The evolution of body shape, locomotion and ecology in tetrapods

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Abstract

A central aim in biology is to understand the underlying mechanisms that cause morphological evolution and subsequently lead to vast phenotypic diversity amongst species. Body shape has a fundamental effect on all aspects of an organism’s biology and therefore ultimately impacts survival. Through natural selection the shape of a species has been moulded through time to reflect the adaptations needed to survive in environments where they live. As vertebrates evolved onto land, they have dispersed into a wide range of body shapes to best increase their survival rates and have evolved to adapt to different dietary and locomotor niches. Although body shape has a huge impact on a species, most research has focused on ‘singular’ aspects of body shape change such as relative proportions of the limbs, particularly in phylogenetic groups such as the Carnivora, or have focused on specific ecological or locomotory modes. Changes of body shape across tetrapods as a whole, have not been currently researched, which is surprising considering the wide range of influences body shape evolution has ultimately had across species. The aim of this thesis is to understand how overall body shape has been modified by size, locomotor and trophic ecology across tetrapods. A dataset of 410 extinct and extant tetrapods spanning most major taxonomic, locomotor and trophic groups were analysed, with several body shape and size metrics quantified from 3D skeletal models. The data set also separated species into locomotor and dietary categories. A variety of linear and volumetric measures of body segment proportions were derived and volumetric size metrics using convex hulls were generated. A newly modified version of the
Wheatsheaf index (w) was used to provide measures of convergence in extinct and extant taxa. The size of the head and neck was also quantified using multiple metrics representative of a range of methods used across previous studies including traditional morphometrics, convex hulls and the use of alpha shapes.

In summary this thesis found that most body segments in terrestrial tetrapods scale with negative allometry or isometry. Overall, as body size increases: relative head size decreases, the torso becomes wider but anteroposteriorly shorter and the hind limb becomes strongly graviportal, but the forelimb only weakly so. Quadratic relationships between small-medium and large tetrapods are supported, particularly in quadrupedal striding species, which suggests that graviportal allometric trends occur in limb proportions in quadrupedal taxa with different patterns in proximal versus distal limb segments as body size increases. Locomotion was associated with changes in multiple body segments for the clear adaptation of moving through different media, examples of this include limb reduction and a more elongate body form in aquatic and scansorial taxa, elongation of the forelimb in flying and arboreal taxa and long hindlimbs in saltatorial taxa. Ecological convergence exists in tetrapods, and the evolution of body segments seems to be driven by locomotor ecology rather than trophic habits overall. Specialised forms of locomotion such as fossoriality, flight, quadrupedal and scansorial show much stronger magnitudes of convergence in key adaptive aspects of their morphology with scansorial species showing the highest rates of convergence in limb morphology.

As body size increases in tetrapods, skull volume, length and width scale with negative allometry, with neck length scaling with positive allometry and isometry in
neck volume with body size. Allometric patterns in linear size metrics are better described by a linear model overall, whilst quadratic models fit volumetric measurements better. Species over 100kg tend to show greater negative allometry in skull volume, whilst the neck shows strong positive allometry. Taxonomy provides large constraints on the evolution of skull and neck size, with skull width being more highly influenced by diet. The results show that differing methodologies and statistical techniques in the literature to quantify skull and neck size in relation to body size leads to conflicting results and therefore impacts qualitatively on the allometric pattern recovered. Future work would benefit from increasing the number of species in this dataset, as well as filling in key gaps of the fossil record, to further untangle the complex interplay between phylogeny, locomotor and trophic ecology influences. More sophisticated phylogenetic statistics such as evolutionary rates analysis is suggested to test for the presence of adaptive signals, to further explain the overall ecological diversification of tetrapod body shape.
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Chapter 1
Chapter 1- Introduction and overview

1.1 Introduction and thesis structure

Tetrapods have continually adapted to terrestrial life since their ancestors originally left the water over 300 million years ago (Linzey 2020) and in doing so have occupied an impressive variety of ecological niches. One way in which tetrapods have achieved this is through varying their body size and shape (See figure 1.1). Organismal function is fundamentally impacted by body shape (Cuvier 1817; Russel 1916; Lauder 1981), including accommodating major organ systems (e.g., the blood, heart, lungs and skeleton) (Rushton and Rushton 2004; Clauss et al 2016), affecting physiological functions such as increasing, or decreasing body surface area for heat exchange (Phillips and Heath 1995; Henderson 2013), maximising oxygen supply (Schmidt-Nielsen and Knut 1984), enabling axial flexibility and locomotor performance (Lindsey 1975; Webb 1982; Brainerd and Patek 1998; Porter et al 2009) and finally influencing ecological and evolutionary aspects of life such as feeding ecology, sexual selection, habitat use, home range size, bite force and prey size (Eisenberg 1981; McMahon and Bonner 1983; Peters and Wassenberg 1983; Schmidt-Nielson and Knut 1984; Calder 1996; LaBarbera 1986; Blackburn and Gaston 1994). The diversity of body shape has consequently long captured many functional and comparative morphologists’ interests.
Natural selection has acted upon body shape through major functional and ecological transitions in the history of life. It would be expected therefore that body shape should vary adaptively across animals occupying different ecological niches because different environments and behaviours place different demands on the functional mechanics and physiologies of organisms, (Werner and Gilliam 1984; LaBarbera 1986; Ebenham 1992; Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Edgington and Taylor 2019; Law 2021), whilst also being constrained by other factors such as body size (Gould 1966; Penrose et al 2016), phylogenetic history and the ecological trajectory of evolutionary change (Lauder 1981; Huey and Bennet 1987; Wainwright 1991).

Animal body shape has therefore evolved with environmental pressures to enhance survival and reproductive success and has created a variation of shapes and sizes from the smallest terrestrial vertebrates, the microhylid frogs (Kraus 2011) to the largest known terrestrial vertebrates, the extinct titanosaur (Balter 2015). Many studies have sought to investigate the factors that drive evolutionary body shape changes amongst these diverse organisms (Werner and Gilliam 1984; LaBarbera 1986; Ebenham 1992; Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Bates et al 2016; Edgington and Taylor 2019; Law 2021). Whilst such studies have regularly identified important adaptive trends in body shape evolution, they have leant towards a focus on specific taxonomic or ecological groups because of the difficulties in sampling a broad number of taxa, or they have tended to focus on an individual aspect of body shape (e.g., limb length, body length etc.) or a region of the
body (e.g., the skull, the forelimb). Currently, no study to date has systematically investigated patterns of whole-body shape change across tetrapods using modern phylogenetic comparative approaches, which hinders our abilities to predict how body shape has influenced locomotion and ecology and vice versa through time.
Figure 1.1: A variety of different body shapes in tetrapods. Image credit: Unsplash; Woolly mammoth image courtesy of giant screen films © 2012 d3d ice age, llc.
In this thesis I therefore seek to quantify the links between body shape, locomotor and dietary diversity across a broad variation of taxa during the evolution of tetrapods by means of modern phylogenetic comparative methods. This chapter (Chapter 1) will provide a summary of the relevant literature to date and identify key gaps in knowledge related to this body shape evolution and will finish on a list of aims and objectives, followed by a brief outline of chapters (2-4). Chapter 2 will provide additional detail on the data and methods used in this thesis. Immediately following chapter 2, each of the three objectives will be addressed in individual research chapters (3-5), which are formatted as stand-alone manuscripts with a self-contained introduction and discussion. A general discussion chapter (Chapter 6) will provide a summary and retrospective evaluation of the analysis of each data chapter and comment on future studies to improve research into this field. To close, chapter 7 will provide final conclusions to the major findings in this thesis. An appendix is provided in electronic format attached to the back of this thesis.
1.2 Background

1.2.1 Literature review: body shape, locomotion and diet

The body of vertebrates integrates an endoskeleton, connective tissue and muscle. A typical endoskeleton consists of a vertebral column, cranium, limbs, limb girdles and visceral arches and has typically evolved to enable specific interactions with the environment. Identifying associations between skeletal form, trophic and locomotor function are critical to determining how skeletons adapt to the biological roles they must perform. This is especially important for reconstructing ecological behaviours in extinct species. Obtaining body size information from fossils is of great interest to many palaeobiologists (Campione 2017) and understanding its evolution is a common objective in modelling past ecosystems (Begon and Townsend, 2020).

How can we begin to understand how and why the remarkable diversity of vertebrate body shapes evolved? Body shape selective pressures are highly interconnected and complex, but taking biomechanical principles into consideration can help, as they link shape and performance, which is tied to evolutionary fitness and acted upon by natural selection (Arnold 1983). The performance that maximizes fitness depends on the ecological context, reflecting the ecological diversity vertebrates exhibit using a spectacular variety of body shapes, which range from the extremes of elongated (e.g., salamanders, mustelids), to stout and short (e.g., frogs and toads).
Most aquatic and semi-aquatic species, for example, are fusiform or elongated to pass through the dense medium of water with minimal resistance. Swimming performance depends on the net balance between thrust and resistance, which is determined by hydrodynamic properties of body shape (as reviewed by Webb 1984, 1997; Domenici 2002; Langerhans and Reznick 2010). Biomechanically, extreme elongation therefore offers maneuverability and flexibility that allow the occupation of narrow crevices seen in lungless salamanders for example, which has a resulting effect on gut morphology (Ward and Kley 2012), imposing structural constraints on the feeding apparatus affecting both feeding ecology and physiology.

As vertebrates evolved onto land, changes to terrestrial locomotion led to further major modifications in body form and shape. Terrestrial limbs are segmented into three parts, proximal, intermediate and distal with joints in-between (Anderson et al 2018). They contain large amounts of muscle to deal with gravity and movement on land, therefore the posterior limbs are usually larger than the anterior to support greater body weights and provide rapid acceleration for hunting or fleeing purposes (Domenici et al 2011).

Terrestrial invasion has led to the exploitation of many ecological niches, exhibiting varying locomotor morphologies such as a more upright, non-sprawling quadrupedal gait (adapted to support larger weights e.g., elephants, rhinos, sauropods); saltatorial
(jumping e.g., frogs and kangaroos); semi-aquatic (swimming and moving on land e.g., seals, salamanders); fossorial (digging e.g., moles); arboreal (movement through trees e.g. monkeys); aerial (flying and soaring e.g. bats and birds); bipedalism (movement on two legs e.g. humans and theropods) and scansorial (climbing e.g. squirrels and rats) whilst also exploring trophic niches such as carnivory (meat-eaters), herbivory (vegetation), omnivory (consuming both meat and vegetation), insectivory (consuming an insect dominant diet) and piscivory (consuming a fish dominant diet).

Quadrupedal locomotion is found in the majority of tetrapods both living and extinct and represents the primitive condition for fully terrestrial species, having been found in the earliest known forms and retained in known descendants. Vertebrates made this significant evolutionary move in the Devonian when the first tetrapods stepped onto the land, with one of the main issues being that of gravity (Benton 2014). In water, body mass has less impact owing to the density of the medium, but on land the body is held up by the limbs and must cope with the stronger downward pull of gravity (Benton 2014). Features of quadrupedal locomotion are therefore correlated with dealing with gravity and consequently affect body shape, such as a columnar, graviportal limb posture, increased limb robusticity, shortened distal limb segments and increased femoral midshaft eccentricity (Alexander et al 1979; Wilson and Carrano 1999; Carrano 2001). These features also increase with body size (Alexander et al 1979; Carrano 2001), with the length of the distal limb decreasing with body size in dinosaurs and terrestrial mammals (where several measures of limb bone
dimensions such as femur length were used as proxies for body mass; Carrano 2001; Carrano 1999). Increases in body size impose greater bending moments on the spine being supported between the limbs. In Quadrupedal sauropod species, a shorter spine and larger torso has been suggested as an adaptive response to minimise increasing bending moments in the spine as body size increases (Hallett and Weddel, 2016). A large torso therefore also influences gut morphology and dietary needs, with a longer gut and a larger torso allowing for a larger intake of a low-quality food (usually found with herbivores) and to maintain some minimum retention time of food in the gut (Ribble and Smith 1983; Sibly and Calow 1986; Horn 1989; Starck 2005), thereby increasing digestive efficiency (Clements and Raubenheimer 2006).

Bipedalism has evolved from quadrupedal ancestors in several tetrapod lineages independently e.g., in ancestral reptiles and in some mammals and archosaurs (Archosaurs inherited bipedalism and many hindlimb morphological features from theropod dinosaurs, which first appeared around 230 million years ago (Gatesy and Middleton 1997)). Bipedalism has been hypothesised to evolve to promote endurance running (Coombs, 1978; Garland, 1983; Carrano, 1999), which brought about further body shape changes. Hindlimb elements distal to the femur are consistently longer amongst bipeds than in quadrupeds, when proximal vs distal limb segments are taken into account (Coombs 1978; Berman 1985; Grinham and Norman 2020). Functionally, this facilitates longer stride lengths and greater ground clearance, all of which contribute to more efficient strides and greater support for the body on two limbs (Snyder 1954, 1962; Hsieh 2003; Clemente and Wu 2018). For
example, dinosaur bipedality is viewed as one of the adaptations underpinning their success, as it enabled greater stride lengths and speeds than quadrupedal reptiles living at the same time (e.g., Alexander 1976) and facilitated the later evolution of gigantism in some lineages (e.g., Sander et al 2011). Most bipedal species, particularly theropods, exhibit carnivory due to their long legs being used to maximize top speed, potentially helping run down faster prey items and therefore increasing hunting efficiency (Dececchi et al 2020). To move bipedally the pectoral girdle is caudally displaced (Alexander 2004; Hutchinson 2004a, 2004b; Clemente and Wu 2018), which leads to the torso section being shorter in bipeds than quadrupeds also complementing the use of carnivory, as meat is easier to digest than foliage (Milton 1999), enabling meat eaters to have smaller guts and therefore torsos than herbivorous eaters of a similar size (Milton 1999).

Locomotion through saltatorial movement is found in modern anuran amphibians and some mammal species (e.g., kangaroo rats) and is used for bursts of efficient high-speed locomotion (Alexander and Vernon 1975; Clancy and Croft 1991). Saltatorial locomotion has been associated morphologically with a shortening of the body, developmentally increased posterior appendages and loss of the tail (except in some mammal species such as the kangaroo, which kept the tail for balance (O’Conner et al 2014)). Frogs and toads (anurans) feature short bodies, with well-developed hindlimbs commonly associated with their jumping locomotion, the ilium is extremely long, and the caudal vertebrae are fused into the urostyle, a rod-like structure that acts as a propulsion mechanism leading to short and stocky bodies,
with longer legs relative to body length resulting in increased leaping performance (Zug and Altig 1978; Choi et al. 2003; Gomes et al 2009). Saltatorial locomotion is an efficient way of moving in smaller species and anurans display some of the smallest sizes and masses found in tetrapods. A smaller mass, however, requires a higher metabolic rate that can be met by the demands of having a predominantly insect-based diet (Voigt and Dechmann 2010). Insects represent high-energy, high-quality food sources, providing an animal with protein and are easier to digest than plant foods and provide more protein for a high metabolism. However, they must be consumed in large quantities (Rothman et al 2014), due to the allometric effects of body size on basal metabolic rate, most insect eating species are therefore small, typically with a body mass of less than 1 kg (Kay 1984).

From purely terrestrial tetrapods, animals then further branched out into broader ecological niches they could exploit. The ability to climb is a key behaviour that facilitates access to above ground resources and therefore additional ecological opportunities become accessible (Jenkins and McClearn 1984; Scheffers et al 2013; Bars-Closel et al 2017). Skeletal features previously associated with scansorial locomotion in rodents and other small mammals include equally proportioned limbs, a long humerus, and grasping hands and feet (Sargis 2002, 2007; Samuels and Van Valkenburgh 2008; Tulli et al 2016). When exploiting the arboreal niche body shape must allow for navigation between the trunks and branches of trees, while simultaneously performing basic activities, such as feeding. In general, arboreal primates rely on increased grip and claw purchase and therefore longer fingers and
the use of the tail for balance, stability and grasping (Cartmill et al 1985; Tulli et al 2016). The transition from the ground to the trees in arboreal species has also been investigated in various small, non-primate species (e.g. Micromys minutus; Didelphis marsupialis), which vary in size from ~15 g to 6 kg (Argot, 2002; Urbani and Youlatos, 2013). Most studies to date focus on the numerous independent origins of arboreality in mammals, mainly in primates however (Jenkins, 2017; Gebo, 1996, 2004; Bloch and Boyer, 2002; Kirk et al 2008) and therefore adaptations to arboreality can be seen as a clade specific adaptation rather than across tetrapods using this locomotor mode.

It has been hypothesised that when arboreal species evolved the ability to glide, this was the beginning of the evolution of flight (Norberg 1985). Birds are the most taxonomically and ecologically diverse group of tetrapods and generally most can fly. Their forelimbs have been modified as wings used mainly for aerial locomotion, whereas their legs act as a locomotory apparatus for obligatory bipedal locomotion (Gatesy and Dial 1996; Gatesy and Middleton 1997, 2007). Much research has been done on the association between the skeletal proportion of the limbs and locomotor modes and its functional aspects in birds (e.g., Middleton and Gatesy 2000; Zeffer et al. 2003; Nudds 2007; Hinić-Frlog and Motani 2010; Kilbourne 2014) and the only other extant flying clade alive today, bats (Simmons, 2005; Simmons et al., 2008).

The body shapes of flying vertebrates tend to be shortened and relatively rigid, although the neck can be long in birds (Bohmer et al 2019), with differing forelimb
shapes to terrestrial vertebrates. In bats, the forelimbs are overly enlarged with elongated skeletal elements and large flight membranes (Simmons et al 2008). In birds, forelimbs proportionally increased in size in association with the origin of flight, the pectoral appendages are modified as wings, and the sternum is broad and keeled to provide a large surface area for flight muscles. There is also fusion in the pelvic bones, sacral, lumbar, and thoracic vertebrae and bones of the hindlimbs, which has resulted in birds having a short and ridged back shape (Galbusera and Bassani 2019).

The neck in birds is used in a variety of environmental manipulation tasks (van der Leeuw et al 2001; Bohmer et al 2019; Marek et al 2021) and has therefore been suggested to be adapted for feeding, preening, manipulation, sexual display and combat behaviours (Zweers et al 1994; Van der Leeuw et al 2001; Hansell 2005; Bohmer et al 2019), affecting the overall body shape of a species. Lengthening of the neck in birds is often associated with habitat and diet (Bohmer et al 2019), this is due to the functional constraint of the forelimb being adapted for flight in birds (Gatesy and Dial 1996). Shorter necks tend to be found in terrestrial species such as the Kiwi or woodcock species, whilst longer necks are generally found in semi-aquatic species of birds such as flamingos and swans (Bohmer et al 2019). A long neck and a streamlined skull have an advantage to predating on fish and reaching vegetation below the surface of the water. Long necks create a more streamlined body shape for plunge diving species (for example frigatebirds and gannets), most likely due to the need to support the high forces the bird experiences with the impact of water (Chang et al 2016).
As expected, interactions between different aspects of ecology (e.g., diet and habitat) as well as trade-offs between various performances (e.g., locomotor and feeding performance), results in body shape ultimately representing a compromise between competing forces constrained by historical factors. Overall, previous studies have identified important adaptive trends in body shape evolution, but they have tended to focus on individual taxonomic or ecological groups and on an individual aspect of body shape leading to the inability to compare whole body shape change in tetrapods reliably across the literature.

1.2.2 Body shape and studies of convergent evolution

Convergent evolution is the pattern of evolution in which species in two independently evolving lineages become phenotypically similar and has long been considered as an important area of research in evolutionary biology (Stayton 2015). For instance, one of the most cited examples of convergence is that of the evolution of flight. Flight has evolved in three separate tetrapod groups; the extinct Pterosaurs and the extant bats and birds, which have all adapted their forelimbs for this mode of locomotion (McGhee 2011). The wings of flying tetrapods are all very similar in appearance, due to the functional constraints of locomotion via flying. Examining convergent evolution allows for patterns of adaptation, constraint and questions about the predictability of evolution to be assessed (Wake et al 2011; Stayton 2015). Convergence therefore occupies a key position in the quantitative evaluation of
macroevolutionary patterns (Arbuckle and Speed 2016) and the rate of convergence of traits in a study can be a limiting constraint on the power of phylogenetic estimations (Maddison and FitzJohn 2015).

Evolutionary impacts of transitions in certain aspects of body shape and size in tetrapods are well documented (Sidlauskas 2008; Bhullar et al. 2012; Muschick et al. 2012; Mahler et al. 2013; Vidal Garcia and Keough 2015; Lingham-Soliar 2016; Johnson et al. 2017; Tierney et al. 2017; Morris et al. 2018; Watanabe et al. 2019; Sansalone et al. 2020), with different configurations of body shapes best suited to different locomotor and dietary specializations.

Rigorous phylogenetically controlled tests of convergence effects across vertebrates body shapes are rare, with few exceptions, such as convergence on similar body plans in some fast-swimming marine vertebrates (Lingham-Soliar 2016). Past research has looked at either specific taxonomies regarding convergent evolution e.g. African great lake cichlid fish (Muschick et al. 2012), Caribbean anole lizards (Mahler et al. 2013), Australian frogs (Vidal Garcia and Keough 2015), Swallows (Johnson et al. 2017), marine angelfishes (Tierney et al. 2017) and Euarchantoglires (Morris et al. 2018), or specific body parts e.g. skulls (McCurry et al. 2017, Godoy and Turner 2020), long necks (Sander et al. 2011) and wings (Pigot et al. 2020), or specific ecological modes e.g. trophic functional morphology in marsupial and placental carnivores (Jones et al. 2003), locomotion in Jerboas and kangaroo rats (Webster and Dawson 2004), locomotion in the lamiid shark and tuna (Donley et al. 2004) and body size and shape
difference as an adaptation to environmental niche in Anoles (Harmon et al 2005). Comparisons across a diverse array of taxa and ecotypes are required to determine whether similar ecological opportunities can lead to equivalent outcomes.

1.2.3 Background summary

- Body shape is fundamentally important to a species survival and is heavily influenced by natural selection.

- Interactions on the effects of body shape between different aspects of ecology and locomotion represents a compromise between competing forces constrained by historical factors.

- Previously published studies evaluating the evolution of body shape in tetrapods are limited to specific taxonomic and ecological groupings or look at individual aspects of body shape. This hinders their ability to predict how body shape in tetrapods has influenced locomotion and ecology and vice versa through time.

- Comparisons in convergent evolution across a diverse array of taxa and ecotypes are needed to determine whether similar ecological opportunities can lead to equivalent outcomes.
Recent phylogenetic comparative methods now allow for explicit consideration of patterns of whole-body shape change across tetrapods through time.

1.2.4 Aims

The overall aim of this thesis is to quantify the links between body shape, locomotor and dietary diversity during the evolution of tetrapods. This aim will be achieved through several smaller objectives that address key gaps in knowledge currently present in the field. These specific objectives include:

1) To understand how overall body shape has been modified by size, locomotor, and trophic ecology across tetrapods.

2) To quantify the degree of convergent evolution of relative body segment proportions in different locomotor and dietary groups across tetrapods.

3) To survey and quantitively compare the metrics used for analysing allometry in the head and neck across tetrapods.
1.2.5 Chapter structure

1.2.5.1 Chapter 2 overview

This chapter includes additional detail on the data and methods used in this study (Chapters 4-6) including data collection and phylogenetic analysis.

1.2.5.2 Chapter 3 overview

This chapter is based on a manuscript which is currently in review at Nature Communications. While I carried out the majority of the research, the work benefited from the contribution of a number of co-authors as follows:

Dr Karl Bates (KTB), Dr Philip Cox (PGC) and Dr Thomas Maddox (TWM) conceived the study.

KTB, PGC, Dr Emma Schachner (SCRM) and Alice Maher (AEM) designed the study.

KTB, PGC, TWM, ERS and AEM collected the scan data.

AEM processed the scan data, made the measurements, and conducted the statistical analysis with guidance from all co-authors.

All authors contributed to writing the manuscript.
1.2.5.3 Chapter 4 overview

I am currently developing this chapter for publication. While I carried out the majority of the research, the work benefited from the contribution of a number of co-authors as follows:

A.E.M, KTB and Dr Christopher Mitchell (C.M) conceived the study.

A.E.M, KTB and C.M. designed the study.


C.M. developed the statistical approach.

A.E.M processed the scan data, made the measurements and conducted the statistical analysis with guidance from all co-authors.

The present thesis version was drafted by AEM and benefited from editorial suggestions from all other co-authors.

1.2.5.4 Chapter 5 overview

I am currently developing this chapter for publication. While I carried out the majority of the research, the work benefited from the contribution of a number of co-authors as follows:

AEM, KTB, PGC, TWM conceived the study.

KTB, PGC and AEM designed the study.
KTB, PGC, TWM and AEM collected the scan data.

AEM processed the scan data, made the measurements, and conducted the statistical analysis with guidance from all co-authors.

The present thesis version was drafted by AEM and benefited from editorial suggestions from all other co-authors.

1.3 References


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Chapter 2
Chapter 2 - Additional detail on the data and methods used in this thesis

2.1 Measuring linear parameters.

Size is measured in a variety of ways among animals and is commonly described in terms of either linear, volume or mass-based metrics, as these parameters offer the greatest predictive values for ecology. One of the most important approaches for assessing variation in body shape over evolutionary time is allometry, the study of changes in shape associated with changes in size (Klingenberg 2016). Evolutionary allometry concerns variation in individuals within a single ontogenetic stage, from multiple evolutionary lineages that share a similar common ancestor (Cock 1966). A variety of skeletal proxies are available for the estimation of body mass, which depend on isometric or allometric relationships between various skeletal dimensions. This is examined further in section 2.2, while here I first focus on measurements of skeletal dimensions for the analysis of body shape.

Measuring morphometric data across a tremendous diversity of body shapes in species, clades and vertebrates, has led to quantifying this variation to be a challenge. Many previous researchers have summarized morphological shape variation using multivariate statistical approaches, such as principal components or discriminant function analysis (e.g., Losos 1990; Walker and Bell 2000; Rüber and Adams 2001; Langerhans et al 2007; Brandley et al 2008; Bergmann et al 2009). Another method
of quantifying shape variation among vertebrates is using landmark-based morphometrics, which has been highly effective at identifying shape aspects that differentiate between taxa (Rohlf and Marcus 1993; Adams et al 2004), but this method is restricted in quantifying broad scale body shape patterns across vertebrates due to a variety of forms being restricted by the presence of homologous structures and landmarks (Collar et al 2013).

An alternative approach has been to devise and apply metrics that combine body measurements in ways that capture variation along the elongation continuum (Webb 1975, Ward and Azizi 2004, Ward and Brainerd 2007, Helfman et al 2009). Collar et al (2013) offered a revised metric for quantifying body shape, named the vertebrate shape index (VSI), which combines four major anatomical components that contribute to body shape variation among vertebrates: secondary axis reduction + head elongation + precaudal elongation + caudal elongation. These ratios, however, provide only limited insight into the morphological basis of shape variation and transformations along the elongation continuum and may result from modifications of several morphological components. They also do not always work well for fossils as they require an accurate body depth or width, which is not always present in fossilised material.

