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Inferring the physiological regimes of extinct vertebrates: methods, limits and framework

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What can we know of the physiological regimes of ancient vertebrates? Essential to the exploration of this question are several epistemological tools: (i) a phylogenetic framework for interpreting whole animals and individual tissues, (ii) reliable knowledge of variation in populations and among climates and geographies, (iii) an understanding of phenotypic variation during ontogeny and between sexes, and (iv) a sense of the patterns of body size change, both phyletically and ontogenetically. Palaeobiologists are historically bound to a dichotomous set of terms developed long ago to describe the relatively depauperate living vertebrate fauna. This system sees only binary categories of five major groupings: the 'cold-blooded' fishes, amphibians, and reptiles, and the 'warm-blooded' birds and mammals. The integration of histoanatomical data with patterns of size, growth and phylogeny provides an opportunity to re-imagine not only vertebrate palaeophysiology, but vertebrate physiology in general. Here, we discuss how four 'signals' or 'influences' on bone tissues—phylogeny, ontogeny, mechanics and environment—can help to address these questions.

This article is part of the theme issue 'Vertebrate palaeophysiology'.

1. Introduction

It is a truism that metabolic parameters cannot be measured directly in dead and extinct organisms. This circumstance puts palaeobiologists at a distinct disadvantage. Inferences about the physiology of dead and extinct organisms have to be drawn from correlative factors in preserved tissues—almost always hard tissues, but occasionally soft tissues (e.g. [1–3]). But how strong are such correlations? When can correlative factors be confused with or compounded by other biological properties of organisms? And what are the rules, if any, for determining a reliable palaeophysiological signal?

It is one thing to interpret the palaeobiological features of an extinct Pliocene horse. Perhaps smaller or larger than today's horses, this one probably shared most of the same dental, dietary, growth, reproductive and locomotory features. Little in its biology would surprise us unless its skeleton showed clearly aberrant characteristics, which then would be of interest for their own sake and analysed accordingly. However, when we try to do the same for an ichthyosaur, a plesiosaur or a pterosaur, as early palaeontologists did [4], we lose our compass: here are ancient beasts only distantly and often uncertainly related to living animals, with lifestyles vaguely similar to but in other ways unlike anything alive today. Our guide to interpreting these animals cannot be merely analogical, appealing to us because they remind us of today's familiar shapes [5]. We need close phylogenetic context when we can get it, and when we cannot, we need diagnostic anatomical criteria that determine physiological factors.

The great Victorian anatomist and palaeontologist Richard Owen (1809–1892) attempted to interpret the metabolic regimes of ancient animals in indirect ways, mostly related to two features: their taxonomic position, and key characters that he thought he could relate to physiology. In the first case, once an extinct creature was classified into a taxonomic group with living relatives, it bore the characteristics of that group. Owen once erected a group called the Haemothermia (birds and mammals) on the basis of their high metabolic rates, not upon any evolutionary relationships (Owen did not accept the common descent of all organisms in a Darwinian sense, although he accepted a kind of ‘evolution’, and did not accept natural selection as a strong influence on the evolution of life: [6–8]). His criteria were frankly typological. For example, *Archaeopteryx* was classified as a bird (because it had feathers), so it had to be warm-blooded, because birds are warm-blooded. As to the second criterion, Owen [9, p. 73] stated ‘we may, with certainty infer that *Archaeopteryx* was hot-blooded, because it had feathers, not because it could fly’. In this case, the possession of feathers provided the kind of insulatory covering correlated with homeothermy that insured that the animal was ‘warm-blooded’. So, the dual criteria of taxonomic position and the possession of key characters provided the metabolic regime of the extinct animal to be deduced [4].

We do not reason in the same way today. Birds and mammals evolved their high metabolic rates independently, as we know from phylogenetic methods not available to Owen and his colleagues; and more importantly, we ground-truth ideas about metabolic rates in data that relate to growth rates, in the context of developmental stage, adult body size, phylogenetic context, biomechanics and environmental factors [10]. Most importantly, in contrast with Owen and his predecessors, we accept evolution as the ultimate explanation of both variation and unity in the features of organisms ([11], summary of chapter VI).

