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RESEARCH ARTICLE

Near-equal compressibility of liver oil and seawater minimises buoyancy changes in deep-sea sharks and chimaeras

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ABSTRACT

Whereas upper ocean pelagic sharks are negatively buoyant and must swim continuously to generate lift from their fins, deep-sea sharks float or swim slowly buoyed up by large volumes of low-density oils in their livers. Investigation of the pressure, volume, temperature (PVT) relationships for liver oils of 10 species of deep-sea Chondrichthyes shows that the density difference between oil and seawater, $\Delta\rho$, remains almost constant with pressure down to full ocean depth (11 km, 1100 bar), theoretically providing buoyancy far beyond the maximum depth of occurrence (3700 m) of sharks. However, $\Delta\rho$ does change significantly with temperature and we show that the combined effects of pressure and temperature can decrease buoyancy of oil by up to 10% between the surface and 3500 m depth across interfaces between warm southern and cold polar waters in the Rockall Trough in the NE Atlantic. This increases drag more than 10-fold compared with neutral buoyancy during horizontal slow swimming (0.1 m s^{-1}), but the effect becomes negligible at high speeds. Chondrichthyes generally experience positive buoyancy change during ascent and negative buoyancy change during descent, but contrary effects can occur at interfaces between waters of different densities. During normal vertical migrations buoyancy changes are small, increasing slow-speed drag no more than 2- to 3-fold. Equations and tables of density, pressure and temperature are provided for squalene and liver oils of Chimaeriformes (*Harriotta raleighana*, *Chimaera monstrosa*, *Hydrolagus affinis*), Squaliformes (*Centrophorus squamosus*, *Deania calcea*, *Centroscymnus coelolepis*, *Centroscyllium fabricii*, *Etmopterus spinax*) and Carcharhiniformes (*Apristurus laurussonii*, *Galeus murinus*).

KEY WORDS: Buoyancy, Chimaeras, Deep-sea, Liver oil, Sharks, Squalene

INTRODUCTION

Near-neutral buoyancy is important for aquatic animals to move efficiently in a three-dimensional environment (Denton, 1962; Alexander, 1990). In chondrichthyan fishes such as sharks, most buoyancy is provided by low-density oils in the liver (Bone and Roberts, 1969; Treberg and Speers-Roesch, 2016), offsetting the weight of body tissues that are generally denser than seawater. Nevertheless, upper-ocean pelagic sharks such as the blue shark *Prionace glauca* and porbeagle *Lamna nasus* tend to be negatively buoyant and compensate for their underwater weight (1.6–4.3% of weight in air) by generating hydrodynamic lift from wing-like fins during continuous swimming (Iosilevskii and Papastamatiou, 2016). Deep-sea sharks are much closer to neutral buoyancy with enlarged livers (Corner et al., 1969) enabling them to swim slowly without sinking. They also have smaller fins (Gleiss et al., 2017) and reduced red muscle mass (Pinte et al., 2019) as hydrodynamic lift is less important. Neutral buoyancy enables deep-sea species such as the leafscale gulper shark *Centrophorus squamosus* to migrate long distances in mid-water at around 1000 m depth, far above the abyssal sea floor (Rodríguez-Cabello et al., 2016). As sharks rarely occur at depths >3000 m and may be physiologically constrained to shallower depths (Priede et al., 2006; Treberg and Speers-Roesch, 2016), such mid-water swimming capability is essential for movement between widely separated patches of bathyal habitat (200–3000 m depths) on continental slopes, ocean ridges and seamounts (Priede, 2017).

Compared with fishes that use gas-filled bladders for buoyancy (Priede, 2018), liver oil is considered advantageous because oils are relatively incompressible so there is little change in buoyancy if the fish moves up or down in the water column. However, density of oils does change in response to temperature and pressure but there are no relevant published values for fish oils. Corner et al. (1969) found that most deep-sea sharks become positively buoyant when retrieved to the surface and Nakamura et al. (2015) suggested that positive buoyancy may aid vertical migration or capture of prey by stealthy upward gliding. In deep-sea sharks, small changes in liver oil density are likely to be critically important in view of their reduced ability to create lift by hydrodynamic lift using their fins or body. Here we evaluate the composition and pressure, volume, temperature (PVT) relationship of liver oils from three orders of deep-sea Chondrichthyes, the Chimaeriformes (chimaeras), Squaliformes (dogfish sharks) and Carcharhiniformes (ground sharks). The Chimaeriformes are representatives of the subclass Holocephali that survived the end of Palaeozoic mass extinction event 250 million years ago by retreat into the deep sea (Priede, 2017). The Squaliformes appeared in the early Triassic about 230 million years ago as the main evolutionary diversification of sharks into the deep sea, evolving bioluminescence in some families (Klug and Kriwet, 2010). The Carcharhiniformes are predominantly shallow-water sharks that originated in the Jurassic about 170 million years ago; the family Scyliorhinidae (catsharks) has secondarily radiated into the deep sea since the late Cretaceous. The aim is to test two main hypotheses. Firstly, we aim to determine if changes in density of liver oils at deep-sea pressures and temperatures in the natural environment can influence buoyancy

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equilibrium and swimming performance. Secondly, we test oils up to pressures of 1100 bar (110 MPa), corresponding to maximum ocean depth (11,000 m), to determine if there is a depth beyond which oil-based buoyancy cannot function and examine if this constraint can partially explain the maximum depth limits of Chondrichthyes.

MATERIALS AND METHODS

Capture of fish and extraction of liver oil

Twenty-one species of sharks and chimaeras were captured from the NE Atlantic Ocean from 24 August to 1 September 2018 at depths from 350 to 1830 m on the continental slopes west of Scotland (2.4–9.9°W, 59.1–60.1°N) by the FRV *Scotia V* using a BT184 otter trawl (Jackson, Peterhead, UK) (Neat et al., 2015). Fish were weighed, measured and the livers removed and frozen. Ten species, one from each genus in the collection, were selected for analysis (Table 1). Livers were thawed, homogenised at room temperature and centrifuged for 10 min at 10,000 r.p.m. (15,880 ***g***) at 20°C (Kubota 7780 centrifuge, Tokyo, Japan). The clear supernatant oil was decanted and stored at 3°C. Generally, oil was extracted from a single individual but in the case of small species, livers were pooled from several fishes as noted in Table 1.

Lipid analysis

Oils were analysed using standardized nuclear magnetic resonance (NMR) spectroscopy. Two hundred microlitres of oil were dissolved in 400 µl of deuterated chloroform $CDCl_3$ and transferred into 5 mm NMR tubes after brief shaking. All 1D and 2D NMR experiments were performed in a Bruker Avance III NMR spectrometer operating at 500.13 MHz for the proton nucleus at a constant temperature of 298K, using standard Bruker pulse program libraries. 1D ^{1}H NMR spectra were obtained with the following parameters: pulse program zg30, SW 20 p.p.m., AQ 3.3 s, TD 64K, ns 128. Spectral processing and analysis were performed using TopSpin 4.0 software. Assignment of triacylglycerols (TAG), diacylglycerol ethers (DAGE) and squalene signals was performed via 2D NMR spectroscopy (gCOSY, multiplicity-edited gHSQC, gHMBC) experiments, analysis of standard compounds and by comparison with literature values, where available. Quantification of shark liver oil constituents (% molar) was afforded by the integration of suitable NMR signals for each chemical compound.

