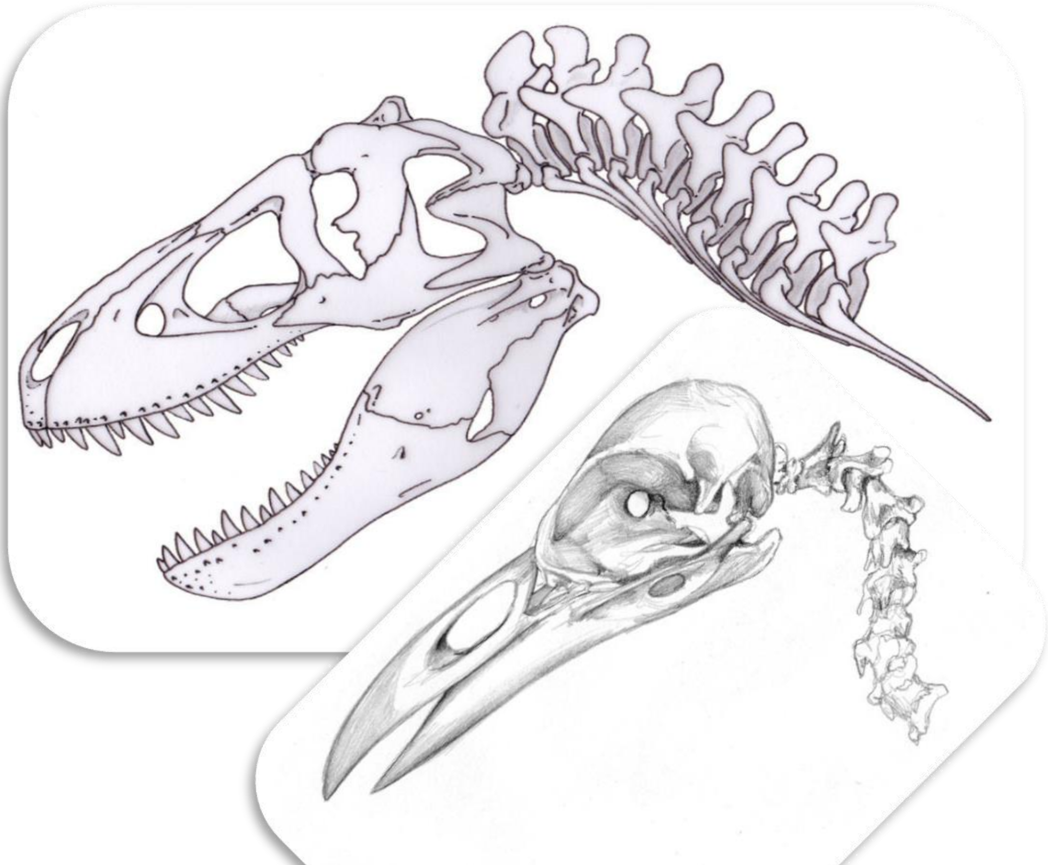

The Surrogate Arm: Functional Morphology of the Avian Cervical Column

Thesis submitted in accordance with the requirements of the University of Liverpool
for the degree of Doctor in Philosophy by

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Abstract

The avian neck allows the head to perform a variety of tasks that would be carried out by the forelimbs in other vertebrates, as the forelimbs are primarily adapted for flight in birds. This has created a strong additional selection pressure on the cervical column and has resulted in the evolution of a vast array of neck morphologies throughout extant birds. This diversity is most evident in the large variation observed in vertebral counts of the neck, and the lack of known homology between species with differing cervical counts has hindered research into morphological variation of the avian cervical spine. The lack of quantitative assessment of this variation has stunted our understanding of how the neck became such an integral component of avian biology. Recent work has shown that *Hox* gene patterning is conserved within the neck across Aves and that five cervical regions exist within the avian spine, and homologous interspecific comparisons can now be made by comparing aspects of regional morphology.

Iterating on previous work, this thesis uses 3D geometric morphometrics as a proxy to delineate and analyse these cervical regions within extant birds. Here I use a holistic approach to understand functional and ecological drivers in avian neck shape and length in a diverse selection of 52 specimens (46 species) of modern birds by combining three-dimensional geometric morphometrics with multivariate statistics and quantitative dissection as part of the following four objectives. The first objective sought to determine if patterns of cervical regionalisation exist within extant Aves by assessing the variation in regional vertebral counts and by using Phenotypic Trajectory Analysis to examine factors that affect morphological variation across the entire cervical spine. Results indicated that increases to cervical counts are not responsible for neck elongation in birds and that few ecological factors have a significant correlation with regional vertebral counts. Vertebral morphology across the entire cervical column only displays significant variation in birds with extreme cervical kinematics, such as carnivores. The second objective was to quantify the relationship between neck length, head mass, and body mass across extant birds. Neck length and head mass scale according to isometry. The relationship between neck length and head mass is also isometric and this indicates that neck length in birds is not as tightly constrained by head mass when compared to other vertebrates. Relative neck lengths are shorter in birds that locomote using powered flight and head mass is lower in terrestrial birds. This may indicate that powered flight is a constraint on cervical morphological variation. The third objective was to quantify factors affecting variation in the morphology and length of individual regions. Region lengths strongly correlated with neck length and this suggests that avian neck elongation is a product of increases to vertebral length rather than increases to vertebral counts, and that regions 2 and 5 are responsible for this elongation. Body mass and neck length were revealed to have the strongest correlation with morphological variation in regions across 4 out of 5 cervical regions. Only ecological factors associated with specialised cervical kinematics, i.e. adaptations to carnivory or aquatic foraging, displayed significant variation in regional vertebral morphology. The final objective was to quantify variation in avian muscle architecture and I found that muscle architecture and mass scaled predominantly according to positive allometry. Ecology has a weak correlation with muscle architecture and mass variation and may accommodate fast head strikes and adaptations to underwater foraging in certain taxa. Cranial muscles that support the head appear to be more morpho-functionally restricted, whilst caudal muscles display much more variation in architecture and mass.

This thesis has shown that across multiple aspects of morphology, the avian cervical column is a generalised musculoskeletal system, only adapting to specialised patterns of cervical kinematics. The avian cervical column is often referred to as a surrogate arm due to its role in positioning the head to manipulate the environment in the stead of the forelimbs which are heavily adapted for flight and the generalised pattern of morphological variation recovered here supports the hypothesis that the avian neck is a surrogate arm. Future work is needed to be done in the wake of this thesis to ensure that more birds are sampled for their variation in muscle architecture, as well as undertaking projects that seek to quantify variation in avian cervical kinematics and patterns of intervertebral flexion.

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"…Our emphasis on necks may be an anthropomorphic preoccupation. They imply that either the trunk or the head is the 'key' feature, so that any shifts of one must be viewed relative to the other. Such a conception is reminiscent of the pre-Medieval discussion on whether the Earth or the sun rotate about the other. We consider necks to be more important than other joints because the organs being shifted when the head moves are critical to us as mammals … However, we must remember that the role of the neck inevitably differs with the species being considered"

— **Carl Gans**, from 'Why Develop A Neck?' 1992

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"In the midst of winter, I found there was, within me, an invincible summer."

— **Albert Camus**

∞

"I have enough trouble with useful information, never mind being burdened with what is useless."

— **Erlend Loe**, from 'Naïve. Super'

Chapter 1: Introduction

The relationship between form and function is a fundamental aspect of vertebrate morphology as it represents a means of studying how musculoskeletal systems adapt to specific ecological niches (Hutchinson 2012; Irschick 2002; Ercoli, Prevosti, and Álvarez 2012; Stayton 2006, 2008; McInnes et al. 2011; Lauder and Thomason 1995; Randau and Goswami 2018). Our understanding of this relationship spans a wide diversity of vertebrates, but there exists a clear directional bias in the study of form-function relationships as cranial and appendicular anatomical systems dominate the literature (Stayton 2006; Pierce, Angielczyk, and Rayfield 2009; Goswami and Polly 2010; Bell, Andres, and Goswami 2011; Ercoli, Prevosti, and Álvarez 2012; Foth and Rauhut 2013; Walmsley et al. 2013; Alvarez, Ercoli, and Prevosti 2013; Martín-Serra, Figueirido, and Palmqvist 2014; Felice et al. 2019). The axial skeleton serves to support appendicular and cranial elements and underpins locomotion, respiration and environmental interaction across Vertebrata (Slijper 1942; Long et al. 1997; Shapiro 1995; Pierce, Clack, and Hutchinson 2011), yet is underrepresented in studies of functional morphology. Recent efforts are rectifying this issue and are discovering that changes to the axial column and its patterns of regionalisation can underpin the success of large vertebrate clades such as in mammals (Jones et al. 2018; Arnold, Esteve-Altava, and Fischer 2017; Randau and Goswami 2018).

Regionalisation is the differentiation of vertebral morphology across the axial column whereby groups of morphologically similar vertebrae form a distinct region (Pourquié 2003; Dequéant and Pourquié 2008; Gomez et al. 2008; Wellik 2007). This allows for the axial column to perform multiple functions along its length. This disparity in function leads to disparity in the strength of ecological signal that each axial region displays: thoracic and lumbar vertebrae have been found to display more ecological signal in vertebral shape due to their involvement in the support of appendicular elements (Randau, Goswami, et al. 2016; Randau, Cuff, et al. 2016; Randau and Goswami 2017, 2018), while the cervical column appears to be generalised in its construction (Arnold et al. 2016; Arnold, Amson, and Fischer 2017; Arnold, Esteve-Altava, and Fischer 2017; Van der Leeuw, Bout, and Zweers 2001).

The neck of vertebrates functions primarily to support the weight of the head and to provide it with a degree of movement that is independent of the trunk (Gans 1992; Wilkinson and Ruxton 2012), in an array of activities that range from observance and vigilance through to feeding and conspecific displays of dominance (Gans 1992; Wilkinson and Ruxton 2012; Boas 1929; Zweers, Bout, and Heidweiller 1994; Van der Leeuw, Bout, and Zweers 2001; Kress, Van Bokhorst, and Lentink 2015; Pete et al. 2015). Many of these activities are performed regularly and it is believed that this often causes the neck to adapt to the 'economics of continuous movement' rather than to a specific behaviour (Van der Leeuw, Bout, and Zweers 2001; Wilkinson and Ruxton 2012). Given that the nature of functional tasks is similar across a diversity of terrestrial vertebrates it might be expected that cervical morphology would be relatively conservative. However, at least qualitatively, the morphological variation of the cervical column is disparate in its distribution across vertebrates (Barrett et al. 2010). Mammals display a highly restricted cervical morphology owing to strict biomechanical and developmental restraints and this has resulted in seven cervical vertebrae being present in almost all extant mammals (Galis 1999; Buchholtz et al. 2012; Hirasawa, Fujimoto, and Kuratani 2016; Buchholtz 2014; Galis and Metz 2003; Arnold, Amson, and Fischer 2017). This restriction to cervical counts across Mammalia evolved as forelimb muscles began to attach to caudal elements of the cervical spine over mammalian evolution, and has been instrumental to a key mammalian synapomorphy, the muscularised diaphragm (Buchholtz et al. 2012; Hirasawa, Fujimoto, and Kuratani 2016; Arnold, Esteve-Altava, and Fischer 2017; Jones et al. 2018). Many of the recent studies that seek to understand the form-function relationship of the cervical column focus on mammals for this reason as the restriction to cervical counts ensures that interspecific comparisons are homologous (Buchholtz et al. 2012; Buchholtz 2012; Arnold, Amson, and Fischer 2017; Arnold, Esteve-Altava, and Fischer 2017; Randau, Goswami, et al. 2016; Jones et al. 2018).

The axial column of Sauropsida is far more diverse than that of Synapsida, with vertebral counts reaching up to 300 in some snakes and cervical counts surpassing 70 in extinct marine reptiles (Head and David Polly 2007; O'Keefe and Hiller 2006; Barrett et al. 2010). Amongst extant sauropsids, birds display a very high level of cervical variability, with vertebral morphology, total number of vertebrae, and neck length all having high levels of diversity across Aves (Benoit et al. 1950; Boas 1929; Baumel, Evans, and Berge 1993). In relation to other skeletal elements the cervical column of birds is somewhat modular as very few extrinsic or external (non-cervical) muscle groups attach to the cervical column (Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). This is in stark contrast to mammals whereby forelimb muscles attaching to caudal cervical elements has restricted variability in the mammalian cervical column (Arnold, Esteve-Altava, and Fischer 2017). With such widespread levels of cervical variability birds are not bound by the same genetic and developmental constraints that are ubiquitous across mammals and this could allow the avian neck to be more adaptable to ecological factors than necks of other vertebrates. Despite this abundance of cervical diversity very few studies have quantified this variation across a phylogenetically broad sample of extant birds (Böhmer et al. 2019).

However this diversity of avian cervical morphology has itself hindered research into this area due to the unknown homology of vertebrae between species with differing cervical counts. This has shaped the study of avian cervical variability immensely as much of the previous literature is either entirely qualitative or restricted to a small sample of phylogenetically similar species. As of 2019 (the submission of this thesis) there is only one quantitative study of avian neck length (Bohmer 2019), other literature is limited in its scope: an ontogenetic study of *Gallus gallus domesticus* (Heidweiller and Zweers 1992) and an evaluation of neck allometry in a small subset of Anseriformes (Van der Leeuw, Bout, and Zweers 2001; van der Leeuw 2002). Böhmer et al. 2019 represents the most comprehensive study of avian cervical variability to date and it there is some overlap with this thesis. Whilst an excellent initial exploration of the scaling relationship of cervical length in extant avians, Böhmer et al. 2019 focuses heavily on dietary ecology and disregards the effect of locomotor mode on neck length. Not only will this thesis explore the correlation between cervical length and ecology across a broader spectrum, it will also place these results into a wider context by also exploring how both scaling factors and ecology correlate with other aspects of neck morphology; namely vertebral morphology and muscle architecture variation. This allows for a holistic investigation into correlates of variation across multiple aspects of avian cervical anatomy, not just neck length and cervical count.

Shape variation in avian cervical vertebrae has not been quantified across any group of birds, despite the plethora of tools that are now available to functional morphologists, such as geometric morphometrics (Adams and Collyer 2009; Adams, Rohlf, and Slice 2013; Adams 2014; Collyer and Adams 2013; Adams et al. 2017; Adams and Felice 2014). Variation in cervical musculature, whilst more broadly sampled, is restricted to the study of variation in attachment sites and is entirely qualitative (Kuroda 1962; Landolt and Zweers 1985; Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). Thus the huge variation in cervical morphology observed in birds, at both the muscular and skeletal level, has not been quantitatively examined across a phylogenetically broad sample. At present, the unknown homology of cervical vertebrae between species is limiting our understanding of neck evolution across vertebrates because only groups with fixed vertebral counts are being researched (Randau and Goswami 2018; Arnold, Esteve-Altava, and Fischer 2017; Jones et al. 2018). The apparent lack of intrinsic constraints imposed upon the avian neck in tandem with its heightened role in ecological endeavours due to the neck acting as a surrogate forelimb makes the avian cervical column an ideal system to study the adaptability of the vertebrate neck.

Axial regions can be further split into subregions and the boundaries between them are delineated by *Hox* gene expression limits (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015a). These *Hox* gene expression limits have been studied in the cervical column of birds and previous literature has documented that five cervical regions may be conserved across Aves (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015a). One of these studies (Böhmer, Rauhut, and Wörheide 2015b) used 3D geometric morphometrics (GMM) to delineate cervical regions in *G. g. domesticus* and found that GMM recovers the same cervical region boundaries that are recovered by *Hox* gene expression limits (Böhmer, Rauhut, and Wörheide 2015a). With this, the single largest roadblock in the study of avian cervical variation has been lifted. As these cervical regions appear to be conserved across Aves, interspecific comparisons of regional vertebral shape change are valid as there is underlying homology between the same regions in different species. However this previous study was restricted to one domesticated species, and this thesis aims to test this methodology more rigorously. If this methodology holds true, no longer are studies hampered by differences in cervical counts, and by utilising GMM as a proxy for *Hox* gene expression limits, the methodology is both efficient and affordable. Using regional vertebral shape as a metric of study, this thesis seeks to understand the factors that affect vertebral morphology across the avian cervical column. By utilising recent techniques that allow 3D shape data to be incorporated into rigorous statistical models (Adams 2014; Adams and Collyer 2009; Collyer and Adams 2013) this thesis aims to understand how both ecological factors (diet and locomotory mode) and scaling factors (body mass, neck length and head mass) govern variation in cervical morphology and musculature across Aves. This over-arching aim will be pursued through a number of specific objectives:

1. To test previous hypotheses concerning patterns of cervical regionalisation within extant Aves.
2. To quantify factors that affect variation in neck length and head mass across extant Aves.
3. To quantify factors that affect variation in regional vertebral morphology and region length in the cervical column of extant Aves.
4. To quantify factors affecting variation in cervical muscle architecture in extant Aves and to determine if this variation is linked to variation in regional vertebral morphology.

The layout of this thesis will be as follows. Immediately following this chapter (Chapter 2) will be a summary of relevant literature to date and will review regionalisation, methods to study regionalisation and shape variation, as well as a general introduction to the avian cervical column as a musculoskeletal system. Following this, each of the four objectives above will be addressed in individual data chapters (Chapters 3-6). Each of these data chapters will be formatted as standalone manuscript with a self-contained introduction and discussion. A discussion chapter (Chapter 7) will follow these four data chapters and will provide an overarching analysis on patterns of morphological variation and regionalisation within the avian cervical column as well as comparing results and methodologies to other recent studies. The final chapter (Chapter 8) will provide conclusions for all the major findings of the thesis and will comment on future studies. An appendix is provided in electronic form which is contained within a USB flash drive and is attached to the insert at the back of physical copies of this thesis.

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Chapter 2: Background

This thesis investigates the regionalisation and morphology of the avian cervical column. In this chapter I aim to provide a general background into the main research themes present throughout this thesis and it is split into 3 sections. The first section reviews existing research on regionalisation in the axial column and its underlying genetics, as well as the methodology best suited for its study, geometric morphometrics. The last portion of the first section reviews avian neck length allometry. The second section includes a general introduction to musculoskeletal biomechanics and then provides an overview of the kinematics that are specific the avian cervical column. The final section reviews the musculoskeletal anatomy of the avian cervical spine.

The vertebrate axial column and regionalisation

By providing flexibility across the length of the body and attachment sites that serve to anchor the appendicular skeleton, the axial column underpins almost all locomotion that vertebrates undertake (Slijper 1942; Long et al. 1997; Shapiro 1995; Pierce, Clack, and Hutchinson 2011). The axial column is comprised of multiple repeated anatomical units, vertebrae (and dorsal ribs), the number of which are determined by somitogenesis (Dequéant and Pourquié 2008; Dubrulle and Pourquié 2002; Tam et al. 1999). Somites are segmental units that form either side of the neural tube and notochord from the presomitic mesoderm during development (Richardson et al. 1998; Gomez et al. 2008) and somitogenesis is the periodic formation of somites from the presomitic mesoderm. This periodicity is controlled by a molecular "segmentation clock" (Dequéant and Pourquié 2008; Pourquié 2003). Depending upon the speed of the "segmentation clock", more or less somites will be formed during somitogenesis (Gomez et al. 2008). The clock's speed varies between vertebrate lineages, leading to meristic changes (changes in the number of vertebrae, otherwise called the vertebral formula) throughout Vertebrata (Gomez et al. 2008; Barrett et al. 2010).

The morphology of individual vertebrae is controlled by sequential *Hox* gene expression in the somitic mesoderm (Pourquié 2003; Dequéant and Pourquié 2008; Gomez et al. 2008; Wellik 2007). Multiple overlapping *Hox* genes are expressed throughout development which leads to multiple vertebral morphologies within a single vertebral column (Pourquié 2003; Dequéant and Pourquié 2008; Gomez et al. 2008; Wellik 2007). The expression limits of these *Hox* genes denote boundaries between groups of vertebrae with distinct morphologies (Fig. 1), and are common to many amniotes, despite a disparity in vertebral counts (Burke et al. 1995; Burke and Nowicki 2001; Wellik and Capecchi 2003). This creates a regionalised vertebral column which consists of multiple regions of morphologically distinct vertebrae (Fig. 1). The axial skeleton of amniotes is split into 4 distinct regions: cervical, dorsal, sacral and caudal. Discrete morphological units of vertebrae within the axial column allow it to perform a variety of functions; for example; cervical vertebrae have adapted for increased flexion to provide large head movements whilst sacral vertebrae are often fused to aid in load bearing.

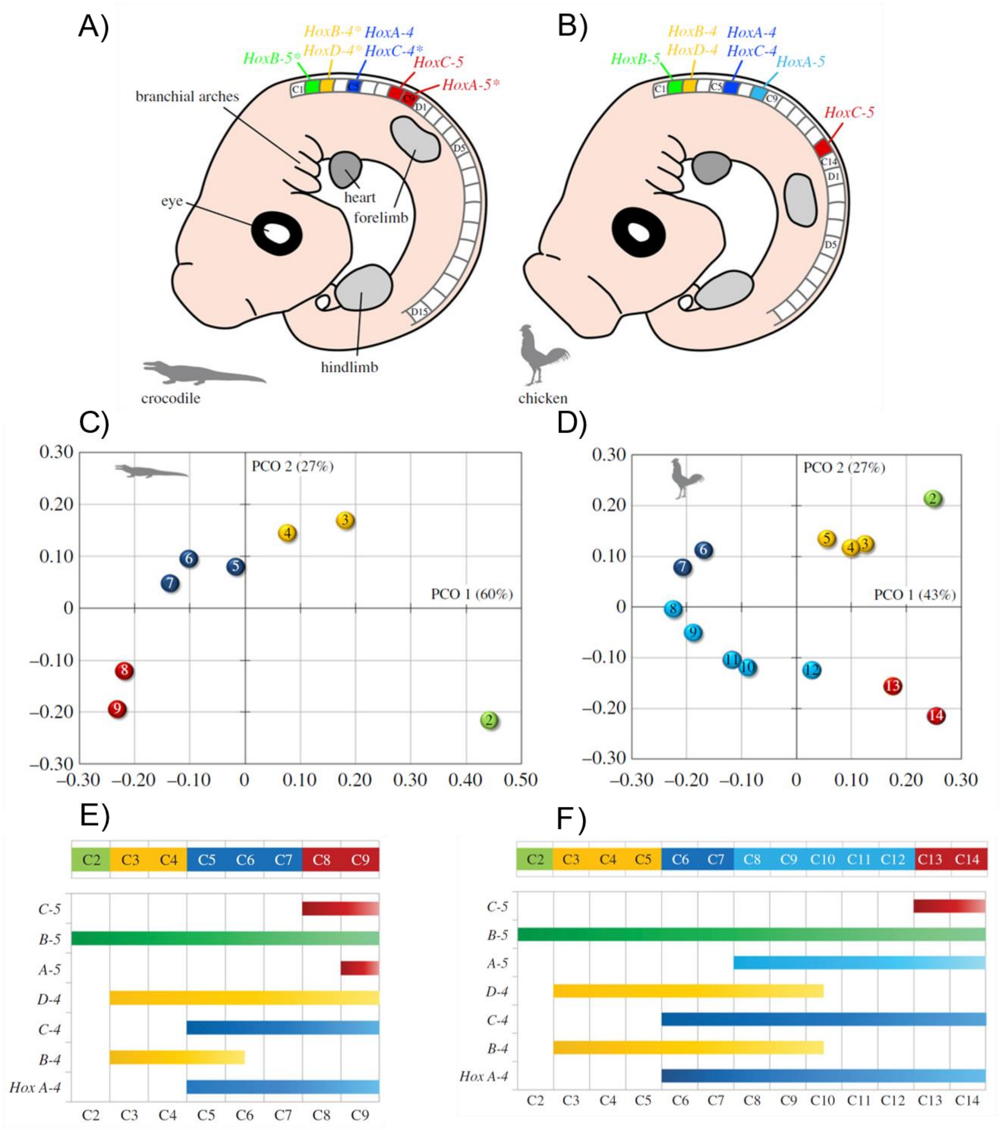

Figure 1 Hox gene expression limits within the cervical columns of an embryonic crocodile (*Crocodylus niloticus*, A) and chicken (*Gallus gallus domesticus*, B). These same regional boundaries are recovered when 3D geometric morphometric analysis is carried out on either species (C, D) or when Hox gene expression limits are studied directly (E, F). Modified from Böhmer et al. (2015).

Many vertebrate lineages have key traits that underpin the success of the clade, and in many clades shifts in regional morphology and/or vertebral count over evolutionary time can be associated with these innovations. The stabilisation of the number of cervical vertebrae in mammals is associated with the muscularisation of the diaphragm (Buchholtz et al. 2012), whilst an increase in vertebrae independent from primaxial *Hox* boundaries gave rise to limblessness in snakes (Head and Polly 2015). Despite this fundamental importance few studies have investigated variation in axial morphology and function, with many studies focusing on cranial and appendicular elements for their more apparent role in feeding and locomotion respectively (Pierce, Angielczyk, and Rayfield 2008; Pierce, Angielczyk, and Rayfield 2009; Walmsley et al. 2013; Alvarez, Ercoli, and Prevosti 2013; Drake and Klingenberg 2010; Meachen, O'Keefe, and Sadleir 2014). Some recent work has provided insight into the large role of adaptations to the axial column in important shifts to the vertebrate body plan (Jones et al. 2018; Böhmer, Rauhut, and Wörheide 2015a; Arnold, Esteve-Altava, and Fischer 2017; Randau, Cuff, et al. 2016). However much more work is needed to understand the factors affecting the evolution, variation and function of the axial column.

Morphological differences may also exist between vertebrae within the same axial region as a single axial region can perform many tasks. The cervical column is a clear example of this 'subregionalisation' (Fig. 1); cranial vertebrae support the head, middle vertebrae must provide a higher degree of flexion to move the head and neck, and caudal vertebrae must provide support via strong musculature and tendons/ligaments (Boas 1929; Boumans, Krings, and Wagner 2015; Zweers, Bout, and Heidweiller 1994; Van der Leeuw, Bout, and Zweers 2001). These 'subregions' can now been formally identified with advanced techniques that can associate *Hox* gene expression limits with specific vertebrae (Fig. 1) (Burke et al. 1995; Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015b). Whilst adding more complexity to the study of axial regions, the problem of regionalisation becomes more granular; factors influencing the evolution of vertebral phenotype can be understood in the context of how they also effect the genotype (specific *Hox* gene expression sites associated with individual vertebrae, Fig. 1a, b).

Recent improvements to gene expression identification techniques and the widespread use of quantitative shape analysis (geometric morphometrics) has led to axial regionalisation becoming a burgeoning topic within functional morphology (Böhmer, Rauhut, and Wörheide 2015b; Randau, Cuff, et al. 2016; Arnold, Esteve-Altava, and Fischer 2017; Jones et al. 2018). Current work suggests that variation in vertebral morphology and formulae within subregions display links to the success and diversification of disparate vertebrate clades (Böhmer, Rauhut, and Wörheide 2015b; Randau, Cuff, et al. 2016; Jones et al. 2018). For example, different subregions may undergo different selection pressures in felids (Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016), whilst morphological adaptations in specific dorsal regions may have facilitated species of varanid lizards to become aquatic (Burnell, Collins, and Young 2012). Due to the expensive nature of genetic analysis this contemporary work relies largely on geometric morphometrics to study *Hox* expression underlying axial regions indirectly. Recent morphometrics data has suggested that GMM can be used to study cervical regionalisation in extant birds and correctly predicts *Hox* gene expression boundaries (Böhmer, Rauhut, and Wörheide 2015a, see below for a thorough explanation). However this work has only been carried out on one species and more work is needed to fully understand the variation in patterns of avian cervicalisation. By understanding this variation, we can begin to understand how external factors influence the underlying genetic patterning of the avian cervical column.

The avian cervical column: a model for regionalisation

The evolutionary transition from large terrestrial ancestors (dinosaurs) has resulted in the acquisition of many features that allowed birds to become such a successful vertebrate clade; extensive postcranial skeletal pneumaticity, a short rigid trunk, and forelimbs primarily used for flight (Gans 1992). With such heavily modified forelimbs it has been hypothesised that the avian neck has evolved to position the head in various ways as a substitute for the forelimbs' inability to manipulate their surroundings effectively (Kuroda 1962; Starck

1978; Clarke and Middleton 2008). Birds display an enormous amount of variation in cervical number and morphology, and it is this role as a 'surrogate arm' that is cited (qualitatively) as the cause of this variation (Kuroda 1962; Starck 1978; Clarke and Middleton 2008). This 'surrogate arm' hypothesis is not necessarily restricted to birds as this phenomenon is observed to a lesser degree in quadrupedal vertebrates whose forelimbs are adapted to terrestrial locomotion. For example; various species of mammal have adapted their cervical morphology to accommodate unique browsing strategies (e.g. gerenuks, Gunji & Endo 2019), defensive strategies involving the neck itself (as in giraffes) as well as morphological adaptations of the neck to support different male combat behaviours in bovids (Vander Linden & Dumont 2019). Avians potentially represent an extreme example of the 'surrogate arm' hypothesis amongst extant vertebrates as they display a wider diversity of cervical morphologies, neck length and cervical count owing to the fewer biomechanical and developmental restraints placed upon the avian cervical column (Galis 1999; Buchholtz et al. 2012; Hirasawa, Fujimoto, and Kuratani 2016; Buchholtz 2014; Galis and Metz 2003; Arnold, Amson, and Fischer 2017), this thesis aims to observe the extent to which morphological characteristics correlate with external factors and indeed if this hypothesis is true.

A tripartite pattern of cervical regionalisation is common amongst many vertebrates and can be found in birds when gross vertebral anatomy and patterns of cervical flexion are examined (Boas 1929; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013; Boumans, Krings, and Wagner 2015; Krings et al. 2017; Kambic, Biewener, and Pierce 2017). This scheme partitions the avian cervical column into 3 regions: cranial, medial and caudal. Intervertebral range of motion varies between these regions with the cranial region displaying average levels of both dorsoventral and lateral flexion, the medial region accommodates high dorsoventral flexion whilst the caudal region has average levels of dorsoventral flexion and the highest display of lateroflexion (Boas 1929; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013; Kambic, Biewener, and Pierce 2017). Partitioning of intervertebral range of motion leads to functional partitioning of the neck; the cranial region is associated with providing stability and accuracy for the head and it’s positioning, the medial region is responsible for moving the head and neck to ground-level and the caudal region provides large lateral movements of the entire cervical column (e.g. for use in preening) (Boas 1929; Zweers, Bout, and Heidweiller 1994; Bout 1997; Van der Leeuw, Bout, and Zweers 2001; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013). Tripartitioning of the avian cervical column is widely supported by studies focusing on the flexion properties of the avian neck (Boas 1929; Christian and Dzemski 2007; Cobley, Rayfield, and Barrett 2013; Krings et al. 2014, 2017; Kambic, Biewener, and Pierce 2017). However this only considers the subset of vertebral anatomy that is associated with range of motion, namely centrum length and morphology of the zygapophyses. The number of cervical regions recovered when more features of anatomy are considered grows from 3 to 5, 6 or even 7, creating differences between methods of study (range of motion versus traditional morphometrics) (Boas 1929; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013; Guinard et al. 2010) If the number of regions is found to vary amongst birds, it would represent the first case of cervical region number variation in Vertebrata.

As outlined above, vertebral morphology and identity is controlled by *Hox* gene patterning and by the position of any particular somite (vertebra precursor) along the anterioposterior body axis. Regionalisation is controlled by the genotype and thus to resolve this dichotomy the genetic basis of avian cervical regionalisation must be studied. In extant avians patterns of *Hox* gene expression have been studied for 1 species only: *Gallus gallus domesticus* (Fig. 1b) (Burke et al. 1995; Böhmer, Rauhut, and Wörheide 2015a) and have found that the expression of *Hox A-4, B-4,C-4, D-4, A-5, B-5,* and *C-5* are responsible for creating the boundaries of 5 cervical regions (Fig. 1b, f). *G. g. domesticus* has 14 cervical vertebrae and the borders between the regions are determined by anterior expression limits of the aforementioned *Hox* genes. The first cervical vertebrae (C1, atlas) is not included in these studies as it displays very few features of anatomy which are homologous with other cervical vertebrae

(Böhmer, Rauhut, and Wörheide 2015a; Baumel, Evans, and Berge 1993). Region 1 is formed of just cervical vertebrae 2 (C2) and is delineated by the anterior expression limit of *Hox B-4* and *D-4* (Fig. 1b, f). Region 2 consists of the next 3 vertebrae, C3-5, the posterior limit of which are denoted by the anterior expression limits of *Hox A-4* and *C-4* which fall at the level of C5 (Fig. 1 b, f). The anterior expression limit of *Hox A-5* is at the level of C7 and creates the third cervical region consisting of C6 and 7 (Fig. 1 b, f). Region 4 is the largest cervical region and contains C8-C12 and the posterior boundary is formed of the *Hox C-5* anterior expression limit at the level of (Fig. 1b, f). The fifth and final region is composed of C13 and C14 and is separated from the thoracic vertebrae via the anterior expression limits of *Hox C-6* (Fig. 1b, f).

Recent work using three-dimensional geometric morphometrics (GMM, Fig 1d) has also recovered five cervical regions in *Gallus* and the boundaries between regions match those presented by previous *Hox* expression limits (Böhmer, Rauhut, and Wörheide 2010, 2015a, 2015b). This has led to the proposal

(Böhmer, Rauhut, and Wörheide 2015a) that 3D geometric morphometrics can be used as a proxy when studying the number and size of axial regions in extant birds (Fig. 1). This strong link between morphology (via GMM) and *Hox* gene expression provides the basis of this thesis as now the large amount variation in cervical morphology across extant Aves can be studied within the framework of cervical regionalisation. Cervical *Hox* gene boundaries have also been studied in crocodilians (Mansfield and Abzhanov 2010) which are, along with birds, the only extant members of Archosaurs (Fig. 1a, c, e). Crocodilians have a much stricter axial formula than birds, rarely deviating from 9 cervical vertebrae and subsequently it has been found that they possess only 4 cervical regions (Fig. 1a, c, e) (Böhmer, Rauhut, and Wörheide 2010, 2015a, 2015b). Using the geometric morphometric framework that will be used in this thesis, previous authors have postured that the early sauropodomorph *Plateosaurus* had 4 cervical regions and have thus hypothesised that the crocodilian condition of 4 cervical regions is the ancestral condition for Archosauria (Böhmer, Rauhut, and Wörheide 2015a). The same study postulates that regions 1, 2, 3 and 5 are homologous across Archosauria and that avian region

4 is an evolutionary novelty unique to modern birds (Fig. 1) (Böhmer, Rauhut, and Wörheide 2015a). By analysing the factors that affect variation in the shape of avian region 4 relative to other regions we can begin to understand the evolutionary impetus behind the emergence of region 4 in modern birds.

Geometric morphometrics: a quantitative proxy for regionalisation

Hox genotypes underlie axial regionalisation in that they control the overall morphology of any given vertebra (Fig. 1) (Burke et al. 1995; Burke and Nowicki 2001; Wellik 2007). Conventional methods of studying regionalisation of the avian cervical column observe patterns of intervertebral joint angles whereby the vertebral anatomy being analysed is restricted to zygapophyseal shape and centrum length (Boas 1929; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013; Krings et al. 2017). To gain a deeper understanding of how external factors may correlate with both morphological regionalisation and the underlying *Hox* genotype a more complete representation of vertebral shape is required. Quantitative assessment of biological shape variation is the definition of morphometrics, and until recently was largely restricted to studies of linear measurements of a particular biological shape (Slijper 1946; Shapiro 1995; Koob and Long 2000). Linear measurements are not inherently multidimensional and may fail to accurately describe three-dimensional shape. As such, traditional morphometrics has been superseded by a landmark based approach. Each landmark is described with a set of x, y and z coordinates (otherwise known as a Cartesian coordinate system) and when multiple landmarks are placed on anatomically discrete loci across an object, the shape of that object can be described in three dimensions (Bookstein 1991). This more comprehensive representation of three-dimensional space makes geometric morphometrics a more suitable choice to study regionalisation when compared to analysing patterns of cervical intervertebral joint patterns (Guinard et al. 2010; Böhmer, Rauhut, and Wörheide 2015a; Kambic, Biewener, and Pierce 2017).

Acquiring a landmark configuration is the foundation of all geometric morphometric analyses and as such landmarks must be chosen with care. The total configuration of landmarks must be representative of the shape being described, whilst individual landmarks must be anatomically discrete and homologous across the entire sample. Landmarks can be classified into 3 categories based on their anatomical definition (Bookstein 1991). Type 1 landmarks are those placed at a meeting point of 2 or more biological structures (such as sutures), type 2 landmarks are defined by local minima or maxima of a structure, for example the dorsal most point of a neural spine or at the tip of a tooth. Type 3 landmarks can be either points of global maxima or minima (e.g. the dorsal/ventral/lateral-most point of anatomy) or located in the middle of two other landmarks (Zelditch, Swiderski, and Sheets 2012). Type 1 and 2 landmarks are preferred as the homology they represent is more apparent than that presented by type 3 landmark and as such, only types 1 and 2 are used throughout this thesis.

Variation in shape can be calculated after landmark data is collected for all samples in the study, but at this stage of the analysis shape differences are compounded by differences in form. Shape has a specific definition, it is "… all the geometrical information that remains when location, scale and rotational effects are filtered out from an object" (Kendall 1977). This definition has led to the creation of a tool which is the cornerstone of geometric morphometrics: Procrustes superimposition (Fig. 2) (Rohlf 1990; Rohlf and Slice 1990; Bookstein 1991). This technique consists of 3 stages and the end result is the isolation of shape data from effects of location, rotation and scale (Rohlf 1990; Rohlf and Slice 1990; Bookstein 1991). Location is normalised by ensuring all landmark configurations originate at a central point (Fig. 2). This is done by subtracting the centroid (landmark configuration mean) coordinates from the coordinates of the initial landmark configuration (Fig. 2). Centroid size can then be calculated as the squared root of the summed squared distances of each landmark to the central origin. Scale is normalised by dividing each landmark by the centroid size for its landmark coordination (Fig. 2a) (Zelditch, Swiderski, and Sheets 2012; Adams, Rohlf, and Slice 2013). Finally landmark configurations are rotated relative to one another to minimise the distance between homologous landmarks (Fig. 2c). This produces a set of Procrustes coordinates whereby the only difference between each landmark configuration is that solely of shape (Fig. 2) (Rohlf 1990; Rohlf and Slice 1990; Bookstein 1991). It is these Procrustes Coordinates that are used to perform further analysis.

Assessing the shape variation within 3D data is cumbersome as this variation is multidimensional and difficult to visualise using 2D graphic aids. Recent advancements to geometric morphometrics have allowed for 3D shape variation to be easily visualised in two dimensions and now high dimensional

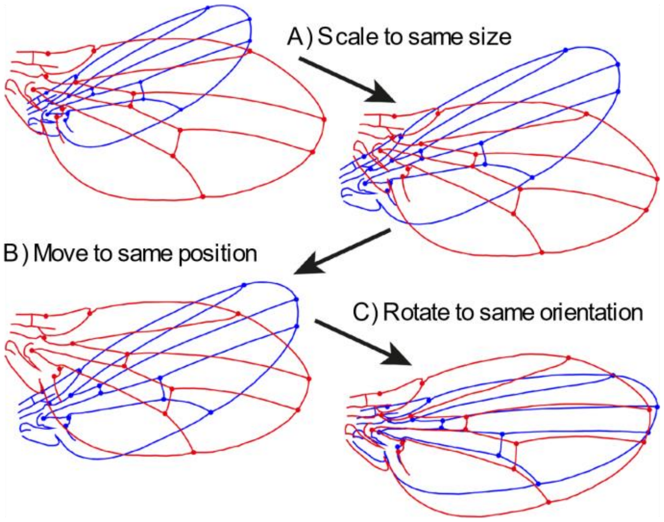

Figure 2. Visual simplification of the 3 processes involved in Procrustes superimposition. Landmark data is scaled to the same size (A), then is shifted to originate at a fixed position (B). The final step involves rotation of all samples to the same orientation (C). Modified from Klingenberg (2015).

data can be readily incorporated into robust statistical models. Principal Components Analysis (PCA) is often used as an initial assessment of shape variation within a dataset as it reduces this dimensionality by calculating the 2 axes on which the majority of shape variation occurs. Each individual landmark configuration is represented as a single point on a set of principal component (PC) axes and the scatter of PC plot represents the variation in shape of the dataset. By representing the 2 largest sources of morphological variation within a dataset, a PC graph can be used to quickly visualise the variation of large datasets of 3D shape data. An initial assessment of shape variation via PCA is usually the first of multiple stages in a geometric morphometrics study, and further work often incorporates this 3D shape data into a statistical model to observe the effects of external variables on shape variation. Often this is performing either a multivariate ANOVA or a multivariate regression using shape data as the dependent variable (Martin and Maes 1979; Goodall 1991; Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016). However this often causes the number of species (N) to be dwarfed by the number of trait dimensions (p) due to the highly multivariate nature of 3D shape data, rendering these parametric approaches invalid when testing for significance between the dependent and independent variable(s) (Adams 2014). This problem has been overcome by incorporating 3D shape data into phylogenetic least squares models, a technique that is now termed Procrustes-Distance phylogenetic generalised least squares (D-PGLS) (Adams 2014). Within this thesis I have used D-PGLS to assess the relationship between mean regional cervical shape change and external factors. D-PGLS when applied this way tests how these factors affect vertebral shape change within regions, i.e. at a local level (within a specific region).

To assess the impact of these factors on global cervical shape change, shape change variation must be quantified across all five regions simultaneously, and this can be achieved by adapting an approach termed Phenotypic Trajectory Analysis (PTA, Fig. 3) (Adams and Collyer 2009; Collyer and Adams 2013). PTA is a tool that allows for the quantification of phenotypic change across a dataset with multiple levels (for example; time steps, evolutionary events etc.)

(Fig. 3b). These multiple levels have previously been set as evolutionary levels representing multiple time stages over a populations evolutionary history (Adams and Collyer 2009). A trajectory is drawn in multivariate trait space between the levels of a population and this trajectory can be compared to trajectories of other populations according to 3 aspects of trajectory morphology: magnitude, direction and shape (Fig. 3a) (Adams and Collyer 2009; Collyer and Adams 2013). The magnitude (Fig. 3a) of a trajectory is its total path length, and if a population displays an isolated increase in trajectory magnitude compared to another population (i.e. trajectory direction and shape are identical between these two populations) then the former population (the population with the highest increase in pure magnitude) is inferred to have undergone a greater amount of phenotypic evolution (Adams and Collyer 2009; Collyer and Adams 2013). Comparisons of trajectory direction can be used to determine if different populations are undergoing convergence,

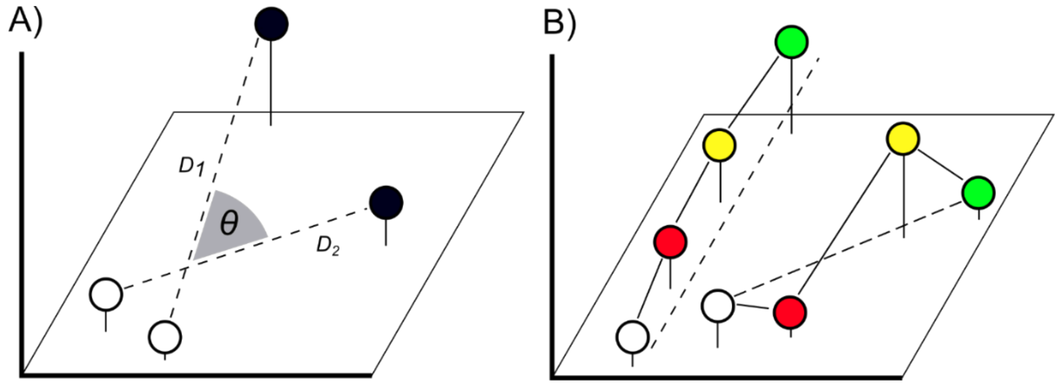

Figure 3. Example of phenotypic trajectory analysis (PTA). A) Two phenotypic vectors between 2 phenotypic stages (white and black dots). Dashed lines D_1 and D_2 denote the magnitude (length) of the vector through phenotype space whilst theta represents direction. B) Displays an example of PTA across multiple phenotypic levels. Dashed line represents the magnitude of the phenotypic change. Based on Adams & Collyer (2009).

divergence or parallelism (Adams and Collyer 2009; Collyer and Adams 2013; Randau, Cuff, et al. 2016).

Trajectory shape is a measure of the complexity of trait evolution across the multiple stages (Fig. 3b). PTA has been recently adapted to observe the effects of external factors on vertebral shape across multiple levels (Randau, Cuff, et al. 2016). These multiple levels are no longer evolutionary stages, but rather specified vertebrae along the vertebral column (Randau, Cuff, et al. 2016) and this methodology will be adapted for this thesis to observe the correlation between ecological factors and the variation of the avian cervical column. Within this thesis each trajectory level is the mean shape of one of the cervical sub-regions and a singular trajectory now represents an ecological group within a larger factor (e.g. terrestrial birds within locomotor type). The trajectory of a single group represents the mean shape change across the entire cervical spine for that group and in this way, trajectory magnitude, direction and shape can be calculated to quantitatively test for ecological differences in shape between different ecological groups.

Allometric scaling in the avian cervical column

Neck length is a hugely diverse trait amongst vertebrates reaching up to 2.4 metres in extant giraffes and well beyond 10 metres in many extinct sauropod dinosaurs (Taylor and Wedel 2013a). There are two predominant factors that contribute to neck length variation in vertebrates: head mass and body mass (Christiansen 1999; McGarrity, Campione, and Evans 2013). The primary function of the vertebrate neck is to safely support the mass of the head and this has given rise to the notion that neck length must decrease with an increase in head mass (Gans 1992; Wilkinson and Ruxton

2012; Christiansen 1999; McGarrity, Campione, and Evans 2013). In mammals this relationship has been quantified and appears to be related to the disparity between the body mass scaling exponents of head mass and neck cross-sectional area (Cardini and Polly 2013; Preuschoft and Klein 2013; Arnold, Amson, and Fischer 2017). Head mass scales with body mass to the power of three however the cross sectional area of the neck only scales with the power of 2, meaning that across Mammalia the length of the neck must be decreased for it to safely resist the load of the head by bringing the mass of the head closer to the animals centre of mass and the fulcrum of the neck (Preuschoft and Klein 2013; Cardini and Polly 2013; Arnold, Amson, and Fischer 2017). Mammalian neck elongation must occur via increases to the lengths of cervical vertebrae as vertebral counts in the cervical spine of mammals are often restricted to seven cervical vertebral due to strict biomechanical and developmental constraints (Arnold, Amson, and Fischer 2017; Galis 1999; Burke et al. 1995; Wellik 2007; Wellik and Capecchi 2003; Jones et al. 2018).

The avian neck displays much more variation in both neck length and counts of cervical vertebrae (Benoit et al. 1950; Van der Leeuw, Bout, and Zweers 2001; Wilkinson and Ruxton 2012) than mammals yet the scaling relationships of neck length and head mass have rarely been studied amongst the clade. It initially appears logical to equate the variation in neck length to the large meristic variation in cervical vertebrae (cervicalisation). Recent work has qualified that across birds there appears much variation in size corrected neck length for any given number of cervical vertebrae (Böhmer et al. 2019). However this relationship was not investigated with any statistical tests. Until the final year of this doctoral program (2019) no literature had quantified the scaling relationships of avian head mass or neck length in a phylogenetically broad group of birds. The small number of studies that had quantified the scaling relationships of head mass and neck length were restricted to ontogenetic studies of a single species (Heidweiller et al. 1992; Heidweiller and Zweers 1992) or allometric studies of a single clade of birds (Anseriformes) (van der Leeuw 2002). These studies suggested that head mass in birds, unlike in mammals, displayed a negative allometric relationship with body mass and that this may be due to the negative allometry displayed in many cranial soft tissues (sensory organs) across birds (Brooke, Hanley, and Laughlin 1999). If this negative scaling of head mass is representative for all birds then it may have implications for the scaling of avian neck length, and recent work has found that neck length scaling may be also different in birds compared to mammals.

This thesis aims to increase our understanding of neck elongation in Aves by quantifying the scaling relationships of both neck length and head mass, and examining the ecological factors that may cause variation in these relationships. The relationship between cervical count and neck length will also be quantified to statistically assess the contribution of cervicalisation to avian neck elongation. As this thesis will use GMM to delineate regions within the cervical column, this data can be utilised to observe the correlation between regionalisation and neck elongation and cervicalisation in extant birds. By studying variation in region lengths and regional vertebral counts this thesis could highlight which regions are responsible for neck elongation and which regions are responsible for increases in meristic changes to the entire cervical column.

Kinematics of the Avian Craniocervical System

Introduction to musculoskeletal kinematics

The musculoskeletal system allows an organism to interact with its environment and the objects within it. Newton's Second Law states that Force (*F*) is the product of mass (m) and acceleration (a). The musculoskeletal system is underpinned by this law with the soft muscular component of this system generating the required forces for movement (Biewener 2003; Nigg and Herzog 2007; Alexander 2003). The rigid skeleton acts as a supportive framework that performs two main functions; protection of internal organs and to act as an attachment site for muscles. This creates a lever system where forces can be transferred over articulations of bones at joints. Muscles generate these forces to perform work which is carried out when they shorten (contract), as work (*W* measured in joules) is the product of force and the change in length of the muscle:

$$W = F \times \delta L$$

The power (P) of a muscle, the work per unit time,

$$P = \mathbf{F \times \delta L/\delta t)}$$

and is positive when a muscle shortens, and contraction is termed isometric when a muscle generates force with little or no change in length. Muscles largely generate tensile (pulling) forces, thus for both flexion and extension to occur around the same joint, a flexor muscles(s) must be accompanied by an antagonistic extensor muscle(s). It must also be noted that passive elastic structures may act as antagonists by storing elastic strain energy as the agonist contracts and then releasing this energy as the agonist relaxes. Such structures are evident in the avian cervical column, and examples include the ligamentum elasticum and ligamentum nuchae (Tsuihiji 2004; Baumel, Evans, and Berge 1993; Dzemski and Christian 2007), which function as energy stores and braces for the cervical column of long-necked members of avians (Alexander 1985; Dzemski and Christian 2007; Tsuihiji 2004; Stevens 1999).

Kinematics of the avian cervical column

With such a vast quantity of cervical muscles and joints supporting the avian head there are a large number of degrees of freedom within the system. This leads to kinematic redundancy whereby there is an enormous set of possible combinations of muscle forces and neck joint angles that can produce any particular head displacement. Kinematic redundancy presents problems for modelling the function of the neck in extinct taxa, especially as many of these taxa have neck lengths unparalleled by any extant terrestrial organism (Taylor and Wedel 2013b). One of these exemplar extinct taxa is the sauropod dinosaurs. The addition of cervical vertebrae to the neck in sauropods allowed for a large feeding envelope whereby even small changes in cervical joint angles can represent a movement of the head over many metres. With such long necks, understanding neck function is integral to the understanding of feeding ecologies (Gans 1992; Wilkinson and Ruxton 2012).

Kinematic redundancy states that there are multiple ways in which the musculoskeletal system of the cervical column can position the head for any particular task. Many of these possibilities, whilst mathematically possible, can be immediately excluded due to biological unfeasibility of large angles between successive vertebrae (Bout 1997). By treating the cervical column as a series of one-dimensional rigid bars previous workers (Bout 1997) have asserted that large angles at a single joint are avoided in the avian neck and that an even distribution of rotation along the entire chain allows for the characteristic resting ‘S-shaped’ curvature seen in most extant avians (Fig. 4).

Classically (Boas 1929) the cervical column is divided into 3 regions that are delineated by both mobility and vertebral morphology, and vary between species and studies (Boas 1929; Boumans, Krings, and Wagner 2015; Krings et al. 2014; Landolt and Zweers 1985). The cranial region allows for predominantly ventral flexion, the medial region accommodates dorsal flexion and the caudal region facilitates both to a restricted degree, with transitions between the regions marked by joints displaying intermediate flexion values (Fig. 4) (van der Leeuw, Bout, and Zweers 2001; Van der Leeuw, Bout, and Zweers 2001). As an example that is commonplace within the literature, the ostrich (*Struthio camelus*) has a cranial region consisting of cervical vertebrae 1-7, the middle region C8-11, and the caudal region C12-16 (Dzemski and Christian 2007). There are discrepancies in the number of regions found when using different methodologies. This is exemplified in the American barn owl (Boumans, Krings, and Wagner 2015) (*Tyto furcata pratincola*) by which an osteology-based approach results in seven subdivisions of the neck (Krings et al. 2014), whilst a myology-based definition produces only 4 subdivisions (Baumel, Evans, and Berge 1993).

The neck in most birds is thought to have evolved in response to the 'economics of continuous movement' rather than to any extreme functions (notable exceptions include the Picidae, woodpeckers, and the Ardeidae, herons) (Van der Leeuw 1992; van der Leeuw, Bout, and Zweers 2001; Van der

Leeuw, Bout, and Zweers 2001). This has led to a similar fundamental design of the avian cervical system, particularly the musculature, across a wide range of species and across a wide range of body masses (Palmgren 1949). However current studies only note the conserved nature of muscle attachment sites and no work has studied quantitative variation in the size and architectural properties, which are determinant factors in functional and contractile behaviour of muscles. Many similarities exist in the number and arrangement of cervical musculature between mallards and chickens (Landolt and Zweers 1985; Zweers, Vanden, and Koppendraier 1986; van der Leeuw, Bout, and Zweers 2001; Van der Leeuw, Bout, and Zweers 2001) and despite broad similarities in the musculoskeletal system of the cervical column, these two species have fundamentally different kinematics. In mallards two waves of vertebral rotation occur that result in a rolling pattern of the rostral curve of the neck (Fig. 4a) whereby the external outline of the curve remains similar despite vertebral movements through the outline of the curve (van der Leeuw, Bout, and Zweers 2001). A lever-like pattern occurs in chickens (Fig. 4b) where some vertebrae in the caudal curve are involved in rotation, and others are kept straight in relation to adjacent vertebrae (Heidweiller et al. 1992). During the majority of cervical movements ratites employ a rolling pattern in the caudal loop of the cervical column due to large vertical head trajectories. However during movements such as pecking (involving a more horizontal head trajectory) the kinematics of the caudal loop in ratites changes to a more chicken-like lever pattern (Fig. 4b) (Van der Leeuw, Bout, and Zweers 2001).

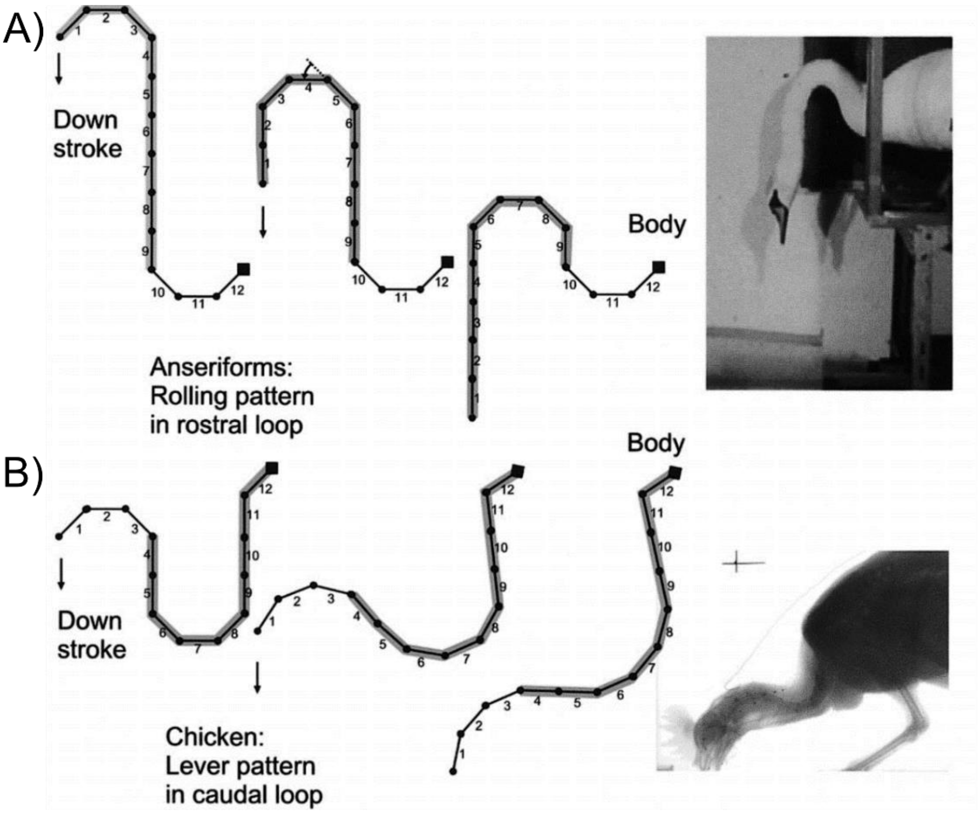

Figure 4. Kinematic patterns of neck movement during pecking and drinking in Anseriforms (A) and chickens (B). The predominant differences between these two kinematic patterns is that Anseriforms (A) utilise a rolling pattern in the rostral portion of the cervical column, whilst chickens utilise a lever-like pattern in the caudal portion of the neck. Adapted from Van der Leeuw et al. (2001).