Here, we review the evidence for and interpretations of metabolic status and physiological parameters in extinct vertebrates through the analysis of hard tissues. We examine the four principal factors (we do not call them ‘causes’, but ‘influences’ may be an appropriate term) that are reflected in the histological features of hard tissues in living and extinct vertebrates. These are ontogeny, phylogeny, mechanics and environment (the history of this framework is reviewed in [12], p. 2).

Bones and teeth are all that usually remain of vertebrates in the fossil record, and they grow in very different ways. All vertebrates except mammals replace their (premaxillary, maxillary, dentary) teeth more or less continually throughout their lives, whereas mammals produce a partial set of ‘deciduous teeth’ and a permanent complement of tooth forms from incisors to molars. The regular growth of teeth is marked by von Ebner lines [13]. There is no secondary reworking of dental tissue in any vertebrate, but bone tissue can be more or less reworked depending on size, age, growth rate, metabolic levels and mechanical loading.

2. The physiological regimes of ancient vertebrates: terminology and standardization

Interpretations of the biology of ancient organisms are rooted to some extent in the principle of *actualism*, often expressed as

‘the present is the key to the past’, and sometimes interpreted as a kind of uniformitarianism (e.g. [14]). This rationale can be perilous because, although scientists accept that the laws of the Universe have been constant since shortly after nucleogenesis, the amounts and rates of many natural processes and phenomena, ranging from the intensity of the Sun’s radiation to the rate of erosion of terrestrial soils, have varied greatly through geological time. Empirically, chemical and physical processes and reactions should occur at the same rates, given the same conditions, anywhere in the Universe, but the rates of those processes vary predictably with conditions in time and space (e.g. the precipitation rate of many salts in the ocean depends directly on water temperature).

The deposition of skeletal hard tissues is a chemical process moderated by the biological factors of growth rate and metabolic rate, which are related to levels of body temperature and nutrient supply that determine the maximum rates at which tissues can be deposited [15–18]. It is an inference of Amprino’s rule [19–21], as it has been historically interpreted, that microanatomical features of bone tissue reveal the rate at which it was deposited, and therefore the growth rate of the tissue in question [22]. It does not follow, however, that growth rate of an individual tissue or element is a direct indicator of metabolic rate of the taxon. Humans, for example, have relatively high metabolic rates, but they grow relatively slowly compared to other mammalian groups of similar sizes, as do most other apes. Within a single skeleton, some bony elements often grow more rapidly than others, for reasons related to their ecology and locomotion; smaller, more slowly growing elements may experience more secondary reworking of their cortices than larger, more rapidly growing elements [23]. Inferences of overall growth rate, to say nothing of metabolism, are better drawn from relatively complete ontogenetic series of an animal, and comparisons among taxa should be made at comparable ontogenetic stages [24].

In English, a set of terms has been developed historically by physiologists to describe the metabolic regimes of animals. The contrasting terms of *homeothermy* (*homiothermy*) and *poikilothermy* denote, respectively, whether an animal’s body temperature is relatively constant, irrespective of ambient temperature, or whether it tends to vary mostly or entirely with ambient temperature. The contrasting terms of *endothermy* and *ectothermy* denote whether an animal mainly manufactures its own body heat through metabolic processes, or whether it is more or less dependent on external, environmental sources of warming (e.g. the Sun, or objects that the Sun has warmed). The use of the ‘warm-blooded’ and ‘cold-blooded’ dichotomy goes back at least informally to Aristotle (*De Partibus Animalium*), and has been in use since the nineteenth century. Although interpretations can vary (the dichotomy is sometimes equated with homeothermy/poikilothermy), it is sensibly interpreted as contrasting relatively high and relatively low blood temperatures, the former being characterized by mammals and birds and the latter by fishes, amphibians and non-avian reptiles, although the ranges of temperatures of the contrasting terms have not been defined [25,26].