Viscosity measurement

Viscosity measurements were conducted at 25°C on 8 ml aliquots using a digital rotational viscosimeter (DV-2P; Anton Paar, Graz, Austria) equipped with a small sample adapter (L-model) and a low-viscosity spindle (TL5). After thermal equilibration, 18 replicate measurements were made over 3 min and the mean was recorded.

Energy content

The gross calorific value (GCV) was determined on 1 g samples of oil using an XRY-1A Oxygen Bomb Calorimeter (Shanghai Changji Geological Instrument Co. Ltd, Shanghai, People's Republic of China) using standard methods.

Density measurement

PVT measurements were made using a 2 ml U-tube vibrating densitometer cell (Lipták et al., 2003) (DMA-HPM; Anton Paar) enclosed within a temperature-controlled chamber (MKT-115; Binder, Tuttlingen, Germany). Pressure was applied by a mechanical screw (Sitec, Zurich, Switzerland) via a mercury column and measured to 0.07 bar resolution with a quartz sensor (QS30K-B; Quartzdyne, Salt Lake City, UT, USA). Temperature within the cell was measured to <0.1°C and the period of vibration (P, µs) of the U-tube was converted to density (ρ) by:

$$\rho = A \times P^2 - B, \quad (1)$$

where A and B are constants derived using two standards of known density (Paar, 2012), water and decane (457116; Sigma-Aldrich), at each temperature. After equilibration to temperature, ascending pressure increments were applied allowing time to stabilise at each new pressure. All samples were tested at 0.1°C and 15°C while *C. coelolepis* was additionally tested at 5 and 10°C. A temperature of 0.1°C was chosen instead of 0.0°C used in a previous study on gas buoyancy (Priede, 2018) because of the need to avoid freezing of the water standard used for calibrating the apparatus.

Data analysis

All data analysis and calculations were performed using the standard functions in Microsoft Excel for Office 365, version 1911. To the measurements of density as a function of pressure, second order polynomial equations were fitted:

$$\rho_P = aP^2 + bP + c, \quad (2)$$

where ρ_P is density at pressure P (bar) and a, b and c are constants. Pressure units followed the oceanographic convention with sea surface (atmospheric) pressure as zero. The fitted value of c (intercept of the density–pressure curve) was taken as the value for oil density at atmospheric pressure (1 bar≈10 m depth of seawater).

Table 1. List of species sampled

Order	Family	Species	Common name	Depth (m)	
				Range[a]	Capture[b]
Chimaeriformes	Rhinochimaeridae	*Harriotta raleighana* Goode and Bean 1895*	Bentnose rabbitfish	200–3100	1400
	Chimaeridae	*Chimaera monstrosa* Linnaeus 1758	Rabbit ratfish	40–1400	720
		Hydrolagus affinis (de Brito Capello 1868)	Smalleye rabbitfish	300–3000	1830
Squaliformes	Centrophoridae	*Centrophorus squamosus* (Bonnaterre 1788)	Leafscale gulper shark	145–2400	720
		Deania calcea (Lowe 1839)	Shovelnosed shark	60–1490	720
	Somniosidae	*Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello 1864	Portuguese shark	150–3700	1625
	Etmopteridae	*Centroscyllium fabricii* (Reinhardt 1825)*	Black dogfish	180–1600	1400
		Etmopterus spinax (Linnaeus 1758)*	Velvet belly dogfish	200–2490	520
Carcharhiniformes	Scyliorhinidae	*Apristurus laurussonii* (Saemundsson 1922)	Iceland catshark	560–1550	1110
		Galeus murinus (Collett 1904)*	Mouse catshark	475–1200	1095

[a]Minimum and maximum depths recorded in FishBase (Froese and Pauly, 2019). [b]Median depth of the trawl haul in which the specimens were caught. *Oil pooled from more than one individual.

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Similar polynomials were fitted to density-temperature relationships for *C. coelolepis* and a combined equation was derived:

$$\rho_{P,T} = (a_1T^2 - a_2T - a_3)P^2 + (b_1T^2 + b_2T + b_3)P + (c_1T^2 - c_2T + c_3), \quad (3)$$

where $\rho_{P,T}$ is the density at pressure P (bar) and temperature T (°C) and a_1, a_2, a_3, b_1, b_2, b_3, c_1, c_2 and c_3 are constants. This enabled calculation of oil density at any temperature or pressure. For other species where measurements were only made at two temperatures, a simple linear approximation of the density–temperature relationship was used giving a combined equation with just six constants:

$$\rho_{P,T} = (a_1T + a_2)P^2 + (b_1T + b_2)P + (c_1T + c_2). \quad (4)$$

Calculation of buoyancy

According to Archimedes' principle, the static buoyancy (B) or upward force experienced by a body submerged in seawater is proportional to the density difference ($\Delta\rho$) between the body and that of seawater:

$$\Delta\rho = \rho_{sw} - \rho_{oil}, \quad (5)$$

and

$$B = (\Delta\rho)V\boldsymbol{g}, \quad (6)$$

where V is the volume (m^3), ρ_{sw} is the density of seawater, ρ_{oil} is the density of oil (kg m^{-3}), $\boldsymbol{g}$ is the gravitational acceleration (9.81 m s^{-2}) and B is buoyancy force (in Newtons, N). When a fish moves up or down in the water column, in the short term the mass of oil remains constant but the volume changes from the initial value V_1 in response to pressure and/or temperature. If ρ_1 is the density at the initial temperature t_1 and pressure P_1, and V_2 and ρ_2 are the volume and density, respectively, at pressure P_2 and temperature t_2 then:

$$V_2 = V_1\frac{\rho_2}{\rho_1}, \quad (7)$$

and

$$B_2 = (\Delta\rho)_2\, V_2\boldsymbol{g}. \quad (8)$$

B_2 is then buoyancy force at pressure P_2 and temperature t_2.

Buoyancy in the Rockall Trough

We considered the case of fish in the Rockall Trough region of the NE Atlantic where there are eight water masses with different salinities and temperatures (McGarth et al., 2012) and hence different densities. For simplicity, we assume these waters are stacked one above the other according to relative density (Table S5). The change in buoyancy of the liver oil was calculated (Eqns 3–8) taking initial P_1 and t_1 at the approximate depth of capture of each of the three species considered: *C. coelolepis* (1600 m), *H. affinis* (1800 m) and *A. laurussonii* (1100 m). It was assumed that the fishes were neutrally buoyant at the initial depth:

$$\%\ \text{Change} = 100 \times \frac{(B_n - B_1)}{B_1}, \quad (9)$$

where B_n is the buoyancy force in Newtons per kilogram of oil at a given depth, temperature and salinity and B_1 is the buoyancy force (N kg^{-1}) at the initial depth (1600 m for *C. coelolepis*).

