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RESEARCH

Tonic immobility in cartilaginous fishes (Chondrichthyes): function, evolutionary history, and future directions

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Abstract Tonic immobility, a temporary cessation of voluntary movements, is a widespread phenomenon within the animal kingdom. While commonly regarded as an ‘anti-predator’ defence strategy, empirical evidence supporting this hypothesis is predominantly limited to terrestrial vertebrates. In Chondrichthyes (i.e., sharks, rays, and chimaeras), tonic immobility has also been proposed as an anti-predator mechanism, despite a dearth of evidence and fundamental physiological distinctions from terrestrial counterparts. Here, we present data regarding tonic immobility induced by dorsoventral inversion across 13 chondrichthyan species. We also perform a review of the existing literature to identify temporal, taxonomic, and methodological trends in TI research. Then, using phylogenetic comparative methods, we demonstrate that this trait is plesiomorphic to Chondrichthyes and has been independently lost multiple times throughout chondrichthyan phylogeny. Contrary to expectations, we find no significant correlation between the evolutionary transitions in tonic immobility and major ecological axes of variation in Chondrichthyes. Our findings challenge existing hypotheses and suggest that tonic immobility may lack adaptive significance in extant chondrichthyans, persisting through chondrichthyan phylogeny due to minimal net fitness costs. Additionally, we propose an association between the loss of tonic immobility and entanglement risk in shallow-water benthic ecosystems. Our results provide unprecedented insights into the evolutionary history of this enigmatic behaviour within Chondrichthyes and across the phylogeny of jawed vertebrates.

Keywords Plesiomorphy · Thanatosis · Sharks · Evolution · Elasmobranchii · Limp response

Introduction

Tonic immobility (TI), a temporarily induced cessation of voluntary movement, is widespread amongst vertebrate and invertebrate animals (Hoagland 1928; Rusli 2022). Whilst TI in vertebrates is generally considered an innate reflex (Carli and Farabollini 2022; Hoagland 1928), there is substantial interspecific variation in the nature of tonic responses and their hypothesised functions (Jones 1986; Webster et al. 1981; Yoshida 2021). In some taxa (e.g., mammals) TI is associated with rigid muscle hypertonicity; whereas, in others (e.g., fishes), muscle relaxation (tonic limp response, herein TLR) is observed (Brooks et al. 2011; Páez et al. 2023; Wells et al. 2005). Moreover, a range of different stimuli are

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known to trigger TI, and responses can range in duration from seconds to hours (Jones 1986; Páez et al. 2023; Webster et al. 1981; Yoshida 2021). The only unifying feature of these responses is that they result in a reversible cessation of all movement aside from rhythmic breathing (Watsky and Gruber 1990). The prevailing hypothesis for the adaptive value of TI posits that it is a defensive tactic against predation (Carli and Farabollini 2022). Certainly, there is some empirical evidence for this 'playing dead' hypothesis. In several species tonic responses have been found to reduce the probability of death during predatory attacks (Carli and Farabollini 2022; Miyatake et al. 2009). This is not the only hypothesis, however. Other studies have proposed functions in reproductive behaviour and even predation itself (Williamson et al. 2018; Yoshida et al. 2021). Still, TI remains one of the least-understood behaviours across the animal kingdom. Despite its presence across a wide range of taxa, there are surprisingly few empirical studies examining TI from an evolutionary perspective. This paucity of empirical data, coupled with the interspecific and intraspecific variation in causal stimuli and TI responses, underscores the need for further studies across a phylogenetically diverse range of taxa.

Chondrichthyes (sharks, rays, and chimaera) is a large, ecologically diverse radiation with an extensive evolutionary history (Grogan et al. 2012; Heinicke et al. 2009). Researchers often use TI (specifically the tonic limp response, TLR) as technique during fieldwork to restrain individuals during data collection and surgical procedures, and as a result, TLR has been observed in numerous chondrichthyan species (Páez et al. 2023; Yoshida et al. 2021; Kessel and Hussey 2015). Comparatively few experimental studies specifically describing tonic responses, however, exist in the literature. Even from this small number of studies, it is clear that chondrichthyans display a wide range of tonic responses (Páez et al. 2023). For example, TI can be induced by horizontally inverting individuals, applying pressure to the caudal fin, or by stimulating the ampullae of Lorenzini (Henningsen 1994; Páez et al. 2023; Whitman et al. 1986; Williamson et al. 2018). The efficacy of each of these techniques, the behaviours they elicit, and the duration of the tonic response all vary substantially among species (Henningsen 1994; Páez et al. 2023; Yoshida et al. 2021). Indeed, there are some species that appear to exhibit greatly reduced tonic responses or even lack TI altogether (Henningsen 1994). Unfortunately, existing data are restricted to a small number of species, with a complete absence of data from several major chondrichthyan clades, including chimaera.

Three main hypotheses exist regarding the adaptive function of TI in Chondrichthyes. Firstly, it has been suggested that TI may be a plesiomorphic response to predator attacks that is ancestral to all vertebrates (Carli and Farabollini 2022; Páez et al. 2023). Whilst there is evidence for TI improving the probability of survival following predation attempts in terrestrial vertebrates (Humphreys and Ruxton 2018; Miyatake et al. 2009), there is little empirical evidence for this among fishes, and none whatsoever in chondrichthyans (Yoshida et al. 2021). Secondly, a role for TI in copulatory behaviour has been posed, predominantly based on observations of male zebra sharks (*Stegostoma fasciatum*) inverting (and thus immobilising) females prior to copulation, consequently reducing reproductive aggression (Páez et al. 2023; Williamson et al. 2018; Yoshida 2021). This hypothesis also has several major theoretical flaws, including the fact that tonic responses are not known to differ between sexes (Williamson et al. 2018; Yoshida 2021). If TI were commonly involved in copulation, it would leave females vulnerable to unwanted, costly, and maladaptive mating events, and there would likely be strong selection against this trait in females. Moreover, physiological studies suggest that TI actually *increases* the production of stress hormones (Brooks et al. 2011). Finally, a third hypothesis suggests that TI may limit the effects of overwhelming sensory stimulation, but this also remains untested (Páez et al. 2023). Indeed, the true function and evolutionary origins of TI in Chondrichthyes are poorly understood. While tonic responses in chondrichthyans may superficially resemble those observed in other vertebrates (i.e., particularly Osteichthyes), the significant evolutionary distance between these clades and the fact that some chondrichthyan species exhibit minimal or no TI behaviours renders assuming plesiomorphy or equivalence between TI in chondrichthyans and other vertebrates unwise. Rather, quantitative analyses incorporating consistent definitions of TI are necessary to determine the trait's evolutionary history and possible adaptive functions.

Here, we provide novel experimental data revealing the presence or absence of TI upon horizontal inversion (as in Fig. 1)—the most common method

Fig. 1 A neonatal sicklefin lemon shark (Negaprion acutidens) being dorsoventrally inverted to induce TI in Moorea, French Polynesia. Image credit is given to Rachel Moore

of TI induction in Chondrichthyes, and from here on referred to as TLR—of 13 chondrichthyan species, including the first ever empirical data on TI from a chimaera species. We subsequently perform a review of the literature, identifying all existing studies that provide verifiable reports of TLR in chondrichthyan species and identifying major qualitative trends in TI research. Combining our novel experimental data with data gathered from this literature review, we search for relationships within a phylogenetic context between TI and major ecological axes of variation between the taxa included in this study to provide insight into the environmental conditions that might favour the presence or absence of tonic responses in Chondrichthyes. Finally, we model the evolutionary history of TI in chondrichthyans, provide estimates of the ancestral state of tonic capabilities in the clade, and aim to uncover the evolutionary dynamics of subsequent gains/losses of TI. We discuss our results in the context of existing adaptive hypotheses and propose future avenues for research that may uncover new information regarding the prevalence and function of this complex, yet poorly understood behaviour.