One problem with these sets of terms is that they are traditionally treated as dichotomous when each contrasting set is a continuum. That the traditional dichotomies could be formulated at all is an artefact of the process of selective extinction through geological time that has removed forms

with intermediate or mosaic states of these conditions. (Darwin's figure in *The Origin of Species* [11] illustrates this concept succinctly.) An important contribution of palaeobiological research has been to reconstruct and expand our knowledge and concepts of how extinct animals filled metabolic 'spaces' that do not exist today. To complicate matters further, subclades of vertebrates have evolved specialized adaptations to climate or energetic regime, such as torpor, hibernation, shivering and daily heterothermic regulation of body temperature; but we regard these as departures from general physiological syndromes and do not have space to explore them here.

A second problem with these sets of terms is that the variables of different sets are often seen to be inextricably linked. For example, mammals and birds are homeothermic, endothermic and warm-blooded, whereas other vertebrates are poikilothermic, ectothermic and cold-blooded. Examples to the contrary can be found in several papers in Thomas & Olson [27].

A third problem with these sets of terms is that they are often generalized taxonomically (as in the previous paragraph) when there are exceptions that show the importance of evolution (in addition to the 'lost evolution' represented by extinct forms) within groups. For example, mammals are said to be endothermic and homeothermic, but the body temperature of the echidna varies considerably with ambient temperature (and that of marsupials somewhat less so) [28]; many birds are heterothermic, a condition in which they lower their body temperatures and shut down some metabolic functions on a daily basis to save energy [29,30]; and endothermy has evolved independently in several lineages of fishes [31].

Palaeobiological investigations into the metabolic regimes of ancient animals have blurred some of these traditional distinctions and questioned the universal acceptance of the 'actualistic' method when assessing ancient animals (e.g. [16,32]). It would be uncontroversial to presume that the metabolism of a Pliocene or Pleistocene deer was more or less similar to that of living deer, unless unusual features in its gross or histological morphology led us to suspect otherwise. It is more complex with animals far removed from anything living today. Although birds evolved from other Mesozoic theropod dinosaurs, it is not clear whether the first known birds (e.g. *Archaeopteryx*; see discussion of Owen's view above) were physiologically identical to living birds in general (discussed below), although it is generally accepted that dinosaurs in general, as well as all bird-line (ornithodiran) archosaurs, were metabolically distinct from living reptiles [33,34]. Pterosaurs, the flying reptiles of the Mesozoic Era, who were also ornithodirans and the closest major sister group to dinosaurs, have long figured in this general controversy. Owen [9] reasoned that because pterosaurs show osteological features of reptiles, they must have had a reptilian physiology, and therefore, their wings and their hollow skeletons were 'merely adaptive' for flight. Seeley [35, p. 150] countered him by maintaining that it was not only the hollow, thin-walled bones, but the presence of pneumatic foramina extending the respiratory tissue into the medullary cavities that allied them to the physiological condition of birds, noting that the main function of such a system was to 'cool the blood' and that to ignore this fact was 'to discard the only known clue to their interpretation'. More recently, Bernard *et al.* [36] used carbon isotope

compositions of the teeth of extinct Mesozoic reptiles (plesiosaurs, ichthyosaurs and mosasaurs, the last being a lineage of sea-going lizards) to determine that the body temperatures of these animals was in the range of about 35–39°C, well within the range of mammals. If these body temperatures were significantly higher than those of the ambient ocean waters, it follows that these animals were at least endothermic and 'warm-blooded' by any reasonable definition.

So far, we have concentrated on showing that the typological categories of modern physiology do not adequately encompass the regimes of some ancient animals. In the following sections, we discuss how palaeobiologists 'read' the hard tissues of extinct vertebrates to assess their metabolic attributes, and we suggest methods and approaches that are likely to provide reliable results. We also discuss sources of conflict in the interpretation of palaeohistological data and suggest some solutions.