One way to measure body shape changes across a diverse set of species and clades is to apply morphometric analysis to measure the maximum lengths (and/ or widths, circumference etc.) of each bone (See figure 2), which can be used to build a larger
picture of whole-body shape changes. Most measurements of forelimb and hind limb long bones in the literature, such as the humerus (HL), femur (FL), forearm (FaL), and shank (SL), are measured using the greatest length of each bone in a variety of species (Gingerich 1990; Demes et al 1991; Christiansen and Farina 2004; Onar and Belli 2005; Bonnan 2007; Seymour et al 2012; Demiraslan et al 2014; Watanabe 2017). Therefore, the long bones in this study were measured in a similar way (See figure 2.1). Regarding the metatarsal (MtL) and metacarpal (McL), the greatest length was regarded as the longest individual metacarpal or metatarsal the species exhibited, as seen in these studies (Gingerich 1990; Revell et al 2007).

Measurements of the manus (ML) and pes (PL) are more variable across the literature, with most studies measuring the 3rd or 4th digit, which tends to be the longest digit in most animals. Some published indices use measurements of small elements, such as claws (e.g., Revell et al 2007; Samuels and Van Valkenburgh 2008) and where claws where present, this study also adhered to this and measured from the proximal end of the bone to the most distal point including the claw (See figure 2).

Trunk length was measured as gleno-acetabular distance. This is the distance from the centre of the shoulder joint to the centre of the hip joint. All measurements were obtained using digital callipers in Geomagic (https://uk.3dsystems.com) and were reported in millimetres (See electronic supplementary material, ESM1.xlsx).
Figure 2.1: Osteological measurements taken in this study in a variety of taxa including (from top left to bottom right); *Acris crepitans, Hyllobates agilis, Pelcanus occidentalis, Phrynosoma cornutum, Caenagnathus, Caenagnathus, Mata mata, Acanthixalus spinosus, Cathartes aura, Nycteres grandis*. Linear measurements included gleno-acetabular distance (GA), femur length (FL), shank segment length (SL), metatarsal segment length (MtL), pes segment length (PL), humerus length (HL), forearm segment length (FaL), metacarpal segment length (McL) and manus segment length (ML).
2.2 Volumetric measurements for size normalisation

Mass is correlated more than any other biological variable to many aspects of an animal’s biology. Mass has demonstrated correlations with diet (Tucker et al 2014), rates of evolution (Evans et al 2012), habitat use (Polo and Carrascal 1999), metabolic rates (Gillooly et al 2001; Kozlowski et al 2020), home range size (Tucker et al 2014; Kroshko et al 2016) and life history traits (Schmidt-Nielson and Knut 1984). A robust estimate of body mass therefore has the capacity to predict other aspects of biology that may not be otherwise available.

When dealing with extinct taxa, a proxy for body mass is proposed to combat the significant challenges of using physical methodologies applied to extinct species that may have body proportions and indeed body sizes outside the range seen in living species. The volumetric approach is an alternative to only using extant scaling equations to estimate body mass and can be accomplished using physical scale models, two-dimensional mathematical models, or three-dimensional virtual models.

Here we focus on the method of convex hulling, a three-dimensional virtual model (Sellers et al 2012) using mathematical shape fitting methods that have been developed and grounded in quantitative data from extant taxa (Sellers et al 2012; Brassey and Sellers 2014; Brassey et al 2015). The grounding of data from living
animals enables the error within the method of convex hulling to be confidently quantified and improves the repeatability and objectivity in the derivation of segment mass properties (Brassey 2016). Convex hulling is an alternative approach to body mass estimation, combining both aspects of volumetric modelling and linear regression (Sellers et al 2012), which has been applied in various studies (Sellers et al 2012, Brassey and Sellers 2014, Bates et al 2015, Brassey et al 2015, Brassey 2016, Brassey et al 2018).

This method has benefits over physical and two-dimensional models in several ways. Firstly, by being based directly on the original skeletal material; secondly, its ease of alteration due to the digital process and thirdly; this technique aims to include the maximum amount of information from the skeleton into an estimate for mass or overall body size. This avoids the single bone problem seen in regression analysis of isolated limb bone dimensions. It must be noted however that convex hulls require a lot of investment in generating 3D articulated skeletons through either photogrammetry or Computed tomography (CT) scanning and is also sensitive to the effects of errors in the original reconstructions of the skeletal material (Brassey et al 2013).

A convex hull is formed when 3D shapes are wrapped around the points at the extremes of the skeletal material using automated, mathematical shape fitting (See figure 2.2 and figure 2.3A). Convex hulling is useful because it gives a more meaningful measure of size for certain body segments (e.g., the torso, the skull) than
a single linear measurement, which can then be used to investigate the measure of a particular body components size against whole body size. However, some elements of the body such as the skull can be difficult to convex hull due to the issue of achieving reliability in ‘missing mass’ such as in strange crests and beaks. A potentially better alternative to this is to use alpha shapes (See figure 2.3 C, D and E). Convex hulling is essentially an alpha shape with an infinitely high $\alpha$ value. Alpha shapes are generalizations of the concept of convex hulls, with their ultimate form depending on the value of alpha. Alpha values can range anywhere from 0 to infinity. Depending on the value chosen resulting shapes can be concave in places and more closely sculpted in others to the underlying geometry, creating the potential to achieve a tighter shrink wrap effect (see figure 2.3).

They further improve on convex hulling by removing the need to split the skeleton into subunits, which saves time, human error, and reduces the influence of potential outliers (Brassey 2016). The technique, however, is sensitive to the postures of a skeleton, and requires selection of an appropriate alpha value for every object (which is a time-consuming and subjective process) and is more computationally expensive than convex hulling. The fit of the a-shape is also defined by the refinement coefficient, $k$, which must be subjectively set by the investigator (versus no user defined subjectivity in the simpler convex hulling approach). In this study for consistency, all species were therefore convex hulled, and the sum of all volumetric segments used as the proxy for overall body size.
However, in chapter 5, In addition to convex hulls, new measures of skull and neck volumes using an a-shape fitting algorithm were also generated (Brassey and Gardiner 2015; Marek et al 2021). As noted above, the a-shape approach allows the investigator to refine the fit of the mathematically generated volume, thereby potentially yielding a more realistic 3D shape. To investigate the impact of this subjectivity on skull size patterns across the data set, three different a-shape iterations, where k was set to values 0.5, 1 and 2 were produced.
Figure 2.2: 3D digital skeletal models of taxa used in this study from top left to bottom right: *Acris crepitans*, *Equus ferrus caballus*, *Aptenodytes forsteri*, *Xenosaurus rectocollaris*, *Crocodylus johnstoni*, *Giraffatitan*, *Australopithecus afarensis*, *Nycteris grandis*.
Figure 2.3: Alpha shapes as fitted to the *Accipiter cooperii* skull in dorsal and lateral view:
A: CT scan of the skull; B: convex hull fit of alpha shape when $\alpha = \infty$; C: ‘coarser’ fit of alpha shape when $\alpha = 2.0$; D: ‘finer’ fit of alpha shape when $\alpha = 1.5$ and E: ‘finest’ fit of alpha shape when $\alpha = 0.5$. 
2.3 Phylogenetics statistical analysis

2.3.1 Building the tree.

The recent development of a diverse toolkit of phylogenetic comparative methods now allows explicit consideration of phylogenetic history into statistical assessments of body shape change. It is important to understand whether evolutionary history has a substantial influence on the outcome of body size and shape reconstructions, to obtain a better understanding of both patterns and processes of evolution.

Due to the nature of the evolutionary branching process, some pairs of species are closely related, and others are distantly related. This violates an important assumption of standard statistical methods, being the independence of data points. Before phylogenetic comparative methodology was used, common statistical tests for the evolutionary correlation of discrete characters were prone to reporting a significant association, even when a pattern was driven by a single or very few independent transitions from one character state to another (Maddison 1990, Pagel 1994; Maddison and FitzJohn, 2015). Phylogeny is therefore known to strongly alter the regression coefficients of allometric equations compared to ordinary regression analysis (e.g., Harvey and Pagel 1991; Clauss et al 2014; Lemaître et al 2014).

“Phylogenies and the Comparative Method”, which was published by Joseph Felsenstein in 1985 reported the first phylogenetic comparative method. His method
is referred to as Phylogenetically Independent Contrasts and is essentially a reformulation of linear (regression) models, which biologists routinely use (Cornwell and Nakagawa 2017), in which each evolutionary branching point or ‘divergence’ is treated as a replicate in a statistical sense and the individual species are not seen as the data point. Unlike species themselves, evolutionary divergences are independent events, and classical statistical models can therefore be applied.

Since the innovation of phylogenetic comparative methods, the field has grown exponentially, and new methodologies have been made more flexible by introducing both discrete and continuous data, allowing a diverse array of evolutionary questions to be answered, including the association between ecology and shape (Bhullar et al 2012; Sidlauskas 2008; Watanabe et al 2019). Given the relationship between phenotypic similarities created by convergence, the use of phylogenetic comparative methods has become almost imperative on analyses of evolutionary shape changes (Felsentein 1985; Harvey and Pagel 1991; Losos 2011; Rohlf 2002).

In this thesis, phylogenetic informed statistical approaches were applied to analyse body proportions (represented by linear and volumetric measures, see above) in a phylogenetic context. A number of body shape and size metrics were quantified from 3D skeletal models of 116 amphibians, 47 birds, 55 non-avian dinosaurs, 143 mammals, 46 non-avian reptiles and 3 reptiliomorphs spread across the major groups within these clades. The data set consisted of 318 extant and 92 extinct species.
Table 1: Percentage representation of groups across the dataset compared to known numbers of species, families and orders

<table>
<thead>
<tr>
<th>Order</th>
<th>Order (%) representation</th>
<th>Family (%) representation</th>
<th>Species (%) representation</th>
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<tbody>
<tr>
<td>Mammals</td>
<td>46%</td>
<td>60%</td>
<td>2.60%</td>
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<td>Reptiles</td>
<td>100%</td>
<td>27.17%</td>
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<td>Dinosaurs</td>
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</tbody>
</table>

By order of importance, I aimed to cover a large percentage representation of orders, families and then species in this dataset (See table 1). The goals in achieving a broad phylogenetic spread and a range of body size were limited by the availability of scans and photogrammetric models. The criteria for the dataset included animals that could live on land and locomote successfully, therefore fish and families such as the Cetecea were not included in this dataset. If a model was not reconstructed successfully or if a fossil scanned was too damaged they were removed from the dataset.

Whole body scans of specimens were obtained from a variety of sources including CT scans and photogrammetric models collected by the authors, as well as models from previous studies (Bates et al 2009a, b; Sellers et al 2012; Allen et al 2013; Clauss et al 2016; Bates et al 2016; Macaulay et al 2017; Schachner et al 2014, 2017) and online digital repositories (Morphosource, KUPRI, Digimorph, Sketchfab, animalsimulation.org). LiDAR and photogrammetric scans are of lower quality than CT scans, however, a previous study by Bates et al 2015, found that as long as the
extremities are present in low quality scans then the convex hull volume should be a good representation of the hull you would get from a high-resolution scan. All body scans of specimens in this dataset therefore had to have clear extremities for the outline of each bone and structure, to enable competent convex hulling of the specimen.

A full list of the models, including their source information, can be found in the electronic supplementary material (ESM2.xlsx). Each tetrapod species was then added to a phylogenetic tree, which was built by merging recent, taxon-rich cladograms of major tetrapod groups using Mesquite (www.https://www.mesquiteproject.org) (See figure 2.4 and 2.4.1 to 2.4.10). A full list of the phylogenetic trees merged, including their source information, can also be found in the electronic supplementary material (ESM2.xlsx). First and last occurrences of each species were noted to calculate branch length and time calibrate the tree by taking the stratigraphic range of the formation in which the fossil was found in the Palaeobiology Database (www.paleobiodb.org)
Figure 2.4: A phylogenetic tree created in Mesquite showing in the full range of tetrapods used in this study. Figure 2.4.1, 2.4.2, 2.4.3, 2.4.4, 2.4.5, 2.4.6, 2.4.7, 2.4.8, 2.4.9, 2.4.10 show the individual tetrapod tree highlighted in each section to show the scope of species used in this study.
Figure 2.4.1: A phylogenetic tree created in Mesquite showing the full range of Caudata used in this study. Species images from iStock.
Figure 2.4.2: A phylogenetic tree created in Mesquite showing the full range of Anurans used in this study. Species images from iStock.
Figure 2.4.3: A phylogenetic tree created in Mesquite showing the Fereuungulata part of the mammalian tree used in this study. Species images from Biorender.
Figure 2.4.4: A phylogenetic tree created in Mesquite showing the Chiroptera part of the mammalian tree used in this study. Species images from Biorender and iStock.
Figure 2.4.5: A phylogenetic tree created in Mesquite showing the Euarchontoglires part of the mammalian tree used in this study. Species images from Biorender.
Figure 2.4.6: A phylogenetic tree created in Mesquite showing the Atlantogenata part of the mammalian tree used in this study. Images from Biorender.com.
Figure 2.4.7: A phylogenetic tree created in Mesquite showing the full range of Aves used in this study. Species images from Biorender and iStock.
Figure 2.4.8: A phylogenetic tree created in Mesquite showing the range of theropods and sauropods used in this study. Species images from phylopoc.
Figure 2.4.9: A phylogenetic tree created in Mesquite showing the range of Dinosauriformes used in this study. Species images from phylopic.
Figure 2.4.10: A phylogenetic tree created in Mesquite showing the range of Lepidosauromorpha used in this study. Species images from iStock.
These data were then combined with the phylogenetic tree to produce a time calibrated tree (See figure 2.5). There are two main approaches to time calibrate a tree; tip-dating or ‘a posteri’ time scaling (APT), which are each both a necessary and important step to perform a variety of macroevolutionary analysis. Tip-dating infers both relationships and divergence dates at the same time for a set of taxa and APT methods use a set of stratigraphic data for the taxa involved to date a pre-existing unscaled topology (Lloyd et al 2016; Bapst et al 2016). APT is the best method for producing a time calibrated tree for topologies that are created through super tree approaches or by combining taxonomic and phylogenetic data and therefore the APT approach was used. To achieve this, the ‘strap’ package (Bell and Lloyd 2015) for R software was used. This package enables the user to time scale cladograms and compare the resulting topologies with their fit to the stratigraphic record (Chronostratigraphic chart (Cohen et al 2020)) (See figure 2.5).
Figure 2.5: To investigate the evolution of body shape and ecology in tetrapods a data set of 410 extinct and extant terrestrial vertebrates was assembled from across Tetrapoda, spanning from the Permian to the Quaternary.
Each species was also assigned to a trophic and locomotor category. Trophic categories included Carnivores \( (n = 123) \), feeding on tetrapods; herbivores \( (n = 120) \), feeding on vegetation; insectivores \( (n = 95) \), feeding on insects; omnivores \( (n = 64) \), feeding on both tetrapods and vegetation and piscivores \( (n = 9) \), feeding on fish (See figure 2.6). The five trophic categories allowed the exploration of major trophic niches whilst also allowing for a large enough sample size in each category to perform robust statistical tests. The partitioning of species into these five categories was met by following the consensus for the diet in the literature (See electronic supplementary material (ESM1.xlsx)).

The locomotor habit of each taxon was classified as either active flight \( (n = 33) \), repeated wing movements to stay aerial; aquatic \( (n = 12) \), locomotion in water; arboreal \( (n = 42) \), locomotion in trees; bipedal striding \( (n = 32) \), locomotion using the rear limbs; fossorial \( (n = 28) \), locomotion through sand or soil, with an adaptation for burrowing; quadrupedal striding \( (n = 131) \), locomotion using all four limbs; saltatorial \( (n = 79) \), locomotion through hopping, jumping or leaping; scansorial \( (n = 14) \), locomotion through climbing; semi-aquatic \( (n = 30) \), equal locomotion in both terrestrial and aquatic media and soaring flight \( (n = 9) \), majority of aerial flight performed without moving the wings or using power (See figure 2.6). The ten locomotor categories allowed the exploration of major locomotor niches whilst also allowing for a large enough sample size in each category to perform robust statistical tests. The partitioning of species into these ten categories was met by following the consensus for locomotion habits from the literature (See the electronic supplementary material (ESM1.xlsx)).
Figure 2.6: To investigate the evolution of body shape and ecology in tetrapods, a data set that captured major evolution changes in trophic ecology and locomotor ecology was assembled.
2.3.2 Scaling and regression analysis

To examine how each body segment scaled with body size across the data set, a regression analyses using phylogenetic generalised least squares (pGLS) in the R package caper was conducted (Freckleton 2002). This approach follows a general linear model calculating the slope, intercept, confidence, and prediction intervals, adjusting the expected covariance according to phylogenetic signal (Symonds and Blomberg 2014). The method of phylogenetic generalized least squares (PGLS) is an expansion of the general linear model. One of the crucial assumptions of the general linear model is that the residuals show independence to one another. Taxa that share larger or smaller fractions of their evolutionary history are likely to be similar to one another and this therefore violates the need for independence in the data leading to non-independent residuals (Felsenstein 1985). PGLS accounts for phylogenetic history and leads to independent residuals and therefore essentially ‘downweights’ points that derive from species with shared phylogenetic history (Symonds and Blomberg 2014).

Phylogenetic ANCOVAs (phylANCOVA) were used to test for the influence of phylogeny and the differences in the allometric relationships of body shape between locomotor and dietary groups using the approach of Smaers and Rohlf (2016) implemented in the R package evomap (Smaers and Mongle 2014). PhylANCOVA mathematically addresses phylogenetically distant specimens or size outliers that would require separate, semi-quantitative exploration in a non-phylANCOVA.
Whether quadratic models provided a statistically better fit to scaling trends than linear fits in log10 parameter v body size analyses were also tested for. All analysis was carried out in R studio using the packages qpcr, ape, Geiger and nlme (Andrej-Nikolai 2014; Campione et al 2014; Pennell et al 2014; Pinheiro et al 2015). A statistically significant second-degree coefficient established the nonlinear nature of the data if present. Models were compared using associates Akaike information criteria for limited sample sizes (AICc) and standard errors of the estimate. A cut off value of 2 for AIC scores was used, which is seen as an informative model in terms of ‘goodness of fit’ (Symonds and Moussalli 2010). However, it is not possible to say which fit is better for some segments than others, in particular models with values of up to 6 are suggested as not to be discounted either (Richards 2005). Pagal’s lambda (λ) was used to estimate the strength of the phylogenetic signal in the analyses.

2.3.3 Testing for differences in body shape between locomotor and trophic categories.

To test for differences in body shape between locomotor and dietary categories and to visualise changes in body shape across major evolutionary transitions, phylogenetic ANOVAs in the R package RRPP were used where size-normalised comparison was required (Adams and Collyer, 2018). The package RRPP exhibits higher power than the alternative methods based on phylogenetic simulation (Garland et al 1993; Adams and Collyer, 2018) and has been demonstrated to display
appropriate statistical properties such as a low error rate and high statistical power under diverse scenarios and these properties hold when examining various types of response variables, including both regression designs and analysis of variance [ANOVA] (Adams and Collyer 2018).

2.3.4 Measuring convergence

Recent developments in testing convergence quantitatively rather than qualitatively have enabled researchers to identify both convergent evolution and its statistical significance (Zelditch et al 2012; Arbuckle et al 2014; Friedman et al 2016; Page and Cooper 2017; Speed and Arbuckle 2017). The simplest way of quantifying convergent evolution in a trait is to identify it via a method such as ancestral state reconstruction (ASR) that can be implemented in standalone packages such as Bayes Traits (Pagel et al 2004) and Mesquite (Maddison and Maddison 2015) (see figure 5) or through R packages such as ape (Paradis et al., 2004), phytools (Revell 2012), surface (Ingram and Mahler 2013) and windex (Arbuckle and Minter 2015) with the number of independent origins of the trait then counted (e.g. Foote et al. 2015).

Looking at the strength of convergence is also another important factor for a better understanding of the convergence in a system. To analyse the strength of convergence in morphology across the dataset the Wheatsheaf index was used, as implemented in the R package Windex (Arbuckle et al 2014; Arbuckle and Minter 2015). The Wheatsheaf index compares the mean distance in phenotypic space
between species to the overall average distance between all pairs of species and scales those comparisons by the phylogenetic variance–covariance matrix. However, the original Wheatsheaf index cannot be used on fossil data because phylogenetic relatedness is penalised based on the distance from the root to the node where two species diverge. Following the advice from Arbuckle et al (2014) a cophenetic distance matrix was used to penalise for phylogenetic distance rather than a variance covariance matrix. This was achieved using the function cophenetic.phylo in the package ape (Paradis and Schliep 2018), where pairwise distances between pairs of tips using branch length were used, which is a more accurate representation of a non-ultrametric tree.

The Wheatsheaf index however, has been criticised because it can struggle to distinguish convergence from long term stasis (Stayton 2015). For example, taxa such as omnivores that have not undergone much evolutionary change compared to other taxa with diets such as herbivory and carnivory could be convergent according to these measures. Future work could address this by doing a modified version of a second convergence metric.
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Chapter 3- The evolution of body shape in tetrapods

This chapter is based on a manuscript which is currently in review at Nature communications.

Author contributions. KTB, PGC, TWM conceived the study. KTB, PGC, SCRM & AEM designed the study. KTB, PGC, TWM, ERS & AEM collected the scan data. AEM processed the scan data, made the measurements, and conducted the statistical analysis with guidance from all co-authors. All authors contributed to writing the manuscript.

3.1 Abstract

Body shape plays a fundamental role in organismal function, and it is expected that animals have evolved body proportions to best exploit their ecological niche. Tetrapods evolved a diverse array of body plans, but to date this diversity in body shape and its relationship to ecology have not been systematically quantified. Here, body proportions in 410 extinct and extant tetrapods spanning most major taxonomic, locomotor and trophic groups were analysed. Most body segments scale with negative allometry or isometry across Tetrapoda, and statistical support for quadratic relationships indicative of differential scaling in small-medium versus large animals were also found. Recovered differences between locomotor and dietary groups highlight key adaptations in body proportions that mechanistically underlie
the exploitation of major ecological niches, as well as revealing patterns of change across major macroevolutionary events. These results emphasise the pivotal role of body proportions in the broad-scale ecological diversification of tetrapods.

3.2 Introduction

Body shape plays a universal and fundamental role in the mechanical and physiological function of all organisms (Cuvier 1817; Russell 1916; Lauder 1981). At the most basic level, the motion of terrestrial vertebrates is constrained by Newtonian mechanics; that is, acceleration is a function of force and mass. Body proportions describe the distribution of mass within the moving body and the lengths of levers responsible for generating that movement. Body shape also plays a determinant role at multiple physiological levels; for example, in describing the space available for accommodating major organ systems (Rushton and Rushton 2004; Clauss et al 2016), and body surface area for heat exchange (Phillips and Heath 1995; Henderson 2013). Because different environments and behaviours place different demands on the functional mechanics and physiologies of organisms, it is expected that body shape should vary adaptively across animals occupying different ecological niches (Werner and Gilliam 1984; LaBarbera 1986; Ebenman 1992; Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Edgington and Taylor 2019; Law 2021). However, adaptive modification of body shape by ecological pressures may also be constrained by other factors, notably body size (Gould 1966; Penrose et al 2016), phylogenetic history and the ecological trajectory of evolutionary change.
Given the universal potential for natural selection to act upon body shape, it is not surprising that many studies have sought to investigate associations between body proportions and ecological niche occupation (Werner and Gilliam 1984; LaBarbera 1986; Ebenman 1992; Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Edgington and Taylor 2019; Law 2021). While these studies have regularly identified important adaptive trends in body shape evolution (Werner and Gilliam 1984; LaBarbera 1986; Ebenman 1992; Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Edgington and Taylor 2019; Law 2021), they have tended to focus on individual taxonomic or ecological groups, or on an individual aspect of body shape. The wider availability of imaging approaches allowing three-dimensional (3D) quantification of whole-organism shape, and the relatively recent development of a diverse toolkit of phylogenetic comparative methods, now allows explicit consideration of the role played by multiple ‘confounding’ factors like body size and phylogenetic history into statistical analyses of whole-body shape change. However, to date, no study has systematically investigated patterns of whole-body shape change across tetrapods using modern phylogenetic comparative approaches.

Here the first systematic statistical analysis of whole-body proportions across tetrapod evolution using a new dataset of 410 digital skeletons is presented (Figure 3.1). By using a broad taxonomic sample of extinct and extant tetrapods, the complex
interaction between body proportions, size, locomotor and trophic diversity and how these traits have contributed to macroevolutionary dynamics over the past 350 million years are examined. Using a novel data set three major questions are tackled: how have (1) body size, (2) locomotor, and (3) trophic ecology influenced the evolution of tetrapod body shape? To address these questions, not only are linear measurements of body segment size from 3D skeletal models extracted, but also mathematical shape-fitting was used to generate 3D volumetric representations of body proportions (Sellers et al 2012; Brassey and Sellers 2014; Bates et al 2016; Brassey 2016) (Figure 3.1). This allows the examination of changes in body segments whose overall size and shapes are poorly captured by linear measurements, and also provides a whole organism measure of body size against which to normalise body segment dimensions and assess allometric changes in proportions. These results provide the first statistically driven analysis of how the vertebrate body plan has been shaped as a multi-element or modular system during the broad-scale ecological diversification of tetrapods.
Figure 3.1: To investigate the evolution of body shape and ecology in tetrapods, a data set of (A) 410 extinct and extant terrestrial vertebrates from across Tetrapoda that captured major evolutionary changes in (B) locomotor, (C) trophic ecology and (D) body size was assembled. (E) From 3D digital skeletal models of these taxa a range of linear and volumetric measures were extracted and used to derive measures of body size and shape using phylogenetic comparative approaches. Linear measurements included gleno-acetabular distance (GA), femur length (FL), shank segment length (SL), metatarsal segment length (MtL), pes segment length (PL), humerus length (HL), forearm segment length (FaL), metacarpal segment length (McL) and manus segment length (ML).
3.3 Methodology

3.3.1 Data collection

A number of body shape and size metrics were quantified from 3D skeletal models of 116 amphibians, 47 birds, 55 non-avian dinosaurs, 143 mammals, 46 non-avian reptiles and 3 reptiliomorphs spread across the major groups within these clades. The data set consisted of 318 extant and 92 extinct species. Whole body scans of specimens were obtained from a variety of sources including CT scans and photogrammetric models collected by the authors, as well as models from previous studies (Bates 2009a; Bates et al 2009b; Sellers et al 2012; Allen et al 2013; Schachner et al 2014; Clauss et al 2016; Bates et al 2016; Macaulay et al 2017; Schachner et al 2017; Brassey et al 2018) and online digital repositories (Morphosource, KUPRI, Digimorph, Sketchfab, animalsimulation.org). A full list of the models, including their source information, can be found in the electronic supplementary material (ESM1.xlsx). The precise ontogenetic age and gender of most of the specimens are unknown. However, based on overall size it was inferred that most of the extant specimens are adults.

