Journal of Fish Biology (2017)
doi:10.1111/jfb.13361, available online at wileyonlinelibrary.com

No support for Heincke's law in hagfish (Myxinidae): lack of an association between body size and the depth of species occurrence

E. L. Schumacher*, B. D. Owens*, T. A. Uyeno*, A. J. Clark† and J. S. Reece‡§

**Valdosta State University, Department of Biology, 1500 N Patterson Street, Valdosta, GA 31698, U.S.A., †College of Charleston, Department of Biology, 58 Coming Street, Rm 214, Charleston, SC 29401, U.S.A. and ‡California State University at Fresno, Department of Biology, 2555 East San Ramon Ave MS/73, Fresno, CA 93740, U.S.A.*

(Received 7 March 2016, Accepted 24 May 2017)

This study tests for interspecific evidence of Heincke's law among hagfishes and advances the field of research on body size and depth of occurrence in fishes by including a phylogenetic correction and by examining depth in four ways: maximum depth, minimum depth, mean depth of recorded specimens and the average of maximum and minimum depths of occurrence. Results yield no evidence for Heincke's law in hagfishes, no phylogenetic signal for the depth at which species occur, but moderate to weak phylogenetic signal for body size, suggesting that phylogeny may play a role in determining body size in this group.

Key words: body size evolution; ecomorphology; Myxinidae; phylogenetic inertia; phylogenetic signal.

INTRODUCTION

Body size is one of the most important morphological features that affects how an organism interacts with its environment (Haldane, 1928; Thiel, 1975; Peters, 1983; Schmidt-Nielsen, 1984; Rex & Etter, 1998). Body size has been shown in previous work to be associated with, or influenced by, latitude (Jordan, 1892), temperature (Atkinson & Sibly, 1997), anguilliform swimming (Neat & Campbell, 2013), diet (Warburton, 1989; Kulbicki *et al.*, 2005), evolutionary history (Warburton, 1989), the abundance of food (Thiel, 1975), physiological constraints (Pauly, 1997; Chapelle & Peck, 1999) and in fishes, the depth at which species live (Smith & Brown, 2002). As with most morphological variables, no single ecological correlate explains all, or even a majority, of the variation in fish body size. Ecological and life-history correlations often vary within and among lineages or functional groups (Rex & Etter, 1998; Smith & Brown, 2002; Albert & Johnson, 2012; Drazen & Haedrich, 2012). One of the most

§Author to whom correspondence should be addressed. Tel.: +1 559 278 8440; email: joshua_reece@csufresno.edu

widely cited and debated trends in body size and shape, first observed in the fossil record of mammals, is Cope's rule, which posits a progression to larger body sizes in a given lineage over macroevolutionary timescales (Cope, 1877; Maurer *et al.*, 1992). In many terrestrial species, there is a positive correlation between body size and range size (Lindstedt *et al.*, 1986), but this pattern does not seem to hold for fishes (Warburton, 1989; Jacquemin & Doll, 2014). In marine fishes, a similar pattern known as Heincke's law (Heincke, 1913) describes the pattern of increasing body size in species that live at greater depth; however, this relationship is not universal. Elasmobranchs, *e.g.* exhibit an inverse correlation between size and depth (Smith & Brown, 2002; Drazen & Haedrich, 2012).

A variety of mechanisms potentially explain the general pattern of increasing size with depth in marine fishes and these explanations may vary among lineages (Smith & Brown, 2002). A widely accepted explanation is that large fish can benefit from a significantly lowered metabolism in deeper, colder waters due to decreased respiration rate and enzymatic activity (Smith, 1978; Childress & Somero, 1979). One alternative explanation suggests benefits of large size for scavenging species that live at great depth because larger species can swim over longer distances with a lower mass-specific metabolic rate, which is beneficial when food items are sporadically available (Collins *et al.*, 2005). Another explanation is that large species tend to have elongate bodies and elongation may provide a kinematic advantage to swimming under the high hydrostatic pressure found in the deep ocean (Neat & Campbell, 2013). Several elongate species have been shown to consume less oxygen than non-elongate fishes while swimming at a constant rate as water pressure increases (Sébert *et al.*, 2009; Neat & Campbell, 2013).

Hagfishes (Myxinidae) are a successful lineage of elongate demersal fishes that inhabit nearly all of the world's oceans and their ecomorphology, including size–depth relationships, is poorly understood. There are 78 recognized species of hagfishes, which vary substantially in maximum body length from 18 cm total length (L_T) in the dwarf hagfish *Myxine pequenoi* Wisner & McMillan 1995 to 127 cm total length in the goliath hagfish *Eptatretus goliath* Mincarone & Stewart, 2006 and live at depths ranging from 30 to 5000 m (Fernholm, 1998; Martini, 1998; Mincarone & Stewart, 2006). Hagfishes lack several phenotypic traits occurring in most other fish taxa (*e.g.* dermal scales, image-forming eyes, jaws and vertebrae, but see Ota *et al.*, 2013; Miyashita & Coates, 2015) (Hart, 1973; Fernholm, 1998). They are also known for possessing highly plesiomorphic features: their ability to produce huge quantities of thick, proteinaceous mucus when agitated (Fudge *et al.*, 2005) and the ability to tie themselves into overhand, figure-of-eight, or even more complicated knots depending on the species (Jørgensen *et al.*, 1998; Clark & Summers, 2012; Uyeno & Clark, 2015). Hagfishes are soft animals possessing only a few poorly articulated unmineralized cartilages within the cranial region and tail tip. Hagfishes are primarily recognized as opportunistic scavengers capable of feeding on exceedingly large marine carcasses as well as live prey subdued by other animals (Auster & Barber, 2006). Hagfishes (*e.g. Neomyxine* Richardson 1953), however, may take on predatory behaviours by which they actively seek, capture and feed on free-swimming prey (Martini, 1998; Zintzen *et al.*, 2011). Despite their jawless condition, hagfishes use a rapacious feeding mechanism involving three-dimensionally complex arrangements of muscle and connective tissues that power dynamic toothplates with dentition that can be forcefully driven into large food items (Dawson, 1963; Clark & Summers, 2007). Hagfishes initially rely on olfaction for finding food items and then once close enough

