REVIEW / SYNTHÈSE

Variability in the growth patterns of the cornified claw sheath among vertebrates: implications for using biogeochemistry to study animal movement

Danielle M. Ethier, Christopher J. Kyle, T. Kurt Kyser, and Joseph J. Nocera

Abstract: We review the role of biogeochemical signatures, such as stable isotopes and trace elements, in the cornified claw tissue as a means of studying movement and foraging behaviour of vertebrates because this approach is noninvasive and can capture contemporary and historic signatures. Because biogeochemical techniques are still relatively new in studies of animal movement, we are only beginning to understand how the growth patterns of the cornified claw sheath may affect our ability to interpret the biogeochemical signals in these tissues. To move towards resolving this, we review the morphology of the epidermal cornified claw sheath in several taxa that illustrate substantial variation in growth patterns both between taxa and between individual distinct claw regions. For instance, in mammalian claws, deposition of keratinizing cells from the epidermis is nonlinear because the claw tip is composed of old and new cornified epidermal cells, whereas the cornified blade horn covering the claw's lateral walls is deposited continuously and without assortment, providing unbroken time-series data. We also consider patterns of growth in mammalian hooves, as well as reptilian, avian, and amphibian cornified claw sheaths, and address the need for expanded research in this field. We conclude this synthesis by describing a noninvasive technique for monitoring growth rates in a model mammal, the American badger (*Taxidea taxus* (Schreber, 1777)), and provide guidelines for future sampling of claw keratin, which will improve our ability to back-calculate the time of biogeochemical integration into this tissue.

Résumé : Nous faisons une revue du rôle des signatures biogéochimiques, telles que les isotopes stables et les éléments en traces, dans le tissu corné des griffes comme moyens d'étudier les déplacements et le comportement alimentaire chez les vertébrés, puisqu'il s'agit d'une méthodologie non invasive qui peut retenir des signatures actuelles et passées. Puisque les techniques biogéochimiques sont d'usage relativement récent dans les études des déplacements animaux, nous commençons tout juste à comprendre comment les patrons de croissance de l'étui corné des griffes peuvent affecter notre capacité à interpréter les signaux biogéochimiques dans ces tissus. Pour faire du progrès dans ce domaine, nous passons en revue la morphologie de l'étui corné épidermique de la griffe chez divers taxons, ce qui révèle une importante variation dans les patrons de croissance, tant entre les taxons qu'entre les différentes régions distinctes de la griffe. Par exemple, dans les griffes de mammifères, la déposition des cellules kératinisantes par l'épiderme ne se fait pas de façon linéaire parce que la pointe de la griffe est composée de cellules épidermiques cornées anciennes et récentes, alors que la lame cornée qui couvre les parois latérales de la griffe est déposée de manière continue et sans assortiment, ce qui fournit une série chronologique continue. Nous examinons aussi les patrons de croissance des sabots de mammifères et des étuis cornés des griffes de reptiles, d'oiseaux et d'amphibiens. Nous terminons notre synthèse en décrivant une technique non invasive pour suivre les taux de croissance chez un mammifère modèle, le blaireau d'Amérique (*Taxidea taxus* (Schreber, 1777)), et fournissant des règles pour l'échantillonnage futur de la kératine des griffes, qui permettront d'améliorer notre capacité de rétrocalculer le moment de l'intégration biogéochimique dans ce tissu.

[Traduit par la Rédaction]

Received 17 February 2010. Accepted 20 August 2010. Published on the NRC Research Press Web site at cjz.nrc.ca on 20 October 2010.

D.M. Ethier. Environmental and Life Sciences Graduate Program, Trent University, DNA Building, 2140 East Bank Drive, Peterborough, ON K9J 7B8, Canada.
C.J. Kyle. Forensic Science Department, Natural Resources and DNA Profiling Forensic Centre, Trent University, DNA Building, 2140 East Bank Drive, Peterborough, ON K9J 7B8, Canada.
T.K. Kyser. Department of Geological Sciences and Geological Engineering, Queen's University, Kingston, ON K7L 3N6, Canada.
J.J. Nocera.[1] Environmental and Life Sciences Graduate Program, Trent University, DNA Building, 2140 East Bank Drive, Peterborough, ON K9J 7B8, Canada; Ontario Ministry of Natural Resources, Trent University, DNA Building, 2140 East Bank Drive, Peterborough, ON K9J 7B8, Canada.

[1]Corresponding author (e-mail: Joe.Nocera@ontario.ca).

Introduction

Understanding movement and foraging behaviour of animals on a daily, seasonal, or annual basis is fundamental to ecological, evolutionary, and conservation studies. Until recently, studying animal movement required trapping and tracking extrinsically marked individuals. These extrinsic markers may be either passive (e.g., ear piercings, tattoos, or bands), or active (e.g., radio or satellite transmitters). Though these techniques have contributed to our understanding of movement, foraging behaviour, and habitat selection, they are often burdened with low recovery rates, high costs, and substantial logistical barriers (Webster et al. 2002). Recent technological advancements and new analytical techniques have allowed researchers to overcome some of these obstacles. For instance, intrinsic markers, such as biological (e.g., genetic) and biogeochemical (e.g., stable isotopes and trace elements) signatures have become important tools in studies of animal movement.

Stable isotopes and trace elements have begun to revolutionize the way we approach and execute research in ecology and behaviour. Chemical analysis of animal tissues can provide valuable data on an animal's location history, improving our understanding of seasonal foraging behaviour, dispersal, and habitat selection in species that have traditionally been difficult to study. The chemical composition of certain tissues reflects that of ingested food and water and can therefore serve as an indicator of diet (e.g., see Ben-David et al. 1997) and geographic origin (e.g., see Oppel and Powell 2008). Different tissues turn over (i.e., are renewed through growth) at different rates and therefore reflect dietary and habitat information from different temporal and spatial scales (Tieszen et al. 1983; Bearhop et al. 2003; Rubenstein and Hobson 2004; Dalerum and Angerbjorn 2005). For example, some metabolically active tissues turn over in a matter of hours or days (e.g., blood plasma or liver), whereas other tissues can take several months (e.g., muscle) to several years (e.g., bone collagen) (Hobson et al. 1993; Chamberlain et al. 1997; Bearhop et al. 2002; Pearson et al. 2003; Voigt et al. 2003). In metabolically inactive tissues (e.g., teeth enamel, keratinized hair, feathers, and claw sheaths), biogeochemical signatures remain unchanged following synthesis (Caumette et al. 2007), incorporating information on habitat and foraging behaviour over the time the tissue was synthesized (Hobson and Clark 1992; Chamberlain et al. 1997; Hobson and Wassenaar 1997; Bearhop et al. 2002). There are several advantages of using keratinized tissues for chemical analysis. Small samples can be taken relatively noninvasively from living specimens (feather sampling and claw clipping, e.g., Mazerolle and Hobson 2005), allowing for serial sampling to provide temporal assessment of chemical integration from the same individual and from ecologically sensitive species with little demographic consequence. Also, the chemical signatures in these metabolically inactive tissues do not dilute over time (Caumette et al. 2007), thus analyses can be performed on tissues from archival specimens (Struck et al. 2002).

