

An allometric analysis of the cardiac and
pulmonary systems of the blue wildebeest
(*Connochaetes taurinus taurinus*)

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Declaration

I, David Gordon Roberts, declare that the thesis, which I hereby submit for the degree Master of Science (Veterinary Science) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Abstract

This study describes the growth of the blue wildebeest (*Connochaetes taurinus taurinus*) cardiac and pulmonary systems with ontogenetic allometric equations, contributing an additional species to the small collection of ontogenetic allometric studies on mammals.

Allometric equations of the form $y = ax^b$ were derived to describe the changes in cardiac or pulmonary measurements (y), with change in body mass and heart or lung mass (x). The body masses used in these equations were calculated from external body measurements, to compensate for temporary changes in individual body mass. Different mass estimation equations were investigated to choose the most suitable method.

Forty-three post-natal blue wildebeest (22 male, 21 female; body mass 74 - 258 kg), and five foetuses, with (12 and 15 kg; gestational age approximately 7 months) were measured. All animals were sourced from culling operations on the Buby Valley Conservancy in southern Zimbabwe, in different seasons, over three years.

Body mass (M_b) in kilograms was most accurately predicted from body length (L) and girth (G) in meters: $M_b = 39.08(LG^2)^{0.909}$ ($R^2 = 0.935$, $PPE_A=8.2\%$) or $M_b = 64.34G^{2.58}$ ($R^2 = 0.911$, $PPE_A=9.4\%$). These equations are mildly affected by both sex and season. The equations predict significantly lower body masses ($p<0.001$) than similar equations for East African subspecies.

Blue wildebeest heart mass (M_h) scales hypoallometrically with body mass and ranges from approximately 0.8% of body mass in the foetus to 0.5% of body mass in adult. Heart growth follows the allometric equation: $M_h = 0.0118M_b^{0.832}$ ($R^2 = 0.846$, $PPE_A=10.9\%$) in the post-natal animal.

While the dimensions of the left heart grow isometrically with heart mass, the thickness of the right ventricular wall does not appear to change significantly after birth. This change in thickness may be due to the change in the function of the right ventricle at birth.

The lung mass in kg (M_l) and tracheal volume in ml (V_t) follow the allometric equations: $M_l = 0.0260M_b^{0.816}$ ($R^2 = 0.71$, $PPE_A=16$) and $V_t = 3.31M_b^{0.905}$ ($R^2 = 0.78$, $PPE_A=16$) in the post-natal animal, which are not significantly different from isometry ($p>0.05$). The relative lung masses of foetal wildebeest are significantly greater and the foetal trachea relatively narrower than in post-natal animals.

Growing wildebeest heart masses are lower than found in horses and giraffes, larger than in buffalo and similar to those of cattle of the same body mass. Their lung masses are smaller than those of horses but larger than those of giraffes.

Interspecific equations for mammals predict larger heart and lung masses than measured in post-natal wildebeest, but not significantly so (heart: $p=0.11$ and lung: $p=0.83$). However, the difference between measured and predicted heart mass, when only adult wildebeest are considered, is statistically significant ($p=0.016$).

In conclusion, blue wildebeest body mass can be predicted from external body measurements and heart, lung and tracheal measurements scale with body mass. Heart growth is hypoallometric to body mass and lung mass scales isometrically with body mass. Throughout growth, the heart and lung masses of wildebeest are not different from those predicted for average mammals.

Dedication

This dissertation is dedicated to the memory of Professor John Skinner, a research icon, a mentor and a friend.



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Ethics Statement

The author, whose name appears on the title page of this thesis, has obtained, for the research described in this work, the applicable research ethics approval from the Animal Ethics Committee of the University of Pretoria. The project approval was granted under project number: V077-13.

The author declares that he has observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the policy guidelines for responsible research.



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Chapter 1: Introduction

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The Blue Wildebeest

Five subspecies of blue wildebeest (*Connochaetes taurinus* Burchell, 1823) are recognised, each occurring in different areas. Four of these occur in Central and East Africa: Cookson's wildebeest, *C. t. cooksoni* (Blaine 1914); the Nyassa or Johnston's wildebeest, *C. t. johnstoni* (Sclater 1896); the eastern white-bearded wildebeest, *C. t. albojubatus* (Tomas 1892), and the western white-bearded wildebeest, *C. t. mearnsi* (Heller 1913). *C. t. taurinus*, the blue wildebeest or brindled gnu, is the only one of the sub species to naturally occur in Southern Africa. They occur mainly south and west of the Zambezi River, from southern Angola and south-western Zambia, through north-eastern Namibia Botswana and south-western Zimbabwe to Mozambique and South Africa, north of the Orange river (Whyte, 1985; Grubb, 2005; Skinner & Chimimba, 2005; Estes, 2014).

In this study, the term wildebeest should be taken to refer to the blue wildebeest (*Connochaetes taurinus*) and not the related, black wildebeest (*Connochaetes gnou*). Much of the research on blue wildebeest does not refer to the subspecies directly, although it can usually be deduced from the location of each study, most aspects of their behaviour and physiology are likely to be common to the entire species.

Blue wildebeest play a key role in the grassland ecosystems which they inhabit. Due to their high population numbers and body sizes. The biomass of blue wildebeest in their preferred habitat is greater than that of any other mammal (Talbot & Talbot, 1963). Historically, populations were far higher than today. Massive reduction in local wildebeest populations, of up to 90% in a single season have occurred on separate occasions in different parts of their range, caused by several interconnected factors (Whyte & Joubert, 1988; East, 1999; Apps, 2000; Mbaiwa & Mbaiwa, 2006). The most important of these include the erection of fences, hunting and competition with livestock; compounded by the natural phenomena of drought, disease and predation. (Spinage, 1992; East, 1999; Skinner & Chimimba, 2005; Tambling & du Toit, 2005; Bolger & Newmark, 2008). Wildebeest are particularly susceptible to fencing and other interruptions to historical migratory routes, which is in part because wildebeest are dependent on surface water, needing to drink daily or at least every second day and therefore can only graze up to 15 km from water (Estes, 2012).

Blue wildebeest are well known for their impressive migrations. The most well-known of these which still occur today are those in the Serengeti ecosystem of east Africa which cover approximately 1500km a year, an average of 4.25km per day (Estes, 2014). Although many wildebeest populations are now forced to be more sedentary due to the loss of natural migration routes, their physiology and anatomy is still that of an animal capable of and well-adapted to travelling long distances. Wildebeest are capable of sustained cantering over a several kilometres at speeds of 3-6m/s (Pennycuick, 1975; Perry *et al.*,

1988) and can reach top speeds of between 14 and 22m/s (Howell, 1965; Alexander, Langman & Jayes, 1977; Elliott, Cowan & Holling, 1977).

The blue wildebeest has a remarkably well-synchronised breeding and calving season. In a study of twelve African ungulates (Sinclair, Mduma & Arcese, 2000) the wildebeest had the highest birth synchrony. In East Africa 80-90% of calves are born in a three week period during the month before the most reliable rainfall (Estes, 1976, 2012). In southern Africa births are also highly synchronised but not as tightly as in East Africa (Sinclair, 1977).

The remarkable birth synchrony of the blue wildebeest is achieved through a tight synchrony of oestrus and high conception rates during the first oestrus cycle of the season. Breeding occurs at the end of the rainy season approximately 240 days before calving (Estes, 1966; Clay *et al.*, 2010). Oestrus synchronisation in blue wildebeest cows has been linked to photoperiod, rainfall and available nutrition, lunar cycles and male behaviour and vocalisation (Leuthold & Leuthold, 1975; Sinclair, 1977; Clay *et al.*, 2010). Females conceive for the first time at either 16 or 28 months (Estes, 2012).

Blue wildebeest calves are the most precocious of all ungulates at birth (Lent, 1974). All alcelaphine antelope, except the hartebeest, have calves that can follow the mother from the first hour after birth, but the wildebeest calf is exceptional. A calf will stand and can run to keep up with the herd within an average of 7 minutes from birth (Estes, 1966, 1976).

Both the short calving season and the follower calf strategy can be explained as adaptations to the migratory habits of the blue wildebeest, their preference for short grass and their tendency to form large herds. These characteristics make concealing young calves impractical (Estes, 2012). Calves born before the peak calving season or out of the calving season are unlikely to survive predation. Calves born after the calving peak have higher chances of survival than those born before as it is harder for predators to distinguish them from the older calves present in the herd (Estes, 1966, 1976). The light fawn colour of the calves gradually changes to the adult colouration between two and three months of age. The chief predator of wildebeest calves, the spotted hyena (*Crocuta crocuta*), almost exclusively hunts calves younger than 2 days old. After this age, they are far more difficult to catch. The main predator avoidance technique employed by wildebeest calves is for them to run from predators and become lost among their conspecifics. Small herds have far lower calf survival rates (50%) than large herds (84%) (Estes, 1976).

Other research on the wildebeest has been primarily on behaviour (Estes, 1966, 1969; Leuthold & Leuthold, 1975; Berry, Siegfried & Crowe, 1982; Whyte, 1985; Murray, 1995), migration (Williamson, Williamson & Ngwamotsoko, 1988; Murray, 1995; Bolger & Newmark, 2008) and population ecology, often concentrating on massive die offs (Talbot & Talbot, 1963; Watson, 1967; Child, 1972; Braack, 1973; Berry, 1981; Attwell, 1982a; Whyte, 1985; Williamson & Williamson, 1985; Parry, 1987; Whyte

& Joubert, 1988; Williamson *et al.*, 1988; Spinage, 1992; Mduma, Sinclair & Hilborn, 1999; Tambling & du Toit, 2005; Grubb, 2005; Mbaiwa & Mbaiwa, 2006; Hopcraft, 2010; Kohn, Curry & Noakes, 2011; Hopcraft *et al.*, 2013).

The growth and body size of the blue wildebeest has been studied in relation to age determination (Spinage, 1973; Attwell, 1980; Attwell & Jeffery, 1981) and the plotting of growth with age (Talbot & Talbot, 1963; Attwell, 1982b; Hopcraft, 2010). Little else is known about the anatomy or physiology of the blue wildebeest. No studies on the cardiovascular physiology of wildebeest and no records of cardiovascular variables for wildebeest have been published.

Only three references to the respiratory system of the wildebeest could be found. Two are reported from a single set of related studies. Two wildebeest of body masses 92 and 104 kg were trained to run on a treadmill and the maximum rate of oxygen consumption that they achieved ($\text{Vo}_2 \text{ max}$) was measured along with other physiological data (Taylor *et al.*, 1980; Gehr *et al.*, 1981; Taylor & Weibel, 1981). A study on thermal panting recorded respiratory rate, tidal volume and dead space in two female wildebeest, each weighing 140 kg (Taylor, Robertshaw & Hofmann, 1969).

Blue wildebeest are a good candidate as an example of a normal African antelope for comparative research. They occur in large numbers, in their natural habitat, in functional natural ecosystems and are considered a species of least concern by the International Union for the Conservation of Nature (IUCN SSC Antelope Specialist Group, 2016)

Allometry

Allometry (literally translated as ‘of another measure’) is a tool used to compare the disproportionate changes in one variable with those of a second (Lindstedt & Schaeffer, 2002). Traditionally it is used to compare the growth of a variable or part of an organism (y) with change in the absolute magnitude of the organism, often measured as body mass (x), using a power function. The terminology used in allometric studies was initially formally described by Huxley and Teisser (1936) who suggested the following notation: $y = bx^\alpha$. More recently the commonly-used notation is: $y = ax^b$ (Schmidt-Nielsen, 1985) which is the notation which will be used in this dissertation.

The constant a is the value of y when $b = 1$ and, although attempts have been made to attribute significance to it, generally it is considered to have little practical value. It can be described as the mean ratio of y/x , or approximately the percentage change in y corresponding to a 1% change in x . (Huxley & Teissier, 1936; Brody, 1945; Gayon & Gimblett, 2002). The value of b , referred to as the scaling exponent or allometric exponent, is highly significant and refers to the rate at which y scales with x or ‘the ratio of the specific growth rates of x and y ’ (Huxley, 1950).

When any change in size of the variable under consideration, y , is directly proportional to the change in x , a special case of allometry, referred to as isometry or geometric similarity, occurs. If y and x have the same dimensions, then isometry will occur when $b = 1$. In this case, y scales linearly with x and so is always a constant fraction of x . If the dimensions of x and y differ, then other values of b can represent an isometric relationship. For example, if x is a measure of body mass and y is a length measurement, then $b = 1/3$ would indicate isometry. This is because the mass of an object is proportional to volume, which is proportional to length raised to the power of three (Gould, 1966).

If b is greater than the value expected for an isometric relationship, the relationship is then described as hyperallometric or positively allometric and where b is less than the isometric value, the relationship is hypoallometric or negatively allometric.

Many different forms of allometry have been described by various authors, based on how the animals compared are related to one another, and the terminology to describe them is not universally accepted. The terminology used by several authors is compared by Gould (1966) and five main forms of allometric study are described. These definitions will be followed in this dissertation. Allometric studies can be classified into five main groups: ontogenetic, evolutionary, intraspecific individual allomorphy, intraspecific race allomorphy and interspecific allometry.

Ontogenetic allometry describes the change in one variable with development, usually in a single species. Individuals of different ages are sampled, or one individual is repeatedly measured at different life stages, so that the change in one variable in relation to that of another can be studied.

Evolutionary allometry refers to relative change with evolution of a species. Attempts are made to follow a single line of descent and animals of the same age are used. Intraspecific allometry compares individuals of the same species; either differently sized animals of the same growth stage in a single population (individual allomorphy) or different populations, races or subspecies of different sizes at the same growth stage (race allomorphy). Interspecific allometry is the comparison of differential growth between species of different sizes at the same growth stage, usually adult. It is important to consider the type of allometric study used when comparing results. The relative size of an organ in a small adult animal is not necessarily comparable to that of a young animal of a larger species (Gould, 1966).

Interspecific allometry is used to describe general trends between species, usually in a particular phylogenetic group; for example, heart mass relative to body mass in eutherian mammals. In this case, the heart masses and body masses of many species of mammals would be plotted against each other. Each data point usually represents a single species, which is ideally an average value for the species but often a single measurement from one animal is used. Clearly, all the individuals of a certain species do

not have the same body or heart mass. Therefore, intraspecific variation in both variables, due to age as well as individual variation, can affect the accuracy of each datum.

Body mass (the most common independent variable) is an indicator of the extent of growth and has several advantages over age. Firstly, the age of animals is often not known and is hard to determine accurately and, secondly, the same factors that affect changes in growth (such as food availability) also affect body mass to a greater extent than age.

Derivation of Allometric Equations

Allometric equations are derived by fitting a power equation to bivariate data plotted on a scatterplot. The most common method is to plot a straight line through a scatterplot of the log-transformed data, to derive an equation in the form:

$$\log y = m \log x + c$$

This equation can be converted to an allometric (power) equation in the original units:

$$y = a(x)^b$$

Here, slope of the linear regression line (m) becomes the allometric exponent (b) and the antilog of the intercept (c) the allometric coefficient (a).

There has been extensive debate about the appropriateness of this method. The opponents of the method state that it is often unnecessary and that it can affect the data and the resulting relationship and therefore, the statistical analysis and conclusions drawn from the analysis. They advise that log-transformation should only be used when the data and research question warrant it (Packard, 2009, 2011; Xiao *et al.*, 2011). Supporters of log transformation argue, convincingly, that many of the apparent limitations of the method are useful advantages, mainly because variation in biology is multiplicative rather than additive (Kerkhoff & Enquist, 2009).

Fitting straight lines through log-transformed data automatically assumes that a power relationship is the best model for describing the data and often this is not the case; often linear relationships fit allometric data as well as or better than power relationships. For this reason, data should first be evaluated in the untransformed units to determine whether log-transformation is appropriate or necessary (Smith, 1980; Packard, 2009, 2013).

Log-transformation causes multiplicative rather than additive error, which can affect further statistical analysis of the relationship. This multiplicative error is seen as a limitation of the method by opponents to log-transformation (Packard, 2009). However, the natural variation in many biological parameters is proportional to the magnitude of the parameter and therefore error is multiplicative (proportional) rather than additive, especially when the range of the data is large, as is the case in many allometric samples

(Kerkhoff & Enquist, 2009; Xiao *et al.*, 2011). For example, a naturally occurring variation of 100g in animals with a 1kg heart is plausible whereas variations of 100g in animals with a heart that only weighs 200g is not.

Fitting a line through log-transformed data reduces the effect that large values have on the fitted line and increases the relative effect of small values. Although this effect has been seen as a limitation of log-transformation because it can falsely hide the effects of influential outliers (Packard, 2009), it has been shown to be an advantage of the method because it reduces the effects of large values which have far greater absolute natural variation than small values. Additionally, log-transformation can highlight potential outliers among smaller values which are not apparent if untransformed data are evaluated (Kerkhoff & Enquist, 2009).

Log-transformation is also useful for meaningful statistical evaluation of relationships because it can significantly improve the normality and homoscedasticity of many datasets (Smith, 1980), allowing the data to conform to the assumptions of linear regression.

There are several alternative methods of plotting straight lines through data. The most commonly used method is linear regression, by the method of ordinary least squares. Alternative, commonly-used include major axis and standardised major axis (reduced major axis) regression (Warton *et al.*, 2006). Linear regression by ordinary least squares is recommended for equations designed to predict y from x or to test for associations between the two. Major axis and standardised major axis regression is recommended where lines are fitted to describe the bivariate scatter of y and x test if the slope is equal to a certain value (Warton *et al.*, 2006). However, linear regression, major axis regression and standardised major axis regression methods do not differ significantly in relationships with high correlation coefficients (Smith, 2009). One disadvantage of linear regression by ordinary least squares is that of slope attenuation; the tendency for lines fitted with this method to have shallower slopes. Slope attenuation is a function of measurement error in the x variable. If measurement error is kept low, then the effects on the slope of the regression equation are minimal and do not affect the interpretation of the data. Standardised major axis methods are susceptible to over-estimating the slope of a regression if the scatter of data is large, which may be caused by measurement error or natural variation. For these reasons, ordinary least squares regression has recently been recommended for studies of allometry in preference to standardised major axis (reduced major axis) methods (Kilmer & Rodríguez, 2017).

Manipulation of Allometric Equations

Algebraic manipulation of allometric equations is often useful to compare two equations, or place an equation in a different format for simpler use or better understanding.

Change units of x or y

The units of the y term of an allometric equation can be altered easily. For example, the mass of a wildebeest in pounds (W) can be estimated from its girth (G) (Talbot & McCulloch, 1965). The equation is:

$$W = 146G^{2.78}$$

The equation can easily be converted to one for body mass in kilograms (M_b) by multiplying both sides of the equation by 0.4536, the conversion factor between pounds and kilograms, so that the equation becomes:

$$M_b = 66.2G^{2.78}$$

If the units of the x term of the equation need to be altered, the manipulation is more difficult. For example, the body mass of an elephant in kilograms (M_b) can be estimated from its shoulder height in cm (h_s), according to the equation (Hanks, 1972):

$$\log(M_b) = 3.11 \log h_s - 3.99$$

In this format, the equation is very cumbersome to work with but it can be expressed as the power equation:

$$M_b = 0.00010233h_s^{3.11}$$

Expressing this relationship in term of shoulder height in meters (H_s), involves more complex manipulations of the equation (Schmidt-Nielsen, 1985).

$$M_b/0.00010233 = h_s^{3.11}$$

$$(M_b/0.00010233)^{1/3.11} = h_s$$

$$\frac{(M_b/0.00010233)^{1/3.11}}{100} = H_s$$

$$\frac{M_b/0.00010233}{100^{3.11}} = H_s^{3.11}$$

$$M_b = (100^{3.11})(0.00010233H_s^{3.11})$$

$$M_b = 169.8H_s^{3.11}$$

The mass estimation equations in these examples are not good examples of allometric equations because they predict body mass from a body measurement, rather than describe the scaling of a body part with change in mass. However, they are good examples of power equations that require transformation and the same algebraic transformations are applicable to all power equations, including more traditional allometric relationships.

Relative growth

Allometric manipulation can be useful in expressing a growth relationship in terms of relative growth. For example, the relationship between heart mass (M_h) and body mass (M_b) in mammals is described by Brody (1945). This relationship can be manipulated to describe the change in relative heart mass with change in body mass:

$$M_h = 0.00588M_b^{0.984}$$

$$\frac{M_h}{M_b} = \frac{0.00588M_b^{0.984}}{M_b}$$

$$\frac{M_h}{M_b} = 0.00588M_b^{0.984-1}$$

$$\frac{M_h}{M_b} = 0.00588M_b^{-0.016}$$

Heart mass as a percentage of body mass is:

$$\frac{100 M_h}{M_b} = 0.588M_b^{-0.016}$$

Allometric cancellation

A method of comparing two different allometric equations was developed and extensively used by Stahl (1962, 1967). In short, the method derives an allometric equation for the ratio between two variables by dividing the allometric equations for those variables by each other. For example, an allometric equation for the ratio of tidal volume / total lung capacity can be derived from the equations for each:

$$\begin{aligned} \frac{\textit{Tidal Volume}}{\textit{Total Lung Capacity}} &= \frac{7.69M_b^{1.04}}{53.5M_b^{1.06}} \\ &= 0.14M_b^{-0.02} \end{aligned}$$

In this relationship, the exponents of either original equation are very similar and so the exponent of the derived equation is close to zero. Because of this, the ratio of tidal volume to total lung capacity is shown to vary very little with change in body mass (Stahl, 1967). However, the rounding of exponents can lead to large errors in prediction, especially if the equation is used over large body mass ranges (Prothero, 1986).

Express y in terms of x

An allometric equation with y expressed as a function of x can be re-arranged, to express x as a function of y :

$$y = ax^b$$

$$x^b = \frac{y}{a}$$

$$x = \left(\frac{y}{a}\right)^{\frac{1}{b}}$$

$$x = \left(a^{-\frac{1}{b}}\right) \left(y^{\frac{1}{b}}\right)$$

Although this method is algebraically sound, it is not always correct. If the ordinary least squares method is used to derive an allometric equation for a dataset with x as the indeterminate variable and y as the determinate and then the same analysis is done after swapping the determinate and indeterminate variables, the relationships are not the same. This effect is caused by the way in which the ordinary least squares method fits a trend-line to the data. If a symmetrical line-fitting method such as reduced major axis is used, then this transformation is valid (Smith, 2009).

Derive new equations

Two allometric equations can be used to estimate a third new relationship. For example, the equation for the relationships between body mass and lung mass in kilograms (M_l) and that for the relationship between lung volume in millilitres (V_l) and body mass can be used to estimate the relationship between lung mass and lung volume. Using the interspecific mammalian equations for mammals from Stahl (1967):

If:

$$M_l = 0.0113 M_b^{0.99} \text{ and } V_l = 53.5 M_b^{1.06}$$

Both equations can be expressed in terms of body mass and then equated:

$$M_b = \left(0.0113^{\frac{-1}{0.99}}\right) \left(M_l^{\frac{1}{0.99}}\right) \text{ and } M_b = \left(53.5^{\frac{-1}{1.06}}\right) \left(V_l^{\frac{1}{1.06}}\right)$$

$$\left(0.0113^{\frac{-1}{0.99}}\right) \left(M_l^{\frac{1}{0.99}}\right) = \left(53.5^{\frac{-1}{1.06}}\right) \left(V_l^{\frac{1}{1.06}}\right)$$

$$V_l^{\frac{1}{1.06}} = \frac{\left(0.0113^{\frac{-1}{0.99}}\right) \left(M_l^{\frac{1}{0.99}}\right)}{\left(53.5^{\frac{-1}{1.06}}\right)}$$

$$V_l = \frac{\left(0.0113^{\frac{-1}{0.99}}\right)^{1.06} \left(M_l^{\frac{1}{0.99}}\right)^{1.06}}{\left(53.5^{\frac{-1}{1.06}}\right)^{1.06}}$$

$$V_l = \frac{121.5 M_l^{\frac{1.06}{0.99}}}{0.0187}$$

$$V_l = 6500 M_l^{1.07}$$

From this equation, it can be deduced that, in the average mammal, lung volume is approximately 6.5 litres per kg of lung tissue and that the relationship is slightly hyperallometric, so that as lung mass increases, the change in lung volume per kg change in lung mass also increases.

Comparison of Allometric Equations

Allometric equations can be used to compare organisms or groups of organisms to answer questions such as: Do wildebeest have larger hearts than other mammals? Or, is foetal heart growth different from post-natal heart growth?

The first response of a scientist trying to answer the first question is most likely to be, 'It depends on the size of the wildebeest and the other mammals'. It would be logical to compare wildebeest with other, similarly-sized animals. Since wildebeest are not all the same size, more accurate comparison could be achieved if only adult wildebeest are used. If only adult wildebeest are used, should they be compared with only similarly sized adult mammals or can juveniles of larger species also be used for the comparison? The most accurate comparison would probably be between adult wildebeest and other adult mammals with body masses close to those of the wildebeest. However, these requirements can severely restrict the sample size and, in a field where reliable data on most species is scarce, are often not practical.

In most situations, it is necessary to use data collected from animals with greatly varying body sizes. The simplest comparison that can be done is to compare the average heart mass in the mammal sample with the average of the wildebeest sample. However, this method is inappropriate because the average heart masses in both sample have little meaning. The average heart mass of a sample of growing animals is not a useful statistic unless the animals in the sample are all of similar size. Similarly, the average heart mass for mammals, spanning a body mass range of eight orders of magnitude (Prothero, 1980), is meaningless.

Therefore, comparing mean *relative* heart mass is more meaningful. However, in any relationship that is not isometric, relative organ masses are not constant, making this approach inappropriate for the majority of allometric comparisons. Even in the relationship between heart mass and body mass in mammals, which is often considered to be isometric, the difference from isometry is large enough that mammals weighing 1kg are expected to have average relative heart masses of 0.6% of body mass and those weighing 100kg have average relative heart masses of 0.52% of body mass.

Therefore, in this example of comparing wildebeest heart mass with that of other mammals, the best model for mammalian heart mass is not the mean heart mass for mammals, or even the mean relative heart mass, but rather the allometric equation for heart mass in mammals. The allometric equation is an estimation of the average value of the y variable at any given value of x .

One of the simplest methods of comparing measurements with an allometric equation is to plot the data on a scatterplot, along with trend-lines representing the best-fit lines through the data. This approach has the advantage that the relationships between the trend-lines and datasets can easily be evaluated, and obvious trends can be seen, before more advanced statistical analysis is done. If the 95% confidence and prediction intervals of the trendlines are also plotted they can be useful to summarise the scatter of the data and allow more informed comparison of the datasets. Unfortunately, the statistics necessary for the calculation of these intervals is often not published along with allometric equations.

A less subjective method of comparing two datasets, which both show allometric regression, is to compare the measured values (in our example, wildebeest heart masses) with those predicted for an animal of the same body mass by the other allometric equation (heart mass in mammals). If the differences between the measured and predicted values are calculated for each measured animal, the mean of these differences would be significantly different from zero in significantly different populations. The significance of this difference can be tested by performing a paired, two-tailed, t-test on the measured and predicted values. It is important that a paired t-test is used and not a 2-sample test between the measured and predicted values. A two-sample t-test only compares the mean of the predicted values with that of the measured values and, if the range of the sample is large, this method is less sensitive.

If the range of data is large it is likely that the percentage difference between the measured and predicted values is more meaningful than the absolute difference. This percentage difference, referred to as the percent prediction error (PPE) is calculated as:

$$PPE = \left[\frac{\text{Measured} - \text{Predicted}}{\text{Predicted}} \right] * 100$$

The mean of the PPE values for a sample will also be significantly different from zero in different populations and this difference can be tested with a single-sample, two-tailed t-test.

The disadvantage of tests which compare measured values with those predicted by an allometric equation is that any variation in the dataset from which the prediction equation was derived is ignored. All the predicted values follow the equation exactly. A better method is to compare the datasets with each other directly. In our example, it would be better to compare a large dataset for body and heart masses in mammals with the wildebeest dataset, rather than relying on only the equation which summarises the mammalian dataset.

One method to compare two datasets directly (for example heart masses and body masses for cattle and wildebeest) is through multiple linear regression. The effect of species on the relationship between heart and body mass is determined by evaluation of the interaction between species and heart mass. In this case, the interaction term is (*species* × *heart mass*). If the coefficient of the interaction term is not significantly different from zero it can be concluded that there is no interaction between the variables and the effect of one variable on the outcome is the same at different levels of the other dependant variable. The significance of the difference from zero answers the question ‘does the species of animal affect the way in which heart mass changes with body mass’. It is similar to testing for a difference between the exponents of the allometric equations for each species.

Significant differences in the exponents of two allometric equations do not confirm that the populations are significantly different from each other. Two populations can overlap significantly while having significantly different exponents. In fact, if two equations have different exponents, then the trendlines must intercept at some point and, depending on the distance from that point, the populations will be significantly different from each other or not. It is easier to visualise this relationship if the power equations are drawn onto log-transformed axes; in this form, the power equations can be represented as straight lines where the exponent of the power equation is equal to the slope of the straight line. Any two straight lines with different slopes will cross at some point. Therefore, if the exponents of two allometric equations are significantly different from each other the best way to compare whether the populations are different is by comparing the populations, or sections of the populations at different points, to determine whether there is a significant difference at that point.

For example, when comparing the heart and body masses of wildebeest and cattle, one could find that the exponents of the two allometric equations are different and that the equations intersect at a body mass of 50kg but diverge widely at 200kg. One cannot conclude that wildebeest and cattle have significantly different body masses at all ages. For body masses of approximately 50kg the two populations overlap. However, if only animals larger than 200kg are compared, a significant difference could be concluded in that age group.

In cases where the exponents of two allometric equations are not significantly different from each other, the lines representing the equations on log-transformed axes are nearly parallel. In this case, the vertical difference between the lines describes whether or not there is a significant difference between the samples. This vertical difference is the difference between the elevations (*c*) of the two linear equations of the log-transformed equations. A significant difference between the elevations of two relationships with the same exponent indicates that the two populations are significantly different from each other at all values of *x* or *y* (Warton *et al.*, 2006).

A final way in which two populations with the same allometric exponent may differ from each other is if the members of one population are significantly larger than the other in terms of both x and y variables. This difference has been described as a shift along the axis (Warton *et al.*, 2006).

Objectives, Research questions and Hypotheses

This study aims to describe the ontogenetic allometry of the cardiac and respiratory systems of the blue wildebeest, to describe a method of predicting the body mass of blue wildebeest from external body measurements and to use these relationships to compare the blue wildebeest with other mammals. A secondary aim is to compare foetal and post-natal growth.

To achieve these aims, several research questions and hypotheses have been formulated for testing:

1. What are the patterns describing the relative growth of body mass and external body measurements in blue wildebeest?
 - a. $H_0: b(\text{girth, length or shoulder height vs body mass}) = 1/3$
 - b. $H_0: b(\text{length} \times \text{girth}^2 \text{ vs body mass}) = 1$
2. Can blue wildebeest body mass be predicted from external body measurements?
 - a. $H_0: \text{PPE}_A(\text{prediction equation}) < 10\%$
3. Are these prediction equations affected by sex or season?
 - a. $H_0: b(\text{male prediction equation}) = b(\text{female prediction equation})$
 - b. $H_0: a(\text{male prediction equation}) = a(\text{female prediction equation})$
 - c. $H_0: \text{mean PPE}_A(\text{male sample vs female prediction equation}) = 0$
 - d. $H_0: \text{mean PPE}_A(\text{female sample vs male prediction equation}) = 0$

The same hypotheses as a-d ere repeated for the wet and dry season samples and for the relationships in points 4,7,8 and 10 - 12.

4. Do the prediction equations for Southern African blue wildebeest differ from those formulated for East African subspecies?
5. What are the patterns of ontogenetic allometry in the wildebeest heart?
 - a. $H_0: b(\text{heart mass vs body mass}) = 1$
 - b. $H_0: b(\text{relative heart mass vs body mass}) = 0$
 - c. $H_0: b(\text{linear heart measurements vs body mass}) = 1/3$
 - d. $H_0: b(\text{linear heart measurements vs heart mass}) = 1/3$
6. Are the hearts of foetal wildebeest different from those of post-natal animals?
 - a. $H_0: b(\text{Entire Sample Equation}) = b(\text{Post-Natal Sample Equation})$
 - b. $H_0: a(\text{Entire Sample Equation}) = a(\text{Post-Natal Sample Equation})$
7. Are the ontogenetic allometric equations which describe the wildebeest heart different from those of other mammal species?
8. Are the hearts of blue wildebeest different from those of other mammals?
9. What are the patterns of ontogenetic allometry in the wildebeest trachea and lung?
 - a. $H_0: b(\text{lung mass vs body mass}) = 1$

- b. $H_0: b \text{ (relative lung mass vs body mass)} = 0$
 - c. $H_0: b \text{ (linear tracheal measurements vs body mass)} = 1/3$
 - d. $H_0: b \text{ (linear tracheal measurements vs heart mass)} = 1/3$
 - e. $H_0: b \text{ (tracheal volume vs body mass)} = 1$
 - f. $H_0: b \text{ (tracheal volume vs lung mass)} = 1$
 - g. $H_0: b \text{ (right ventricular wall thickness vs lung mass)} = 1$
10. Are the tracheas and lungs of foetal wildebeest different from those of post-natal animals?
11. Are the ontogenetic allometric equations which describe the wildebeest trachea and lungs different from those of other mammal species?
12. Are the tracheas and lungs of blue wildebeest different from those of other mammals?

General Materials and Methods

Experimental Design

This study describes the growth of the blue wildebeest cardiac and respiratory systems with ontogenetic allometric equations, contributing an additional species to the small collection of ontogenetic allometric studies on mammals.

The best method for estimating the body mass from external body measurements is evaluated and the effect of sex and season on these prediction equations is tested. The body mass estimation equations are compared with those for East African subspecies of blue wildebeest.

The allometric equations describing the cardiac and respiratory systems are used to test the differences between foetal and post-natal growth and the differences between wildebeest and other mammals.

Data Collection

The data used in this study was collected as part of a larger study by the Centre for Veterinary Wildlife Studies on the anatomy of the giraffe, wildebeest and African buffalo, under the supervision of Professor John Skinner including project: (V043/08). Data collection occurred prior to the registration of the author as an MSc student. However, the author played a central role in planning and carrying out the data collection.

The animals used were from the resident wildebeest population of the Buby Valley Conservancy, Matabeleland, southern Zimbabwe. The Buby Valley Conservancy is situated around 30°7'E, 21°30'S, at about 550m above sea level and covers an area of 3743km³ (Du Preez, Loveridge & Macdonald, 2014).

Data were collected on four different occasions from November 2008 until December 2010, which allowed measurements to be made over different seasons. The rainy season is in the summer, usually from November to March, when an average of 350mm rain falls (Du Preez *et al.*, 2014). There was a severe drought in 2008, rains were delayed and only 236mm fell (Mitchell, van Sittert & Skinner, 2010). The driest months are usually in Winter, between May and August (Mitchell *et al.*, 2010; Du Preez *et al.*, 2014).



Figure 1-1: Location of Buby Valley Conservancy¹

Animals were sampled to ensure that the final sample included a roughly similar number of animals, of each sex, spanning the complete body mass range. Individual animals were sampled at random. No visibly unhealthy animals were shot and each animal was inspected during dissection to ensure that it was not suffering from disease or any anatomical abnormalities.

All animals used in the study were culled by a professional hunter, as part of routine population management. The animals were preferentially shot through the top of the spine, below the skull. If this was not sufficient to ensure a quick death the animals were shot through the heart and lungs. Any animals shot through the heart were excluded from the heart sample. Measurements were made as soon as possible after each animal was shot and transported to a central processing area, to prevent changes associated with *rigor mortis*.

The body mass and external body measurements of each animal were measured and then each animal was dissected and the specific organs measured. Measurements were made by the author, or another student, and recorded by an assistant, on data collection sheets for each animal (Appendix 1).

The methods for each measurement are described in full in the relevant chapters.

1

Maps modified from:
TUBS (https://commons.wikimedia.org/wiki/File:Zimbabwe_in_Africa.svg), „Zimbabwe in Africa“ NordNordWest, Lizenz: (https://commons.wikimedia.org/wiki/File:Zimbabwe_adm_location_map.svg), „Zimbabwe adm location map“, <https://creativecommons.org/licenses/by-sa/3.0/legalcode>

Data Analysis

All data were entered into a Microsoft Excel® spreadsheet and analysed using SPSS Version 23.0 and 24.0 (IBM Corp. Released 2015, 2016), Standard Major Axis Tests and Routines (SMATR), Version 2.0 (Falster, Warton & Wright, 2006) and Microsoft Excel® 2016.

Ontogenetic Allometric Regression

Scatterplots of each comparison were created in Microsoft Excel, to allow visual inspection of the data for the shape of the curve and for outliers.

Linear regression was performed using the method of ordinary least squares. This method fitted trend-lines on the scatterplots and produced linear regression equations, in the form:

$$y = m x + c$$

Power relationships were derived in the same manner, but the variables were first log-transformed. Linear equations derived from the log-transformed data are in the form:

$$\log y = m \log x + c$$

Which can then be converted to power equations in the form:

$$y = a(x)^b$$

Here, the slope of the linear regression line (m) becomes the allometric (scaling) exponent (b) and the antilog of the intercept (c) becomes the allometric coefficient (a).

$$m = b$$

$$a = 10^c$$

When discussing allometric equations it is often more convenient to compare the corresponding linear relationship between log-transformed units. Differences in the slopes and intercepts of two linear equations are simpler to visualise. Therefore, the terms slope (m) and exponent (b) may be used interchangeably. In the same way comparison between allometric coefficients (a) may be done by comparing the intercepts (c) of the corresponding linear equations even though they are not equal in value (Prothero, 1986). Comparison of intercepts can also be referred to as comparison of the elevation of the equations (Warton *et al.*, 2006).

Model fit and validity

Each relationship was explored further using regression in SPSS, which provided the standard errors for the model coefficients and allowed assessment of model fit and validity. Assumptions in place for linear regression include a linear relationship between variables, a lack of outliers, independence of

residuals, homoscedasticity of residuals and normal distribution of residuals. Cases with a standardized residual greater than ± 3 standard deviations were considered outliers. All outliers and cases with a Cook's distance greater than one or Leverage value greater than 0.2 were individually evaluated to determine whether they should be removed from the dataset or retained. Only outliers that were so far from the predicted values that they seemed biologically impossible were removed from the dataset. Linearity and homoscedasticity were evaluated by inspecting a scatterplot of the standardized residuals plotted against the standardized predicted values for each relationship. To assess the normality of the distribution of the residuals, histograms of residuals and normal probability plots were examined, and the Shapiro-Wilk normality test performed.

The adjusted R^2 value or coefficient of determination and the absolute mean percent prediction error (PPE_A) for each relationship were assessed to gauge goodness of fit. R^2 values are commonly used as a predictor of goodness of fit but have the disadvantage of being strongly associated with sample size (Smith, 1984 cited by Campione and Evans, 2012;), range of dependent and independent variables (Smith, 1980), and slope (White & Kearney, 2014). It is also possible to obtain a large R^2 with large residuals (Smith, 1980; White & Kearney, 2014). Percent prediction error was calculated for each data point as:

$$PPE = \left[\frac{\text{Measured} - \text{Predicted}}{\text{Predicted}} \right] * 100$$

The positive (absolute) value of the PPE for each data point was then calculated (PPE_A) and the mean of these reported for each equation.

The PPE_A uses the data in its original non-logged units, and the PPE_A values for two equations can be compared using the mean and confidence intervals of the PPE_A values calculated for each data point. It is therefore considered a superior method of measuring regression strength (Campione & Evans, 2012).

ANOVA was used to test whether the equations were appropriate models for representing the data by testing whether the slopes of the equations (m) differed significantly from zero, which tests whether the regression line results in a statistically significantly better prediction of the dependent variable than the mean of the measured (independent) variables. If $m = 0$ in the linear equation, then $y = c$ and y does not change with change in x . If $m = 0$ in the equation with log-transformed variables, then $b = 0$ in the power equation: $y = ax^b$, and so $y = b$ and therefore the value of y is not affected by changes in x . In this case, it can be concluded that y does not scale with x and the regression is not significant.

Body mass calculated for use in organ equations

A calculated body mass was used, rather than the measured body mass, to minimise the individual, daily and seasonal variations due to factors such as gut-fill and body condition and individual errors in mass

measurement caused by blood and fluid loss. Errors due to blood and fluid loss are expected to have affected the animals which could not be weighed whole. Body mass was calculated from equations formulated in this study and described in Chapter 2.

The body mass for foetal animals is expected to be less variable than that of post-natal individuals and easier to measure accurately. The body mass prediction equations are also not recommended for foetal animals. Therefore, the measured body masses of the foetuses were used without any adjustment.

Sub-samples

To describe the growth of the wildebeest heart and lungs, allometric regressions were performed using three overlapping datasets. The primary dataset consisted of only the post-natal animals (Post-Natal Sample). A second regression was performed on all the animals in the study, including the foetuses (Entire Sample). This second regression was compared with the Post-Natal regression to assess the difference between foetal and post-natal organs. A third regression was done using only animals within the range of body masses shown by mature animals (Adult Sample) to account for possible changes in growth at maturity. Change in body mass in juvenile wildebeest is expected to be strongly correlated with age, whereas mass variation in adults is more likely to be due to other factors such as sex and individual variation. Therefore, an equation based only on adult animals is an example of intraspecific allometry (individual allomorphy) rather than ontogenetic allometry (Gould, 1966).

Wildebeest body mass begins to plateau at approximately two years of age but full adult weight is only achieved at three to four years old in females and six years in males (Attwell, 1982b; Watson, 1967 cited by Hopcraft, 2011; Talbot and Talbot, 1963). Wildebeest body mass begins to plateau at approximately two years of age but full adult weight is only achieved at three to four years old in females and six years in males (Attwell, 1982b; Watson, 1967 in Hopcraft, 2011; Talbot and Talbot, 1963). Both male and female wildebeest can theoretically reach sexual maturity between in their second or third year. However, most females only breed after three years, and males do not mate until four or five (Estes, 2014; Watson, 1967 cited by Hopcraft, 2011).

Tooth eruption was recorded in all animals and animals were assigned to the Adult Sample based on having a calculated body mass larger than that which corresponded to the eruption of the second adult incisor, an age of approximately two years (Attwell, 1980).

Tests for Isometry

The equations were assessed for isometry by testing whether the allometric exponents (b) were significantly different from those of an isometric relationship ($\frac{1}{3}$ for linear dimensions, 0 for relative heart mass and 1 for heart mass), using the standard errors of the exponents and a two-tailed t-test.

Comparison of Foetal and Post-Natal Organ Size

The foetal sample was not large enough to directly compare the allometry of foetal and post-natal animals or perform meaningful tests for statistically significant differences between the foetal and post-natal measurements. Instead, the difference between the organs of foetal and post-natal wildebeest was assessed using three methods.

Firstly, scatterplots of the data were inspected to determine whether the foetal data fell within the 95% prediction intervals of the post-natal equation. Secondly, the measurements of the foetal organs were compared with the predictions of the allometric equations derived from post-natal animals. The heart and lung mass and cardiac and tracheal dimensions for each foetus were calculated using the body mass of each foetus and the Post-Natal equations (predicted values) and these values were compared with the physical measurements made on the foetuses (measured values). The differences between the measured and predicted values were calculated (*Measured – Predicted*) as well as the percentage prediction error (PPE) between the measured and predicted values. The means of these values describe the mean absolute and percentage difference between the foetal animals and the predictions made by the post-natal equations and are presented with no attempt to determine if they are statistically significant, because such tests would be inappropriate with a sample of only four or five foetal wildebeest.

Thirdly, the SMATR software program was used to test for significant differences between the Post-Natal and Entire Sample allometric equations for each measurement. It tested whether the addition of the foetal data had a significant effect on the ontogenetic equations.

Comparing equations

The allometric equations derived for wildebeest were compared with other ontogenetic allometric equations and interspecific equations for mammals using three different methods, similar to those used to compare foetal and post-natal animals.

Ninety-five percent confidence intervals were calculated for each regression equation using Microsoft Excel (Zaiontz, 2016) and used to compare trend-lines visually. Where the trend-line for a specific equation falls outside these 95% confidence intervals it suggests that the average wildebeest is significantly different from that of the average animal represented by that equation.

The mean of the differences between each measurement made in this study and that predicted by the alternative equation was calculated, as well as the mean percent prediction error (PPE). A two-tailed t-test was used to test for a statistically significant difference between these means and a hypothesised value of zero.

T-tests were performed on the PPE values (percentages) rather than the difference between measured and predicted values in the original units of measurement because the mean of percentage differences is equally applicable to organs of different sizes. For example, a mean difference of 50g may be

significant in an animal with a 500g heart but not in one with a heart weighing 1500g whereas a difference of 5% is equally applicable to any heart size.

Finally, the SMATR software package was used to directly compare the prediction equations from different datasets. SMATR can be used to test for a difference between slopes or intercepts of linear equations, as well as for a shift along a common slope.

When the original data for two relationships are available, the SMATR software package allows trendlines to be fitted to the datasets, as well as construction of confidence intervals for the slope and elevation of the equations. SMATR also allows the comparison of equations, by comparing their slopes and elevations and testing for a shift along a common slope. Tests for a common slope are done through a likelihood ratio test compared to a chi-squared distribution and tests for a common elevation or shift along a slope use a Wald statistic (Warton *et al.*, 2006).

In some cases, original data were not available for analysis. However, close approximations could be extracted from published figures. Data Thief III, Version 1.7 (Tummers, 2006) was used to extract raw data. The images of the figures are inserted into the Data Thief program and the axes are marked and labelled before the data points are marked. The coordinates for each data point can then be exported as comma-separated values which can then be used for further analysis.

Where original data were not available in any form the SMATR software package was used to test whether the slope and elevations of the wildebeest equations were significantly different from those of the published equations, by testing if the residual and fitted axis scores are uncorrelated.

The mean of the PPE values, used to compare measured values with those predicted by an equation for a different sample, as used in this case, is not equivalent to the absolute mean of the PPE values (PPE_A) used to describe the fit of a trendline to data. PPE_A is always larger than PPE. This is because, mean PPE_A is calculated from absolute (positive) PPE values to describe the total scatter of points around a trendline. Whereas, the mean PPE, used to compare values with those predicted by another equation, uses the negative and positive values, to indicate whether the measured values are larger or smaller than the predicted values on average.

Chapter 2: Size and Mass of the Blue Wildebeest

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Summary

The relationship between several external body measurements and the body mass of blue wildebeest (*Connochaetes taurinus taurinus*) was investigated to derive equations for the prediction of body mass. Data were obtained from 21 female and 22 male post-natal animals and five foetuses. The relationship between body mass and measurements is described better by power than by linear equations. Body mass (M_b) is most accurately predicted from body length (L) and girth (G) using: $M_b = 39.1(LG^2)^{0.909}$ ($R^2=0.935$, PPE=8.21%). Similar equations using only body length or girth were only slightly less accurate. Sex and season have a small but significant effect on all the equations. Equations previously published for East African subspecies of blue wildebeest predict body masses approximately 7.5% larger than masses measured in this study, a difference which is statistically significant ($p<0.001$).

Introduction

The body size, and therefore the mass, of an animal plays a central role in all aspects of its physiology, including behaviour, metabolic rate, anatomical transport networks, population density and size of home range (Calder, 1996).

Body mass is necessary in medical fields to determine the quantity of drug to administer in order to achieve the recommended dosage, which is most often recorded as mg drug per kg body mass. An accurate body mass prediction is useful in studies of nutrition, growth rates, body condition and estimating consumption by carnivores (Hall-Martin, 1977a) as well as sport hunting (Jansen & Jenks, 2011). In game management, the ideal body mass of animals, predicted from body measurements, can be compared to their actual body mass to assess their condition and therefore also the condition and quality of the food source.

It is important to realise that the body mass of an individual animal is far from constant. It may vary by as much as 10% per day in extensive conditions, especially where animals walk long distances between grazing and water. (McCulloch & Talbot, 1965). This variation is mainly due to the consumption of large volumes of water at times when it is available under conditions where it is not available every day. Mass also varies due to the body condition of an animal and therefore with season as food availability changes. This variation is more pronounced in mammals living in temperate and polar regions than those in the tropics (McCulloch & Talbot, 1965; Cattet & Atkinson, 1997).

Often the mass of an animal is not easy to measure, especially in wildlife species or remote locations where live, conscious animals cannot be easily weighed. Large animals are difficult to hoist to the level of a hanging scale. Scales with the capacity to weigh whole animals and places to hang them are often unavailable under field conditions and platform scales capable of weighing large animals are expensive and difficult to transport. These constraints often cause animal mass to either be estimated or to go unmeasured.

Allometry is the study of differential growth. In studies of allometry, the growth of an organ or body part is compared with that of another growing body part or, more commonly, body mass. If this mass is measured by weighing the animal only once, the relationship may not be accurately represented due to the variability of body mass. A less variable measurement of animal size than body mass, such as a linear body measurement, is therefore expected to show a more accurate correlation with certain anatomical, physiological and behavioural variables. When conducting an allometric study of a measurement that is not expected to vary with short term fluctuations in body mass, such as heart mass, a more accurate description of differential growth can be achieved using body mass predicted from linear body measurements rather than that measured by weighing. This method was successfully used by Mitchell and Skinner (Mitchell & Skinner, 2009, 2011) for giraffes.

Body mass can only be reliably estimated from body measurements if there is a strong correlation between the two variables. The relationships between body mass and body measurements are most often described by either linear (in the form $y = mx + c$) or power equations (in the form $y = bx^a$). Predictions based on linear relationships assume that the ratio between the change in the dependent variable and the change in the independent variable remains constant. In situations where this is not the case they are less accurate than power equations but can still be useful. Power equations assume that the ratio between the dependant and independent variable changes predictably with change in the independent variable.

Linear equations are most appropriate in situations which predict length measurements from other length measurements, areas from measured areas, masses from other masses or masses from volumes if densities remain unchanged. This is because, in a series of objects of different sizes but unchanging shape, the ratios between two different lengths, areas or volumes of the object will always be the same. However, the ratio between any area on the object and any length will change as size increases; areas will scale with length squared. Similarly, volumes (and therefore mass) will scale with length cubed.

When predicting the mass of an animal from the measurement of a length (assuming that the animal only changes in size and not shape or density), the mass would scale with the length cubed. Because of this relationship, power equations are usually more appropriate for comparing lengths and masses. However, animals are more complex than this theoretical example and do change shape as they grow. Furthermore, natural variation between individuals also affects the accuracy with which a single parameter, such as body mass, can be predicted from any other measurement. It is therefore the case that, in some situations and species, linear equations can be used to predict body mass. Several authors have concluded that body mass can be estimated as accurately by using linear equations as by direct weighing and recommend them over more accurate power equations because they are simpler to model and to use (McCulloch & Talbot, 1965; Rideout & Worthen, 1975; Hall-Martin, 1977b) which was an important consideration at the time of these studies, before the widespread use of personal computers. Even today some authors still prefer linear to power equations (Packard, 2009, 2014; Packard & Boardman, 2015).

Different single body measurements have been used to formulate linear equations for mass estimation, including girth (Talbot & McCulloch, 1965; Smart, Giles & Guynn, 1973; Hall-Martin, 1977a; Weckerly, Leberg & Van Den Bussche, 1987; Fandos, Vigal & Fernandez-Lopez, 1989; Millspaugh & Brundige, 1996; Cook, Cook & Irwin, 2003), body length, shoulder height (Hall-Martin, 1977a) and even horn length (Fandos *et al.*, 1989).

A combined variable of body length multiplied by the square of the girth (LxG^2) is occasionally used for the prediction of body mass. Equations in this form work well for rhinoceroses (Freeman & King, 1969), mountain goats (Rideout & Worthen, 1975), giraffes (Hall-Martin, 1977a) and other antelope,

including wildebeest (McCulloch & Talbot, 1965). Length times girth squared gives the volume of a rectangular cuboid much larger than the volume of the animal measured. However, this volume is still more likely to show a linear relationship with body mass, because length cubed (volume) is directly proportional to mass in an object of changing size but unchanging shape and density. Relying on two variables instead of one also makes the predictive power of the equation more robust.

Despite the success and ease-of-use of linear relationships, power equations are theoretically more appropriate and usually fit the data more accurately because animals do change shape as they grow. As modern technology improves the ease with which power equations can be used, these have been recommended over linear relationships (Wallin, Cederlund & Pehrson, 1996). Power equations have the added advantage that they can be used for allometric studies.

For some species, a single formula can be used to predict the body mass of all individuals (Freeman & King, 1969; Rideout & Worthen, 1975; Millspaugh & Brundige, 1996). For other species, separate formulae are needed for subsections of the population. For example, separate equations may be needed for different age groups or different sexes. Occasionally, different equations are also needed for different seasons or locations (Smart *et al.*, 1973; Weckerly *et al.*, 1987; Wallin *et al.*, 1996). Furthermore, in some species, such as polar bears and ibex, accurate predictions of body mass cannot be made from body measurements (Cattet & Atkinson, 1997; Bassano, Bergero & Peracino, 2003).

In their study on the use of morphometry for the prediction of body mass in polar bears, Cattet & Atkinson (1997) concluded that there was no single equation that was applicable to the entire species. They found that the equations previously derived for polar bears were neither accurate nor precise and recommended that similar studies be carried out, testing the accuracy and precision of morphometric methods of mass estimation, in other large animals. Polar bears are less suited than other animals to this type of study because they have thick coats, which make measurements difficult. The most significant factor causing inaccuracy in Cattet and Atkinson's study was measurement error. Polar bears also undergo substantial changes in body mass between seasons, due to changes in food availability. McCulloch & Talbot (1965) point out that ungulates in East Africa undergo far less seasonal variation in mass than animals living in temperate zones, and that wild animals show far less variation than domestic animals. Southern African ungulates may differ from East African ones, because they face greater seasonal differences.

In a study of East African antelope, including blue wildebeest, McCulloch and Talbot (1965) concluded that neither separating the animals into age and sex classes, nor excluding stomach contents or viscera from body mass increased the accuracy of the equations. They also concluded that including the tail length in the body length measurement increased the accuracy of the equations.

The mass of a female mammal also changes with her breeding cycle, especially when she is carrying a near term foetus. The extent to which the foetus affects the relationship between body mass and linear measurements is hard to quantify. Hall-Martin, (1977) found that when body mass was calculated by subtracting the mass of the uterus and foetus of pregnant female giraffes from their total mass, the resulting formulae were less accurate than when using entire body mass. He attributed this to the reduced gut-fill of pregnant females. In elk, however, pregnancy did affect the relationship between mass and chest girth (Cook *et al.*, 2003).

When weighing dead animals, even those freshly killed, it is important to note that carcass mass is not necessarily equal to live mass. Blood and body fluids are easily lost during handling and butchering and are often not weighed. Mass losses also occur due to evaporation and the loss of scraps that are not weighed (Hall-Martin, 1977a). Most studies do not make allowance for this difference, although, in some cases, the difference is estimated to be approximately five percent (Crile & Quiring, 1940).

The only attempt to derive formulae for predicting the body mass of wildebeest was carried out in East Africa (McCulloch & Talbot, 1965; Talbot & McCulloch, 1965). The animals measured were recorded as being of the subspecies *Gorgon taurinus hecki* and *Gorgon taurinus albojubatus*, now called *Connochaetes taurinus mearnsi* and *Connochaetes taurinus albojubatus*, the Eastern and Western white-bearded wildebeest. These subspecies are genetically distinct from the Southern African blue wildebeest (*Connochaetes taurinus taurinus*) (Skinner & Chimimba, 2005; Estes, 2014). Attwell (1982b) mentions an equation for body mass prediction from girth in adult wildebeest, in a paper on *C. t. taurinus* in Zululand, South Africa. The equation is $M_b = 2.97G - 242.24$ ($r=0.9133$), with body mass (M_b) in kilograms and chest girth (G) in centimetres. However, the source of the data, sample size and method of calculation are unclear.