3. Factors of bone histology that reflect growth rate and physiology

(a) Ontogeny

Growth rates change through ontogeny; oddly, very young birds and mammals grow very quickly but are not *sensu stricto* endothermic or homeothermic (e.g. [37–39]). They have to attain a high basal metabolic rate that produces enough heat to maintain functions of growth and development, an external insulatory layer to retain this heat and internal neurophysiological mechanisms that will regulate it. This may take from days to weeks in living birds and mammals; we cannot estimate this duration in extinct animals.

As growth proceeds, individuals in a species tend to follow characteristic curves that differ between length-related (the broadly sigmoid Gompertz curve) and mass-related (the more asymptotic von Bertalanffy) accumulations (figure 1). With growth in body length, initial rates are low after birth or eclosion but soon accelerate; they tend to reach an inflection point at sexual maturity, which often occurs at attainment of about two-thirds the body length at skeletal maturity. Following this inflection point, growth slows and tends to be asymptotic (e.g. [33]). This pattern of slow growth at either end of a curve with more rapid growth in between fits the classic definition of a Gompertz curve. With mass growth, initial rates are again low after birth and eclosion but rapidly accelerate; sexual maturity is reached at about half the mass at skeletal maturity, and thereafter, accumulation of mass decreases until it becomes asymptotic around the point of attainment of skeletal maturity. The resulting curve tends to be sigmoidal, but technically, it also fits the definition of a Gompertz curve by having lower growth rates at both ends. It should be noted that animals which reach full skeletal maturity before the onset of sexual maturity, such as nearly all living birds and small mammals, may not follow these curves exactly.

The reflection of these ontogenetic rates in bone tissue is manifested mainly in the type of tissue deposited. Generally speaking, the most rapidly growing tissues of the fibro-lamellar complex (FLC) have vascular canals that are predominantly oriented radially, although they proceed in all directions; the density of these canals is very high and the bone tissue that they produce tends to be ephemeral, being resorbed as the medullary cavity expands [16–18,22]. This

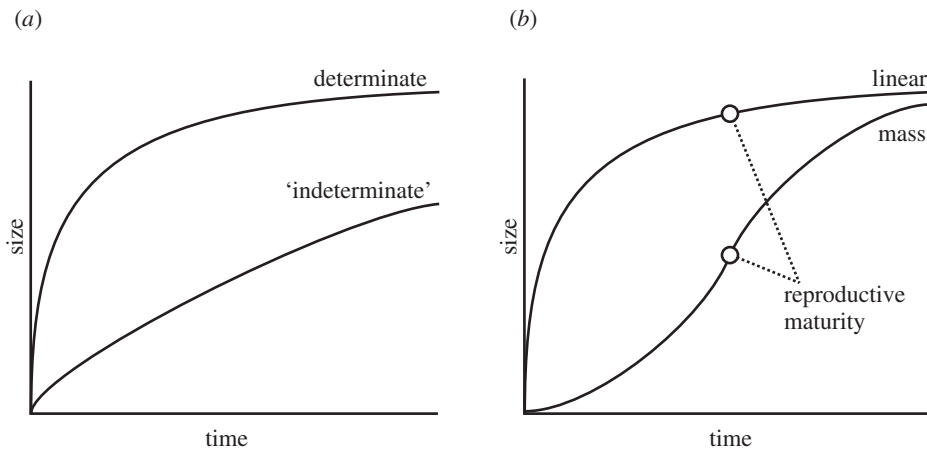


Figure 1. (a) A comparison between determinate and 'indeterminate' growth modes. The contention here is that there is no thing such as 'indeterminate' growth: all taxa slow and eventually cease growing at some point. However, in slowly growing (usually ectothermic, poikilothermic) vertebrates, growth tails off so slowly that life usually ends for an individual before growth does. Hence, 'indeterminate' is a misnomer. (b) A contrast between growth in body length and body mass in vertebrates. These curves represent cumulative increases in length and mass, but instantaneous growth rates that underlie these accumulations are reflected in the instantaneous slope of the line. Reproductive maturity occurs typically at about half the attainment of full adult mass, but at a far higher proportion of growth in length. Exceptions occur in animals that reach full size in less than a year, such as birds and small mammals. Figure by Sarah Werning, from Padian & Lamm [10].

tissue is normally deposited during the earliest stages of growth, after thermoregulatory capacity is established. As growth rates begin to settle, the deposition of tissue becomes more regular, and tends to follow the continuum of stages between the classical 'fibro-lamellar' and 'lamellar-zonal' categories. As a general pattern, more rapid deposition is characterized by higher vascularity, multi-directional orientations of vascular canals (often with frequent anastomoses) and greater density of osteocytes, whereas slower deposition is characterized by lower vascularity and osteocyte density, and the predominance of longitudinally oriented canals [16–18,22].