Recently, cornified claw capsules have been increasingly used as a source of intrinsic markers for biogeochemical analysis (Hobson et al. 1999; Kielland 2001; Struck et al. 2002; Bearhop et al. 2003, 2005; Mazerolle and Hobson 2005; Belant et al. 2006; Clark et al. 2006; Hobson et al. 2006; Harrison et al. 2007*a*; DeVink et al. 2008; Fraser et al. 2008; Hobson and Robbins 2009; Reudink et al. 2009). Unlike feathers, claws are grown continuously, representing an unbroken time series of ecological data (Fraser et al. 2008). The biogeochemical signature contained in claws can be obtained either by examining the total isotopic or elemental content of an excised portion (often the claw tip, e.g., Mazerolle and Hobson 2005; Oppel and Powell 2008) to determine the animal's point of origin, or by examining the isotopic or elemental content at points along the length of the claw capsule in an attempt to track fine-scale shifts in foraging behaviour and habitat use over time (e.g., Kielland 2001; Belant et al. 2006; Harrison et al. 2007*a*; Fraser et al. 2008); we hereafter refer to these approaches separately as "claw tip sampling" and "time-series sampling". Regardless of the approach, it is important to note that the exact time of chemical integration into claw keratin is often difficult to determine, as rates and patterns of claw growth are poorly described (Oppel and Powell 2008).

To further explore the advantages and disadvantages of examining biogeochemical markers in claw keratin, we (*i*) provide a review on the development of the cornified claw sheath in several taxa (prenatal and postnatal), (*ii*) present a noninvasive technique for monitoring patterns and rates of claw growth from a model mammalian species, the American badger (*Taxidea taxus* (Schreber, 1777)), and (*iii*) provide suggestions for improving claw sampling strategies that will reduce the introduction of artefacts caused by the mixing of keratin of different ages. We focus on mammals because our understanding of biogeochemical signals in their tissues is dramatically underdeveloped compared with other taxa, such as birds. Our goal is to provide clarification and guidelines for future sampling of claw keratin that will enhance our ability to back-calculate the time of biogeochemical integration into this tissue.

Growth patterns of the epidermal cornified claw sheath

A typical vertebrate claw sheath is a hard keratinized and cornified modification of the epidermis that protects the top, sides, and tip of a digit, extending beyond the apex of the underlying phalanx. A claw is a narrow, downward curving structure common to many mammals, reptiles, birds, and some amphibians. A claw is composed primarily of a thick keratinous and cornified sheath, which is tightly associated with the dermis, subcutis, and periosteum of the distal phalanx. Claws can be manipulated by muscular actions and function as sensory organs, weapons, and tools used for scratching, digging, and holding (Chapman 1986; Hamrick 2003). Variability in claw morphology is likely related to their functional evolution (Spearman and Hardy 1985).

The embryonic development of a claw is associated with the growth of the terminal phalanx (Kato 1977; Alibardi 2008*a*), thus the shape and size of a claw capsule often reflect that of the underlying bone (Clark 1936). Mammalian claw sheaths contain hard α-keratins and glycine–tyrosine-rich or sulphur-rich keratin filament associated proteins, whereas reptilian and avian claws contain β-keratins and associated proteins (Alibardi 2008*b*). The basic architecture of

mammalian, avian, and reptilian claws is similar and consists of two unequally developed keratinous parts: the dorsal unguis (wall) and the ventral subunguis (sole). The unguis, covering the dorsal and lateral surfaces of the claw, is formed of tougher and more compact keratinized strata compared with the ventral subunguis (Mercer 1961). The different rates of growth and wear of these two parts maintains the shape, functional efficiency, and sharpness of the claw (Mercer 1961; Homberger et al. 2009).

Mammals

In mammalian claws, hard keratin contributes to the formation of the unguis (i.e., the wall of the claw sheath) and consists of six layers, as described in detail by Homberger et al. (2009). The cells of the epidermal germinal matrix are responsible for the generation of new epidermal cells, which will cornify and thus form the growth of the claw sheath. If the cornified claw sheath is mechanically detached using a scalpel from the underlying dermis, an opaque pale pink triangular-shaped area of living epidermis is visible, which extends forward along the dorsal aspect of the distal phalanx and marks the extent of the basal germinal (at the claw base, often partially covered by a skin fold) and terminal matrices (epidermis of the dorsal dermal papilla along the central dorsal ridge) (Clark 1936; Fig. 1).

Two thin superficial layers of hard-cornified tissues (i.e., external coronary and proximal cone horn) originate from a germinal matrix that surrounds the base of the claw or coronary band: the basal germinal matrix (Fig. 1). These superficial layers form a thin covering on the dorsal and lateral surfaces of the claw and are thinned and eventually removed by wear (Clark 1936; Homberger et al. 2009; Figs. 2, 3). A soft-cornified perioplic horn is deposited by the epidermis on the inner side of the unguicular pleat (skin fold) and likely acts to protect the superficial layers as they form (Homberger et al. 2009). The perioplic horn may be quickly removed by abrasive forces once beyond the margins of the unguicular pleat. The deeper keratinized layers are generated from either the distal portion of the basal germinal matrix in the coronary band or by the terminal matrix, which runs along the dorsal dermal papilla (Clark 1936; Thorndike 1968; Kato 1977; Homberger et al. 2009; Fig. 1). This epidermal terminal matrix generates keratinizing cells continuously along the length of the dorsal ridge (Clark 1936; Kato 1977; Homberger et al. 2009), causing variation in the dorsoventral thickness of the claw unguis as the number of layers of cornified cells in the apical cone horn increase from base to tip (Hamrick 1999; Fig. 2). The lateral walls of the claw are formed of the thick blade horn and the thinner underlying parietal horn. The blade horn is deposited by the basal matrix on the distal margin of the coronary band (Homberger et al. 2009). The parietal horn is produced by the claw bed epidermis covering the phalanx, distal to the coronary band (similar to the nail bed or hoof bed epidermis) (Figs. 1, 2). The epidermis covering the tip of the phalanx also deposits the soft terminal horn, while the ventral surface deposits the sole horn (subunguis), which is soft in mammalian claws but hard in ungulate hoofs. Both of these layers can be easily mechanically separated from the overlying hard cornified layers using a scalpel.

