

## RESEARCH ARTICLE

# Blood flow for bone remodelling correlates with locomotion in living and extinct birds

Georgina H. Allan<sup>1</sup>, Phillip Cassey<sup>1</sup>, Edward P. Snelling<sup>1</sup>, Shane K. Maloney<sup>2</sup> and Roger S. Seymour<sup>1,\*</sup>

## ABSTRACT

Nutrient arteries enter limb bones through discrete foramina on the shafts. They are required for bone remodelling in response to mechanical loading and dynamic forces imposed by locomotion. The cross-sectional area of the nutrient foramen of the femur represents an index of blood flow rate to the shaft and thus provides insight into the animal's level of activity. Morphometric data on femoral length, mass and foramen size from 100 extant bird species and eight extinct moa species were analysed allometrically and phylogenetically. The nutrient foramen blood flow index ( $Q_i$ ) and femur mass ( $M_f$ ) increase with body mass ( $M_b$ ). At 1 kg body mass, cursorial species have approximately 2.1 times higher  $Q_i$  and 1.9 times heavier  $M_f$  than volant species. The scaling of  $Q_i$  on  $M_f$  is independent of the primary mode of locomotion, but the ratio  $Q_i/M_f$  decreases significantly in larger birds, although absolute  $Q_i$  increases. The overall avian equation for  $Q_i$  on  $M_b$  is not significantly different from previous data from mammals, but when differences in blood pressure are accounted for, estimated blood flow to the femur is approximately 1.9 times higher in cursorial birds than in mammals, possibly in relation to bipedalism and quadrupedalism, respectively. Femoral bone blood flow in both endothermic groups is estimated to be 50–100 times higher than in ectothermic reptiles.

**KEY WORDS:** Bird, Locomotion, Blood flow, Bone remodelling, Nutrient foramen, Allometry

## INTRODUCTION

The nutrient foramen is a discrete hole located along the shaft of bones that provides an opening through which the nutrient artery can service the living bone tissue. The nutrient foramen on the shaft of the femur is of particular interest, because its size can be used as a gauge of the maximum aerobic metabolic rate (MMR) of mammals (Seymour et al., 2012). The functional connection between this tiny anatomical feature and an important physiological trait is locomotion. Locomotion results in micro-damage of bone, which is repaired during bone remodelling that is supported by blood flow. Blood vessel size is adaptively related to flow rate, and consequently the size of the foramen is related to the maximum size of the nutrient artery. This study examines the relationships between locomotion, femur bone size and blood flow through the femoral nutrient foramen of birds. It is hypothesised that birds that put more stress on their bones during locomotion have larger bones and greater blood flow in relation to body size. Such correlations would indicate a dynamic interplay between morphology, physiology and function.

More than a century ago, Wolff recognised the relationship between mechanical loading and bone formation, concluding that exercise increases bone mass, whereas unloading results in bone atrophy (Wolff, 1892). This was later reinforced by Foote, who showed that the femur is particularly responsive to the functional demands of the animal, including the stresses and strains to which the bone is exposed and the position of the femur relative to the body (Foote, 1911). Major limb bones, such as the femur, support the weight of an individual's body and must also contend with more acute forces experienced during locomotion.

Bone remodelling functions not only to normalise stresses, but also to repair micro-fractures that are caused by the bending and torsion of bones during locomotion (Burr, 2002). Remodelling occurs continually by the removal of pre-existing bone and the laying down of new bone, both on the external periosteum and the osteons of the Haversian system inside cortical bone (Parfitt, 1994; Robling et al., 2006). Haversian remodelling is a stepwise process in which capillary loops burrow through old bone, led by a 'cutting cone' of osteoclasts that dissolve old bone and followed by osteoblasts that replace it. Increased loading or exercise immediately increases the frequency of micro-fractures and stimulates bone remodelling over an extended period (Currey, 2003; Lieberman et al., 2003; Robling et al., 2006). Therefore, highly active vertebrates are more likely to put a greater range of forces on their bones than sedentary ones, increasing bone size and hence the incidence of micro-fractures that must be repaired. Most of our knowledge concerning bone remodelling comes from experiments on mammals, but we assume that similar mechanisms exist in birds. For example, the furculae of birds show Haversian remodelling, apparently in response to strain-related micro-damage during flight (Ponton et al., 2007), but there are no studies on remodelling of avian hind-limb bones.

The efficiency of the cutting cone is limited by the supply of nutrients and oxygen delivered by the blood vessels in the bone (Jaworski, 1991). Bone cells have high respiratory rates (Schirmacher et al., 1997), and the interior of bones is highly oxygenated (Bingmann and Wiemann, 2007). During remodelling, oxygen consumption in bone tissue increases (Sim and Kelly, 1970), and so an adequate blood supply is required to transport oxygen to the site of remodelling. More active vertebrates would therefore be expected to require a greater blood supply to support remodelling.

The long bones of the limbs have three sources of blood: the nutrient artery, and the periosteal and epiphyseal blood supplies. The nutrient artery is the primary blood source of long bones, providing 50–70% of total blood flow (Trueta, 1963). The nutrient artery enters the long bone through the nutrient foramen on the shaft (diaphysis). The foramen sets a limit to the maximum size of the vessel and so should reflect the extent of blood flow through it. The size of the blood vessel, and consequently the foramen, is affected by rheological properties of blood in the vessel – stretch and shear stress (Berry, 1978; Birchard, 1997; Ward et al., 2000). Stretch is

<sup>1</sup>School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia. <sup>2</sup>School of Anatomy, Physiology and Human Biology, The University of Western Australia, Crawley, WA 6009, Australia.