As noted earlier, Amprino's rule [19–21] infers that the type of tissue laid down at any given point in the development of a bone mainly reflects its rate of growth. Rates of growth can be generally quantified in bone tissue, regardless of taxon and whether from living or fossil animals, by actualistic comparison to data obtained from various extant vertebrates. Classic categories of cortical bone, for example, in the FLC, display fairly circumscribed ranges of growth rate corresponding to the characteristics of bone tissue discussed in the previous paragraph, regardless of taxon. Generally speaking, sustained high rates of growth imply *sustained* high metabolic rates that underlie the production of bone tissue. We stress the adjective 'sustained'. Especially in the early stages of growth, 'ectothermic' animals can produce tissues that appear to reflect anomalously high growth rates (turtle, alligator; varanid lizards: e.g. [40,41]), but the production of these tissues is not sustained for long; as a result, it should not be inferred that the animal is endothermic, either during part or all of its ontogeny.

Similarly, an aged endothermic individual may produce bone tissue that is growing slowly, but that does not indicate that it has become ectothermic. Moreover, some animals such as humans have relatively high metabolic rates and are both endothermic and homeothermic, but they grow relatively slowly compared to other mammals of their size. This growth pattern would never be taken for evidence that humans are ectotherms.

We caution that as a general rule the physiological regime of an animal cannot be read directly from a single section of bone tissue.

Isolated sections of bone do not directly reflect the overall physiology of an animal; nor, in many cases, does the full section of a single bone. The most reliable results are obtained from full ontogenetic series of several bones in a skeleton, if possible. For example, smaller limb bones may grow more slowly than larger limb bones, and may show more remodeling [23], so larger bones may provide more accurate estimates of growth rates and inferences of the underlying physiological regime, as well as of skeletochronology.

Given that in individuals of various taxa and growth/metabolic rates, those rates may be generally high in early stages of growth and low with advancing age, it follows that neither of these stages is optimal for comparing instantaneous rates. Assessment of growth rates and inferences about metabolic rates should be taken throughout growth, but it is at later stages (e.g. subadult) that comparability among taxa is most meaningful [24,34,42]. Figure 1a compares two taxa of equal body size, one (determinate) with high growth rates in linear dimensions and the other (indeterminate) with lower rates. By the end of the time interval represented at the right side of the graph, the determinate taxon has long since reached full size, but the 'indeterminate' animal is still growing (at a progressively lower rate). At any point in this curve, the former animal is growing more rapidly than the latter; it is merely a question of the slope of the line. If high growth rates are persisting at later stages, it is a good indication of sustained high metabolic rates throughout somatic growth, and this is why we prefer a 'subadult' stage for comparisons between taxa. However, we stress that growth rates, although well tested in a variety of taxa, are only a proxy for the inference of metabolic rates.

(b) Mechanics

Osteological indicators of mechanical loads usually tell us little about the metabolic features of an organism. Sometimes loading is inferred by the presence of secondary osteons in cortical bone, and at one time, this was considered a possible direct indicator of metabolic rate (e.g. [43,44]), but growth rate and bone size have more to do with this than loading *per se* [23]. Humans are a notable exception because they grow slowly and evince extensive micro-fracture repair, which has

been cited as a main function of their presence in humans ([45]; but see [46]). In some cases, particulars of metabolic rate may be inferred from the histological details of secondary tissue that repairs fractures or breaks in bone, but these must be interpreted with caution, especially in extinct animals [47].