Methodology

Ethics statement

All animal care and experimental protocols were approved by James Cook University Animal Ethics Committee regulations (permit: A2841, approved for this study) according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and the Queensland Animal Care and Protection Act, 2001.

Data collection

We collected novel data regarding tonic limp response (TLR) from the following 13 chondrichthyan species: *Callorhinchus milii* (n = 4), *Pseudobatos lentiginosus* (n = 1), *Heterodontus francisci* (n = 1), *Squalus suckleyi* (n = 9), *Ginglymostoma cirratum* (n = 1), *Hemiscyllium ocellatum* (n = 2), *Hemiscyllium freycineti* (n = 10), *Chiloscyllium punctatum* (n = 3), *Chiloscyllium plagiosum* (n = 4), *Scyliorhinus canicula* (n = 6), *Mustelus mustelus* (n = 1), *Carcharhinus melanopterus* (n = 3) and *Rhizoprionodon longurio* (n = 2). All individuals included in the study were housed in aquaria, aside from *Rhizoprionodon longurio*, which were tested during routine data collection procedures in the field.

To maximise the comparative power of our results, we selected TLR as our measure of tonic response. Muscle relaxation, deep rhythmic breathing, and cessation of struggling can be observed in chondrichthyan taxa upon dorsoventral inversion if they exhibit TLR (Henningsen 1994). To test for this, individuals were restrained and inverted dorsoventrally as quickly and gently as possible, whilst keeping the individual fully submerged (Fig. 1). While maintaining contact to ensure stability, the time until the individual exhibited deep rhythmic breathing and muscle relaxation was measured. If the limp response was detected, the individual was released but not reinverted, and the time taken for the individual to regain mobility was measured (i.e., up to five minutes, after which the individual was re-inverted manually). If no limp response was detected within 90 s, the individual was reinverted and released for one minute. The test was then repeated until the limp response was detected, or until each individual had been tested a total of three times. Video footage was recorded for all individuals to verify induction times and the duration of tonic responses.

To supplement our novel experimental data, we performed a literature review of TLR from existing literature. We conducted two independent searches of the primary literature in Web of Science and Google Scholar databases, using combinations of the following keywords: “Chondrichthyes” or “Shark” or “ray” or “chimaera” or “elasmobranch” and “tonic immobility” or “limp response”. We then manually filtered the literature identified to discard any unverifiable reports, or those not explicitly describing the process used to induce (or attempt to induce) TLR. The presence/absence of TLR in each species included in these studies was noted, alongside additional biological information regarding the individuals used to test for TLR. Only studies that used a method of TLR induction comparable to that described above in our experimental protocol were incorporated into later analyses.

To facilitate subsequent evolutionary analyses, phylogenetic data for Chondrichthyes were obtained from Stein et al. (2018). A pruned maximum clade credibility (MCC) tree was then inferred using the R packages picante (Kembel et al. 2010) and phangorn (Schliep 2011). The 13 species for which we collected data were included alongside taxa for which existing data are available (see results). All evolutionary analyses used this MCC tree, which was visualised prior to use to ensure that pruning did not significantly alter phylogenetic interrelationships.

To test for relationships between TLR and ecology, latitude (tropical, subtropical or temperate), trophic level and maximum total length data for each species were gathered from Fishbase (Frouse and Pauly 2024). We also gathered depth and habitat information from Fishbase and the reference guide ‘Sharks of the World: a complete guide’ (Ebert et al. 2021). Following the approach used by Sternes et al. (2024), we coded species as one of three habitat types, ‘benthic’, ‘benthopelagic’, or ‘pelagic’ based on their habitat descriptions. The habitat type ‘benthic’ was assigned based on keywords of ‘benthic,’ ‘on muddy bottom,’ ‘on sediment,’ ‘bottom on insular continental shelves.’ The habitat type of ‘benthopelagic’ was assigned based on the key terms of ‘demersal,’ ‘near bottom,’ or ‘near continental shelves.’ The habitat type ‘pelagic’ was assigned based on the keywords of ‘pelagic,’ ‘epipelagic,’ ‘bathypelagic,’ ‘open ocean,’ or ‘oceanic’. We coded depth as one of three depth types, ‘shallow’, ‘intermediate’ and ‘deep’. We selected these 5 ecological variables, as they encompass the major axes of variation that distinguish the ecological niches occupied by the species included in this study. Consequently, any significant relationships between TI and these variables would provide valuable insight into the function(s) of TLR, and the conditions favouring retention/gain/loss of the TLR response, despite not providing direct tests of the existing functional hypotheses.

The full dataset utilised in this study can be found in the supplementary materials associated with this article.

Data analyses

Prior to analysis, all discrete traits were coded as binary variables (Table 1). Due to uncertainty as to which category ‘benthopelagic’ and ‘intermediate’ species should be included in, two separate coding regimes were used for each of ‘depth’ and ‘habitat type’. Maximum total length and trophic level were both log10 transformed to minimise the effects of skewness. Note that preliminary analyses performed without log transformation did not yield qualitatively different results.

Table 1 Coding protocol used to convert discrete variables to binary variables

Variable	Coded as 0	Coded as 1
TLR (Yes, No)	No	Yes
Latitude (Tropical, Subtropical, Temperate)	Subtropical, Temperate	Tropical
Habitat type 1 (Benthic, Benthopelagic, Pelagic)	Pelagic, Benthopelagic	Benthic
Habitat type 2 (Benthic, Benthopelagic, Pelagic)	Pelagic	Benthic, Benthopelagic
Depth 1 (Shallow, Intermediate, Deep)	Shallow	Deep, Intermediate
Depth type 2 (Shallow, Intermediate, Deep)	Shallow, Intermediate	Deep

To test for relationships between TLR and the two continuous ecological variables (i.e., maximum total length and trophic level), we fit phylogenetic logistic regression models between TLR (coded as a binary variable), maximum total length, and trophic level, in the R package phylolm (Ho et al. 2016). Specifically, we fit logistic regression models following the approach of Ives and Garland (2010), employing the 'logistic_MPLE' method to maximise penalised likelihood. Statistically significant results here would indicate that the evolutionary history of TLR in chondrichthyans is correlated to the evolutionary history of body size and/or trophic level. For model comparison, we also fit a third logistic regression model lacking covariates (the null model). Models were selected based on AIC values, as described previously. Due to the relatively low number of observations per species (see results) it was not possible to test quantitatively for ontogenetic or sex-based differences in TLR.

To test for evolutionary correlation between TLR and discrete ecological variables (i.e., depth, habitat, and latitude), we instead used Pagel's correlation method (Pagel 1994) in the R package Geiger (Harmon et al. 2015). This test differs subtly from the phylogenetic independent contrast method and PGLS approaches; significant results suggest that the rate of evolution of one trait depends on that of the other, rather than implying a strict evolutionary correlation (Pagel 1994).

