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RESEARCH ARTICLE

Growth rate and stable carbon and nitrogen isotope trophic discrimination factors of lion and leopard whiskers

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Rationale: Stable isotope analysis (SIA) of whiskers has been used to identify temporal feeding habits, intra-population diet variation, as well as individual dietary specialisation of marine and terrestrial carnivores. However, the potential of the method to disclose such dietary information for large wild felids is hampered by lack of information on species-specific whisker growth rates, whisker growth patterns and whisker-diet trophic discrimination factors (TDFs).

Methods: Whisker growth rates and growth patterns were measured for four lions (*Panthera leo*) and one leopard (*Panthera pardus*) held at the National Zoological Gardens, Pretoria, South Africa. Actively growing whiskers of the felids were 'marked' four times over 185 days using ^{13}C-depleted, C_3-based giraffe (*Giraffa camelopardalis*) meat. The periods with low $\delta^{13}C$ values, identified following serial sectioning of the regrown whiskers at 1 mm intervals and isotopic analysis, were then correlated to specific giraffe meat feeding bouts and hence growth periods. $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs were estimated for five lions whose diet remained consistent over multiple years.

Results: The whisker growth rates of three lionesses and the leopard were similar (mean = 0.65 mm day^{-1}), despite species, sex and age differences. There was a decrease in whisker growth rate over time, suggesting a non-linear whisker growth pattern. However, linear and non-linear growth simulations showed slight differences between the two growth patterns for the proximal ~50 mm of whiskers. $\delta^{13}C$ and $\delta^{15}N$ lion whisker-diet TDFs were also similar amongst individuals (mean = 2.7 ± 0.12 ‰ for $\delta^{13}C$ values and 2.5 ± 0.08 ‰ for $\delta^{15}N$ values), irrespective of age and sex.

Conclusions: The whisker growth rate and $\delta^{13}C$ and $\delta^{15}N$ lion whisker-diet TDFs obtained in this study can be applied in future studies to assign dietary information contained in analysed felid whiskers to the correct time period and improve deductions of prey species consumed by wild felids.

1 | INTRODUCTION

Large felids, like other mammalian carnivores, play an integral role in maintaining biodiversity and ecosystem functioning, yet they are among the most threatened carnivores.[1-3] Novel measures that can aid informed decision-making for the successful conservation of large carnivore species *in situ* and restoration in areas where they have been extirpated are needed.[1,4] Such measures include in-depth understanding of their feeding ecology.[5,6] The diets of wild felids have been broadly explored using an array of traditional methods such as faecal analysis (e.g.[7-9]), continuous follows (e.g.[10-12]), global positioning system (GPS) cluster analysis (e.g.[13,14]), and spoor tracking (e.g.[15-17]). These methods contribute to a better understanding of the feeding habits of large felids in their respective natural habitats. However, limitations inherent in these methods make it difficult to acquire longitudinal dietary records of individuals, which can identify temporal dietary patterns, as well as intra-population diet variations.[18,19] Stable carbon and nitrogen isotope analyses of animal tissues provide an approach to obtain novel insights into the diets of felids at spatial and temporal scales that were previously unobtainable.[20,21]

The utility of stable carbon and nitrogen isotopes in dietary studies is based on the concept that isotope ratios assimilated in consumer tissues reflect those of diet and habitat(s) exploited by the respective individuals.[22,23] These isotopes are incorporated in a predictable manner which can be traced through analysis of both consumer and prey tissues.[21] Stable carbon isotopes (presented by convention as $\delta^{13}C$ values) can directly identify the source of primary production at the base of the food chain (e.g. plants with either a C_3 or C_4

photosynthetic pathway) mainly because there is little isotopic discrimination between an animal and its diet.[24-26] Conversely, stable nitrogen isotope ratios ($\delta^{15}N$ values) are generally used to determine the trophic level of a consumer due to the predictable increase in $\delta^{15}N$ values at each trophic step.[27-29]

Animal tissues such as blood plasma, red blood cells, muscle and bone can be analysed to provide dietary information integrated at different time periods, from days to years.[30] Information provided by these metabolically active tissues is dependent on the tissue turnover rate, which is the length of time over which the molecules of a specific tissue are lost and replaced.[31,32] Inert keratinous tissues (i.e. baleen, claws, hair and whiskers), in which no turnover takes place, archive foraging habits of an individual at the time of their growth and this information remains unaltered over time.[33] Serial isotopic sampling of such inert tissues, at least those which grow incrementally, therefore provides a longitudinal record of dietary variation within individuals,[19,33] enabling investigations of diet switching patterns and individual niche specialisation.[18,34,35] Amongst mammalian carnivores, whiskers are a viable material for such investigations because they archive dietary information at relatively short, ecologically relevant time scales of weeks to months.[36] Moreover, whiskers are anatomically thicker than pelage hair[37] making it possible to section and analyse the tissue at high-resolution temporal scales.[31] The stable isotope analysis of whiskers has revealed information about the dietary niche structure of populations of both aquatic (various pinnipeds and sea otters) (e.g.[19,38-40]) and terrestrial (badgers, minks, foxes and coyotes) carnivore species.[35,41-43] Stable isotope analysis of large felid whiskers should be equally informative for studying the dietary habits of this key group of apex predators.

To accurately interpret large carnivore whisker isotope series, knowledge of whisker growth rates and growth patterns, and how these vary with time and within populations, is required.[36,38,44] Understanding growth rates and growth patterns is necessary to inform the appropriate timeframe of the dietary history contained in a whisker of a specific length.[39] Whiskers, like other tissues, may grow either in a linear or non-linear pattern. In linear growth, segments of equal length represent an approximate equal time interval anywhere along the whisker.[45] In non-linear growth (e.g. the asymptotic von Bertalanffy growth pattern), segments near the distal tip of the whisker probably represent higher temporal resolution than segments of equal length near the proximal root, as expected under typical non-linear growth patterns.[46]