\*Author for correspondence (roger.seymour@adelaide.edu.au)

**List of symbols and abbreviations**

AICc	Akaike information criterion corrected for finite sample size
$L$	femur length (mm)
$M_b$	body mass (g)
$M_f$	femur mass (g)
MMR	maximum aerobic metabolic rate
$Q_i$	index of blood flow derived from foramen area and femur length
$R$	foramen radius (mm)
$\dot{V}_{O_2}$	oxygen consumption rate ( $\text{ml min}^{-1}$ )

related to the radial force exerted on a vessel wall due to the balance between blood pressure and the mechanical properties of the wall. Shear stress is related to the friction between the blood and the vessel wall, which is usually considered proportional to the product of blood viscosity and mean velocity (Lehoux and Tedgui, 2003). These mechanical forces lead to alterations of vessel circumference and thickness, which promote laminar flow and reduce the work for blood perfusion. Assuming laminar flow, it is possible to estimate an index of flow rate ( $Q_i$ ) from the radius ( $r$ ) of the nutrient foramen and the length ( $L$ ) of the bone, through a simplification of the Hagen–Poiseuille equation for laminar flow:  $Q_i = r^4/L$  (Seymour et al., 2012).  $Q_i$  is not a flow rate, because blood pressure and viscosity are not represented, the radius is for the foramen, not the artery lumen, both the artery and vein usually pass through the foramen and the length does not represent a uniform tube. Nevertheless  $Q_i$  is based on principles of laminar flow and is therefore comparable between species.

A previous allometric analysis of femur nutrient artery  $Q_i$  in relation to body mass in mammals and reptiles revealed that although  $Q_i$  is approximately 10-fold higher in mammals than in reptiles, the scaling exponents are indistinguishable. Furthermore, when blood pressure differences are taken into account, the estimated perfusion of the interior of the bones of mammals is approximately 50 times higher than that in reptiles (Seymour et al., 2012). Furthermore, the exponent of  $Q_i$  in mammals (0.86) is indistinguishable from the exponent (0.87) for exercise-induced MMR in mammals, suggesting that blood flow to the bone increases in proportion to exercise-induced bone damage. The low blood flow into reptilian bones is consistent with the fact that reptiles do not remodel their bones in response to mechanical loading. The earlier study also indicated that blood flow to the femora of dinosaurs was even higher than in mammals, after body size is accounted for

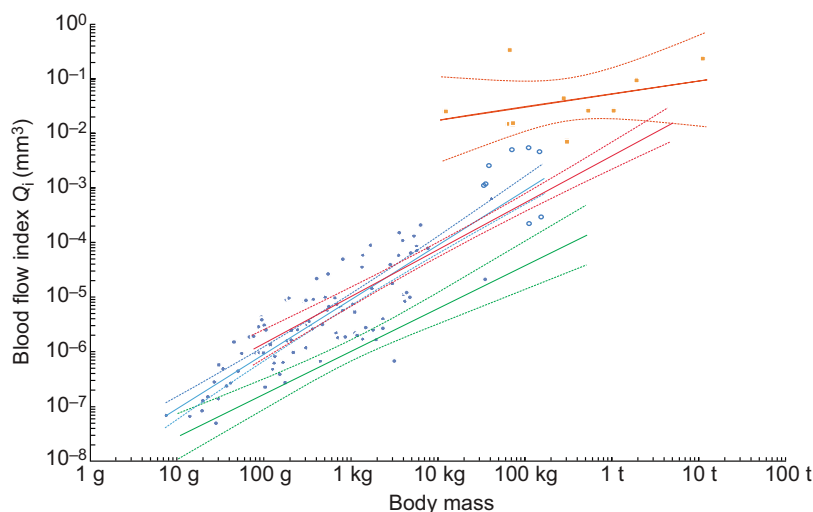
allometrically. Birds were not included in that study because of uncertainties associated with additional blood flow through pneumatic foramina and the decoupling of flight locomotion and the leg bones.

Here, we analyse the relationships between locomotion, femur bone size and blood flow through the femoral nutrient foramen of birds. We hypothesise that birds that put greater forces on their leg bones during terrestrial locomotion will have larger bones and greater blood flow in relation to body size. We focus on the femur because it provides a relatively consistent blood flow, it usually has only one foramen and it is involved primarily in terrestrial locomotion (Brookes and Revell, 1998). We clarify the role of blood flow via the pneumatic foramen and evaluate differences between primarily cursorial and volant species. Our phylogenetically-informed allometric study concerns living birds varying in body mass over 3 orders of magnitude (7.5 g–40.6 kg). To increase the mass range further, we include the fossil femora of moa, the extinct giant birds of New Zealand, estimated to weigh up to 157 kg. Finally, we compare our results for birds with mammals, reptiles and dinosaurs.

**RESULTS**

One hundred species of extant birds and eight species of extinct moa were analysed (see supplementary material Table S1 for raw data). A strong correlation existed between blood flow index ( $Q_i$ ) and body mass ( $M_b$ ) in birds (Fig. 1). The equation for all birds was  $Q_i = 8.90 \times 10^{-9} M_b^{1.00 \pm 0.10}$ . (All exponent error statistics are 95% confidence intervals.) When moa were excluded, the equation for extant birds was  $Q_i = 1.47 \times 10^{-8} M_b^{0.91 \pm 0.13}$ . ANCOVA showed that the exponent of the bird regression was not significantly different from the mammal ( $F_{1,148} = 3.25$ ,  $P = 0.073$ ) and reptile ( $F_{1,124} = 3.36$ ,  $P = 0.060$ ) regressions in our previous study (Seymour et al., 2012). However, the elevations of the bird ( $F_{1,125} = 57.73$ ,  $P < 0.001$ ) and mammal ( $F_{1,61} = 64.78$ ,  $P < 0.001$ ) regressions were significantly higher than the reptile regression. The  $Q_i$  of dinosaurs appeared higher than that of birds and mammals, especially the smaller dinosaurs, but there was high variability in the dinosaur data. The Johnson–Neyman test indicated that the regressions for birds and dinosaurs were not significantly different above a body mass of 1688 kg.