Talbot and McCulloch (1965) used several different methods to compare body mass (M) with the linear measurements of girth (G), total body length including the tail (L) and shoulder height (S). Starting with a linear equation in the form:

$$M = a + bG + cL + dS$$

They concluded that girth contributed far more to the variation in body mass than the other variables. Therefore, linear and power equations with girth as the only variable were derived. A linear equation using length multiplied by girth squared was also derived (Table 2-1).

Even though the data showed a clear curvilinear relationship, McCulloch and Talbot concluded that the linear equation was best suited to predicting body mass in the field. The more complicated equations did not provide sufficiently greater accuracy to justify the added difficulty in using them.



Published Equation	Equation in metric units	n	Coefficient of determination (r^2)	Residual standard deviation (pounds)	Coefficient of Variation (%)
$W = 644G - 517$	$M_b = 292G - 235$	158	0.91*	36	10.4
$W = 79.9LG^2 + 6$	$M_b = 36.2LG^2 + 2.72$	158	0.92*	34	9.8
$W = 146G^{2.78}$	$M_b = 66.2G^{2.78}$	158	0.94		

Weight (W) in pounds, Body Mass (M_b) in kilograms, Girth (G) and total body length (L) in meters.
Data from Talbot and McCulloch (1965) * $p < 0.001$

Table 2-1: Formulae for estimating body mass of East African wildebeest (*Connochaetes taurinus mearnsi* and *C. t. albojubatus*) from body measurements.

In this study, the relationship between body mass and linear dimensions in Southern African blue wildebeest (*C.t. taurinus*) is reported. The relationships are used to determine which external body measurements can be used to estimate body mass accurately. The effect of sex and season on the prediction equations is evaluated. The relationships are also used to compare the Southern African blue wildebeest with the East African eastern and western white-bearded wildebeest.

Materials and Methods

Data Collection

Measurements

Body measurements followed the methods described in (Sachs, 1967), with slight adjustments, as described below.

1. *Body mass* (M_b , kg) Body mass was measured to the nearest 500g using a Salter suspended spring balance with a capacity of 200kg. Animals with a mass less than 200kg were weighed whole and larger animals were weighed in pieces. Female body mass includes the mass of the reproductive tract and that of any foetus carried.
2. *Body Length* (L , mm). Length measurements were made, to the nearest 5mm, along the contour of the body, using a flexible measuring tape. The following measurements were summed to give the full body length: length from the tip of the nose to the occipital crest, occipital crest to base of neck, base of neck to caudal sacrum and caudal sacrum to the tip of the tail, excluding the hairs.
3. *Shoulder height* (H_S , mm). Shoulder height was measured from the the middle of the base of the lateral hoof wall, with the carpus maximally extended, along the lateral surface of the foreleg, to the highest point of the withers. In a recently dead ungulate the hoof is often flexed, so the hoof was forced into a normal standing position so that the sole was perpendicular to the leg.
4. *Girth* (G , mm). Chest girth was measured immediately caudal to the thoracic limb, around the xiphoid process of the sternum and over the highest point of the withers.

Seasons were defined by rainfall records collected on the conservancy and available vegetation, rather than calendar dates, and defined as dry, medium or wet. According to historical rainfall records from the study site, rain is expected to fall during the summer months, between October and March.

Data Analysis

Formulae were derived to correlate body mass with each of the three measurements made, as well as with the value calculated from length multiplied by girth squared ($L \times G^2$).

Ontogenetic Allometric Regression of Linear Body Measurements of the Wildebeest

Linear and power equations were derived for each body measurement and length times girth squared ($L \times G^2$), for the Entire Sample (including foetal data) and the Post-Natal Sample (excluding foetal data).

Scatterplots of each comparison were created and linear regression of body mass against the body measurements and $L \times G^2$ was performed. Through linear regression trend-lines were fitted on the scatterplots and produced linear equations for the untransformed data, and power equations were derived from lines fitted to the log-transformed data.

Model fit and validity

Each relationship was explored further using regression in SPSS which provided the standard errors for the model coefficients and allowed assessment of model fit and validity.

The adjusted R^2 value or coefficient of determination and the absolute mean percent prediction error (PPE_A) for each relationship were assessed to gauge goodness of fit and ANOVA was used to test whether the equations were appropriate models for representing the data by testing whether the slopes of the equations (m) differed significantly from zero.

Investigation of the Effects of Sex and Season

The effect of sex and season was tested on the post-natal power equations for each body measurement. The Post-Natal Sample was divided into two samples according to sex and three samples according to season, and regression analysis was performed on each individual sample.

Ninety-five percent confidence and prediction intervals were calculated for each regression equation and used to compare trend-lines visually. The mean difference between the body masses measured for one sex or season and that predicted by the equation for the other sex or season was calculated and then the mean percent prediction error (PPE). Finally, the SMATR software package was used to directly compare the slopes (exponents) and elevations of the prediction equations from different datasets.

Comparison of Southern African Blue Wildebeest with East African subspecies

The measurements and regression equations derived for Southern African blue wildebeest in this study were compared with those published for East African subspecies by McCulloch and Talbot (1965) in an attempt to detect any existing race allomorphosis.

The original data for the East African animals were not available for analysis. However, close approximations of the measurements made could be extracted from the published figures (McCulloch & Talbot, 1965) using Data Thief. These data were used to plot 95% confidence intervals for the East African equations and compare the equations using SMATR, with the same methods as the comparison between different sexes and seasons.

The mean differences between the body masses measured in the Post-Natal sample and those predicted by the East African equations (Table 2-1) were investigated.

Results

Forty-three post-natal wildebeest (21 female and 22 male) with body masses ranging from 74 to 258kg and five foetuses (two female and three male) with body masses between 12 and 15kg were sampled. The body mass recorded for pregnant females included that of the foetus. No animals were excluded due to disease, anatomical abnormalities or *rigor mortis*. All animals were processed within an hour of being shot. The measured body mass and dimension medians and ranges are summarised in Table 2-2. Adult animals are defined as those animals with a full set of adult incisors to allow comparison with other similar studies.

Measurement	Foetus (n=5)	n	Post-Natal Male (n=22)	n	Post-Natal Female (n=21)	n	Adult Male (n=10)	n	Adult Female (n=8)	n
Body Mass (kg)	13.5 (12-15)	5	174 (74-258)	22	149 (74-225)	21	233 (193-258)	10	217 (192-225)	8
Girth (m)	0.535 (0.49-0.58)	4	1.48 (1.08-1.76)	21	1.42 (1.00-1.64)	21	1.64 (1.50-1.76)	9	1.54 (1.47-1.64)	8
Shoulder Height (m)	0.69 (0.63-0.72)	5	1.36 (1.11-1.54)	22	1.22 (0.97-1.36)	21	1.43 (1.37-1.54)	10	1.32 (1.22-1.36)	8
Body Length (m)	1.1 (1.02-1.2)	5	2.54 (1.74-2.84)	22	2.32 (1.73-2.61)	21	2.67 (2.59-2.84)	10	2.47 (2.36-2.61)	8

Table 2-2: Median and range of measured body mass and body measurements for wildebeest measured in this study.

Ontogenetic Allometric Regression

Evaluation of Scatterplots

Visual inspection of scatterplots of the relationships between each body measurement and mass showed an approximately linear relationship for the Post-Natal Sample. Both linear and power trend-lines fitted the post-natal data well (Figure 2-1, 2-3, 2-5 and 2-7). However, if the trend-lines were extrapolated to the foetal body mass range, the power relationships predicted foetal body masses closely, whereas the linear relationships greatly underestimated them. When trend-lines were fitted to the Entire Sample (including foetuses), power relationships fitted the data better than linear ones (Figure 2-2, 2-4, 2-6 and 2-8).

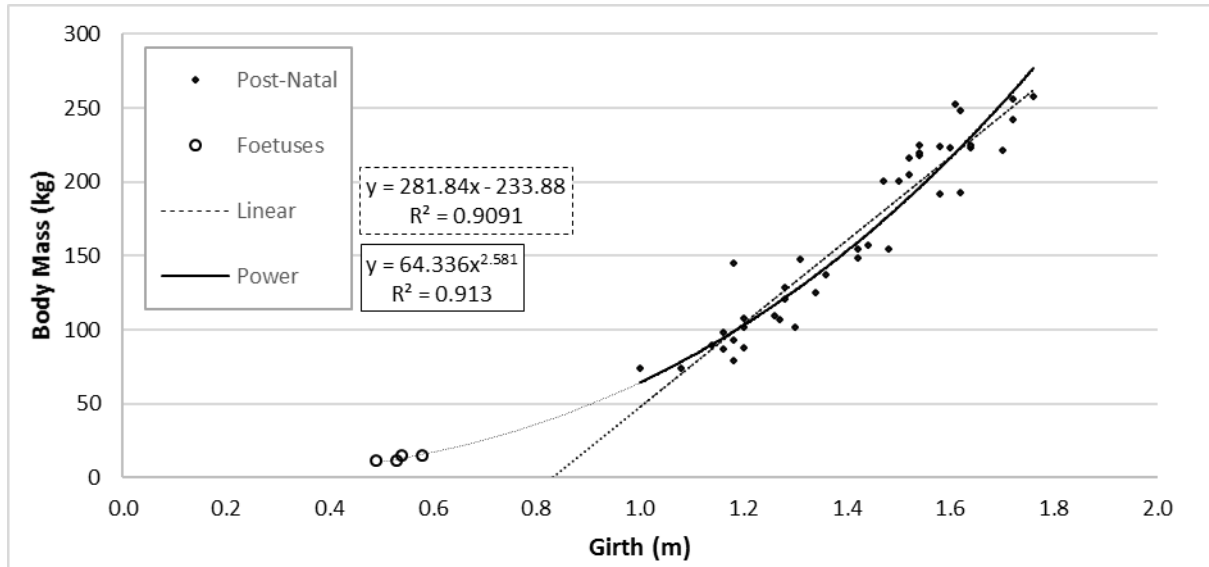


Figure 2-1: Linear and power trend-lines describing the relationship between girth and body mass in post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation. Dotted lines represent extrapolation of the Post-Natal relationships.

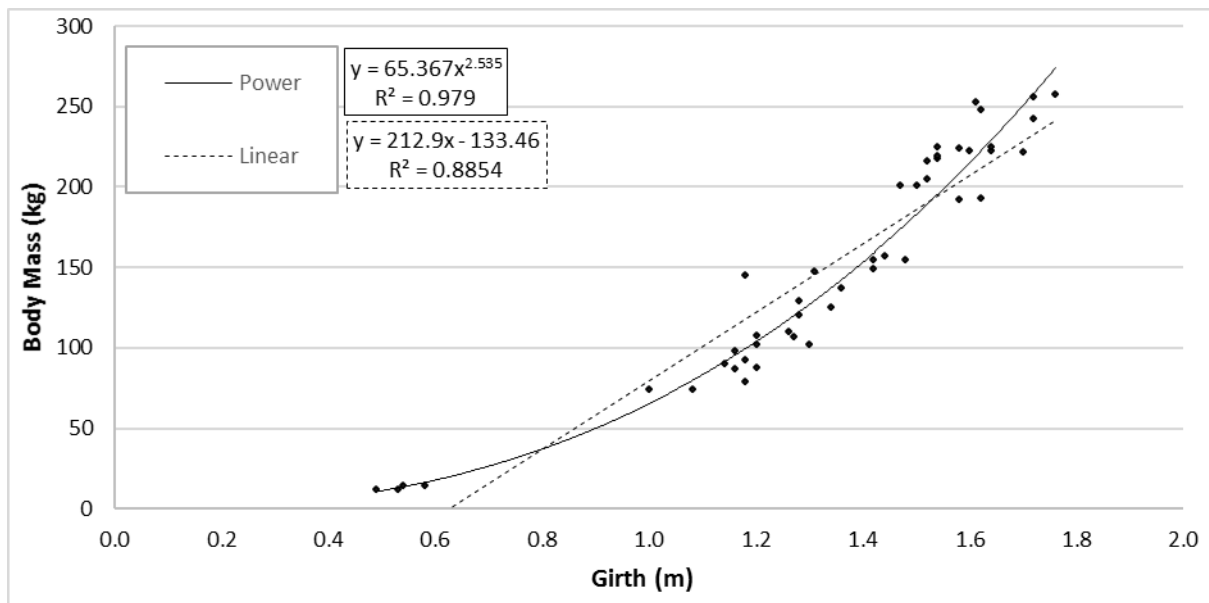


Figure 2-2: Linear and power trend-lines describing the relationship between girth and body mass in foetal and post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation.

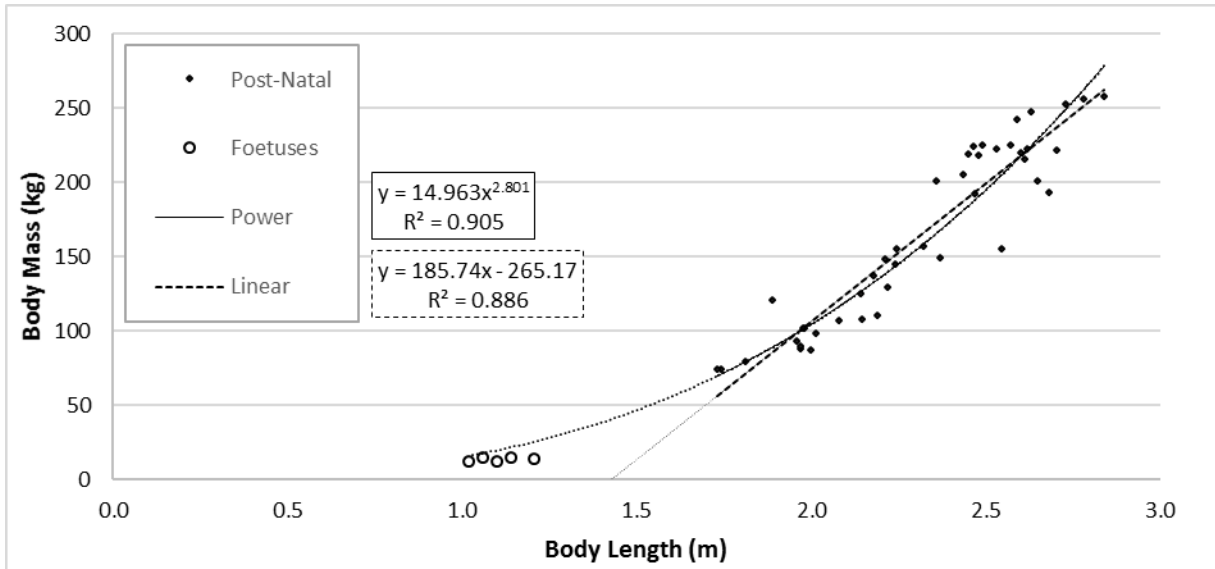


Figure 2-3: Linear and power trend-lines describing the relationship between body length and body mass in post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation. Dotted lines represent extrapolation of the Post-Natal relationships.

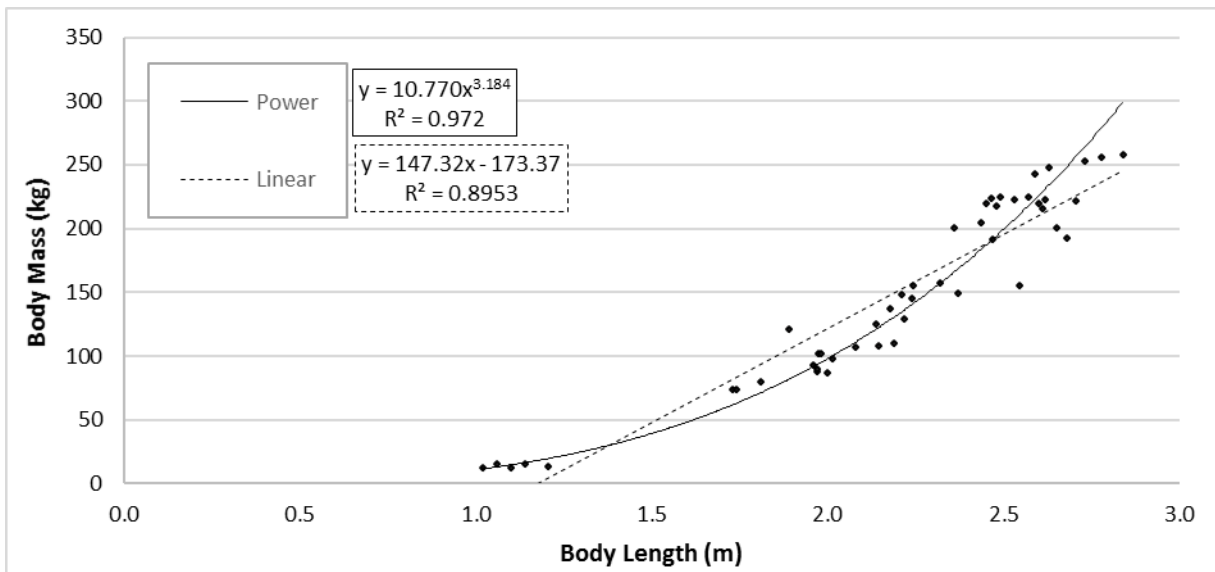


Figure 2-4: Linear and power trend-lines describing the relationship between body length and body mass in foetal and post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation.

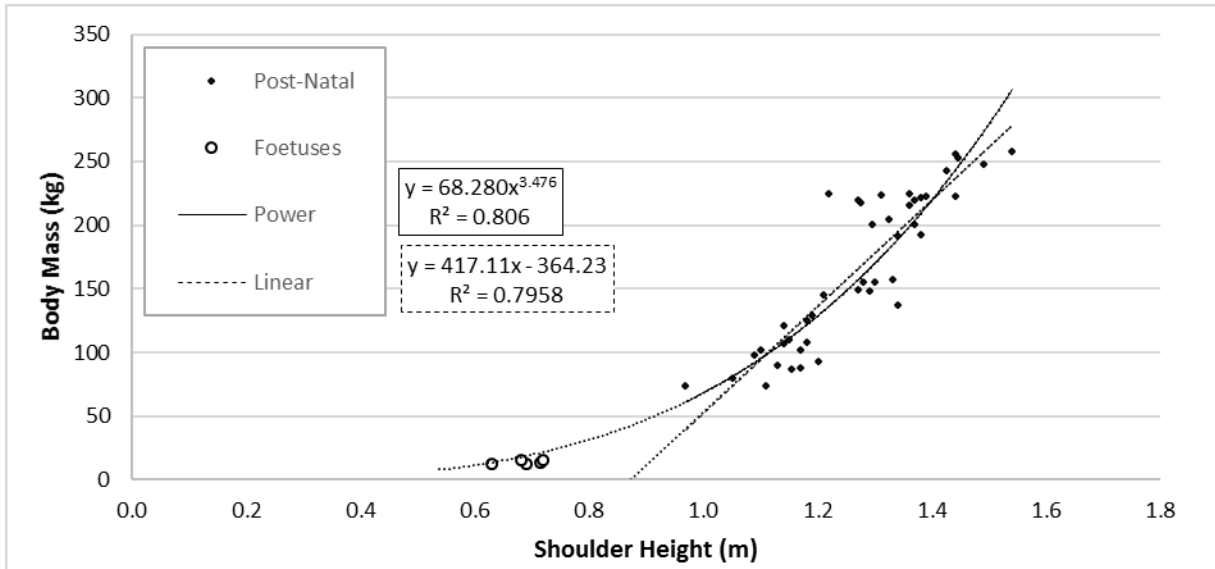


Figure 2-5: Linear and power trend-lines describing the relationship between shoulder height and body mass in post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation. Dotted lines represent extrapolation of the Post-Natal relationships

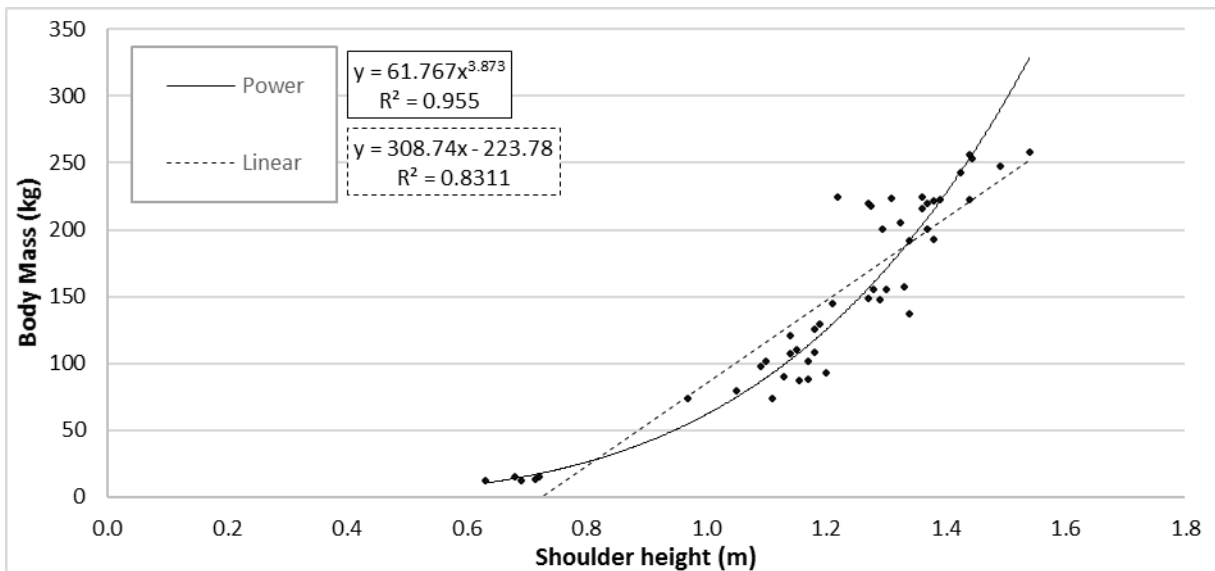


Figure 2-6: Linear and power trend-lines describing the relationship between shoulder height and body mass in foetal and post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation.

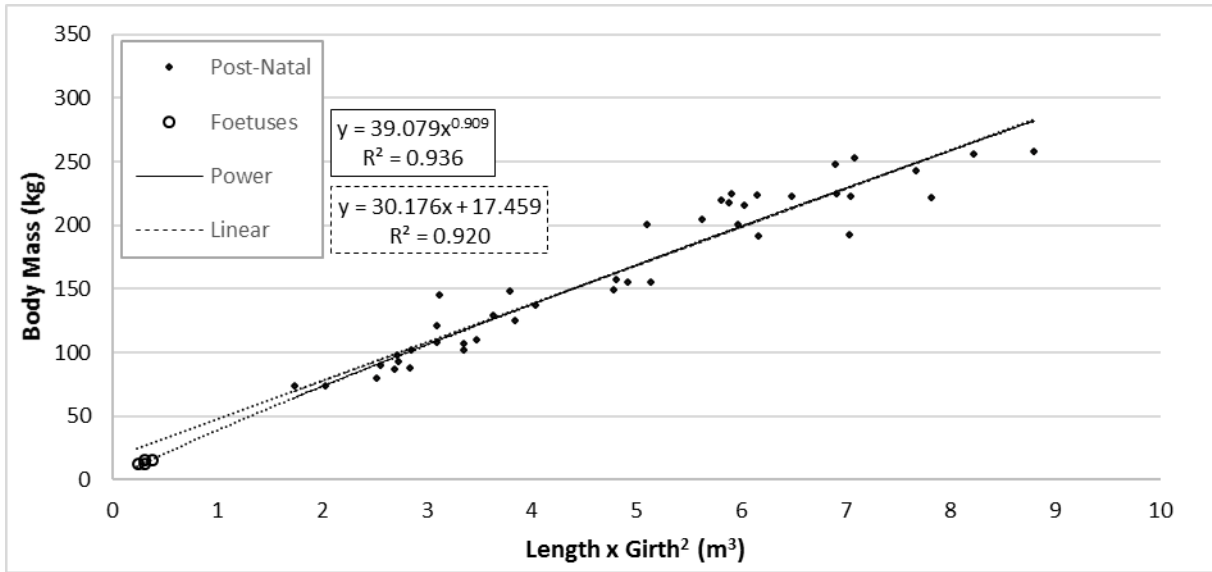


Figure 2-7: Linear and power trend-lines describing the relationship between length times girth squared and body mass in post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation. Dotted lines represent extrapolation of the Post-Natal relationships

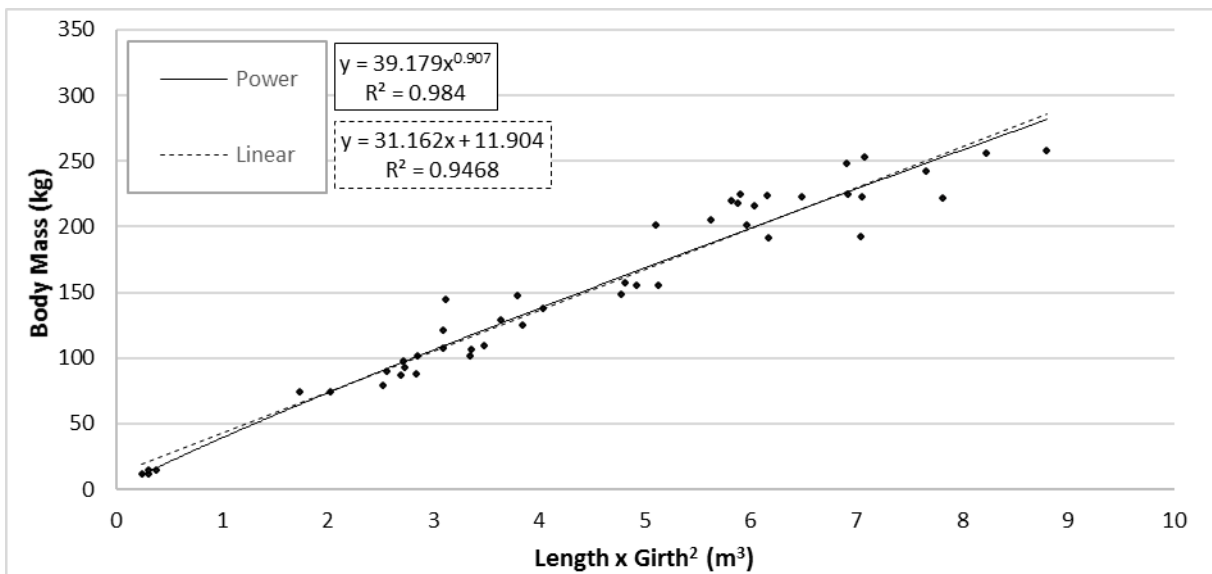


Figure 2-8: Linear and power trend-lines describing the relationship between length times girth squared and body mass in foetal and post-natal wildebeest.

The dashed line and equation with the dashed border represent the linear equation and the solid line and equation with the solid border describe the power equation.

Allometric Equations

The regression equations represented by the trend-lines in Figures 2-1 to 8 are summarised in Table 2-3 and 2-4 for the linear and power relationships respectively. The regression between body mass and each of the external body measurements is statistically significant, as the slopes of the linear equations (m) and the exponents of the power equations (b) are significantly different from 0 (Appendix 2).

In all cases, a power equation described the data better than a linear equation, as judged by the adjusted R^2 and PPE. Inclusion of the foetal data increased the R^2 values and PPEs in all cases, except for the linear girth relationship, where it decreased the R^2 value.

The power equations are significantly different from isometry, except for the post-natal relationships for shoulder height and body length. The relationships for body length and shoulder height in the Entire sample are hyperallometric and those for girth and LxG^2 are hypoallometric. In the Post-Natal Sample, power equations for girth and LxG^2 are hypoallometric but the exponents of the equations for body length and shoulder height were not significantly different from the isometric exponent of three.

Sample	Equation $M_b = mx + c$					Goodness of fit		n	p ^a
	x	m	SE (m)	c	SE (c)	Adjusted R ²	PPE _A (95% CI)		
E	Body length (m)	147	7.43	-173	16.6	0.893	35.5 (15.3:55.8)	48	<0.001
PN		186	10.4	-265	24.3	0.883	10.8 (8.2:13.4)	43	<0.001
E	Body length x Girth ² (m ³)	31.2	1.11	11.9	5.54	0.946	10.8 (7.9:13.7)	46	<0.001
PN		30.2	1.41	17.5	7.31	0.918	8.51 (6.9:10.1)	42	<0.001
E	Girth (m)	213	11.6	-133	15.8	0.883	28.4 (14.0:42.7)	46	<0.001
PN		282	14.1	-234	20.1	0.907	10.4 (7.4:13.3)	42	<0.001
E	Shoulder height (m)	309	20.5	-224	25.2	0.827	59.7 (10.8:109)	48	<0.001
PN		417	33.0	-364	42.1	0.791	14.8 (10.3:19.3)	43	<0.001

SE: Standard Error

E: Entire Sample (Foetal, Juvenile and Adult animals); PN: Post-Natal Sample (Juvenile and Adult animals)

PPE_A: absolute mean percent prediction error.

^a ($H_0: m = 0$) The null hypothesis is that the regression equation is not significantly different from the mean of the y values.

Table 2-3: A comparison of the linear body mass prediction formulae, derived using different linear body measurements, either using all animals measured or only the post-natal animals.

Sample	Equation $M_b = a(x)^b$					Goodness of fit		n	p ^a	p ^b
	x	b	SE (b)	a	SE (a)	Adjusted R ²	PPE _A (95% CI)			
E	Body length (m)	3.18	0.0804	10.8	0.690	0.971	11.1 (8.58:13.6)	48	<0.001	0.027
PN		2.80	0.142	15.0	1.78	0.903	9.47 (7.26:11.7)	43	<0.001	0.169
E	Body Length x Girth ² (m ³)	0.907	0.0175	39.2	1.05	0.983	8.38 (6.72:10.0)	46	<0.001	<0.001
PN		0.909	0.0374	39.1	2.27	0.935	8.21 (6.49:9.94)	42	<0.001	<0.001
E	Girth (m)	2.54	0.0566	65.4	1.46	0.978	9.49 (7.4:11.58)	46	<0.001	<0.001
PN		2.58	0.126	64.3	2.96	0.911	9.44 (7.21:11.7)	42	<0.001	0.002
E	Shoulder height (m)	3.87	0.124	61.8	2.06	0.954	14.2 (10.6:17.8)	48	<0.001	<0.001
PN		3.48	0.266	68.3	4.62	0.802	13.4 (9.75:17.0)	43	<0.001	0.081

SE: Standard Error

E: Entire Sample (Foetal, Juvenile and Adult animals); PN: Post-Natal Sample (Juvenile and Adult animals)

PPE_A: absolute mean percent prediction error

P-values are for the tests of the following two null hypotheses:

^a 1) H₀: $b = 0$. The null hypothesis is that the regression equation is not significantly different from the mean of the y values.

^b 2) H₀: $b = isometric$. The null hypothesis is that the equation is isometric, i.e. that the exponent (b) is equal to the value of the exponent of the isometric equation (3 for all length measurements and 1 for LxG²)

P-values smaller than 0.05 are shown in bold, in these instances the null hypothesis can be rejected.

Table 2-4: A comparison of the power relationship body mass prediction formulae, derived using different linear body measurements, either using all animals measured or only the post-natal animals.

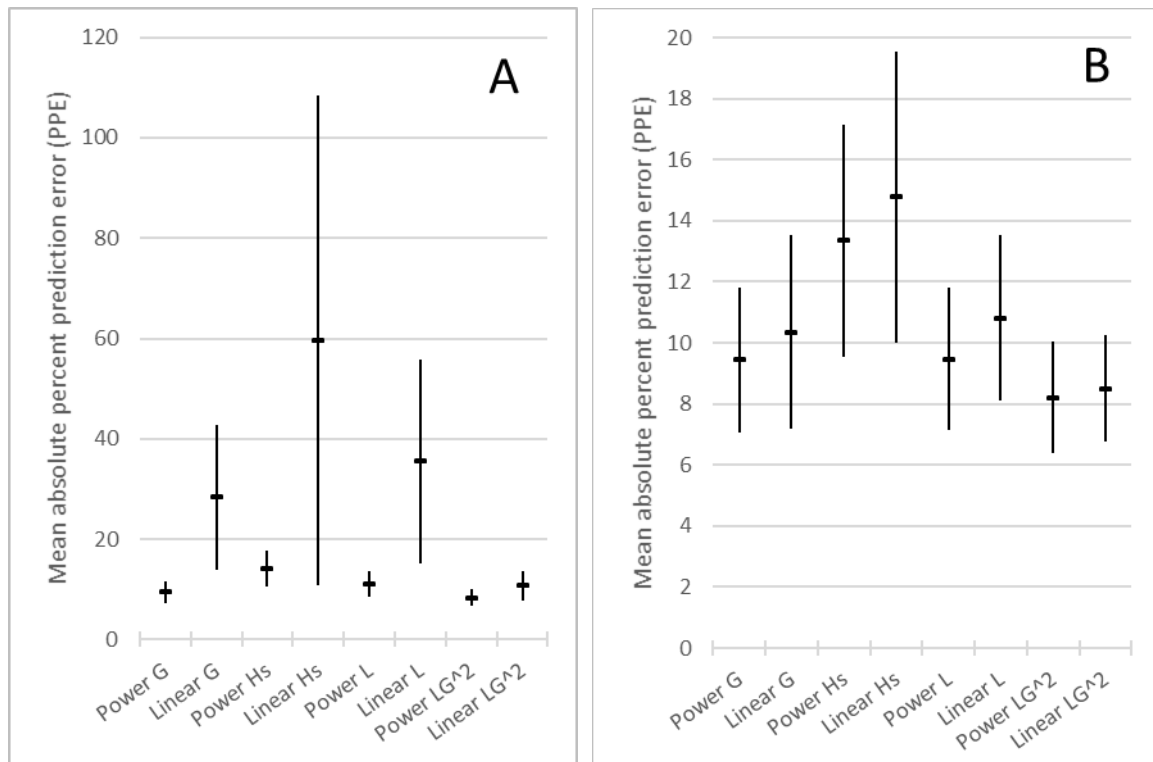


Figure 2-9: Comparison of the Percent Prediction Error of the power and linear equations.

The horizontal bars represent the mean absolute percent prediction error for each equation and the vertical lines the 95% confidence intervals of the means. Figure A represents the Entire Sample equations and Figure B the Post-Natal Sample equations. G, Girth; Hs, Shoulder Height; L, Length; LG², Length x Girth².

Comparison of the absolute mean percent prediction error (PPE_A) of the equations (Table 2-3 and 2-4 and Figure 2-9) supports the observations made from the scatter plots. Power equations fit the Entire Sample data (Figure 2-9A) far better than linear equations for the girth, shoulder height and length equations. However, the difference between the two forms of equations is less extreme for the $L \times G^2$ equation for the Entire Sample, and for all the Post-Natal Sample equations. The power equations still have lower PPE_A values than the linear equations, however. In all cases the PPE_A of the Post-Natal equation is smaller than that of the Entire-Sample equation.

The Effect of Sex

When the data are divided into separate samples for male and female animals, the separate equations can be compared to determine whether sex has a significant effect on the mass prediction equations.

Visual inspection of scatterplots of the data (Figure 2-10 to 2-13) show that although the equations for female animals predict larger body masses than the corresponding male equations, for all external body measurements; the data are interspersed and the 95% Confidence intervals of the equations overlap.

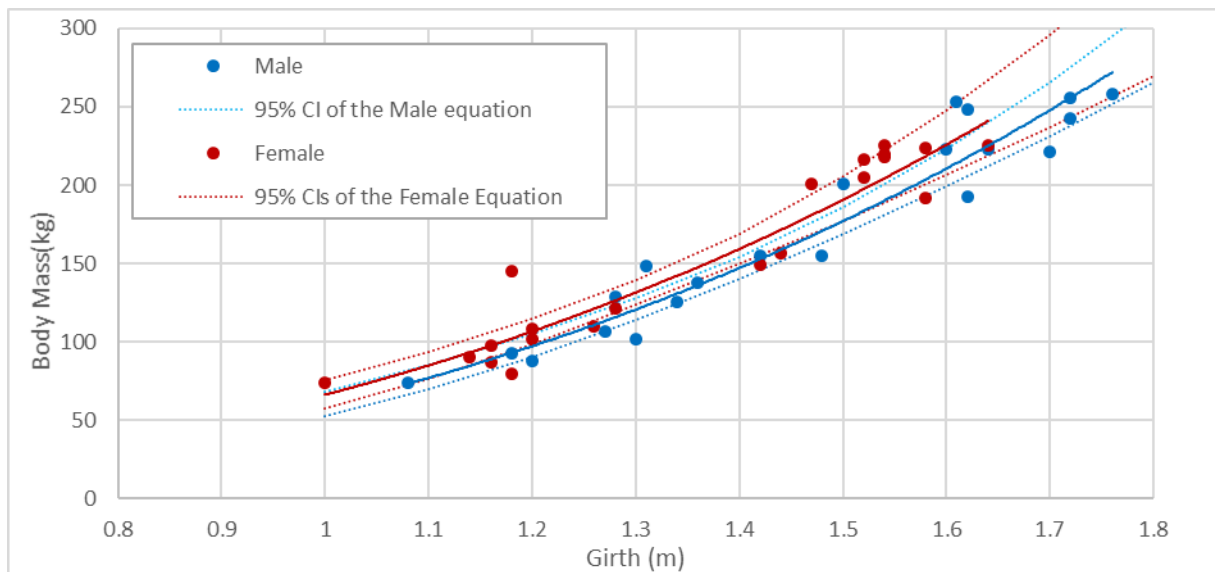


Figure 2-10: Sex effect on the girth equation.

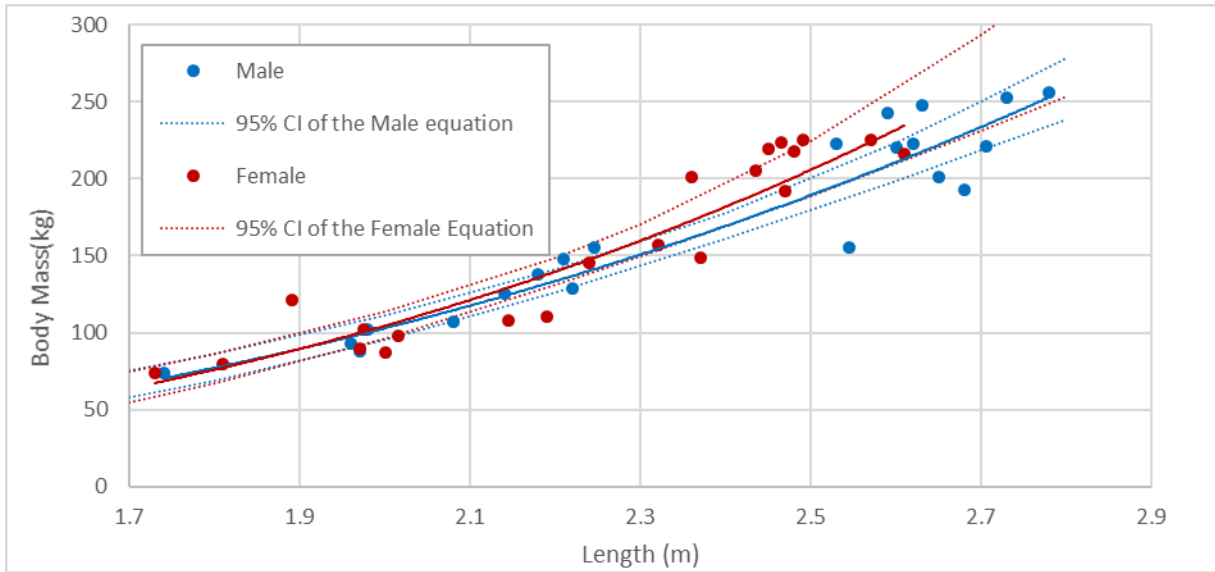


Figure 2-11: Sex effect on the body length equation

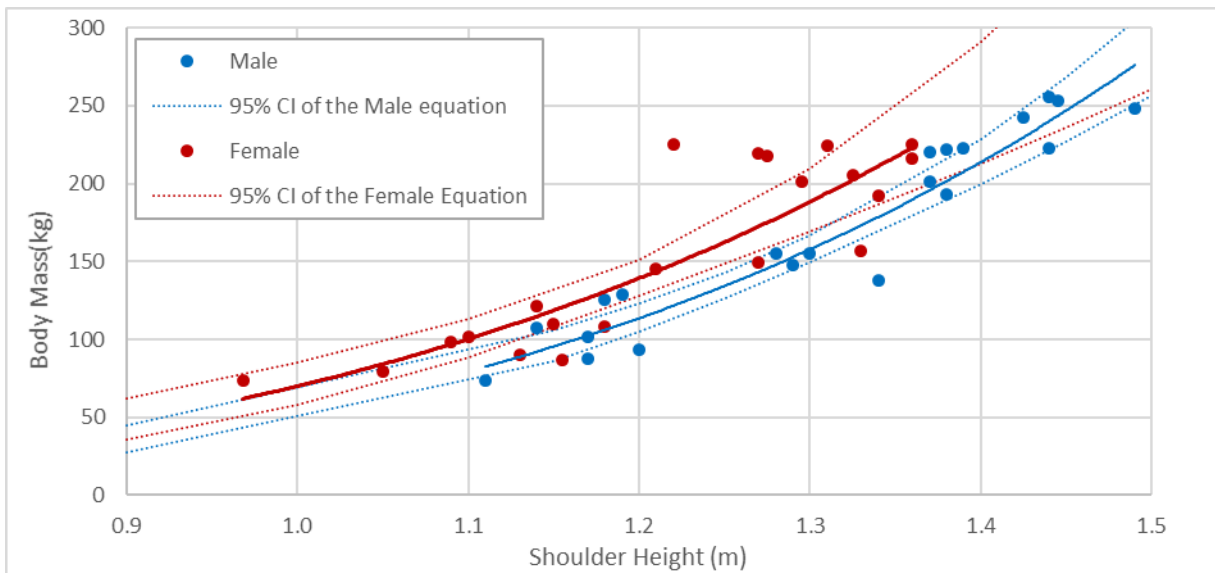


Figure 2-12: Sex effect on the shoulder height equation

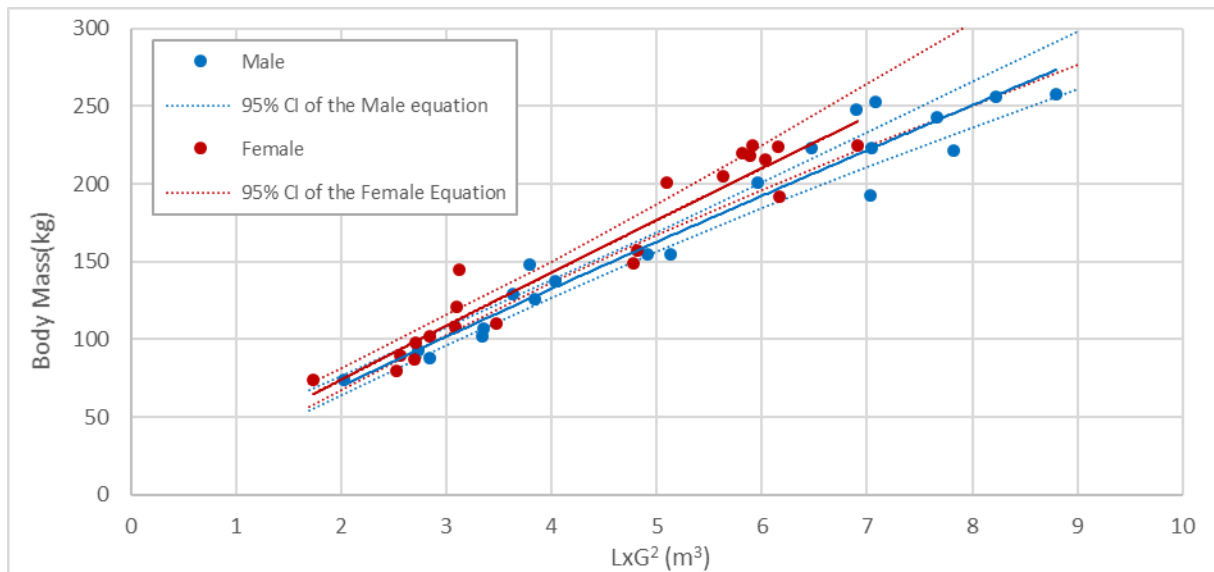


Figure 2-13: Sex effect on the length times girth squared equation

The power equations derived from post-natal male and female wildebeest data are summarised in Table 2-5.

For all body measurements, the exponents of the equations are not significantly different from each other, as shown by the SMATR test for similar slope (Table 2-6). This means that for any given change in any of the linear measurements, the change in body mass is the same, irrespective of the sex of the animal.

The elevation of the equations, other than the body length equation, are significantly different from each other. This significant difference indicates that for any given girth or shoulder height, the female equation predicts a significantly larger body mass than the male equation.

There are significant differences between body masses measured for the two sexes and those predicted by the equation for the opposite sex (Table 2-6). The smallest difference between the two sexes is seen in the body length equation, where the mean mass difference between measured females and masses predicted by the male equation is approximately 10kg (5.5% of predicted) and is not statistically significant ($p=0.104$). Despite the statistical significance of the differences, mean differences are only greater than 10% of the predicted body mass for the shoulder height equations.

Sample	Equation $M_b = a(x)^b$					Goodness of fit		n
	x	b	SE (b)	a	SE (a)	Adjusted R ²	PPE _A	
Male	Body length (m)	2.72	0.161	15.7	2.21	0.931	9.07	22
Female		3.04	0.250	12.7	2.57	0.880	12.4	21
Male	Body length x Girth ² (m ³)	0.918	0.0439	37.1	2.70	0.956	9.04	21
Female		0.948	0.0588	38.4	3.27	0.928	11.0	21
Male	Girth (m)	2.67	0.158	59.6	3.72	0.935	10.2	21
Female		2.61	0.196	66.1	4.27	0.898	12.2	21
Male	Shoulder height (m)	3.93	0.0286	55.8	4.61	0.900	16.1	22
Female		3.77	0.437	70.0	6.49	0.786	25.5	21

The regressions are statistically significant in all cases. $P(b = 0) < 0.001$
SE: Standard Error
PPE_A: absolute mean percent prediction error.

Table 2-5: Sex-specific mass prediction equations for blue wildebeest

Body Measurement	SMATR ^a		Mean Difference ^d					
	Exponent ^b	Intercept ^c	Female measured - Male prediction			Male measured - Female prediction		
			kg	PPE ^e	p	kg	PPE	p
Body length (m)	0.280	0.148	9.85	5.49	0.104	-14.4	-6.09	0.010
Body length x Girth ² (m ³)	0.693	0.010	12.7	8.54	0.003	-15.1	-7.56	<0.001
Girth (m)	0.772	0.028	13.6	10.0	0.005	-12.2	-6.83	0.003
Shoulder height (m)	0.758	<0.001	29.0	23.6	<0.001	-33.0	-16.1	<0.001

a. SMATR: Results are p-values for the test for heterogeneity in slopes^b and the test for shift in elevation^c between the samples. $H_0: \text{male Sample} = \text{female Sample}$.

d. Mean differences were calculated when comparing the measurements made on one sex and the values predicted by the equations for the other sex.

e. PPE: mean percent prediction error. Two-tailed t-test, $H_0: \text{Mean PPE} = 0$

Table 2-6: Comparison of the sex-specific equations for body-mass prediction

The Effect of Season

Rainfall records and observation of the vegetation growth allowed three different seasons to be defined as follows:

‘Dry’ describes samples that were collected at the end of a prolonged dry season. Data for this season were collected in late November and early December 2008. The first rains fell late, twenty-two days before data collection. The spring rains commonly start in September or October but at the time of collection only 45.5 mm had fallen and vegetation was scarce.

‘Mid’ describes data collected in November and December of 2009 and 2010. More than 75 mm had fallen in the month before data collection took place and normal vegetation growth expected in spring had begun.

‘Wet’ describes data collected in April 2009, when grazing was abundant, after the end of the rainy season which lasted from November 2008 to February 2009.

When regression equations are derived for each season, the Mid and Dry Season equations are very similar and Wet Season Equations consistently predict larger body masses than the Mid and Dry Season Equations (Appendix 3). Subjective examination of scatterplots of the relationships between the two extreme seasons (Wet and Dry) for each body measurement, (Figure 2-14 to 17) show overlapping datasets and 95% confidence intervals.

Despite the overlap, formal evaluation of the datasets and power equations (Table 2-8) show significant differences between body masses in the Wet and Dry season. The slopes of the regression equations for the Wet and Dry season are not significantly different from each other but this allows comparison of the elevations of the equations, which shows a significant difference in all cases except for the body length equation.

The PPEs for body masses predicted by the equation for the opposite season are larger than between the two sexes, with only the body length differences less than 10% (Table 2-8). All the differences are statistically significant and this supports the findings of the SMATR analysis.

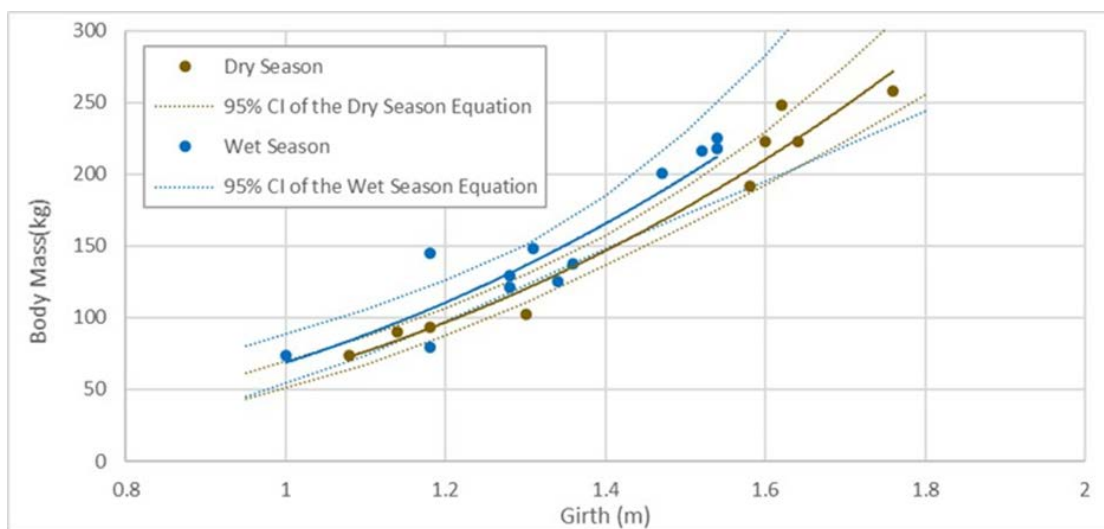


Figure 2-14:Season effect on the girth equation

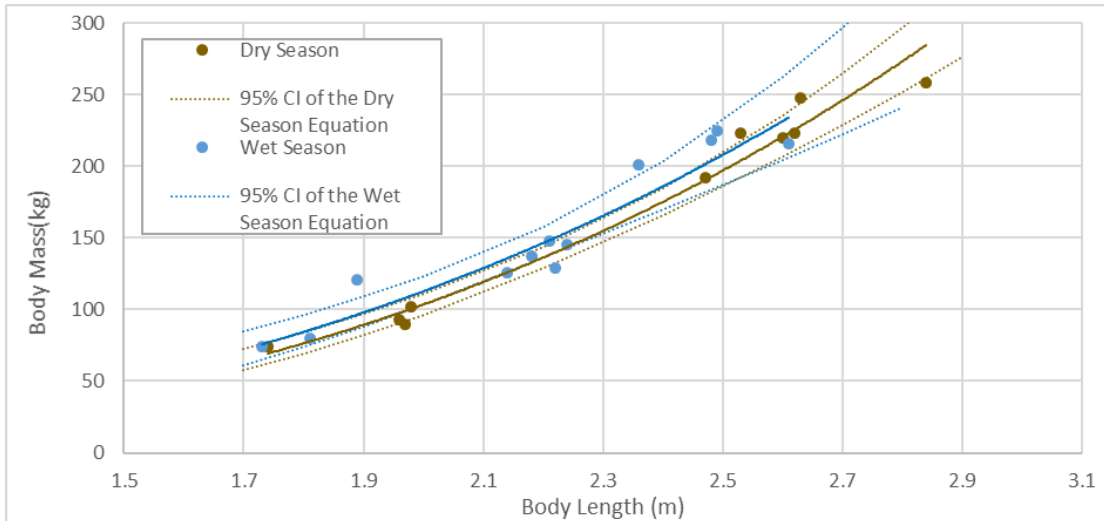


Figure 2-15: Season effect on the body length equation

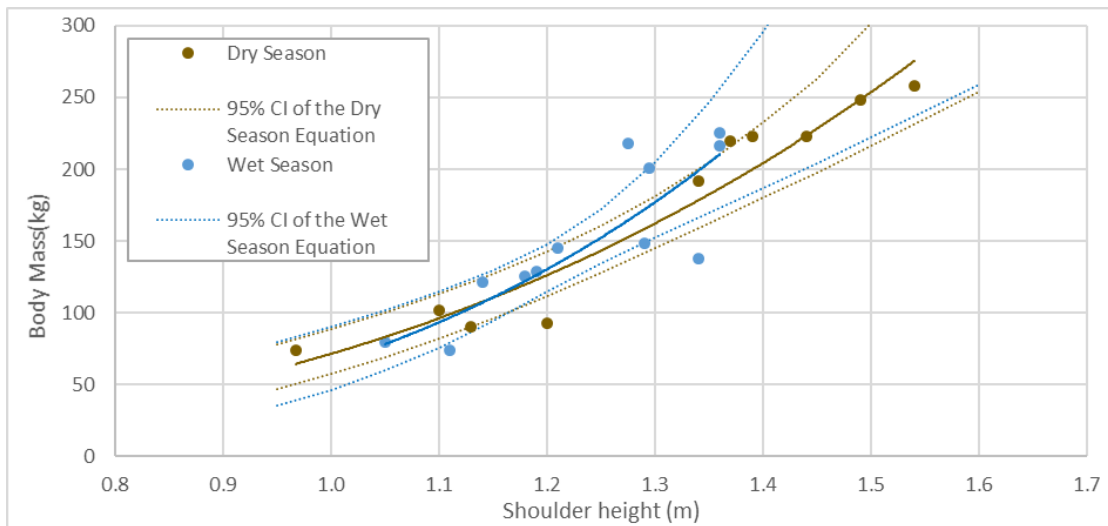


Figure 2-16: Season effect on the shoulder height equation

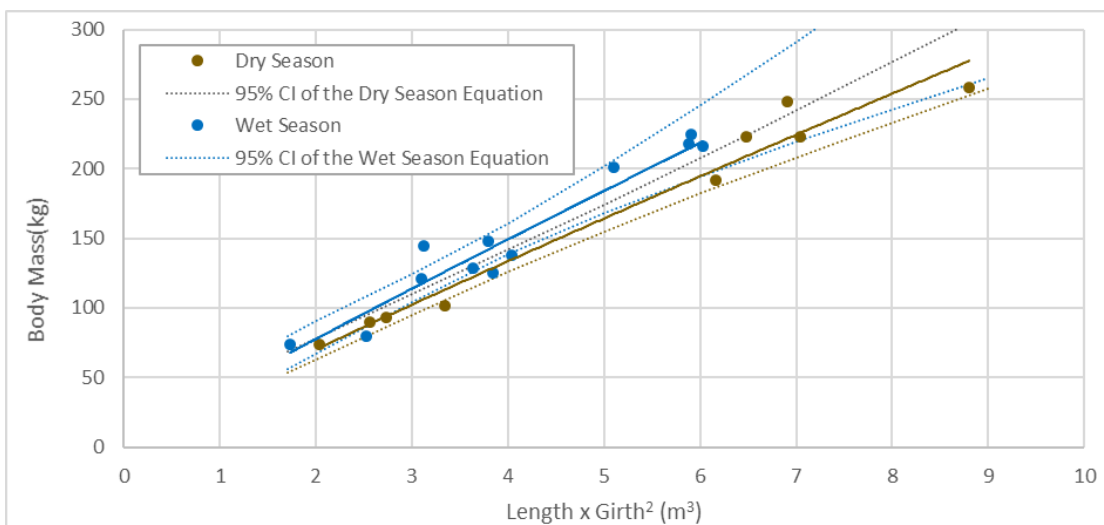


Figure 2-17: Season effect on the length times girth squared equation

Sample	x	Equation $M_b = a(x)^b$				Goodness of fit		n
		b	SE (b)	a	SE (a)	Adjusted R ²	PPE _A	
Wet	Body length (m)	2.75	0.262	16.80	3.48	0.908	7.73	12
Dry		2.88	0.141	14.03	1.68	0.979	10.2	10
Wet	Body length x Girth ² (m ³)	0.941	0.0904	40.52	5.08	0.907	10.5	12
Dry		0.927	0.0472	36.96	2.78	0.980	14.1	9
Wet	Girth (m)	2.62	0.366	68.66	7.69	0.820	11.7	12
Dry		2.70	0.174	59.13	3.98	0.968	17.5	9
Wet	Shoulder height (m)	3.16	0.471	77.11	7.99	0.800	14.4	12
Dry		4.04	0.337	52.50	5.18	0.940	28.9	10

The regressions are statistically significant in all cases. $P(b = 0) < 0.001$
SE: Standard Error,
PPE_A: absolute mean percent prediction error.