Calculation of drag

Total drag experienced by a shark during swimming is the sum of three components, parasite drag D_p, induced drag D_i and acceleration reaction drag (Gleiss et al., 2017). Here we ignored the latter as we only consider swimming at constant velocity. Parasite drag (D_p) of a body moving through a fluid in accordance with the standard drag equation is:

$$D_p = \frac{1}{2}\,\rho_w \cdot SA \cdot C_D \cdot U^2, \quad (10)$$

where ρ_w is density of seawater (kg m^{-3}), SA is body wetted area (m^2), C_D is drag coefficient and U is velocity (m s^{-1}). Induced drag (D_i) is additional drag created from generation of lift if the body is not neutrally buoyant:

$$D_i = \frac{\gamma\, W^2}{\pi(TM/SL)\ (1/2\rho_w U^2)(SL \cdot TM)}, \quad (11)$$

where γ is a correction factor, here assumed to be 1, W is negative buoyancy or weight underwater (N), TM is body maximal width (m) and SL is standard length (m). Drag coefficient (C_D) was calculated as (Gleiss et al., 2017):

$$C_D = \left\{0.072\left(\frac{v}{SL \cdot U}\right)^{\frac{1}{5}}\right\} \cdot \left[1 + \frac{1.5}{FR^{3/2}} + \frac{7}{FR^3}\right], \quad (12)$$

where FR is body fineness ratio and v is kinematic viscosity of seawater. Calculations were based on the example of *C. coelolepis* assuming the following values: ρ_w = 1033 kg m^{-3}, SA = 0.300 m^2, TM = 0.135 m, SL = 0.85 m, FR = 6.3 and v = 1.15. Calculations were performed for different values of underwater weight (W). The standard length was from the fish sampled in this study, SA was calculated from the equation given by Musick et al. (1990) and FR was taken from Gleiss et al. (2017) for a short buoyant shark. SL, FR, TM and SA were assumed to remain constant.

RESULTS

Oil properties

At 3°C, typical of deep-sea temperatures, all the oils were clear transparent liquids except for slight cloudiness in the chimaeras *H. raleighana* and *C. monstrosa*, waxy deposits in *E.* spinax, and waxy consistency in the Carcharhiniformes. NMR analysis identified three main components, namely squalene, TAGs and DAGE, plus small quantities of sterols (0.5–3.6%) with other minor constituents amounting to <0.5% (Table 2). The three orders of fishes showed distinct compositional differences: >70% DAGE in the Chimaeriformes, >80% TAG in the Carcharhiniformes and elevated squalene content in the Squaliformes (33–94%) (Table 2). The density of squalene (859.7 kg m^{-3}) is lower than the other oils and its percentage is a significant (P<0.001) determinant of differences in oil density (Fig. 1) with no significant correlations with depths of occurrence or capture. Squalene also has a lower viscosity than TAG and DAGE, resulting in a significant (P<0.001) linear relationship between viscosity and percentage of squalene (Fig. 1). The Chacharhiniformes, with high TAG content, had the highest densities and viscosities. The mean energy content of the oils was 41.60 MJ kg^{-1} (s.d.=1.15) with no significant correlations with composition or depth.

Pressure, volume, temperature (PVT) relationships and buoyancy

The density of the oils was lower at 15°C than at 0.1°C and all showed increase in density with pressure (Fig. 2; Tables S1, S2, S3).

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Table 2. Analysis and properties of liver oils

Order	Genus species	Analysis (%)					Density (kg m^{-3})	Viscosity (mPa s)	Energy (GCV) (MJ kg^{-1})
		Squalene	TAG	DAGE	Sterols	Total			
Chimaeriformes	*Harriotta raleighana*	0	27.7	71.6	0.5	99.8	905.92	57.3	40.81
	Chimaera monstrosa	0.0	20.1	78.8	1.0	99.9	904.34	57.4	41.57
	Hydrolagus affinis	0.0	27.3	71.5	1.1	99.9	907.38	55.3	40.67
Squaliformes	*Centrophorus squamosus*	94.0	1.3	3.9	0.8	100.0	864.79	16.2	41.08
	Deania calcea	61.7	15.1	22.5	0.7	100.0	884.92	31.9	42.86
	Centroscymnus coelolepis	65.4	12.6	21.5	0.5	100.0	884.19	28.1	44.36
	Centroscyllium fabricii	33.6	10.8	52.0	3.6	100.0	893.25	50.7	42.18
	Etmopterus spinax	50.6	22.2	26.2	1.0	100.0	891.30	38.2	41.13
Carcharhiniformes	*Apristurus laurussonii*	2.1	81.9	15.0	0.7	99.7	911.57	69.1	40.48
	Galeus murinus	0.0	81.7	14.7	3.0	99.4	912.96	73.4	40.86
	Squalene*	≥98%					859.66		

*Squalene reference standard: S3626, Sigma-Aldrich; TAG, triacylglycerols; DAG, diacylglycerol ethers; GCV, gross calorific value. Density at 15°C and atmospheric pressure; viscosity at 25°C.

The density–pressure curves are very similar, nearly parallel to one another and to the corresponding curve for seawater of salinity (35 p.s.u.). Consequently, Δρ (the density difference between oil and seawater) was almost constant regardless of pressure applied (Fig. 3). For squalene at 0.1°C, there was virtually no change in Δρ from 158.1 kg m^{-3} at the surface to 158.3 kg m^{-3} at 500 bar (5000 m depth), close to the limits of resolution of our density measurement equipment. For most species at 0.1°C, Δρ changed by <0.4% except for *C. monstrosa* and *A. laurussonii* in which we observed greater changes (Fig. 3A). At 15°C there was a small but consistent decrease in Δρ with pressure. For squalene at 15°C, Δρ decreased from 166.3 kg m^{-3} at the surface to 163.6 kg m^{-3} at 500 bar. The mean decrease across all the fish species at 15°C was 2.05% (s.d.=0.216) between 0 and 500 bar (Fig. 3B).

The change in buoyancy of 1 m^3 of oil during pressure increase from 0 to 500 bar was calculated according to Eqns 7 and 8. At 0.1°C, the predicted loss in buoyancy from the surface to 500 bar was a mean of 2.57% (s.d.=0.22) except for two outliers *C. monstrosa* and *A. laurusonii* with greater buoyancy loss (Fig. 4A). At 15°C, the data were consistent across all species, with a mean buoyancy loss of 4.58% (s.d.=0.18) (Fig. 4B). As

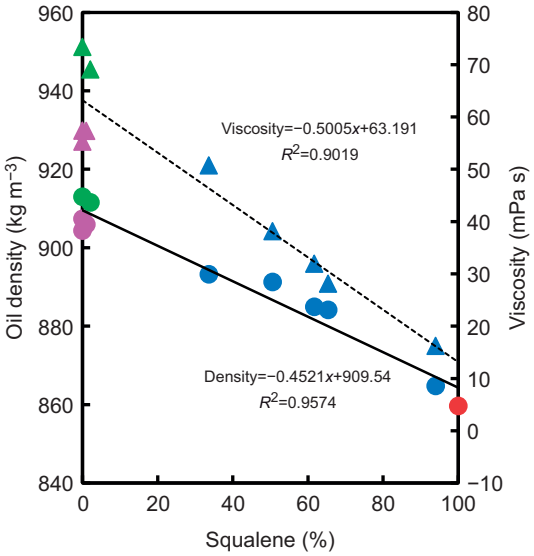

Fig. 1. Density (circles) and viscosity (triangles) of liver oils in relation to squalene content. Red symbol, pure squalene; pink symbols, Chimaeriformes; blue symbols, Squaliformes; green symbols, Carcharhiniformes.

sharks rarely occur deeper than 3000 m, it is useful to note the mean buoyancy losses at 300 bar: 1.84% (s.d.=0.12) and 3.17% (s.d.=0.11) at 0.1 and 15°C, respectively.