Despite the broad similarities in anatomy between these taxa, specific differences in both osteology and myology may influence the kinematic patterns of the avian cervical column. The longer first region of the neck in anseriformes may facilitate the rolling pattern, alongside longer individual vertebrae compared to chickens and rheas (van der Leeuw, Bout and Zweers 2001). Differences in the long dorsal neck musculature between the chicken and mallard combined with the large ligaments associated with the rhea may account for the differences seen in the kinematic patterns of the cervical column in these species. However, electromyographical studies show that the control of neck muscles is fundamentally different between chickens and mallards (van der Leeuw, Bout, and Zweers 2001) and that anatomy alone cannot explain the disparity in kinematic patterns. Kinematic patterns are substantially different between terrestrial and aquatic clades of avians, yet despite this many of the feeding mechanisms within aquatic clades (such as anseriformes which can feed on both land and water) still display similar overall movement patterns (Van der Leeuw, Bout, and Zweers 2001). This suggests that the economics of movement does constrain cervical kinematic patterns to a large degree (at least in some birds), and cervical kinematics may only adapt to ecologies that require entirely different kinematic regimes, such as the transition from terrestrial feeding to water feeding (Heidweiller et al. 1992; van der Leeuw, Bout, and Zweers 2001; Van der Leeuw, Bout, and Zweers 2001). The lack of variability in the cervical kinematics within the various feeding mechanisms displayed within anseriformes may suggest that the cervical system is versatile and highly flexible (Van der Leeuw, Bout, and Zweers 2001).

Changes to cervical kinematics have been documented over ontogeny (Heidweiller and Zweers 1992; Heidweiller et al. 1992). The increase in size of the oropharynx through ontogeny influences cervical kinematic schemes in domestic chickens (Heidweiller and Zweers 1992). Differences in motion patterns are also present between anatids of differing size (Van der Leeuw 1992), and alongside changes caused by ontogeny it can be hypothesised that scaling effects can determine kinematic patterns in birds. The kinematics of the cervical column in Galloanserae (Prum et al. 2015) have been widely studied, but within Neoaves (Hackett et al. 2008) there are many different behaviours that involve the neck, specifically during feeding. Extant raptors, for example, consume live prey whilst restraining it with their hindlimbs. Using videography the six stage feeding mechanism that raptorial birds employ has allowed cervical movements to be linked to gross body kinematics (Snively et al. 2014). The stages of this mechanism are as follows (Snively et al. 2014): The first stage involves the sighting of the prey, in which the head is ventroflexed. This is followed by the pre-strike stage: the head is raised by raising the trunk relative to the femur or by extending the posterior curve of the cervical column whilst flexing the head further ventrally. The third stage ('pecking') involves the extension of both anterior and posterior curves of the neck, as well as the lowering of the trunk relative to the femur to bring the head closer to prey. Stage four involves contact of the beak with the prey (initial biting), whereby the anterior portion of the neck moves to position the head in the appropriate orientation to engage with the prey. The trunk is then raised relative to the femur and the posterior curvature of the neck is dorsiflexed during the fifth 'pull' stage. Finally, the anterior portion of the neck is dorsiflexed, this raises the head and shifts the gaze away from the prey. There are interspecific variations between raptors, predominantly on the basis of varying levels of leg involvement in the stages leading up to the fifth pull stage.

Whilst initially seeming complex due to kinematic redundancy and an especially intricate musculoskeletal system, the adaptation of the avian neck to the economics of movement has ensured that the kinematics of the cervical column are somewhat similar between large groups of extant avians. This would suggest that in extinct avians, such as dinosaurs, that kinematic patterns within clades of considerable size may be similar, and only change under an extensive change in the economics of movement.

Anatomy of the avian cervical column

Introduction to animal bone and avian cervical vertebrae osteology

Animal bone consists of two primary constituents: collagen and the mineral hydroxyapatite. The proportions (between 63% and 70% hydroxyapatite) of which have been limited by natural selection to balance stiffness (ability to resist deformation under force) and strength (the stress needed to cause a material to break) (Currey 2002). Individual cervical vertebrae can vary in size and morphology which reflects their position in the cervical column, the size and morphology of the vertebrae are integral to the flexibility of the neck (Van der Leeuw 1992; Stevens 1999; Tambussi et al. 2012; Cobley, Rayfield, and Barrett 2013). Soft tissues play a significant determinant role in the mobility of the avian neck (Cobley, Rayfield, and Barrett 2013; Hutson and Hutson 2012; Hutson and Hutson 2013; Hutson and Hutson 2014; Dzemski and Christian 2007) and thus osteology should not be considered in isolation. The neck has been historically split into three regions (Boas 1929) often delineated using vertebral morphology and intervertebral joint motion, with more (up to seven) regions documented in extreme examples (Boumans, Krings, and Wagner 2015). The cervical column begins at the interface between the atlas (the first cervical vertebrae) and the occipital condyle, and transitions to the thoracic component of the vertebral column with the acquisition of true ribs (not fused with the transverse process, as is the case for cervical ribs).

Components common to many cervical vertebrae are the vertebral body (corpus vertebrae or centrum) and the vertebral arch (arcus vertebrae) which both surround the vertebral canal (foramen vertebrale) that protects the spinal column (Fig. 5a). Two further canals lie lateral to the foramen vertebrae (one sinistral and one dextral), the foramina transversaria (Fig. 5a). Projecting laterally from the vertebral arch are the transverse processes (processus transversi, Fig. 5a), that may be divided into a diapophysis and a parapophysis in some cervical vertebrae (present in all archosaurs except avians). In between the central vertebral foramen and the transverse processes lie the ansa costotranversaria which lateral culminate into tuberculum ansae (Fig. 5a), of which the number may vary across the cervical column. Slender processes project posteriorly from the transverse processes, these are the cervical ribs (processus costalis, Fig. 5b). Cervical ribs usually do not extend further posteriorly than the ends of the centra, and rib length appears to be correlated with body size (Sanders and Wedel 2002). From the base of the transverse processes emerge the zygapophyses, of which there are two types: anteriorly projecting prezygapophyses (Fig. 5a) and posteriorly projecting postzygapophyses (Fig. 5b). The prezygapophyses of one vertebra articulate with the postzygapophyses of the previous vertebra, and between the postzygapophyses is the lacuna interzygapophysialis, a V-shaped notch. From the dorsal surface of the ramus of the postzygapophyses is an extruding rugosity referred to as the torus dorsalis. Mediodorsally is the neural spine (processus spinosus, Fig. 5a), which in some birds (such as ratites and in extinct relatives, the sauropods) may be bifurcated, and running between the torus dorsalis and neural spine is a low crest (crista transverso-obliqua, Fig. 5b). From the midline of the centrum is ventrally projecting crest is the ventral process (processus ventralis corporis), which is more pronounced in more posteriorly oriented cervical vertebrae.

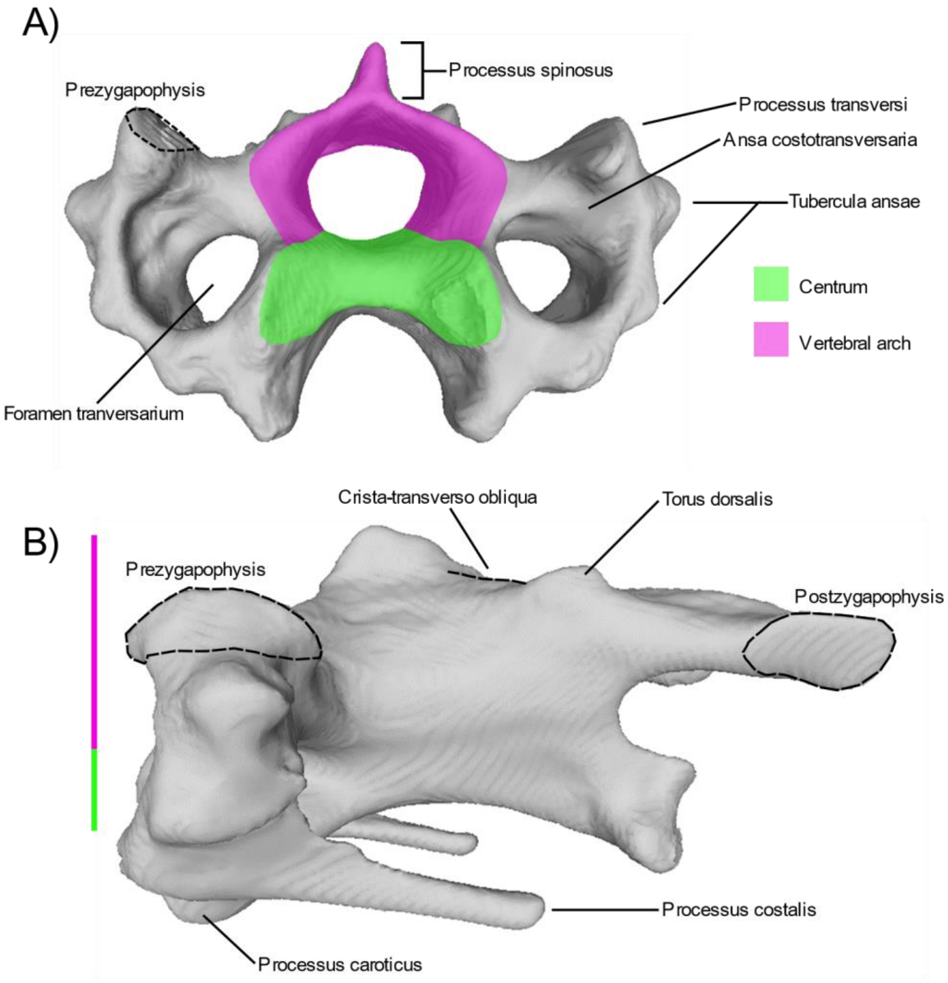

Figure 5. Osteological anatomy of an avian cervical vertebrae (C6 of a red-legged partridge, Alectoris rufa) in anterior view (A) and lateral view (B). Green colouration indicates the extent of the centrum, purple colouration indicates the extent of the vertebral arch.

Transverse processes and neural spines provide the origination sites for the long dorso-lateral muscles of the neck (m. longus colli dorsalis and m. ascendens cervicis) and insert onto the tori dorsalis. The processes carotici (Fig. 5b) and costales are the origination sites for ventral muscles such as m. flexor colli lateralis, m. flexor colli medialis and the m. longus colli ventralis and then insert onto the processes costales and ventrales of the centra. Smaller, often deeper muscle groups such as the mm. intercristales, mm. interspinales and mm. intertransversarii attach and connect the neural spines, foramen transversaria and the crista transverso-obliqua of connecting vertebrae.

Myology

Introduction to vertebrate skeletal muscle

Individual muscle fibres form bundles of fascicles which then group to form the gross morphology of a muscle. Within all muscle fibres are multiple myofibrils that contain an arrangement of proteins common to all vertebrate muscle that allow for the sliding filament model of muscle contraction. It is the arrangement of the myofibrils that create an external patterning for the muscle as a whole, giving it a striated appearance (Fig. 6). This gives rise to the division of myological nomenclature between striated and non-striated muscle. Striated muscle is studied exclusively as part of this thesis as it is this subdivision that skeletal muscle falls within. In skeletal muscle, groups of fibres that are innervated by the same motor neuron form motor units, and these can vary in number of fibres and size.

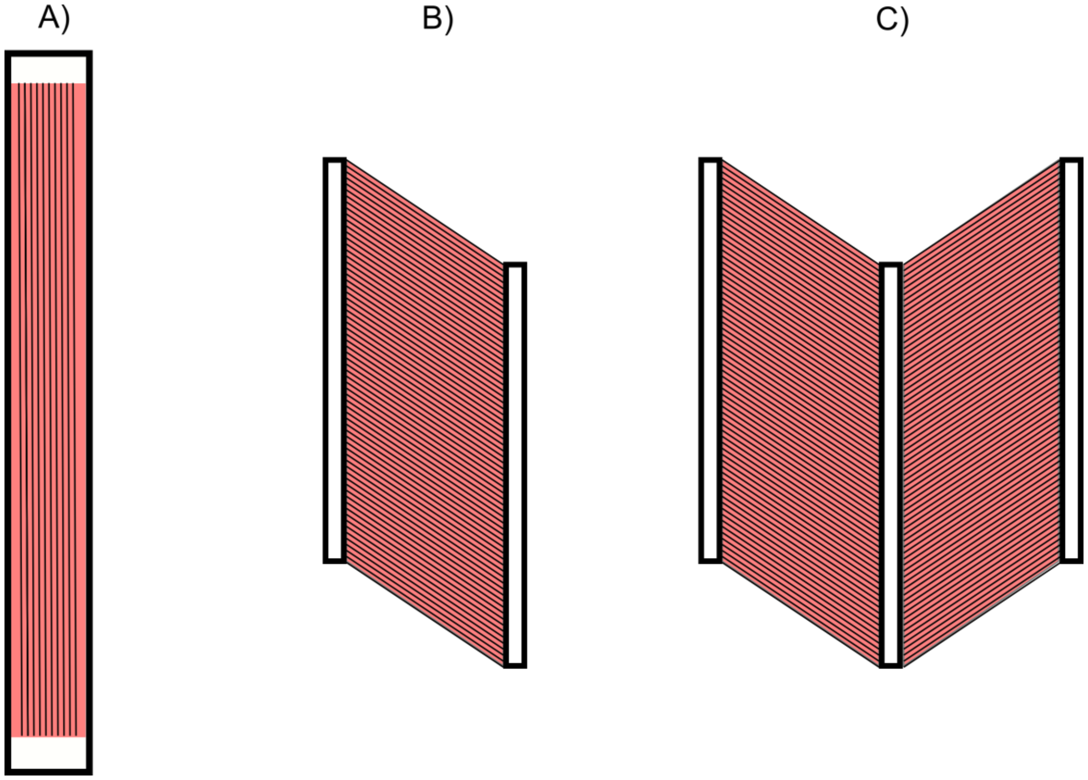

Figure 6. Examples of different vertebrate muscle architecture: A) parallel fibred, B) unipennate and C) bipennate muscles. Adapted from Biewener (2003).

Smaller motor units are formed by slow twitch fibres that contract slowly but over a greater time. Larger motor units are composed of fast twitch fibres that contract more rapidly over a shorter time. Fibre morphology can used to classify muscles into pinnate- and parallel-fibred muscle (Fig. 6). Pinnate muscles (Fig. 6b, c) are more complex in their architecture and are composed of shorter fibres that run at an angle to a muscle’s primary axis of force transmission (Biewener 2003). Pinnate fibres often attach to the skeleton via a tendon and can be subdivided into uni- and bi-pennate muscles (Fig. 6b, c). The fibres of uni-pinnate muscles attach to a distal tendon and are oriented at similar angles in one plane (Fig. 6b), whilst bi-pinnate muscles have two sets of fibres that mirror each other at an angle (Fig. 6c). Parallel-fibres attach directly to the skeleton (Fig. 6a), have longer fibres that are organised so that they lie end-to-end parallel to the axis of force transmission (Nigg and Herzog 2007; Biewener 2003).

The sliding filament theory is common to all striated muscle, thus the cross-sectional area is proportional to a muscle’s force output. A pinnate-fibred muscle is capable of producing a greater force than a parallel-fibred muscle of similar mass due to its larger physiological cross-sectional area (Fig. 6b, c). The longer fibres of parallel-fibred muscles account for this lower force by providing a greater range of shortening. This framework provides a relationship between muscle architecture, contractile properties and force output of a muscle. Alongside the length and architecture of a muscle, the geometry and location of the attachment site of a muscle can also influence muscle shortening, as well as any tendon involved with the attachment site. Tendons are present primarily to attach muscle to bone and can both transmit forces and store elastic energy (Nigg and Herzog 2007; Alexander and Vernon 1975). When tendons stretch and recoil, they can allow for a muscle to shorten at lower velocities, to remain at a constant length and allow the muscle to modulate its energy requirements and force production (Nigg and Herzog 2007; Alexander and Vernon 1975). By providing muscle with an intermediate tissue between itself and bone, tendons allow muscle to be present in specific areas located away from joints, allowing for elaborate movements, such intricate movements are vital in the avian cervical system (Boas 1929; Kuroda 1962; Landolt and Zweers 1985; Dzemski and Christian 2007).

Fundamental properties of muscle contraction involve positive work production during shortening and energy absorption during negative work associated with lengthening. *In vivo* the situation is more complex and is best described in terms of ‘work loops’ (Alexander 2003). Variations *in vivo* between length changes relative to the force production are more dynamic and can be best described observing the fluctuations between these two variables over time. If the force-length relationship can be described by a counter-clockwise loop it represents the positive work during shortening a muscle undertakes during each contraction cycle. Conversely muscle lengthening is represented by a clockwise loop, which details the negative work occurring during each contraction cycle. Clockwise loops are associated with power generating muscles and counter-clockwise loops denote muscles that act as breaks or energy absorbing shunts. Crucial to a muscle’s performance is the timing of its activation relative to the change in its length, as peak efficiency occurs when activation occurs immediately prior to its shortening and lasts through until midway through this shortening phase. With a broad overview to the fundamental architecture and contraction properties, the next section will proceed to discuss the intricacies of extant avian cervical musculature.

Extant archosaur cervical myology

i) Introduction

Muscles of the cervical column serve to position the head (via lateral and sagittal movements) in order to locate sources of stimuli and to acquire food and nourishment (Berthoz, Graf, and Vidal 1992; Schwenk 2000). Cervical musculature is herein defined following the scheme from Boumans et al. (Boumans, Krings, and Wagner 2015), in that any muscle that attaches to a cervical vertebrae is a cervical muscle. The muscles of the avian cervical column can be regionalised by their locations and points of attachment throughout the cervical column (Fig. 7), and four myological regions have long been established (Boas 1929), these are: 1) Mm. craniocervicales; 2) Mm. cervicales dorsales; 3) Mm. cervicales laterales and 4) Mm. cervicales ventrales. For individual avian muscles, nomenclature shall follow the scheme presented in

Nomina Anatomica Avium (Baumel, Evans, and Berge 1993). As it lacks a thorough grounding in homology, the myological regions present in Boumans et al. (Boumans, Krings, and Wagner 2015) shall not be used here, and instead divisions shall be based on inferences of serial homology and patterns of innervation (Tsuihiji 2005, 2007, 2010). When homology and patterns of innervation are considered, 4 muscle regions can be defined: the transversospinalis, longissimus, iliocostalis and longus systems respectively. Transversospinalis muscles are located dorsolaterally, aiding with dorsal and lateral flexion. Muscles associated with the longissimus system are involved in lateral and some dorsal movement of the neck and are positioned laterally. The longus and iliocostalis muscles assist in ventrolateral flexion and form the ventral most portion of the cervical musculature.

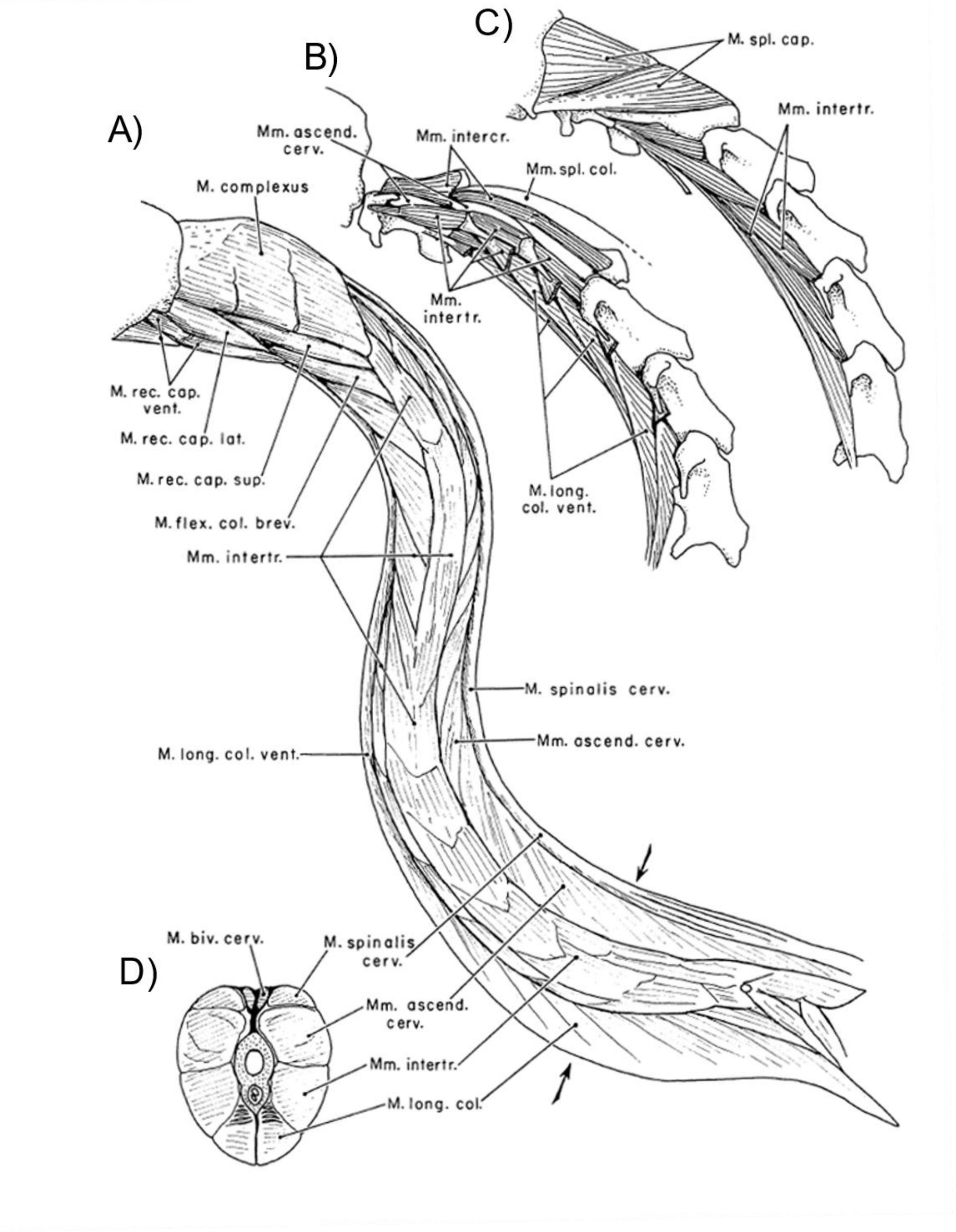

Figure 7. Overview of avian cervical myology (in *Podilymbus podiceps*). A) Superficial musculature in left lateral view. B) Cranially positioned deep ventral musculature. C) Cranially positioned deep dorsal musculature. D) Caudally positioned deep musculature. Modified from Zusi & Storer (1969).

ii) M. tranverspinalis system

M. biventer cervicis

Discounting the sites of origin and insertion, the M. biventer cervicis (Fig. 7d, 8f) is the most dorsally located muscle within the avian cervical column. It is elongate and thin, bisected and connected by a tendon (intersectio tendinea Fig. 8f) that creates two parallel fusiform bellies. Both bellies originate tendinously and caudally from the spinous processus of the posteriormost cervical vertebrae and rostrally from a connecting tendon that covers the third to the ninth cervical vertebrae (Fig. 11) (Jenni 1981; Snively and Russell 2007; Zusi and Bentz 1984; Lautenschlager, Bright, and Rayfield 2014). A degree of interspecies variation can be observed in terms of the degree of separation the rostral belly experiences with the immediately lateral M. complexus. In larger birds (*Aquila chryseatos, Pelicanus occidentalis* and *Struthio camelus* (Snively and Russell 2007)) it is discernible from M. complexus, but must be dissected free of it in the case of some smaller birds (such as *Pica pica* (Snively and Russell

2007)). The presence of the M. biventer cervicis can also vary and in some cases can be absent entirely (*Plotus anhinga, Phalacrocorax harrisi,* and *Ardea comerea* (Boas 1929; Kuroda 1962; Boumans, Krings, and Wagner 2015)). Homologous to the M. biventer cervicis is the medial portion of the M. transversospinalis capitis (Seidel 1978; Tsuihiji 2005) in extant crocodilians. M. transversospinalis capitis shares many similarities with its avian homologue, including a comparable tendinous insertion onto the dorsomedial portion of the occiput and dorsal placement above other cervical muscles. Variations from the avian morphology include the absence of an intersectio tendinea, and an origin divided into lateral and medial sections. The lateral portion originates from a fascia on the neural spines which is likely homologous to the aponeurotic origin found in some birds (Tsuihiji 2004, 2005, 2007, 2010; Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). Medially the origin is divided into multiple slips spanning the dorsal tips of the second to the ninth cervical vertebrae (Cleuren and de Vree 2000). The dorsal insertion on the occipital condyle suggests the M. biventer cervicis and M. transversospinalis capitis is involved in dorsiflexion of the head relative to the level of the posterior cervical vertebrae (Frey 1988; Burton 1974) and to extend the head relative to the trunk (Kaupp 1918). A lesser involvement in lateral flexion (Cleuren and de Vree 2000) has also been hypothesised. Electromyography has confirmed the participation of the M. transversospinalis capitis in head-neck dorsiflexion (Cleuren and de Vree 2000).

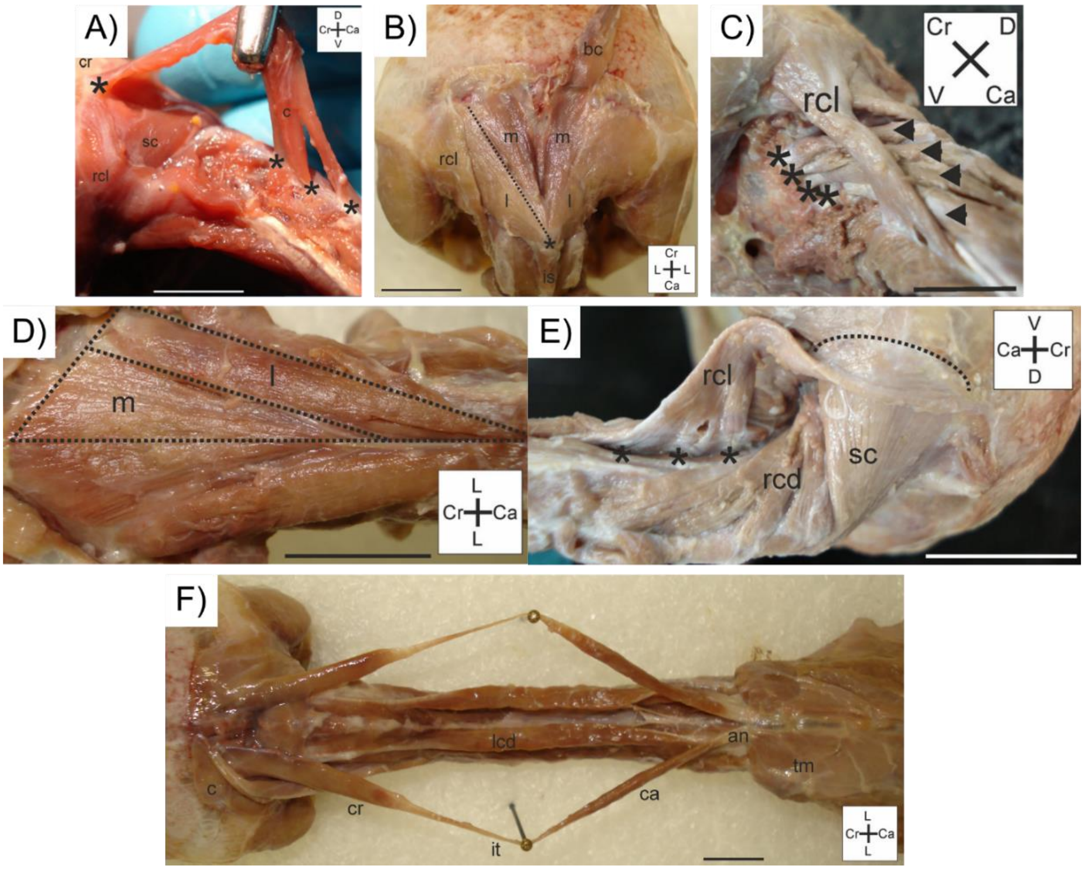

Figure 8. Cranially positioned muscles as found in *Tyto furcata pratincola*: A) M. complexus in left lateral view, B) M. splenius capitis in dorsal view, C) M. rectus capitis dorsalis in left lateral view, D) M. rectus capitis ventralis in ventral view, E) M. rectus capitis lateralis in left lateral view, F) M. biventer cervicis in dorsal view. Abbreviations: cr = cranium, c = M. complexus, sc = M. splenius capitis, rcl = M. rectus capitis lateralis, m = pars medialis, l = pars lateralis, bc = M. biventer cervicis, rcd = M. rectus capitis dorsalis, cr (F) = M. biventer cervicis cranial portion, it = intersection tendineo, ca = M. biventer cervicis caudal portion, lcd = M. longus colli pars dorsalis, an = aponeurosis notarii. Scale bars represents one centimetre. Asterisks note sites of muscle attachment. Collated and modified from Boumans et al. (2015). Asterisks represent sites of origin

M. complexus

M. complexus is the anterior-most cervical muscle, it is broad, flat and parallel-fibred (Fig. 7a, 8a) that is subject to much inter- and intraspecific variation. Three slips form the points of origin from the lateral tubercles associated with the transverse processes of the third to the fifth cervical vertebrae (Fig. 11) (Zusi and Bentz 1984; Landolt and Zweers 1985; Snively and Russell 2007), or from the diapophyses of the cervical vertebrae 4 through 6 in woodpeckers (Jenni 1981), corvids (Shufeldt 1890), pigeons (Burton 1974) and chickens (Kaupp 1918). Four slips are found in some strigids and accipitrids (Boas 1929; Boumans, Krings, and Wagner 2015) (*Strix aluco* and *Haliaeetus albicilla*) and originate from the processus dorsalis of C3, the processus costalis of C4 and C5 and the tuberculum ansae of C6. M. complexus inserts onto the medial section of the parietals and in some instances onto the posterior portion of the squamosal in birds. Insertions are predominantly fleshy or aponeurotic, or in the unique example of the Brown Pelican, (*Pelicanus occidentalis*) tendinous (Snively and Russell 2007). The lateral portion of M. transversospinalis capitis shares homology with the avian M. complexus (Cleuren and de Vree 2000)'

(Tsuihiji 2005). M. complexus has another homologue in crocodilians which is part of entirely different muscle system, M. longissimus capitis superficialis. This muscle in crocodilians originates further posteriorly than its anteriorly restricted avian counterpart, from the ventrolateral portions of neural arches of cervical vertebrae 5 through 9 (compared to C1-4 in birds). The insertion of M. longissimus capitis superficialis is the most lateral of any crocodilian craniocervical muscle, inserting ventral to the origin of the M. depressor mandibulae and dorsolateral to the insertion of M. rectus capitis lateralis on the lateral extremity of the paroccipital process. M. complexus and its homologue participate in the dorso- and latero-flexion of the head relative to the axis, as confirmed in electromyographical studies (Snively et al. 2014), as such this muscle plays a large role in drinking in adult birds (such as *Gallus* (Heidweiller et al. 1992)).

M. splenius capitis

M. splenius capitis (Fig. 7c, 8b) is similar to the M. complexus in that it is a short, broad, cranially positioned cervical muscle that displays variation between different groups of birds. Often divided in to a pars lateralis and pars medialis (Snively and Russell 2007; Boumans, Krings, and Wagner 2015; Lautenschlager, Bright, and Rayfield 2014) (in *Haliaeetus leucocephalus, Struthio camelus, Buteo buteo* and *Tyto furcata pratincola*) M. splenius capitis forms a teardrop-shaped origination site on the neural spines of C2 and in some cases, C3 (Fig. 10) (Snively and Russell 2007; Lautenschlager, Bright, and Rayfield 2014). From this origin both divisions of the muscle increase in size proximally and have a large insertion area over the parietal, the dorsolateral portion of the paroccipital process and a lateral section of the supraoccipital. The lateral part of M. splenius capitis may insert on to the lateral portion of the paroccipital process (as per *Struthio camelus* (Tsuihiji 2005)). Two key variations occur in anatids and adopiformes; the M. splenius capitis gains a third ventrolateral division in the mallard (Landolt and Zweers 1985) and adopiformes display a cruciform morphology (Burton 1971; Brause, Gasse, and Mayr 2009) that is hypothesised to account for the restricted movement of the head joint in swifts and for the lack of a muscular tongue in hummingbirds (Brause, Gasse, and Mayr 2009). Homologous to the medial portion of the M. splenius capitis is the crocodilian M. epistropheo-capitis medialis (Seidel 1978; Cleuren and de Vree 2000) which originates from the long axial spinous process and inserts medial and deep to M. complexus, dorsal to the occipital condyle. It has been previously suggested that the M. splenius capitis is responsible in part for head dorsiflexion relative to the neck alongside and provides some capacity for lateral movement (Burton 1974), but with shorter moment arms than M. complexus. This would limit its function to a role in stabilising the atlanto-occipital joint (Snively and Russell 2007). Electromyography supports this role in dorsiflexion for the M. splenius capitis, however, as a prerequisite, the muscle must be unstretched for this to occur (Snively and Russell 2007). If both parts of the M. splenius capitis are contracted, then the head is flexed dorsally, if only unilateral contraction occurs then the head is moved laterally to one side (Zusi 1962). As the M. epistropheo-capitis medialis is amongst the smallest cervical muscle in crocodilians, it is concluded that it performs a similar function to its avian homologue (Cleuren and de Vree 2000; Snively and Russell 2007).

M. longus colli dorsalis

Perhaps the most complex cervical muscle is the dorsally located M. longus colli dorsalis (Fig. 9a-c). It is often comprised of four subdivisions: pars cranialis (Fig. 9b), caudalis (Fig. 9a), profunda (Fig. 9c) and thoracica (Baumel, Evans, and Berge 1993), however there are few descriptions that elucidate the status of pars thoracica, as it may form a part of M. longissimus dorsi. Due to this debate surrounding this subdivision, the view that three subdivisions create the M. longus colli dorsalis is taken here after Landolt & Zweers (Landolt and Zweers 1985).

Numerous slips of pars cranialis (Fig. 9b) originate aponeurotically or tendinously from the neural spines of dorsally positioned cervical vertebrae (C3-7 (Boumans, Krings, and Wagner 2015)). The number of slips of cranialis can vary between five and six, with five being common to a variety of distantly related birds (Fig. 10) (such as owls (Boumans, Krings, and Wagner 2015; Boas 1929), scrub birds and lyrebirds (Zusi 1985)). Pars cranialis inserts via a tendon onto the posterior or dorsal surface of the epipophyses of C2 or C3 (Snively and Russell 2007), or in the case of *Buteo buteo*, partly onto the axis (Lautenschlager, Bright, and Rayfield 2014).

Largest of the subdivisions of the M. longus colli dorsalis is pars caudalis (Fig. 9a). Similar to the pars cranialis, it is composed of several separate slips that originate from the aponeurosis notarii on neural arches and transverse processes of the last few cervical vertebrae and the most anterior thoracic vertebrae (Snively and Russell 2007; Boumans, Krings, and Wagner 2015). Insertion sites are located at the processes/torus dorsalis of multiple vertebrae spanning regions 2 and 3 of the cervical column in birds (as denoted by Boas (Boas 1929)). Slips of the pars caudalis may merge with certain bellies of Mm. cervicales ascendentes (as found in *Struthio camelus*) or share a tendon with the M. cervicales ascendens (as per *Pelicanus occidentalis*) (Snively and Russell 2007). Ventral to the pars caudalis is the pars profunda (Fig. 9c) and splits into several individual muscle slips that are oriented parallel to each other.

Pars profunda can span many vertebrae as in owls (C5-12, Fig. 9c (Boumans, Krings, and Wagner 2015; Boas 1929)) or comparatively few, as in the case of mallards where the pars profunda spans two or three vertebrae (Landolt and Zweers 1985). The processes spinosus of C5-12 (in the case of the tawny owl, *Strix aluco*) are origination sites for pars profunda, whilst the transverse processes of C4-11 provide sites of insertion (Boumans, Krings, and Wagner 2015; Boas 1929). Aside from strigiformes, other avians either display a more restricted range of origin and insertion sites, often limited to more caudal vertebrae (as in the huia (Burton 1974)) or lack the pars profunda entirely (as in some woodpeckers (Jenni 1981)). With 4 subdivisions the M. longus colli dorsalis has 4 corresponding homologues in crocodilians: pars cranialis with M. spino-capitis posticus; pars caudalis with M. transversospinalis capitis (lateral portion); pars thoracica with M. articulospinalis dorsi and pars profunda with M. transversospinalis cervicis (Seidel 1978; Cong et al. 1998; Cleuren and de Vree 2000; Tsuihiji 2005; Frey 1988). M. transverspinalis cervicis originates aponeurotically on to the lateral surfaces of the neural spines of C3-9 just anterior to the prezygapophyses and immediately ventral to the origins of M. transversospinalis cervicis and M. spinocapitis posticus.

The aponeurosis involved with the origin of M. transversospinalis cervicis is connected with the intermuscular septum that separates the longissimus and transversospinalis muscle systems. M. longissimus cervicis also originates via aponeuroses on the neural spines of C4-7. Both muscles insert onto the posterodorsal section of the postzygapophyses of C1 with M. transversospinalis cervicis also inserting onto a similar position on C3 and C4. This insertion is similar to that displayed in avians yet in crocodilians the insertions onto C2 are skipped entirely (in birds C2 it is the most conspicuous area of insertion). The dorsal insertion of the pars cranialis onto the axis suggests a role in the dorsiflexion of the concave cranial portion of the cervical column (Snively and Russell 2007; Boumans, Krings, and Wagner 2015), with EMG studies confirming this alongside suggestions of aiding neck retraction and dampening during ventroflexion (van der Leeuw, Bout, and Zweers 2001). Electromyography has also confirmed a dorsiflexive role for the M. transversospinalis of crocodiles (Cleuren and de Vree 2000). Pars profunda is assumed to assist in upward flexion of the medial region of the cervical column in birds, with this muscle being highly developed in Strigidae and underdeveloped and even absent in distantly related avians. It is thought that M. longus colli dorsalis pars profunda underlies owl-specific movements of the neck (Boas 1929; Boumans, Krings, and Wagner 2015).

Mm. intercristales and Mm. interspinales

Covering much of the dorsal portion of the second cervical vertebrae is the expansive Mm.intercristales. This muscle group connects transverse oblique crests (crista transverso-obliqua) of adjacent vertebrae and are often mistaken for slips of M. longus colli dorsalis pars cranialis, as they are difficult to separate from each other (Baumel, Evans, and Berge 1993). Each slip of Mm. intercristales originates from the anterior surface of the transverse oblique crests of the posterior vertebrae of each pair (Snively and Russell 2007), and inserts into the posterior edge of this crest on the anterior vertebra of the pair. The Mm. interspinales connect spinous processes of adjacent vertebrae and lie deep to the M. longus colli dorsalis pars cranialis. Mm. interspinales is also found in crocodilians, whilst Mm. intercristales is homologous to the Mm. interarticulares (Seidel 1978; Tsuihiji 2005), that only connect postzygapophyses. Mm. intercristales is hypothesised to function during intervertebral dorsiflexion and stabilisation of intervertebral joints (Snively and Russell 2007).

Mm. ascendens cervicalis

Forming a singular functional unit from 2-4 consecutive slips is the cervical extension of the M. ascendens thoracicus, M. ascendens cervicalis (Fig. 7a)

(Baumel, Evans, and Berge 1993). These slips can originate from the dorsal portions of transverse processes, or aponeuroses from this area (Snively and Russell 2007), and in birds they converge onto the processus dorsalis and/or epipophyses of vertebrae that are two vertebrae anterior to this origin. M. ascendens cervicalis inserts dorsolateral to the centre of rotation for each vertebral pair, which is shared with the insertions for M. longus colli dorsalis pars cranialis, placing this insertion at the transverse oblique crest. The lateral portion of M. transversospinalis cervicis (Seidel 1978; Tsuihiji 2005) is the crocodilian homologue to M. ascendens cervicalis. Studies involving electromyography have reported M. ascendens cervicalis to be involved in neck dorsiflexion in chickens (Heidweiller and Zweers 1992). Due to this function, this muscle unit is enlarged in raptorial birds such as *Aquila chrysaetos*, as strong dorsiflexion of the neck is required for tearing of flesh from prey (Snively and Russell 2007).

iii) M. longissimus system

M. rectus capitis dorsalis

Of the rectus capitis muscle complex, M. rectus capitis dorsalis (Fig. 8c) is the deepest. It is formed of 4 to 5 individuals slips (4 in owls (Boumans, Krings, and Wagner 2015), 5 in mallards (Landolt and Zweers 1985)) that run parallel and longitudinal down the ipsilateral portion of the cervical column. The lateral and anterolateral portions of costal and transverse processes of C1-6 (Fig. 11) form the origination sites of M. rectus capitis dorsalis (Snively and Russell 2007; Boumans, Krings, and Wagner 2015; Jenni 1981; Zusi and Bentz 1984). Some slips may also originate on the lateral (as in *Buteo buteo* (Lautenschlager, Bright, and Rayfield 2014)) or anterolateral (Snively and Russell 2007) surface of the atlas (C1). It inserts anteroventral to the occipital condyle onto the basioccipital. The crocodilian homologue to M. rectus capitis dorsalis is the M. longus capitis profundus which originates immediately dorsal to the articulations between the transverse processes with the cervical ribs of C1-6 (Snively and Russell 2007). It has a similar insertion to M. rectus capitis dorsalis, inserting onto the basioccipital ventrolaterally (compared to the anteroventral insertion in birds). This insertion site would provide for an antagonistic function to M. rectus capitis lateralis, ventroflexing the head relative to the neck, which has been corroborated by crocodilian electromyography studies (Cleuren and de Vree 2000).

Mm. intertransversarii and Mm. inclusii

The muscles that form Mm. intertransversarii (Baumel, Evans, and Berge 1993; Cong et al. 1998) are present in many sauropsids, including birds and crocodilians, whilst Mm. inclusii are found exclusively in birds. Mm. intertranversarii are uniarticular components of the longissimus system that connect consecutive transverse processes in both birds and crocodilians. Variation exists between the avian and crocodilian Mm. intertransversarii, with complex subdivsions that vary along the neck between bird species and a dorsoventrally thicker band present in crocodilians (Cong et al. 1998). It is the posterior transverse process of one vertebrae that forms the origin of Mm.

intertransversarii, and the anterior transverse process that provides the site of insertion. In birds the origination is via an aponeurosis from the lateral and dorsolateral tubercles, whilst in crocodilians Mm. intertransversarii originates deeper from the anterior surface of the transverse processes. Occupying the posterior, dorsally concave section of the cervical column is the Mm. inclusii which is formed from numerous short muscle bellies. Aponeuroses on the anterior surfaces of costal processes are the sites of origin for Mm. inclusii. Anteriorly, long superficial bellies of Mm. inclusii insert onto the costal processes, multiple cervical vertebrae anterior to the origin. This is found in only a few birds and is the exception to the usual uniarticular morphology. In more posterior sections Mm. inclusii inserts onto dorsolateral and lateral crests of the posterior neural arch. The position of Mm. intertransversarii eludes to a role in the lateral flexion of vertebrae relative to each other, which is especially important in birds who lack large superficial longissimus muscles that can laterally flex the whole neck (Heidweiller and Zweers 1992), similar function is proposed for crocodiles (Cleuren and de Vree 2000). This function has been confirmed by electromyography of anterior slips of Mm. intertransversarii of adult chickens (Heidweiller and Zweers 1992) during intervertebral dorsiflexion.

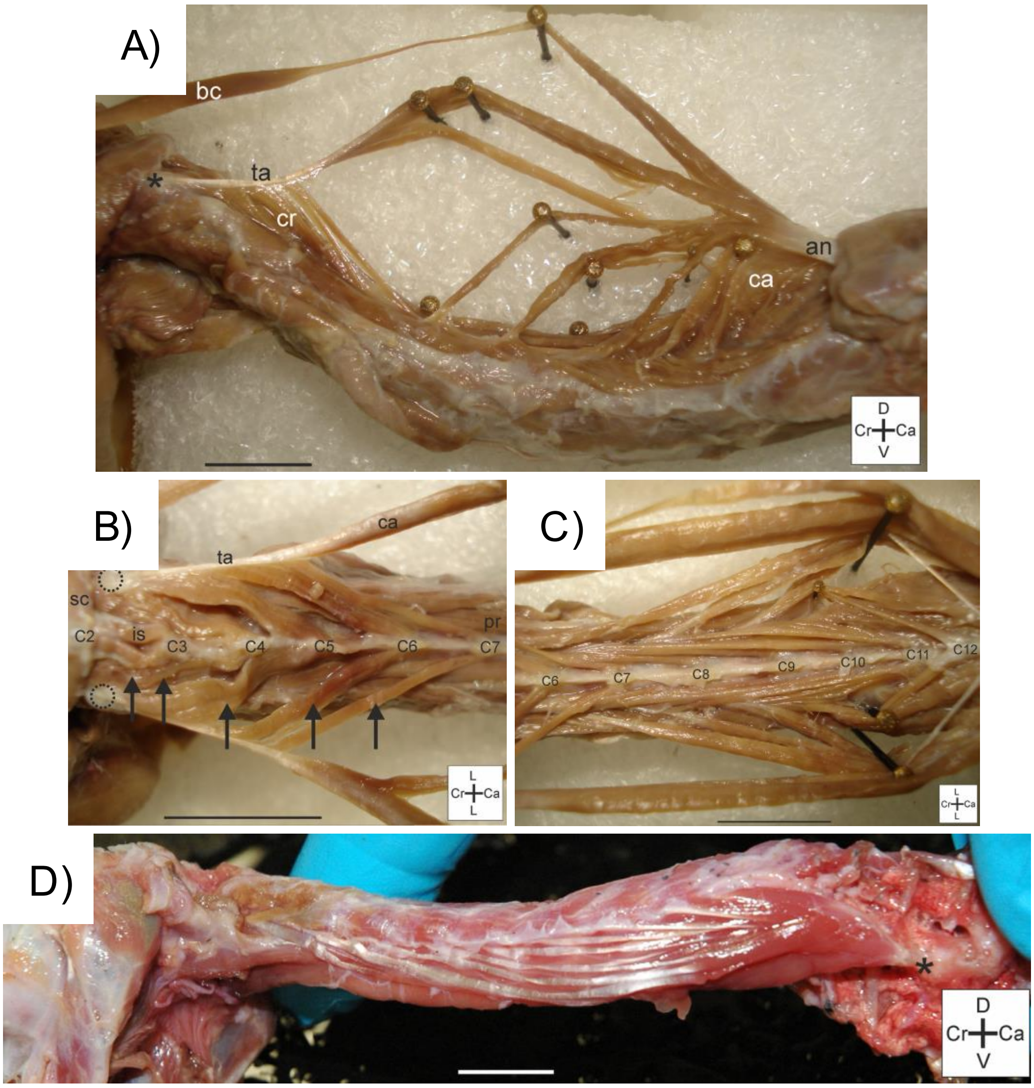

Figure 9. Caudal cervical musculature of *Tyto furcata pratincola.* A) M. longus colli dorsalis pars caudalis in left lateral view, B) M. longus colli dorsalis pars cranialis in dorsal view, C) M. longus colli dorsalis pars cranialis in dorsal view, D). M. longus colli ventralis in left lateral view. Scale bars represent one centimetre. Abbreviations: bc = M. biventer cervicis, ta = tendo axialis, ca = M. longus colli pars caudalis, an = aponeurosis notarii, pr = M. longus colli dorsalis pars profunda. Collated and modified from Boumans et al. (2015). Asterisk in A) indicates the insertion of the axial tendon of the M. longus colli dorsalis muscle group. Asterisk in D) indicates the origination of M. longus colli ventralis.

iv) M. iliocostalis and M. longus systems

M. rectus capitis lateralis

Lateral to the M. rectus capitis dorsalis is the flattened M. rectus capitis lateralis (Fig. 8e), which is found in both birds and crocodiles. Origins of this muscle are all ventromedial; from ventral processes (Lautenschlager, Bright, and Rayfield 2014), enlarged hypophyses or from ventrolateral portions of the centrum (Snively and Russell 2007) of C2 and one or more posterior vertebrae (Tsuihiji 2007). Mallards display sites of origination from C2-4 (Fig. 11) (Landolt and Zweers 1985), whilst some gulls and woodpeckers have an additional origin from C5 (Boas 1929; Tsuihiji 2007; Jenni 1981; Snively and Russell 2007). Origins on C3-5 (Fig. 11) occur in owls (Boumans, Krings, and Wagner 2015), pigeons and some corvids (Shufeldt 1890) In other amniotes (including crocodilians) it is the cervical ribs (homologous to costal processes in birds) that provide sites of origin for M. rectus capitis dorsalis. Birds lack this site of origin whilst crocodiles display an origin on the cervical ribs. In birds this represents a medial shift of the origin from the plesiomorphic origination site of archosaurs on the cervical ribs/costal processes (Snively and Russell 2007). The insertion of M. rectus capitis lateralis is dorsoventrally stretched onto the lateral rim of the paroccipital process (Snively and Russell 2007; Lautenschlager, Bright, and Rayfield 2014). Homologous to sections of the avian M. rectus capitis lateralis is the M. iliocostalis capitis (Seidel 1978; Cleuren and de Vree 2000), which in crocodiles originates from the white fascia surrounding the cervical ribs of the atlas and inserts in a similar fashion to the M. rectus capitis lateralis in birds. The location of M. rectus capitis lateralis and M. iliocostalis suggests they are involved with lateral flexion of the head versus the neck, which is corroborated by electromyographical studies on crocodiles (Cleuren and de Vree 2000).

M. rectus capitis ventralis

This muscle is found in both birds and crocodilians (Baumel, Evans, and Berge 1993; Cong et al. 1998) and is formed from two parallel and interconnected subdivisions (medial and lateral). Both parts originate from ventral surfaces and processes spinosus ventralis (Fig. 8d). Pars medialis originates from C1-3 (Fig.

10) in many birds, the precise arrangement varies between species; in owls C1 and C2 provide the site of origin (Fig. 10), and in *Buteo buteo* it originates from C2 (Boumans, Krings, and Wagner 2015) and C3 (Lautenschlager, Bright, and Rayfield 2014). Pars lateralis originates from C3-6 (as in mallards (Landolt and Zweers 1985)) and fuses with pars medialis at the level of the third cervical vertebrae (Boumans, Krings, and Wagner 2015; Lautenschlager, Bright, and Rayfield 2014) to form a large muscle mass in birds (Snively and Russell 2007). In crocodilians the origin of slips of m. rectus capitis ventralis is limited to the ventrolateral surfaces of the centra of C1 and C2. The fused parts of M. rectus capitis ventralis insert onto the basitemporal plate (Eric Snively and Russell 2007) in birds, and in crocodilians it forms a continuous insertion with the M. longissimus capitis profundus (Snively and Russell 2007). Ventroflexion of the head relative to the cervical column is the proposed function of M. rectus capitis ventralis due to its attachments spanning multiple myological regions of the neck (Snively et al. 2014). The combined actions of M. rectus capitis ventralis and M. longus capitis profundus serve to stabilise the head-neck joint during energetic feeding (Snively and Russell 2007) in crocodilians.

M. longus colli ventralis

M. longus colli ventralis (Fig. 9d) is a ventrally oriented muscle with multiple slips and bellies that have complex origins and insertions which is often split into a pars cranialis and caudalis (Landolt and Zweers 1985). It is an expansive muscle that connects anterior cervical vertebrae to the notarial vertebrae (Fig. 10) (Snively and Russell 2007; Lautenschlager, Bright, and Rayfield 2014). M. longus colli ventralis originates from the processus spinalis ventralis or sublateral processes of thoracic (notarial) vertebrae. Multiple slips can originate from the same site and some slips may originate from the body of a centrum on more anterior vertebrae (Fig. 10). Slips of M. longus colli ventralis insert tendinously 3-10 veterbrae anterior to their origin, with one slip detaching from C10-3 and inserting onto a lateroventral point on the processus transversus (Fig. 10). The tendons insert onto cervical ribs or onto postlateral processes if the cervical ribs are underdeveloped. It is the M. iliocostalis cervicis and the closely associated M. longus colli that serve as homologues to M.

longus colli ventralis in crocodilians (Cong et al. 1998; Seidel 1978). Both muscles in crocodilians originate from ventral portions of the centra and hypophyses of anterior thoracic and posterior cervical vertebrae. Insertion sites for the M. iliocostalis cervicis are located at the posterior processes of cervical ribs and more dorsally onto myosepta or ribs immediately anterior to their origin (Snively and Russell 2007). M. longus colli ventralis functions as the antagonist to M. longus colli dorsalis, yet this is an oversimplification for such a complex muscle. The numerous slips allow for intricate intervertebral ventroflexion, and can be involved during both head retraction and approach, allowing for ventroflexion of specific portions of the cervical column during these movement, whilst also acting to dampen the actions of dorsiflexive musculature (Snively and Russell 2007; van der Leeuw, Bout, and Zweers 2001; Van der Leeuw, Bout, and Zweers 2001). Electromyography has shown that in crocodiles the M. iliocostalis cervicis functions in lateroflexion of the neck (rather than ventroflexion as in the avian homologue) (Cleuren and de Vree 2000). However similarities do exist between the function of M. iliocostalis cervicis and M. longus colli ventralis, as they both operate to dampen and stabilise during the contraction of dorsiflexors (Cleuren and de Vree 2000).

M. flexor colli

This muscle is often cited as being subdivided into a medial and lateral part (Fig. 7a), however this is not a feature that is universal to all birds as this subdivision is absent in the *Buteo buteo* (Lautenschlager, Bright, and Rayfield 2014). Both parts of this muscle are either continuous or share an attachment with another nearby muscle. Pars medialis is often a continuation of anterior portions of the M. longus colli ventralis (Zusi and Storer 1969), and pars lateralis shares attachment sites with M. rectus capitis dorsalis (which partly conceals M. flexor colli) (Baumel, Evans, and Berge 1993). M. flexor colli originates ventrolaterally onto posterior vertebrae via an aponeurosis and inserts posteriorly onto the processus spinosus ventralis of the vertebrae anterior to its origin (Snively and Russell 2007). With each portion of M. flexor colli spanning at least 2 intercorporeal articulations, this muscle to ventroflex any intervertebral joints that it crosses (Snively and Russell 2007).