Table 2-7: Regression equations for prediction of body mass in different seasons and tests for significant differences between the equations

Body Measurement	SMATR ^a		Mean Difference ^d					
	Exponent ^b	Intercept ^c	Wet season measured - dry prediction			Dry season measured - wet prediction		
			kg	PPE ^e	p	kg	PPE	p
Body length (m)	0.659	0.080	11.1	8.5	0.036	-10.3	-6.4	0.014
Body length x Girth ² (m ³)	0.892	0.007	16.8	12.2	0.005	-20.2	-10.5	<0.001
Girth (m)	0.837	0.025	18.9	14.6	0.015	-19.9	-11.2	0.002
Shoulder height (m)	0.160	0.007	27.3	25.6	0.002	-20.0	-12.9	0.013

a. SMATR: Results are p-values for the test for heterogeneity in slopes^b and the test for shift in elevation^c between the samples. $H_0: \text{wet season sample} = \text{dry season sample}$.

d. Mean differences were calculated when comparing the measurements made on wildebeest in one season and the values predicted by the equations for the other season.

e. PPE: mean percent prediction error. Two-tailed t-test, $H_0: \text{Mean PPE} = 0$

Table 2-8: Comparison of the season-specific equations for body-mass prediction

Comparison of East and Southern African Wildebeest

When the body mass prediction equations for Southern African blue wildebeest are compared with those derived for East African subspecies, the East African equations generally predict higher body masses. Visual inspection of scatterplots of the data (Figure 2-18 to 2-20), shows that the data overlap and that the 95% confidence intervals of the equations overlap at body masses below approximately 130kg.

There is a significant difference between the slopes of the LxG^2 equations (Table 2-9). For both girth equations, the slopes are not significantly different but the elevations are. Therefore, for any given girth, the body mass of an average East African Wildebeest is significantly different ($p < 0.001$) from that of an average Southern African Wildebeest.

When the measured body masses were compared with the masses predicted by the East African equations (Table 2-9) the body masses of the Southern African wildebeest were on average, approximately 7.5% smaller than predicted. The differences are statistically significant in all cases ($p < 0.001$).

The equation mentioned by Attwell (Table 2-1) for Southern African wildebeest, appears very similar to the East African Equation, but there is insufficient information to explore reasons for the apparent similarity and to compare this equation with the linear equation formulated in this study.

Equation	SMATR ^a		Mean Difference ^d (measured – predicted)		
	Exponent ^b	Intercept ^c	kg	PPE ^e	p
Body length x Girth ² (m ³)	<0.001	-	-14.5	-6.97	<0.001
Girth (m) - linear	0.476	<0.001	-13.2	-7.54	<0.001
Girth (m) - power	0.210	<0.001	-17.2	-8.43	<0.001

a. SMATR: Results are p-values for the test for heterogeneity in slopes^b and the test for shift in elevation^c between the samples. H_0 : *Southern African sample = East African sample*.

d. Mean differences were calculated when comparing the body masses of the wildebeest in this study and the values predicted by the East African equations.

e. PPE: mean percent prediction error. Two-tailed t-test, H_0 : *Mean PPE = 0*

Table 2-9: Comparison of the southern African wildebeest with the East African equations for body mass prediction

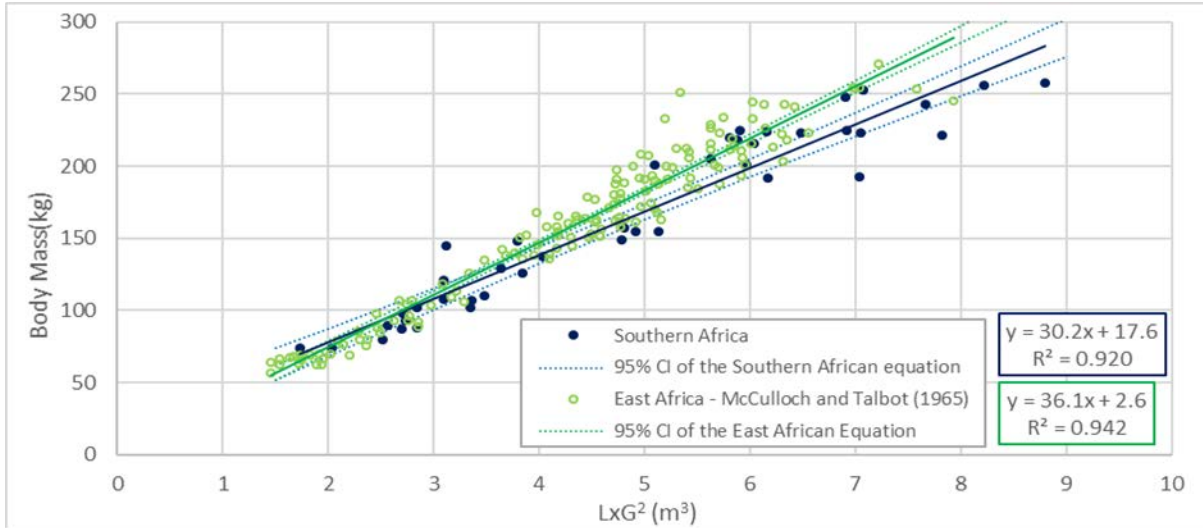


Figure 2-18: Comparison of the Southern and East African length times girth squared data and linear equations.

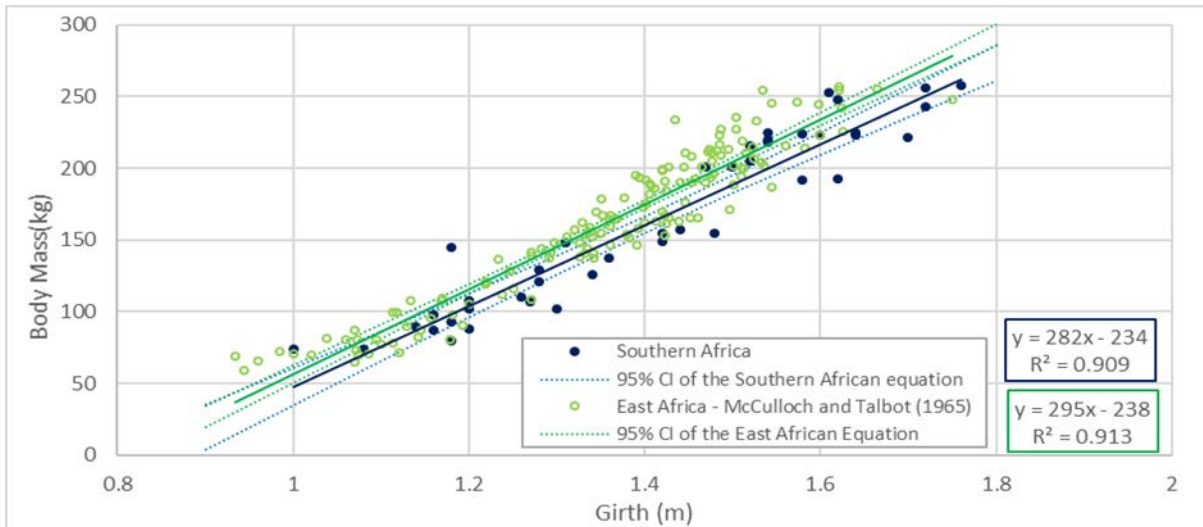


Figure 2-19: Comparison of the Southern and East African girth data and linear equations

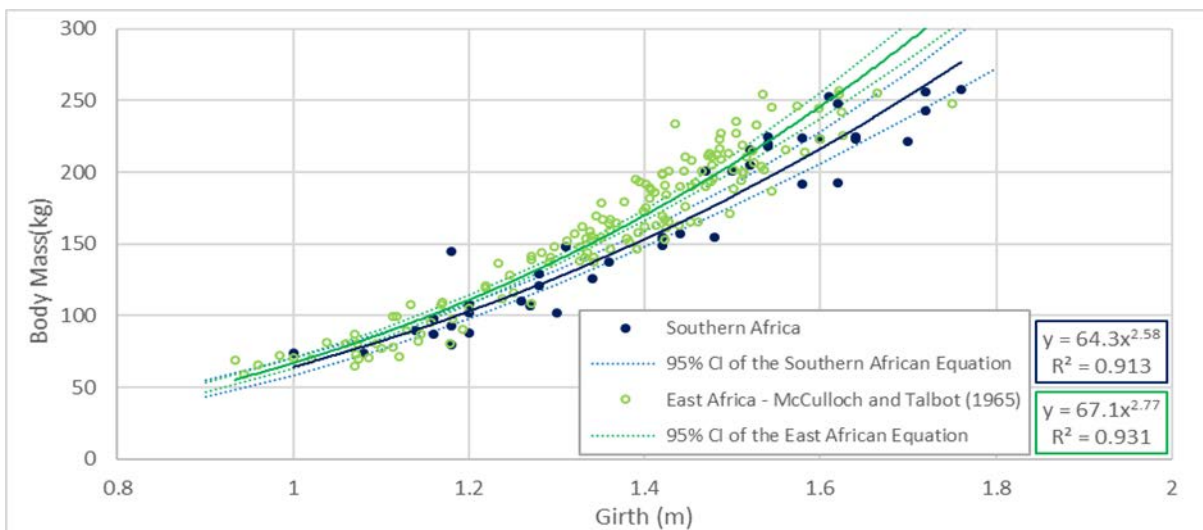


Figure 2-20: Comparison of the Southern and East African girth data and power equations

Discussion

Prediction of Body Mass

The suitability of linear or power equations

This study aimed to determine whether blue wildebeest body mass could be calculated from body measurements, to establish which measurements predict mass most accurately, and to decide what form of equation is most suitable.

The mass of an animal can be predicted from other measurements only if the relationship between those measurements and body mass remains constant as the animal grows. If the ratio between the change in the measurement and change in mass remains constant, then a linear equation can be used to calculate mass accurately. If the ratio changes and that change is proportional to the animal's mass, then a power equation is more appropriate. Since the ratio between any length measurement and mass changes with size, power equations are expected to be more appropriate than linear equations in these cases.

The data from this study show that power equations are more appropriate. For the Post-Natal sample, the difference between the two forms of equation is not immediately apparent. However, on scatterplots of the Entire Sample, the relationship between any single measurement and body mass is clearly curvilinear. When the indicators of goodness of fit, R^2 and PPE_A , are considered, power equations are shown to be better predictors of body mass than linear equations, in all cases.

The only linear relationship which is theoretically justified is that for length times girth squared. This is because the units of this variable are m^3 which are expected to scale with body mass in a linear fashion. Although a linear relationship between the other variables and body mass is theoretically flawed, in the Post-Natal sample, the practical difference between a linear and a power equation is minor. The mean difference between measured and predicted body masses for these relationships (PPE_A) is approximately 10% and so linear equations can be used to predict body mass from both body length and girth with reasonable accuracy, given that daily variation in body mass can exceed 10% (McCulloch & Talbot, 1965).

Suitability of different body measurements for predicting body mass

Body mass can be estimated most accurately with the length times girth squared ($L \times G^2$) power equations, which have smallest PPE_A values and the largest R^2 values, indicating the best fit to the data. The reliability of the $L \times G^2$ equation is increased because it relies on two measurements and so reduces inaccuracy caused by possible errors in the measurement of one variable. The equations using body length alone and girth alone are only slightly less accurate, with the measured body masses and those calculated by the Post-Natal equations within 10% of each other. If only one measurement is used to estimate body mass, girth is more suitable than body length.

Shoulder height was found to be the least suitable measurement for the estimation of body mass. The differences between measured body masses and those predicted by the shoulder height equations are larger than 10%. This is most likely due to measurement error rather than greater natural variation in shoulder height compared with other measurements. Shoulder height was measured along the contours of the body, from the middle of the base of the lateral hoof wall (held in a natural standing position) to the highest point of the withers. The measurement is difficult to make, requiring an assistant to hold the leg in position. Additionally, the method of measurement is subjective and prone to error because it is not a straight-line measurement; the withers are not directly dorsal to the long axis of the leg. For these reasons, this equation, using shoulder height, is not recommended for body mass estimation. A more accurate method may be Sachs' (1967) 'shoulder height between pegs', which attempts to simulate the vertical distance from the ground to the top of the withers in a standing animal. An equation using this method of measuring shoulder height would also be useful to estimate the body mass of live animals from photographs.

Inclusion of foetal measurements

The foetal animals which were measured in this study were all between 12 and 15kg and had approximate gestational ages of seven months. Calving season in the Bubyane Conservancy is between December and January and the foetuses were collected in late November and early December. The length of gestation in the wildebeest is approximately eight months and calves are born weighing approximately 22kg (Skinner & Chimimba, 2005; Estes, 2014). The finding that the extrapolated power relationships for the Post-Natal sample can closely predict the body masses of these foetuses suggests that the relationships between body measurements and mass in the near-term foetus similar to that in the post-natal animal. If so, it is reasonable to assume that the power equations are suitable for estimating the body mass of juvenile wildebeest smaller than the smallest post-natal wildebeest measured in this study (74kg).

Although the Entire Sample equations have higher R^2 values than the Post-Natal equations, this does not confirm that they are better models. The high R^2 values are influenced by the larger sample size and greater range of the Entire Sample. The PPE_A values are also higher in the Entire Sample equations than in those for the Post-Natal sample, indicating that the Post-Natal equations are better predictors of body mass for animals within their range.

Allometry

According to equations for the Entire Sample, body mass scales hyperallometrically with both body length and shoulder height and hypoallometrically with girth. This means that the relative proportions of the wildebeest change with growth. Expressed differently, as the wildebeest grows, body length and shoulder height become relatively shorter and girth relatively larger. In the Post-Natal equations, body mass still scales hypoallometrically with girth but the relationship between body mass and both body

length and shoulder height are not significantly different from isometry. This allometry suggests that in the growing post-natal animal, body length and shoulder height remain the same relative to body mass but girth increases disproportionately. However, as sample size decreases, the probability of incorrectly concluding an isometric relationship (type II error) increases for allometric relationships that are close to isometry (Brown & Vavrek, 2015). Therefore, the relationships between body mass and both body length and shoulder height can be referred to as soft isometry to indicate uncertainty in the conclusion of an isometric relationship.

The Effect of Sex and Season

Sex

Separate mass prediction equations for male and female animals were found to be unnecessary in East African antelope, including wildebeest (McCulloch & Talbot, 1965). Although wildebeest exhibit limited sexual dimorphism (Lent, 1974; Georgiadis, 1985; Estes, 2014), males achieve greater adult body masses than females and at greater ages than females (Attwell, 1982b; Hopcraft, 2010). According to Attwell (1982b), female wildebeest in Zululand, South Africa, reach adult body mass and girth at approximately three years old but shoulder height continues to grow until approximately four-and-a-half years. In males, adult girth is reached at three-and-a-half years and shoulder height at four years but body mass increases until approximately six years old. In this case, the relationship between body mass, shoulder height and girth would be different in adult male and female wildebeest, especially in animals older than approximately four years old (approximately 200kg). However, Attwell's study used small sample sizes (11-17 animals) and it is difficult to determine the age of wild animals accurately and therefore the asymptote of the growth curves (Attwell, 1982b). The data from the current study, do not appear to support Attwell's data. Body measurements continue to change with body mass in animals weighing more than 200kg. There is a slight tendency for the equations to overestimate the body masses of the heaviest animals, but a larger sample size would be necessary to explore this. Further studies may still show that an animal's adult mass and body dimensions are achieved at roughly the same time.

The significant difference between the body masses of male and female animals of a given body measurement for girth, shoulder height and LxG^2 measurements (significant differences in elevation) (Table 2-6) and the significant differences between the measured body masses of the animals of each sex and those predicted by the equations for the opposite sex, show that the relationship between body mass and body measurements is affected by sex. However, the mean differences between measured and predicted masses are less than or equal to 10% for the length, girth and LxG^2 relationships.

Using separate equations for male and female animals does not necessarily improve the accuracy of the equations. The male equations have greater R^2 values than the combined equation but the R^2 values of the female equations are lower and, in all cases but one, the percent prediction error of the sex specific

equations is higher than for the combined equation. These differences are likely due to the sex-specific equations having half the sample size of the combined equation. Therefore, although sex affects the relationship between body mass and external body measurements, there is no benefit in predicting body mass from the sex-specific equations derived in this study. There may, however, be an advantage to sex-specific equations if they can be derived from larger sample sizes in future.

Season

In many animals, season affects body mass significantly. If seasonal variation in mass is not accompanied by similar changes in the variable from which mass is predicted (such as body length) then the relationship between that variable and mass will be dependent on season. In such a case, either the mass prediction equation must take season into account or separate equations must be used for different seasons.

A disadvantage of using different equations for different seasons is that changes in body mass based on season are gradual and continuous. Animals that increase body mass in seasons with plentiful resources and then lose body mass in resource-poor seasons do so gradually. Therefore, separate equations for predicting body mass in summer and winter or wet and dry seasons will be inaccurate, unless used with caution. Additionally, seasonal changes which affect body mass are not necessarily the same each year which is especially true for the savanna ecosystem, which is prone to highly variable and unreliable rainfall, causing large differences in available vegetation from year to year. Seasonal effects have been shown to be less extreme for wild ungulates living in the tropical areas of East Africa than for those living in temperate areas (McCulloch & Talbot, 1965).

The variability in available vegetation in different seasons and years was apparent during the data collection for this study, prompting the investigation into how season affects body mass. Three distinct seasons were identified and compared because of the clear difference in available vegetation between the Dry and Mid seasons, although the data were collected in the same months, in successive years.

Comparison of the wet and dry season equations show evidence for a significant season effect. SMATR tests for common elevation show that the body mass of the average animal (of a given girth, shoulder height or LxG^2 measurement), is significantly greater in the wet season. The difference between body masses measured in each season and those predicted by the equations derived from the data in the other season is greater than 10% for the girth, shoulder height and LxG^2 equations and, despite the small sample sizes and large confidence intervals of each equation, the differences are statistically significant for all equations (Table 2-8).

Sub-division of the sample into three separate seasons drastically reduced the sample sizes; the Wet and Dry season equations are based on between 9 and 12 animals. The small samples are likely to reduce the accuracy of the prediction equations and increase the chance of type II errors (conclusion of no

difference when one exists). It is likely that with a larger sample size, a significant difference between the elevation of the body length equations could become apparent.

The season-specific equations are not able to predict the body masses of individual wildebeest more accurately than the combined equations, based on the comparison of mean absolute PPEs. It is also difficult to decide which season-specific equations would be most appropriate to use in a practical situation, because of the continuous variation in seasons and the complex effects that they have on vegetation and animals. The season-specific equations are therefore of little practical value.

Interaction between sex and season

A limitation of the sex or season-specific equations is possible interaction between sex and season. Of the 10 animals measured in the dry season, only two were female and of the 12 animals measured in the wet season, only four were male. In the sex-specific equations, the effect of season is less likely to have an effect because approximately half of the animals of each sex were sampled in the Mid season, reducing the effect of the Wet and Dry season biases.

Comparisons of the sex-specific equations using only Mid season data (Appendix 4) show significant differences between the elevation of the male and female equations in the Shoulder height and LxG^2 but not the individual length and girth equations. The lack of a significant difference between the girth equations may be due to the reduced ability to distinguish small differences when sample sizes are small. The effect of sex on the season-specific equations is more difficult to test because of the small samples sizes in the Wet and Dry Season. Separate sex-specific equations plotted for the Wet and Dry season do suggest that sex has little impact on the difference between the season-specific equations (Appendix 5). However, the samples are not large enough to test the interaction in a statistically meaningful way.

Comparison of Southern and East African Blue Wildebeest

This study attempted to detect any existing race allomorphy; differences in size between animals of different subspecies at the same growth stage. The southern African blue wildebeest, *C. t. taurinus*, and the East African subspecies, *C.t. mearnsi* and *C.t. albojubatus*, were compared.

The body masses and measurements for adult blue wildebeest measured in this study are similar to those measured in other populations of *C.t. taurinus* and summarised in Attwell (1982b).

The East African wildebeest studied by McCulloch and Talbot (1965) were of two different subspecies (*C.t. mearnsi* and *C.t. albojubatus*), which are genetically distinct from the blue wildebeest found in Southern Africa (*C.t. taurinus*).

Misclassification and changing nomenclature has confused comparisons of the subspecies. For example, Talbot and Talbot (1963) recorded body measurements for *C.t. hecki*, which are reported by Attwell (1982b) under the more recently accepted name *C.t. mearnsi*. Measurements by Sachs (1967) are reported by Attwell (1982b) for *C.t. albojubatus*. However, although the subspecies are referred to as *C.t. albojubatus* in the original paper, the animals were collected in the ‘Grumeti Controlled Area, bordering on the Western Corridor of the Serengeti National Park, Mara Region, Tanzania’ which is outside the range of *C.t. albojubatus*. The subspecies sampled was most likely the local *C.t. mearnsi* and these measurements have subsequently been used to describe *C.t. mearnsi* (Estes, 2014). The East African wildebeest, especially *C.t. mearnsi*, are considered smaller than the Southern African subspecies, on average (Sachs, 1967; Attwell, 1982b; Estes, 2014).

Comparison of average body masses for different animals should be done with caution, even when the comparison is done between only adult animals, which are presumed to have stopped growing. It is difficult to assess when animals have reached their full adult weights, even when they are aged according to dentition (Sachs, 1967). If only the incisors are inspected or even if an animal is considered sexually mature, it may not yet have achieved its full body mass (Attwell, 1980, 1982b; Hopcraft, 2010). Eruption of teeth is only useful in aging an animal less than approximately three years old (Attwell, 1980). *C. mearnsi* females reach adult body mass around this age, but males continue to grow between four and six years old (Watson, 1967 in Hopcraft, 2011; Talbot and Talbot, 1963). *C. t. taurinus* males grow until about six years old (Attwell, 1982b). Both male and female wildebeest are theoretically sexually mature between two and three years old, although most females only breed in their third year, and males do not mate until four or five (Watson, 1967 in Hopcraft, 2011; Estes, 2014).

For any given girth, the average East African wildebeest has a significantly larger body mass than the corresponding Southern African animal. Change in body mass per unit change in length times girth squared ($L \times G^2$) is different in East and Southern African wildebeest; for a given measurement of $L \times G^2$, the slopes of the East and Southern African equations differ. For animals weighing less than 150kg, a difference cannot be distinguished, but, for larger body masses, the difference becomes progressively larger. From these results, it can be concluded that adult Southern African blue wildebeest have greater body lengths relative to mass than the East African subspecies or that, for a given body length, the East African subspecies are heavier.

The differences between the subspecies may be genetic or environmental and are likely to be due to a combination of both factors. The animals studied by McCulloch and Talbot were collected in the Maasailand of Southern Kenya and Northern Tanzania. Differing annual rainfall, soil fertility, vegetation types and competition with other grazers are among the ecological factors which could lead to differences in relative differences in body mass. Anthropogenic factors such as fencing and culling

also affect the two populations of wildebeest differently and may affect the ecology of the two populations enough to affect relative body masses.

The differences between the Southern African blue wildebeest which were studied in this study and the East African subspecies studied by McCulloch and Talbot (1965) may not necessarily describe the differences between the average members of each subspecies. Both studies only sampled animals from a specific subpopulation from each area and the differences found may only represent differences between these two subpopulations. These subpopulations could easily differ from the averages of the subspecies which they represent, due to habitat or genetic differences or differences in anthropogenic factors.

Conclusion

Wildebeest body mass can be predicted from external body dimension, with an error of less than 10%. The relationships between external measurements and body mass are curvilinear and so power equations are more appropriate than linear equations for predicting body mass. By combining the measurements of length (L) and girth (G) into a single term ($L \times G^2$), body mass can be predicted more accurately than by using each term individually. Although measurements made on late-term fetuses fit the trends found in post-natal animals, equations based on both foetal and post-natal data are less accurate and are not recommended for estimating body mass. Both sex and season have statistically significant effects on the relationship between body dimensions and mass. However, sex and season-specific equations are not recommended for body mass prediction because the subdivision of the sample needed to form these equations reduces the sample size and accuracy of the equations. Southern African blue wildebeest have relatively lower body masses compared to body dimensions than previously-studied subspecies from East Africa.

Chapter 3: Ontogenetic Allometry of the Blue Wildebeest Heart

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Summary

The growth of the blue wildebeest heart is modelled with ontogenetic allometric equations, contributing an additional species to the small collection of ontogenetic allometric studies on mammals. Heart mass scales hypoallometrically with body mass in the post-natal animal and can be estimated using the equation: $M_h = 0.0118M_b^{0.832}$ ($R^2 = 0.846$, $PPE_A = 10.9\%$). While the dimensions of the left heart grow isometrically with heart mass, the thickness of the right ventricular wall does not appear to change significantly after birth. Including heart measurements made on near-term fetuses did not alter the allometric equations significantly for heart mass or dimensions. The measured post-natal wildebeest heart masses are, on average, 5.7 and 4.1% smaller than predicted by the interspecific equations for mammals. The ontogenetic equation for wildebeest heart mass follows that for domestic cattle closely. Wildebeest have lower heart masses than giraffes and horses of the same body mass and larger hearts than African buffalo. The thickness of the left ventricular wall in wildebeest is not unusual for a mammal.

Introduction

The overwhelming abundance of blue wildebeest (*Connochaetes taurinus*) is an indication that their anatomy and physiology are particularly well suited to their natural habitat. Wildebeest do not reach the extreme body masses of Africa's mega herbivores, nor do they have any outstanding anatomical features which would have an unusual effect on their cardiovascular systems (such as giraffe have). Wildebeest, unlike the more-thoroughly-studied domestic species, have not undergone artificial selection for increased size, muscle mass or athletic ability. Therefore, blue wildebeest are expected to have unexceptional cardiovascular systems and are good candidates for studying normal mammalian heart growth.

Wildebeest are not entirely unremarkable: they are famous for their mass migrations in which they can move approximately 1500km a year (Estes, 2014). Although many wildebeest populations are now forced to be more sedentary due to the loss of natural migration routes, their physiology and anatomy is still that of an animal capable of and well-adapted to travelling long distances.

The wildebeest calf is the most precocious of all antelope (Lent, 1974) and can stand and keep up with the herd within seven minutes of birth (Estes, 1966, 1976). To enable this, many of the calf's organ systems, especially the locomotory, respiratory and cardiovascular systems, should be fully functional at birth and therefore more mature than the same organ systems of other mammals at that age. It is therefore interesting to model both foetal and post-natal organ growth.

This study aims to describe the growth of the blue wildebeest heart by formulating ontogenetic allometric equations for heart mass and several cardiac dimensions. These equations describe whether

relative heart mass and size remain the same throughout life or change with growth. These equations also form the basis for testing the difference between foetal and post-natal growth, and between wildebeest and other species. The latter includes the differences between the ontogenetic growth of the heart and predictions made by interspecific allometric equations based on mainly adult animals. Physiological values for the wildebeest are predicted from previously published allometric equations.

Allometry of the Heart

The size and structure of the mammalian heart is closely correlated with body mass. Heart mass makes up approximately 0.6% of body mass in most mammals, but smaller mammals have proportionally larger hearts than larger mammals (Prothero, 1979) (Table 3-1). Relative heart mass is also influenced by activity: more athletic species and breeds have relatively larger hearts than those that are considered less athletic. Additionally, exercise and training also increase relative heart size within a species or breed (Joseph, 1908; Brody, 1945; Hanson, Kline & Foreman, 1994; Poole & Erickson, 2011; Østergaard *et al.*, 2013).

The majority of research on the allometry of the mammalian heart has been interspecific: the relationship between the variable of interest and body mass for adult animals across multiple species (Brody, 1945; Holt, Rhode & Kines, 1968; Martin & Haines, 1970; Prothero, 1979; Woodall, 1992; Calder, 1996; Seymour & Blaylock, 2000; Lindstedt & Schaeffer, 2002; White & Seymour, 2014, 2015). In contrast, intraspecific allometric equations for heart mass are rare but available for mature animals in a few species (Brody, 1945; Mitchell & Skinner, 2009). No examples of ontogenetic equations that model heart growth from foetus to adult have been published for mammals of similar size to the wildebeest. However, data from which equations can be derived are available for the horse and giraffe (Crile & Quiring, 1940; Mitchell & Skinner, 2009).

Intraspecific and Ontogenetic Allometry

Intraspecific equations on the allometry of heart mass for several species were calculated and published by Brody (1945), along with an interspecific mammalian equation. The species with similar body masses to wildebeest were hogs, cattle and horses. Brody's equations show that adult horses have proportionally larger hearts, and steers and hogs proportionally smaller hearts, than the average mammal. Brody's equations were calculated only from measurements of relatively mature animals. These intraspecific adult equations are useful for comparison with his interspecific mammalian equation, which was also based on adult animals, but they do not necessarily represent the ontogenetic allometric relationships for any of the species.

The only studies to have published ontogenetic allometric equations of the cardiovascular system wild mammals in roughly the same body mass range as the wildebeest are one study on giraffes (Mitchell & Skinner, 2009) and one on kangaroos (Snelling *et al.*, 2015). This study on giraffes showed that,

although they have highly specialised cardiovascular systems that maintain a high blood pressure, their average relative heart mass is not different from that of other mammals (Mitchell & Skinner, 2009). The equations for foetal and post-natal heart growth were reported separately.

The work on kangaroos compared the allometry of heart growth of joey's in the pouch and after emerging from the pouch and showed that heart growth was hyperallometric to body mass in the pouch and hypoallometric in free-roaming juveniles and adults.

Because of the lack of ontogenetic studies, interspecific studies are used to estimate ontogenetic relationships. However, the suitability of interspecific equations for making predictions for immature animals is questionable (Gould, 1966). Furthermore, foetal and post-natal growth rates may be similar or differ significantly but the question has not been well studied, a fact that was observed more than 60 years ago but which still has not yet been properly investigated (von Bertalanffy & Pirozynski, 1952).

The differences between the ontogenetic and interspecific allometry of an organ depend on the characteristic under investigation. If the characteristic is specialised in the studied species (such as giraffe neck length) or if it is well developed only in adult animals (such as secondary sexual characteristics) then the differences between ontogenetic intraspecific and interspecific equations should be greater than for a characteristic that is physiologically closely related to body mass in all species and age groups (such as heart mass) (Gould, 1966). Hence, a marked difference between the ontogenetic equation for one species and the interspecific equation can indicate specialisation of the trait in that species.

Interspecific Allometry

Heart Mass

The interspecific allometry of the mammalian cardiovascular system has been thoroughly studied, especially the allometry of heart mass. Heart mass scales almost isometrically with body mass in adult mammals of different species and several authors have postulated that the relationship is truly isometric and can be treated as such for the purpose of further calculations (Holt *et al.*, 1968; Wilson, Schmidt & Hanks, 1984; Schmidt-Nielsen, 1985; Calder, 1996; Lindstedt & Schaeffer, 2002). In many cases an isometric relationship can be assumed for the sake of convenience, although this may be inaccurate and lead to significant errors, especially if the range over which the equations is used is large (Prothero, 1986). When a relationship is close to isometric, large samples are required to confidently conclude that the relationship is indeed isometric (Brown & Vavrek, 2015) and the studies with larger sample sizes report a slight but significant hypoallometric relationship (Prothero, 1979).

One of the more famous equations for heart mass was published by Brody (1945) as $M_h = 5.88M_b^{0.984}$, with heart mass (M_h) in grams and body mass (M_b) in kilograms. It was derived from approximately 200 data points representing mature mammals, ranging in mass from 0.01 kg to

100 000kg. Ninety-five percent confidence intervals for the exponent (0.984) are 0.966 to 1.002 and therefore it can be considered as not significantly different from an isometric equation. This equation is quoted by Stahl (1965) who in turn was quoted by Calder (1996).

Prothero (1979) used the data published by several authors, including Brody (1945), to derive a similar equation from 213 data points. The data represented 104 terrestrial mammal species, ranging in size from the mouse to the Indian elephant and produced the most broadly substantiated interspecific allometric equation for the heart mass of terrestrial mammals ($M_h = 6.2M_b^{0.97}$). In the same publication, Prothero also included five species of cetacean to produce a combined equation for both terrestrial and aquatic mammals ($M_h = 6.0M_b^{0.98}$). Neither of Prothero's equations are affected by sex or habitat. The standard error of the exponents of both equations is reported to be 0.01. The equations can therefore be considered significantly hypoallometric, since the upper 95% confidence intervals for the exponents are less than one.

Heart mass equations can be transformed to solve for relative heart mass by converting the equation to give heart mass in kilograms and dividing by body mass. For example, using Brody's equation, with heart mass in kilograms:

Solving for: Relative heart mass, (heart mass as a fraction of body mass)

$$M_h = 0.00588M_b^{0.984}$$

$$\frac{M_h}{M_b} = \frac{0.00588M_b^{0.984}}{M_b}$$

$$\frac{M_h}{M_b} = 0.00588M_b^{0.984-1}$$

$$\frac{M_h}{M_b} = 0.00588M_b^{-0.016}$$

Heart mass as a percentage of body mass:

$$\frac{100 M_h}{M_b} = 0.588M_b^{-0.016}$$

Heart mass as a percentage of body mass ($\frac{100 M_h}{M_b}$) is $0.62M_b^{-0.03}$ for Prothero's terrestrial mammal equation and $0.60M_b^{-0.02}$ for the equation including aquatic mammals.

Body Mass (kg)	0.01	0.1	1	10	100	1000	Equation
Heart Mass as a percentage of Body Mass	0.63	0.61	0.59	0.57	0.55	0.53	Terrestrial Mammals Brody (1945)
	0.71	0.66	0.62	0.58	0.54	0.50	Terrestrial Mammals Prothero (1979)
	0.66	0.63	0.60	0.57	0.55	0.52	All Mammals Prothero (1979)

Table 3-1. Mammalian relative heart mass (M_h) (heart mass as a percentage of body mass (M_b)) as calculated for different interspecific mammalian equations, for body masses over six orders of magnitude.

According to a study of 13 species of adult African antelope with body masses ranging from eight to 540kg, African antelope have relatively large hearts compared to the average mammal (mean 0.71%). The study derived the equation $M_h=0.01104M_b^{0.884}$ for the group. Small antelope species were found to have proportionally larger hearts (0.82% of body mass for a 10kg animal) than larger species (Woodall, 1992).

Cardiac Anatomy

Several equations have been published to describe the interspecific allometry of anatomical features of the mammalian heart. These include equations for left and right ventricular end diastolic volume (Holt *et al.*, 1968; Seymour & Blaylock, 2000) and left ventricular mass (Holt *et al.*, 1968). Equations for the thickness of the left ventricular wall can be derived from the data published from two studies on blood pressure and ventricular wall stress (Martin & Haines, 1970; Seymour & Blaylock, 2000).

Cardiovascular Physiology

The interspecific allometry of physiological cardiovascular variables has been well studied in mammals. Equations have been published for heart rate (Stahl, 1967; Holt *et al.*, 1968; Calder, 1996; Seymour & Blaylock, 2000), cardiac output (Stahl, 1967; Holt *et al.*, 1968; Seymour & Blaylock, 2000; Lindstedt & Schaeffer, 2002), stroke volume (Holt *et al.*, 1968; Calder, 1996; Seymour & Blaylock, 2000), total peripheral resistance (Holt *et al.*, 1968) and blood pressure (Calder, 1996; Seymour & Blaylock, 2000; White & Seymour, 2014, 2015).

The allometry of blood pressure in 24 species of mammals was described by Seymour and Blaylock (2000). White and Seymour (2014, 2015) increased the sample from 24 to 47 species. This analysis improved the accuracy of the predictive equations and showed that blood pressure increases with body size in mammals, systolic blood pressure is clearly affected by body mass and diastolic pressure less so. Their studies further showed that simple allometric equations are not necessarily the best method of modelling blood pressure.

Holt, Rhode and Kines (1968) conducted an in-depth study of ventricular volumes in 43 anaesthetised and unanaesthetised animals, representing nine species, ranging from rats to cattle (0.49 – 518kg). They derived allometric equations for several cardiovascular variables, relating them not only to body mass but also heart mass, ventricular mass, body surface area, metabolic weight and end diastolic volume. The authors noted that the exponents of several of their allometric equations were very close to exact fractions. The exponent of the allometric equations for heart mass, ventricular volume and ventricular

mass were close to 1, that for heart rate was approximately $\frac{1}{4}$, for peripheral resistance $\frac{-3}{4}$ and for cardiac output $\frac{3}{4}$. They therefore derived further, theoretical equations using these exponents.

The interspecific allometric equations are summarised in Table 3-2. To aid in selecting the most appropriate formula for any variable, the sample size, number of species used, and the approximate mass ranges of the animals studied are summarised in Table 3-3.

Variable	Formulae relating each variable to body mass (M_b) or heart mass (M_h) in kg:	
	Body Mass	Heart Mass
Heart Mass (g)	5.88 $M_b^{0.98}$ (1,2), 2.61 $M_b^{1.10}$ (3), 4.34 $M_b^{1.0}$ (3*), 5.68 $M_b^{1.00}$ (4), 4 $M_b^{1.05}$ (6), 6.2 $M_b^{0.97}$ (7), 6.0 $M_b^{0.98}$ (7), 4.04 $M_b^{1.06}$ (8), 11.04 $M_b^{0.884}$ (11)	
Left Ventricular Mass (g)	1.65 $M_b^{1.11}$ (3), 2.83 $M_b^{1.0}$ (3*)	630 $M_h^{1.00}$ (3)
Left Ventricular Wall Thickness (cm)	2.50 $M_b^{0.46}$ (5), 0.26 $M_b^{0.42}$ (6), 0.28 $M_b^{0.35}$ (8)	
Left Ventricular End Diastolic Volume (ml)	1.76 $M_b^{1.02}$ (3), 2.25 $M_b^{1.0}$ (3*), 1.48 $M_b^{1.1}$ (8)	449 $M_h^{0.94}$ (3)
Right Ventricular End Diastolic Volume (ml)	2.02 $M_b^{1.02}$ (3), 2.37 $M_b^{1.0}$ (3*)	469 $M_h^{0.94}$ (3)
Heart Rate (beats/min)	236 $M_b^{-0.25}$ (3), 246 $M_b^{-0.25}$ (3*), 218.8 $M_b^{-0.24}$ (6), 227 $M_b^{-0.23}$ (8), 241 $M_b^{-0.25}$ (9)	10.8 $M_h^{-0.26}$ (2), 61.4 $M_h^{-0.24}$ (3)
Cardiac Output (ml/min)	187 $M_b^{0.81}$ (9), 166 $M_b^{0.79}$ (3), 209 $M_b^{0.75}$ (3*), 223 $M_b^{0.75}$ (4), 211 $M_b^{0.78}$ (6), 225 $M_b^{0.8}$ (8)	11200 $M_h^{0.70}$ (3)
Stroke Volume (ml)	0.78 $M_b^{1.06}$ (2**), 0.66 $M_b^{1.05}$ (3), 0.92 $M_b^{1.0}$ (3*), 1.05 $M_b^{1.0}$ (6), 0.99 $M_b^{1.03}$ (8)	197 $M_h^{0.96}$ (3)
Total Peripheral Resistance (Pa·s/m ³)	4872 $M_b^{-0.76}$ (3 [†]), 4913 $M_b^{-0.75}$ (3* [†]), 3638 $M_b^{-0.76}$ (6)	
Mean Blood Pressure (mmHg)	90.0 $M_b^{0.032}$ (2 [†]), 89.3 $M_b^{0.04}$ (6), 99 $M_b^{0.03}$ (10), 6.9 $M_b^{0.24}$ + 93 (10)	
Systolic Pressure (mmHg)	115.2 $M_b^{0.05}$ (8 [†]), 116 $M_b^{0.04}$ (10), 19 $M_b^{0.17}$ + 98 (10)	
Diastolic Pressure (mmHg)	78.7 $M_b^{0.05}$ (8 [†]), 84 $M_b^{0.03}$ (10), 0.75 $M_b^{0.47}$ + 47 (10)	

References are given in brackets after each equation: ¹Brody (1945), ²Calder (1984, 1996), ³Holt, Rhode & Kines (1968), ⁴Lindstedt & Schaeffer (2002), ⁵Martin and Haines (1970), ⁶Mitchell & Skinner (2009), ⁷Prothero (1979), ⁸Seymour & Blaylock (2000), ⁹Stahl (1967), ¹⁰White and Seymour, (2014), ¹¹Woodall (1992)

* Theoretical equations assuming that ventricular volumes, weight and heart mass are isometric to body mass, heart rate scales with body mass raised to the -0.25 power, peripheral resistance, the -0.75 power and cardiac output, the 0.75 power (Holt *et al.*, 1968).

** Calculated from Stahl's equations (Stroke Volume = Cardiac Output / Heart Rate)

† For consistency, units have been altered from the format in which they were originally published.

Table 3-2: Phylogenetic allometric equations describing the cardiovascular system.

Reference	Sample Size	Number of Species	Body Mass range (kg)
Brody (Brody, 1945)	~200		0.01 - 100 000
Stahl (1967)	447 - 568		0.1 - 800
Holt, Rhode & Kines (1968)	43	9	0.49 - 518
Martin and Haines (1970)	12	6	0.31 - 252
Prothero (1979)	213	104	0.1 - 10 000
Woodall (1992)	24	13	8.4 - 185
Seymour and Blaylock (2000)	24	24	0.03 - 4 080
Lindstedt and Schaeffer (2002)	4 to 14	4 to 14	0.04 - 100
White and Seymour (2014)	39 - 44	39 - 44	0.03 – 4 080

Table 3-3: Comparison of studies on the interspecific allometry of the mammalian cardiovascular system.

Foetal and Post-Natal Hearts

Evidence from giraffes (Mitchell & Skinner, 2009) and humans (Hieokawa, 1972) suggests that the allometry of the heart mass differs before and after birth, with heart mass increasing more per unit change in body mass before birth than post-natally. The same trend is seen in western grey kangaroos before and after the joey leaves the pouch (Snelling *et al.*, 2015).

The anatomy and function on the foetal and post-natal mammalian heart are different and change suddenly at birth. The foetal heart supplies the necessary pressure to perfuse both the body and the placenta. At birth, perfusion of the placenta ceases, suddenly reducing the volume of the system. When breathing starts, pressure in the pulmonary artery drops, leading to the closure of the *ductus arteriosus* and the *foramen ovale* and thus changing the function of the right heart. Changes in activity, posture, nutrition and homeothermy all increase the workload on the heart after birth.

The foetal heart does not need to only provide the needs of the foetal body, it also must develop the potential or latent ability to adequately supply the needs of the neonate immediately after birth. In precocious animals, like the wildebeest, the change from foetal physiology to a functional post-natal one is rapid.

Materials and Methods

Data Collection

To describe cardiac growth in the wildebeest, heart mass and dimensions were measured on a sample of the wildebeest collected for the body dimension study in Chapter 2, following the methods of Mitchell and Skinner (2009).

- For all post-natal animals, *body mass* (M_b) was calculated from length and girth measurements, using the equations formulated in this study and described in Chapter 2. For most animals, both body length (L) and girth (G) were recorded and the equation $M_b = 39.08(LG^2)^{0.9091}$ was used to estimate body mass (M_b). For one animal, for which girth was not measured, mass was estimated from the equation $M_b = 14.96(L)^{2.801}$.
- *Heart mass* (M_h) was measured to an accuracy of two grams, after removal of as much blood as possible from the ventricles, removal of the pericardium and adnexal fat and transection of the major blood vessels at the level of the atria.
- *Heart circumference* (C_h) was measured around the coronary groove and heart diameter across the interventricular septum, after removing the atria, while holding the heart so that it retained a roughly circular cross section.
- *Heart length* (H_l) was measured from the coronary groove to the apex, in a straight line, perpendicular to the plane of the coronary groove (not along the curvature of the heart).
- *Internal left and right ventricular depths* were measured from the atrio-ventricular valves to the deepest recess of the ventricle, using a probe.
- Mid ventricular wall thicknesses (of the outer walls of the left and right ventricles and the interventricular wall) were measured after the ventricles were transected, at a point half way between the atrio-ventricular groove and the apex (calculated by halving the measured heart length and measuring that distance from the coronary groove). The measurements are referred to as: *Left ventricular wall thickness* (WT_l), *Right ventricular wall thickness* (WT_r) and *Interventricular wall thickness* (WT_i). The width of the left ventricular wall was calculated as the mean of the widths at the widest and narrowest points and the thicknesses of the interventricular septum and right ventricular wall were measured at their midpoints (Figure 3-1). Wall thicknesses were measured with a Vernier calliper.

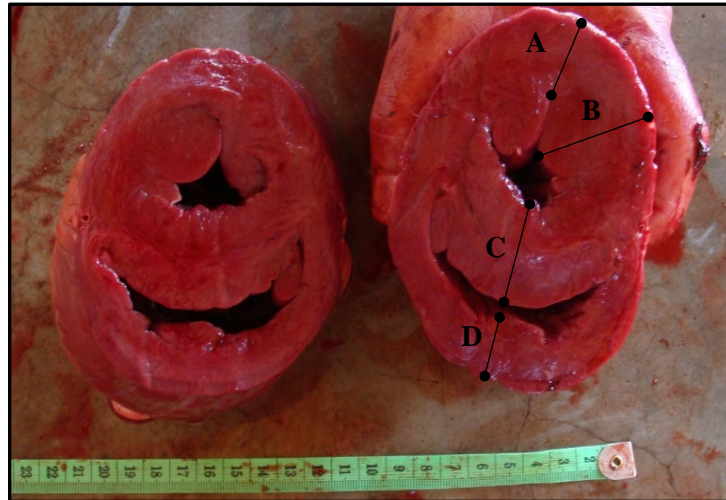


Figure 3-1: Ventricular wall thickness measurements.

Left ventricular wall thickness at the narrowest (A) and widest point (B), Inter ventricular wall thickness (C) and Right ventricular wall thickness (D).

Data Analysis

Ontogenetic Allometric Regression of Wildebeest Heart Size

To describe the growth of the wildebeest heart, allometric equations were derived for the change in heart mass compared with change in body mass and the change in each cardiac measurement compared with both body and heart mass. Allometric regressions were performed for each variable, for the Post-Natal, Entire and Adult samples.

Scatterplots of each comparison were created to allow visual inspection of the data and allometric equations were derived by plotting the log-transformed data and fitting trend-lines and converting the linear equations to allometric equations. Each relationship was explored further using regression in SPSS, to assess model fit and validity and the adjusted coefficient of determination (adjusted R^2) and absolute mean percentage predictive error (PPE_A) for each relationship were assessed to gauge goodness of fit. ANOVA was used to test whether power equations were appropriate models for representing the data, by testing whether the slopes of the linear equations (m) (derived from the log-transformed data) differed significantly from zero.

Tests for Isometry

The equations were assessed for isometry, which was done by testing whether the slopes of the linear equations (m) were significantly different from the slope of an isometric relationship ($\frac{1}{3}$ for linear dimensions, 0 for relative heart mass and 1 for heart mass).

Comparison of Foetal and Post-Natal Heart Size in Wildebeest

The foetal sample was not large enough to directly compare the allometry of foetal and post-natal animals. Instead, the difference between the hearts of foetal and post-natal wildebeest was assessed

using scatterplots and 95% prediction intervals, comparison of measurements of the foetal hearts and the predictions of the allometric equations derived from post-natal animals and the SMATR software program was used to test for significant differences between the Post-Natal and Entire Sample equations for each cardiac measurement.

Comparison of Ventricular Wall Thicknesses in Wildebeest

Scatterplots and the SMATR software package were used to test for significant differences between the thickness of the left, right and interventricular walls by testing whether the allometric equations for each wall thickness were significantly different from each other.

Comparison of Heart Size in Wildebeest with other Mammals

The heart sizes measured in wildebeest were compared with those of other mammals with the aid of interspecific equations for mammals and intraspecific equations for other species. Where possible, published equations were used and if these were not available equations were derived from published raw data.

The wildebeest heart mass and left ventricular wall thickness (LVWT) equations and their 95% confidence intervals were subjectively compared to the inter- and intraspecific heart and LVWT equations. The difference between the heart mass and left ventricular wall thickness (LVWT) measured for each post-natal wildebeest in this study and that predicted by other allometric equations was investigated by calculating the mean differences and mean PPE. Comparison of the allometric equations was done with the use of the SMATR software package, to test for common slope and elevation.

The heart mass equations with which the wildebeest equation was compared were: the interspecific equations for mammals in general (Brody, 1945; Prothero, 1979) and African antelope (Woodall, 1992) and the intraspecific equations for steers, horses (Brody, 1945) and post-natal giraffes (Mitchell & Skinner, 2009). In addition, published data were used to derive ontogenetic equations for cattle, horses (Crile & Quiring, 1940) and giraffes (Mitchell and Skinner, 2009 and unpublished data). The giraffe data included the addition of unpublished measurements made using the same methods and on the same giraffe population as the 2009 study. An equation for adult buffalo was derived from previously unpublished measurements made using the same methods employed in this study (Mitchell 2016, personal communication).

The Left Ventricular Wall Thickness equations with which the wildebeest equation was compared were two interspecific equations for mammals, derived from the data presented by Martin and Haines (1970) and Seymour and Blaylock (2000), and an equation for giraffe (Mitchell & Skinner, 2009) (Table 3-9). In addition to the data used in Mitchell and Skinner (2009), unpublished data from a continuation of that study is also used for the calculation of the giraffe equations.

Prediction of Physiological Cardiovascular Parameters for Wildebeest

Several physiological parameters for wildebeest were calculated from interspecific allometric equations found in the literature (see Table 3-2). If more than one equation was available, the most appropriate equation was chosen, based on the total number of data points and species used in the study (see Table 3-3).

Physiological values were calculated from both body mass and heart mass, when the necessary equations were available. The heart masses that were used were calculated specifically for wildebeest of each assigned body mass, using the equation from this study. All the equations from the literature were edited to use body mass and heart mass in kilograms, heart rate in beats per minute, blood pressure in millimetres mercury (mmHg), volumes in millilitres, cardiac output in millilitres per minute, and total peripheral resistance in resistance (Wood's) units ($\text{mmHg}\cdot\text{min}\cdot\text{L}^{-1}$).

Results

Heart data were collected from 35 wildebeest ranging from 12 to 258kg measured body mass. The sample comprised: four fetuses with body masses between 12 and 15 kg and an approximate gestational age of seven months, 15 juveniles of between 64 and 139 kg calculated body mass and 16 adults of between 162 and 282kg calculated mass. No animals were excluded due to disease, anatomical abnormalities or *rigor mortis*. All animals were processed within an hour of being shot. The calculated body mass and cardiac measurement medians and ranges for these three age groups are summarised in Table 3-4.

Measurement	Foetus (n=4)	n	Juvenile (n=15)	n	Adult (n=16)	n
Calculated Body Mass (kg)	12.8 (12 – 15)	4	110 (64– 139)	15	209 (162 - 282)	16
Heart Mass (kg)	0.111(0.096 - 0.120)	4	0.516 (0.426 - 0.780)	15	1.042 (0.726- 1.402)	16
Relative Heart Mass (% M _b)	0.81 (0.75-1.0)	4	0.57 (0.41-0.72)	15	0.48 (0.37-0.61)	16
Heart Circumference (mm)	155 (135 – 160)	4	273 (250 – 320)	14	340 (300 – 390)	15
Heart Diameter (mm)	50 (49 – 65)	4	85 (80 – 100)	15	113 (100 – 140)	14
Heart Length (mm)	65 (65-70)	4	120 (110-160)	15	150 (105-190)	16
Left Ventricular Wall Thickness (mm)	11.6 (11.5 - 13.0)	4	25.0 (19.5 - 30.4)	15	29.6 (23.8 - 35.9)	16
Right Ventricular Wall Thickness (mm)	11.4 (8.0 - 14.0)	4	15.2 (10.0 - 19.0)	14	18.1 (10.0 - 24.2)	16
Interventricular Wall Thickness (mm)	12.3 (11.0 - 14.0)	4	23.8 (18.0 – 27.0)	15	30.0 (22.0 - 41.0)	16
Left Ventricular Depth (mm)	66.3 (65 – 70)	4	114 (85.0 – 120)	14	133 (100 – 160)	16
Right Ventricular Depth (mm)	53.8 (50 – 62)	4	80 (65 – 100)	14	102 (70 – 125)	16

able 3-4: Median and range of body mass and heart measurements of the wildebeest measured for this study.

Model Validity

Ventricular depths were not measured in one specimen and heart circumference was omitted in two others. Three measurements were discarded as extreme outliers; two heart diameters and one right ventricular wall thickness. These values were considered to be either measurement errors or exceptionally unusual animals. In either case, including the outliers in the analysis would not have been useful. After removal of these outliers, in all the adult body mass equations, the largest wildebeest measured had a leverage value of >0.25 . In the Entire Sample heart mass equations, the foetus with the smallest heart mass had a leverage value between 0.199 and 0.201 and in the Adult heart mass equations the two smallest adult animals and the largest animal had leverage values of >0.2 . These animals were not removed from the sample but may affect some of the allometric equations. No extreme leverage values were found in the Post-Natal equations.

Tests for normality showed that the residuals of most relationships were not significantly different from normality. Exceptions in the body mass equations were the residuals for left ventricular depth in all three samples (Entire, $p=0.004$; Post-Natal, $p=0.019$; Adult, 0.029) and right ventricular depth in the Adult sample ($p=0.011$). For the heart mass equations, residuals for heart circumference and diameter were not normal for the Entire sample ($p=0.005$).

Ontogenetic Allometric Regression of Wildebeest Heart Size

Evaluation of Scatter Plots

A scatter plot of the relationship between heart mass and body mass (Figure 3-2) suggests a linear and isometric relationship between the two. However, the relationship is not quite linear, as shown by the scatter plot of relative heart mass compared with body mass (Figure 3-3). Relative heart mass decreases

with increase in body mass, from approximately 0.8% in a 12kg foetus to 0.5% in an adult, indicating that a power equation is more suitable.