Numerous models of trait evolution have been proposed in the literature, each with differing sets of assumptions and limitations (Blomberg et al. 2020). Comparisons of fit and performance between different models of trait evolution can provide valuable insight into the selective regime, or lack thereof, that underlies trait evolution and the distribution of trait values. Given that TLR is a binary trait (presence/absence), we incorporated binary-specific models, such as phylogenetic logistic regression, to enhance the robustness of our analyses. In addition to continuous trait models, we employed phylogenetic logistic regression within the R package 'phylolm' (Ho et al. 2016) to assess the evolutionary association between TLR and species-level ecological traits while accounting for phylogeny. This approach directly addresses the binary nature of TLR.

For further model comparison, we fit two models of trait evolution to our data in the R package geiger (Harmon et al. 2015) to examine evolutionary transitions. The fist model assumed equal transition rates between the presence and absence of TLR ('equal rates' model, also referred to as the symmetric model for binary traits). The second model allowed for unequal transition rates ('all rates differ model), providing flexibility to capture potential asymmetries in trait evolution. Model selection was based on AICc values, which are found to be more accurate for smaller phylogenetic trees (Posada and Buckley 2004). Ultimately, this model-fitting approach allows us to contextualise the selective pressures influencing the evolution of the tonic immobility and to ensure that our analyses align with best practices for binary trait analysis.

To reconstruct the evolutionary history of TLR in chondrichthyans, predict the ancestral state, and estimate the number of independent losses and gains of TLR, we performed ancestral state reconstruction using the 'ace' function in the R package ape (Paradis and Schliep 2019). Ancestral state reconstruction calculates the probability of each internal node of the phylogeny exhibiting a given trait value (i.e., in this case, presence or absence of TLR). Following standard procedures (Maritz et al. 2021), we performed two separate ancestral state reconstructions, using both the 'equal rates' model and the 'all rates different' model, as outlined above. Note that the combined dataset (novel experimental data and previously published records) includes both positive and negative TLR observations, thereby minimising potential selection bias.

To provide a measure of the phylogenetic dependence of TLR, we performed a test for phylogenetic signal (measured as the 'D statistic' per Fritz and Purvis 2010) using the R package caper (Orme et al. 2013). This metric describes the sum of changes in estimated nodal values of a binary trait along the edges in a phylogeny, where values significantly greater than 1 indicate trait lability (Fritz and Purvis 2010). We compared the observed value of D to that expected under phylogenetic randomness and Brownian Motion threshold models, using 10,000 independent permutations.

Results

We collected novel data regarding tonic limp response (TLR) from 13 chondrichthyan species by restraining and inverting individuals dorsoventrally as quickly and gently as possible, and observing for muscle relaxation, deep rhythmic breathing, and cessation of struggling. Of these 13 species, seven exhibited TLR upon dorsoventral inversion, whereas six did not (Table 2). Where induction time could be measured, it varied from seven seconds in *Mustelus mustelus* to 25 s in *Carcharhinus melanopterus* (Table 2). Duration of the tonic response varied from 12 s in *Mustelus mustelus* to 131 s in *Rhinobatos lentiginosus* (Table 2). No major intraspecific differences in TLR were observed, and where multiple individuals of a given species were tested, they all displayed qualitatively equivalent responses (e.g., either all individuals or no individuals exhibited TLR).

A review of the literature produced verifiable data regarding the presence/absence of TLR in 29 species, from seven elasmobranch orders (Table 3). Carcharhiniformes was the order best represented, with 16 (over 50%) of the total species for which TLR data exists in the literature. Contrastingly, other orders such as Rajiformes and Lamniformes are represented by a single species (Table 3). Some groups, such as Chimaeriformes, Squaliformes, and Torpediniformes are entirely unrepresented. Only five published manuscripts provide empirical data regarding TLR induction or duration, with the remaining studies instead noting the presence of TLR as justification for its use as a field technique. Only two reports indicate species lacking TLR (Table 3) and no notable intraspecific trends were observed in any species.

Phylogenetic logistic regression models recovered no significant evolutionary relationship between TLR and either maximum total length or trophic level, with the null model having the lowest AIC value (Table 4).

Pagel's correlation test for rate dependency between TLR and latitude, habitat, and depth recovered no evidence of significant relationships (Table 5).

Comparison of model fit for equal rates (ER) and all rates different (ARD) models failed to provide conclusive support for one model over the other (the difference in AIC values between models was lower than 2), although the equal rates model had a lower AIC value ($AIC_{ER} = 31.08$, $AIC_{ARD} = 32.89$).

Ancestral state reconstruction of the presence/absence of TLR assuming either equal or different evolutionary transition rates produced qualitatively similar results, suggesting that the ancestral state of Chondrichthyes is the presence of TLR upon dorsoventral inversion (Fig. 2). Both models also suggest that TLR has been independently lost at least five times through chondrichthyan phylogeny (Fig. 2). According to both models, TLR has been lost at least once within each of Chimaeriformes, Myliobatiformes, Hexanchiformes, Orectolobiformes, and Carcharhiniformes (Fig. 2). However, the two models differ in their support for the presence of TLR at various nodes within the phylogeny;

Table 2 Presence or absence of TLR upon horizontal inversion, including the induction time and duration of TI where relevant

Order	Scientific name	N	TLR (Yes/No)	Mean induction time (s)	Mean duration (s)
Chimaeriformes	*Callorhinchus milii*	4	No	N/A	N/A
Rhinopristiformes	*Pseudobatos lentiginosus*	1	Yes	17	131
Heterodontiformes	*Heterodontus francisci*	1	Yes	15	74
Squaliformes	*Squalus suckleyi*	9	Yes	NA	NA
Orectolobiformes	*Ginglymostoma cirratum*	1	Yes	N/A	N/A
	Hemiscyllium ocellatum	2	No	N/A	N/A
	Hemiscyllium freycineti	10	No	N/A	N/A
	Chiloscyllium punctatum	3	No	N/A	N/A
	Chiloscyllium plagiosum	4	No	N/A	N/A
Carcharhiniformes	*Scyliorhinus canicula*	6	No	N/A	N/A
	Mustelus mustelus	1	Yes	7	12
	Carcharhinus melanopterus	3	Yes	25	92
	Rhizoprionodon longurio	2	Yes	N/A	N/A

Table 3 Chondrichthyan species identified by a literature review for which the nature of TI responses observed is consistent with our definition of TLR. Note that observations or lack thereof of TI responses to dorsoventral inversion do not preclude alternative results existing for different methods of TI induction