to contact the food, will use their barbels for sensing the physical qualities of the food (*e.g.* size, texture and shape) before biting into it (Jensen, 1966). Little is known about the life history of hagfishes, but many species are thought to spawn year round and deposit large eggs (Barss, 1993; Grant, 2006; Ota & Kuratani, 2006). Between 2000 and 2008, commercial harvests of hagfishes increased dramatically, especially in the North Atlantic Ocean (Grant, 2006), but recently the market has shown signs of contracting (Grant, 2015). Several species of *Myxine* are also harvested for their skin, which makes tough, supple leather often marketed as eel leather and for Asian markets where both the fish and their slime are consumed (Gorbman *et al.*, 1990; Barss, 1993).

New species of hagfish are described annually, including 19 new species described between 1998 (Fernholm, 1998) and 2015 (Froese & Pauly, 2015). This influx of new taxa presents problems for determining the taxonomy of the Myxinidae, but recent genetic evaluations of this lineage have resolved some of the interrelationships amongst the six genera. A study by Fernholm *et al.* (2013) compared 32 unique taxa by using two mitochondrial genes and Bayesian phylogenetic methods to clarify the placement of genera and provide support for the newly described genus, *Rubicundus* (Fernholm *et al.*, 2013). The goal of the present study is to determine if species of hagfishes conform to an interspecific pattern consistent with Heincke's law. Phylogenetic relationships were reconstructed from the sequences used by Fernholm *et al.* (2013) to test for associations between depth and body size. This study is novel in three ways. First, this study tests for evidence of Heincke's law in a lineage of elongate fishes that includes species of large and small body size and that inhabit depths from shallow to extremely deep environs. Second, this test was executed with and without a phylogenetic correction, an approach that is lacking in many similar studies despite the importance of accounting for phylogeny in ecomorphological studies (Garland *et al.*, 1992; Freckleton, 2000; Alfaro *et al.*, 2009; Revell, 2009, 2010; Reece & Mehta, 2013). Third, in response to criticisms that tests of this type often oversimplify depth as a single value, when for many species it may be more complicated, association tests were executed using a variety of metrics of the depth at which each species lives.

MATERIALS AND METHODS

PHYLOGENETIC CONSTRUCTION

To investigate the relationship of the depth inhabited and the maximum body length among species of hagfishes, sequences were downloaded from GenBank for 652 bp of cytochrome c oxidase subunit I (*coI*) and 565 bp of *16s* (sometimes referred to as the small subunit, *ssu*) regions of mitochondrial DNA for 27 of the 78 known species of hagfishes that have sequences available on GenBank (www.ncbi.nlm.nih.gov/genbank). The analysis consisted of a total of 99 individuals from 27 species, with one to twelve representatives of each species; *coI* analysis included 61 individuals with one to nine representatives of each of 17 species, while *16s* had 70 individuals with one to 10 representatives of each of 26 species. Fernholm *et al.* (2013) included 35 species-level entities, but five of these were only identified to genus. A total of 27 named species was included in the current combined dataset of *16s* and *coI*. Sequences were manually aligned using GeneDoc (Nicholas & Nicholas, 1997) to ensure that homologous nucleotide positions were being used to infer patterns of ancestry and that *coI* formed an open reading frame. jModelTest (Darriba *et al.*, 2012) was used to determine the model of nucleotide evolution *via* Akaike information criterion for finite samples (AICc) scores. This analysis identifies the pattern and complexity of the nucleotide evolution model that should be used to reconstruct the evolutionary history of different lineages based on DNA sequence data. The programme

BEAST 1.8.2 (Drummond *et al.*, 2012) was used to generate the tree after formatting the run in BEAUti (Drummond *et al.*, 2012). Both separate gene trees and a concatenated dataset for *coI* and *16s* Markov chain Monte-Carlo runs consisted of 200 million chain lengths with a sampling interval every 10 000 trees. All phylogenies were constructed with a relaxed, uncorrelated lognormal clock and the Yule speciation process (typical for interspecific phylogenetic reconstructions; Drummond *et al.*, 2012). In both the gene tree and concatenated analyses the *coI* model of nucleotide evolution was partitioned by codon position. In the concatenated analyses substitution and clock models were unlinked and trees were linked. The phylogeny was rooted with three closely related out-group species: the lancelet *Branchiostoma lanceolatum*, European river lamprey *Lampetra fluviatilis* (L. 1758) and sea lamprey *Petromyzon marinus* L. 1758. The output file generated by BEAST was viewed using Tracer 1.6 (Rambaut *et al.*, 2014) to determine whether phylogenetic reconstructions were consistently producing reliable and equally likely trees. Runs were considered consistent when all estimated parameters had an effective sample size value greater than 200. All BEAST runs were duplicated to ensure reliable results. Replicate runs were combined using LogCombiner (also in the BEAST package) and a single Bayesian maximum clade credibility tree was then selected by Tree Annotator (Drummond *et al.*, 2012) from all trees generated after a burn in of 10%. The constructed phylogeny was then viewed in FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