To date, studies of whisker growth rate have been limited to several pinniped species,[38-40,45,47-50] the southern sea otter (*Enhydra lutris nereis*),[44] and only a few terrestrial species, i.e. laboratory rats (*Rattus norvegicus*) and mice (*Mus domesticus*),[51] stoats (*Mustela ermine*)[52] and Eurasian badgers.[36] Most whisker growth rate experiments have been conducted on small numbers of captive individuals and have used different approaches including ^{15}N-enriched glycine labelling (e.g.[44,45]) and Rhodamine B fluorescent biomarking (e.g.[36,52]), to endogenously mark whiskers for growth rate calculations. Terrestrial species whose whisker growth has been explored displayed variable growth rates and growth patterns, either linear or non-linear, followed by varying degrees of whisker retention and shedding. Apart from being species-specific, whisker and other hair growth rates can vary within populations due to factors such as age, sex, disease and food deprivation.[51,53,54] The method of whisker removal employed when conducting experiments can also affect growth rate. For example, plucking whiskers during the growth phase can lead to delays in the emergence of a new whisker.[51]

As with any consumer tissue, the use of whisker stable isotope ratios to reconstruct diets also requires knowledge of $\delta^{13}C$ and $\delta^{15}N$ whisker-diet trophic discrimination factors (TDFs), which is the isotopic difference between a consumer's bulk tissue (in this case whisker) and its diet.[55,56] TDFs are usually derived from captive feeding experiments[57] and, in a few cases, under well-constrained field conditions (e.g.[55]). TDFs are known to vary across species, individuals of the same species, tissues, ages and growth rates.[58,59] Studies where experimental determination of TDFs was not carried out commonly use surrogate values obtained from closely related species or consumer's whole body $\delta^{13}C$ and $\delta^{15}N$ TDFs of 1 and 3 ‰, respectively.[24,27] Few studies have estimated TDFs for hair of terrestrial carnivore species, and in these cases pelage hair has mainly been studied (e.g.[6,60,61]). Experiments on whisker-diet TDFs have only been carried out for marine carnivores such as the southern sea otter,[44,55] Steller sea lion (*Eumetopias jubatus*)[56] and elephant seal (*Mirounga angustirostris*).[62] The lack of species-specific terrestrial carnivore whisker-diet TDFs currently limits the correct interpretation of stable isotope patterns in whiskers when making quantitative diet statements.

In this study, whisker growth rate, growth patterns, and $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of large-bodied felids were investigated in order to provide baseline information and suggest protocols for use of stable isotope analysis of whiskers to infer diets of free-ranging populations. Whisker growth rates and growth patterns were measured for four captive lions (*Panthera leo*) and one leopard (*Panthera pardus*) using an endogenous biomarker (giraffe (*Giraffa camelopardalis*) meat, which was isotopically distinct from their regular diet) to consecutively mark whiskers as they grew. Whisker-diet $\delta^{13}C$ and $\delta^{15}N$ TDFs were estimated for lion based on average stable isotope compositions of their diets in captivity. Comparisons of how these parameters differ across individuals, as well as between the lion and leopard, are made, and protocols and limitations for future investigations are discussed.

2 | EXPERIMENTAL

2.1 | Whisker growth rate and growth pattern

The growth of whiskers of four lions and one leopard was monitored in a controlled biomarker feeding experiment at the National Zoological Gardens (hereafter, Zoo), Pretoria, South Africa, for 6 months, from 3 June to 5 December 2014. Three female lions known by Zoo staff as Emma, Bianca and Tess, one male lion known as Boesman and one male leopard known as Diesel, were available for the study. Table 1 provides details on individual weights, heights and ages.

The standard diet of lions at the Zoo comprised 0.5 kg of chicken (with feathers) daily, and 4 kg beef every second day. The leopard was fed 1.5 kg of chicken, and 2 kg beef every second day. To determine the isotopic consistency of the Zoo diets, a pilot study was conducted

TABLE 1 Weight, height and age of the study felids measured at the beginning of the experiment

Study animals	Weight (kg)	Shoulder/Height (cm)	Age (years)
Emma	104.8	84	2.5
Bianca	111.5	80	2.5
Tess	120.1	89	2.6
Boesman	164.9	101	5
Diesel	113	82	4

on a female lion (known as Amber). The proximal 60 mm of a 130 mm whisker from this individual was sectioned at 2 mm intervals and the resultant 30 whisker sections were analysed for their stable carbon isotope compositions. The $\delta^{13}C$ values revealed a consistent C_4-derived signal with little variation (mean = −11.3 ‰; standard deviation (*SD*) = 0.40; range = −12.1 to −10.7 ‰). This consistency in diet provided the opportunity to 'isotopically' mark regrowing whiskers at predetermined intervals with ^{13}C-depleted, C_3-plant-derived giraffe meat sourced from a game farm in northern KwaZulu-Natal (KZN) to sufficiently alter whisker ^{13}C profiles. The identified periods with low $\delta^{13}C$ values would then be correlated to specific giraffe meat feeding bouts, and hence growth periods.

On 3 June 2014, the study animals were intramuscularly administered a combination of zoletil 100 (composition: 250 mg tiletamine hydrochloride and 250 mg zolazepam hydrochloride; Virbac RSA (Pty) Ltd, Centurion, South Africa) and medetomidine hydrochloride (Kyron Laboratories, Johannesburg, South Africa) to anaesthetise the felids following standard zoological protocols. The capture and handling protocol of these felids was approved by the Cape Peninsula University of Technology Ethics Committee (Ref. 01/2014) and the National Zoological Gardens Research Ethics and Scientific Committee (P14/03). The longest upper lip whisker available on both cheeks of each individual was plucked with its roots using steel forceps. The position of these whiskers was carefully recorded as the focus was on measuring the growth of the whiskers replacing those removed. Felid whiskers are positioned in well-defined rows; therefore, each whisker was allocated a unique identification number, L*x*/*y* or R*x*/*y*.[63] The prefixes L and R were used to refer to the left and right cheeks, respectively, and *x* and *y* were the row and column position, in sequence, numbered from the posterior end (Figure 1). The longest whisker (LD1 or RD1), determined after a whisker length profile had been conducted on two individuals, was removed. Where this whisker was absent, whisker LC1/RC1 or LE1/RE1 was removed.

Felids were fed the giraffe meat at four predetermined feeding bouts and each feeding bout comprised four consecutive days. The four giraffe meat feeding bouts took place 34 days (7–10 July), 105 days (16–19 September), 147 days (28–31 October) and 181 days (1–4 December 2014) after the initial whisker removal. On each of the four days of a feeding bout, the individual felids received 2.5 kg of giraffe meat. It was confirmed visually that the animals consumed the ration in total. The study animals were sedated a second time on 5 December 2014, 185 days after commencement of the experiment, to remove the regrown whiskers. During the second removal of whiskers, it was discovered that all Boesman's whiskers had broken.

