

COMMENTARY

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

Jason R. Treberg^{1,2,*} and Ben Speers-Roesch³

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

¹Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2. ²Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2. ³Department of Ocean Sciences, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1C 5S7.

*Author for correspondence (Jason.Treberg@umanitoba.ca)

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"> ⎵ Vast (the deep sea deeper than 1000 m comprises ~1 billion km³, or 88% of the world's ocean). ⎵ Dark (the photic zone typically ends at 200 m but some light may penetrate to 1000 m).
Can interfere with physiological function and limit physiological or ecological capacity	<ul style="list-style-type: none"> ⎵ Cold (4–20°C at 200–1000 m depending on latitude and thermocline depth; typically 1–4°C below 1000 m). ⎵ High hydrostatic pressure (increases at 1 atm per 10 m). ⎵ Oligotrophic (nutrient availability generally declines markedly with depth).

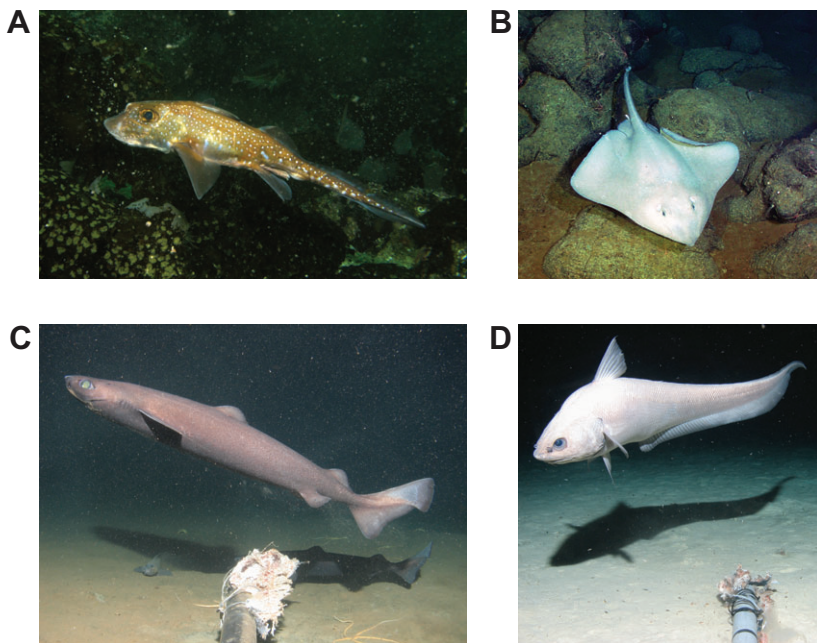


Fig. 1. Representative species belonging to three chondrichthyan groups common in the deep sea and a teleost group common in the deep sea. (A) A chimaeroid, the spotted ratfish (*Hydrolagus colliei*). Northeast Pacific Ocean (Barkley Sound, Canada), 30 m. Species depth range: 0–971 m. Photo: Ben Speers-Roesch. (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 diacylglycerol 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	↓ ^a	↓
Growth rate	↓	↓
Muscle function and metabolism		
Protein content	↔	↓ ^b
WM metabolic capacity		
Glycolytic	↓	↓ ^b
Aerobic	↔	↓ ^b
RM metabolic capacity	↔	↓
Liver		
HSI (% body mass)	↑	↔ ^c
Liver lipid (% of organ mass)	↑	↔ ^c
Protein stabilization		
Extrinsic (organic osmolytes)	↑	↑
Intrinsic (protein structure)	?	↑

^aPresumed based on white muscle enzyme activities. ^bExceptions occur with more active species. ^cMay 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⁻¹ 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⁻¹ 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 ^a (g l ⁻¹)	Lift ^a (g per ml lipid)	Functional role ^b
Triacylglycerols (TAG)	0.93	+0.095	Primarily a metabolic fuel (released as free fatty acids or converted and released as ketone bodies)
Diacylglycerol ethers (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.

^aValues for density (at 1 atm, 20°C) and lift (versus seawater of density 1.025 g ml⁻¹) are taken from Phleger (1998).

^bSee Ballantyne (1997); Nevenzel (1989); Speers-Roesch and Treberg (2010).