CT data were segmented using Mimics research 20.0(www.materialise.com/mimics), in order to generate 3D models of the skeletal material. Photogrammetric reconstructions were carried out in AgiSoft Photoscan. The resulting surface models were processed in Meshlab. Any unwanted (non-skeletal) material was removed, and
the skeleton of each specimen was split into segments (i.e., skull, neck, trunk, tail, humerus, forearm, metacarpal, manus, femur, shank, metatarsal and pes) to aid measurements and volumetric reconstructions (see below). Some segments were further subdivided such as the neck if postural issues effected the overall hull.

3.3.2 Body shape and size metrics

To examine patterns of body shape variation across tetrapods in this data set, a variety of linear and volumetric measures of body segment proportions were derived. The linear morphometric data collected consisted of gleno-acetabular (GA) distance, humerus length, forearm segment length, metacarpal segment length, manus segment length, femur length, shank segment length, metatarsal segment length, and pes segment length. The length of the forelimbs and hind limbs were calculated by summing the lengths of the four individual segments. Measurements were taken from approximate joint centres rather than the absolute length of the bones; this was preferred as some species have elongated areas of bone that do not contribute to the overall segment length or overall limb length in life. The overall size of some body segments (e.g., the torso) are not well captured by a single linear measurement and therefore volumetric size metrics using convex hulls, as in previous studies (Sellers et al 2012; Brassey and Sellers 2014; Bates et al 2016; Brassey 2016) were also generated. This yields an approximation of the skeletal volume of each individual body segment in the models, which can be summed to generate a whole-
body skeletal volume (herein referred to as whole-body convex hull volume [WBCHV]).

For regression analyses (see below) WBCHV was used as a proxy for whole body size. WBCHV was preferred as a proxy for overall body size because it utilises the entire skeleton rather than relying on a measure from a single body segment, which may bias any further analyses due to potential allometric signals in that one body segment. Using WBCHV also allowed all linear and volumetric parameters to be assessed or normalised by the same size metric (e.g., if femur length were used as the body size metric then a second body size metric would need to be found to size-normalise femur length). Also, variability in scan/model resolution meant that popular alternative metrics (e.g., long bone circumference (Campione 2017)) could not be accurately and/or repeatably measured across the data set.

3.3.3 Phylogenetic statistical analysis

To analyse body proportions in a phylogenetic context, each tetrapod species was added to a phylogenetic tree, which was built by merging recent, taxon-rich cladograms of major tetrapod groups using Mesquite (www.https://www.mesquiteproject.org). A full list of the phylogenetic trees merged, including their source information, can be found in the electronic supplementary material (ESM2.xlsx). To calculate branch lengths and time-calibrate the tree, first and last occurrences of each species were taken as the stratigraphic range of the
formation in which the fossil was found in the Palaeobiology Database (www.paleobiodb.org). These data were then combined with the phylogenetic tree in R studio using the package *strap* (Bell and Lloyd 2015) to produce a time calibrated tree. To examine variations and correlations in body shape and ecological variables taxa were classified into locomotor and dietary categories based on the current consensus in the literature about their primary mode of locomotion and diet (ESM1.xlsx). The locomotor habit of each taxon was classified as either active flight \((n = 33)\), aquatic \((n = 12)\), arboreal \((n = 42)\), bipedal striding \((n = 32)\), fossorial \((n = 28)\), quadrupedal striding \((n = 131)\), saltatorial \((n = 79)\), scansorial \((n=14)\), semi aquatic \((n = 30)\) or soaring flight \((n = 9)\). The dietary habit of each taxon was classified as either carnivore \((n = 123)\), herbivore \((n = 120)\), insectivore \((n = 95)\), omnivore \((n = 64)\), or piscivore \((n = 9)\).

To examine how each body segment metric scaled with body size across the dataset, regression analyses were conducted using phylogenetic generalised least squares (pGLS) in the R package *caper* (Orme et al 2013). This approach follows a general linear model calculating the slope, intercept, confidence, and prediction intervals, adjusting the expected covariance according to phylogenetic signal (Symonds and Blomberg 2014). It was also tested whether quadratic models provided a statistically better fit to scaling trends than linear fits in log transformed parameter \(v\) WBCHV data sets (Campione 2017). A statistically significant second-degree coefficient established, if present, the nonlinear nature of the data. Models were compared using Akaike weights and associated Akaike information criteria for limited sample
sizes (AICc). Shapiro-Wilks tests indicated that data for all body segment parameters were not normally distributed, and therefore all data were log-transformed prior to these regression analyses. Corrections for multiple comparisons (Bonferroni corrections) were not used due to the underlying variables being grounded in theory from previous empirical literature studies whereby correlations between locomotor and dietary types have been seen before. Bonferroni corrections also lead to a less powerful test, it will decrease the chances of type 1 error whilst increasing the chance of type 2 error, it is therefore not recommended by the package ape which was used, for being overly conservative (Armstrong 2014).

Pagel’s lambda (λ) was used to estimate the strength of the phylogenetic signal in the analyses. All analysis was carried out in R studio using the packages qpcr, ape, Geiger and nlme (Andrej-Nikolai 2014; Pennell et al 2014; Pinheiro et al 2015; Smaers and Rohlf 2016). To examine the nature of non-linearity in body segment allometry we compared the linear (PGLS) slopes of a taxa within a series of size thresholds (or size bins), where the data set was split at above versus below ~25 kg body mass, above versus below ~100 kg body mass, and above versus below ~500 kg body mass. These thresholds were chosen in part based on a combination of size-thresholds recovered in previous studies (Economos 1983; Silva 1998, Ren et al 2010) and in part because they allowed for a reasonable sample size in our largest size category. Phylogenetic ANCOVAs (phylANCOVA) were used to test for differences in body shape and the allometric relationships between locomotor and dietary groups using the approach of Smaers and Rohlf (2016) (Smaers and Mongle 2017) implemented in the R package
evomap (Paradis et al 2004). To visualise changes in body shape across major evolution transitions (Figure 3.3-3.7) the Brownian motion model was used to apply ancestral state reconstruction using the ‘phytools’ function ‘contmap’.

3.4 Results and discussion

3.4.1 Body size and the evolution of tetrapod body shape

Phylogenetically 108nrecognize least squares regressions (pGLS) (Orme et al 2013) were used to examine scaling patterns from all major body segments relative to overall body size in the broad sample of tetrapods used in this study (Figure 3.2, Fig. S1 in ESM3.docx), which includes some of the smallest tetrapods (e.g., Sorex monticolos; Selasophorus sasin, Takydromus sexlineatus) with approximate masses of 0.005 kg, and the largest mammals and dinosaurs (Loxodonta 108nrecogn, Tyrannosaurus rex, Dreadnoughtus schrani), with estimated masses of up to ~40,000 kg (Bates et al 2016; Campione 2017).

Linear allometric relationships versus body size for all body segments were statistically significant (Figure 3.2, Tables S1-2 in ESM3.xlsx, Fig. S1 in ESM3.docx), with a relatively strong phylogenetic signal (Lambda values varied between 0.819 to 0.938). pGLS slopes for the femur, humerus, forearm, metacarpal, overall forelimb length, torso and neck volume were scaled with isometry, providing support for
broad geometric similarity in these segments across the full body size range seen in tetrapods (Figure 3.2, Tables S1-2 in ESM3.xlsx). Negative allometry was recovered for the shank, metatarsal, pes, manus, gleno-acetabular (GA) distance and overall hind limb length, and skull volume (Figure 3.2, Tables S1-2 in ESM3.xlsx, Fig. S1 in ESM3.docx). The magnitude of negative allometry increases from proximal to distal segments within the hindlimbs of tetrapods, consistent with more ‘graviportal’ limb proportions in large versus small animals (Coombs 1978; Christiansen 2002). However, little support for this proximal to distal trend in the forelimb was found. This suggests that, across tetrapods as a whole, size-based constraints on forelimb construction are matched by selective pressures associated with highly disparate functional mechanics in different locomotor ecologies (e.g., flight, burrowing, arboreality; see below). The metatarsal and pes segments show the strongest negative allometry (e.g., metatarsal segment lower 95% CI = 0.278). Isometric scaling of torso volume, but negative allometry in GA distance suggests a change in torso shape 109nrecognized109 by mediolateral expansion of the ribcage and pelvis as body size increases in tetrapods. It has been suggested that large-bodied sauropod and ornithischian dinosaurs evolved mediolaterally wider bodies and limb girdles and that this impacted on locomotor kinematics (Mannion et al 2010; Bates et al 2016; Maidment et al 2014), but to our knowledge such trends have not previously been noted in other groups.

Therefore, on average, tetrapods change body shape as size increases: relative head size decreases, the torso becomes wider but anteroposteriorly shorter, the hind limb
becomes strongly graviportal, but the forelimb only weakly so (Figure 3.2, Fig. S1 in ESM3.docx). However, studies of specific body segments in certain tetrapod subgroups have proposed size thresholds in animal allometry, above which aspects of anatomy and locomotor biomechanics, such as effective limb mechanical advantage and maximum performance (e.g., running speed), differ in smaller vs larger animals (Coombs 1978; Biewener et al 1989; Christiansen 2002; Hutchinson 2004; Ren et al 2010; Fuentes 2016; Hirt et al 2017; Sellers et al 2017). A recent study addressed this issue in the scaling of femoral and humeral minimum shaft circumference and recovered greater support for a linear model, with quadratic models yielding a nonsignificant second-degree term (Campione 2017). However, other studies have suggested that some aspects of animal shape, such as body length, show size-dependent allometry (or size-thresholds) well recognized by second-degree quadratic relationships (Economos 1983; Silva 1998), but to date these studies have been restricted to specific taxonomic orders and individual body segments.

Statistically significant (p < 0.05) second-degree coefficients for phylogenetically informed quadratic models fit through linear and volumetric body segment measurements were also found (Figure 3.2, Tables S3-4 in ESM3.xlsx, Fig. S1 in ESM3.docx). However, in most cases, lower AICc values suggest that phylogenetically informed linear models are slightly better supported than quadratic models across tetrapods as a whole (Figure 3.2; Tables S3-4 in ESM3.xlsx, Fig. S1 in ESM3.docx). In almost all cases, AICc values are very similar, and values for shank length, metatarsal length, skull volume and neck volume are lower for the phylogenetically informed
quadratic models, providing statistical support for non-linear scaling in these body segments across tetrapods as a whole.

Tetrapods with upright striding gaits define the larger extremes of body size in this data set, and their locomotor bauplans are under the strongest or narrowest selection pressure in terms of first-order Newtonian mechanics; that is, locomotion in these groups is mostly dedicated to anti-gravity support on the ground, while other groups are subject to additional selective forces as associated with flight, swimming, climbing and burrowing. Linear and quadratic models fitted through data for bipedal striding and quadrupedal striding categories are again all statistically significant (p < 0.05; Tables S5-8 in ESM3.xlsx) with very similar AICc values in all cases. In bipedal striding tetrapods, phylogenetically informed linear models are statistically better supported than quadratic models for hind limb segments and overall hind limb length, while phylogenetically informed quadratic models better describe scaling trends in most forelimb segments and the forelimb overall (Tables S5-6 in ESM3.xlsx).
Figure 3.2. Scaling relationships between major body segment size and overall body size (total whole body skeletal convex hull volume) in 410 terrestrial tetrapods using phylogenetically informed linear (thick dashed lines) and quadratic (thin dotted lines) fits. The (A) head, (B) neck and (C) torso are represented by volumes, while (D) gleno-acetabular (GA) distance, © total forelimb and (F) total hind limb size is represented by lengths. Isometry in (A-C) would therefore be a slope of 1, and in (D-F) a slope of 0.33. Full breakdowns of the regression model information can be found in Tables S1-14 in ESM3.xlsx, including additional comparisons of scaling in individual limb segment lengths (Fig. S1 in ESM3.docx) and volumes. Taxa have been colour-coded by taxonomic order for display purposes.
These trends are likely driven by the highly varied ecological function of the forelimb across bipedal striding taxa; smaller bipedal taxa in this dataset are predominantly extant flightless birds which relatively short forelimb segments most likely due to flight loss, while larger bipedal taxa are generally non-avian theropod dinosaurs with short forelimbs, which may have been actively used in prey capture rather than body support during locomotion. In upright quadrupedal striding tetrapods, which include the largest mammals and dinosaurs, strong statistical evidence that quadratic models best describe scaling of the locomotor body plan was found (Tables S7-8 in ESM3.xlsx). Lengths and skeletal volumes for the hind limb, forelimb and all individual limb segments with the exception of the femur (length and volume) and humerus (length) are better described by phylogenetically informed quadratic rather than linear models (Tables S7-8 in ESM3.xlsx). To our knowledge this is the first evidence for near-ubiquitous differential size-based scaling in gross locomotor anatomy in tetrapods. To examine the nature of this non-linearity in body segment allometry, the slopes of a series of size thresholds (or bins) within the full data set and within upright quadrupedal striding taxa were compared (Tables S9-14 in ESM3.xlsx; Fig. S2 in ESM3.docx). This analysis again reveals differences between segments that define a strong proximal-to-distal scaling trend in terms of selection for increasingly graviportal limb proportions at larger body sizes. For the more distal limb segments the qualitative difference between slopes remains similar regardless of the specific body size chosen to split the data set into ‘smaller’ vs ‘larger’ animals; that is, in all these limb segments (metatarsal, pes, metacarpal and manus) the “larger” size group (>25 kg, >100 kg or >500 kg) always displays stronger negative allometry than the corresponding “smaller” size group (<25 kg, <100 kg, <500 kg) (Tables S9-14 in
ESM3.xlsx; Fig. S2 in ESM3.docx). The next most proximal segments in the forelimb (i.e., forearm segment) and hind limb (i.e., thigh and shank segments) show more negative allometry in the “larger” size bin when animals are split at thresholds 25 kg or 100 kg body mass. However, when split at a threshold of 500 kg, a reversal of the scaling pattern is found in the forearm, thigh and shank segments, with animals larger than 500 kg scaling with less negative allometry than animals smaller than 500 kg. In the humerus the relative reversal of slopes occurs at the lower mass threshold of 100 kg (Tables S9-14 in ESM3.xlsx; Fig. S2 in ESM3.docx). This reversal is such that animals over 500 kg actually show positive allometry in these segments, particularly so in the humerus (Tables S9-14 in ESM3.xlsx; Figure 3.2, Fig. S2 in ESM3.docx).

This size-dependent proximal-to-distal pattern of non-linearity refines our understanding of the morphological consequences of a suite of selective factors thought to act on vertebrate limbs to maintain locomotor efficiency whilst coping with increasing mechanical demands of large body size. Functionally, larger animals maintain similar peak stresses to smaller animals by adopting more upright postures and limiting joint excursions during habitual motions (Coombs 1978; Biewener 1989; Christiansen 2002; Biewener 2005). More extended joint postures reduce bending stresses acting on limb bones and the necessary forces that must be generated by muscles to support limb joints (Biewener 1989; Biewener 2005). Externally derived bending stresses in any given joint posture will also be reduced by shortening segment length, which may be the selective pressure driving stronger negative allometric signals in more distal limb segments (Figure 3.2; Tables S1-14 in ESM3.xlsx;
Biomechanical models have suggested that more distal limb segments and their connective joints may have lower safety factors in terms of peak muscle forces (Biewener 1989; Hutchinson 2004; Biewener 2005; Ren et al 2010) and bending stresses (Biewener 1989; Biewener 2005; Sellers et al 2017). The fact that larger animals always appear to scale more negatively than smaller animals in distal segments suggests that, as a first approximation, this selective response is broadly continuous in nature across the body size range exhibited by tetrapods, particularly for quadrupedal striding taxa (Figure 3.2; Tables S1-14 in ESM3.xlsx; Fig. S1-2 in ESM3.docx). However, more proximal segments in the largest tetrapods scale either near isometrically or with positive allometry at larger sizes. Relative lengthening of these more robust segments (that might logically be assumed to have inherently higher safety factors) may represent a compensatory mechanism to maintain stride length at more extended joint postures and thus minimize the cost of locomotion by recognizing a reduction in overall limb length at larger body sizes (Figure 3.2; Tables S1-14 in ESM3.xlsx; Fig. S1-2 in ESM3.docx).
3.4.2 Body shape and the evolution of locomotor ecology in tetrapods

For the analysis of the evolution of locomotor ecology and body proportions, the limbs were focused on, which are primarily responsible for powering differing types of locomotion, and the torso, the proportions of which determine the separation between the forelimbs and hind limbs. Large scale differences recovered across tetrapod locomotor ecology, which are centered around the mechanical requirements of moving through different media (water, air, substrates) and major evolutionary transitions were also focused on (Figure 3.3-3.6; Fig. S3-4 in ESM3.docx and Tables S15-45 in ESM3.xlsx).

The evolution of flight appears to have had the most significant and wide-scale effects on body proportions in tetrapods (Figures 3.3-3.4; Figs S3-4 in ESM3.docx; Tables S15-45 in ESM3.xlsx). Active fliers and soarers have significantly larger humeral, forearm and metacarpal segments and overall forelimb lengths than almost all other locomotor categories in phylANOVA comparisons (Figure 3.3A-D, Tables S21-23 in ESM3.xlsx). Manus lengths are also larger on average in flying taxa, but differences in their pGLS slopes and intercepts with other locomotor groups are not statistically significant (Figure 3.3D Tables S23-24 in ESM3.xlsx). Flying groups also show the strongest positive allometry in proximal forelimb segments and these are significantly different to most other groups (Figure 3.3A-D, Figs S3-4 in ESM3.docx; Tables S42 in ESM3.xlsx). These trends most likely relate to a greater evolutionary
pressure to increase wing area and muscle mass in flying birds, not only to maintain flight ability under the allometric constraints of increasing body mass, but also to produce both lift and thrust for take-off (Tobalske 2007; Rader et al 2020). Soarers and active fliers have broadly similar overall body size ranges and show similar humeral and forearm lengths at their largest overall body sizes, but active fliers have smaller humeral lengths at smaller body sizes, which is reflected in the statistically significant differences in their pGLS slopes and intercepts (Figure 3.3A Fig. S4 in ESM3.docx; Tables S21-22 in ESM3.xlsx). Forelimb elongation in the dinosaurian ancestors of birds appears to have been initiated in dromaeosaurs (Microraptor, Velociraptor), with the earliest taxa 118nrecognized to have had modest powered flight capabilities (Yixianornis, Archaeopteryx) showing overall forearm and forelimb segment sizes that are similar to extant flying birds (Figure 3.3A-D).

Distal limb segments (the manus) are not statistically longer in flying taxa, with high levels of variation seen in the majority of locomotor categories (Figure 3.3A-D, Fig. S4 in ESM3.docx; Tables S18-20, 22-24 in ESM3.xlsx). This could indicate greater ecological 118nrecognized118n in distal limb segments of tetrapods generally, as these segments are directly responsible for environmental manipulation, the nature of which may be highly varied even within locomotor groups. For example, within flying taxa, distal limb 118nrecognized118n may have evolved to better 118nrecogn locomotion for a variety of different substrates (branches, perches, wetland soils, water) and trophic niches (e.g., carnivory, herbivory, piscivory). The largest manus segments within flying taxa are found in the Chiroptera (bats), which evolved longer
forelimbs through the elongation of the forearm and manus rather than the humerus, as in birds (Figure 3.3 A-D). The manus in bats is highly modified for climbing and hanging from the walls of caves, whereas the hind limb shows comparatively little recognized relative to birds (Figure 3.4 A-D). Within birds, however, hind limb recognized does occur (Tobalske et al 2007; Stoessel et al 2013) for example, within active fliers and soaring species, the largest metatarsal and pes lengths are found in predominantly semi-terrestrial species (Antigone canadensis or Phoenicopterus roseus), and most likely represent an adaptive response to increase stride length, and thus efficiency, during bipedal locomotion.
In addition to flight, the evolution of locomotion in bird-line theropods was also marked by systematic changes in the relative lengths of hind limb bones, thought to be mechanistically linked to walking and running mechanics (Gatesy et al 1997; Allen et al 2013). Basal theropods had a long femur relative to distal limb segments (particularly the metatarsal segment) and are thought to have powered the stride from the hip (Gatesy et al 1997; Allen et al 2013). Along the lineage to birds, the fossil record demonstrates a reversal in the relative lengths of limb bones with the femur making up a relatively small proportion of limb length and the metatarsal segment greatly enlarged (Gatsey et al 1997). This is thought to be causatively linked to a shift in joint power away from the hip in favour of the knee and ankle joints (Gatesy et al 1997; Allen et al 2013). This data sheds light on how limb segments changed relative to overall body size during this evolutionary transition (Figure 3.4A-D). **pGLS slopes**
and intercepts suggest that femoral length did increase in certain derived clades of non-avian theropod dinosaurs like dromaeosaurs (*Microraptor, Velociraptor*) and transitional taxa (*Yixianornis, Archaeopteryx*) and remained relatively longer in specific extant avian sub-clades (e.g., *Apterygidae*). However, this data also suggests that a reversal occurred in early avian evolution and that the relative size of the femur observed in basal theropods is equaled in many extant avian groups, including flying taxa (Figure 3.4A). Shank length is fairly conservative relative to body size across the dinosaur-bird transition and indeed within extant groups (although it must be noted here that the sample size is relatively small), though with noticeable lengthening in certain lineages, such as Gruiformes and Cariamidae (Figure 3.4B). Similar conservatism is seen in metatarsal segment length relative to overall body size during theropod dinosaur evolution, but with much greater differentiation in modern groups (as noted above in the context of ecological 122), with many groups showing relatively short metatarsal lengths similar to basal theropod dinosaurs (Figure 3.4C).

Flying categories retain relatively long hind limbs overall and are statistically significant in their pGLS slopes and intercepts than any other locomotor category (Figure 3.4D, Tables S27 in ESM3.xlsx). Saltatorial species have the longest hind limbs of all locomotor categories and are significantly longer than fossorial and semi-aquatic taxa (Table S27 in ESM3.xlsx), whilst also differing significantly in their pGLS slopes and intercepts from these groups and quadrupedal striding taxa in certain individual hind limb segments (Tables S17-20, S28 in ESM3.xlsx). It appears that hind
limb elongation in saltatorial taxa is primarily achieved by lengthening of the proximal segments (femur, shank), though this category also shows a statistically longer pes compared to some other locomotor categories. The metatarsal segment is not statistically longer in saltatorial taxa than other locomotor groups (Tables S19 in ESM3.xlsx), which is perhaps unexpected given anecdotal observations that many specialist jumpers, like bushbabies and frogs, have elongated distal segments (Emerson 1978; Alexander 1995). By contrast, saltatorial taxa are unremarkable in their forelimb segments (Fig. S4 in ESM3.docx; Tables S21-26, S37- S40 in ESM3.xlsx), and their noticeably enlarged hind limbs are wholly consistent with adaptation for enhanced jumping performance (Alexander 1995). Biomechanical simulations of jumping have demonstrated that longer legs enable the animal to accelerate over a greater distance, meaning that limb extensor muscles have a longer time to shorten (increasing force output) and thus to accelerate the animal to a given speed (Alexander 1995). In an evolutionary context, these results are consistent with the hypothesis that the body plan of saltatorial anurans has been relatively fixed since the Triassic (Shubin and Jenkins 1995; Reilly and Jorgensen 2011), with relatively elongate limbs and the reduction of trunk vertebrae (Shubin and Jenkins 1995) leading to a relatively short GA length (Figure 3.5). Although extant anurans exhibit a broad range of locomotor modes, the anuran ancestor was optimised for hopping and eventually jumping (Reilly and Jorgenson 2011).
Figure 3.4. Colour-shaded phylogenetic trees to show the evolution of hind limb segment proportions during the evolution of locomotion in bats and across the non-avian to avian theropod transition using ancestral state reconstruction. Comparison of size normalised (A) femur, (B) shank, (C) metatarsal, and (D) pes segment lengths.
Figure 3.5. Conservatism in the anuran body plan (A) from the Triassic to present, particularly with respect to (B) GA length and (C) hind limb length. This conservatism underpins the low levels of variability seen in limb and body proportions in saltatorial taxa.
Fossorial and arboreal species are recognized to exhibit enlarged forelimbs for digging and locomotion through trees respectively (Samuels and Valkenburgh 2008; Selby et al 2020). Qualitatively, some support is found for this, with both groups demonstrating some of the largest forelimb segments on average, but in neither case are they statistically different in their pGLS slopes and intercepts to other locomotor groups (See figure 3.3, Tables S21-26 in ESM3.xlsx). Both groups show the same contrasting allometric patterns in the forelimb versus hind limb, with positive allometry in the forelimb and negative allometry in the hind limb (Fig. S3-4 in ESM3.docx; Tables S26-27 in ESM3.xlsx). Positive forelimb allometry may be associated with a need to redistribute greater soil volumes under isometric scaling of the neck and torso segments also observed in fossorial taxa (Tables S32,S41 in ESM3.xlsx). Positive allometry in the forelimb of arboreal species represents an obvious adaptative response to the demands of forelimb-driven locomotion through trees (Selby et al 2020). Changes in body shape, such as a streamlined torso and reduced limbs, might confer obvious functional advantages for animals that habitually move through water. Aquatic and semi aquatic species have the smallest limb segments relative to body size (Figure 3.6, Tables S17-45 in ESM3.xlsx). They also show the greatest negative allometry in individual limb segments and overall limb lengths and are regularly statistically different from other groups in phylANCOVAs in this respect (Fig. S3-4 in ESM3.docx; Tables S17-45 in ESM3.xlsx). Shortening of the femur, along with increasing bone density, has previously been noted as a common skeletal modification seen in vertebrates transitioning to a semi-aquatic existence (Amson et al 2014). Short limbs increase the capacity of limb muscles to produce backward thrust and propel the body forward and reduce drag forces, while a higher
bone mass increases robustness to accommodate larger muscles and cope with hydrodynamic forces (Fish 2004; Amson et al 2014).

Aquatic, semi-aquatic and scansorial taxa might be expected to have greater GA distances (indicative of a more streamlined body form) as an adaptation for movement through water and climbing (i.e., proximity of torso mass to the substrate). However, none of these groups are statistically ‘longer-bodied’ in their intercepts for GA distance (Fig. 3K) or average leg length (Fig. S3 in ESM3.docx) (Figure 3.6). However, these groups do appear, on average, to have the highest GA distances, and indeed contain taxa with the highest values (e.g., *Mustela erminea*, *Amphiuma means*, *Cryptobranchus alleganiensis*) (Figure 3.6E-G). The allometric patterns recovered for GA distance across locomotor categories make adaptive sense in terms of structural support and locomotion: striding quadrupeds display the strongest negative allometry (Table S25 in ESM3.xlsx), which may be mechanistically related to the fact that increases in body size impose greater bending moments on the spine cantilevered between the limbs. Thus, allometry in GA distance may represent an adaptive response to increasing bending moments in the spine as body size increases in striding quadrupeds. In contrast, animals that move habitually through air and water demonstrate the highest positive allometries in GA distance (Fig. S4 in ESM3.docx; Table S25 in ESM3.xlsx), which is likely to be advantageous by reducing whole-body drag through air or water.
Figure 3.6. Limb reduction and torso elongation in aquatic, semi-aquatic and fossorial tetrapods. Phylogenetic-informed regression provides support for relatively small (A) hind limbs and (B) forelimbs, and large GA distance relative to (C) overall size and particularly (D) average limb length in these locomotor groups. This tendency
towards reduced limbs and an elongate torso can be seen within major taxonomic sub-groups that contain aquatic, semi-aquatic and fossorial species, including € Testudines (turtles and tortoises), (F) lizards and (G) rodents.