MODEL FITTING AND PGLS

Data for depth (m) and maximum body length (L_T, cm) were obtained from the Global Biodiversity Information Facility (www.gbif.org) and FishBase (Froese & Pauly, 2015). When data on individual specimens was available from museum specimens, the exact depth records were used (when a range of depths was reported for a specimen from a trawl, *e.g.* the mean of that range was used). When no specimen data were available from museums, the depth range reported in FishBase was used. For each species, depth was calculated in four ways: average depth of occurrence for museum specimens; minimum depth; maximum depth; the mean of maximum and minimum depths. Shapiro–Wilk tests (utilizing the nlme package in R; www.r-project.org; Pinheiro *et al.*, 2015) determine the normality of values for maximum L_T ($P > 0{\cdot}05$). Values for maximum depth were not normally distributed ($P < 0{\cdot}05$) due to some very large values for deep-water species, but were normally distributed after being log-transformed ($P > 0{\cdot}05$). Both the greatest L_T and maximum body depth were log-transformed in all subsequent analyses to ensure normality of the data and comparability of depth with maximum L_T data recorded for each species. To determine the most likely model of evolution for depth inhabited and maximum L_T across the phylogeny, packages geiger (Harmon *et al.*, 2008) and ape (Paradis *et al.*, 2004) were used in R to compare the following models [Brownian motion (BM), lambda, kappa, drift, Ornstein–Uhlenbeck (OU) and white noise] and select the best-fit model based upon AICc scores. Owing to the phylogenetic signal indicated in model analyses, a phylogenetic generalized least square (PGLS) regression was used to determine the degree to which phylogenetic relatedness explained the evolution of maximum L_T and maximum depth (analyses repeated for each of the four metrics of depth), using the caper library (Orme *et al.*, 2015) in R. To address any concerns about species with few specimen records, all analyses were rerun on a dataset reduced to those species with data from five or more specimens.

RESULTS

PHYLOGENETIC RECONSTRUCTION

The best model of nucleotide evolution for *coI* and *16s* was identified as GTR (general time reversible) + I (invariant sites) + G (discretized γ-distribution) (Table I). This model allows for certain nucleotide positions to have changed due to multiple mutations, for other positions to have had no mutations and for certain nucleotide positions to evolve at higher or lower rates than others. The gene trees for *coI* and *16s* are largely

TABLE I. Models of hagfish nucleotide evolution for *coI* and *16s* genes evaluated using jModelTest. The lowest value for the negative log-likelihood score (−lnL) represents the preferred model

Model (*coI*)	−lnL (*coI*)	Model (*16s*)	−lnL (*16s*)
GTR + I + G	5763	GTR + I + G	4011·7
TIM2 + I + G	5764	TIM3 + I + G	4012·2
TIM1 + I + G	5765·9	TIM3 + G	4012·3
TIM3 + I + G	5766·1	GTR + G	4012·7
TrN + I + G	5766·2	TIM1 + I + G	4016·9

GTR, general time reversible; I, invariant sites; G, discretized γ-distribution; TIM, transition model with 1, 2, or 3 transversion rates; TrN, Tamura-Nei model.

concordant (Figs S1 and S2, Supporting information). The phylogeny generated from the concatenated *coI* and *16s* genes (Fig. 1) places the genus *Rubicundus* as sister to the Eptatretinae (Bayesian posterior probability, Bpp 0.58) with low support (alternative topologies place *Rubicundus* as sister to Eptatretinae and Myxininae), while *Neomyxine* is determined to be the earliest branching point within the family Myxinidae (Bpp 1.0). *Eptatretus* and *Myxine* are placed as sister to each other (Bpp 1.0) and *Notomyxine* is placed as a branching point within the clade containing *Myxine* (Bpp 0.53). This reconstruction differs from that of Fernholm *et al.* (2013) only in the placement of *Neomyxine*. While not shown here, species-tree reconstruction using *BEAST to address any differences between gene-tree topologies yielded qualitatively similar results for all phylogenetic analyses.

DEPTH AND BODY SIZE

Maximum L_T and maximum depth at which each species can be found are given in Table II. Information was assimilated for 2910 unique specimens, collected from 541 different depths; this information was compiled from museum records with an average of 108 specimens per species (median = 9; some species were very heavily represented). Maximum L_T ranged from 20 to 85 cm (average = 52 cm) and maximum collection depths ranged from 10 to 2743 m. Often, the average depth at which a species was collected is quite different from the average of the minimum and maximum depths at which that species had been recorded. As such, all four metrics were used in subsequent tests (reported below), although there is no change in the significance of any test, no matter which metric of depth was used. No qualitative differences were found in significance for any test rerun on a dataset reduced to those species with five or more specimen records, which included 16 species.

MODEL FITTING AND PGLS

Model fitting determines white-noise and lambda ($\lambda = 0{\cdot}58$) to be the best models of evolution for maximum L_T, suggesting moderate to weak effects of phylogeny on body size. Models of BM, drift, OU and kappa are all refuted (Table III). The best model for maximum depth inhabited is also white-noise. The white-noise model fit is consistent across all four metrics of depth (average depth of specimens collected, minimum depth, maximum depth and the average of minimum and maximum depths).