We examined the effect of temperature in more detail in *C. coelolepis* (Fig. 5A). Across the range of pressures investigated, increase in temperature resulted in parallel decreases in oil density. The overall relationship between density, pressure and temperature in *C. coelolepis* is described by Eqn 3, and in Table S4. In contrast to the density–pressure relationships (Fig. 2), the density–temperature curves are not parallel to the corresponding curves for seawater (Fig. 5A). Temperature therefore has a greater effect on Δρ and buoyancy than pressure. The loss in buoyancy, at constant pressure, resulting from a fixed mass of oil cooled from a typical NE Atlantic surface temperature of 15°C to a deep-water temperature of 0.1°C, ranges from 6.88% at 0 bar to 5.74% at 300 bar (Fig. 5B). The combined effect of change from surface 15°C and 0 bar, to deep water 0.1°C and 300 bar is a buoyancy loss of 7.17%.

Model of buoyancy change in the Rockall Trough

In real life, buoyancy is determined by a combination of pressure and temperature of the oil itself and also by the density of the surrounding seawater, which depends on salinity as well as temperature and pressure resulting in an infinite set of possibilities. Here we consider the Rockall Trough area of the NE Atlantic, from which our fish were caught, where waters from the Arctic, Mediterranean, Atlantic and Antarctic converge to create an area of complex oceanography with eight recognisable water masses (McGarth et al., 2012) (Fig. 6A; Table S5). From Eqns 3, 7 and 8 we calculated the oil buoyancy change in *C. coelolepis*, ascending or descending from its capture depth 1600 m, assuming constant mass of liver oil and temperature equilibrium with the surrounding water. The starting point at 1600 m depth is in Labrador Seawater at 3.4°C and buoyancy of the liver oil is 1.47 N kg^{-1} (Fig. 6B). We assume that at this depth the fish is neutrally buoyant, i.e. the buoyancy of the oil is equal to the total underwater weight of all the other tissues. If the fish ascends, it encounters colder fresher Wyville Thomson Overflow Water originating from the Arctic Ocean and the oil loses buoyancy, initially decreasing to 1.45 N kg^{-1}. The buoyancy then increases as the shark moves upwards, meeting warm, high salinity Mediterranean Water at 700 m depth. There is then a further loss of buoyancy in the colder fresher Eastern North Atlantic Water, but an increase in buoyancy as the surface is approached where the oil buoyancy reaches 1.58 N kg^{-1}. If the fish moves downwards from 1600 m there is a continuous decrease in buoyancy with depth reaching −2.44% (1.44 N kg^{-1}) at maximum depth in the Antarctic Bottom Water. The

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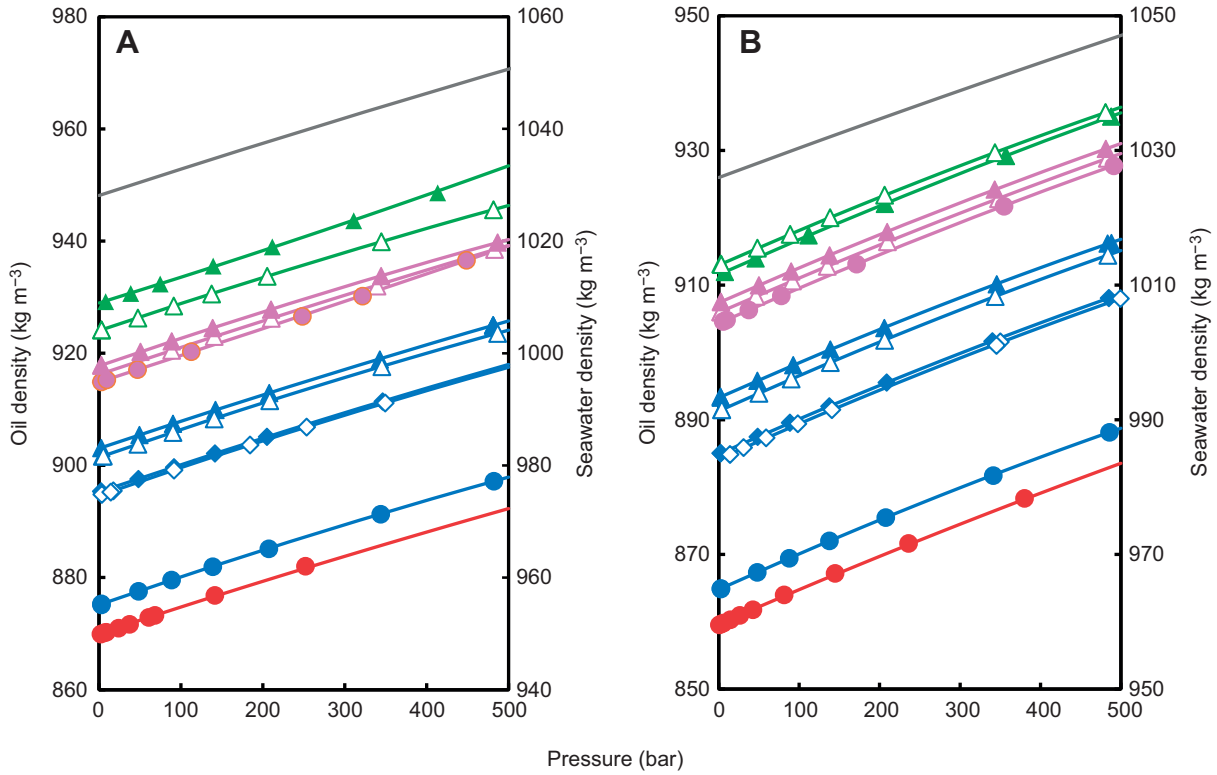

Fig. 2. Effect of pressure on density of liver oils compared with squalene (red circles) and 35 p.s.u. seawater (grey line plotted on right-hand scale). (A) 0.1°C; (B) 15°C. Green symbols: Carcharhiniformes (filled triangles, *A. laurussonii*; open triangles, *G. murinus*). Pink symbols: Chimaeriformes (filled triangles, *H. affinis*; open triangles, *C. monstrosa*; circles, *H. raleighana*). Blue symbols: Squaliformes (filled triangles, *C. fabricii*; open triangles, *E. spinax*; filled diamonds, *D. calcea*; open diamonds, *C. coelolepis*; filled circles, *C. squamosus*. Zero is sea surface (atmospheric) pressure; 1 bar is approximately 10 m depth of seawater.

amplitude of buoyancy change over the depth range of this species is 9%. Similar calculations were done for *H. affinis* and *A. laurussonii* using Eqns 4, 7 and 8 (Table S6) and the pattern of buoyancy change was similar with the curve for *H. affinis*, closely following that of *C. coelolepis*. *Apristurus laurussonii* starts at a shallower depth and experiences a buoyancy change of <6.0% over its known depth range (Fig. 6B).

