267-275.
- Baldrige, H. D., Jr.** (1970). Sinking factors and average densities of florida sharks as functions of liver buoyancy. *Copeia* **1970**, 744-754.
- Batista, I., and Nunes, M. L.** (1992). Characterisation of shark liver oils. *Fish. Res.* **14**, 329-334.
- Blumer, M.** (1967). Hydrocarbons in digestive tract and liver of a basking shark. *Science* **156**, 390-391.
- Bone, Q., Roberts B. L.** (1969). The density of elasmobranchs. *J. Mar. Biol. Assoc. UK* **49**, 913-937.
- Bustamante, C., Vargas-Caro, C., Oddone, M. C., Concha, F., Flores, H., Lamilla, J., and Bennett, M. B.** (2012). Reproductive biology of *Zearaja chilensis* (Chondrichthyes: Rajidae) in the south-east Pacific Ocean. *J. Fish Biol.* **80**, 1213-1226.
- Capapé, C., Reynaud, C., Vergne, Y., and Quignard, J. P.** (2008). Biological observations on the smallspotted catshark *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) off the Languedocian coast (southern France, northern Mediterranean). *Pan-Am. J. Aquat. Sci.* **3**, 282-289.
- Chen, W. K., and Liu, K. M.** (2006). Reproductive biology of whitespotted bamboo shark *Chiloscyllium plagiosum* in northern waters off Taiwan. *Fish. Sci.* **72**, 1215-1224.
- Clarke, M. W., Connolly, P. L., and Bracken, J. J.** (2001). Aspects of reproduction of the deep water sharks *Centroscyllium coelelepis* and *Centrophorus squamosus* from west of Ireland and Scotland. *J. Mar. Biol. Assoc. UK* **81**, 1019-1029.
- Coelho, R., and Erzini, K.** (2007). Population parameters of the smooth lantern shark, *Etmopterus pusillus*, in southern Portugal (NE Atlantic). *Fish. Res.* **86**, 42-57.
- Coelho, R., and Erzini, K.** (2010). Depth distribution of the velvet belly, *Etmopterus spinax*, in relation to growth and reproductive cycle: The case study of a deep-water lantern shark with a wide-ranging critical habitat. *Mar. Biol. Res.* **6**, 381-389.
- Colonello, J. H., Lucifora, L. O., and Massa, A. M.** (2007). Reproduction of the angular angel shark (*Squatina guggenheim*): geographic differences, reproductive cycle, and sexual dimorphism. *ICES J. Mar. Sci.* **64**, 131-140.
- Colonello, J. H., García, M. L., and Lasta, C. A.** (2009). Reproductive biology of *Rioraja agassizi* from the coastal southwestern Atlantic ecosystem between northern Uruguay (34 S) and northern Argentina (42 S). In *Biology of Skates* (pp. 171-178). Springer Netherlands.
- Colonello, J. C., García, M. L., Lasta, C. A., and Menni, R. C.** (2012). Reproductive biology of the spotback skate *Atlantoraja castelnaui* in the south-west Atlantic Ocean. *J. Fish Biol.* **80**, 2405-2419.

**Corner, E.D.S., Denton, E.J., and Forster, G.R.** (1969). On the buoyancy of some deep-sea sharks. *Proc. Roy. Soc. Lond. B* **171**, 415-429.

**Cowper, T. R., and Downie, R. J.** (1957). *A Line-Fishing Survey of the Fishes of the South-Eastern Australian Continental Slope*. Report No. 6, Division of Fisheries and Oceanography. Melbourne: CSIRO.

**Craik, J. C. A.** (1978). An annual cycle of vitellogenesis in the elasmobranch *Scyliorhinus canicula*. *J. Mar. Biol. Assoc. UK* **58**, 719-726.

**Davidson, B., and Cliff, G.** (2002). The liver lipid fatty acid profiles of seven Indian Ocean shark species. *Fish Physiol. Biochem.* **26**, 171-175.

**Ebert, D. A., and Cowley, P. D.** (2009). Reproduction and embryonic development of the blue stingray, *Dasyatis chrysonota*, in southern African waters. *J. Mar. Biol. Assoc. UK* **89**, 809-815.