Order	Scientific name	TLR (Yes/No)	References
Rajiformes	*Rostroraja eglanteria*	Yes	Henningsen (1994)
Rhinopristiformes	*Pseudobatos productus*	Yes	Henningsen (1994)
Myliobatiformes	*Urobatis halleri*	Yes	Henningsen (1994)
	Urolobatis jamaicensis	No	Henningsen (1994)
	Urogymnus dalyensis	Yes	Campbell et al. (2012)
	Pateobatis fai	Yes	Gaspar et al. (2008)
	Hypanus americanus	Yes	Henningsen (1994)
	Rhinoptera bonasus	Yes	Henningsen (1994)
Hexanchiformes	*Notorynchus cepedianus*	No	Henningsen (1994)
Orectolobiformes	*Stegostoma fasciatum*	Yes	Williamson et al. (2018)
	Ginglymostoma cirratum	Yes	Chapman et al. (2005)
	Cephaloscyllium ventriosum	Yes	Henningsen (1994)
Carcharhiniformes	*Galeocerdo cuvier*	Yes	Holland et al. (1999)
	Carcharhinus melanopterus	Yes	Davie et al. (1993); Henningsen (1994)
	Carcharhinus plumbeus	Yes	Papastamatiou et al. (2010)
	Carcharhinus leucas	Yes	Werry et al. (2011)
	Triaenodon obesus	Yes	Henningsen (1994)
	Carcharhinus amblyrhynchos	Yes	Speed et al. (2011)
	Carcharhinus galapagensis	Yes	Meyer et al. (2010)
	Carcharhinus perezii	Yes	Henningsen 1994
	Negaprion acutidens	Yes	Speed et al. 2011
	Negaprion brevirostris	Yes	Brooks et al. (2011); Watsky and Gruber (1990)
	Triakis semifasciata	Yes	Henningsen (1994)
	Mustelus canis	Yes	Whitman et al. (1986)
	Mustelus californicus	Yes	Espinoza et al. (2011)
	Mustelus lenticulatus	Yes	Francis (2013)
	Haploblepharus pictus	Yes	Sesbastian (2020)
	Haploblepharus edwardsii	Yes	Sesbastian (2020)
Lamniformes	*Carcharhinus taurus*	Yes	Kneebone et al. (2012)

Table 4 Output from phylogenetic logistic regression models of TLR (0 = absence, 1 = presence) and the potential ecological covariates. Log lik refers to the penalised log likelihood score

Model covariate	Correlation coefficient	Alpha	Log lik	AIC
Maximum total length	0.5115467	0.003656925	− 13.39	34.24
Trophic level	0.006451849	0.004706463	− 15.29	35.25
Null	N/A	0.00632504	− 14.31	33.41

at the root of the tree, TLR is predicted with 77% scaled likelihood in the equal rates model (Fig. 2a), but with only 62% scaled likelihood in the 'all rates differ' model (Fig. 2b). Transition rates were ignored, as neither model predicted any cases of TLR being gained.

The presence or absence of TLR was underlain by a D metric of − 0.2576763 (Fig. 3). This value

Table 5 Output from Pagel's correlation test for evolutionary rate dependency between TLR and each of 5 discrete ecological traits respectively

Model covariate	Likelihood ratio	*P* value
Latitude	2.38	0.67
Habitat 1	2.07	0.72
Habitat 2	2.35	0.67
Depth 1	3.73	0.44
Depth 2	9.61E−10	1.00

was significantly lower than expected under a phylogenetic randomness model ($p = 0.004$), but did not differ significantly from the value expected under a Brownian Motion threshold model ($p = 0.694$).

Discussion

The primary aim of this study was to explore the prevalence, potential adaptive function, and evolutionary history of tonic immobility (TI) in cartilaginous fishes. Whilst the number of species included may seem modest compared to some other comparative phylogenetic analyses, collecting empirical data from live chondrichthyan species poses unique logistical challenges, particularly in the case of rare species or those that live at great depths. Nevertheless, the new data presented here represent a substantial increase the sample size available in the literature. Our expanded dataset includes taxa from many of the major elasmobranch radiations (providing a breadth and diversity sufficient for comparative phylogenetic analyses), including several that were entirely unrepresented in the literature prior to this study (Tables 2, 3).

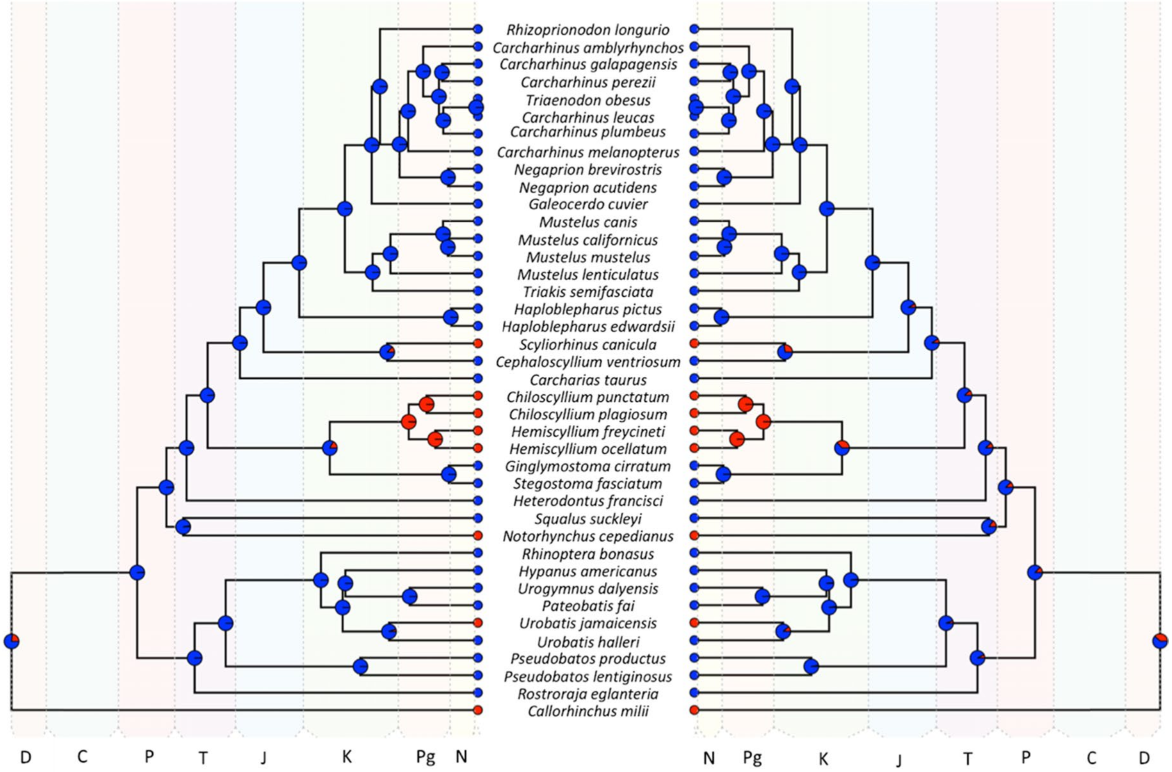

Fig. 2 Time-calibrated phylogenies overlain with graphical representations of ancestral state reconstructions using a) 'equal rates' model and b) 'all rates differ' model. Pie charts represent the probability of a given node exhibiting TLR (blue) or not exhibiting TLR (red). Geological timescale labels refer to the Devonian (D), Carboniferous (C), Permian (P), Triassic (T), Jurassic (J), Cretaceous (K), Paleogene (Pg), and Neogene (N)