Effect of buoyancy change on drag during swimming

Here we consider our sampled specimen of *C. coelolepis* with standard length of 0.85 m and a total body weight in air of 4.4 kg. If we assume that 20% of this is liver oil (Treberg and Speers-Roesch, 2016), at a depth of 1000 m (100 bar and 15°C) buoyancy provided is ∼140 g. Inspection of Fig. 6 shows that the maximum possible change in buoyancy in the Rockall Trough between the sea surface and maximum depth is 10%, equivalent to 14 g or 0.14 N. A more probable scenario is a buoyancy change of 5%: 7 g or 0.07 N. From Eqns 9, 10 and 11, the effect on drag is most important at slow swimming speeds (<0.2 m s^{-1}) (Fig. 7). For negative buoyancy of 0.14 N at 0.1 m s^{-1}, drag is 11 times higher than for neutral buoyancy. At the cruising speed of 20 km per day (0.23 m s^{-1}) observed in deep-sea species during migration (Rodríguez-Cabello et al., 2016), drag is 1.36 times higher and at 1 m s^{-1} the effect becomes negligible. The same equations show that a force of 0.14 N is sufficient to propel the fish in a vertical glide at 0.46 m s^{-1}, upwards or downwards depending on whether the buoyancy is positive or negative. Negative buoyancy of 0.07 N which would increase drag by a factor of 3.5 at 0.1 m s^{-1} and 1.09 at 0.23 m s^{-1} cruising and give a vertical glide speed of 0.32 m s^{-1}.

Here it was assumed that body shape parameters SL, TM, FR and SA remain constant in the face of pressure and temperature changes. To check the magnitude of likely effects, we calculated the change in FR over the maximum depth change considered, assuming a cylindrical body form with the same PVT properties as the liver oil, and found that with no change in SL, FR increased from 6.3 to 6.39. This decreased the drag coefficient C_D by 0.3% resulting in small changes to the curves in Fig. 7, which for the purposes of the present study we concluded could be ignored.

Extreme pressures

Tests were undertaken on pure squalene and liver oils from three species (*C. monstrosa*, *C. coelolepis* and *A. laurussonii*) up to over 1100 bar (110 MPa), corresponding to maximum ocean depth (11,000 m) at temperatures of 0.1 and 15°C (Fig. 8). The density curves continue to parallel the seawater curve, indicating potential for positive buoyancy down to full ocean depth. In *A. laurusonnii* there is evidence of phase change characterised by a discontinuity in the density–pressure curve at 500 bar at 0.1°C and 900 bar at 15°C where density increased. The change manifested itself as a decrease in pressure over 10–30 min after a pressure increment had been applied, which then stabilised and a reading could be taken.

DISCUSSION

The compressibility of the liver oils and pure squalene was found to be very similar to that of seawater (Fig. 2) so that density difference between oil and sea water (Δρ) was almost constant. For example, for squalene at 0.1°C, Δρ was 158.1 kg m^{-3} at the surface and 160.1 kg m^{-3} at 1100 bar (full ocean depth); there was remarkably little variation (<1.5%) over such a large pressure range. At 15°C

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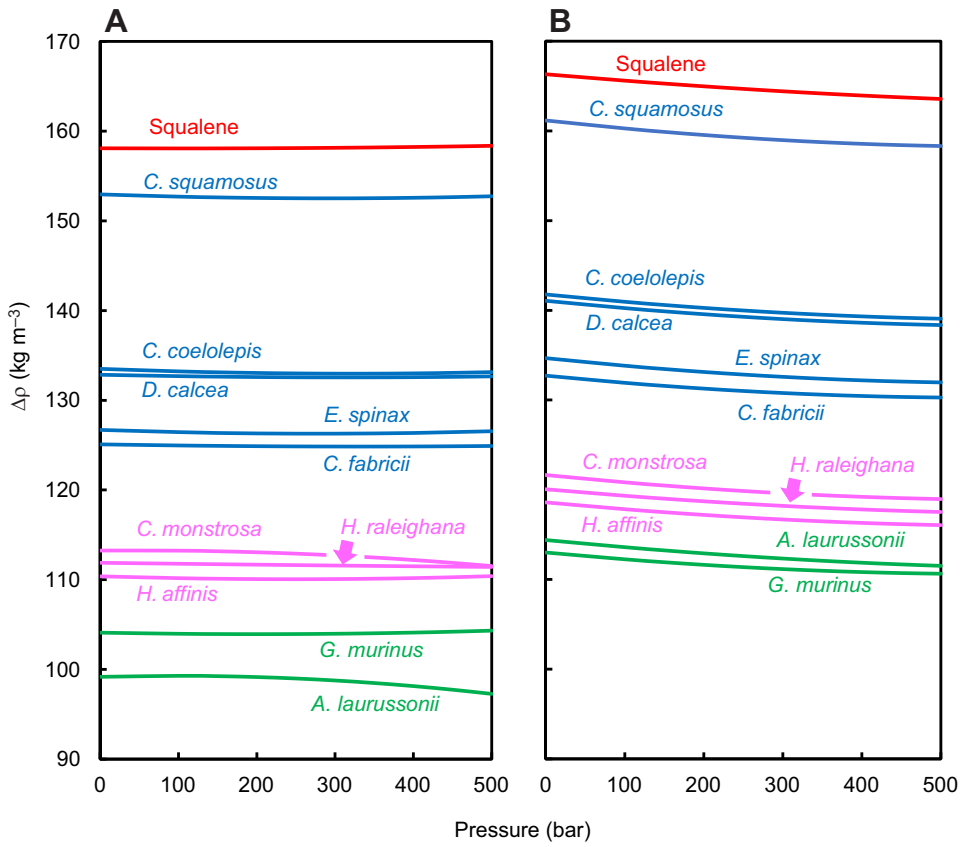

Fig. 3. The effect of pressure on the density difference (Δρ) between liver oil and seawater (35 p.s.u.). (A) 0.1°C; (B) 15°C. Green lines, Carcharhiniformes; pink lines, Chimaeriformes; blue lines, Squaliformes; red line, squalene.

there was a small but consistent decrease in Δρ with pressure. No fishes live at depths greater than 8400 m (Yancey et al., 2014) and no sharks or chimaeras live deeper than 3700 m (Priede et al., 2006). However, our results show that, in principle, an oil-filled liver could provide buoyancy at full ocean depth in the absence of other physiological constraints. It is of interest to note that at 0.1°C, the value of Δρ for squalene (160.1 kg m^{-3} at 1100 bar) is comparable to that of oxygen (i.e. 228 kg m^{-3}) in a hypothetical gas-filled buoyancy bladder at the same pressure and temperature in 35 p.s.u. seawater (Priede, 2018).