The evolution of quadrupedality from a bipedal ancestor is very rare in tetrapod evolutionary history but occurred on four independent occasions within dinosaurs; three times within Ornithischia and once within sauropodomorphs (Maidment et al 2012; Maidment et al 2014). This data highlights how quadrupedality impacted dinosaur body proportions, and the differential changes that occurred in independent quadrupedal lineages (Figure 3.7A-C). The shift in forelimb use to active locomotory support does not appear to be universally associated with its elongation relative to overall body size across quadrupedal dinosaurs (Figure 3.7B). Indeed, within striding quadrupeds only the fan-throated lizard (*Sitana ponticeriana*) has a shorter relative forelimb length than armoured thyreophorans (Ankylosauria, Stegosauria), which have among the shortest forelimbs, relative to overall body size, of any tetrapod in this data set (Figure 3.7). This raises the possibility that relative forelimb length may have decreased during the evolution of quadrupedality in some ornithischian clades (Figure 3.7B). As a result of these trends, and due to stronger negative allometry in hind limb length within sauropods, quadrupedal dinosaurs also exhibit notable disparity in their ratios of hind limb to forelimb length, with relatively equal lengths in ornithischians, but a clear progressive trend towards relatively longer forelimbs than hind limbs during sauropodomorph evolution (Figure 3.7C). This shift in relative limb proportions in sauropod dinosaurs may be causatively linked
to neck enlargement in sauropods and a craniad shift in centre of mass position (Bates et al 2016), which in turn has been linked to changes in locomotion and environmental distributions in the late Jurassic and early Cretaceous (Mannion and Upchurch 2010).

Previously recognized disparity in relative trunk size across different bipedal to quadrupedal transitions were also recovered (Figure 3.7A). While all quadrupedal lineages evolved slightly enlarged torsos for their size, relatively small increases in ceratopsians (Marginocephalia), and hadrosaurids (Ornithopoda), moderate increases in sauropods, and much larger increases in armoured thyreophorans were found (Figure 3.6B). Together with the changes in limb proportions (Figure 3.7B, C), this suggests complex and varied selective pressures behind the evolution of quadrupedal locomotion across dinosaurs. For example, torso enlargement in quadrupedal dinosaurs may be closely linked with increasing gut size and mega-herbivory across dinosaurs generally (Maidment et al 2012; Maidment et al 2014; Bates et al 2016). The most extreme investment in relative torso size is seen in armoured thyreophorans, which also show short forelimbs for their body size relative to other quadrupedal dinosaurs (Figure 3.7) and quadrupeds generally, and together these traits are likely to have negatively impacted on locomotor performance. It is possible that extensive dermal armour evolved in concert with these changes to provide active or passive predator defense, given an investment in torso size above locomotor performance. In contrast, hadrosaurids, which lacked the more elaborate dermal and cranial ornamentation seen in marginocephalians and thyreophorans,
retained more cursorial limb proportions and overall limb size and a smaller torso, and presumably therefore more superior locomotor performance to outrun predators (Maidment et al 2012; Maidment et al 2014).

Figure 3.7. The evolution of quadrupedality in Dinosauria showing evolutionary trends in (A) forelimb length, (B) trunk volume, (C) hind limb to forelimb length ratio and (D) neck to forelimb length ratio. The patterns seen during the independent
acquisition of quadrupedality in ornithischians and sauropods in the relative proportions of the torso and forelimb mirror the wider allometric patterns seen in quadrupeds and herbivores generally. Relatively short forelimbs in quadrupedal dinosaurs may relate to coupling of forelimb and neck lengths to maintain the ability to graze near ground level. This is indirectly supported by the narrow and uniform range of neck to forelimb length ratio observed across quadrupedal dinosaurs with very different overall body plans (D). Circled numbers represent: 1= Ornithiscia; 2=Ceratopsia; 3=Ornithopoda; 4= Thyreophora; 5= Sauropods; 6= Neosauropoda; 7= Titanisauriformes; 8= Theropoda; 9=Eumaniraptora.

3.4.3 Body shape and the evolution of trophic ecology in tetrapods

For the analysis of trophic ecology and body proportions the focus is on the head, neck and torso segments, which are primarily responsible for processing food. This analysis emphasises the importance of viewing the tetrapod body plan as a multimodal system and analysing multiple segments concurrently to understand the nature and extent of ecological adaptation in body proportions. This is particularly important for characterising the evolution of carnivory and herbivory (Figure 3.7; Tables S46-47 in ESM3.xlsx).
However, phylANCOVAs do reveal significant differences in the allometry of skull, neck and torso size between carnivores and herbivores and all other trophic groups (Fig. 8D-G; Tables S49-51 in ESM3.xlsx). Negative allometry in skull volume was recovered for all trophic groups but is least marked in carnivores, which scale closest to isometry (slope = 0.966), while herbivores scale with greatest negative allometry (slope = 0.889; Figure 3.7A; Tables S47 in ESM3.docx). Greater selective pressure to maintain relative head size in carnivores likely relates to hyper-carnivory and large prey specialisation, particularly in the biggest carnivores. Indeed, the carnivores with the largest relative skull sizes in this dataset include frogs that specialise in eating other vertebrates of similar or larger size (Lepidobatrachus laevis) as well as reptiles, birds, dinosaurs and mammals that specialize in prey of a similar size to themselves (Crocodilus niloticus; Sphenodon punctatus; Strix aluco; Falco columbarius; Velociraptor mongolinensis and Mustela erminea). A larger skull size gives these species a relatively bigger gape and stronger bite force with which to subdue large struggling prey and greater ability to subsequently reduce food mass (Erickson et al 2003; Anderson et al 2008; Cornette et al 2015). In line with these results, the evolution of carnivory is consistently marked by an increase in relative head size (Figure 3.7 E-F), and herbivory with decreases in head sizes in most taxonomic orders.

The relative allometric signal observed in head size between carnivores and herbivores is reversed in the neck: carnivores exhibit negative allometry in neck relative to overall body Figure 3.7 C, F-G) and isometry relative to head size (slope = 1.004; Figure 3.7 B), and are statistically different to herbivores (and some other
groups; see Tables S47-S52) whose necks scale with positive allometry relative to overall body size (slope = 1.04; Figure 3.7C, G) and negative allometry to head size (slope = 0.990; Figure 3.7B). These patterns likely reflect intrinsically linked mechanical and ecological selective pressures; an absolutely and relatively larger head may necessitate a shorter neck to minimise the first mass moment of the head in carnivores, while an absolutely and relatively smaller head may remove this constraint and enable longer necks in herbivores. Equally, longer necks increase the feeding envelope and total range of motion accessible to the head-neck system, which may be beneficial to herbivores but undesirable for carnivores where the more inherent structural stability of a shorter neck is beneficial to prey capture. This ecomorphological distinction is exemplified in non-avian dinosaurs: the relatively longest necks and smallest heads are found in herbivorous sauropod dinosaurs, while carnivorous theropod dinosaurs have relatively short necks and large heads for their body size relative to many terrestrial vertebrates (Figure 3.7F).
Figure 3.8. Trophic ecology and body shape tetrapods. (A-D) phylANCOVA comparisons do reveal statistically significant differences in allometric signals in these body segments between carnivores and herbivores and most other dietary types. The tendency towards larger heads and smaller necks in carnivores compared to herbivores (particularly at larger body sizes) is reflected in evolutionary transitions between these dietary ecologies in (E) mammals, (F) dinosaurs and (G) birds. Circled numbers in (E) represent: 1= Pecora; 2= Suina; 3= Arctoidea; 4= Canidae; 5= Felidae). Circled numbers in (F) represent: 1= Sauropoda; 2= Neosauropoda; 3= Titanasauriformes; 4= Theropoda; 5= Eumaniraptora). Circled numbers in (G) represent: 1= Aves; 2=Palaeognathae; 3=Galliformes; 4=Aequorlitornithes; 5=Aequornithes; 6= Accipitriformes; 7=Afroaves; 8= Australaves)
Because torso volume makes up such a large proportion of total body volume, its allometric signal relative to average limb length was also assessed (Figure 3.7D). Herbivores exhibit the greatest positive allometry (slope = 3.209; Figure 3.7D) and are statistically different to carnivores (and some other groups; ESM3.xlsx, Tables S47, S54) which also scale with positive allometry but to a lesser extent (slope = 3.169; Figure 3.7D). Collectively, relatively larger torsos and necks and smaller heads in herbivores suggests selection towards food processing shifting from the mouth to the gut, particularly in mega- herbivores like sauropod dinosaurs.

3.5. Conclusion

These results highlight that the complex interaction of body size (Figure 3.2), locomotor (Figures 3.3-3.6) and trophic ecology (Figure 3.7) has adaptively modified whole-body proportions during tetrapod evolution. The analysis suggests that the geometric similarity model is the best linear descriptor of how much of tetrapod body shape scales across body sizes of approximately 0.005-40,000 kg, but significant negative allometry in some segments indicates a change in body shape as size increases (Figure 3.2, Figs S1-2 in ESM3.docx). Indeed, graviportal allometric trends often noted in limb proportions was found to be non-linear in quadrupedal taxa with differential patterns in proximal versus distal segments developing as body size increases (Figure 3.2, Figs S1-2 in ESM3.docx). Biomechanical analyses that combine morphological data with measures of in-vivo muscle and bone stress and metabolic
energy expenditure would illuminate the exact interaction of these changes as body size increases.

Phylogenetically informed statistics recovered numerous significant differences between locomotor (Figure 3.3-3.6) and dietary (Figure 3.7) groups. In particular, locomotion through different media is associated with changes in multiple body segments with clear adaptive benefits: for example, elongation of the forelimb in flying and arboreal taxa (Figs 3-5), limb reduction and a more elongate body form in aquatic and scansorial taxa (Figure 3.3) and long hind limbs in saltatorial tetrapods (Figure 3.3). Inclusion of fossils in the data set allows us to visualise quantitative changes to whole-body proportions across major evolutionary transitions between locomotor and trophic categories, illuminating synchronous changes in multiple body segments during transitions to flight (Figure 3.4 and 3.5), and between bipedality quadrupedality (Figure 3.6) and carnivory-herbivory (Figure 3.7). Despite the differences between locomotor and dietary categories, the analyses showed that phylogenetic history also has an important influence on tetrapod body proportions (e.g., ESM3.xlsx tables S1-2). It would be interesting for future work to consider directionality and evolutionary pathways of body shape change more explicitly than is achieved here (and with expanded taxonomic coverage), and indeed to assess statistically the extent to which different ecologies are converging upon similar overall body shapes.
3.6 References


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Chapter 4- Convergent evolution of body shape in tetrapods

I am currently developing this chapter for publication.

Author contributions. A.E.M, KTB & C.M. conceived the study. A.E.M, KTB & C.M. designed the study. K.T.B, P.G.C, T.W.M, & A.E.M collected the data. C.M. developed the statistical approach. A.E.M processed the scan data, made the measurements and conducted the statistical analysis with guidance from all co-authors. The present thesis version was drafted by AEM and benefited from editorial suggestions from all other co-authors.

4.1 Abstract

Morphological convergence is often explained as adaptation to similar mechanical functions and ecological niches. Body shape plays a fundamental role in organismal function and previous work has recovered clear ecological signals in the evolution of body proportions across terrestrial tetrapods. However, to date, there has been no formal test of the hypothesis that, across tetrapods generally, animals that have independently evolved into analogous ecological niches have concurrently converged upon similar body proportions. Here, we use phylogenetic comparative methods to quantitatively assess the nature and magnitude of convergence in body proportions in different dietary and locomotor ecotypes in a broad sample (410
species) of extinct and extant terrestrial tetrapods. Using a newly modified version of the Wheatsheaf index (w) to provide measures of convergence in extinct and extant taxa, our results indicate statistically significant levels of convergence in body proportions throughout most dietary and locomotor categories. However, contrary to our over-arching hypothesis, the magnitude of this convergence is predominantly low, particularly within dietary ecotypes. However, we find that mechanically demanding or specialised forms of locomotion such as fossoriality and flight show much stronger magnitudes of convergence in key adaptive aspects of their morphology. Overall, our findings suggest that locomotor ecology has exerted stronger convergent selective pressure on the evolution of body proportions in terrestrial tetrapods than trophic habits.

4.2 Introduction

An important aim in evolutionary biology is to identify the mechanisms responsible for morphological and ecological diversity (Werner and Gilliam 1984; LaBarbera 1986; Ebenman 1992; Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Edgington and Taylor 2019; Law 2021). Convergent evolution is the process whereby organisms that are not closely related, independently evolve similar traits or ‘convergent’ morphologies. In many instances, such convergent morphologies have been hypothesised to represent the interaction between genotype and external environmental pressures: in other words, given genetic plasticity, natural selection
favours specific morphologies that function well mechanically and physiologically in
a given behaviour or ecological niche (Werner and Gilliam 1984; LaBarbera 1986;
Harmon et al 2005; Allen et al 2013; Claverie and Wainwright 2014; Edgington and
Taylor 2019; Law 2021). A classic example is the evolution of flight, a locomotor type
that has evolved independently in three tetrapod groups, birds, bats and pterosaurs,
each group having adapted their forelimbs to be similar in appearance, due to the
functional aerodynamic constraints (McGhee 2011). Measures of convergent
evolution are therefore extremely valuable for understanding the relationship
between genotypic and functional factors and morphological diversity, and how
these relate to macroevolutionary patterns and ecological diversification. Increasing
recognition of this importance has seen considerable research investment into the
development of phylogenetic statistical approaches to quantify patterns of
convergent evolution (e.g., Arbuckle et al 2014; Stayton 2015; Friedman et al 2016;
Rowe et al 2016; Page and Cooper 2017; Zelditch et al 2017; Speed and Arbuckle
2017).

Body size and shape play a fundamental role in multiple aspects of organismal
function (Schmidt-Nielsen and Knut 1984; Phillips et al 1995; Rushton and Rushton
2004; Porter et al 2009; Henderson 2013; Clauss et al 2016). The relative lengths and
masses of body segments influence the forces and energetics of motion (Biewener
and Patek 2018), while body size and shape determine physiological factors such as
thermoregulation and the space available for major organs (Rushton and Rushton
2004; Clauss et al 2016). One might therefore expect that behaviours and ecologies
that place specific mechanical and physiological demands on organisms may have led to evolutionary convergence in body proportions through deep time. To date, most studies of convergence in body proportions have focused on relatively closely related species or specific sub-groups of vertebrates, within which the independent evolution of specific ecologies has seemingly resulted in similar adaptive changes to body shape. For example, environmentally associated convergence in relative body segment proportions and body size has been suggested within island anole radiations (Harmon et al 2005; Mahler et al 2013) and African great lake cichlid fish (Muschick et al 2012); or between lineages that share a similar ecotype, such as trophic ecology in marsupial and placental carnivores (Jones et al 2013); hopping locomotion in jerboas and kangaroo rats (Webster and Dawson 2004) and fast, continuous swimming in lamnid sharks and tuna (Donley et al 2004). However, broader-scale statistical examinations of the nature and magnitude of convergence in body shape across terrestrial tetrapods more generally are rare in the literature.

In recent years the more widespread availability of low-cost approaches for digitising animal morphology (Bates et al 2010; Falkingham 2012) and the development of semi-automated methods for measuring body proportions (e.g Sellers et al 2012; Brassey and Sellers 2014, Bates et al 2015, Brassey and Gardiner 2015, Brassey et al 2015, Brassey et al 2016, Brassey et al 2018) have made such large-scale or broad comparative studies of whole-organism morphology more feasible. In chapter 3, a study of 410 extant and extinct terrestrial tetrapods spanning most major taxonomic, locomotor and trophic groups recovered differences between locomotor and dietary
groups that highlight key adaptations in body proportions that mechanistically underlie the exploitation of major ecological niches, as well as revealing patterns of change across major macroevolutionary events. These results emphasise the pivotal role of body proportions in the broad-scale ecological diversification of tetrapods (See chapter 3). Here this data set on body proportions was used to assess ecologically associated patterns of convergence in terrestrial tetrapods at a broad taxonomic scale for the first time.

4.3 Materials and methods

The data set of 410 3D skeletal models assembled in chapter 3 consists of 116 amphibians, 47 birds, 55 non-avian dinosaurs, 143 mammals, 46 non-avian reptiles and 3 reptiliomorphs spread across the major groups within these clades, with 318 extant and 92 extinct species (Figure 4.1). Whole body scans of specimens were obtained from a variety of sources including CT scans and photogrammetric models collected by the authors, as well as models from previous studies (Bates et al.2009a, b; Sellers et al 2012; Bates et al 2012; Allen et al 2013; Lyson et al 2014; Schachner et al 2014; Bates et al 2015; Bates et al 2016; Clauss et al 2016; Macaulay et al 2017; Schachner et al 2017) and online digital repositories (Morphosource, KUPRI, Digimorph, Sketchfab, animalsimulation.org), as well as a small number of additional models provided by colleagues. A full list of the models, including their source information, can be found in the electronic supplementary material (ESM1.xlsx). The
precise ontogenetic age and sex of many of the specimens are unknown. However, based on overall size, most of the extant specimens were inferred to be adults. To examine convergent evolution in body shape within ecotypes, taxa were classified into locomotor and dietary categories based on the current consensus in the literature about their primary mode of locomotion and diet (Figure 4.1, C-D, ESM1.xlsx). The locomotor habit (Figure 4.1D) of each taxon was classified as either active flight (n = 33), aquatic (n = 12), arboreal (n = 42), bipedal striding (n = 32), fossorial (n = 28), quadrupedal striding (n = 131), saltatorial (n = 79), scansorial (n=14), semi aquatic (n = 30) or soaring flight (n = 9). The dietary habit (Figure 4.1C) of each taxon was classified as either carnivore (n = 123), herbivore (n = 120), insectivore (n = 95), omnivore (n = 64), or piscivore (n = 9).

The metrics used to quantify aspects of body shape or body proportions are those measured in chapter 3 (Figure 4.1). The linear morphometric data collected consisted of gleno-acetabular (GA) distance, humerus length, forearm segment length, metacarpal segment length, manus segment length, femur length, shank segment length, metatarsal segment length, and pes segment length. The length of the forelimbs and hind limbs were calculated by summing the lengths of the four individual segments. The overall size of some body segments (e.g., the torso) are not well captured by a single linear measurement and therefore volumetric size metrics using convex hulls were also generated, as in previous studies (Brassey and Gardiner 2015, Bates et al 2009a, b, 2012; 2016; Brassey and Sellers 2014; Brassey et al 2018). This yields an approximation of the skeletal volume of each individual body segment.
in the models, which can be summed to generate a whole-body skeletal volume (herein referred to as whole-body convex hull volume [WBCHV]). To examine convergence in relative body segment proportions across taxa it was necessary to size-normalise these linear and volumetric metrics. For various comparisons, body segment linear measurements were normalised by WBCHV$^{0.33}$ (e.g., femur length/WBCHV$^{0.33}$) and volumetric segment measurements were normalised by WBCHV (e.g., torso volume/WBCHV). WBCHV was preferred as a proxy for overall body size and subsequently size-normalisation because it utilises the entire skeleton rather than relying on a measure from a single body segment, which may bias any further analyses due to potential allometric signals in that one body segment. Using WBCHV also allowed all linear and volumetric parameters to be assessed or normalised by the same size metric (e.g., if humerus length were used as the body size metric then a second body size metric would need to be found to size-normalise humerus length). Also, variability in scan/model resolution meant that popular alternative metrics (e.g., long bone circumference; Campione and Evans 2012) could not be accurately and/or repeatably measured across the data set. However, in addition to examining body segments relative to overall body size (WBCHV), convergence in ratio of certain segments sizes to each other were also tested for. Specifically, evolutionary patterns in the ratio of GA length to limb length, and the ratio of proximal limb segments (i.e., femur and humerus) to their respective distal limb segments (i.e., metatarsal and pes segments; metacarpal and manus segments) were also examined. These ratios were considered due to possible mechanical adaptations to specific ecologies. For example, it might be predicted that aquatic and semi-aquatic ecologies would converge on more elongate or streamlined body shapes (i.e. higher GA/leg length),
while the relative proportions of proximal versus distal limb lengths have long been recognized as influential or even predictive of differences in locomotor mechanics (e.g., Coombs 1978; Gatesy and Middleton 1997; Middleton and Gatesy 2000; Christiansen 2002; Carrano 2006).

To analyse size-normalised body proportions in a phylogenetic context, each tetrapod species was added to a phylogenetic tree, which was built by merging recent, taxon-rich cladograms of major tetrapod groups using Mesquite (www.https://www.mesquiteproject.org). A full list of the phylogenetic trees merged, including their source information, can be found in chapter 2 and ESM2.docx. To analyse convergence in morphology across the dataset the Wheatsheaf index was used, as implemented in the R package WINDEX (Arbuckle et al 2014; Arbuckle and Minter 2015). The Wheatsheaf index compares the mean distance in phenotypic space between species to the overall average distance between all pairs of species and scales those comparisons by the phylogenetic variance–covariance matrix. However, the original Wheatsheaf index cannot be used on non-ultrametric trees because it uses a variance-covariance matrix to penalise for phylogenetic distance. Following recommendations in Arbuckle et al (2014), the calculation of the Wheatsheaf index was modified to use a cophenetic distance matrix. This was achieved using the function cophenetic.phylo in the package ape (Paradis and Schliep 2018), which computes pairwise distances between pairs of tips using branch lengths and is therefore a more accurate representation of a non-ultrametric tree (see Figure S1 in ESM4.docx). If a pair of species are closely related and therefore the shared
proportional distance between species obtained from the phylogeny is close to 1, then the $w$ value or phenotypic distance between species corrected for phylogeny will be much larger than the phenotypic (Euclidean) distance between species. Because $w$ increases as convergence becomes stronger, we interpret a value of less than 1 as no convergence, 1 as convergence and anything above 1 as strong convergence as seen in previous papers such as Aguilar-Puntriano et al 2018 (See Figure S2 in ESM4.docx of histograms showing the distributions of Wheatsheaf index values; we can see that the example in (a) is more strongly convergent than (b) both in absolute terms (calculated value is higher) and with respect to the constraints of the tree (further to the right of the distribution).

To complement the assessment of evolutionary convergence, statistical differences between locomotor and dietary categories were tested for in the central tendency of each normalised metric using phylogenetic ANOVAs (pANOVAs) in the R package RRPP (Adams and Collyer, 2018).
Figure 4.1. To investigate convergent evolution of body shape and ecology in tetrapods, a data set of (A) 410 extinct and extant terrestrial vertebrates from across Tetrapoda that captured major evolution changes in (C) locomotor and (D) trophic ecology was assembled. (B) From 3D digital skeletal models of these taxa a range of linear and volumetric measures were extracted and used to derive measures of body size and shape using phylogenetic comparative approaches. Linear measurements included gleno-acetabular distance (GA), femur length (FL), shank segment length (SL), metatarsal segment length (MtL), pes segment length (PL), humerus length (HL), forearm segment length (FaL), metacarpal segment length (McL) and manus segment length (ML).
4.4 Results

4.4.1 Convergence in trophic categories

Statistical assessment of normalised body segment proportions within trophic categories yielded relatively few statistically significant results (Tables S2-S3, ESM4.xlsx). No statistically significant occurrences of convergence were recovered in the normalised size of the head or neck within dietary categories, which represent the body segments primarily responsible for gathering food. Carnivores, however, yielded significant convergence (p = 0.048, w = 1.2) in relative manus length (Table S2, ESM4.xlsx). pANOVAs indicated that carnivores have a significantly longer manus (including claw length) on average than herbivores (See Figure 4.33 F, Table S7, ESM4.xlsx) and a shorter manus length compared to piscivores (Figure 4.3 F, Table S7, ESM4.xlsx).

Herbivores showed statistically significant convergence in GA length (p = 0.04, w = 1.28) and WBCHV (p = 0.03, w = 2.18) (Table S2, ESM4.xlsx). pANOVAs further revealed herbivores have a significantly larger whole-body size (WBCHV) on average than all other dietary groupings (Figure 4.3A, Table S11, ESM4.xlsx) and significantly shorter GA lengths when compared to carnivores (Figure 4.3E, Table S8, ESM4.xlsx). Omnivores showed statistically significant (p = 0.02) convergence (w = 1.34) in relative torso volume (Table S3, ESM4.xlsx) and were found to have significantly
smaller torsos than herbivores, and significantly larger torsos than insectivores (Figure 4.3d, Table S10, ESM4.xlsx).
Figure 4.2. Bar chart of Wheatsheaf index values for body proportion parameters that show statistically significant ($p = <0.05$) levels of convergence with (A) dietary and (B) locomotor categories. Labels include AF, active flight, FO, fossorial, QS, quadrupedal striding, SC, scansorial and SO, soaring.
Figure 4.3. Box plots of size-normalised body segment sizes in different trophic categories, with statistical differences between them examined using pANOVA comparisons. These results highlight considerable variation within and between dietary categories in size-normalised (a) WBCHV, (b) skull volume, (c) neck volume, (d) torso volume, (e) GA length and (f) manus length and as a result these segments show statistically significant levels of convergence or significant differences in pANOVA comparisons. Dietary categories that are statistically different (P < 0.05) to other dietary categories are indicated by the coloured squares.
4.4.2 Convergence in locomotor categories

Statistical assessment of normalised body segment proportions within locomotor categories yielded a greater number of significantly convergent morphologies than trophic categories, and also higher $w$ values indicating stronger convergence in body proportions (Fig. 4.2, Table S1, ESM4.xlsx). Active fliers showed statistically significant convergence in relative humeral ($p = 0.01, w = 1.52$) and forearm segment lengths ($p = 0.05, w = 1.49$), and strong convergence in the ratio of humeral to manus segment lengths ($p = 0.05, w = 2.02; \text{Fig.4.2, Table S1, ESM4.xlsx}$). pANOVA comparisons confirm that active flying species have longer humeri and forearms than all other locomotor categories (Figure 4.4a and b; Table S18-S19, ESM4.xlsx) and significantly larger humerus to manus ratios than fossorial, saltatorial and quadrupedal striding species (Figure 4.5h; Table S33, ESM4.xlsx). Soaring species, however, do not yield any statistically significant incidences of convergence in relative forelimb segment proportions, but do show significant convergence in relative shank ($p = 0.02, w = 2.58$), metatarsal ($p = 0.03, w = 2.48$) and overall hind limb lengths ($p = 0.05, w = 2.14; \text{Fig.4.2,Table S1, ESM4.xlsx}$), as well as being convergent in torso volume ($p = 0.05, w = 2.40; \text{Table S4, ESM4.xlsx}$) and strongly convergent in WBCHV ($p < 0.005, w = 4653.89$). pANOVA comparisons reveal that soaring species have a significantly longer hindlimbs than aquatic, quadrupedal striding, scansorial and semi-aquatic species (Figure 4.4j; Table S23, ESM4.xlsx). In particular, the shank is relatively longer than aquatic, quadrupedal striding and fossorial species (Figure 4.4f; Table S15, ESM4.xlsx), with the metatarsal
being significantly longer relative to body size than in all other locomotor categories except for active flying and bipedal striding (Figure 4.4 g; Table S16, ESM4.xlsx).