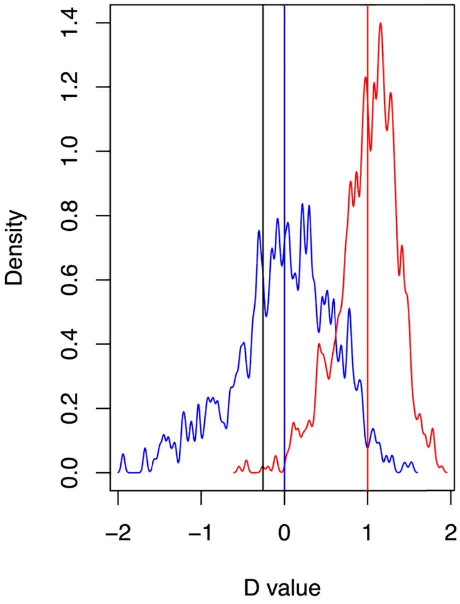

Fig. 3 Observed (black line) and expected (red and blue lines) values for D under phylogenetic randomness and Brownian Motion evolution respectively

Our findings have important implications for understanding TI. We observed the absence of TLR in 6 species (Table 2), increasing the total number of species known not to exhibit TLR by 300%. Notably, this includes *Callorhinchus milii*, the first chimaeriform to be formally tested for TLR, which holds significance as a sister taxon to Elasmobranchii and contributes to our understanding of the ancestral state of TI in Chondrichthyes and gnathostomes more broadly. Beyond elucidating the evolutionary history of a complex behaviour, our results indicate that TI may not be universally applicable as an alternative to anaesthesia in research and husbandry. Absence of TLR in some species indicates that dorsoventral inversion may not convey pain relief or an anaesthetic effect consistently. Importantly, studies have shown that various stimuli can elicit TI-like responses in chondrichthyans (Páez et al. 2023), and thus the absence of TLR in this study does not preclude the possibility of similar physiological responses under different conditions. Additionally, both our literature review (Table 3) and experimental data (Table 2) indicate that there are no clear intraspecific trends in TLR, and that it is a relatively static trait through ontogeny. In the following sections, we discuss these results and our phylogenetically corrected analyses in the context of trait evolution, as well as the potential origins and functions of TLR. Overall, this study underscores the importance of species-specific research on tonic responses and provides novel insights into the adaptive value of tonic immobility in Chondrichthyes and gnathostomes more broadly.

The adaptive significance (or lack thereof) of TLR

Whilst several hypotheses have posited the adaptive significance of TI in vertebrates, there is little to no empirical support for any of these hypotheses in Chondrichthyes. The hypothesis that TI represents an anti-predator defence appears to be valid in at least some terrestrial vertebrates (Humphreys and Ruxton 2018), but there is no evidence for this hypothesis in Chondrichthyes and only limited support in Actinopterygian fishes (Yoshida 2021). Moreover, there are multiple theoretical issues with this hypothesis. Namely, the maintenance of a dorsoventral position for extended periods of time during predation events is unlikely (Yoshida 2021), as most predators of chondrichthyan species either utilise suction feeding or cutting/gouging to handle prey (Heithaus and Vaudo 2004; Wilga and Ferry 2015), neither of which are conducive to inducing TI. In the rare cases where predators do induce TI in chondrichthyans (e.g., Orca predation upon *Mobula* rays), it seems that TI is being exploited by the predator rather than improving the probability of survival in the chondrichthyan taxon (Higuera-Rivas et al. 2023). Finally, most chondrichthyan species known that do not exhibit TLR are relatively small-bodied (Table 2; Ebert et al. 2021) and are thus likely subject to greater predation pressure than many of the large-bodied species in which TLR has been observed. Alternatively, it has been suggested that TLR in Chondrichthyes may play a role in copulation (Páez et al. 2023). However, there is no evidence that TLR differs between the sexes (Williamson et al. 2018). Moreover, this hypothesis is flawed because it would leave females susceptible to unwanted mating events at a great cost to evolutionary fitness. In actinopterygian fishes, TLR may be used as a predatory tactic (Yoshida 2021); however, no chondrichthyan has ever been observed engaging in such behaviour. Consequently, there is no evidence to support either of the proposed adaptive functions of TLR in chondrichthyans, and to the contrary, both have major theoretical limitations that contradict our current understanding of evolutionary ecology in the clade.

Our results suggest that neither depth ranges, body size, biogeography, nor habitat preferences can satisfactorily predict the phylogenetic distribution of TLR in Chondrichthyes (Tables 4, 5). Admittedly, these variables do not provide a direct test of existing functional hypotheses for TLR. Relationships between trophic level (or body size) and TLR could theoretically have provided evidence for or against TLR as a predator evasion strategy; however, empirical support for a consistent relationship between size or trophic level and predation pressure in chondrichthyans does not yet exist. Rather, these variables represent the major axes of ecological variation between taxa that are included in the present study. Moreover, the shallow-deep, pelagic-benthic, small-large, and tropical-boreal gradients are all known to have influenced the diversification of various morphological and physiological traits in Chondrichthyes (Gayford et al. 2024; López-Romero et al. 2023; Sorensen et al. 2014; Sternes et al. 2024). This, combined with the criticism of existing hypotheses above leads us to suggest that TLR in Chondrichthyes may not be adaptive at all. Rather, TLR may simply be a plesiomorphic trait that served some adaptive function in the past but has been retained in many extant chondrichthyans despite no longer serving any significant adaptive function. Further circumstantial evidence for this comes from our finding that the evolution of TLR in Chondrichthyes is highly conserved and underlain by a strong phylogenetic signal (Fig. 3). We are therefore unable to speculate as to the original function of TLR or its relation to TLR functions seen in actinopterygian fishes. However, considering the issues with existing adaptive hypotheses and the absence of support for significant ecological signal or evolution toward trait optima in Chondrichthyes, it appears equally, if not more parsimonious to suggest that TLR is simply a retained plesiomorphic trait that serves no clear function in extant species.

Whilst our results do not resolve the uncertainty regarding the possible adaptive significance of TLR, they do reveal an intriguing qualitative trend underlying evolutionarily independent losses of TLR. If TLR in extant chondrichthyans represents a functionally obsolete plesiomorphic trait, this raises the question of why TLR has been lost in some lineages but not others. Of the eight chondrichthyan species known not to exhibit TLR, none are found in deep waters, and none occupy predominantly pelagic waters (Ebert et al. 2021). We found no significant correlation between the evolutionary histories of TLR and these ecological variables (Tables 4, 5); however, there are valid reasons to suggest that TLR could incur fitness costs in shallow-water benthic environments. These areas frequently host blue carbon ecosystems including reefs, kelp forests, and mangrove forests, resulting in complex biogeomorphological features relative to deeper or open-ocean ecosystems (Emery 1978; Gratwicke and Speight 2005; Larouche et al. 2020). Chondrichthyan species often live-in close association with biogeomorphological structures in these environments—particularly in the case of small-bodied benthic sharks that feed within and around reef complexes (Heupel and Bennett 1998; Heupel and Simpfendorfer 2014). The risk of entanglement within narrow reef structures presents an issue for these species—indeed the structural and hydrodynamic properties of complex reef ecosystems are thought to exert strong selection on morphology and body form in fishes (Larouche et al. 2020). If a small-bodied shark were to become obstructed within a reef structure in a dorsoventrally inverted position, TLR could be disastrous, substantially increasing the chances of injury and/or death. Potential fitness costs of TLR would be negligible in a pelagic or deep-water context, where obstacles of this nature are minimal or non-existent (Larouche et al. 2020). Thus, in the absence of any significant fitness benefits or costs, there is no particular reason why TLR should be lost in other lineages. Of course, this does not account for small-bodied, coral reef-associated species such as *H. halmahera* that may exhibit TLR. It must be emphasised, however, that TI in this species was not induced in the same way as in the taxa included in this study, but instead by stimulation of the ampullae of Lorenzini (Mukharror et al. 2020). Ultimately, additional data will be necessary to improve the statistical power of correlation analyses. However, we hypothesise that TLR may be lost more frequently in the context of structurally complex, shallow benthic ecosystems due to the potential fitness costs associated of TI induction whilst entangled in narrow reef structures or similar biogeomorphological features.