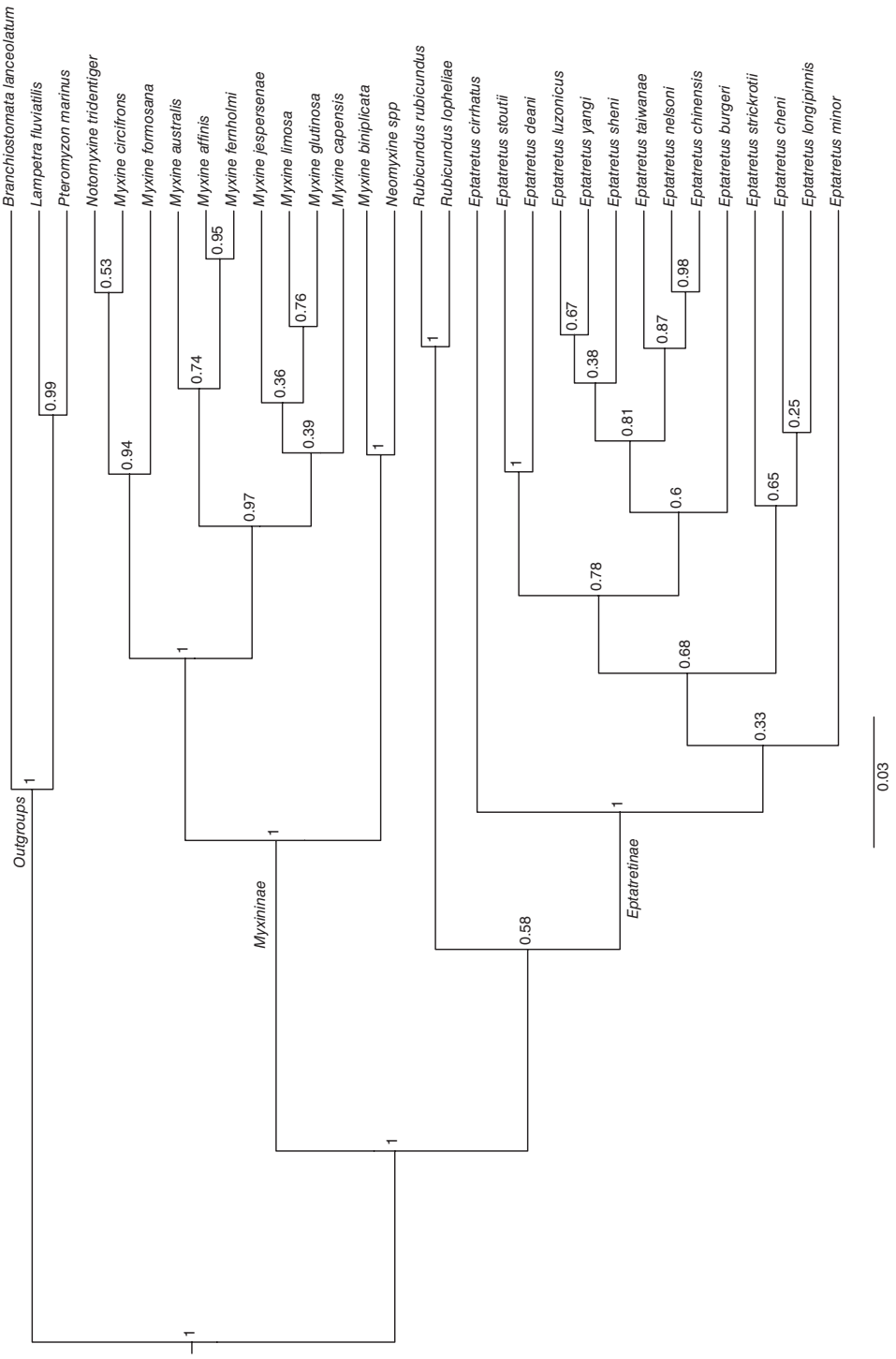

FIG. 1. Phylogeny of hagfishes created using concatenated *coI* and *16s* gene sequences. Node labels are Bayesian posterior probability values.

TABLE II. Numbers of individual hagfish sampled (n), independent depth records, total body length (L_T), and four metrics of depth of occurrence: calculated average depth from specimen records ($D_C \pm$ S.D.); minimum depth from specimen records (D_{min}); maximum depth from specimen records (D_{max}); the mean of the maximum and minimum recorded depths for that species ($D_{min:max}$)

Species	n	Depth records	L_T (cm)	D_C (m)	D_{min} (m)	D_{max} (m)	$D_{min:max}$ (m)
Eptatretus burgeri	15	15	60	156·5 ± 158·9	10	270	140
Eptatretus luzonicus	136	5	29·5	464·4 ± 90·6	200	400	300
Eptatretus cheni	312	8	71·4	229 ± 34·4	156	260	208
Eptatretus chinensis	1	1	37·5	590	–	600	–
Eptatretus cirrhatus	25	25	83	416·6 ± 149·9	–	1100	–
Eptatretus deani	43	43	63·5	606·2 ± 339·7	103	2743	1423
Eptatretus longipinnis	0	0	62·7	–	14	40	27
Eptatretus minor	10	10	39·5	371·6 ± 49·7	300	400	350
Eptatretus nelsoni	9	2	25·9	184 ± 7·8	50	250	150
Eptatretus sheni	394	9	43·6	466·7 ± 130·3	200	800	500
Eptatretus stoutii	31	31	63·5	235·2 ± 230·2	16	966	491
Eptatretus strickrotti	0	0	31·4	–	2100	2300	2200
Eptatretus taiwanae	154	3	33·4	243·3 ± 162·1	20	427	223·5
Eptatretus yangi	1401	10	29·6	240·4 ± 116·6	20	547	283·5
Myxine affinis	0	0	65·9	–	30	150	90
Myxine australis	14	14	60	106·3 ± 35·7	10	100	55
Myxine capensis	170	170	40	342·5 ± 167·7	175	460	317·5
Myxine circifrons	7	7	65	1310·0 ± 215·6	700	1860	1280
Myxine fernholmi	4	4	84·6	219·0 ± 125·4	135	1480	807·5
Myxine formosana	2	2	76·8	839·5 ± 4·9	588	1500	1044
Myxine glutinosa	170	170	80	112·3 ± 63·2	30	1200	615
Myxine jespersenae	1	1	49·8	905·0	752	1556	1154
Myxine limosa	1	1	51	932·7	75	1006	540·5
Neomyxine biniplicata	4	4	41·2	509·3 ± 303·7	–	73	–
Notomyxine tridentiger	6	6	57·5	137·6 ± 25·1	11	106	58·5
Rubicundus lopheliae	0	0	20·1	–	382	700	541
Rubicundus rubicundus	0		46·4	–	–	800	–