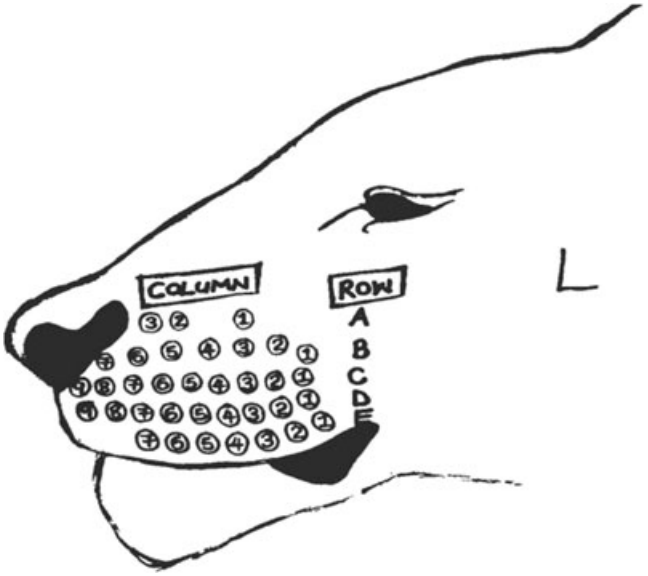

FIGURE 1 An illustration of well-defined rows of felid whiskers and how each removed whisker was allocated an identification number

Hence, available whiskers that were not plucked initially, but long enough to potentially provide some information, were removed from this male lion at the end of the study.

Samples of giraffe, chicken and beef meat were collected at each feeding session for calculations of meat type average stable carbon and nitrogen isotope compositions. These were used to estimate whisker-diet TDFs. Meat samples were individually obtained from different meat packets and chunks. Six samples (two of each meat type) were collected during the first feeding bout and 36 samples (12 of each meat type) during the second, third and fourth feeding bouts. In total, 114 meat samples were collected during the experiment. The collected meat samples were kept frozen until arrival in the laboratory where they were selected and processed.

2.2 | Whisker-diet TDFs

Stable carbon and nitrogen whisker-diet TDFs could only be determined for the lions as their diet remained constant over time. The whisker removed from Amber in June 2013 for the pilot study, and those removed from Emma, Bianca, Tess and Boesman at the beginning of the growth rate experiment in June 2014, were used to estimate isotopic whisker-diet differences. Comparing the stable isotope compositions of a whisker removed a year before the commencement of the experiment, and those of whiskers removed at the time of the experiment, provided an opportunity to validate the consistency of the diet fed to the lions. Meat samples collected during the experiment for SIA were compared with data from whiskers to calculate TDFs.

2.3 | Laboratory sample preparation

2.3.1 | Growth rate experiment samples

The lengths of all the plucked whiskers were measured on a flat surface using a metal ruler and their masses were weighed on a GH series analytical balance (A & D Instruments, Oxford, UK). Individual whiskers were inspected for the presence of any club roots signifying

cessation of growth.[64] Five whiskers (one from each study animal) were washed in a 2:1 methanol/chloroform (99.5%: 99%, Scienceworld, Cape Town, South Africa) solution to remove surface contaminants, further cleaned in a DG-600 ultrasonic bath (MRC, Holon, Israel) using distilled water for 5 min and air-dried. To obtain high-resolution dietary information, whiskers were sectioned at 1 mm intervals from the root towards the tip using a scalpel. Sections were weighed and packed in tin capsules for stable carbon and nitrogen isotope analysis. Precautionary measures, such as handling both samples and packaging material with forceps, were employed during the weighing process to prevent any sample contamination.

Of the 114 meat samples, 42 (14 of each meat type i.e. chicken, beef and giraffe) were randomly selected, cut into 2 g pieces, packed in small labelled plastic containers and placed in a freezer at −20 °C. These were then freeze-dried using a ScanVac CoolSafe 55-4 freeze dryer (LaboGene, Lynge, Denmark) without extracting lipids at −55 °C for 48 h. All dried samples were finely ground into homogenous powder using an A11 basic analysis mill (IKA, Staufen, Germany), weighed to 0.5 mg and packed in tin capsules for stable carbon and nitrogen isotope analysis.

2.3.2 | Whisker-diet discrimination samples

Lion whiskers were cleaned and weighed following the procedure described above. The proximal 60 mm of the whiskers of Bianca, Emma, Tess and Boesman were each cut into six 10 mm segments. From each of these six segments, the distal 2 mm were cut for analysis resulting in six whisker samples per individual. In total, 54 whisker samples including the 30 samples obtained from Amber for the pilot study were weighed, packed in tin capsules and sent for stable carbon and nitrogen isotope analysis.

Lipids are usually depleted in ^{13}C relative to the diet, resulting in more negative $\delta^{13}C$ values.[30] Therefore, the isotope compositions of 16 meat samples with and without lipids were compared. Eight beef and eight chicken samples were each cut into two, and half of the samples were analysed with lipids as described earlier. The other half of the meat samples were treated for lipid extraction following the method of Lee et al.[65] All the visible fat tissue was trimmed and samples were cut into 2 g pieces, individually weighed off into 205 × 30 mm extraction tubes with 20 mg of 2:1 chloroform/methanol solution. Meat samples were homogenised together with the extraction solvent for 10–20 s using a PT 2500 E polytron homogeniser (Kinematica AG, Lucerne, Switzerland) set at 7–8 × 1000 speed. To prevent sample contamination, the polytron was thoroughly cleaned between samples by removing residue sinews and tissue with tweezers, rinsed with water followed by chloroform/methanol solution then wiped clean. The meat and solution mixture was filtered through Whatman grade 1 filter paper into Erlenmeyer flasks. Meat sample residue was brushed from the filter paper into small labelled plastic containers and placed in a freezer at −20 °C. Samples were freeze-dried, ground, weighed and packed in tin capsules following the same procedure as the non-lipid-extracted meat.