The evolutionary history of TLR in Chondrichthyes (and other gnathostomes)

Ancestral state reconstruction suggests that TLR would have likely been present in basal chondrichthyans and has subsequently been lost on at least five separate occasions (Fig. 2). Combined with the apparent absence of secondary gains of TLR (Fig. 2), this suggests that TLR is plesiomorphic to Chondrichthyes (confirming the findings of Páez et al. 2023). Yet, there are several caveats to this finding. Firstly, we excluded *Hemiscyllium halmahera* from our analyses, as the method of TI induction (Mukharror et al. 2020) differed from that utilised in this study. *H. halmahera* is nested within Hemiscylliidae (Dudgeon et al. 2020), one of the lineages representing independent losses of TLR (Table 2). *H. halmahera* has, however, been recorded as exhibiting TI, and given that both techniques (i.e., snout stimulation and dorsoventral inversion) were used, we cannot confirm that *H. halmahera* does not exhibit TLR, unlike other species within Hemiscylliidae (Mukharror et al. 2020). Therefore, the number of independent losses and gains of the trait may still be equivocal. If the ancestral state of *Hemiscyllium* and/or Hemiscyllidae is TLR, this would require TLR to have been lost independently in both *Chiloscyllium* and *Hemiscyllium* genera, thus increasing the minimum number of independent losses of TI responsivity to six. If, as our models suggest, the ancestral state of Hemiscyllidae and/or *Hemiscyllium* is absence of TLR (Fig. 2), this suggests that there may have been at least one secondary gain of TLR within the genus *Hemiscyllium*. The second caveat with the suggestion of TI plesiomorphy in Chondrichthyes is the fact that only one Chimaeriform species was included (Table 2). Both ancestral state reconstruction models show strong support for TLR as the ancestral state of Elasmobranchii; however, there is much greater uncertainty regarding the ancestral state of Chondrichthyes (Fig. 2). This uncertainty could be reduced by including additional Chimaeriform taxa, as it is impossible to determine whether absence of TLR is an ancestral or derived state within Holocephali based on just one species. Lastly, as TLR is a complex physiological trait that is measured behaviourally, it was impossible to include any fossil data in our analyses, increasing the level of uncertainty associated with ancestral state estimations at basal nodes in the phylogeny.

Despite these potential limitations, based on existing data, there is substantial support for TLR being ancestral to Chondrichthyes, and at the very least to Elasmobranchii (Fig. 2). Chondrichthyes are the earliest diverging crown gnathostome lineage (Brazeau and Friedman 2015); therefore, if TLR is ancestral to Chondrichthyes, this would provide evidence to suggest that this TI response may be ancestral to gnathostomes as well. This is by no means to say that all TI responses are equivalent and ancestral to jawed vertebrates. It is important to note that the stiff muscle hypertonicity that characterises TI in terrestrial vertebrates differs fundamentally from the muscle relaxation (TLR) observed in Chondrichthyes. Chondrichthyans are not unique in displaying a limp TI response (referred to here as TLR)—bony fishes (and some lissamphibians) display TI responses that are at least superficially similar to TLR in Chondrichthyes (Sweckard 2021). We tentatively suggest that TLR may represent a symplesiomorphy—a shared ancestral state—between Chondrichthyes and Osteichthyes that was subsequently lost (or modified) at some point following the divergence of ray-finned fishes (Fig. 4a). Alternatively, TLR would have to have evolved independently in at least three major gnathostome clades: Elasmobranchii, Actinopterygii, and Lissamphibia (Fig. 4b). Whilst this second hypotheses requires fewer character changes and could thus be considered more parsimonious, given the complex physiological nature of TI, we argue that independent gains of TLR in phylogenetically distant lineages are less probable than losses of TLR. We cannot comment on the potential for TI responses in Lissamphibia being plesiomorphic to Chondrichthyes and/or Actinopterygia, as most lissamphibians exhibit a more amniote-like TI response than TLR, and the TI character state of non-tetrapod sarcopterygian fishes remains entirely unknown. Regardless, determining that TLR is ancestral, at least to Elasmobranchii and likely to Chondrichthyes, substantially improves our ability to understand macroevolutionary shifts in TI responses through gnathostome phylogeny. Through similar studies in other major gnathostome clades, we may be able to discern between competing hypotheses, such as those shown in Fig. 4.

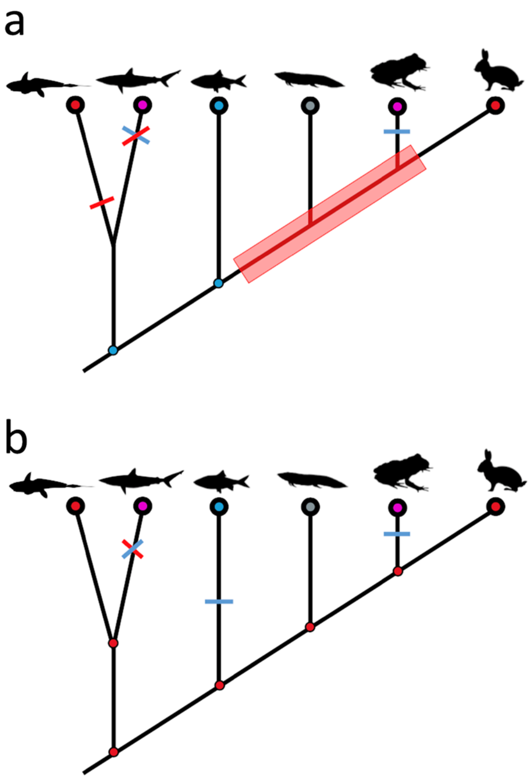

Fig. 4 Alternative hypotheses for macroevolutionary trends in TLR across gnathostome phylogeny (Diverging lineages represent Holocephali, Elasmobranchii, Actinopterygii, Sarcopterygii, Lissamphibia, Amniota from left to right respectively). Coloured circles represent the character state at a given node (blue = TLR, red = absence of TLR, pink = a combination of taxa with and without TLR, grey = uncertain character state). Coloured lines represent character transitions (red = TLR lost in this lineage, blue = TLR gained in this lineage, crossed lines = TLR gained and then secondarily lost in this lineage). The large red line in Fig. 4a indicates uncertainty regarding where loss of TI responsivity could have occurred (the gain of TI responsivity in the lissamphibian lineage only occurs where this loss occurs prior to the divergence of Lissamphibia). Importantly this phylogeny is not time-scaled, and character transitions do not imply a given character state in all extant members of a lineage