This model is also consistently identified as the best-fit model in the dataset of 16 species that all have five or more specimen records. These results imply that, for both maximum depth inhabited and for maximum L_T, evolution in trait values as recorded here are best described by random changes irrespective of phylogeny (white-noise). It is not possible, however, to rule out a moderate to weak phylogenetic signal only for maximum L_T ($\lambda = 0{\cdot}58$), where $\lambda = 0$ implies no phylogenetic signal and a $\lambda = 1$ implies strong phylogenetic signal.

A generalized least-squares (GLS) regression shows no correlation between maximum L_T and maximum depth inhabited ($P > 0{\cdot}05$, d.f. 27). Again, these results are consistent across all four metrics of depth. Because body size displays some phylogenetic signal ($\lambda = 0{\cdot}58$), it is necessary to determine whether or not any correlation between body size and depth also shows phylogenetic signal. Accordingly, a phylogenetically corrected GLS regression was performed. The results of this analysis are also non-significant ($P > 0{\cdot}05$, d.f. 25; Fig. 2) for all four metrics of depth and when

TABLE III. Size-corrected Akaike information criterion (AIC) scores (AICc) and size-corrected ΔAIC scores (ΔAICc) for maximum depth inhabited and total body length of hagfishes. A score for ΔAICc of 0 indicates the best model. Models are statistically indistinguishable when less than two units apart

	BM	Drift	Lambda	Kappa	OU	White noise
Maximum depth inhabited						
AICc	54·6	57·1	42·7	53·2	54·1	40·2
ΔAICc	14·4	16·9	2·5	13·0	13·9	0
Total body length						
AICc	−14·6	−12·0	−16·9	−13·4	−14·4	−16·2
ΔAICc	2·3	4·8	0·0	3·5	2·5	0·7

BM, Brownian motion; OU, Ornstein–Uhlenbeck.

repeated on the reduced dataset of 16 species with five or more specimen records. The same tests executed on the phylogeny in Fernholm *et al.* (2013), which differ only in the placement of the genus *Neomyxine*, are also not qualitatively different.

DISCUSSION

Variation in maximum L_T across species of hagfishes appears to evolve independent of ocean depth and thus hagfishes do not follow Heincke's law. This finding

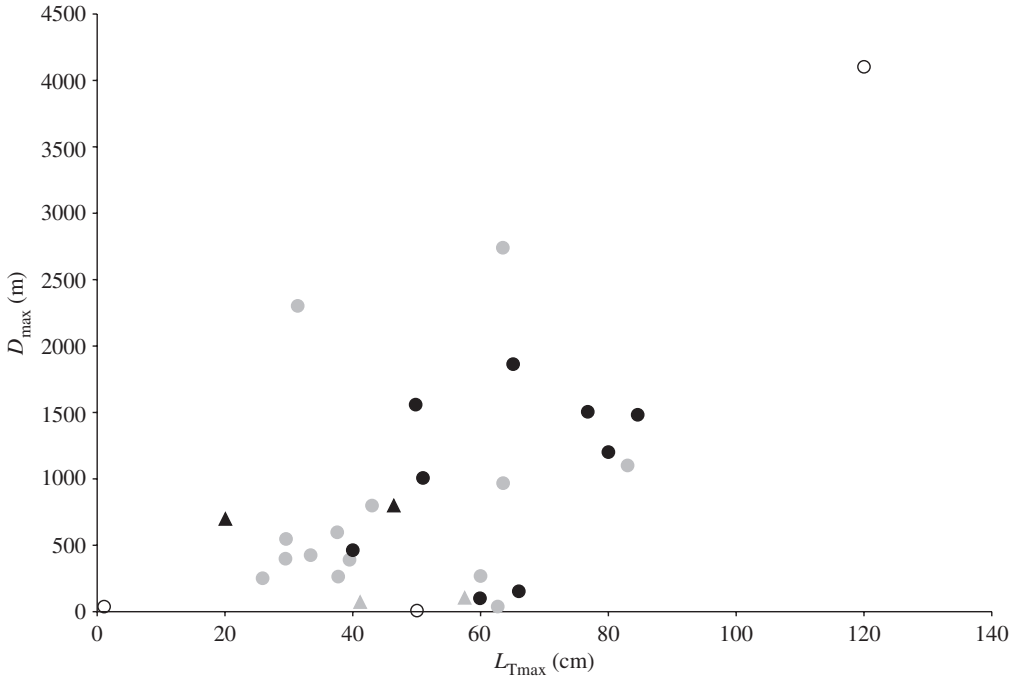

FIG. 2. Relationship between hagfish maximum total length (L_{Tmax}) and maximum depth inhabited (D_{max}) split into in-group and out-group genera. Generalized least-squares regression revealed no correlation ($P > 0{\cdot}05$, d.f. 27). A phylogenetic generalized least-squares regression was also non-significant ($P > 0{\cdot}05$, d.f. 25). ○, Out-group; ▲, *Neomyxine*; ▲, *Rubicundus*; ●, *Eptatretus*; ●, *Myxine*.