2.4 | Stable isotope analysis

Samples were individually combusted in a Flash 2000 organic elemental analyser (Thermo Scientific, Bremen, Germany) and the resultant CO_2 and N_2 gases introduced into a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific) via a continuous Conflo IV flow-through inlet system (Thermo Scientific). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are presented in delta (δ) notation in parts per thousand or per mil (‰) relative to the Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 standards, respectively, derived from the expression:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard deviations of repeated measures of laboratory standards (Chocolate powder, Seal and Valine) were ≤0.3 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

2.5 | Data analysis

2.5.1 | Identification of feeding bouts along whisker lengths

Isotopic profiles of whiskers were plotted with whisker length (mm) on the *x* axis and $\delta^{13}C$ values on the *y* axis. The four giraffe meat feeding bouts, illustrated by immediate transitions to low $\delta^{13}C$ values, had to be identified, in order to determine the point along each whisker where experimental feeding bouts took place. These transitions represent the change from a ^{13}C-enriched, C_4-derived chicken and beef diet to an experimental ^{13}C-depleted, C_3-derived giraffe diet.

2.5.2 | Whisker growth rate estimations

Whisker growth rate was investigated by two methods. First, the interval growth rate between two consecutive measurements was calculated (Δlength/Δtime) using the beginning of the C_3 peak at each feeding bout. This revealed the range of growth rates that occurred within an individual whisker at different phases during the experiment and made it possible to discern the whisker growth pattern.[48] It also revealed individual and mean felid whisker growth rates. Whisker growth rates were calculated for (i) whisker length grown for the entire duration of the experiment (185 days) and (ii) whisker length grown over the last 76 days of the experiment (from 109 to 185 days).

Second, the relationship between length of whiskers (mm) and growth rate (mm day^{-1}) with time (days) was modelled using mixed effects linear models (MELMs) performed in R software (version 3.1.3[66]) with add-on ImerTest package.[67] The effect 'individual' was included as a random effect to ensure that correct error terms were compared considering there were multiple measurements (i.e. non-independent measures) for each individual. However, only three empirical observations were available for each individual (experimental feeds 2, 3 and 4, from days 109 to 185). Therefore, the models were rerun including growth from day 1 of the experiment. Comparisons of growth rates (slopes) derived from the analysis of the two data sets were used to determine whether growth was linear or not. A significant decrease in growth rate between the latter and former models would indicate non-linear growth. Comparisons of whisker growth amongst individuals were based on 95% confidence intervals of slopes and intercepts of the random effects. Despite the small sample size (three or 'four'

observations per individual), assumptions of normality and homogeneity of variance of the data were not violated, based on Shapiro-Wilk's W[68] and Levene's tests,[69] respectively. A significance level of 0.05 was used in all tests.

2.5.3 | Whisker growth pattern: linear vs non-linear

The MELMs used above assume that whisker growth in large felids is linear (apart from the comparison between models fitted to the exact obtained data and those with an assumed growth at day one). Many body tissues, including hair, are expected to exhibit a non-linear growth, generally slowing down until it reaches an asymptote after an initial phase of faster growth.[70] A common physiological growth pattern is described by von Bertalanffy's model,[46] although other non-linear growth patterns such as the Gompertz and logistic are also possible.[71] The small sample obtained for the present study could not allow for fitting of non-linear growth models to the data due to over-parameterisation. Use of parameter values derived from linear growth models to reconstruct growth of whiskers harvested from free-ranging animals could be problematic. It is therefore necessary to consider (i) whether this approach would be appropriate for field studies and (ii) whether changes in growth patterns are such that linear growth can be assumed for certain portions of a whisker (i.e. a portion over which growth is presumably more-or-less constant). To address this issue, comparisons of simulated linear and non-linear (von Bertalanffy) whisker growth patterns were made. Linear growth was simulated using the following equation:

$$l_t = a + bt \tag{2}$$

where l_t is length (mm), a is the intercept (set at 0 assuming zero growth at zero days), b is growth rate and t is days. The growth rate (b) used was the slope of the MELM for millimetres (mm) over days. Non-linear growth was simulated using the following von Bertalanffy equation:

$$l_t = k\left[1-e^{-b(t-to)}\right] \tag{3}$$

where l_t is length (mm), k is asymptotic length when growth rate is equal to zero, b is growth rate, t is days and t_0 is length at zero days.[48] Parameters for the von Bertalanffy simulations were chosen by trial and error until the solution that most closely resembled the linear scenario was found. In this case, b= 0.02 and k = 72 (note that the value of k is equal to the number of days for which reliable growth rate data could be obtained from the whiskers measured in this study, i.e. 105 days; see Figure 2). The trial-and-error solution was used because there was no obvious non-linear trend in observed data for the relevant whisker portions. Thus, no *a priori* assumptions about parameter values could be made.

After the establishment of the two hypothetical curves, the growth period represented in 72 mm (measured from root) of each curve was calculated and compared. Solving for growth rate or time represented within a 72 mm whisker, Equation 2 gives:

$$t = (l_t - a)/b \tag{4}$$

and Equation 3 gives:

$$t = \ln\left(1-\frac{lt}{k}\right)/bt_0 \tag{5}$$

The two scenarios were compared in how they estimated whisker length grown after a specific time period (i.e. number of days represented within a 72 mm whisker). A dependent samples (paired) t-test was then used to compare time intervals between each simulated series, and hence determine whether the two curves differ