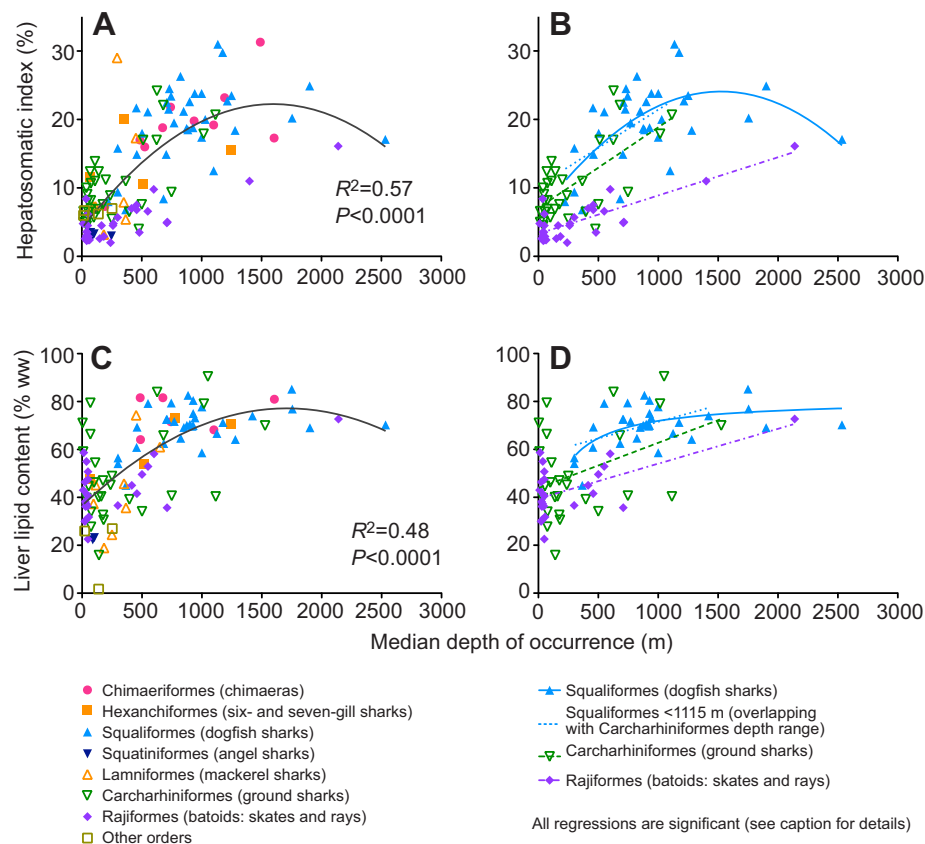


Fig. 2. Hepatosomatic index (HSI, % of body mass) and liver lipid content (% of liver wet mass) as a function of median depth of occurrence among chondrichthyans. HSI and liver lipid in chondrichthyan fishes (A,C) or within each of the three most speciose and successful deep-sea chondrichthyan orders (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