The median phylogenetic signal in  $\log_{10}$ -transformed  $Q_i$  was high (0.84) and non-zero in all phylogenetic generalised least square (pgls) models containing body mass and mode of locomotion



**Fig. 1. Relationship between blood flow index ( $Q_i$ ,  $\text{mm}^3$ ) and body mass ( $M_b$ , g) in extant birds (blue circles,  $Q_i = 8.90 \times 10^{-9} M_b^{1.00 \pm 0.10}$ ) and extinct moa (open circles) plotted on logged axes. Linear regressions with 95% confidence bands (dotted lines) for the regression means are shown for birds and compared with previous results for mammals (red,  $Q_i = 2.63 \times 10^{-8} M_b^{0.86 \pm 0.11}$ ), reptiles (green,  $Q_i = 4.42 \times 10^{-9} M_b^{0.79 \pm 0.19}$ ) and dinosaurs (orange squares,  $Q_i = 0.0020 M_b^{0.24 \pm 0.47}$ ) (Seymour et al., 2012).**

Table 1. Pgl models for the relationship between blood flow index,  $Q_i$ , and body mass and mode of locomotion in extant birds

Model ( $\log_{10} Q_i =$ )	Model rank count	Model weight [95th percentiles]	Pagel's $\lambda$ [95th percentiles]	Model estimates [95th percentiles]
Intercept	3: 851	0.00	0.84 [0.79, 0.89]	-6.60 [-6.66, -6.54]
$\log_{10}$ body mass	2: 1000	0.18 [0.14, 0.24]	0.49 [0.42, 0.57]	0.53 [0.52, 0.54]
Locomotion (cursorial)	4: 851	0.00	0.83 [0.77, 0.87]	1.54 [1.48, 1.59]
$\log_{10}$ body mass $\times$ locomotion	1: 1000	0.82 [0.76, 0.86]	0.36 [0.00, 0.44]	-0.48 [-0.50, -0.47]

Results are summarised for 1000 phylogenetic trees (see Materials and methods). The highest ranked model, on all occasions, was a full model that included both body mass and locomotion mode, as well as their interaction. The count of the model rank is the number of times (out of 1000) that the particular model obtained the observed rank. The model estimates are provided for the terms from this highest ranked model. Values for the relative model weight, Pagel's  $\lambda$ , and model estimates (pgls slopes) are medians from pgls models across 1000 trees, including 95th percentile ranges.

(Table 1). In a set of pgls models accounting for the non-independent evolutionary associations among related species, the increasing  $Q_i$  of extant birds was strongly associated with both increasing body mass and the difference in mode of locomotion (Table 1). The interaction between body mass and mode of locomotion was significant (with cursorial species having significantly higher  $Q_i$  than volant species), and the best-fitting model included this interaction term (relative model support [95th percentiles]=0.82 [0.76, 0.86]). The change in the corrected Akaike information criterion (AICc) between the two highest ranked models was greater than 2 ( $\Delta AICc$  [95th percentiles]=3.01 [2.32, 3.57]), indicating considerable support for the interaction model.

When birds, including moa, were classified as primarily cursorial or volant, ANCOVA revealed no significant difference in exponent, but a significant difference in elevation. For any given body mass, cursorial birds had relatively heavier femora ( $F_{1,98}=27.79$ ,  $P<0.001$ ; Fig. 2) and significantly higher blood flow indices ( $F_{1,98}=4.29$ ,  $P=0.041$ ; Fig. 3). For example, at a body mass of 1 kg, the calculated  $M_f$  for cursorial birds was 1.9 times heavier than for volant birds, and  $Q_i$  was 2.1 times greater. If moa were excluded from the analysis, femur mass remained significantly higher in cursorial species ( $F_{1,90}=15.83$ ,  $P<0.001$ ), whereas the  $Q_i$  relationships produced significantly different exponents, yet the cursorial data were significantly higher below a body mass of 431 g, according to the Johnson–Neyman test. If  $Q_i$  was regressed on femur mass ( $M_f$ ), the equations were not significantly different for cursorial ( $Q_i=6.31\times10^{-6}M_f^{0.82}$ ) and volant birds ( $Q_i=5.91\times10^{-6}M_f^{0.89}$ ), indicating that  $Q_i$  depends directly on the amount of bone, rather than the primary mode of locomotion. The relationship for all 108 birds was  $Q_i=5.96\times10^{-6}M_f^{0.87\pm0.07}$ , which indicates that  $Q_i$  decreases

significantly in relation to the amount of bone as the birds become larger (Fig. 4).

DISCUSSION  
Scaling of avian femur size

It is well established that the musculoskeletal structure of the limbs of birds is strongly correlated with functional lifestyle (Alexander et al., 1981; Bennett, 1996). Birds fill a diverse array of niches and rely on different modes of locomotion, which impart differential forces on their legs, and these differences can be observed in the architecture of the bone (Doubé et al., 2012). Therefore, cursorial species might be expected to have relatively heavier bones than volant species, an outcome that would normalise the strain. The parallel scaling of femur mass supports this hypothesis, with the femora of cursorial species approximately twice as heavy as those from volant species (Fig. 2). Primarily volant birds put comparatively little pressure on their leg bones, except during take-off and landing. When not flying, the hind-limb bones adapt mainly to weightbearing. Natural selection on flight energetics may favour loss of hind-limb mass at the risk of reducing the safety margin for catastrophic failure. Consequently, volant birds have lighter femora in response to an extreme reduction in hind-limb loading (Habib and Ruff, 2008). Primarily cursorial birds are terrestrial organisms and so their bones are built strong enough to support their weight and withstand mechanical stresses during locomotion (McMahon, 1973). Cursorial birds are usually on their feet and, unlike volant birds that can take flight at the sight of danger, cursors must flee on foot. To escape predation, flightless birds must be agile and have a great capacity to run. Cursorial femora must be sufficiently large and robust to resist the bending, torsion and compression stresses associated with fast terrestrial locomotion.