In the fetal claws of domestic cats (*Felis catus* L., 1758), the terminal matrix extends distally from the coronary band, along the dorsal ridge, comprising 46% of the total matrix length (Kato 1977). The terminal matrix in a claw of a marmoset (*Callithrix jacchus* (L., 1758)) is small relative to the basal matrix and comprises 19%–22% of the matrix length (Clark 1936; Thorndike 1968). Mice and some neotropical opossums have a highly reduced terminal matrix (Hamrick 2001, 2003). Clark (1936) noted that the three-dimensional extent and shape of the epidermal germinal matrix could influence the shape of the claw sheath by influencing the rate of keratinized cell proliferation from the epidermis. Morphological modifications in the length and diameter of the dermal papilla and flexibility or rigidity of the keratinized and cornified cells together influence the shape of the claw sheath (Siedamgrotzky 1871; Clark 1936). Our own morphological observations support this assertion. Using a compound microscope, we examined the disarticulated claws of American badger (n = 10 claw) and black bear (*Ursus americanus* Pallas, 1780) (n = 4 claws). We noted that the thickness of the cornified claw sheath increased with the three-dimensional extent and thickness of the dorsal dermal papilla. The dermal papilla in badger claws is visibly thicker than in bear claws, resulting in a more highly developed apical cone horn, which likely facilitates their semi-fossorial life style. This morphological variability in the structure of the claw sheath resulting from differences in an animal's life history was also noted in some breeds of domestic dogs (*Canis lupus familiaris* L., 1758) that frequently dig versus those that do not (Siedamgrotzky 1871). The thickness of the lateral walls (blade and parietal horn) of the claw capsule remains constant throughout the length of the claw, as keratin deposition in the lateral walls occurs predominantly from the coronary band and less substantially from the epidermis overlying the dermis of the phalanx.

Fig. 1. Digital photograph of a disarticulated and dismantled distal phalanx of a claw from a black bear (*Ursus americanus*) and the associated dermal structures (cornified claw sheath removed with a scalpel). The basal epidermal germinal matrix surrounds the dermis of the coronary band at the claw base depositing the superficial keratin layers (external coronary and proximal cone horn) proximally and the thicker blade horn distally. The terminal matrix (living epidermis) extends along the dermis of the central dorsal ridge and deposits the apical cone horn. The epidermis covering the underlying phalanx deposits the sole, parietal, terminal, and crest horn.

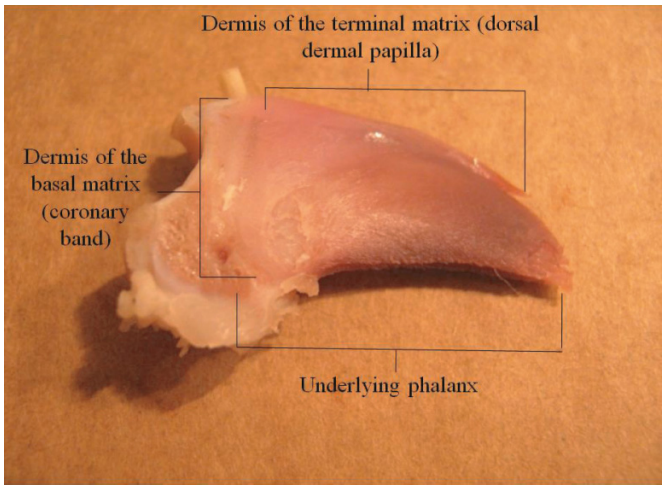


Fig. 2. Diagram of a sagitally sectioned and serial cross-sectioned mammalian claw depicting the nine keratinized layers. The soft keratinized parts of the claw capsule are indicated with an asterisk and the extent of the underlying epidermal germinal matrix is depicted with a broken line in the sagital section. Variation in the thickness of the apical cone horn and disappearance of the superficial keratinized layers (i.e., perioplic, external coronary horn, and proximal cone horn) from the base to the tip are illustrated in the series of cross-sections. At the tip of the claw, the terminal horn and crest horn are deposited by the underlying epidermis of the phalanx. Arrows indicate level and relative angle of cross-section. Figure modified from Homberger et al. (2009) and reproduced with permission of J. Anat., vol. 214, issue 4, p. 638,