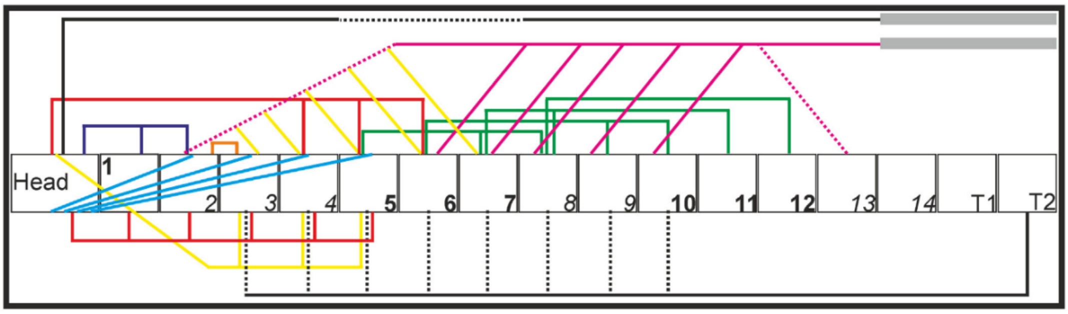

Figure 10. Connection diagram of cervical musculature in *Tyto furcata pratincola*. Number boxes denote cervical vertebrae (in sequence, C1-C14). Coloured lines denote different muscles: M. complexus (red), M. biventer cervicis (black), M. splenius capitis (purple), M. rectus capitis dorsalis (blue), M. longus colli dorsalis, pars caudalis (pink), M. longus colli dorsalis, pars cranialis (yellow), pars profunda (green), M. interspinalis (orange), M. rectus capitis lateralis (yellow), M. rectus capitis ventralis (red), M. longus colli ventralis (black). Dashed lines indicate tendinous or aponeurotic attachments. Modified from Boumans et al. (2015).

Summary

This chapter has summarised the current state of the literature for each of the four data chapters present in this thesis. The avian cervical column is split into five distinct regions and the boundaries between these regions are controlled by *Hox* gene expression patterns (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015b). Geometric morphometrics can be used as a proxy to delineate these regional boundaries in the cervical column of birds (Böhmer, Rauhut, and Wörheide 2015b), and by studying variation in regional morphology, problems associated with unknown homology of vertebrae between species with different counts of cervical vertebrae can be overcome. Techniques that utilise geometric morphometrics data can be adapted to study factors that influence variation in cervical morphology across the entire cervical spine (PTA) and within individual cervical regions. (D-PGLS models) (Adams et al. 2017; Adams 2014; Adams and Collyer 2009; Collyer and Adams 2013). Kinematics of the avian cervical column are somewhat conserved across Aves due to constraints imposed by the economics of continuous movement (Boas 1929; Bout 1997; Van der Leeuw, Bout, and Zweers 2001), however when extreme functional demands occur (such as when adapting to life in water) cervical kinematics adapt accordingly (Van der Leeuw, Bout, and Zweers 2001; Chang et al. 2016). Vertebral morphology varies across the length of the cervical column and many aspects osteology are conserved across extant birds (Baumel, Evans, and Berge 1993; Van der Leeuw, Bout, and Zweers 2001; Krings et al. 2014). Neck length is much more variable in birds than mammals and it is unclear whether changes to vertebral counts or vertebral lengths are responsible for this variation. The effect of ecological factors and regionalisation on neck elongation are currently also unclear in the avian cervical column. Variation in cervical musculature has only been studying in the context of muscle attachment sites and many cervical muscles display a similar organisation across birds (Boas 1929; Landolt and Zweers 1985; Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015), however some differences do occur and the reasons behind these variations have not yet been quantified. As has been stated above (and throughout Chapter 1) much of the literature devoted to the avian cervical column is qualitative in nature and this thesis aims to quantify the factors affecting variation in avian cervical morphology and the mechanisms that underlie this variation.

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Chapter 3: Patterns of avian cervical regionalisation

Introduction

Avians utilise the cervical column like a surrogate arm as the forelimbs are heavily adapted for flight rather than to manipulate the surrounding environment (Starck 1978; Clarke and Middleton 2008; Bhullar et al. 2012). This has led to the involvement of the avian neck in many disparate behaviours such as feeding, preening and conspecific display. Across Aves, neck morphology varies considerably and this may be attributed to the many functional tasks the neck can be involved in. The drivers behind this immense diversity are not well understood and to-date no study has attempted to quantify this morphofunctional diversity in a broad systematic regime.

The cervical column of birds is a kinematically redundant chain of vertebrae that refutes large angles of motion at any particular joint by favouring a system where joint motion favours an even distribution across all joints in the chain (Bout 1997). This accounts for the 'S-shaped' curvature observed in the avian cervical column (Bout

1997; van der Leeuw, Bout, and Zweers 2001). Cervical vertebral counts range from 11 (parrots) to 26 in larger birds such as swans (Dietrich Starck 1979). Such plasticity of cervical vertebral counts is rare in higher tetrapods. Mammals are especially restricted with only seven cervical vertebrae in the majority of species (Gadow 1933; Slijper 1946; Galis 1999). *Hox* gene patterning is responsible for stabilising the developing nervous system and is linked to variation in cervical counts, but variation is restricted in mammals as the *Hox* gene patterning behind it is related to the emergence of neonatal cancers (Galis 1999). As birds represent a unique case in variation of cervical vertebral counts, understanding the underlying mechanics of cervical variation and neck elongation in birds may allow for a deeper understanding of how *Hox* genes adapt and change between lineages and over evolutionary time.

Regionalisation is a vital feature of the axial column allowing the cervical, dorsal, sacral and caudal regions of the spine to perform different functions owing to their different morphologies and vertebral counts. Functional differentiation of the axial column has been vital to the success of vertebrates as it underpins locomotion, breathing and prey capture (Buchholtz 2012; Hirasawa, Fujimoto, and Kuratani 2016; Pierce, Clack, and Hutchinson 2011; Koob and Long Jr 2000; Shapiro 1995). Understanding the causes and consequences of regionalisation across vertebrates will allow for a better understanding of large scale changes to the vertebrate body plan over deep time. *Hox* gene expression boundaries can elucidate borders between these major vertebral regions and the *Hox* code responsible for this is highly conserved amongst amniotes (Burke et al. 1995). Recent work has focused upon refining this technique to observe how *Hox* expression limits correspond to individual vertebrae (Böhmer, Rauhut, and Wörheide 2015b; Burke et al. 1995), allowing variation of vertebral morphology within a single axial region to be equated to gene expression that is conserved within a lineage. This would provide a framework where regionalisation could be studied within the avian cervical column. This could allow for the inference of the genetic basis behind neck elongation in modern birds.

It has long been thought that subregions exist within the cervical region of modern birds, with range of motion (ROM) of cervical joints being used as a proxy for boundaries of functional regions (Boas 1929). This technique has hypothesised that extant birds have 3 cervical regions; anterior, middle and posterior (Boas 1929; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013; Boumans, Krings, and Wagner 2015; Krings et al. 2017). These regions appear to correspond with the S-shaped curvature of avian neck at rest. Whilst supported in the wider literature (Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013; Boumans, Krings, and Wagner 2015; Krings et al. 2014) this scheme is often derived from work on single species, and does not provide a framework that can support the comparison of multiple bird species with varying cervical counts. Nevertheless ROM studies are still valuable as previous work suggests that certain regions may be expanded according to specific kinematic patterns that are used in daily behaviours (such as drinking) (van der Leeuw, Bout, and Zweers 2001; Van der Leeuw, Bout, and Zweers 2001).

Studies that use vertebral morphology (instead of ROM) to delineate cervical regions conclude that the avian neck is comprised of upwards of 5 or 6 distinct morphological regions (Van der Leeuw, Bout, and Zweers 2001; Guinard et al. 2010). Between the two techniques lies an unresolved dichotomy between the number of regions within the avian cervical column. Morphological studies are still unable to robustly compare multiple species of birds with different cervical counts. Without a systematic study of cervical regionalisation the evolutionary drivers and underlying genetics behind cervical regionalisation are poorly understood for Aves. By equating *Hox* gene expression limits to vertebral morphology in the domesticated chicken, *Gallus gallus* *domesticus* (analysed using geometric morphometrics), recent work (Böhmer, Rauhut, and Wörheide 2015b) has provided a methodology where regionalisation and neck elongation can be studied across Aves. In this study it was found that birds display an extra cervical region when compared to more basal archosaurs such as crocodilians and dinosaurs. Expanding upon this study to closer examine regionalisation of the cervical column of modern birds could provide insights into the potential relationships between regionalisation, neck elongation, ecology and phylogeny.

This current study aims to use this new methodology to explore how genetics-based cervical regionalisation changes across Aves by addressing the following questions:

> Q1: Is the number of cervical regions constant across extant birds or does it vary with external factors?
>
> Q2: Are cervical regions defined by specific morphological traits?
>
> Q3: Do these traits vary across Aves in accordance with external factors?
>
> Q4: Do variations in cervical region size (e.g. number of vertebrae) across Aves correlate with external factors?

Q5: What are the functional mechanistic links between correlations in region size and shape and external factors?

By addressing these questions and comparing results to recent work (Böhmer, Rauhut, and Wörheide 2015b) a deeper insight into what regions (and thus, the *Hox* gene expression that controls these regions) are responsible for neck diversity in birds can be gained. By investigating the response of ecological signals on cervical morphology we can begin to understand the drivers behind the evolution of the cervical column, the most complex musculoskeletal system in extant avians. Understanding these links in extant archosaurs will allow for future work to focus on if these links hold true for extinct archosaurs. Recent work has analysed a single species for each branch of Archosauria (Böhmer, Rauhut, and Wörheide 2015) and expanding to identify patterns of regionalisation and *Hox* gene patterning throughout archosaur evolution, whilst not the focus of this study, this would be vital in understanding the evolution of neck morphology and biomechanics in bird-line theropod dinosaurs but also other dinosaurs groups, particularly neck elongation in sauropods, which have some of the longest cervical columns in evolutionary history (Sander et al. 2011).

Methods

Specimen information and 3D digitisation

For 52 extant birds (46 species across 25 orders, Table 1), 3D digital models for all vertebrae in the cervical column of each bird were sourced either directly by micro-CT or from digital collections of colleagues (K. T. Bates, R. B. J. Benson and E. R. Schachner, specimen numbers are located in Appendix Table 3.1). Specimens newly scanned for this project were imaged at the University of Manchester's Henry Moseley X-Ray Imaging facility (Manchester HMXIF) using the 320/225 kV custom bay Nikon XTEK with system settings for kV set between 50 kV and 90 kV, and µA ranged between 58 µA and 140 µA. Initial scan data was reconstructed using CT Pro 3D (Metris XT 2, version 2.4365.28608), and a TIFF stack was created using TomoTools v1.0 for ease of import into analysis software. All scans were segmented using Amira 5.6 or Avizo 7.1, and each cervical vertebrae were individually exported as OBJ mesh files. Meshes were then cleaned and converted to PLY mesh files using Geomagic Studio 10 for use in Landmark Editor.

Species	Common name	Diet	Flight style	Order	Estimated body mass (kg)
Cathartes aura	Turkey vulture	Carnivore	S	Accipitriformes	1.893
Hieraaetus morphnoides	Little Eagle	Carnivore	S	Accipitriformes	0.811
Necrosyrtes monachus	Hooded Vulture	Carnivore	S	Accipitriformes	2.050
Branta leucopsis	Barnacle goose	Herbivore	CF	Anseriformes	N/A
Apteryx owenii	Little spotted kiwi	Insectivore	Fl	Apterygiformes	1.118
Bucorvus abyssinicus	Northern Ground-hornbill	Carnivore	CF	Bucerotiformes	4.000
Cariama cristata	Red-legged Seriema	Insectivore	BAF	Cariamiformes	1.862
Dromaius novaehollandiae	Emu	Generalist	Fl	Casuariiformes	13.150
Glareola pratincola	Collared Pratincole	Insectivore	CF	Charadriiformes	0.079
Larus canus	Seagull	Piscivore	FG	Charadriiformes	0.868
Scolopax rusticola	Woodcock	Insectivore	CF	Charadriiformes	0.276
Turnix varius	Painted buttonquail	Generalist	CF	Charadriiformes	0.073
Uria aalge	Murre	Piscivore	CF	Charadriiformes	0.509
Goura cristata	Western Crowned-pigeon	Frugivore	CF	Columbiformes	2.100
Geococcyx californianus	Greater roadrunner	Insectivore	CF	Cuculiformes	0.305
Microhierax caerulescens	Collared Falconet	Insectivore	FG	Falconiformes	0.040
Agelastes niger	Guineafowl	Insectivore	BAF	Galliformes	1.352
Alectorus rufa	Red-legged partridge	Generalist	BAF	Galliformes	0.444
Gallus gallus domesticus	Chicken	Generalist	BAF	Galliformes	N/A
Lagopus lagopus	Ptarmigan	Herbivore	BAF	Galliformes	0.589
Porphyrio hochstetteri	South Island takahe	Herbivore	Fl	Gruiformes	N/A
Porphyrio poliocephalus	Grey-headed swamphen	Generalist	CF	Gruiformes	N/A
Opisthocomus hoatzin	Hoatzin	Herbivore	BAF	Opisthocomiformes	N/A
Certhia familiaris	Eurasian treecreeper	Insectivore	IB	Passeriformes	N/A
Cinclus cinclus	White-throated dipper	Insectivore	IB	Passeriformes	0.065
Lonchura striata domestica	Bengalese finch	Herbivore	IB	Passeriformes	0.014
Xenicus gilviventris	New Zealand Rockwren	Insectivore	IB	Passeriformes	0.018
Xenicus longipes	Bushwren	Insectivore	Fl	Passeriformes	0.018
Pelecanus occidentalis	Brown pelican	Piscivore	S	Pelecaniformes	2.449
Phoenicopterus chilensis	Flamingo	Filter	CF	Phoenicopteriformes	2.250
Pteroglossus torquatus	Collared Araçari	Frugivore	CF	Piciformes	0.238
Sphyrapicus varius	Yellow-bellied Sapsucker	Insectivore	CF	Piciformes	0.050
Podilymbus podiceps	Grebe	Piscivore	CF	Podicipediformes	1.021
Nestor notabilis	Kea	Generalist	CF	Psittaciformes	0.922
Strigops habroptilus	Kakapo	Herbivore	Fl	Psittaciformes	1.975
Trichoglossus moluccanus	Rainbow lorikeet	Herbivore	CF	Psittaciformes	0.144
Rhea americana	Rhea	Herbivore	Fl	Rheiformes	7.850
Spheniscus humboldti	Penguin	Piscivore	SUB	Sphenisciformes	4.010
Bubo virginianus	Great horned owl	Carnivore	FG	Strigiformes	N/A
Strix aluco	Tawny owl	Carnivore	FG	Strigiformes	N/A
Struthio camelus	Ostrich	Generalist	Fl	Struthioniformes	3.538
Anhinga anhinga	Darter	Piscivore	S	Suliformes	1.085
Fregata aquila	Ascension Frigatebird	Piscivore	S	Suliformes	1.250
Phalacrocorax harrisi	Flightless cormorant	Piscivore	Fl	Suliformes	3.250
Sula dactylatra	Masked booby	Piscivore	S	Suliformes	1.450
Crypturellus tataupa	Tataupa tinamou	Frugivore	BAF	Tinamiformes	0.199

Table 1 List of species studied alongside associated diet, flight style and phylogenetic order. Flight style abbreviations are as follows: burst adapted flying (BAF), continual flapping (CF), flightless (Fl), flap gliding (FG), intermittent bounding (IB), soaring (S), subaqueous (SUB). Body mass was estimated using scaling equations (see methods section).

Morphological analysis of 3D landmarks

A combination of quantitative, landmark-based, 3D geometric morphometrics and qualitative characters were used to assess regionalisation of the avian cervical column. Homologous landmarks (Figure 1, Table 2) and qualitative characters (see Appendix Table 3.2a) were derived from previous studies (Böhmer, Rauhut, and Wörheide 2015b, 2010). This scheme of landmarks relies on features that characterise the gross shape of vertebrae throughout the cervical column from C2 to the last cervical in any given species. The anatomical features that are specific to certain vertebrae (such as cervical ribs and the presence of a hypophysis) are documented by the qualitative characters. The landmark scheme captures variation in major features of vertebral

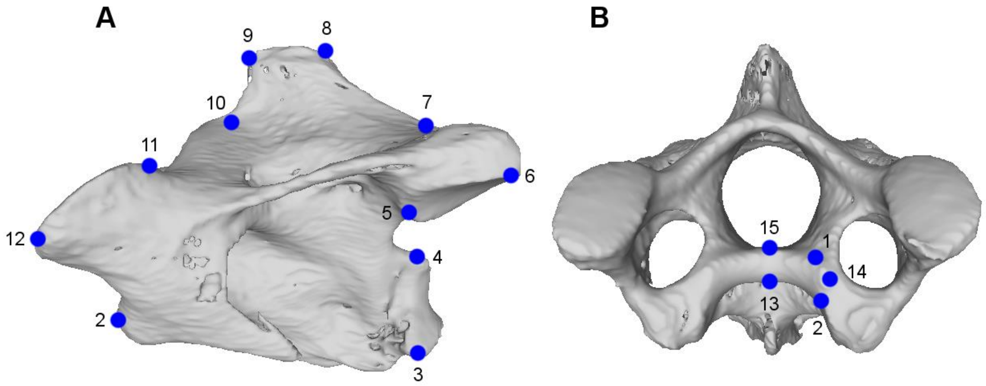

Figure 1 Lateral (A) and anterior view (B) of cervical 4 of *Fregata aquila* denoting the landmark scheme used in the geometric morphometric analyses.

anatomy across the cervical spine such as the height and width of the neural spine and centrum as well as the orientation of the pre- and post-zygapophyses. A sizeable amount of morphological variation in the avian cervical spine is caused by features of vertebral anatomy that disappear and reappear across the cervical column (features such as cervical ribs and the processus caroticus). These features cannot be landmarked and are covered by the list of qualitative characters. The atlas (C1) of all specimens was excluded as it displays few landmarks that are homologous with vertebrae from the rest of the cervical column (Böhmer, Rauhut, and Wörheide 2015b; Böhmer 2017). For

Landmark	Definition	Type
1	Dorsal-anterior edge of the centrum	II
2	Ventral-anterior edge of the centrum	II
3	Ventral-posterior edge of the centrum	II
4	Dorsal-posterior edge of the centrum	II
5	Anteriormost edge of the articular facet of the postzygapophysis	II
6	Dorsal-posterior edge of the articular facet of the postzygapophysis	II
7	Point of maximum curvature between postzygapophysis and neural spine	II
8	Posterior edge of the neural spine	II
9	Anterior edge of the neural spine	II
10	Point of maximum curvature between neural spine and prezygapophysis	II
11	Posteriormost point of the articular facet of the prezygapophysis	II
12	Dorsal-anterior edge of the articular facet of the prezygapophysis	II
13	Ventralmost point of the centrum	II
14	Lateralmost point of the centrum	II
15	Dorsalmost point of the centrum	II

Table 2 Definitions of landmarks (alongside the type of Bookstein coordinate they represent) for all 15 landmarks in the 3D geometric morphometrics analyses used throughout this thesis.

each bird, all vertebrae in the cervical column (except the atlas) were landmarked using Landmark version 3.0 (Wiley 2006) and landmark coordinates were exported into MorphoJ (Klingenberg 2011).

Analysing morphological regionalisation in the avian cervical column

Q1: Is the number of cervical regions constant across extant Aves or does it vary with external factors?

The landmark coordinates described above were subjected to a Generalised Procrustes Analysis (GPA), from which a Principal Coordinates Analysis (PCA) was performed to assess shape change within the cervical column of each bird. Regionalisation was then assessed by performing a Principle Coordinates Analysis (PCO) on the Procrustes coordinates from the GPA combined with the qualitative characters using a Gower index (Gower 1966, 1971) in PAST (Hammer, Harper, and Ryan 2001). Alongside these PCO analyses, regions were founded on minimum distance measures between vertebral levels via single linkage cluster analyses in PAST. Minimum distance measures (nodes of vertebrae on a cladogram produced by the cluster analysis which had the smallest distance between them) were utilised in conjunction with PCO morphospace to delineate regions. No further tests to discern potential relationships between the number of cervical regions and ecology and/or neck length are needed as no variation in region number was found.

Q2: Are cervical regions defined by specific morphological traits? Do these traits vary according to external factors?

Following an initial PCA shape analysis in MorphoJ, the correlation between ecology and phylogeny on individual region shape was assessed using the 'geomorph' package (Adams et al. 2017) in R version 5.0 (R Core Team 2018). Based on the results from the PCO and cluster analysis, a dataset consisting of the mean vertebral shape for each of the cervical regions for all birds in the study was created. This dataset was then subject to an initial GPA and PCA within 'geomorph'. An application of phenotypic trajectory analysis (PTA) (Adams and Collyer 2007; Adams and Collyer 2009; Collyer and Adams 2013) similar to that of Randau et al. 2016 (Randau, Cuff, et al. 2016) was used to quantify ecological and phylogenetic effects on shape across the entire cervical column. PTA in this instance plots a trajectory through shape space for a specific group within a factor (flightless birds for example) by connecting the mean shape for each cervical region for that specific group with the mean shape of the next region (e.g. from region 1 to region 2, then from region 2 to region 3 etc.) until all cervical regions are connected and form a trajectory that represents the shape change across the entirety of the cervical column.

Q3: Do variations in cervical region size across Aves correlate with external factors?

Region number and size (number of cervical vertebrae per region), along with ecological parameters (diet, flight style), gross morphological parameters (neck length and body mass) were collected for each bird. Ecological parameters were based on categorisations used in previous literature (Bruderer et al. 2010; Close and Rayfield 2012; Martin-Silverstone et al. 2015). Neck length was measured digitally as the summed length of each individual cervical vertebrae of each bird. Where possible body masses were measured by directly weighing the specimens that were CT scanned. Where this was not possible body masses were estimated using scaling equations. Scaling equations for femoral length, minimal circumference of the femoral shaft and humeral articulation facet on the coracoid were taken from the literature (Field et al. 2013) and an average of all three was taken. To account for differences in cervical number and body size, region size was assessed using percentage cervical vertebrae per region (as opposed to singular vertebrae), and neck length was normalised using the following equation:

$$Neck\ length\ (normalised) = \frac{Neck\ length}{Body\ mass^{0.33}}$$

To assess the effect of these factors on the number of vertebrae per region a phylogenetic ANOVA using the ‘geomorph’ package (Adams et al. 2017) in R version 5.0 (R Core Team 2018). The coefficients of these relationships were examined to assess the effect of each factor on region size in each region.

Results

Q1: Is the number of cervical regions across extant Aves constant?

Principle Coordinate Analysis and cluster analysis suggests that all 52 birds display 5 regions within their cervical column (Figs 2-3, Appendix Figs. 3.0) with 70% - 90% of morphological variation being accounted for by the first three Principle Coordinate axes (see Appendix Table 3.2b). Despite region size (the number of cervical vertebrae per region, figure 2) varying substantially across species, PCO morphospace occupation of each region was conserved for all birds studied (Fig. 3, Appendix Figs 3.0). The anteriormost cervical (C2) always occupied a distinct region of morphospace, followed by a small number of anterior cervicals forming the second cervical region. Regions 3 and 4 were also separate in morphospace, but to a lesser degree than regions 1 and 2. Similar to the first region, region 5 occupied its own discrete area of morphospace (Fig. 3).

Landmark geometric morphometrics in isolation (PCA, without qualitative characters) revealed consistent shape change across the cervical column of all birds studied (Appendix Fig. 3.0). For each individual bird, the first three principal component axis accounted for 70% - 90% of the observed morphological variation (Appendix Table 3.2). Although not the primary aim of this PCA analysis, the aforementioned five regions can be discerned in PCA plots of individuals (Appendix Figs. 3.0). Region 1 retains its unique morphospace occupation, as does region 5. The middle three regions are less distinct than in the combined study (GMM landmarks with qualitative characters) above, owing to the lack of qualitative characters included in the PCO analyses.

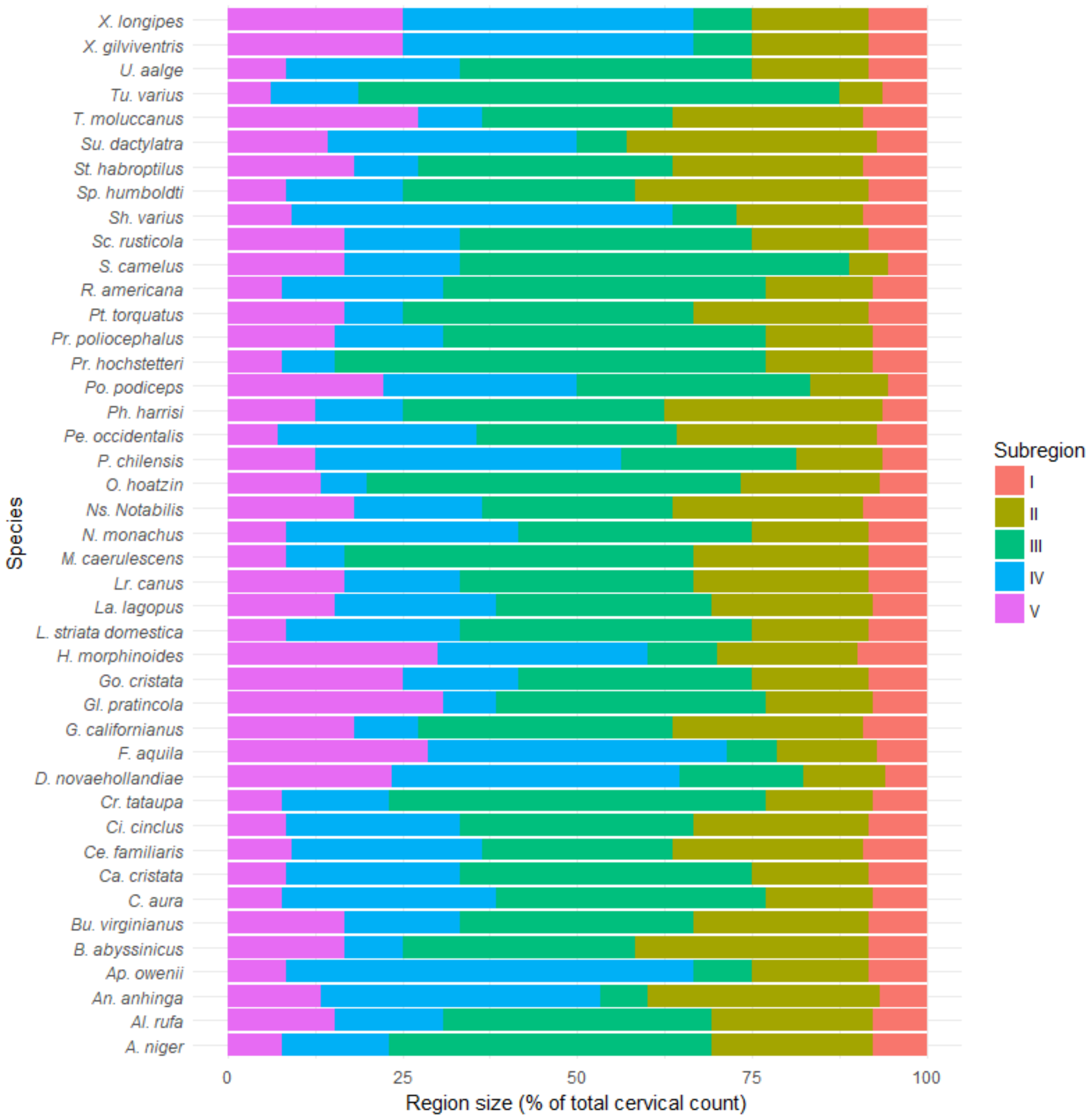

Figure 2 Region size (as a normalised measure of percentage of total cervical vertebrae) variation for all birds studied. Colours denote region number. All extant avians have five cervical regions. Regions 1 and 5 are stable in their number of vertebrae, where as regions 3 and 4 display the largest variations in vertebrae per region.

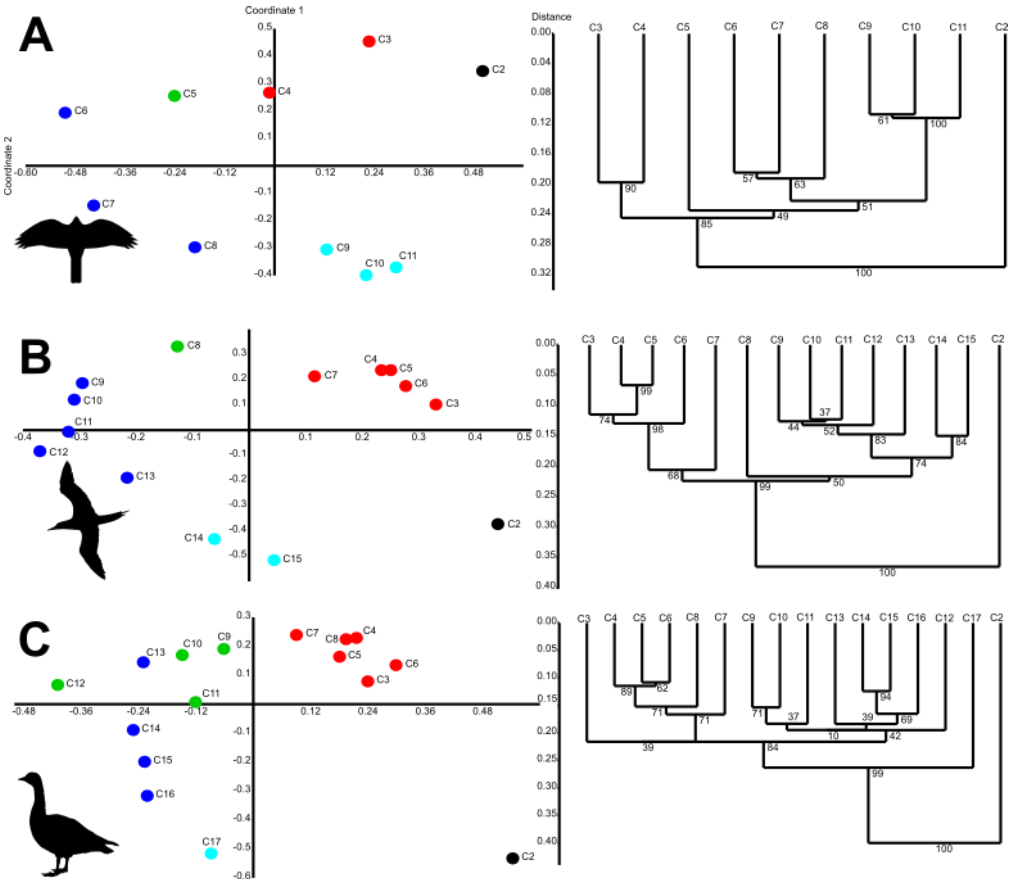

Figure 3 Principle Coordinate graphs (left) and cluster analysis charts (right) depicting the delineations between cervical regions in 3 taxa; A) *Hieraaetus morphnoides*, B) *Sula dactylatra*, C) *Branta leucopsis*. Colours on Principle Coordinate graphs denote cervical regions. Numerical values underneath cluster branches denote bootstrap support after 1000 replicates. Despite changes to total number of cervical vertebrae and ecology, all birds display 5 cervical regions when PCO and cluster analyses are used together to designate regions.

Q2: Are cervical regions defined by specific morphological traits? Do these traits vary across Aves in accordance with external factors?

For all species, shape change along the first principal component involves a variation in the height of the neural spine, rotation of both pre- and postzygophyseal articulation facets, and an anterioposterior variation in the length of the centrum (Fig.

4). Shape change across PC2 predominantly consisted of the anterioposterior positioning of the neural spine along with changes to centrum height (Fig. 4). Collating region boundary data (PCO and cluster analysis) and region shape data (individual PCA plots) allows for a broad scale comparison of mean region shape between species (Fig. 4). Region 1 is defined by an anterioposteriorly restricted centrum length, a deepened centrum, a tall neural spine, a small prezygopophysis with an anteriorly facing articular facet and a larger postzygopophysis ventroposteriorly facing articular facet. The second region retains the enlarged neural spine but displays a longer, thinner centrum and a larger more anteriorly positioned prezygopophysis. Region 3 displays the smallest neural spine of all 5 regions, as well as the longest centrum. The articular facet of the prezygopophysis now faces dorsoanteriorly, whilst the facet of the postzygopophysis is oriented ventroposteriorly. Neural spine height increases slightly within region 4, whilst the centrum is shorter and deeper than the previous region. The articular facet of the prezygopophysis is more dorsally oriented in this region. Region 5 displays a larger neural spine still, with a shorter and deeper centrum with the articular facet of the prezygopophysis facing more anteriorly than in region 4.

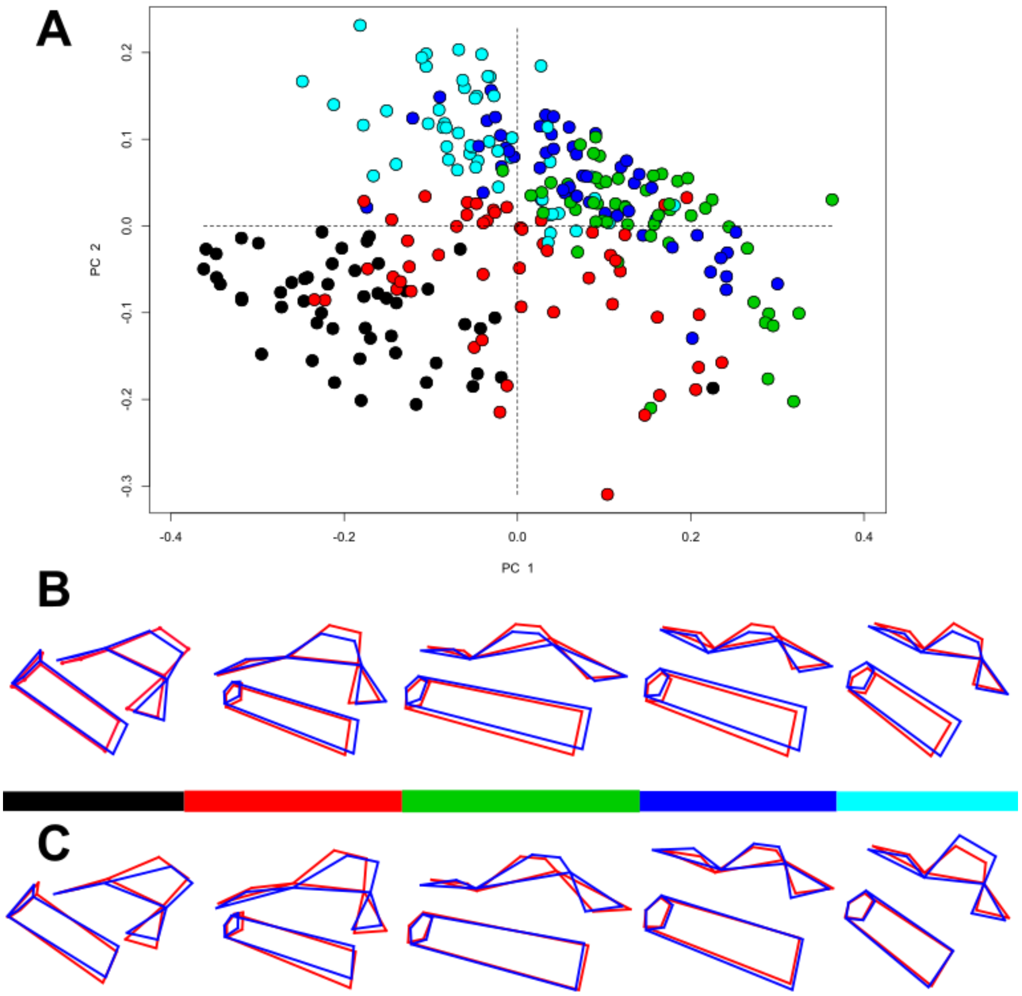

Figure 4 A) PCA graph of the first two principal components of mean region shape for all five regions of all birds studied, colours denote regions. B) Shape change across PC1 for all 5 regions (the colour bar indicates region number, anterior regions are on the left). Red outline denotes mean shape, blue outline displays the maximum shape change across PC1. C) Shape change across PC2 for all 5 regions (colour notations are as in B). For all birds, shape change along the first principal component involves a variation in the height of the neural spine, rotation of both pre- and postzygophyseal articulation facets, and an anterioposterior variation in the length of the centrum

Q3: Do these traits vary across Aves in accordance with external factors?

PTA was performed for each ecological factor and taxonomic group. Pairwise comparisons of dietary groups revealed that diet had little effect on shape variation across the entire cervical column (Figure 5, Table 3), with only carnivores and insectivores being significantly different from each other in trajectory direction and shape ($P = 0.025$ and $P = 0.025$ respectively), and other dietary groups displaying no significant variation between each other ($P >> 0.05$). Similarly, only soaring and continual flapping flight styles were found to be significantly different in trajectory direction when compared to each other ($P = 0.045$), with no other groups displaying significant differences in trajectory direction, size or shape (Figure 6, Table 3). Taxonomic groupings displayed no significant differences between all three trajectory descriptors (Appendix Table 3.2c, Appendix Fig 3.1).

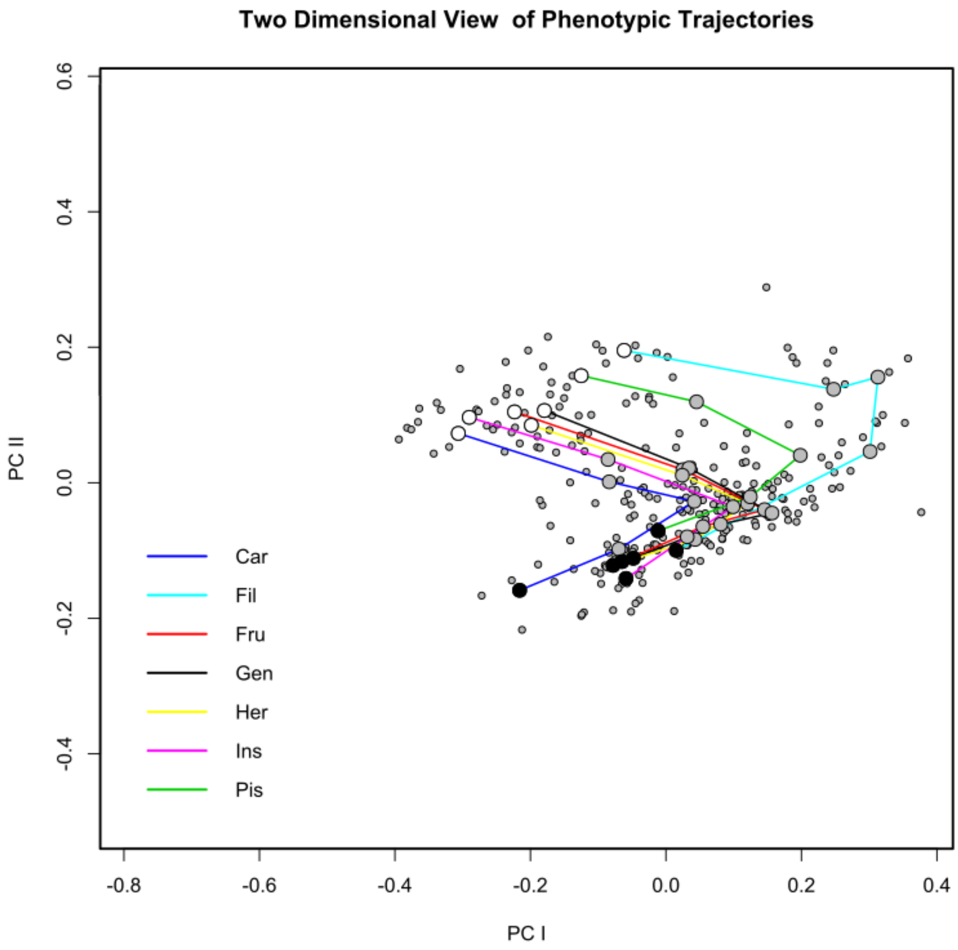

Figure 5 Phenotypic trajectories of dietary ecologies. Colours denote diet. Car = carnivory, Fil = filter feeding, Fru = frugivore, Gen = generalist, Her = herbivore, Ins = insectivore, Pis = piscivore. PTA analyses shows that despite large differences in dietary ecology, the gross morphology of the entire cervical column does not change (except between ecologies that are extremely divergent, see table 3 below). Black circles represent the group mean region shape for region 1, white circles represent group mean region shape for region 5. Grey circles represent the group mean region shape for regions 2-4.

Between dietary groups (Figure 7b, c) region 1 of carnivores has an enlarged, more upright neural spine compared to insectivores. The morphology of regions 2 and 3 is similar for carnivores and insectivores. Morphological variation between region 3 and 4 appears lower in insectivorous taxa. Insectivores have a much shallower neural spine than carnivorous birds in region 4. Carnivores display more variation in centrum length and height between regions 3 and 4 than insectivores. A similar trend can be seen in inter- and intraregional variation between regions 4 and 5 in carnivores and insectivores.

Table 3 Pairwise comparisons of phenotypic trajectories for diet and flight style categories. Significant P values are emboldened, italicised and starred. BAF = burst adapted flyer, CoF = continual flapping, FlG = flap gliding, InB = intermittent bounding, Soa = soaring, Ter = terrestrial. Most birds studied do not show a difference in gross vertebral morphology across the entire cervical column, except in examples of extreme divergence such as between carnivores and insectivores, as well as soarers and continual flappers.

Ecology	**Direction *P*-value**	**Size *P*-value**	**Shape *P*-value**
Carnivore vs Filter	0.370	0.870	0.815
Carnivore vs Frugivore	0.380	0.745	0.755
Carnivore vs Generalist	0.060	0.560	0.710
Carnivore vs Herbivore	0.070	0.275	0.845
Carnivore vs Insectivore*	***0.025****	***0.705***	***0.045****
Carnivore vs Piscivore	0.070	0.270	0.430
Filter vs Frugivore	0.345	0.815	0.380
Filter vs Generalist	0.260	0.860	0.515
Filter vs Herbivore	0.330	0.740	0.630
Filter vs Insectivore	0.235	0.875	0.155
Filter vs Piscivore	0.250	0.780	0.480
Frugivore vs Generalist	0.965	0.545	1.000
Frugivore vs Herbivore	0.890	0.305	0.975
Frugivore vs Insectivore	0.895	0.615	0.815
Frugivore vs Piscivore	0.620	0.375	0.825
Generalist vs Herbivore	0.605	0.525	0.995
Generalist vs Insectivore	0.435	0.820	0.495
Generalist vs Piscivore	0.340	0.480	0.755
Herbivore vs Insectivore	0.495	0.440	0.355
Herbivore vs Piscivore	0.280	0.995	0.730
Insectivore vs Piscivore	0.085	0.330	0.725
BAF vs CoF	0.695	0.615	0.545
BAF vs FlG	0.260	0.860	0.645
BAF vs InB	0.195	0.510	0.100
BAF vs Soa	0.080	1.000	0.650
BAF vs Sub	0.195	0.950	0.795
BAF vs Ter	0.470	0.055	0.470
CoF vs FlG	0.340	0.930	0.985
CoF vs InB	0.455	0.430	0.500
CoF vs Soa*	***0.045****	***0.610***	***0.375***
CoF vs Sub	0.265	0.945	0.995
CoF vs Ter	0.470	0.130	0.970
FlG vs InB	0.300	0.410	0.750
FlG vs Soa	0.085	0.855	0.400
FlG vs Sub	0.550	0.835	1.000
FlG vs Ter	0.335	0.285	0.900
InB vs Soa	0.055	0.515	0.075
InB vs Sub	0.355	0.955	0.990
InB vs Ter	0.190	0.065	0.775
Soa vs Sub	0.145	0.935	0.800
Soa vs Ter	0.085	0.070	0.400
Sub vs Ter	0.280	0.660	0.950

Inter- and intraregional differences between soaring and continual flapping are less than those recovered for dietary groupings (Figure 7d, e). Continual flappers have a shorter neural spine than soaring birds and display less inter-regional variation between regions 3 and 4, these patterns are similar to those displayed in dietary groupings (Fig. 7). There appears to be other aspects of inter-regional variation that are specific between soarers and continual flappers, with the angle of orientation of the prezygopophyseal articular facet changing to a greater degree between all 5 regions, as well as the anterio-posterior enlargement of the neural spine of region 1 in soarers (Fig. 7).

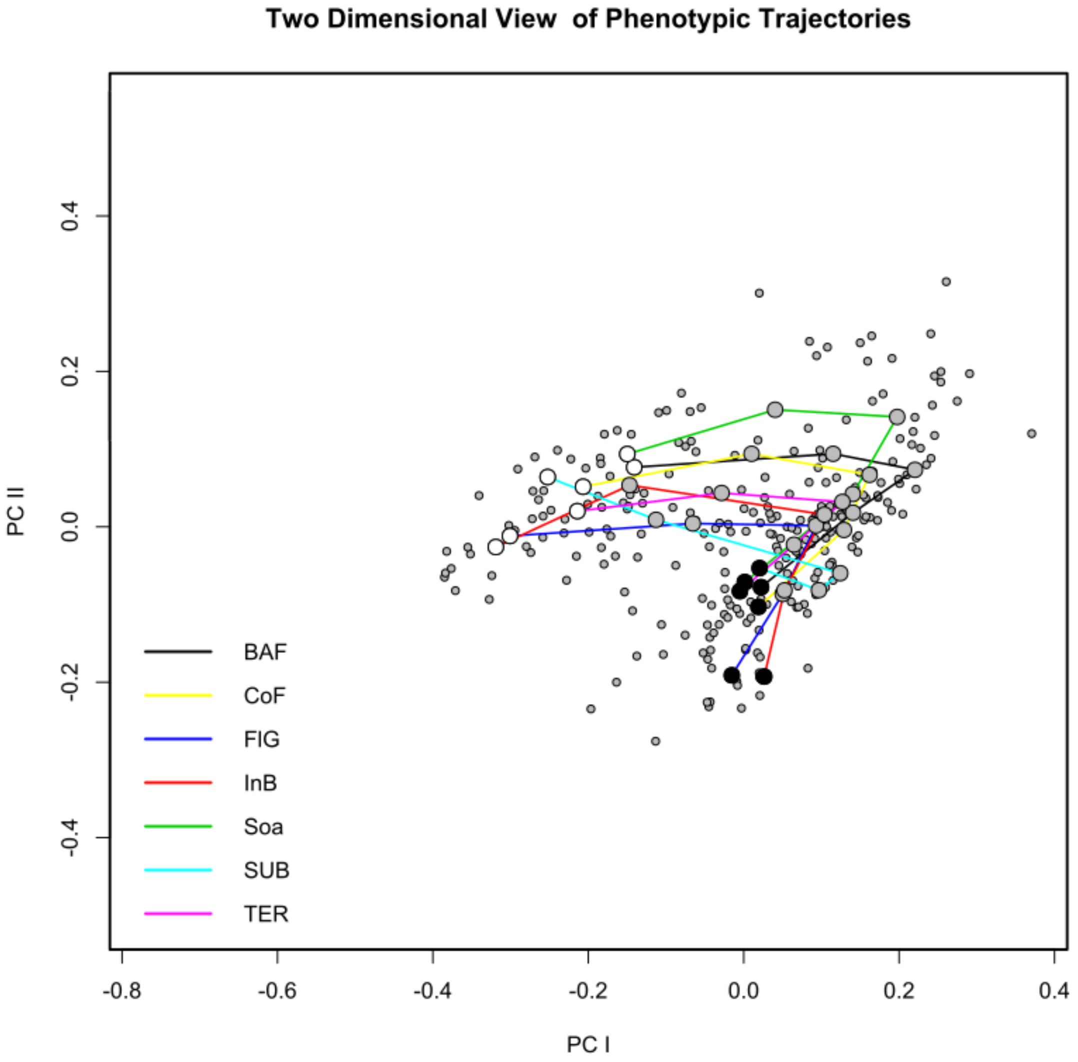

Figure 6 Phenotypic trajectories of different flight styles. Colours denote flight. BAF = burst-adapted flight, CoF = continual flapping, FIG = flap gliding, InB = intermittent bounding, Soa = soaring, SUB = subaqueous, TER = terrestrial. As for dietary ecologies (Fig 5 above), locomotory mode (flight style) has little impact on gross morphology across the entire cervical column (except in extremely divergent taxa, see table 3 above). Black circles represent the group mean region shape for region 1, white circles represent group mean region shape for region 5. Grey circles represent the group mean region shape for regions 2-4.

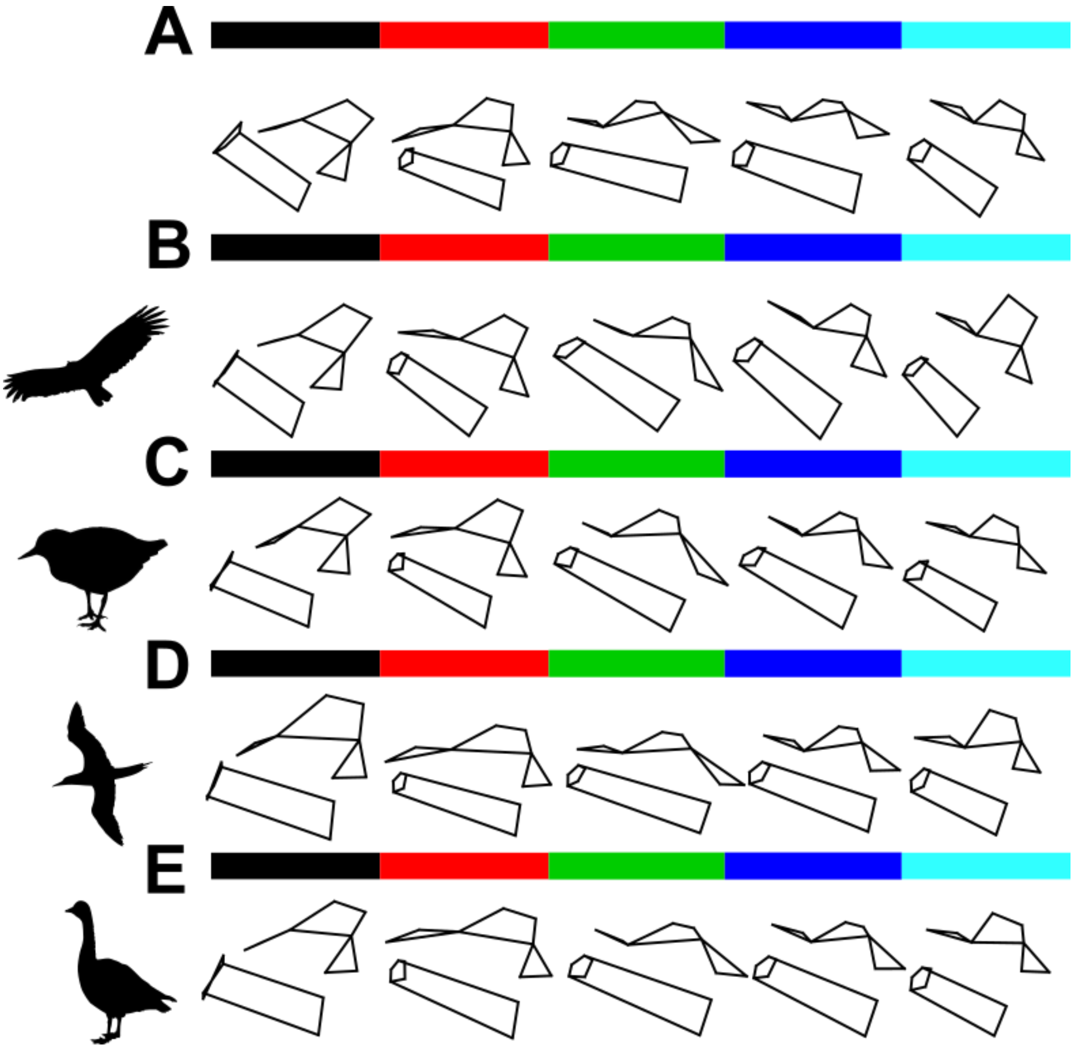

Figure 7 Mean regional shape change across the cervical column. Lateral view of each region mean vertebral shape, colour indicates region, anterior regions are towards the left, posterior regions are to the right. A) Mean region shapes for all birds, B) mean region shapes for carnivorous birds, C) mean region shapes for insectivorous birds, D) mean region shapes for soaring birds, E) mean region shapes for continual flapping birds. In ecologies that are extremely divergent, the vertebral morphology across the entire cervical column changes. However, aspects of the 'general pattern' of cervical morphology are still present in these 'extreme' taxa.

Q4: Do variations in cervical region size across Aves correlate with external factors?

ANOVAs determine if differences between means of multiple independent groups are statistically signficant. Phylogenetic ANOVAs have two predominant assumptions that must be met in order subsequent tests to remain valid: data is normally distributed and homogeneity of variance (variance among groups must be approximately equal). For all signficant models (Table 4, 5) these assumptions were met (Appendix Figs 3.2) The phylogenetic ANOVA revealed that only frugivory and soaring significantly correlated withh region counts (*P* = 0.024 and 0.020 respectively, Table 4). Neck length has no signficant correlation with the number of cervical vertebrae per region (*P* = 0.811, Table 4). Frugivorous birds have relatively more vertebrae in region 3 and 5 and relatively less in regions 2 and 4 (Table 5, coefficients). Soaring birds appear to have relatively more vertebrae in regions 2, 4 and 5 and relatively less vertebrae in region 3 (Table 5, coefficients).

Factor	DF	SS	MS	R-squared	F	Z	*P*-value
Neck length	1	1.895	1.895	0.007	0.258	-0.823	0.811
Body mass	1	6.871	6.871	0.025	0.934	0.353	0.374
Frugivore**	***1***	***28.625***	***28.625***	***0.106***	***3.890***	***1.663***	***0.024*****
Insectivore	1	0.994	0.994	0.004	0.135	-1.289	0.896
Soaring**	***1***	***30.334***	***30.334***	***0.112***	***4.123***	***1.850***	***0.02*****
Predator	1	3.005	3.005	0.011	0.408	-0.142	0.606
Generalist	1	4.445	4.445	0.016	0.604	0.165	0.480
Herbivore	1	9.308	9.308	0.034	1.265	0.996	0.139
Filter feeding	1	7.042	7.043	0.026	0.957	0.769	0.220
Continual flapping	1	1.117	1.117	0.004	0.152	-0.957	0.848
Burst adapted flying	1	3.782	3.782	0.014	0.514	0.199	0.472
Terrestrial	1	0.528	0.528	0.002	0.717	-1.432	0.909
Intermittant bounding	1	4.018	4.018	0.015	0.546	0.278	0.412
Flap gliding	1	0.122	0.122	0.000	0.017	-2.910	0.986

Table 4 ANOVA table for analysing the correlation between ecological (diet and flight style) and functional (neck length and body mass) factors on regional vertebral counts in a selection of extant birds. Significant factors are emboldened, italicised and starred. Only two factors significantly correlate with region size: frugivory and soaring.

Factor	Region 1	Region 2	Region 3	Region 4	Region 5
Intercept	7.845	20.144	33.898	23.119	13.994
Frugivore	0.275	-1.127	9.051	-9.657	1.459
Soaring	-0.113	3.278	-16.159	12.365	0.629

Table 5 Coeffiencts from the significant factors of the phylogenetic ANOVA, frugivory and soaring. Frugivorous birds have relatively more vertebrae in region 3 and 5, and relatively less vertebrae in regions 2 and 4. Soaring birds have relatively more vertebrae in regions 2, 4 and 5 and relatively less vertebrae in region 3.

Discussion

This study is the first to quantitatively demonstrate that extant avians from a wide spectrum of taxonomic, ecological and locomotor groups, universally exhibit 5 morphological cervical regions (Fig 2, 3), the boundaries of which have been found previously to correspond to specific *Hox* gene expression sites (Böhmer, Rauhut, and Wörheide 2015a). Previous work that demonstrated the direct links between these morphological and genetic sub-regions in the chicken (Bohmer et al. 2015) did not examine the nature of morphological variation between regions, examine its functional implications nor attempt to examine how shape variation within and between species may be linked to factors like phylogeny, diet and locomotion. By applying systematic, quantitative techniques to a large sample of birds, this study has attempted this for the first time (Figs. 2 – 7). Addressing these issues for the first time (Q1-4) provides a new basis to interpret address the fifth research question posed in this chapter: (Q5) What are the functional mechanistic links between correlations in region size and shape and external factors? These links will be discussed herein.

Cervical regions are defined by specific morphologies which don’t respond to external factors: a ‘general pattern’

Results from the PCA of mean region shape can be used to show the defining features of anatomy for each cervical region (Fig. 4). Regional shape change along the cervical column corresponds to a decrease in vertebral height (predominantly neural spine height) from anterior to middle cervical regions and increases towards the posteriormost region 5, whilst centrum length increases from anterior to middle regions and decreases towards the posterior end of the cervical column (Fig 4). These morphological trends are consistent with qualitative descriptions of waterfowl (Van der Leeuw 1992; Van der Leeuw, Bout, and Zweers 2001; van der Leeuw, Bout, and Zweers 2001) and palaeognaths (Böhmer, Rauhut, and Wörheide 2015; Van der Leeuw, Bout, and Zweers 2001).