**Ebert, D. A., Fowler, S. L., Compagno, L. J., and Dando, M.** (2013). *Sharks of the World: a Fully Illustrated Guide*. Plymouth: Wild Nature Press.

**El Kebir, M. V. O., Barnathan, G., Siau, Y., Miralles, J., and Gaydou, E. M.** (2003). Fatty acid distribution in muscle, liver, and gonads of rays (*Dasyatis marmorata*, *Rhinobatos cemiculus*, and *Rhinoptera marginata*) from the East Tropical Atlantic Ocean. *J. Agric. Food Chem.* **51**, 1942-1947.

**Francis, M. P.** (2006). Distribution and biology of the New Zealand endemic catshark, *Halaaelurus dawsoni*. *Environ. Biol. Fishes* **75**, 295-306.

**Froese, R. and Pauly, D.** Editors. (2014). FishBase. World Wide Web electronic publication. [www.fishbase.org](http://www.fishbase.org), (08/2014).

**González, C., Teruel, J., López, E., and Paz, X.** (2007). Feeding habits and biological features of deep-sea species of the Northwest Atlantic: large-eyed Rabbitfish (*Hydrolagus mirabilis*), Narrownose Chimaera (*Harriotta raleighana*) and Black Dogfish (*Centroscyllium fabricii*). *Northwest Atlantic Fisheries Organization Scientific Council Meeting* **5423**, 1-9.

**Hamm, W. S.** (1950). *Liver Oil Oroperties of Philippine Sharks and Rays*. Research Report 23. Washington, DC: US Government Printing Office.

**Hayashi, K.** (1983). Component glyceryl ethers in liver lipids of spiny dogfish and Japanese cat shark. *Bull. Facul. Fish. Hokkaido Univ.* **34**, 250-259.

**Hayashi, K., and Takagi, T.** (1980). Composition of diacyl glyceryl ethers in the liver lipids of ratfish, *Hydrolagus novaezealandiae*. *Bull. Jap. Soc. Sci. Fish.* **46**, 855-861.

**Hayashi, K., and Takagi, T.** (1981). Distribution of squalene and diacyl glyceryl ethers in the different tissues of deep-sea shark, *Dalatias licha*. *Bull. Jap. Soc. Sci. Fish.* **47**, 281-288.

**Hayashi, K., Takagi, T., and Kitagawa, M.** (1983). Compositions of ether-linked lipids in livers of two species of ratfish [*Hydrolagus barbouri* and *Rhinochimaera pacifica*]. *Bull. Jap. Soc. Sci. Fish.* **49**: 777-782.

**Heller, J. H., Heller, M. S., Springer, S., and Clark, E.** (1957). Squalene content of various shark livers. *Nature* **179**, 919-920.

**Hernández-Pérez, M., Gallego, R. M. R., Alayón, P. J. P., and Hernández, A. B.** (1997). Squalene content in livers from deep-sea sharks caught in Canary Island waters. *Mar. Fresh. Res.* **48**, 573-576.