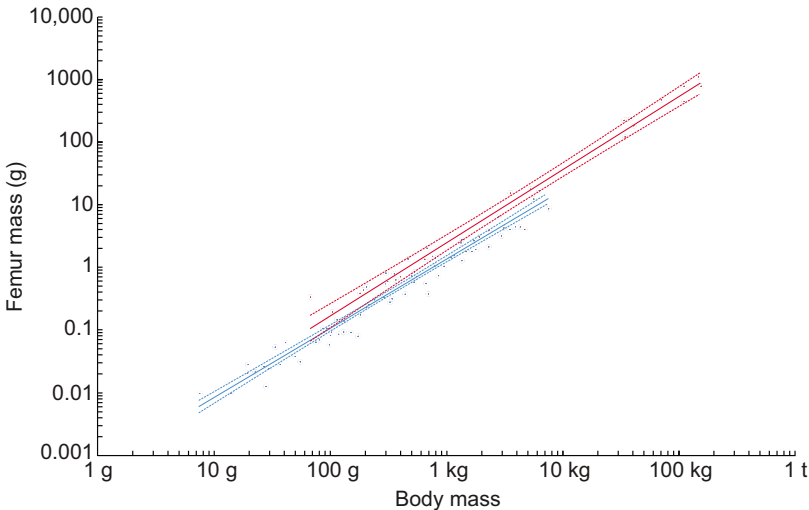
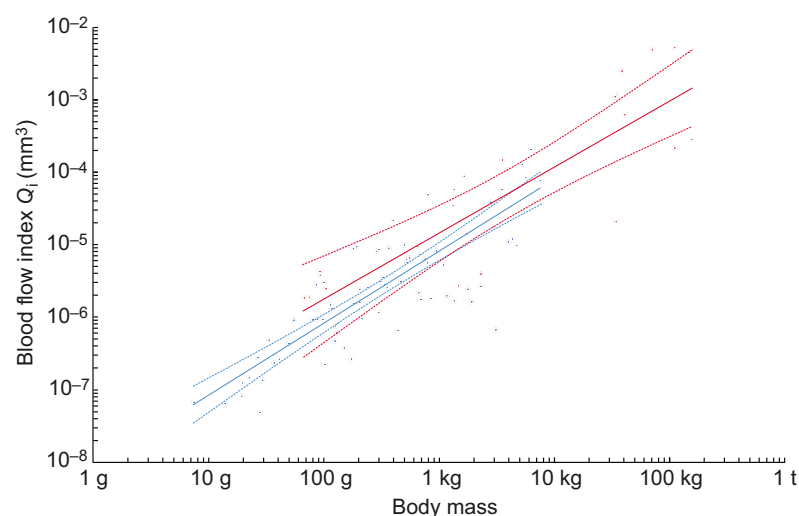


Fig. 2. Relationship between femur mass ( $M_f$ , g) and body mass ( $M_b$ , g) in cursorial birds (red circles,  $M_f=8.34\times10^{-4}M_b^{1.16\pm0.08}$ ) and volant birds (blue circles,  $M_f=6.68\times10^{-4}M_b^{1.10\pm0.06}$ ). Statistics as in Fig. 1.



**Fig. 3. Relationship between blood flow index ( $Q_i$ , mm<sup>3</sup>) and body mass ( $M_b$ , g) in cursorial birds (red circles,  $Q_i = 3.61 \times 10^{-8} M_b^{0.89 \pm 0.25}$ ) and volant birds (blue circles,  $Q_i = 8.49 \times 10^{-9} M_b^{0.99 \pm 0.14}$ ). Statistics as in Fig. 1.**

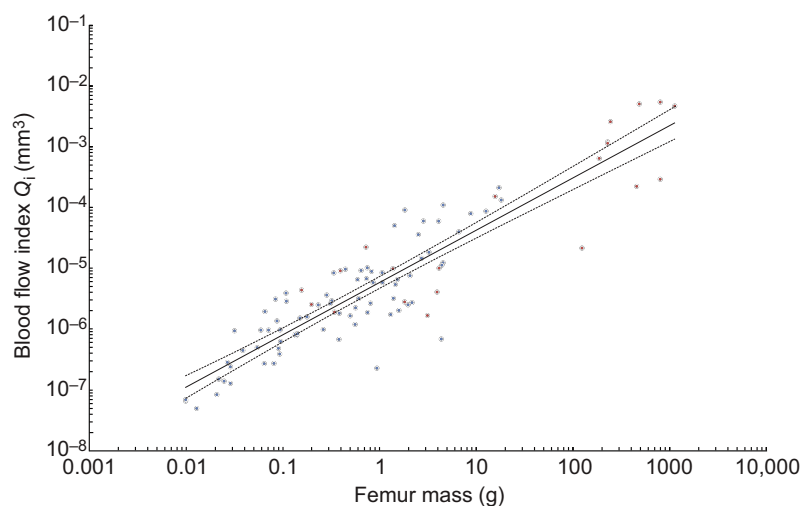
### Scaling of femur perfusion

The allometric equation from 108 species of extant and extinct birds showed that the femoral blood flow index  $Q_i$  increases with body mass,  $M_b$ , with an exponent of 1.00, indicating a direct proportionality (Fig. 1). However, when the species are divided into groups on the basis of the primary mode of locomotion, the exponent appears an artefact of an uneven distribution of locomotory modes among the species, with primarily volant species being smaller than primarily cursorial ones. When the locomotor modes were analysed separately, there was no significant difference in exponents, but cursorial species had significantly higher  $Q_i$  (Fig. 3).

A higher  $Q_i$  at a given body mass in cursorial birds is consistent with larger bones and possibly a higher frequency of micro-fractures that require repair. When  $Q_i$  was related to femur mass  $M_f$ , the difference between cursorial and volant birds was lost, and  $Q_i$  is related to the amount of bone rather than mode of locomotion (Fig. 4). The exponent of  $Q_i$  on  $M_f$  for all birds (0.87) is significantly less than 1.0, indicating that  $Q_i$  is not directly proportional to bone mass. This suggests that the mass-specific frequency of micro-fractures decreases in birds with larger femora, but the absolute frequency increases.

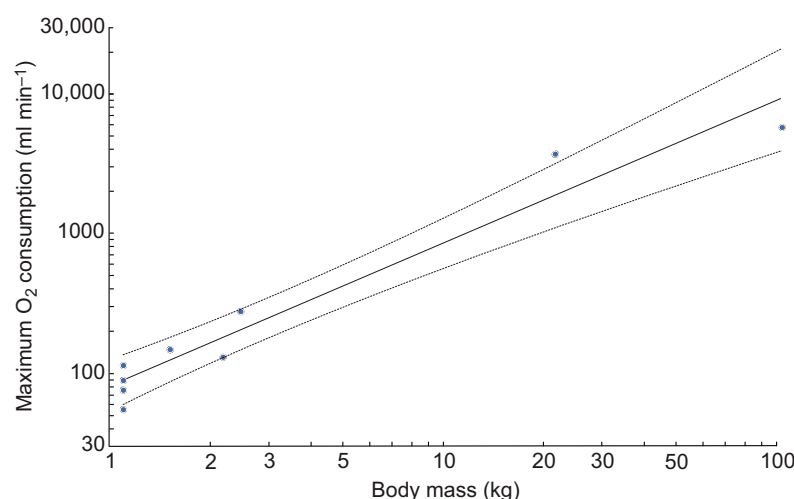
The scaling exponent of  $Q_i$  on  $M_b$  in cursorial birds (0.89) is similar to the exponent in mammals (0.86), which is indistinguishable from the exponent for MMR during terrestrial