Fossorial taxa show convergence in multiple parameters, including all four hind limb segment lengths ($p = 0.01$-$0.03, w = 1.44$-$1.55$), forearm segment length ($p = 0.05, w = 1.77$), total hind limb length ($p = 0.01, w = 1.51$) and average leg length ($p = 0.01, w = 1.58$; Table S1, ESM4.xlsx). pANOVAs reveal the hind limb of fossorial species to be significantly shorter relative to body size than active flying, bipedal striding and saltatorial species, but also longer than semi-aquatic species (Figure 4.4 f-j; Table S23, ESM4.xlsx). The forearm segment is also significantly smaller than active flying, arboreal and soaring species, but longer again than semi-aquatic species relative to body size (Figure 4.4b; Table S19, ESM4.xlsx).

Quadrupedal striding animals also show widespread convergence in the length ratios of individual body and limb segments (Fig.4.2, Table S3, ESM4.xlsx). Specifically, statistically significant convergence is recovered for ratios of GA to hind limb length ($p < 0.005, w = 1.99$), GA to forelimb length ($p < 0.005, w = 1.78$), GA to average leg length ($p = 0.01, w = 1.91$), hind limb to forelimb length ($p < 0.04, w = 1.27$), femur to metatarsal segment length ($p = 0.01, w = 1.29$), femur to pes segment lengths ($p = 0.01, w = 1.347$), humerus to metacarpal segment lengths ($p = 0.04, w = 1.28$) and humerus to manus segment lengths ($p = 0.04, w = 1.43$; Fig.4.2, Table S1, ESM4.xlsx). Quadrupedal striding taxa also show significant convergence in relative metacarpal ($p = 0.04, w = 1.299$), manus ($p = 0.01, w = 1.40$) and overall forelimb length ($p = 0.03,$
w = 1.26; Table S1, ESM4.xlsx). Femur to metatarsal and the femur to pes length ratios in quadrupedal striding taxa were significantly larger than all other locomotor groupings apart from active flying and scansorial species (Figure 4.5e and f; Tables S29-S30, ESM4.xlsx). The humerus to metacarpal ratio was larger than all other groupings also, except for arboreal, bipedal striding, scansorial and fossorial species, with quadrupedal striders having longer metacarpals on average (Figure 4.5g; Table S32, ESM4.xlsx), whilst the humerus to manus length was also larger than all other groups apart from soaring, scansorial, saltatorial and fossorial species (Figure 4.5h; Table S33, ESM4.xlsx).

The highest w values are recovered within scansorial taxa, which again show multiple incidences of statistically significant convergence in the length ratios of body segments (Fig.4.2, Table S1, ESM4.xlsx). As in quadrupedal striding taxa, statistically significant, but much higher, levels of convergence are recovered for ratios of GA to hind limb length (p < 0.005, w = 12.63), GA to forelimb length (p < 0.005, w = 17.92) and GA to average leg length (p < 0.005, w = 14.67), with pANOVAs revealing scansorial species have significantly larger GA to hind limb and GA to forelimb ratios than all locomotor groupings but semi aquatic species, and larger GA to average leg length than all locomotor categories but aquatic species (Figure 4.5b-d; Table S27-S28, S32, ESM4.xlsx). Hind limb to forelimb length (p < 0.005, w = 13.59), femur to metatarsal segment length (p = 0.01, w = 10.43), femur to pes segment lengths (p < 0.005, w = 8.65), humerus to metacarpal segment lengths (p < 0.005, w = 8.72) and humerus to manus segment lengths (p < 0.005, w = 13.99) also had statistically
significant and high levels of convergence (See figure 4.2). However, despite appearing to converge strongly on specific morphologies, pANOVAs suggest that scansorial taxa show fewer significant differences to other locomotor groups in these parameters, with only the femur to pes ratio recovered as statistically larger in scansorial species compared to active flying, arboreal and soaring species (Figure 4.5f; Table S30, ESM4.xlsx) and the humerus being longer to the metacarpal compared to bipedal striding and arboreal species (Figure 4.5g; Table S32, ESM4.xlsx). Scansorial taxa also showed statistically significant convergence in relative femur length (p < 0.005, w = 2.95; Table S1, ESM4.xlsx), and are recovered as having significantly longer femora than flying species and shorter femora than saltatorial species (Figure 4.4e, Table S14, ESM4.xlsx).

The aquatic taxa in our data set showed strong convergence in WBCHV (p = 0.02, w = 283.42), which perhaps represents an artifact of restricted sampling in this category. Arboreal, bipedal striding, semi-aquatic and saltatorial categories did not show any statistically significant incidences of convergence in body segment proportions (Table S3, ESM4.xlsx).
Figure 4.4. Box plots of size-normalised body segment lengths in different locomotor categories, that reached statistical significance for evolutionary convergence, with statistical differences between them examined using pANOVA comparisons. The results highlight considerable variation within and between locomotor categories in size-normalised (a) humerus, (b) forearm, (c) metacarpal, (d) manus, (e) femur, (f) shank, (g) metatarsal (h) pes, (i) total forelimb and (j) total hind limb, (k) GA length and (l) average leg length. Locomotor categories that are statistically different to other locomotor categories are indicated by the coloured circles. Labels include AF, active flight, AQ, aquatic, AR, arboreal, BS, bipedal striding, FO, fossorial, QS, quadrupedal striding, SAL, saltatorial, SC, scansorial, SA, semi-aquatic, SO, soaring.
Figure 4.5. Box plots of ratios of body segments lengths in different locomotor categories that reached statistical significance for evolutionary convergence. Additionally, differences between locomotor groups using pANOVA comparisons were tested for. The results highlight considerable variation within and between
locomotor categories in size-normalised (a) hind limb by forelimb, (b) GA by average leg length, (c) GA by hind limb length, (d) GA by forelimb length, (e) femur by metatarsal length, (f) femur by pes length, (g) humerus by metacarpal length (h) humerus by manus length. Locomotor categories that are statistically different to other locomotor categories are indicated by the coloured circles. Labels include AF, active flight, AQ, aquatic, AR, arboreal, BS, bipedal striding, FO, fossorial, QS, quadrupedal striding, SAL, saltatorial, SC, scansorial, SA, semi-aquatic, SO, soaring.
4.5 Discussion

4.5.1 Body proportions show little convergence with trophic categories

The analyses yielded relatively low Wheatsheaf index values \((w)\) for body segment proportions amongst dietary categories, suggesting trophic ecology has exerted relatively weak convergence in body proportions across tetrapods generally. Despite the input of traits most strongly correlated with diet (skull volume, neck volume and torso volume), low \(w\) values and high \(P\) values \((p > 0.05)\) were recovered for most dietary categories. This does not imply that convergence has not occurred, but that it is not very strong in most linear and volumetric measurements of relative body segment size within diet categories. There are however a few exceptions to this, notably that omnivores spread across a breadth of tetrapod groups (e.g., birds, mammals, and non-avian reptiles) have converged on a similar torso size. Omnivory is relatively complex and functionally demanding because it involves feeding at multiple trophic levels and on different types of food that vary in digestibility and nutrient content (Rudnick and Resh 2005). Our results suggest that omnivores converge on an ‘average’ or ‘intermediate’ torso size for tetrapods and the different dietary groups studied here (Figs 4.2-4.3). Omnivores have statistically smaller torsos for their size that herbivores (the group with the largest torsos) and significantly larger torsos than insectivores (the group with the smallest torsos), suggesting that convergence on an intermediate torso size suitable for a highly varied diet.
It was expected herbivores would also converge on a large torso volume (Maidment et al. 2012; Clauss et al. 2016; Bates et al. 2016) but this was not the case here. However, herbivores were seen to converge on GA lengths relative to body size that are relatively short for tetrapods generally (Fig. 4.3). This convergence may be the product of a mechanical constraint on efficient support of the horizontal spine cantilevered between the forelimb and hind limbs in quadrupeds that habitually use upright striding gaits. Specifically, shorter GA distance may represent an adaptive response to minimise increasing dorsoventral (anti-gravity) bending moments in the spine (Hallett and Weddel, 2016). This interpretation is supported by similar statistically significant convergence in GA distance in quadrupedal striding tetrapods (Figure 4.4-4.5, see below).

Herbivores show convergence in overall body size and are recovered as significantly larger bodied overall than all other dietary categories (Figs 4.2-4.3). The influence of body size on the directionality of macroevolutionary radiations and as a constraint on morphological diversity is a classic area of evolutionary biology (Stanley 1973; Geist 1974; Wilson 1975; Cooper and Vitt 2002; Smith et al. 2010; Benson et al. 2014; Price and Hopkins 2015; Olsen 2015) and it has often been inferred that animals will tend to evolve towards a mechanically or physiologically optimum body size for a given ecological niche (Stanley 1973; Geist 1974; Wilson 1975; Cooper and Vitt 2002; Smith et al. 2010; Benson et al. 2014; Price and Hopkins 2015; Olsen 2015). The association between herbivory and large body size has been noted in mammals (Smith et al. 2010; Price and Hopkins 2015), dinosaurs (Benson et al. 2014) and lizards
(Cooper and Vitt 2002) and is often explained by adaptive mechanisms like the Jarman-Bell Principle (Geist 1974) and the abundance-packet size hypothesis (Olsen 2015). Quantitative tests of patterns in body size evolution have largely been restricted to mammals, where analyses across different taxonomic and temporal scales have generally concluded that herbivores evolve towards larger body sizes, which exceed those of carnivores and (when analysed) other dietary groups (Smith et al 2010; Price and Hopkins 2015). Our results not only suggest that herbivory is associated with larger body size across tetrapods generally (Fig. 4.3), but that herbivory exerts moderately strong pressures leading to convergent body sizes (Fig. 4.2)

The only other body proportion measure to exhibit statistically significant convergence was relative manus length in carnivores (Fig. 4.2). Carnivores appear to show convergence on a relative manus length that is fairly average for tetrapods generally, although the group does contain a number of taxa with particularly long manus lengths (Fig. 4.3). While the basic mechanical role of proximal limb segments remains relatively uniform across tetrapods, distal limb segments are required to interact directly with a disparate environment in a range of locomotor and non-locomotor functions, which may potentially lead to greater adaptive variation. It is therefore perhaps not surprising that this is the only statistically significance instance of convergence in the manus or pes across all dietary and locomotor groups. In carnivores, the manus serves to support body weight during locomotion and in the majority of taxa is involved in dispatching and/or subduing prey. As a result, many
carnivores have convergently evolved claws or talons, (Ben-David et al 1991; Pellis and Officer 1987), and convergence in relative manus length recovered here may reflect the interaction between the demands of locomotion and prey capture.

4.5.2 Stronger convergence of body proportions in locomotor ecologies

Animal movement is limited by its morphology and therefore provides a constraint to locomotor activities. Here we find evidence that locomotion with very different mechanical demands has resulted in patterns of differing convergent body shape evolution across tetrapods (Figs 4.2-4.5). Furthermore, this convergence is considerably stronger and more widespread than seen in trophic categories.

The relative proportions of individual component bones represent a fundamental aspect of overall limb design (Middleton and Gatesy 2000). In their examination of relative forelimb element proportions, excluding the manual digits, Middleton and Gatesy (2000) noted that large areas of potential forelimb proportion morphospace have never been occupied by birds or their non-avian theropod ancestors. These authors proposed several biological and mechanical factors that might constrain relative proportions of forelimb segments in birds, including limb folding, inertia, spatial access, and developmental constraints (Middleton and Gatesy 2000). Middleton and Gatesy (2000) did not include manual digit lengths in their analyses,
but theoretical consideration of adaptive design for these independent mechanical constraints led them to speculate that, in each case, the optimal design may centre around roughly equal lengths for the three major forelimb segments (humerus, forearm and carpometacarpal+manual digits) in birds (Middleton and Gatesy 2000).

Our results for the active flight locomotor category, which includes species of both birds and bats, are approximately consistent with this ratio, and statistically significant convergence in humerus to manus ratio (with humerus to metacarpal ratio only slightly above significance; p=0.07) strongly suggests that mechanical constraints (Middleton and Gatesy 2000) are acting on overall forelimb design in tetrapods that utilize active flight. Soaring tetrapods have broadly similar forelimb proportions and variances to those that engage in active flight, but do not show significant convergence. This is perhaps not surprising given this category contains just nine species, all of which are birds. However, soaring birds are recovered as convergent in hindlimb, shank and metatarsal length, and in overall body size (Fig 4.2). Because of the low n number however, it seems prudent not to over-interpret the adaptive significance of convergence in other body proportions with soaring birds (Figs 4.2-4.3) based on the present data.

Fossorial mammals, particularly rodents, have often been cited as a textbook example of convergent evolution (Nevo 1979, Sansalone et al 2020) and evidence suggests that the requirements of moving through a subterranean environment requires a high degree of specialisation, leading to comparable morphological forms
(Nevo 1979; Marcy et al 2016; Sansalone et al 2018), although not all studies have supported homoplasy in skeletal proportions (e.g., Wolfer et al 2019). Here, fossorial species showed significant convergence on relatively short hind limb segments and forearm length. These results are consistent with previous findings in mammals, were reduced limb elements are consistent with a force-based (rather than velocity-based) lever system adapted to move and redistribute large volumes of soil and to stabilize the torso during digging, whilst also reducing bending stresses in the limb bones (Samuels and Van Valkenburgh 2008; Stein et al 2000; Wolfer et al 2019). Enlarged manual digits (claws) have also been suggested to have evolved convergently in some fossorial mammals (Samuels and Van Valkenburgh 2008), but we recover neither evidence for convergence (Figs 4.2, Tables S1, ESM4.xlsx) or a large manus relative to body size (Fig. 4.3) across our broad sample of fossorial tetrapods. Indeed, relative manus length in fossorial taxa is significantly shorter than actively flying, arboreal, bipedal striding and soaring groups (Fig. 4.3, Table S21, ESM4.xlsx)

In quadrupeds, relative the proportions of limb segments and the distance between the limbs (i.e., GA distance) defines lengths of the levers responsible for locomotor movements, and arguably this basic body plan might be under the narrowest selection pressure in terms of first-order Newtonian mechanics. That is, locomotion in these quadrupedal striders is mostly dedicated to anti-gravity support on the ground, while other groups are subject to additional selective pressures (e.g., flight, swimming, climbing, burrowing). It is therefore significant that the gross locomotor
body plan of quadrupedal striding species showed strong convergence within both the forelimb and hind limb (Fig. 4.2), and in the ratio of GA distance to limb lengths (Fig. 4.2). As noted above for herbivores, convergence in relative GA length may represent a mechanical constraint on efficient support of the horizontal spine cantilevered between the forelimb and hind limbs in quadrupeds to minimise increasing dorsoventral (anti-gravity) bending moments in the spine (Hallett and Weddel 2016). In the forelimb, strong convergence was seen in the metacarpal, manus and total forelimb segments, and each case the mean and median values for these segment lengths are relatively modest in quadrupeds compared to other tetrapods and are statistically shorter than a number of other locomotor ecotypes (Fig 4.3). However, the convergence in these individual segments may not be independent of other limb segments, as strong convergence is also recovered the ratio of proximal to distal segment lengths in both the forelimb and hind limb in quadrupedal striding taxa (Figs 4.2-4.4). In all cases, ratio values are relatively high compared to other locomotor groups indicating a relatively long proximal segment (femur; humerus) relative to distal segments (metatarsal, pes; metacarpal, manus). Convergence in these ratios is perhaps surprising given that a large body of literature has identified systematic variation in these parameters associated with both locomotor ecology and performance (Coombs 1978; Carrano 1999; Janis et al 2019; Janis et al 2020; Hutchinson 2021) and body size (Carrano 1998; Carrano 1999; Christiansen 1999; Christiansen 2002; Campione 2012) in mammals and dinosaurs. Reconciling these established trends with our finding that quadrupedal striding tetrapods do tend to converge on specific limb proportions perhaps suggests that modifications to the relative proportions of proximal versus distal limb segments
associated with body size and locomotor performance occur around an area of limb morphospace that is mechanical optimal for striding quadrupedalism.

Scansorial (climbing) species show the strongest convergence of all ecotypes studied here, with w values ranging from 2.95 to 17.92 (Fig 4.2). Our scansorial data set is extremely diverse taxonomically, and includes squamates, anurans and four mammalian orders. The frequency and strength of convergence across this taxonomically diverse sample is surprising given some studies have suggested scansorial ecotypes are difficult to diagnose based on morphology alone (Polly, 2007). Particularly strong convergence is seen the ratios between GA distance and limb lengths, and between forelimb and hind limb length (Fig 4.2). The strongest homoplasy is recovered in the ratio of GA to limb lengths (Fig 4.5), with scansorial species converging on relatively long bodies relative to the limbs (Fig 4.2). A longer spine length to limb length would be beneficial to climbing by distributing the mass over a larger area and maintaining proximity between the centre of mass and the substrate, facilitating rapid maneuverability in the trees (Law et al 2018). Strong convergence on a forelimb to hind limb ratio close 1 (i.e., sub-equal limb lengths; scansorial median = 1.19) reflects the use of both limbs in climbing and has been quantitatively demonstrated in climbing and arboreal rodents (Samuels and Van Valkenburgh 2008). However, convergence on elongate manual digits, or a large manus segment generally, are not supported seen mammalian climbers (Samuels and Van Valkenburgh 2008; Samuels et al 2013) is not supported in our broader taxonomic data set.
A number of previous studies of skeletal adaptations and convergence in various scansorial mammals have suggested that elongation of all major limb segment may characterize this locomotor ecotype (e.g., Samuels and Van Valkenburgh 2008; Samuels et al 2013; Kilbourne 2017). In contrast our data suggests that scansorial tetrapods generally do not have particularly elongate limb segments and have not converged strongly on any specific limb segment lengths relative to overall body size. However, we do recover strong convergence in the relative proportions of proximal to distal limb bones within both the forelimb and hind limb (Fig 4.2). Specifically, scansorial taxa appear to have converged proximal to distal limb segment ratios (femur:metatarsal, femur:pes, humerus:metacarpal, humerus:manus) that are moderately high for tetrapods (Fig 4.2).

4.6 Conclusion

In this study the first formal statistical examination of broad-scale ecologically correlated patterns of convergence in body proportions across extinct and extant terrestrial tetrapods is provided (Figs 4.1-4.5). This unique analysis of all major body segments across such a broad taxonomic sample has shown that ecological homoplasy in major body segment proportions exists in tetrapods, and that this convergence is driven primarily by locomotor ecology rather than trophic habits (Figs 4.2). Adaptively important convergence in trophic categories was seen in torso volume in omnivores, GA length in herbivores and manus length in carnivores.
However, specialised form of locomotion such as fossoriality and flight show much stronger magnitudes of convergence in key adaptive aspects of their morphology. Somewhat expectedly, scansorial species, which including species widely dispersed across the tetrapod tree, show the highest rates of convergence in body segment proportions. Scansorial, fossorial and quadrupedal striding ecotypes also show strong convergence in relative body lengths (e.g., proximal to distal segment lengths), emphasizing the inter-dependance or modular constraints on ecological adaptation of the locomotor system. It must also be noted that the Wheatsheaf index, has been criticised because it can struggle to distinguish convergence from long term stasis (Stayton 2015). For example, taxa such as omnivores that have not undergone much evolutionary change compared to other taxa with differing diets could be convergent according to these measures. Future work could address this by the creation of a modified version of a second strength of convergence metric.

4.7 References


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Chapter 5 - New perspectives on the evolution of head and neck allometry in tetrapods

I am currently developing this chapter for publication.

Author contributions. AEM, KTB, PGC, TWM conceived the study. KTB, PGC & AEM designed the study. KTB, PGC, TWM & AEM collected the scan data. AEM processed the scan data, made the measurements, and conducted the statistical analysis with guidance from all co-authors. The present thesis version was drafted by AEM and benefited from editorial suggestions from all other co-authors.

5.1 Abstract

The skull and neck are important parts of the body, influencing feeding ecology, habitat use and locomotion. However, the use of varied methodologies for measuring skull and neck size across tetrapods currently makes it challenging to directly compare inferences of adaptive trends seen in different vertebrate clades in different studies. Here, the methods used for quantifying and analysing head and neck size evolution in terrestrial tetrapods were surveyed, as well as the current points of consensus and controversy within the literature. Currently, studies use different metrics to represent both head and neck size and different normalisation for body size, with varied statistical approaches. It is therefore difficult to join or
compare past studies to come up with a clear picture of ecological and taxonomic differences in head and neck evolution. To address this issue a new analysis of head and neck proportions, using a dataset of 410 3D digital skeletons that samples the taxonomic breadth of extinct and extant terrestrial tetrapods is presented. The size of the head and neck was quantified using multiple metrics representative of a range of methods used across previous studies. Allometric and ecological patterns were also analysed using phylogenetically informed statistical approaches. Results revealed that as body size increases in tetrapods, skull volume, length and width all show negative allometry, while neck length exhibits positive allometry compared to isometric scaling in neck volume. This suggests that in tetrapods as a whole, regardless of the metric used, the same pattern of allometry is seen in the skull length, width and volume, whereas for the neck, the result can be influenced by the metric that is used (length vs volume). Allometric patterns in linear size metrics (skull length, width and neck length) are better described by a linear model overall, whilst volumetric measurements (skull volume, neck volume and alpha shapes) are better fit by a quadratic model. Statistical support for quadratic models appears to be driven by species over 100kg tending to show greater negative allometry in skull volume, whilst the neck shows strong positive allometry above this approximate size-threshold. Taxonomy appears to enact considerable constraints on the evolution of skull and neck size, however, within these constraints’ skull width is influenced by differing diets. Convergent evolution was not found in dietary ecotypes. Future studies should conform on a similar measurement to assess body size, as well as similar landmarks on skulls and necks used to measure lengths and widths. Future work would also benefit from increasing the number of species in this dataset to
further untangle the complex interplay between phylogeny and trophic ecology influences on skull and neck size.

5.2 Introduction

The structure and function of the head and neck in tetrapods has been the subject of considerable academic interest for a long time (Emerson 1985; Christiansen 1999; Xu et al 2002; Goswami, 2006; Snively and Russell 2007; Cox, 2008; Kulemeyer et al., 2009; Slater and Van Valkenburgh 2009; Sander et al 2011; Baab et al., 2014; Bohmer et al 2015; VanBuren and Evans 2015; Arnold et al 2017; Felice et al 2019; Terray et al 2020; Marek et al 2021). The head is an important part of the body, containing the brain and sensory organs which together act as a processing centre for the body. The head is therefore an important influence on brain size (Henneberg 1998); sexual selection (Gould 1974; Weston et al 2007; Rackovic et al 2019; Morris et al 2020); habitat use and locomotion (Trueb 1973; Herrel et al 2008, Vanhooydack et al 2011; Fabre et al 2016) and feeding ecology (Emerson 1985; Christiansen 1999; Goswami, 2006; Cox, 2008; Kulemeyer et al., 2009; Slater and Van Valkenburg, 2009; Baab et al., 2014; Felice et al 2019). Feeding ecology in particular, has been shown to influence the gross size and structure of the skull through deep time (Goswami, 2006; Cox, 2008; Kulemeyer et al., 2009; Slater and Van Valkenburg, 2009; Baab et al., 2014; Dumont 2016); for example, a larger skull allows for larger bite forces which facilitates increased foraging efficiency and net energy intake (Herrel et al., 2001; Verwaijen et al., 2002; Van der Meij and Bout, 2006; Anderson et al., 2008; Law et al
affording animals the ability to consume otherwise unobtainable resources (Herrel et al., 2007; Anderson et al., 2008; Bulté et al., 2008; Santana et al., 2010; Gignac and Erickson, 2016).

The vertebrate head must be fully supported by the neck. The neck in all animals is an intrinsic link between the head and the body and is morphologically defined as the association of multiple cervical vertebrae (Romer 1950). The neck is a complex structure and needs to be able to provide adequate support, flexion, extension and rotation of the head about the spine to aid in a variety of actions such as: feeding (Sander et al 2011; Arnold et al 2017; Bohmer et al 2019; Marek et al 2021); the manipulation of objects (Diliger et al 1960); combat (Senter 2007; Laurin 2010; Vander Linden and Dumont 2019; Guevara and Hurme 2019); sexual display (Simmons and Scheepers 1996; Senter 2007; Bohmer et al 2019) and the stabilisation of the head during locomotion (Warwick et al 2002; Pete et al 2015; Marek et al. 2021). The fundamental role of the neck maintaining functionality of the head means that the evolutionary ecomorphology of these two body regions have often been said to be intrinsically linked; for example, changes in neck length have been observed to correlate with ecologically adaptive changes in head size (e.g., Bohmer 2019; Marek et al 2021; Arnold 2021).

Evolutionary variation in neck length and size can be achieved by either adding or removing cervical vertebrae, or through elongating or shortening cervical vertebrae. For example, birds and sauropod dinosaurs have a highly variable number of cervical
vertebrae (Boas 1929; Zweers et al 1994; Taylor and Wedel 2013a; Krings et al 2014). Amphibians have only one (Vidal et al 1986; Wake 2009), and mammals have a fixed number of seven cervical vertebrae (Galis 1999; Narita and Kuratani 2005) apart from extant sloths and the manatee (Buchholtz and Stepień 2009; Hautier et al 2010; Buchholtz et al 2014). Reptiles have a highly variable number of cervical vertebrae between species and families. For example, modern chameleons have five; true lizards, the Rhynchocephalia and Chelonia, have eight; the monitor lizards, Crocodilia, Theropoda, Iguanodontia, and Ceratopsia, nine; the Phytosauria and Pterosauria, eight or nine; and the Pseudosuchia, eight to ten (Müller et al 2010; Vidal et al 1986; Williston 1925). Overall neck length can also vary due to variation in the size of individual vertebrae. With a fixed set of seven cervical vertebrae, neck elongation in mammals (exemplified by species with exceptionally long necks such as giraffes) is achieved through the elongation of each individual cervical vertebra (Galis 1999; Narita and Kuratani 2005; Arnold 2020) and recently a similar mode of variation was proposed to underpin the highly disparate neck lengths seen in birds (Marek et al. 2021).

Across tetrapods, head and neck size vary considerably and in many different taxa the question has been posed as to what factors have primarily structured this variation. Identifying the factors that drive evolutionary changes in the heads and necks of tetrapods has been a challenge, however, because of the difficulties in quantifying complex morphologies, sampling a broad number of taxa and identifying mechanisms responsible for generating macroevolutionary patterns. In this chapter
the methods for quantifying and analysing head and neck size evolution in terrestrial tetrapods are surveyed, and the current points of consensus and controversy within the literature. This review highlights previous studies have used a variety of methods to quantify head and neck size in relation to body size in different sub-groups, and subsequently employed different statistical techniques to examine macroevolutionary patterns. This currently makes it challenging to compare results across studies, and in some cases to compare adaptive trends seen in different vertebrate clades.