(c) Environment

Relatively endothermic, homeothermic animals are less affected by the ambient conditions of the environment than relatively ectothermic, poikilothermic animals are. Some living endotherms appear virtually unaffected in seasonal bone growth by ecological conditions (e.g. [48]) that vary geographically. Animals traditionally considered ectothermic and poikilothermic may respond with higher growth rates when exposed to a more congenial environment (e.g. high temperatures and abundant food for farmed crocodiles: [49]). However, under normal conditions, their growth is measurably slower than in relatively endothermic, homeothermic animals. The effects of environment are best measured using the same taxon in a variety of controlled environments, tracking its growth and metabolic rates. Comparative growth rates can be measured in fossil specimens, and variations may reflect environmental fluctuations. However, individual variation is great in populations, even in those lineages inferred to have relatively high metabolic rates (e.g. [50] on *Maiasaura*). We cannot expect extinct animals to behave exactly like living ones with respect to their growth and metabolic rates and features, especially if they have no close living relatives. As a result, it is often difficult to determine the direct effects of environmental factors on physiology, measured through growth. In relatively constant environments, one might expect that metabolic processes would not be variably affected and so growth would proceed regularly in a skeleton. In this way, yearly deposits of cortical bone between annual growth marks would decrease regularly in thickness as growth rate slowed naturally through life (e.g. *Tyrannosaurus rex*: [51]). However, in many vertebrates, the thickness of these annual cortical layers may fluctuate between higher and lower intervals as growth progresses, even as the overall trend is to slow growth throughout life. This pattern suggests that even 'endothermic' animals can have 'fat and lean' years, much like the variable growth of trees expressed in their annual accumulations of wood rings.

Nevertheless, environmental conditions can have direct effects on metabolic rates of animals, and these can be translated directly into patterns of growth. This is well known for ectothermic animals because they rely on external sources of heat for most of their metabolic processes, and at lower temperatures, many of these processes run more slowly. It is not always appreciated that in birds and mammals, including domestic breeds, excessively high or low temperatures can also affect physiological performance ranging from decrease in appetite to the over-production of hormones and metabolites [52].

(d) Phylogeny

Organisms inherit their tendency to express the mode of growth and their metabolic rates, just as they inherit other phenotypic features. However, these modes and rates evolve, as we have seen in the estimates of dinosaurs compared to more basal reptiles, so we cannot accept a typological view of the effects of evolutionary legacy

(phylogeny). The classic case of this begins with Owen's declaration that dinosaurs must be cold-blooded because they are reptiles [6], and it extends to the dichotomous treatment of physiological syndromes that still characterize textbooks on animal physiology (see above).

Given the four factors reflected in bone histology (ontology, phylogeny, mechanics and environment), we can move beyond a typological approach to a quantitative assessment of phylogenetic signal, providing that we can account for other influences. The challenge in interpreting the effects of phylogenetic legacy on physiology in extinct animals is to identify and separate by quantification the role of phylogenetic heritage in the expression of bone tissue types. Cubo and his co-workers (e.g. [53,54]) have pioneered this approach, although we have reservations about methodology: for best results, bones at comparable ontogenetic stages should be used, and 'phylogenetic contrasts' become problematic among distantly related taxa (e.g. above the 'genus' level at most: [55]). Even when care is taken to control variables, within a taxon, metabolic rates will vary with size, age to maturity (which independently reflects growth rate), environmental roles and climates (e.g. mustelids versus other carnivorans: [56]).

Apart from these concerns, there is much to be gained from phylogenetic optimization of traits taken from bone histology (e.g. [57–60]). Least-squares regression allows the optimization of character states on phylogenies, and for basal and derived character states to be inferred at any given node. These traits can be related to rates of growth and metabolic activity by actualistic comparison: that is, if the density and orientation of vascular canals and osteocytes, for example, are correlated with these rates in living animals, the same rates per unit tissue can be inferred for the extinct animal in question.