Scatter plots of cardiac dimensions (Figure 3-4 to Figure 3-12) show similar power relationships for both body mass and heart mass, with varying scatter around the trend-lines, depending on the measured variable. Scatter is greatest for the heart wall thickness measurements, particularly right ventricular wall thickness (Figure 3-6).

All the trend-lines derived from the Entire Sample closely resemble those derived using the Post-Natal Sample. The foetal heart masses and measurements of overall heart shape (heart length, circumference and left ventricular depth) lie very close to extrapolation of the Post-Natal trend-lines. The Adult trend-lines follow the other two curves less closely. On visual inspection, Adult trend-lines for the thickness of the right ventricular walls, heart length, and the ventricular depths when regressed against body mass, deviate widely from the other trend-lines. The Adult Sample also shows greater scatter than the other samples.

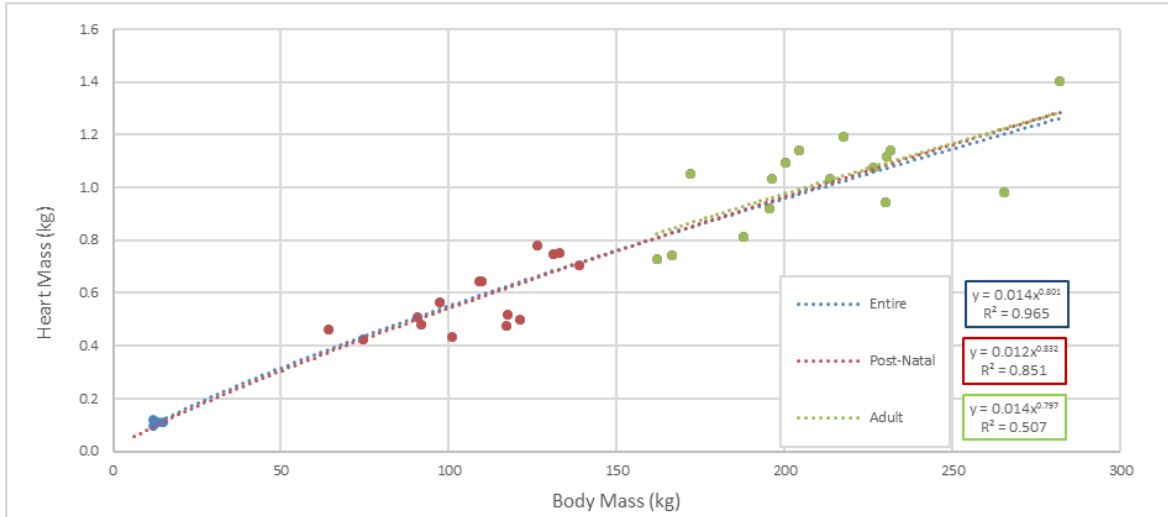


Figure 3-2: Heart Mass versus Body Mass.

Blue data points represent foetuses, red: juvenile animals and green: adults. The blue trend-line is calculated using all the data points (Entire Sample), the red: only Post-Natal animals (both juveniles and adults: Post-Natal Sample) and the green: only adult animals (Adult Sample). The trend-line for the Post-Natal data is extrapolated backwards for comparison with the foetal measurements.

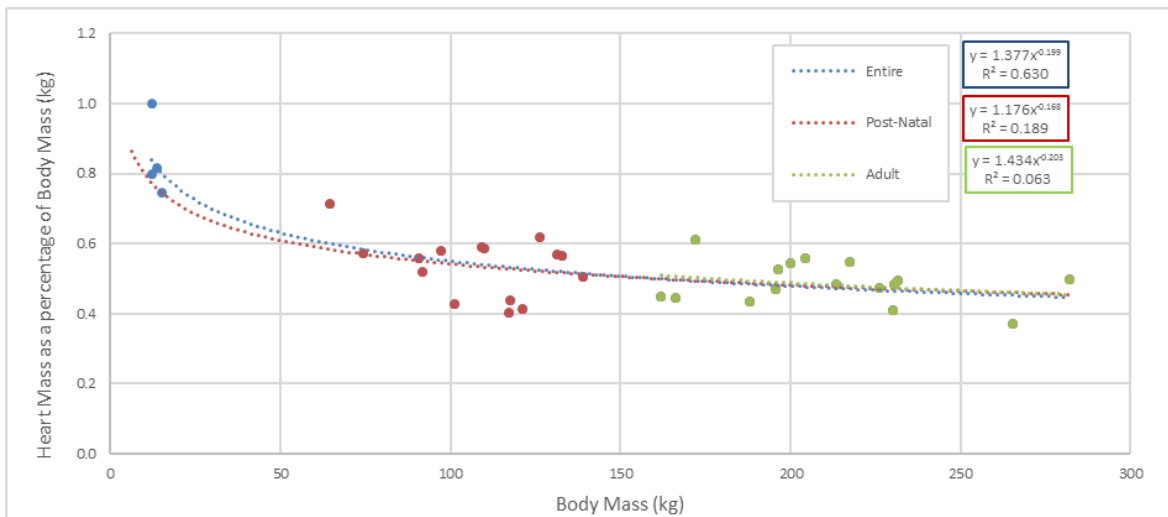


Figure 3-3: Relative Heart Mass versus Body Mass.

Lines and data points follow the format in Figure 1. The regression model for adult animals is not statistically significantly different from the mean ($p > 0.05$).

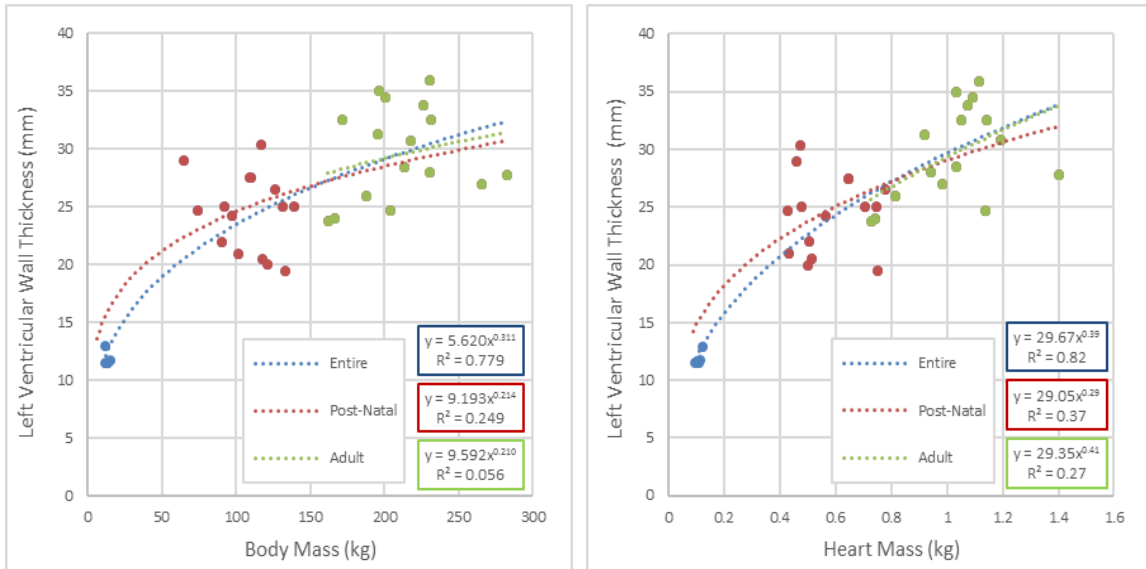


Figure 3-4: Left Ventricular Wall Thickness versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. The body mass regression model for adult animals is not statistically significantly different from the mean ($p > 0.05$).

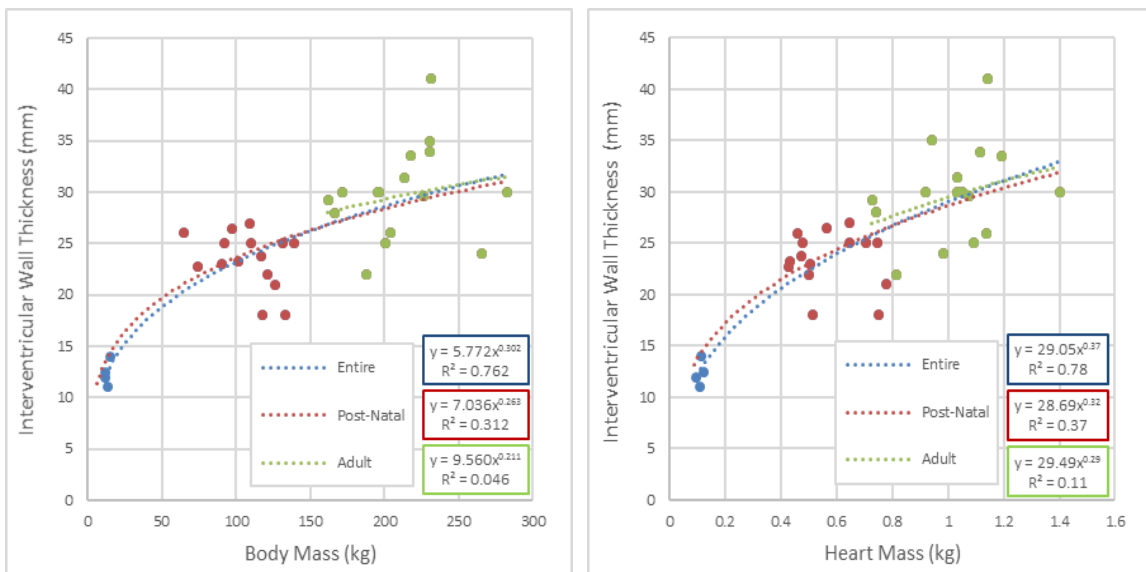


Figure 3-5: Interventricular Wall Thickness versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. The regression models for adult animals are not statistically significantly different from the mean ($p > 0.05$).

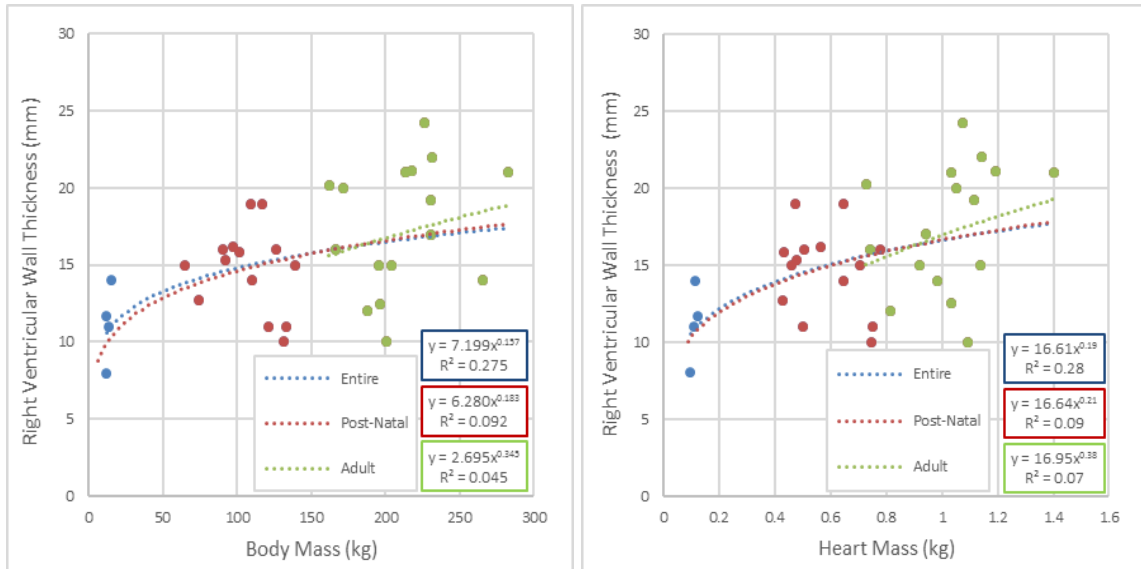


Figure 3-6: Right Ventricular Wall Thickness versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. Both Post-Natal and Adult regressions are not statistically significantly different from the mean ($p > 0.05$).

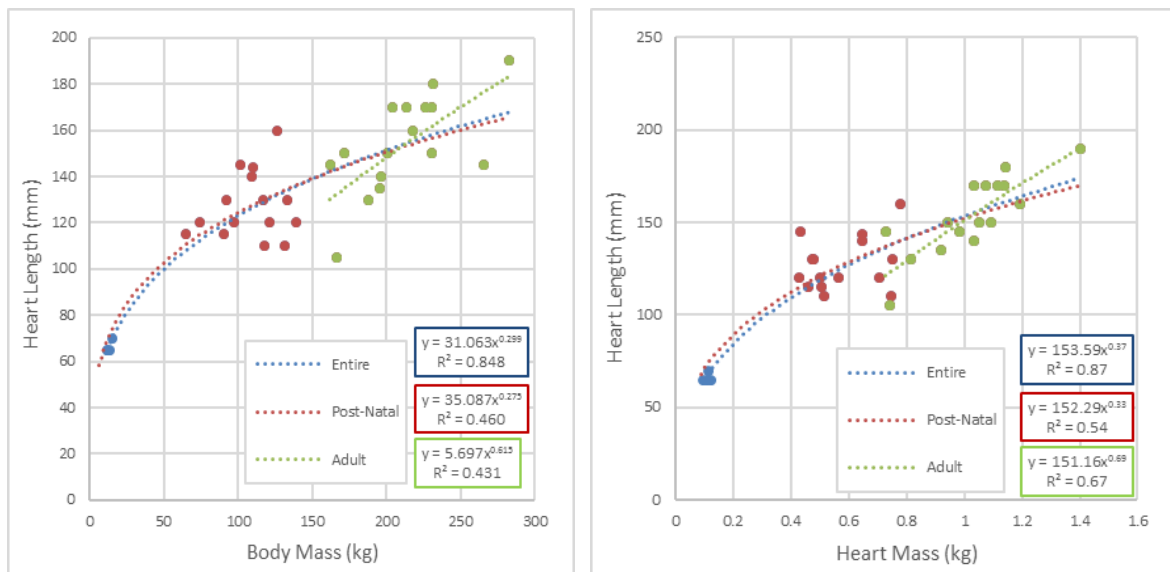


Figure 3-7: Heart Length versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1.

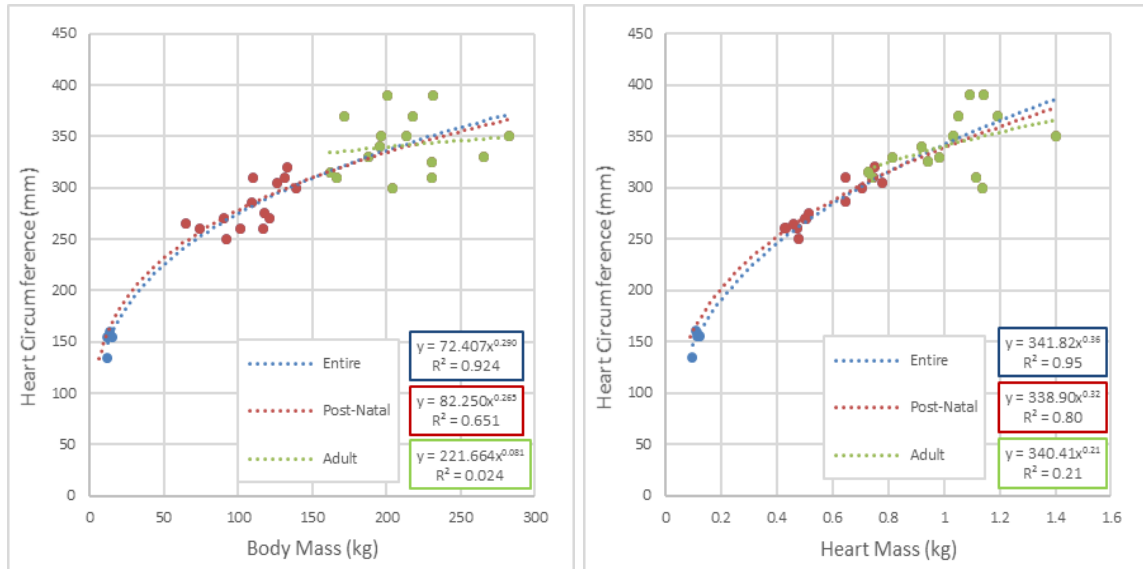


Figure 3-8: Heart Circumference versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. The regression models for adult animals are not statistically significantly different from the mean ($p > 0.05$).

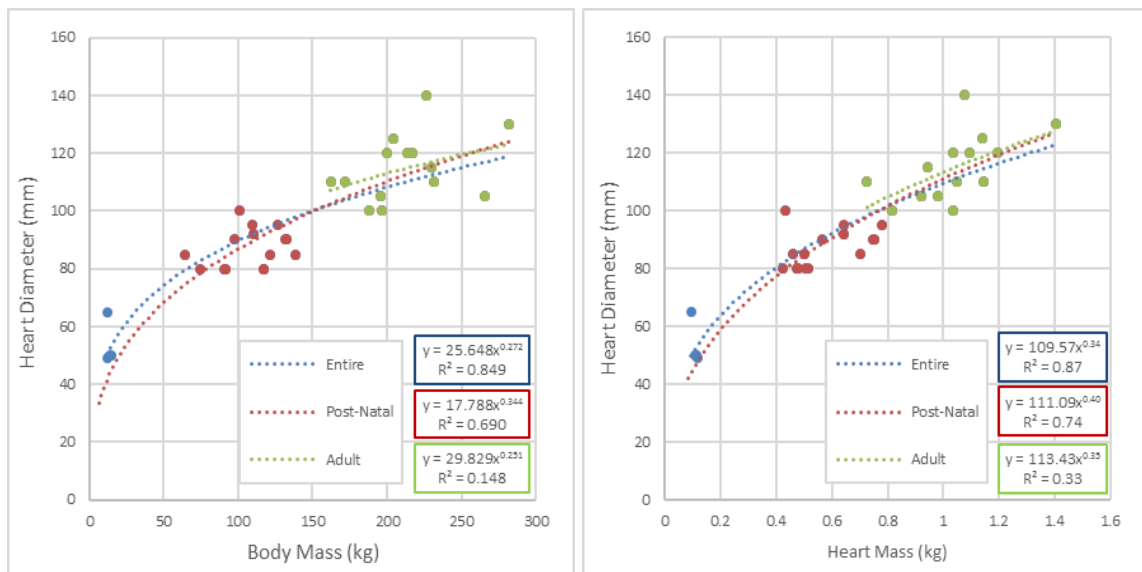


Figure 3-9: Heart Diameter versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. The body mass regression model for adult animals is not statistically significantly different from the mean ($p > 0.05$).

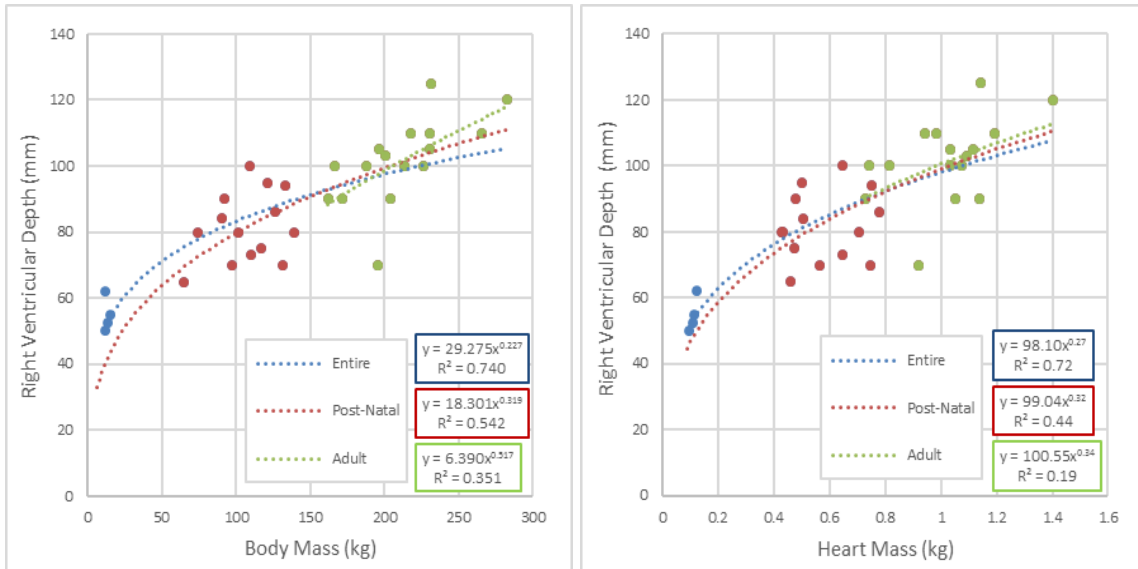


Figure 3-10: Right Ventricular Depth versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. The heart mass regression model for adult animals is not statistically significantly different from the mean ($p > 0.05$).

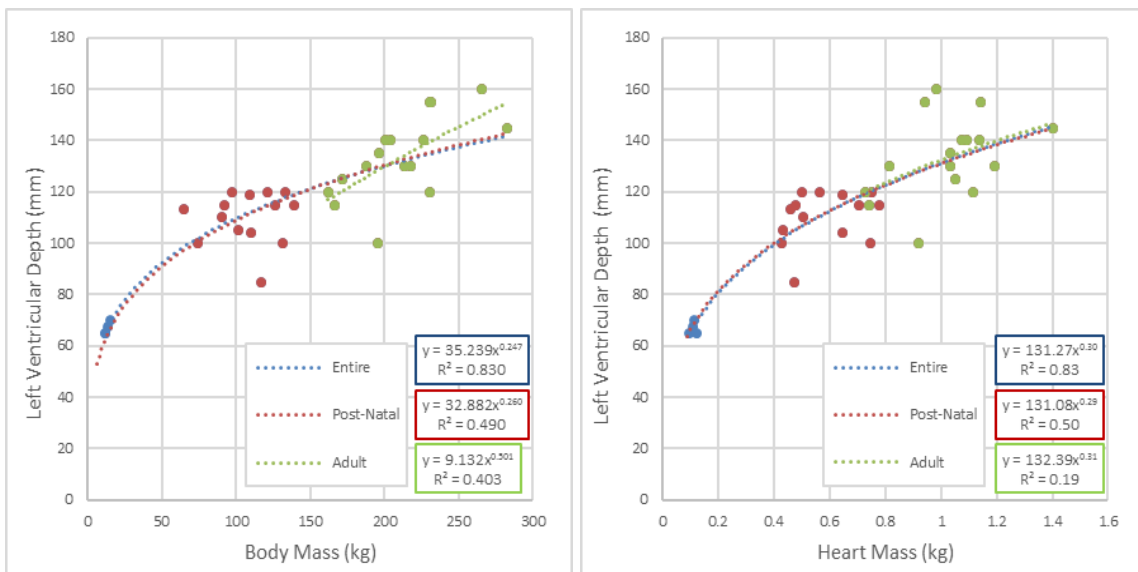


Figure 3-11: Left Ventricular Depth versus Body Mass and Heart Mass.

Lines and data points follow the format in Figure 1. The heart mass regression model for adult animals is not statistically significantly different from the mean ($p > 0.05$).

Allometric Equations

The trend-lines in Figure 3-1 to Figure 3-12 represent regression equations which describe changes in the dimensions of the heart with changes in body mass and heart mass. The key statistics for the Body Mass (M_b) equations are summarised in Table 3-5 and the Heart Mass (M_h) equations in Table 3-6.



Sample	Equation $y = a(M_b)^b$					Adjusted R ²	PPE _A (%)	n	p*	p†
	y	b	SE (b)	a	SE (a)					
E		0.801	0.0266	0.0138	0.00176	0.964	10.7	35	<0.001	<0.001
PN	Heart Mass (kg)	0.832	0.0647	0.0118	0.00383	0.846	10.9	31	<0.001	0.015
A		0.797	0.210	0.0143	0.0161	0.472	10.0	16	0.002	0.350
E	Relative Heart	-0.199	0.0266	1.377	0.176	0.619	10.7	35	<0.001	<0.001
PN	Mass (% of	-0.168	0.0647	1.176	0.382	0.161	11.0	31	0.014	0.014
A	Body Mass)	-0.203	0.210	1.434	1.61	-0.00418	9.9	16	0.349	0.349
E	Left Ventricular	0.311	0.0288	5.620	0.779	0.772	12.3	35	<0.001	0.444
PN	Wall Thickness	0.214	0.0690	9.193	3.19	0.223	12.3	31	0.004	0.094
A	(mm)	0.210	0.231	9.592	11.8	-0.0117	12.2	16	0.378	0.602
E	Right	0.157	0.0449	7.199	1.56	0.253	17.9	34	0.001	<0.001
PN	Ventricular Wall	0.183	0.109	6.280	3.45	0.0591	18.4	30	0.104	0.179
A	Thickness (mm)	0.345	0.427	2.695	6.15	-0.0237	20.4	16	0.433	0.979
E	Interventricular	0.302	0.0294	5.772	0.816	0.755	11.1	35	<0.001	0.294
PN	Wall Thickness	0.263	0.0726	7.036	2.57	0.288	11.6	31	0.001	0.341
A	(mm)	0.211	0.258	9.560	13.2	-0.0222	11.1	16	0.426	0.643
E	Heart	0.290	0.0150	72.4	5.20	0.921	6.1	33	<0.001	0.007
PN	Circumference	0.265	0.0373	82.3	15.4	0.638	6.1	29	<0.001	0.078
A	(mm)	0.081	0.143	222	169	-0.0512	6.7	15	0.582	0.101
E		0.272	0.0206	25.7	2.53	0.844	8.3	33	<0.001	0.006
PN	Heart Diameter	0.344	0.0444	17.8	3.96	0.678	7.8	29	<0.001	0.812
A	(mm)	0.251	0.174	29.8	27.8	0.0774	7.4	14	0.174	0.645
E		0.299	0.0220	31.1	3.29	0.844	8.5	35	<0.001	0.128
PN	Heart Length	0.275	0.0553	35.1	9.76	0.441	9.4	31	<0.001	0.300
A	(mm)	0.615	0.189	5.70	5.75	0.390	8.9	16	0.006	0.158
E		0.247	0.0197	35.2	3.35	0.825	6.7	34	<0.001	<0.001
PN	Left Ventricular	0.260	0.0502	32.9	8.31	0.471	7.5	30	<0.001	0.155
A	Depth (mm)	0.501	0.163	9.13	7.95	0.361	7.0	16	0.008	0.321
E	Right	0.227	0.0238	29.3	3.35	0.732	9.0	34	<0.001	<0.001
PN	Ventricular	0.319	0.0555	18.3	5.12	0.525	8.7	30	<0.001	0.798
A	Depth (mm)	0.517	0.188	6.39	6.41	0.305	6.9	16	0.016	0.345

P-values for the tests of the following two null hypotheses:

* 1) $H_0: b = 0$. The null hypothesis is that the regression equation is not significantly different from the mean of the y values

† 2) $H_0: b = isometric$. The null hypothesis is that the equation is isometric, i.e. that the exponent (b) is equal to the value of the exponent of the isometric equation (1 for heart mass, 0 for Relative Heart Mass and 1/3 for all length measurements)

P-values smaller than 0.05 are shown in bold, in these instances the null hypothesis can be rejected.

SE: Standard Error

E: Entire Sample (Foetal, Juvenile and Adult animals); PN: Post-Natal Sample (Juvenile and Adult animals); A: Adult Sample

PPE_A: mean absolute percent prediction error

Table 3-5: Ontogenetic allometric equations relating cardiac measurements to Body Mass (M_b)



Sample	Equation $y = a(M_h)^b$					Adjusted R ²	PPE _A	n	p*	p†
	y	b	SE (b)	a	SE (a)					
E	Left Ventricular Wall Thickness (mm)	0.390	0.0322	29.7	0.818	0.811	10.3	35	< 0.001	0.878
PN		0.289	0.0701	29.0	0.898	0.348	10.9	31	< 0.001	0.532
A		0.415	0.181	29.3	0.898	0.220	9.7	16	0.038	0.659
E	Right Ventricular Wall Thickness (mm)	0.193	0.0550	16.6	0.788	0.255	17.8	34	0.001	0.016
PN		0.206	0.123	16.6	0.883	0.0586	18.3	30	0.105	0.309
A		0.382	0.377	16.9	1.077	0.00196	19.6	16	0.327	0.899
E	Interventricular Wall Thickness (mm)	0.374	0.0350	29.0	0.871	0.769	11.0	35	< 0.001	0.254
PN		0.316	0.0773	28.7	0.979	0.344	11.1	31	< 0.001	0.824
A		0.288	0.223	29.5	1.108	0.0431	11.1	16	0.216	0.842
E	Heart Circumference (mm)	0.361	0.0143	341.8	4.297	0.952	4.0	33	< 0.001	0.062
PN		0.321	0.0311	338.9	4.682	0.790	3.9	29	< 0.001	0.695
A		0.214	0.114	340.4	6.735	0.152	5.5	15	0.084	0.314
E	Heart Diameter (mm)	0.337	0.0237	109.6	2.283	0.863	7.4	33	< 0.001	0.878
PN		0.396	0.0448	111.1	2.249	0.734	6.3	29	< 0.001	0.173
A		0.351	0.144	113.4	2.587	0.277	6.3	14	0.031	0.904
E	Heart Length (mm)	0.370	0.0254	153.6	3.341	0.862	8.1	35	< 0.001	0.158
PN		0.329	0.0568	152.3	3.818	0.521	8.6	31	< 0.001	0.940
A		0.685	0.129	151.2	3.280	0.646	6.6	16	< 0.001	0.016
E	Left Ventricular Depth (mm)	0.303	0.0242	131.3	2.738	0.825	7.1	34	< 0.001	0.219
PN		0.295	0.0561	131.1	3.176	0.479	7.7	30	< 0.001	0.500
A		0.306	0.170	132.4	3.794	0.130	7.8	16	0.093	0.875
E	Right Ventricular Depth (mm)	0.275	0.0303	98.1	2.563	0.711	9.5	34	< 0.001	0.063
PN		0.325	0.0690	99.0	2.951	0.422	10.1	30	< 0.001	0.905
A		0.338	0.188	100.5	3.182	0.130	8.2	16	0.093	0.981

P-values for the tests of the following two null hypotheses:

* 1) $H_0: b = 0$, That the regression equation is not significantly different from the mean of the y values.

† 2) $H_0: b = 1/3$, That the equation is not significantly different from isometry.

P-values smaller than 0.05 are shown in bold, in these instances the null hypothesis can be rejected.

SE: Standard Error

E: Entire Sample (Foetal, Juvenile and Adult animals); PN: Post-Natal Sample (Juvenile and Adult animals); A: Adult Sample

PPE_A: mean absolute percent prediction error

Table 3-6: Ontogenetic allometric equations relating cardiac measurements to Heart Mass (M_h).

Suitability of Power Equations

When heart growth from foetus to adult is assessed (Entire Sample equations) there is a significant regression between each of the cardiac measurements and both heart mass and body mass. In the Post-Natal sample the regression is significant for all the relationships except for those for the thickness of the right ventricular wall. In contrast, in the Adult Sample the regressions for many heart dimensions are not significant. The only cardiac measurements which scale with body mass are heart mass, heart length and the depth of the ventricles and the only measurements which scale with heart mass are the thickness of the left ventricular wall, heart diameter and heart length. For the other relationships, the values predicted by the regression equation were not significantly different from the mean of the sample ($p > 0.05$), as shown in Appendix 6.

Goodness of Fit

Adjusted coefficients of determination (adjusted R^2 values) were generally larger for the Entire sample, smaller for the Post-Natal Sample and smallest for the Adult Sample. Both the body mass and heart mass equations for the Entire Sample show R^2 values greater than 0.7, except for the equation for the thickness of the right ventricular wall (adjusted $R^2=0.253$ and 0.255) and relative heart mass ($R^2=0.619$). The equation for heart mass shows the greatest adjusted R^2 (0.964) of all the cardiac equations calculated in this study.

The mean absolute percentage prediction errors (PPE_A) show that on average, the heart masses measured in the sample are within 11% of those predicted by the equations. Other heart measurements are within 10% of the predicted values, except for the ventricular wall thicknesses which have larger PPE_A values. The PPE_A results support those of the R^2 values in showing that the equations for the right ventricular wall are less accurate than for the other heart measurements.

Most of the heart mass equations have slightly higher adjusted R^2 and smaller PPE_A values than the analogous body mass equations. The exceptions for R^2 are: Right Ventricular Depth, the Post-Natal equation for Right Ventricular Wall Thickness and the Adult equation for Left Ventricular Depth, and for PPE_A , the equations for both Left and Right Ventricular Depth.

Tests for Isometry

In the Post-Natal Sample, heart mass and relative heart mass scale hypoallometrically with body mass, indicated by the exponents being significantly smaller than one. However, the relationships between other heart dimensions and body mass for the Post-Natal sample are not significantly different from isometry (Table 3-5). For the Entire Sample, the exponents (b) of all the body mass equations are smaller than, and, in most cases, significantly different from the exponent expected by isometry ($p<0.05$) and the relationships are therefore hypoallometric. The exceptions are the equations for heart length and left and interventricular wall thickness. In the Adult Sample, the exponents of all the body mass equations cannot be distinguished from those of an isometric relationship ($p>0.05$).

The dimensions of the heart scale isometrically with heart mass in all instances except two (Table 3-6). The thickness of the right ventricular wall scales hypoallometrically with heart mass when foetal measurements are included in the equation and heart length scales hyperallometrically with heart mass in the adult animal.

Comparison of Foetal and Post-Natal Heart Size in Wildebeest

Heart masses measured in the foetal wildebeest were on average 9g or 9.6% larger than those predicted by the Post-Natal equation and the measured foetal heart dimensions ranged from a mean of 25% less than predicted to 32% greater than predicted. Many foetal heart measurements differed from the predicted values by more than 10% (Table 3-7). Despite these differences, the measured values fell within the 95% prediction intervals (PIs) of the Post-Natal equations in all but one case (Appendix 8). For this measurement (Heart Diameter) only one measurement fell outside the prediction intervals.

Addition of the data from the four near-term fetuses to the samples did not significantly alter the ontogenetic equations for heart measurements. When the Entire Sample and Post-Natal Sample equations were compared using the SMATR software package, it failed to show any significant difference between either the exponents or intercepts of any of the equations, as shown in Table 3-7, despite the equations being mostly isometric for the Post-Natal Sample and hypoallometric for the Entire-Sample.

Cardiac Measurement	Difference (Measured – Predicted)		Do foetal measurements fall outside the 95% PI?	SMATR		
	Mean (measured units)	Mean PPE# (%)		Exponent*	Intercept†	
Heart Mass (kg)	0.00907	9.6	No	0.65	0.94	
Relative heart mass (%Mb)	0.0766	9.9	No	0.66	0.94	
Body Mass Equations	Left Ventricular Wall Thickness (mm)	-4.01	-25.1	No	0.21	0.78
	Right Ventricular Wall Thickness (mm)	1.12	10.9	No	0.83	0.95
	Interventricular Wall Thickness (mm)	-1.46	-10.6	No	0.62	0.92
	Heart Circumference (mm)	-11.3	-7.0	No	0.54	0.90
	Heart Diameter (mm)	10.4	24.5	Yes	0.14	0.66
	Heart Length (mm)	-4.91	-6.9	No	0.70	0.93
	Left Ventricular Depth (mm)	2.71	4.2	No	0.80	0.96
	Right Ventricular Depth (mm)	13.3	32.2	No	0.13	0.75
Heart Mass Equations	Left Ventricular Wall Thickness (mm)	-3.39	-22.2	No	0.20	0.76
	Right Ventricular Wall Thickness (mm)	0.63	5.8	No	0.93	0.97
	Interventricular Wall Thickness (mm)	-1.88	-13.2	No	0.49	0.87
	Heart Circumference (mm)	-15.3	-9.2	No	0.25	0.80
	Heart Diameter (mm)	7.27	16.3	Yes	0.25	0.80
	Heart Length (mm)	-7.26	-9.8	No	0.52	0.87
	Left Ventricular Depth (mm)	-1.34	-1.9	No	0.90	0.97
	Right Ventricular Depth (mm)	6.64	13.6	No	0.53	0.87

Differences represent the mean of the differences between the cardiac measurements made on each of four foetal wildebeest and the values predicted by the Post-Natal equations for that animal.

*PPE: mean Percentage Prediction Error= [(measured-predicted)/predicted]*100.

SMATR: (H_0 : Entire Sample = PostNatal Sample) P-Values compare the Entire Sample and Post-Natal Sample and represent the SMATR test for heterogeneity in slopes* and the test for shift in elevation between the samples†.

PI: Prediction Interval of the Post-Natal equation

Table 3-7: Evaluation of the suitability of predicting foetal cardiac parameters from Post-Natal equations.

Comparison of Ventricular Wall Thicknesses in Wildebeest

Figure 3-3 to Figure 3-5 describe each ventricular wall separately but it is also interesting to compare the walls with each other. Figure 3-12 compares the equations for the three different wall thicknesses derived from the Post-Natal Sample. The left, right and interventricular walls are of similar thickness in the foetal wildebeest but as the animal grows, the left and interventricular walls increase in thickness more rapidly than the right ventricular wall. The allometric equations for interventricular and left ventricular wall thickness are not significantly different from each other; tests of the log-transformed equations showed no difference in slope ($p=0.793$ for the heart mass equation and $p=0.634$ for the body mass equation) or elevation ($p=0.591$ for the heart mass equation and $p=0.614$ for the body mass equation) between the equations. Left and interventricular wall thicknesses measured in each wildebeest were not significantly different from each other ($p=0.592$, paired, 2-tailed t-test) but were significantly different from the thickness of the right ventricle wall ($p<0.001$).

The slopes of the right ventricular wall thickness equations do not differ from those of the other two equations significantly but the elevations are significantly different ($p<0.001$).

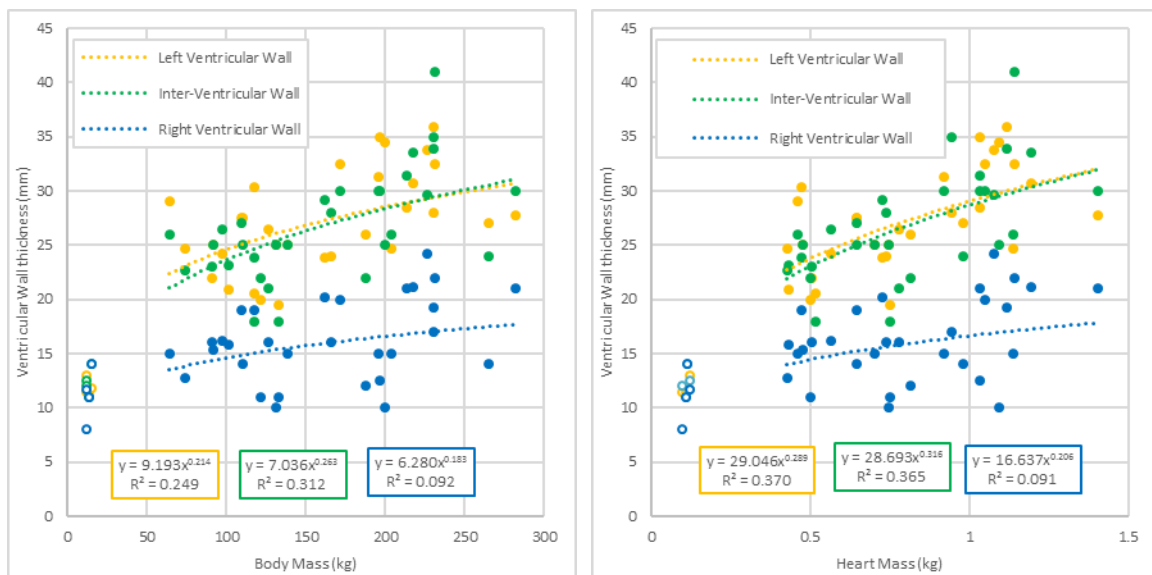


Figure 3-12: Relative Heart Wall Thickness versus Body Mass and Heart Mass.

Yellow points and trend-line represent the left ventricular wall; green, the inter-ventricular wall and blue, the right. Foetal animals are shown by unfilled points.

Comparison of Heart Size in Wildebeest with other Mammals

Heart Mass

Intraspecific Equations

The ontogenetic allometric equation which relates heart mass to body mass in post-natal blue wildebeest is $M_h = 0.0118M_b^{0.832}$ with heart mass (M_h) and body mass (M_b) in kilograms. When this equation and its 95% confidence intervals are plotted together with similar equations for other species, visual inspection of the chart can be used to compare wildebeest with other animals (Figure 3-13). Subjective evaluation reveals that the hearts of wildebeest are smaller than those of horses or giraffe of the same body mass. Wildebeest and cattle have similar heart masses and wildebeest have larger hearts than buffalo of the same body mass until they attain a body mass of approximately 220kg.

The allometric equations which describe the relationship between heart mass and body mass for horses, cattle, giraffe and buffalo are summarised in Table 3-8. The exponents of these equations are all significantly different from that of the blue wildebeest equation (SMATR test for common slope $p < 0.05$), indicating that per kilogram change in body mass, the change in heart mass in wildebeest is significantly less than that in horses, giraffes and buffalos and more than in cattle.

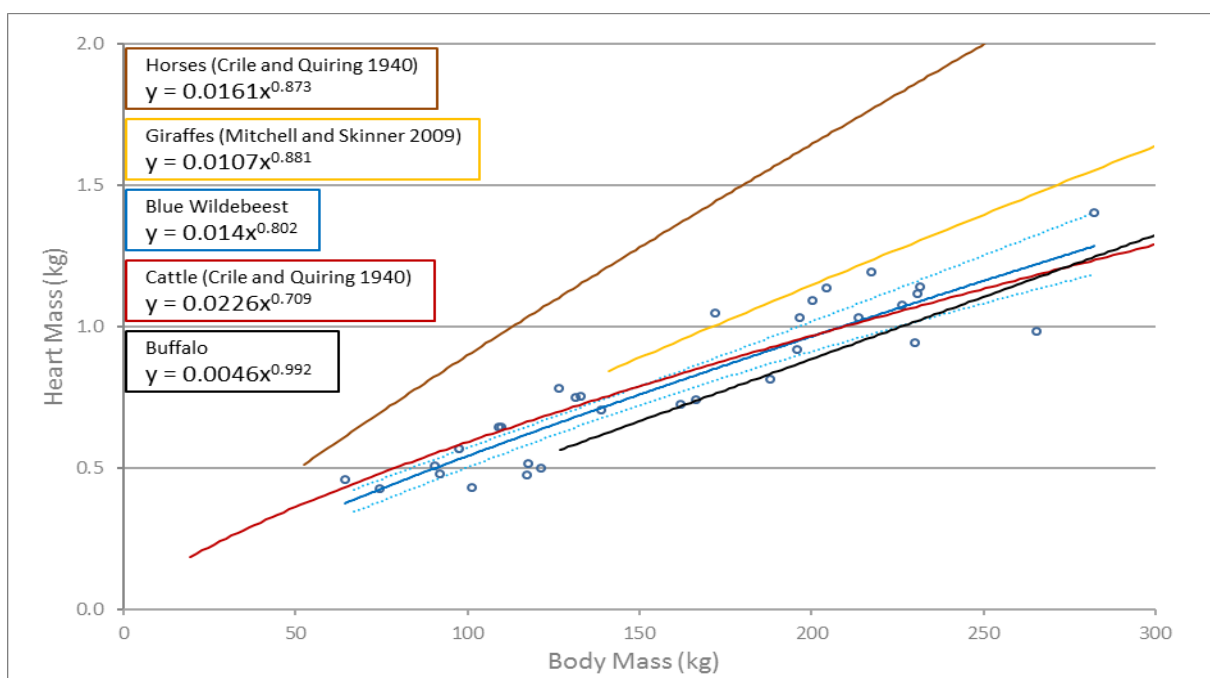


Figure 3-13: Ontogenetic allometry of blue wildebeest compared with that of other mammals.

Data points show the individuals from this study from which the blue wildebeest line was calculated. The dotted blue lines represent the 95% confidence intervals of the blue wildebeest equation.

Table 3-9 shows the mean differences between the heart masses measured in wildebeest and those predicted by the intraspecific equations for cattle, horses, giraffe and buffalo and the mean PPE for each comparison. There are significant differences between measured heart masses and those predicted by the horse, giraffe and buffalo equations ($p < 0.001$). In contrast, the heart masses measured in wildebeest differ from those measured in cattle by less than three percent on average, a difference which is not statistically significant ($p = 0.276$).

Interspecific Equations

Comparison of the post-natal ontogenetic equation for wildebeest heart mass with the two interspecific equations for mammals and one for African antelope is depicted in Figure 3-14. Subjective evaluation suggests that for any given body mass, the average heart mass for a wildebeest is less than that of the average African antelope and, for animals over approximately 120kg body mass, it is also less than that predicted for the average mammal.

Formal comparison, using the SMATR software, shows that the exponent of the wildebeest ontogenetic equation (0.832) is significantly different from the exponents of the mammalian equations: 0.984 ($p = 0.026$) (Brody, 1945) and 0.97 ($p = 0.043$) (Prothero, 1979). The exponents of the wildebeest and African antelope equations are not significantly different ($p = 0.603$) but the intercepts differ ($p = 0.005$), indicating that, at all body masses, the heart mass of a wildebeest is significantly lower than that predicted for an average African antelope by Woodall's equation.

The differences between the heart masses measured in the post-natal wildebeest and those predicted by the interspecific equations are summarised in Table 3-9, as both the mean of the differences calculated for each animal and the mean percentage difference. For example, the heart masses measured in wildebeest were on average 70g or 5.7%, smaller than predicted by Brody's mammal equation, a difference which is statistically significant ($p = 0.030$).

The differences between the measured heart masses and those predicted by the mammal equations changes with changes in heart mass, as can be seen in Figure 3-14 and confirmed by the SMATR test for difference in slope. The difference is more pronounced in adult animals than in juveniles, which is supported by comparing the differences between measured and predicted values for only the adult animals (Table 3-9). Adult wildebeest have significantly smaller heart masses than predicted by both the interspecific equations for mammals (Brody: $p = 0.003$, Prothero: $p = 0.016$). However, the exponents and multipliers of the Adult Sample equation are not significantly different from those of the mammal equations.

Comparison of measured post-natal heart masses and those predicted by Woodall's equation for African antelope show a larger and more significant difference ($p < 0.001$) than the comparison with the mammal equations.

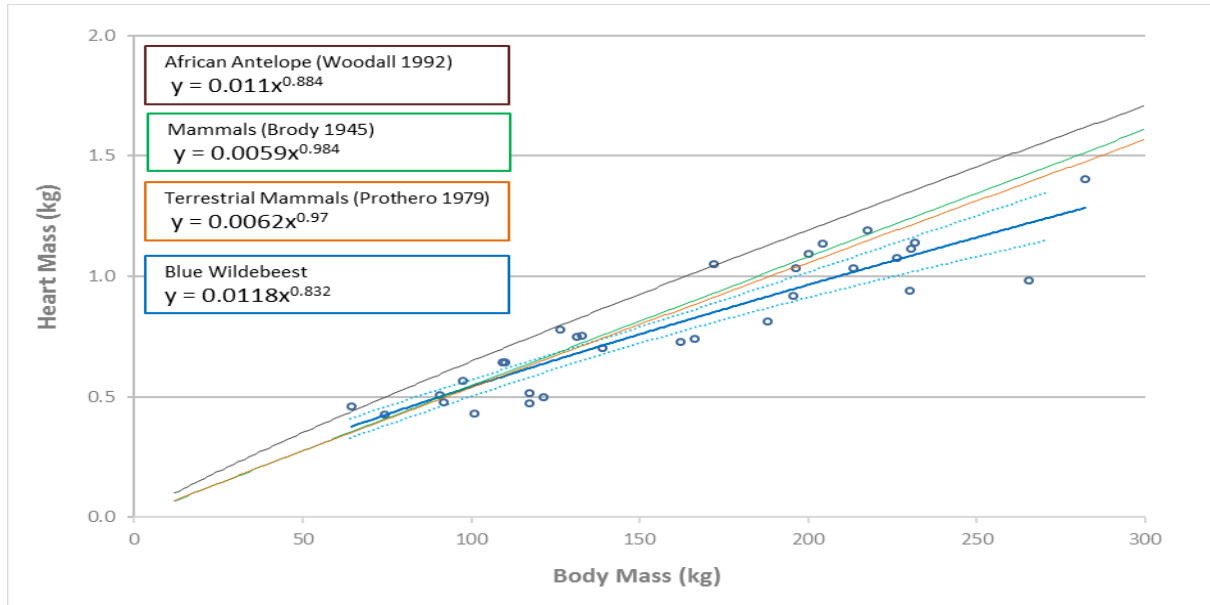


Figure 3-14: Ontogenetic allometry of blue wildebeest heart mass compared with mammalian interspecific equations.

Formatting follows that in Figure 12

Species	Allometric Equation: $y = a(x)^b$					Adjusted R^2	n	Ages	M_b Range (kg)	Data Source	
	y	x	b	SE (b)	a						SE (a)
Horse			0.873	0.0494	0.0161	0.0045	0.909	32	Juvenile and Adult	52.5-771	Crile and Quiring (1940)
Cattle	Heart Mass (kg)	Body Mass (kg)	0.709	0.0375	0.0226	0.00487	0.947	21	Juvenile and Adult	19.5-888	
Giraffe			0.881	0.035	0.0107	0.00246	0.938	43	Juvenile and Adult	141-1512	
Buffalo			0.992	0.0374	0.0046	0.00106	0.945	42	Juvenile and Adult	127-779	
Mammals	Left Ventricular Wall Thickness (mm)	Body Mass (kg)	0.457	0.0249	2.5	0.153	0.969	13	Adult	0.31-252	Martin and Haines (1970)
Mammals			0.358	0.0225	2.76	0.269	0.92	24	Adult	0.03-4080	Seymour and Blaylock (2000)
Giraffe			0.413	0.0361	3.674	0.865	0.761	42	Juvenile and Adult	141-1512	Mitchell and Skinner (2009)*
Mammals	Heart Mass (kg)		0.468	0.274	27.3	3.481	0.96	13	Adult	0.31-252	Martin and Haines (1970)
Mammals			0.34	0.0149	17.854	1.024	0.958	24	Adult	0.03-4080	Seymour and Blaylock (2000)
Giraffe			0.446	0.0429	31.687	1.777	0.723	42	Juvenile and Adult	141-1512	Mitchell and Skinner (2009)*

In addition to the data used in Mitchell and Skinner (2009) unpublished data, from a continuation of that study, is also used for the calculation of the giraffe equations

Table 3-8: Allometric equations for heart mass and left ventricular wall thickness derived from data from other studies.

Left Ventricular Wall Thickness

Interspecific Equations

Two different interspecific equations were derived for the allometry of the left ventricular wall in mammals, one from the data presented by Martin and Haines (1970) and the other from that of Seymour and Blaylock (2000) (Table 3-8).

Visual inspection of the relationship between the thickness of the left ventricular wall and body mass and heart mass in post-natal wildebeest and other mammals (Figure 3-15 and 16) suggests that wildebeest have thicker interventricular walls than other mammals, for body masses lower than 180kg. The wildebeest equation appears to follow the Martin and Haines (1970) equation more closely than that of Seymour and Blaylock (2000) and for body masses greater than approximately 180kg the allometric equation from Martin and Haines (1970) falls within the 95% confidence intervals of the wildebeest equation.

The differences between the wildebeest equation and the other two equations is further illustrated by SMATR analysis (Table 3-9). The exponent of the wildebeest equation is significantly different from the equation of Martin and Haines in both the body mass and heart mass relationships ($p=0.0028$ and $p=0.0258$ respectively, SMATR test for common slope). The equation from Seymour and Blaylock and the wildebeest equation have exponents which are not significantly different in either equation ($p=0.0584$ and $p=0.480$, SMATR test for common slope) but intercepts which are ($p<0.001$ SMATR, test for common elevation).

It is interesting to note that the exponent of the equation derived from the data of Martin and Haines is significantly different from 0.33 ($p<0.001$). The relationship described is therefore hyperallometric. However, both the wildebeest equation and that derived from the data from the study by Seymour and Blaylock are not significantly different from isometry ($p=0.439$ and 0.278 respectively).

Comparison of measured and predicted wall thickness also shows a significant difference between the measurements made on wildebeest and the predictions made by Seymour and Blaylock's and Martin and Haines' equations ($p<0.001$) (Table 3-9).

Using the relationship between ventricular wall thicknesses and heart mass, rather than body mass (Figure 3-16), removes any effect that would be caused by wildebeest having different heart masses from other mammals and shows that, on average, relative wall thicknesses measured in wildebeest are significantly different from those predicted by the heart mass equations for mammals ($p<0.001$).

Intraspecific equation: Giraffe

Figure 3-15 shows that the thickness of the left ventricular wall in giraffe is greater than that of the average wildebeest, for post-natal giraffes. This difference is not only due to wildebeest having relatively smaller hearts than giraffe. As Figure 16 shows, the wall thickness relative to heart mass is

also smaller in the wildebeest than in the giraffe. Comparison of wall thicknesses measured in the wildebeest to those predicted by the giraffe equation (Table 3-9) shows a significant difference for the giraffe body mass equation ($p < 0.001$) but not the heart mass equation ($p = 0.196$). However, this is an extrapolation of the giraffe equation to body masses lower than that of a giraffe at birth.

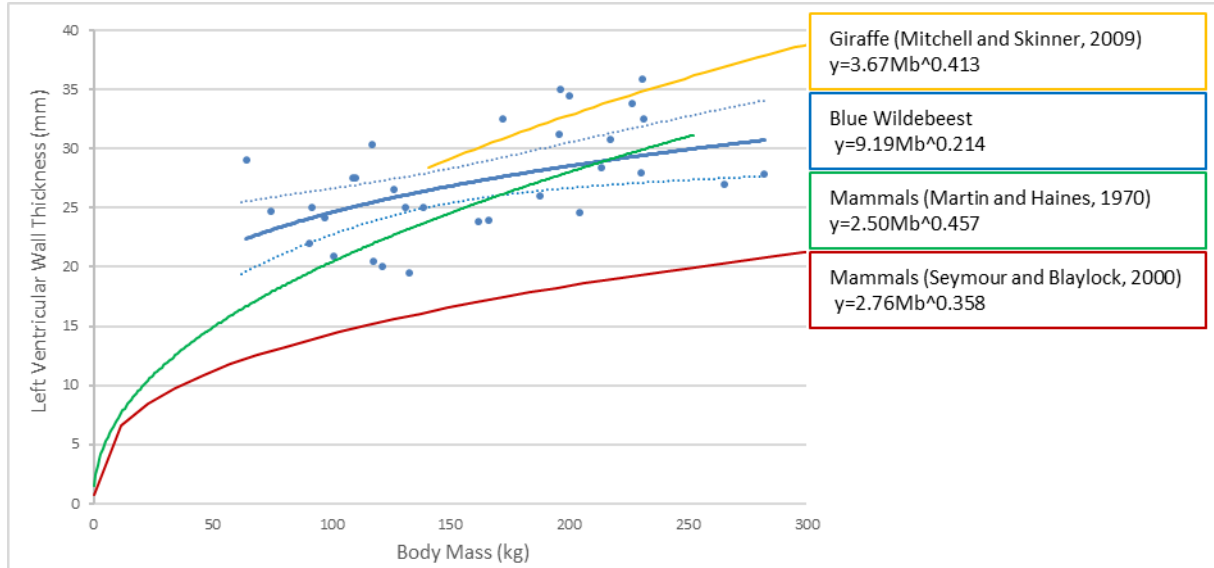


Figure 3-15: Allometry of left ventricular wall thickness in the post-natal blue wildebeest in relation to body mass, compared with two estimates of the interspecific adult mammalian trend and one for the ontogenetic allometric relationship for giraffes.