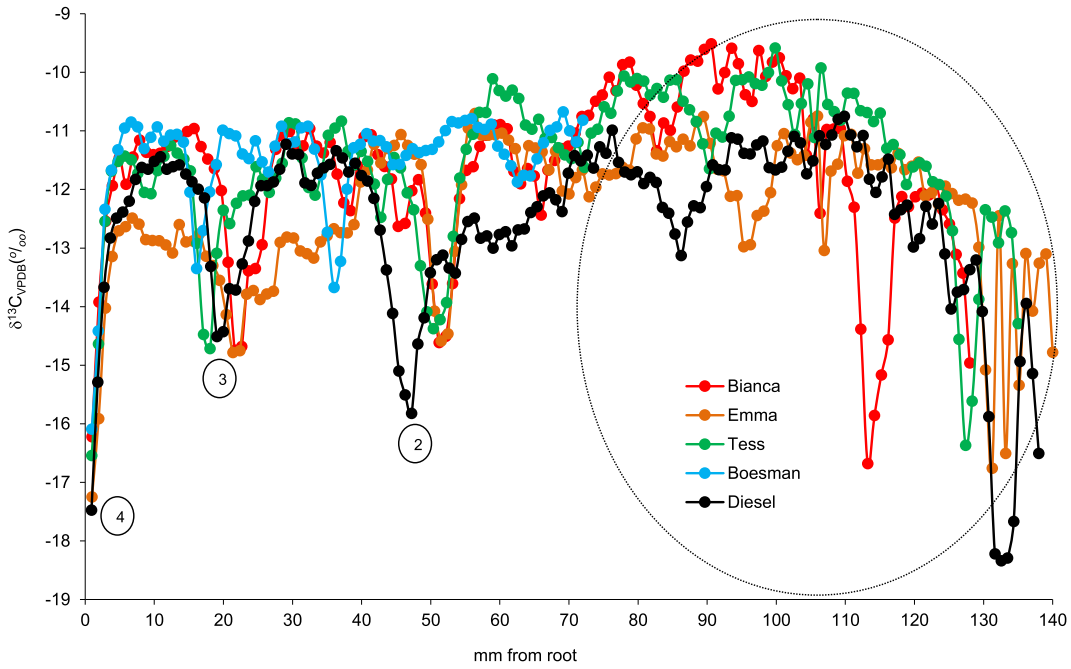

FIGURE 2 $\delta^{13}C$ values for whiskers of the study animals. The circled data includes the first feeding bout and were not used due to uncertainty. The numbered incisions of low $\delta^{13}C$ values represent the last three identifiable feeding bouts [Color figure can be viewed at wileyonlinelibrary.com]

significantly in expected growth rates. This procedure tests whether the application of linear vs non-linear growth models could be expected to have significant impacts on resolving growth rates and time periods represented in 72 mm of a felid whisker harvested in field. Simulations were carried out using the Visual Basic for Applications (VBA) editor of Microsoft Excel 365.

2.5.4 | Lion whisker-diet TDFs

The amount of chicken and beef consumed by the captive animals was converted into percentages, and the weighted mean $\delta^{13}C_{diet}$ and $\delta^{15}N_{diet}$ values were calculated. A two sample *t*-test, with Satterthwaite's approximation of degrees of freedom,[72] performed in R (version 3.1.3[66]), was used to determine whether the means of lipid- and non-lipid-extracted chicken and beef differed. TDFs were then calculated for each point along the whisker series, following Craig:[73]

$$\varepsilon^*_{WD} = \left(\frac{1000 + \delta^{13}C_{whisker}}{1000 + \delta^{13}C_{diet}} - 1\right)^* 1000 \quad (6)$$

where ε^*_{WD} is effectively the difference in delta-values of the whisker (W) and diet (D), but captures the fact that isotope exchange between a source (diet) and sink (whisker) is non-linear and non-reversible. The asterisk denotes that isotopic equilibrium is not assumed. ε^*_{WD} values are sometimes, but not always, equivalent to the more commonly used Δ_{WD} $(=\delta^{13}C/\delta^{15}N_{consumer} - \delta^{13}C/\delta^{15}N_{diet})$ values. The former approach was followed here as it is not scale -dependent.[74]

To determine mean TDFs (i.e. mean ε^*_{WD}), a randomisation procedure was used to account for biases such as pseudo-replication effects that could arise in simple calculations of arithmetic means of data series, including both repeated measures and uneven sample sizes. For example, the ε^*_{WD} series for Amber was much longer (n = 30) than for the other individuals (n = 24). Thus, the means and *SD*s from 1000 bootstraps or random sub-samples of the data set (25% of the sample size) were estimated. The resampling algorithms were coded using VBA editor of Microsoft Excel 365.

3 | RESULTS

3.1 | Lengths and weights of individual whiskers

A total of 644 sections from the five felid whiskers removed at the end of the experiment were analysed. The lengths of the whiskers ranged from 72 to 140 mm, while their weights ranged from 24 to 32.3 mg. Table 2 provides details on individual lengths and weights of the sampled whiskers, and the number of sections obtained from each whisker. As mentioned earlier, all the whiskers of the male lion, Boesman, were broken when the experiment ended; hence, the whisker length provided here for him was not the full length grown for the entire duration of the experiment.

TABLE 2 Lengths, weights and number of sections of the five felid whiskers analysed to determine whisker growth rate

Felid name	Whisker position	Length sampled (mm)	Weight (mg)	Number of sections
Bianca	LD1	128	22.5	130
Emma	LD1	140	29	144
Tess	RF1	135	24	142
Boesman	RC1	72	28	76
Diesel	LD1	138	32.3	152

3.2 | Stable isotope compositions of the experimental diet

Table 3 provides the mean (± *SD*) $\delta^{13}C$ and $\delta^{15}N$ values of the giraffe meat and Zoo chicken and beef samples. The $\delta^{13}C$ values of the giraffe meat were indicative of a C_3-based diet, while those of the Zoo chicken and beef were largely C_4-derived. The meat consumption of the five lions (including the pilot study individual, Amber) consisted of 20% chicken and 80% beef every 2 days. The average $\delta^{13}C$ and $\delta^{15}N$ values of lipid- and non-lipid-extracted chicken were not significantly different from each other (t = −0.07, p = 0.94 for $\delta^{13}C$ and t = −0.13, p = 0.90 for $\delta^{15}N$). Similarly, the average $\delta^{13}C$ and $\delta^{15}N$ values of lipid- and non-lipid-extracted beef were not significantly different from each other (t = 1.02, p = 0.32 for $\delta^{13}C$ values and t = −0.11, p = 0.91 for $\delta^{15}N$ values). The mean (± *SD*) $\delta^{13}C$ and $\delta^{15}N$ values of the non-lipid-extracted diet (chicken and beef combined) were −14.3 ± 1.08 ‰ and 6.8 ± 1.29 ‰, while those of the lipid-extracted diet were −13.9 ± 0.92 ‰ and 6.8 ± 1.06 ‰, respectively.

The C:N ratios of the lipid-extracted chicken (n = 8, mean ± *SD* = 3.1 ± 0.03) did not significantly differ from the C:N ratios of the non-lipid-extracted chicken (n = 8, mean ± *SD* = 3.3 ± 0.07) (t = 2.03, p = 0.082). Similarly, the C:N ratios of the lipid-extracted beef (n = 8, mean ± *SD* = 3.2 ± 0.07) did not significantly differ from the C:N ratios of the non-lipid-extracted beef (n = 8, mean ± *SD* = 3.3 ± 0.08) (t = 2.33, p = 0.052). However, the C:N ratios of the combined chicken and beef showed a significant difference between the lipid-extracted and non-lipid-extracted diet (Wilcoxon signed rank test t = 120, n = 16, p <0.001). This difference in elemental composition, reflecting an expectedly lower carbon content of the non-lipid-extracted (mean ± *SD* % C = 42.6 ± 19.78) than of the lipid-extracted diet (mean ± *SD* % C = 47.3 ± 12.26), is nonetheless quite small (mean % C difference ± *SD* = 0.17 ± 0.11 ‰).