Future directions

While these results provide many novel insights into TI in sharks, many unanswered questions remain for future studies. Although we find evidence of at least five independent losses of TLR, our sample size is small relative to total chondrichthyan diversity, potentially underestimating the number of independent losses or gains. Whilst our analyses incorporated taxa from 10 of the 14 extant chondrichthyan orders, the dataset was nonetheless biased in favour of galeomorphs, with comparatively few squalomorph sharks and chimaera included (Tables 2, 3). Sample size may also have influenced the statistical power of our correlation analyses. For this reason, additional studies clarifying the presence or absence of TLR in other chondrichthyan species are needed—particularly from clades such as Chimaeriformes, where TLR appears to be absent. Phylogenetic studies of TI in other major vertebrate radiations would also be beneficial, particularly in the case of Actinopterygii, non-tetrapod Sarcopterygii, and Lissamphibia. In any case, to improve the comparative potential of studies into TI, a standardised experimental approach is needed—and for this reason we suggest future studies adopt the 'TLR' trait described in our methodology. At least in the case of Chondrichthyes, this is the method of TI induction discussed most prevalently in the literature. Additionally, novel studies into the comparative neurophysiology of extant chondrichthyans may provide insight into the proximate, mechanistic basis of evolutionary shifts in TLR. Finally, additional work is needed to gather the ecological/behavioural data necessary to provide direct tests of functional hypotheses for TLR, including those related to predator evasion and copulation.

Conclusions

Tonic immobility is one of the most enigmatic behavioural traits known in nature. Even in Chondrichthyes, the nature of TI responses varies markedly between species, and no adaptive hypothesis exists to satisfactorily explain the function of TI in extant chondrichthyan species. The data presented here, combined with our comparative phylogenetic analyses, suggest that tonic limp response (TLR) is ancestral to Chondrichthyes and that it has been lost numerous times during the clade's diversification. Based on existing evidence, we hypothesise that, whilst TI may have served some adaptive function in basal chondrichthyans, it represents a functionally obsolete plesiomorphy in extant taxa. This hypothesis does not suffer from the major theoretical contradictions of 'anti-predator' or 'copulatory behaviour' hypotheses that have previously been posited. Furthermore, we speculate that shallow-water benthic ecosystems, which tend to be structurally complex relative to deep-water or pelagic ecosystems, may favour the loss of TLR, which in other environmental contexts conveys negligible net fitness effects. Additional data are needed to test these hypotheses, and we hope that the 'TLR' methodological framework described in this study will result in greater standardisation between future studies of TI in chondrichthyans.

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Author contributions JHG and JLR conceptualised the study and collected the data. JHG analysed the data. JHG and JLR wrote and reviewed the manuscript.

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Data availability All data and code used in this study can be found in the following figshare repository: https://doi.org/10.6084/m9.figshare.27191226.

Declarations

Conflicts of interest The authors declare no conflicts of interest regarding this study.

References

Blomberg SP, Rathnayake SI, Moreau CM (2020) Beyond Brownian motion and the Ornstein-Uhlenbeck process: stochastic diffusion models for the evolution of quantitative characters. Am Nat 195(2):145–165

Brazeau MD, Friedman M (2015) The origin and early phylogenetic history of jawed vertebrates. Nature 520(7548):490–497

Brooks EJ, Sloman KA, Liss S, Hassan-Hassanein L, Danylchuk AJ, Cooke SJ, Mandelman JW, Skomal GB, Sims DW, Suski CD (2011) The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868). J Exp Mar Bio and Ecol 409(1–2):351–360

Campbell HA, Hewitt M, Watts ME, Peverell S, Franklin CE (2012) Short-and long-term movement patterns in the freshwater whipray (*Himantura dalyensis*) determined by the signal processing of passive acoustic telemetry data. Mar Freshwater ReS 63(4):341–350

Carli G, Farabollini F (2022) Tonic immobility as a survival, adaptive response and as a recovery mechanism. Prog Brain Res 271(1):305–329

Chapman DD, Pikitch EK, Babcock E, Shivji MS (2005) Marine reserve design and evaluation using automated acoustic telemetry: a case-study involving coral reef-associated sharks in the Mesoamerican Caribbean. Mar Technol Soc J 39(1):42–55

Davie PS, Franklin CE, Grigg GC (1993) Blood pressure and heart rate during tonic immobility in the black tipped reef shark *Carcharhinus melanoptera*. Fish Physiol Biochem 12:95–100

Dudgeon CL, Corrigan S, Yang L, Allen GR, Erdmann MV, Sugeha HY, White WT, Naylor GJ (2020) Walking, swimming or hitching a ride? Phylogenetics and biogeography of the walking shark genus *Hemiscyllium*. Mar Freshwater Res 71(9):1107–1117

Ebert DA, Dando M, Fowler S (2021) Sharks of the world: a complete guide. Princeton University Press. https://doi.org/10.1515/9780691210872

Emery AR (1978) The basis of fish community structure: marine and freshwater comparisons. Environ Biol Fishes 3:33–47

Espinoza M, Farrugia TJ, Lowe CG (2011) Habitat use, movements and site fidelity of the gray smooth-hound shark (*Mustelus californicus* Gill 1863) in a newly restored southern California estuary. J Exp Mar Biol Ecol 401(1–2):63–74

Francis MP (2013) Temporal and spatial patterns of habitat use by juveniles of a small coastal shark (*Mustelus lenticulatus*) in an estuarine nursery. PLoS One 8(2):e57021

Fritz SA, Purvis A (2010) Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. Conserv Biol 24(4):1042–1051

Froese R and Pauly D (2024) FishBase. https://fishbase.mnhn.fr/

Gaspar C, Chateau O, Galzin R (2008) Feeding sites frequentation by the pink whipray *Himantura fai* in Moorea (French Polynesia) as determined by acoustic telemetry. Cybium 32(2):153–164

Gayford JH, Brazeau MD, Naylor GJ (2024) Evolutionary trends in the elasmobranch neurocranium. Sci Rep 14(1):11471

Gratwicke B, Speight MR (2005) The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. J Fish Biol 66(3):650–667

Grogan E, Lund R, Greenfest-Allen E (2012) The origin and relationships of early chondrichthyans. In: Carrier Jeffrey, Musick John, Heithaus Michael (eds) Biology of sharks and their relatives, second edition. CRC Press, pp 3–29. https://doi.org/10.1201/b11867-3

Harmon L, Weir J, Brock C, Glor R, Challenger W, Hunt G, FitzJohn R, Pennell M, Slater G, Brown J and Uyeda J (2015) Package 'geiger'. R package version 2.0.11. https://cran.r-project.org/web/packages/geiger/index.html [Accessed 16 June 2024]

Heinickea MP, Naylorb GJP, Blair Hedgesa S (2009) Cartilaginous fishes (Chondrichthyes). In: Blair Hedges S, Kumar S (eds) The timetree of life. Oxford University PressOxford, pp 320–327. https://doi.org/10.1093/oso/9780199535033.003.0041

Heithaus M (2004) Predator, prey interactions. In: Musick J, Carrier J, Heithaus M (eds) Biology of sharks and their relatives. CRC Press, pp 487–521. https://doi.org/10.1201/9780203491317.ch17

Henningsen AD (1994) Tonic immobility in 12 elasmobranchs: use as an aid in captive husbandry. Zoo Biol 13(4):325–332