locomotion in mammals (Ellerby et al., 2003; Seymour et al., 2012). Unfortunately, most attempts to determine the scaling of MMR in birds have involved measures made during flight or cold exposure, which are unrelated to terrestrial locomotion (Bishop, 1999; Hinds et al., 1993; Norberg, 1996; Wiersma et al., 2007). Therefore, the scaling exponents from these avian studies (between 0.62 and 0.80) are not comparable. Studies of MMR during treadmill exercise in brush turkeys *Alectura lathami* during ontogeny reveal an exponent of 1.07 (Seymour et al., 2008), but this is also not comparable to our interspecific study on adults. Therefore, we collected data on the highest rates of oxygen consumption ( $\dot{V}_{O_2}$ , ml min<sup>-1</sup>) that had been measured during treadmill exercise in birds weighing 1.1–103 kg. The MMR during treadmill exercise scales according to the allometric equation,  $\dot{V}_{O_2} = 81 M_b^{1.02 \pm 0.22}$  ( $r^2 = 0.95$ ) (Fig. 5). The birds include domestic fowl *Gallus gallus* var. *domesticus* (Brackenbury and Avery, 1980; Brackenbury et al., 1981), red jungle fowl *Gallus gallus* (Hammond et al., 2000), helmeted guineafowl *Numida meleagris* (Ellerby et al., 2003), malleefowl *Leipoa ocellata* (Weathers et al., 1993), brush turkey *Alectura lathami* (Seymour et al., 2008), rhea *Rhea americana* (Bundle et al., 1999) and ostrich *Struthio camelus* (Fedak and Seeherman, 1979). The exponent for  $Q_i$  in cursorial birds (0.89) is not significantly different from this exponent for MMR in cursorial species running on a treadmill, which is similar to the results for mammals.



**Fig. 4. Relationship between blood flow index ( $Q_i$ , mm<sup>3</sup>) and femur mass ( $M_f$ , g) in cursorial birds (red circles) and volant birds (blue circles). The equation for the regression of all species is  $Q_i = 5.96 \times 10^{-6} M_f^{0.87 \pm 0.07}$ . Statistics as in Fig. 1.**





**Fig. 5. Relationship between maximum metabolic rate, measured as oxygen consumption ( $\text{ml min}^{-1}$ ) during treadmill exercise, and body mass in birds.** The equation for the line is  $\dot{V}_{\text{O}_2} = 81M_b^{1.02 \pm 0.22}$ . Statistics as in Fig. 1. Sources of the data are given in the text.

## Moa

The data for femur mass in moa fell around the extrapolated line for living cursorial birds (Fig. 2). To some extent this is an expected result, because body mass is calculated from bone length and circumference. The body mass may be overestimated in *Pachyornis elephantopus*, a moa with such grossly heavy legs that Alexander (Alexander, 1983) proposed that their skeleton might have resulted from a lack of selection for locomotory efficiency in a largely sedentary bird without predators. That idea finds support if femur mass and  $Q_i$  are compared among moa. Although *P. elephantopus* has the largest femur mass, its  $Q_i$  value is approximately the same as in *Emeus crassus*, which has a femur less than half the mass, suggesting that *P. elephantopus* did not have much demand for femur repair. A striking feature of the data from moa is the order of magnitude differences in  $Q_i$  between the eight moa species (Fig. 1). The two lowest  $Q_i$  points are from *Dinornis struthoides* and *Dinornis robustus*, species with relatively long and thin legs (Alexander, 1983). Such great structural differences among moa may relate to the habitats that they occupied. Moa were browsers, consuming herbs and low shrubs (Wood et al., 2008; Worthy and Scofield, 2012). A potential explanation for the large difference in  $Q_i$  is that *Dinornis* may have occupied open habitat, whereas other moa foraged in closed forests (Bunce et al., 2009). We speculate that dodging and weaving through dense vegetation places stresses on the femur that result in heavier legs and higher bone perfusion than moving in the open.

## Comparison with mammals, reptiles and dinosaurs

Our analysis reveals that the size of the femur nutrient foramen of birds increases with body mass in a pattern similar to that of mammals. Under the assumption that the maximum blood flow rate through the foramina is proportional to  $Q_i$ , then the avian data are not significantly different from the mammalian data (Fig. 1). However, the estimation of  $Q_i$  is based on morphological features only, and birds and mammals differ in one important physiological trait that influences blood flow – blood pressure. The mean systemic arterial blood pressure is approximately 135 mmHg in a 1 kg bird and 91 mmHg in a 1 kg mammal (Seymour and Blaylock, 2000). If we multiply together the allometric equations for  $Q_i$  and the mean blood pressure, the estimated femur blood flow in cursorial birds scales with body mass to the 0.90 power, and mammals to the 0.91 power, but the elevation is 1.9 times higher in birds than it is in mammals at 1 kg. In addition, more blood enters the pneumatic foramen in some species. Part of this difference could be explained

by birds being bipedal, supporting their weight on two limbs as opposed to four in quadrupedal mammals. Mammalian posture also changes as body size increases, from crouched to upright, and becomes more affected by bending and compression stresses (Bertram and Biewener, 1990). In contrast, avian femora are held almost horizontally, which makes them more susceptible to torsion (Carrano and Biewener, 1999). The architecture and microstructure in the avian femur must be adapted to withstand these torsional stresses, and probably as a result, avian femora are short and robust, providing structures that resist torsion to a much greater extent than the long, slim tibiotarsus (Gates, 1991).

Our previous study showed that  $Q_i$  is approximately 10-fold higher in mammals than in reptiles and, given differences in blood pressure and  $\text{O}_2$  carrying capacity, bone oxygenation is approximately 50 times higher (Seymour et al., 2012). Because the actual blood flow to the avian femur is estimated to be approximately twice that for mammals, the oxygen flux approaches 100 times higher in birds than in reptiles. The difference is attributed to the fact that, except in varanid lizards, reptiles generally do not remodel their bones in response to loading and exercise. The limb bone safety factor for reptiles is twice as high as those calculated for mammals and birds (Blob and Biewener, 1999). Because reptiles have such high safety factors, and because they spend relatively little time undertaking strenuous exercise, their bones rarely succumb to damage and so would not require as much blood flow for remodelling and repair.