3.3 | Identification of feeding bouts

Only the last three feeding bouts could be clearly identified from the preliminary plots of individual whisker series (Figure 2). The first feeding bout could not be detected as there was noise and uncertainty at the thinner part of whiskers probably because of rapid whisker growth during that phase (circled section in Figure 2). As a result, the first feeding bout, conducted 34 days after the commencement of the experiment, was excluded in whisker growth calculations to avoid

TABLE 3 $\delta^{13}C$ and $\delta^{15}N$ values (mean ± *SD*) of the experimental diet

Diet	Treatment	Sample size (n)	$\delta^{13}C$ ± *SD* (‰)	$\delta^{15}N$ ± *SD* (‰)
Giraffe	Non-lipid extracted	14	−24.6 ± 0.29	5.7 ± 0.25
Chicken	Non-lipid extracted	14	−17.5 ± 0.47	2.4 ± 0.70
	Lipid extracted	8	−17.5 ± 0.48	2.4 ± 0.77
Beef	Non-lipid extracted	14	−13.5 ± 1.23	7.9 ± 1.44
	Lipid extracted	8	−13.0 ± 1.02	7.9 ± 1.14

bias. Thus, reliable and comparable data (i.e. 72 mm of whiskers from root) covering the three identifiable feeding bouts were used for further calculations (Figures 3A–3E).

The positions of the remaining three giraffe meat feeding bouts, represented by low $\delta^{13}C$ values, were consistent for all three lionesses. Although Diesel is a different species, his assimilation rate of the giraffe meat was similar to that of the lionesses. However, Boesman's rate of giraffe meat assimilation appeared to be slower than the other felids (Figure 2). The last feeding bout had slightly lower mean $\delta^{13}C$ values for all five felids (−16.7 ‰) than the third (−14.4 ‰) and second (−14.6 ‰) feeding bouts.

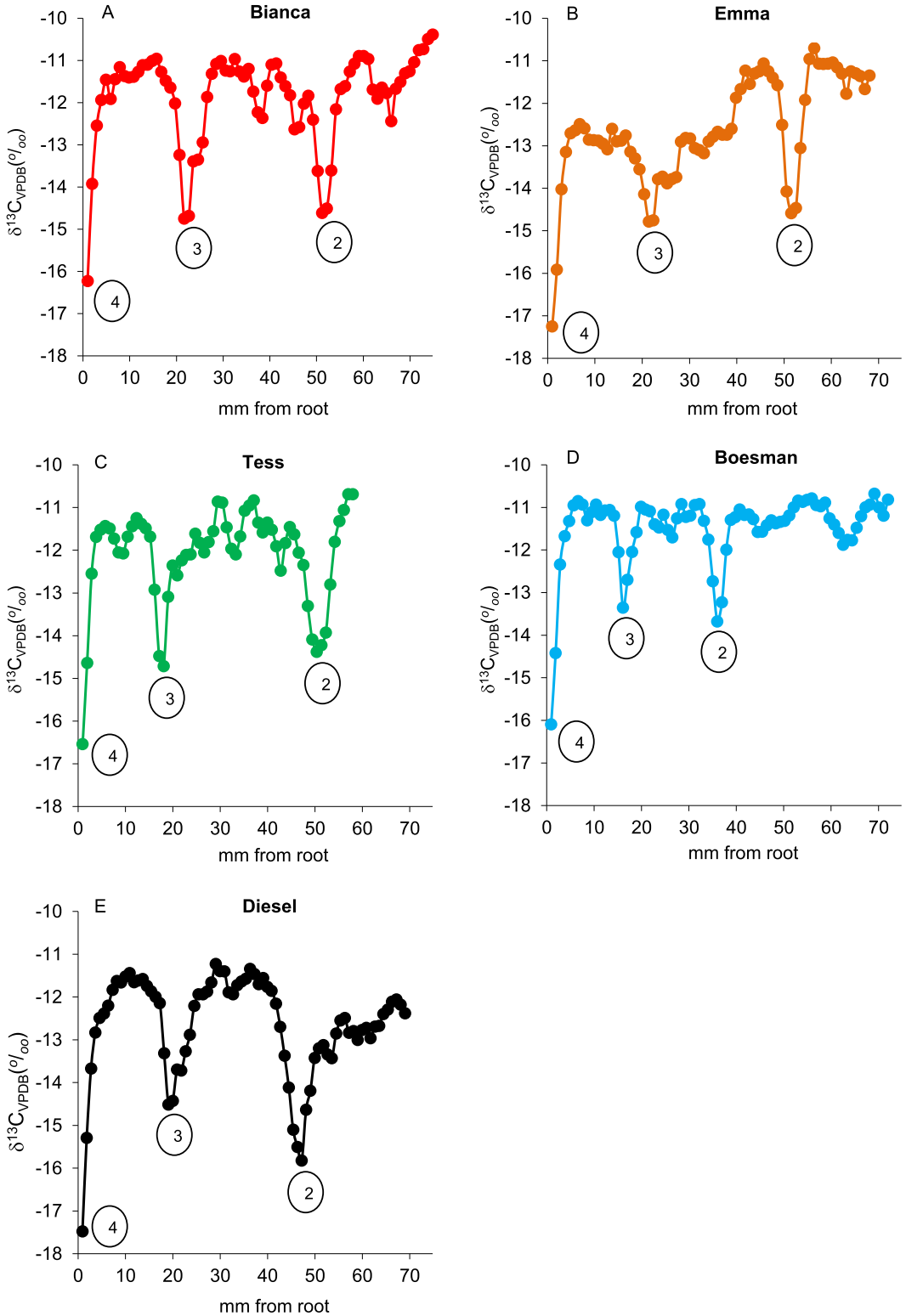

FIGURE 3 (**A**–**E**) Whisker $\delta^{13}C$ values for the four lions (Bianca, Emma, Tess and Boesman) and the leopard (Diesel). The three numbered phases with a sharp decrease in whisker $\delta^{13}C$ values represent the second, third and fourth feeding bouts where felids were fed the experimental C_3-derived giraffe meat [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | Whisker growth rates

The individual whisker $\delta^{13}C$ series were used to estimate the growth rate (mm day^{-1}) because the exact dates of whisker removal at the beginning and end of the experiment were known, as well as the feeding dates. Boesman was not included in whisker growth rate calculations since the total lengths of the initially removed whisker and the one that was removed and analysed at the end of the study were not known. As a result, 288 (72 per individual) of the 644 samples provided usable data.