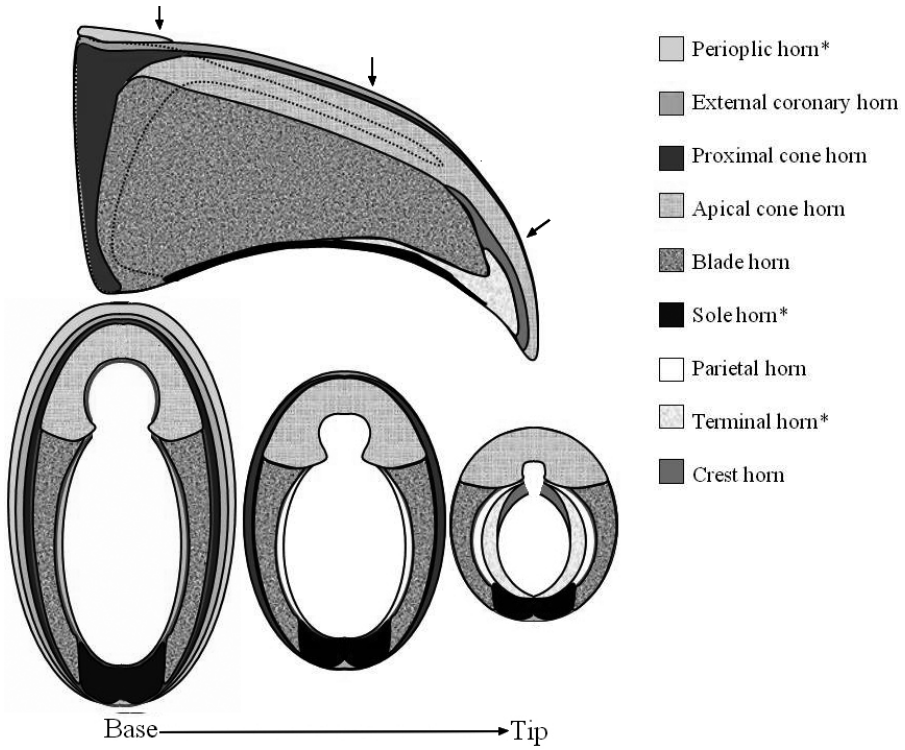


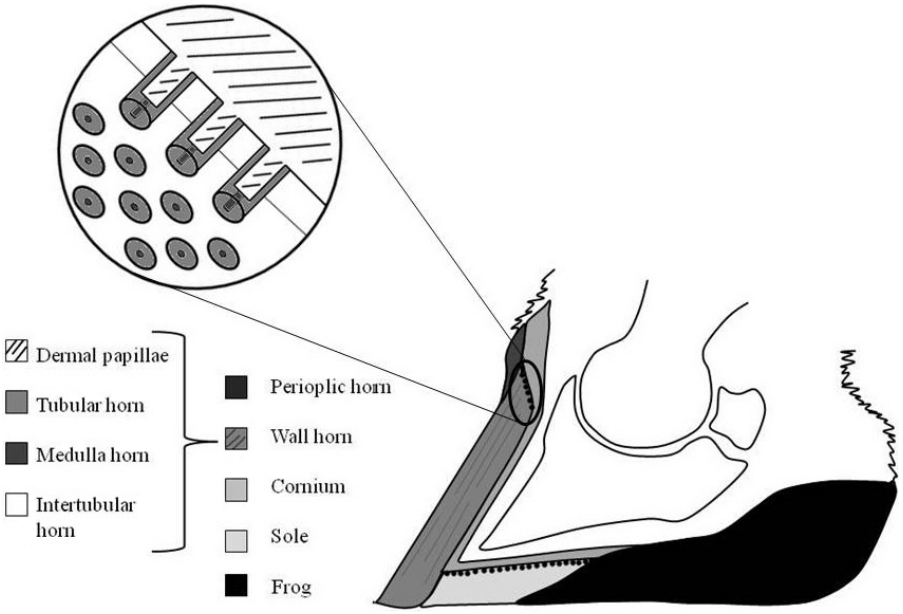
Fig. 3. Photograph of the claws of an American badger (*Taxidea taxus*) with soldering iron markings (arrows) following 40 days of growth. The outward facing surface of the lateral wall and central dorsal ridge were marked to document patterns of growth and variability in growth rates.

Hooves are a keratinized epidermal derivative found in ungulates. Hooves differ from a typical mammalian "claw" in their morphology and growth, and are considered one of the most complex and specialized digital end organs comparable with avian feathers (Bragulla and Hirschberg 2003). The hoof consists of three main parts, the wall, sole, and frog (Fig. 4; Chapman 1986); the structural morphology of the hoof is well described in the literature (see Pollitt 1994; Pellmann 1995; Bragulla 1996; Henke 1997; Douglas and Thomason 2000; Thomason et al. 2001; Bragulla and Hirschberg 2003). At the base of the hoof is a periople or perihoof (similar to the perioplic horn in claws), which is a band of nonpigmented soft keratin. The perihoof protects the developing wall horn and is removed by wear. Cornified cells of the wall horn are produced by the epidermis covering a series of dermal papillae found in the coronary band at the base of the hoof (Fig. 4). Tubules of hard keratin are deposited around the dermal papilla (Maierl and Böhmisch 2001), while a softer keratin fills the medulla space (Maierl and Mülling 2004). The intertubular horn is of intermediate hardness and holds the tubular wall horn together (Fig. 4; Mülling et al. 1999). Because not every layer of wall horn has the same starting point, the wall is very thin at the periople and only reaches its full thickness approximately 15 mm from the top of the hoof. Therefore, the cornified layers of cells are deposited in a linear fashion (like those in human fingernails), but an offset is observed in the age of cornified claw material from the outer (older) to the deeper (younger) cornified layers (Harrison et al. 2007*a*).

Birds and reptiles

Patterns of claw growth in birds have received very little

Fig. 4. Diagram of a sagitally sectioned hoof and schematic of horn production from the dermal papillae. The soft periople horn protects the developing wall horn at the base of the hoof. The wall horn consists of three layers: hard tubular horn deposited around the dermal papillae; a soft medulla horn that fills the medulla space; and an intertubular horn that fills the space surrounding the tubular wall horn. The cornified layers of wall horn have staggered starting points along the length of the coronary band at the base of the claw, creating an offset in the age of keratinized cells from the outer (older) in inner (younger) layers. Figure modified from Harrison et al. (2007*a*) and reproduced with permisson of Rapid Commun. Mass Spectrom., vol. 21, issue 24, p. 3972, © 2007 John Wiley and Sons.



empirical examination (Lucas and Stettenheim 1972; Spearman and Hardy 1985), although the process is thought to be similar to those seen in reptiles (Alibardi 2008*b*). Claws in birds and reptiles have an epidermal matrix zone (Maddin et al. 2007), although the three-dimensional extent and shape of this matrix still remains to be detailed. We were unable to identify a distinct tissue corresponding to the dermis of the basal or terminal matrix upon microscopic examination of samples ($n < 5$ claws in all cases) of claws of Blue Jays (*Cyanocitta cristata* (L., 1758)), American White Pelicans (*Pelecanus erythrorhynchos* J.F. Gmelin, 1789), Canada Geese (*Branta canadensis* (L., 1758)), and Common Map Turtles (*Graptemys geographica* (Le Sueur, 1817)); the claws were sectioned and disarticulated using a rotary saw and scalpel and examined under a compound microscope. The claw sheath thickness increased continuously in the dorsal, lateral, and ventral aspects of the claws of the American White Pelican and Canada Goose, as keratinized cells accumulate distally to the apex. A similar pattern of cell deposition is seen in reptile claws, as old and new keratinized cells accumulate towards the tip (Alibardi 2008*c*; Maddin et al. 2007). These observations suggest the epidermal germinal matrix, in at least some avian and reptilian claws, likely covers the entire extent of the terminal phalanx. A superficial keratin layer (similar to the external coronary horn in mammalian claws) is identifiable in both avian and reptilian claws and can be removed mechanically with a scalpel.

Amphibians

Claws in nonamniotes are present in a few species of urodeles and anurans. They are formed of α-keratins with some associated proteins (Alibardi 2008*b*). In contrast to amniotes, amphibian claws lack a distinct matrix zone as seen in mammals (Maddin et al. 2007) and keratinized cells are deposited by the basal epidermal layer along the entire length of the dermis overlying the phalanx (Maddin et al. 2007, 2009). Keratinized cells are pushed perpendicularly away from the dermis during claw formation rather than moving predominantly parallel towards the claw apex, as seen in mammals and reptiles. This distinct difference in the pattern of claw formation is indicative of an independent acquisition of claws in these taxonomic lineages (Maddin et al. 2007).