In mammals a shorter relative centrum length corresponds to increased intervertebral stiffness due to a shorter range of motion over which vertebrae are not in contact with each other, whilst a longer relative centrum length overcomes this issue to achieve less intervertebral stiffness and a great range of motion (Pierce, Clack, and Hutchinson 2011; Shapiro 1995; Koob and Long Jr 2000; Long et al. 1997). Thus the variation in centrum length and height across PC1 and PC2 (Fig. 4) may be indicative of differences in maximal range of motion within the cervical column of birds. If this interpretation is correct then the shorter relative centrum length in regions 1 and 5 may indicate increased stiffness (or reduced range of motion) relative to regions 2-4, where increased relative centrum length facilitates greater intervertebral flexibility. This is a cautionary interpretation, however, as the mammalian relationship between cervical range of motion and vertebral linear measurements mentioned above have not been found in isolated studies of birds, such as *Meleagris gallopovo* (Kambic, Biewener, and Pierce 2017). More quantitative data is needed to assess the extent of the relationship between vertebral shape and range of motion in extant birds to test this hypothesis.

Significant differences are found between the trajectory direction and shape of carnivores and insectivores, as well as significant differences between trajectory directions of soaring and continual flapping birds (Figures 4, 5, Table 3). However, no significant differences were observed between phylogenetic groups. Differences in trajectory direction have been previously suggested to represent distinct relative covariations of vertebral shape amongst ecological groupings throughout the part of the vertebral column in question (Collyer and Adams 2013; Adams and Collyer 2009;

Randau, Cuff, et al. 2016). In another sense, differences in trajectory direction appear to be correlated with ecological divergence between taxa. In this regard it is interesting to note the wider lack of significant differences in trajectories for other ecological groups in birds (Table 3). Interpreting the results from the phenotypic trajectory analysis suggests that for the majority of extant avians, vertebral shape changes along the cervical column in a similar, general fashion, as shown in the mean shapes in Figures 4 and 7.

Biomechanical studies are grounded in the rhetoric of 'form follows function' with there being a multitude of examples of vertebrate bone morphology adapting to specific functions to accommodate the musculature that powers these functions (Lauder 1995 and references therein). There are many qualitative examples of avian cervical musculature adapting to specific ecologies; more generally in Passeriformes to aid acrobatic capabilities when capturing food items (Palmgren 1949), or more localised myological adaptations as in Apodiformes that cross over the M. splenius capitis to accommodate faster head turning in order to catch insects in-flight (Jenni 1981; Zusi and Bentz 1984; Fritsch and Schuchmann 1988). Cervical musculature can even be adapted to accommodate for entirely new behaviours as in the pecking of wood in Ciconiiformes (Kral 1965). Despite this apparent wide variety of form-function relationship in vertebral anatomy of the cervical column, the previous examples represent extremes within extant Aves. The majority of variation observed in avian cervical musculature is attributed to differences in the number of muscle slips associated with any one muscle group, in itself this is more likely related to the total number of cervical vertebrae (Zusi 1962; Zusi and Storer 1969; Kuroda 1962; Boumans, Krings, and Wagner 2015; Shufeldt 1890; Sanchez et al. 2013; Tsuihiji 2005; Landolt and Zweers 1985). Many previous authors have commented on this general organisation of cervical myology, which has led to the notion that for many birds, the cervical musculature, and thus by association vertebral shape, is adapted to the 'economics of continuous movement (Van der Leeuw, Bout, and Zweers 2001; van der Leeuw, Bout, and Zweers 2001) rather than any extreme functional demand. Birds use their neck as a 'surrogate arm', manoeuvring the head into many different positions in order to perform a wide variety of tasks and behaviours normally executed by the forelimbs. Thus it is perhaps no surprise that ecological factors, in general, display little correlation on the overall anatomy of the cervical column, and that this 'general pattern' of vertebral shape change (Figs 4, 7) is present to accommodate for the plethora of tasks the cervical column is involved with on a daily basis. However PTA in this instance was used to analyse potential relationships between external factors and the shape variation across the entire cervical column. This 'general pattern' hypothesis needs to be tested further to observe the effects of external factors on individual cervical regions within a phylogenetic framework, which the Chapter 5 of this thesis shall address using Procrustes distance-based phylogenetic regression models (Adams 2014).

Shape change across the cervical column deviates from the 'general pattern' for extreme ecologies

The 'general pattern' of vertebral shape change across the cervical column is not universal across Aves and significant differences between cervical column shape trajectories can be observed between certain ecologies (Figs 5 – 7, tables 3, 4). Significant differences exist in region shape between carnivores and insectivores as well as soarers and continual flappers (Fig. 7). The phenotypic trajectories between these ecologies displayed significantly different trajectory directions, which has been previously interpreted as evidence for ecological divergence between groups (Stayton and Ruta 2006; Randau, Cuff, et al. 2016; Collyer and Adams 2013). That only 'extreme' ecologies, which require specialised cervical kinematics and kinetics (see below) vary in shape supports the idea of a 'general pattern' of cervical morphology for the majority of extant Aves.

As the primary interface between predator and prey, cranial (primarily beak) morphology of birds is closely linked to dietary specialisation (Cooney et al. 2017; Sievwright and Higuchi 2011). The neck also contributes heavily to feeding (Palmgren 1949; Zusi and Storer 1969) thus it is unsurprising that ecologies involving complex prey capture methods display differences in shape across the entire cervical column. To remove flesh from prey carnivorous birds must produce large retraction forces with their necks and thus perform more work with their necks than other birds. Such forces may be specific to carnivorous birds as other diets include prey items that can be swallowed whole and do not need to be processed extraorally. In birds, dorsal musculature such as M. longus colli dorsalis pars caudalis is responsible for head and neck retraction (Heidweiller et al. 1992; Van der Leeuw, Bout, and Zweers 2001; van der Leeuw, Bout, and Zweers 2001; Boumans, Krings, and Wagner 2015), and the neural spines of vertebrae in regions 4 and 5 serve as the insertion site for this muscle. Within carnivorous birds the observed enlargement of the neural spine in regions 4 and 5 (Fig 7b, c) may be explained by a potential increase in the mass and attachment site area of M. longus colli dorsalis pars caudalis. More cranially positioned dorsal musculature such as the M. splenius capitis and the cranial portion of M. longus colli dorsalis also aid in feeding by allowing for enlarged movements of the head, and due to the larger forces and work involved in carnivory these muscles may also be enlarged in carnivorous birds, as these muscles attach to the neural spines of regions 1 (M. splenius capitis) and 2 (M. longus colli dorsalis). Further work will be needed to confirm this as description of cervical musculature in carnivorous birds is scant (Boumans, Krings, and Wagner 2015).

In contrast, insectivorous birds feed on much smaller prey that rarely require extraoral processing and thus necessitate less cervical force production. To catch small and fast moving insects on the wing swifts and hummingbirds (Apodidae) display a specialised 'cruciform origin' of the M. splenius capitius muscle (Burton 1971; Fritsch and Schuchmann 1988; Brause, Gasse, and Mayr 2009), which allows for rapid head movement as well as reducing the space occupied by this muscle. This cruciform origin of the M. splenius capitis is specific to Apodidae and Aegothelidae (Burton 1971) (the owlet-nightjars (Burton 1971; Burton 1974)). However the selection pressure to reduce musculoskeletal mass of the cervical column may be common amongst insectivorous birds as insectivory requires fast and precise head strikes. Unlike the high work output of the necks of carnivorous birds, insectivores need higher power, to produce short and precise head strikes. The success of these strikes may be compromised when the cervical column is burdened with extra mass and as such this mass reduction in combination with the low cervical force production required for catching very small prey could have led to the reduction in the neural spines of regions 4 and 5 of insectivorous birds (Fig 7d, e). To account for the loss in muscle mass in the necks of insectivores, muscle architecture may change to increase the power output of cervical muscles. Again, further work is needed to confirm this.

The trajectory of cervical morphology is significantly different between soaring birds and birds that continually flap (Table 3, fig 7d, e). These differences are less than those observed between dietary ecologies, and may relate functionally to head stabilisation. During flight birds must stabilise their gaze in order to safely and efficiently navigate their flight path (Land 1999; Goller and Altshuler 2014) to avoid predators and collision with conspecifics. This stabilisation must occur to counteract the effects of varying wind conditions and directional changes, as well as against the oscillatory movements of the body during each wingbeat. Wingbeats not only interrupt image stabilisation, but the wings may occlude vision during each wingbeat, as such the neck is used to dampen the body’s wingbeat oscillations and can saccade the head rapidly when the wing is occluding vision (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015). The number of wingbeats during flight varies considerably between soarers and continual flappers, with soarers beating their wings far less on average than continual flappers. Although the role of the neck in head stabilisation during flight has not yet been quantified in soaring birds, extrapolation from studies of flapping flight (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015) indicates that with fewer wingbeats, the neck would perform less compensatory movement during soaring. If this is true then there would be potentially less musculature required to achieve this dampening, as has been documented previously between different flight styles (Popova 1972). Reduced muscle mass could be mechanically linked to the osteological differences recovered by phenotypic trajectory analysis between these two flight styles (Fig 7d, e).

Number of vertebrae per cervical region does not correlate with external factors

The 'S' shape of the cervical column is the defining feature of the avian neck and has been suggested to be present to ensure that joint angles are approximately equal across the entire length of the column when held in this 'neutral' habitual posture (Bout 1997). It is postulated that maintaining equal joint angles throughout the neck in this habitual posture serves to minimise the potential for injury during large motions over single joints (Bout 1997). This means that changes in overall neck flexibility are accomplished by the addition of cervical vertebrae (Zweers, Bout, and Heidweiller 1994; Bout 1997; van der Leeuw, Bout, and Zweers 2001). The lack of a significant relationship between regional cervical counts and neck length suggests that cervicalisation (the addition of cervical vertebrae) is not responsible for the elongation of the avian neck, as has stated previously in the literature (Zweers, Bout, and Heidweiller 1994; Bout 1997; van der Leeuw, Bout, and Zweers 2001). As stated above, cervicalisation is most likely present to ensure the neck has adequate neck flexibility for a wide array of tasks whilst also reducing the flexion ranges of any singular joint to avoid injury (Bout 1997). The lack of significant differences between many ecological groups' region sizes further supports this 'general' pattern of neck adaptation in extant Aves. Previous work comparing the kinematic patterns of generalised tasks (drinking and pecking) in Galliformes, Anseriformes and Palaeognaths found that these patterns showed that, despite large size differences, there are similarities between terrestrial taxa (Van der Leeuw, Bout, and Zweers 2001). This notion holds true in this study as neither body mass nor neck length has a significant correlation with the number of vertebrae per cervical region (Table 4).

The coefficients from the phylogenetic ANOVA (Table 5) suggest that frugivorous birds have less vertebrae in regions 2 and 4 and more vertebrae in regions 3 and 5, whereas soaring birds have more vertebrae in regions 2, 4 and 5 and less vertebrae in region 3 when compared to other birds. Since excessive joint angles at any specific joint are avoided and joint angles are more equal across the cervical column in birds (Bout 1997), the coefficients from the phylogenetic ANOVA could potentially be interpreted as a rough proxy for changes to regional range of motion in frugivorous and soaring birds. Thus frugivorous birds could be inferred to have more flexion relative to other birds in regions 3 and 5, and less in regions 2 and 4. ‘Legitimate’ frugivorous birds (i.e. those that primarily eat the pericarp or soft areas of the fruit over its seeds) have larger gapes relative to other birds (Snow 1981), suggesting that frugivorous birds needs to efficiently hold and/or transport these large fruits to where they are eaten. To enable this, it would be beneficial for frugivorous birds to ensure this extra weight is held as close to the centre of mass as possible in order to maintain stability during locomotion. If this is the case then the inferred higher flexion range found in region 3 of frugivorous birds (due to more vertebrae in this region) may allow for the neck to tuck the head closer to the body, thereby carrying the fruit closer to the centre of mass.

In soaring birds the coefficients suggest that they have larger flexion ranges relative to other birds in regions 2, 4 and 5 and lower flexion ranges in region 3. Potential functional or mechanistic links between soaring and the changes to the number of vertebrae in cervical regions are less clear than those present in frugivores. Soaring is the only locomotor mode to significantly affect the number of vertebrae per region, so a larger region 3 may be required for flapping flight to ensure that during each wingbeat vision is stabilised and is not occluded by each flap (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015).

Evolution of regionalisation in archosaurs

Compared to other archosaurs (Böhmer, Rauhut, and Wörheide 2015b) birds display an extra cervical region (region 4) and this region displays a large variation in the number of cervical vertebrae (Fig 2). This extra cervical region (region 4) is defined by a centrum that is anteroposteriorly short, and tall due to an enlarged neural spine. In extant avians the vertebrae within region 4 serve as attachment sites for the M. longus colli dorsalis pars caudalis and pars profunda which are involved in head retraction and neck stabilisation during a variety of tasks, whilst also supporting vertebrae in regions 2 and 3 (Heidweiller and Zweers 1992; Heidweiller et al. 1992; Zweers, Bout, and Heidweiller 1994). Region 4 potentially acts as an anchorage for more anterior regions allowing for the expansion of these regions when coupled with expansion of region 4. The results above suggest that the cervical kinematics associated with carnivory display a selection pressure on the neck morphology in modern birds (Fig. 6b, Table 3). As cervical morphology (as well as a change in cervical vertebral counts) appears to change only in herbivorous theropods (Zanno and Makovicky 2010) (theropods being a dinosaurian clade largely associated with carnivory), this selection pressure may be present ancestrally. This suggests that the novel fourth cervical region may have evolved as the diets of ancestral birds shifted away from carnivory. At present, this idea remains highly speculative.

Extant avians display a remarkable variation in cervical count and relative neck size (Dietrich Starck 1979) and this disparity in neck length is also present in sauropod dinosaurs (Sander et al. 2011). Neck lengths of sauropods are amongst the longest of any terrestrial vertebrate (Stevens 1999; Sander et al. 2011), and revealing the mechanisms behind the evolution of neck elongation will aid in the understanding of extreme evolutionary changes to the axial column. Hypotheses concerning the elongation of the sauropod neck suggest that elongation may have begun by the addition of a small number of vertebrae to region 3 (Böhmer, Rauhut, and Wörheide 2015b). In this study, region 3 is often the region with both the most cervical vertebrae and displays the most variation in vertebral count across species (Figs. 2.8, 2.9). This indicates that the *Hox* gene expression boundaries controlling the size of region 3 are highly variable and may have been the ancestral site of neck elongation in sauropods. These conclusions are solidified by recent works that link vertebral morphology to *Hox* gene expression boundaries in the cervical column of both archosaurs and mammals (Böhmer, Rauhut, and Wörheide 2015a; Böhmer 2017; Böhmer et al. 2018). Regions 1 and 5 display the lowest variation in vertebral counts and this suggests that biomechanical constraints may restrict the addition of cervical vertebrae to regions that support the head (region 1) and support the entire neck (region 5) in archosaurs.

Conclusions

The complexity of the avian cervical column has long prevented systematic study into the potential drivers behind the large variation seen in the neck morphology of extant Aves. By using 3D geometric morphometrics to predict cervical regions and their *Hox* gene expression boundaries, cervical morphology can be statistically compared across a broad spectrum of extant birds. This study has shown that all birds display five cervical subregions (Fig 2), and that each subregion has a consistently identifiable morphology (Figs 4, 7). Unlike past work investigating the axial column of mammals (Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016; Shapiro 1995; Pierce, Clack, and Hutchinson 2011; Koob and Long Jr 2000; Long et al. 1997), birds show little correspondence with many ecological categories (diet and flight style; Figs 5, 6, 2.8, 2.9, Tables 3, 4), and there exists a 'general' pattern of gross neck morphology which appears adapted to the large amount of daily tasks common to most ecologies (Figs 5-7). However, the shape of the cervical column does correlate to 'extreme' ecologies, whereby neck vertebrae, myology and kinematics may need to be specialised for a specific function such as carnivory (Figs 5-7, Table 3).

Surprisingly, the variation in vertebral counts of each region do not correspond to measures of neck length (Table 4), and similar to variation in region shape do not correlate well with ecological factors (Table 4). As avians appear adjust cervical count to aid different kinematic patterns (via changes in cervical range of motion), the results presented here suggest that region size variation may be correlated with birds that transport and/or manipulate large, cumbersome food items such as fruits. Finally, the maximum variation of vertebral count in any single region is observed in region 3 (Fig. 2). This highlights the potential role that this region may have played in the elongation of the sauropod neck.

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Chapter 4: Neck length and head mass variation

Introduction

The head is an important anatomical unit in any given animal. It contains the brain, multiple sensory organs and tools for mastication. It allows an animal to perceive and engage in every activity during its lifetime, thus it is unsurprising that morphological and biomechanical studies of both extant and extinct animals focus mainly upon the head. The size of an organism's head governs its feeding ecology (Christiansen 1999; Bright et al. 2016; Felice et al. 2019), and the size of the head compared to an organism's body mass can be related to brain size (encephalisation quotient (Jerison 2012)). Thus cranial size (both absolute and relative) and morphology have become important metrics in understanding the drivers and developmental pathways behind major evolutionary events (Rowe, Macrini, and Luo 2011), such as the evolution of modern birds from theropod dinosaurs (Bhullar et al. 2012).

Variations in head size do not exist in isolation: head size has direct effects on the neck, the anatomical unit which connects the head to the rest of the body. The stress-resisting cross-sectional area of the neck scales with an exponent of 2 whilst head weight scales with an exponent of 3 in mammals (Cardini and Polly 2013; Cardini et al. 2015; Preuschoft and Klein 2013; Arnold, Amson, and Fischer 2017). Thus neck length tends to decrease as head weight increases in mammals in order to reduce the neck bending moments by bringing the head closer to the fulcrum of the neck. Relationships between head size and neck length in archosaurs have often been hypothesised, with many authors suggesting that a negative scaling relationship between head mass and neck length also exists for dinosaurs (Sander et al. 2011; Taylor and Wedel 2013; Christian 2002). The relationship between head mass and neck length has never been quantified across Aves despite neck construction and morphology displaying obvious differences (due in part to the S-shaped neutral posture common to most birds) to mammalian counterparts.

The loss of grasping capability in the avian forelimb appears to be integrated with changes in head morphology and head size (e.g. a pointed beak from the existing premaxilla (Clarke and Middleton 2008)) early in the evolutionary history of modern birds. Head size is vital in the early evolution of birds, as it is associated with the expansion of the forebrain for enhanced motor control during flight (Balanoff et al. 2013), as well as expansion of eye size to aid in rapid flight (Brooke et al. 1999). Further integration may exist between the avian head and the cervical column, as the neck is used to position the head to perform the tasks usually undertaken by the forelimbs. The evolution of an elongated neck in birds may have been important in the early evolution of the group as they first evolved flight (Kambic, Biewener, and Pierce 2017), and potential relationships between head size and neck elongation warrant quantitative examination. By analysing the relationship between head mass, neck length, and vertebral morphology, we may begin to understand the cascading effects of flight on integrated anatomical units, such as the cervical column. Any relationship found between head size and neck length may also aid in the understanding of the extreme neck elongation seen in other groups of extinct archosaurs, such as the sauropod dinosaurs. To understand the relationship between head mass, neck length and body mass in extant birds, this study will address the following questions:

Q1. How does neck length scale with body mass across extant Aves?

Q2. How does head mass scale with body mass across extant Aves?

Q3. Do ecological factors influence neck length and head mass allometry?

Q4. How does neck length vary with head mass across extant Aves?

Q5. Do ecological factors influence neck length versus head mass relationship?

Methods

Defining gross morphological parameters: head mass, body mass and neck length

Quantifying head size: α-shape fitting and volumetric estimates

Head size was quantified digitally using an α-shape fitting algorithm (Brassey and Gardiner 2015) on the skulls of 38 species of extant birds to produce a volume for the skull. These 38 species were used to assess regionalisation in chapter 3, allowing for direct consideration of the relationship between head size and regionalisation in this sample. 3D digital models of each bird skull were sourced either directly by micro-CT or from digital collections of colleagues (K. T. Bates, R. B. J. Benson and E. R. Schachner). Specimens newly scanned for this project were imaged at the University of Manchester's Henry Moseley X-Ray Imaging facility (Manchester HMXIF) using the

320/225 kV custom bay Nikon XTEK with system settings for kV set between 50 kV and 90 kV, and µA ranged between 58 µA and 140 µA. Initial scan data was reconstructed using CT Pro 3D (Metris XT 2.2, version 2.2.4365.28608), and a TIFF stack was created using TomoTools v1.0 for ease of import into analysis software. All scans were segmented using Amira 5.6 and Avizo 7.1, and each skull was individually exported as an OBJ mesh file. Point clouds of the skull OBJs were downsampled to 50,000 points to reduce computing time. α-shapes were fitted to the skulls using an in-house modified version of the 'alphavol' package (available form MATLAB central file exchange, originally written by Jonas Lundgren: http://www.mathworks.co.uk/matlabcentral/fileexchange/28851-alphashapes) which also calculates the volume of the computed α-shape. The fit of the α-shape is defined by the refinement coefficient, *k*, which was set at 10. A range of different values of *k* were tested on 3 morphologically distinct avian skulls to determine which *k* value best fit the entirety of the skull and produces a α-shape that crosses the orbits, a feature of anatomy that significantly impacts upon the final α-shape volume and is not fitted in lower *k* values (Figure 1, the MatLab script for this method can be seen in Appendix file 4.0). Head mass was estimated by multiplying the α volume by the weighted mean density of soft tissues within the skull (approximated to the density of water, 997 kg m^3), and normalised by taking the head mass as a percentage of total body mass.

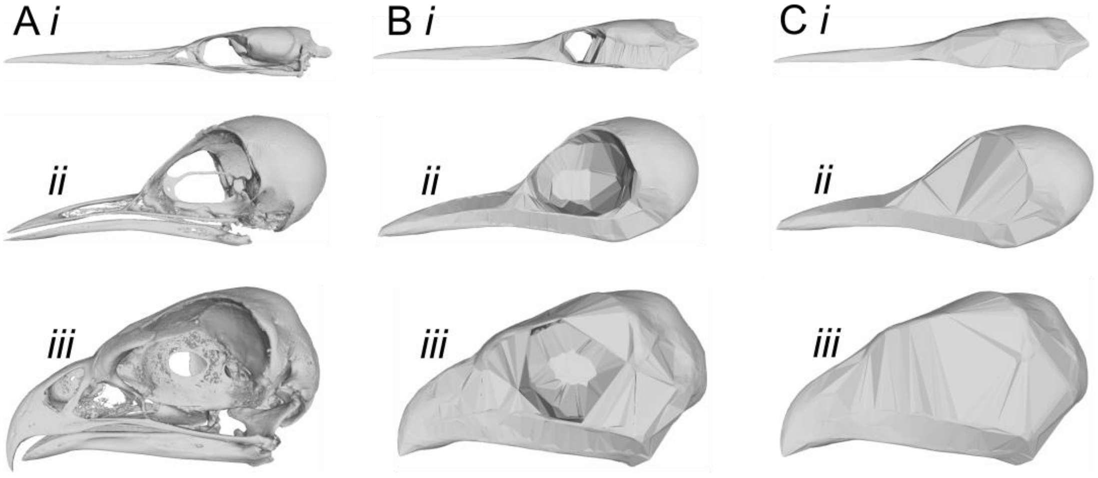

Figure 1 A) 3D digitally rendered skulls of 3 morphologically disparate bird skulls: i) *Anhinga anhinga*, ii) *Xenicus gilviventris*, iii) *Hieraaetus morphnoides*. B) outputted α-shape volume with a refinement coefficient of 5 for each bird in A, C) outputted α-shape volume with a refinement coefficient of 10 for each bird in A. α-shape outputs in C are more appropriate for approximating head volumes as the algorithm successfully covers the orbits, unlike lower inputted values of k.

Defining body mass and neck length

Where possible, body masses were directly measured by weighing the specimens that were CT scanned. Where this was not possible body masses were estimated using scaling equations. Scaling equations for femoral length, minimal circumference of the femoral shaft and humeral articulation facet on the coracoid were taken from the literature (Field et al. 2013) and an average of all three was taken. Neck length was measured digitally as the summed length of each individual cervical vertebrae of each bird. To account for differences in cervical number and body mass, region size was assessed using percentage cervical vertebrae per region (as opposed to singular vertebrae). Overall neck length was normalised using the following equation:

$$Neck\ length\ (normalised) = \frac{Neck\ length}{Body\ mass^{0.33}}$$

Hypothesis testing

Phylogenetic generalised least squares regression (Grafen 1989) was used to test how neck length and head mass scale with body mass, and how neck length varies with head mass (Q1 and Q2 respectively) in birds within a phylogenetic framework. The phylogenetic tree used throughout this analysis is a subset of consensus trees from previous analyses (Jetz et al. 2012, Appendix tree 4.0). Form-function relationships are not usually explained entirely by phylogeny and other factors must be accounted for. In conjunction with pGLS, Pagel's λ (Pagel 1999) was used with a freely varying parameter to assess the impact of phylogeny on statistical models and to scale them accordingly. Pagel's λ is defined by a Brownian motion model combined with a transformation of branch lengths by the value of λ (the branch lengths are multiplied by λ). When λ = 1 the phylogeny is unchanged and when λ = 0 the phylogeny becomes star-shaped (the Brownian Motion model becomes equivalent to independent random walks, meaning that λ values closer to 0 indicate the model has low phylogenetic signal, whereas the inverse is true for values closer to 1). Pagel's λ was used here as it is more robust to polytomies and incomplete phylogenetic data (Molina-Venegas & Rodriguez 2017). To assess the effect of ecology on the relationships between neck length, head size and body mass each bird was assigned a dietary group and a flight style. Diets were assigned based on data from multiple volumes of 'Handbook of the Birds of the World' (Hoyo, Elliott, and Sargatal 1992, 1994; Del Hoyo, Elliot, and Sargatal 1996; Collar et al. 1997; Del Hoyo, Elliott, and Sargatal 1999; Hoyo, Elliott, and Sargatal 2001; Del Hoyo, Elliot, and Sargatal 2002; Del Hoyo, Elliott, and Sargatal 2005, 2006, 2008, 2009, 2010), whilst data on flight style was collated from the literature (Bruderer et al. 2010; Close and Rayfield 2012; Martin-Silverstone et al. 2015). Multiple models are presented as part of each hypothesis in an attempt to observe the effect of multiple explanatory variables (both functional and ecological) on neck length and head mass. To quantitatively compare multiple models for a given data variable, Akaike's information criterion (AICc) was calculated, and comparing AICc values for different models of a certain data variable allows for the most parsimonious model to be estimated, which is the model with the lowest AICc value (Sugiura 1978; Burnham and Anderson 2003).

Results

Q1. How does neck length scale with body mass across extant Aves?

Phylogenetic GLS has similar requirements to the phylogenetic ANOVA performed in Chapter 3 (normal distribution of data, normality of residuals and homogeneity of residuals), and the relationship between neck length and body mass satisfies these criteria (Appendix figs 4.1). Body mass has a significant correlation with neck length (Table 1, $P = < 0.0001$) and the correlation is stronger than that between body mass and head mass (lower AICc value, Table 1). Values for Pagel's λ are > 1 in all but one instance (1.038 – 1.432, Table 1) which indicates that related taxa are more similar than expected based on phylogeny alone (Pagel 1999). Model coefficients reveal that neck length scales close to isometry (coefficient = 0.345, Table 1).

Model	AICc	Coefficient	SE	*P*-value
Neck length ~ body mass + flap gliding	***7.1647***	***-0.3174***	***0.1465***	***0.0371***
Neck length ~ body mass + subaqeuous	7.2172	-0.451	0.2373	0.0656
Neck length ~ body mass	***7.6779***	***0.3453***	***0.0537***	***< 0.0001***
Neck length ~ body mass + continual flapping	***7.9039***	***0.1858***	***0.0811***	***0.0282***
Neck length ~ body mass + filter feeding	10.6116	0.1261	0.2093	0.5508
Neck length ~ body mass + carnivore	10.8447	-0.1159	0.1838	0.5322
Neck length ~ body mass + frugivore	10.9787	0.1691	0.1459	0.2544
Neck length ~ body mass + burst adapted flying	11.0031	0.1073	0.1682	0.5276
Neck length ~ body mass + terrestrial	11.1458	-0.1121	0.0935	0.2387
Neck length ~ body mass + generalist	11.51	-0.1263	0.1024	0.2254
Neck length ~ body mass + piscivore	11.5969	-0.0654	0.1373	0.6371
Neck length ~ body mass + soaring	11.7198	0.071	0.1215	0.5267
Neck length ~ body mass + herbivore	***14.2794***	***0.0215***	***0.0069***	***0.0038***
Neck length ~ body mass + intermittant bounding	16.0004	-0.0959	0.1225	0.4393
Neck length ~ body mass (OLS)	***18.4471***	***0.4786***	***0.0562***	***< 0.0001***
Head mass ~ body mass + herbivore	65.1362	0.4139	0.2146	0.0619
Head mass ~ body mass + insectivore	65.2926	-0.3616	0.1826	0.0556
Head mass ~ body mass	***65.5176***	***1.0149***	***0.1366***	***< 0.0001***
Head mass ~ body mass + subaqueous	66.2274	-0.4521	0.5259	0.3958
Head mass ~ body mass + filter feeding	67.0607	0.1693	0.4728	0.7225
Head mass ~ body mass + continual flapping	67.2942	0.2523	0.1957	0.2057
Head mass ~ body mass + carnivore	67.3033	0.2115	0.3824	0.5836
Head mass ~ body mass + burst adapted flying	67.6092	-0.095	0.3713	0.7995
Head mass ~ body mass + flap gliding	67.6303	-0.2024	0.3022	0.5073
Head mass ~ body mass + frugivore	67.9048	0.09	0.3164	0.7682
Head mass ~ body mass + intermittant bounding	67.9264	-0.4528	0.3447	0.1975
Head mass ~ body mass + soaring	68.1134	0.1399	0.2559	0.588
Head mass ~ body mass + piscivore	68.4711	-0.067	0.2397	0.7814
Head mass ~ body mass + generalist	68.679	-0.0209	0.2238	0.926
Head mass ~ body mass + terrestrial	***69.2554***	***-0.1031***	***0.0069***	***< 0.0001***

Table 1 Results from phylogenetic generalised least-squares (pGLS) analysis of the allometric relationships of head mass and neck length across a selection of extant Aves, ordered according to AICc value (lowest first). Significant ($P = < 0.05$) models are emboldened and italicised. Both neck length and head mass scale with body mass according to isometry. Ecology has a limited association with the allometry of neck length, with only herbivory, flap gliding and continual flapping effecting this relationship and all 3 ecologies showing a negative scaling relationship between neck length and body mass. Ecology has a lower correlation with head mass with only terrestrial birds displaying a significantly different allometric relationship between head mass and body mass, displaying negative allometry.

Q2. How does head mass scale with body mass across extant Aves?

The relationship between head mass and body mass is significant (*P* = < 0.0001, Table 1) and satisfies the requirements of pGLS (normality of residuals and homogeneity of fitted values versus residuals, Appendix Figs 4.2) . Pagel's λ indicated that related taxa are more similar than expected based on phylogeny alone (all λ values are above 1, Table 1, Pagel 1999). The coefficient of the head mass ~ body mass relationship is within the bounds of isometry (1.015, Table 1).

Q3. Do ecological factors correlate with neck length and head mass allometry?

All significant ecological models of allometric relationships of both neck length and head mass satisfy the requirements for pGLS (normality of residuals and homogeneity of fitted values versus residuals, Appendix Figs. 4.3). Ecology is weakly associated with the allometric relationships of neck length and head mass, as very few dietary or locomotory models show significance (Table 1). Herbivorous birds as well as birds that flap glide and continually flap have significant allometric relationships with neck length (Table 1). Two of these ecological groups deviate from isometry as neck length in herbivorous birds and birds that locomote using flap gliding show strong negative allometry (0.022 and -0.317 respectively, Table 1). Continual flappers also display negative allometry between neck length and body mass, but have coefficient values closer to isometry (coefficient = 0.19, SE = 0.081, Table 1). Ecology has a weaker correlation with head mass than neck length as the only grouping to show a significantly different head mass allometry is flightless terrestrial birds (Table 1). Head mass in flightless birds differs from isometry and scales with body mass according to negative allometry (coefficient = -0.103).

Q4. How does neck length vary with head mass across extant Aves?

The relationship between head mass and neck length satisfies the requirements of pGLS (normality of residuals and homogeneity of fitted values versus residuals, Appendix Figs. 4.4). Head mass has a significant (P = < 0.0001, Table 2) correlation with neck length and the relationship between the two is very close to isometric (coefficient = 0.314, SE = 0.036, Table 2). This relationship retains significance when body mass is accounted for (P = < 0.0001, Table 2), although the inclusion of body mass reduces the coefficient and the relationship between neck length and head mass becomes negatively allometric. The addition of body mass results in a less parsimonious relationship between neck length and head mass (AICc of -3.23 compared to an AICc of -6.787 in the neck length ~ head mass model, Table 2).

Q5. Do ecological factors influence neck length versus head mass relationship?

No specific dietary or flight style groups have a significant association with the relationship between neck length and head mass, nor do they have a significant association when body mass is accounted for (Table 2).

Model	AICc	Coefficient	SE	*P*-value
Head mass ~ body mass + neck length	***51.4456***	***1.3141***	***0.2886***	***< 0.0001***
Neck length ~ body mass + head mass	***-3.23***	***0.2297***	***0.0491***	***< 0.0001***
Neck length ~ head mass	***-6.7869***	***0.3135***	***0.0358***	***< 0.0001***
Neck length ~ body mass + head mass + carnivore	-1.7039	-0.2297	0.1433	0.1183
Neck length ~ body mass + head mass + insectivore	-0.4342	0.1083	0.0625	0.092
Neck length ~ body mass + head mass + soaring	1.4129	0.0443	0.0962	0.6482
Neck length ~ body mass + head mass + continual flapping	0.1351	0.1027	0.0695	0.1486
Neck length ~ body mass + head mass + generalist	1.5227	-0.0825	0.0627	0.1967
Neck length ~ body mass + head mass + frugivore	0.9745	0.1106	0.0983	0.2682
Neck length ~ body mass + head mass + herbivore	2.8126	-0.0374	0.0689	0.591
Neck length ~ body mass + head mass + filter feeding	0.1561	0.1021	0.1653	0.5408
Neck length ~ body mass + head mass + piscivore	1.2805	-0.0354	0.1087	0.7468
Neck length ~ body mass + head mass + terrestrial	1.6925	-0.0477	0.0765	0.5367
Neck length ~ body mass + head mass + intermittant bounding	5.2616	-0.0464	0.0879	0.601
Neck length ~ body mass + head mass + flap gliding	-2.1689	-0.2215	0.1194	0.0722
Neck length ~ body mass + head mass + subaqueous	-3.1801	-0.3552	0.1883	0.0677

Table 2 Results from phylogenetic generalised least-squared (pGLS) analysis of scaling relationships between head mass and neck length across a selection of extant Aves. Significant (P = < 0.05) models are emboldened and italicised. Neck length has a negative scaling relationship with head mass when body mass is considered. When body mass is not considered the relationship between neck length and head mass is isometric and displays a more parsimonious AICc score. No ecologies have a significant effect on the relationship between neck length and body mass.

Discussion

Perspectives on the study of the vertebrate cervical column

By using Pagel's λ with a freely varying parameter this study has assessed the impact of phylogeny on the relationships between head mass, neck length and body mass (Tables 1 and 2) and scaled them accordingly (Pagel 1999). Phylogeny can have a large impact on scaling relationships as well as cause a significant amount of variation in skeletal morphology and almost all current large scale studies of functional morphology take phylogeny into consideration in their models (Arnold, Esteve-Altava, and Fischer 2017; Arnold, Amson, and Fischer 2017; Arnold et al. 2016; Randau, Goswami, et al. 2016; Randau, Cuff, et al. 2016; Jones et al. 2018). Recent work on the axial skeleton has taken appropriate measures to take phylogeny into account, and have (along with results presented here; Table 1) shown that excluding phylogeny can lead to wildly different conclusions (Arnold, Amson, and Fischer 2017). Body mass also has a large effect on relative body proportions and the relative scaling of different body segments (e.g. the relationship between neck length and head mass). The effects of body mass on neck length and head mass are examined here as well as throughout the recent literature on the functional morphology of the vertebrate axial skeleton (Arnold, Esteve-Altava, and Fischer 2017; Arnold, Amson, and Fischer 2017; Arnold et al. 2016; Randau, Goswami, et al. 2016; Randau, Cuff, et al. 2016; Jones et al. 2018). Very rarely is body mass excluded as a variable in functional morphology studies. However considerable variation in methods used to measure or estimate body mass potentially creates issues when comparing results across studies. Body mass has been estimated using scaling equations in recent work on the mammalian cervical column (Arnold, Esteve-Altava, and Fischer 2017; Arnold, Amson, and Fischer 2017) or has used centroid size as a proxy where 3D GMM is used (Randau, Cuff, et al. 2016). Scaling equations are frequently used but often vastly overestimate body mass in taxa that have low or high body sizes and are presented with high prediction intervals (Campione and Evans 2012; Bates et al. 2015). Where possible this study has directly measured body mass from the specimen. In cases where this was not possible we used scaling equations based on measurements of the coracoid humeral articulation facet as it has previously been shown to be a more relatively accurate predictor of body mass in volant birds (Field et al. 2013). It must be noted that this scaling equation is different to those used in similar mammalian studies.

Similar inconsistency exists in the approach to incorporating measures of head size in studies examining the relative scaling of the neck and head. Head mass must be properly supported by the neck and represents another functional factor which governs neck morphology and construction. Despite this clear mechanistic link very few studies quantify head mass directly when attempting to assess its scaling relationship with cervical morphology and construction. Amongst recent quantitative studies of the cervical column (Arnold, Amson, and Fischer 2017; Arnold, Esteve-Altava, and Fischer 2017; Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016), this current study is the only one to do so. Studies that do consider the head instead measure head length, width or height as a proxy for head mass (Christiansen 1999; van der Leeuw 2002; Sereno et al. 2007; McGarrity, Campione, and Evans 2013). This current study attempted to model head mass as accurately as possible using volumetric methods (Brassey and Gardiner 2015) by multiplying the α volume by the density of soft tissues within the skull (approximated to the density of water, 997 kg m^3). However this complicates comparisons with older work that uses head lengths.

Further work should directly measure head mass, volume and density across a disparate selection of extant birds in order to quantify the diversity of head mass throughout extant avians. This would provide a comparative platform for future studies that wished to test the accuracy of head mass proxies, either using scaling relationships or volumetric methods.

Ecological groupings are often non-specific in quantitative studies of cervical morphology (Arnold, Esteve-Altava, and Fischer 2017; Randau, Cuff, et al. 2016). Predatory versus non-predatory are amongst the few ecological groups considered in similar studies in mammals (Arnold, Esteve-Altava, and Fischer 2017). This is justified (somewhat) in the mammalian literature as predation involves head-neck movement that resists the movements of prey. Mammals are able to use their forelimbs to aid in predation whereas birds cannot, as such the differences in the head-neck movements between carnivorous and piscivorous birds are extensive, despite being grouped together in the 'predatory' category from mammalian research. More specificity when defining ecological groups leads to lower group sample sizes and is a potential shortfall of this current study, and when compared to other similar studies this one has a lower overall *N*. This relatively low sample size is likely the cause of the low R^2

values that are observed throughout the results of this study (Tables 1-4). This reflects the difficulty in acquiring a wide range of extant avian cadaveric material. Despite some issues common to large scale studies of functional morphology this study is ambitious in its number and variety of species analysed, more than recent mammalian work whereby the overall *N* was high (109) but species diversity was low (9) (Randau, Cuff, et al. 2016). The avian neck is a complicated musculoskeletal system and the only previous attempts to study the factors effecting its morphology have been restricted to a to a few commercially available taxa (Heidweiller et al. 1992; Heidweiller and Zweers 1992; Van der Leeuw 1992; Van der Leeuw, Bout, and Zweers 2001; van der Leeuw 2002). This current study builds on previous avian work to provide a more complete picture of how the avian neck has responded to external factors. Despite relatively small sample sizes and low coefficient of variation values, numerous interesting patterns in neck size scaling and morphological variation occur, all of which appear to have logical biomechanical explanations behind them. Future work must seek to further understand these biomechanical explanations by expanding current knowledge of avian cervical myology and its variation between species.

How does neck length and head size scale with body mass across extant Aves? Do ecological factors correlate with neck length and head size?

Head mass allometry has been studied in many groups of vertebrates (Christiansen 1999; Sander et al. 2011; Georgi, Sipla, and Forster 2013) but little data exists for extant birds (van der Leeuw 2002). The results of this study suggest that avians display an isometric relationship between head mass and body mass (coefficient = 1.015, SE = 0.137, $P = < 0.001$), unlike the positive allometry that has been suggested in mammals and dinosaurs (Christiansen 1999; Sereno et al. 2007; Sander et al. 2011; McGarrity, Campione, and Evans 2013). Proxies for head mass (such as skull length and width) are often used in allometric studies and little has been done to quantify the relative accuracy of these proxies (Christiansen 1999; Sereno et al. 2007; Sander et al. 2011; McGarrity, Campione, and Evans 2013), thus the validity of the previously found positive allometry between head mass and body mass is questioned here. As aforementioned; head mass must be quantified and its variation throughout vertebrates must be studied in greater detail before differences in head mass allometry between groups can be examined thoroughly. Similar issues compound functional interpretations of an isometric relationship between head mass and body mass in birds as widespread truisms exist concerning the lightweight nature of birds and their skeleton. Many features may contribute to the low cranial skeletal mass in birds such as cranial pneumaticity and edentulism (Witmer 1990, 1997; Louchart and Viriot 2011). However when quantified these features don't result in an appreciable mass reduction when compared to other groups of vertebrates (Dumont 2010; Soons et al. 2012; Seki, Mackey, and Meyers 2012). This points to mass reduction in cranial musculature and other soft tissues as a potential functional interpretation for the isometric increase in head mass in extant birds. Previous work lends credence to this claim as both brain size and eye size in birds scale according to negative allometry with body mass (Brooke, Hanley, and Laughlin 1999; Schmidt-Nielsen and Knut 1984). Cranial musculature associated with mastication may be reduced in birds as mechanical food processing is performed by the gizzard, and it is the size and morphology of the gizzard that responds to shifts in diet in extant avians (Battley & Piersma 2005, and references therein). Other studies have recovered a negative scaling relationship between head mass and body mass (van der Leeuw 2002) and whilst head mass is isometric with body mass throughout all birds in this study (coefficient = 1.015, Table 1), negative allometry occurs in models where smaller (ecological) groups of birds are considered (Table 1). This could explain why the former study found negative head mass allometry as they only considered Anseriformes (van der Leeuw 2002).

Studies into avian neck length allometry have been, until very recently, sparse and restricted to ontogenetic studies of a single species (Heidweiller et al. 1992; Heidweiller and Zweers 1992) or allometric studies in just a single order (Van der Leeuw 1992; van der Leeuw 2002). These previous studies did not incorporate a phylogenetic framework into their methodology and when phylogeny was not considered in the neck length ~ body mass model presented here, neck length becomes positively allometric (coefficient = 0.479, SE = 0.056, Table 1). The positive neck length allometry found in these studies may well be the product of the exclusion of phylogenetic considerations. However after this work was completed, neck length allometry was studied in a large sample of extant avians, and corroborates this thesis in its conclusions that neck length scales with body mass according to isometry (Böhmer et al. 2019). This is an important result as it clearly indicates that neck length is not constrained by body mass, a feature which is prevalent within other large clades of vertebrates (mammals). Böhmer and colleagues (Böhmer et al. 2019) elude to reduction in head mass due to cranial pneumatisation as the cause of this isometric scaling of neck length (Dumont 2010, Seki et al. 2010), and this thesis corroborates this speculation in finding that head mass scales isometrically, as opposed to positive allometry in many other vertebrate groups (Arnold et al. 2017, Cardini & Polly 2013, Preuschoft & Klein 2013). A lighter head in conjunction with other factors (e.g. the S-shaped curve of the avian neck) may have allowed birds to evolve such a wide array of neck lengths and morphologies by releasing neck length of body mass constraints.

Cervicalisation (the addition of cervical vertebrae) in avians increases the overall flexion ranges that the cervical column can achieve (Zweers, Bout, and Heidweiller 1994; Bout 1997; Van der Leeuw, Bout, and Zweers 2001). Flexibility and neck length contribute to many daily tasks such as preening, drinking, conspecific interaction, locomotion and posture which are common to all birds, irrespective of dietary or locomotor ecology. This had led to the conclusion that no single adaptive explanation for neck elongation in extant Aves exists (Wilkinson and Ruxton 2012). It has also been recently suggested that the evolution of neck length in Aves is tightly integrated with the evolution of leg length, and only weakly associated with ecological factors (Böhmer et al. 2019). These factors may explain the weak correlation between ecology and neck length within this dataset. Despite a low number of ecological categories with a significant association with neck length and head mass (Table 1), some interesting relationships do arise. Unlike the all birds model, neck length is negatively allometric for birds that locomote using strong powered flight (flap gliding coefficient = -0.317, continual flapping coefficient = 0.186, Table 1). Gaze and vision need to be stabilised during flight (Land 1999; Goller and Altshuler 2014) and the negative neck length allometry displayed in birds with strong powered flight may represent an adaptation to provide greater stability by reducing overall cervical flexion (Zweers, Bout, and Heidweiller 1994; Bout 1997). Negative neck length allometry is also found within herbivorous birds (coefficient = 0.022, Table 1). Herbivory is often hypothesised to be associated with elongated necks as grazing would be more efficient due to a larger feeding envelope (Sander et al. 2011) so the negative neck length allometry presented above is unexpected. However terrestrial foraging birds have been shown to have shorter necks relative to all other birds (Böhmer et al. 2019). Arguments linking neck elongation to herbivory often exemplify large quadrupedal herbivores and may not apply to smaller bipedal animals like birds (Dzemski and Christian 2007; Sander et al. 2011). Thus the relationship between neck elongation and grazing needs to be considered for a wider array of body sizes and locomotor modes.

Ecology has an even weaker correlation with head mass; terrestrial birds are the only ecological group with a statistically significant relationship between head mass and body mass. Head mass allometry appears to be fundamentally different between the all birds model and terrestrial birds as head mass decreases with increasing body mass in terrestrial birds (coefficient = -0.1031, SE = 0.007, Table 1). Many extant terrestrial vertebrates with long necks usually hold their heads above their shoulders at rest and during locomotion to bring the mass of head closer to the centre of mass, enhancing stability (Christian and Dzemski 2007; Dzemski and Christian 2007). There are a multitude of sensory and vestibular adaptations to flight in extant birds and in evolving terrestriality, flightless birds may have lost or reduced these specific adaptations which may lead to a lower head mass (Brooke et al. 1999; Garamszegi, Møller, and Erritzøe 2002; Vincze et al. 2015). Terrestriality also demands larger legs more suited for cursorial locomotion (Gatesy and Biewener 1991; Zeffer and Norberg 2003; Zeffer, Johansson, and Marmebro 2003; Abourachid and Höfling 2012). The decrease in head mass relative to body mass may be a result of other body parts increasing in size at a faster rate than the head, although assessing these rates is outside the scope of this current study. Recent work has discovered that neck length and leg length are integrated in extant birds and that neck length increases with leg length. As terrestrial birds (particularly Paleognaths) have such elongated hindlimbs, a decrease in relative head mass may be present to ensure it is better supported by an elongated neck (Böhmer et al. 2019). However in this current study terrestrial birds do not display a significant specialisations in neck length allometry (Table 1).

How does neck length vary with head mass across extant Aves? Do ecological factors correlate with this relationship?

A reduction in head mass is upheld as an important factor in neck elongation across vertebrates; in mammals neck length decreases with an increased head weight as the stress-resisting cross-sectional area of the neck does not match the scaling exponent of head weight, meaning neck length must decrease in order to decrease loading on the neck by bringing the head closer to the fulcrum of the neck (Cardini and Polly 2013; Preuschoft and Klein 2013; Cardini et al. 2015; Arnold, Amson, and Fischer 2017). This current study suggests that when body mass is considered, birds follow this same pattern of a negative scaling relationship between neck length and head mass (coefficient = 0.229, SE = 0.049, P = < 0.001, Table 2). The only previous work on the scaling relationship between head mass/size and neck length in modern birds also recovered a negative scaling relationship, but head length was used in place of head mass and only anseriforms are considered (van der Leeuw 2002). However the neck length ~ head mass model that excludes body mass is the most parsimonious herein (AICc of -6.787 compared to -23, Table 2) and this model suggests the relationship is isometric (coefficient = 0.314, SE = 0.036, Table 2). This implies that for a given and static body mass, head mass increases at the same rate as neck length in this studied sample of modern birds.

The negative scaling of large cranial organs with body mass may result in birds having a relatively lighter head than other vertebrates (Brooke, Hanley, and Laughlin 1999; Schmidt-Nielsen and Knut 1984). The avian neck has a characteristic 'S' shape and allows for the head to be positioned closer to the centre of mass, lowering the loading on the neck and the energy required to support any given head mass (Bout 1997; Van der Leeuw, Bout, and Zweers 2001). This cervical configuration in tandem with a universally lighter head may allow for birds to overcome the negative scaling relationship between head mass neck length which appears to be present in many other vertebrates and to therefore maintain the same relative neck length as head mass increases. Avian forelimbs are heavily adapted for flight and environmental manipulation largely falls to the head and beak, leading to the head often being referred to a 'surrogate arm' (Clarke and Middleton 2008; Bhullar et al. 2012). By overcoming the restraints on the scaling relationship between head mass and neck length, birds can adapt their head size and morphology with more freedom than other long-necked vertebrates in order to adapt to a wide range of ecological niches. Recent work on raptorial birds have provided some evidence for this, as species modulate their head size to alter their feeding ecology (Bright et al. 2016; Felice et al. 2019).

Conclusions and future work

The cervical column of vertebrates is primarily tasked with supporting the weight of the head whilst providing it access to sufficient flexion ranges in order to best interact with the environment and other parts of the body. This primary role of providing head support places morphological and functional constraints on the vertebrate neck which potentially limits the extent of the adaptive response to external factors such ecology (Arnold et al. 2016; Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016; Arnold, Amson, and Fischer 2017; Arnold, Esteve-Altava, and Fischer 2017). Using multivariate statistics this study suggests that birds are able to adapt neck morphology and construction to accommodate a wide variety of neck lengths, head sizes and specialised dietary ecologies to some degree. Unlike other vertebrates, such as mammals whose head mass has been suggested to scale negatively with increasing neck length, head mass and neck length are isometric in extant avians. The combination of a lighter head (negative allometric scaling of cranial soft tissues) and the S-shaped curve seen in all avian necks, seems to be important in allowing them to overcome the constraint of lowering head mass for increased neck length (Table 2). This allows birds to adapt head size and morphology (e.g. beak size/shape) to fulfil the role of a surrogate arm, interacting with the environment due to the lack of involvement of the forelimb.

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Chapter 5: Variation in regional vertebral morphology and region length

Introduction

Regionalisation is the differentiation of the axial column into morphologically distinct groups of vertebrae and the distinct vertebral morphology of these regions allows each of them to have specific kinematics and functions (Koob and Long Jr 2000; Slijper 1946; Buchholtz 2012). Regional boundaries are controlled by *Hox* gene expression limits (Pourquié 2003; Dequéant and Pourquié 2008; Gomez et al. 2008; Wellik 2007) and changes to these limits have led to patterns of axial regionalisation that have underpinned the evolution of key traits in many groups of vertebrates (Buchholtz 2012; Hirasawa, Fujimoto, and Kuratani 2016; Jones et al. 2018; Arnold, Esteve-Altava, and Fischer 2017). By understanding factors that affect variation in these patterns of axial regionalisation we can begin to understand how ecology interacts with both the morphological phenotype and the genotype across vertebrate evolution. The avian cervical column is itself regionalised, and the involvement of the avian neck in multiple behaviours is potentially aided by this regionalisation (Boas 1929; Dzemski and Christian 2007; Krings et al. 2017; Kambic, Biewener, and Pierce 2017). Recent work has found that 5 regions exist within the avian cervical column (Chapter 3; Böhmer, Rauhut, and Wörheide 2015), and that morphology across all of these regions is generalised across Aves, only correlating with certain ecologies that require specialised muscle force production patterns (e.g. carnivores; see Chapter 3). Thus it has been assumed that the avian neck is adapted for 'general use' rather than to any specific function (Chapter 3, Van der Leeuw 1992; Van der Leeuw, Bout, and Zweers 2001).

This thesis has also found that neck length in birds is less restricted by body mass and head mass than other vertebrates (Chapter 4), potentially due to the S-shaped neutral pose of the neck combined with the decreased mass of cranial soft tissues (Brooke, Hanley, and Laughlin 1999; Garamszegi, Møller, and Erritzøe 2002). The effects of external factors on neck morphology and length have only been studied on a gross level and the generalised nature of the avian cervical column may be a product of this experimental design. This study seeks to study correlations between external factors and vertebral morphology on a smaller, regional scale using multivariate statistical models that properly incorporate 3D geometric morphometric data (Adams and Collyer 2009; Adams and Felice 2014; Adams 2014). Previous work has suggested that scaling of head size, both through ontogeny (Heidweiller and Zweers 1992) and within specific taxonomic groups (Van der Leeuw 1992) can affect the size of cervical regions as well as the musculature needed to support the head. However these studies did not relate head size to neck length, nor did they test the effects of these parameters on vertebral morphology. By further investigating the relationships of functional (head mass, neck length and body mass) and ecological factors on region size and shape this study can quantify the effect of external factors that correlate with the length, overall construction and vertebral morphology of the avian cervical column and its regions by answering the following questions:

Q1: Does regional vertebral morphology correlate with gross morphological factors?

Q2: Do ecological factors have any correlations with regional morphology?

Q3: How do gross morphological and ecological factors correlate with region length?

Methods

Defining functional factors: head mass, body mass and neck length

Quantifying head size: α-shape fitting and volumetric estimates

Head size was quantified digitally using an α-shape fitting algorithm (Brassey and Gardiner 2015) on the skulls of 38 species of extant birds to produce a volume for the skull. These 38 species are the same specimens used to assess regionalisation in chapter 3, allowing for direct consideration of the relationship between head size and regionalisation. 3D digital models of each bird skull were sourced either directly by micro-CT or from digital collections of colleagues (K. T. Bates, R. B. J. Benson and E. R. Schachner). Specimens newly scanned for this project were imaged at the University of Manchester's Henry Moseley X-Ray Imaging facility (Manchester HMXIF) using the 320/225 kV custom bay Nikon XTEK with system settings for kV set between 50 kV and 90 kV, and μA ranged between 58 μA and 140 μA. Initial scan data was reconstructed using CT Pro 3D (Metris XT 2.2, version 2.2.4365.28608), and a TIFF stack was created using TomoTools v1.0 for ease of import into analysis software. All scans were segmented using Amira 5.6 and Avizo 7.1, and each skull was individually exported as an OBJ mesh file. Point clouds of the skull OBJs were downsampled to

50,000 points to reduce computing time. α-shapes were fitted to the skulls using an in-house modified version of the 'alphavol' package (available form MATLAB central file exchange, originally written by Jonas Lundgren: http://www.mathworks.co.uk/matlabcentral/fileexchange/28851-alphashapes) which also calculates the volume of the computed α-shape. The fit of the α-shape is defined by the refinement coefficient, *k*, which was set at 10. A range of different values of *k* were tested on 3 morphologically distinct avian skulls to determine which *k* value best fit the entirety of the skull and produces a α-shape that crosses the orbits, a feature of anatomy that significantly impacts upon the final α-shape volume and is not fitted in lower *k* values (Figure 3.1). Head mass was estimated by multiplying the α volume by the density of soft tissues within the skull (approximated to the density of water, 997 kg m^{3}), and normalised by taking the head mass as a percentage of total body mass.