was robust to corrections for phylogenetic relationships among species and to several metrics of depth of occurrence. No effect of phylogeny on depth of occurrence was found, but a moderate to weak influence of phylogeny was found on maximum body L_T ($\lambda = 0{\cdot}58$). These results add to a growing body of work that demonstrates that Heincke's law (Heincke, 1913) is a failed, or at best an oversimplified, paradigm for explaining variation in body size for marine fishes. Heincke's law holds true for some teleosts including Aulopiformes, Lophiiformes, Stephanoberyciformes and Pleuronectiformes (Smith & Brown, 2002), demersal scavenging teleosts (Collins *et al.*, 2005) and deep-sea gastropods (Rex *et al.*, 1999). In Perciformes, Beryciformes, Notothenioformes, Salmoniformes and Zeiformes, however, there is no significant relationship (Drazen & Haedrich, 2012), or the weak correlation (Gadiformes) was disregarded as being coincidental (Aubry *et al.*, 2009). Elasmobranchs (Smith & Brown, 2002) and predatory deep sea teleosts (Collins *et al.*, 2005) show the inverse of Heincke's law. The current findings add the Myxiniformes to the list of lineages that do not conform to Heincke's law.

Factors other than depth that might explain fish body size variation include phylogeny (Steele & López-Fernández, 2014), body shape and the relationship between shape and habitat depth (Sébert *et al.*, 2009; Neat & Campbell, 2013), the energetics of reproduction (Smith & Brown, 2002; Drazen & Haedrich, 2012), temperature (Ashton, 2001; Hunt & Roy, 2006; Roy, 2008), or food availability (Collins *et al.*, 2005). It is also important to consider how depth and size are measured. Maximum standard length, mass, or combinations of these and other measurements are all commonly used to determine the presence of a size–depth relationship (Smith & Brown, 2002; Grant, 2006; Neat & Campbell, 2013), but these metrics may ignore potentially important components of body size evolution such as sexual dimorphism, average size, or geographic or bathymetric variation in size. Depth of occurrence is often reported only as maximum depth (Collins *et al.*, 2005) at which a species has been sampled (or worse, the maximum depth at which a trawl was conducted that happened to sample a species). Body size and the depth at which species occur are both probably influenced by numerous factors and should be measured in multiple dimensions while also accounting for potential impacts of phylogeny (Steele & López-Fernández, 2014).

There are several potential explanations for why hagfishes do not conform to Heincke's law. Knowledge of wild hagfishes is limited, especially in terms of their occurrences at variable depths. While the present findings were robust to several measures of depth, there are numerous other dimensions that remain to be explored. The depths at which specimens of the inshore hagfish *Eptatretus burgeri* (Girard 1855) can occur range from 10 to 100 m depending on the time of the year (Fernholm, 1974). Sex ratios vary by depth in the Pacific hagfish *Eptatretus stoutii* (Lockington 1878). In the black hagfish *Eptatretus deani* (Evermann & Goldsborough 1907), body size varies by depth (Johnson, 1994; Martini, 1998). It is not currently known which, if any, species of hagfishes spend their entire adult lives at a single depth, or what the range of depths inhabited is for each species. Grant (2006) found that individuals of the Atlantic hagfish *Myxine glutinosa* L. 1758 caught at greater depths tended to be larger in body size. Intraspecific variation in depth occurrence is a particularly interesting issue as representative species of hagfishes can show a large ontogenetic range in L_T (Clark & Summers, 2012). Sampling error due to variation in the number of records available for each species is not likely to influence the results presented here. When the current analyses (data available upon request) were re-run using only

species with five or more records ($N = 16$ species), the results were qualitatively similar across all tests. The influence of temperature as a function of depth may also affect body size evolution in hagfishes, as it does in deep-sea ostracods (Hunt & Roy, 2006). The effect of temperature, however, may be increasingly complex for hagfishes. Because temperatures remain fairly stable below 1000 m (Zelle *et al.*, 2004) and many species of hagfishes have ranges that extend well beyond that depth, any potential relationship between morphology and depth may be different in shallow waters than in deeper waters, even within a single species. Future evaluations of depth and body size should include intraspecific evaluations with thorough intraspecific geographic and depth sampling. Lastly, the evaluation of hagfishes presented here does not include all species of hagfishes and may not reflect the full spectrum of body size and depth preferences in this lineage, especially given the fact that many species were only recently described and inhabit understudied deep ocean environments (Mincarone & Stewart, 2006).

Funding for this work was provided by the National Science Foundation (IOS-1354788) to A.J.C. and T.A.U. and a Valdosta State University grant to J.S.R. and T.A.U.

Supporting Information

Supporting Information may be found in the online version of this paper:

Fig. S1. Hagfish phylogenetic relationships based only on the *coI* gene. Bayesian posterior support values are given at each node.

Fig. S2. Hagfish phylogenetic based only on the *16s* gene. Bayesian posterior support values are given at each node.

References

Albert, J. S. & Johnson, D. M. (2012). Diversity and evolution of body size in fishes. *Evolutionary Biology* **39,** 324–340.

Alfaro, M., Brock, C., Banbury, B. & Wainwright, P. (2009). Does evolutionary innovation in pharyngeal jaws lead to rapid lineage diversification in labrid fishes? *BMC Evolutionary Biology* **9,** 255.

Ashton, K. G. (2001). Body size variation among mainland populations of the western rattlesnakes (*Crotalus viridis*). *Evolution* **55,** 2523–2533.

Atkinson, D. & Sibly, R. M. (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution* **12,** 235–239.

Aubry, E., Methven, D. A. & Hurlbut, T. (2009). Length–depth relations of *Enchelyopus cimbrius* fourbeard rockling (Gadiformes: Phycidae) from the southern Gulf of St Lawrence and Cabot Strait in relation to abiotic factors. *Journal of the Marine Biological Association of the United Kingdom* **89,** 1643–1653.

Auster, P. J. & Barber, K. (2006). Atlantic hagfish exploit prey captures by other taxa. *Journal of Fish Biology* **68,** 618–621.