Techniques for monitoring mammalian claw growth

Hahn et al. (1986) developed a technique to noninvasively monitor growth in hooves of cattle (*Bos taurus* L., 1758), and we modified this technique to monitor growth in a model clawed organism, American badgers. We monitored growth rates of claws of two captive badgers, which were held in a 16′ × 36′ (4.88 m × 10.97 m) outdoor pen where they could dig naturally. On 30 September 2009, we captured and immobilized the badgers following protocol approved by Trent University's Animal Care Committee. Using a soldering iron, we marked five (right digits #2, 3, 4, 5 and left digit #4) of their front claws within 3–6 mm of the skin fold at the claw base (Fig. 3). The claws we sampled were selected based on their similar morphology and size, which facilitates comparisons both within and between animals. We burned the marks to a depth of approximately 0.5–1.0 mm, where total horn thickness in badger claws varies from approximately 1.0–3.0 mm. Markings were made on both the outward facing surface of the lateral wall and central dorsal ridge to better document growth patterns and within-claw variability in growth rates. Using a

standardized protocol to reduce error, measurements to the skin fold at the claw base were made to the nearest 0.1 mm using callipers. On 9 November 2009, we again captured and immobilized the badgers and measured the distance between the marks and the skin fold. We then calculated daily growth rates (Δ length/Δ time) over a 40-day period. The total length (mean ± SD) of all monitored claws was 37.3 ± 12.2 mm (when initially marked) and 40.64 ± 4.8 (after 40 days). Growth rates did not vary substantially between the dorsal ridge (0.262 ± 0.08 mm/day; mean ± SD) and lateral wall (0.281 ± 0.08 mm/day). Therefore, given the growth rate and mean length of a claw, the complete replacement of the claw capsule in American badgers seems to take approximately 130–140 days.

Stable carbon and nitrogen isotope marking (Harrison et al. 2007*b*) and radioactive labelling (Pollitt 1990) have been used to monitor the three-dimensional growth rates of hooves of bovine and horse (*Equus caballus* L., 1758) and to measure the offset in the cornified layers. The major disadvantage to the method is that the animals under study must be euthanized to collect final results. We found marks made with the soldering iron to be a suitable noninvasive technique for use on captive mammals and could easily be deployed on their wild counterparts.

Claws as biogeochemical indicators

Mammals

Because keratin is deposited along the length of the thickened central dorsal aspect of mammalian claws through to the tip (Fig. 2), these portions of the claw do not seem appropriate for obtaining time-series biogeochemical data. However, the blade horn keratin covering the lateral walls of mammalian claws is likely more reliable for time-series biogeochemical analysis than the central dorsal ridge or tip of the claw, because the keratin forming the blade horn appears to be deposited exclusively by the basal germinal matrix in the coronary band. Therefore, the outside wall of the blade horn encapsulates an unbroken time series of ecological data, uncomplicated by additional keratin layers deposited along its length. Our investigation of growth rates of badger claws indicates that replacement of the keratinized claw capsule occurs in 4–4.5 months. The keratinized claw capsules of domestic cats, which grow at a rate of 1.9 mm/week, are replaced in 6–9 months (Verde 2005). Growth rates of mammalian claws need to be better defined in wild and domesticated animals to improve inferences from time-series biogeochemical analyses, as it is very likely that growth rates vary both intra- and inter-specifically owing to differences in nutritional status, age, activity level, and function, although this assertion needs further empirical investigation.

Growth rates of hooves in cattle can vary from 3.2 mm/month (MacCallum et al. 2002) to 10.5 mm/month (Harrison et al. 2007*b*), depending on the breed and type of confinement. A substantial amount of research on variability in growth rates of hooves also indicates that nutrition (Wheeler 1966; Greenough et al. 1972; Mülling et al. 1999), reproductive status (Hahn et al. 1978; Mülling et al. 1999; MacCallum et al. 2002), season (Dowling and Nay 1960; Berman and Volcani 1961; Prentice 1973; Adams 1974), genetics (Greenough et al. 1972; Glicken and Kendrick 1977; Gilmore 1978), and age (Prentice 1973) can all influence the rates of hoof growth and wear. Consideration must also be given to the age of keratin (time since cells cornified) at different depths. An offset of 9.3–11.7 days/mm in depth has been reported in bovine hooves (Harrison et al. 2007*a*).

Birds and reptiles

We were unable to identify a structure in bird or reptilian claws that could unequivocally offer time-series data. Unlike the lateral aspect of mammalian claws, the lateral walls of avian and reptilian claws appear to increase in thickness distally, suggesting continuous keratin deposition towards the claw tip. By assaying only the tip of a bird or reptile claw, investigators are effectively homogenizing old and new keratin that has been differentially deposited over an unknown period, which may explain some of the problems identified by Oppel and Powell (2008) in trying to use claw isotopes to determine the migratory origin of King Eiders (*Somateria spectabilis* (L., 1758)). Variability in growth rates of bird claws shown by Bearhop et al. (2003) and Mazerolle and Hobson (2005) also suggests that using data from one species as a surrogate for those with an outward similarity may be inadvisable, as birds, like other taxa, may experience variable rates of claw growth coinciding with periods of fluctuating energetic demands, such as breeding or migration.

Amphibians

Amphibian claws may present an underappreciated and potentially valuable source tissue for time-series chemical analysis, as growth is uncomplicated and is effectively linear in a perpendicular plane. Thus, a cross-section of the claw would consist of layers very similar to the rings of a tree. How quickly keratin is deposited into these claws, however, still remains to be detailed but could be determined in a laboratory or field setting using claw-marking trials.

Conclusion

We have reviewed patterns of claw growth in several taxa and demonstrated that there is substantial variability in growth within regions of the claw and between species. Therefore, linking biogeochemical signatures in the claw with a time-series of the animal's movement history can only be accurate when we understand and account for patterns of claw development and rates of claw growth in the species under study. Though there has recently been a renewed interest in the study of claw development and patterns of growth, particularly amongst veterinary scientists (Hamrick 2001, 2003; Maddin et al. 2007, 2009; Alibardi 2008*a*, 2008*b*, 2008*c*; Homberger et al. 2009), most investigations do not take place with biogeochemical applications in mind. As a result, there has been a lack of relevant data on patterns of claw growth that are directly applicable to ecologists.