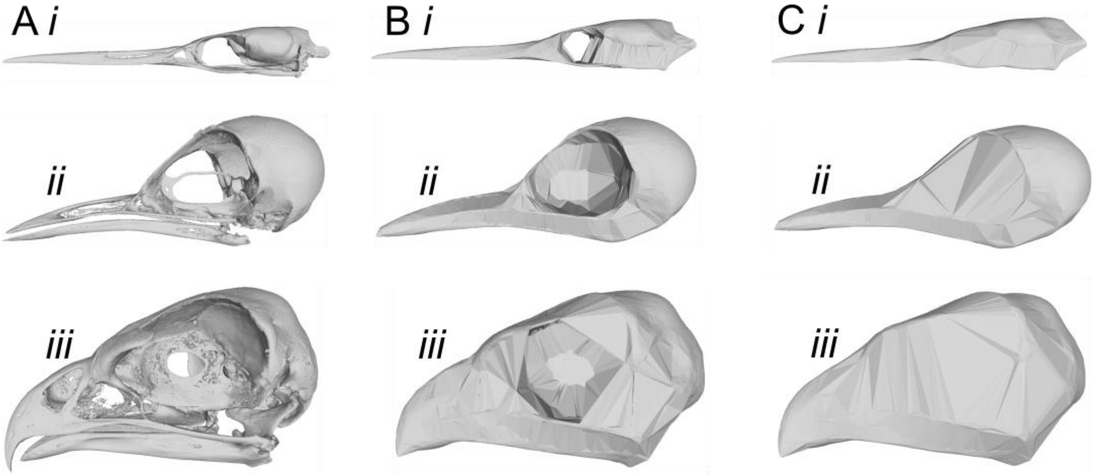

Figure 1 A) 3D digitally rendered skulls of 3 morphologically disparate bird skulls: i) *Anhinga anhinga*, ii) *Xenicus gilviventris*, iii) *Hieraaetus morphnoides*. B) outputted α-shape volume with a refinement coefficient of 5 for each bird in A, C) outputted α-shape volume with a refinement coefficient of 10 for each bird in A. α-shape outputs in C are more appropriate for approximating head volumes as the algorithm successfully covers the orbits, unlike lower inputted values of k.

Defining body mass and neck length

Where possible body masses were directly measured by weighing the specimens that were CT scanned. Where this was not possible body masses were estimated using scaling equations. Scaling equations for femoral length, minimal circumference of the femoral shaft and humeral articulation facet on the coracoid were taken from the literature (Field et al. 2013) and an average of all three was taken. Neck length was measured digitally as the summed length of each individual cervical vertebrae of each bird. To account for differences in cervical number and body mass, region size was assessed using percentage cervical vertebrae per region (as opposed to singular vertebrae). Overall neck length was normalised using the following equation:

$$Neck\ length\ (normalised) = \frac{Neck\ length}{Body\ mass^{0.33}}$$

Geometric morphometrics

3D models were created for all cervical vertebrae in each bird by segmenting individual vertebrae in Amira 5.6 (FEI Visualisation Sciences Group, Berlin, Germany) and exporting them to OBJ and PLY format using MeshLab (Cignoni et al. 2008). Each cervical vertebra is assigned a regional identity using the workflow described in Chapter 3 of this thesis. All cervical vertebrae except vertebrae 1 (C1) for each bird were landmarked using Landmark (Wiley 2006) and three-dimensional geometric morphometrics was carried out using the ‘geomorph’ 3.1.1 (Adams et al. 2017) package in R 3.5.0 (R Core Team 2018). Generalised Procrustes Analysis (GPA) was performed on the set of 15 vertebral landmarks using geomorph. The Procrustes coordinates created by GPA removes differences in position, size and orientation leaving only true shape change as the difference between landmarked vertebrae.

These Procrustes coordinates, alongside the distances between them are then used in multivariate statistical models to assess the effect of external factors such as neck length and ecology. These models are described in ‘Hypothesis testing’ below.

Hypothesis testing

Procrustes Distance phylogenetic Generalised Least Squares regression (D-PGLS) was used to model relationships between vertebral shape and external factors within each cervical region and was chosen because standard PGLS and ANOVA fail to properly incorporate 3D shape data which is both high-dimensional and multivariate (Adams 2014). Phylogenetic relationships were modelled with a tree pruned from published consensus trees (Jetz et al. 2012, Appendix tree 4.0). To observe the shape changes associated within each region for all functional (neck length, head mass and body mass only) models, two terminal end members (e.g. 2 species with the lowest and highest body masses respectively) were chosen for a specific model and a 3D visualisation for each member was created using the fitted values of the Procrustes coordinates used in the D-PGLS using the ‘open3D’ function in R. Question 4 also analyses multivariate models using D-PGLS as it analyses the effect of external factors on the length of each of the 5 cervical regions (length being a multivariate factor across each of the 5 regions).

To assess the effect of ecology on the region shape and region length each bird was assigned a dietary group and a flight style. Diets were assigned based on data from multiple volumes of 'Handbook of the Birds of the World' (Hoyo, Elliott, and Sargatal 1992, 1994; Del Hoyo, Elliot, and Sargatal 1996; Collar et al. 1997; Del Hoyo, Elliott, and Sargatal 1999; Hoyo, Elliott, and Sargatal 2001; Del Hoyo, Elliot, and Sargatal 2002; Del Hoyo, Elliott, and Sargatal 2005, 2006, 2008, 2009, 2010), whilst data on flight style was collated from the literature (Bruderer et al. 2010; Close and Rayfield 2012; Martin-Silverstone et al. 2015). Significant models were assessed using a *P*-value of < 0.05 and correlation coefficients (R^2) were used to attain the strength of the relationship between the variables in the models.

Results

Q1: Does vertebral morphology correlate with functional factors?

Procrustes Distance phylogenetic generalised least squares (D-PGLS) analysis operates under the same assumptions as the GLS models used in Chapter 4; normality of data, normality of residuals and homogeneity of residuals. These criterion were satisfied for all shape ~ functional factor models for all cervical regions (Appendix Figs. 5.1). PC scores for the first 3 principal components were regressed against centroid size for each region to assess the effect of allometric scaling on morphological variation (Appendix Table 3.3). Allometry explains a limited, but significant portion of variation in region 1 ($R^2 = 0.3068$, $P = 0.001$), 2 ($R^2 = 0.4084$, $P = 0.001$) and 4 ($R^2 = 0.1234$, $P = 0.031$). Before ecological factors were assessed for their relationship with vertebral morphology, multivariate models were created to ascertain which of the functional factors (neck length, head mass and body mass) were significant and which ones contributed to the largest amount of variation in vertebral shape. Body mass has the strongest influence on vertebral shape in region 1 ($P = < 0.03$, Table 3). However the variance explained by body mass is low (0.081, Table 1). The shape changes associated with an increase in body mass relate to anterior-posterior elongation of the centrum and neural spine, pre- and post-zygopohyses and an overall deepening of the centrum (Fig. 2a-b). Neck length is the dominant functional factor associated with vertebral shape in region 2 ($P = < 0.05$, Table 1), and explains a larger portion of variance in vertebral shape than body mass in region 1 ($R^2 = 0.120$, Table 1). With increasing neck length vertebral centra elongate (Fig. 2c-d), neural spine height decreases and pre- and post-zygapophyses project further anteriorly and posteriorly respectively (Fig. 2c-d). Similar to the previous region, neck length is the dominant functional factor associated with vertebral shape changes in region 3 ($P = < 0.05$, Table 1). The proportion of variance in region 3 vertebral shape explained by neck length is low ($R^2 = 0.090$, Table 1) and is similar to that of the previous two regions. Region 3 vertebral shape changes associated with increasing neck length are similar to those displayed in region 2: elongation of the centra, reduction in neural spine height and lengthening of the pre- and post-zygapophyses (Fig. 2e-f). Neck length continues to be the most significant functional factor that correlates with vertebral shape in region 4 ($P = 0.044$, Table 1) but still explains a low amount of variance in shape ($R^2 = 0.067$, Table 1). Shape changes of vertebrae in region 4 associated with increasing neck length include elongation and deepening of the centra, an increase in neural spine and pre- and post-zygapophyseal height (Fig. 2g-h). Body mass is the most significant functional factor that correlates with vertebral shape in region 5 (*P* = < 0.02, Table 1) and again explains a low amount of variance in vertebral shape (R^2 = 0.088, Table 1). Increasing body mass leads to a much-heightened neural spine and centrum within vertebrae of region 5, pre- and post-zygapophyses also appear to increase in height (Fig. 2i-j).

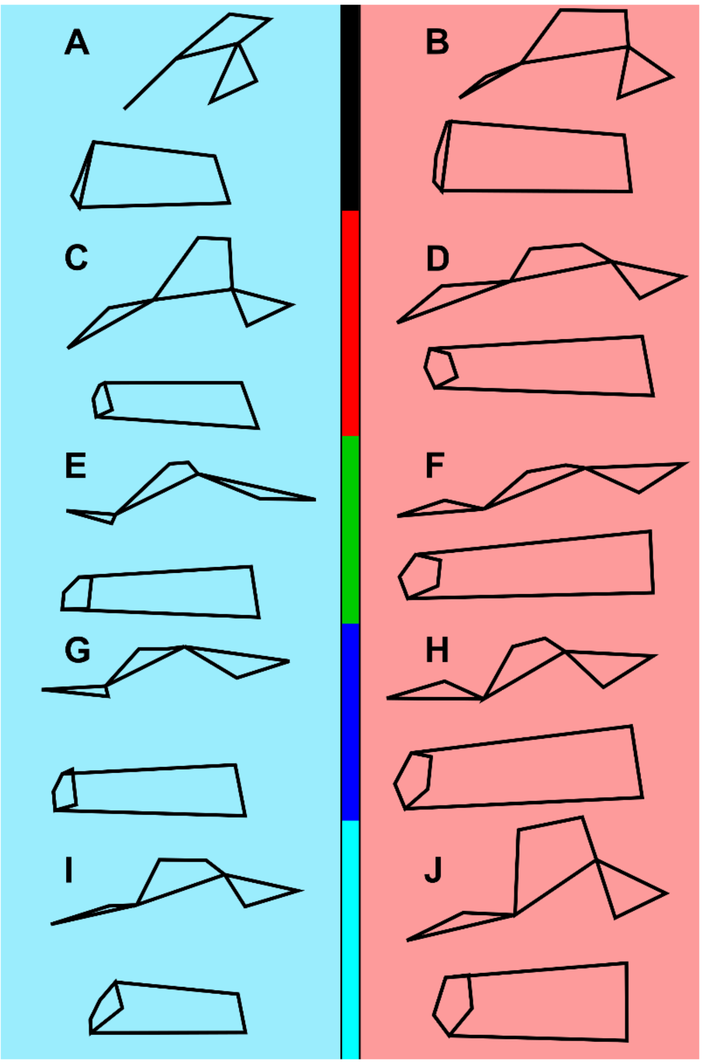

Figure 2 Significant shape differences between minimum (blue) and maximum (red) values of body mass (A, B, I, J) and neck length (C-H) for cervical vertebrae across all 5 regions. Coloured vertical bars denoted region number: black = region 1, red = region 2, green = region 3, dark blue = region 4, teal = region 5. This figure depicts the shape differences of cervical vertebrae (in a simplified, left lateral view) for the significant models of body mass and neck length stated in Table 3. Increasing body mass causes vertebrae in regions 1 and 5 to decrease in centrum length and to increase neural spine height. Increasing neck length causes vertebrae in regions 2-4 to elongate their centrums.

Q2: Do ecological factors have any correlations with morphology?

Across all regions, all models of shape ~ ecology satisfied the requirements of D-PGLS (normality of data, normality of residuals and homogeneity of residuals, Appendix Figs. 5.2). Piscivory is the only ecological factor to have a significant correlation with vertebral shape in region one ($P = < 0.04$, Table 1), and accounts for much less of the variance in shape than body mass ($R^2 = 0.007$-0.058, Table 1). Filter feeding ($P = 0.023$, $R^2 = 0.071$, Table 1) and piscivory ($P = 0.016$, $R^2 = 0.061$, Table 1) also display a significant but weak correlation with vertebral shape in region 2 (Table 1). Both soaring and subaqueous flight have a significant correlation with vertebral shape in region 3 ($P = < 0.02$ and $P = <0.04$ respectively, Table 1), and each explains a low but similar amount of variance in shape (soaring: $R^2 = 0.072$-0.08, subaqueous: $R^2 = 0.063$-0.085, Table 1).

Carnivory has a significant but weak association with vertebral shape ($P = < 0.05$, $R^2 = 0.0723$, Table 1) and is the only ecological factor to cause significant variation in region 4 vertebral shape. Carnivory is again, much like in region 4, the only ecological grouping to significantly correlate with vertebral shape in this region ($P = < 0.002$,

Table 1), but has a stronger correlation with vertebral shape than displayed in region 4 (R^2 = 0.099-0.105, Table 1).

Region	Model	R^2	F	P-value
1	***shape ~ body mass + head mass***	***0.081***	***3.142***	***0.011***
1	shape ~ body mass + head mass	0.0181	0.7035	0.567
1	shape ~ body mass + neck length	0.0809	3.1736	0.11
1	shape ~ body mass + neck length	0.027	1.0593	0.275
1	***shape ~ body mass + piscivory***	***0.075***	***2.987***	***0.017***
1	shape ~ body mass + piscivory	0.0502	2.0086	0.053
1	***shape ~ head mass + body mass***	***0.072***	***2.789***	***0.019***
1	shape ~ head mass + body mass	0.0272	1.0569	0.293
1	***shape ~ neck length + body mass***	***0.077***	***3.012***	***0.022***
1	shape ~ neck length + body mass	0.0311	1.2209	0.211
1	***shape ~ piscivory + body mass***	***0.058***	***2.332***	***0.039***
1	***shape ~ piscivory + body mass***	***0.007***	***2.663***	***0.023***
2	***shape ~ neck length + filter feeding***	***0.12***	***5.044***	***0.003***
2	***shape ~ neck length + filter feeding***	***0.071***	***2.97***	***0.023***
2	***shape ~ body mass + head mass***	***0.084***	***3.209***	***0.02***
2	shape ~ body mass + head mass	0.027	1.0503	0.294
2	***shape ~ head mass + body mass***	***0.095***	***3.642***	***0.008***
2	shape ~ head mass + body mass	0.0161	0.6175	0.613
2	***shape ~ head mass + neck length***	***0.095***	***3.797***	***0.007***
2	***shape ~ head mass + neck length***	***0.053***	***2.092***	***0.044***
2	***shape ~ neck length + head mass***	***0.12***	***4.789***	***0.004***
2	shape ~ neck length + head mass	0.0276	1.1003	0.249
2	***shape ~ neck length + piscivory***	***0.12***	***4.984***	***0.003***
2	***shape ~ neck length + piscivory***	***0.061***	***2.528***	***0.016***
2	***shape ~ piscivory + neck length***	***0.127***	***5.285***	***0.001***
2	shape ~ piscivory + neck length	0.0536	2.2261	0.053
3	***shape ~ body mass + head mass***	***0.061***	***2.297***	***0.044***
3	shape ~ body mass + head mass	0.011	0.4165	0.857
3	shape ~ body mass + neck length	0.0552	2.1853	0.051
3	***shape ~ body mass + neck length***	***0.061***	***2.412***	***0.036***
3	shape ~ head mass + body mass	0.06119	2.3075	0.059
3	shape ~ head mass + body mass	0.0108	0.4058	0.888
3	***shape ~ neck length + body mass***	***0.09***	***3.549***	***0.013***
3	shape ~ neck length + body mass	0.0265	0.4914	0.293
3	***shape ~ neck length + soaring***	***0.09***	***3.701***	***0.01***
3	***shape ~ neck length + soaring***	***0.063***	***2.2***	***0.018***
3	***shape ~ neck length + subaqueous***	***0.09***	***3.681***	***0.011***
3	***shape ~ neck length + subaqueous***	***0.058***	***2.383***	***0.037***
3	***shape ~ soaring + neck length***	***0.08***	***3.304***	***0.009***
3	***shape ~ soaring + neck length***	***0.072***	***2.985***	***0.015***
3	***shape ~ subaqueous + neck length***	***0.085***	***3.49***	***0.008***
3	***shape ~ subaqueous + neck length***	***0.063***	***2.574***	***0.035***
4	shape ~ body mass + head mass	0.0483	1.8092	0.11
4	shape ~ body mass + head mass	0.0168	0.6299	0.627
4	***shape ~ carnivore + neck length***	***0.072***	***2.903***	***0.027***
4	shape ~ carnivore + neck length	0.0564	2.265	0.068
4	shape ~ head mass + body mass	0.0529	1.9786	0.088
4	shape ~ head mass + body mass	0.0123	0.4605	0.815
4	shape ~ head mass + neck length	0.0529	2.0411	0.078
4	shape ~ head mass + neck length	0.0409	1.5816	0.134
4	***shape ~ neck length + carnivore***	***0.067***	***2.686***	***0.039***
4	***shape ~ neck length + carnivore***	***0.062***	***2.483***	***0.042***
4	***shape ~ neck length + head mass***	***0.067***	***2.582***	***0.044***
4	shape ~ neck length + head mass	0.0269	1.0405	0.315
5	***shape ~ body mass + carnivore***	***0.115***	***5.075***	***0.001***
5	***shape ~ body mass + carnivore***	***0.088***	***3.871***	***0.002***
5	***shape ~ body mass + head mass***	***0.088***	***3.413***	***0.005***
5	shape ~ body mass + head mass	0.0085	0.3293	0.988
5	***shape ~ body mass + neck length***	***0.088***	***3.435***	***0.005***
5	shape ~ body mass + neck length	0.0142	0.5533	0.823
5	***shape ~ carnivore + body mass***	***0.105***	***4.593***	***0.001***
5	***shape ~ carnivore + body mass***	***0.099***	***4.353***	***0.001***
5	***shape ~ head mass + body mass***	***0.059***	***2.281***	***0.017***
5	shape ~ head mass + body mass	0.0377	1.4609	0.136
5	***shape ~ neck length + body mass***	***0.062***	***2.407***	***0.015***
5	shape ~ neck length + body mass	0.0406	1.5813	0.1

Table 1 Results from Procrustes distance phylogenetic generalised least-squares regression (DPGLS) of the relationship between external (functional and ecological) factors on regional vertebral morphology of cervical vertebrae. Significant models are emboldened and italicised. Body mass has a significant effect on vertebral shape in regions 1 and 5 whilst neck length has a significant effect on vertebral shape in more central regions (2-4). At least one specialised ecology significantly effects the vertebral morphology of each cervical region: piscivory for region 1, filter feeding and piscivory in region 2, soaring in region 3, carnivory in regions 4 and 5.

Q3: How do functional factors and ecological factors correlate with region length?

PGLS was used to test correlations of ecological and functional factors with region lengths, and all requirements for PGLS (normality of residuals, homogeneity of residuals) were met for all significant models (Appendix Figs. 5.3). All three functional factors (neck length, body mass and head mass) have a significant correlation with region length (*P* = 0.001, *P* = 0.009 and *P* = 0.01 respectively, Table 2). Neck length has the strongest correlation with region length and there is a considerable difference between its association with region length and the second highest coefficient of determination value (neck length: R^2 = 0.669, body mass: R^2 = 0.013). The coefficients for individual regions reveal how factors affect the length of each region. In response to increasing neck length, regions 2 and 5 increase in length, whilst regions 1, 3 and 4 decrease in length (Table 2). An increase in body mass causes a relative decrease in the length of all regions, with region 5 showing the highest decrease in length, followed by regions 1 and 2 (Table 2). With respect to body mass, regions 3 and 4 show the lowest decrease in region length (Table 2). Much like body mass, increasing head mass causes a decrease in all region lengths, with regions 3 and 4 showing the largest decrease in length (Table 2).

Factor	R^2	F	P-value	R1 CE	R2 CE	R3 CE	R4 CE	R5 CE
Neck length	0.6698	77.0583	0.001	0.8057	1.1769	0.9321	0.8569	1.2132
Body mass	0.0131	1.5109	0.009	-0.0831	-0.0438	0.1745	0.1573	-0.2064
Head mass	0.0124	1.4226	0.01	0.1075	0.0161	-0.0226	-0.1316	0.1114
Carnivore	0.0011	0.1222	0.751	-0.0284	0.0277	0.3186	-0.4316	0.1027
Insectivore	0.0013	0.1495	0.683	-0.0557	-0.0803	0.0836	-0.2311	0.1687
Generalist	0.0067	0.7751	0.067	-0.1562	-0.1629	0.2559	-0.338	0.223
Frugivore	0.0239	2.76	0.001	-0.1228	-0.1031	0.3417	-0.4684	0.2367
Herbivore	0.0248	2.8516	0.002	-0.103	-0.0514	0.2942	-0.3391	0.1774
Filter feeding	0.0023	0.2588	0.357	-0.1234	-0.0842	-0.1229	0.2015	0.0111
Continual flapping	0.0029	0.3391	0.254	-0.1953	-0.2119	0.1109	0.3429	-0.1874
Soaring	0.0316	3.6309	0.001	-0.2243	-0.2572	-0.3113	0.6772	-0.1423
Burst adapted flying	0.0058	0.6698	0.032	-0.1815	-0.1825	0.2124	0.3849	-0.4239
Terrestrial	0.0056	0.6456	0.031	-0.2391	-0.2237	0.0467	0.2843	-0.1587
Intermittant bounding	0.0042	0.4802	0.075	-0.2179	-0.1074	0.0117	0.2786	-0.1509
Flap gliding	0.0032	0.3699	0.126	-0.2004	0.0425	0.1639	0.0997	-0.2615

Table 2 Results from Procrustes distance phylogenetic generalised least-squares analysis of the relationship between region length and external (functional and ecological) factors for all 5 cervical regions. R'x' denotes cervical region 'x', CE denotes coefficient. Significant models are emboldened and italicised. Neck length has the largest effect on individual region length, with regions 2 and 5 contributing most to neck elongation in this selection of extant avians. Grazing birds have a significant impact upon region length, with the elongation of regions 3 and 5 seemingly adapted to increase grazing efficiency. Birds that flap less or not at all during flight have elongated regions 3 and 4, and suggests that region length is more adaptable to ecologies when the burden of stabilising vision during wingbeats is lessened.

Numerous ecological factors have a significant correlation with region length. These factors are frugivory, herbivory, soaring, burst adapted flying and flightlessness (P = < 0.04, Table 2). Ecological factors have a much lower correlation with region length than neck length (soaring has the highest coefficient of determination amongst ecological groups: R^2 = 0.032, Table 2). However the majority of ecological factors displayed a greater correlation with region length than the remaining 2 functional factors, body mass and head mass (R^2 = > 0.02 for frugivores, herbivores and soaring birds). The remaining two ecological factors, burst adapted flyers and terrestrial birds have the lowest association with region length of any other significant factor (R^2 =0.0058 and R^2 = 0.0056 respectively, Table 2). Frugivores and herbivores have elongated regions 3 and 5 at the expense of a reduction in length in regions 1, 2 and 4 (Table 2). Soaring birds show a marked increase in the length of region 4, which is compensated by notable decreases in length in all other regions (Table 2). Burst adapted flyers and terrestrial birds display similar responses to region length changes: decreased length in regions 1, 2 and 5 and increased length in regions 3 and 4 (Table 2). However the magnitude of change appears to be slightly higher in regions 1 and 2 in terrestrial birds, and much higher in regions 3-5 in burst adapted flyers (Table 2).

Discussion

Does vertebral morphology correlate with functional factors? Do ecological factors have any correlations with morphology?

Neck length and body mass are responsible for the largest proportion of variation in vertebral morphology across the first 4 cervical regions (Table 1). Head mass, despite having a significant correlation with vertebral morphology in many regions, never explains more variance in morphology than either neck length or body mass (Table 1). Body mass and neck length appear to correlate with different parts of the avian neck: body mass with terminal regions (1 and 5) whilst neck length correlates with central regions (2-4). In regions 1 and 5 increases in body mass significantly correlate ($P = < 0.03$ and $P = < 0.02$ respectively, Table 1) with increases to neural spine height (Figure 2). The neural spines of vertebrae within these regions act as attachment sites for M. longus colli pars caudalis (Heidweiller and Zweers 1992; Van der Leeuw, Bout, and Zweers 2001; Boumans, Krings, and Wagner 2015) as well as dorsal ligaments (Bennett and Alexander 1987; Dzemski and Christian 2007). A large component of active support for the entire cervical column in birds is supplied by the M. longus colli pars caudalis. This active support is aided by passive support structures such as the dorsal ligaments (Heidweiller and Zweers 1992; Van der Leeuw, Bout, and Zweers

2001; Boumans, Krings, and Wagner 2015). Relative increases to neural spine height in regions 1 and 5 will inherently increase the available attachment areas for M. longus colli pars caudalis and the dorsal ligaments, thereby potentially facilitating increases in the size of these supportive structures in larger birds. Whilst not quantified here, previous authors have qualitiatively noted an increase in size to M. longus colli dorsalis pars caudalis and dorsal ligaments in larger birds. However this trend has not been quantified (Boumans, Krings, and Wagner 2015; Dzemski and Christian 2007).

An S-shaped curve is present in the neck of birds and allows for the neck to efficiently support the weight of the head by bringing it closer to the fulcrum of the neck and closer to the centre of mass (Bout 1997). The musculature associated with this (M. longus colli dorsalis pars caudalis and pars profunda) attaches on vertebrae throughout regions 2-4 (Boumans, Krings, and Wagner 2015). Similar to the hypothesised effect of body mass on vertebral shape in regions 1 and 5, muscle mass could correlate to shape in regions 2-4 to ensure that the elongated neck is supported (wider and taller neural spines, Fig. 2 c, d, g, h) as well as to allow birds to retain neck flexibility with increasing neck length.

Phenotypic trajectory analysis (PTA; Adams and Collyer 2007; Adams and Collyer 2009; Collyer and Adams 2013), analyses shape trajectories between fixed groups of data (e.g. average vertebral morphology of a single cervical region) and finds the trajectory between that group and the next fixed group (for example between the mean shape of vertebrae in region 1 and the mean shape of vertebrae in region 2 etc.). Trajectories created in this way act as a method of visualising the pattern of shape change across the entire avian cervical spine, as the trajectory is created from morphological data from each of the 5 cervical regions. By testing for significant differences in size, shape and direction of these trajectories between different ecological groups of birds, PTA has been previously used to assess the impacts of ecology on the morphology of the entire neck (Chapter 3, this thesis) and has suggested that carnivory, insectivory, soaring and continual flapping all have a significant correlation with overall cervical morphology in extant birds (Table 2.2, Figure 2.7, Chapter 3 this thesis). Results from the D-PGLS (Table 1) provide a more granular insight into factors vary with cervical morphology, as they assess factors that correlate with the morphology of individual cervical regions rather than gross neck morphology. Using D-PGLS, carnivory and soaring are again found to significantly correlate with regional cervical morphology, and correlate to specific morphological changes to vertebrae in regions 4 and 5 for carnivory and region 3 for soaring. The effects of carnivory outweigh the effect of functional factors in region 5 and this is the only occurrence of an ecological factor to do so within all 5 cervical regions. Vertebral morphology in regions 4 and 5 is significantly correlated with carnivory ($P = < 0.05$ and $P = < 0.002$ respectively, Table 1). Results from the previous PTA (Fig 2.7, Chapter 3) study have shown that carnivorous birds display larger neural spines in regions 4 and 5. In birds the M. longus colli dorsalis pars caudalis is the most prominent muscle attaching to the neural spines of vertebrae in regions 4 and 5 and is associated with creating retraction forces (Boumans, Krings, and Wagner 2015). As birds lack teeth, carnivorous birds must process food extra-orally and the neck is tasked with creating large retraction forces required for the head to strip small chunks of flesh away from carcasses. To attain these larger retraction forces it is hypothesised that the M. longus colli dorsalis pars caudalis is expanded (Boumans, Krings, and Wagner 2015), meaning the attachment sites on the neural spines must also be enlarged. This may be the cause behind the correlation between vertebral morphology and carnivory in regions 4 and 5 and is similar to the potential correlation between vertebral shape and body mass above.

Vertebral morphology in region 3 is significantly different in soaring birds with PTA finding that vertebral morphology in region 3 of soaring birds had a shortened neural spine and centrum Fig. 2.7, Chapter 3). These shape changes may be correlated with the lower amount of compensatory movements the neck has to make to stop vision being occluded by wingbeats (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015). These muscles associated with compensatory movements (M. longus colli dorsalis pars caudalis and pars profunda) may have a reduced mass in soaring birds, leading to the significant changes to vertebral morphology in region 3.

Two ecologies that previously went undetected by PTA have a significant correlation with variation in vertebral morphology in regions 1 and 2: piscivory and filter feeding. Piscivory in birds has been qualitatively associated with many adaptations of cervical morphology, the most well documented of which is the morphology of the 'hinge-like' joint between cervical 8 and 9 in the genus *Anhinga* (Hoyo, Elliott, and Sargatal 1992; Nelson 2005). Fast, precise head movements allow birds to catch fish and many of the muscles associated with the control of these movements originate on vertebrae from region 1 and 2 (M. complexus, M. rectus capitis dorsalis, M. rectus capitis lateralis and M. capitis rectus ventralis) thus any adaptations of these muscles to piscivory may correlate with the morphology of the vertebrae to which they attach (Boumans, Krings, and Wagner 2015). Filter feeding is represented by one taxon in this dataset: *Phoenicopterus chilensis* (Chilean flamingo) and thus the effect of filter feeding on the vertebral morphology of region 2 may be explained by 'flamingo-specific' feeding behaviour. Flamingos filter feed by inverting their heads in order to place their bills on the water's surface, and do so for minutes at a time (Hoyo, Elliott, and Sargatal 1992). The same muscles that piscivorous birds use for fast head movements (M. complexus and the M. rectus capitis subsystem) may be adapted to providing stability during this behaviour as well as the extra ventral flexion of the head needed to perform such head inversion. However little is currently know about flamingo-specific cervical myology. Modifications to these muscles and their attachment sites may alter the morphology of vertebrae in cervical region 2.

Despite their significance ($P = < 0.05$, Table 1), functional factors have a low correlation with vertebral shape, with R^2 values mostly falling below 0.1 (except for the effect of neck length in region 2, Table 1). Ecological factors, whilst having at least one group significantly correlating with each region, also had low association with vertebral shape throughout the cervical regions, lower in most cases than R^2 values for functional factors (Table 1). External factors have little effect on the morphology and organisation of mammalian necks (Randau et al. 2016; Arnold, Esteve-Altava, and Fischer 2017), which, along with results from this study provide evidence that vertebrate neck organisation and morphology, except in extremely specialised instances, is adapted for general use (Wilkinson and Ruxton 2012). However, the avian cervical column is far less restricted than mammals in terms of its adaptability. Unlike mammals, avians have fewer genetic restrictions to cervicalisation (Galis 1999) and this study provides results that suggest that the morphology of the avian cervical column is more adaptable to external factors than in mammals, where adaptations to locomotion and diet appear to be concentrated in thoracic and lumbar vertebrae (Randau et al. 2016).

How do functional factors and ecological factors correlate with region length?

Neck length is the primary factor that correlates with region length (R^2 = 0.669, Table 2) and the relationship between region length and neck length can inform us to what extent each cervical region contributes to neck elongation (or shortening) in extant avians. Regions 2 and 5 contribute most to neck elongation at the expense of the slight reduction in lengths of regions 1, 3 and 4 (Table 2). Cervicalisation provides both extra flexion (more joints) and more area for muscle attachment in the cervical column (Bout 1997; Van der Leeuw, Bout, and Zweers 2001). In contrast to prior literature, this thesis has previously found that cervicalisation is not responsible for the neck elongation of birds (Chapter 3), and this current study now suggests that lengths of cervical vertebrae, particularly the lengths of the cervical regions, are responsible for neck elongation in extant birds. This study finds that neck elongation is predominantly due to increases in the lengths of regions 2 and 5 and the expansion of these regions specifically may be due to the expansion of musculature associated with providing active support for the head and the entire cervical column. These muscles include M. rectus capitis group (dorsalis, lateralis and ventralis), M. biventer cervicis and the M. longus colli pars caudalis and predominantly attach to vertebrae in regions 2 and 5 (Boumans, Krings, and Wagner 2015). Mammals lengthen more central vertebrae to accommodate neck elongation (Arnold, Amson, and Fischer 2017) and these results further highlight the differences in neck organisation between the two clades. Cervical vertebrae act as load bearing structures and resist axial load placed on the neck by the mass of the head and body (Slijper 1942; Smit 2002; Arnold, Amson, and Fischer 2017). To safely resist these loads, vertebrae must minimise their lengths and this results in negative scaling relationship between both head and body mass in many vertebrates (Slijper 1942; Smit 2002; Arnold, Amson, and Fischer 2017). Birds appear to be no exception to this rule, with region lengths all displaying negative allometry with increasing head and body mass (Table 2).

The relationship between ecological factors and region length can inform how diet and locomotory mode correlate with cervical flexion patterns in extant avians. Grazing birds (herbivores and frugivores) show relative increases in the length of regions 3 and 5 (at the expense of the lengths of the other 3 regions) and these birds spend much of their time feeding at ground level. Region 3 forms the central portion of the neck in extant birds and displays the highest levels of dorsoventral flexion (Bout 1997; Van der Leeuw, Bout, and Zweers 2001; Cobley, Rayfield, and Barrett 2013; Dzemski and Christian 2007), meaning elongations of this region (via increased cervical counts) may increase flexion capabilities further and allow for more efficient ground-level feeding. Region 5 forms the fulcrum of the cervical column and any additions to the flexion properties of this region result in a large increase to the range of positions the head can reach (Bout 1997; Van der Leeuw, Bout, and Zweers 2001; Dzemski and Christian 2007; Cobley, Rayfield, and Barrett 2013), therefore the elongation of this region could be an adaptation to provide a wider feeding envelope in grazing birds, although this needs be tested in future work. Taken together, answers to questions 3 and 4 suggest that there are two methods by which the avian cervical column can adapt to specialised dietary ecologies: by altering vertebral morphology to accommodate a shift in muscle force production for specialised feeding methods or to increase flexion in key cervical regions to allow for efficient foraging and grazing.

Region length patterns are only significantly correlated with flight style when little to no flapping is involved in travel (soaring, burst-adapted flying and terrestrial are all significant, P = < 0.04). During flight each wingbeat can occlude vision and interrupt image stabilisation (Land 1999; Goller and Altshuler 2014) and the neck is used to counteract the movements of the body during each wingbeat (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015) and, although never previously quantified, may act as a limitation on neck construction and flexion patterns. Without these constraints, birds which flap less or not at all during flight may have more freedom with how they adapt region lengths and overall cervical construction. However relationships between cervical kinematics, musculature and locomotor ecology need to be further quantified to properly assess these hypotheses.

Conclusions

This study sought to observe variation in avian neck morphology on a finer scale than chapter 3 of this thesis by investigating the correlation of external factors with regional vertebral morphology and regional length. Neck elongation appears to be fundamentally different in mammals and birds, owing to the lower burden that head mass places on neck length in avians, and is concentrated in terminal rather than central regions. Elongation of the neck is primarily due to the elongation of vertebrae within cervical regions rather than the addition of cervical vertebrae, and neck elongation appears to be concentrated in specific cervical regions (2 and 5). Previous work suggests the avian column is constructed according to a general pattern, and this is largely recovered here, with few dietary groups and flight styles showing significant correlations with regional lengths and morphology. However unlike the cervical spine of mammals, birds may have two methods of adapting the cervical column to specialised dietary ecologies: morphological changes to vertebrae that accommodate musculature that provides specific force production (carnivores, piscivores and filter feeders) and regional length variations that increase flexion to provide efficient grazing for herbivores and frugivores. However further work is needed to quantitatively test for correlations between ecology and changes to cervical musculature. Flight style is more opaque in how exactly it correlates with avian cervical morphology. However powered flight appears to present limits on cervical construction due to the need for image stabilisation during flapping flight. Cervical organisation in mammals has high integration with the forelimb, with major shifts in forelimb function significantly altering the construction and modularity of the cervical column (Arnold, Esteve-Altava, and Fischer 2017). Such integration may exist in modern birds and tracking the relationship between these two musculoskeletal systems over the dinosaur-bird transition may provide a valuable insight into how the cervical column interacts with other musculoskeletal systems throughout Vertebrata.

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Chapter 6: Variation in cervical muscle architecture and muscle mass

Introduction

The neck is one of the most complex musculoskeletal systems in extant avians, with over 200 muscle slips on each sinistral and dextral side (Landolt and Zweers 1985; Baumel, Evans, and Berge 1993). This complexity has resulted in cervical musculature receiving relatively little attention in the literature, and has limited research to qualitative studies of commercially available species common to the western hemisphere (Landolt and Zweers 1985; Heidweiller and Zweers 1992; Van der Leeuw 1992; Van der Leeuw, Bout, and Zweers 2001; Snively and Russell 2007b; Snively et al. 2014; Krings et al. 2017 and references therein). The neck of vertebrates supports the mass of the head whilst simultaneously positioning it to engage in a multitude of different actions from feeding to environmental observation (Gans 1992). Cervical musculature must be able to meet all of these demands at any particular moment and these generalised biomechanical constraints have restricted the patterning of the cervical musculoskeletal system in many groups of vertebrates (Arnold, Esteve-Altava, and Fischer 2017; Arnold, Amson, and Fischer 2017). These generalised biomechanical factors are common amongst vertebrates and often restrict the total number of cervical vertebrae in the axial column. The most notable example of this is the mammalian cervical spine whereby strong biomechanical and developmental constraints have restricted the total number of cervical vertebrae and this has been suggested to be causatively related to conservatism in cranially positioned cervical musculature (Buchholtz 2012; Galis 1999; Buchholtz 2014; Buchholtz et al. 2012; Hirasawa, Fujimoto, and Kuratani 2016; Galis and Metz 2003; Galis et al. 2006).

Cervical count variability is less restricted in birds and as such they display variation in the total number of cervical vertebrae, from 10 to 26 (Boas 1929; Benoit et al. 1950). This release from biomechanical and developmental constraints (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015b) in birds may also release them from constraints to variation in cervical muscle arrangement and architecture. Birds occupy an extremely diverse array of ecological niches and the neck of birds is integral in their participation within these ecosystems; it is involved in activities ranging from head stabilisation during flight (Kress, Van Bokhorst, and Lentink 2015; Pete et al.

2015) and assisting parrots in arboreal locomotion (Dilger 1960). Together the avian head and neck form a 'surrogate arm' to act as a replacement for the forelimbs in the manipulation of their surroundings, as avian forelimbs are so heavily adapted for flight (Clarke and Middleton 2008; Bhullar et al. 2012). Due to the lack of quantitative interspecific study of avian cervical muscle architecture there is little understanding of how the necks involvement in a wider array of daily activities (i.e. being used as a 'surrogate arm') has shaped variation in cervical musculature. Current literature appears conflicted as multiple authors suggest avian cervical musculature is conserved across species due to the generalised tasks the neck is involved in (Heidweiller et al. 1992; Van der Leeuw, Bout, and Zweers 2001; van der Leeuw 2002; Böhmer et al. 2019), whilst many other authors note distinct variations to muscle arrangements in specialised taxa, such as the cruciform origin of the M. splenius capitis in swifts and hummingbirds (Burton 1971; Brause, Gasse, and Mayr 2009) and the expansion of the M. longus colli dorsalis pars profunda in owls (Krings et al. 2014; Boumans, Krings, and Wagner 2015; Krings et al. 2017). The work described in this chapter aims to quantify variation in muscle properties (specifically size and architecture) across a diverse sample of extant birds.

Myological variation may not exist in isolation, as changes to muscle properties (particularly size) may be intrinsically linked to variations in skeletal morphology as the skeleton forms the majority of attachment sites for vertebrate muscle. The effects of extrinsic factors on the skeletal morphological of avian cervical vertebrae has not been previously studied due to the large disparity in counts of cervical vertebrae across Aves, as homology of vertebrae between species is unknown. This problem has been overcome by observing the effect of extrinsic factors on the variation of regional morphology within the cervical column, as five cervical regions are common across Aves due to the conserved patterns of *Hox A-4/5*, *B-4/5*, *C-4/5* and *D-4* expression boundaries (Böhmer, Rauhut, and Wörheide 2015a). Morphological variation of cervical regional morphology has been found to be significantly affected by a limited but specialised few ecological groups (Chapter 5, this thesis). This effect is strongest in the posterior-most two regions of carnivorous birds and has been hypothesised to correspond to an increase in musculature associated with neck retraction, as carnivorous birds use strong neck dorsiflexion to allow the beak to tear flesh from prey (Chapters 3 and 5, this thesis). By quantifying the relationship between extrinsic factors and cervical muscle architecture this study seeks to understand if ecology correlates with significant shifts to avian cervical muscle architecture and whether these shifts could account for the significant variation in regional morphology that are observed in some ecological groups of birds. Evolutionary shifts in axial patterning have been linked to significant changes in muscle properties in other vertebrate groups (Buchholtz et al. 2012; Hirasawa, Fujimoto, and Kuratani 2016; Jones et al. 2018) and these changes have underpinned the success of many modern vertebrate clades, such as mammals. This study seeks to add to this body of work by assessing if variation in regional vertebral morphology in the cervical column of birds is underpinned by changes to muscle size and architecture, and will do so according to the following questions:

1. What are the allometric scaling relationships of architecture variables and mass of cervical muscles in extant avians?
2. Are variations in cervical muscle architecture and mass correlated with changes to dietary or locomotory ecologies in extant avians?
3. Are variations in cervical muscle architecture and mass correlated with variations in the morphology of cervical vertebrae as well as patterns of cervical regionalisation?

Material and methods

Muscle and fibre length measurements

Ten specimens (10 separate species) of extant bird form the materials of this study and were sourced from deceased zoo animals, cadaveric museum collections and wild meat suppliers (Table 1). Within each bird 10 cervical muscles were chosen for dissection based on their contribution to head and neck positioning (Table 2). Before muscles were dissected away for measurement, fascia was removed, cervical muscles were separated from each other, and origin and insertion sites for each muscle were documented. Each individual muscle was then dissected away with any internal or external tendons still attached in full. Digital callipers (±0.01 cm accuracy) were used to measure total muscle tendon unit length, along with internal and/or external tendon length (if present) and muscle length. All measurements were repeated 3 times and an average was taken. Where needed, fascicle length was measured at 3 separate points along the length of a muscle to account for potential variation in fibre length and these measurements, where applicable, were averaged. Muscle mass was measured using a set of digital scales to the nearest 0.01g.

Species	Common name	Diet	Flight style	Order
Anser albifrons	Greater white-fronted goos	Herbivore	CoF	Anseriformes
Cariama cristata	Red-legged seriema	Insectivore	BAF	Cariamiformes
Phoenicopterus chilensis	Chilean flamingo	Filter feeder	CoF	Phoenicopteriformes
Spheniscus humboldti	Humboldt penguin	Piscivore	SUB	Sphenisciformes
Phasianus colchicus	Common pheasant	Herbivore	BAF	Galliformes
Gavia stellata	Red-throated loon	Piscivore	CoF	Gaviiformes
Morus bassanus	Northern gannet	Piscivore	Soa	Suliformes
Tyto alba	Western barn owl	Carnivore	FlG	Strigiformes
Buteo buteo	Common buzzard	Carnivore	Soa	Accipitriformes
Strix aluco	Tawny owl	Carnivore	FlG	Strigiformes

Table 1 All samples that are present throughout this study along with their species, common name, diet, flight style and taxonomic order. Flight style codings: CoF = continual flappers, BAF = burst adapted flyers, SUB = subaqueous flight, Soa = soaring, FlG = flap-giding. All birds were sourced as cadaveric material from zoos, museum collections or from wild meat companies.

Muscle fibre length was determined by first fixing muscles in 40% paraformaldehyde solution for 24 hours. Muscles were then placed in a PBS solution to prevent dehydration before muscle fibres could be digested. Samples were then placed in a 35% nitric acid solution for 48-72 hours (depending on the size of the muscle) until fibre bundles were freed of connective tissue. Fibres were separated and suspended in a 50% glycerol solution and fibre lengths were measured digitally in ImageJ from images of the separated fibres taken under a light microscope at 0.7x magnification, or with a DLSR camera. Physiological cross-sectional area was calculated for each muscle according to:

$$\text{PCSA} = \frac{\cos\ \theta m}{\rho l}$$

Where *m* is muscle belly mass (g), *ρ* is the density of fresh muscle (1.06 g cm^{-3}, Mendez & Keys 1960), and *l* is muscle fibre length. Pennation angle (*θ*) in all samples was < 30 ° which allows for the removal of cos *θ* from the equation as at these angles it approximates 1 (Calow & Alexander 1973).

Muscle	**Origin**	**Insertion**
M. complexus	Diapophyseal processus of processus transversus of C4, C5 and C6	Os supraoccipitale
M. complexus	Diapophyseal processus of processus transversus of C4, C5 and C6	Os supraoccipitale
M. biventer cervicis	Aponeurosis notarii located above C14	Os supraoccipitale
M. splenius capitis	Teardrop-shaped origins on either side of midline of dorsolateral surface of neural arches of C2 and C3	Os supraoccipitale
M. rectus capitis lateralis	Processus ventralis of C3-C5	Os supraoccipitale
M. rectus capitis ventralis	Processus ventralis of C1-C5	Os basioccipitale
M. rectus capitis dorsalis	Lateral processus of C2-C5	Os basioccipitale
M. longus colli dorsalis pars caudalis	Aponeurosis notarii located above C14 and processus spinosus of C13	Os basioccipitale
M. longus colli dorsalis pars cranialis	Processus spinosus of C3-C7	Tendo axialis (attaches to torus dorsalis of C2)
M. longus colli dorsalis pars profunda	Processus spinosus of C7-C12	Processus transversus of C5-C8
M. longus colli ventralis	Processus ventralis of T2	Processus transversus of C3-C10

Table 2 Avian cervical muscles and their origination and insertion sites for an average bird with 14 cervical vertebrae. Modified from Boumans et al. 2015.

Data analysis

Variation in muscle mass and architecture

Subsequent analysis of fibre length, muscle mass and PCSA was split into two discrete analyses and followed previous approaches of assessing variation in muscle architecture within a small data set (Myatt, Crompton, and Thorpe 2011; Myatt et al. 2012). The first step assessed the scaling relationship between fibre length, muscle mass and PCSA with body mass for each muscle. Each architecture variable was log transformed and regressed against log transformed body mass. Previous studies have used ordinary least squares (OLS) regression to calculate the effect of allometry on muscle architecture. However these studies contained closely related species and as such had no need to account for the effect of phylogeny in their regressions (Myatt, Crompton, and Thorpe 2011; Myatt et al. 2012). This study contained a sample of birds from phylogenetic disparate sub-groups. Therefore, to account for phylogeny, log transformed muscle architecture variables were regressed against log transformed body mass using a generalised least squares model that incorporated a pruned-phylogenetic tree of all sampled species (using a subset of trees presented in Jetz et al. 2012, Appendix tree 6.0) using a Brownian motion model of evolution (using the 'corBrownian' tool in R 3.5.0). This has been well documented to be equivalent to an

OLS regression of independent contrasts (Garland & Ives 2000). Linear equations from significant relationships between muscle architecture and body mass were used to scale muscle architecture values according to the equation $Y = aM^b$, where Y is the muscle architecture variable, M is body mass (kg), a and b are constants and b (Table 3) is the coefficient used as the scaling exponent. This scaled data was then used to visually explore the variability in cervical muscle architecture and muscle mass in each of the ten cervical muscles studied (Figs 5-8). Variability was studied by plotting the scaled values for fibre length, muscle mass and PCSA in a bar chart format for each muscle (Fig. 5-7), where variability could be viewed qualitatively. Boxplots were also used to plot variability across all species for each individual muscle's measurements of fibre length, muscle mass and PCSA (Fig. 8a), standard deviations for each muscle fibre length, muscle mass and PCSA were also calculated and plotted (Fig. 8b). The scaling coefficient, b, would also be used to assess if the allometric relationship between each muscle variable (fibre length, muscle mass and PCSA) and body mass differed from isometry by calculating the 95% confidence intervals for all scaling coefficients (Table 3). Assessing allometric relationships and exploring variability in muscle architecture and muscle mass were the only areas where allometrically scaled muscle variables were used. Log transformed raw values of muscle fibre length, muscle mass and PCSA were used to test the effect of external factors on variation in these measurements (Table 4) and geometrically scaled values of fibre length were used when assessing for muscle function variability (Figs. 10-12). To quantitatively compare multiple models for a given data variable, Akaike's information criterion (AICc) was calculated, and comparing AICc values for different models of a certain data variable allows for the most parsimonious model to be estimated, which is the model with the lowest AICc value (Sugiura 1978; Burnham and Anderson 2003).

The second discrete analysis involved the hypothesis testing of factors affecting variation in the three muscle architecture variables. Each species was assigned a dietary and locomotory ecology based on prior literature and the effect of each of these ecologies was tested on all three muscle architecture values for each of the ten muscles (Hoyo, Elliott, and Sargatal 1992; Del Hoyo, Elliot, and Sargatal 1996; Collar et al. 1997; Del Hoyo, Elliott, and Sargatal 1999; Hoyo, Elliott, and Sargatal 2001; Del Hoyo, Elliot, and Sargatal 2002; Del Hoyo, Elliott, and Sargatal 2005, 2006, 2008, 2009, 2010). Previous authors have noted the effect of body mass on muscle architecture in small sample sizes and as a result have tested for significant effects of external factors on muscle architecture variables using body mass as a covariate within an ANCOVA analysis (Myatt, Crompton, and Thorpe 2011; Myatt et al. 2012). These studies did not account for or document the effect of phylogeny on these relationships. Herein a generalised least squares regression model is used to include the effect of evolutionary relationships. Body mass is used as a covariate in GLS models as follows: $\mathbf{MA}^{x} = \mathbf{BM} + E$, where MA is the log transformed raw muscle architecture variables of muscle x, BM is log transformed body mass and E is the ecology of interest (Smaers and Rohlf 2016). Phylogenetic relatedness was accounted for by incorporating a Brownian Motion model of evolution using the 'corBrownian' function in R 3.5.0. This function accounts for phylogenetic relatedness without estimating a value for the phylogenetic signal of the data set. The phylogenetic signal within these GLS models could not be estimated as it has been previously shown that small sample sizes produce highly variable estimates for phylogenetic signal parameters (such as Pagel's λ and Blomberg's K) between species (Boettiger, Coop, and Ralph 2012; Münkemüller et al. 2012). Using phylogenetic GLS models in this way allows for the relationship between muscle architecture and ecology to be studied effectively in a small sample size by using body mass as a covariate and is equivalent to the ANCOVA models used in prior analyses (Myatt, Crompton, and Thorpe 2011; Myatt et al. 2012; Smaers and Rohlf 2016). The coefficients of the pGLS models will be used to understand the effect each ecology has on the muscle architecture value of interest. Again, AICc was used to quantitatively compare multiple ecological models.

Alternative statistical tests for low sample sizes

The sample size of this study was extremely low (*N* = 10) and may break one of the requirements of PGLS, that sample size must considerably exceed the number of predictors. As such Pearson's correlation was used as a parametric alternative to PGLS analysis. Since sample size is too small to accurately model the distributions of variables then non-parametric alternative tests were carried out for all analyses: Spearman's rank and Pearson's correlation in place of scaling models and Kruskal-Wallis tests in place of PGLS models of muscle architecture ~ ecology. For the Kruskal-Wallis tests, the effects of body mass were considered by testing both scaled and unscaled values of fibre length, muscle mass and PCSA. For all alternative tests (Pearson's correlation, Spearman's rank correlation and Kruskal-Wallis tests) phylogenetic relationships were not considered.

Variation in Muscle function

Variation in muscle function was studied by plotting geometrically scaled fibre length (fibre length/body mass$^{0.33}$) versus geometrically scaled PCSA (PCSA/body mass$^{0.67}$) for all muscles in all species (Fig. 9). The relative functional capabilities, or specialisms, of muscles within an anatomical system can be approximated by observing the relative positions on the muscle function chart (i.e. scaled fibre length versus scaled PCSA). Muscle force is directly proportional to PCSA, thus a muscle is considered a relatively force-specialist muscle if it plots into the upper left quadrant of muscle function space (it has relatively high PCSA but relatively short muscle fibres). Fibre length is proportional to muscle shortening velocity and working range, thus when a muscle plots into the lower right quadrant of muscle function space (long muscle fibres and low PCSA) it is deemed a displacement specialised muscle, able to contract over relatively a large distance and relatively high shortening velocity, but with a relatively low force. Muscles with relatively high PCSA and fibre lengths plot in the upper right quadrant of muscle function space and are interpreted as power-specialised as they can produce high force over large working ranges. With no particular specialisations for muscle force, contraction range, contraction speed or power, muscles that occupy the lower left quadrant function as generalists.

Muscle function plots were also used to view variation in muscle function of caudal-only and cranial-only muscles (Fig. 10 and Fig. 11 respectively). Caudal muscles were delineated as muscles that spanned multiple joints and originated distally to the vertebrae in the middle of the cervical spine (M. biventer cervicis, M. longus colli dorsalis pars caudalis, M. longus colli dorsalis pars cranialis, M. longus colli pars profunda and M. longus colli ventralis, Table 2), whilst cranial muscles were those that inserted onto the cranium and originated before the middle cervical vertebra (M. complexus, M. splenius capitis, M. rectus capitis lateralis, M. rectus capitis ventralis, M. rectus capitis dorsalis, Table 2). Chapter 5 revealed that ecologically correlated variation in bone shape was more prevalent in more caudal cervical regions across a broad sample of extant birds and this grouping of cranial and caudal muscles is in part to determine if this pattern holds true for cervical muscle architecture and mass. Cranial cervical muscles primarily support the weight of the head whilst caudal cervical muscles primarily support the weight of the neck and thus grouping cervical muscles in this way allows for the assessment of the impact of head support on variation in cervical muscle architecture. Muscle function was also studied at a broader level by observing the ratio of raw flexor muscle mass to raw extensor muscle mass in all birds

(Fig. 13), and by observing muscle function space variation between these two categories of muscle (Fig. 12).

Results

Myological description of 'extreme' taxa

Most birds in this study displayed modest qualitative variation in the arrangement of cervical muscles (Figs. 2-4). Specifically, small variations in the number of slips in a muscle or the movement of the attachment site of a muscle slip cranially or caudally by a couple of vertebrae (See 'Variation in muscle attachment sites and vertebral span', Figs. 2-4)). However, there were four species in which considerable qualitative variation was noted and these are outlined below (Fig. 1).