Data points represent the animals in this study, from which the blue wildebeest trend-line was calculated. The dotted blue lines represent the 95% confidence intervals of the wildebeest trend-line.

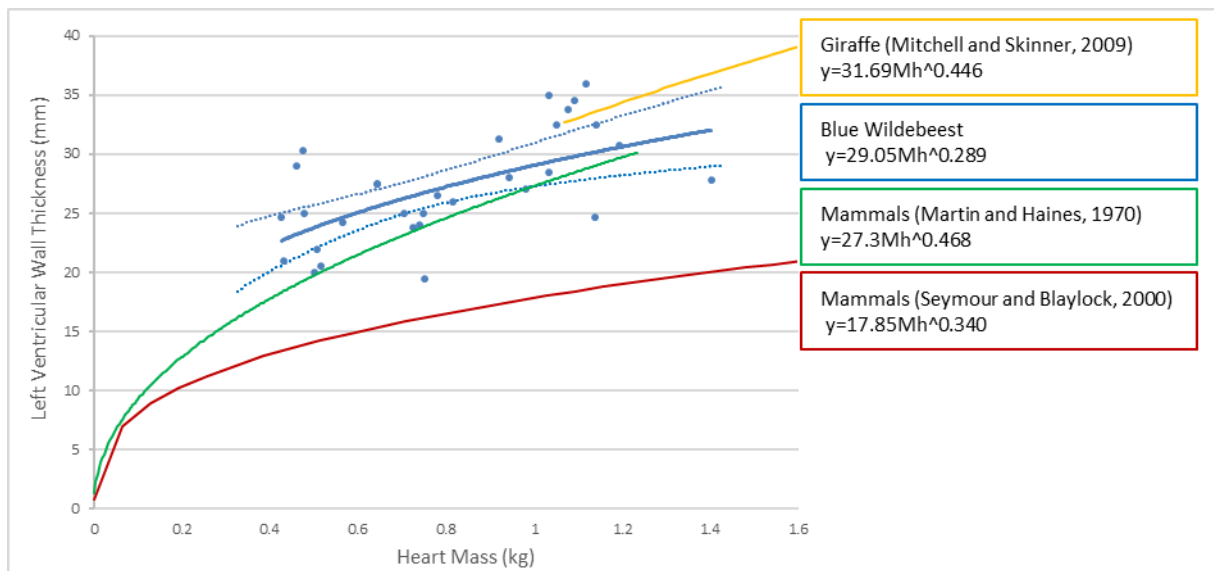


Figure 3-16: Allometry of left ventricular wall thickness in the post-natal blue wildebeest in relation to heart mass, compared with two estimates of the interspecific adult mammalian trend and one for the ontogenetic relationship for giraffes.

Formatting follows that for Figure 14.



y	x	Equation	SMATR ^a		Mean difference ^d			
					measured-predicted		PPE ^e	
			Exponent ^b	Intercept ^c	Mean (kg /mm)	p	Mean (%)	p
Heart mass (kg)	Body mass (kg)	Wilbebest			0.00	0.836	0.469	0.845
		Mammals (Brody)	0.026 ^f	-	-0.067	0.005	-5.73	0.030
		Mammals (Prothero)	0.043 ^f	-	-0.050	0.025	-4.13	0.110
		African antelope	0.603	0.005	-0.174	<0.001	-17.2	<0.001
		Cattle	0.110	0.742	-0.012	0.545	-2.70	0.276
		Horses	0.612	<0.001	-0.546	<0.001	-40.0	<0.001
		Giraffe	0.491	0.002	-0.129	<0.001	-13.3	<0.001
		Buffalo	0.037	-	0.095	<0.001	15.8	<0.001
	Body mass (kg) (Adult sample)	Mammals (Brody)	0.391 ^f	0.444 ^f	-0.116	0.004	-9.73	0.003
		Mammals (Prothero)	0.426 ^f	0.471 ^f	-0.091	<0.001	-7.74	0.016
Left Ventricular Wall Thickness (mm)	Body mass (kg)	Mammals (M&H)	0.0028	-	-24.29	<0.001	-96.9	<0.001
		Mammals (S&B)	0.0584	<0.001	-15.96	<0.001	-95.3	<0.001
		Giraffe	0.0138	-	-28.69	<0.001	-97.3	<0.001
	Heart mass (kg)	Mammals (M&H)	0.0258	-	2.86	<0.001	12.9	<0.001
		Mammals (S&B)	0.480	<0.001	10.82	<0.001	66.4	<0.001
		Giraffe	0.0546	<0.001	-1.19	0.101	-3.36	0.196

a. SMATR: Results are p-values for the test for heterogeneity in slopes^b and the test for shift in elevation^c between the samples.
d. Mean differences were calculated when comparing the measurements made on the post-natal wilbebest and the values predicted by the other mammal equations.
e. PPE: mean percent prediction error. H₀: Mean PPE = 0
f. Tests done without the original data for the mammal equations

Table 3-9: Comparison of heart dimensions measured in wilbebest with values predicted by equations for other mammals.

Predicted Physiological Cardiovascular Parameters for Wildebeest

With the aid of previously published allometric equations, physiological cardiovascular parameters were calculated for wildebeest of various body masses and are shown in Table 3-10. The values calculated from body mass and those calculated from the heart mass calculated for a wildebeest of that body mass are similar.

	Juvenile Wildebeest			Adult Wildebeest			Allometric Equation	Reference
	30	50	100	150	200	250		
Body Mass (kg)	30	50	100	150	200	250		
Heart Mass (kg)	0.200	0.306	0.544	0.763	0.969	1.167	$0.0118M_b^{0.832}$	This study
Mean Blood Pressure (mmHg)	110	111	114	115	116	117	$99M_b^{0.03}$	(White & Seymour, 2014)
	109	111	114	116	118	119	$6.9M_b^{0.24+93}$	
Systolic Blood Pressure (mmHg)	133	136	140	142	143	145	$116M_b^{0.04}$	(White and Seymour, 2014)
	132	135	140	143	145	147	$19M_b^{0.17+98}$	
Diastolic Blood Pressure (mmHg)	85	85	85	85	85	85	$84M_b^{0.003}$	(White and Seymour, 2014)
	51	52	54	55	56	57	$0.75M_b^{0.47+47}$	
Heart Rate (beats/min)	103	91	76	69	64	61	$241M_b^{-0.25}$	(Stahl, 1967)
	98	88	76	70	66	63	$65.1M_h^{-0.255}$	(Calder, 1996)
Stroke Volume (ml)	29	49	103	158	214	272	$0.78M_b^{1.06}$	(Calder, 1996)
	42	63	110	152	191	228	$197M_h^{0.96}$	(Holt <i>et al.</i> , 1968)
Cardiac Output (ml/min)	2 940	4 450	7 800	10 830	13 670	16 400	$187M_b^{0.81}$	(Stahl, 1967)
	3 630	4 890	7 320	9 270	10 960	12 480	$11200M_h^{0.7}$	(Holt <i>et al.</i> , 1968)
Left Ventricular End Diastolic Volume (ml)	57	95	193	292	391	491	$1.76M_b^{1.02}$	(Holt <i>et al.</i> , 1968)
	199	147	253	348	436	519	$449M_h^{0.94}$	
Right Ventricular End Diastolic Volume (ml)	65	109	221	335	449	564	$2.02M_b^{1.02}$	(Holt <i>et al.</i> , 1968)
	103	154	265	364	455	542	$469M_h^{0.94}$	
Left Ventricular Mass (g)	72	127	274	429	591	757	$1.65M_b^{1.11}$	(Holt <i>et al.</i> , 1968)
Total Peripheral Resistance (mmHg·min·L ⁻¹)	45.9	31.1	18.4	13.5	10.9	9.16	$609M_b^{-0.76}$	(Holt <i>et al.</i> , 1968)

Table 3-10: Physiological cardiovascular values predicted for wildebeest of specific body masses.

Predictions based on heart mass are italicised.

These predictions are made assuming the wildebeest does not have a specialised cardiovascular system.

Discussion

The purpose of this study was to describe the growth of the wildebeest heart with the use of allometric equations and to contribute an additional species to the small collection of ontogenetic studies on mammals. The ontogenetic equations were used to compare relative growth of the heart with change in body mass, and the growth of the internal structures of the heart with both body mass and heart mass change, and to compare the heart of the wildebeest with other mammals.

Ontogenetic Allometric Regression of Wildebeest Heart Size

Suitability of Power Equations

Blue wildebeest heart mass increases with body mass in a manner that can be reliably modelled using an allometric power equation. Additionally, significant power relationships occur between body and heart mass and heart dimensions when the Entire Sample is used, and for most relationships involving the Post-Natal sample. The exception is the relationships for the thickness of the right ventricular wall. The wide variation in Right Ventricular Wall Thickness measurements, likely due to measurement error or a possible lack of natural growth of the right ventricular wall after birth, are expected to be responsible for this lack of significance.

In adult wildebeest, many cardiac parameters do not scale significantly with body mass or even heart mass. In the adult sample, larger animals have heavier, longer hearts but the other dimensions do not necessarily scale with body or heart mass. The data show that larger hearts are significantly longer than smaller ones. Heart growth at adult body masses may therefore be more by increase in length than increase in wall thickness, which may be influenced by attachment of the apex of the heart to the sternum by the sternopericardiac ligament. However, inspection of the heart length scatterplots (Figure 3-6) shows that the adult relationships for heart length differ from the other sample equations more than for other cardiac measurements. This difference suggests that this adult equation may be incorrectly influenced by outlying data points and small sample size and not be a true representation of the allometry of heart length in adult animals. Additionally, the adult heart length equations have small R^2 values. In that case, heart length growth may not be that different from the growth of other cardiac dimensions in adult wildebeest.

The lack of significant scaling of many heart dimensions in the Adult Sample suggests that changes in body mass in adult wildebeest are not accompanied by similar changes in the heart. However the lack of significant differences can also be type II error, which is becomes more likely as sample size decreases (Brown & Vavrek, 2015).

It may be tempting to conclude that lack of scaling in the Adult Sample is because growth in mammals slows considerably at maturity, which is not the case in these comparisons. Although growth slows in

mammals after maturity, this is growth in relation to age, not allometric growth. In this analysis, there is no measure of age; only different body masses. As one progresses from left to right along the horizontal axes it can be assumed that in the foetuses and juvenile animals the ones to the right are older than those on the left but at some point, near maturity, this trend stops. In the adult sample, the heavier animals are not necessarily older than the lighter ones but there is still a range of body masses and the allometric relationships compare how this range is correlated with differences in heart size.

Goodness of Fit

Heart mass is the cardiac measurement which shows the strongest correlation with body mass (as measured by the adjusted R^2 value). Ninety-six percent of the variation in heart mass can be accounted for by change in body mass. This high correlation is to be expected if heart function is closely correlated with heart mass and most of the mass of the body consists of tissues which require blood perfusion.

The high R^2 values and low PPE_A of the relationships for heart circumference, diameter, length and the depth of the left ventricle are expected as these measurements determine heart shape and function. The weak correlation between the right ventricular measurements and body mass is also to be expected because the right ventricle is responsible for maintaining pulmonary blood pressure, which is significantly lower than systemic blood pressure and is not well correlated with body mass (Snelling *et al.*, 2015).

The Post-Natal and Adult equations have lower adjusted R^2 values than the equations based on the Entire Sample. This result can be attributed to the reduction in sample size and range of the body masses in the Post-Natal and Adult Samples (White & Kearney, 2014). The body mass range of the Entire Sample is 270kg, the range of the Post-Natal Sample is 218kg and that of the adult animals is only 120kg. The same trend is not seen in the PPE_A values because PPE_A is not affected by reduced sample size and range in the same way as R^2 .

A second source of the differences in R^2 values is that natural variation is greater in larger animals than in smaller ones. A naturally occurring variation of 100g in animals with a 1kg heart is plausible, whereas variations of 100g in animals with a heart that only weighs 200g is not. Natural variation is often proportional to the value being measured, rather than absolute (Kerkhoff & Enquist, 2009). The similar PPE_A values for the three samples supports this observation and shows that; although the absolute natural variation in the measured variable increases with body mass the proportional variation remains the same.

The differences between the PPE_A and R^2 values for the heart mass equations and those for the body mass equations indicate that changes in most of the heart dimensions are better explained by change in heart mass than change in body mass, as would be expected.

Tests for Isometry

Scaling of Heart Mass with Body Mass

The relationship between heart mass and body mass is hypoallometric, meaning that: as body mass increases, the change in heart mass per unit change in body mass gradually decreases. This trend is clearly apparent in Figure 3-2, comparing body mass and relative heart mass. A significant hypoallometric trend is also present in the intraspecific equations for other mammals and Prothero's interspecific mammalian equation. This trend indicates that larger animals require relatively smaller hearts than smaller ones, this may be due to larger animals having proportionally more body mass taken up by tissues which require less perfusion, such as bone, fascia and integument. It also corresponds with the hypoallometric relationship between metabolic rate and body mass.

Mammalian cardiomyocytes have only a limited capacity for post-natal proliferation (Sharma, Zhang & Wu, 2015; Shimizu & Minamino, 2016; Zebrowski, Becker & Engel, 2016). Foetal heart growth is mainly by cell proliferation but, from shortly after birth, heart growth in most mammals is chiefly due to cell hypertrophy (Zebrowski & Engel, 2013; Shimizu & Minamino, 2016; Zebrowski *et al.*, 2016). Although the specialised heart of the giraffe may grow by cardiomyocyte hyperplasia (Østergaard *et al.*, 2013), this is considered unusual in mammals. Adult mammalian heart cells also do not differ greatly in size. There is evidence to suggest that the larger hearts of larger species of animals are made up of more cells, not larger ones (Prothero, 1979; Østergaard *et al.*, 2013). If most of the cells which make up a functional adult heart are present in that of a juvenile, this could cause it to be relatively larger. This explanation may also explain why the relationship between heart mass and body mass in this ontogenetic study is more hypoallometric than for interspecific studies and why, as a rule, ontogenetic allometric equations tend to have smaller exponents than corresponding interspecific ones. Juvenile animals of a large species will have more heart cells and a larger heart than an adult animal of the same body mass, but of a different species.

Comparison of Different Age-Group Equations

The allometry of heart growth can be used to describe the differences in growth of various parts of the heart at different growth stages. If heart growth throughout the life of the wildebeest (including late term foetal growth) is considered, the changes in left ventricular wall thickness, interventricular wall thickness and heart length are isometric with body mass. In contrast, heart mass, right ventricular wall thicknesses, heart circumference, diameter and the depth of both ventricles is hypoallometric with growth. This result suggests that the left ventricular wall grows proportionally with increases in body mass whereas the rest of the heart and the overall structure of the organ does not. This trend would be explained by the left heart continuing to provide the cardiac output required for a growing body and the right heart not needing to increase capacity to the same extent.

If only post-natal growth is considered, heart mass changes hypoallometrically with changing body mass, but heart dimensions are isometric to it. Since heart density is unlikely to change with growth, the difference must be due to unmeasured (but weighed) parts of the heart, such as the atria, having a growth rate which is hypoallometric to body mass. As the atria do not directly contribute to systolic blood pressure, it is likely that their growth rate is indeed hypoallometric to body mass. It is also possible that the measured heart dimensions do grow hypoallometrically compared with body mass but that the relationship was not statistically significant in this study. Further studies with larger sample sizes, larger body mass ranges and smaller measurement error may reduce the standard error of the value of b and therefore show significant hypoallometric relationships.

In contrast, the relative growth of the measured heart dimensions compared with heart mass is not affected by the different age groups except for right ventricular wall thickness and heart length.

Comparison of the Body Mass and Heart Mass Equations

It is the hypoallometric nature of the relationship between heart and body mass which causes the exponents of the body mass equations to be lower (more hypoallometric) than the corresponding heart mass equation. Each of the heart mass equations can be approximated by the body mass equations once the relationship between heart mass and body mass is known, as shown by the following equations:

If the relationship between heart mass and body mass is known:

$$M_h = 0.0118M_b^{0.832}$$

Therefore:

$$M_b^{0.832} \propto M_h$$

$$M_b \propto M_h^{1/0.832}$$

$$M_b \propto M_h^{1.2}$$

Therefore, if one knows the body mass equation for variable y ,

$$y = a(M_b)^b$$

Then one can calculate the heart mass equation,

$$y \propto (M_b)^b$$

$$y \propto (M_h^{1.2})^b$$

$$y \propto M_h^{(1.2 b)}$$

If heart mass scales with body mass raised to the power of $\frac{5}{6}$, as it does in the wildebeest, then the exponent of a heart mass equation will always be approximately $\frac{6}{5}$ or 1.2 times the value of the exponent of the analogous body mass equation. This relationship between the exponents is true for the results from this study, which supports the finding that the heart mass / body mass relationship is hypoallometric.

Comparison of Foetal and Post-Natal Heart Size in Wildebeest

The function and anatomy of the foetal heart is remarkably different from that of the post-natal animal. In eutherian mammals, the foetal heart is responsible for perfusion of both the foetal body and the placenta. As the foetus grows, the size of the placenta relative to the foetus decreases and at birth perfusion of the placenta ceases. After birth, changes in posture and activity also affect the cardiovascular system. In the exceptionally precocious wildebeest, this happens relatively soon after birth compared with other mammals. With a change in posture, blood pressure must overcome gravity and cardiac output must be sufficient to supply the needs of the, increasingly active, body. At birth, there is a net flow of blood from the placenta to the foetus, before placental perfusion stops (Yao, Hirvensalo & Lind, 1968; Rabe, Diaz-Rossello & Duley, 2012; Hooper *et al.*, 2015; Katheria *et al.*, 2017), via a process called placental transfusion. Although placental transfusion increases blood volume, it is accompanied by increased perfusion of the lungs when breathing starts. Therefore, there is a drop in blood pressure soon after birth. The function of the right heart also changes at birth, along with the changes in pulmonary blood flow associated with inflation of the lungs.

Despite the large differences between foetal and post-natal hearts the hearts of the foetal wildebeest measured for this study are remarkably similar to those of post-natal animals in terms of their relative size. The foetal measurements from this study do not fall far from the post-natal trajectories and the Entire Sample and Post-Natal equations are not significantly different from each other. Put another way, if the post-natal wildebeest equations were used to predict the structure of the heart of an animal of the same mass as that of the measured foetuses, the predicted heart would not be significantly different from that measured in the foetal animals.

Although foetal cardiovascular system is clearly very different from that of the post-natal mammal, the structures needed to support the post-natal cardiovascular physiology must be in place at the time of birth, especially in such a precocious animal. Neonatal wildebeest calves can run nearly as fast as adults soon after birth (Howell, 1965). The necessity for a cardiovascular system that supports high-speed running at such a young age would explain a relatively mature heart in the near-term foetus.

This suggests that the growth of the heart follows the same trajectory from at least a month before birth until maturity. If so, the Entire Sample Equations are theoretically valid and could be useful for

describing heart growth from the late term foetus to the adult animal. However, since no wildebeest calves smaller than 64kg were measured and all the measured foetuses fall within the narrow body mass range of 12 to 15kg, it is not certain that the Entire Sample trendlines accurately describe cardiac growth in the wildebeest calf or in the foetus smaller than 12kg or larger than 15kg. It is possible that the foetal and post-natal heart growth follow different allometric equations which intersect at a point close to the body mass of the foetuses measured.

Contrary to what is seen in wildebeest in this study, evidence from other mammals (Giraffes and Kangaroos) (Mitchell & Skinner, 2009; Snelling *et al.*, 2015) suggest that cardiac allometry before and after birth (or before and after leaving the pouch, in the case of the kangaroo) are significantly different. These studies suggest that; because the workload on the heart is different in the foetus and post-natal animal, the factors which influence heart growth are likely to also differ and affect its allometry. For this reason, the data from this study is only used to describe the ontogenetic allometry of the post-natal wildebeest with confidence.

Further evidence that the foetal and post-natal hearts are not as similar as they seem to be on subjective evaluation of the scatter plots, is present in the values of the mean percentage prediction errors (PPE). Many foetal heart measurements differed from the predicted values by more than 10% (Table 3-7) and although the sample size is too small to perform any useful tests of significance on these differences they do suggest that even in these late-term hearts there is an appreciable difference between the foetal and post-natal heart. The data suggest that foetal hearts have relatively narrower left and interventricular walls and smaller circumferences and lengths, but larger diameters and deeper right ventricles than post-natal hearts. The comparison of the foetal and post-natal allometry of the heart is an interesting field for future research. Larger sample sizes and body mass ranges in the foetal dataset are needed to determine the relative growth of the foetal heart and compare it with that of the post-natal animal. Comparison of the differences between foetal and post-natal heart allometry between different species could explore the effect of relative maturity at birth.

In the foetal wildebeest, the left, right and interventricular walls have similar thicknesses. After birth, the left and interventricular walls scale with both heart and body mass while the right ventricular wall does not change significantly. The difference in ventricular growth is related to the change in function of the right heart at birth. In the foetus, blood from both the left and right heart is pumped into the aorta. Consequently, both ventricles are subjected to similar afterload. At birth and the start of respiration, the lungs are inflated, which increases pulmonary circulation and reduces afterload on the right ventricle and increases preload on the left heart. The *ductus arteriosus* and *foramen ovale* close, separating the left and right hearts. After this point, cardiac output from both sides of the heart is the same, but workloads are different. Heart wall thickness adjusts appropriately to the workload imposed on it, as explained the principle of Laplace, leading to a left ventricle which grows more than the right after

birth. The principle of Laplace and its relation to the work of the heart have been well-studied, in short it predicts that cardiac wall thickness increases in proportion to the load imposed on the heart to ensure that wall stress is conserved (Martin & Haines, 1970; Seymour & Blaylock, 2000; Snelling *et al.*, 2015).

Comparison of Ventricular Wall Thicknesses in Wildebeest

The similarity between the thicknesses of the left and interventricular walls may be useful for future heart measurements. The thickness of the left ventricular wall is not uniform, which could lead to measurement error if several measurements are not made. The interventricular wall has a far more uniform thickness, which is easier to measure.

Comparison of Heart Size in Wildebeest with other Mammals

Heart Mass

Intraspecific Equations

Mammalian heart mass is generally closely related to body mass (Prothero, 1979), but more athletic species and breeds tend to have relatively larger hearts (Joseph, 1908; Brody, 1945; Gunn, 1989; Hanson *et al.*, 1994; Poole & Erickson, 2011; Østergaard *et al.*, 2013). However, heart mass is only a crude estimator of heart function. An important example is that of the giraffe which, at a relative mass no different from that of other animals, produces twice the blood pressure (Mitchell & Skinner, 2009; Østergaard *et al.*, 2013).

The relative size of the wildebeest heart in comparison with ontogenetic equations for horses and giraffes is not surprising. The heart mass predicted for a wildebeest of a given body mass is less than that of a horse, which is more athletic, and the giraffe, which has an unusually high systemic blood pressure to support its unusual body shape (Mitchell & Skinner, 2009; White & Seymour, 2015).

The high relative heart masses of horses, as predicted by their ontogenetic allometric equation (Table 3-8 and Figure 3-12), seems exceptional. The equation for relative heart mass as a percentage body mass is $\frac{100 M_h}{M_b} = 1.61 M_b^{-0.127}$, predicts a relative heart mass of 0.90% in a 100kg horse, 0.75 % in a 400kg horse and 0.70% in one weighing 700kg. Although this is high for a mammal, other sources record similar relative heart masses for horses. In popular press and veterinary textbooks (Budras, Sack & Rock, 2009; Marr & Bowen, 2010; Dukes & Reece, 2015) it is reported as accepted fact that race horses have relative heart masses which reach and even exceed 1% of body mass. This high relative heart mass may be true for exceptional animals but it is not supported by available data. Although supporting data is scarce, measurements of the heart and body masses can be used to calculate a mean relative heart mass of 0.88% for thoroughbred horses (n=15, mean $M_h = 457\text{kg}$), 0.73% for Percherons (n=2, mean $M_h=703\text{kg}$) (Altman & Dittmer, 1962) and 0.59% for two horse of unspecified breed (mean

$M_h = 455\text{kg}$) (Joseph, 1908). A study on heart weight and running ability in horses and dogs calculated a mean relative heart mass of 0.86% in thoroughbred horses ($n=11$, mean $M_h=182\text{kg}$) and 0.76% in other breeds ($n=12$, mean $M_h=273\text{kg}$) and concluded that relative heart weight is greater in breeds noted for high speed running than other breeds (Gunn, 1989). A study on unconditioned horses concluded that race horse breeds have significantly larger relative heart masses (0.67%, $n=5$, mean $M_h=537$) than draught horses (0.52%, $n=5$, mean $M_h=710$) (Hanson *et al.*, 1994) and the difference between these relative heart masses and those calculated from the previous studies suggests that exercise and conditioning can significantly increase heart mass in horses, as it can in humans (Shimizu & Minamino, 2016).

Wilbeest and cattle of the same body mass have similarly sized hearts, which is interesting because cattle are considered to have relatively small hearts both because of their lack of athletic ability compared to similarly-sized mammals and as a result of artificial selection for increased muscle mass (Brody, 1945; Gunn, 1989). The unexpectedly similar heart masses of wilbeest and cattle could be because cattle with similar body masses to wilbeest are relatively less mature and younger animals have relatively larger hearts. The median relative heart mass of the adult wilbeest in this study (0.48%) is higher than for the adult cattle (0.38%) in the dataset from Crile and Quiring (1940). Other estimates of relative heart mass in cattle are similar, approximately 0.34% in 2year old pregnant beef heifers weighing on average 460kg (Scheaffer *et al.*, 2001), 0.44% in British cross breeds, weighing on average 400kg (Sainz, De la Torre & Oltjen, 1995) and 0.37% in feeder steers weighing approximately 420kg (Jones, Rompala & Jeremiah, 1985).

The finding that wilbeest have significantly larger hearts than African buffalo, despite buffalo in the wilbeest body mass range being immature, suggests that buffalo have significantly smaller relative heart masses than the other mammals. Adult buffalo ($M_b > 500\text{kg}$) from the sample described in this study had a median relative heart mass of 0.44% of body mass (range 0.37-0.52%, $n=22$) which are within the range found for domestic cattle.

Interspecific Equations

Comparisons between ontogenetic and interspecific equations can be useful to compare a single species with the average for a larger group. However, the comparison must be done with caution because the two types of equations are not directly comparable (Gould, 1966, 1971).

There is an interesting difference between the wilbeest heart masses and those estimated for African antelope. All measured wilbeest heart masses were lower than those predicted for African antelope of the same body masses by the equation derived by Woodall (1992). The difference is not only due to the difference between ontogenetic and interspecific equations, even though intraspecific equations often have smaller exponents than interspecific ones (Brody, 1945; Gould, 1966, 1971). If this was the case, the heart masses of the juvenile animals would be expected to be greater than those predicted by

the interspecific equation and those of adult animals would not be significantly different from the interspecific predictions.

Woodall's equation may not be an accurate model for all African antelope, due to the small sample of 25 individual animals from 13 different species and the fact that most of the species used in the study were relatively small antelope. Although the sample contained several large antelope, which fitted the trendline well, eight of the thirteen species used in the study had adult body masses lower than 100kg and the median body mass was only 46.5kg. The median relative heart mass measured by Woodall is 0.71% of body mass (range: 0.47-0.96%), far larger than predicted for the average mammal of similar size (0.55%). To compare the allometry of heart mass in large and small antelope a larger sample is needed with more species of large antelope such as sable, waterbuck or kudu. Only then can it be concluded whether blue wildebeest have unusually small hearts for antelope or only in comparison to small antelope.

The SMATR software package can compare the coefficients (intercepts) of two different allometric equations but this is only useful if the exponents are not significantly different from each other. For example, the slope of the post-natal ontogenetic equation for heart mass in wildebeest is significantly different from that of Prothero's interspecific equation ($p=0.043$). Therefore, the result that the wildebeest equation for heart mass and Prothero's mammal equation have intercepts that are not significantly different is only useful for comparing the equations at a body mass of 1kg. The heart mass predicted for 1kg wildebeest (by the post-natal allometric equation for wildebeest heart mass) is not significantly different from that predicted for an adult mammal weighing 1kg. An interesting result, however, since no foetal wildebeest that small were measured, the predictions for these 1kg animals are extrapolations and may be incorrect. A study on the foetuses of domestic cattle suggests that the extrapolations are likely to be inaccurate. A 1kg ox foetus has a relative heart mass of 0.72% of body mass (Szuba, Babula & Kalisińska, 1986), which is lower than the 1.2% predicted for wildebeest and closer to the 0.6% percent estimated for adult mammals weighing 1kg.

In contrast, the significant difference between the intercept of the wildebeest and African antelope equation is a useful result. Because the exponents are not significantly different from each other, the different intercepts refer not only to a significant difference between the heart mass predicted for a 1kg animals but to a significant difference between heart masses predicted by the two equations, for any body mass.

When two equations with significantly different exponents are compared, the relationship between the two equations differs depending on body mass, as it does for the relationship between the wildebeest and mammal equations. This difference between exponents indicates that the change in heart mass per unit change in body mass in the growing wildebeest is different from the interspecific mammalian trend. This also means that the difference between wildebeest heart masses and those predicted by the

interspecific equations changes depending on body mass. Because of this, when exponents are significantly different the most appropriate method of comparing allometric equations is to compare measured values in one sample with those predicted by the equation from the other sample. Using differences between measured and predicted values, which are expressed as a percentage of the predicted value (PPE), reduces errors caused by measurements of larger values having greater natural variation than those of smaller values.

Comparison of the measured heart masses with those predicted by the two mammal equations (by using mean differences and mean PPEs) also returns conflicting results depending on which mammal equation is used. The differences between the wildebeest measurements and the predictions for mammals are small. The difference is statistically significant for Brody's equation but not for the more accurate equation derived from a larger sample by Prothero, indicating that, the heart masses measured in post-natal wildebeest are not significantly different from those expected for the average mammal of the same body mass.

However, visual inspection of the scatter plot of the data (Figure 3-13) and the understanding that the exponents of the wildebeest ontogenetic equation and mammal equations differ, show that as wildebeest heart mass increases so does the difference between the heart mass of the wildebeest and that of the average mammal.

Since the interspecific equations for mammals are based on adult animals, comparisons with adult wildebeest are indicated, to determine if wildebeest heart masses are different from those of other mammals. These comparisons show that the heart mass of the average mature wildebeest is approximately 90 to 116g (7.7 to 9.7%) lighter than that of the average mammal of the same body mass, a difference which is statistically significant for both mammal equations (Brody, $p=0.003$; Prothero, $p=0.016$). The evidence for a significant difference is not conclusive though, it is contradicted by the finding that the exponent (b) and multiplier (or intercept of the log-transformed equation) (a) of the Adult Sample equation for heart mass in wildebeest are not significantly different from those of either of the mammal equations (Table 3-9). This lack of a significant difference and the conflicting results seem counter-intuitive at first. Especially since, the Adult Sample is clearly further from the mammalian trend lines on average than the Post-Natal sample is and the exponent of the Adult Sample (0.797) is smaller than that of the Post-Natal Sample (0.832) and further from that of the mammal equations (0.984 and 0.97). The reason for the lack of a significant difference in this case is that the adult sample size is smaller and the variance greater than in the Post-Natal Sample, leading to larger standard errors so that a larger difference between values of the allometric exponent of multiplier of the equations is needed before the difference can be considered significant. Therefore, the data suggest that although the average difference between the hearts of adult wildebeest and those of the average mammal of the same body mass is significant, a larger sample of mature wildebeest is required to test whether the allometric

relationship between heart and body mass in the adult wildebeest is significantly different from that found in mammals in general.

One common explanation given for differences in relative heart mass between species is that more athletic species and breeds have larger hearts than those which are less athletic (Joseph, 1908; Brody, 1945; Gunn, 1989; Poole & Erickson, 2011). However, relative athleticism in these cases not well defined and is based on a subjective understanding of the behaviour of different species.

If prolonged physical exertion is a criterion for athleticism, then the migratory blue wildebeest must be classified as an athletic species. Wildebeest are capable of sustained cantering over a several kilometres at speeds of 3-6m/s (Pennycuick, 1975; Perry *et al.*, 1988), which is comparable to the average speeds achieved by horses in endurance races (3.9m/s) (Hargreaves *et al.*, 2002; Marlin *et al.*, 2002; Barton *et al.*, 2003; Castejón *et al.*, 2006)(Hargreaves *et al.*, 2002; Marlin *et al.*, 2002; Barton *et al.*, 2003; Castejón *et al.*, 2006) and equine postal systems (4.4m/s) (Minetti, 2003).

Wildebeest also achieve relatively high maximum running speeds. Wildebeest are often reported to be able to achieve a maximum speed of 80km/h (22m/s) which they can sustain for approximately a quarter of a mile (400m). Although this speed is reported in an authoritative text on animal running speeds (Howell, 1965), the original source is from the observations made by an early wildlife film maker (Johnson, 1929). More recent measurements of wildebeest top speeds are lower, 11m/s when being chased in a vehicle (Alexander *et al.*, 1977) and 14.3 m/s when chased by a lion (*Panthera leo*) (Elliott *et al.*, 1977) but are still within the range of athletic antelope (Christiansen, 2002).

In contrast to the conclusions drawn from their running ability, blue wildebeest are classified as 'non-athletic' based on their relatively low V_{O_2max} of 0.75ml/s/kg recorded while running on a treadmill at 9m/s (Taylor *et al.*, 1980; Weibel *et al.*, 2004). However, since V_{O_2max} is influenced by cardiac output and therefore heart mass, the unexceptional V_{O_2max} of the wildebeest is more likely to be as result of their heart mass rather than an explanation for it.

Differences in relative heart mass can also be the result of differences in the relative masses of other body parts (Joseph, 1908). It is possible that the relatively large digestive tract of large ruminants, especially bulk grazers, affects the relationship between heart and body mass. The limited data on relative digestive system weights of African ruminants show that the mass of the contents of the ruminoreticulum scales hyperallometrically with body mass in African ungulates, for example the mass of the contents of the ruminoreticulum of an African buffalo (*Syncerus caffer*) is approximately 20% of body mass and that of wildebeest is 14% whereas it makes up between 7 and 8% in small, browsing antelope such as the bushbuck (*Tragelaphus scriptus*) and steenbok (*Raphicerus campestris*) (Demment, 1982). Additionally, the viscera make up a far larger fraction of total carcass weight in the wildebeest (28%) than the Impala (*Aepyceros melampus*), Thomson's gazelle (*Eudorcas thomsonii*) or

Grant's gazelle (*Nanger granti*) (19%) (Blumenschine & Caro, 2008). This explanation seems to explain the low relative heart masses of cattle, buffalo and wildebeest. However, not all large ruminants seem to have low relative heart sizes, for example, Woodall (1992) reports a mean relative heart size of 0.62% for two eland (*Taurotragus oryx*) and one of 0.71% for a single black wildebeest (*Connochaetes gnou*). Therefore, neither relative athleticism nor gut mass provide a simple, definitive explanation for the apparent relatively small heart of the adult wildebeest.

If the low relative heart mass found in blue wildebeest is not a biproduct of other organs which are relatively heavier, then the myocardium must be more efficient than that of other mammals, as it is in the giraffe (Mitchell & Skinner, 2009). A more efficient myocardium is likely to be an adaptation to a migratory lifestyle. However, further research is needed determine whether this is a trait which found in other large migratory mammals.

Left Ventricular Wall Thickness

Interspecific equations

The two Left Ventricular Wall Thickness equations that were compared with the wildebeest equation were derived from measurements of ventricular wall thickness made using very different methods. Neither was published as an allometric equation for wall thickness; the equations were formulated during this study from data published in the original references.

In the study by Martin and Haines (1970), “five replicate measures of wall thickness were taken” at between two and four locations chosen at random on the left ventricle. The study by Seymour and Blaylock (2000) published measurements of the inner and outer radius of the left ventricle, from which wall thickness was calculated by subtraction. The equations for ventricular radii were based on either direct measurement of the radii using imaging studies or calculation of the radii from end diastolic volume and left ventricular myocardial volume, which in some cases were themselves calculated from other cardiac measurements and allometric equations. The Martin and Haines equation is more likely to be accurate and is, unsurprisingly, closer to the wildebeest equation. If this equation is an accurate representation of the average mammal, then immature wildebeest have thicker left ventricular walls than the average mammal and adult wildebeest do not differ from other mammals. This is the pattern that is expected when comparing an ontogenetic allometric equation with an interspecific equation derived from only adult animals; the ontogenetic equation has a smaller exponent but the adult animals fit the prediction of the interspecific equation. It therefore suggests that wildebeest do not have unusual left ventricular wall thicknesses.

Intraspecific equation: Giraffe

The giraffe, possesses left ventricular walls which are thicker than those of the average mammal, to provide the high blood pressure necessary for its extreme height (Mitchell & Skinner, 2009). Therefore, it is surprising to find that the mean percentage prediction error (PPE), between the wall thicknesses

measured in wildebeest and the giraffe heart mass equation, is not significantly different from zero. Closer inspection of the comparison reveals the reason for this unexpected result. Firstly, comparison between the giraffe and wildebeest is hampered by their very different sizes. Giraffe weigh approximately 100kg at birth and the smallest post-natal giraffe in the sample weighed 141kg. Since, the PPE compares the wall thicknesses measured in wildebeest with those predicted by the giraffe equation, the PPE of any wildebeest weighing less than 141kg are based on extrapolations of the giraffe equation. A better appreciation of the relative thicknesses of wildebeest and giraffe left ventricular walls can be gained by inspection of in Appendix 7. Therefore, the results do not show that wildebeest and giraffe have similar left ventricular wall thicknesses. Giraffe and wildebeest foetuses of similar mass have similarly sized left ventricular walls but, where wildebeest hearts develop isometrically, the wall of the giraffe heart grows hyperallometrically to body mass.

Conclusion

In the post-natal blue wildebeest, heart mass scales hypoallometrically with body mass, meaning that relative heart and lung size decreases with increasing body mass. Heart mass ranges from approximately 0.8% of body mass in the foetus to approximately 0.5% in adult animals. The dimensions of the heart scale isometrically with both heart and body mass.

Although the heart mass and dimensions of late-term foetuses can be predicted with reasonable accuracy from the post-natal allometric relationships, a larger foetal sample is required to test for a significant difference between foetal and post-natal heart growth.

Blue wildebeest have similarly sized hearts to those of domestic cattle, smaller hearts than horses and giraffe and larger hearts than African buffalo of the same body mass.

Blue wildebeest have smaller hearts than predicted for adult African antelope and the average adult mammal of the same body mass, which is unlikely to be due to a difference in athletic ability between wildebeest and other species but may be due to a relatively heavier digestive tract contents in the wildebeest.

The thickness of the left ventricular wall in wildebeest is not unusual for a mammal.

Chapter 4: Ontogenetic Allometry of the Blue Wildebeest Respiratory System

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Summary

Lung mass, tracheal length, tracheal diameter and tracheal volume scale isometrically with body mass in post-natal wildebeest. The lung mass in kg (M_l) and tracheal volume in ml (V_t) of the post-natal blue wildebeest can be estimated from the equations: $M_l = 0.0260M_b^{0.816}$ ($R^2 = 0.71$, $PPE_A=16$) and $V_t = 3.31M_b^{0.905}$ ($R^2 = 0.78$, $PPE_A=16$). The lung masses of foetal wildebeest (approximately 3.3% of body mass) are greater than predicted by the post-natal relationships (approximately 1%), due to fluid in the lungs before birth. The diameter of the foetal trachea (average 9mm) is on average 10mm (53%) narrower than predicted by the post-natal allometric relationship, while the length of the foetal trachea is the same as predicted.

Wildebeest lung masses are smaller than those of horses and larger than those of giraffes of the same body mass. Comparison of the wildebeest equations with interspecific equations for mammals showed that there is no significant difference between the ontogenetic equation for lung mass in post-natal wildebeest and the interspecific equations for African ruminants (exponent $p=0.287$, multiplier $p=0.191$) or other mammals (exponent $p=0.054$, multiplier $p=0.07$).

Introduction

The function of the respiratory system is chiefly the exchange of oxygen and carbon dioxide between the atmosphere and the blood. It functions in unison with the cardiovascular system and the growth of both systems is expected to be closely related to and highly correlated with body mass. In the foetal animal, the trends may be different because, in contrast to the circulatory system, the respiratory system is not functional until after birth.

Allometry of the Respiratory System

Interspecific Allometry

When different mammalian species are compared, respiratory variables scale well with body mass, producing allometric equations with correlation coefficients greater than 0.9 (Stahl, 1967).

The interspecific allometry of lung mass has been well-studied (Brody, 1945; Bennett & Tenney, 1982; Calder, 1996; Lindstedt & Schaeffer, 2002). The most accurate interspecific equation comparing lung mass (M_l) and body mass (M_b) (both in kg) is: $M_l = 11.3M_b^{0.986}$ (Brody, 1945). This equation was derived from approximately 150 data points, each representing a different mammal species. It is this equation that was later quoted by Stahl (1967) and, incorrectly, is occasionally attributed to him. The allometric exponents for the equations relating lung mass to body mass are close to one and so lung mass is generally expected to scale isometrically with body mass. In most cases, lung mass can be assumed to be equal to 1% of body mass in mammals. However, rounding of exponents, even slightly,

can have a large impact on the accuracy of allometric equations, especially those used over large ranges (Prothero, 1986). Table 4-1 summarises the three best estimates for the allometric equation for lung mass, and the predicted relative lung mass for mammals of different body masses. It emphasises how slight variation in the exponent can have a major effect on predicted relative lung mass.

Body Mass (kg)	0.01	0.1	1	10	100	1000	Equation and reference
Lung Mass as a percentage of Body Mass	1.21	1.17	1.13	1.09	1.06	1.03	$11.3 M_b^{0.986}$ Brody (1945)
	0.67	0.72	0.77	0.83	0.89	0.95	$7.72 M_b^{1.03}$ Bennett and Tenney (1982)
	0.93	0.93	0.93	0.93	0.93	0.93	$9.27 M_b^{1.00}$ Lindstedt and Schaeffer (2002)

Table 4-1: Mammalian relative lung mass as a percentage of body mass (M_b) as calculated for different interspecific mammalian equations, for body masses spanning six orders of magnitude.

Allometric equations for lung volume have been published from several different studies (Tenney & Remmers, 1963; Gehr *et al.*, 1981; Calder, 1996; Lindstedt & Schaeffer, 2002). Like lung mass, lung volume is also expected to scale isometrically with body mass. Lung volume is measured by fluid displacement of the inflated lung (Tenney & Remmers, 1963; Gehr *et al.*, 1981) and so includes both total lung capacity and the volume of the lung tissues. Stahl (1967) used the data of Tenney and Remmers (1963) to formulate his equation for total lung capacity. This method probably overestimates total lung capacity because no attempt was made to compensate for the fact that lung tissue makes up approximately 10% of the fully inflated lung (Calder, 1996). The equation is reported here as one for lung volume, as it is in Calder (1996), rather than total lung capacity.

The good correlation between respiratory variables and body mass ensures that the interspecific allometric equations are also useful for the prediction of respiratory variables for individual animals, if body mass is known (Stahl, 1967).

The various interspecific allometric equations available for mammals are summarised in Table 4-1 and Table 4-2. To assist in choosing between the various equations, the sample sizes of each study (when known or estimated) are summarised in Table 4-3.



Variable	Formulae relating each variable to body mass (M_b) (kg)
Lung Mass (g)	$7.72 M_b^{1.03}$ (1), $11.3 M_b^{0.986}$ (2 [†]), $11.3 M_b^{0.99}$ (3,6), $9.27 M_b^{1.00}$ (5)
Lung volume (ml)	$53.5 M_b^{1.06}$ (3,6), $46.0 M_b^{1.06}$ (4), $66.13 M_b^{0.986}$ (4**), $48.1 M_b^{1.10}$ (5), $a M_b^{1.02}$ (8*)
Total System volume (ml)	$54 M_b^{1.06}$ (3)
Dead Space (ml)	$2.76 M_b^{0.96}$ (6)
Vital Capacity (ml)	$56.7 M_b^{1.03}$ (6)
Functional residual capacity (ml)	$24.1 M_b^{1.13}$ (6)
Inspiratory reserve volume (ml)	$50 M_b^{0.79}$ (6)
Expiratory reserve volume (ml)	$24 M_b^{1.10}$ (6)
Respiratory rate (breaths/min)	$53.5 M_b^{-0.26}$ (6)
Tidal Volume (ml)	$7.69 M_b^{1.04}$ (6)
Minute Volume (L/min)	$0.463 M_b^{0.960}$ (5 [†]), $0.379 M_b^{0.80}$ (6 [†]), $0.499 M_b^{0.809}$ (9 [†])
Tracheal Length (cm)	$6.2 M_b^{0.4}$ (3), $a M_b^{0.27}$ (7*)
Tracheal Diameter (cm)	$0.41 M_b^{0.39}$ (3*), $a M_b^{0.39}$ (7*)
Tracheal Cross-Sectional Area (cm ²)	$0.10 M_b^{0.78}$ (3)
Tracheal Volume (ml)	$0.82 M_b^{1.18}$ (3*), $a M_b^{1.05}$ (7*)

References are given in brackets after each equation:

¹Bennett & Tenney (1982); ²Brody (1945); ³Calder (1996); ⁴Gehr *et al.* (1981); ⁵Lindstedt & Schaeffer (2002);
⁶Stahl (1967); ⁷Tenney & Bartlett (1967); ⁸Tenney and Remmers (1963); ⁹Bide *et al.* (2000).

* Tenney and Bartlett (1967) and Tenney and Remmers (1963) did not report the value of the multiplier, however, the equations published by Calder (1996) are based on these studies and personal communication with the authors.

** Equation for African antelope and viverrids

† For consistency, the units have been altered from the format in which they were originally published.

Table 4-2: Interspecific allometric equations describing the respiratory system.

Reference	Number of Animals	Number of Species	Body Mass range (kg)
Brody (1945)	~ 150	~ 150	0.01 – 100 000
Tenney and Remmers (1963)		20	~ 0.01 – 2 000
Tenney & Bartlett (1967)	~ 35 - 44	19 - 23	0.01 – 2 000
Stahl (1967)	52 - 688		0.01 - 300
Gehr <i>et al.</i> (1981)	27	15	0.42 - 251
Bennett & Tenney (1982)	31	5	0.0006 - 26
Bide <i>et al.</i> (Bide <i>et al.</i> , 2000)	2616	18	0.02 - 540
Lindstedt & Schaeffer (2002)		4 - 12	~ 0.04 – 1 000

Table 4-3: Comparison of studies on the interspecific allometry of the mammalian respiratory system.

Intraspecific and Ontogenetic Allometry

Studies of intraspecific and ontogenetic allometry are rare in mammals and the respiratory system is no exception. Allometric relationships for some growing animals are reported by Brody (1945). Along with his calculation for the interspecific equations, ontogenetic equations for lung mass (M_l) in growing rats ($M_l=0.0038M_b^{0.75}$), dogs ($M_l=0.0138M_b^{0.82}$) and horses ($M_l=0.133M_b^{0.58}$) were published.

The only large mammal in which the allometry of the respiratory system has been well-studied is the giraffe, which has a specialised respiratory system because of its unusual morphology. Giraffes have smaller lungs and larger dead space than other mammals of the same body mass, but maintain the same dynamic lung volumes. The ontogenetic allometric equation for giraffe lung mass is $M_l=0.018M_b^{0.84}$ (Mitchell & Skinner, 2011). The exponents of the interspecific equations are all hypoallometric, indicating that young mammals have relatively larger hearts than older animals of the same species.

The Respiratory System of the Blue Wildebeest

The respiratory system of the blue wildebeest has not been well-studied until now but some measurements on small samples of animals give useful approximate values. Published measurements of respiratory parameters are limited to one set of papers on the design of the mammalian respiratory system (Taylor *et al.*, 1980; Taylor & Weibel, 1981). This study was based in Kenya and the animals used were of the local subspecies, either *C. t. mearnsi* or *C. t. albojubatus* but not *C. t. taurinus*. Two juvenile wildebeest (body masses 92 and 104kg) were trained to run on treadmills and achieved maximum rates of oxygen consumption of 69.5 and 75.7 ml/s (45.3 and 43.67ml/kg/min) at maximum speeds of 8.1 and 9.1 m/s. In an associated study, a lung volume of 7678ml was recorded for a wildebeest weighing 102kg (Gehr *et al.*, 1981; Weibel *et al.*, 1981). Respiratory parameters measured on two wildebeest of 140kg body mass at 20 degrees Celsius were: respiratory rate 14 breaths per minute, tidal volume 1.7 litres per breath and ventilation volume 24 litres per minute (Taylor *et al.*, 1969). Mean respiratory dead space (entire dead space volume, excluding the oral cavity, sinuses and alveoli) was 630ml in a sample of three wildebeest of body masses between 130 and 140kg; 1.4 times that measured in zebu cattle of similar size. This result was attributed to the larger nasal passages of the wildebeest (Taylor *et al.*, 1969).

This study aims to describe the growth of the blue wildebeest lungs and trachea by formulating ontogenetic allometric equations for lung mass and tracheal dimensions. These equations therefore describe whether relative lung mass and tracheal dimensions remain the same throughout life or change with growth. They also describe the relationship between the growth of the lungs and growth of the trachea. The equations form the basis for testing the difference between wildebeest and other species. In addition, unmeasured physiological values for the wildebeest are predicted from published interspecific equations for mammals.

Materials and Methods

Data Collection

Lung mass and tracheal dimensions were measured on a sample of the wildebeest collected for the body dimension study in Chapter 2, following the methods of Mitchell and Skinner (2011). Tracheal dimensions were measured in situ, to prevent elastic recoil. Tracheal length was measured from the first tracheal ring below the pharynx to the tracheal bifurcation, with a flexible measuring tape. The internal tracheal diameter was measured with a Vernier calliper after partially transecting the trachea between the tracheal rings. This diameter was measured at three locations: below the pharynx, just cranial to the tracheal bifurcation and midway between these points. Tracheal volume was calculated as the volume of a cylinder, using the mean tracheal diameter and tracheal length. The lungs were separated from the bronchi at the surface of each lung and lung mass was measured to an accuracy of two grams.

Right ventricular wall thickness was measured after the ventricles were transected, at a point half way between the atrio-ventricular groove and the apex (calculated by halving the measured heart length and measuring that distance from the coronary groove). Right ventricular wall thickness was measured at the midpoint of the wall with a Vernier calliper.

For all post-natal animals, a calculated body mass was used for comparison with organ measurements. The body mass was calculated from the equations formulated in this study and described in Chapter 2. For most animals, both body length (L) and girth (G) were recorded and the equation $M_b = 39.08(LG^2)^{0.9091}$ was used to estimate body mass (M_b). For one animal, for which girth was not measured, mass was estimated from the equation $M_b = 14.96(L)^{2.801}$.

Data Analysis

To describe the growth of the wildebeest trachea and lungs, allometric equations were derived for the change in lung mass compared with change in body mass and the change in tracheal dimensions compared with both body and lung mass. Right ventricular wall thickness was regressed against lung mass.

Ontogenetic Allometric Regression of the Wildebeest Respiratory System

Allometric regressions were performed for each variable, using the Entire Sample, the Post-Natal Sample and the Adult Sample. The Post-Natal Sample was compared with the Entire Sample to assess the difference between foetal and post-natal organs.

Scatterplots of each comparison were created and allometric equations were derived by plotting the log-transformed data and fitting linear trend-lines. The linear equations from the log-transformed data were converted to allometric equations.

Each relationship was then explored further using regression analysis in SPSS, which provided the standard errors for the model coefficients and allowed assessment of model fit and validity. The adjusted coefficient of determination (adjusted R^2) and absolute mean percentage predictive error (PPE_A) for each relationship were assessed to gauge goodness of fit and ANOVA was used to test whether power equations were appropriate models for representing the data.

Tests for Isometry

The equations derived for tracheal measurements and lung mass were used to test whether tracheal dimensions changed isometrically with body and lung mass and lung mass with body mass.

Comparison of Wildebeest Tracheal Diameter at Different Sites.

Scatterplots and the SMATR software package were used to test for significant differences between the diameter of the trachea below the pharynx, above the tracheal bifurcation and midway between these points. This was done to determine whether the mean of the three measurements could be used to represent tracheal diameter accurately.

Comparison of the Foetal and Post-Natal Respiratory System in Wildebeest

The foetal sample was not large enough to directly compare the allometry of foetal and post-natal animals. Instead, the difference between the lungs and tracheas of foetal and post-natal wildebeest was assessed using scatterplots and 95% prediction intervals, comparison of measurements of the foetal hearts and the predictions of the allometric equations derived from post-natal animals, and the SMATR software program was used to test for significant differences between the Post-Natal and Entire Sample equations for each cardiac measurement.

Comparison of the Wildebeest Respiratory System with other Mammals

The ontogenetic equation for lung mass in wildebeest was compared with ontogenetic equations derived from data on horses (Crile & Quiring, 1940) and giraffes (Mitchell and Skinner, 2009 and unpublished data). The latter data included the data used in the 2009 study, as well as the addition of unpublished giraffe measurements made using the same methods and on the same giraffe population. The data of Crile and Quiring (1940) are compiled from several studies carried out by different researchers for different purposes and include animals representing different breeds and ages of horses.

The interspecific lung mass equations to which the wildebeest equation was compared were: equations for mammals in general (Brody, 1945; Stahl, 1967) and for African ruminants, calculated from the data compiled by Crile and Quiring (1940). Tracheal dimensions in wildebeest were compared with interspecific equations for mammals (Calder, 1996).

The equations were first compared by plotting the 95% confidence intervals of the wildebeest equation on a scatterplot of the wildebeest data with trend-lines representing the other equations.

Where the raw data were available (horses, giraffe and African ruminants), comparisons were made with the wildebeest raw data and tested for significant differences in the slope and elevation of the allometric equations, using the SMATR software package. Where the raw data were not available (interspecific mammalian equations), this software package was used to test if the slope and elevation values from the mammalian equation were significantly different from those in the wildebeest equation, which was calculated by SMATR from the raw wildebeest data.

The mean differences between the lung masses and tracheal dimensions measured for the post-natal wildebeest in this study and those predicted by other allometric equations were also investigated. The mean difference between measured and predicted was calculated, and then the mean percentage prediction error (PPE).

Prediction of Physiological Values for Wildebeest

Several physiological parameters for wildebeest were calculated from interspecific allometric equations found in the literature (see Table 4-2). If more than one equation was available, the most appropriate equation was chosen, based on the total number of data points and species used in the study (see Table 4-3). All the equations from the literature were edited to use body mass and lung mass in kilograms, volumes in millilitres, respiratory rates in breaths per minute and ventilation rates in litres per minute.

Derived Allometric Equations

An allometric equation for tracheal cross-sectional area was estimated from that for tracheal diameter, assuming that the cross-section of the trachea is circular. The ontogenetic equation for lung volume in wildebeest was estimated from the wildebeest lung mass equation and the relationship between lung mass and volume in mammals (Stahl, 1967; Mitchell & Skinner, 2011). The allometric equations for tracheal and lung volume were compared for wildebeest and mammals, using the “allometric cancellation technique” of Stahl (Stahl, 1962, 1967).

Results

Measurements were made on 37 wildebeest (20 males and 17 females) with measured body masses between 12 and 258kg. The sample included four foetuses (with body masses between 12 to 15kg and an approximate gestational age of seven months), 15 juveniles (ranging in calculated mass from 64 to 139kg) and 18 adults (with calculated body masses ranging from 162 to 282kg). No animals were excluded due to disease, anatomical abnormalities or *rigor mortis*. All animals were processed within an hour of being shot. The sample size, median and range for each measurement for each age group are summarised in Table 4-4.