- Jakobsdóttir, K. B.** (2001). Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii* (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. *Fish. Res.* **51**, 247-265.
- Jayasinghe, C., Gotoh, N., Tokairin, S., Ehara, H., and Wada, S.** (2003). Interspecies changes of lipid compositions in liver of shallow-water sharks from the Indian Ocean. *Fish. Sci.* **69**, 644-653.
- Jayasinghe, C., Gotoh, N., and Wada, S.** (2003b). Variation in lipid classes and fatty acid composition of salmon shark (*Lamna ditropis*) liver with season and gender. *Comp. Biochem. Physiol. B* **134**, 287-295.
- Kadri, H., Marouani, S., Saïdi, B., Bradai, M. N., Ghorbel, M., Bouaïn, A., and Morize, E.** (2012). Age, growth and reproduction of *Raja miraletus* (Linnaeus, 1758) (Chondrichthyes: Rajidae) of the Gulf of Gabès (Tunisia, Central Mediterranean Sea). *Mar. Biol. Res.* **8**, 388-396.
- Kayama, M., Tsuchiya, Y., and Nevenzel, J. C.** (1969). The hydrocarbons of shark liver oils. *Bull. Jap. Soc. Sci. Fish.* **35**, 653-664.
- Kyne, P. M., and Simpfendorfer, C. A.** (2010). Deepwater chondrichthyans. In *Sharks and their Relatives II* (ed. J. C. Carrier, J. A. Musick, and M. R. Heithaus), pp. 37-113. Boca Raton: CRC Press.
- Last, P. R., and Stevens, J. D.** (2009). *Sharks and rays of Australia*. Canberra: CSIRO Press.
- Lewis, R. W.** (1969). Studies on the stomach oils of marine animals—I. Oils of the black shark *Dalatias licha* (Bonnaterre). *Comp. Biochem. Physiol.* **31**, 715-724.
- Lucifora, L. O., Menni, R. C., and Escalante, A. H.** (2002). Reproductive ecology and abundance of the sand tiger shark, *Carcharias taurus*, from the southwestern Atlantic. *ICES J. Mar. Sci.* **59**, 553-561.
- Maia, C., Erzini, K., Serra-Pereira, B., and Figueiredo, I.** (2012). Reproductive biology of cuckoo ray *Leucoraja naevus*. *J. Fish Biol.* **81**, 1285-1296.
- Malins, D. C., and Barone, A.** (1970). Glycerol ether metabolism: regulation of buoyancy in dogfish *Squalus acanthias*. *Science* **167**, 79-80.
- Malins, D. C., Wekell, J. C., and Houle, C. R.** (1965). Composition of the diacyl glycerol ethers and triglycerides of the flesh and liver of the dogfish (*Squalus acanthias*). *J. Lipid Res.* **6**, 100-105.
- Morris, R. J., Ballantine, J. A., and Roberts, J. C.** (1983). The sterol composition of some shark livers. *J. Mar. Biol. Assoc. UK* **63**, 295-299.
- Navarro-Garcia, G., Pacheco-Aguilar, R., Vallejo-Cordova, B., Ramirez-Suarez, J. C., and Bolanos, A.** (2000). Lipid composition of the liver oil of shark species from the Caribbean and Gulf of California waters. *J. Food Comp. Anal.* **13**, 791-798.
- Navarro-Garcia, G., Pacheco-Aguilar, R., Bringas-Alvarado, L., and Ortega-Garcia, J.** (2004). Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnura marmorata* rays. *Food Chem.* **87**, 89-96.
- Navarro-García, G., Ramirez-Suarez, J. C., Ortega-García, J., García-Camarena, R., Márquez-Farías, F., Santos-Valencia, J., and Bringas-Alvarado, L.** (2009). Lipid composition, natural antioxidants and physicochemical characteristics in liver oil from rajiforms from the Gulf of Mexico. *J. Amer. Oil Chem. Soc.* **86**, 323-328.
- Navarro-García, G., González-Félix, M. L., Márquez-Farías, F., Bringas-Alvarado, L., Pérez-Velazquez, M., Montoya-Laos, J. M., and Moreno-Silva, B.** (2014). Lipid content and fatty acid composition of the liver from the rajiforms *Urotrygon chilensis*, *Urobatis halleri*, *Rhinobatos glaucostigma*, *Rhinoptera steindachneri* and *Dasyatis dipeteura* captured in Sinaloa, México. *Int. Food Res. J.* **21**, 229-235.