M. biventer cervicis is a long and thin muscle than runs the entire length of the cervical spine (Table 2), inserting on the cranium (os suppraoccipitale) and originating at an aponeurotic sheet which covers the vertebrae across the cervico-thoracic transition (Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015, Figs. 2, 3). This muscle is usually split into cranial and caudal bellies that are joined by a long interconnecting tendon (Fig. 1c). However in the Humboldt penguin (*Spheniscus humboldti*) M. biventer cervicis is lacking this tendon and is fleshy along the entirety

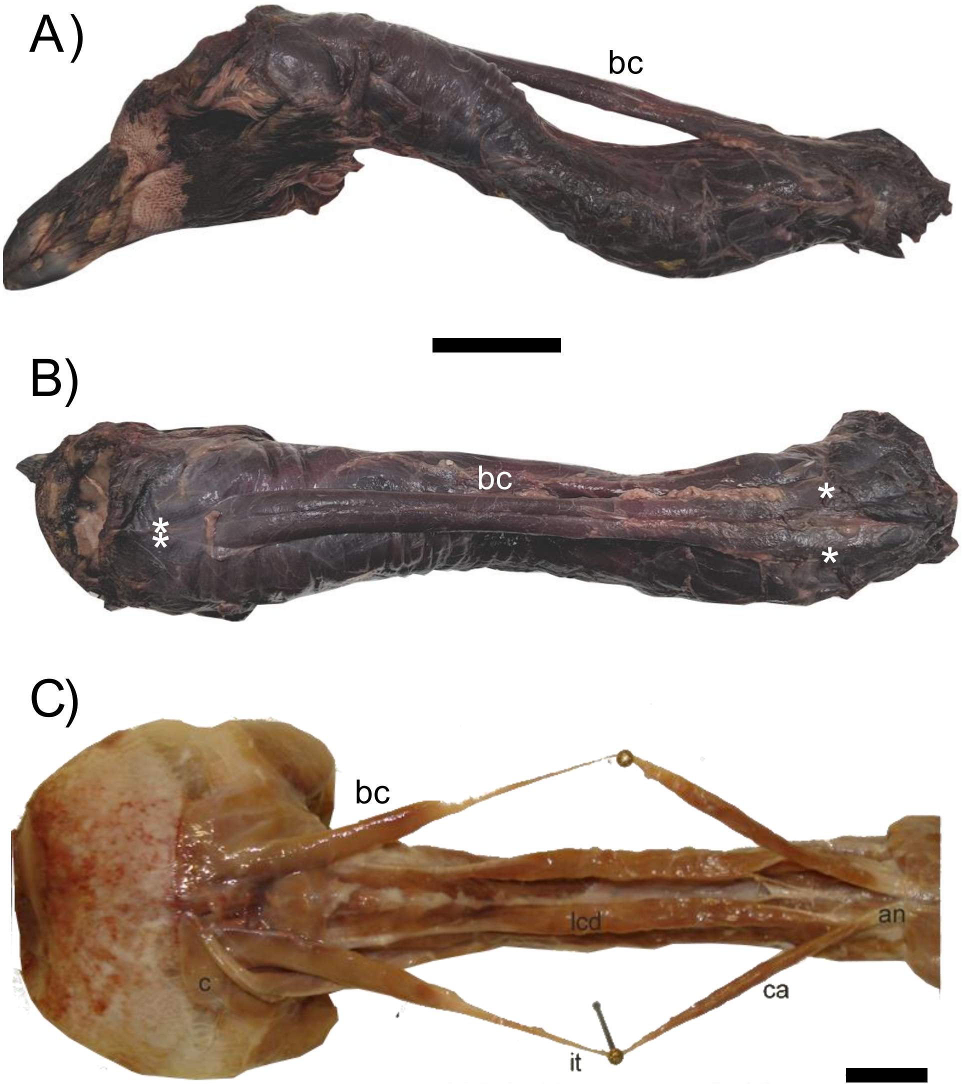

Figure 1 Photographs displaying the unique configuration of M. biventer cervicis in penguins (*Spheniscus humboldti*, A and B) compared to the generalised avian condition (C*, Tyto furcata pratincola*). M. biventer cervicis of penguins does not have an intersecting tendon between cranial and caudal bellies as in all other birds studied. A) Neck of *S. humboldti* in left lateral view, B) neck of *S. humboldti* in dorsal view, C) neck of *T. f. pratincola* in dorsal view. Bc = M. biventer cervicis, it = intersecting tendon, lcd = M. longus colli dorsalis, ca = caudal belly of M. biventer cervicis, an = aponeurosis notarii. Scale bars represent 5 cm for A and B (shared scale bar) and 1 cm in C. White stars denote origin and insertion sites for M. biventer cervicis.

of its length (Fig. 1a, b). This has been previously documented in other penguins (Kuroda 1962) and appears to be unique to the clade.

M. complexus is a cranially positioned muscle (Table 2) inserting onto the os supraoccipitale and originates on the transverse process of cervical vertebrae 3-7 (Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015, Figs 2, 3). Most of the specimens in this sample had 3 origination slips of M. complexus that attached to C3-C5 (Figs 2, 3). However the northern gannet (*Morus bassanus*, Fig. 3c) only had 1 origination slip that attached much more posteriorly at C7 (Appendix Fig. 6.1). M. complexus was far more apparent in *M. bassanus* due to its much larger size compared to all other birds in the dataset (Appendix Fig. 6.1). Whilst no anatomical description has corroborated this result, previous research presenting the effect of cervical muscle forces on impact force negation in plunge-diving gannets has noted the enlargement of dorsal musculature attaching to the head, the most noticeable muscle of which is M. complexus (Chang et al. 2016).

Finally, there are distinct differences to M. longus colli dorsalis pars profunda in both of the owl species in this study, *Strix aluco* (Fig. 2e) and *Tyto alba* (3b). M. longus colli dorsalis pars profunda is a small muscle that is not ubiquitously present across all birds (Table 2). It lies deep to M. longus colli dorsalis pars cranialis and caudalis and inserts and originates onto transverse and spinous processes respectively. In both of the owl species studied M. longus colli dorsalis pars profunda has 4 muscle slips, noticeably more than in other taxa and are also much thicker and easily spotted. This does not appear to be related to carnivory as a relative decrease in both muscle size and number of slips occurs in *Buteo* and so this appears to be an owl specific adaptation, suggested to aid in large lateral head rotations that typify owls (Baumel, Evans, and Berge 1993; Krings et al. 2014; Boumans, Krings, and Wagner 2015; Krings et al. 2017).

Variation in muscle attachment sites and vertebral span

Myological variation between the ten species studied (outside of the aforementioned 'extreme' examples) consisted of variation in the number of muscle slips, attachment sites (Figs. 2, 3) and total number of vertebrae spanned (Fig. 4). Initial comparisons of muscle attachment patterns across the entire neck indicate that that cranial muscles (muscles involved in head flexion and insert directly onto the cranium) display much less variation in the number of attachment sites and vertebral span than caudal muscles (multi-slipped muscles which originate and insert onto caudally positioned cervical vertebrae, Figs. 2-4).

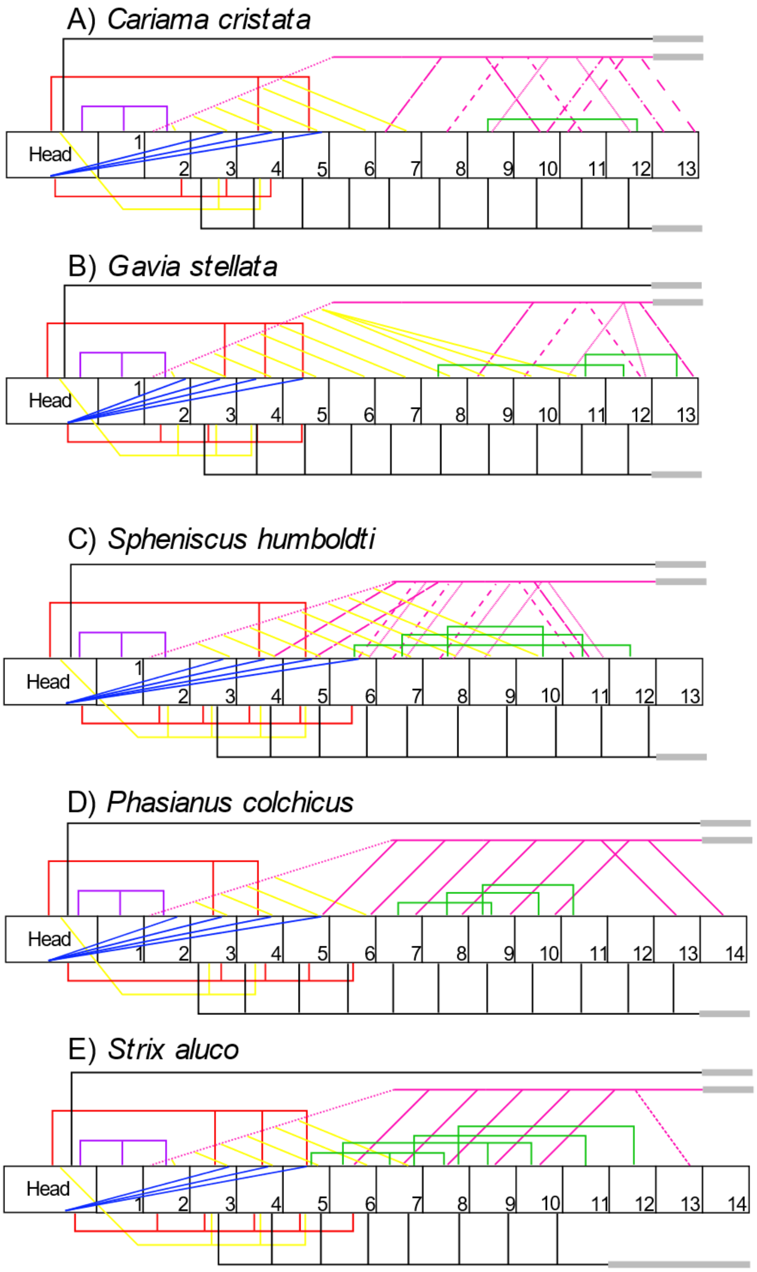

Figure 2 Muscle attachment diagrams for five species of birds with 13-14 cervical vertebrae. Numbered boxes represent the head and cervical vertebrae. Coloured lines are representations of muscles. Dorsal musculature: M. complexus (red), M. biventer cervicis (black), M. splenius capitis (purple), M. longus colli dorsalis pars caudalis (pink), M. longus dorsalis pars cranialis (yellow), M. longus colli dorsalis pars profunda (green). Ventral musculature: M. rectus capitis ventralis (red, M. rectus capitis lateralis (Yellow), M. rectus capitis dorsalis (blue), M. longus colli ventralis (black). Dashed lines for M. longus colli dorsalis pars caudalis represent discrete origination slips. Thicker grey bars represent aponeurotic insertions.

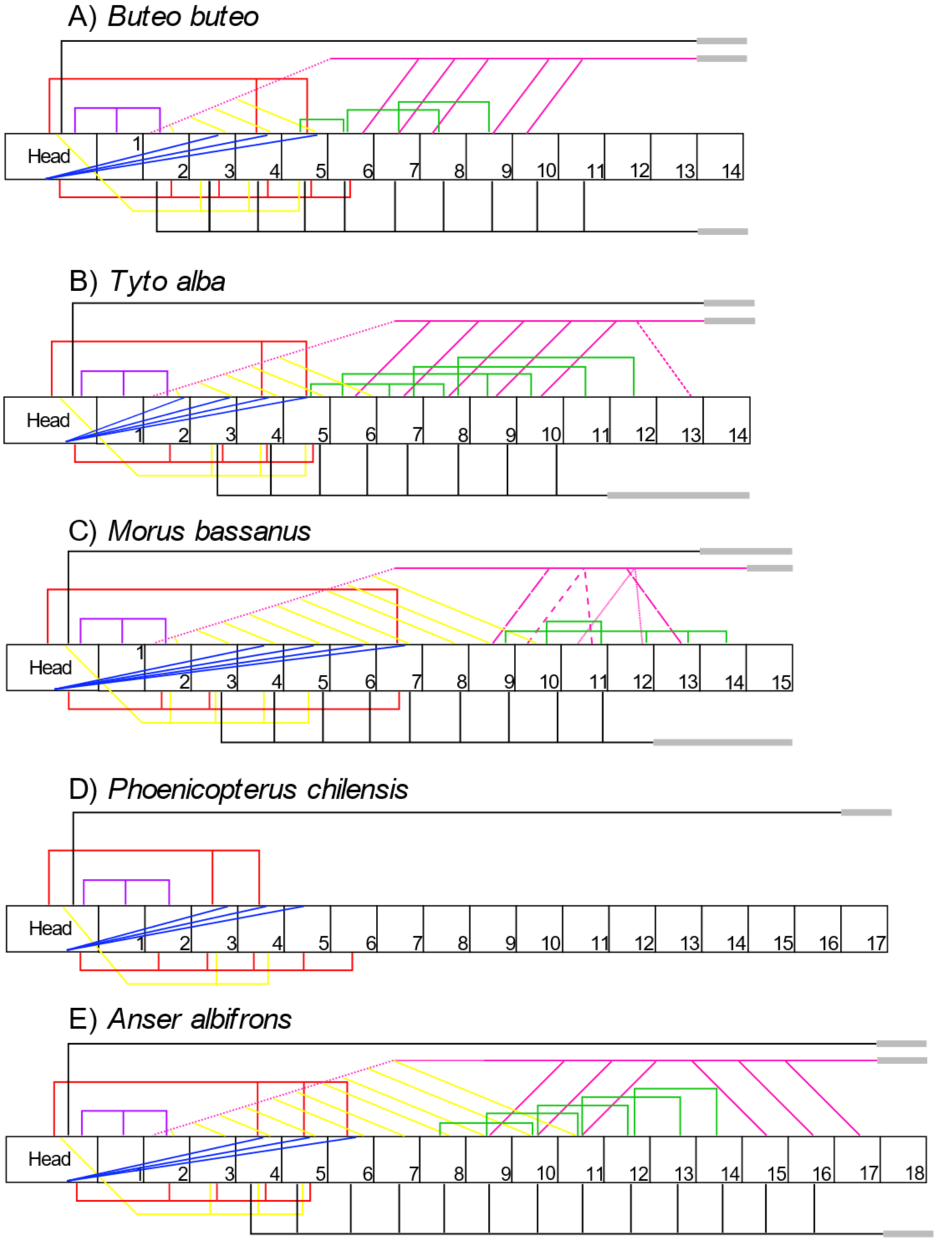

Figure 3 Muscle attachment diagrams for five species of birds with 13-14 cervical vertebrae. Numbered boxes represent the head and cervical vertebrae. Coloured lines are representations of muscles. Dorsal musculature: M. complexus (red), M. biventer cervicis (black), M. splenius capitis (purple), M. longus colli dorsalis pars caudalis (pink), M. longus dorsalis pars cranialis (yellow), M. longus colli dorsalis pars profunda (green). Ventral musculature: M. rectus capitis ventralis (red, M. rectus capitis lateralis (Yellow), M. rectus capitis dorsalis (blue), M. longus colli ventralis (black). Dashed lines for M. longus colli dorsalis pars caudalis represent discrete origination slips. Thicker grey bars represent aponeurotic insertions.

'Cranial' muscles

M. complexus is a dorsally positioned cranial muscle that inserts onto the os supraoccipitale of the cranium and originates onto the transverse processes of cervical vertebrae C3 to C7 (Figs. 2, 3). There is no variation in the site of insertion, however the number of origination slips and total vertebral span displays limited variation across all species (Figs. 2, 3). The number of origination slips varies from 1 (*M. bassanus*, Fig. 3c) to 3 (*A. albifrons,* Fig. 3e *G. stellata,* Fig. 2b *St. aluco* Fig. 2e), with two origination slips that attach to C3 and C4 or C4 and C5 representing the most common condition amongst all birds studied (Figs. 2, 3). Vertebral span ranged from 4 (*P. colchicus* Fig. 2d, *Ph. chilensis*, Fig. 3d) to 7 (*M. bassanus*, Fig. 3c), with 5 being the most common vertebral span amongst all birds studied (Figs. 2, 3).

M. splenius capitis is small, cranially positioned muscle that inserts onto the os supraoccipitale and originates onto the dorsolateral surfaces of the neural arches of C1 and C2. No variation in the number of attachment sites or vertebrae spanned was recorded within this dataset (Figs. 2-4).

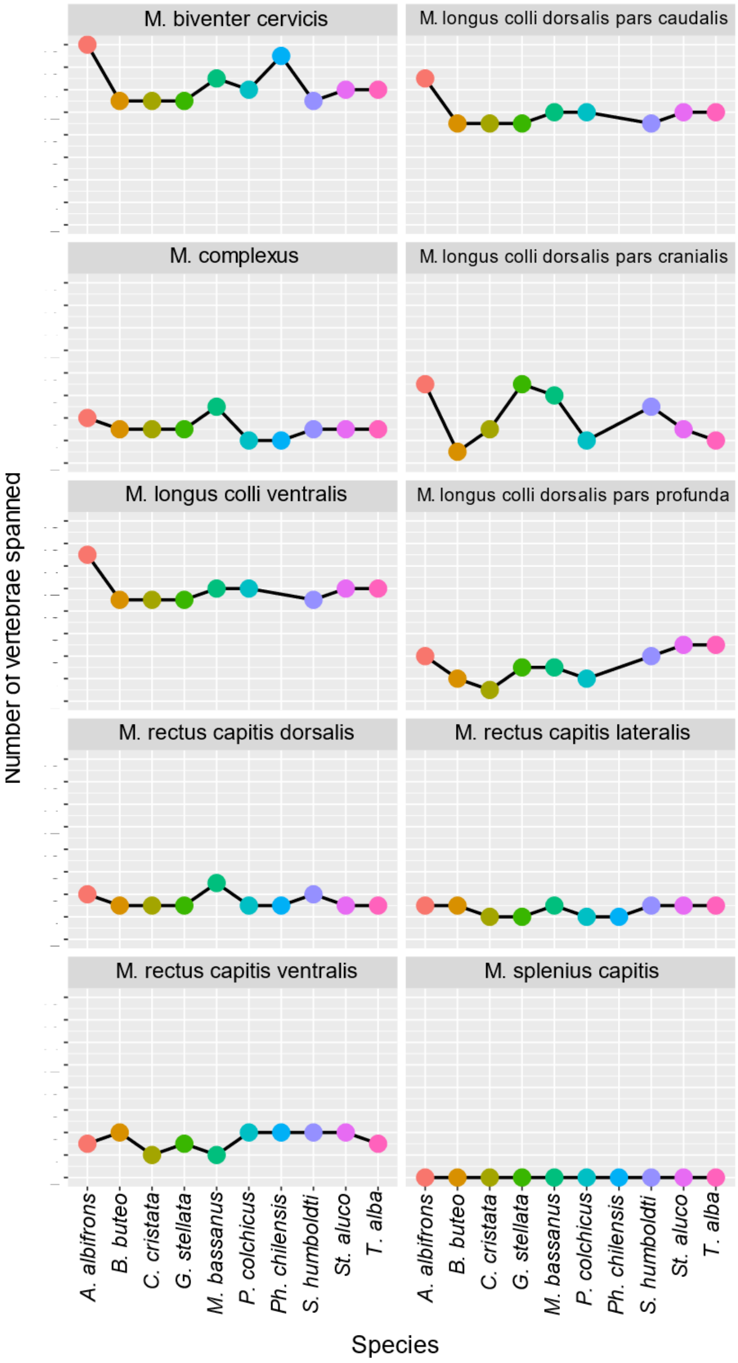

Figure 4 Total number of cervical vertebra spanned in each of the ten cervical muscles studied for all ten species of bird. Colours represent species.

M. rectus capitis lateralis is a laterally positioned muscle that inserts onto a lateral portion of the os supraoccipitale and originates ventrally onto the processus ventralis of C4-5. Variation in number of vertebrae spanned is small, only varying by one (4 or 5 total, Fig. 4), whilst variation in the number of origination slips is higher, between 2 (*C. cristata,* Fig. 2a *P. colchicus,* Fig. 2d, *Ph. chilensis,* Fig. 3d) and 4 (*Sp. humboldti, M. bassanus*). There is no variation for the location or number of insertion sites of M. rectus capitis lateralis (Figs. 2, 3).

M. rectus capitis ventralis is ventrally positioned muscle that inserts onto the cranium (os basioccipitale) and originates onto the processus ventralis of C2-C7. The number of origination slips varies between 3 (*C. cristata,* Fig. 2a, *G. stellata,* Fig. 2b, *M. bassanus,* Fig. 3c) and 5 (*St. aluco,* Fig. 2e, *Sp. humboldti,* Fig. 2f, *Ph. chilensis,* Fig. 3d). Vertebral span varies between 4 (*C. cristata*, Fig. 2a *M. bassanus,* Fig. 3c) and 6 (*B. buteo,* Fig. 3a, *P. colchicus,* Fig. 2d, *Ph. chilensis,* Fig. 3d, *Sp, humboldti,* Fig. 2c, *St. aluco*). There was no variation in the location or number of attaching muscle slips for the insertion of M. rectus capitis ventralis (Figs. 2, 3).

M. rectus capitis dorsalis is positioned on the ipsilateral portion of the cervical column, inserts onto the cranium (os supraoccipitale) and originates on the lateral processes of C2-C7. The number of origination slips varies between 3 (*C. cristata,* Fig. 2a, *St. aluco*, Fig. 2e, *B. buteo,* Fig. 3a, *Ph. chilensis,* Fig. 3d, *A. albifrons,* Fig. 3e) and 4 and attach around C4 and C5, with the cranial most origination slip attaching to C2 (*G. stellata,* Fig. 2b, *P. colchicus,* Fig. 2d, *T. alba,* Fig. 3b), C3 (*C. cristata,* Fig. 2a, *Sp. humboldti,* Fig. 2c, *St. aluco,* Fig. 2e, *B. buteo,* Fig. 3a, *Ph. chilensis,* Fig. 3d) or C4 (*M. bassanus,* Fig. 3c, *A. albifrons,* Fig. 3e) and the caudal most origination slip attaching to C5 (*C. cristata,* Fig. 2a, *G. stellata,* Fig. 2b, *P. colchicus,* Fig. 2d, *St. aluco,* Fig. 2e), C6 (*Sp. humboldti,* Fig. 2c, *A. albifrons,* Fig. 3e) or C7 (*M. bassanus,* Fig. 3c). There is no variation in the number or location of insertion muscle slips (Figs. 2, 3).

'Caudal musles'

M. biventer cervicis is a dorsally positioned muscle that inserts onto the os supraoccipitale, spans the entire length of the cervical column and originates onto an aponeurotic sheet above the cervicothoracic transition. Across all birds studied there is no variation in the location or number of insertion slips (Figs. 2,3), however the number of vertebrae this muscle spans varies with the maximum number of cervical vertebrae of each species (13 to 18, Fig. 4).

M. longus colli dorsalis pars caudalis is the caudal portion of the M. longus colli dorsalis muscle group and is a dorsally positioned, multislipped muscle which originates onto an aponeurosis notarii above the cervicothoracic transistion and the most cranial insertion site is the torus dorsalis of C2 via an axial tendon (tendo axialis). Whilst there is no variation in the location and number of attachment sites for the cranialmost insertion (Figs. 2,3), there is considerable variation in the number of caudally positioned insertion slips that all originate from the aponeurosis notarii but insert onto C4 (*Sp. humboldti,* Fig. 2c) to C17 (*A. albifrons,* Fig. 3e). The number of these slips can vary between 3 (*G. stellata,* Fig. 2b) to 6 (*Sp. humboldti,* Fig. 2c). As this muscle originates onto the aponeurosis notarii above the cervicothoracic transition the vertebral span varies with the total number of cervical vertebrae in each species, as in M. biventer cervicis (13-17, Fig. 4).

M. longus colli dorsalis pars cranialis is the cranialmost portion of the M. longus colli dorsalis muscle, it is a dorsally positioned, multislipped muscle that inserts onto the torus dorsalis of C2 (via the tendo axialis) and has multiple slips that originate on C3-C11 (Figs. 2, 3). The number of origination slips varies between 3 (*B. buteo,* Fig. 3a) and 9 (*G. stellata,* Fig. 2b, *A. albifrons,* Fig. 3e) and correlates with the caudal most origination site as muscle slips originate on every vertebrae between the insertion and caudalmost origin slip. Excluding the insertion site at C2, the vertebral span of M. longus colli dorsalis pars cranialis varies between 3 (*B. buteo*) and 9 (*A. albifrons*, Fig. 4).

M. longus colli dorsalis pars profunda is a dorsally located muscle that is positioned ventral to M. longus colli dorsalis pars caudalis. It is formed of multiple slips that each originate onto the processus transversus of a caudally positioned vertebrae and insert a onto the processus spinalis of a more cranially positioned vertebrae. Both the number of slips and vertebral span of each slip displays considerable diversity among the species studied (Figs. 2-4). The number of slips can varies between 1 (*C. cristata,* Fig. 2a) and 4-5 (*St. aluco,* Fig. 2e, *T. alba,* Fig. 3b, *A. albifrons,* Fig. 3e), whilst the vertebral span of a single slip can vary between 1 and 7 (Fig. 4), and sometime this variation occurs between slips of a single species (as in *Sp. humboldti,* Fig. 2c, and *M. bassanus*, Fig. 3c).

M. longus colli ventralis is a ventrally oriented muscle that spans almost the entirety of the cervical column as it originates on early thoracic vertebrae and inserts onto the processus transversus of C3-16. Between the cranial- and caudal-most insertion points there is an insertion muscle slip for each cervical vertebrae that M. longus colli ventralis spans (Figs. 2, 3). The cranialmost insertion slip is positioned at C3 across all species studied (Figs. 2, 3), however the vertebrae at which the caudalmost insertion slip occurs varies between C10 (*St. aluco,* Fig. 2e, *T. alba,* Fig. 3b) and C16 (A*. albifrons,* Fig. 3e). Vertebral span ranges from 11 vertebrae in (*B. buteo, C. cristata, G. stellata*) to 16 vertebrae (*A. albifrons,* Fig. 4).

Muscle architecture and muscle mass allometry

Fibre length, muscle mass and PCSA all have significant linear relationships with body mass for at least some muscles (Table 3). However significant relationships between muscle architecture variables and body mass were not ubiquitous across all ten cervical muscles. Cervical fibre lengths display a low level of significance with body mass, with only 4 out of the 10 cervical muscles studied displaying a significant linear relationship with body mass (Table 3). The muscles with a significant linear relationship between scaled fibre length and body mass were M. complexus (P = < 0.001, AICc = -4.589, Table 3), M. splenius capitis (P = < 0.001, AICc = -7.807, Table 3), M. rectus capitis lateralis (P = < 0.001, AICc = -11.362, Table 3) and M. rectus capitis dorsalis (P = < 0.001, AICc = -2.083, Table 3). Amongst these muscles, M. rectus capitis lateralis has the strongest linear relationship with body mass as it has the lowest AICc value (-11.362, Table 3), and M. rectus capitis dorsalis has the weakest linear relationship with body mass (highest AICc value, AICc = -2.083, Table 3).

Muscle	Dependent variable	Independent variable	a	CI ± (a)	b	CI ± (b)	P	AIC
M. complexus	Fibre length	Body mass	0.403	0.1194386	0.545	0.2259542	< 0.001	-4.589
M. biventer cervicis	Fibre length	Body mass	0.486	0.3470744	0.607	0.6565963	0.066	12.479
M. splenius capitis	Fibre length	Body mass	0.06	0.0976765	0.528	0.1847846	< 0.001	-7.807
M. rectus capitis lateralis	Fibre length	Body mass	0.171	0.0782193	0.573	0.1479755	< 0.001	-11.362
M. rectus capitis ventralis	Fibre length	Body mass	0.312	0.2133266	0.349	0.4035719	0.081	-1.569
M. rectus capitis dorsalis	Fibre length	Body mass	0.184	0.1396932	0.651	0.2642718	< 0.001	-2.083
M. longus colli dorsalis pars caudalis	Fibre length	Body mass	0.513	0.2994328	0.341	0.5664679	0.203	10.116
M. longus colli dorsalis pars cranialis	Fibre length	Body mass	0.292	0.3732979	0.466	0.7062061	0.167	13.644
M. longus colli dorsalis pars profunda	Fibre length	Body mass	0.106	0.2335544	0.351	0.4418389	0.104	6.141
M. longus colli ventralis	Fibre length	Body mass	0.449	0.2800178	0.368	0.5297384	0.148	9.044
M. complexus	Muscle mass	Body mass	-0.365	0.4240697	1.272	0.8022561	0.006	15.685
M. biventer cervicis	Muscle mass	Body mass	-0.095	0.2591284	0.844	0.4902198	0.004	7.803
M. splenius capitis	Muscle mass	Body mass	-0.399	0.244743	1.209	0.4630056	< 0.001	6.889
M. rectus capitis lateralis	Muscle mass	Body mass	-0.589	0.2785473	1.856	0.5269566	< 0.001	8.959
M. rectus capitis ventralis	Muscle mass	Body mass	-0.206	0.3387451	0.814	0.6408389	0.019	12.09
M. rectus capitis dorsalis	Muscle mass	Body mass	-0.304	0.3561775	1.438	0.6738176	0.001	12.893
M. longus colli dorsalis pars caudalis	Muscle mass	Body mass	0.117	0.3473673	1.374	0.6571505	0.001	12.492
M. longus colli dorsalis pars cranialis	Muscle mass	Body mass	-0.349	0.4865283	1.457	0.9204158	0.007	17.883
M. longus colli dorsalis pars profunda	Muscle mass	Body mass	-1.597	0.8665315	0.878	1.639308	0.252	27.118
M. longus colli ventralis	Muscle mass	Body mass	0.149	0.3473459	1.328	0.6571099	0.002	12.491
M. complexus	PCSA	Body mass	-0.794	0.321889	0.727	0.6089505	0.025	11.273
M. biventer cervicis	PCSA	Body mass	-0.599	0.3201786	0.255	0.6057146	0.359	11.188
M. splenius capitis	PCSA	Body mass	-0.485	0.1758927	0.681	0.3327543	0.002	1.604
M. rectus capitis lateralis	PCSA	Body mass	-0.785	0.2300724	1.282	0.4352516	< 0.001	5.9
M. rectus capitis ventralis	PCSA	Body mass	-0.544	0.3119797	0.464	0.5902041	0.107	10.773
M. rectus capitis dorsalis	PCSA	Body mass	-0.214	0.3649023	0.787	0.690323	0.03	13.28
M. longus colli dorsalis pars caudalis	PCSA	Body mass	-0.419	0.2797171	1.038	0.5291696	0.002	9.0267
M. longus colli dorsalis pars cranialis	PCSA	Body mass	-0.665	0.2707747	0.995	0.5122524	0.002	8.507
M. longus colli dorsalis pars profunda	PCSA	Body mass	-1.726	0.8931871	0.531	1.689733	0.489	27.903
M. longus colli ventralis	PCSA	Body mass	-0.324	0.2924525	0.965	0.5532625	0.004	9.729

Table 3 Results from phylogenetic generalised least squares (pGLS) regression models of muscle architecture variables (fibre length, muscle mass and PCSA) versus body mass. 'b' represents the scaling coefficient used to muscle architecture data seen in figures 1-3. Significant P-values are < 0.05. Scaling effects of body mass do not effect muscle architecture variables evenly, muscle mass and PCSA display a significant allometric relationship in double the number of muscles than fibre length. Across all muscles, all architecture variables predominantly display positive allometry, with a small subset displaying an isometric relationship with body mass.

The effect of body mass is relatively stronger on muscle masses and PCSA of cervical muscles with eight and seven muscles respectively displaying a significant relationship with body mass (Table 3). The only muscles not to display a significant relationship between muscle mass and body mass were M. biventer cervicis and M. longus colli dorsalis pars profunda (Table 3). The linear relationship between body mass and cervical muscle mass was strongest in M. splenius capitis and M. rectus capitis lateralis (lowest AICc values, AICc = 6.889 and AICc = 8.959 respectively, Table 3). M. longus colli dorsalis pars cranialis and M. complexus had comparatively the weakest linear relationships with body mass of all cervical muscles (highest AICc values , AICc = 17.883 and AICc = 15.685 respectively, Table 3). M. biventer cervicis, M. rectus capitis ventralis and M. longus colli dorsalis pars profunda are the only three muscles not to display a significant relationship between PCSA and body mass. The linear relationship between cervical PCSA and body mass appears to be strongest in M. splenius capitis (lowest AICc value, AICc = 1.604, Table 3) and weakest in M. rectus capitis dorsalis and M. complexus (highest AICc values, AICc = 13.280 and AICc = 11.273 respectively, Table 3).

The coefficients (b) from linear relationships between cervical muscle architecture variables and body mass are used to scale fibre length, muscle mass and PCSA in muscles that display a significant allometric relationship between these variables and body mass (Myatt, Crompton, and Thorpe 2011; Myatt et al. 2012). These coefficients can also be used to assess any deviations from isometric scaling within cervical muscle architecture and muscle mass when stated with their 95% confidence intervals.

Muscles that displayed significant allometric scaling of fibre length have coefficients that suggest either isometry or positive allometry, i.e. a coefficient value and 95% confidence interval around or above 0.33. M. complexus displays an isometric scaling relationship (b = 0.545, CI = 0.226, Table 3), whilst the other 3 muscles that had a significant relationship between fibre length and body mass display positive allometry (M. splenius capitis = 0.528, CI = 0.185, M. rectus capitis lateralis = 0.573, CI = 0.148, M. rectus capitis dorsalis = 0.651, CI = 0.264, Table 3).

Deviations from coefficient values of 1.00 (including confidence interval estimates) represent deviations from isometry for allometric relationships of cervical muscle mass. Five of the eight muscles that display significant muscle mass allometry have coefficients that suggest positive allometry (M. rectus capitis lateralis = 1.856, CI , M. rectus capitis dorsalis = 1.438, CI = 0.674, M longus colli dorsalis pars caudalis = 1.374, CI = 0.657, M. longus colli dorsalis pars cranialis = 1.457, CI = 0.920, M longus colli ventralis = 1.328, CI = 0.657, Table 3). Muscles that display an isometric scaling relationship between cervical muscle mass and body mass are M. complexus (b = 1.272, CI = 0.802, Table 3), M. splenius capitis (b = 1.209, CI = 0.463, Table 3) and M. rectus capitis ventralis (b = 0.814, CI = 0.641, Table 1).

As per fibre length and muscle mass, cervical PCSA displays either an isometric or positive allometric scaling relationship with body mass. Out of the seven muscles that had a significant linear relationship, 3 were close to isometric (M. complexus = 0.727, CI = 0.609, M. splenius capitis = 0.681, CI = 0.333, M. rectus capitis dorsalis = 0.787, CI = 0.690, Table 3). The remaining four cervical muscles scaled with body mass according to positive allometry (M. rectus capitis lateralis = 1.282, CI = 0.435, M. longus colli dorsalis pars caudalis = 1.038, CI = 0.529, M. longus colli dorsalis pars cranialis = 0.995, CI 0.512, M longus colli ventralis = 0.965, CI = 0.553 Table 3).

All muscles with significant scaling relationships of fibre length, muscle mass and PCSA were also significant (*P* = < 0.05, Appendix Table 6.1) in alternative parametric tests (Pearson's correlation). Non-parametric alternatives to PGLS modelling of the scaling relationships of fibre length, muscle mass and PCSA were more conservative, with only M. complexus , M. splenius capitis, M. rectus capitis lateralis and M. rectus capitis dorsalis (*P* = < 0.05, Appendix Table 3.1) returning significant relationships between body mass and all 3 measured variables (fibre length, muscle mass and PCSA).

Variation in cervical muscle architecture

Variation amongst species

Scaled fibre length displays a larger variation amongst species in more caudally positioned muscles that span multiple joints (M. biventer cervicis, M. longus colli dorsalis pars caudalis, pars cranialis, pars profunda and M. longus colli ventralis, Fig. 5 and Fig. 8 respectively). The standard deviation of fibre lengths of these large caudally positioned muscles are between 2 and 4 times higher than those calculated for cranially positioned muscles (Fig. 8b). More cranially positioned muscles that control head movements displayed lower interspecific variation in fibre length (M. complexus, M. splenius capitis and M. rectus capitis lateralis especially, Fig. 5 and 8a respectively). Amongst these cranially positioned muscles, M. complexus (SD = 6.40, Fig. 8b) and M. rectus capitis ventralis (SD = 1.198, Fig. 8b) displayed the highest levels of variation. Large, caudally positioned muscles also displayed a larger amount of variation in muscle mass (Fig. 7, Fig. 8). However this pattern was much less pronounced than in fibre length variability. Disparity in the variation of PCSA between cranial and caudal muscles is much less pronounced than in fibre length and muscle mass (Fig. 6, Fig. 8).

Morus bassanus represents an outlier as in all cranial muscles except M. splenius capitis (M. complexus and the M. rectus capitis complex, Fig. 6), *M. bassanus* has the largest PCSA by a sizeable margin. *M.* bassanus also has substantially larger values of muscle mass in M. complexus (Appendix Fig 6.1) and the muscles of the M. rectus capitis complex (Fig. 7).

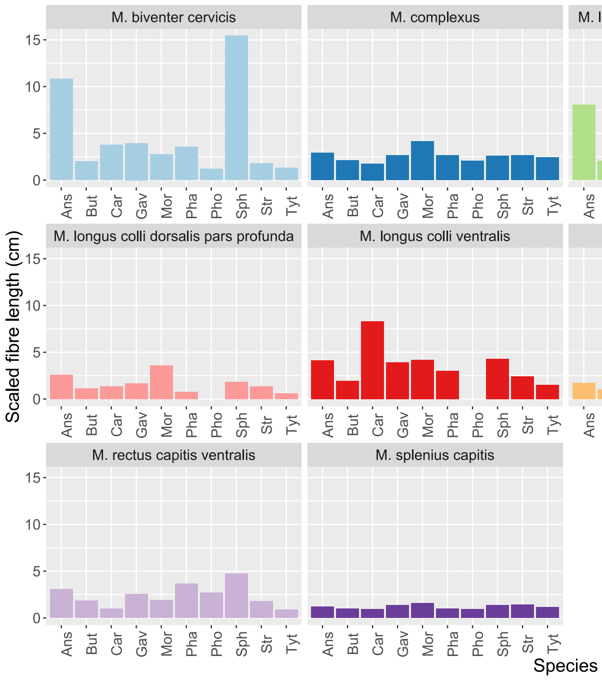

Figure 5 Bar charts comparing scaled fibre length values for all 10 species in this study. Fibre length is more variable in multi-joint spanning caudal muscles (M. biventer cervicis, M. longus colli dorsalis pars cranialis, pars caudalis, pars profunda and M. longus colli ventralis). Among more cranially positioned muscles, M. rectus capitis ventralis and M. rectus capitis dorsalis displayed higher levels of fibre length variation. Linear equations from significant relationships between muscle architecture and body mass were used to scale muscle architecture values according to the equation $Y = aM^b$, where Y is the muscle architecture variable, M is body mass (kg), b is the scaling coefficient. Ans = *A. albifrons*, But = *B. buteo*, Car = *C. cristata*, Gav = *G. stellata*, Mor = *M. bassanus*, Pha = *P. colchicus*, Pho = *Ph. chilensis*, Sph = *S. humboldti*, Str = *S. aluco*, Tyt = *T. alba*.

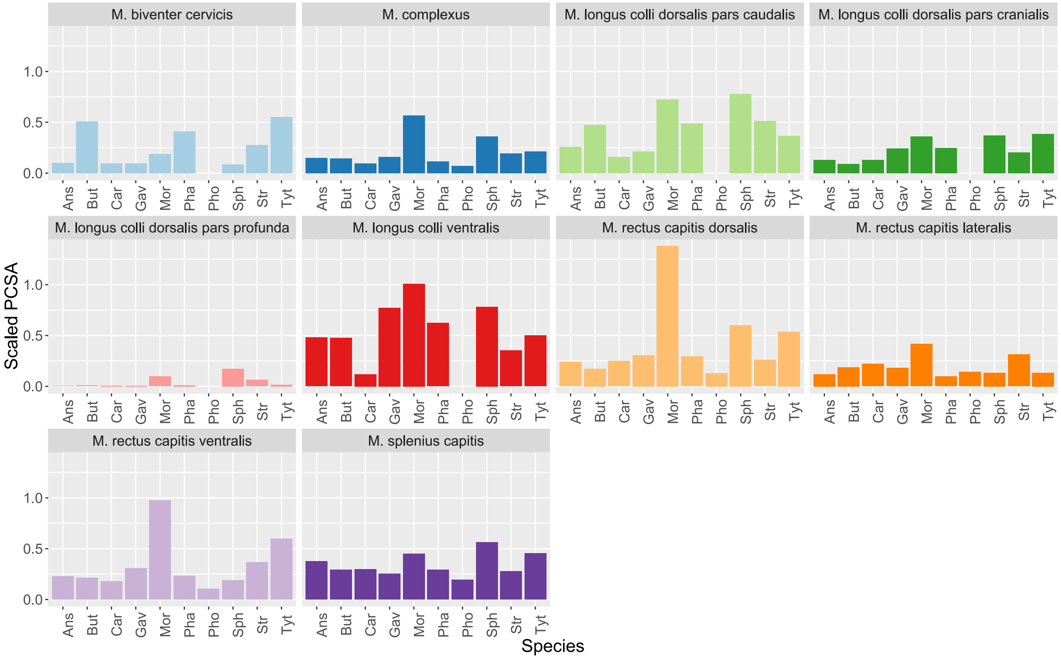

Figure 6 Bar charts comparing scaled PCSA for all 10 species in this study. PCSA is more variable in caudal muscles that span multiple joints (M. biventer cervicis, M. longus colli dorsalis pars cranialis, pars caudalis, pars profunda and M. longus colli ventralis). Cranially positioned muscles displayed lower variability in PCSA, except for M. splenius capitis. *M. bassanus* displays the largest PCSA by some margin in M. complexus and all three muscles in the M. rectus capitis complex.

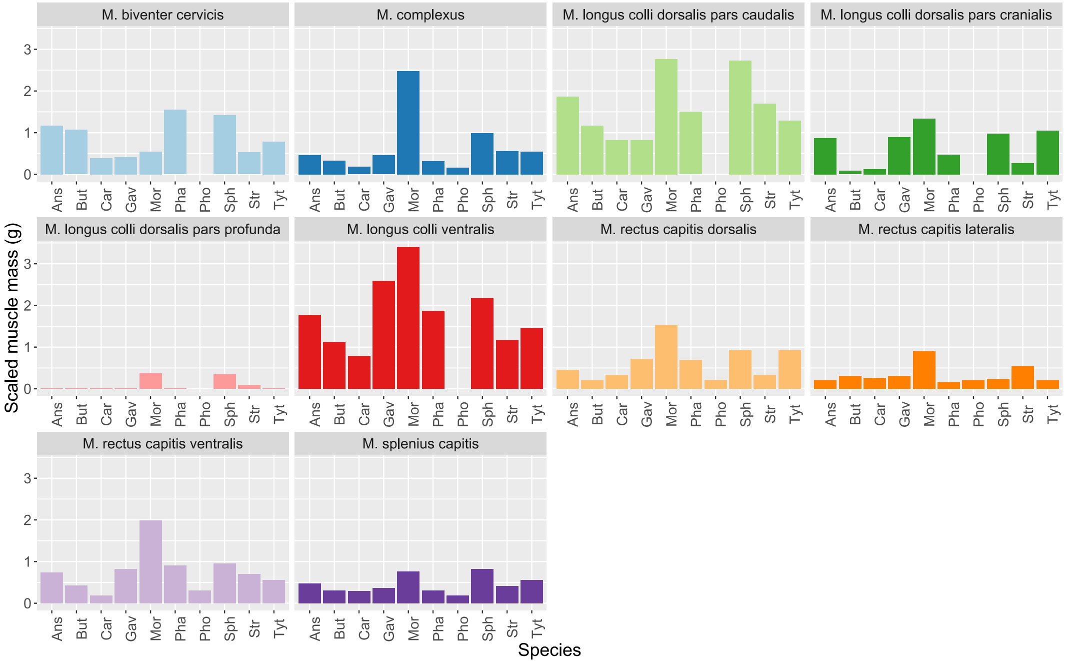

Figure 7 Bar charts comparing scaled muscle mass for all 10 species in this study. Interspecific variation in muscle mass is split evenly between cranial and caudal muscles. *M. bassanus* again displays a the highest muscle mass of the head flexors M. complexus, M. rectus capitis lateralis, M. rectus capitis ventralis and M. rectus capitis dorsalis

Muscle	Dependent variable	Covariate	Independent variable	Coefficient^{CV}	CI ± (CV)	P^{CV}	Coefficient^{I}	CI ± (I)	P^{I}	AIC
M. biventer cervicis	Fibre length	Body mass	SUB	0.3642	0.622624	0.2091	0.607	0.6751839	0.0711	11.2308
M. biventer cervicis	Muscle mass	Body mass	SUB	0.6543	0.450193	0.0109	0.4745	0.488197	0.0551	6.6911
M. longus colli dorsalis pars caudalis	Muscle mass	Body mass	CoF	1.4891	0.592323	0.0006	-0.3608	0.4298596	0.0876	12.5607
M. longus colli dorsalis pars caudalis	PCSA	Body mass	insectivore	1.1576	0.474769	0.0007	-0.4873	0.5506892	0.0747	8.2852
M. longus colli ventralis	PCSA	Body mass	insectivore	1.1441	0.29402	< 0.001	-0.7273	0.3410363	0.0015	1.5767
M. rectus capitis dorsalis	Fibre length	Body mass	predator	0.3081	0.440962	0.1425	-0.3267	0.3608524	0.0696	-2.121
M. rectus capitis dorsalis	Fibre length	Body mass	soaring	0.6455	0.212298	0.0002	-0.1815	0.1745839	0.0436	-1.6197
M. rectus capitis lateralis	Fibre length	Body mass	insectivore	0.6101	0.124301	< 0.001	-0.1491	0.1441785	0.0444	-10.4765
M. rectus capitis lateralis	Muscle mass	Body mass	soaring	1.8662	0.455891	< 0.001	0.3267	0.3749043	0.0783	9.08
M. rectus capitis ventralis	Fibre length	Body mass	insectivore	0.4715	0.261049	0.0037	-0.4941	0.3027934	0.0062	-0.0885
M. rectus capitis ventralis	Muscle mass	Body mass	insectivore	0.9642	0.562872	0.0049	-0.6094	0.6528811	0.0631	10.6683
M. splenius capitis	Muscle mass	Body mass	filter	1.2798	0.393415	0.0001	-4.174	0.4378666	0.0588	6.1266
M. splenius capitis	PCSA	Body mass	filter	0.7281	0.296546	0.0007	-0.2786	0.3300524	0.0862	2.1694
M. splenius capitis	PCSA	Body mass	SUB	0.5497	0.301174	0.0035	0.3281	0.3265981	0.0492	1.0632

Table 4 Significant ($P = < 0.05$) and close to significant ($P = 0.05 – 0.09$) results from phylogenetic generalised least squares (pGLS) regression models test for the effect of ecological variables on cervical muscle architecture (fibre length, muscle mass and PCSA) values. Significant reductions to muscle fibre lengths can be observed in M. rectus capitis lateralis and M. rectus capitis ventralis of insectivorous taxa as well as in M. rectus capitis dorsalis of soaring taxa. Insectivorous also display a significant decrease of PCSA in M. longus colli ventralis. Subaqueous taxa display a significant increase to the PCSA of M. splenius capitis.

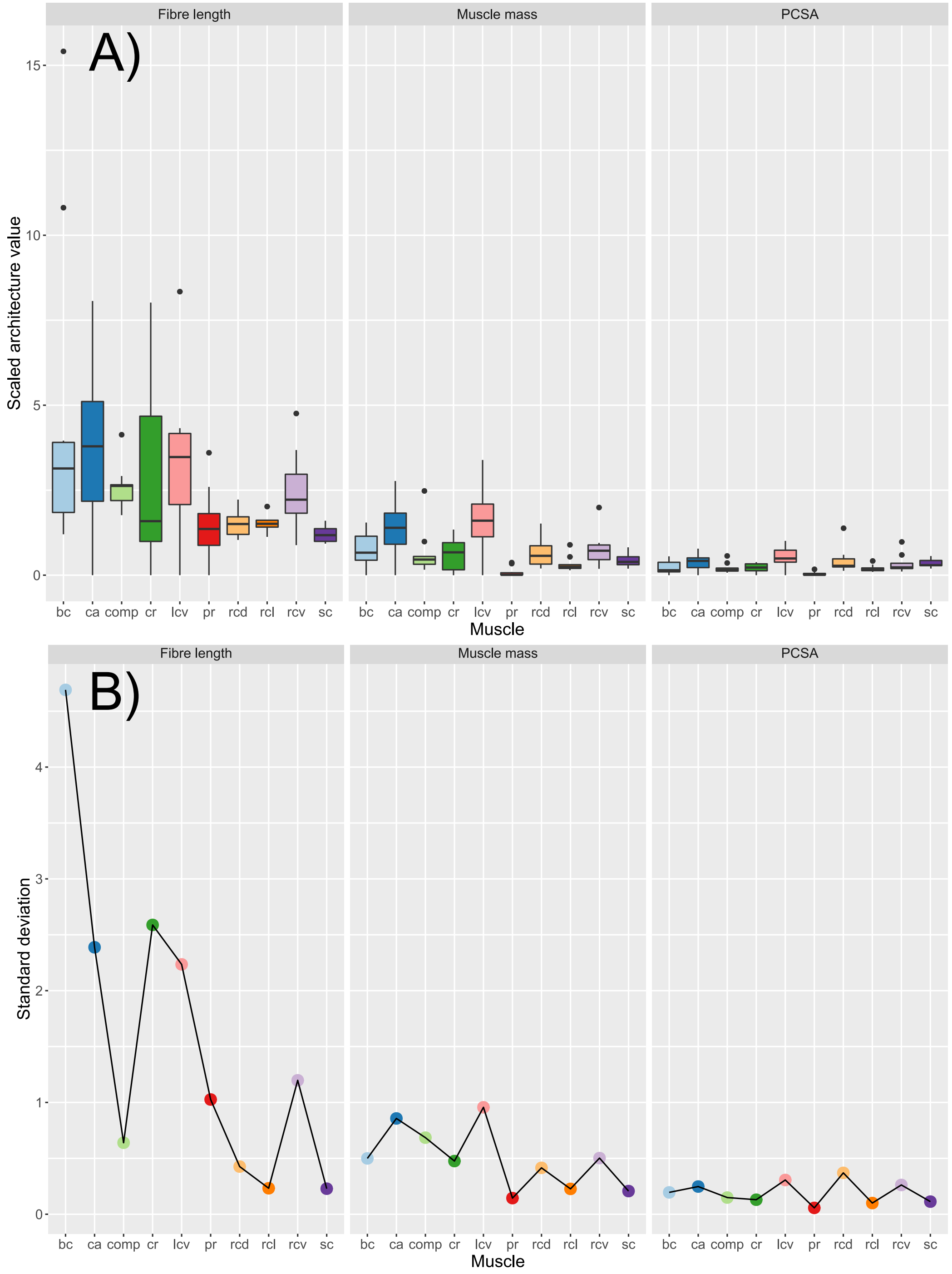

Figure 8 A) Box plots denoting total interspecific variation for each cervical muscle for measurements of fibre length, muscle mass and PCSA. B) Standard deviation for interspecific variation for each cervical muscle for measurements of fibre length, muscle mass an PCSA. Caudal muscles display more variation than cranial muscles in fibre length and muscle mass but this disparity is much less apparent in PCSA.

Variation amongst ecologies

Across all ten cervical muscles only five displayed a significant relationship between muscle architecture and ecology (Table 4). Fibre length and PCSA were the only two muscle architecture variables to display a significant correlation with ecological variables, with cervical muscle mass never achieving significance across all models for all muscles (Table 4). Subaqueous locomotion was found to have a significant correlation with the PCSA of M. splenius capitis (*P* = 0.0492, AICc = 1.0632, Table 4), and the coefficients indicate that PCSA is higher in taxa that display subaqueous locomotion (coefficient = 0.3281, SE = 0.1381, Table 4). The fibre lengths of M. rectus capitis lateralis and M. rectus ventralis both have a significant correlation with insectivory (*P* = 0.0444, AICc = -10.4765 and *P* = 0.0062, AICc = -0.0885 respectively, Table 4). Fibre lengths of these muscles both appear to be reduced in insectivorous taxa (M. rectus capitis lateralis coefficient = -0.1481, SE = 0.061, M. rectus capitis ventralis coefficient = -0.4941, SE = 0.1281, Table 4). Insectivory also has a significant correlation with PCSA in M. longus colli ventralis (*P* = 0.0015, AICc = 1.5767, Table 4) and insectivorous taxa appear to have a lower PCSA value for this muscle (coefficient = -0.7273, SE = 0.1442, Table 4). Soaring has a significant correlation with fibre length in M. rectus capitis dorsalis (*P* = 0.0436, AICc = -1.6197, Table 4) and soaring taxa display decreased fibre lengths (coefficient = -0.1815, SE = 0.0738, Table 4).

While the number of significant (*P* = < 0.05) relationships between muscle architecture variables and ecological variables is low (5, Table 4) there are nine further models which lie just outside significance (*P* = 0.05 – 0.09, Table 4) that may provide insight into factors controlling cervical muscle variation in birds and areas for future studies with larger sample sizes. As above, each model with significance values between *P* = 0.05 and *P* = 0.09 will be noted along with its coefficients. Fibre lengths of M. biventer cervicis and M. rectus capitis dorsalis display a non-significant correlation with subaqueous (*P* = 0.0711, AICc = 11.2308, Table 4) and carnivorous (and *P* = 0.0696, AICc = -2.121, Table 4) taxa respectively, with subaqueous species displaying relatively elongated fibre lengths in M. biventer cervicis (coefficient = 0.607, SE = 0.2855, Table 4) and carnivorous species displaying relatively shorter fibre lengths in M. rectus capitis dorsalis (coefficient = -0.3267, SE = 0.1526, Table 4). Five muscles display a correlation between muscle mass and ecology with a *P*-value range between 0.05 and 0.09; M. biventer cervicis (subaqueous, *P* = 0.0551, AICc = 6.6911, Table 4), M. longus colli dorsalis pars caudalis (continual flappers, *P* = 0.0876, AICc = 12.5607, Table 4), M.

rectus capitis lateralis (soaring, *P* = 0.0783, AICc = 9..08, Table 4), M. rectus capitis ventralis (insectivore, *P* = 0.0631, AICc = 10.6683, Table 4), and M. splenius capitis (filter, *P* = 0.0588, AICc = 6.1266, Table 4). Muscle mass is increased in M. biventer cervicis of subaqueous taxa (coefficient = 0.4745, SE = 0.2065, Table 4) as well as in M. rectus capitis lateralis of soaring species (coefficient = 0.3267, SE = 0.1585, Table 4). Muscle mass is decreased in M. longus colli dorsalis caudalis of continual flappers (coefficient = 0.3608, SE = 0.1818, Table 4), M. rectus capitis ventralis of insectivorous taxa (coefficient = -0.6094, SE = 0.2761, Table 4 and in M. splenius capitis of filter feeding species (coefficient = -4.174, SE = 0.1852, Table 4). Two muscles display a correlation between PCSA and ecology with a significance value between *P* = 0.05 and *P* = 0.09: M longus colli dorsalis pars caudalis (insectivores, *P* = 0.0747, AICc = 8.2852, Table 4) and M. splenius capitis (filter feeders, *P* = 0.0862, AICc = 2.1694, Table 4) and PCSA is decreased in both muscles for both ecologies (insectivore coefficient = -0.4873, SE = 0.2329 and filter feeder coefficient = -0.2786, SE = 0.1396 respectively, Table 4).

Kruskal-Wallis tests were used as a non-parametric alternative to PGLS models and returned some notable differences to the aforementioned parametric PGLS results.

Flap-gliding and carnivory had a significant correlation with fibre length, muscle mass and PCSA in many of the ten cervical muscles studied (P = < 0.05, Appendix Table 6.2). Fibre length had a significant correlation with flap gliding in M. rectus capitis lateralis (P = 0.03671, Appendix Table 6.2) and M. rectus capitis dorsalis (P = 0.03671, Appendix Table 6.2). Carnivory had a more widespread correlation with fibre length, with M. complexus (P = 0.0167, Appendix Table 6.2), M. longus colli dorsalis pars cranialis (P = 0.03037, Appendix Table 6.2), M. rectus capitis dorsalis (P = 0.0167, Appendix Table 6.2), M. rectus capitis lateralis (P = 0.0167, Appendix Table 6.2) and M. splenius capitis (P = 0.0167, Appendix Table 6.2) all displaying a significant correlation with carnivory. Muscle mass displays the highest level of correlation with flap gliding and carnivory with 8 and 9 out of 10 cervical muscles displaying significant correlations with these specific ecologies (P = < 0.05, Appendix Table 6.2). Flap gliding is significantly correlated with muscle mass in all cervical muscles studied aside from M. longus colli dorsalis pars cranialis and pars profunda (P = < 0.05, Appendix Table 6.2). Carnivory is significantly correlated with muscle mass in all cervical muscles studied aside from M. biventer cervicis (P = < 0.05, Appendix Table 6.2). Four out of ten cervical muscles (M. biventer cervicis P = 0.03671 M. longus colli dorsalis pars caudalis P = 0.03671, M. longus colli ventralis P = 0.03671, M. splenius capitis P = 0.03671) display a significant correlation between PCSA and flap gliding. Five cervical muscles out of ten studied (M. longus colli dorsalis pars caudalis *P* = 0.03671, M. complexus *P* = 0.0167, M. longus colli dorsalis pars cranialis *P* = 0.0167, M. longus colli ventralis *P* = 0.03671, M. rectus capitis dorsalis *P* = 0.0167, M. splenius capitis *P* = 0.0167) displayed a significant correlation between PCSA and carnivory. None of these relationships were resolved as significant when muscle architecture and mass values were scaled according to body mass. Only 3 significant relationships between muscle architecture and mass were present once body mass was accounted for (*P* = < 0.05, Appendix Table 6.2). Muscle mass and PCSA of M. rectus capitis lateralis both had a significant correlation with herbivory, whilst fibre length in M. rectus capitis dorsalis is significantly correlated with soaring (*P* = < 0.05, Appendix Table 6.2).