Heupel MR, Bennett MB (1998) Observations on the diet and feeding habits of the epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre), on Heron Island Reef, Great Barrier Reef Australia. Mar Freshwater Res 49(7):753–756

Heupel MR, Simpfendorfer CA (2014) Importance of environmental and biological drivers in the presence and space use of a reef-associated shark. Mar Ecol Prog Ser 496:47–57

Higuera-Rivas JE, Hoyos-Padilla EM, Elorriaga-Verplancken FR, Rosales-Nanduca H, Rosenthal R (2023) Orcas (*Orcinus orca*) use different strategies to prey on rays in the Gulf of California. Aquat Mamm 49(1)

Ho LST, Ane C, Lachlan R, Tarpinian K, Feldman R, Yu Q, van der Bijl W, Maspons J, Vos R and Ho MLST (2016). Package 'phylolm'. See http://cran.r-project.org/web/packages/phylolm/index

Hoagland H (1928) On the mechanism of tonic immobility in vertebrates. J Gen Physiol 11(6):715–741

Holland KN, Wetherbee BM, Lowe CG, Meyer CG (1999) Movements of tiger sharks (*Galeocerdo cuvier*) in coastal Hawaiian waters. Mar Biol 134:665–673

Humphreys RK, Ruxton GD (2018) A review of thanatosis (death feigning) as an anti-predator behaviour. Behav Ecol Sociobiol 72:1–16

Ives AR, Garland Jr T (2010) Phylogenetic logistic regression for binary dependent variables. Syst Biol 59(1):9–26

Jones RB (1986) The tonic immobility reaction of the domestic fowl: a review. J World's Poult Sci 42(1):82–96

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26(11):1463–1464

Kessel ST, Hussey NE (2015) Tonic immobility as an anaesthetic for elasmobranchs during surgical implantation procedures. Can J Fish Aquat Sci 72(9):1287–1291

Kneebone J, Chisholm J, Skomal GB (2012) Seasonal residency, habitat use, and site fidelity of juvenile sand tiger sharks *Carcharias taurus* in a Massachusetts estuary. Mar Ecol Prog Ser 471:165–181

Larouche O, Benton B, Corn KA, Friedman ST, Gross D, Iwan M, Kessler B, Martinez CM, Rodriguez S, Whelpley H, Wainwright PC (2020) Reef-associated fishes have more maneuverable body shapes at a macroevolutionary scale. Coral Reefs 39:1427–1439

López-Romero FA, Stumpf S, Kamminga P, Böhmer C, Pradel A, Brazeau MD, Kriwet J (2023) Shark mandible evolution reveals patterns of trophic and habitat-mediated diversification. Commun Biol 6(1):496

Maritz B, Barends JM, Mohamed R, Maritz RA, Alexander GJ (2021) Repeated dietary shifts in elapid snakes (Squamata: Elapidae) revealed by ancestral state reconstruction. Biol J Linn Soc 134(4):975–986

Meyer CG, Papastamatiou YP, Holland KN (2010) A multiple instrument approach to quantifying the movement patterns and habitat use of tiger (*Galeocerdo cuvier*) and Galapagos sharks (*Carcharhinus galapagensis*) at French Frigate Shoals Hawaii. Mar Biol 157:1857–1868

Miyatake T, Nakayama S, Nishi Y, Nakajima S (2009) Tonically immobilized selfish prey can survive by sacrificing others. Proc R Soc B Biol Sci 276(1668):2763–2767. https://doi.org/10.1098/rspb.2009.0558

Mukharror DA, Susiloningtyas D, Ichsan M (2020) Tonic immobility induction and duration on halmahera walking shark (*Hemischyllium halmahera*). IOP Conf Ser Earth Environ Sci 404(1):012080

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2013) The caper package: comparative analysis of phylogenetics and evolution in R. R Package Version 5(2):1–36

Páez AM, Padilla EMH, Klimley AP (2023) A review of tonic immobility as an adaptive behavior in sharks. Environ Biol Fishes 106(6):1–8

Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc R Soc Lond B Biol Sci. 255(1342):37–45

Papastamatiou YP, Itano DG, Dale JJ, Meyer CG, Holland KN (2010) Site fidelity and movements of sharks associated with ocean-farming cages in Hawaii. Mar Freshwater Res 61(12):1366–1375

Paradis E, Schliep K (2019) Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35(3):526–528

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst Biol 53(5):793–808

Rusli MU (2022) Tonic immobility. In: Encyclopedia of Animal Cognition and Behavior, Cham: Springer International Publishing, pp 7008-7009

Schliep KP (2011) Phangorn: phylogenetic analysis in R. Bioinformatics 27(4):592–593

Sebastian LM (2020) Tonic immobility in two species of Southern African catsharks. Doctoral dissertation, University of the Western Cape

Sorenson L, Santini F, Alfaro ME (2014) The effect of habitat on modern shark diversification. J Evol Biol 27(8):1536–1548

Speed CW, Meekan MG, Field IC, McMahon CR, Stevens JD, McGregor F, Huveneers C, Berger Y, Bradshaw CJ (2011) Spatial and temporal movement patterns of a multi-species coastal reef shark aggregation. Mar Ecol Prog Ser 429:261–275

Stein RW, Mull CG, Kuhn TS, Aschliman NC, Davidson LN, Joy JB, Smith GJ, Dulvy NK, Mooers AO (2018) Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. Nat Ecol Evol 2(2):288–298

Sternes PC, Schmitz L, Higham TE (2024) The rise of pelagic sharks and adaptive evolution of pectoral fin morphology during the Cretaceous. Curr Biol 34(12):2764–2772

Sweckard LJ (2021) Tonic immobility in amphibians: a literature overview. Honours thesis, Northern Illinois University

Watsky MA, Gruber SH (1990) Induction and duration of tonic immobility in the lemon shark *Negaprion brevirostris*. Fish Physiol Biochem 8:207–210

Webster DG, Lanthorn TH, Dewsbury DA, Meyer ME (1981) Tonic immobility and the dorsal immobility response in twelve species of muroid rodents. Behav Neur Biol 31(1):32–41

Wells RM, McNeil H, MacDonald JA (2005) Fish hypnosis: induction of an atonic immobility reflex. Mar Freshw Behav Physiol 38(1):71–78

Werry JM, Lee SY, Otway NM, Hu Y, Sumpton W (2011) A multi-faceted approach for quantifying the estuarine–nearshore transition in the life cycle of the bull shark *Carcharhinus leucas*. Mar Freshwater Res 62(12):1421–1431

Whitman PA, Marshall JA, Keller EC (1986) Tonic immobility in the smooth dogfish shark, *Mustelus canis* (Pisces, Carcharhinidae). Copeia 1986(3):829–832

Wilga CA and Ferry LA (2015) Functional anatomy and biomechanics of feeding in elasmobranchs. In: Fish physiology, Academic Press, vol 34, pp 153–187

Williamson MJ, Dudgeon C, Slade R (2018) Tonic immobility in the zebra shark, *Stegostoma fasciatum*, and its use for capture methodology. Environ Biol Fishes 101:741–748

Yoshida M (2021) Immobility behaviors in fish: a comparison with other vertebrates. In: Death-feigning in insects: mechanism and function of tonic immobility, Springer Singapore, pp 159–178