Barss, W. H. (1993). Pacific hagfish, *Eptatretus stouti* and black hagfish, *E. deani*: the Oregon fishery and port sampling observations, 1988–92. *Marine Fisheries Review* **55,** 19–30.

Chapelle, G. & Peck, L. S. (1999). Polar gigantism dictated by oxygen availability. *Nature* **399,** 114–115.

Childress, J. J. & Somero, G. N. (1979). Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Marine Biology* **52,** 273–283.

Clark, A. J. & Summers, A. P. (2007). Morphology and kinematics of feeding in hagfish: possible functional advantages of jaws. *Journal of Experimental Biology* **210,** 3897–3909.

Clark, A. J. & Summers, A. P. (2012). Ontogenetic scaling of the morphology and biomechanics of the feeding apparatus in the Pacific hagfish *Eptatretus stoutii*. *Journal of Fish Biology* **80,** 86–99.
Collins, M. A., Bailey, D. M., Ruxton, G. D. & Priede, I. G. (2005). Trends in body size across an environmental gradient: a differential response in scavenging and non-scavenging demersal deep-sea fish. *Proceedings of the Royal Society B* **272,** 2051–2057.
Cope, E. D. (1877). Synopsis of the cold blooded vertebrata, procured by Prof. James Orton during his exploration of Peru in 1876–77. *Proceedings of the American Philosophical Society* **17,** 33–49. https://doi.org/10.2307/982276
Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012). jModeltest 2: more models, new heuristics and parallel computing. *Nature Methods* **9,** 772.
Dawson, J. A. (1963). The oral cavity, the 'jaws' and the horny teeth of *Myxine glutinosa*. In *The Biology of Myxine* (Brodal, A. & Fange, R., eds), pp. 231–255. Oslo: Scandinavian University Books.
Drazen, J. C. & Haedrich, R. L. (2012). A continuum of life histories in deep-sea demersal fishes. *Deep-Sea Research. Part I, Oceanographic Research Papers* **61,** 34–42.
Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29,** 1969–1973. https://doi.org/10.1093/molbev/mss075
Fernholm, B. (1974). Diurnal variations in the behaviour of the hagfish *Eptatretus burgeri*. *Marine Biology* **27,** 351–356.
Fernholm, B. (1998). Hagfish systematics. In *The Biology of Hagfishes* (Jørgensen, J. M., Lomholt, J. P., Weber, R. E. & Malte, H., eds), pp. 34–44. London: Chapman & Hall.
Fernholm, B., Noren, M., Kullander, S. O., Quattrini, A. M., Zintzen, V., Roberts, C. D., Mok, H.-K. & Kuo, C.-H. (2013). Hagfish phylogeny and taxonomy, with description of the new genus *Rubicundus* (Craniata, Myxinidae). *Journal of Zoological Systematics and Evolutionary Research* **51,** 296–307.
Freckleton, R. P. (2000). Phylogenetic tests of ecological and evolutionary hypotheses: checking for phylogenetic independence. *Functional Ecology* **14,** 129–134.
Fudge, D. S., Levy, N., Chiu, S. & Gosline, J. M. (2005). Composition, morphology and mechanics of hagfish slime. *Journal of Experimental Biology* **208,** 4613–4625.
Garland, T., Harvey, P. H. & Ives, A. R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* **41,** 18–32.
Gorbman, A., Kobayashi, H., Honma, Y. & Matsuyama, M. (1990). The hagfishery of Japan. *Fisheries* **15,** 12–18.
Grant, S. M. (2006). An exploratory fishing survey and biological resource assessment of Atlantic hagfish (*Myxine glutinosa*) occurring on the southwest slope of the Newfoundland Grand Bank. *Journal of Northwest Atlantic Fishery Science* **36,** 91–110.
Grant, S. M. (2015). Hagfish fisheries research. In *Hagfish Biology* (Edwards, S. L. & Goss, G. G., eds). Boca Raton, FL: CRC Press.
Haldane, J. B. S. (1928). On being the right size. In *Possible Worlds and Other Papers* (Haldane, J. B. S., ed), pp. 20–28. New York, NY: Harper.
Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. (2008). GEIGER: investigating evolutionary radiations. *Bioinformatics* **24,** 129–131.
Hart, J. L. (1973). Pacific fishes of Canada. *Bulletin of Fisheries Reserach Board of Canada* **180**, 740.
Heincke, F. (1913). Untersuchungen über die Scholle – Generalbericht I. Schollenfischerei und Schonmassregeln. Vorlaeufige kurze Uebersicht über die wichtigsten Ergebnisse des Berichts. *Rapport et Procès-verbaux des Réunions du Conseil international pour l'Exploratio de la Mer* **16**, 1–70.
Hunt, G. & Roy, K. (2006). Climate change, body size evolution and Cope's rule in deep-sea ostracods. *Proceedings of the National Academy of Sciences of the United States of America* **103,** 1347–1352.
Jacquemin, S. J. & Doll, J. C. (2014). Body size and geographic range do not explain long term variation in fish populations: a Bayesian phylogenetic approach to testing assembly processes in stream fish assemblages. *PLoS One* **9,** 1–7. https://doi.org/10.1371/journal.pone.0093522
Jensen, D. (1966). The hagfish. *Scientific American* **214,** 82–90.