To begin to address this issue a new analysis of head and neck proportions is presented using a dataset of 410 3D digital skeletons that samples the taxonomic breadth of extinct and extant terrestrial tetrapods. The size of the head and neck in this data set is quantified using multiple metrics representative of a range of methods used across previous studies and is subsequently used to analyse allometric and ecological patterns using phylogenetically informed statistical approaches. The results emphasise that similar methodologies across the literature are needed to understand the complex interplay in skull and neck size with form and function. To accurately assess skull and neck size in tetrapods, multiple ecological processes should be assessed simultaneously.
5.3 Quantifying head and neck size: A review of measurements and methods

5.3.1 Linear measurements

5.3.1.1 Head size

Previous studies have used various measures such as length, width, or height as a proxy for head mass and/or the overall size of the head (e.g., Christiansen 1999; Van der Leeuw 2002; Sereno et al 2007; McGarrity et al 2013). Head length is generally defined as the greatest length of the skull from the most posterior to the most anterior points, usually identified as the distance between the occipital condyle and the anterior most projection (e.g., Radinsky 1981; Christiansen 2005; Lazaro 2017). Thus, while the anatomical locations of measurement points vary across studies, these reflect variation in cranial anatomy and the fact different morphological landmarks represent the most posterior and anterior extremes of the head in different animal groups. For example, in therapod dinosaurs, skull length was measured as the distance between the tip of the premaxillae and the occipital condyle (Therrien and Henderson 2007). In birds, skull length has been measured as the greatest length from the tip of the bill to the most caudal end of the skull (e.g., Hallgrímsson et al 2016; Kass et al 2018; Süzer et al 2018; Angst et al 2020), from the occipital protuberance to the distal tip of the premaxilla (e.g. Thompson et al 1999; Verdiglione and Rizzi 2018), the foramen magnum to the tip of the bill (Setiawan et al 2004) and in other studies from the occipital bone to the insertion of the beak into the skull (Francesch et al 2011) (See figure 5.1); In amphibians, skull length is measured as the distance from the anterior tip of the skull to the posterior of the
condyles (Emerson 1985; Birch 1999; Lappin et al 2017) and in Rhynchocephalia, along the midline of the tip of the rostrum to a point level with the back of the jaw points (Jones 2008) and snout tip to dorsal supraoccipital border in crocodiles (Erickson et al 2003).

At least in some instances, differences in the specific way that head length has been measured within animal groups may represent investigator preferences and/or the specific goals of the study. Within mammals there is variation in the precise approach used to measure skull length (Figure 5.1). For example, Gould (1974) defined skull length as the length from the anterior border of the premaxillary bones to the lip of the foramen magnum; Radinsky (1981) used the distance from the back of the occipital condyles to the anterior tip of the premaxilla; He et al (2002) used the distance between the rostral end of the inter-incisive structure (located between the roots of the upper central incisor teeth) and the central surface point on the occipital protuberance. Christiansen (2005) defined skull length from the occipital condyle to the dentary tip; Lieberman et al (2000) used basicranial length suggested by Radinsky (1984) as a proxy for skull length to avoid the confounding effects of jaw length variation that can arise when total skull length is used. Lázaro (2017) defined skull length from the anterior most projection to the occipital condyle and Choudhary et al (2020) used the distance between the highest points of the parietal bones to the rostral margin of the incisive bone, whilst Timm-Davis et al (2015) measured the maximum length of the skull from the tip of the rostrum to the nuchal crest.
In contrast with this methodological variation, head width is nearly universally measured as the greatest width of the skull, which is generally the maximum distance between the two zygomatic arches, across all species including mammals (Gould 1974; Radinsky 1981; He et al 2002; Timm-Davis et al 2015; Choudhary et al 2020); birds (Süzer et al 2018; Verdiglione and Rizzi 2018); reptiles (Herrel and O’Reilly, 2006) and dinosaurs (Christiansen 1999). In extant amphibia where the zygomatic arch is not present, skull width has also been measured as the widest part of the skull, which is the lateral extent of the jaw joints (Emerson 1985; Lappin et al 2017).
Figure 5.1: Common anatomical areas on the skull of birds and mammals used to measure skull length and width in the literature.
5.3.1.2 Neck size

Most studies calculate neck size as the sum of centrum lengths from individual cervical vertebrae regardless of the taxonomic group under study (Marek et al 2021; Bohmer 2019; Arnold 2017; Jones 2015; Dzemski and Christian 2007; O’Keefe and Hiller 2006; Taylor and Wedel 2013a). Most measurements in the literature for neck length did not consider the contribution of cartilage and intervertebral discs to neck length as most studies have taken measurements from skeletons and/or fossils (Bohmer et al 2019; Arnold et al 2017; Jones 2015; O’Keefe and Hiller 2006; Taylor and Wedel 2013a). However, some extant species have neck lengths measured by adding in the length of cartilage and intervertebral disc lengths (Dzemski and Christian 2007). The contribution of unpreserved joint tissues is difficult to account for in extinct species and where intervertebral disc length has been estimated in sauropod species the length of the neck had drastically changed (Taylor and Wedel 2013b).

5.3.2 Mass and volumetric measurements

An alternative approach to using linear measurements of the head and neck as a proxy for overall segment mass or overall size is to use a volumetric approach. This can be achieved through physical scale models (e.g., Gregory 1905; Colbert 1962; Alexander 1985) or three-dimensional virtual models (e.g., Allen et al 2009, 2013; Sellers et al 2012; Henderson 2013; Brassey and Gardiner 2015; Bates et al 2009a, b,
2012; 2016; Snively et al 2013; Brassey and Sellers 2014; Maidment et al 2014; Bates et al 2016; Brassey 2016; Macaulay et al 2017; Marek et al 2021). Briefly, these approaches either directly extract skin volume from medical image data (e.g., Allen et al 2009; Macaulay et al 2017; Coatham et al 2021) or estimate it through manual sculpture (e.g., Allen et al 2013; Bates et al 2009a, b; Henderson 2013) or mathematical shape-fitting (e.g. Sellers et al 2012; Brassey and Gardiner 2015; Bates et al 2016; Marek et al 2021) to derive a volumetric measure of neck and/or head size. This volumetric measure is then used analytically or converted to a mass through multiplication by a density value depending on the goal of the analysis. Indeed, the use of such models are typically highly driven by the analytical goals due to the logistical challenges of volumetric modelling approaches. While hardware and software advances (and cost effectiveness) have made digitisation techniques more accessible in recent decades (e.g., Bates et al 2010; Falkingham 2012), volumetric approaches rely on upon more complete skeletal material and are typically far more time, labour and skill intensive to carry out compared to linear measurements (Campione and Evans 2020). As a result, volumetric approaches have tended to be used in studies driven by those that specifically require representation of the head and/or neck by volume or mass. Examples include assessment of the role of the head and neck in the evolution of whole-body shape (e.g., Allen et al 2013; Maidment et al 2014; Bates et al 2016), estimation of aspects of organism thermal biology (e.g., Henderson 2013), the relationship between head mass and neck length allometries (e.g., Marek et al 2021) and to facilitate biomechanical assessments of neck-head function (e.g., Snively et al 2013).
5.3.3 Metrics for size normalisation

A measure of overall animal body size is required to assess allometric patterns and to normalise measures of head and neck size to evaluate relative similarities and differences in body proportions across animals. Arguably the most fundamental measure of the overall body size of an individual is their body mass and it is widely considered as the gold standard size-normalisation metric (Campione and Evans 2012), particularly in functional and biomechanical studies. As a result, many studies have used direct measures of body mass or estimates of body mass to size-normalise head and neck size for comparative purposes (e.g., Kleiber 1947; Hemmingsen 1960; Jerison 1969; Peters and Peters 1986; Brown et al 1993; Burness et al 2001; Gillooly et al 2001; Gillooly et al 2002; Capellini and Gosling 2007; McClain and Boyer 2009).

However, in many instances the body mass of specimens has been unavailable to researchers. For example, the body mass of osteological specimens in museums is often not recorded, while for extinct species direct measures of mass are fundamentally not possible. In such instances, a variety of approaches have been utilised to derive representative measures of body mass to normalise head and neck size. By far the most common approaches are extant skeletal scaling relationships and volumetric reconstructions. A brief overview of the methodology, benefits, and limitations of these approaches is provided next. The reader is directed to Campione and Evans (2020) and Brassey (2016) for more exhaustive reviews.
5.3.3.1 Extant scaling approach

Regression-based predictive models, based on the relationship between specific osteological measurements and body mass in extant animals, are the most common approach to estimating body mass in fossil specimens (e.g., Greenewalt 1975; Campell Jr and Tonni 1983; Damuth et al 1990; Gingerich 1990; Campbell and Marcus 1992; Finarelli and Flynn, 2006; Butler and Goswami 2008; Rinderknecht and Blanco, 2008; Millien and Bovy 2010; Field et al 2013; Ghizzoni 2014). The popularity of this approach stems from its wide utility as it is possible to derive body mass from highly incomplete skeletal remains, compared to volumetric approaches, which require fairly complete skeletons (Campione and Evans 2020). Most scaling question-based approaches use elements experiencing weight bearing during locomotion such as femoral and humeral circumference (Anderson et al 1985; Campione and Evans 2012) or femoral head width (Ruff et al 1991). Cranial metrics are also used (Wroe et al 2003; Spoctor and Manger 2007), but these cranial metrics can be highly variable especially when diverse phylogenetic histories come into play, which leads to biases in final mass estimation if interspecific scaling patterns are not properly assessed (Damuth et al 1990; Millien and Bovy 2010).

Using regression-based models therefore offers an efficient method with which to generate large data sets of body mass and subsequently for examining broad-scale patterns of evolution (Laurin 2004; Carrano 2006; Finarelli and Flynn 2006; Sookias
et al 2012; Benson et al 2014, 2018; Puttick et al 2014; Kubo and Kubo 2016). This approach is also a relatively simple to use, as linear measurements are taken using calliper based morphometric analysis either by hand or digitally, therefore requiring very little training, allowing for minimal user input techniques and no subjective assumptions when applied to fossil specimens. As a statistical approach, this method also provides confidence or prediction intervals that can provide broad error bounds for use in macroevolutionary hypothesis testing, although actual application of this is relatively rare (Campione and Evans 2012; Serrano et al 2015).

5.3.3.2 Volumetric measurements

As explained in section 2.2 of this thesis, in the context of head and neck size, volumetric methods approximate or estimate body mass by multiplying a value for body volume by density. The same diversity of physical and computational approaches to approximating or estimating body volume noted above for the head and neck have been applied to estimate overall body mass, particularly in extinct species. Historically, physical approaches were favoured (Colbert 1962), but these have now largely been replaced by computational approaches to volume reconstruction. Within computational volumetric approaches there has been a gradual shift from manual volume sculpture, where an investigator subjectively decides upon the sizes and shapes of body volumes (e.g. Hutchinson et al 2007; Bates et al 2009a, b, 2012; Henderson 2013; Snively et al 2013; Maidment et al 2014), to
more automated approaches where the final body volume is partially (e.g. Allen et al 2009) or wholly guided by soft tissue proportions measured in living animals (e.g. Sellers et al 2012; Bates et al 2016; Coatham et al 2021). These approaches circumvent the single bone problem seen in regression analysis of isolated limb bone dimensions by including the maximum amount of information from the skeleton into the mass estimate. However, the approach is restricted by the need for relatively complete skeletons, which limits its capacity in larger scale macroevolutionary studies (Campione and Evans 2020).

5.3.4 An overview of macroevolutionary studies of head and neck evolution

Generally, across tetrapod taxa, the skull shows a reduction in the size of the neurocranium and an increase in size of the face and snout area as body size increases (Hanken and Hall 1993). Most studies on size changes of the skull have examined mammals (Caumul and Polly 2005; Marroig 2007; Cardini and Elton 2008; Wilson and Sánchez-Villagra 2010) which show a strong influence of allometry on the evolution of skull shape (Cardini and Polly 2013; Cardini et al 2015; Tamagnini et al 2017). It has been suggested that larger species of mammals tend to evolve greater skull lengths, therefore exhibiting positive craniofacial allometry (CREA) (Radinsky 1985). CREA has therefore been proposed as a ‘rule’ for cranial evolution in mammals having been recovered in placentals (Cardini and Polly 2013; Tamagnini et al 2017;
Andrea 2019), marsupials (Radinsky 1985) and kangaroos (Cardini et al 2015). Indeed, it has even been suggested to have occurred in some bird species (Galliformes and raptors) although this is less conclusive (Linde-Medina 2016; Bright et al 2016). Studies on CREA, however tend to use geometric morphometric approaches with differing landmarks in which to measure skull length and width and varying ways of normalising the data either through generic species average body masses from the literature (Radinsky 1985), convex hulls (Andrea 2019) or by centroid size (Cardini and Polly 2013; Linde-Medina 2016; Bright et al 2016; Tamagnini et al 2017). As pointed out by Linde-medina (2016), the use of centroid size can lead to problems due to the issue of covariation between the face and the braincase.

In certain subgroups of mammals and dinosaurs head mass has previously been suggested to have a positive allometric relationship with body mass, despite the use of different measures (Christiansen 1999; Sereno et al 2007; Sander et al 2011; McGarrity et al 2013; Cardini and Polly, 2013; Cardini et al 2015; Tamagnini et al 2017), although variation likely exists within sub-groups of dinosaurs. For example, Lobón and Buscalioni (2003) suggested that head size in non-avian theropods scaled close to isometry, Therrien and Henderson (2007) suggested that skull length increases more slowly than body mass with increasing body size, whilst Bates et al (2016) suggested that head size in sauropods scaled with negative allometry and Sereno et al (2007) found positive allometry in the skull length of ceratopsians. Differences in measurement methods also confound comparisons of the interaction between head and neck size across major vertebrate groups, and subsequently upon
inferences about constraints on the co-evolution of these body segments. For example, Therrien and Henderson (2007) measured skull length from the tip of the pre-maxillae to the occipital condyle from previous literature or physically from museum specimens and used three dimensional mathematical slicing as a proxy for body mass (Henderson 1999), whilst Sereno et al (2007) measured the tip of the snout to the posterior point on the occiput with the maxillary tooth row orientated horizontally for skull length and measured body mass estimates based on the “polynomial method”. This is a method that uses cross-sections to estimate axial volume and cylinders for limbs (Sereno et al 2007).

The mass of the vertebrate head must be supported by the neck and previous researchers have proposed that this may impose a mechanical constraint of relative head to neck length (Preuschoft and Klein 2013; Cardini and Polly 2013; Cardini et al 2015; Arnold et al 2017a ,b); for example the evolution of larger heads will tend to be causatively linked to reduced neck lengths in order to reduce the neck bending moments by bringing the larger head closer to the fulcrum of the neck. Studies of mammalian head size that typically use head length or width as a proxy for head mass (Gould 1974; Radinsky 1985; Christiansen 2005; Cardini and Poll, 2013; Cardini et al 2015; Tamagnini et al 2017; Andrea 2019), have concluded mammalian necks do indeed scale with negative allometry relative to head size, consistent with the mechanical theory that larger heads tend to evolve in concert with shorter necks (Preuschoft and Klein 2013; Cardini and Polly 2013; Cardini et al 2015; Arnold et al 2017a ,b).
Dinosaurs exhibit considerably variation in absolute and relative neck length (Gauthier 1986; Sereno 1991; Pisani et al 2002). Like some groups of birds, sauropod dinosaurs had very long necks, and possessed epiphyses similar to that of birds suggesting they were myologically similar (Taylor and Wedel 2013). Neck length has been suggested to exhibit positive allometry in sauropods (Parrish 2006), where quadrupedality provided a platform for both large body sizes and longer necks, which likely increased herbivorous feeding envelopes (Wedel et al 2000). Dinosaurs of comparable sizes to sauropods such as Tyrannosaurus rex, did not evolve necks as long as sauropods with similar mass, and part of this reason could have been because of the increased moment caused by neck elongation in a biped must be counteracted by an equal moment caused by a longer or more massive tail, increasing physiological cost (Taylor and Wedel 2013). Another reason is due to the need for a larger head for killing prey, as is the case with the Tyrannosaurus rex. With a larger head a shorter neck provides stability to predate upon other large dinosaur species and acts as a powerful instrument for wielding the jaws during feeding (Snively et al 2014).

Previous work has suggested that a shift away from carnivory may have facilitated shifts in longer neck lengths and smaller skull sizes (Zanno and Makovicky 2011). A smaller head size was possibly the result of lack of dentition because of no need to orally process their food (Chure et al 2010). However, within ornithischians such as ceratopsians, massive dentition was needed for the oral processing of their food which may have resulted in larger heads developed on shorter necks, or an
intermediate head on an intermediate sized neck in hadrosaurs (Taylor and Wedel 2013). As a result of large head size in ceratopsids, the centre of mass was located further anteriorly (Maidment et al 2014), implying that the evolution of a larger head size might also result in a structural need to adopt a quadrupedal pose (Maidment et al 2014). Smaller head sizes in sauropods may also allow for longer neck lengths because of the reduction in head weight, reducing the power needed to lift the head and therefore reducing subsequent muscle mass that would be needed allowing the necks to be longer (Taylor and Wedel 2013).

In birds, studies have suggested either a negative allometric relationship between head mass and body mass (Van der Leeuw 2001) or an isometric relationship (Marek et al 2021), but negative allometry when smaller groups of birds where considered (Bohmer et al 2019; Marek et al 2021). The differences in the relationships quantified here within birds, dinosaurs and mammals appear to be related to differences between the methodologies of head mass and neck mass with body mass scaling exponents (Christiansen 1999; Van der Leeuw 2001; Sander et al 2011; McGarrity et al 2013; Cardini and Polly, 2013; Preuschoft and Klein 2013; Cardini et al 2015; Tamagnini et al 2017; Arnold et al 2017; Bohmer et al 2019; Marek et al 2021). Independent studies have recovered positive allometry in neck length relative to body mass in birds, despite using different data sets and different means of estimating body mass in their samples (Bohmer et al 2019; Marek et al 2021). These authors concluded that aspects of avian anatomy and biology, such as negative scaling of the brain and eye size with body mass, the reduction of jaw musculature
and the widespread pneumatization of the skull, may have weakened the mechanical constraint on relative head-neck size seen in mammals. Also, the constraint in most bird species to use the forelimbs for flying, means freeing up the neck allows this body segment to act as a surrogate arm (Marek et al. 2021). Interestingly, Bohmer et al. (2019) also found that neck length scaled isometrically with total hind limb length in their sample of birds. They inferred that this pattern suggests correlated evolution between these body regions, noting the basic mechanical benefit of maintaining a neck length capable of allowing the head to reach and forage on the ground as hind limb length evolves (Bohmer et al. 2019).

In non-avian reptiles such as turtles and crocodylomorphs the skull generally becomes shorter and wider as body sizes increase (Foth and Joyce 2016; Gignac and O’Brien 2016; Souza 2021), with feeding ecology having a considerable impact on the shape and size of the skull (Godoy 2020; Souza 2021). In some non-avian reptiles with a fossorial lifestyle, species with narrower heads have been shown to be more efficient at digging, but are also associated with a weaker bite force, potentially restricting the dietary spectrum to softer prey (Baeckens et al. 2017). These conflicting selective pressures are thought to limit the evolution of skull shape in head-first burrowers, and potentially implies that diet may have a more important role in skull shape than locomotory performance in some species of non-avian reptiles (Vanhooydock et al. 2011; Kazi and Hipsley 2018).
The skulls of carnivorous taxa tend to be short and wide with a large volume. These features enable the skull to increase the mechanical advantage of their bite force and to deal with large torsional loadings from struggling prey (Radinsky 1981, 1984; Wroe and Milne 2007; Felice et al 2021). Previous work from chapter 3, also found an association between skull volume and carnivory in that these dietary group exhibited the least strong negative allometry in skull volume, which was suggested to be related to hypercarnivory and large prey specialisation (Erickson et al 2003; Anderson et al 2008; Cornette et al 2015). In non-avian reptiles the skull becoming shorter and wider with body size in some species is most likely due to adaptation towards capturing and crushing large prey (Herrel et al 2018). For example, greatly enlarged heads in map turtles was correlated to predation rates on molluscs (Lindeman 2000). In line with biomechanical evidence, the convergent evolution of long snouts in crocodiles, was also driven by dietary specialisation (McCurry et al 2017), a longer skull increases the speeds of attack and prey capture in water associated with a diet of soft and fast-moving prey such as fish (Balle et al 2019).

In amphibians allometric trajectories of the skull and neck have been rarely studied (Ponssa and Candiot, 2012; Sherratt et al 2014). This may be due the assumption that the skull and skeletal morphology of this clade are tightly conserved (Handrigan and Wassersug 2007). Skull allometry in the few studies that exist have been found to vary extensively among amphibian species (Ivanović and Arntzen 2018), with skull size generally associated isometrically with body size in frogs when centroid size was used as a proxy for skull size (Trueb and Alberch 1985; Paluh et al 2020; Dos Reis et
al 2021), in particular, cranial width is found to increase with body size and is highly influenced by microhabitat (Bardua et al. 2021). A developmentally plastic increase in relative head width in response to encountering small prey such as insects in anurans may also be a factor (Hudson et al. 2018). Morphologies that enable high bite forces are also found in other insectivorous species, which may face even more challenging mechanical demands if they feed on insects with tough cuticles and could therefore possess specific skull specializations that may be more adapted to demands for high bite force when compared with carnivorans, for example in bats (Santana and Cheung 2016).

Throughout this chapter so far we have seen the difficulties in comparing studies of skull and neck size allometry within major taxonomic groups, it is therefore not surprising that it is extremely difficult to also compare differences between major taxonomic groupings. Assessing the magnitude of allometric differences between major taxonomic groups, such as birds and mammals is currently difficult due to multiple methodological differences across these studies. For example, positive allometry of neck length with body mass in birds contrasts with negative allometry recovered in mammals (Preuschoft and Klein 2013; Cardini and Polly 2013; Cardini et al. 2015; Arnold et al. 2017a, b), but studies on birds use either mean literature masses (Bohmer et al. 2019) or a mixture of bird-specific scaling equations and measured masses for the studied specimens (Marek et al. 2021), while mammalian studies use quadrupedal scaling equations to generate body masses against which to judge allometric trends. Regarding the skull within mammal studies skull size typically uses
head length or width as a proxy for head mass (Gould 1974; Radinsky 1985; Christiansen 2005; Cardini and Poll, 2013; Cardini et al 2015; Tamagnini et al 2017; Andrea 2019), whereas bird and some dinosaur studies have used volumes and length of the head as proxies for head mass (Sereno et al 2007; Therrien and Henderson 2007; Bohmer et al 2019; Marek et al 2021) making it very hard to compare the magnitude of allometric differences in head and neck size across major taxonomic groupings.

5.4 New perspectives on head and neck evolution

5.4.1 Aims and objectives

This review highlights that varied metrics and analyses have been carried to study head and neck size in terrestrial tetrapods. This disparity in metrics and analytical approaches means that it is currently difficult to reconcile allometric patterns and ecological adaptations across studies and subsequently across major taxonomic groups. The goal of this study is therefore to examine head and neck evolution across all major terrestrial tetrapods using a diverse range of metrics for quantifying the size of these measurements, and a single holistic measure of body size against which to judge allometric patterns. The specific aims of this chapter are therefore:

1) To quantify absolute and relative head and neck size across a large, taxonomically and ecologically diverse range of extinct and extant
terrestrial tetrapods using a range of metrics representative of the breadth of parameters used in the literature (see section 2.4).

2) To use this data set to determine how terrestrial tetrapods scale in head and neck size parameters.

3) To determine how head and neck size differ across major taxonomic and trophic groupings.

4) To quantify the nature and magnitude of convergent evolution in head and neck size across major trophic groupings.

5.4.2 Methodology

5.4.2.1 Data set

Several head and neck shape and size metrics were quantified from 3D skeletal models of 116 amphibians, 47 birds, 55 non-avian dinosaurs, 143 mammals, 46 non-avian reptiles and 3 reptiliomorphs spread across the major groups within these clades, using the data set of chapters 2 and 4. This data set consisted of 318 extant and 92 extinct species. Chapters 2 and 3 obtained whole body scans of specimens were obtained from a variety of sources including CT scans and photogrammetric models collected by the authors, as well as models from previous studies (Bates et al
2009a, b; Sellers et al 2012; Bates et al 2012; Allen et al 2013; Lyson et al 2014; Schachner et al 2014; Bates et al 2015; Bates et al 2016; Clauss et al 2016; Macaulay et al 2017; Schachner et al 2017; Brassey et al 2018) and online digital repositories (Morphosource, KUPRI, Digimorph, Sketchfab, animalsimulation.org), as well as a small number of additional models provided by colleagues. A full list of the models, including their source information, can be found in the electronic supplementary material for reference (ESM1.xlsx), but also see chapter 3 and 4. The precise ontogenetic age and sex of many of the specimens are unknown. However, based on overall size it is inferred that most of the extant specimens are sub-adults or adults (ESM1.xlsx).

5.4.2.2 Metrics for head and neck size

A variety of linear and volumetric measures of head and neck proportions were generated to examine how allometric and ecological patterns of head and neck size variation are influenced by different metrics. The linear morphometric data collected consisted of skull length, skull width and neck length (See figure 5.2 and 5.3). Based on the broad consensus of previous studies (see section 5.3.3 above), skull length was taken as the most posterior to the most anterior points, usually the distance between the occipital condyle and the anterior most projection or to the end of the beak in birds. Skull width was measured as the largest width of the skull, which
tended to be the distance between the two zygomatic arches. The neck was considered as the skeletally defined cervical region. That is, all vertebrae from the cranium to the first thoracic vertebrae or rib (Romer 1950). Neck length was calculated across this skeletal region by the adding of centrum lengths and did not include any estimates of size of intervertebral discs or cartilage (see figure 5.3).
Figure 5.2: Maximum skull length (blue) and width (red) measurements taken in this study in a variety of taxa including (A) *Agalychnis calcarifer*, (B) *Condylura cristata cristata*, (C) *Edmontosaurus* (D) *Gopherus agassizii*, (E) *Homo sapien*, (F) *Mustela erminea*, (G) *Nycteris grandis*, (H) *Pelecanus occidentalis*.

Figure 5.3: Maximum neck length measurements taken in this study in a variety of taxa including (A) *Agalychnis calcarifer*, (B) *Canis familiaris*. 
Skull and neck size have also been quantified using volumes and/or mass in previous studies (e.g. Hutchinson et al 2007; Bates et al 2009a, b, 2012; Sellers et al 2012; Henderson 2013; Snively et al 2013; Maidment et al 2014; Bates et al 2016; Coatham et al 2021), including the convex hull volume metrics used previously in chapter 3. In addition to these data, new measures of head volume were generated using an a-shape fitting algorithm (Figure 5.4) similar to previous studies (e.g. Brassey and Gardiner 2015; Marek et al 2021). The a-shape approach allows the investigator to refine the fit of the mathematically generated volume, thereby potentially yielding a more realistic 3D shape. All the skulls were down sampled to 10,000 vertices to be uniform across photogrammetric and CT models. Skulls with less than 10,000 vertices were noted and the code reapplied to skulls with <10,000 vertices. Two skulls were less than 1000 vertices in the dataset and were subsequently removed from the analysis. The fit of the a-shape is defined by the refinement coefficient, k, which must be subjectively set by the investigator (versus no user defined subjectivity in the simpler convex hulling approach). To investigate the impact of this subjectivity on skull size patterns across the data set, three different a-shape iterations were produced, where k was set to values 0.5, 1 and 2 (Fig. 5.4).