4. Potential sources of confusion in using hard tissue histology to interpret physiology

(a) Interpreting physiology from a single histological section

A typical osteohistological section captures the deposition rate of tissue for a given interval of growth. Few sections capture the entire history of growth because resorption or secondary reworking of the internal cortex may have erased earlier growth stages, the growth stage of the sampled section may not be known, and (or) the ultimate size of the individual may not be represented. The security of inferences increases with greater ontogenetic sampling and greater populational sampling. Consequently, it is unwise to interpret physiological parameters from a single section of bone, unless it comprises nearly all the history of growth of the animal. This may apply to animals with very small medullary cavities and little secondary reworking of bone, in which nearly the entire ontogenetic history is visible. Such animals are usually slow-growing and show no evidence of high metabolic rates.

(b) Within a clade, growth rate is usually correlated with adult body size

Case [61] showed that within vertebrate groups, generally the larger the adult body size, the higher the growth rate of the

species. We should expect, therefore, that smaller species of a clade would produce bone tissue that does not reflect growth rates as high as those reflected by the tissues of larger species. Gorillas are larger than humans and grow more rapidly (they mature in their early teens), and as a correlate humans have lower metabolic rates than gorillas. Consequently, if the rates of bone tissue deposition of a small species of a clade appear lower than in larger forms, it should not be inferred that this species had a lower metabolic rate than those of larger species in its clade.

(c) Growth and metabolic rates slow through ontogeny

The trajectory of growth for most vertebrates is represented by a Gompertz curve for growth in body length and a von Bertalanffy curve for mass. Because these rates change extensively through growth, it is critical to know the ontogenetic stage of a histological section before making inferences about physiology. Instantaneous growth rates represented by individual sections may be inferred, but without a fuller ontogenetic sequence, only one point on a complex curve is being represented, and it cannot be extrapolated.

(d) Secondary tissue and the interpretation of physiology

As a skeleton grows, its individual elements change in proportion: some become relatively larger, some relatively smaller. In the course of growth, especially in subadult stages and beyond, secondary osteons (Haversian bone) are brought in to replace primary tissue, especially in the inner cortex, in some vertebrate clades. This phenomenon has been linked to high metabolic rates, and whereas that inference may not be entirely untrue, other factors may be equally or more important within individual clades. For example, in humans, Haversian replacement has been linked to the repair of microfractures within long bones [45,46]: there is a strong correlational pattern even if a precise causal mechanism has not yet been well established. The slow growth of human skeletons suggests that mechanical stresses on bones weaken their internal structure, and that secondary bone replacement is the optimal solution. However, this says nothing about the metabolic rate of humans, which is high. Moreover, in larger and more rapidly growing taxa, such as hadrosaurian dinosaurs, Haversian replacement appears to predominate in smaller bones of the skeleton, which grow relatively slowly compared to larger

elements. Padian *et al.* [23] interpreted this pattern as the result of differential growth rates: smaller elements cannot refuse the mineral nutrients brought by the vascular supply, and so rather than grow, they simply remodel their tissues—whereas larger elements use this supply for primary growth.

5. Conclusion

The most important thing to take away from this review is that physiological and metabolic regimes of animals cannot be read from single sections of bone that do not encompass the full ontogenetic history of the animal. Even if a full diametric section of a bone can fit on a single histological slide, if there are internal erosions of the inner cortex that expand the medullary cavity or if the cortex is obscured by the development of secondary (Haversian) osteons, the picture will not be complete. Equally important in the comparative assessment of tissue types, growth rates and inferred metabolic rates is the need to use equivalent ontogenetic stages (e.g. subadult), and if possible to understand the growth trajectory that provides comparative skeletochronological data. This is particularly important in comparing species of different sizes within a clade, because larger species generally grow more rapidly than smaller species, so their relative sizes at, e.g. 3 years of age should reflect different tissue types.

It is easier to estimate growth rates of an element at a particular ontogenetic stage, using actualistic data from growth rates of the tissues of living animals, than it is to infer underlying metabolic rates. In general terms, inferences will be more secure if they incorporate a variety of data from both the ontogenies of their component species and a range of species within the clade in question. In this way, ontogeny and phylogeny help us to discover macroevolutionary patterns that can test palaeobiological inferences.

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