Measurement	Foetus (n=4)	n	Juvenile (n=15)	n	Adult (n=18)	n
Calculated Body Mass (kg)	12.8 (12-15)	4	110 (64-139)	15	209 (162-282)	18
Lung Mass (kg)	0.420 (0.386-0.482)	4	1.18 (0.838-1.61)	14	2.09 (1.35-3.18)	18
Relative Lung Mass (% of Body Mass)	3.2 (3.1-3.6)	4	1.1 (0.8-1.5)	14	0.9 (0.7-1.4)	18
Tracheal Length (mm)	230 (230-230)	2	440 (295-510)	15	520 (410-690)	16
Tracheal Diameter (mm)	9.2 (8.6-9.2)	3	25.7(21.0-29.2)	14	31.8 (26.8-36.5)	18
Tracheal Volume (ml)	15.2 (15.2-15.2)	2	236 (114-305)	14	399 (271-608)	16

Table 4-4: Median and range of body mass and lung measurements of the wildebeest measured for this study

Model Validity

Three recorded measurements were excluded as outliers. One measurement each of lung mass, tracheal length and tracheal diameter were excluded. The excluded measurements were most likely incorrect due to measurement or recording error. Both tracheal measurements were missing for one animal and tracheal length for one animal, contributing to a total of five missing tracheal volumes.

Three of the wildebeest were shot through the lungs, the lung masses of these animals were included in the study since the recorded lung masses were not found to be outliers in any of the analyses.

Of the measurements which were retained in the sample, at least one foetus had a leverage value greater than 0.2 (maximum 0.36) in every relationship, except for that between lung mass and Right Ventricular Wall Thickness. Additionally, the Cook's Distances were elevated for two foetuses (1.25 and 1.35) for the tracheal volume vs lung mass equation.

For the Adult body mass equations, the wildebeest with the largest recorded body mass had a large leverage value (maximum 0.26), and two animals had large leverage values for the lung mass equations, maximum 0.23.

The residuals for most equations were normally distributed. However, the Shapiro-Wilk test for normality produced low p-values for the Body mass equations for Tracheal diameter for the Entire

sample ($p=0.026$) and Tracheal Volume for the Post-Natal sample ($p=0.043$), and for the Lung Mass equations for Tracheal length in adults ($p=0.048$) and Right Ventricular Wall Thickness for the Entire sample ($p=0.047$), representing a small but significant deviation from normality. Since the analyses were performed on log-transformed data, to reduce the deviations from normality and the methods used are considered robust to deviations from normality, no other manipulation of the data to compensate for deviations from normality were considered necessary.

Ontogenetic Allometric Regression of the Wildebeest Respiratory System

Evaluation of Scatter Plots

Visual inspection of scatter plots (Figure 3-1-6) shows that lung mass scales positively with body mass and that the dimensions of the trachea scale positively with both body and lung mass. The relative lung mass of foetal wildebeest (approximately 3%) is greater than for post-natal animals (approximately 1%) (Figure 3-3 and Table 4-4).

The relationship between the thickness of the right ventricular wall and both body and lung mass (Figure 4-6) is less obvious than for the respiratory system measurements. The data points are widely scattered, and form a roughly horizontal band. There is large variation in wall thickness that is not directly related to change in either body or lung mass ($R^2 = 0.275$ and 0.25 respectively).

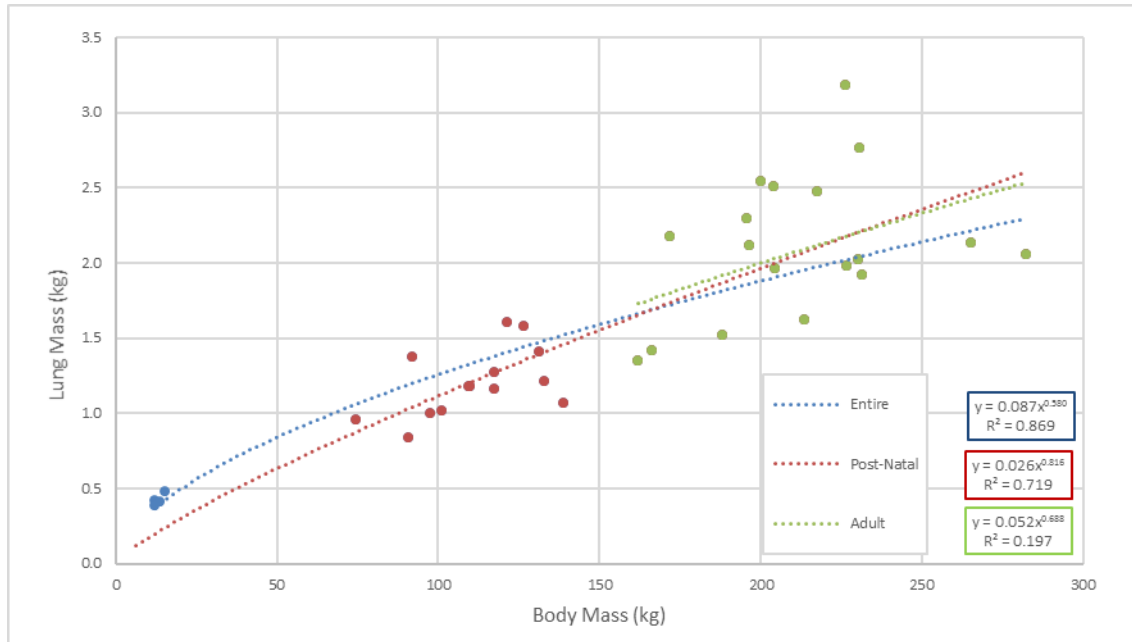


Figure 4-1: Lung Mass versus Body Mass.

Blue data points represent foetuses, red: juvenile animals and green: adults. The blue trend-line is calculated using all the data points (Entire Sample), the red: only Post-Natal animals (Post-Natal Sample) and the green: only adult animals (Adult Sample). The trend-line for the Post-Natal data is extrapolated backwards for comparison with the foetal measurements.

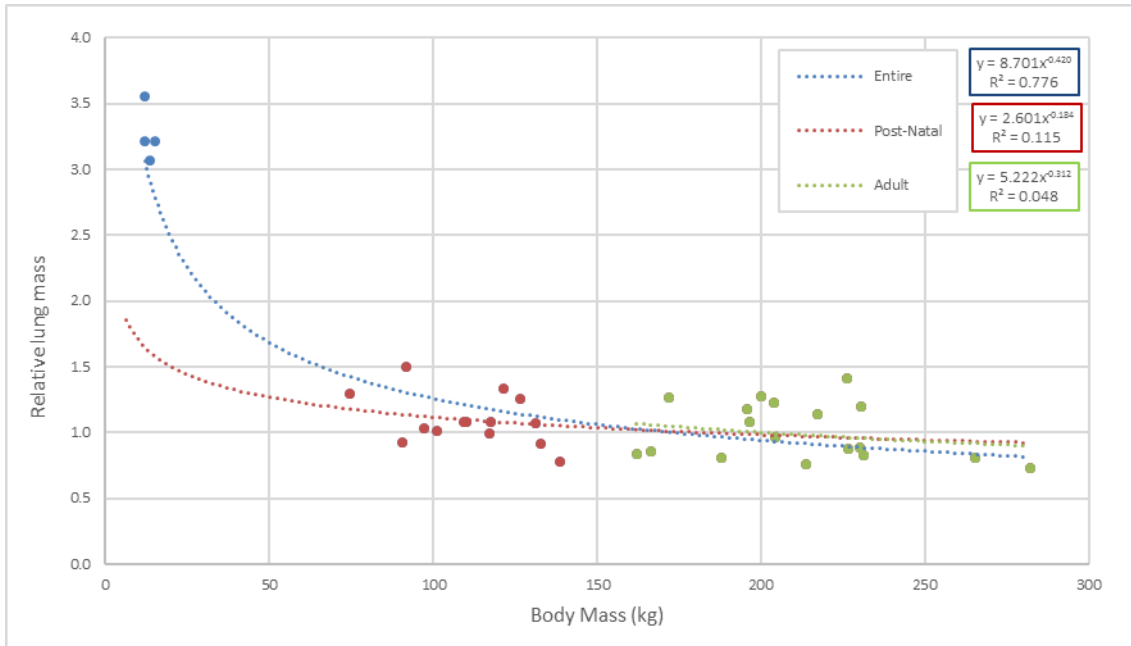


Figure 4-2: Change in Relative Lung Mass with growth
Lines and data points follow the format in Figure 1.

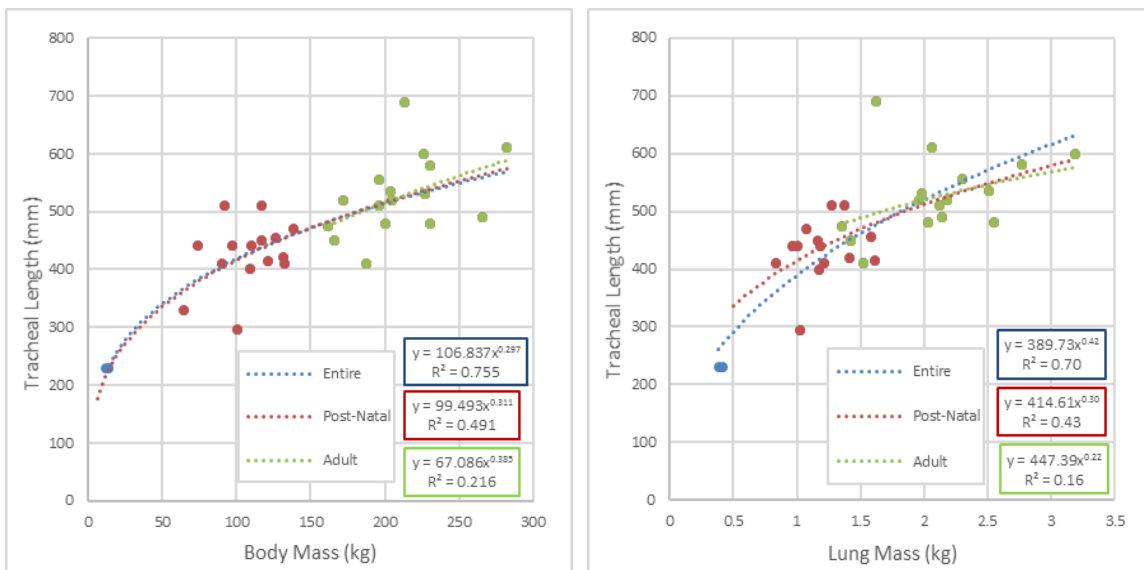


Figure 4-3: Tracheal Length versus Body Mass and Lung Mass.
Lines and data points follow the format in Figure 1.

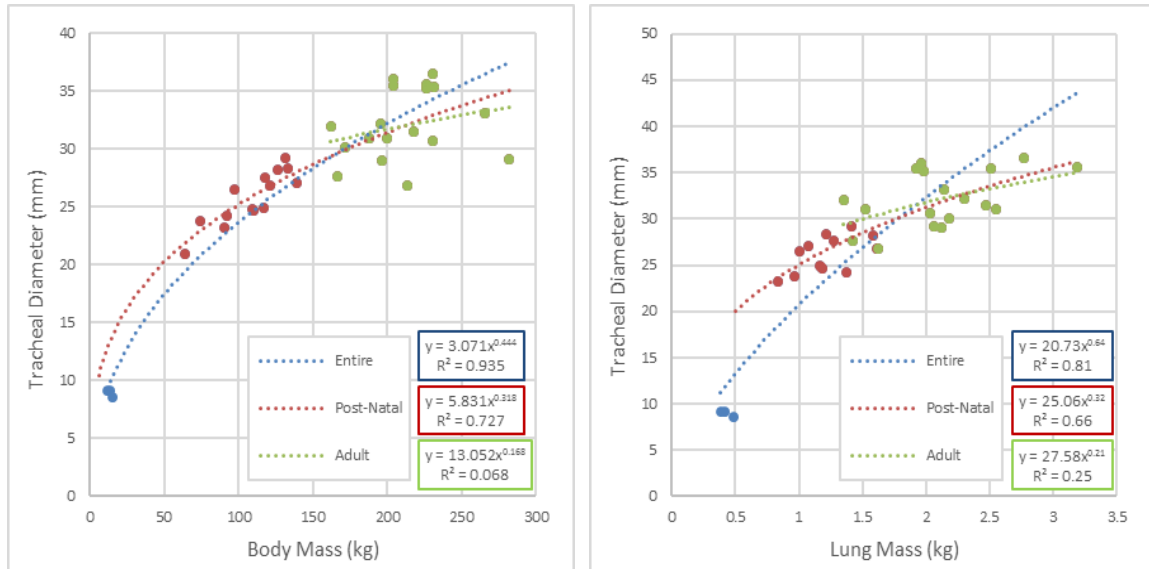


Figure 4-4: Tracheal Diameter versus Body Mass and Lung Mass.
Lines and data points follow the format in Figure 1.

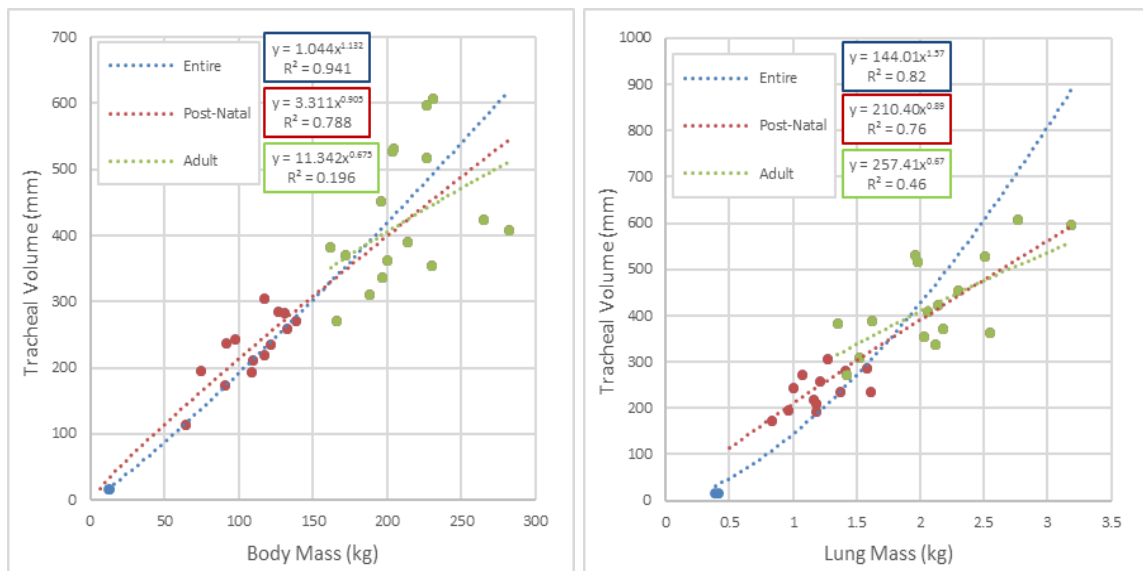


Figure 4-5: Tracheal Volume versus Body Mass and Lung Mass.
Lines and data points follow the format in Figure 1.

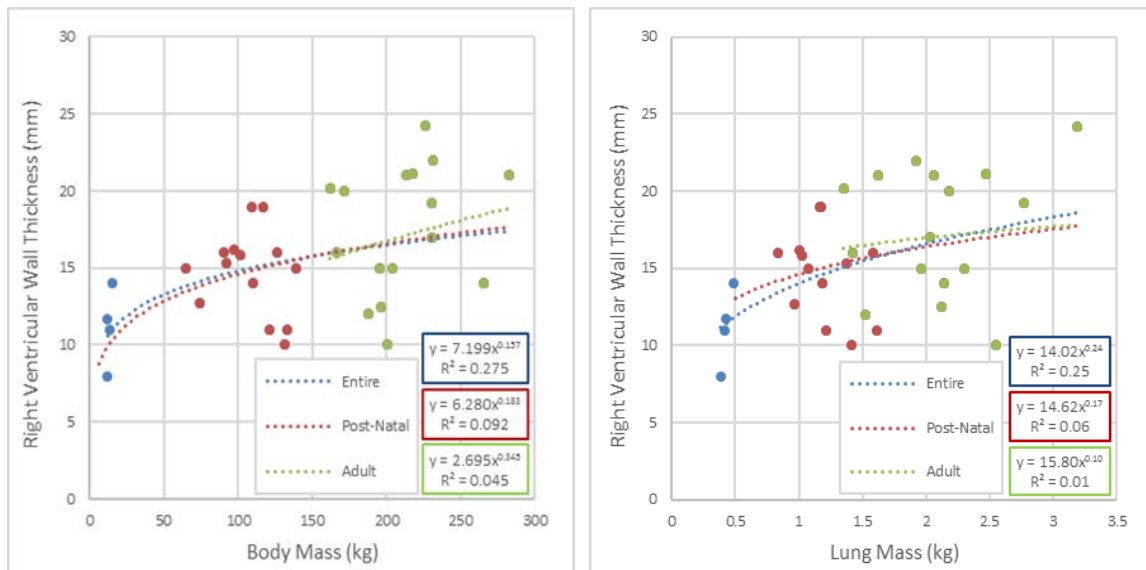


Figure 4-6: Right Ventricular Wall Thickness versus Body Mass and Lung Mass.
 Lines and data points follow the format in Figure 1.

When inspected visually, the trend-line plotted through the entire sample and the line plotted through only the post-natal sample (and extrapolated to foetal body mass) diverge noticeably, in both the lung mass and relative lung mass graphs (Figures 4-1 and 4-2). This trend is also apparent on the graph comparing tracheal diameter to body mass (Figure 4-4), which suggests that the foetal data do not fall on the same trajectory as the post-natal data. If so, the Entire Sample equations in these cases would be inappropriate. In contrast, the length and volume of the foetal trachea fall close to the extrapolated post-natal trend-line.

Allometric Equations

The allometric equations which are represented by the trend-lines in Figure 4-1 to 4-6 are summarised in Table 4-6 and 4-7.

Suitability of Power Equations

A significant regression between the variables in each equation was concluded if the regression equation is a better predictor of the measurements than the mean of those measurements. The results of the ANOVA test for a significant regression showed that the regression between lung mass and body mass is significant in the Entire and Post-Natal samples, but not in the Adult sample ($p=0.065$). The relationship between body mass and relative lung mass is only significant for the Entire Sample. The size of the trachea also scales significantly with both body mass and lung mass in the Entire and Post-Natal samples. In the adult wildebeest, neither lung mass nor tracheal dimensions scale with body mass. The results for the ANOVA test for the non-significant regression are summarised in Appendix 9.

Goodness of Fit

Adjusted coefficients of determination (R^2) are largest for the Entire Sample equation and smallest for the Adult Sample equation for each measurement. The Post-Natal sample had the lowest mean absolute percentage prediction error (PPE_A) in all cases, except for the tracheal length equations. Change in body mass explains a greater proportion of the change in tracheal dimensions than change in lung mass does, as judged from the R^2 values; except for tracheal diameter and volume in adult animals. However, the PPE_A results do not show the same trend. There is a poor correlation between lung mass and the thickness for the right ventricle, showing high PPE_A values and the smallest R^2 values in each age sample.

Sample	Equation $y = a(M_b)^b$					Adjusted R^2	PPE_A	n	p ($H_0: b = 0$)*	p ($H_0: b = isometric$)†
	y	b	SE (b)	a	SE (a)					
E		0.580	0.0387	0.0870	0.0164	0.865	16.6	36	<0.001	<0.001
PN	Lung Mass (kg)	0.816	0.0931	0.0260	0.0123	0.710	16.0	32	<0.001	0.058
A		0.688	0.347	0.0522	0.0969	0.147	19.3	18	0.065	0.382
E		-0.420	0.0387	8.70	1.64	0.769	16.6	36	<0.001	<0.001
PN	Relative Lung Mass (% of Body Mass)	-0.184	0.0931	2.60	1.23	0.085	16.0	32	0.058	0.058
A		-0.312	0.347	5.22	9.69	-0.0114	19.3	18	0.382	0.382
E		0.297	0.0303	106.8	15.9	0.747	8.5	33	<0.001	0.240
PN	Tracheal Length (mm)	0.311	0.0587	99.5	29.3	0.474	9.0	31	<0.001	0.706
A		0.385	0.196	67.1	70.2	0.160	8.4	16	0.070	0.796
E		0.444	0.0203	3.07	0.305	0.933	6.9	35	<0.001	<0.001
PN	Tracheal Diameter (mm)	0.318	0.3560	5.83	1.050	0.718	6.0	32	<0.001	0.966
A		0.168	0.155	13.1	10.8	0.0100	7.3	18	0.295	0.302
E		1.132	0.0518	1.04	0.266	0.939	16.0	32	<0.001	0.016
PN	Tracheal Volume (ml)	0.905	0.0886	3.31	1.48	0.781	15.8	30	<0.001	0.293
A		0.675	0.366	11.3	22.1	0.138	17.8	16	0.086	0.390

P-values for the tests of the following two null hypotheses:

* 1) $H_0: b = 0$. The null hypothesis is that the regression equation is not significantly different from the mean of the y values.

† 2) $H_0: b = isometric$. The null hypothesis is that the equation is isometric, i.e. that the exponent (b) is equal to the value of the exponent of the isometric equation (1 for lung mass and tracheal volume, 0 for Relative Lung Mass and $\frac{1}{3}$ for all length measurements)

P-values smaller than 0.05 are shown in bold, in these instances the null hypothesis can be rejected.

SE: Standard Error

E: Entire Sample (Foetal, Juvenile and Adult animals); PN: Post-Natal Sample (Juvenile and Adult animals); A: Adult Sample

PPE_A : mean absolute percent prediction error

Table 4-5: Ontogenetic allometric equations relating respiratory system measurements to Body Mass (M_b)



Sample	Equation $y = a(M_l)^b$					Adjusted R ²	PPE _A	n	p (H ₀ : $b = 0$)*	p (H ₀ : $b = isometric$)†
	y	b	SE (b)	a	SE (a)					
E		0.417	0.0494	389.7	11.63	0.694	10.2	33	< 0.001	0.100
PN	Tracheal Length (mm)	0.304	0.0659	415	15.8	0.412	8.6	31	< 0.001	0.660
A		0.218	0.131	447	44.3	0.104	8.3	16	0.120	0.394
E		0.643	0.0558	20.7	0.728	0.799	13.5	35	< 0.001	< 0.001
PN	Tracheal Diameter (mm)	0.318	0.0420	25.1	0.633	0.653	6.6	32	< 0.001	0.718
A		0.205	0.0901	27.6	1.88	0.198	10.5	18	0.037	0.174
E		1.570	0.136	144.	12.0	0.814	28.7	32	< 0.001	< 0.001
PN	Tracheal Volume (ml)	0.894	0.0959	210	11.8	0.754	14.6	30	< 0.001	0.278
A		0.669	0.195	257	37.8	0.419	20.2	16	0.004	0.112
E		0.242	0.0761	14.0	0.664	0.222	18.5	34	0.003	0.239
PN	Right Ventricular Wall Thickness (mm)	0.166	0.129	14.6	1.10	0.0222	18.9	30	0.212	0.205
A		0.104	0.285	15.8	3.38	-0.0614	20.7	16	0.721	0.434

P-values for the tests of the following two null hypotheses:

* 1) H₀: $b = 0$. The null hypothesis is that the regression equation is not significantly different from the mean of the y values.

† 2) H₀: $b = isometric$. The null hypothesis is that the equation is isometric, i.e. that the exponent (b) is equal to the value of the exponent of the isometric equation (1 for tracheal volume and 1/3 for all length measurements)

P-values smaller than 0.05 are shown in bold, in these instances the null hypothesis can be rejected.

SE: Standard Error

E: Entire Sample (Foetal, Juvenile and Adult animals); PN: Post-Natal Sample (Juvenile and Adult animals); A: Adult Sample

PPE_A: mean absolute percent prediction error

Table 4-6: Ontogenetic allometric equations relating tracheal measurements to Lung Mass (M_l).

Tests for Isometry

In the post-natal and adult wildebeest, the relationships between lung and body mass are not significantly different from isometric and the relationships between tracheal dimensions and both body mass and lung mass are also not significantly different from isometric. If foetal measurements are included in the relationship, then the relationship between lung mass and body mass and both the body and lung mass equations for tracheal diameter and tracheal volume are significantly different from isometry.

Comparison of Wildebeest Tracheal Diameters at Different Sites

A scatterplot relating tracheal diameters, measured at the pharynx, mid trachea and at the carina, to body mass (Figure 4-7) shows that no tracheal diameter is consistently larger or smaller than another. Analysis of the allometric equations for tracheal diameters at each position, using the SMATR software, showed no significant differences in the slope ($p=0.199$) or elevation ($p=0.074$) of the equations.

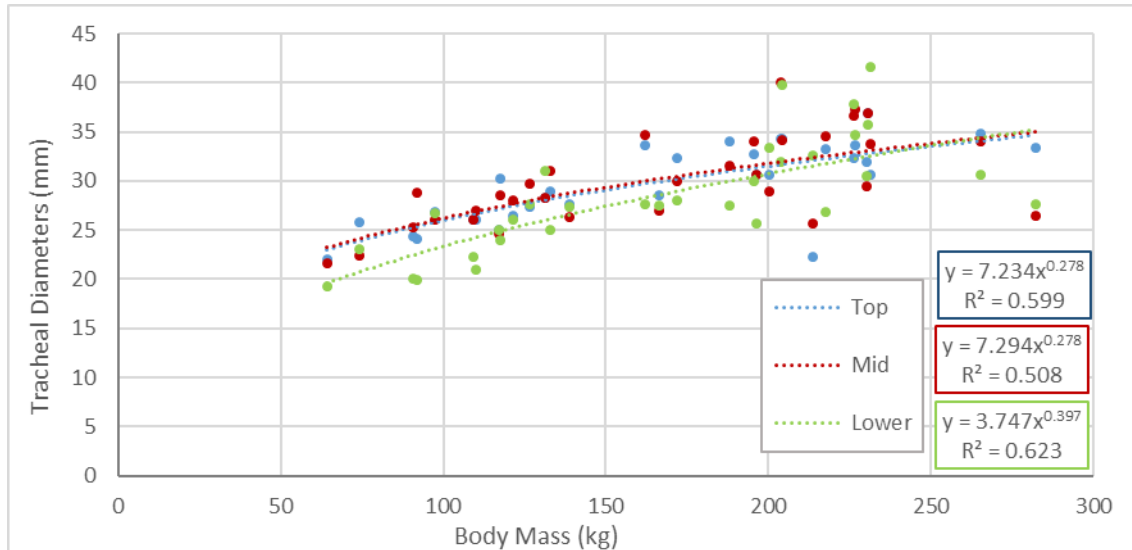


Figure 4-7: Comparison of tracheal diameters in wildebeest, measured at the pharynx (top), mid trachea (mid) and tracheal bifurcation (lower)

Comparison of Foetal and Post-Natal Respiratory System

Table 4-7 summarises the three methods used to compare the respiratory system measurements made on the foetal wildebeest with the values predicted by the data from post-natal animals.

Measured foetal lung masses were on average 215g (101.2% of the predicted mass) heavier than predicted. Relative lung mass in foetuses is significantly larger than predicted by the post-natal allometric relationship between lung and body mass. Additionally, when comparing the allometric equations of the Entire Sample and Post-Natal sample, the exponents are significantly different for both the lung mass equation ($p=0.023$) and the relative lung mass equation ($p=0.025$).

Foetal blue wildebeest also have significantly narrower tracheas than predicted by both the body mass and lung mass post-natal allometric equations and, consequently, smaller tracheal volumes. There is, however, no significant difference in the relationship between tracheal length and body mass in foetuses and post-natal blue wildebeest, as assessed using the SMATR software. Table 4-7 also shows that tracheal lengths, predicted from body mass, were the only foetal tracheal measurements that fell within the 95% prediction intervals of the post-natal equations (see scatterplots and 95% prediction intervals in Appendix 10).



Measurement	Do foetal measurements fall outside the 95% PI? ^a	SMATR ^b		Mean difference ^e (Measured – Predicted)		
		Exponent ^c	Intercept ^d	Mean (measured units)	Mean PPE (%)	
Body Mass Equations	Lung mass (kg)	Yes	0.0228	n/a	0.215	101.2
	Relative lung mass (% Mb)	Yes	0.0246	n/a	1.64	101.2
	Tracheal length (mm)	No	0.836	0.967	10.5	4.8
	Tracheal diameter (mm)	Yes	0.003	n/a	-4.37	-32.6
	Tracheal volume (ml)	Yes	0.033	n/a	-18.0	-54.1
Lung Mass Equations	Tracheal length (mm)	Slightly	0.183	0.690	-83.8	-26.7
	Tracheal diameter (mm)	Yes	<0.001	n/a	-10.1	-53.0
	Tracheal volume (ml)	Yes	<0.001	n/a	-77.6	-83.6
	Right ventricular wall thickness (mm)	No	0.611	0.882	-1.51	-12.1

a. 95% PI: Prediction Interval of the Post-Natal equation.

b. SMATR: Results are p-values for the test for heterogeneity in slopes ^c and the test for shift in elevation ^d between the samples. H₀: *Entire Sample = PostNatal Sample*.

e. Mean differences were calculated when comparing the cardiac measurements made on each of four foetal wildebeest and the values predicted by the Post-Natal equations for that animal. PPE: percent prediction error.

Table 4-7: Evaluation of the suitability of predicting foetal respiratory parameters from Post-Natal equations

Comparison of the Wildebeest with other Mammals

Lung Mass

Ontogenetic allometric equations for lung mass were derived for the giraffe and horse. These were the only species, within the body mass range of wildebeest, for which more than ten measurements could be found. An interspecific equation for African ruminants was derived from measurements of 12 individual animals of different species, made during a single study (Crile & Quiring, 1940). Table 4-8 summarises these three equations.

Intraspecific Equations

The equations for the growth of lung mass in horses and giraffe are compared with the equation for wildebeest in Figure 4-8. Giraffe lung mass scales with $M_b^{0.83}$, which is not significantly different from the wildebeest ($M_b^{0.82}$) ($p=0.938$) (Table 4-9). However, the elevation of the equations is significantly different ($p=0.002$). Therefore, a wildebeest has a significantly larger lung mass than a giraffe of the same body mass. Horse lung mass scales with $M_b^{0.59}$ which is significantly different from the wildebeest ($p=0.037$). Per unit change in body mass, there is a greater change in wildebeest than horse lung mass. However, in the wildebeest body mass range, the average horse has a greater lung mass than an average wildebeest of the same body mass. The lung masses measured for the wildebeest in this study are significantly less than predicted by the equation for horses ($p<0.001$) and more than those predicted by the giraffe equation ($p<0.001$).

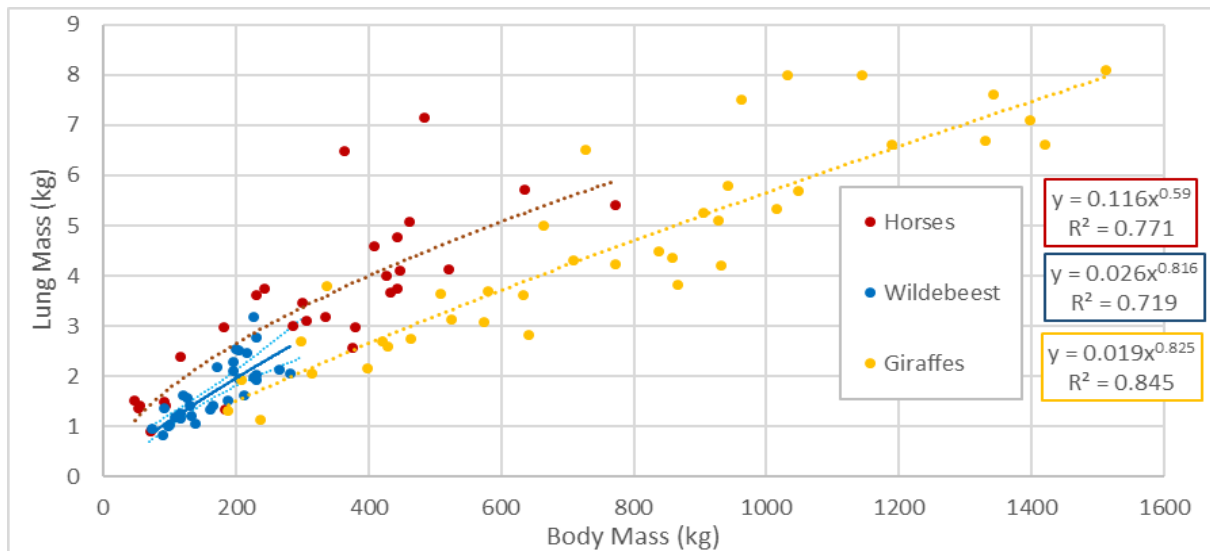


Figure 4-8: Comparison of the ontogenetic equations for post-natal lung growth in wildebeest, horses and giraffes.

Thin blue lines represent the 95% confidence intervals of the wildebeest equation.

Interspecific Equations

The wildebeest ontogenetic equation for lung mass was compared with the interspecific lung mass equations for African ruminants (calculated from the data of Crile and Quiring (1940)) and the equation for all mammals (Brody, 1945) in Table 4-9 and Figure 4-9. Brody's equation is $M_l = 0.0113M_b^{0.986}$, where M_l is lung mass in kilograms and M_b is body mass in kilograms.

Predicted mammalian lung mass fits within the 95% confidence intervals of the wildebeest equation, which suggests that the average wildebeest has a lung mass that is not significantly different from that of an average mammal of the same body size. Neither the exponent (0.816) nor the multiplier (0.0259) of the wildebeest lung mass equation are significantly different from the exponent (0.986) and multiplier (0.0113) of the mammal equation ($p=0.054$ and 0.07 respectively). Lung masses measured for the post-natal wildebeest in this study were on average 80g (2.7%) smaller than predicted by the interspecific mammalian equation, a difference which is not statistically significant ($p=0.421$) (Table 4-9).

Visual inspection of Figure 4-9 suggests that wildebeest have lower lung masses than the average for African ruminants of the same body mass. However, the data points for wildebeest do overlap the ruminant equation and, at body masses below approximately 100kg, the African ruminant equation falls within the 95% confidence intervals of the wildebeest equation. The close relationship between the wildebeest measurements and those made on other ruminants, and the variation in relative lung mass between African ruminants, can be appreciated more easily when the data are plotted on logarithmic axes (Figure 4-10). Lung masses measured for the post-natal wildebeest are on average 274g (12.7%) smaller than the African ruminant equation predicts, a difference which is statistically significant

($p < 0.001$) (Table 4-9). In contrast, evaluation of the raw data with SMATR shows that the exponents are not significantly different ($p = 0.287$) and neither are the multipliers ($p = 0.190$).

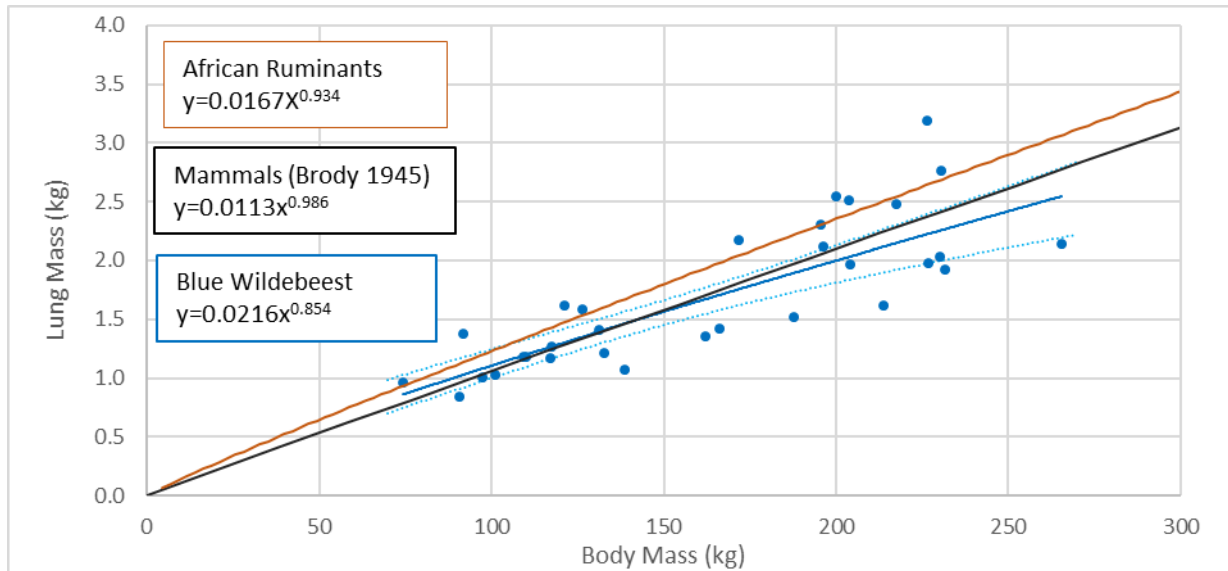


Figure 4-9: Comparison of the ontogenetic equation for lung mass in wildebeest with the interspecific equations for mammals and African ruminants.

Blue dots represent individual wildebeest. Thin blue lines represent the 95% confidence intervals of the wildebeest equation.

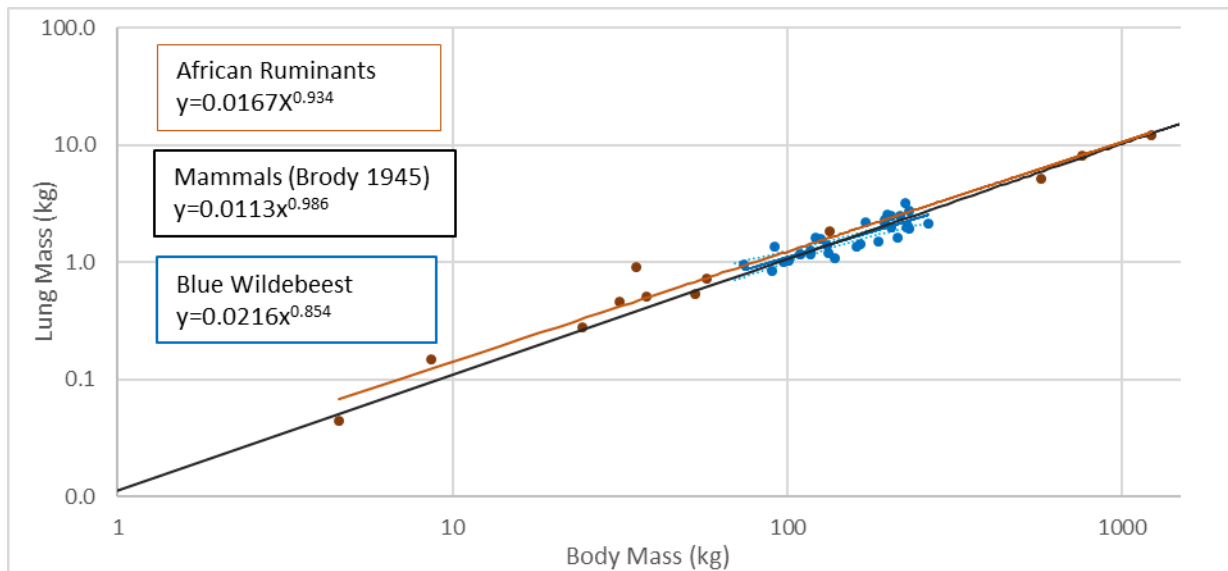


Figure 4-10: Comparison of the ontogenetic equation for lung mass in wildebeest with the interspecific equations for mammals and African ruminants, on logarithmic axes.

Blue dots represent individual wildebeest, and brown indicate other African ruminants.



Species	Allometric Equation: $y = a(M_b)^b$					Adjusted R ²	n	Ages	M _b Range (kg)	Data Source
	y	b	SE (b)	a	SE (a)					
Giraffe	Lung Mass (kg)	0.825	0.0613	0.0190	0.00731	0.841	38	Post-Natal	189 - 1512	Mitchell and Skinner (2009)*
Horse		0.591	0.0620	0.116	0.401	0.762	29	Post-Natal	48 - 771	Crile & Quiring (1940)
African Ruminants		0.934	0.0500	0.0167	0.00378	0.969	12	Adult	4.57 - 1220	Crile & Quiring (1940)

*In addition to the data used in Mitchell and Skinner (2009), unpublished data from a continuation of that study is also used for the calculation of the giraffe equations

Table 4-8: Ontogenetic allometric equations for lung mass that were calculated for giraffes and horses and an interspecific equation for African ruminants.

y	Equation	SMATR ^a		Mean difference ^d			
		Exponent ^b	Intercept ^c	measured-predicted		PPE ^e	
				Mean (kg)	p	Mean (%)	p
Lung mass (kg)	Mammals (Brody)	0.054	0.07	-0.0832	0.230	-2.74	0.421
	African Ruminants	0.287	0.190	-0.274	< 0.001	-12.7	< 0.001
	Giraffe	0.938	0.002	0.428	< 0.001	33.1	< 0.001
	Horses	0.037	-	-0.644	< 0.001	-28.4	< 0.001
Tracheal length (cm)		0.128	< 0.001	1.29	0.232	3.32	0.172
Tracheal diameter (cm)	Mammals (Calder)	0.046	< 0.001	-0.0344	0.445	-0.781	0.569
Tracheal volume (ml)		0.004	< 0.001	-3.27	0.829	3.36	0.393

a. SMATR: Results are p-values for the test for heterogeneity in slopes^c and the test for shift in elevation^d between the samples.

d. Mean differences were calculated when comparing the measurements made on the post-natal wildebeest and the values predicted by the other mammal equations.

e. PPE: mean percent prediction error. H₀: Mean PPE = 0

Table 4-9: Comparison of the lung and tracheal equations for wildebeest with equations for other mammals

Tracheal Dimensions

The ontogenetic equations for the post-natal wildebeest trachea are compared with interspecific equations for mammals (Calder, 1996) in Figure 4-11 to 13 and Table 4-9. Calder's equations are $D_t = 4.1M_b^{0.39}$, $L_t = 62M_b^{0.4}$ and $V_t = 0.82M_b^{1.18}$, where M_b is body mass in kilograms, D_t is tracheal diameter in mm, L_t is tracheal length in mm and V_t is tracheal volume in millilitres.

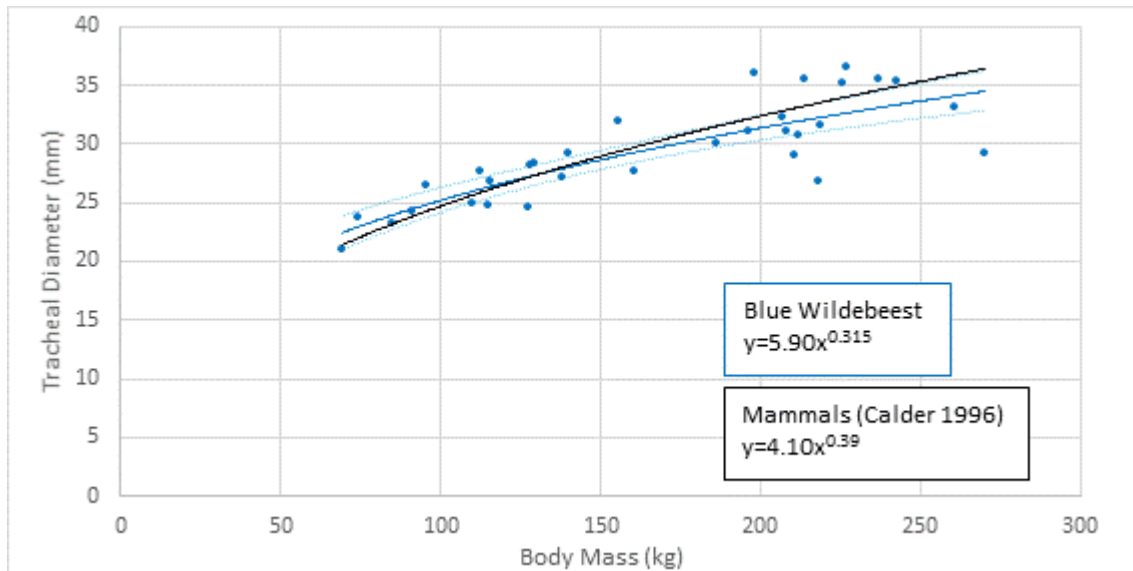


Figure 4-11: Comparison of the ontogenetic equation for post-natal growth of the diameter of the trachea in wildebeest with the interspecific equation for mammals.

Blue dots represent individual wildebeest. The blue lines represent the 95% confidence intervals of the wildebeest equation.

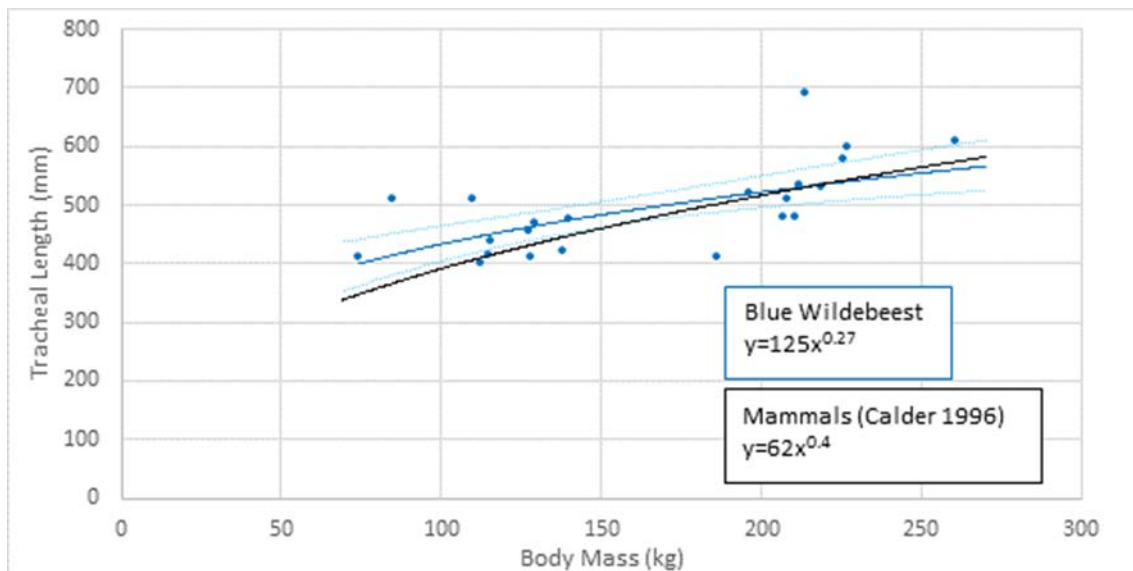


Figure 4-12: Comparison of the ontogenetic equation for post-natal growth of the length of the trachea in wildebeest with the interspecific equation for mammals.

Blue dots represent individual wildebeest. The blue lines represent the 95% confidence intervals of the wildebeest equation.

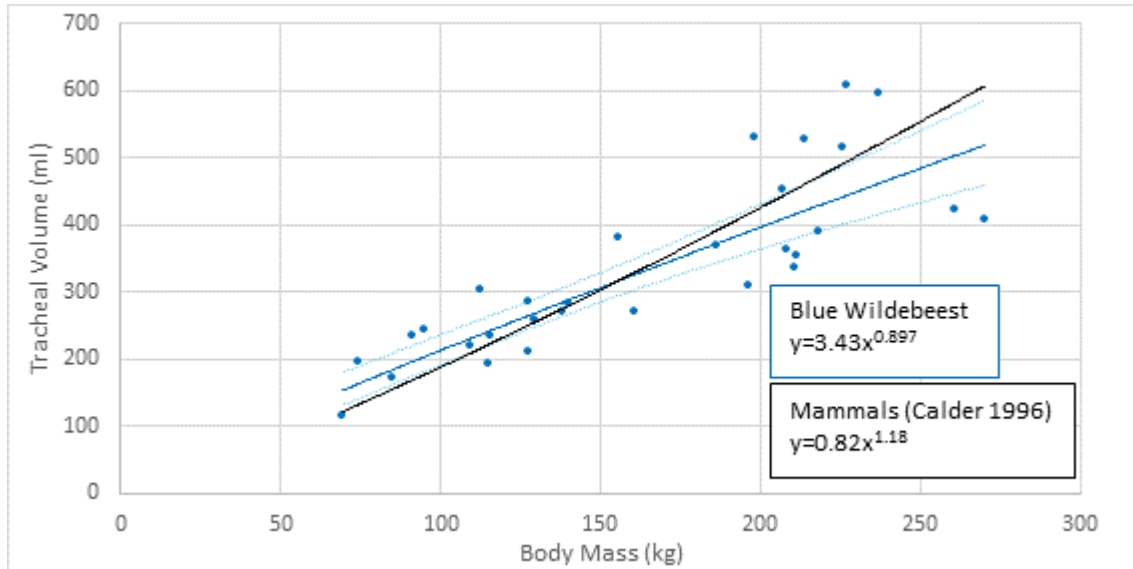


Figure 4-13: Comparison of the ontogenetic equation for post-natal growth of the volume of the trachea in wildebeest with the interspecific equation for mammals.

Blue dots represent individual wildebeest. The blue lines represent the 95% confidence intervals of the wildebeest equation.

The ontogenetic allometric equations for tracheal dimensions in wildebeest are significantly different from the mammalian interspecific equations. As shown in Table 4-9, the exponents of the diameter and volume equations are significantly smaller in the wildebeest equations and all the intercepts are significantly larger. Despite this, the interspecific equations mostly fall within the 95% confidence intervals of the wildebeest data, within the wildebeest body mass range (Figure 4-11 to 13) and predict similar tracheal dimensions to those measured in wildebeest.

On average, the tracheal dimensions measured on the post-natal wildebeest in this study were within 3.5% of those predicted by interspecific allometric equations. None of the differences are statistically significant ($p=0.172$ length, $p=0.569$ diameter and $p=0.393$ volume) (Table 4-9).

Predicted Physiological Respiratory Parameters for Wildebeest

The wildebeest trachea and lungs are not significantly different from those of the average mammal of the same body mass. It is therefore reasonable to assume that other respiratory parameters also closely follow those of the average mammal. If so, they can be estimated from the published interspecific equations for mammals. Table 4-10 predicts some respiratory variables for wildebeest. using allometric equations from the literature.

	Juvenile Wildebeest			Adult Wildebeest			Allometric Equation	Reference
	30	50	100	150	200	250		
Body Mass (kg)	30	50	100	150	200	250		
Lung Volume (ml)	1 968	3 383	7 053	10 840	14 704	18 628	$53.5M_b^{1.06}$	Stahl (1967)
	1 892	3 130	6 200	9 248	12 280	15 303	$66.13M_b^{0.986}$	Gehr <i>et al.</i> (1981) *
	1 692	2 908	6 064	9 320	12 643	16 017	$46M_b^{1.06}$	Gehr <i>et al.</i> (1981)
Dead Space (ml)	72	118	230	339	447	553	$2.76M_b^{0.96}$	Stahl (1967)
Vital Capacity (ml)	1 884	3 188	6 510	9 885	13 294	16 729	$56.7M_b^{1.03}$	Stahl (1967)
Total System Volume (ml)	1 987	3 414	7 119	10 941	14 842	18 802	$54M_b^{1.06}$	Calder (1996)
Functional Residual Capacity (ml)	1 125	2 004	4 385	6 934	9 598	12 351	$24.1M_b^{1.13}$	Stahl (1967)
Inspiratory Reserve Volume (ml)	734	1 099	1 901	2 619	3 287	3 921	$50M_b^{0.79}$	Stahl (1967)
Expiratory Reserve Volume (ml)	1 012	1 775	3 804	5 942	8 154	10 422	$24M_b^{1.1}$	Stahl (1967)
Respiratory Rate (breaths/min)	22	19	16	15	13	13	$53.5M_b^{-0.26}$	Stahl (1967)
Tidal Volume (ml)	264	450	925	1 409	1 901	2 398	$7.69M_b^{1.04}$	Stahl (1967)
Ventilation Rate (l/min)	5.76	8.67	15.1	20.9	26.3	31.4	$0.379M_b^{0.8}$	Stahl (1967)
	7.82	11.8	20.7	28.7	36.3	43.5	$0.499M_b^{0.809}$	Bide <i>et al.</i> (2000)

* Equation for African antelope and viverrids

Table 4-10: Predicted respiratory parameters for mammals in the wildebeest body mass range

Derived Allometric Equations

Tracheal Cross-Sectional Area

The cross section of the trachea is roughly circular and so the cross-sectional area of the trachea (A_t) can be calculated from the diameter of the trachea (D_t): $A_t = \frac{1}{4} \pi D_t^2$ and the ontogenetic allometric equation for wildebeest tracheal diameter (D_t): $D_t = 5.83M_b^{0.318}$.

Therefore, the equation for the cross-sectional area of the wildebeest trachea in mm^2 is: $A_t = 26.7M_b^{0.636}$. For comparison the interspecific equation for mammals, calculated from Calder's (1996) equation for tracheal diameter, is: $A_t = 13.2M_b^{0.78}$.

Lung Volume

The volume of wildebeest lungs can be estimated from their mass, if the relationship between mass and volume is known. This can be achieved by using the density of inflated lungs, if this density does not change with lung mass. Alternatively, the interspecific mammalian allometric equations for lung mass and volume can be equated to achieve an allometric relationship between the two. From Stahl's (1967) equations for lung mass in kg (M_l) and lung volume in ml (V_l): $M_l = 0.0113 M_b^{0.99}$ and $V_l = 53.5 M_b^{1.06}$, an equation for the relationship between volume and mass can be calculated: $V_l = 6500 M_l^{1.07}$. If this relationship between lung volume and mass is true for wildebeest, then the relationship between lung volume and body mass can be calculated from the relationship between lung mass and body mass: $M_l = 0.0260 M_b^{0.816}$:

$$V_l = 6500 M_l^{1.07}$$

$$V_l = 6500 (0.0260 M_b^{0.816})^{1.07}$$

$$V_l = 130.9 M_b^{0.873}$$

Scaling of Tracheal Dimensions with Body and Lung Mass

The relationship between tracheal and lung volume can be identified by comparing the allometric equations using the "allometric cancellation technique" of Stahl (Stahl, 1962, 1967). The allometric relationship for the ratio of tracheal volume to lung volume is:

$$\begin{aligned} \frac{V_t}{V_l} &= \frac{3.311M_b^{0.905}}{130.9M_b^{0.873}} \\ &= 0.0253M_b^{0.032} \end{aligned}$$

In comparison, the interspecific allometric equations for tracheal and lung volumes (Stahl, 1967; Calder, 1996) give the equation:

$$\begin{aligned} \frac{V_t}{V_l} &= \frac{0.82M_b^{1.18}}{53.5M_b^{1.06}} \\ &= 0.0153M_b^{0.12} \end{aligned}$$

Discussion

The purpose of this study was to formulate ontogenetic allometric equations which describe the growth of the respiratory system in the blue wildebeest and contribute an additional species to the small collection in ontogenetic studies in mammals. The equations were used to compare the relative growth of the lungs and trachea and compare wildebeest with other mammals.

Ontogenetic Allometric Regression of the Wildebeest Respiratory System

Suitability of Power Equations

The regressions between lung mass and body mass are significant in the Post-Natal sample, as are the regressions between the tracheal dimensions and both body and lung mass. The lack of significant scaling between the respiratory system and body mass in the Adult Sample is due to a combination of the reduced sample size in adults and the large variation in lung mass and tracheal dimensions. This is also the case for the relationship between tracheal length and lung mass in the Adult Sample.