- Néchet, S. L., Dubois, N., Gouygou, J. P., and Bergé, J. P.** (2007). Lipid composition of the liver oil of the ray, *Himantura bleekeri*. *Food Chem.* **104**, 559-564.
- Nevenzel, J.C.** 1989. Biogenic hydrocarbons of marine organisms. In *Marine Biogenic Lipids, Fats, and Oils Vol. 1* (ed. R.G. Ackman), pp. 3-72. Boca Raton: CRC Press.
- Nibam, A. H.** (2011). *Reproductive biology and diet of the St. Joseph (Callorhinchus capensis) in South Africa*. Unpublished M.Sc. thesis, University of Cape Town.
- Nichols, P. D., Bakes, M. J., and Elliott, N. G.** (1998). Oils rich in docosahexaenoic acid in livers of sharks from temperate Australian waters. *Mar. Freshw. Res.* **49**, 763-767.
- Oddone, M. C., and Velasco, G.** (2006). Relationship between liver weight, body size and reproductive activity in *Atlantoraja cyclophora* (Elasmobranchii: Rajidae: Arhynchobatinae) in oceanic waters off Rio Grande do Sul, Brazil. *Neotropical Biol. Conserv.* **1**, 12-16.
- Oguri M.** (1978). On the hepatosomatic index of holocephalian fish. *Bull. Jap. Soc. Sci. Fish.* **44**, 131-134.
- Oliveira, P., Hazin, F.H.V., Carvalho, F., Rego, M., Coelho, R., Piercy, A., and Burgess, G.** (2010). Reproductive biology of the crocodile shark *Pseudocarcharias kamoharai*. *J. Fish Biol.* **76**, 1655-1670.
- Pal, D., Banerjee, D., Patra, T. K., Patra, A., and Ghosh, A.** (1998). Liver lipids and fatty acids of the sting ray *Dasyatis bleekeri* (Blyth). *J. Am. Oil Chem. Soc.* **75**, 1373-1378.
- Patent, G. J.** (1970). Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch (*Squalus acanthias*) and a holocephalan (*Hydrolagus colliei*). *Gen. Comp. Endocrin.* **14**, 215-242.
- Perez-Brazen, C. R., Cailliet, G. M., and Ebert, D. A.** (In press). Reproduction of the sandpaper skate, *Bathyraja kincaidii* (Garman 1908) in the eastern North Pacific. *Environ. Biol. Fishes* DOI 10.1007/s10641-014-0220-4
- Pethybridge, H., Daley, R., Virtue, P., and Nichols, P.** (2010). Lipid composition and partitioning of deepwater chondrichthyans: inferences of feeding ecology and distribution. *Mar. Biol.* **157**, 1367-1384.
- Peyronel, D., Artaud, J., Iatrides, M. C., Rancurel, P., and Chevalier, J. L.** (1984). Fatty acid and squalene compositions of Mediterranean *Centrophorus* spp. egg and liver oils in relation to age. *Lipids* **19**, 643-648.
- Powter, D. M., and Gladstone, W.** (2008). The reproductive biology and ecology of the Port Jackson shark *Heterodontus portusjacksoni* in the coastal waters of eastern Australia. *J. Fish Biol.* **72**, 2615-2633.
- Richardson, A. J., Maharaj, G., Compagno, L. J. V., Leslie, R. W., Ebert, D. A., and Gibbons, M. J.** (2000). Abundance, distribution, morphometrics, reproduction and diet of the Izak catshark. *J. Fish Biol.* **56**, 552-576.
- Rocha, F., and Gadig, O. B. F.** (2013). Reproductive biology of the guitarfish *Rhinobatos percellens* (Chondrichthyes, Rhinobatidae) from the São Paulo Coast, Brazil, western South Atlantic Ocean. *J. Fish Biol.* **82**, 306-317.
- Rossouw, G. J.** (1987). Function of the liver and hepatic lipids of the lesser sand shark, *Rhinobatos annulatus* (Müller and Henle). *Comp. Biochem. Physiol. B* **86**, 785-790.
- Ruocco, N. L., Lucifora, L. O., de Astarloa, J. M. D., and Wöhler, O.** (2006). Reproductive biology and abundance of the white-dotted skate, *Bathyraja albomaculata*, in the Southwest Atlantic. *ICES J. Mar. Sci.* **63**, 105-116.
- Saglam, H., and Bascinar, N. S.** (2008). Feeding ecology of thornback ray (*Raja clavata* Linnaeus, 1758) on the Turkish coast of the south-eastern Black Sea. *Mar. Biol. Res.* **4**, 451-457.