3.4.1 | Whisker growth rates at different intervals

The Δlength/Δtime metric showed that whisker length increased with time (Figures 4A and 4B), whereas growth slowed down (and differed between intervals) as the experiment progressed (Figure 5; Table 4). For all four felids, whisker growth was fastest during the first 109 days of the experiment, although growth varied amongst individuals. During this period, Diesel's whisker grew faster than those of the other felids reaching a length of 91.70 mm, while Bianca had the slowest growing whisker, measuring 77.78 mm. Whisker growth rates for this interval for the four felids ranged from 0.71 to 0.84 mm day^{-1}. From 109 to 151 days, individual whisker length grown ranged from 28.24 to 32.33 mm. Tess had a faster whisker growth (0.77 mm day^{-1}) than the other felids, while Diesel's whisker growth was the slowest (0.67 mm day^{-1}). Bianca and Emma had similar growth rates of 0.70 and 0.72 mm day^{-1}, respectively. The slowest whisker growth rates for all four felids were recorded for the last 34 days of the experiment (from 151 to 185 days). During this period, Bianca and Emma had similar whisker growth rates (0.61 and 0.60 mm day^{-1}, respectively), while growth rates for Tess and Diesel were slower (0.50 and 0.53 mm day^{-1}, respectively). Individual whisker growth rates were similar for the total duration of the experiment (i.e. 185 days) as well as the period where detailed growth data was obtained (i.e. 76 days). Mean felid whisker growth rates were 0.73 ± 0.03 mm day^{-1} over 185 days and 0.65 ± 0.03 mm day^{-1} for the last 76 days of the experiment where the thicker section of whiskers was formed.

3.4.2 | Mixed effects linear models

Comparison of two models, one including empirical data only (last 76 days of the experiment, from 109 to 185 days) and another forcing the model through the origin (i.e. assuming zero growth at day zero, encompassing 185 days), differed in respective whisker growth rates

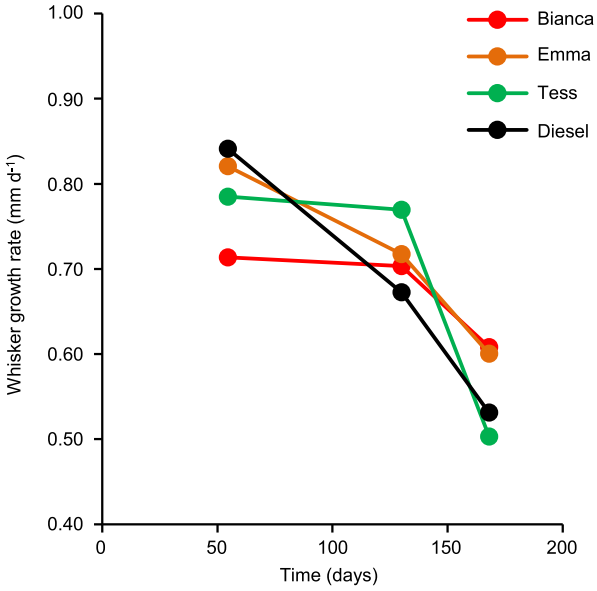

FIGURE 5 Calculated average whisker growth rates, plotted at the midpoint between giraffe meat feeding bouts, suggest non-linear growth pattern [Color figure can be viewed at wileyonlinelibrary.com]

(mean ± *SD* = 0.65 ± 0.01 and 0.74 ± 0.03 mm day^{-1}, respectively). This difference implies that whiskers grew faster at the beginning of the experiment, but growth slowed down as the experiment progressed, supporting the observations discussed above.

Whisker growth was broadly similar across individuals (range = 0.70–0.76 mm day^{-1} for 185 days, and 0.64–0.66 mm day^{-1} for the last 76 days of the experiment), despite the presence of two species and two sexes in the data (Table 5). However, some differences were evident, and the lack of significant differences between individuals (overlapping 95% confidence intervals of growth rate estimates) may be due to low statistical power associated with the small sample size incorporated. Changes in growth rates through time may also have obscured individual-level effects. For instance, Emma and Diesel exhibited the highest whisker growth rates over 185 days (0.76 mm day^{-1}), but were the slowest growing individuals over the last 76 days (0.60 mm day^{-1}). Bianca had the slowest growing whisker for the duration of the experiment (0.70 mm day^{-1}); however, in the last 76 days, her whisker grew faster than those of the other felids (0.66 mm day^{-1}).

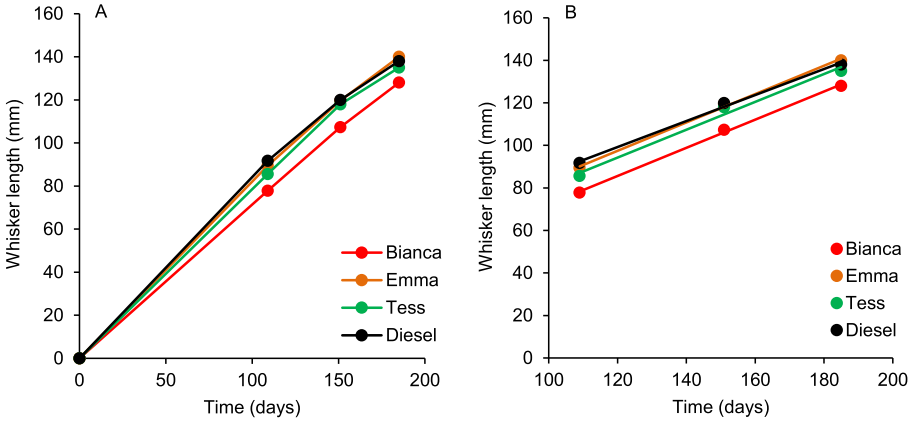

FIGURE 4 Whisker length grown over the entire duration of the experiment, i.e. 185 days (A) and over the last 76 days of the experiment (B) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Whisker length grown between feeding intervals, growth rates per interval and individual whisker growth rates for the three female lions and a male leopard