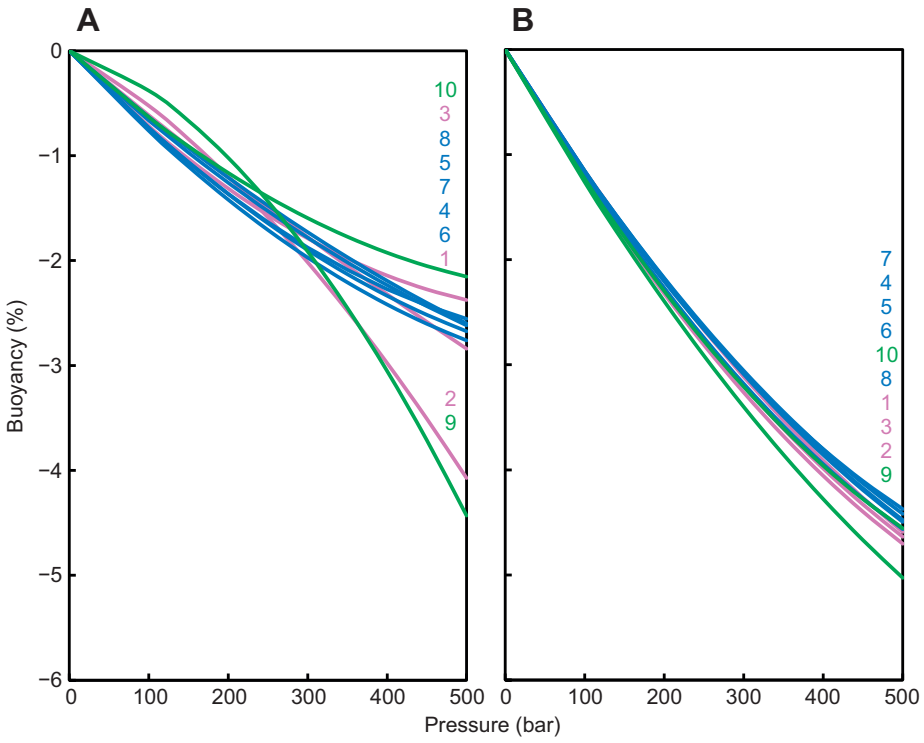

Fig. 4. Change in buoyancy of oils in 35 p.s.u. seawater assuming compression of a constant mass of oil from the surface (0 bar). (A) 0.1°C; (B) 15°C. Pink lines: Chimaeriformes (1, *H. raleighana*; 2, *C. monstrosa*; 3, *H. affinis*). Blue lines: Squaliformes (4, *C. squamosus*; 5, *D. calcea*; 6, *C. coelolepis*; 7, *C. fabricii*; 8, *E. spinax*). Green lines: Carcharhiniformes (9, *A. laurussonii*; 10, *G. murinus*).

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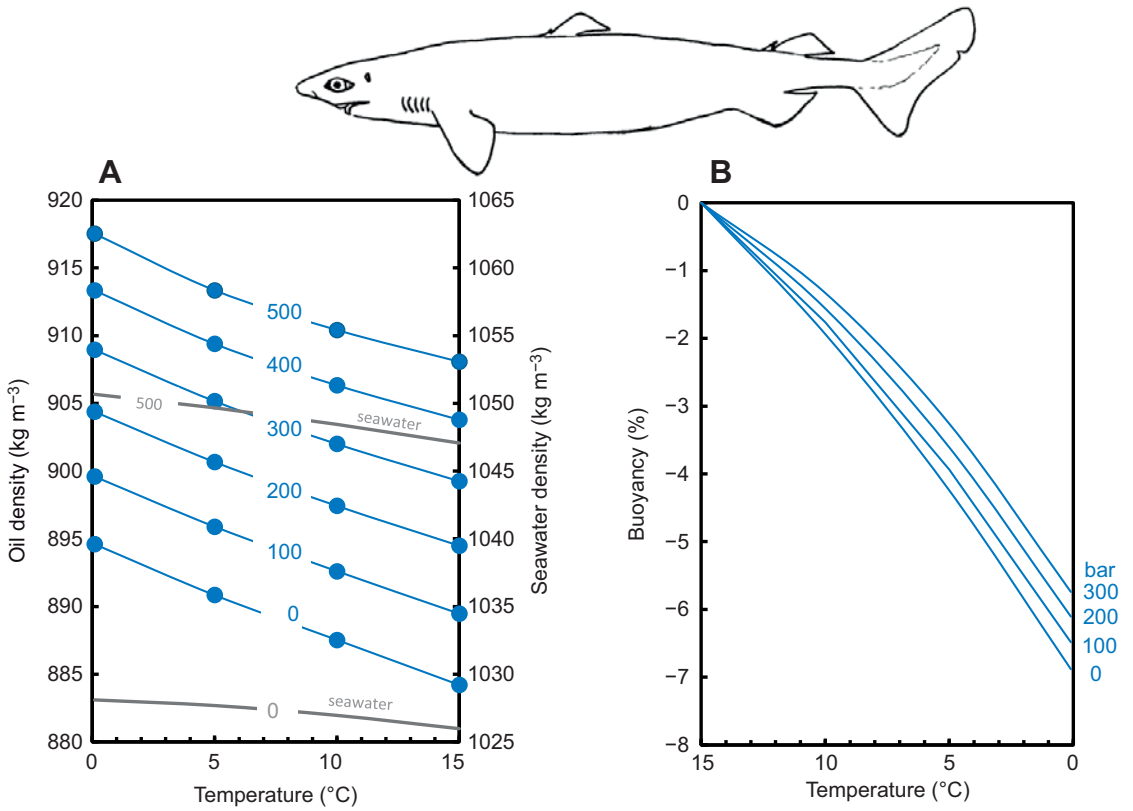

Fig. 5. Density and buoyancy of liver oil of *Centroscymnus coelolepis*. (A) Density in relation to temperature at the pressures (bar) indicated on each line (blue). Grey lines denote density of seawater (35 p.s.u.) at 0 and 500 bar. (B) Change in buoyancy of a constant mass of oil cooled from 15 to 0°C at constant pressures 0, 100, 200 and 300 bar as indicated on the right-hand side. Curves are calculated from the data in panel A.

In the tests to full ocean depth, squalene and the oils of *C. monstrosa* and *C. coelolepis* had smooth PV curves with small or gradual change in Δρ, but in *A. laurussonii* there was evidence of phase change, with the oil probably solidifying at 400–500 bar at 0.1°C and 900 bar at 15°C. In small planktonic crustacean copepods, wax esters in the oil sacs show a phase transition from around 400 bar in temperate species (Yayanos et al., 1978) and 50 bar in Antarctic species (Pond and Tarling, 2011), possibly regulating buoyancy during descent to great depths. Clarke (1978) also proposed that sperm whales may use phase changes in their spermaceti oil to regulate buoyancy. The maximum depths of the Carcharhiniformes, *A. laurussonii* (1550 m) and *G. murinus* (1095 m), are much more shallow than the putative phase transitions, so this mechanism is unlikely in these sharks. Generally, phase changes in liver oils appear to be unimportant in deep-sea Chondrichthyes.

The observation of almost constant Δρ suggests a remarkable automatic regulation of buoyancy in relation to pressure change. The oil nevertheless changes in volume when compressed, and buoyancy is lost despite constant Δρ (Fig. 4). A fish could compensate by increasing the quantity of oil in the liver, but this may be a slow process. There is evidence that sharks can alter the composition of oil in the liver in order to regulate buoyancy. Experiments with small dogfish sharks, *Squalus acanthias*, show that 50 h after weights were attached, the ratio of DAGE/TAG in the liver increased by over 75%, compensating for loss of buoyancy (Malins and Barrone, 1970). Temperature causes greater buoyancy changes than pressure changes alone (Fig. 5) (Yayanos et al., 1978). Assuming the composition and quantity of oil in the liver remain constant, there are three factors influencing the buoyancy: (1) density of the surrounding seawater, (2) pressure and (3) temperature. These act on different time scales. Density of the surrounding water has an instantaneous effect when the fish moves from one water mass to another. The response to pressure change is essentially instantaneous, but may be delayed at low temperatures due to increased viscosity of the oil hindering the necessary molecular rearrangements to achieve equilibrium (Yayanos et al., 1978). Temperature change depends on the rate of heat transfer between the exterior and interior of the fish. If there is a delay before the liver oil reaches thermal equilibrium with the surrounding water, it may be possible for a shark to make rapid adiabatic dives or ascents with no change in liver temperature. In the example of *C. coelolepis* in the Rockall Trough, displacement from 1600 m to the surface would increase the buoyancy by only 0.18% (compared with 7% for the isothermal ascent), as increase in buoyancy from the liver swelling under pressure decrease is almost cancelled out by the reduced density of the warm seawater at the surface. Once on the surface, the fish would gradually warm up and its buoyancy would increase to the value at thermal equilibrium. The fish may be able to modulate blood flow to and from the liver to alter the rate of heat transfer. Several surface-dwelling Lamniform sharks achieve regional endothermy by directing blood flow through counter-current heat exchangers, *retia mirabile* (Bernal et al., 2012; Dickson and Graham, 2004), enabling the viscera to be maintained at up to 21°C above ambient water temperature (Goldman et al., 2004). The primary advantage of this is assumed to be the ability to sustain a high metabolic rate regardless of ambient water temperature. A secondary advantage for such species may be reduction in buoyancy changes when diving into cold deep water in pursuit