The data for  $Q_i$  in extant and extinct birds sets a trajectory that can be extrapolated to the body mass of dinosaurs (Fig. 1). The avian regression becomes insignificantly different from dinosaurs at body masses greater than approximately 1.7 tonnes. It is clear that the data from dinosaurs are presently sparse and variable, and that, with the limited statistical power, the exponent is not different from zero. More data from dinosaurs are required to confirm the high  $Q_i$  values of the smaller species and to test whether the dinosaur pattern is indeed different from the pattern observed in living and extinct birds.

## Limitations in the use of $Q_i$ for birds

The present study is the first to relate foreman size to metabolic rate and locomotory mode in birds. Our inductive approach provides hypotheses that reasonably describe the relationships observed in the data. However, further research is required to underpin the functional relationships between locomotion, bone remodelling and bone perfusion. The introduction considers some general limitations

associated with the use of  $Q_i$  as an index of blood flow rate, but there are two other specific aspects of avian life history that should be considered.

One of the important functions of bone perfusion that separates birds and mammals is the production of calcareous eggs in birds (Dacke et al., 1993). Bone is the major calcium store in the body, and egg production requires mobilization of those stores. Foramen size may be influenced by this requirement in females that lay down and resorb medullary bone with each reproductive cycle. If so, some of the variability in our data may be a gender effect. Unfortunately, our study could not identify males and females, because the gender of museum specimens was rarely indicated. Further work on a few species could clarify the issue. If there are significant differences between the foramina of males and females, then foramina size could potentially be used as a gender indicator for extinct species such as the moa.

The pneumatic foramen in the long bones of birds is large, and the airways are accompanied by a pneumatic artery (Beaumont, 1968), which raises the possibility that the pneumatic artery could supply the long bone with a significant amount of blood. During ontogeny, the pneumatic artery sends blood to the metaphyseal and epiphyseal growth cartilages, but once pneumatic invasion of the bone has ceased, the pneumatic artery becomes restricted to the top one-third of the bone. Thus it is likely that the nutrient artery remains the primary source of blood for remodelling and repair in pneumatized femora (Beaumont, 1968). Furthermore, a study of 24 avian species revealed that, although 70% of humeri were pneumatized, only 39% of femora were pneumatized (Cubo and Casinos, 2000). Future studies could better test the influence of pneumatization on nutrient foramina by comparing birds that are known to have pneumatized bones to those that do not undergo pneumatization, such as the Charadriiformes (Cubo and Casinos, 2000).

## Conclusion

Our study provides evidence of a functional relationship between the size of the nutrient foramen on the femoral shaft and the locomotor activity of birds. The scaling exponent of the blood flow index on body mass is similar in birds and mammals, and is parallel to the relation between MMR during terrestrial locomotion and body mass. The elevations for the two endothermic groups overlap, but when the higher blood pressure in birds is considered, the femora of bipedal birds are potentially perfused at approximately twice the rate of the femora of quadrupedal mammals, presumably in relation to the extent of bone remodelling after exercise. The internal shafts of reptile femora receive approximately 2 orders of magnitude less blood than do bird femora, as most reptiles do not remodel their long bones with exercise.

## MATERIALS AND METHODS

Morphometric data were obtained from museum specimens of 100 extant bird species (268 individuals) and eight extinct New Zealand moa species (55 individuals). Femur maximum length, mass and volume (by displacement of glass beads or rice in a cylinder) were determined, and the area of the nutrient foramen was measured from digital photographs of known scale, according to previous methods (Seymour et al., 2012). To minimise measurement error due to the nutrient artery's oblique angle of entry into the bone, the femur was angled so that the foramen appeared as circular as possible. The left and right femora were measured for each individual, and where possible replicate measurements for each species were taken. Each datum for a species is a grand mean that represents one femur. Only adult bones were measured to circumvent error due to ontogenetic changes in blood supply via bone growth and haematopoiesis that occur, as

has been demonstrated in rats (Brookes, 1967). Sexual dimorphism is present in many avian species, so both males and females were measured when possible. Data on adult body mass and mode of locomotion were taken from literature on extant birds (del Hoyo et al., 1992; Higgins et al., 2006; <http://www.parrots.org/>). Body mass for moa was calculated as the mean of three allometric equations based on femur length (Prange et al., 1979) and circumference (Campbell and Marcus, 1992; Dickison, 2007).

Birds were classified as primarily volant or cursorial based on their descriptions in the Handbook of Australian, New Zealand and Antarctic Birds, known as HANZAB (Higgins et al., 2006). Species that could not be conclusively categorised were excluded from the locomotion analyses. Measured femur variables ( $Y$ ) were analysed allometrically in relation to body mass ( $M_b$ ) to produce power equations of the form  $Y = aM_b^b$ , where  $a$  is the elevation and  $b$  is the exponent. Ordinary least square regressions were performed on  $\log_{10}$ -transformed data and 95% confidence intervals for the regression means were obtained. Regressions for birds were compared with mammals, reptiles and dinosaurs from our previous study (Seymour et al., 2012) using ANCOVA (Zar, 1998) and, if the exponents differed, the Johnson–Neyman technique identified the body mass range over which the regressions differed (White, 2003).

Avian phylogenetic trees were constructed online (birdtree.org) based on data from the complete avian phylogeny of Jetz et al. (Jetz et al., 2012) and using the primary backbone tree of Hackett et al. (Hackett et al., 2008). One thousand trees were constructed, and pgls models were conducted using the package 'caper' (Orme et al., 2011) in the statistical software program R (R Software, version 2.12.0, Vienna, Austria, <http://www.R-project.org>). Five phylogenetic models were compared in which  $\log_{10} Q_i$  was the response variable and the model set included all combinations of the following explanatory variables:  $\log_{10}$  body mass, locomotion (flying or cursorial) and the interaction of  $\log_{10}$  body mass and locomotion. The Akaike information criterion corrected for small sample sizes (AICc) and was used to assess the best relative model (Burnham and Anderson, 2002). The phylogenetic signal was measured using Pagel's  $\lambda$  (Pagel, 1999). This value indicates the strength of the phylogenetic relationship, where  $\lambda$  lies between 0 and 1. If  $\lambda$  lies near 0, it indicates phylogenetic independence and values near 1 indicate that the variable is related to evolutionary history (Freckleton et al., 2002).