Variation in muscle function

The area occupied by any given muscle on a muscle function chart (scaled PCSA versus scaled fibre length, Fig. 9 gives an indication of potential functional specialisation relative to other muscles in its anatomical system (for a thorough explanation, see Materials and Methods), and variability of a particular muscle's function can be studied by observing the range of function space a particular muscle occupies when multiple samples (in this case species) are plotted (Figs. 9-12).

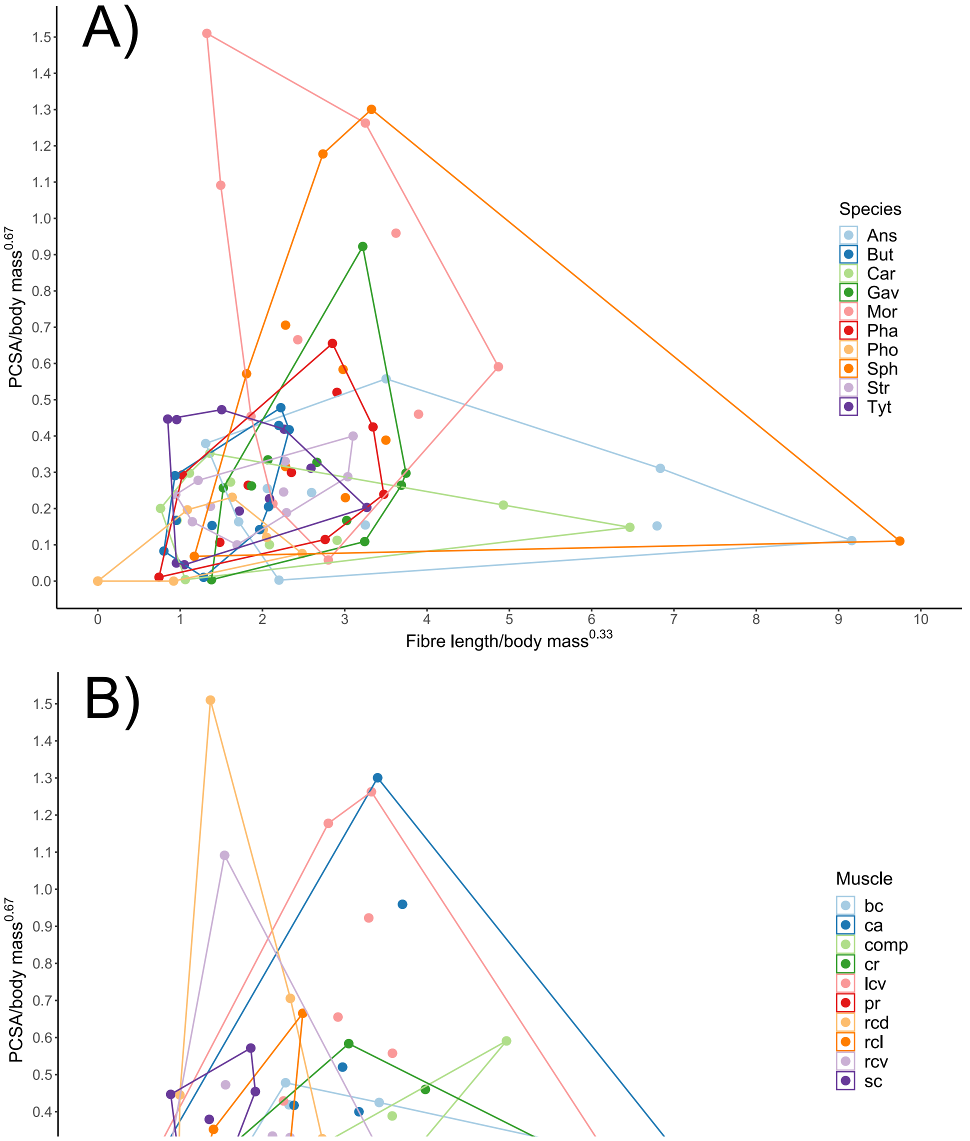

Figure 9 Muscle function plot (scaled PCSA vs scaled fibre length) of all ten studied muscles. A) Grouped by species (Ans = *A. albifrons*, But = *B. buteo*, Car = *C. cristata*, Gav = *G. stellata*, Mor = *M. bassanus*, Pha = *P. colchicus*, Pho = *P. chilensis*, Sph = *S. humboldti*, Str = *S. aluco*, Tyt = *T. alba*, B) grouped by muscle bc = biventer cervicis, ca = M. longus colli dorsalis pars caudalis, cr = M. longus colli dorsalis pars cranialis, lcv = M. longus colli ventralis, pr = M. longus colli dorsalis pars profunda. All muscles display variation in either muscle force or contraction speed, no muscles display a coupling of high force over long contraction ranges. Caudal muscles display more variability in function than cranial muscles, as cranial muscles cluster towards the bottom left of the muscle-function plot (low forces acting over a small range).

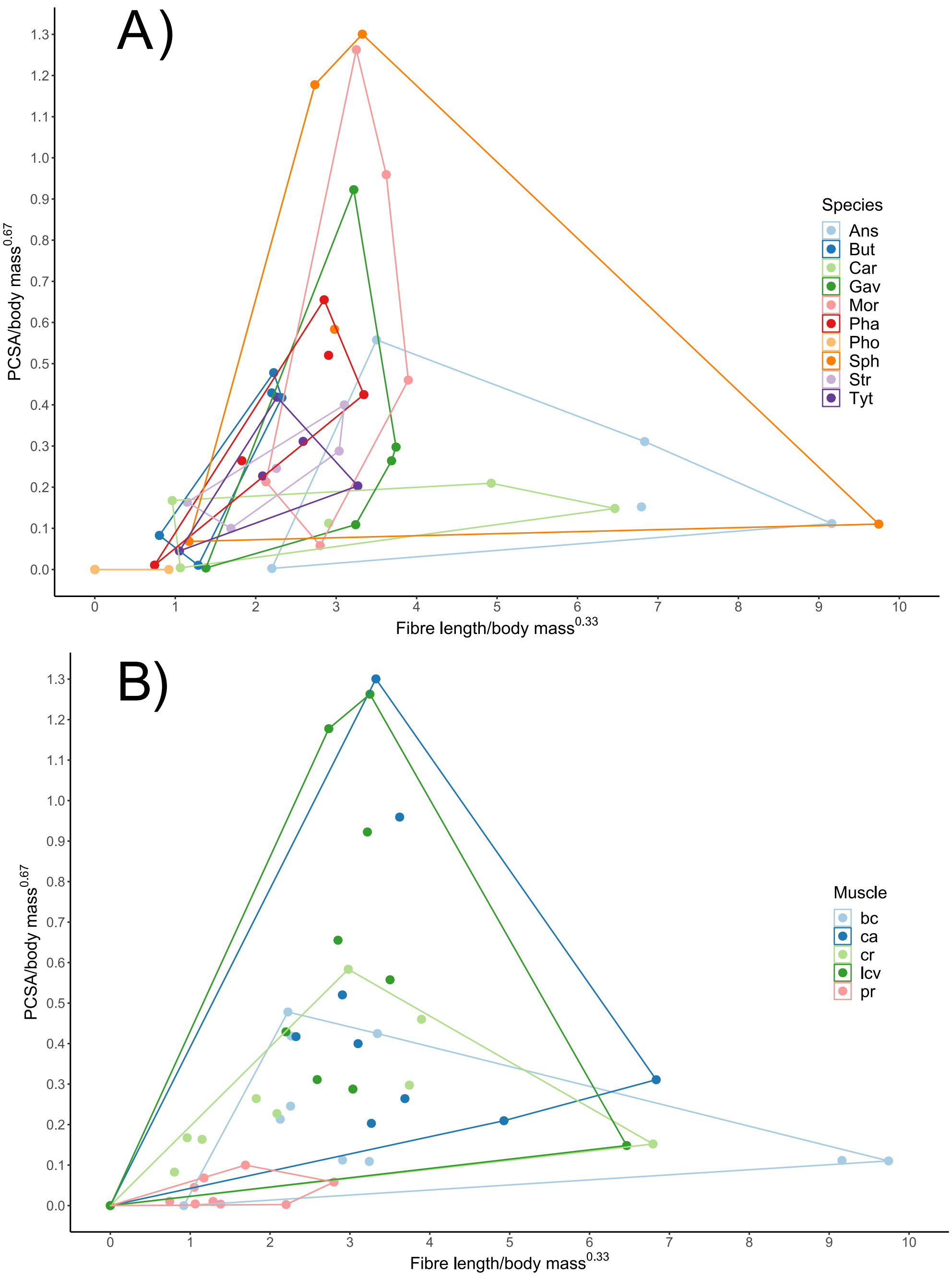

Figure 10 Muscle function plot of all caudal muscles studied grouped by A) species and B) muscle. Caudal muscles display much more variation in muscle function than cranial muscles (Fig. 7).

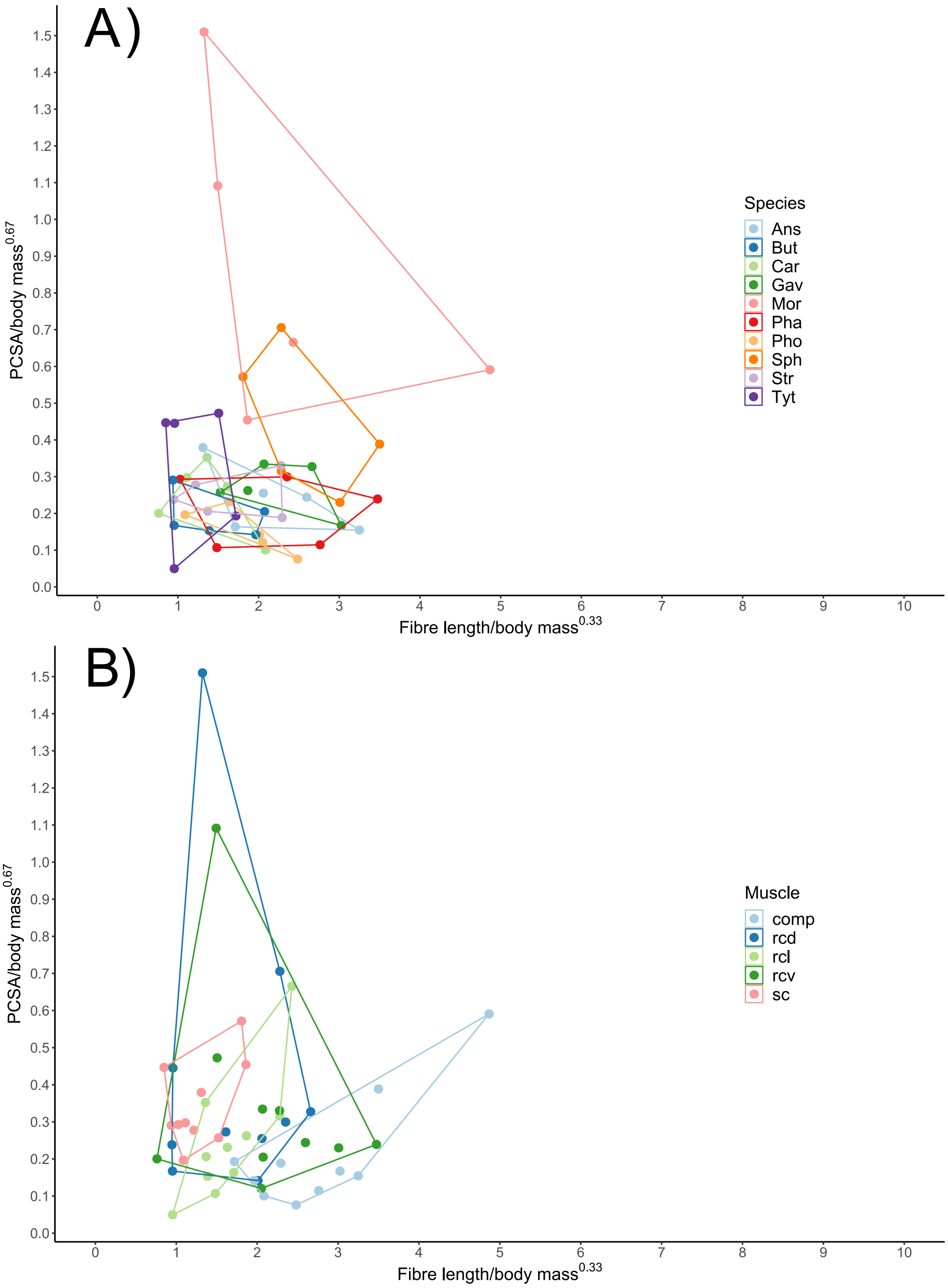

Figure 11 Muscle function plot of all cranial muscles studied grouped by A) species and B) muscle. Cranial muscles display much less variation in muscle function than caudal muscles (Fig. 6).

When all muscles from all species are considered there is a sizeable amount of variation on both axes of the muscle function plot (Fig. 9). The total range variation along the x axis (fibre length/body mass$^{0.33}$) is 9.744 whilst the total variation along the y axis (PCSA/body mass$^{0.67}$) is 1.510 (Fig. 9). Variation in muscle PCSA is higher (coefficient of variation = 94.072%) than that of muscle fibre length (coefficient of variation = 69.474%). This suggests that both across species (Fig. 9a) and within individuals (Fig. 9b) there is a variation in functional specialisation of muscles, in terms of both their force generating capacity (PCSA) and the lengths over which they generate force (fibre length). The lower coefficient of variation value for muscle fibre length may be caused by the clustering observed in cranial muscles across all species (Fig. 10). Both at the level of the individual and at a species level, variation in PCSA and fibre length is much more restricted in cranial muscles (Fig. 11 than in caudal muscles (Fig. 10). Variation in muscle power (combined fibre length and PCSA) is also sizeable, but no muscle in any species studied occupies the 'power-specialised' area of muscle function space (upper-right quadrant) (Figs. 9-12). Across all muscles, flexors have a lower variability in their occupation of muscle function space (Fig. 12a) and this trend does not change when only caudal muscles (Fig. 12b) or only cranial muscles are considered (Fig. 12c). The ratio of flexor muscle mass to extensor muscle mass varies from an even split of 50% mass each in *Gavia stellata* (Fig. 13) to an extensor dominated neck muscle mass in *Spheniscus humboldti* (71% extensor muscle mass, Fig. 13). The majority of species (7) vary between 29% and 39% flexor muscle mass, making extensor muscle mass the dominant component of neck muscle mass across all species studied (Fig. 13).

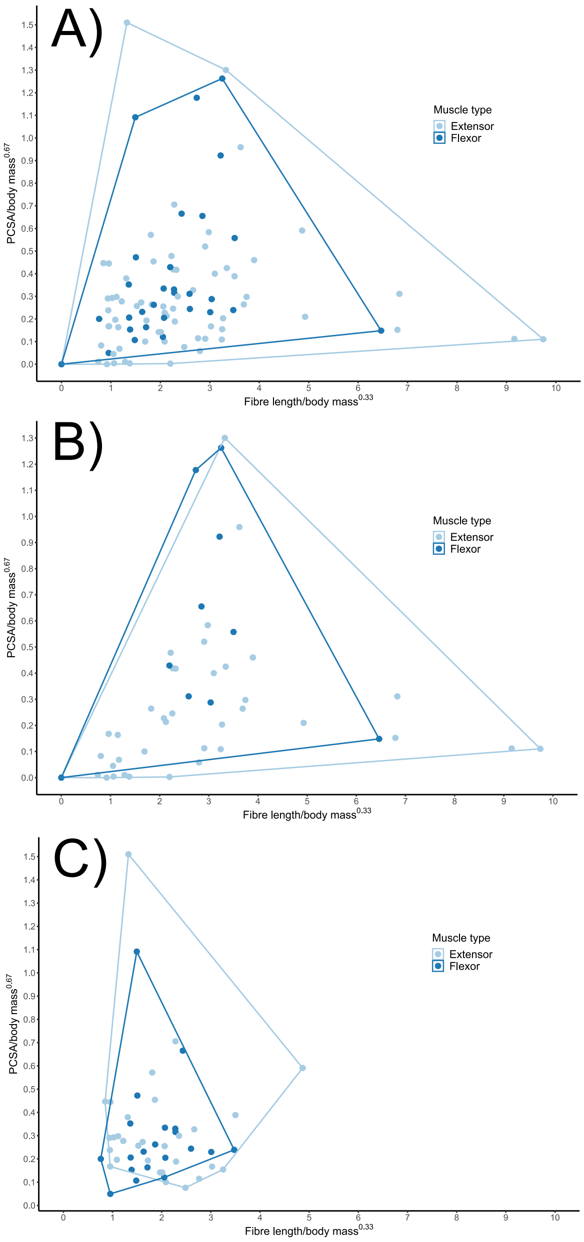

Figure 12 Muscle function plot of flexor muscles (dark blue) and extensor muscles (light blue) for A) all ten cervical muscles, B) caudal muscles and C) cranial muscles. Across all levels, flexor muscle variability is less than extensor variability.

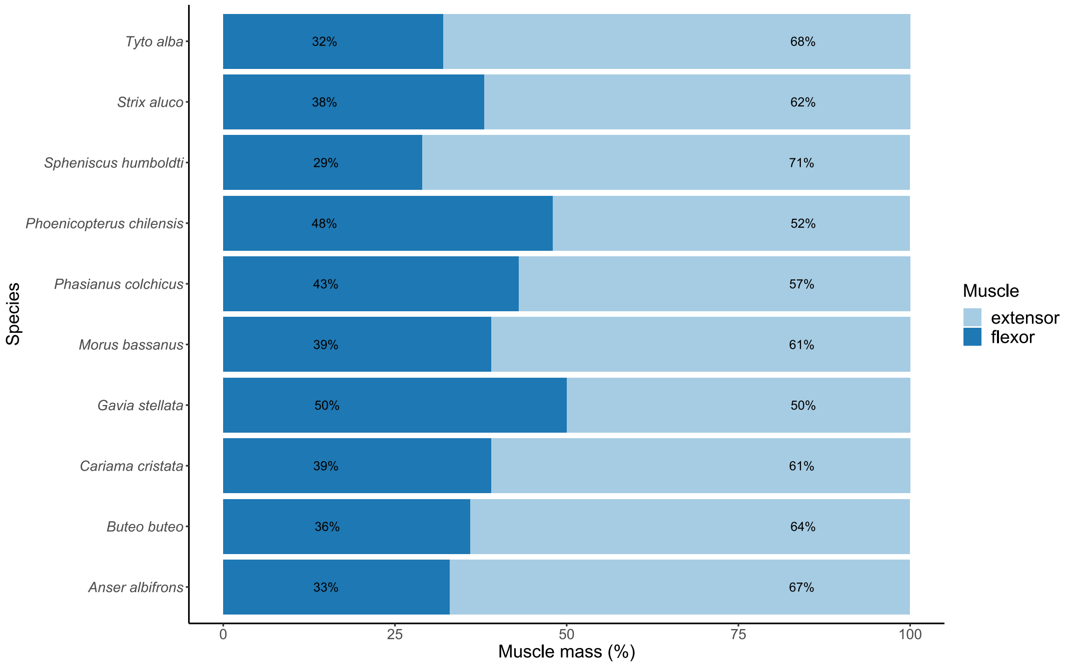

Figure 13 Percentage flexor muscle mass (dark blue) and percentage extensor muscle mass for all species studied. Extensor muscles are the predominant component of neck muscle mass across all species of birds studied

Discussion

Allometric scaling of cervical muscle architecture is predominantly positive

This is the first study to assess the scaling relationships of cervical muscles across a phylogenetically disparate group of extant avians. Previous literature has focused on ontogenetic scaling relationships within one species, *Gallus gallus domesticus* (Heidweiller and Zweers 1989; Heidweiller and Zweers 1992; Heidweiller et al. 1992). Comparisons between this work and previous literature are limited due to this focus on ontogenetic scaling and also due to the fact that these earlier studies measure (predominantly) muscle belly lengths rather than fibre lengths directly (Heidweiller and Zweers 1989).

Of all 19 significant muscle architecture scaling relationships only 5 do not display positive allometry (all of which display isometry, Table 3), leading to the conclusion that, generally, cervical muscle architecture and muscle mass scales with body mass according to positive allometry (although isometry almost always falls within 95% confidence intervals). Cervical muscle mass, by scaling according to positive allometry in adult birds, can provide adequate support for the mass of the head across a wide range of body masses. Many of the muscles that support the weight of the head (M. longus colli dorsalis pars caudalis, pars cranialis, pars profunda and M. biventer cervicis) are extensors that are positioned on the dorsal side of the neck and have been shown to account for the largest proponent of neck mass (compared to neck flexor masses, Fig. 13). Therefore these muscles must be able to support the combined effect of isometric head mass scaling (Chapter 4, this thesis), and does so via scaling cervical muscle mass (and PCSA, Table 1) according to positive allometry.

A general pattern: ecology correlates with significant variation in cervical muscle architecture only in specialised taxa

Only five of the ten of cervical muscles studied display significant ecological variation in muscle architecture and within these 5 muscles only 3 specific ecologies were responsible for this variation (Table 4). Non-parametric tests show that two further ecologies display significant variation in muscle architecture and mass, carnivory and flap gliding (Appendix Table 6.2). In contrast to ecology, body mass recurrently has a significant correlation with cervical fibre lengths, muscle mass and PCSA when included as a coefficient in pGLS regressions testing for the effects of ecology on muscle variables (Table 3, Table 4). The inclusion of body mass in the non-parametric tests entirely nullifies the significance of flag gliding and carnivory (Appendix Table 6.2). These both suggest that body mass may have a larger impact upon variation in cervical fibre length, muscle mass and PCSA in extant avians than ecological factors. Similar patterns have been in observed in scaling relationships of other aspects of cranio-cervical morphology. For example, avian head mass and neck length also show very few instances where ecological factors display significant variation in these metrics (Chapter 4, this thesis), whereas the relationships between head mass and neck length with body mass is stronger (lower AICc values for both, Chapter 4) than with any of the few significant ecological factors. Variation in cervical fibre lengths, muscle mass and PCSA appear to be more widely correlated with body mass than ecological factors, and this may suggest that cervical muscle architecture and muscle mass primarily responds to general scaling relationships in order to best support an elongate neck across a wide range of head masses (indicated by the small number of ecologies with significant variation, Table 4). However this conclusion should be viewed with caution this study has a restricted sample size.

With only specialised ecologies (insectivore, subaqueous fliers, carnivores) displaying a significant correlation with the muscle architecture in a low number of cervical muscles it is suggested that many cervical muscles are adapted for general use. However this pattern may not be the case for all muscles, and cranially positioned cervical muscles may be more generalised than caudally positioned ones. Cranially positioned muscles display less variation in muscle arrangement (Figs 2-4) and function (Fig. 10, Fig. 11). Compared to caudal counterparts, cranial muscles vary less in the number of origination slips (Fig 2, 3) and vertebral span (Fig 4) and cluster together in an area of muscle function space commonly associated with generalised functions (Allen et al. 2010), without relative specialisations for extreme force, power or contraction length/speed (Fig. 9, Fig. 11). The avian neck has to meet a large and diverse number of daily functional demands (Zweers, Bout, and Heidweiller 1994; Van der Leeuw, Bout, and Zweers 2001) and prior work has determined that for general tasks such as drinking and pecking the cervical column adopts similar kinematic patterns even across disparate taxa (Van der Leeuw 1992; Van der Leeuw, Bout, and Zweers 2001; van der Leeuw 2002). The conformity of avian cervical kinematics may be linked to the relative lack of variation seen in cranially positioned muscle architecture and muscle mass. The avian forelimb is heavily adapted for flight and many of the environmental interaction tasks are now undertaken by the head and neck, and this has led to the avian neck being referred to as a 'surrogate arm' (Clarke and Middleton 2008; Bhullar et al. 2012). The comparatively low variation of cranially positioned cervical muscle architecture, mass and arrangement amongst a diverse group of birds (Fig. 2, Fig. 3, Fig. 9, Fig. 11) potentially indicates that this musculature is less specialised than caudally placed muscles, and this may be a widespread adaptation amongst avians to allow the neck to become a 'surrogate arm' (Clarke and Middleton 2008; Bhullar et al. 2012; Böhmer et al. 2019). Biomechanical and developmental factors constrain variability in cranially positioned cervical musculature in other groups of vertebrates, most notably in mammals (Buchholtz 2012; Galis 1999; Buchholtz 2014; Buchholtz et al. 2012; Hirasawa, Fujimoto, and Kuratani 2016; Galis and Metz 2003; Galis et al. 2006). Supporting and positioning the head is the primary function of these cranially positioned cervical muscles across vertebrates (Gans 1992), and as such head mass may be a large constraint on the variability of cranial muscle architecture and function in birds.

As previously indicated there appears to be a disparity in muscle function variation between cranially and caudally positioned cervical muscles (Fig.10, Fig. 11). Some of this variation in muscle function may be due to specific adaptations of muscle architecture to certain ecologies, such as the large expansion of dorsal neck extensors (such as M. biventer cervicis) in response to subaqueous flight (Fig. 1, Fig. 10, Fig. 13). Subaqueous fliers are represented solely by *Spheniscus humboldti*, and many other ecological groups are represented by a low number of species in the current data set. This represents a clear sample size issue, and must be rectified in future studies.

Muscles that are classified as caudal cervical muscles are all large muscles with multiple slips that span and attach to multiple cervical vertebrae. This study and multiple previous bodies of work have noted that qualitative differences in caudal cervical musculature is associated with the number of muscle slips present (Kuroda

1962; Van der Leeuw, Bout, and Zweers 2001; Boumans, Krings, and Wagner 2015). In Chapter 3 of this thesis it was found that across a wide sample of modern birds (46 species) variation in counts of cervical vertebrae were concentrated largely in regions 3 and 4. Vertebrae of these regions form the attachment sites for all of the muscles classified as caudal cervical muscles, therefore variations in counts of cervical vertebrae across birds may be linked to the variation in muscle function observed for caudal cervical muscles. Two examples from the results presented above show that this hypothesis is an oversimplification: M. longus colli dorsalis pars caudalis and M. longus colli dorsalis pars profunda (Figs. 2, 3). Both of these muscles display an increase in the number of muscle slips when species with minimal (13, *P. colchicus,* Fig. 2d) and maximal (17, *A. albifrons,* Fig. 3e) cervical counts are compared, however an increase in number of slips of both these muscles can be observed in species with lower numbers of cervical vertebrae, and these species indicate that differences in muscle attachment patterns may contain an ecological signal. The highest number of origination slips of M. longus colli dorsalis pars cranialis occurs in *A. albifrons* with 17 cervical vertebrae (Fig. 3e), but also *G. stellata* (Fig. 2b) and *Sp. humboldti* (Fig. 2c) with 13 cervical vertebrae and *M. bassanus* (Fig. 3c) with 15 cervical vertebrae. All of these species feed or forage underwater and this pattern may be indicative of how avian cervical muscle arrangement adapts to the aquatic environment. Aquatic foraging has been linked to shifts in kinematic patterns of neck movement in Anseriformes (Van der Leeuw 2001a, b) and this potential shift in muscle attachment organisation may explain this pattern, future work should investigate this hypothesis. A similar pattern can be observed for M. longus colli dorsalis pars profunda as the highest number of slips for this muscle is displayed in *A. albifrons* (Fig. 3e) and for two species with a more typical number of cervical vertebrae (14, *St. aluco,* Fig. 2e, *T. alba,* Fig. 3b). These two further species are both owls (*St. aluco* and *T. alba*) and it has been previously postulated that an expansion of M. longus colli dorsalis pars profunda is responsible for the extreme lateral neck flexion that is synonymous with owls (Boumans et al. 2015). With no clear consensus on what factors affect the arrangement of cervical muscle insertions, these instances exemplify the need for an expansion of this small dataset to properly examine the causes of variation in cervical muscle anatomy and function.

Functional interpretations of significant ecological variation in muscle architecture

Functional interpretations based on the statistical models presented in Table 4 (and Appendix Table 6.2) should be carefully considered due to the low sample size of this study and the conflicting results of parametric and non-parametric tests. However, in order to generate potential hypotheses of musculoskeletal function of avian cervical musculature, significant ecological signals should undergo preliminary interpretation. There are a few ecological variables that vary significantly in their muscle architecture (Table 4, Appendix Table 6.2). By interpreting a muscle function space plot (Fig. 9) alongside comparisons to previous work of avian cervical muscle function, hypotheses surrounding the functional consequences of ecological specialisations of muscle architecture can be proposed. Insectivorous taxa display a significant decrease in relative fibre lengths of both M. rectus capitis lateralis and M. rectus capitis ventralis (Table 4). These muscles are responsible for lateral and ventral movements of the head relative to the neck (Boumans, Krings, and Wagner 2015; Snively and Russell

2007c, 2007b, 2007a). Shorter fibre lengths are associated with a reduction in the absolute maximum contraction velocity (V_{max}) of a muscle, meaning that shortening velocity is likely lower in M. rectus capitis lateralis and M. rectus capitis ventralis of insectivorous birds, and force generating capacity limited to a smaller range of joint excursions (i.e. absolute muscle lengths or working range). Insectivorous birds often feed on small ground dwelling insects and this reduction to contraction speed may be due to less selective pressure for force generation across a large range of lateral and ventral joint angles for M. rectus capitis lateralis and M. rectus capitis ventralis. Insectivorous taxa also display a significant decrease to the PCSA of M. longus colli ventralis which spans almost the entirety of the ventral side of the cervical column. This relatively low value of PCSA is caused by a relatively high fibre length (Fig. 9) and can be interpreted as a contraction-speed specialised muscle. As M. longus colli ventralis functions as an antagonist to the dorsally positioned M. longus colli dorsalis to ventrally rotate the entire cervical column (Zusi 1962; Zusi 1985; Boumans, Krings, and Wagner 2015). This muscle may be adapted in insectivores to provide a fast ventral flexion of the entire neck to secure a successful prey capture after the beak has been carefully placed over prey by M. rectus capitis lateralis and M. rectus capitis ventralis. This could be tested with *in vivo* kinematic data as part of future work.

Subaqueous fliers display a significant positive increase in the PCSA of M. splenius capitis (Table 4). M. splenius capitis is responsible for dorsal head movements when both sinistral and dextral counterparts are contracted simultaneously and lateral head movements when contracted unilaterally (Zusi 1962; Burton 1971; Fritsch and Schuchmann 1988; Boumans, Krings, and Wagner 2015). Subaqueous fliers are represented in this study solely by penguins and during underwater movement, this group tucks the head close to the body in order to reduce drag. This renders much of the neck stationary during underwater locomotion (pers. obvs) and head position may be modified largely by head flexors that attach to the cranium: M. complexus, M. biventer cervicis, M. splenius capitis, M. rectus capitis lateralis, M. rectus capitis ventralis and M. rectus capitis dorsalis. Of these muscles it is M. splenius capitis that has the cranial most origination site at C2 and as such is relatively unaffected by the dorsiflexion involved in more caudally positioned vertebrae that allow for the head to be tucked close to the body and may increase M. splenius capitis' involvement in head movement. Movement of the head underwater requires a larger force compared to completing the same movement on land as the surrounding water must be displaced in order to move the head. The increased PCSA of M. splenius capitis in penguins may be due to a combination of the extra burden M. splenius capitis experiences due to the stationary dorsiflexed position of more caudally positioned vertebrae and their associated muscles, as well as the force required to displace both the surrounding water and the mass of the head. The dorsiflexion of cervical vertebrae which tucks the head close to the body in penguins may be achieved by the modifications of the M. biventer cervicis (RDM pers. obvs. during dissections and data collection, Fig. 1). The M. biventer cervicis in birds is usually split into a cranial muscle belly and a caudal muscle belly that are connected by an intersecting tendon (Landolt and Zweers 1985; Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). However in penguins this intersecting tendon is entirely absent and M. biventer cervicis is completely fleshy (see Fig. 1). This change to M. biventer cervicis alters the neutral posture of the cervical column so that the head is closer to the body. This effect is displayed very clearly in cadaveric material (Fig. 1).

The northern gannet (*Morus bassanus*) displays the highest values for relative fibre length in M. complexus and relative PCSA in both M. rectus capitis ventralis and M. rectus capitis dorsalis (Fig. 9). This bird catches fish using plunge-diving whereby the bird dives to catch near-surface dwelling fish, entering the water at up to speeds of

20 m/s (Shealer 2002; Wodzicki and Robertson 1955; Garthe et al. 2014; Garthe, Benvenuti, and Montevecchi 2000; Ropert‐Coudert et al. 2004). These high-speed dives place considerable forces on the neck of *M. bassanus* and can be fatal (Machovsky Capuska et al. 2011). Previous work has calculated that the muscle force of cranially positioned head flexors act against impact force to allow *M. bassanus* to plunge-dive safely at speed (Chang et al. 2016), highlighting the role of cranially positioned muscles attached to head. The muscles that display increases to fibre length and PCSA in this study are also cranial head flexors (M. complexus, M. rectus capitis ventralis and M. rectus capitis dorsalis) and it is suggested that these increases are adaptations to plunge-diving in *M. bassanus.*

The results of alternative non-parametric tests are difficult to interpret as they are nullified at the inclusion of body mass and conflict with PGLS results, yet they suggest that carnivory and flap gliding are both significantly correlated with fibre length, muscle mass and PCSA in numerous cervical muscles. Three taxa are classified as carnivores: *B. buteo, St. aluco* and *T. alba* and of these, the two owls (*St. aluco* and *T. alba*) are also the only flap gliders in the dataset. This overlap indicates that the signal detected by the non-parametric tests may be owl-specific. Previous work has noted that owls possess a degree of myological specialisation in the cervical column (Boumans et al. 2015), and the results presented here may suggest that this specialisation may be apparent in more cervical muscles than previously thought, future work should address these results in more detail. Whilst many birds display a homogeneous organisation of cranially positioned cervical musculature these outliers suggest that in specific circumstances where cervical kinematics have radically different requirements, the avian neck must adapt and overcome these new challenges with substantial changes to muscle architecture.

Variations in cervical muscle architecture may not underly variation in vertebral morphology or patterns in cervical regionalisation

Regionalisation is the differentiation of the axial column into morphologically distinct groups of vertebrae. This morphological differentiation allows for the axial column to have disparate functions along its length and changes to axial regionalisation patterns have underpinned the success of many large vertebrate clades (Buchholtz et al. 2012; Head and Polly 2015; Jones et al. 2018). The avian cervical column is divided into five distinct morphological regions (Böhmer, Rauhut, and Wörheide 2015a; Chapter 3) and prior work has investigated the factors effecting the morphology of these regions (Chapters 3, 4 and 5 of this thesis). This previous work has quantified the effect of external factors on regional morphology across two scales: between cervical regions (i.e. across the entire cervical spine, chapter 3) and within each individual cervical region (chapter 5). Similar to variation in cervical muscle architecture, the effect of ecological factors on regional morphology across these two levels are restricted to a small number of specialised taxa. Patterns of morphological regionalisation across the entire cervical spine only significantly differ between carnivorous and insectivorous taxa. However ecology appears to have a more substantial effect on a smaller scale as more ecological groups display significant variation of morphology within individual regions. Muscles require a supportive skeletal framework over which they can transmit forces and as such the skeleton forms the main attachment sites of many vertebrate muscles. Because of this relationship between bone and muscle, the morphology of a bone is influenced by the muscles attaching to it. This simple notion can be used to inform hypotheses on what underlies the variation in regional vertebral morphology by observing what muscles attach to vertebrae in regions that show significant ecological variation and how they might change in response to the ecology that has shown significant variation in regional cervical morphology. Hypotheses based on these within region results are outlined below.

Vertebral morphology in regions 1 and 2 displays significant variation in piscivorous birds and this may be related to adaptations in cervical muscles that directly attach to the skull that span vertebrae across regions 1 and 2 and operate in head flexion relative to the neck such as M. complexus, M. splenius capitis and the three muscles of the M. rectus capitis group (Hoyo, Elliott, and Sargatal 1992; Boumans, Krings, and Wagner 2015; Snively and Russell 2007a, 2007b; Snively et al. 2014). These adaptations may be present to overcome the drag induced by head movement underwater or to allow for fast and precise head movements during the catching of fish (Hoyo, Elliott, and Sargatal 1992). Soaring birds display significant variation in morphology of vertebrae in region 3 and this is hypothesised to be a product of muscles involved with head stabilisation during wingbeats (M. longus colli dorsalis pars caudalis and pars profunda) reducing in size as soaring birds beat their wings less during flight (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015). The final and most prominent ecological factor to cause variation in regional morphology is carnivory as it causes significant variation in the morphology of vertebrae in both regions 4 and 5.

Carnivorous birds process food extraorally by ripping chunks of flesh from prey with their beak and this movement requires a suitably high level of power produced by cervical muscles involved in neck retraction (Boumans, Krings, and Wagner 2015; Snively and Russell 2007a, 2007b, 2007c; Snively et al. 2014). These muscles (M. longus colli dorsalis pars caudalis and M. longus colli dorsalis pars profunda) attach to vertebrae in regions 4 and 5 (Boumans, Krings, and Wagner 2015; Snively and Russell 2007a, 2007b, 2007c; Snively et al. 2014) and are hypothesised to show architecture more suited to high force production.

Upon analysing the variation of cervical muscle architecture across a diverse range of extant birds none of the aforementioned hypotheses can be upheld as no significant relationship is found between the ecologies stated above and any aspect of muscle architecture that is hypothetically responsible for the significant variation in regional vertebral morphology. Before the role of muscle architecture in underpinning patterns of cervical regionalisation birds is entirely dismissed it must be made clear that problems in gaining access to a diverse range of avian cadaveric material in high numbers significantly reduced the sample size that was available in the current analysis. Predation is an important factor effecting morphological variation in cervical regions 4 and 5 as it outweighs even the effects of body mass in these regions and despite a lack of significance, muscles attaching to these areas that are hypothesised to create high neck retraction forces, namely M. longus colli dorsalis pars caudalis and pars profunda, both display high muscle mass and PCSA values when body mass accounted for (Figs. 6f, 6g, 7f, 7g). This in combination with the many other ecologies which had *P* values close to significance (*P* = 0.05 – 0.09, Table 4) as well as the significant correlation between fibre length, muscle mass and PCSA and carnivory found in non-parametric tests indicates that a study with a larger sample size must be conducted in order to confidently dismiss muscle architecture as a cause of variation in patterns of morphological regionalisation in the avian cervical spine.

With current evidence suggesting muscle architecture does not underpin patterns of regional morphological variation, alternative hypotheses for what processes govern regional morphology must be theorized. Previous research has stated that kinematic patterns of the cervical column during general tasks are conserved across a diverse range of extant avians. However these patterns can be changed in response to large changes in function, as has been found in birds that feed predominantly in water (Van der Leeuw, Bout, and Zweers 2001; van der Leeuw 2002; Zweers, Bout, and

Heidweiller 1994). Changes to patterns of cervical kinematics have been attributed to the restriction of movement in specific areas of the cervical column (Heidweiller et al. 1992; Van der Leeuw 1992; Van der Leeuw, Bout, and Zweers 2001; van der Leeuw 2002) and changes to regional morphology may accommodate this. In carnivorous birds it was shown that centrum length decreases whilst neural spine height increases in regions 4 and 5 and this was used as the basis to postulate that muscles attaching to neural spines in these regions (M. longus colli dorsalis pars caudalis and pars profunda) were increasing in mass and subsequently PCSA. However, these adaptations of cervical morphology may be accommodating a cervical kinematic pattern that is unique to carnivores. Both of these adaptations in cervical morphology have been associated with increased intervertebral stiffness in other groups of vertebrates (Pierce, Clack, and Hutchinson 2011; Shapiro 1995; Koob and Long Jr 2000; Long et al. 1997) and may be present to accommodate the potentially unique cervical kinematic patterns of carnivorous birds. Previous studies lend some credence to this hypothesis as owls display restricted movement in medial and posterior regions during head turning (Krings et al. 2017) and raptorial birds favour trunk movements over movements of the posterior cervical column during the pre-strike phase (Snively et al. 2014). However, these are currently the only available data on *in vivo* kinematic patterns of carnivorous birds, more work is needed to understand the relationship between patterns of cervical regionalisation and cervical kinematics.

Conclusions

This study sought to understand the variation in the cervical muscle architecture of extant birds within three frameworks: allometric scaling, ecological variation and patterns of cervical regionalisation. Avian cervical muscles predominantly scale with body mass according to positive allometry and this is due to the need to support a wide range of head masses and sizes in adult birds and is in contrast to the pattern of negative allometry of cervical muscle lengths seen in avian ontogeny (Heidweiller and Zweers 1989; Heidweiller and Zweers 1992), however confidence intervals are relatively large (perhaps due to a restricted sample size) and isometry regularly falls within them. Ecology has a weak correlation with avian cervical muscle architecture with only a small number of specialised taxa displaying a significant correlation with the variation of the fibre length, muscle mass and PCSA of avian neck musculature. Finally, no evidence is recovered to support the hypothesis that variation in muscle architecture underpins changes to cervical regionalisation patterns in birds. This study presents data that suggests cranially positioned avian cervical musculature is more homogeneous in its construction. However specific outliers and observations from dissection data suggests that when extrinsic factors require the neck to adopt entirely different kinematics or exposure the neck to very different external forces (as in insectivorous and taxa that locomote underwater), cervical musculature adapts accordingly. This relative lack of variation in cranially positioned cervical muscle architecture may allow the neck to act as an effective substitute for forelimbs that are highly modified for powered flight. Caudally positioned cervical musculature is much more variable in its functional morphology, and this variation may, in part, be effected by the variation in cervical counts in caudally positioned cervical regions. This study acknowledges its low sample sizes and it is clear that much more work is needed in order to truly understand patterns of variation in avian cervical musculature. More work is also needed to understand the variation in both cervical muscle architecture and kinematics to observe their combined effect on the regionalisation of the avian cervical column, and in doing so we will gain a clearer understanding on the links between form, function and genetic patterning within the axial column of vertebrates.

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Chapter 7: Discussion

This chapter will be split into three sections. The first section will contain a summary of the main discussion areas for each of the four data chapters (Chapters 3-6) in this thesis. This will be followed by a section discussing the results of this thesis and how it contributes to our understanding of factors affecting variation in the avian cervical column. It will also discuss implications for the evolution of the avian cervical column and a broader discussion on the implications for factors effecting cervical variation across vertebrates. The final section of this chapter will critique the methodologies used within this thesis and review alternative methods and potential avenues for future research.

Summary of previous data chapters

Each of the previous four data chapters were focused upon one of the four central aims and objectives of this thesis, which were:

1. To test previous hypotheses concerning patterns of cervical regionalisation within extant Aves.

2. To quantify factors that affect variation neck length and head mass across extant Aves.
3. To quantify factors that affect variation in regional vertebral morphology and region length in the cervical column of extant Aves.
4. To quantify factors effecting variation in cervical muscle architecture in extant Aves and to determine if this variation is linked to variation in regional vertebral morphology.

Patterns of cervical regionalisation within Aves

Chapter 3 attempted to address Objective 1 and concluded that five cervical regions are common amongst all extant Aves and that a common pattern of vertebral shape change across the cervical spine (i.e. across all five regions) is present amongst many birds (Chapter 3 Figs 2-4;). PCA reveals that this pattern corresponds to a decrease in vertebral height (via a decrease in neural spine height) from cranial to middle regions and a further increase in vertebral/neural spine height from middle to caudal regions (Chapter 3 Fig. 4). A similar, but inverted, pattern can be found for centrum length where it decreases in both cranial and caudal directions from the middle region (region 3) (Chapter 3 Fig. 4). This pattern of vertebral shape change across the cervical spine may suggest that the middle regions (regions 2-4) display increased flexibility compared to the stiffer cranial- and caudal-most regions (1 and 5 respectively) and may allow for a highly flexible neck, as well as a stiff supporting framework for the head, and the head and neck as a whole (Shapiro 1995; Koob and Long Jr 2000; Buchholtz 2012). PTA was then used to statistically compare shape variation across the cervical spine (i.e. across all of the five regions) between ecological groups and found that only two comparisons were statistically significant: in the context of diet carnivorous taxa appear to be distinct from insectivorous taxa, and the context of locomotion soaring taxa appear to be distinct to continual-flapping taxa (Chapter 3 Fig. 5-7; Table 2).

The forelimbs of birds are heavily adapted for flight and as such the head and the neck take on many environmental manipulation tasks that would be usually carried out by the hands, this has led to the avian neck being often referred to as a 'surrogate arm' (Clarke and Middleton 2008; Bhullar et al. 2012). This would make the avian cervical column a generalised musculoskeletal system, whereby specialisation can only occur when the demands of a specialised cervical kinematic regime outweighs the need for the system to perform generalised daily tasks. This surrogate arm hypothesis is initially supported by lack of ecologies that display a significant deviation from the generalised pattern of regional morphology across the avian cervical spine. Ecologies that do deviate from this pattern either require large cervical retraction forces to rip flesh from prey or require fast and precise head movements to catch small, fast moving insects.

To further address Objective 1 of the thesis, patterns of cervical regionalisation were also evaluated by quantifying the correlation of scaling and ecological factors on the vertebral counts of each cervical region (Chapter 3 Fig. 2; Tables 3-4). Again, a generalised pattern of regionalisation was found as only 2 ecological factors (soaring and frugivory) appear to significantly correlate with variation in regional vertebral counts (Chapter 3 Tables 3, 4). The lack of a significant correlation between vertebral counts within regions and neck length (Chapter 3 Table 3) suggests that the extreme elongation seen in neck length across Aves is not due to cervicalisation (the addition of cervical vertebrae), as previous literature has often suggested (Zweers, Bout, and Heidweiller 1994; Bout 1997; Van der Leeuw, Bout, and Zweers 2001). Extant avians display an extra cervical region when compared to other members of Archosauria (5 compared to 4; Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015b) and much of the variation in regional vertebral counts was localised to the region that has been previously found to be unique to Aves, region 4 as well as region 3. Region 4 is defined by a shortened centrum and an enlarged neural spine, and *in vivo* serves as the attachment site for M. longus colli dorsalis pars caudalis and pars profunda. These morphological features perhaps serve to increase the overall stiffness and stability of region 4 and provide larger attachment sites for muscles that support the entire neck and head (Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). Thus the evolution and subsequent expansion of this region in modern birds may have allowed for the avian neck to evolve such a wide diversity of neck lengths. Finally, cervical vertebral counts are restricted in regions 1 and 5 and may represent strong constraints on underlying *Hox* patterning in this region due to the need to support the weight of the head (region 1) and the overall weight of the head and neck (region 5).

Variation in head mass, neck length and region length allometry across extant Aves

Chapter 4 addressed objective 2 and it was discovered that head mass scales isometrically with body mass (Chapter 4 Table 1), which is different from the widespread positive allometry that is observed for head mass scaling in other groups of vertebrates (Christiansen 1999a; Sereno et al. 2007; Sander et al. 2011; McGarrity, Campione, and Evans 2013). It was noted that methodological differences in estimating head size and head mass exist between this study and previous work (and indeed a lack of standardisation in general exists within previous work) and this may contribute to the difference observed in head mass allometry between birds and other groups of vertebrates (Christiansen 1999a; Sereno et al. 2007; Sander et al. 2011; McGarrity, Campione, and Evans 2013). Previous work has identified that many cranial soft tissues (such as brain and eye size) scale negatively with increasing body mass (Brooke, Hanley, and Laughlin 1999; Schmidt-Nielsen and Knut 1984) and this may be responsible for the isometric scaling relationship of head mass that is observed in Chapter 4 (Chapter 4 Table 1). Avian head mass allometry has been studied previously and prior work has suggested that the relationship is negatively allometric (van der Leeuw 2002). This current study found the scaling relationship of head mass to be isometric when all birds in the data set are studied (Chapter 4 Table 1), although when smaller groups are considered in isolation some negative allometric relationships are recovered (Chapter 4 Table 1). The aforementioned study into avian head mass allometry only considered a small, phylogenetically similar, group of individuals (van der Leeuw 2002) and this may account for the differences in head mass scaling relationships between the two studies. The only ecological group to show a statistically different scaling relationship between head and body mass to other groups are terrestrial birds (Chapter 4 Table 1), meaning that ecological factors appear display a weak correlation with head-body size scaling. The strong negative allometry in head mass recovered for terrestrial birds may be due to the loss of cranially located sensory and vestibular adaptations for flight (Brooke, Hanley, and Laughlin 1999; Garamszegi, Møller, and Erritzøe 2002).

Prior work has suggested that neck length scales with body mass according to positive allometry in extant avians (Heidweiller and Zweers 1992; Heidweiller et al. 1992; van der Leeuw 2002). However this study, along with other recent work (Böhmer et al. 2019) suggests that the relationship is isometric (Chapter 4 Table 1). Phylogenetic relationships were not accounted for in previous studies of avian neck length allometry (Heidweiller et al. 1992; Heidweiller and Zweers 1992; van der Leeuw 2002) and when phylogeny is discounted, this work recovers a positive scaling relationship between neck length and body mass (Chapter 4 Table 1). This is a clear indicator that phylogeny has a some effect on neck elongation in extant birds and is supported by recent work that has indicated that phylogeny has a significant effect on the distribution of total cervical vertebrae in birds (Böhmer et al. 2019). Isometric scaling of neck length in avians has been recovered as part of this thesis and is corroborated in Böhmer et al. 2019, and demonstrates that neck length is not constrained by body mass as is the case for other large clades of vertebrates (Arnold et al. 2017, Cardini & Polly 2013, Preuschoft & Klein 2013). Reduction in avian body mass is achieved by high levels of skeletal pneumatization (Dumont 2010, Seki et al. 2010) and negative scaling of cranial soft tissues (Brooke, Hanley, and Laughlin 1999; Garamszegi, Møller, and Erritzøe 2002), and thus the reduction in body mass may have led to the release of body mass as a constraint on neck length. This release of constraint from body mass has potentially allowed the avian neck to more freely adapt to a diverse array of behaviours and kinematic patterns. Body mass reduction is a key component in avian evolution and reductions to body size occurred in a stepwise fashion along the stem lineage (Benson et al. 2018). If a reduction in body mass is responsible for the release of constraint on neck length, then perhaps future work could document the relationship between body size and neck length across the avian stem lineage to determine when this constraint was released.

The allometric scaling relationship of neck length is significantly different in a select few ecological groups. Birds that locomote using strong powered flight (flap-gliding and continual flapping) have shorter necks (Chapter 4 Table 1). It is possible that head and gaze stabilisation during flight may be responsible for this trend due to the higher stability present in a shorter neck with less overall cervical flexion (Zweers, Bout, and Heidweiller 1994; Bout 1997). Herbivorous birds also display a relatively shorter neck length for their size (Chapter 4 Table 1). Whilst this is unexpected due to previous studies (Sander et al. 2011; Taylor and Wedel 2013; Dzemski and Christian 2007; Button, Rayfield, and Barrett 2014; Wilkinson and Ruxton 2012) linking increased neck length to increased feeding envelope size and grazing efficiency in other herbivorous vertebrates, it is consistent with a recent study that found neck length to be shorter in terrestrial foraging birds in other recent studies (Böhmer et al. 2019).

The relationship between head mass and neck length was also studied, with all birds sampled displaying an isometric relationship between the two variables (Chapter 4 Table 2). The primary role of the neck across vertebrates is to support the weight of the head and neck stability is increased by shortening the neck as head size increases (Cardini and Polly 2013; Preuschoft and Klein 2013; Cardini et al. 2015; Arnold, Amson, and Fischer 2017). Birds may escape this constraint with the combination of the following traits: a lighter head mass due to the negative allometry of cranial soft tissues (Brooke, Hanley, and Laughlin 1999; Schmidt-Nielsen and Knut 1984; Garamszegi, Møller, and Erritzøe 2002) and the 'S-shaped' posture of the neck that allows the head to be positioned closer to the centre of mass (Bout 1997; Van der Leeuw, Bout, and Zweers 2001). These factors may mean that head mass is perhaps less of a constraint in birds relative to other terrestrial vertebrates, and this may explain why birds display such a wide variety of head shapes and sizes (Bright et al. 2016; Felice et al. 2019).

Localised variation in regional shape and length of avian cervical vertebrae

Chapter 5 sought to address Objective 3 by observing what factors correlate with variation in cervical morphology and length on a regional level. Functional factors (body mass and neck length) display the strongest correlation with vertebral morphology across all cervical regions except for region 5 (Chapter 5 Fig. 2; Table 1). Despite its significant effect on morphological variation, head mass did not produce a coefficient of determination higher than those produced by either body mass or neck length, leading to the conclusion that head mass has a weaker direct correlation with vertebral morphology than either neck length or body mass (Chapter 5 Table 1). The correlation of body mass and neck length with regional morphology appears to be partitioned, with neck length having the dominant correlation in the middle regions 2 – 4 and body mass in the cranial- and caudal-most regions (1 and 5 respectively). Vertebrae in regions 2 – 4 elongate and display a decreased neural spine height as neck length increases, both of these features have been linked to increases in intervertebral flexion ranges (Shapiro 1995; Koob and Long Jr 2000; Buchholtz 2012). The neck of birds when at rest displays a characteristic S-shaped morphology (Boas 1929; Bout 1997) and so by adapting vertebrae in middle regions 2 – 4 to increase flexibility in this portion of the neck, the S-shaped curve can be drawn tighter and closer to the base of the neck and the birds centre of mass. This would result in the recruitment of less muscle force to support an elongated neck and it is hypothesised that this is the reason behind the partitioning of the correlation of functional factors with regional vertebral morphology in the cervical column of birds. The dominant correlation of body mass with vertebral morphology in regions 1 and 5 may be associated with an increase in size of dorsal ligaments attaching to the enlarged neural spines of vertebrae in these regions to increase the effect of passive weight bearing structures as body mass increases.

Out of the ten ecological factors present in the analysis, only five had a significant correlation with regional vertebral morphology. Piscivorous taxa displayed significant variation in the morphology of vertebrae in regions 1 and 2, filter feeding taxa displayed significant variation in region 2, soaring and subaqueous taxa in region 3, whilst regions 4 and 5 displayed significant morphological variation in carnivorous taxa (Fig. 2; Table 1). All of these occurrences, except for carnivory in region 5, displayed a much weaker correlation with regional vertebral morphology than scaling factors (body mass and neck length) (Chapter 5 Fig. 2; Table 1). In conjunction with the low number of ecological factors that display significant variation in regional vertebral morphology, Chapter 5 provides further evidence that the avian cervical column is a generalised musculoskeletal system, and is adapted for use as a 'surrogate arm' that is required to carry out a range of different tasks (e.g. feeding, vigilance and locomotion). This chapter also suggests that this 'generalised pattern' of avian cervical regionalisation can be overcome if the avian neck is placed under 'extreme' functional selective pressure, as observed in carnivorous birds. The high neck retraction forces needed to tear flesh from carcasses may be a selective force responsible for the significant changes observed in the vertebral morphology of vertebrae in regions 4 and 5 of the neck (Chapter 5 Fig 2; Table 1). Powerful neck dorsiflexor muscles such as M. longus colli dorsalis pars caudalis and pars profunda attach to vertebrae in this region and thus it is hypothesised that these muscles will be expanded in carnivorous birds. An attempt was made to test this hypothesis, alongside others related to Objective 4, in Chapter 6 of this thesis (see below).

Variation in the length of cervical regions was found to be strongly associated with neck length, and with previous results suggesting that cervicalisation is not responsible for neck elongation in extant birds (Chapter 3) it appears that changes to vertebral length are the primary factors responsible for neck elongation throughout Aves (Table 2). Increases to the lengths of regions 2 and 5 are responsible for neck elongation across birds and account for the slight decrease in length to regions 1, 3 and 4 (Chapter 5 Table 2). Muscles attaching to vertebrae in regions 2 and 5 (M. rectus capitis muscles, M. biventer cervicis and M. longus colli dorsalis pars caudalis) provide support to both the head and neck and may allow these regions to act as loci for neck elongation across Aves. Head mass and body mass also have a significant effect on the scaling of regional length (Chapter 5 Table 2), but these factors have a weaker effect than neck length. Scaling relationships between head and body mass and region lengths were both negative and this may represent a constraint on cervical length as throughout many other groups of vertebrates it has been observed that the axial column increases stability and weight-bearing ability by decreasing vertebral lengths (Slijper 1942; Smit 2002; Arnold, Amson, and Fischer 2017).