Johnson, E. W. (1994). Aspects of the biology of the Pacific (*Eptatretus stoutii*) and black (*Eptatretus deani*) hagfishes from Monterey Bay, California. In *Biology*. Fresno, CA: California State University at Fresno.
Jordan, D. S. (1892). Relations of temperature to vertebrae among fishes. *Proceedings of the United States National Museum* **14,** 107–120.
Jørgensen, J. M., Lomholt, J. P., Weber, R. E. & Malte, H. (1998). *The Biology of Hagfishes*. London: Chapman & Hall.
Kulbicki, M., Bozec, Y.-M., Labrosse, P., Letourneur, Y., Mout-Tham, G. & Wantiez, L. (2005). Diet composition of carnivorous fishes from coral reef lagoons of New Caledonia. *Aquatic Living Resources* **18,** 231–250.
Lindstedt, S. L., Miller, B. J. & Buskirk, S. W. (1986). Home range, time and body size in mammals. *Ecology* **67,** 413–418.
Martini, F. H. (1998). The ecology of hagfishes. In *The Biology of Hagfishes* (Jørgensen, J. M., Lomholt, J. P., Weber, R. E. & Malte, H., eds). London: Chapman & Hall.
Maurer, B. A., Brown, J. H. & Rusler, R. D. (1992). The micro and macro in body size evolution. *Evolution* **46,** 939–953.
Mincarone, M. M. & Stewart, A. L. (2006). A new species of giant seven-gilled hagfish (Myxinidae: *Eptatretus*) from New Zealand. *Copeia* **2,** 225–229.
Miyashita, T. & Coates, M. I. (2015). Hagfish emryology: staging table and relevance to the evolution and development of vertebrates. In *Hagfish Biology* (Edwards, S. L. & Goss, G. G., eds), pp. 95–121. Boca Raton, FL: CRC Press.
Neat, F. C. & Campbell, N. (2013). Proliferation of elongate fishes in the deep sea. *Journal of Fish Biology* **83,** 1576–1591.
Ota, K. G. & Kuratani, S. (2006). The history of scientific endeavors towards understanding hagfish embryology. *Zoological Science* **23,** 403–418.
Ota, K. G., Fujimoto, S., Oisi, Y. & Kuratani, S. (2013). Late development of hagfish vertebral elements. *Journal of Experimental Zoology. B* **320,** 129–139. https://doi.org/10.1002/jez.b.22489
Paradis, E., Claude, J. & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20,** 289–290.
Pauly, D. (1997). Geometrical constraints on body size. *Trends in Ecology & Evolution* **12,** 442.
Peters, R. H. (1983). *The Ecological Implications of Body Size*. Cambridge, MA: Cambridge University Press.
Reece, J. S. & Mehta, R. S. (2013). Evolutionary history of elongation and maximum body length in moray eels (Anguilliformes: Muraenidae). *Biological Journal of the Linnean Society* **109,** 861–875.
Revell, L. J. (2009). Size-correction and principal components for interspecific comparative studies. *Evolution* **63,** 3258–3268.
Revell, L. J. (2010). Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution* **1,** 319–329.
Rex, M. A. & Etter, R. J. (1998). Bathymetric patterns of body size: implications for deep-sea biodiversity. *Deep-Sea Research Part II: Topical Studies in Oceanography* **45,** 103–127.
Rex, M. A., Etter, R. J., Clain, A. J. & Hill, M. S. (1999). Bathymetric patterns of body size in deep-sea gastropods. *Evolution* **53,** 1298–1301.
Roy, K. (2008). Dynamics of body size evolution. *Science* **321,** 1451–1452.
Schmidt-Nielsen, K. (1984). *Scaling, Why is Animal Size So Important?* New York, NY: Cambridge University Press.
Sébert, P., Scaion, D. & Belhomme, M. (2009). High hydrostatic pressure improves the swimming efficiency of European migrating silver eel. *Respiratory Physiology & Neurobiology* **165,** 112–114.
Smith, K. F. & Brown, J. H. (2002). Patterns of diversity, depth range and body size among pelagic fishes along a gradient of depth. *Global Ecology and Biogeography* **11,** 313–322.
Smith, K. L. J. (1978). Metabolism of the abyssopelagic rattail *Coryphaenoides armatus* measured *in situ*. *Nature* **274,** 362–364.
Steele, S. E. & López-Fernández, H. (2014). Body size diversity and frequency distributions of neotropical cichlid fishes (Cichliformes: Cichlidae: Cichlinae). *PLoS One* **9,** e106336. https://doi.org/10.1371/journal.pone.0106336

Thiel, H. (1975). The size structure of the deep-sea benthos. *Internationale Revue der gesamten Hydrobiologie* **60,** 575–606.
Uyeno, T. A. & Clark, A. J. (2015). Muscle articulations: flexible jaw joints made of soft tissues. *Integrative and Comparative Biology* **55,** 193–204.
Warburton, K. (1989). Ecological and phylogenetic constraints on body size in Indo-Pacific fishes. *Environmental Biology of Fishes* **24,** 13–22.
Zelle, H., Appeldoorn, G., Burgers, G. & Van Oldenborgh, G. J. (2004). The relationship between sea surface temperature and thermocline depth in the eastern equatorial Pacific. *American Meteorological Society* **34,** 643–655.
Zintzen, V., Roberts, C. D., Anderson, M. J., Stewart, A. L., Struthers, C. D. & Harvey, E. S. (2011). Hagfish predatory behaviour and slime defence mechanism. *Scientific Reports* **1,** 131.

Electronic References

Froese, R. & Pauly, D. (2015). *FishBase*. Available at www.fishbase.org
Nicholas, K. B. & Nicholas, H. B. (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments. Available at http://www.nrbsc.org/old/gfx/genedoc/
Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. & Pearse, W. (2015). Comparative analyses of phylogenetics and evolution in R: 'caper'. Available at https://cran.r-project.org/web/packages/caper/index.html
Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2015). R package nlme: linear and nonlinear mixed effects models. Available at http://CRAN.R-project.org/package=nlme/
Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). Tracer v1.6. Available at http://beast.bio.ed.ac.uk/Tracer/