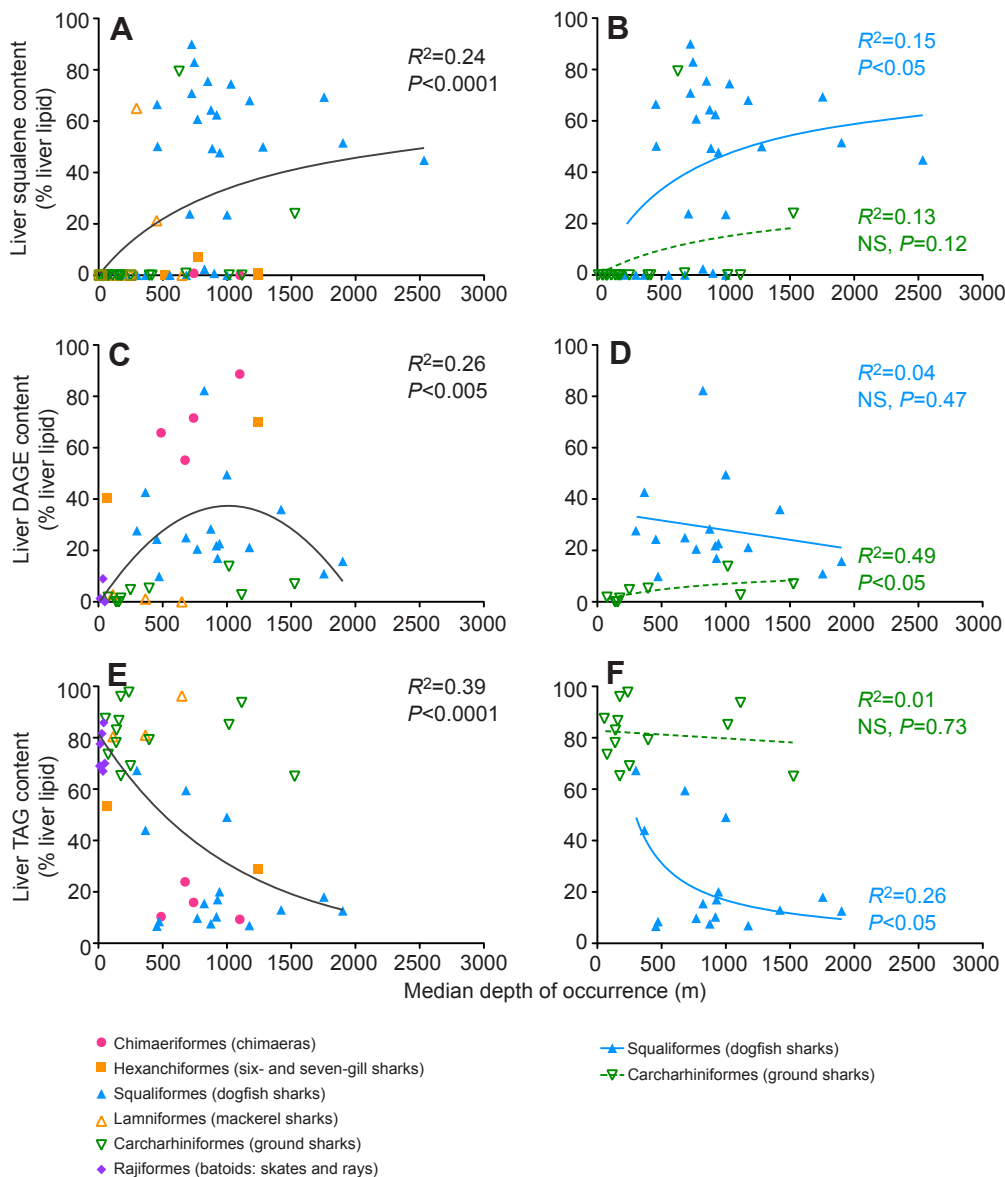


Fig. 3. Liver triacylglycerol, diacylglycerol ether and squalene contents as a function of median depth of occurrence among chondrichthyans. TAG, DAGE and squalene levels in chondrichthyan fishes (A,C,E) and within each of the two most speciose and successful deep-sea chondrichthyan orders (B,D,F). Data 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-

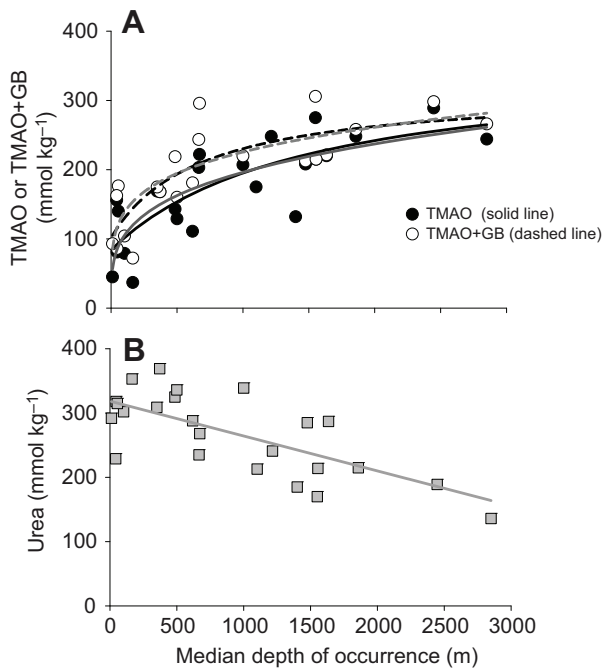


Fig. 4. Relationships between muscle trimethylamines or urea and median depth of occurrence (MDO) among chondrichthyan fishes. (A) Muscle trimethylamine and (B) urea contents. Data points represent individual species. There is no clear intraspecific pattern of increasing muscle trimethylamine content 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⁻¹; TMAO: 202, 149 mmol kg⁻¹; 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⁻¹; TMAO: 132±8 mmol kg⁻¹; 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 ~ 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.

Acknowledgements

We dedicate this paper to our colleague and friend R. Aidan Martin. Much of J.R.T.'s contribution to this work was initially catalysed by Aidan's question, 'Is there really such a thing as a deep-sea shark?' While the answer continues to be debated, his insightful questions and challenges were influential to us. We apologize to the authors of the many important papers that we could not cite due to space limitations. Data listed as Treberg, Atchison, Reist, unpublished or Brandt, Anderson, Reist and Treberg, unpublished were made available from samples collected during the Beaufort Regional Environmental Assessment (BREA) Marine Fishes Project conducted by Fisheries and Oceans Canada. We thank the crew of the F/V Frosti and other members of the Science crew (A. Majewski, W. Walkusz, S. MacPhee, J. Eert) for their efforts. We thank Ocean Networks Canada and Dr Alan Jamieson (Oceanlab, University of Aberdeen) for allowing us to reproduce their images of deep-sea fishes.

Competing interests

The authors declare no competing or financial interests.

Funding

J.R.T. is currently funded by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) [418503] and is the Canada Research Chair in *Ecosystem Dynamics and Metabolism* (NSERC tier 2) [223744]. B.S.-R. was supported by an NSERC postdoctoral fellowship. Data included as 'J.R.T. and W. R. Driedzic, unpublished observations' were funded by NSERC Discovery grants to W.R.D.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.128108/-/DC1>

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 chondrichthyan: 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 chondrichthyan. 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 chondrichthyan: 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 chondrichthyan 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 chondrichthyan: 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 chondrichthyan. *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., Hefter, 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.