Claw keratin holds a great deal of promise for investigations of previous movement and foraging environments. However, homogenization of old and new keratin layers at the tip of bird and reptile claws, and the dorsal ridge and tip of mammal claws is considerable. This characteristic has important implications for studies using these tissues de-

pending on the nature of the study and the questions being asked. If the goal of an investigation using claw keratin is to use claw tip sampling to assess a single point of origin of an animal that is actively migrating (e.g., birds), then perhaps the problems associated with keratin homogenization are not yet relevant. However, if migration is protracted or an animal has already reached its destination, then claws may have grown to a point where keratin homogenization would interfere with such an investigation and another tissue source for isotopes should be considered (e.g., Oppel and Powell 2008). In short, we urge caution in using claw tip sampling to determine the origin of a migratory animal and emphasize that the claw needs to be sampled very shortly after migratory departure. We further advise against sampling a claw tip from an individual that has resided for any length of time at either the wintering or breeding grounds, as keratin homogenization will influence the validity of the isotopic signal.

If an investigation seeks to gain time-series location data from claws, then keratin homogenization presents a major obstacle. Amphibian claws, ungulate hooves, and the lateral portions of some mammal claws may be more reliable temporal indicators of previous foraging environments, as their growth appears to be fairly predictable once the growth rate is known and offset considered (in the case of hooves). Using these portions of the claw would also allow for finer-scale studies of movement during the life of individual animals. In all cases, there is a need for better documentation of growth rates and keratin deposition through cell-labelling, scanning electron microscopy, and three-dimensional reconstruction based on X-ray computer tomography. Claw growth is a complex and variable process, both between and within taxa and warrants further attention prior to biogeochemical assay of these tissues.

While the application of biogeochemistry to ecological research is further improved and developed, we make the appeal that greater attention should be paid to the physiology of the structures under study, as there are crucial gaps in our foundational knowledge of anatomy and morphology. Nonetheless, we should be encouraged by the significant potential for biogeochemical techniques to continue to revolutionize the way we approach and execute studies in ecology. Despite the challenges presented here, the possibilities for further novel and influential research are very exciting.

Acknowledgements

We thank Josh Sayers for extensive input and editorial assistance. Chris Sharp, the Ontario Badger Recovery Team, and the Ontario Ministry of Natural Resources provided claws for dissection. Laurene Ratcliffe provided comments and editorial assistance on early drafts of this manuscript. The final manuscript was improved by the constructive comments of two anonymous reviewers. Permission to reproduce modified figures was kindly provided by Dominique Homberger, Sabine Harrison, and their co-authors. We are grateful to the Muskoka Wildlife Centre, who gave us access to their two captive badgers and assisted with claw marking trials. This work was supported financially and logistically by the Ontario Ministry of Natural Resources, Queen's University Facility for Isotope Research, and the Natural Sciences and Engineering Research Council of Canada.

References

Adams, O.R. 1974. Lameness in horses. Lea and Febiger, Philadelphia, Pa.

Alibardi, L. 2008*a*. Ultrastructural characteristics of the process of cornification in developing claws of the bushtail possum (*Trichosurus vulpecula*). Acta Zool. **89**: 1–15. doi:10.1111/j.1463-6395.2008.0360.x.

Alibardi, L. 2008*b*. Microscopic analysis of lizard claws morphogenesis and hypothesis on its evolution. Acta Zool. **89**(2): 169–178. doi:10.1111/j.1463-6395.2007.00312.x.

Alibardi, L. 2008*c*. Cornification in developing claws of the common Australian skink (*Lampropholis guichenoti*) (Squamata, Lacertidae). Ital. J. Zool. **75**(4): 327–336. doi:10.1080/11250000801973334.

Bearhop, S., Waldron, S., Votier, S.C., and Furness, R.W. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol. Biochem. Zool. **75**(5): 451–458. doi:10.1086/342800. PMID:12529846.

Bearhop, S., Furness, R.W., Hilton, G.M., Votier, S.C., and Waldron, S. 2003. A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. Funct. Ecol. **17**(2): 270–275. doi:10.1046/j.1365-2435.2003.00725.x.

Bearhop, S., Fiedler, W., Furness, R.W., Votier, S.C., Waldron, S., Newton, J., Bowen, G.J., Berthold, P., and Farnsworth, K. 2005. Assortative mating as a mechanism for rapid evolution of a migratory divide. Science (Washington, D.C.), **310**(5747): 502–504. doi:10.1126/science.1115661. PMID:16239479.

Belant, J.L., Kielland, K., Follmann, E.H., and Adams, L.G. 2006. Interspecific resource partitioning in sympatric ursids. Ecol. Appl. **16**(6): 2333–2343. doi:10.1890/1051-0761(2006)016[2333:IRPISU]2.0.CO;2. PMID:17205908.

Ben-David, M., Flynn, R.W., and Schell, D.M. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia (Berl.), **111**(2): 280–291. doi:10.1007/s004420050236.

Berman, A., and Volcani, R. 1961. Seasonal and regional variation in coat characteristics of dairy cattle. Aust. J. Agric. Res. **12**(3): 528–538. doi:10.1071/AR9610528.

Bragulla, H. 1996. Zur fetalen Entwicklung des Pferdehufes. Doctoral dissertation, Faculty of Veterinary Medicine, Free University of Berlin, Berlin, Germany.

Bragulla, H., and Hirschberg, R.M. 2003. Horse hooves and bird feathers: two model systems for studying the structure and development of highly adapted integumentary accessory organs—the role of the dermo-epidermal interface for the microarchitecture of complex epidermal structures. J. Exp. Zool. B Mol. Dev. Evol. **298B**(1): 140–151. doi:10.1002/jez.b.31.

Caumette, G., Ouypornkochagorn, S., Scrimgeour, C.M., Raab, A., and Feldmann, J. 2007. Monitoring the arsenic and iodine exposure of seaweed-eating North Ronaldsay sheep from the gestational and suckling periods to adulthood by using horns as a dietary archive. Environ. Sci. Technol. **41**(8): 2673–2679. doi:10.1021/es062241y. PMID:17533823.

Chamberlain, C.P., Blum, J.D., Holmes, R.T., Feng, X., Sherry, T.W., and Graves, G.R. 1997. The use of isotope tracers for identifying populations of migrating birds. Oecologia (Berl.), **109**(1): 132–141. doi:10.1007/s004420050067.

Chapman, R.E. 1986. Hair, wool, quill, nail, claw, hoof and horn. *In* Biology of the integument. *Edited by* J. Bereither-Hahn, G.A. Matoltsy, and K. Sylvia-Richards. Springer-Verlag, New York. pp. 293–312.