Although the Entire Sample equations showed significant regression in all cases, significant differences between foetal and post-natal lung masses and tracheal diameters occur. Because of these differences, the Entire Sample equations do not describe the growth of either variable accurately.

The lack of a significant regression between relative lung mass and body mass in the Post-Natal and Adult Samples indicates that relative lung mass does not change significantly after birth, which indicates that the relationship between lung mass and body mass in these populations is not significantly different from isometry. The p-values for the test for significant regression in the relative lung mass equations and those for isometry of the lung mass equation are identical because the tests are essentially the same.

The lack of significant scaling between the right ventricular wall and lung mass, in both the Post-Natal and Adult samples, indicates that the thickness of the right ventricular wall does not change appreciably after birth. Since heart wall thickness increases with the load placed on the heart, according to the law of Laplace (Holt *et al.*, 1968; Seymour & Blaylock, 2000), the lack of change in right ventricular wall thickness with changing lung mass suggests that pulmonary blood pressure does not increase with increasing lung size. However, accurate measurements of right ventricular wall thickness were difficult to achieve and significant measurement error is suspected in this variable. Therefore, conclusions drawn from this measurement should be treated with caution.

Goodness of Fit

The large coefficients of determination (R^2) found in the Entire Sample equations are influenced by the larger sample sizes and body mass and lung mass ranges. In the relationships that include lung mass

and tracheal diameter, they are also influenced by the large difference between foetal and post-natal animals. The low R^2 value for the relationship between relative lung mass and body mass in the post-natal and adult samples is due to the exponent of the lung mass equations being close to 1, therefore relative lung mass hardly changes with change in body mass.

Tests for Isometry

In the post-natal and adult wildebeest, lung mass scales isometrically with body mass and the dimensions of the trachea scale isometrically with both body mass and lung mass. If foetal measurements are included in the relationship, lung mass scales hypoallometrically with body mass and tracheal diameter and tracheal volume scale hyperallometrically with both body mass and lung mass. The differences between the Entire Sample and Post-Natal Sample equations are affected by significant differences between foetal and post-natal lung mass and trachea diameter which are discussed in more detail below.

Scaling of Lung Mass with Body Mass

In the post-natal blue wildebeest, lung mass scales with $M_b^{0.816}$, but, although the exponent is less than one and the relationship is likely to be hypoallometric, the null hypothesis of isometry cannot be rejected ($p=0.058$). Hypoallometric relationships are seen in the ontogenetic relationships between many organs and body mass (Brody, 1945) and may be due to a combination of a greater mass of less metabolically active tissue in adults relative to total body mass, reduced metabolic rate in adult animals compared to juveniles, and more efficient homeothermy in larger animals.

The results suggest that, in the adult sample, lung mass is also isometric to body mass. If the relationships are isometric, then relative lung mass is approximately 1% of body mass, irrespective of total body mass. However, conclusions of isometry in small samples must be treated with caution because the chance of type II errors (incorrectly concluding no difference when one exists) increases as sample size decreases. Because the lung mass relationships have been concluded with a low level of confidence, they should be referred to as soft isometry, according to the recommendations of Brown and Vavrek (2015).

Scaling of Tracheal Dimensions with Body and Lung Mass

The relationships between the tracheal dimensions and body mass are not significantly different from isometry and neither is the relationship between the trachea and lung mass. Change in body mass does account for more of the change in tracheal dimensions than change in lung mass.

Tracheal volume also scales isometrically with lung volume. It makes biological sense for lung volume to scale isometrically with dead space, of which a large proportion is tracheal volume (Stahl, 1965).

Comparison of the Foetal and Post-Natal Respiratory System

Comparison of the Foetal and Post-Natal Lungs

The results show that the relative lung masses measured in foetal wildebeest are significantly higher than in post-natal animals and that this is not only due to a possible hypoallometric relationship between lung and body mass. The result is not unexpected, because foetal lungs are not only un-inflated but contain proportionally larger volumes of surfactant, which is expelled or absorbed when breathing begins. To compensate for this difference, the dry mass of the lungs should be used for any comparison of foetal and post-natal lung mass.

Because of these differences, plotting an allometric relationship using both foetal and post-natal lung masses is inappropriate. Therefore, both the Entire-Sample equations for lung mass and relative lung mass as well as the Entire Sample equations which use lung mass as the independent variable are also affected and are not biologically useful comparisons.

Comparison of the Foetal and Post-Natal Trachea

The data suggest that the diameter of the trachea is significantly smaller in the foetal wildebeest, although a larger foetal sample is needed to confirm this finding. If so, an equation derived from both foetal and post-natal data points is not useful for modelling tracheal diameter. The equation is useful to show the hyperallometric relationship between tracheal diameter and lung and body mass. In practical terms this means that the change in diameter per unit change in body mass increases as lung mass increases. Since the post-natal relationship is isometric, tracheal diameter or the rate of growth of the trachea must change close to the time of birth. Tracheal diameter could grow at a constantly high rate in the foetus and then at a more isometric pace after birth, or it could grow isometrically both before and after birth with a sudden increase at the time of birth. The second scenario seems more likely because of the extreme changes which occur in the respiratory system at birth. However, there is no reference to sudden changes in tracheal diameter occurring near the time of birth in other animals, and the necessary measurements are scarce. A study on tracheal diameter in rabbits (Bhutani, Rubenstein & Shaffer, 1981) shows no sudden change in relative tracheal diameter at birth, neither do measurements made in human foetuses and infants (Hall, 1955; Kalache *et al.*, 1999; Harjeet & Jit, 2004).

Tracheal diameter may change rapidly after the onset of breathing if the trachea is physically affected by the pressures exerted on it by respiration. Physical air pressure acting on the trachea may cause it to conform to the optimum dimensions to minimise dead space and resistance to air flow. If so, the foetal tracheal diameter would be a function of the genotype, whereas the post-natal tracheal diameter is phenotypically derived. However, it is likely that the evolutionary pressures determining tracheal diameter also affect the development of the trachea on a genetic level.

Tracheal diameter affects volume, and hence all the Entire Sample allometric equations on tracheal diameter and volume are inappropriate if there is a significant change in the allometry of the diameter of the trachea at birth.

The only measured variable which is not significantly different in the foetal and post-natal wildebeest is tracheal length and so the only appropriate Entire Sample Equations are those describing it.

Comparison of the Wildebeest with other Mammals

Intraspecific equations

It is not surprising that the ontogenetic equation for wildebeest lung mass falls between that of the horse and giraffe. The giraffe has an unusually low lung mass for its body mass: an adaptation to its unusual height (Mitchell & Skinner, 2011). In the comparison between the lung masses measured in wildebeest and those predicted by the giraffe equation, the values predicted for wildebeest with body masses less than 100kg are based on extrapolations of the giraffe equation and therefore may not be accurate.

The respiratory system of the horse is also known to be exceptional, which allows it to be such an athletic animal (Poole & Erickson, 2011). The allometric equation for horses is likely to be a poorer representation of the ontogenetic allometry of lung mass in this species than the equations for other animals because the data for horses includes animals from several different breeds, which have very different adult body masses. Juveniles of one breed may be larger than adults of another and so increase in body mass is not necessarily linked to age. The data for horses is also sourced from several studies, which may have used different methods. Comparison with other species would be interesting but it was not possible in this study because no reliable datasets of both lung and body mass could be found for any other species with body masses which included those of the blue wildebeest. Comparisons with domestic cattle and other ruminants would be of particular interest.

Interspecific equations

The comparisons of the ontogenetic equations in wildebeest with the mammalian interspecific equations are interesting because they show that at all ages wildebeest have similar lung masses and tracheal dimensions to an average adult mammal of the same body mass. Therefore, predictions of lung mass and tracheal dimensions made by the interspecific equation for mammals are useful for predicting these values in individual wildebeest. Consequently, the predictions made by the interspecific equations for other respiratory parameters in mammals are also likely to be useful for individual wildebeest.

When wildebeest lung mass is compared with the interspecific equation for African ruminants, the different tests for a significant difference give different results. Visual inspection of the dataset is inconclusive, the mean percentage difference between the wildebeest measurements and the predictions of the African ruminant equation are statistically significant at 12.7%, but SMATR analysis shown no

significant difference between the elevations of the equations. The difference in results is because SMATR analysis uses the raw data for the African ruminant equation whereas comparison between measured and predicted values does not. The interspecific equation for African ruminant lung mass is derived from only 12 data points, which do not all follow the trend line (Figure 4-10) and therefore the SMATR results should be used, which fails to show a significant difference between the lung masses of wildebeest and other African ruminants.

Comparison of values predicted by interspecific respiratory equations with measurements from literature

It seems that most respiratory parameters that have been measured in wildebeest are close to those predicted for mammals of the same body mass. The lung volume of 7 678ml measured in a 102kg wildebeest (Weibel *et al.*, 1981) is only 6.2% larger than predicted by Stahl's equation. The tidal volume of approximately 1.7 litres measured in a 140kg wildebeest (Taylor *et al.*, 1969) is 23% greater than predicted by Stahl's equation. Along with a respiratory rate of approximately 14 breaths per minute (close to the predicted 14.8) measured in the same animal, this tidal volume gives a measured ventilation rate of approximately 24 l/min, where Stahl's equation predicts approximately 20 l/min. The differences between these values may be relatively large but given the high natural variation in tidal volume and respiratory rate in animals, due to excitement, exercise or ambient temperature, these differences are probably not significant.

In contrast to the other findings, respiratory dead space measured in the blue wildebeest is larger than predicted by Stahl's interspecific equation. The average dead space measured in three wildebeest of between 130 and 140 kg was 630ml (Taylor *et al.*, 1969). This is 51% greater than predicted for a 135kg mammal. In the same study, the dead space in zebu cattle of the same body mass was measured as 450ml, smaller than that of the wildebeest but also 32% larger than predicted by the allometric equation for mammals. The difference between the dead space measured in the wildebeest and that in the zebu cattle was at least in part due to the far larger nasal passages of the wildebeest.

The differences between the dead space measured in Taylor's study and that expected for mammals suggests that the dead space from which the mammalian equation was derived may be different from that measured in this study, which is defined as 'the anatomical dead space (excluding: oral cavity, sinuses, and alveoli)' and included the nasal passages and bronchial tree (Taylor *et al.*, 1969). The methods for measuring the dead space volumes for the mammalian equation are not described, but in several similar studies only tracheal dead space is considered (Tenney & Bartlett, 1967; Calder, 1996; Mitchell & Skinner, 2011).

Regardless of the accuracy of Stahl's dead space equation, wildebeest do have relatively larger nasal passages than cattle. The large nasal passages in wildebeest reduce airflow resistance and the large dead space, due to the large nasal passages and ventilation of the paranasal sinuses, are adaptations which

allow thermoregulation through panting, even at high temperatures and tidal volumes, without causing respiratory alkalosis (Taylor *et al.*, 1969).

Derived Allometric Equations

The derived allometric equations are useful but must be used with caution. Because they are based on other equations which are not necessarily accurate and the assumption that all equations involved are equally applicable to the population in question, they are less accurate than the equations from which they are derived (Prothero, 1986).

The derived equation for tracheal cross sectional area is likely to overestimate the area slightly, because the cross section of the trachea is not exactly circular and tracheal diameter was measured across the widest diameter. This difference may be significant if tracheal cross-sectional area is used for calculating resistance to airflow.

The equation for lung volume is only accurate if lung volume and mass in wildebeest are related in the same way as other mammals, on average. The relationship in mammals is only as accurate as the original mammal equations. The calculated lung volume is an estimation of the volume of the inflated lung, including lung tissue, which will be greater than total lung capacity.

The derived equation for tracheal volume as a fraction of lung volume has an exponent of 0.032. Although this is close to zero, and may not be significantly different from zero based on the accuracy of the equations from which it is derived, it may be an oversimplification to conclude that it is zero (Prothero, 1986). The positive value of this exponent indicates that the scaling exponent of the tracheal volume equation is larger than that for lung volume and therefore the ratio of tracheal to lung volume increases with increase in body mass. If the exponent were equal to zero, tracheal volume would be equal to approximately 2.5% of lung volume in wildebeest, independent of body mass. If the exponent of 0.032 is used, the ratio changes from one of 2.9% in a 65kg animal to 3.0% in one weighing 280kg. The equation derived for the same relationship in mammals has a larger allometric exponent of 0.12 which suggests significant scaling with body mass. This mammalian equation predicts tracheal volumes of between 2.5 and 3.0% of lung volume in 65-280kg body mass range, similar to those found in growing wildebeest.

Conclusion

Ontogenetic allometric equations for lung mass and tracheal dimensions were formulated and show isometric relationships with body mass. This means that, at all ages, the relative sizes of the trachea and lungs do not change significantly with growth. Measurements of foetal wildebeest indicate that the wet mass of the lungs is relatively higher and the diameter of the trachea is relatively smaller in foetal animals. Blue wildebeest have significantly smaller lungs than horses and larger lungs than giraffes of

the same body mass. The anatomy of the wildebeest respiratory system is shown to be similar to that of the average mammals at all stages of post-natal growth.

Chapter 5: General Discussion

Limitations

Several limitations are apparent in the study design and dataset of this study and should be taken into consideration when drawing conclusions from the results.

This study distinguished between juvenile and adult wildebeest based on body mass, supported by data on tooth eruption. Scrutiny of the scatter-plots shows, however, that there is no obvious, sudden point that distinguished juvenile from adult wildebeest. The body mass cut-off point which was used was 140kg calculated body mass, which fitted the tooth eruption data well (Appendix 11). All animals with two or more erupted incisors had calculated body masses greater than 140 kg and only one animal with one erupted incisor had a body mass greater than 140 kg. This body mass therefore approximates an animal of two years old (Attwell, 1980). At this age the animals have reached physiological sexual maturity but are unlikely to have started breeding or reached full body size (Attwell, 1982b; Watson, 1967 cited by Hopcraft, 2011; Talbot and Talbot, 1963). Therefore, the averages of the Adult Sample, used in the chapters on cardiac and respiratory physiology underestimate measurements of fully mature animals in the population. For this reason, the average adult body measurements reported for adult male and female wildebeest in Chapter 2 (Table 2-2) are reported for animals with a full set of adult incisors, corresponding to animals older than approximately three years and allows better comparison with body measurements that have been reported for other wildebeest populations (Ledger, 1963; McCulloch & Talbot, 1965; Sachs, 1967; Attwell, 1982b; Skinner & Chimimba, 2005; Estes, 2014).

The Adult equations are influenced by having relatively small sample sizes ($n \leq 16$). Small sample sizes increase the chance of type II errors, where a lack of difference is concluded when a difference exists. For example, incorrect conclusions of isometry are more likely in small samples. This is because, when testing for an allometric relationship between two variables, isometry is the null hypothesis and rejection of the null hypothesis is more difficult with smaller samples, especially when the true relationship between the variables is close to isometry (Brown & Vavrek, 2015). Therefore, the results that adult cardiac measurements scale isometrically with heart and body mass may be incorrect. It can only be concluded that the available data do not show a significant difference from isometry. Similarly, the results that certain cardiac measurements do not scale with body mass in the adult animal may also be due to the small sample size.

Only five foetal wildebeest were measured for this study; far fewer than post-natal animals. The foetal body masses also only cover a narrow range compared with the other animals. For this reason, no relationships were described for foetuses alone. To assess the differences between foetal and post-natal growth more thoroughly, accurate allometric equations, based only on foetal data should be used. This

comparison would need a larger sample of fetuses with as wide a range of body masses as possible. This is a promising area for further investigation, not only in wildebeest but in all species, as data and analyses of this sort are rare.

There is also a large gap in the body masses of the measured wildebeest, between the largest foetus and the smallest juvenile. This gap in the data may hide any sudden changes that occur around birth and in early post-natal life. The interpretation of the foetal data only shows how fetuses between 12 and 15 kg with a gestational age of approximately 7 months (wildebeest gestation length is eight months and fetuses are born weighing 22kg) affect or fit the allometric relationships. It is possible that smaller or larger fetuses or young animals do not conform in the same way as the sampled fetuses.

The measurements made on foetal hearts are not sufficient evidence that the allometry of foetal hearts is the same as in post-natal animals or that relative cardiac growth does not change between fetuses and adults. However, those data are not able to disprove this hypothesis either. In contrast, the significant difference between foetal and post-natal lung mass and tracheal diameter are able to disprove the hypothesis that the allometry of these measurements is the same in the foetal and post-natal animal.

Body size in a natural population is not normally distributed. If a variable such as heart mass is measured for several animals with the same body mass, one would expect the values to be normally distributed. In contrast; if the heart masses of the entire population, from the smallest fetuses to the largest bulls, are considered, then this information would not be normally distributed because adult, fully grown, animals always greatly outnumber growing juveniles. The juvenile population is also not normally distributed. Juvenile mortality is high and so in any year there are more young juveniles than older ones (Attwell, 1982a; Hopcraft, 2010). This study attempted to sample animals from all body mass groups evenly, which helped to compensate for the lack of normality in the population, which would have been more apparent if a random sampling method were used. Log-transformation helps to improve the normality of residuals (Kerkhoff & Enquist, 2009), linear regression is robust to deviations from normality and tests for normality of the residuals of the regressions showed no major deviations from normality for the regressions in this study. Therefore, it is unlikely that deviations from normality have affected the derived allometric equations. However, since body size is not normally distributed in the population and the sampling method attempted to evenly sample all body sizes, the body and organ sizes in each sample show no central tendency. Any averages reported such as the medians in Tables 2-2, 3-4 and 4-4 are not necessarily useful as approximations for the average animal in that age group in the natural population.

Measurement error in recording the thicknesses of the heart walls may obscure the true relationships between wall thickness and heart or body mass. Accurate measurement of the ventricular walls was difficult to achieve. The walls were between 8 and 41mm thick and errors of a few mm were possible due to distortion of the thin muscle. Consequently, the heart wall data are expected to have greater

measurement error than the other cardiac dimensions, leading to the widely-scattered points which are visible on the corresponding scatter plots (Figure 3-3 to Figure 3-5). The error in these measurements may disguise any actual relationship between these dimensions and body mass. Therefore, the result that the thickness of the right ventricular wall does not scale with body or heart mass in the post-natal animal may be inaccurate. In future studies, it is recommended that an estimate of entire ventricle wall size, such as wall mass or volume is used to gain a better understanding of changes in ventricular function with growth.

Contribution

Body mass estimation

This study provides a method to estimate the body mass of an individual wildebeest from measurements of either its body length or girth or both. Accurate mass estimation should prove useful to researchers, wildlife managers, veterinarians and hunters.

The equations for mass estimation estimate the average body mass for an animal of a particular size. Therefore, they are useful for estimating average mass in situations where repeated measurements are not possible but where temporary fluctuations in mass (for example from gut fill) should be avoided; for example, in allometric studies. The mass estimation equations will also be useful for the comparison of the morphology of different wildebeest subspecies. Comparison of predicted and measured masses of individual animals is a better method of determining differences between populations than the use of average masses because, in a natural population mass, is not normally distributed.

Allometry of the Cardiac and Respiratory System

Studies of the ontogenetic allometry of mammalian organs are rare. For the heart, the only published equations that could be found for both juvenile and adult animals were those for the giraffe (Mitchell & Skinner, 2009) and the western grey kangaroo (Snelling *et al.*, 2015). For the lungs, only the giraffe has been studied (Mitchell & Skinner, 2011). Other interspecific allometric equations were available for a few other mammals but these were based on mainly adult animals (Brody, 1945). Even data on relative heart and lung masses, reported as a proportion of body mass, are hard to find for any mammals in the wildebeest body mass range. In order to gain an idea of normal values for cattle and horses, relative heart masses were calculated from raw data which was reported in studies which were most often designed for other purposes. An example of the lack of reliable data on relative organ size is the commonly-repeated, but incorrect, assumption that racehorses have hearts which weigh 1% of their body mass.

This study is a unique evaluation of the ontogenetic allometry of the heart and respiratory system of a mammal. This study is useful for comparison with those on other animals because the heart and respiratory system of the wildebeest are relatively unspecialised. Additionally, the blue wildebeest in this study are from an endemic wild population which has undergone minimal artificial selection and therefore should make them useful examples of normal mammals in comparison with highly selected domestic species.

Publications

A paper on the estimation blue wildebeest body mass, based on Chapter 2 of this dissertation was submitted to the South African Journal of Wildlife Research in May 2014 (Ms 5263). It was rejected as not being an appropriate research topic for the journal. Comments from the reviewers were useful to improve the chapter and the statistical methods throughout this study.

Three future publications are planned from this dissertation: an updated version of the paper on the estimation of blue wildebeest body mass, and separate papers on the allometry of the cardiac and respiratory systems of the blue wildebeest.

Conclusion

The body mass of the blue wildebeest (*Connochaetes taurinus taurinus*) can be estimated from external body measurements to within 10% of measured body mass. This is best achieved by a power equation which incorporates measurements of both total body length and girth. Equations based on either length or girth alone are less accurate but also useful. Although both sex and season affect the relationship between external body measurements and mass, sex and season-specific equations were not more accurate than combined equations. Southern African blue wildebeest *C.t. taurinus* have lower body masses than East African subspecies (*C.t. hecki* and *C.t. albojubatus*) of a given length or girth.

The growth of the cardiac and respiratory systems of the blue wildebeest can be described by ontogenetic allometric equations. These equations provide a rare example of a study of ontogenetic allometry of visceral organs in a mammal.

In the post-natal blue wildebeest, heart mass scales hypoallometrically with body mass which ranges from approximately 0.8% of body mass in the foetus to approximately 0.5% in adult animals. The relationships between body mass and, heart dimensions, lung mass and tracheal dimensions are not significantly different from isometry. Neither are the relationships between heart mass and heart dimensions or lung mass and tracheal dimensions.

When compared with ontogenetic equations for other mammals, blue wildebeest have similarly sized hearts to those of domestic cattle, smaller hearts than horses and giraffe and larger hearts than African buffalo of the same body mass. Blue wildebeest lung mass is smaller than that of horses but larger than that predicted for giraffes of the same body mass.

In comparison with the general mammalian trends described by interspecific equations, blue wildebeest have smaller hearts than predicted for adult African antelope the average adult mammal of the same body mass. This difference is unlikely to be due to a difference in athletic ability between wildebeest and other species and may be due to a relatively heavier digestive tract, due to relatively greater gut fill in the bulk-feeding wildebeest. Tracheal dimensions and the thickness of the left ventricular wall in wildebeest are not significantly different from predictions for the average mammal.

The relationships between body mass and external body measurements, cardiac measurements and tracheal length, are similar in the foetal and post-natal animal. However, a larger sample of foetal and young wildebeest is required to formally test for a statistically significant difference between foetal and post-natal growth. The differences between foetal and post-natal lung mass and tracheal diameter are large and likely to be significant.

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Appendices

Appendix 1: Data Collection Sheets

DATA COLLECTION SHEET GIRAFFE, WILDEBEEST, BUFFALO PROJECTS

Date _____ Time: _____ Species: _____ Number: _____

Gender: _____ Age: (juvenile, young adult, old adult?) _____

Teeth (Teeth in both left and right lower jaws to be noted. Numbers to refer to one side of the jaw. Note if the teeth are permanent or deciduous)

Incisors: _____ Canines: _____ Premolars: _____ Molars: _____

BASIC DIMENSIONS

Before dissection

Body Lengths (cm):

Head: Tip of nose to occipital crest (cm): _____

Neck: a) Occipital crest to base of neck (cm): _____

b) Angle of jaw to point of shoulder (cm): _____

c) Length = (a) + (b) ÷ 2 (cm): _____

Body: base of neck to base of tail (cm): _____

Tail: base of tail to tip of tail (cm): _____

Total Length: (Head+(a)+Body+Tail): _____ (cm)

Body Heights (cm):

- Sole of front foot to withers. _____

- Sole of front foot to axilla. _____

- Sole of back foot to rump. _____

- Sole of back foot to groin. _____

Total height: (ht to withers + occipital crest to base of neck): _____ (cm)

Girth

Thoracic girth measured behind the elbow (cm): _____

Neck measurements

Circumference of the neck at the jaw : _____ (cm).

Circumference of the neck at its base : _____ (cm).

After dissection

Comments: *Removal of the neck and head should follow Hall-Martin's method in his "Carcass composition" paper. It says:*

"The neck was severed from the thorax by a cut running between the seventh cervical and first thoracic vertebrae. When dealing with large bulls the neck also had to be divided between the fourth and fifth cervical vertebrae as it was too unwieldy to be handled in one piece."

"The head was separated from the neck at the atlanto-occipital joint"



ANIMAL NUMBER: _____

NECK DATA

Before skinning

a) Total neck mass: _____ (kg)

After skinning (skin removed in one piece or two halves. If halves then dimensions below must be doubled)

b) Neck skin mass: _____ (kg)

Width of the base of the neck skin _____ (cm)

Width of the apex of the neck skin _____ (cm)

Length of the neck skin from apex to base _____ (cm)

Body Mass

Total body mass

Piecemeal weight: _____ (If the animal is weighed piecemeal then identify and record the component weights on the back of this sheet. See guideline list on page 3).

Unless the recorder is fully familiar with the equations leave the following blank)

Calculated weight from length and girth: _____

Calculated weight from length and girth: _____

Calculated weight from length and girth: _____

Combined body mass: _____

CARDIOVASCULAR DATA

Heart weight: (grams) _____

Heart dimensions (cms):

- diameter at the coronary groove. _____
- length from coronary groove to apex. _____
- mid-length left ventricular wall thickness. _____
- mid-length right ventricular wall thickness. _____
- mid-length interventricular wall thickness. _____
- mid-length left ventricle diameter (vertical part of T) _____
- (Horizontal part of T) _____
- mid-length right ventricle diameter. _____
- left ventricle depth (coronary groove to apex). _____
- right ventricle depth (coronary groove to apex). _____

RESPIRATION DATA

Lung mass: (grams) _____

Total trachea length(cm). _____ (cm)

Trachea diameter

- at pharynx. _____ (cm)
- at mid-cervical. _____ (cm)
- at thorax. _____ (cm)



ANIMAL NUMBER: _____

HEAD, EYE, AND SKULL DATA

Masses

a) Total Head: _____ (kg). b) Head skin: _____ (kg).

c) Parotid glands (both): _____ (grams). d) Tongue: _____ (grams) _____ (cm)

e) Eyes (L+R): _____ (grams). f) Brain (entire): _____ (grams).

g) Masseter muscles (L+R): _____ (grams). h) Mandible: _____ (grams)

h) Horns/Ossicones: Left _____ (grams). Right: _____ (grams)

i) Skull: _____ (kg)

Skull

Length:

Fronto-parietal junction to fronto-nasal junction: _____ cm

(Measure along the surface of the skull over the median ossicone. Shortest distance if junctions are uneven)

Width:

Anterior orbit width (mm): _____ (mm)

Posterior orbit width (mm): _____ (mm)

Orbit

Dorso-ventral diameter (mm): Left: _____ Right: _____ Mean: _____

Lateral diameter (mm): Left: _____ Right: _____ Mean: _____

Eye:

Eye Volume: (ml): Left: _____ Right: _____ Mean: _____

Eye Mass: (g): Left: _____ Right: _____ Mean: _____

Dorso-ventral Diameter (mm): Left: _____ Right: _____ Mean: _____

Lateral Diameter (mm): Left: _____ Right: _____ Mean: _____

Cranio-caudal Diameter (mm): Left: _____ Right: _____ Mean: _____

Weight of body parts (kg)

Head: _____ Neck: _____ Forelegs: _____ Hindlegs: _____

Trunk: _____ Skin: _____ Rumen+contents: _____

Reticulum + contents: _____ Omasum: _____ Abomasum: _____

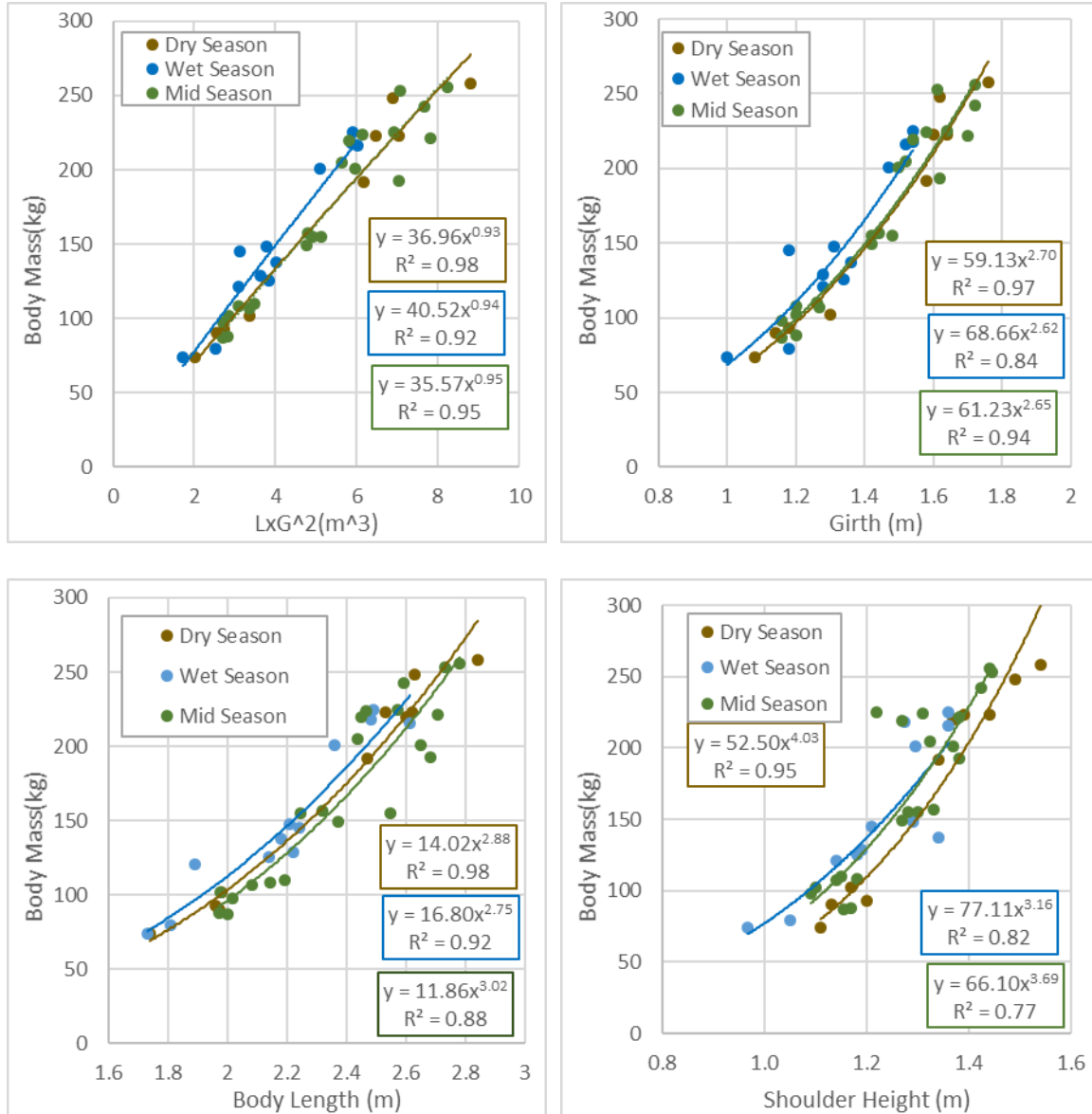
Small intestine: _____ Large intestine: _____ Foetus: _____

Appendix 2: Results of ANOVA test for the significance of the Power and Linear regressions between body mass and body measurements.

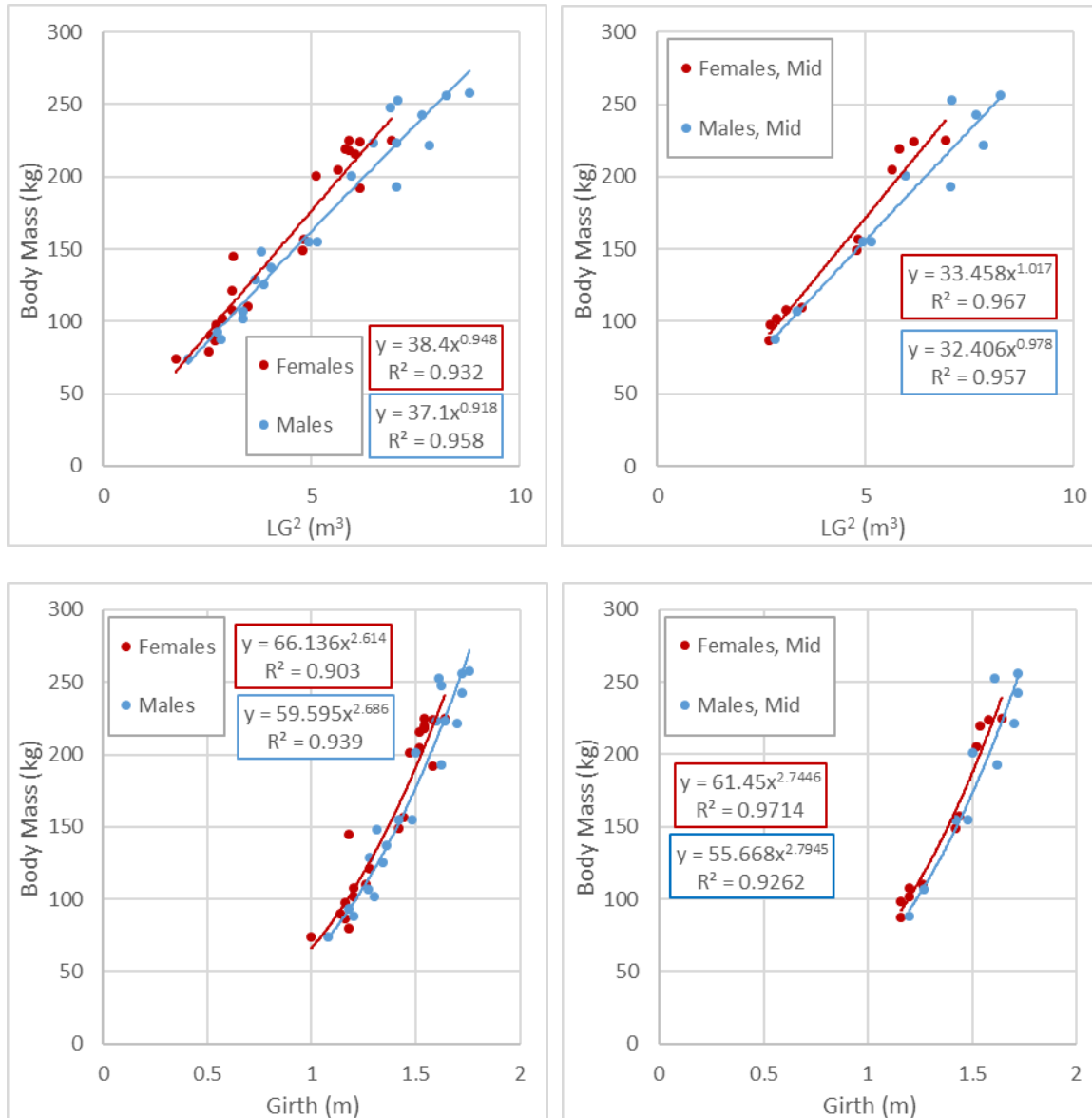
Sample	Equation $y = a(x)^b$		F - statistic	P ($H_0: \alpha=0$)
	y	x		
With foetuses	Body	Shoulder Height	F (1-46) = 979.6	<0.001
		Body Length	F (1-46) = 1569	<0.001
	Mass	Girth	F (1-44) = 2003	<0.001
		LxG^2	F (1-44) = 2682	<0.001
Without foetuses	Body	Shoulder Height	F (1-41) = 170.6	<0.001
		Body Length	F (1-41) = 391.7	<0.001
	Mass	Girth	F (1-40) = 418.5	<0.001
		LxG^2	F (1-40) = 589.8	<0.001

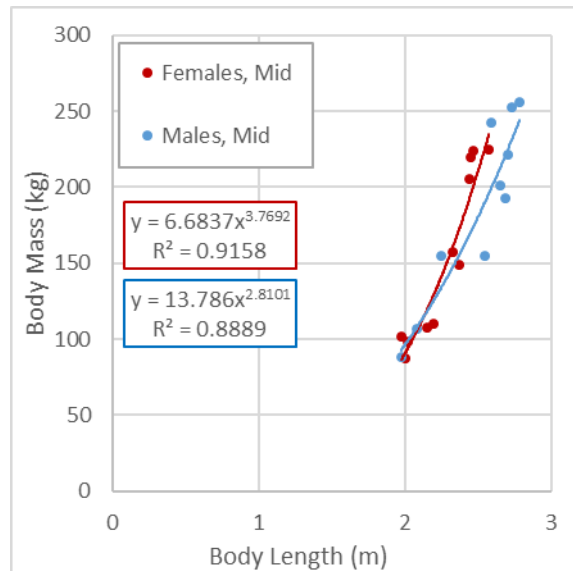
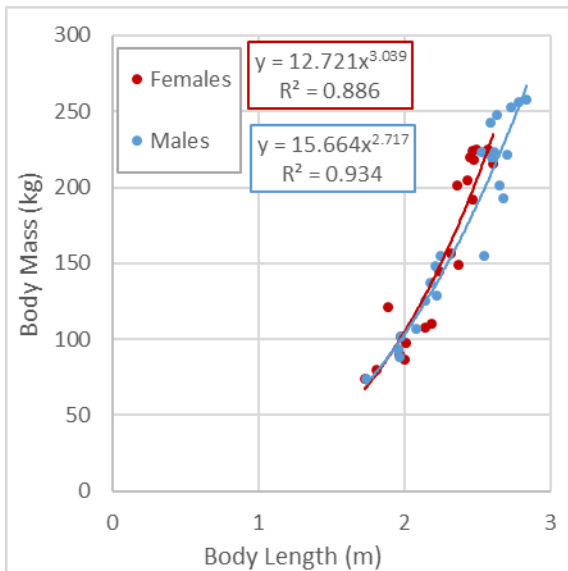
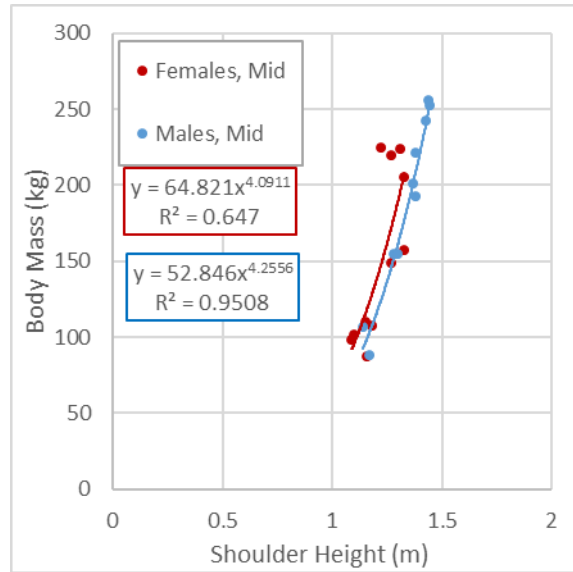
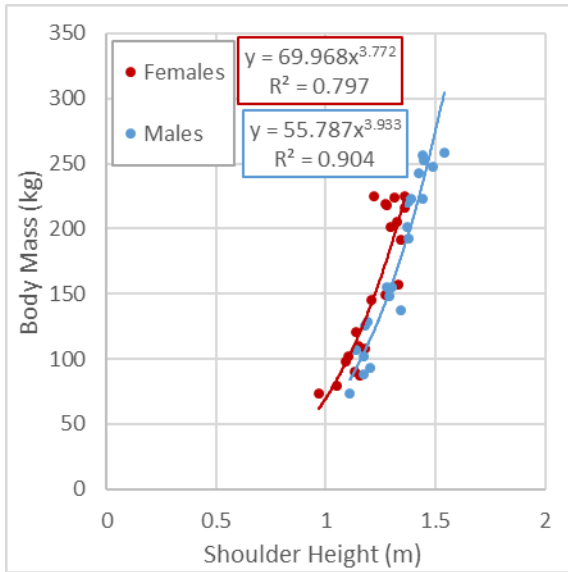
Sample	Equation $y = mx + c$		F - statistic	P ($H_0: \alpha=0$)
	y	x		
With foetuses	Body	Shoulder Height	F (1-46) = 226.3	<0.001
		Body Length	F (1-46) = 393.3	<0.001
	Mass	Girth	F (1-44) = 340.0	<0.001
		LxG^2	F (1-44) = 783.7	<0.001
Without foetuses	Body	Shoulder Height	F (1-41) = 159.7	<0.001
		Body Length	F (1-41) = 318.5	<0.001
	Mass	Girth	F (1-40) = 400.2	<0.001
		LxG^2	F (1-40) = 460.8	<0.001

Appendix 3: Scatterplots of the difference between Wet, Mid and Dry Season equations predicting body mass from external body measurements

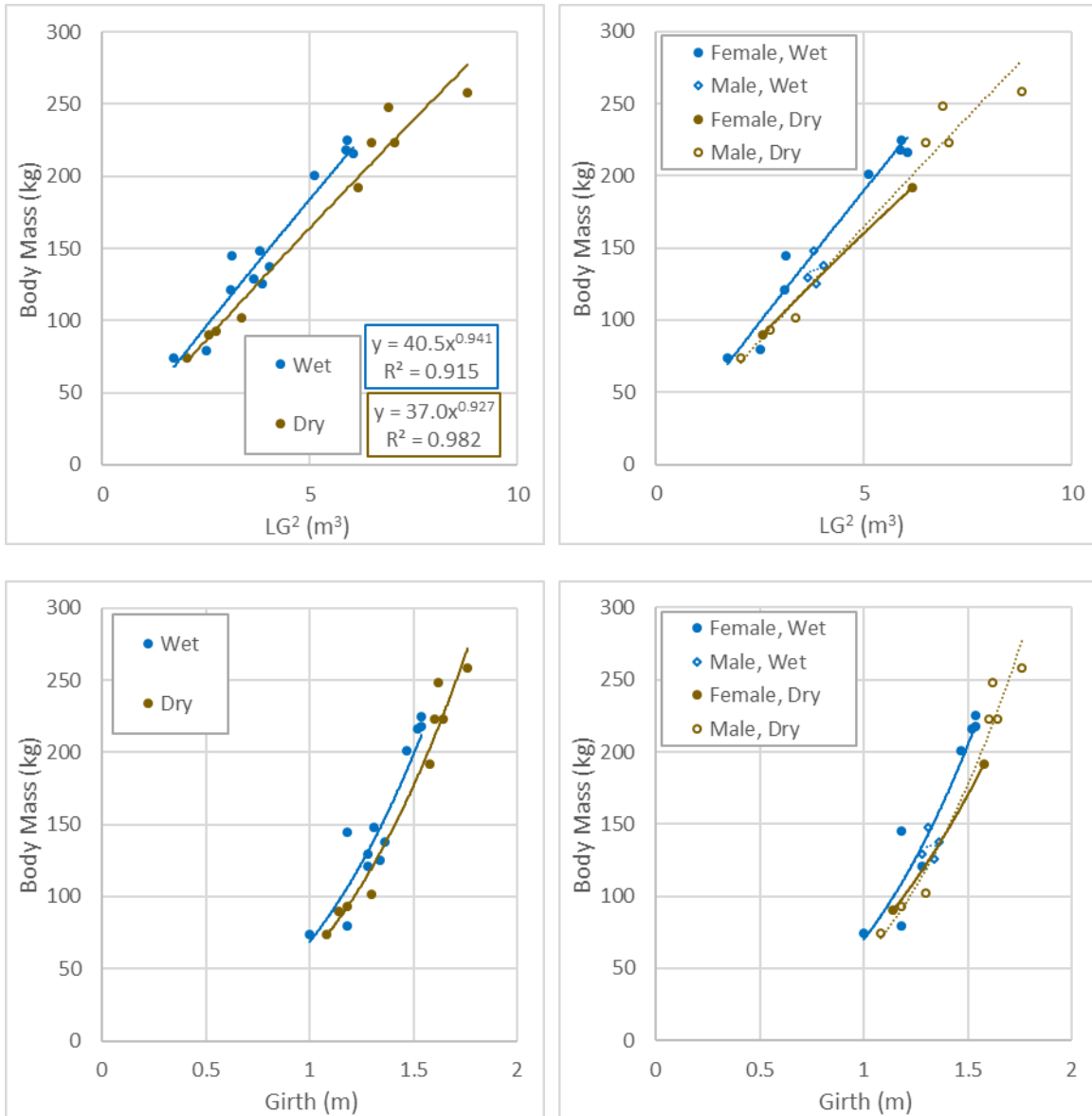


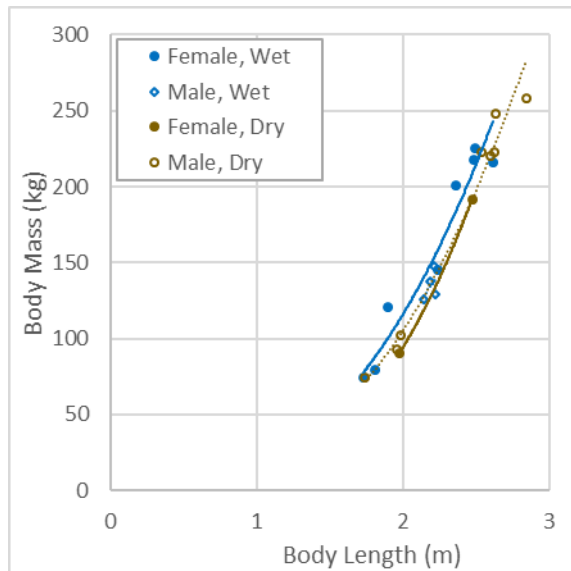
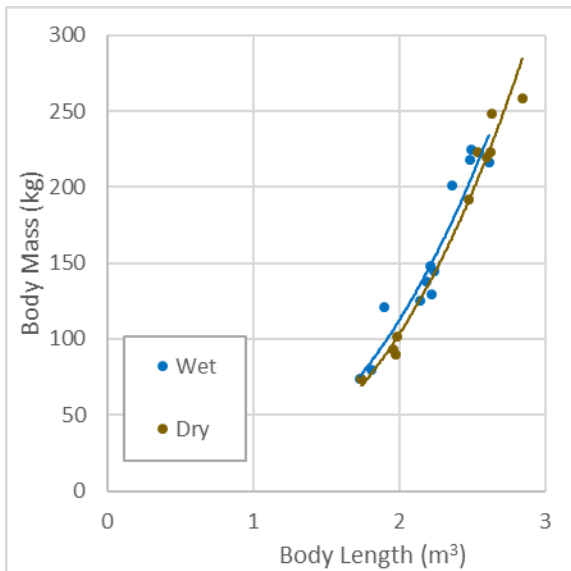
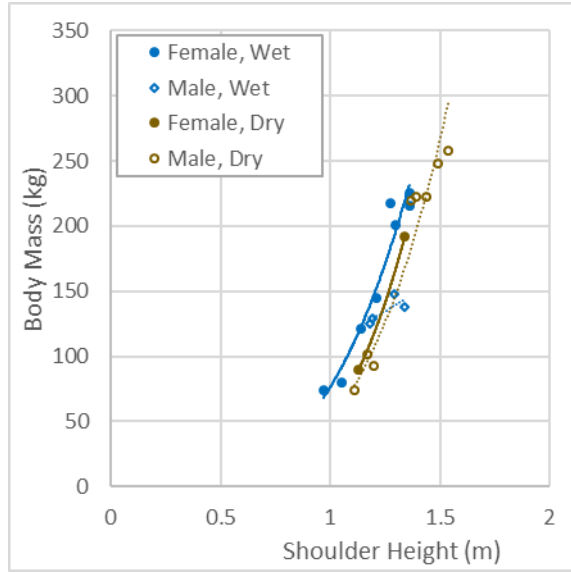
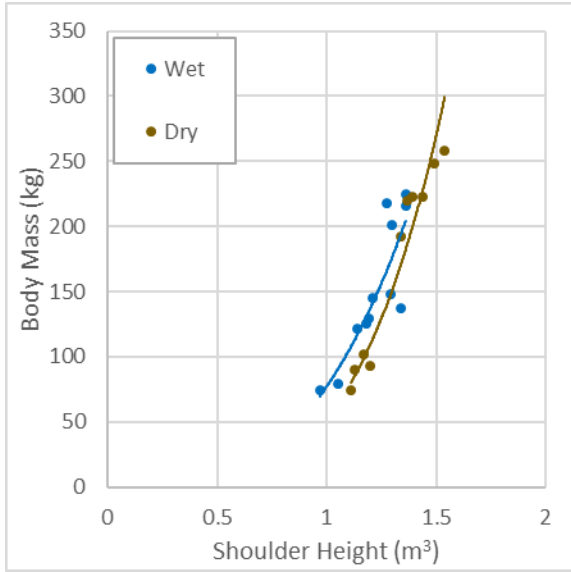
Appendix 4: Interaction between Sex and Season: separate equations for males and females using all post-natal animals and using only animals collected in the Mid season to predict body mass from body measurements





Appendix 5: Interaction between sex and season: equations for the wet and dry season, and also for the sexes separately in these seasons, to predict body mass from external body measurements.



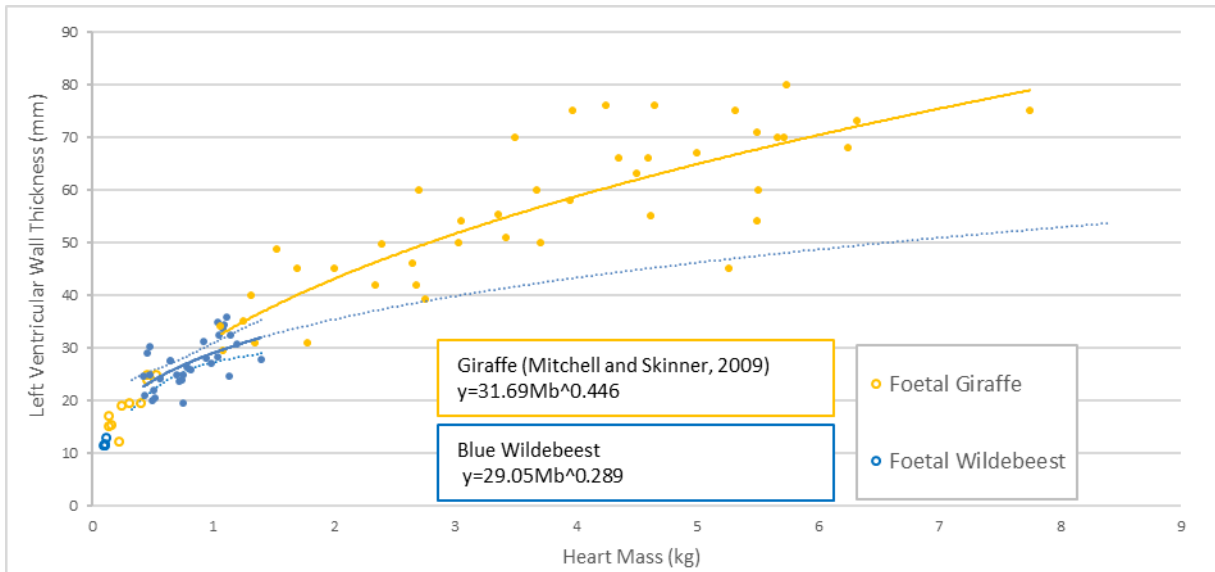


Appendix 6: ANOVA results for regression of body mass and heart mass against heart dimensions (y).

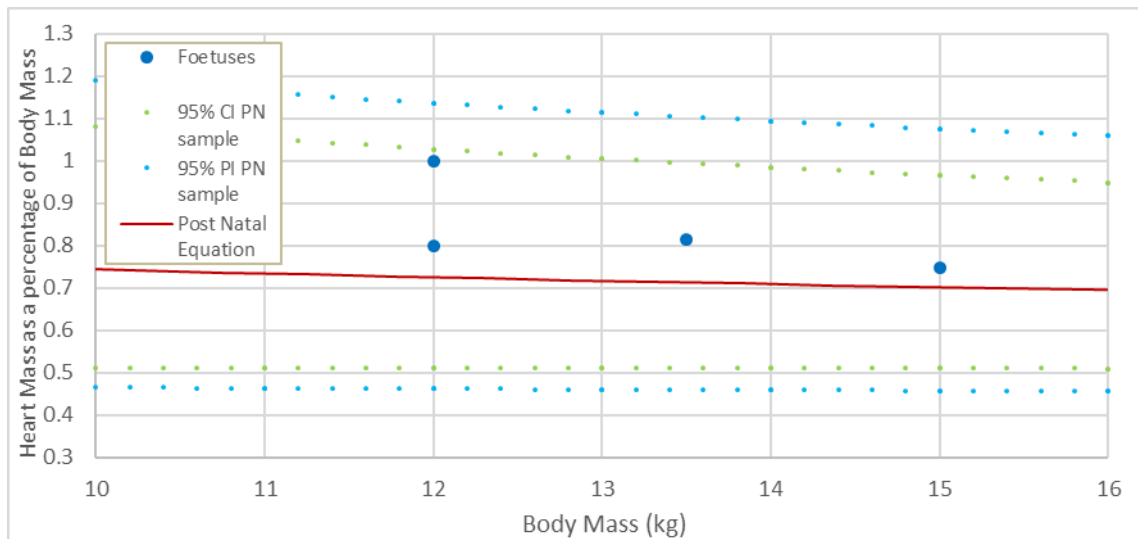
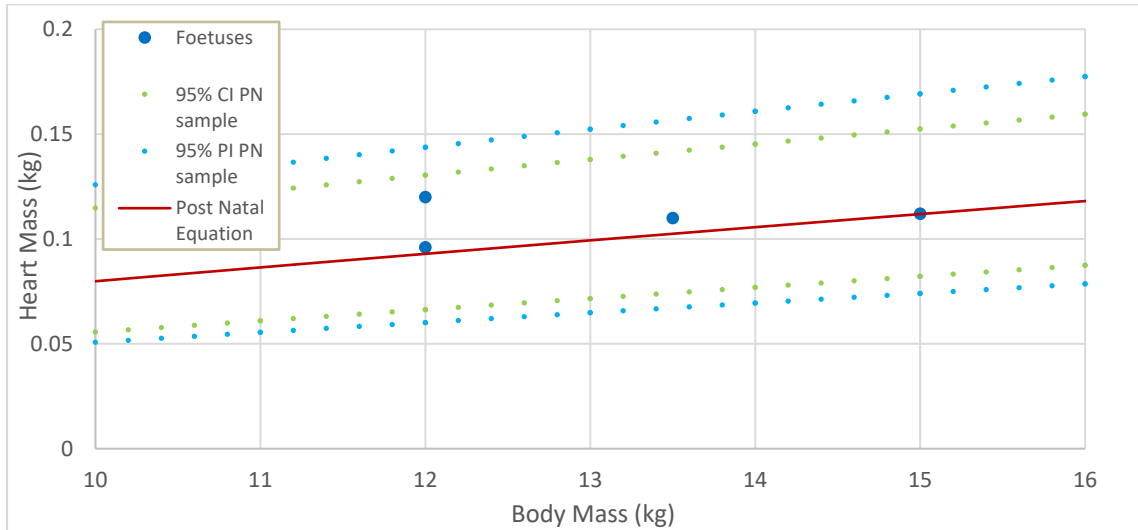
Results of ANOVA test where the regression model is not a significantly better estimator of the value of y than the mean of the y values.