As in chapter 3, whole-body convex hull volume (WBCHV) was used as the measure of whole-body size to normalise data and against which to judge allometric patterns. WHCHV is simple the sum of the minimum skeletal convex hull volume of all body segments in each 3D model. For various size-normalised comparisons the skull and neck linear measurements were normalised by $WBCHV^{0.33}$ (e.g., neck length/
WBCHV0.33) and volumetric segment measurements by WBCHV (e.g., skull volume/WBCHV). For regression analyses (see below) WBCHV was used as the proxy for whole body size on the x axis. WBCHV is preferred as a proxy for overall body size because it utilises the entire skeleton rather than relying on a measure from a single body segment, which may bias any further analyses due to potential allometric signals in that one body segment. Using WBCHV also allowed all linear and volumetric parameters to be assessed or normalised by the same size metric. Also, variability in scan/model resolution meant that popular alternative metrics (e.g., long bone circumference; Campione and Evans 2012) could not be accurately and/or repeatably measured across the data set.

Figure 5.4: Alpha shapes as fitted to the *Barbourula busuangensis* skull in dorsal and lateral view: (left to right) CT scan of the skull; convex hull fit of alpha shape when α...
= infinite, ‘coarser’ fit of alpha shape when $\alpha = 2.0$, ‘finer’ fit of alpha shape when $\alpha = 1.0$, ‘finest’ fit of alpha shape when $\alpha = 0.5$.

5.4.2.3 Statistical analysis

To analyse trends in the skull and neck proportions using phylogenetic informed statistics, the existing tree for this data set from Chapter 2, 3 and 4 were used. Full description of the consensus phylogenetic trees merged to build these trees, can be found in chapter 2, but a brief description is given here, and source information can be found in the electronic supplementary material (ESM2.xlsx), Branch lengths were calculated by time calibrating the tree using first and last occurrences of each species from the PBDB Palaeobiological database (www.paleobiodb.org) and fossilworks (www.Fossilworks.org). This data was then combined with the phylogenetic tree in R studio using the package strap (Bell and Lloyd 2015) to produce a time calibrated tree. To examine variations and correlations in body shape and ecological variables taxa, were classified dietary categories based on the current consensus in the literature (see supplementary information). The dietary type of each species was classified as either carnivore ($n = 124$), herbivore ($n = 120$), insectivore ($n = 94$), omnivore ($n = 64$) or piscivore ($n = 8$).
To examine how each skull and neck metric scaled with body size (WBCHV) across the data set, a regression analyses using phylogenetic generalised least squares (pGLS) in the R package caper was conducted (Freckleton 2002). This approach follows a general linear model calculating the slope, intercept, confidence, and prediction intervals, adjusting the expected covariance according to phylogenetic signal (Symonds and Blomberg 2014). Quadratic models were also tested so see if they provided a statistically better fit to scaling trends than linear fits in log10 parameter v body mass data set (Campione 2017) as in chapter 3. All analysis was carried out in R studio using the packages qpcr, ape, Geiger and nlme (Andrej-Nikolai 2014; Pennell et al 2014; Pinheiro et al 2015; Campione et al 2017). A statistically significant second-degree coefficient established the nonlinear nature of the data if present. Models were compared using associates Akaike information criteria for limited sample sizes (AICc) and standard errors of the estimate. Pagal’s lambda (λ) was used to estimate the strength of the phylogenetic signal in the analyses. Phylogenetic ANCOVAs (phylANCOVA) were used to test for differences in the allometric relationships and body shape between dietary groups using the approach of Smaers and Rohlf (2016) implemented in the R package evomap (Smaers and Mongle 2014).

To analyse convergence in morphology across normalised (WBCHV) head and neck size the Wheatsheaf index was used (See chapter 4), as implemented in the R package WINDEX (Arbuckle et al 2014; Arbuckle and Minter 2015). The Wheatsheaf
index compares the mean distance in phenotypic space between species to the overall average distance between all pairs of species and scales those comparisons by the phylogenetic variance–covariance matrix. However, the original wheatsheaf index cannot be used on fossil data because phylogenetic relatedness is penalised based on the distance from the root to the node where two species diverge. Following the advice from Arbuckle et al (2014) a cophenetic distance matrix was calculated to penalise for phylogenetic distance rather than a variance covariance matrix. This was achieved using the function cophenetic.phylo in the package ape (Paradis and Schliep 2018), cophenetic.phylo computes pairwise distances between pairs of tips using branch lengths is therefore a more accurate representation of a non-ultrametric tree.
5.5 Results

5.5.1 Allometric patterns for different head and neck metrics across terrestrial tetrapods

Here, the scaling patterns for skull length, skull width, neck length, skull volume, neck volume and alpha shape volumes in the sample of terrestrial tetrapods are examined, using phylogenetically informed regression analysis (PGLS; Harvey and Pagal 1991). Linear allometric relationships for all variables were statistically significant (Figure 5.5; Table S1, see ESM5.xlsx) with a strong phylogenetic signal for the data (Lambda values varied between 0.97 to 0.99; Table S1, ESM5.xlsx). All linear and volumetric metrics for skull size scale with negative allometry, with isometry not included with confidence intervals in any case (skull length; slope = 0.29+/-0.01, skull width; slope=0.31 +/-0.01, skull volume; slope=0.92+/-0.03; Tables S1-S2, ESM5.xlsx; Figure 5.5). Therefore, all metrics suggest a relative decrease in head size as overall body size increases in terrestrial tetrapods. However, neck volume scales isometrically with body size (slope = 1.00+/-0.03; Table S2, ESM5.xlsx), while neck length scales with positive allometry with confidence intervals excluding isometry (slope = 0.36+/-0.02; Table S1, ESM5.xlsx). Therefore, these metrics suggest different qualitative patterns in neck size scaling across terrestrial tetrapods as a whole; necks become longer as overall body size increases, while neck volume remains a relatively constant proportion of overall skeletal volume at all body sizes (Tables S1-S2, see ESM5.xlsx).
Figure 5.5: Scaling relationships between major body segment size and overall body size (total whole-body skeletal convex hull volume) in 410 terrestrial tetrapods using phylogenetically informed linear (thick dashed lines) and quadratic (thin dotted lines) fits. (A) skull length, (B) skull width, (C) Head volume (MC), (D) Alpha volume 0.5, (E) Alpha volume 1.0, (F) Alpha volume 2.0, (G) neck length and (H) neck volume. Isometry in (A, B, G and H) would be a slope of 0.33. Full breakdowns of the regression model information can be found in ESM5.xlsx; tables S1-S2, including additional comparisons of scaling in limb segment volumes. Taxa have been colour-coded by taxonomic order for display purposes.
5.5.2 Differential scaling in head and neck size in large versus small bodied terrestrial tetrapods

Here, statistically significant (P<0.05) second-degree coefficients for phylogenetically informed quadratic models fit through all volumetric head and neck measurements were examined (Tables S3-S4, ESM5.xlsx). Phylogenetically informed quadratics models for skull width and neck length are statistically significant (Table S3, ESM5.xlsx), but the second-degree coefficient for skull length falls slightly above significance (p=0.0532; Table S3, ESM5.xlsx). AICc values are similar in linear versus quadratic fits for all parameters, they are lower for linear fits for all linear size metrics (skull length and width, and neck length) but are lower for quadratic models for volume metrics (skull and neck convex hull volume, and the three skull alpha shape volumes). In other words, allometric patterns in linear size metrics are slightly better described by a simple linear model, while a non-linear quadratic fit better describes the scaling of volumetric size metrics.

To examine the nature of this non-linearity on head and neck segment allometry, the slopes of a series of size thresholds (or bins) within the full data set were compared (Fig 5.6). The qualitative pattern of pGLS slopes across the size bins in skull length and skull width show that greater negative allometry is always recovered in the larger size group regardless of whether the size threshold is drawn at 25kg or 100kg,
however at 500kg a reverse in this trend is seen, whereby skull length and width are now more positively allometric at the larger size threshold (Tables S5-S10, ESM5.xlsx; Figure 5.6). For skull convex hull volume and alpha shape volume 1 (i.e., $k = 0.5$, the a-shape most dissimilar to a convex hull) greater negative allometry is always recovered in the larger size group regardless of whether the size threshold is drawn at 25kg, 100kg or 500kg (Tables S5-S10, ESM5.xlsx; Figure 5.6). For alpha skull volumes 2 ($k=1.0$) and 3 ($k=2.0$), greater negative allometry is recovered in taxa above 25kg versus below 25kg. However, when taxa at split at 100kg and 500kg the magnitudes of negative allometry are much more similar (Tables S5-S10, ESM5.xlsx; Figure 5.6). For neck volume, taxa above and below 25kg both scale close to isometry. However, when taxa are split at larger body sizes, the >100kg and >500kg taxa show strong positive allometry, while the smaller size-bin remain isometric below 100kg and decrease into negative allometry below 500kg (Table S5-S10, ESM5.xlsx; Figure 5.6).
Figure 5.6: pGLS regression slopes for individual body segments with taxa split into body size bins (<25kg vs. >25kg; <100kg vs. >100kg; <500kg vs. >500kg).
5.6 Taxonomic and trophic patterns in head and neck size

5.6.1 Taxonomic patterns in head and neck size.

5.6.1.1 Head size

Linear allometric relationships for all variables in all major taxonomic sub-groups were statistically significant (Figure 5.7). Skull length shows negative allometry in all taxonomic classes, ranging from a slope of 0.254 in birds to 0.294 in amphibians. Only the upper 95% confidence interval of the slope for amphibia includes isometry (Table S11, ESM5.xlsx). The difference in slope between birds and amphibians are not recovered as statistically different by phylANCOVA tests (Table S12, ESM5.xlsx). However, amphibians are statistically closer to isometry than non-avian dinosaurs, mammals and non-avian reptiles when differences in body size are accounted for (Table S12, ESM5.xlsx; Figure 5.7). Birds shows statistically greater negative allometry than non-avian dinosaurs, mammals and non-avian reptiles when differences in body size are and are not taken in account (Table S12, ESM5.xlsx; Figure 5.7).

Taxonomic classes are qualitatively more divergent in skull width allometry (Figure 5.7; Table S11, ESM5.xlsx). Amphibians scale with positive allometry (slope = 0.351), although negative allometry is narrowly included within 95% confident intervals. Non-avian reptiles scale with isometry, while mammals (slope = 0.289), birds (slope = 0.265) and non-avian dinosaurs (slope = 0.26) scale with negative allometry with
isometry not included within 95% confidence intervals (Figure 5.7; Table S13, ESM5.xlsx). PhylANCOVA’s suggest that non-avian dinosaurs are not statistically different to mammals or non-avian reptiles in skull width, and that mammals and non-avian reptiles are not statistically different (Table S13, ESM5.xlsx). However, positive allometry in amphibians is statistically different to the isometry and negative allometry seen in the other groups (Table S11, ESM5.xlsx). Birds scale with significantly greater negative allometry to non-avian reptiles and mammals in skull width, while non-avian dinosaurs show statistically greater negative allometry to birds (Tables S13, ESM5.xlsx).

Skull convex hull volume scales isometrically in amphibians (Table S11, ESM5.xlsx; Figure 5.7). While negative allometry is recovered in non-avian reptiles, isometry is included within the 95% confidence interval for the group (Table S14, ESM5.xlsx). Mammals (slope = 0.923), birds (slope = 0.856) and non-avian dinosaurs (slope = 0.77) scale negatively and isometry is not included within 95% confidence intervals (Table S14, ESM5.xlsx). PhylANCOVA’s suggest that non-avian dinosaurs are not statistically different to mammals or non-avian reptiles in skull convex hull volume, and that mammals and non-avian reptiles are also not statistically different (Table S14, ESM5.xlsx). Isometric scaling in amphibians is only statistically different to negative allometry in birds when the contribution of differing intercepts is accounted for (Table S14, ESM5.xlsx). However, amphibia are recovered as statistically different to all other groups with and without consideration of body size (Table S14, ESM5.xlsx). As with skull width, birds scale with significantly greater negative allometry to non-
avian reptiles and mammals in skull convex hull volume, while non-avian dinosaurs show statistically greater negative allometry to birds (Table S14, ESM5.xlsx).

The three alpha shape iterations show the same pattern relative to each other in each taxonomic group; that is, as k increases the slopes of each taxonomic group increase and move closer to isometry (Table S11, ESM5.xlsx). In contrast, birds and amphibians are recovered with both low and high values in the different alpha volume iterations (birds lambda = 0.6-0.84; amphibia lambda = 0.43-0.81). Amphibia show the least marked negative allometry (slopes = 0.945, 0.961 and 0.987), while birds (Slope = 0.691, 0.791, and 0.789) show the strongest negative allometry (Figure 5.7; Tables S17-S19, ESM5.xlsx). In all three alpha volume iterations, non-avian dinosaurs are not statistically different to mammals or non-avian reptiles, and mammals and non-avian reptiles are not statistically different in phylANCOVA comparisons (Tables S17-S19, ESM5.xlsx). Across all three alpha volume iterations, amphibia are recovered with a statistically greater slope and birds with a significantly smaller slope than all other groups with and without consideration of body size (Table S17-S19, ESM5.xlsx).
Figure 5.7: Scaling relationships between major body segment size and overall body size (total whole-body skeletal convex hull volume [WBCHV]) in different taxonomic categories, with statistical differences between phylogenetically informed linear fits tested using a phlyANCOVA. Relationships shown are (A) Skull length, (B) Skull width, (C) Skull volume, (D) Alpha shape 0.5, (E) Alpha shape 1.0, (F) Alpha shape 2.0. Taxa have been colour-coded by taxonomic order for display purposes.
Overall, therefore, the different metrics mostly yield the same qualitative picture of allometric patterns within and across major taxonomic groups. All length measures of head size suggest isometry in birds, non-avian dinosaurs and mammals, but positive allometry in non-avian reptiles and amphibians (Figure 5.7; Tables S11, S17-S19, ESM5.xlsx). The same qualitative allometric patterns are recovered in all volumetric measures of head size for birds, non-avian dinosaurs and mammals. However, a different qualitative pattern of head size allometry is recovered when volumetric metrics are used in non-avian reptiles and amphibians (Figure 5.7; Tables S11, S17-S19, ESM5.xlsx). While convex hull volume also scales with positive allometry in non-avian reptiles (qualitatively similar to skull length and width), all three alpha shape volumes scale with negative allometry in this group. In amphibians convex hull volume scales isometrically while all three alpha volumes scale with negative allometry, contrasting with the positive allometry seen in length metrics. The choice of metric (length versus width versus volume) chosen to represent skull size does not impact qualitatively on the allometric pattern recovered for non-avian dinosaurs, non-avian reptiles, birds or mammals but does have a qualitative impact on the pattern recovered for amphibia.
5.6.1.2 Neck size

Taxonomic classes are highly disparate in their neck length allometries (Figure 5.8; Table S11, ESM5.xlsx). Birds scale with strong positive allometry (slope = 0.464) and mammals with more modest positive allometry (slope = 0.365), with neither confidence intervals including isometry (Table S11, ESM5.xlsx). Neck length in non-avian dinosaurs’ scales very close to isometry (slope = 0.323), while amphibians and non-avian reptiles scale with negative allometry but with isometry included within 95% confidence intervals (Table S11, ESM5.xlsx). PhylANOVA’s suggest that non-avian dinosaurs are not statistically different to mammals or non-avian reptiles in neck length allometry, and that mammals and non-avian reptiles are also not statistically different (Table S15, ESM5.xlsx). When size is accounted for, amphibia scale with greater negative allometry than birds, mammals and non-avian dinosaurs, while birds scale with greater positive allometry than all other groups (Tables S15, ESM5.xlsx).

Neck volume scales with isometry in mammals, non-avian dinosaurs and birds (slopes 1.03-1.07), and with negative allometry in amphibia (slope = 0.89) and non-avian reptiles (slope = 0.993) (Figure 5.8; Table S11, ESM5.xlsx). PhylANOVA’s suggest that non-avian reptiles are not statistically different to mammals or non-avian dinosaurs in neck volume allometry, which might potentially be attributed to the relatively small sample size and/or high levels of neck length variation in the sample of non-
avian reptiles. When size is accounted for, amphibia show greater negative allometry to all other groups (Tables S16, ESM5.xlsx). Non-avian dinosaurs are recovered with a significantly greater slope to birds and mammals when size is accounted for (Table S16, ESM5.xlsx).

Overall, all length measures of neck size suggest positive allometry in birds and mammals, with non-avian dinosaurs scaling close to isometry, but negative allometry in non-avian reptiles and amphibians (Figure 5.8; Tables S11, S15-S16, ESM5.xlsx). Volumetric measures of neck size, birds, mammals and non-avian dinosaurs scale with isometry, whilst amphibia and non-avian reptiles scale with negative allometry (Figure 5.8; Tables S11, S15-S16, ESM5.xlsx). Therefore, the choice the metric (length versus volume) chosen to represent neck size does not impact qualitatively on the allometric pattern recovered for non-avian dinosaurs, amphibia or non-avian reptiles, but does have a qualitative impact on the pattern recovered for birds and mammals.
Figure 5.8: Scaling relationships between major body segment size and overall body size (total whole-body skeletal convex hull volume [WBCHV]) in different taxonomic categories, with statistical differences between phylogenetically informed linear fits.
tested using a phlyANCOVA. Relationships shown are (A) Neck length, (B) Neck volume. Taxa have been colour-coded by taxonomic order for display purposes.

5.6.2 Trophic patterns in head and neck size

5.6.2.1 Head size

Linear allometric relationships for all variables were statistically significant (Figure 5.9). Skull length shows negative allometry in carnivores (slope = 0.316); herbivores (slope = 0.293); insectivores (slope = 0.322) and omnivores (slope = 0.259), whilst piscivores show positive allometry (slope = 0.389) (Table S21, ESM5.xlsx). Carnivores, herbivores and omnivores have a high phylogenetic signal (lambda = 0.967, 0.946 and 0.929), whilst insectivores and piscivores shown less phylogenetic influence (lambda = 0.127, 0.437). Carnivores and herbivores were recovered as significantly different to all other trophic groupings by phylANCOVA tests (Table S22, ESM5.xlsx). Despite the large differences in their best of fit slopes, piscivores were recovered as statistically significant to carnivores, herbivores and omnivores but not insectivores (Table S22, ESM5.xlsx).

For skull width, carnivores (slope = 0.327) and insectivores (slope = 0.33) scaled close to isometry, whilst herbivores (slope = 0.301), omnivores (Slope = 0.274) and piscivores (slope = 0.265) scaled with negative allometry (Table S21, ESM5.xlsx).
Carnivores, herbivores and omnivores shown the strongest phylogenetic signal (lambda = 0.969, 0.942 and 0.917), whilst insectivores and piscivores revealed lower phylogenetic signal (lambda = 0.567, -0.235). Carnivores and herbivores were recovered as significantly different to all other trophic groupings by phylANCOVA tests (Table S23, ESM5.xlsx). Despite piscivores and omnivores differing in their slopes with other trophic groups they are not recovered as statistically different by phylANCOVA to any other trophic groupings other than carnivores and herbivores (Table S23, ESM5.xlsx).

Skull convex hull volume scales with negative allometry for all trophic groupings, ranging from a slope of 0.833 in omnivores to 0.966 in carnivores (Table S21, ESM5.xlsx). All groups display strong phylogenetic signal (lambda = 0.751 to 0.965), apart from piscivores which show low phylogenetic signal (lambda = -0.091). phylANCOVA’s suggest that only carnivores and herbivores are statistically different to all other groupings and that omnivores, insectivores and piscivores are not statistically different to each other (Table S24, ESM5.xlsx).

The three alpha shape iterations show the same pattern relative to each trophic groups, similar to taxonomic categories. That is, as k increases the slopes of each trophic group increases and moves closer to isometry (Table S21, ESM5.xlsx). The phylogenetic signal recovered across the alpha volume iterations is comparable to skull convex hull volume, with carnivores, herbivores, insectivores and omnivores exhibiting high lambda values (0.745-0.953) and piscivores showing very low lambda
values (-0.091-0.072). Carnivores show the least marked negative allometry (Slope = 0.932, 0.951, 0.959), with omnivores showing the most marked negative allometry (Slopes = 0.776, 0.831, 0.836) (Figure 5.9; Tables S23, S27-29, ESM5.xlsx). In all alpha shape iterations carnivores and herbivores are statistically different to all other trophic groupings and insectivores, omnivores and piscivores are not statistically different to one another (Tables S27-29, ESM5.xlsx).

Overall, head length measures suggest negative allometry in carnivores, herbivores, insectivores and omnivores, but positive allometry in piscivores (Figure 5.9; Tables S21-S22 ESM5.xlsx). All width measures of head size suggest negative allometry in herbivores, omnivores and piscivores, with carnivores and insectivores scaling with isometry (Figure 5.9; Tables S21, S23 ESM5.xlsx). The same qualitative allometric patterns are recovered in all convex hull volume measures of head size with all trophic groupings scaling with negative allometry. (Figure 5.9; Tables S21, S24 ESM5.xlsx). Alpha shapes shown negative allometry for all trophic grouping also, but with a value increasing towards isometry with an increase in k value. Therefore, the choice of size metric impacts on the qualitative allometric pattern recovered for trophic groups, with skull length versus width and linear versus volumetric metrics yielding different allometric patterns in carnivores, insectivores and piscivores. The choice of metric (length versus width versus volume) chosen to represent skull size does not impact qualitatively on the allometric pattern recovered for herbivores or omnivores but does have a qualitative impact on the pattern recovered for carnivores, insectivores and piscivores.
Figure 5.9: Scaling relationships between major body segment size and overall body size (total whole-body skeletal convex hull volume [WBCHV]) in different trophic
categories, with statistical differences between phylogenetically informed linear fits tested using a phlyANCOVA. Relationships shown are (A) Skull length, (B) Skull width, (C) Skull volume, (D) Alpha shape 0.5, (E) Alpha shape 1.0, (F) Alpha shape 2.0. Taxa have been colour-coded by trophic category for display purposes.

5.6.2.2 Neck size

Neck length scaled with positive allometry across all trophic groupings (Figure 6.0; Table S25, ESM5.xlsx). Piscivores scale with the highest positive allometry (slope = 0.455) and carnivores with the lowest positive allometry (slope = 0.340). Carnivores, herbivores and omnivores all show high phylogenetic signal (lambda = 0.937 to 0.979), with insectivores and piscivores showing the lowest phylogenetic signal (lambda = 0.426 and 0.119). phylANCOVA’s suggest a similar pattern to the skull in which only carnivores and herbivores (slope = 0.370) showing significant differences to all other trophic groupings (See table S25, ESM5.xlsx).

Neck convex hull volume scales with positive allometry in herbivores (slope = 1.036), omnivores (slope = 1.040) and piscivores (slope = 1.039), whilst carnivores (slope = 0.983) and insectivores (slope = 0.961) scale with negative allometry. High phylogenetic signal was found in carnivores, herbivores and insectivores (Lambda = 0.974, 0.939, 0.921) and low phylogenetic signal was found in omnivores and piscivores (Lambda = 0.149, 0.410) (See table S21, ESM5.xlsx).
Overall, all length measures of neck size including volumetric measures suggest negative allometry in all trophic groupings (Figure 6.0; Tables S11, S25-S26, ESM5.xlsx). Deviations from this occur in carnivores and insectivores in which positive allometry is found in neck volume (Figure 6.0; Tables S11, S25-S26, ESM5.xlsx). Therefore, the choice the metric (length versus volume) chosen to represent neck size does not impact qualitatively on the allometric pattern recovered for herbivores, omnivores or piscivores, but does have a qualitative impact on the pattern recovered for carnivores and insectivores.
Figure 6.0: Scaling relationships between major body segment size and overall body size (total whole-body skeletal convex hull volume [WBCHV]) in different trophic categories, with statistical differences between phylogenetically informed linear fits tested using a phlyANCOVA. Relationships shown are (A) Neck length, (B) Neck volume. Taxa have been colour-coded by trophic category for display purposes.
5.7 Do different dietary categories converge on specific head and neck morphologies?

The wheatsheaf tests did not recover any statistically significant evidence for convergence in skull or neck size metrics across major dietary categories (See table S31, ESM5.xlsx).

5.8 Discussion

It is widely accepted that a variety of intrinsic and extrinsic factors have acted upon the head and neck to shape their absolute and relative proportions across tetrapod evolution (e.g., Emerson 1985; Christiansen 1999; Goswami 2006; Slater and Van Valkenburgh 2009; Bohmer et al 2015; Terray et al 2020; Arnold 2021). However, linking patterns of head and neck size evolution across diverse taxonomic groupings to mechanical, physiological and ecological drivers is difficult at present due to varying uses of methodologies and differences in techniques for size normalisation across past studies, which have also tended to focus on specific tetrapod sub-groups. Here we have taken a different approach by analysing head and neck size across tetrapods more broadly using several different metrics to represent the size of these body segments. This allows a more direct comparison of relative head and neck size across major taxonomic and ecological groups, and furthermore allows us to directly determine how the choice of metric used to represent head and neck size (which
varies considerably in the literature) impacts on upon deductions made about patterns of evolution in these important body segments.

5.8.1 Methodology comparisons

In this paper, the methods used for quantifying and analysing head and neck size evolution in terrestrial tetrapods were surveyed, as well as the current points of consensus and controversy within the literature. Here we also compared head and neck size using different metrics. Overall, conflicting results were found in the differing metrics used. This suggests that results would change if you represented head and neck size using the different size metrics that previous studies have used. In taxonomic groups, the choice of metric (length versus volume) chosen to represent neck size does have a qualitative impact on the pattern recovered for birds and mammals and the choice of metric (length versus width versus volume) chosen to represent skull size does impact qualitatively on the allometric pattern recovered for amphibia. In trophic groupings, the choice of metric for skull size impacts qualitatively on the allometric pattern recovered for carnivores, insectivores and piscivores. Like the skull, the metric (length versus volume) chosen to represent neck size also has a qualitative impact on the pattern recovered for carnivores and insectivores.

We also compared convex hull volumetrics to alphas shapes and most of the time no qualitative differences were found here, however the allometric slope given by the
alpha shapes did change quantitatively as the k value increased. Specifically, a pattern in both taxonomic and trophic analysis showed skull volume moved closer to a more isometric slope with an increase in k value within alpha shape analysis. There were exceptions to this such as in amphibians the convex hull volume scaled isometrically while all three alpha volumes scaled with negative allometry, contrasting with the positive allometry seen in length metrics.