Clark, W.E.L. 1936. The problem of the claw in primates. Proc. Zool. Soc. Lond. **1**: 1–24.

Clark, R.G., Hobson, K.A., and Wassenaar, L.I. 2006. Geographic variation in the isotopic (δD, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. Can. J. Zool. **84**(10): 1395–1401. doi:10.1139/Z06-135.

Dalerum, F., and Angerbjorn, A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia (Berl.), **144**(4): 647–658. doi:10.1007/s00442-005-0118-0.

DeVink, J.-M.A., Clark, R.G., Slattery, S.M., and Scheuhammer, A. 2008. Cross-seasonal association between winter trophic status and breeding ground selenium levels in boreal white-winged scoters. Avian Conserv. Ecol. **3**(1): 3. Available from http://www.ace-eco.org/vol3/iss1/art3/.

Douglas, J.E., and Thomason, J.J. 2000. Shape, orientation and spacing of the primary epidermal laminae in the hooves of neonatal and adult horses (*Equus caballus*). Cells Tissues Organs, **166**(3): 304–318. doi:10.1159/000016744. PMID:10765026.

Dowling, D.G., and Nay, T. 1960. Cyclic changes in the follicles and hair coat in cattle. Aust. J. Agric. Res. **11**(6): 1064–1071. doi:10.1071/AR9601064.

Fraser, K.C., Kyser, T.K., Robertson, R.J., and Ratcliffe, L.M. 2008. Seasonal patterns in hydrogen isotopes of claws from breeding wood-warblers (Parulidae): utility for estimating migratory origins. Avian Conserv. Ecol. **3**(1): 2. Available from http://www.ace-eco.org/vol3/iss1/art2/.

Gilmore, J.A. 1978. The effects of housing, age, breed and time after trimming on hoof measurements. J. Dairy Sci. **61**(Suppl. 1): 83. [Abstr.]

Glicken, A., and Kendrick, J.W. 1977. Hoof overgrowth in Holstein–Friesian dairy cattle. J. Hered. **68**(6): 386–390. PMID: 608944.

Greenough, P.R., Maccallum, F.J., and Weaver, A.D. 1972. Lameness in cattle. J.B. Lippincott and Co., Philadelphia, Pa.

Hahn, M.V., Wilk, J.C., and McDaniel, B.T. 1978. Effects of lactation number and stage of lactation changes in foot characteristics of Jersey cows. J. Dairy Sci. **61**(Suppl. 1): 84. [Abstr.]

Hahn, M.V., McDaniel, B.T., and Wilk, J.C. 1986. Rates of hoof growth and wear in Holstein cattle. J. Dairy Sci. **69**(8): 2148–2156. doi:10.3168/jds.S0022-0302(86)80647-6. PMID:3760302.

Hamrick, M.W. 1999. Pattern and process in the evolution of primate nails and claws. J. Hum. Evol. **37**(2): 293–297. doi:10.1006/jhev.1999.0330. PMID:10444354.

Hamrick, M.W. 2001. Development and evolution of the mammalian limb: adaptive diversification of nails, hooves, and claws. Evol. Dev. **3**(5): 355–363. doi:10.1046/j.1525-142X.2001.01032.x. PMID:11710767.

Hamrick, M.W. 2003. Evolution and development of mammalian limb integumentary structures. J. Exp. Zool. B Mol. Dev. Evol. **298**(1): 152–163. doi:10.1002/jez.b.32.

Harrison, S.M., Monahan, F.J., Zazzo, A., Bahar, B., Moloney, A.P., Scrimgeour, C.M., and Schmidt, O. 2007*a*. Three-dimensional growth of bovine hoof as recorded by carbon stable isotope ratios. Rapid Commun. Mass Spectrom. **21**(24): 3971–3976. doi:10.1002/rcm.3309. PMID:17994529.

Harrison, S.M., Zazzo, A., Bahar, B., Monahan, F.J., Moloney, A.P., Scrimgeour, C.M., and Schmidt, O. 2007*b*. Using hooves for high-resolution isotopic reconstruction of bovine dietary history. Rapid Commun. Mass Spectrom. **21**(4): 479–486. doi:10.1002/rcm.2861. PMID:17230438.

Henke, F. 1997. Hufbeinträger und Hufmechanismus im Seiten-, Trachten- und Eckstrebenteil des Pferdehufes. Doctoral dissertation, Faculty of Veterinary Medicine, Free University of Berlin, Berlin, Germany.

Hobson, K.A., and Clark, R.G. 1992. Assessing avian diets using stable isotopes I: Turnover of $\delta^{13}C$ in tissues. Condor, **94**(1): 181–188. doi:10.2307/1368807.

Hobson, K.A., and Robbins, M.B. 2009. Origins of late-breeding nomadic sedge wrens in North America: limitations and potential of hydrogen-isotope analyses of soft tissues. Condor, **111**(1): 188–192. doi:10.1525/cond.2009.080001.

Hobson, K.A., and Wassenaar, L.I. 1997. Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia (Berl.), **109**(1): 142–148. doi:10.1007/s004420050068.

Hobson, K.A., Alisauskas, R.T., and Clark, R.G. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor, **95**(2): 388–394. doi:10.2307/1369361.

Hobson, K.A., Atwell, L., and Wassenaar, L.I. 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. Proc. Natl. Acad. Sci. U.S.A. **96**(14): 8003–8006. doi:10.1073/pnas.96.14.8003. PMID:10393937.

Hobson, K.A., Van Wilgenburg, S., Wassenaar, L.I., Hands, H., Johnson, W.P., O'Meilia, M., and Taylor, P. 2006. Using stable hydrogen isotope analysis of feathers to delineate origins of harvested sandhill cranes in the central flyway of North America. Waterbirds, **29**(2): 137–147. doi:10.1675/1524-4695(2006)29[137:USHIAO]2.0.CO;2.

Homberger, D.G., Ham, K., Ogunbakin, T., Bonin, J.A., Hopkins, B.A., Osborn, M.L., Hossain, I., Barnett, H.A., Matthews, K.L., II, Butler, L.G., and Bragulla, H.H. 2009. The structure of the cornified claw sheath in the domesticated cat (*Felis catus*): implications for the claw-shedding mechanism and the evolution of cornified digital end organs. J. Anat. **214**(4): 620–643. doi:10.1111/j.1469-7580.2009.01068.x. PMID:19422432.

Kato, T. 1977. A study on the development of the cat claw. Hiroshima J. Med. Sci. **26**(2–3): 103–126. PMID:591378.