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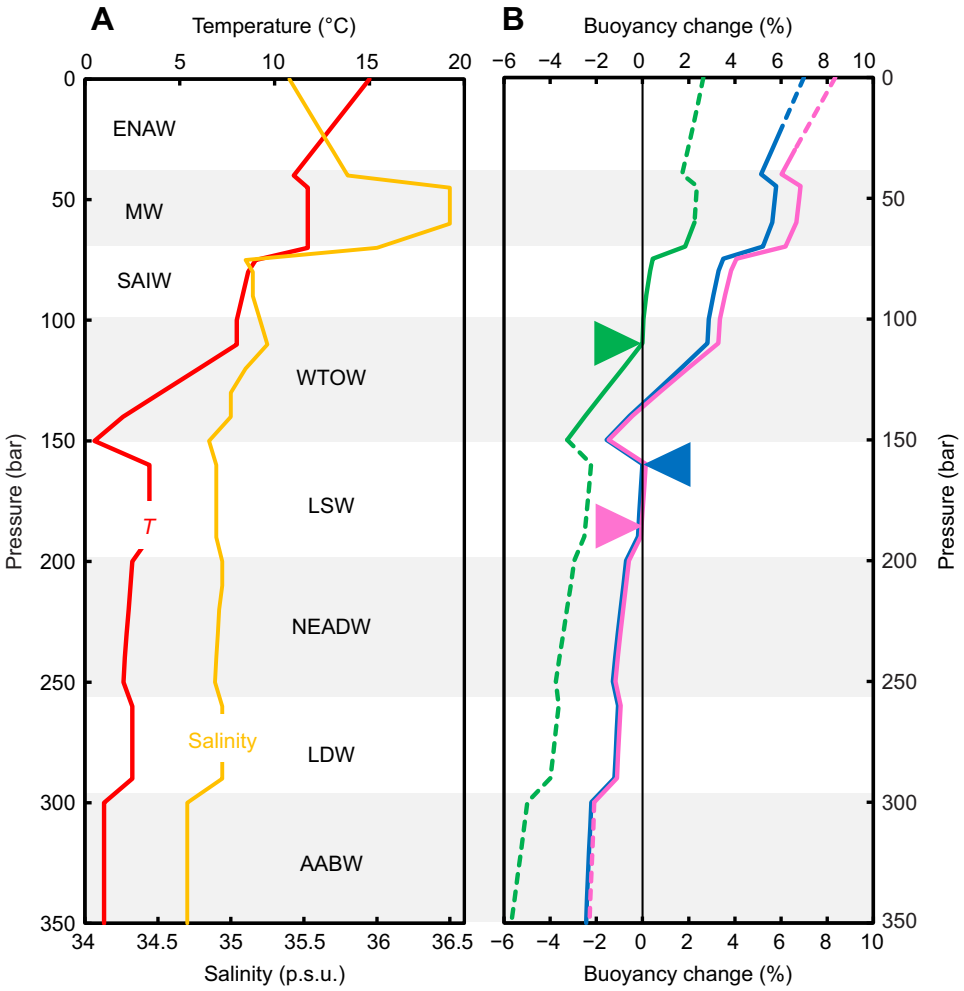

Fig. 6. Change in liver oil buoyancy for fishes moving through the water column of the Rockall Trough, NE Atlantic Ocean. (A) Salinity, temperature (*T*) and water masses are shown. ENAW, Eastern North Atlantic Water; MW, Mediterranean Water; SAIW, Sub-Arctic Intermediate Water; WTOW, Wyville Thomson Overflow Water; LSW, Labrador Sea Water; NEADW, Northeast Atlantic Deep Water; LDW, Lower Deep Water; AABW, Antarctic Bottom Water. (B) Changes in buoyancy during vertical movements from a starting depth indicated by the triangles. Blue line, *C. coelolepis* starting at 1600 m; pink line, *H. affinis* starting at 1800 m; green line, *A. laurussonii* starting at 1100 m. Continuous lines represent the species depth range (Froese and Pauly, 2019).

of prey. However, these fast swimming species are probably little affected by buoyancy changes. There is no evidence that deep-sea Chimaeriformes, Squaliformes or Carcharhiniformes have *retia mirabile* but by vasoconstriction they may be able to restrict blood flow to make short-term adiabatic movements between water masses.

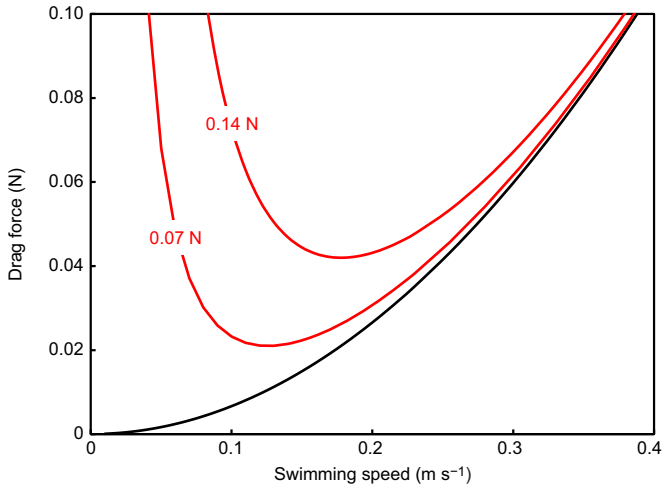

Fig. 7. *Centroscymnus coelolepis* drag in relation to swimming speed, assuming uniform horizontal velocity. Black line, neutrally buoyant; red lines, negative buoyancy, 0.07 and 0.14 N.

The composition of the liver oils with DAGE dominant in the Chimaeriformes, TAG in the Carcharhiniformes and squalene in the Squaliformes confirms previous findings (Wetherbee and Nichols, 2000; Treberg and Speers-Roesch, 2016). The Carcharhiniformes with >80% TAG have the highest density (Table 2). The TAG content in deep-sea *Apristurus* species from New Zealand waters varies between 99 and 65% (Pethybridge et al., 2010). With high density and high viscosity, the TAG-rich oils of the Carcharhiniformes appear to be maladapted for providing buoyancy in deep cold waters. In most oceanic shark species, TAG accounts for over 90% of total lipid, whereas liver lipids of species living deeper than 200 m are dominated by DAGE and squalene (Pethybridge et al., 2014). The Squaliformes, with high volumes of low-density, low-viscosity squalene oil in the liver, are well adapted for effective buoyancy at the greatest depths. The exact quantity of the different lipid constituents in our samples probably reflects the feeding, developmental and environmental history of the individual and may not be representative of each species. In *C. squamosus* from New Zealand, the squalene content varied between <30 and >90% with an inverse correlation with DAGE content. The range of values for squalene in the squaliformes sampled here (33.6–94.0%) reflects the variation generally found in previous studies on deep-sea representatives of this order (Wetherbee and Nichols, 2000; Pethybridge et al., 2010).