- San Martín, M. J., Perez, J. E., and Chiaramonte, G. E.** (2005). Reproductive biology of the south west Atlantic marbled sand skate *Psammobatis bergi* Marini, 1932 (Elasmobranchii, Rajidae). *J. Appl. Ichthyol.* **21**, 504-510.
- Sargent, J. R., Gatten, R. R., and McIntosh, R.** (1973). The distribution of neutral lipids in shark tissues. *J. Mar. Biol. Assoc. UK* **53**, 649-656.
- Schmidt-Nielsen, S., Flood, A., and Stene, J.** (1934). On the size of the liver of some gristly fishes, their content of fat and vitamin A. *Kongelige Norske videnskabers selskab Forhandling* **7**, 47-50.
- Shelmerdine, R. L., and Cliff, G.** (2006). Sharks caught in the protective gill nets off KwaZulu-Natal, South Africa. 12. The African angel shark *Squatina africana* (Regan). *Afr. J. Mar. Sci.* **28**, 581-588.
- Shimma, Y., and Shimma, H.** (1966). On liver oil of deep-sea sharks of Suruga Bay. *Bull. Tokai Reg. Fish. Res. Lah* **48**, 53-61.
- Shimma, H., and Shimma, Y.** (1970). Studies on liver oil of a frill shark. *Bull. Jap. Soc. Sci. Fish.* **36**, 1157-1162.
- Sulikowski, J. A., Kneebone, J., Elzey, S., Jurek, J., Danley, P. D., Howell, W. H., and Tsang, P. C.** (2005). The reproductive cycle of the thorny skate (Amblyraja radiata) in the western Gulf of Maine. *Fish. Bull.* **103**, 536-543.
- Sulikowski, J. A., Elzey, S., Kneebone, J., Jurek, J., Howell, W. H., and Tsang, P. C.** (2007). The reproductive cycle of the smooth skate, *Malacoraja senta*, in the Gulf of Maine. *Mar. Freshw. Res.* **58**, 98-103.
- Templeman, W.** (1973). First records, description, distribution, and notes on the biology of *Bathyraja richardsoni* (Garrick) from the Northwest Atlantic. *J. Fish. Board Can.* **30**, 1831-1840.
- Thorson, T. B.** (1962). Partitioning of body fluids in the Lake Nicaragua shark and three marine sharks. *Science* **138**, 688-690.
- Treberg, J. R. and Driedzic, W. R.** (2007). The accumulation and synthesis of betaine in winter skate (*Leucoraja ocellata*). *Comp. Biochem. Physiol. A* **147**, 475-483.
- Trinnie, F. I., Walker, T. I., Jones, P. L., and Laurenson, L. J.** (2012). Biennial reproductive cycle in an extensive matrotrophic viviparous batoid: the sandyback stingaree *Urolophus bucculentus* from south-eastern Australia. *J. Fish Biol.* **80**, 1267-1291.
- Tsujimoto, M.** (1920). Squalene: a highly unsaturated hydrocarbon in shark liver oil. *Indust. Engin. Chem.* **12**, 63-72.
- Tufan, B., Koral, S., and Köse, S.** (2013). The variations in proximate chemical composition and fatty acid profile in different parts of the thornback ray (*Raja clavata*) caught from Black Sea, Turkey. *J. Aquat. Food Prod. Tech.* **22**, 83-95.
- Van Vleet, E. S., Candileri, S., McNeillie, J., Reinhardt, S. B., Conkright, M. E., and Zwissler, A.** (1984). Neutral lipid components of eleven species of Caribbean sharks. *Comp. Biochem. Physiol. B* **79**, 549-554.
- Wetherbee, B. M., and Nichols, P. D.** (2000). Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand. *Comp. Biochem. Physiol.* **125**, 511-521.
- White, W. T., and Sommerville, E.** (2010). Elasmobranchs of tropical marine ecosystems. *Sharks and their Relatives II* (ed. J. C. Carrier, J. A. Musick, and M. R. Heithaus), pp. 159-239. Boca Raton: CRC Press.
- Withers, P. C., Morrison, G., and Guppy, M.** (1994a). Buoyancy role of urea and TMAO in an elasmobranch fish, the Port Jackson shark, *Heterodontus portusjacksoni*. *Physiol. Zool.* **67**, 693-705.



**Withers, P. C., Hefter, G., and Pang, T. S.** (1994). Role of urea and methylamines in buoyancy of elasmobranchs. *J. Exp. Biol.* **188**, 175-189.

**Wu, T. H., Stine, J. J., and Bechtel, P. J.** (2011). Preliminary chemical and nutritional characterization of liver from longnose skates (*Raja rhina*). *J. Food Compos. Anal.* **24**, 356-361.