Region lengths show significant variation in multiple dietary and locomotory ecologies. In grazing birds (herbivores and frugivores), regions 3 and 5 increased in length, while all other regions decreased in lengths (Chapter 5 Table 2). Region 5 represents the fulcrum of the entire cervical spine, thus increases to overall intervertebral flexion via increasing length in this region will return multiplicative increases to the total range that the head can reach, i.e. the feeding envelope (Bout 1997; Van der Leeuw, Bout, and Zweers 2001; Cobley, Rayfield, and Barrett 2013; Dzemski and Christian 2007). Previously in Chapter 4 and in recent work (Böhmer et al. 2019) it was shown that grazing birds (particularly herbivorous birds) have shorter necks relative to body size when compared to other birds (Chapter 4 Table 1), and so increases to the length and flexion of this region may be present to account for the decrease in total neck length present in grazing taxa. Region lengths are significantly different in birds that locomote terrestrially or incur fewer wingbeats during flight, such as soaring and burst-adapted flyers (Chapter 5 Table 2). Wingbeats occlude vision and has resulted in counteracting movements of the neck to occur in time with each wingbeat (Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015). With less or no wingbeats at all, the constraints placed on the flexion patterns of neck by flapping flight are lessened and this may be the reason why non-flapping birds display significant patterns of region length variation (Chapter 5 Table 2).

Patterns of variation in avian cervical muscle architecture and muscle mass

Chapter 6 addressed objective 4 and measurements of fibre length, muscle mass and PCSA were taken for ten avian cervical muscles across ten different species of bird and the causes of interspecific variation in these variables were determined. However the conclusions drawn from this chapter must be regarded as preliminary due to the low sample size (10 species, Chapter 6 Table 1). Fibre length, muscle mass and PCSA all displayed a significant linear scaling relationship with body mass across all ten species and all three factors displayed an isometric or a positive allometric scaling relationship with body mass (Chapter 6 Table 3). Muscle architecture and muscle mass across all ten cervical muscles trended towards a positive scaling relationship with body mass (Chapter 6 Table 3) and this may be to provide active (muscular) support for the mass of the head and neck. Ecological causes of variation in muscle architecture and muscle mass were investigated and had a low impact on this variation as only half of the cervical muscles studied displayed a significant relationship between muscle variables and ecological groups, with only three ecological variables (insectivory, subaqueous flight and soaring) responsible for this variation (Chapter 6 Table 4). The scaling effects of body mass were included as a coefficient in the analysis that assessed the effect of ecological groupings on muscle variables and was often the only significant term. The recurrent signal of body mass on muscle architecture and mass throughout the analysis, combined with the restricted correlation with ecology indicates that body mass scaling has a larger correlation with variation in cervical fibre length, muscle mass and PCSA. Again, caution must be placed in these conclusions as the sample size within this chapter was small. This pattern is also seen in Chapter 4 as head mass and neck length variation is more strongly correlated with body mass than it is ecological factors.

Variation in muscle function, revealed by muscle function plots using scaled PCSA vs scaled fibre length (Chapter 6 Figs 6-9), appears to be more restricted in cranial muscles that are responsible for head flexion and support, relative to the variation seen in caudal, neck-supporting, muscles. Cranially positioned cervical musculature clusters together in an area of muscle function space associated with generalised muscle function (i.e. relatively limited specialisations for force production or contraction speed; Allen et al. 2010) and clustering in this area may indicate that cranially positioned cervical muscles are potentially constrained by their involvement in head support as well as their involvement in cervical kinematics that are shared across a broad phylogenetic spectrum of Aves (Bout 1997; Van der Leeuw, Bout, and Zweers 2001). The variation in caudal muscle function space occupation is comparatively much larger and may be due to the interspecific variation in the number of muscle slips which has been qualitatively observed in other studies (Kuroda 1962; Landolt and Zweers 1985; Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). This would be caused by increases to vertebral counts in more caudally positioned cervical regions, and this pattern is recovered; regions 3 and 4 display the largest variation in cervical counts across a wide sample of extant Aves (Chapter 3).

Another explanation for the large variation in caudal muscle function is that muscle architecture is adapting to certain ecologies, such as the fleshy expansion of M. biventer cervicis in birds that locomote via subaqueous flight (penguins, Chapter 6 Fig. 1). However as the sample size used in this chapter was limited to 10 species, these ecological variations (despite their obvious deviations from the generalised pattern of avian cervical muscle organisation) were not detected as significant. Future work should increase the sample size of this study to more robustly test if ecological parameters have a significant impact upon muscle architecture and muscle mass variation in the cervical column on birds.

Insectivorous birds display a significant decrease in the fibre lengths of M. rectus capitis lateralis and M. rectus capitis ventralis (Chapter 6 Table 4). A decrease in fibre length is associated with a decrease to absolute maximum contraction velocity (V_{max}) and the absolute excursions over which the muscle can effectively generate force. The significant decrease of fibre lengths in these muscles in ground-dwelling insectivorous birds may be present to facilitate a slow and controlled head movement to ensure ground-level insects do not flee before a successful prey capture event. Insectivorous birds also display a significant lower PCSA in M. longus colli ventralis than other birds

(Chapter 6 Table 4) and this coupled with the relatively long fibre length of this muscle may be to facilitate a fast ventral head strike to catch insects when the head is positioned over the prey. Birds that locomote underwater (subaqueous fliers, penguins) display a significant increase to the PCSA of M. splenius capitis which could represent an adaptation to head flexion forces underwater, as greater muscle force would be required to counteract the pressure exerted on the head and neck by the surrounding water. Whilst not an ecological group defined by this study, the one plunge-diving bird (*Morus bassanus*) in this dataset displayed the longest fibre lengths, largest mass and PCSA in multiple head flexor muscles (M. complexus, M. rectus capitis ventralis and M. rectus capitis dorsalis) and may be present to act against the force of the diving impact (Chang et al. 2016). These three groups of birds (insectivores, subaqueous fliers and plunge divers) may represent functional extremes whereby kinematic pressures placed on the cervical columns of these birds require the usually generalised architecture of cranially-positioned cervical musculature to adapt to these pressures.

In chapters 3 and 5 the correlation between ecology and variation of regional cervical vertebral morphology was demonstrated; certain ecologies correlated with vertebral morphology in different cervical regions. Piscivory was related to specific vertebral shape changes in regions 1 and 2, soaring was related to vertebral shape in region 3 and finally carnivory had the largest correlation with vertebral shape in regions 4 and 5 (Chapter 3, Fig. 7, Chapter 5 Table 1). As skeletal elements form the bony attachments sites for muscles, it was hypothesised that changes to muscle proportions may underpin this ecological variation and that these ecological groups would display significant changes in muscle architecture for muscles that attached to the vertebrae found to be associated with that particular ecological group in chapter 5. For example, carnivorous birds require strong neck retraction forces in order to rip flesh from prey and these neck retractor muscles (M. longus colli dorsalis pars cranialis, pars caudalis and pars profunda) attach to vertebrae in regions 4 and 5, which are the regions in which carnivory has a significant effect on vertebral morphology. If muscle mass is underlying ecological shifts in regional vertebral morphology one would expect carnivores to also display a significant shift in muscle architecture and/or muscle mass for these specific muscles. No ecological groups displayed significant variation in muscle architecture or muscle mass in the muscles hypothesised to be responsible for this variation. Whilst sample sizes are small, the lack of any links between muscle architecture and previous results of vertebral shape variation suggest that muscle architecture is not the dominant factor that underlies regional vertebral shape variation in the avian neck and that other factors (e.g. joint range of motion) must be tested for their effect on regional vertebral shape.

A surrogate arm: the avian cervical spine as a generalised musculoskeletal system

This thesis has quantified variation in multiple aspects of anatomy in the avian cervical column and has repeatedly shown that the correlation with ecological factors are restricted when compared to the effects of scaling factors such as body mass. The effect of body mass on musculoskeletal morphology is well documented throughout many anatomical units and across vertebrate lineages (Biewener 1983; Biewener 1989; Christiansen 1999b; Blob and Biewener 2001; Biewener 2005), and its effect on certain aspects of the avian cervical column have been recently quantified (Böhmer et al. 2019). The lack of a robust relationship between avian cervical morphology and ecological factors has been noted in recent work regarding counts of cervical vertebrae and neck length (Böhmer et al. 2019). However this thesis broadens our understanding of variation in the avian neck by also quantifying variation in cervical muscle architecture (Chapter 6) and vertebral shape (Chapters 3 and 5). Variation across all of these variables is more strongly linked with scaling factors (body mass, neck length) and suggests that overall the morphology of the avian cervical column is somewhat generalised. The relatively modest correlation between ecology and cervical morphology in birds is echoed in mammals, as studies have shown that both vertebral morphology and musculoskeletal organisation are unaffected by ecological factors except in extremely specialised taxa (Arnold, Esteve-Altava, and Fischer 2017; Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016; Randau and Goswami 2017, 2018). The neck of vertebrates is primarily constructed to support the mass of the head whilst positioning it to partake in activities ranging from feeding to conspecific interaction and vigilance (Gans 1992; Wilkinson and Ruxton 2012). The results of this thesis alongside recent work on morphological variation in the mammalian axial column suggests that variation in the cervical column of vertebrates responds to generalised factors such as body mass scaling (Arnold, Esteve-Altava, and Fischer 2017; Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016; Randau and Goswami 2017, 2018). The forelimbs of birds are highly specialised in their adaptations for flight and are rarely utilised in environmental manipulation. The avian neck is often referred to as a 'surrogate arm' as it, along with the head, is involved with many daily tasks that would, in other vertebrates, be carried out by the forelimb (Clarke and Middleton 2008; Bhullar et al. 2012). These tasks range from pecking and drinking, to complex behaviours such as tool use and locomotion support (Boas 1929; Zweers, Bout, and Heidweiller 1994; Van der Leeuw, Bout, and Zweers 2001; Nyakatura and Andrada 2014; Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015). Many of these tasks are shared by a diverse array of extant avians and this 'surrogate arm' constraint may be the reason why so few ecological specialisations exist for the morphology of the avian cervical column (Zweers, Bout, and Heidweiller 1994; Bout 1997; Van der Leeuw, Bout, and Zweers 2001).

Due to its involvement in many activities, the neck aids in the interaction with a birds entire environment (Boas 1929; Zweers, Bout, and Heidweiller 1994; Van der Leeuw, Bout, and Zweers 2001; Nyakatura and Andrada 2014; Pete et al. 2015; Kress, Van Bokhorst, and Lentink 2015) and as such it must adapt when faced with large shifts in ecology (Van der Leeuw, Bout, and Zweers 2001). Neck construction and kinematics have been previously shown to deviate in aquatic environments (Van der Leeuw, Bout, and Zweers 2001), and this has been recovered within this thesis as multiple ecologies that have adapted to life in the water (subaqueous fliers, plunge-divers, general water feeding birds) have significantly different vertebral morphology and muscle properties. The aquatic realm requires multiple adaptations for a group of organisms to become successful within it, and of these it is the ability to efficiently move through an entirely different medium (water) that is paramount. The limited scope of previous work on the avian neck has concluded that adaptations to life in water may be the only ecological pressure that requires a generalised musculoskeletal system such as the avian neck to adapt widespread specialisations (Van der Leeuw, Bout, and Zweers 2001). However this thesis has revealed that there are other ecologies that have resulted in specialisations to occur in multiple aspects of avian cervical morphology. Vertebral morphology both across and within regions has been shown to be significantly different in carnivorous birds. Numerous morphological specialisations to carnivory can be seen across vertebrates, often located in the skull due to the need to restrain prey and orally process meat (Radinsky 1981; Biknevicius and Van Valkenburgh 1996; Raia 2004). Carnivorous birds often process food extraorally and this requires large retraction forces of the neck to raise the head with sufficient force to remove chunks of flesh from prey (Snively and Russell 2007a, 2007b; Snively et al. 2014; Boumans, Krings, and Wagner 2015). Shortening of the centrum and an increase to the height of the neural spines of vertebrae in regions 4 and 5 may provide extra stability for the neck during these movements by reducing intervertebral flexion (Shapiro 1995; Koob and Long Jr 2000; Buchholtz 2012). The increased neural spine height may also act to increase the attachment area for dorsal neck extensor muscles and dorsal ligaments, however further work is needed to rigorously test this.

Across this thesis, insectivory displays significant variation in both vertebral morphology across cervical regions and within cervical muscle architecture and may indicate that the rapid, precise head strikes associated with catching small and fast prey require specialised kinematic patterns of cervical motion. This is consistent with recent findings that terrestrial foraging and probing birds both display a significant decrease to neck length (Böhmer et al. 2019). Powered flight has a widespread effect on both hard and soft tissue morphology throughout extant avians. The cervical column appears to be impacted by powered flight as it acts against the movement of the wings and body during each wingbeat to provide the bird with a stabilized gaze. This thesis has found that birds which utilise strong flapping flight tend to have relatively shorter neck lengths and do not show any specialisations to the length or number of vertebrae in any cervical region. These birds also do not display a significant variation in vertebral morphology or muscle architecture, while birds that utilise less flapping or are truly terrestrial display adaptations to both vertebral morphology and muscle architecture. Combined, these studies indicate that locomotory ecologies that entail relatively little flapping during flight have a more adaptable neck morphology. This hypothesis is partly supported by recent findings that suggest deviations in neck length allometry only occur in either terrestrial or aquatic avians (Böhmer et al. 2019; Van der Leeuw, Bout, and Zweers 2001).

By quantifying how function and ecology govern variation in regional morphology as well as muscle architecture, this thesis can provide hypotheses concerning the evolution of the avian and archosaurian neck, and the role of regionalisation in this process. The ancestral number of cervical regions in Archosauria is inferred to be 4, and is shared by two of the three members of the clade, extant crocodilians and extinct non-avian dinosaurs (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015b). Birds possess five cervical regions, with the avian Region 4 identified as the novel region not found in non-avian archosaurs (Böhmer, Rauhut, and Wörheide 2015b). Regions 1, 2, 3 and 5 are thus homologous across Archosauria and stepwise increases to vertebral counts in region 3 have been hypothesised to be the precursor to the fourth cervical region in non-avian dinosaurs (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015b). The third cervical region in birds displays the highest disparity in vertebral count of all 5 regions (Chapter 3 Fig. 2) which suggests that meristic increases to the cervical column of archosaurs may be ancestrally concentrated in region 3.

The morphology of vertebrae within region 4 is dominated by features that enhance stability (reduce flexibility; shorter centrum length and taller centrum height) in mammalian vertebrae (Koob and Long Jr 2000; Long et al. 1997; Buchholtz 2012; Pierce, Clack, and Hutchinson 2011), as well osteology that is well suited to providing attachment sites for dorsal neck musculature that supports the head and entire neck (enlarged neural spines). It is therefore possible that region 4 evolved as a response to the increased burden placed on the dinosaurian neck with increases to vertebral counts in region 3. That the primary role of region 4 as a group of vertebrae is to enhance overall neck support is indirectly suggested by the disparity of variation observed between cranial and caudal cervical muscle function. Caudally positioned muscles that attach on to regions 3, 4 and 5 that support the entire neck (M. longus colli dorsalis pars caudalis, cranialis and profunda) display more variation in functional specialisation than cranially positioned muscles that act as head flexors. This disparity is echoed in regional vertebral counts as regions 3, 4 and 5 (caudal cervical regions) display much more variation than cranial regions (regions 1 and 2). Taken together these occurrences may explain the rise of the huge variability in cervical counts that has evolved in extant avians, in that region 4 allowed for further meristic changes to occur in the avian cervical column as this new region provided increased support for the neck. Region 4 has morphological adaptations (increased neural spine height/surface area) to provide larger areas of attachment for muscles that support the neck (M. longus colli dorsalis) which vertebrae in region 3 do not have. By acquiring a morphologically distinct extra region which can provide extra muscular support for the neck, rather than simply adding more vertebrae to region 3 (which lack an enlarged neural spine), the avian cervical spine is better supported over a wider range of neck lengths and morphologies.

Two factors may have contributed to the evolution of a an extra cervical region in birds: dietary shifts away from carnivory in theropod dinosaurs, and a decrease in integration between cervical and forelimb modules across the dinosaur-bird transition. Carnivorous birds display distinct differences in the morphology of their cervical vertebrae (chapters 3 and 5, this thesis) and carnivory may constrain cervical morphology as it requires different kinematic patterns when compared to other avian dietary ecologies (Snively and Russell 2007b, 2007a; Snively et al. 2014; Boumans, Krings, and Wagner 2015). This constraint hasn't been directly quantified in Theropoda. However previous research suggests that meristic changes to vertebral counts in theropods only occurred in herbivorous taxa (Zanno and Makovicky 2010). Together, this indicates that dietary shifts away from carnivory may have released the neck from constraints associated with carnivory along the theropod-bird transition.

High levels of integration between anatomical units constrains variation in one or both of the involved units (Goswami and Polly 2010; Goswami et al. 2014; Randau and Goswami 2017, 2018; Arnold, Esteve-Altava, and Fischer 2017). The degree of integration between the cervical column and forelimb in mammals has increased during the evolution of the clade (Arnold, Esteve-Altava, and Fischer 2017). This is the result of forelimb muscles attaching to the pectoral girdle and caudal vertebrae in the cervical column (Arnold, Esteve-Altava, and Fischer 2017. Alongside developmental constraints, this has led to a decrease in cervical variability over mammalian evolution (Arnold, Amson, and Fischer 2017; Galis 1999; Burke et al. 1995; Wellik 2007; Wellik and Capecchi 2003; Jones et al. 2018). The evolution of a novel avian cervical region may have been facilitated by one, or a combination, of the following patterns of integration: decreased integration with the forelimbs, or increased integration with the hindlimbs. The evolution of a keel as the primary attachment sites for the two main flight muscles in birds (M. pectoralis and M. supracoracoideus) ensured that no muscles directly involved in locomotion attach to the cervical column (Prum and Brush 2002; Makovicky and Zanno 2011; Norell and Xu 2005; Wang, Nudds, and Dyke 2011; Kenneth P Dial, Jackson, and Segre 2008; Heers and Dial 2012). This may have led to decreased integration between these units, which has been documented for the forelimb and many other parts of the skeleton in birds (Gatesy and Dial 1996; Bell, Andres, and Goswami 2011). This decreased integration (increased modularity) between the neck and forelimb in birds may have allowed the neck to be utilised as a surrogate forelimb. This hypothesis is as of yet untested and future studies should focus on the evolution of cervical integration across dinosaurs and across the theropod-bird transition. Changes to the morphology of the hindlimb has facilitated changes to many aspects of avian anatomy (Gatesy and Middleton 1997; Zeffer, Johansson, and Marmebro 2003; Cau 2018; Stoessel, Kilbourne, and Fischer 2013) and it has been recently documented that neck length may be correlated with total leg length in extant birds (Böhmer et al. 2019). Animals with shorter necks can lower their head to ground level by rotating the trunk relative to the hindlimbs, and in bipedal animals this is achieved by utilising a long tail as a counterbalance (Grossi et al. 2014). The reduction of the tail throughout the theropod-bird transition may have led to a reduction in stability when lowering the head to ground level in this way, which could have facilitated the evolution of a longer neck (Grossi et al. 2014; Böhmer et al. 2019). This co-elongation between the cervical column of hindlimbs has been documented in other animals, such as the giraffe (Cameron and du Toit 2006). However this recent study of birds (Böhmer et al. 2019) has issues associated with sampling bias and body mass as a potential conflating factor.

These three possible drivers of increased regionalisation across the theropod-bird transition (release of constraint from hypercarnivory, decreased integration of cervical and forelimb units, increased integration between cervical and hindlimb units) are highly speculative, yet they may allow for initial conjecture on the emergence time of the fourth cervical region and the 'surrogate arm' condition. Live observation and myological data is required to categorise a cervical column as a surrogate forelimb, meaning that this emergence of this feature cannot be accurately observed in the fossil record of theropods and stem-group birds. As the fourth cervical region in extant birds forms the attachment site for many muscles that both support the entire neck and provide the neck with a wide flexion range (e.g. M. longus colli dorsalis pars caudalis and M. longus colli dorsalis pars profunda, Chapter 3) it may allow the neck to function as a surrogate arm, yet this link needs to be investigated thoroughly in future work. If this link holds true in future studies then it provides a platform for investigating the emergence of both the fourth cervical region and the surrogate arm (herein called the 'avian cervical condition') as patterns of cervical regionalisation can be identified using vertebral anatomy which is available in the fossil record of the theropod-bird transition.

Five cervical regions is, at the time of writing, a condition that is restricted to crown group avians and is not shared by any other extant or extinct members of Archosauria (Böhmer, Rauhut, and Wörheide 2015) thus a conservative estimate for the emergence of the 'avian cervical condition' is at the base of crown group Aves. However the study of cervical regionalisation in extinct archosaurs is restricted to one species, *Plateosaurus engelhardti*, and has concluded that dinosaurs, like their crocodilian relatives, have four cervical regions (Böhmer, Rauhut, and Wörheide 2015). *P.*

engelhardti is a basal sauropodomorph from the Late Triassic and provides little information on the evolution of cervical regionalisation across the theropod-bird transition. A preliminary study into the patterns of cervical regionalisation of dinosaurs was undertaken as part of this thesis suggests that non-avialan theropods did not possess five cervical regions (they all possessed four regions), however these results are preliminary and only 3 species were studied (*Allosaurus* and two dromaeosaurids). If these results are corroborated by future work then the 'avian cervical condition' may be restricted to, and originate within, Avialae. Further research into the origination of the 'avian cervical condition' within Avialae may prove difficult as techniques used to determine vertebral regionalisation (both within this thesis and as part of other research groups' efforts) rely on 3D vertebral geometry as input data; not only are cervical series of many avialans incomplete, but are preserved largely in two-dimensions. However Avialae do show many features of cervical anatomy that are present only in extant avians, thus despite the aforementioned difficulties future work should investigate regionalisation in this group (Sanz et al. 1997). Heterocoelus centra are a key innovation of avian cervical anatomy and provide the avian neck with a high degree of flexibility. This feature was thought to be restricted to crown-Aves, however discoveries made over the last 20 years have found that incipient heterocoely is widespread amongst avialans (Sanz et al. 1997, Chiappe 1996), even amongst basal taxa (Kurochkin 1995, Imai et al. 2019). Avialans possess both derived features of cervical anatomy and postcranial traits that may drive the evolution of the 'avian cervical condition' (reduced head size, shifts away from hypercarnivory, a highly specialised forelimb), thus it is not inconceivable that the fifth cervical region and the utilisation of the neck as a surrogate arm emerged in Avialae, and is not restricted entirely to crown-Aves.

Distance-based measures as a method for investigating axial regionalisation

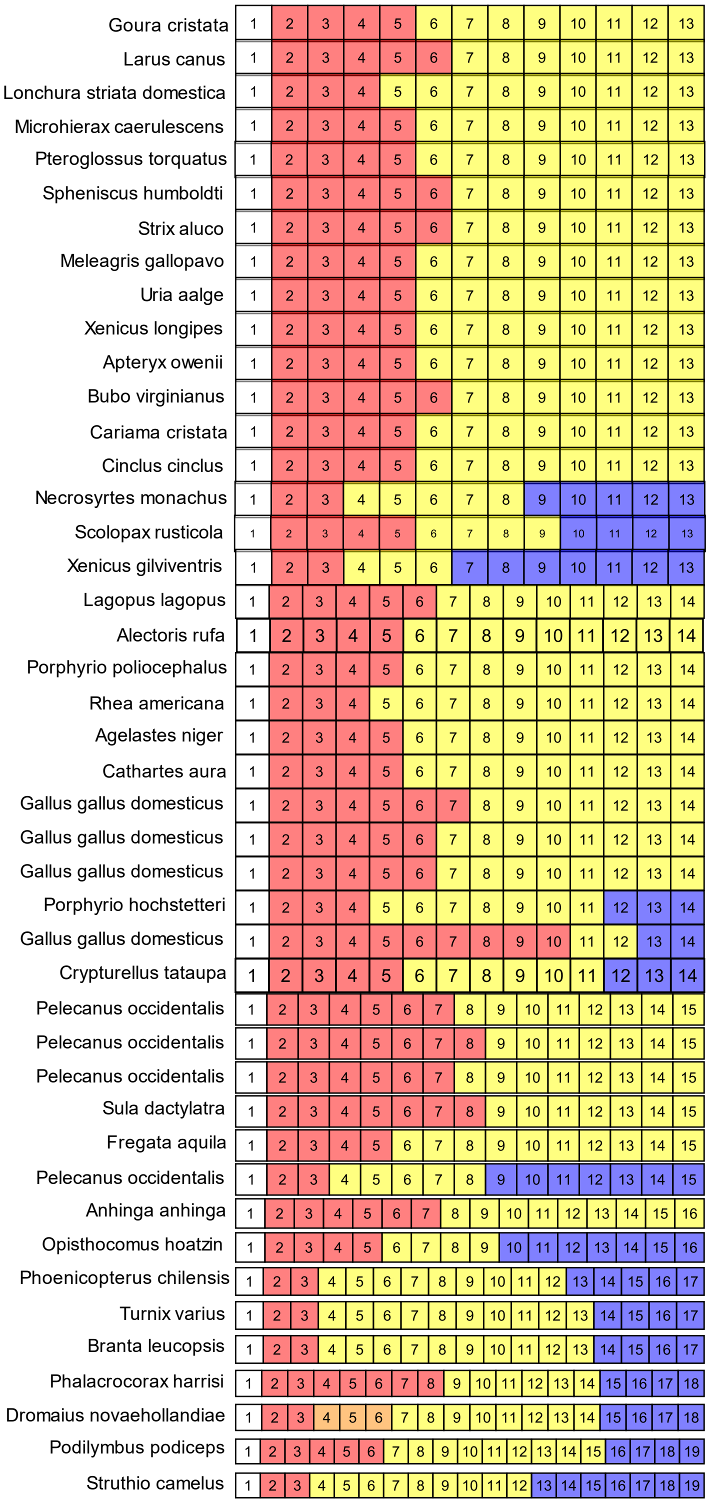
The foundation of much of the work presented in this thesis is formed of the cervical regions themselves, and thus methodology involved in assigning the boundaries between these regions must be scrutinised. The methodology for assigning regional identities to cervical vertebrae was presented in Chapter 3 and is adapted from methodologies which have been peer-reviewed by other authors (Böhmer, Rauhut, and Wörheide 2015b; Böhmer 2017; Böhmer et al. 2018). This method uses vertebral shape to assign regional identity by placing 3D morphometric landmark data into a cluster analysis. Vertebrae of the same region form a distinct group on the cladogram, and have a smaller measured distance between vertebrae of that 'clade' than other vertebral clusters/regions. This methodology has two potential shortcomings: a low number of 3D landmarks and a semi-quantitative assignment of regional boundaries.

Landmark configurations form the basis of all geometric morphometric studies and must be chosen with care, ensuring that all landmarks are homologous across the entire sample (Bookstein 1991; Zelditch, Swiderski, and Sheets 2012). Vertebral morphology within the avian cervical spine displays a large amount of variation with many features of osteology (such as the hypapophysis and processus caroticus) appearing and disappearing along the length of the cervical column (Baumel, Evans, and Berge 1993; Krings et al. 2014) and this has led to the relatively low number (15) of landmarks used throughout this thesis. A character matrix was constructed to note the presence or absence of these fluctuating osteological features for each cervical vertebra (Böhmer, Rauhut, and Wörheide 2015b). The character matrix was then combined with the Procrustes coordinates produced by the 3D geometric morphometrics analysis using a combination of a single linkage algorithm and the Gower similarity index (Gower 1966, 1971). This approach has been used to delineate regional boundaries in peer-reviewed publications and accounts for much of the anatomical variation not captured by the original 15 landmarks (Böhmer, Rauhut, and Wörheide 2015b; Böhmer 2017; Böhmer et al. 2018). Geometric morphometrics is currently undergoing a large shift in methodology, abandoning the strict use of discrete, homologous landmarks in favour of high-density, semiautomated landmark meshes that cover the entirety of a biological structure (Felice et al. 2019; Bardua et al. 2019; Goswami et al. 2019). This new approach is much more effective at accurately depicting patterns of shape variation in complex biological shapes, and reduces subjectivity of landmark placement (Goswami et al. 2019). This semiautomated approach would more efficiently overcome the hurdle of fluctuating features of cervical osteology and should this work continue, a semiautomated landmark approach will be utilised.

Within this thesis regions are delineated based on minimum-distance measures on a cluster diagram of vertebral morphology for each vertebrae within a single cervical column (Böhmer, Rauhut, and Wörheide 2015b; Böhmer 2017; Böhmer et al. 2018). Principle coordinates analysis (PCO) is used to visualise the shape space occupation of each cervical vertebrae to aid in identification of regional boundaries within the cervical spine. Whilst regions are defined numerically and cluster analysis does provide Bremer support values for each region, no quantitative statistical method assesses if this is either a significant regional arrangement or the most supported model of regionalisation. New methodologies that can quantitatively assess models of regionalisation within the axial column have been published since the inception of this project due to the large increase in the study of modularity and integration in recent years (Randau, Cuff, et al. 2016; Randau, Goswami, et al. 2016; Jones et al. 2018; Goswami and Finarelli 2016; Arnold, Esteve-Altava, and Fischer 2017; Goswami et al. 2019; Bardua et al. 2019). These new methodologies have a distinct advantage over distance-based measures as they utilise quantitative statistics or maximum likelihood methods to assign regional boundaries within a rigorous quantitative framework. Recent work has expanded upon the methodology presented here and in Böhmer et al. (2015) by combining PCO with a segmented regression approach to determine the most likely model of axial regionalisation (Head and Polly 2015; Jones et al. 2018). After PCO is performed, axes of shape variation (PCO1, PCO2 etc.) are plotted against vertebral number and via the segmented regression approach, different axial regions should display distinct gradients of shape variation in their respective portion of the axial column (Head and Polly 2015; Jones et al. 2018). Multiple models of regionalisation are presented by this approach, up to a user-defined maximum number of regions (based upon previous hypotheses on axial regionalisation in the area/taxa of interest). Likelihood methods are then used to compare between models using Akaike Information Criterion (AIC) to observe which model best fits the data (Sugiura 1978; Jones et al. 2018). This method, unlike cluster-based approaches, better models patterns of vertebral integration, and allows some confidence in the regionalisation patterns it produces as model selection processes (via likelihood-based approaches) are part of the methodology. This method is now also freely available as part of the 'regions' package in R (Jones et al. 2018). Both landmark data and character data were used in combination to assign regional boundaries in this thesis using PCO (via Gower single linkage algorithms (Gower 1966, 1971)) and since 'regions' main input data is PCO axes, the data from this thesis is primed for use with 'regions'.

Different patterns of cervical regionalisation are produced when this dataset is analysed using 'regions' (Fig. 1), with many species displaying either 2, 3 or 4 cervical regions. When this thesis' dataset is analysed using the 'regions' package there appears to be a relationship between the number of vertebrae and the number of regions as there is increased regionalisation with increasing vertebral counts. The ‘regions’ package was initially designed to assess regionalisation across all presacral vertebrae, whereby vertebral counts exceed the maximum number of cervicals studied as part of this thesis (Head & Polly 2015, Jones et al. 2018) and results from prior work have returned between 3 and 5 regions for these datasets with elevated presacral counts (Jones et al. 2018). This in tandem with the trend found between the number of cervical vertebrae and regionalisation indicates that the ‘regions’ package may need to be modified to avoid biases associated with lower maximum vertebral counts. There are also differences between this thesis and prior applications of the ‘regions’ package in terms of the scale at which regional boundaries are delineated. Prior usage of ‘regions’ has been to identify regional boundaries across the entire presacral portion of the vertebral column (Jones et al. 2018), whereas this thesis sought to observe patterns of regionalisation within just one region of the presacral spine, the cervical column. Anatomical signifiers of vertebral regions are much clearer between larger vertebral regions (such as the differences between cervical and thoracic vertebrae), and are much less conspicuous when analysing patterns of regionalisation within the avian cervical spine. As such the anatomical heterogeneity of the entire presacral spine is much higher when compared to that of the avian cervical column and this difference may lead to the low regionalisation score produced when the landmark data is subjected to analyses by the 'regions' package. It is telling that amongst many birds 'regions' delineates only one regional boundary between C5/C6, C6/C7 or C7/8 (Fig. 1), as this often correlates between the boundary of regions 2 and 3 when cluster-based approaches are used. Large changes to neural spine height and centrum length occur over this boundary and it is often this boundary over which the anatomical changes are most conspicuous across the entire cervical spine (Chapter 3 Fig. 4). This could suggest that the threshold for delineating boundaries with the segmented regression approach in the current version of 'regions' is not appropriate for analysing the relatively homogeneous nature of vertebral anatomy across the avian cervical column.

As stated earlier in this section the number of landmarks used as a basis for the GMM study in this thesis is relatively low when compared to recent studies that utilise a semi-automated landmarking approach (Bardua et al. 2019). This may exacerbate issues associated with the low heterogeneity amongst avian cervical vertebrae, and it would be interesting if increased landmark coverage altered the results produced by 'regions'. A key conclusion of this thesis was that cluster-based methodologies supported the hypothesis that the avian cervical column is divided into five regions (Chapter 3 Fig. 1, Chapter 3 Fig. 2), and the noticeable mismatch between the outputs of the cluster-based and segmented regression-based approaches (Fig. 1) may, at first glance, jeopardise this conclusion. However when investigated further these differences may be due to avian cervical regionalisation occurring on much smaller scale (both in terms of the number of vertebrae and anatomical heterogeneity) than the 'regions' package is currently constructed to examine under the segmented regression approach. As both methodologies pick out similar regional boundaries between regions 2 and 3 (of the five region model presented in this thesis), but 'regions' rarely delineates any other regions it remains that 'regions' must be adjusted to better differentiate regions with less conspicuous features of anatomy and these adjustments must be made alongside an increased landmark coverage. These adjustments should be tied to further quantitative work that assesses the impact of total vertebral counts and landmark coverage on regionalisation scores when utilising the 'regions' package. As both approaches produce such a similar boundary between regions 2 and 3 at this current stage of 'regions' development I am confident that with these adjustments the patterns of regionalisation will more closely match those produced by the cluster-based approach in future builds of the 'regions' package.



Goura cristata	1	2	3	4	5	6	7	8	9	10	11	12	13						
Larus canus	1	2	3	4	5	6	7	8	9	10	11	12	13						
Lonchura striata domestica	1	2	3	4	5	6	7	8	9	10	11	12	13						
Microhierax caerulescens	1	2	3	4	5	6	7	8	9	10	11	12	13						
Pteroglossus torquatus	1	2	3	4	5	6	7	8	9	10	11	12	13						
Spheniscus humboldti	1	2	3	4	5	6	7	8	9	10	11	12	13						
Strix aluco	1	2	3	4	5	6	7	8	9	10	11	12	13						
Meleagris gallopavo	1	2	3	4	5	6	7	8	9	10	11	12	13						
Uria aalge	1	2	3	4	5	6	7	8	9	10	11	12	13						
Xenicus longipes	1	2	3	4	5	6	7	8	9	10	11	12	13						
Apteryx owenii	1	2	3	4	5	6	7	8	9	10	11	12	13						
Bubo virginianus	1	2	3	4	5	6	7	8	9	10	11	12	13						
Cariama cristata	1	2	3	4	5	6	7	8	9	10	11	12	13						
Cinclus cinclus	1	2	3	4	5	6	7	8	9	10	11	12	13						
Necrosyrtes monachus	1	2	3	4	5	6	7	8	9	10	11	12	13						
Scolopax rusticola	1	2	3	4	5	6	7	8	9	10	11	12	13						
Xenicus gilviventris	1	2	3	4	5	6	7	8	9	10	11	12	13						
Lagopus lagopus	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Alectoris rufa	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Porphyrio poliocephalus	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Rhea americana	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Agelastes niger	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Cathartes aura	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Gallus gallus domesticus	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Gallus gallus domesticus	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Gallus gallus domesticus	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Porphyrio hochstetteri	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Gallus gallus domesticus	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Crypturellus tataupa	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
Pelecanus occidentalis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Pelecanus occidentalis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Pelecanus occidentalis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Sula dactylatra	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Fregata aquila	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Pelecanus occidentalis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Anhinga anhinga	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
Opisthocomus hoatzin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
Phoenicopterus chilensis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Turnix varius	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Branta leucopsis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Phalacrocorax harrisi	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Dromaius novaehollandiae	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Podilymbus podiceps	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Struthio camelus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

Figure 1 Cervical regionalisation patterns produced by the ‘regions’ package. Species depicted are the same as those analysed in Chapters 3-5. Numbered squares represent cervical vertebrae. Colours represent individual regions. There appears to be a trend of increased number in regions in taxa with higher counts of cervical vertebrae.

Other recent work has used partial least squares analysis to assess morphological integration (i.e. shape covariation) between vertebrae to delineate regions within the axial column (Randau and Goswami 2017). As per this thesis and the ‘regions’ package, this method uses 3D geometric morphometrics as a foundation. This method uses pairwise comparisons of 3D shape data to test for a significant degree of integration between two separate vertebrae (Randau and Goswami 2017; Bookstein et al. 2003; Rohlf and Corti 2000). Multiple comparisons are performed so that all combinations of vertebral pairs are tested for the segment of axial column being analysed. Multiple comparisons must be accounted for and thus each pairwise comparison is corrected using the Benjamini-Hochberg correction (Benjamini and Hochberg 1995). Each pairwise comparison also has a value for the degree of correlation and a significance value. Regions can then be determined based on localised areas of significant interaction terms with high correlation values (Randau and Goswami 2017; Bookstein et al. 2003; Rohlf and Corti 2000). This method was not utilised as part of this thesis due to its reliance on landmark data alone. Due to the large variability in vertebral shape across the avian cervical column landmark data was used alongside character data to characterise shape variation of cervical vertebrae. This method does not currently allow for the combination of landmark and character data and so was not used.

Techniques assessing modularity and regionalisation hypotheses do not require 3D shape data or geometric morphometrics to be used as a foundational framework. By analysing the physical anatomical connections between vertebrae as well as connecting muscles, Anatomical Network Analysis (AnNA) can delineate regions/modules based on the connectivity patterns of a musculoskeletal area of interest (Esteve‐Altava et al. 2013; Rasskin-Gutman and Esteve-Altava 2014; Esteve‐Altava 2017b). This method does not require a priori assumptions of modularity and can incorporate a wealth of soft tissue data that is usually not directly assessed by geometric morphometrics that primarily focuses on shape variation of osteological elements (Arnold, Esteve-Altava, and Fischer 2017). Modules (regions) can be determined statistically within AnNa by performing a Wilcoxon rank-sum test on internal versus external links of the modules’ nodes (Arnold, Esteve-Altava, and Fischer

2017; Esteve‐Altava et al. 2013; Rasskin-Gutman and Esteve-Altava 2014; Esteve‐Altava 2017b). AnNa provides many numerical parameters that can be used to mathematically describe a given musculoskeletal network such as morphological complexity, degree of modularity and integration, and these parameters can be easily used in a phylogenetic comparative framework to observe the evolution of modularity, regionalisation and integration within a clade (Esteve‐Altava et al. 2013; Esteve‐Altava 2017b; Rasskin-Gutman and Buscalioni 2001; Müller et al. 2003; Esteve‐Altava 2017a; Arnold, Esteve-Altava, and Fischer 2017). Whilst providing an informative framework to investigate modularity using both hard and soft tissue data, AnNa requires connection data for all bones and muscles in an area of interest in a large and diverse sample (Arnold, Esteve-Altava, and Fischer 2017). The current literature on avian cervical anatomical descriptions is limited to widely available taxa that are found predominantly in the western hemisphere (Baumel, Evans, and Berge 1993; Boumans, Krings, and Wagner 2015). Even after conducting dissections on a further ten species on extant birds (chapter 5, this thesis), the number of species only equates to approximately half of those available to geometric morphometrics analysis due to the higher availability of skeletal material from museum collections.

The aim of assessing regionalisation in this project was to provide an aspect of homology so that vertebral anatomy could be compared across Aves. Comparing vertebral anatomy across birds had previously been hindered by the complete lack of understanding of vertebral homology in a cervical column which can vary in size from 11 to 26 cervical vertebrae across Aves. The method used to define cervical regions in this thesis has been previously found to assign regional boundaries in exactly the same vertebral positions as when defined by *Hox* gene expression patterns (Böhmer, Rauhut, and Wörheide 2015a). As the *Hox* gene expression patterns involved in cervical regionalisation are thought to be conserved across all extant birds (Böhmer, Rauhut, and Wörheide 2015a), assigning regional boundaries using geometric morphometrics and distanced-based cluster analysis allows for the assessment of regionalisation across Aves within a homologous, five-region framework. By following this methodology it also allows for a direct comparison to previous work on extant avian regionalisation as the foundational methodology is the same across both studies (Böhmer, Rauhut, and Wörheide 2015a; Böhmer 2017; Böhmer et al. 2018). Working within a homologous framework of cervical regions was the most important factor when choosing the methodology to assign regional boundaries, and as distance-based measures was the only method that was able to match geometric morphometric data with gene expression results, we felt it necessary to delineate regions using a distance-based cluster analysis approach.

Limitations of low sample sizes and broad ecological classifications

Chapters where raw data was collected from skeletal material (to be used in geometric morphometrics and allometric scaling datasets, chapters 3-5) contained relatively large samples sizes of 53 (chapter 3) and 38 species (chapters 4 and 5) due to the assistance of museum collections staff and collaborators, and have been considerably larger than recent work on mammals (Randau, Goswami, et al. 2016; Randau, Cuff, et al. 2016; Randau and Goswami 2017, 2018). However, due to the limited availability of avian cadaveric material, the sample size when investigating variation in avian cervical muscle architecture was limited to 10 individuals (10 species). Clear deviations in muscle architecture were observed in certain species during dissection, such as the enlarged size of M. complexus in *Morus bassanus*, a singular, enlarged fleshy belly of M. biventer cervicis in *Spheniscus humboldti*, and a marked increase in the number of slips associated with M. longus colli dorsalis pars profunda in both species of owl (*Strix aluco* and *Tyto alba*). However, quantitative measures of muscle properties across ecological groupings were rarely found to be significant, and only a handful of ecological variables appear to have a small correlation with the variation in cervical muscle architecture variables. As difficulty in acquiring animal cadaveric material is common amongst studies of muscle architecture variation, many protocols exist for improving the power of statistical tests and the one used in thesis ensures that body mass is accounted for by including it as a covariate in a phylogenetic ANCOVA analysis (Myatt, Crompton, and Thorpe 2011; Myatt et al. 2012). However as many of the ecological groups were only represented by 2 species or less, it must be acknowledged that sample size is at present a fundamental issue, limiting statistical comparisons of ecological or locomotor groupings of birds.

Chapter 6 was hampered by small sample sizes as well a restriction to the number of cervical muscles studied. The number of separate muscles studied in each individual was ten and these ten are the most commonly associated with specific movements of the head and neck (Boumans, Krings, and Wagner 2015; Baumel, Evans, and Berge 1993; Snively and Russell 2007b). However this scheme excludes many muscles due to their small size and deep placement (Boumans, Krings, and Wagner 2015). Many of these muscles are small, but are repeated along the length of the cervical column (such as M. interspinalis) so may have a larger impact upon cervical kinematics than previously thought and should be considered in future work. The metrics used to study cervical muscle variation were limited to gross measurements of fibre length, muscle mass and PCSA. There are many other muscle parameters on which natural selection can act and variation in these parameters may not cause significant variation in the gross measures of muscle morphology and architecture utilised in chapter 6. Some of these other parameters include maximum shortening velocity (V_{max}), maximum isometric stress (a muscles peak force when activated at its optimal length) and the proportion of fast twitch to slow twitch fibres (fast twitch fibres provide a larger force over a shorter time, whilst slow twitch fibres are designed to provide a low force over a longer time period). Changes in one or multiple of these parameters have been associated with locomotory and dietary ecologies in previous studies (Dial and Biewener 1993; Biewener and Gillis 1999; Biewener and Corning 2001; Bonine, Gleeson, and Garland 2005; Brainerd and Azizi 2005; Maie et al. 2011) and future work should include these parameters.

Bird flight is a complicated mode of locomotion, and a vast diversity of flight modes exist within extant Aves between the extremes of fully terrestrial and powered flight

(Rayner 1988; Pennycuick 2008). Many studies categorise a bird's locomotory mode by noting the most frequently utilised flight mode according to previously name flight categories (Pennycuick 2008; Bruderer et al. 2010; Close and Rayfield 2012; Wang, McGowan, and Dyke 2011). This static approach to categorising bird flight was adopted throughout this thesis, but as an approach it is not without limitations. Bird flight is not static and as such a single species of birds can utilise multiple modes of flight to participate in a multitude of behaviours (Taylor and Thomas 2014; Benson et al. 2017). All of the flight modes that a particular species can undertake will also not apply the same selection pressure on the morphology of the avian neck. For example the northern gannet (*M. bassanus*) is classified as a soaring bird in this thesis. However it feeds by catching fish close to the surface of the water by diving into the water at speeds of up to 20 m/s in a behaviour called 'plunge-diving' (Ropert-Coudert et al. 2004; Machovsky Capuska et al. 2011; Chang et al. 2016). Plunge-diving in *M. bassanus* has led to an increase in size in cervical musculature associated with supporting the head and the cranial portion of the neck during a high speed impact with the surface of the water (Chang et al. 2016). Despite soaring flight being the most frequently utilised mode of flight in *M. bassanus* it is clear that other flight modes have a larger impact on the morphology of the cervical column (Chang et al. 2016). Previous work has overcome this issue of static flight mode assignment by scoring multiple flight behaviours on a presence/absence basis for each species in the dataset (Taylor and Thomas 2014; Benson et al. 2017). It would be interesting to see if this multivariate approach resulted in more flight modes displaying a significant correlation with the variation of cervical musculoskeletal morphology in birds as part of future studies.

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Chapter 8: Conclusions and future work

Conclusions and significance of work

The overall aim of this thesis was to quantify variation of the avian cervical column and its patterns of cervical regionalisation. This over-arching aim was pursued through 4 specific objectives. The major conclusions of this thesis are grouped below beneath the relevant thesis objective.

Objective 1: To test previous hypotheses concerning patterns of avian cervical regionalisation within extant Aves

1. **Three dimensional geometric morphometrics has revealed that five cervical regions are shared across extant Aves and each of these regions has an identifiable morphology**.

2. **Patterns of morphological change across these regions are conserved throughout many extant avians**. This pattern only deviates in extreme ecologies where cervical kinematics are specialised, such as carnivorous and insectivorous birds.

3. **Patterns of vertebral counts within each cervical region do not correlate significantly with scaling factors such as body mass or neck length**. The lack of a significant relationship between regional vertebral counts and neck length indicates that cervicalisation is not driving neck elongation across Aves. Regional vertebral counts are rarely affected by ecological factors and appear to adapt for efficient grazing.

4. **Variability in vertebral counts is greatest in regions 3 and 4**. It has been previously hypothesised that stepwise additions of vertebrae to region 3 was the ancestral condition which eventually gave rise to the evolution of a novel region (region 4) in birds (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015). These results suggest that region 4 may have evolved and

expanded to provide attachment sites for muscles to allow increased support for higher vertebral counts in region 3.

Objective 2: To quantify factors that affect variation in neck length and head mass across extant Aves.

5. **Across a phylogenetically broad sample of extant birds, neck length and head mass scale with body mass according to isometry.** This indicates that body mass does not constrain neck length in birds, unlike in other vertebrate clades. Relative neck lengths are shorter in birds that locomote using strong powered flight and this may represent an adaptation to stabilise vision during flight. Relative head mass is decreased in terrestrial birds and this may be due to the decrease in size of sensory organs associated with flight.

6. **The relationship between head mass and neck length in birds is isometric**. This is in contrast to other vertebrates where head mass has a negative scaling relationship with neck length (Christiansen 1999; McGarrity, Campione, and

Evans 2013), and this may be due to lower cranial soft tissue mass of large cranial organs (Brooke, Hanley, and Laughlin 1999). An elongate neck that allows for the characteristic S-shaped habitual neck posture in combination with lighter cranial soft tissues may allow birds to adapt a wide array of head shapes to specific ecological niches.

Objective 3: To quantify factors that affect variation in regional vertebral morphology and region length in the cervical column of extant Aves.

7. **Body mass and neck length are the two dominant factors that influence vertebral morphology within each of the five cervical regions throughout extant Aves.** Each of these factors controls morphological variation in different regions of the cervical spine: body mass for terminal regions (1 and 5) and neck length for middle regions (2-4).

8. **Variation in vertebral morphology within each of the five cervical regions is significantly affected by a few specialised ecologies:** piscivory for region and 2, filter feeding for region 2, soaring and subaqueous flight for region 3,

carnivory for regions 4 and 5. These relationships between ecology and morphology can be used to create hypotheses concerning how certain changes to muscle architecture within these groups may underpin changes to regional vertebral morphology.

9. **The effect of these ecologies on regional vertebral morphology is much weaker than the effect of body mass and neck length.** The one exception is within region 5, where the effect of carnivory outweighs the impact of body mass.

10. **The lengths of cervical regions strongly correlate with overall neck length, more so than any other scaling factor (body mass or head mass).** Regions 2 and 5 scale positively with neck length and this suggests that the lengths of these regions are responsible for neck elongation across Aves. Alongside the conclusion that cervicalisation does not facilitate neck elongation, this result

suggests that increases in length of cervical vertebrae are responsible for avian neck elongation.

11. **Significant variation in regional lengths is only observed in a select view ecological groups** of grazing birds and birds that locomote terrestrially or with weaker powered flight. This again suggests that powered flight has some degree of constraint over cervical morphology in birds.

Objective 4: To quantify factors affecting variation in cervical muscle architecture in extant Aves and to determine if this variation is linked to variation in regional vertebral morphology

12. **Muscle architecture and mass across ten of the most prominent cervical muscles scaled predominantly with body mass according to positive allometry**. This is hypothesised to allow the neck to support the mass of the head and the increased mass of the dorsally positioned cervical muscles that

support the head and the neck. It may also support the isometric scaling of avian head mass and neck length.

13. **Ecology has a weak effect on the variation of fibre length, muscle mass and PCSA in avian cervical musculature.** Insectivorous taxa may decrease PCSA in long ventral muscles to allow for weak but fast head strikes to catch small fast moving prey and subaqueous fliers may increase the PCSA of M. splenius capitis to allow efficient head movement underwater. However these results are affected by a low sample size

14. **There is a disparity in the functional variation of cervical muscles, with cranially positioned head flexors being more restricted to a generalist function (i.e. less specialised for force versus power versus force-length production) relative to caudally positioned neck flexors**, which display large variation in the level of functional specialisation. This suggests that head mass and kinematics associated with generalised tasks (i.e. the neck acting as a surrogate arm) constrain the evolution of cranially positioned cervical

musculature. Caudally positioned musculature may be either responding to large changes in cervical counts in caudal cervical regions or ecological signals. However the sample size was too low to recover any significant variation caused by ecological factors.

15. **None of the ecological groups that exhibited significant variation in regional vertebral morphology demonstrated significant variation in muscle mass or PCSA of muscles that were hypothesised to cause the variation in vertebral morphology**. This indicates that changes to muscle mass do not appear to underpin changes to vertebral morphology in the avian column, and may in fact be adaptations to accommodate different patterns of cervical kinematics. However, as the sample size of this study was restricted to 10 individuals, this relationship must be tested with more samples.

Directions for future work

One of the major limitations of chapter 6 was the low sample size of taxa (10). As such the true extent of the effect of ecological factors on variation in cervical muscle architecture is still relatively unknown. The variation in caudally positioned muscles appears to be correlated with extreme ecologies such as plunge diving and subaqueous flight, however this variation was not recovered as significant. This qualitative suggestion that caudal muscles are adapting to specific ecologies must be properly investigated with an appropriately sized sample. The initial impetus of any future work should thus concentrate on increasing the sample sizes of analyses performed in chapter 6. The low sample sizes within chapter 6 may have also obscured a possible link of muscle architecture variation and regional morphological variation, and because of this it is currently inferred that no such link exists. As suggested in chapter 6, specialised taxa could display patterns of cervical kinematics that are specific to that ecological group, thus it was hypothesised that variation in cervical kinematic patterns that was underlying changes to regional vertebral morphology. Future work should test these assumptions by observing variation in intervertebral flexion in a phylogenetically and ecologically diverse sample of extant birds. Recent work has indicated that this can be achieved by studying variations in maximum joint angle excursions across the entire cervical column in cadaveric birds (Grytsyshina, Kuznetsov, and Panyutina 2016; Krings et al. 2014, 2017; Kambic, Biewener, and Pierce 2017).

This thesis has focused entirely on variation in cervical morphology of extant birds however important questions remain regarding the evolution of the avian neck. Five cervical regions remains a condition unique to modern birds as all other members of Archosauria (extant and extinct) are inferred to have four cervical regions (Mansfield and Abzhanov 2010; Böhmer, Rauhut, and Wörheide 2015). It has been hypothesised that region 3 is an ancestral cite of cervicalisation in non-avian dinosaurs (Böhmer, Rauhut, and Wörheide 2015). In conjunction with the data presented in chapter 3, that regions 3 and 4 display the highest variability in vertebral counts, this suggests that region 4 may have evolved to provide support for an elongated neck, as vertebrae from region 4 form the attachment sites of many neck supporting muscles. The methodology used to delineate cervical regions in chapter 3 has been used elsewhere to infer regional boundaries in the neck of one dinosaur, *Plateosaurus engelhardti*, however to date this remains the only non-avian dinosaur in which cervical regions have been identified (Böhmer, Rauhut, and Wörheide 2015). This severely hinders our knowledge of neck evolution in archosaurs as *Plateosaurus* is a basal member of

Dinosauria. Future work should identify cervical regions in a much broader sample to determine if non-avian dinosaurs display any deviations from the 4-region condition in more derived taxa.

Perhaps the most interesting gap in current literature is the precise reasons behind the evolution of a novel cervical regions in birds. Changes to the degree of integration between the cervical column and appendicular elements have been shown to increase or decrease cervical evolvability depending on the degree of integration between the units (Randau and Goswami 2018; Jones et al. 2018; Arnold, Esteve-Altava, and Fischer 2017). This has been exemplified in mammals whereby expansion of forelimb musculature into the caudal region of the cervical column has restricted the variation of the neck of the clades evolution (Randau and Goswami 2018; Jones et al. 2018; Arnold, Esteve-Altava, and Fischer 2017). As flight evolved across the theropod-bird transition, the forelimb became increasingly less integrated with the hindlimb and other anatomical modules and modularity (decreased integration) may have increased between the forelimb and cervical column of birds (Gatesy and Dial 1996; Bell, Andres, and Goswami 2011). This would increase the adaptability of the early avian neck and allow it to better position the head to manipulate the surrounding environment (i.e.

becoming a 'surrogate arm') in the wake of the forelimb becoming specialised for flight. Recent work has highlighted a potential high degree of integration between leg length and neck length in extant birds (Böhmer et al. 2019). As bipeds, theropods could lower their head to the ground via flexion of the torso around the hip joint, using the tail as a counterbalance (Grossi et al. 2014). As tail length decreased over the theropod-bird transition this action would have become increasingly unstable and as such neck flexibility must increase to allow for important ground-level interactions such as feeding and drinking (Grossi et al. 2014). Cervicalisation in birds has been linked to an increase in total neck flexion ranges (Bout 1997; Zweers, Bout, and Heidweiller 1994; Van der Leeuw, Bout, and Zweers 2001), and region 4 may have evolved to support the increases to vertebral counts as hindlimb and tail proportions changed across the theropod-bird transition. Regardless of which, if any, of these patterns of integration may have facilitated the evolution of a novel region unique to Aves, patterns of integration between the cervical column and other parts of the skeleton must be studied both for extant birds and across their early evolution.

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