The calorific value measurements imply no difference between TAG, DAGE and squalene in terms of energy content per unit mass. Assuming a mean specific energy of 41.5 MJ kg^{-1},

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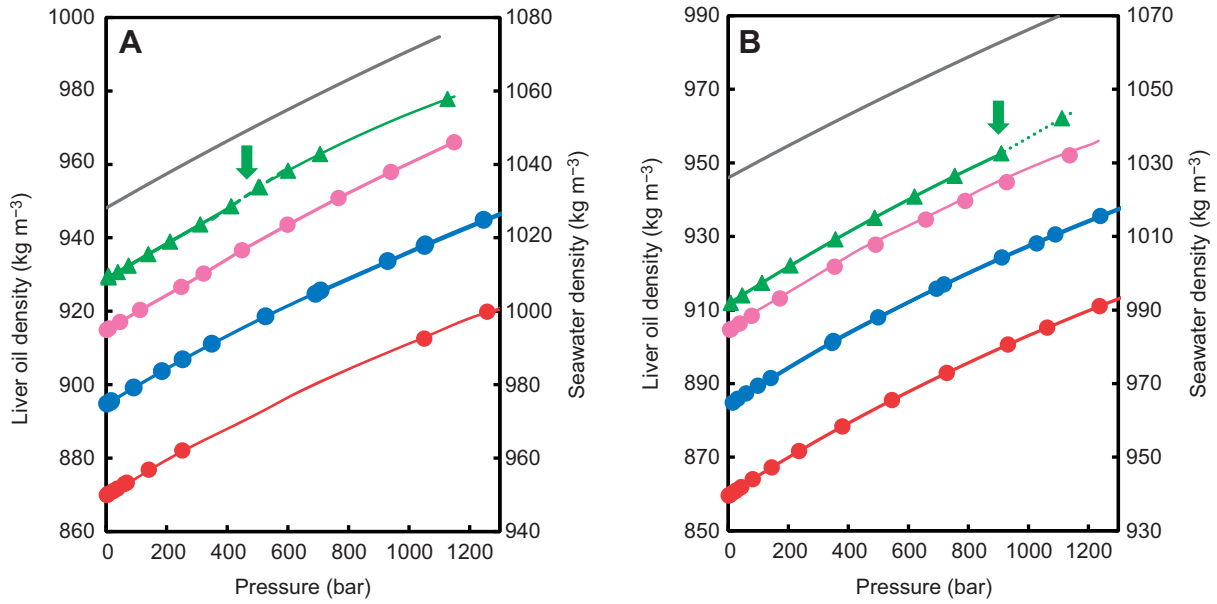

Fig. 8. The effect of high pressures to full ocean depth (1100 bar≈11,000 m) on density of liver oils compared with squalene (red circles) and 35 p.s.u. seawater (grey line plotted on the right-hand scale). (A) 0.1°C; (B) 15°C. Green symbols: Carcharhiniformes (*A. laurussonii*); pink symbols: Chimaeriformes (*C. monstrosa*); blue symbols: Squaliformes (*C. coelolepis*). Zero is sea surface (atmospheric) pressure; 1 bar is approximately 10 m depth of seawater. The green arrows indicate the onset of a phase change during increasing pressure in *A. laurussonii* manifested as discontinuity in the density curve.

the energy cost of buoyancy is therefore simply proportional to the densities of the different oils. At 150 bar (1500 m depth) and 0.1°C, the values are 23.5 MJ N^{-1} for pure squalene, 34.5 MJ N^{-1} for *C. monstrosa*, 28.7 MJ N^{-1} for *C. coelolepis* and 39.9 MJ N^{-1} for *A. laurussonii*, the latter requiring 1.7 times more energy compared with squalene. These values represent the energy content of the oil sequestered in the liver and do not consider energy costs of synthesis and mobilization.

Deviations from neutral buoyancy resulting from changes in density of liver oils are small (Fig. 6) but are enough to increase the energy cost of slow swimming (Fig. 7). Furthermore, positive or negative buoyancy may be used to power slow upward or downward gliding in the water column. Alexander (1990) showed that neutral buoyancy in fishes is most beneficial at low swimming speeds, whereas the extra drag of a buoyancy organ becomes disadvantageous at high swimming speeds, so making negative buoyancy prevalent in fast-swimming species. Pinte et al. (2020) indicated that deep-sea sharks generally swim at slower cruising speeds than their shallow-water counterparts. Gleiss et al. (2017) further examined the trade-offs for sharks and showed that species in which rapid acceleration is important have negative buoyancy, but in steady slow-swimming deep-sea species neutral buoyancy is prevalent. Sharks ascending into warm surface waters in the tropics are likely to experience much greater changes in buoyancy than described here, but that is not relevant to their normal life in the deep sea below the thermocline. Conversely in the Eastern Mediterranean Sea, the deep sea is almost uniformly warm (Priede, 2017), so sharks such as *Etmopterus*, *Centrophorus* and *Galeus* spp. that occur there (Jones et al., 2013) would experience very small buoyancy changes. Here we show that sharks tend to lose buoyancy when entering low-density, low-salinity Eastern North Atlantic Water in the Rockall Trough (Fig. 6). In line with this observation, Gleiss et al. (2015) propose that for sharks entering freshwater the density of the surrounding medium is so low that there is a 2- to 3-fold increase in negative buoyancy, which probably precludes extensive invasion of freshwater habitats by Chondrichthyes.

Compared with gas-filled buoyancy bladders (Priede, 2018), oil-filled buoyancy organs are often regarded as incompressible so that buoyancy remains constant in the face of pressure changes during vertical migration (Phleger, 1998). This is a reasonable approximation for small depth changes, but here we show that for greater depths the true explanation is that oil and seawater are almost equal in compressibility, resulting in very small net change in buoyancy. This conclusion was anticipated by Corner et al. (1969), who speculated that ‘compressibilities of natural oils are not usually very different from that of sea water’, but they had no information on squalene or other oils found in Chondrichthyes. The data presented here permit precise evaluation of the effects of pressure and temperature changes on buoyancy of sharks and chimeras in the deep sea. In turn, this will permit new evaluations of cost of transport and energy expenditure under different ecological conditions in the deep sea.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: I.G.P., M.M.; Methodology: R.W.B., M.M., A.S., P.G., F.B., J.D.; Formal analysis: I.G.P.; Investigation: I.G.P., R.W.B., M.M., A.S., P.G., F.B., J.D.; Resources: A.S., F.B., J.D.; Data curation: I.G.P., F.B., J.D.; Writing - original draft: I.G.P.; Writing - review & editing: I.G.P., M.M., A.S., P.G., F.B., J.D.; Supervision: I.G.P.; Project administration: I.G.P., R.W.B.; Funding acquisition: I.G.P.

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Supplementary information
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