The main normalisation techniques used and compared in this work however were both based on a volumetric approach, in comparison the literature was heavily based not only on the use of volumetrics (See chapter 5.3.4) but on extant scaling approaches too (See chapter 5.3.4). Further analysis should therefore compare differing extant scaling techniques and volumetric methods to fully reveal if studies using different metrics effects a clear picture of ecological and taxonomic differences in head and neck evolution. Another issue regarding metrics was researchers’ perceptions and measurements of skull length. Skull width is generally universally agreed upon (See chapter 5.3.1.1) as well as neck length (See chapter 5.3.1.2), but how skull length is measured has varied, particularly where past studies have focused on different taxonomic groups (See chapter 5.3). It is therefore also suggested for different measurements of skull lengths to be compared to look for the effects this would have on the allometric results that have previously been identified in the literature.
5.8.2 Taxonomic patterns in head and neck size

Within mammals and birds, skull length, width and volume scale with negative allometry relative to overall body size, with skull volume scaling close to isometry in mammals (slope = 0.923). This is consistent with previous inferences that the mechanical demands of disparate feeding ecologies exert a stronger selective pressure on skull size than phylogenetic constraints in mammals (e.g., Cardini and Polly 2013; Cardini et al 2015). In birds, previous studies by Van der Leeuw (2002) and Marek et al. (2021) also found negative allometry in skull volumes of smaller groupings of birds. Skull volume has been assumed to be reduced in birds due to the widespread pneumatisation of the skull (Witmer 1990), reduction of jaw musculature due to most food processing occurring in the gizzard (Battley and Piersma 2005) and negative scaling of the brain and eye size with body mass (Brooke et al 1999), all ways to decrease mass to enable birds to fly (King 1979).

In previous research skull length scaled with negative allometry to body mass with increasing body size in theropods (Therrien and Henderson 2007), with negative allometry suggested in sauropods (Bates et al 2016) and positive allometry seen regarding skull length in ceratopsians (Sereno et al 2007). Overall, when taking all these subgroups into account in this extensive dataset non-avian dinosaurs scaled with negative allometry for skull size overall in this study, showing a general pattern of decreasing skull mass, skull length and width with increases in body size (Fig 5.7).
Regarding reptiles and amphibians, the width of the skull increases with body mass at a quicker rate than skull length. This correlates to other studies in reptiles that find as body size increases the skull becomes shorter and wider (Foth et al 2016; Gignae and O’Brien 2016; Souza 2021) and is comparable to previous findings in amphibia by Bardua et al (2019), Paluh et al (2020) and Bardua et al (2021). Although studies on skull size in Amphibia are less common (Ponssa and Candioti, 2012; Sherratt et al 2014), most studies agree that the adult shape of the skull is strongly influenced by phylogeny rather than lifestyle (Perez Ben et al 2020), with the skull also being constrained structurally to be large enough to accommodate the brain and sensory organs (Paluh et al 2020) and the requirement of a larger skull width potentially because of gape limitations and to enable suction feeding (Fernandez et al 2017; Bardua et al 2021). Bigger reptiles are also known to have wider skulls for relatively larger bite forces to include larger and harder prey in their diet (See 5.8.2) (Herrel et al 2001; Verwaijen et al 2002).

As body size increases in mammals the neck becomes proportionally longer but neck volume scales isometrically (Figure 5.8; Table S11; ESM5.xlsx). Most studies on neck length in mammals recover negative allometry for neck length against skull size (Preuschoft and Klein 2013; Cardini and Polly 2013; Cardini et al 2015; Arnold et al 2017a,b). These patterns, are proposed to likely reflect intrinsically linked mechanical and ecological selective pressures; skull size was found to decrease with body size in mammals overall and an absolutely and relatively smaller head may allow for a longer
neck resulting in the constraint to minimise the first mass moment of the head being removed and enabling longer necks overall as body size increases (Preuschoft and Klein 2013; Cardini and Polly 2013; Cardini et al 2015; Arnold et al 2017a,b).

Neck length scales isometrically in non-avian dinosaurs and is positively allometric to body size in birds, with birds increasing neck length with size at a significantly quicker rate to other clades (See Figure 5.8), including significantly to non-avian dinosaurs. This is similar to previous research which found in extant bird’s, neck length scales with body mass according to positive allometry (Bohmer et al 2019; Marek et al 2021) and therefore complements the idea that neck length scales bigger with body mass in birds than in other tetrapods (Bohmer et al 2019; Marek et al 2021). This could potentially indicate that unlike their non-avian dinosaur relatives’ birds have used their neck as an important tool in evolution to manipulate the environment around them, most likely due to the constraint of the forelimbs to be evolutionary adapted for flying purposes (Marek et al 2021).

Within non-avian reptile’s neck length and volume decreases with increases in body size. Reptiles have a highly variable number of cervical vertebrae between species and families with most reptiles having seven mobile cervical vertebrae including a three-part atlas and axis. Skull size increasing with body size in reptiles would mean a shorter neck would be required to minimise the first mass moment of the skull, allowing a larger skull to maximise bite force (Felice et al 2021), indeed most reptiles in this dataset are either carnivores, insectivores or omnivores. Amphibia however
are conserved to one cervical vertebra and therefore very few studies on allometry in neck size in amphibia have previously appeared in the literature (Arntzen et al 2015; Slijepčević et al 2015; Urošević et al 2016), however we have found that neck size here is unique, where neck length is significantly different to all other clades and orders, being the shortest for an animal of their size, with neck volume and length increasing in size much slower than other taxonomic groupings as body size increases. This is probably due to neck morphology in amphibia being heavily constrained to having only one cervical vertebra, therefore the ability to increase the length of the neck is difficult (Vidal et al 1986; Wake 2009).

5.8.3 Trophic patterns in head and neck size

Previous research has identified greater association between diet and the morphology of the skull including in clades such as non-avian reptiles (Stayton 2003; Claude et al 2004; Herrel and O’Reilly 2006; Hipsley and Muller 2017; brooke et al 2019; Ballele et al 2019; Godoy 2020) mammals (Wilson and Sanchez-Villagra 2010; Maestri et al 2016; Campbell and Santana 2017; Law et al 2018; Arbour et al 2019; Hendges et al 2019; Machado 2020), birds (Sun et al 2018; Pecsics et al 2018; Felice et al 2019; Pigot et al 2019), amphibians (Emerson 1985; Vidal-Garcia and Keough 2017; Hudson et al 2018) and non-avian dinosaurs (King 1996).
In carnivores, skull width scale with positive allometry relative to overall body size. size than skull length and volume which scale with isometry. A wider skull enables bite force to deal with large torsional loadings from struggling prey (Radinsky 1981, 1984; Wroe and Milne 2007; Felice et al 2021). This relationship has been documented in amphibians (Deban et al 2001; Vidal and Keough 2017; Bardua et al 2021), non-avian dinosaurs (King 1996, Taylor and Wedel 2013), non-avian reptiles (Vanhooydonck et al 2011; Vitt and Caldwell 2014; Foth et al 2016; Gignae and O’Brien 2016; Herrel et al 2018; Godoy 2020; Souza 2021) and mammals (Radinsky 1981, 1984; Cardini and Polly 2013; Cardini et al 2015; Jones et al 2015; Marshall and Pyenson 2019). Chapter three also revealed that carnivores have a reduced neck volume and new results here suggest that neck length increased with body size in carnivores but at a significantly slower rate to herbivores whose neck length and volume scale with positive allometry relative to overall body size. Shorter necks in carnivores reduces the feeding envelope and total range of motion accessible to the head neck system, giving the head the structural stability needed to capture prey (Taylor and Wedel 2013; Felice et al 2021), whilst longer necks in herbivores increases the feeding envelope (Taylor and Wedel 2013). Previous work has suggested that a shift away from carnivory may have facilitated shifts in longer neck lengths and smaller skull sizes (Zanno and Makovicky 2011). Smaller skull sizes in herbivores have been related to the lack of dentition within most species, because most of the food processing is done in the gut and not the mouth (Sues and Reisz 1998). This would therefore lead to a reduction in the size and weight of the skull, reducing the power needed to lift the skull and therefore reducing subsequent muscle mass that would be needed allowing the necks to be longer (Taylor and Wedel 2013).
Insectivores shown similar patterns to carnivores with skull width scaling with greater positive allometry to body size than skull length and volume which scaled with isometry. A developmentally plastic increase in relative head width in response to encountering small prey such as insects in anurans may be adaptive, compensating for slow growth in absolute body size (Hudson et al 2018) and therefore enabling high bite forces, which may face even more challenging mechanical demands if they feed on insects with tough cuticles. This pattern is also found in other insectivorous species and could therefore suggest that insectivores possess specific skull specializations where the skull becomes shorter and wider to adapt towards capturing and crushing large insectivorous prey (Herrel et al 2018). However, some insectivorous species have been noted to have a more elongate skull, particularly in mammalian insectivores (Christiansen and Wroe 2007; Samuels 2009; Maestri et al 2016). This could be due to these species tending to predate on softer bodied insects and therefore a further suggestion for this work would be to split the insectivores into soft bodied and hard bodied insect predators. The relative size of the neck of insectivores increases with body size in tetrapods overall. In insectivorous birds it has been suggested that a long neck length is important in capturing fast escaping prey such as insects through high velocity protraction or extension of the neck (Marek et al 2021). Insects tend to live in the ground or on trees, and a long neck is useful in reaching higher up places in trees for ground dwelling species and reaching deeper areas under the surface, without the energetic costs of moving larger bodyweights (Sander et al 2011).
Skull width in omnivores scaled with greater positive allometry to body size than skull length, while skull volume decreased in size (Figure 5.9; Table S21; ESM5.xlsx). Omnivores must deal with the processing of both meat and vegetation, and it is perhaps therefore not surprising that their head and neck proportions and allometric patterns are recovered as intermediate between more specialist dietary categories and ‘average’ for tetrapods as a whole (Figure 5.9, 6.0; Table S11; ESM5.xlsx). Omnivores tend to predate on smaller size prey items and therefore do not have to increase their skull size to larger sizes as seen in carnivores, however as they are still eating meat they need strong bite forces, to subdue and kill struggling prey. To enable them to do this, omnivores need a wide skull, to house a relatively large volume of muscles to increase bite force (Maestri et al 2016).

In piscivores skull length scaled with greater positive allometry to body size than skull width which scaled with negative allometry and skull volume which scaled with isometry. In line with biomechanical evidence, a longer skull increases the speeds of attack and prey capture in water associated with a diet of soft and fast-moving prey such as fish (Ballel et al 2019). In crocodiles a longer skull length is commonly used to infer an active swimming and piscivorous lifestyle (Stayton and Ruta 2006; Witzmann and Schoch 2006; Fortuny et al 2011), with long and flat snouts associated with lateral head swipes to catch prey (Nishukawa and Schwenk 2002). Less resistance is generated in water by a narrow snout allowing it to move faster (Maganuco and Pasini 2009), so this characteristic is often seen hand in hand in piscivores (Witzmann
and Scholz 2007). Neck length in piscivores scaled with positive allometry to body size, with neck volume showing a similar rate. Usually in aquatic piscivorous species such as some crocodiles, the catching of fish with rapid lateral head swipes is an action that can be hastened with a short neck, not a longer one (Rhinehart et al 2015). Fish-eating birds make up a large proportion of taxa in our piscivore dataset. These animals have been suggested to use their neck as an important tool in evolution to manipulate the environment around them (Marek et al 2021), and particularly in feeding due to the constraints of flying on the forelimb. A more streamlined and longer neck and skull would therefore allow for reaching fish in deeper areas of water as seen in herons, spoonbills and cormorants (Wilkinson and Ruxton 2012). However, many birds which hunt fish underwater (e.g., penguins) have not evolved long necks, illustrating there could be a range of evolutionary solutions to this problem (Wilkinson and Ruxton 2012).

5.8.4 Convergent evolution of head and neck size

Convergent evolution of head and neck size has been proposed across many vertebrate clades and may reflect an evolutionary response to shared selective pressures in different dietary ecologies (Nevo 1979; Stayton 2005; Wroe and Milne 2007; McGhee 2011; McCurry et al 2017; Sansalone et al 2018; Fournier et al 2021). Statistically significant convergence was not found in the size of the skull and neck between trophic categories. Strictly, this does not mean convergence has not
occurred but that it is not very strong in most linear and volumetric measurements regarding diet.

**5.8.5 Conclusion**

This study is the first to quantify head and neck size across a large, taxonomically and ecologically diverse range of extinct and extant terrestrial tetrapods to determine how terrestrial tetrapods scale in head and neck size parameters across the range of different size metrics used. This included differences regarding taxonomy and trophic categories in terrestrial tetrapods. The skull and neck are an important influence on feeding ecology; sexual selection; habitat use and locomotion and therefore must conform to many different needs to maximise survival within a species. The results here, highlight how form and function are intimately linked and how the skull and neck are structures that can be preserved phylogenetically and influenced by diet. As body size increases in tetrapods, skull volume, length and width all show negative allometry as a rule, with neck length getting longer and neck volume scaling geometrically with body size. Allometric patterns in linear size metrics (skull length, width and neck length) are better described by a linear model overall, whilst volumetric measurements (skull volume, neck volume and alpha shapes) fit a quadratic model better. Species over 100kg tend to show greater negative allometry in skull volume, whilst the neck shows strong positive allometry. Taxonomy provides large constraints on the evolution of skull and neck size, with skull width being more highly influenced by diet. Convergent evolution however was not found in dietary
ecotypes. The results also show that differing methodologies and statistical techniques in the literature to quantify skull and neck size in relation to body size has led to the challenge of comparing results across studies and to visualise macroevolutionary trends in different vertebrate clades. It is advised for future studies to conform on a similar measurement to assess body size, as well as similar landmarks on skulls and necks used to measure lengths and widths. Future work would also benefit from more species in this dataset, particularly where key gaps in the fossil record occur and where an ecotype such as piscivory groups have smaller numbers of species present to further untangle the complex interplay between phylogeny and trophic grouping influences on skull and neck size.

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Chapter 6
Chapter 6- Discussion

6.1 Summary of previous data chapters

This thesis set out to address the following objectives:

1) To understand how overall body shape has been modified by size, locomotor, and trophic ecology across tetrapods.

2) To quantify the degree of convergent evolution of body segment proportions in different locomotor and dietary groups across tetrapods.

3) To survey and quantitively compare the metrics used for analysing allometry in the head and neck across tetrapods.

In summary this thesis found that:

1) Most body segments in terrestrial tetrapods scale with negative allometry or isometry. Quadratic relationships between small-medium and large tetrapods are supported, particularly in quadrupedal striding species, which suggests that graviportal allometric trends occur in limb proportions in quadrupedal taxa with different patterns in proximal versus distal limb segments as body size increases. Differences in the relative proportions of body segments across locomotor and dietary groupings highlight key adaptations that underlie the exploitation of major ecological niches. Locomotion was correlated with changes in multiple body segments for the clear adaptation of moving through different media. Examples of this include limb reduction and a more elongate body form in aquatic and scansorial
taxa, elongation of the forelimb in flying and arboreal taxa and long hind limbs in saltatorial taxa (Chapter 3).

2) Ecological convergence exists in tetrapods, and the evolution of body segments seems to be driven more strongly and frequently by locomotor ecology rather than trophic habits. Although convergence in trophic categories was seen in torso volume in omnivores, body size and GA length in herbivores and manus length in carnivores, convergence in locomotor ecotypes dominated the findings. Specialised forms of locomotion such as fossoriality, flight, quadrupedal and scansorial show much stronger magnitudes of convergence in key adaptive aspects of their morphology with scansorial species showing the highest rates of convergence in limb morphology (Chapter 4).

3) As body size increases in tetrapods, skull volume, length and width all show patterns of decreasing, with neck length getting longer and neck volume scaling geometrically with body size. Allometric patterns in linear size metrics are better described by a linear model overall, whilst volumetric measurements fit a quadratic model better. Species over 100kg tend to show greater negative allometry in skull volume, whilst the neck shows strong positive allometry. Taxonomy provides large constraints on the evolution of skull and neck size, with skull width being more highly influenced by diet. Convergent evolution however was not found in dietary ecotypes. The results show that differing methodologies and statistical techniques to quantify skull and neck size in relation to body size leads to conflicting results and therefore impacts qualitatively on the allometric pattern recovered (Chapter 5).
6.2 General discussion

For all three chapters, a dataset of 410 extinct and extant taxa spanning most major taxonomic, locomotor and trophic groupings was used. Although the sample size here was relatively large, future work would benefit from including a broader sample of tetrapods. This would allow the further investigation as to what extent adaptation to locomotor or trophic ecology might explain differences in body segment proportions across tetrapods. Tetrapods encompass many species spanning several hundred million years, it would therefore be advised to increase the number of species, particularly within certain groups, including piscivore (n=9), aquatic (n=12) and soaring flight (n=9) species within this dataset.

Piscivorous species consisted of nine individuals, all of those being birds. In chapter three, piscivores did not show many statistical differences in body segments. This is further reflected in chapter four and five, in which a lack of convergent evolution was seen in body segments including the head and neck. This is unusual as skull length and size in piscivores has been shown in other studies to be highly significant to other dietary categories (Fortuny et al 2011; Santana and Cheung 2016; McCurry et al 2017; Ballel et al 2019) and show high levels of convergence in the skull for example in crocodiles (Fortuny et al 2011; McCurry et al 2017; Ballel et al 2019) and even in bats (Santana and Cheung 2016). Some research however has also suggested the splitting of dietary categories further since it may be unrealistic to predict that broad dietary categories such as the ones considered here, would have consistent morphology of
body segments. For example, Felice et al (2019), found that during a study on cranial morphology in birds, a piscivorous lifestyle showed no changes in cranial morphology compared to other dietary groupings, only when categories where further broken down into plunge-diving piscivores (pelicans) and terrestrial piscivores (herons) did significant differences in cranial structure reveal itself.

Aquatic species also had a low number of species within the dataset, however different clades were included and ranged from mammals to reptiles to amphibians. From previous literature it would have been expected to see longer spine lengths for streamlining purposes (Langerhans and Reznick 2010; Ward and Kley 2012), especially when species that move habitually through water demonstrated the highest positive allometries in GA distance (e.g., aquatic salamander species). However, statistical differences in body segments regarding smaller limb sizes relative to body size did show convergence. Similar convergence was also found in the skull and body mass in chapter four and five, but these results would become more clearer and could be further investigated with a larger sample size. Finally, although soaring species did show significant differences seen in hind limb and forelimb segments, a larger sample size would have allowed the further extrication of significant differences. A larger sample size would have also made comparisons to actively flying species more meaningful, especially as other studies have found large differences in the hind limb and forelimb size of actively flying species compared to soaring species (Heers 2018; Voeten et al 2018; Bribiesca-Contreras et al 2019).
However, this could be due to these studies only reviewing birds and not bats, which were added to this dataset under the category of active flight.

To further allow for more robust analysis over the several hundred million years tetrapods have existed, key gaps in the fossil record of this dataset from the Permian to the Quaternary should be included. In particular, more species from the Permian, Triassic and Jurassic eras, would be beneficial due to the huge shifts in the diversity, extinctions and dominance of different species on earth within these eras (Hallam 1996). However, it must be noted that skeletons from these eras are hard to get access to and are very rarely complete to provide analysis within this dataset. It would, however, be recommended to include ancient Crocodilian lineages (Pseudosuchia), and very early archosaurs, for which there are currently no species present in this dataset. As well as filling an important and large evolutionary gap, this group are thought to have been ecologically diverse showing both bipedal and quadrupedal forms of locomotion, with bipedal species having convergent body shapes similar to theropods, as well as similar terrestrial, semi-aquatic and aquatic transitions with a variation of diets (Nesbitt and Norell 2006; Zanno et al 2015; Schrachner et al 2020). They would therefore be an interesting group for the study of body shape evolution.

Larger sample sizes would also allow for various phylogenetic comparative methods developed specifically to test for the presence of adaptive evolution that would have been interesting and informative to explore within this type of dataset. For example,
to test for the selection towards different allometric slopes or locomotor and dietary optima, using the R packages SLOUCH (Kopperud et al 2012), OUwie (Beaulieu and O’Meara 2016) and mvMorph (Clavel et al 2015). This thesis does not test for the presence of adaptive signals and hypothesis about evolution, which would back up the “pivotal role” of body proportions described here and would further explain the ecological diversification of tetrapods. Instead, the focus of this thesis is on the nature of similarities and differences in body shape and allometry across tetrapods, and across major locomotor and trophic ecological groups.

Branch lengths used in phylogenetic trees can largely influence the results of a phylogenetic comparative analysis. Since the start of this thesis new methods for time scaling phylogenies have been developed. The package ‘strap’ is now seen as slightly outdated, and most studies today use the ‘cal3’ method implemented in the ‘palaeotree’ R package (Lloyd et al 2016; Bapst and Hopkins 2017) or the fossilized birth-death model implemented in MrBayes (Button and Zanno 2019; Godoy 2020). Both ‘cal3’ and the fossilized birth death model incorporate uncertainty into the dating algorithm and have been shown to be better than the ‘equal’ algorithm at estimating the ‘true’ time scale of phylogenies. However, ‘strap’ is therefore considered to be sophisticated enough for its purpose within this dataset, since, to my knowledge there is no robust way to choose between the different methods and the ‘timePaleoPhy’ function in ‘palaeotree’ is designed for cases where precise first appearance dates (FADs) and last appearance dates (LADs) are known.
Results using the Strap ‘equal’ and ‘ruta’ methods could have been compared to the various functions in Paleotree such as ‘timePaleophy’ or ‘cal3TimePaleoPhy function’, all of which come with a variety of differing methods for avoiding zero branch lengths. However, exploring the different time calibration methods was outside the scope of this thesis, due to the creation of a very large amount of different time calibrated trees to run all the analysis on. In a more complete and further refined phylogeny, the different time-calibration methods may well have an effect, but at this scale, the time-calibration method used is very unlikely to make any real difference to the results. It is sceptical that doing any of this would lead to such significant branch length changes that the results of the PGLS analysis would be different. Indeed, a much bigger uncertainty in the time-calibration is most likely the raw data themselves and the lack of resolution on the dating of fossils, which impacts all studies of this type.

Regarding results found in chapter three, further biomechanical analysis combined with morphological data measuring in-vivo muscle, bone stress and metabolic energy expenditure would be advised. This would highlight the exact interaction of the changes that most body segments in terrestrial tetrapods scaled with negative allometry or isometry. Further indicating body segments show positive allometry with body size or are geometrically similar across body sizes of approximately 0.005kg-40,000kg as body size increases.
In chapter four a new form of the wheatsheaf index was used to show that ecological convergence exists in various aspects of tetrapod body proportions, and that the evolution of body segments seems to be driven by locomotor ecology rather than trophic habits overall. The new version of the wheatsheaf index can be used on non-ultrametric trees because it uses a cophentic distance matrix, which computes pairwise distances between pairs of tips using branch lengths and is therefore a more accurate representation of a non-ultrametric tree (Arbuckle et al 2014). The use of multiple methodologies for calculating the strength of convergence is however recommended, due to that fact that different measures of convergence address different features (Speed and Arbuckle 2016). Previous studies using multiple methodologies (Freidman et al 2016), found that through quantifying the frequency and strength of convergence and comparing methodologies, a richer source of information was provided. For example, in a study by Freidman et al (2016), when looking at the ecomorphological convergence in planktivorous sturgeon fishes, in one of the strengths of convergence analysis tests there was a lack of statistical power available to accurately assess the strength of convergence. However, because multiple tests were used, the wheatsheaf index managed to confirm convergence within the zooplankton ectomorph was not significantly different than would be expected, reinforcing multiple methodologies of convergence provide more information for the researcher to interpret results appropriately. Multiple methodologies for convergence were not used in this thesis, due to time constraints and the knowledge that many convergence methods are fraught with caveats and limitations such as the considerations of scale and sample size e.g., in larger groups of species the maximum possible number of convergent events is larger, so we would
expect more instances of convergence in larger groups just by chance (Arbuckle and Speed 2016). Future work should therefore consider directionality and evolutionary pathways of body shape change more explicitly than is achieved here and include more extensive use of convergent methodologies and taxonomic coverage, including more species for each locomotor and dietary categories to further assess statistically the extent to which different ecologies are converging upon similar overall body shapes and sizes. Chapter four would also benefit from further biomechanical analysis combined with morphological data measuring in-vivo muscle, bone stress and metabolic energy expenditure to understand whether morphologies that certain groups converge on represent mechanical optima for a given ecotype or behaviour.

In chapter five, it was discussed how differing methodologies and statistical techniques in the literature to quantify skull and neck size in relation to body size has led to the challenge of comparing results across studies and to visualise macroevolutionary trends in different vertebrate clades. Results revealed that as body size increases in tetrapods, skull volume, length and width all show patterns of negative allometry, with neck length getting longer and neck volume scaling according to geometric similarity. This suggests that in tetrapods, regardless of the metric used, the same pattern of allometry is seen in skull length, width and volume, whereas for the neck, the result can be influenced by the metric that is used (length vs volume). Future studies should conform on a similar measurement to assess body size, particularly as varied statistical approaches effect a clear picture of ecological and taxonomic differences in head and neck evolution. It is also suggested for
different measurements of skull lengths to be compared to look at the effects this would have on the allometric results that have previously been identified in the literature.

The vastness of this dataset can also be used in other contexts and analysis for other interesting biological questions, for example this dataset gives us the basis to examine how selectively bred animals now deviate from what is normal for their closest relatives and similar ecotypes. An interesting selectively bred species in this dataset was the dachshund, which was one of the most elongate mammals and quadrupedal striding taxa in this dataset. Through analysis the dachshund had a GA distance to average leg length only seen in extinct mammal like reptiles such as *Diadectes phaseolinus* and *Chiniquodon theotonicus*, which are thought to have walked with somewhat abducted limb postures and potentially some lateral bending to the spine (Mocke et al 2019; Jones et al 2021). Dachshund, and other ‘sausage dogs’ are clearly an outlier among quadrupedal striding vertebrates in terms of relative GA, and this is likely mechanistically linked with epidemiological studies that have shown that long-bodied canine breeds show high prevalence for musculoskeletal dysfunction in the limb girdles and axial column (Roberts & McGreevy 2010). It would be extremely interesting to analyse further selectively bred species against this dataset, not only due to the ability to further understand the enormous impacts selective breeding has had on the morphology of animal species but also to further understand the evolution of body shape, locomotion and ecology in terrestrial vertebrates and the patterns that shape them.
6.3 References


Chapter 7
Chapter 7- Conclusions and future work

7.1 Conclusions and significance of work

Overall, this thesis set out to understand the evolution of body shape, locomotion and ecology in tetrapods. How overall body shape has been modified by size, locomotor and trophic ecology has been identified. The degree of convergence in tetrapod body shape has been quantified and a review of the range of methods used to quantify head and neck size was compared.

In conclusion:

- Most body segments scale with negative allometry or isometry across tetrapods.
- Statistical support for quadratic relationships is indicative of differential scaling in small-medium versus larger animals.
- Allometric patterns in linear size metrics are better described by a linear model and volumetric measurements by a quadratic model.
- Locomotion was associated with changes in multiple body segments for the clear adaptation of moving through different media.
- Convergence in body segments also centered around the mechanical requirements of moving through different media.
- Dietary influence has large effects on the size of the skull and neck.
- Future work should increase the number of species in this dataset, in particular piscivores, soaring and aquatic species for more robust analysis.
- Filling key gaps in the fossil record such as including the Pseudosuchia would help to untangle the complex interplay between phylogeny, locomotor and trophic influences further.
- Evolutionary rates analysis to test for the presence of adaptive signals would help to further explain the ecological diversification of tetrapods.
7.2 Thesis references


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