Kielland, K. 2001. Stable isotope signatures of moose in relation to seasonal forage composition: a hypothesis. Alces, **37**(2): 329–337.

Lucas, A.M., and Stettenheim, P.R. 1972. Growth of follicles and feathers. *In* Avian anatomy integument. Agriculture Handbook, U.S. Department of Agriculture, Washington, D.C. pp. 341–419.

MacCallum, A.J., Knight, C.H., Hendry, K.A.K., Wilde, C.J., Logue, D.N., and Offer, J.E. 2002. Effects of time of year and reproductive state on the proliferation and keratinisation of bovine hoof cells. Vet. Rec. **151**(10): 285–289. PMID:12243269.

Maddin, H.C., Musat-Marcu, S., and Reisz, R.R. 2007. Histological microstructure of the claws of the African clawed frog, *Xenopus laevis* (Anura: Pipidae): implications for the evolution of the claw in tetrapods. J. Exp. Zool. Part B Mol. Dev. Evol. **308**(3): 259–268. doi:10.1002/jez.b.21145.

Maddin, H.C., Eckhart, L., Jaeger, K., Russell, A.P., and Ghannadan, M. 2009. The anatomy and development of the claws of *Xenopus laevis* (Lissamphibia: Anura) reveal alternate pathways of structural evolution in the integument of tetrapods. J. Anat. **214**(4): 607–619. doi:10.1111/j.1469-7580.2009.01052.x. PMID:19422431.

Maierl, J., and Böhmisch, R. 2001. Grundlagenkenntnisse für eine fachmännische Klauenpflege: Funktionelle anatomie der rinderklaue. Großtierpraxis, **2**: 5–13.

Maierl, J., and Mülling, C. 2004. Funktionelle anatomie. *In* Erkrankungen der klauen und zehen des rindes. *Edited by* A. Fiedler, J. Maierl, and K. Nuss. Schattauer, Stuttgart, Germany. pp. 1–28.

Mazerolle, D.F., and Hobson, K.A. 2005. Estimating origins of

short-distance migrant songbirds in North America: contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. Condor, **107**(2): 280–288. doi:10.1650/7681.

Mercer, E.H. 1961. Keratin and keratinization. Pergamon Press, Oxford.

Mülling, C.K., Bragulla, H.H., Reese, S., Budras, K.-D., and Steinberg, W. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anat. Histol. Embryol. **28**(2): 103–108. doi:10.1046/j.1439-0264.1999.00180.x. PMID:10386004.

Oppel, S., and Powell, A.N. 2008. Assigning king eiders to wintering regions in the Bering Sea using stable isotopes of feathers and claws. Mar. Ecol. Prog. Ser. **373**: 149–156. doi:10.3354/meps07744.

Pearson, S.F., Scott, F., Levey, D.J., Greenberg, C.H., and Martínez del Rio, C. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia (Berl.), **135**: 516–523. doi:10.1007/s00442-003-1221-8. PMID:16228250.

Pellmann, R. 1995. Struktur und Funktion des Hufbeinträgers beim Pferd. Doctoral dissertation, Faculty of Veterinary Medicine, Free University of Berlin, Berlin, Germany.

Pollitt, C.C. 1990. An autoradiographic study of equine hoof growth. Equine Vet. J. **22**(5): 366–368. doi:10.1111/j.2042-3306.1990.tb04293.x. PMID:2226405.

Pollitt, C.C. 1994. The basement membrane at the equine hoof dermal epidermal junction. Equine Vet. J. **26**(5): 399–407. doi:10.1111/j.2042-3306.1994.tb04410.x. PMID:7527338.

Prentice, D.E. 1973. Growth and wear rates of hoof horn in Ayrshire cattle. Res. Vet. Sci. **14**(3): 285–290. PMID:4805151.

Reudink, M.W., Studds, C.E., Marra, P.P., Kyser, T.K., and Ratcliffe, L.M. 2009. Plumage brightness predicts non-breeding season territory quality in a long-distance migratory songbird, the American redstart *Setophaga ruticilla*. J. Avian Biol. **40**(1): 34–41. doi:10.1111/j.1600-048X.2008.04377.x.

Rubenstein, D.R., and Hobson, K.A. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol. Evol. **19**(5): 256–263. doi:10.1016/j.tree.2004.03.017. PMID:16701265.

Siedamgrotzky, O. 1871. Ueber die Struktur und das Wachstum der Hornscheide, der Wiederkäuer, und der Fleischfresser. G. Schönfeld's Verlagsbuchhandlung, Dresden, Germany (C.A. Werner).

Spearman, R.I.C., and Hardy, J.A. 1985. Integument. *In* Form and function of birds. *Edited by* A.S. King and J. McLelland. Academic Press Inc., London. pp. 1–56.

Struck, U., Altenbach, A.V., Gaulke, M., and Glaw, F. 2002. Tracing the diet of the monitor lizard *Varanus mabitang* by stable isotope analyses (δ^{15}N, δ^{13}C). Naturwissenschaften, **89**(10): 470–473. doi:10.1007/s00114-002-0361-8. PMID:12384723.

Thomason, J.J., Douglas, J.E., and Sears, W. 2001. Morphology of the laminar junction in relation to the shape of the hoof capsule and distal phalanx in adult horses (*Equus caballus*). Cells Tissues Organs, **168**(4): 295–311. doi:10.1159/000047846. PMID:11275696.

Thorndike, E.E. 1968. A microscopic study of the marmoset claw and nail. Am. J. Phys. Anthropol. **28**(3): 247–261. doi:10.1002/ajpa.1330280314. PMID:4970938.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia (Berl.), **57**(1–2): 32–37. doi:10.1007/BF00379558.

Verde, M. 2005. Canine and feline nail diseases. *In* Proceeding of the NAVC North American Veterinary Conference, Orlando, Fla., 8–12 June 2005. North American Veterinary Conference, Gainesville, Fla. Available online from www.ivis.org [accessed 1 November 2009]

Voigt, C.C., Matt, F., Michener, R., and Kunz, T.H. 2003. Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. J. Exp. Biol. **206**(8): 1419–1427. doi:10.1242/jeb.00274. PMID:12624176.

Webster, M.S., Marra, P.P., Haig, S.M., Bensch, S., and Holmes, R.T. 2002. Links between worlds: unravelling migratory connectivity. Trends Ecol. Evol. **17**(2): 76–83. doi:10.1016/S0169-5347(01)02380-1.

Wheeler, J.L. 1966. Hoof growth, possible index of nutrition in grazing animals. Proc. Aust. Soc. Anim. Prod. **6**: 350–353.