## Acknowledgements

We extend our gratitude to Phillipa Horton, Trevor Worthy and Mary-Anne Binnie from the South Australian Museum and Ross Sadler and Yong Yi Zhen from the Australian Museum for access to the collections. Matthew Golebiowski and Shannyn Siemens helped obtain some of the data. Trevor Worthy provided advice concerning the biology of moa.

## Competing interests

The authors declare no competing financial interests.

## Author contributions

G.H.A. is largely responsible for the whole project, working from a concept from R.S.S. P.C. carried out the phylogenetic analyses. All authors drafted and edited the manuscript.

## Funding

The project was funded by the Australian Research Council Discovery grant [DP-120102081 to R.S.S. and S.K.M.].

## Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.102889/-/DC1>

## References

- Alexander, R. M. (1983). Allometry of the leg bone of moas (Dinornithes) and other birds. *J. Zool.* **200**, 215–231.
- Alexander, R. M., Jayes, A. S., Maloiy, G. M. O. and Wathuta, E. M. (1981). Allometry of the leg muscles of mammals. *J. Zool.* **194**, 539–552.
- Beaumont, G. D. (1968). Vascular factors in pneumatization. *J. Laryngol. Otol.* **82**, 1067–1082.
- Bennett, M. B. (1996). Allometry of the leg muscles of birds. *J. Zool.* **238**, 435–443.
- Berry, C. L. (1978). Hypertension and arterial development. Long-term considerations. *Br. Heart J.* **40**, 709–717.
- Bertram, J. E. A. and Biewener, A. A. (1990). Differential scaling of the long bones in the terrestrial Carnivora and other mammals. *J. Morphol.* **204**, 157–169.