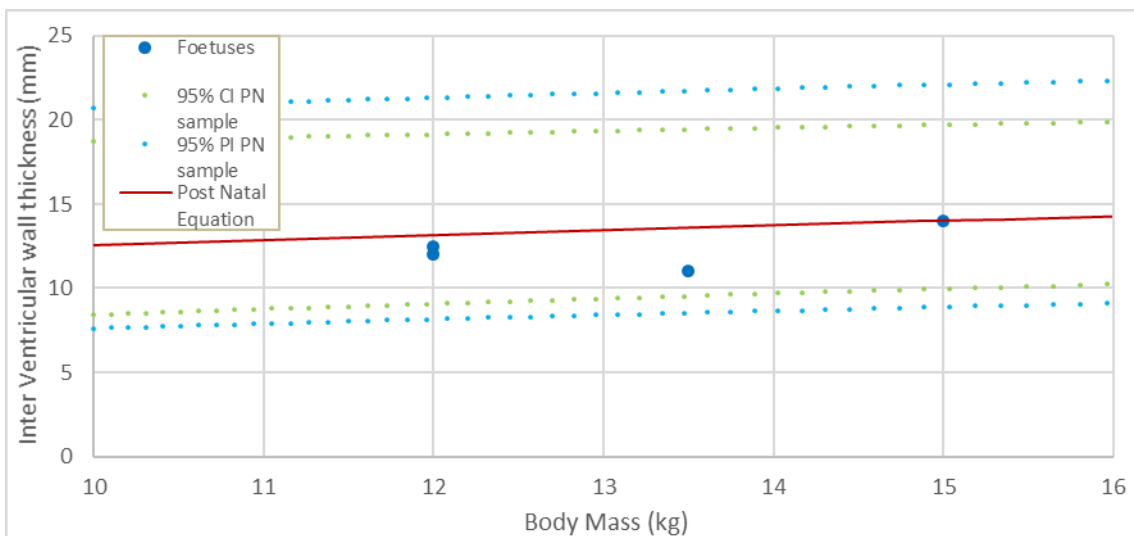
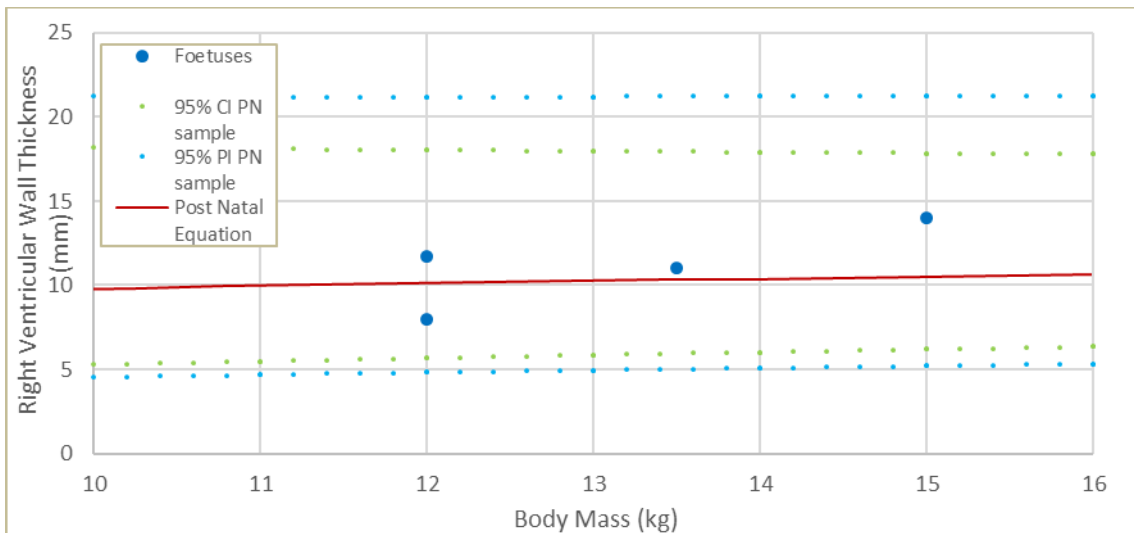
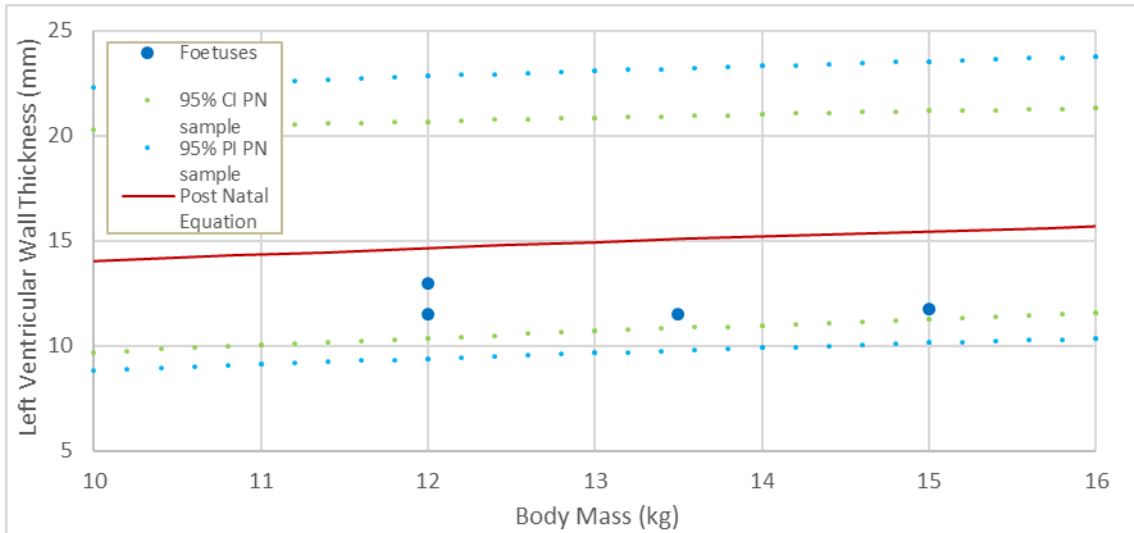
Sample	Equation $y = a(x)^b$		F - statistic	p ($H_0: b=0$)
	y	x		
Post-Natal	Right Ventricular wall thickness	Body Mass	F (1-28) = 2.822	0.104
		Heart Mass	F (1-28) = 2.804	0.105
Adult	Relative Heart Mass	Body Mass	F (1-14) = 0.938	0.349
	Left Ventricular wall thickness	Body Mass	F (1-14) = 0.827	0.378
	Right Ventricular wall thickness	Body Mass	F (1-14) = 0.652	0.433
		Heart Mass	F (1-14) = 1.029	0.327
	Interventricular wall thickness	Body Mass	F (1-14) = 0.674	0.426
		Heart Mass	F (1-14) = 1.675	0.216
	Heart Circumference	Body Mass	F (1-13) = 0.318	0.582
		Heart Mass	F (1-13) = 3.501	0.084
	Heart Diameter	Body Mass	F (1-12) = 2.090	0.174
	Left Ventricular depth	Heart Mass	F (1-14) = 3.243	0.093
Right Ventricular depth	Heart Mass	F (1-14) = 3.245	0.093	

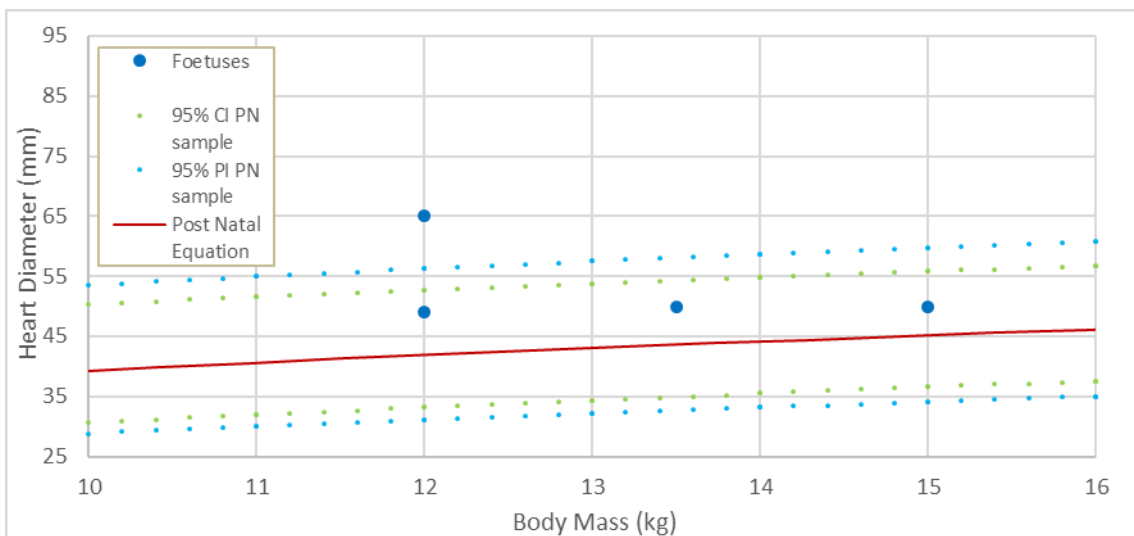
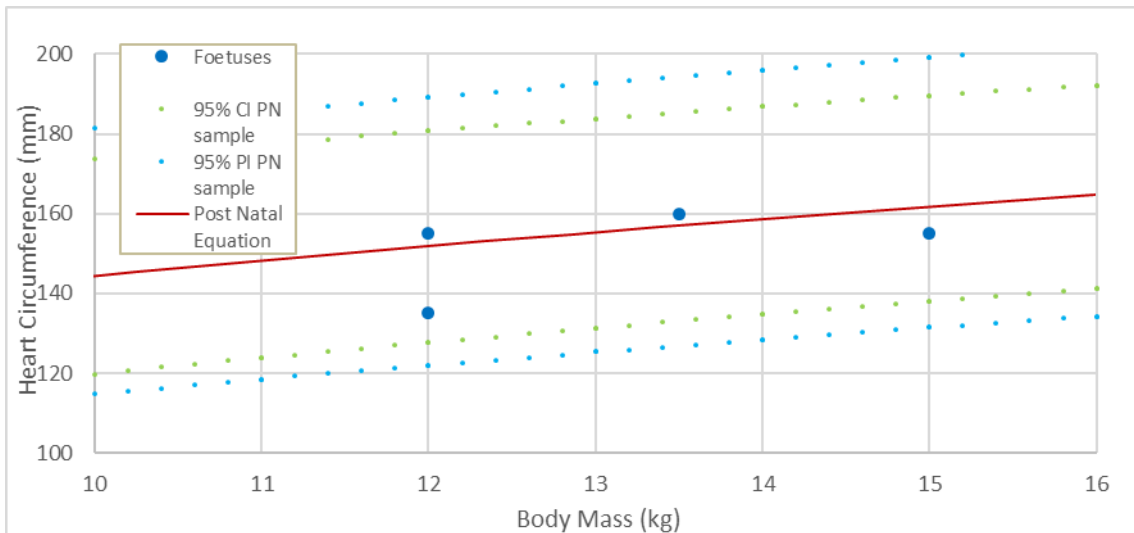
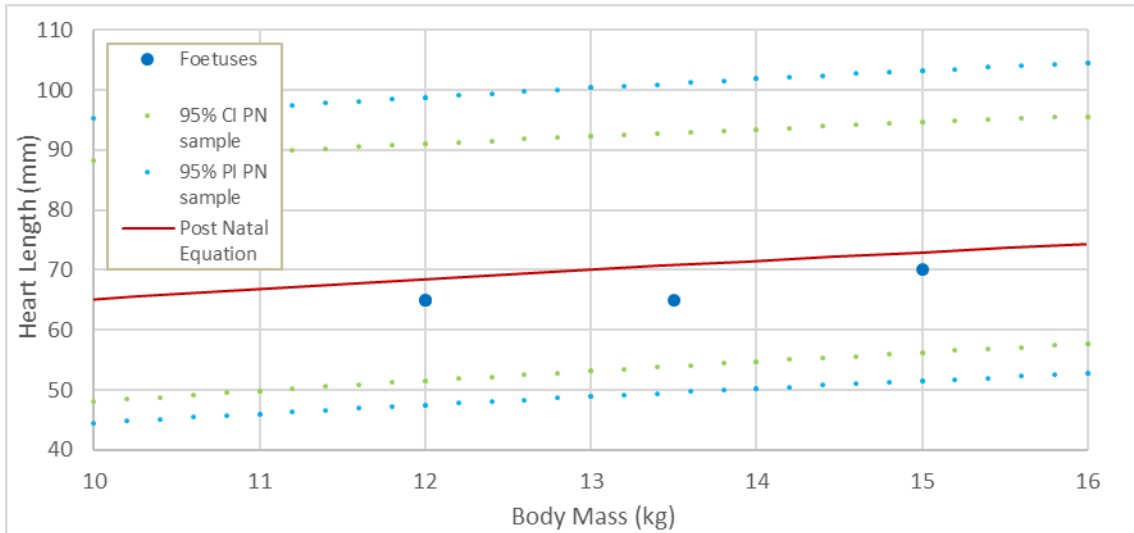
Appendix 7: Comparison of the thickness of the left ventricular wall in giraffe and blue wildebeest.

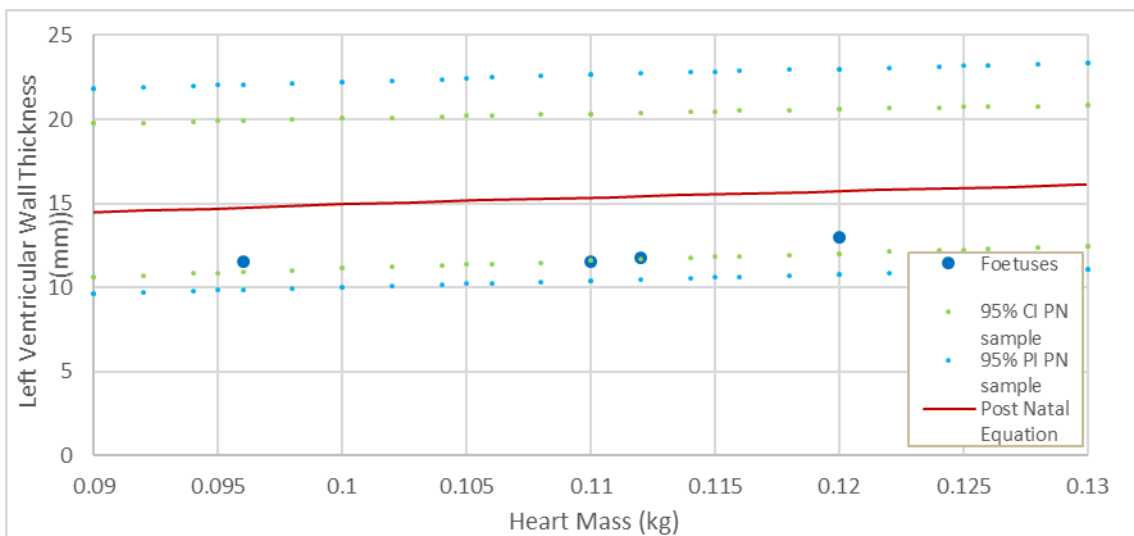
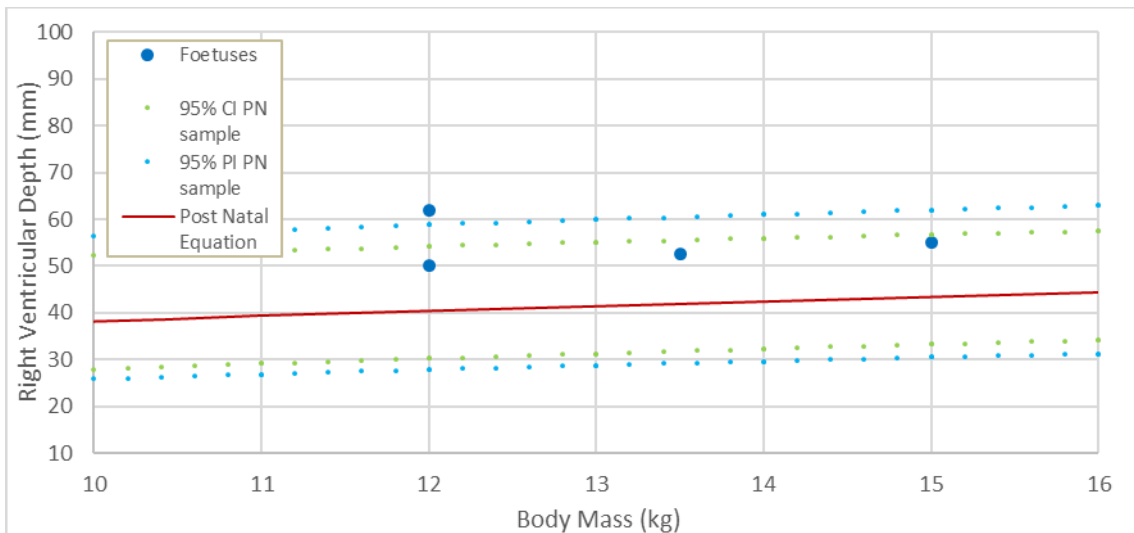
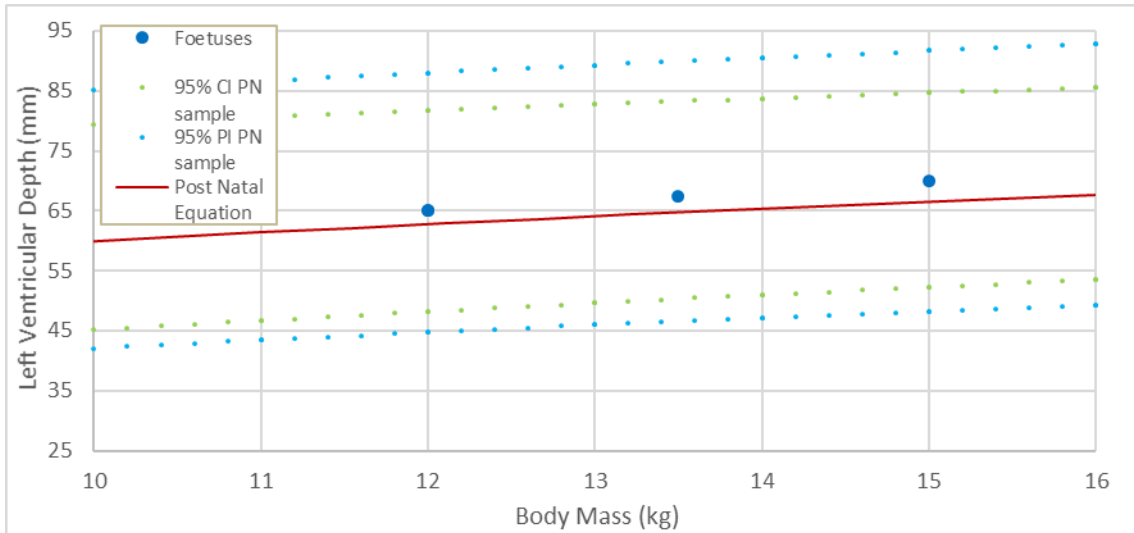


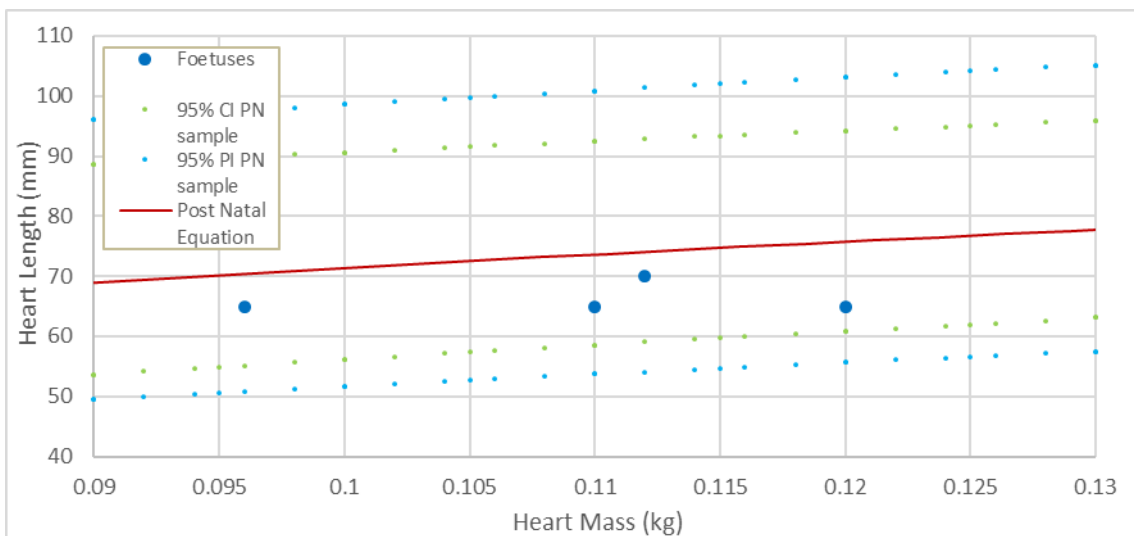
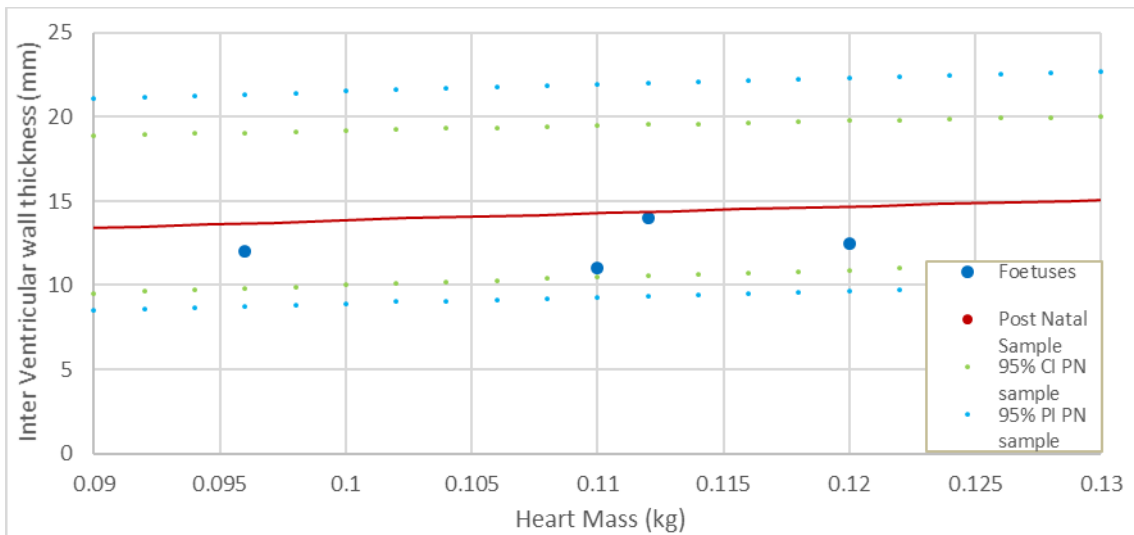
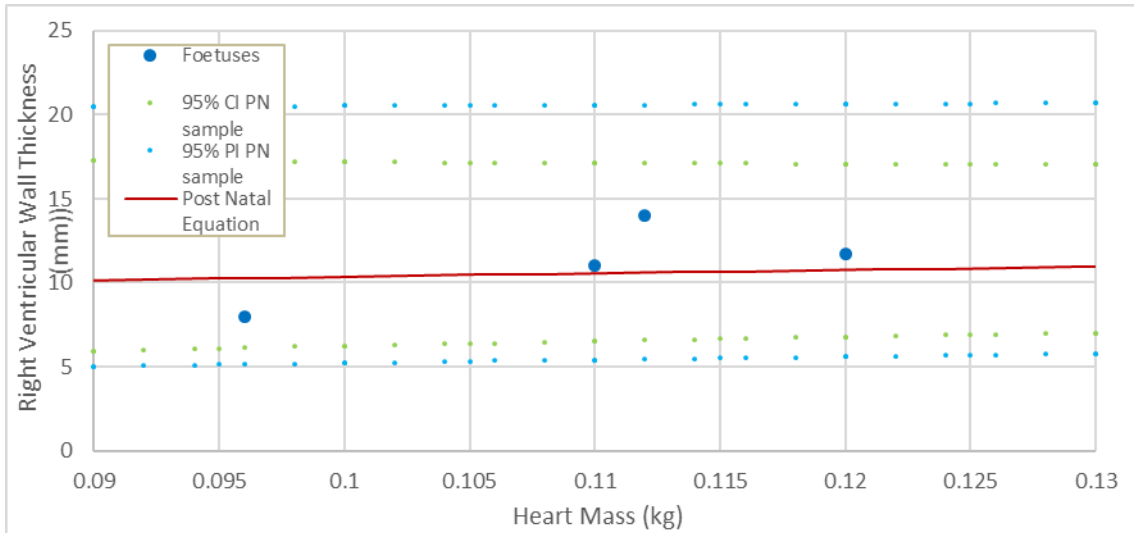
Appendix 8: Figures showing comparison of foetal data with the post-natal equations for the heart. 95% confidence intervals (95%CI) and prediction intervals (95% PI) for the post-natal equations are indicated.

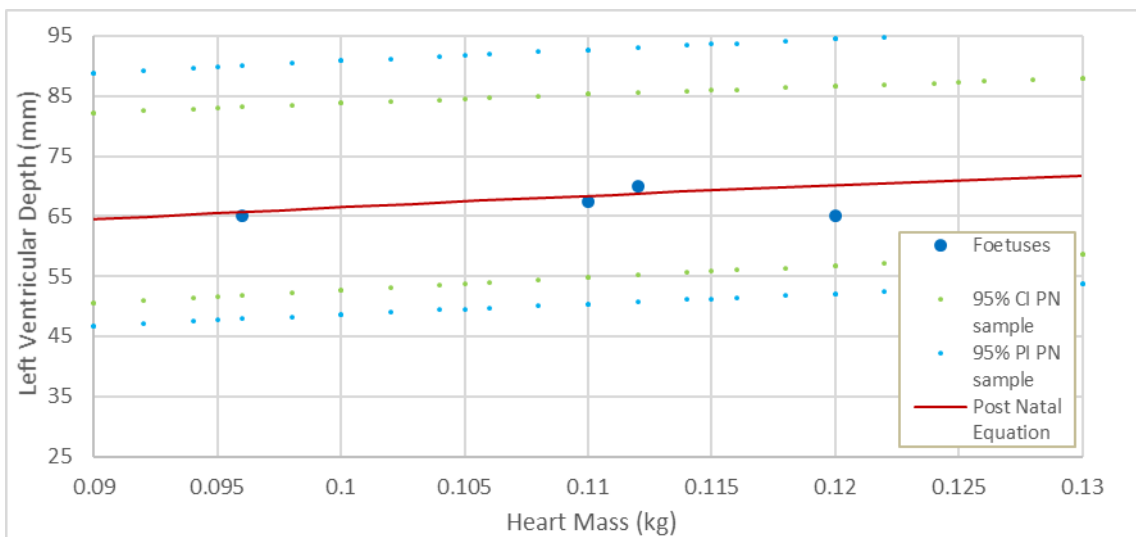
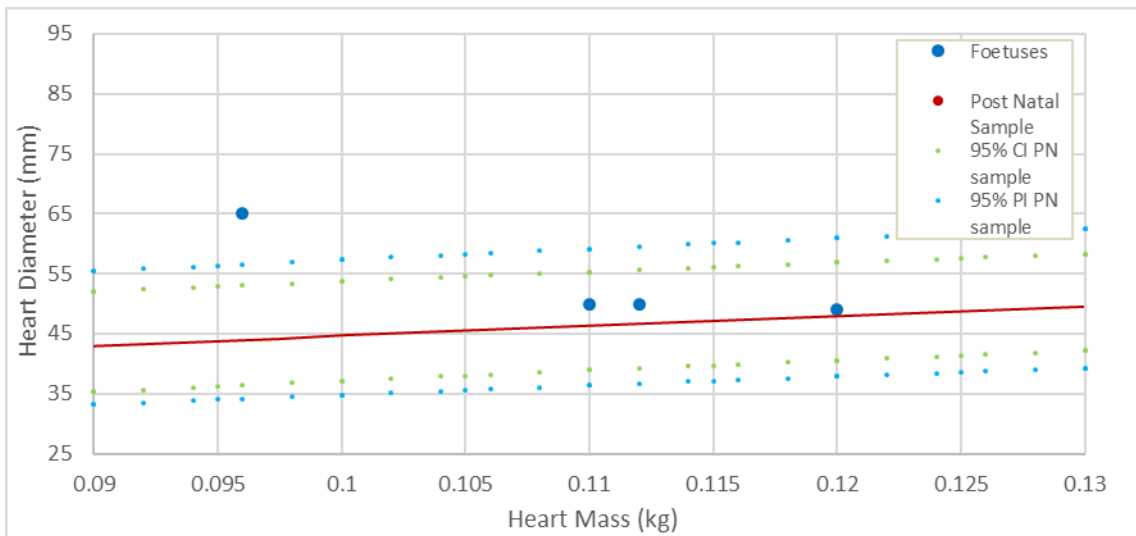
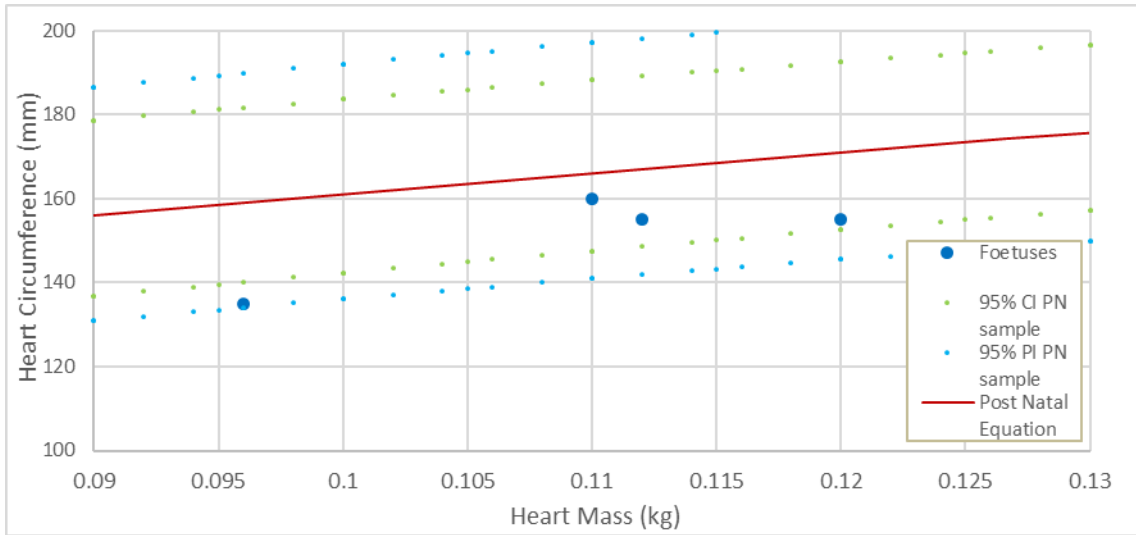


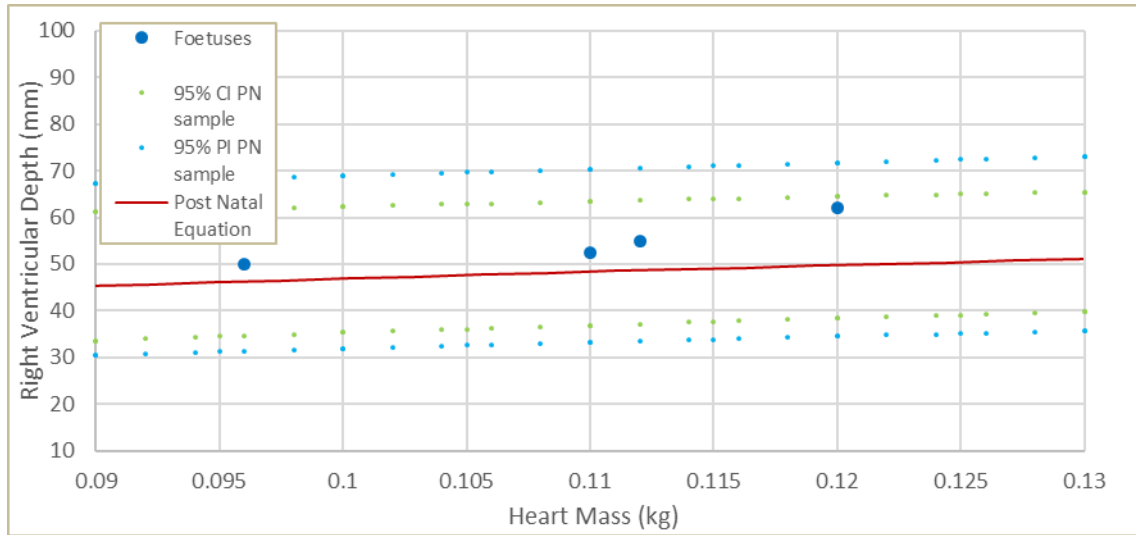












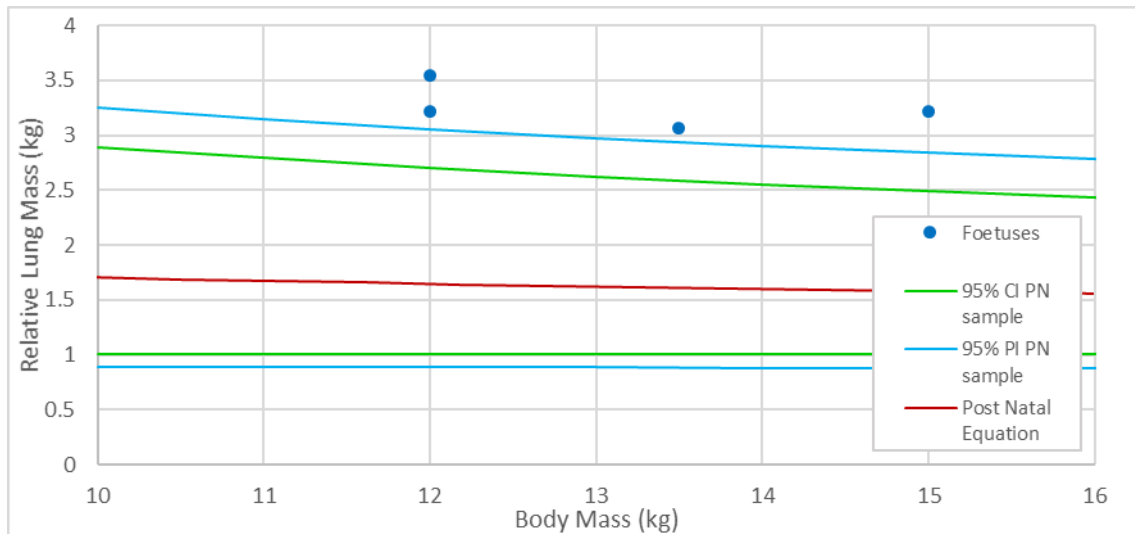
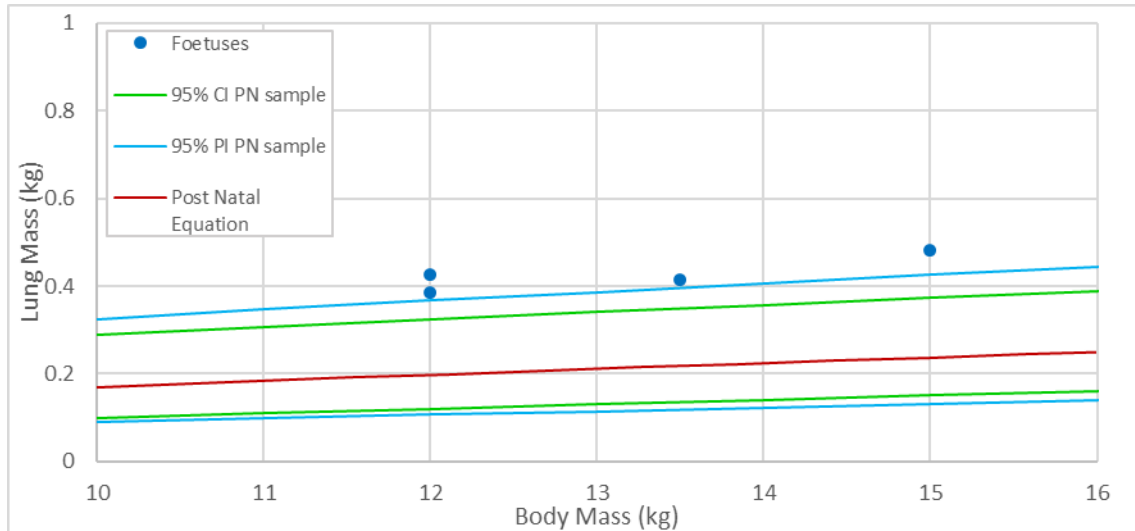
Appendix 9: ANOVA results for regression of body mass and lung mass against respiratory measurements (y).

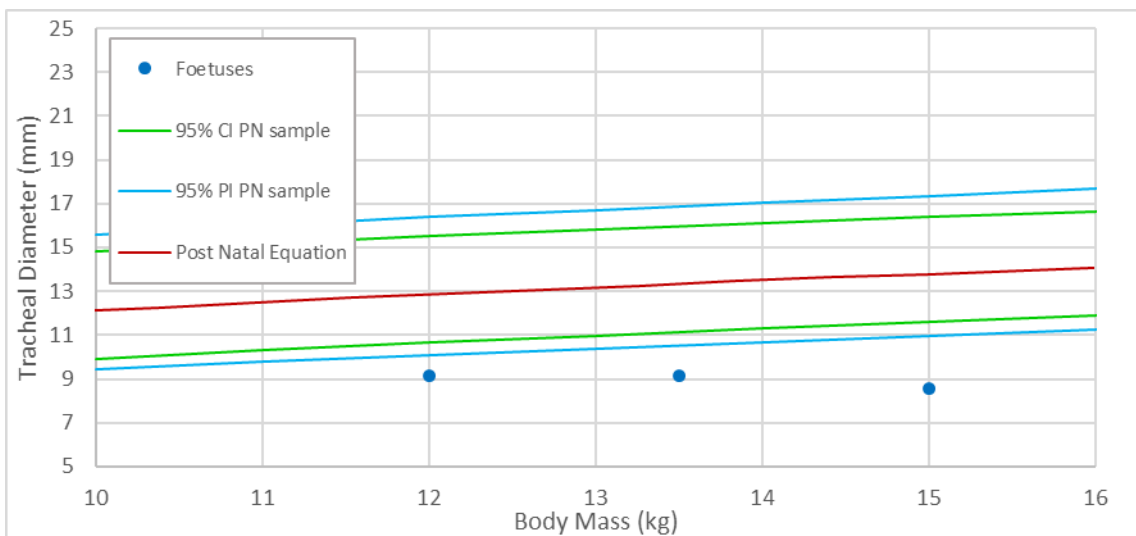
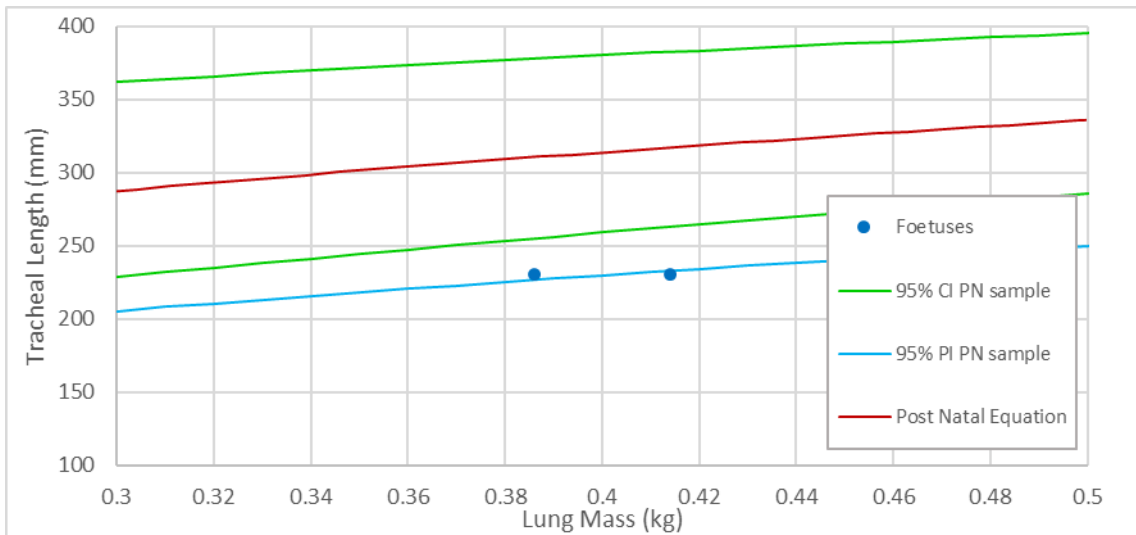
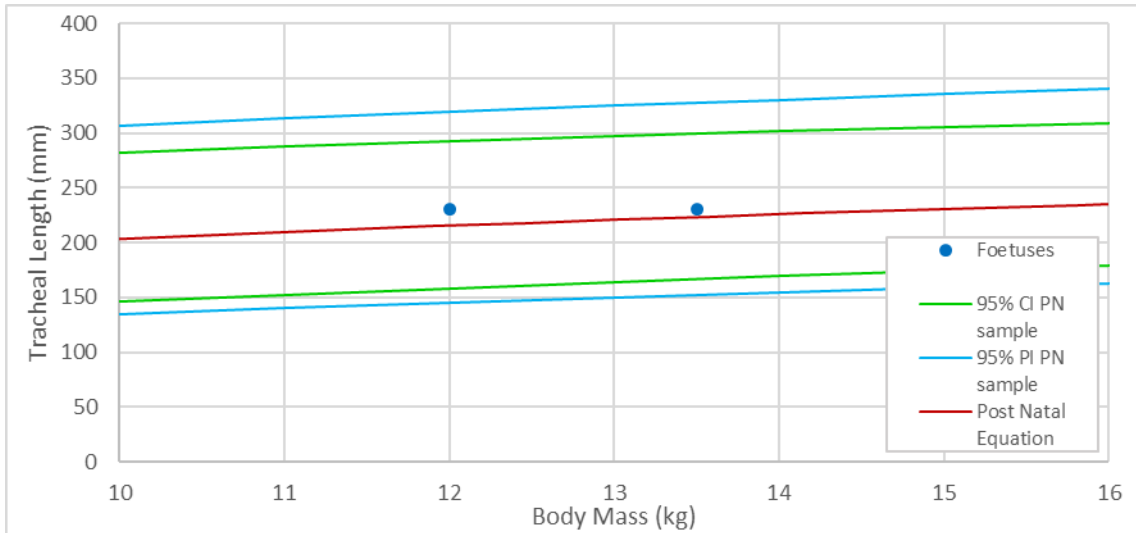
Results of the ANOVA test for significant regression in the cases where the regression model was not a significantly better estimator of the value of y than the mean of the y values.

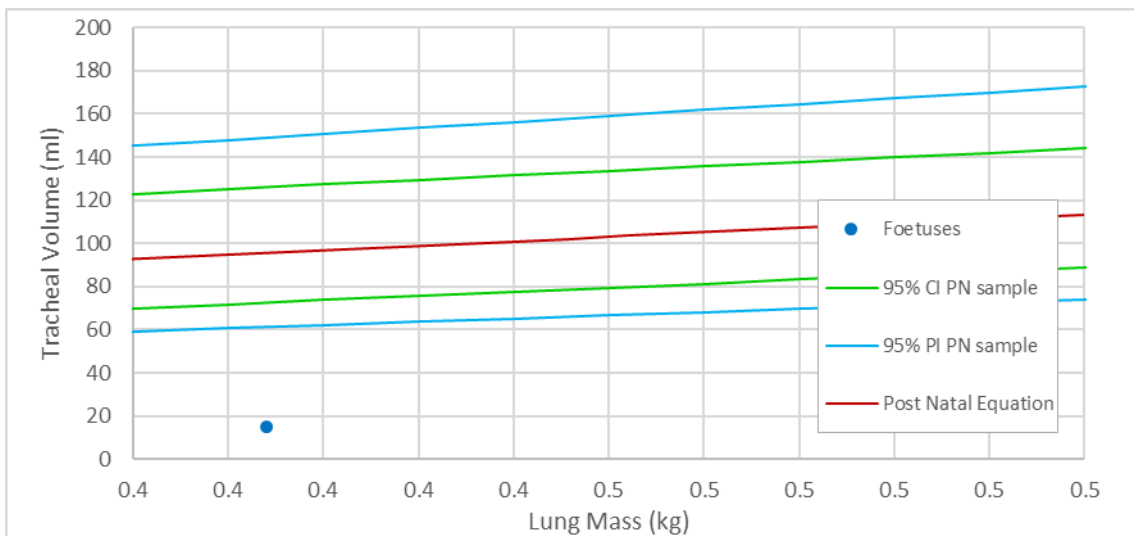
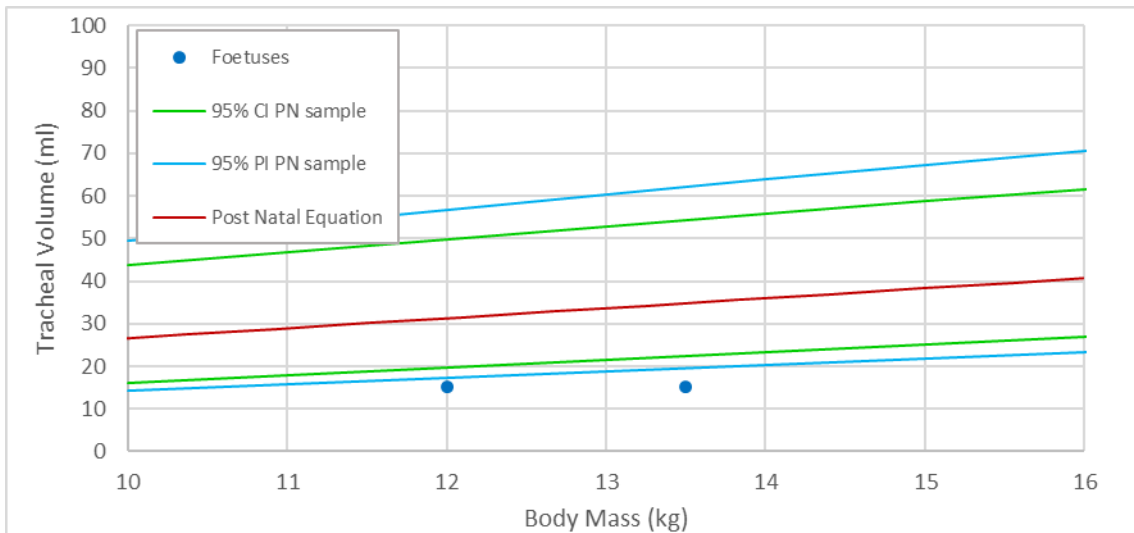
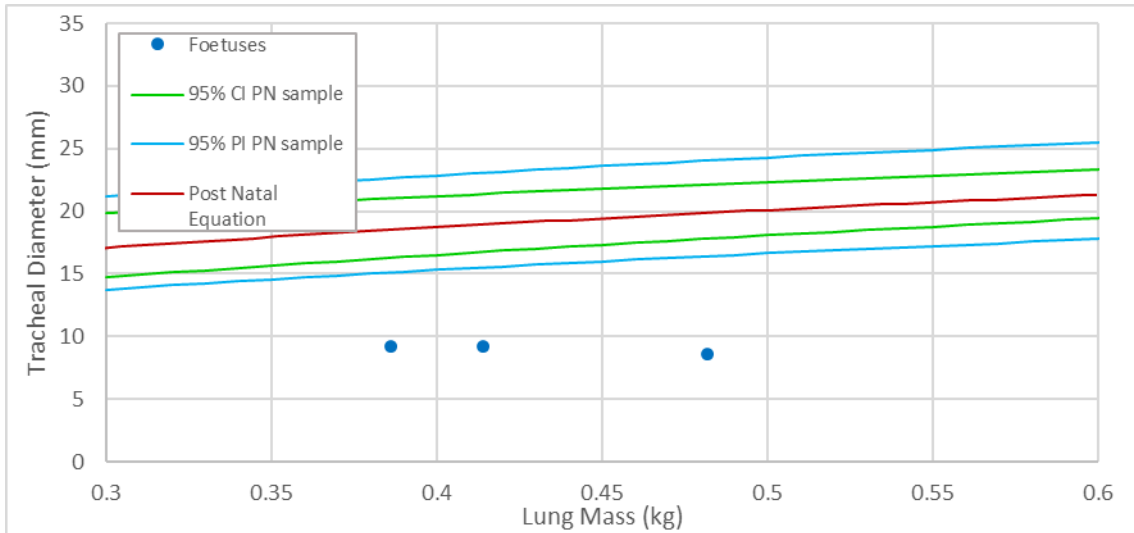
Sample	Equation $y = a(x)^b$		F - statistic	P ($H_0: b=0$)
	y	x		
Post-Natal	Relative lung mass	Body Mass	F (1-30) = 3.896	0.058
	Right ventricular wall thickness	Lung Mass	F (1-27) = 1.637	0.212
Adult	Lung Mass	Body Mass	F (1-16) = 3.930	0.065
	Relative lung mass	Body Mass	F (1-16) = 0.808	0.382
	Tracheal Diameter	Body Mass	F (1-16) = 1.171	0.295
	Tracheal Volume	Body Mass	F (1-14) = 3.410	0.086
	Tracheal Length	Lung Mass	F (1-14) = 2.744	0.120
		Body Mass	F (1-14) = 3.851	0.070
Right ventricular wall thickness	Lung Mass	F (1-16) = 1.121	0.721	

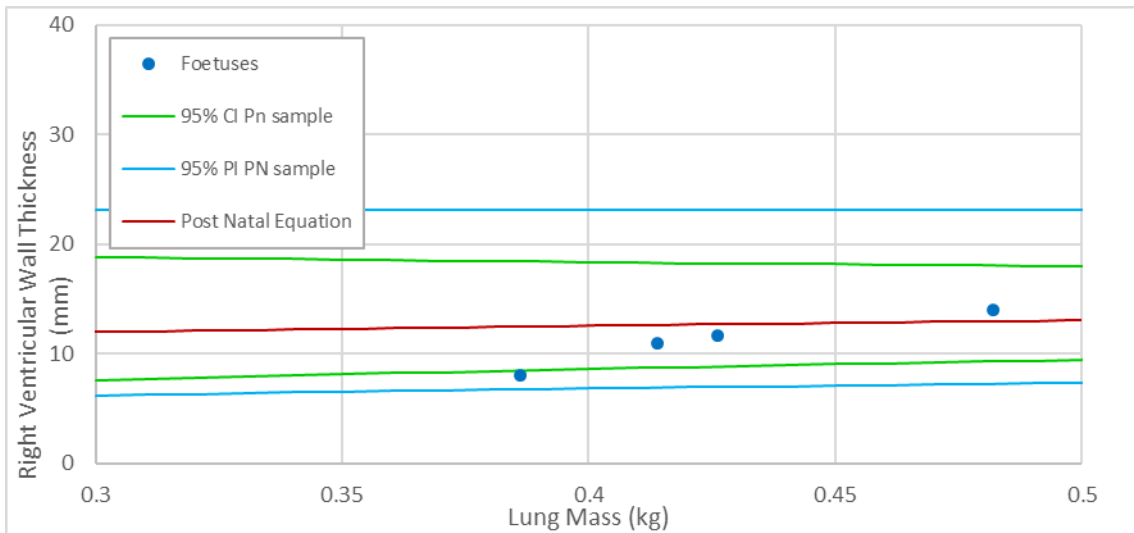
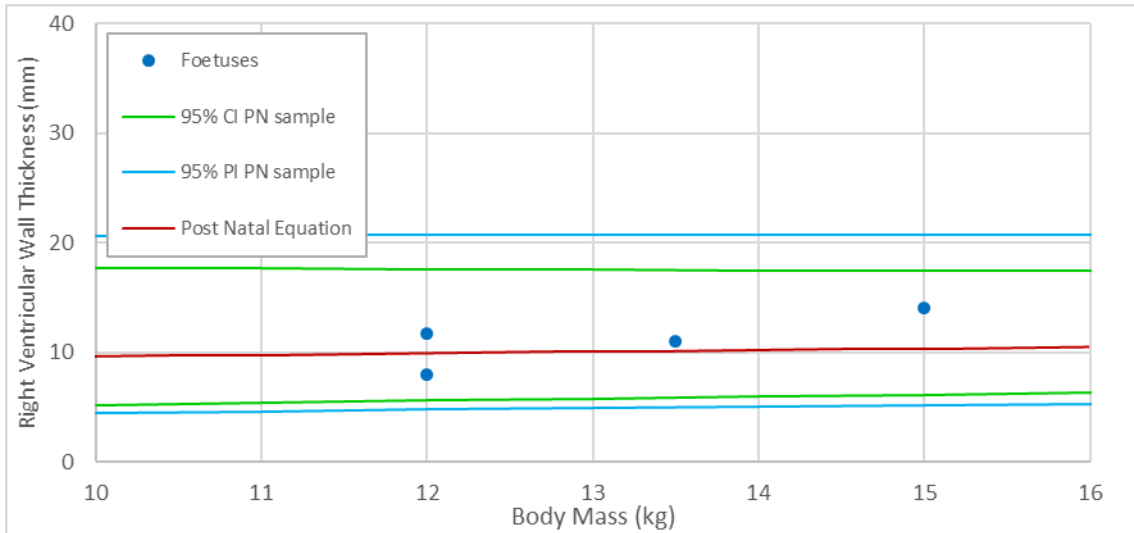
Appendix 10: Figures showing comparison of foetal data with the post-natal equations for the lungs and trachea.

95% confidence intervals (95%CI) and prediction intervals (95% PI) for the post-natal equations are indicated.











Appendix 11: Body Mass and Dentition

Calculated Body Mass (kg)	Body mass as weighed (kg)	Sex	Number of Erupted Incisors /2	Horn Mass (kg)
12.0	12	Male	0	0
12.0	12	Male	0	0
13.5	13.5	Female	0	0
15.0	15	Female	0	0
15.0	15	Male	0	0
64.3	74	Female	0	143
74.4	74	Male	0	
90.6	79.5	Female	0	78
91.9	90	Female	0	
96.1	87	Female	0	552
96.8	98	Female	0	464
97.4	93	Male	0	
100.8	88	Male	0	652
101.1	102	Female	0	374
108.9	108	Female	0	538
109.2	122	Female	1	562
109.9	146	Female	1	798
117.2	102	Male	0	
117.4	107	Male	0	1202
121.3	110	Female	0	482
126.4	130	Male	1	964
131.3	148	Male	1	1592
132.9	125.5	Male		125
138.8	137.5	Male	0.5	1060
162.0	149	Female	2	1038
163.0	157	Female	2	862
166.3	155	Male	1	1402
171.9	201	Female	3	1198
172.8	155	Male	2	2074
187.9	205	Female	3	1058
193.5	219.5	Female	3	1156
195.7	219	Female	2	996
196.4	225	Female	3	1038
198.1	201	Male	3	2736
200.1	216	Female	3	1118
203.9	224	Female	3	
204.2	192	Female	3	
213.6	231	Male	2	
217.4	220	Male	3	
226.3	248	Male	3	
226.6	225	Female	3	1230
230.2	193	Male	3	2338
230.6	223	Male	3	
231.5	253	Male	3	2602
248.8	242.5	Male	3	2314
253.4	221.5	Male	3	1598
265.4	256	Male	3	1786
282.1	258	Male	3	