- Bingmann, D. and Wiemann, M. (2007). O<sub>2</sub>-consumption, blood flow and PO<sub>2</sub> in bone. *Materialwiss. Werkstofftech.* **38**, 950-954.
- Birchard, G. F. (1997). Optimal hematocrit: theory, regulation and implications. *Am. Zool.* **37**, 65-72.
- Bishop, C. M. (1999). The maximum oxygen consumption and aerobic scope of birds and mammals: getting to the heart of the matter. *Proc. R. Soc. B* **266**, 2275-2281.
- Blob, R. W. and Biewener, A. A. (1999). *In vivo* locomotor strain in the hindlimb bones of *Alligator mississippiensis* and *Iguana iguana*: implications for the evolution of limb bone safety factor and non-sprawling limb posture. *J. Exp. Biol.* **202**, 1023-1046.
- Brackenburg, J. H. and Avery, P. (1980). Energy consumption and ventilatory mechanisms in the exercising fowl. *Comp. Biochem. Physiol.* **66A**, 439-445.
- Brackenburg, J. H., Avery, P. and Gleeson, M. (1981). Respiration in exercising fowl. I. Oxygen consumption, respiratory rate and respired gases. *J. Exp. Biol.* **93**, 317-325.
- Brookes, M. (1967). Blood flow rates in compact and cancellous bone, and bone marrow. *J. Anat.* **101**, 533-541.
- Brookes, M. and Revell, W. J. (1998). *Blood Supply of Bone*. London: Springer.
- Bunce, B., Worthy, T. H., Phillips, M. J., Holdaway, R. N., Willerslev, E., Haile, J., Shapiro, B., Scofield, R. P., Drummond, A., Kamp, P. J. et al. (2009). The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. *Proc. Natl. Acad. Sci. USA* **106**, 20646-20651.
- Bundle, M. W., Hoppeler, H., Vock, R., Tester, J. M. and Weyand, P. G. (1999). High metabolic rates in running birds. *Nature* **397**, 31-32.
- Burr, D. B. (2002). Targeted and nontargeted remodeling. *Bone* **30**, 2-4.
- Burnham, K. P. and Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach*. New York: Springer.
- Campbell, K. E., Jr and Marcus, L. (1992). The relationship of hindlimb bone dimensions to body weight in birds. *Natural History Museum of Los Angeles County Science Series* **36**, 395-412.
- Carrano, M. T. and Biewener, A. A. (1999). Experimental alteration of limb posture in the chicken (*Gallus gallus*) and its bearing on the use of birds as analogs for dinosaur locomotion. *J. Morphol.* **240**, 237-249.
- Cubo, J. and Casinos, A. (2000). Incidence and mechanical significance of pneumatization in the long bones of birds. *Zool. J. Linn. Soc.* **130**, 499-510.
- Currey, J. D. (2003). The many adaptations of bone. *J. Biomech.* **36**, 1487-1495.
- Dacke, C. G., Arkle, S., Cook, D. J., Wormstone, I. M., Jones, S., Zaidi, M. and Bascal, Z. A. (1993). Medullary bone and avian calcium regulation. *J. Exp. Biol.* **184**, 63-88.
- del Hoyo, J., Elliott, A. and Sargatal, J. (1992). *Handbook of the Birds of the World*, Volume 1. Barcelona: Lynx Edicions.
- Dickson, M. R. (2007). The allometry of giant flightless birds. In *Department of Biology*, pp. 20-57. PhD thesis, Duke University, Durham, NC, USA.
- Doube, M., Yen, S. C. W., Kłosowski, M. M., Farke, A. A., Hutchinson, J. R. and Shafelbine, S. J. (2012). Whole-bone scaling of the avian pelvic limb. *J. Anat.* **221**, 21-29.
- Ellerby, D. J., Cleary, M., Marsh, R. L. and Buchanan, C. I. (2003). Measurement of maximum oxygen consumption in Guinea fowl *Numida meleagris* indicates that birds and mammals display a similar diversity of aerobic scopes during running. *Physiol. Biochem. Zool.* **76**, 695-703.
- Fedak, M. A. and Seeherman, H. J. (1979). Reappraisal of energetics of locomotion shows identical cost in bipeds and quadrupeds including ostrich and horse. *Nature* **282**, 713-716.
- Foot, J. S. (1911). The comparative histology of femoral bones. *Trans. Am. Microsc. Soc.* **30**, 87-140.
- Freckleton, R. P., Harvey, P. H. and Pagel, M. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**, 712-726.
- Gatesy, S. M. (1991). Hind-limb scaling in birds and other theropods: implications for terrestrial locomotion. *J. Morphol.* **209**, 83-96.
- Habib, M. B. and Ruff, C. B. (2008). The effects of locomotion on the structural characteristics of avian limb bones. *Zool. J. Linn. Soc.* **153**, 601-624.
- Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C. K., Braun, E. L., Braun, M. J., Chojnowski, J. L., Cox, W. A., Han, K. L., Harshman, J. et al. (2008). A phylogenomic study of birds reveals their evolutionary history. *Science* **320**, 1763-1768.
- Hammond, K. A., Chappell, M. A., Cardullo, R. A., Lin, R. and Johnsen, T. S. (2000). The mechanistic basis of aerobic performance variation in red junglefowl. *J. Exp. Biol.* **203**, 2053-2064.
- Higgins, P., Peter, J., Cowling, S., Steele, W. and Davies, S. (2006). *Handbook of Australian, New Zealand and Antarctic Birds (HANZAB)*. Melbourne: Oxford University Press.
- Hinds, D. S., Baudinette, R. V., MacMillen, R. E. and Halpern, E. A. (1993). Maximum metabolism and the aerobic factorial scope of endotherms. *J. Exp. Biol.* **182**, 41-56.
- Jaworski, Z. F. G. (1991). Haversian systems and haversian bone. In *Bone*, Vol. 4 (ed. B. K. Hall), pp. 21-45. Boca Raton, FL: CRC Press.
- Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. and Moores, A. O. (2012). The global diversity of birds in space and time. *Nature* **491**, 444-448.
- Lehoux, S. and Tedgui, A. (2003). Cellular mechanics and gene expression in blood vessels. *J. Biomech.* **36**, 631-643.
- Lieberman, D. E., Pearson, O. M., Polk, J. D., Demes, B. and Crompton, A. W. (2003). Optimization of bone growth and remodeling in response to loading in tapered mammalian limbs. *J. Exp. Biol.* **206**, 3125-3138.
- McMahon, T. (1973). Size and shape in biology. *Science* **179**, 1201-1204.
- Norberg, U. M. (1996). Energetics of flight. In *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 199-249. New York, NY: Chapman & Hall.
- Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S. and Isaac, N. (2011). Caper: Comparative Analyses of Phylogenetics and Evolution in R. Available at <http://CRAN.R-project.org/package=caper>.
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature* **401**, 877-884.
- Parfitt, A. M. (1994). Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone. *J. Cell. Biochem.* **55**, 273-286.
- Pontón, F., Montes, L., Castanet, J. and Cubo, J. (2007). Bone histological correlates of high-frequency flapping flight and body mass in the furculae of birds: a phylogenetic approach. *Biol. J. Linn. Soc. Lond.* **91**, 729-738.
- Prange, H. D., Anderson, J. F. and Rahn, H. (1979). Scaling of skeletal mass to body mass in birds and mammals. *Am. Nat.* **113**, 103-122.
- Robling, A. G., Castillo, A. B. and Turner, C. H. (2006). Biomechanical and molecular regulation of bone remodeling. *Annu. Rev. Biomed. Eng.* **8**, 455-498.
- Schirmacher, K., Lauterbach, S. and Bingmann, D. (1997). Oxygen consumption of calvarial bone cells in vitro. *J. Orthop. Res.* **15**, 558-562.
- Seymour, R. S. and Blaylock, A. J. (2000). The principle of laplace and scaling of ventricular wall stress and blood pressure in mammals and birds. *Physiol. Biochem. Zool.* **73**, 389-405.
- Seymour, R. S., Runciman, S. and Baudinette, R. V. (2008). Development of maximum metabolic rate and pulmonary diffusing capacity in the superprecocial Australian Brush Turkey *Alectura lathami*: an allometric and morphometric study. *Comp. Biochem. Physiol.* **150A**, 169-175.
- Seymour, R. S., Smith, S. L., White, C. R., Henderson, D. M. and Schwarz-Wings, D. (2012). Blood flow to long bones indicates activity metabolism in mammals, reptiles and dinosaurs. *Proc. R. Soc. B* **279**, 451-456.
- Sim, F. H. and Kelly, P. J. (1970). Relationship of bone remodeling, oxygen consumption, and blood flow in bone. *J. Bone Joint. Surg. Am.* **52**, 1377-1389.
- Trueta, J. (1963). The role of the vessels in osteogenesis. *J. Bone Joint. Surg. Am.* **45**, 402-418.
- Ward, M. R., Pasterkamp, G., Yeung, A. C. and Borst, C. (2000). Arterial remodeling. Mechanisms and clinical implications. *Circulation* **102**, 1186-1191.
- Weathers, W. W., Seymour, R. S. and Baudinette, R. V. (1993). Energetics of mound-tending behaviour in the malleefowl, *Leipoa ocellata* (Megapodiidae). *Anim. Behav.* **45**, 333-341.
- White, C. R. (2003). Allometric analysis beyond heterogeneous regression slopes: use of the Johnson-Neyman technique in comparative biology. *Physiol. Biochem. Zool.* **76**, 135-140.
- Wiersma, P., Chappell, M. A. and Williams, J. B. (2007). Cold- and exercise-induced peak metabolic rates in tropical birds. *Proc. Natl. Acad. Sci. USA* **104**, 20866-20871.
- Wolff, J. (1892). *Das Gesetz der Transformation der Knochen*. Berlin: A. Hirshwald.
- Wood, J. R., Rawlence, N. J., Rogers, G. M., Austin, J. J., Worthy, T. H. and Cooper, A. (2008). Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes). *Quat. Sci. Rev.* **27**, 2593-2602.
- Worthy, T. H. and Scofield, R. P. (2012). Twenty-first century advances in knowledge of the biology of moa (Aves: Dinornithiformes): a new morphological analysis and moa diagnoses revised. *N. Z. J. Zool.* **39**, 87-153.
- Zar, J. H. (1998). *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall.

**Table S1.**

**[Download Table S1](#)**