Individual	Date	Activity	Days after initial removal	Day of expected C_3 feed peak	Days between giraffe meat feeding	Length from root (mm)	Length grown between C_3 feed peaks (mm)	Growth rate between C_3 feed peaks (mm day^{-1})	Individual growth rates over 185 days (mm day^{-1})	Individual growth rates for the last 76 days (mm d^{-1})
Bianca	3/06/2014	Whisker removal	0			0			0.69	0.66
	16-19/09/2014	2nd feed	105-108	109	109	77.78	77.78	0.71		
	28-31/10/2014	3rd feed	147-150	151	42	107.32	29.54	0.70		
	1-4/12/2014	4th feed	181-184	185	34	128.00	20.68	0.61		
Emma	3/06/2014	Whisker removal	0			0			0.76	0.67
	16-19/09/2014	2nd feed	105-108	109	109	89.44	89.44	0.82		
	28-31/10/2014	3rd feed	147-150	151	42	119.58	30.14	0.72		
	1-4/12/2014	4th feed	181-184	185	34	140.00	20.42	0.60		
Tess	3/06/2014	Whisker removal	0			0			0.73	0.65
	16-19/09/2014	2nd feed	105-108	109	109	85.56	85.56	0.78		
	28-31/10/2014	3rd feed	147-150	151	42	117.89	32.33	0.77		
	1-4/12/2014	4th feed	181-184	185	34	135.00	17.11	0.50		
Diesel	3/06/2014	Whisker removal	0			0			0.75	0.61
	16-19/09/2014	2nd feed	105-108	109	109	91.70	91.70	0.84		
	28-31/10/2014	3rd feed	147-150	151	42	119.94	28.24	0.67		
	1-4/12/2014	4th feed	181-184	185	34	138.00	18.06	0.53		
Mean ± *SD*									0.73 ± 0.03	0.65 ± 0.03

TABLE 5 Individual whisker growth rates (mm day^{-1}) and confidence intervals (CI) for whisker length grown over 185 days and the last 76 days of the experiment

Felid	185 days			76 days		
	Growth rate	−95% CI	+95% CI	Growth rate	−95% CI	+95% CI
Bianca	0.70	0.66	0.74	0.66	0.62	0.70
Emma	0.76	0.72	0.80	0.64	0.60	0.68
Tess	0.74	0.70	0.78	0.65	0.61	0.69
Diesel	0.76	0.72	0.80	0.64	0.60	0.68
Mean ± *SD*	0.74 ± 0.03			0.65 ± 0.01		

3.5 | Whisker growth patterns

The interval growth rates and the MELMs revealed that whisker growth decreased with time, suggesting a non-linear growth pattern (Figure 5). However, sample sizes of known growth intervals were too small for non-linear model fitting (n = 3 per individual). Thus, simulations were used to compare effect of linear vs non-linear (von Bertalanffy) growth. Outcomes of these simulations indicate that the two types of growth patterns would appear similar for the proximal ~50 mm or last 75 days of growth (Figure 6A). Beyond this length, as one progresses more towards the whisker tip, the two types of growth trajectories would differ substantially. Therefore, reconstructing growth of free-ranging lion and leopard whiskers could be based on assumptions of linear growth with a reasonably high degree of confidence for the thicker proximal 50–60 mm. Most such reconstructions would, however, be aimed at inferring a represented time interval. As a result, more useful comparisons can be made by plotting time as a function of whisker length (Figure 6B). Simulated time intervals for 105 days (the number of days for which 72 mm – measured from root – of reliable growth rate data could be obtained from whiskers of this study) did not differ significantly between the two hypothetical series ($t_{(34)}$ = −1.5; n = 36; mean (± *SD*) = −7.72 ± 30.83; p = 0.28). Visually, however, it appears that slower growth under non-linear conditions may complicate timeframe reconstructions at whisker lengths that are above 60 mm (measured from the root) (Figure 6B).

3.6 | Lion whisker-diet TDFs

Although there were no significant differences between the isotopic signatures of lipid- and non-lipid-extracted chicken and beef (Table 3), only the $\delta^{13}C$ and $\delta^{15}N$ values of lipid-extracted meat were used to calculate ε^*_{WD} (Equation 6) for the lion in this study. This is because whiskers are protein-based (keratinous) tissue and are therefore expected to incorporate only (for the most part) proteinaceous components of the diet.[75,76]

The $\delta^{13}C$ and $\delta^{15}N$ values of whisker segments of the five lion whiskers ranged from −10.6 to −12.7 ‰ and from 8.8 to 10.1 ‰, respectively (Figures 7A and 7B). The whisker-diet TDFs (i.e. ε^*_{WD}) of these segments ranged from 1.2 to 3.4 ‰ for $\delta^{13}C$ values, and from 1.9 to 3.3 ‰ for $\delta^{15}N$ values (Figures 7C and 7D). The mean ε^*_{WD} values were similar amongst individuals, ranging from 2.5 to 2.9 ‰ for $\delta^{13}C$ values and from 2.4 to 2.8 ‰ for $\delta^{15}N$

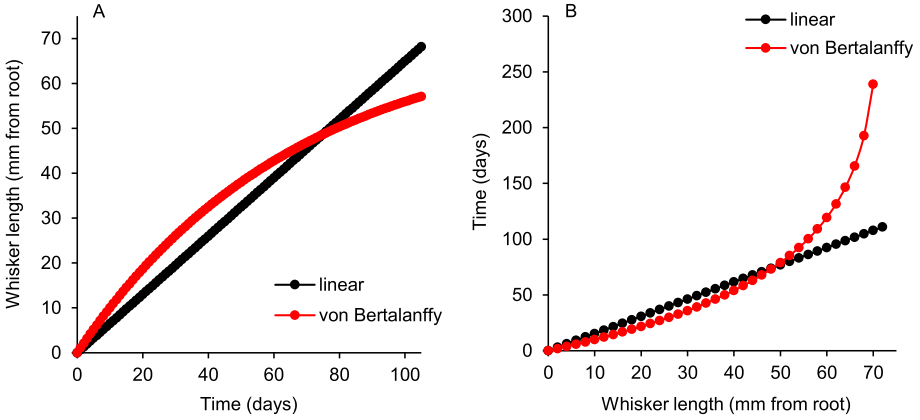

FIGURE 6 The differences in how the simulated linear and von Bertalanffy growth curves estimates whisker length grown over 105 days (A) and amount of time taken to reach a whisker length of 72 mm (B) [Color figure can be viewed at wileyonlinelibrary.com]

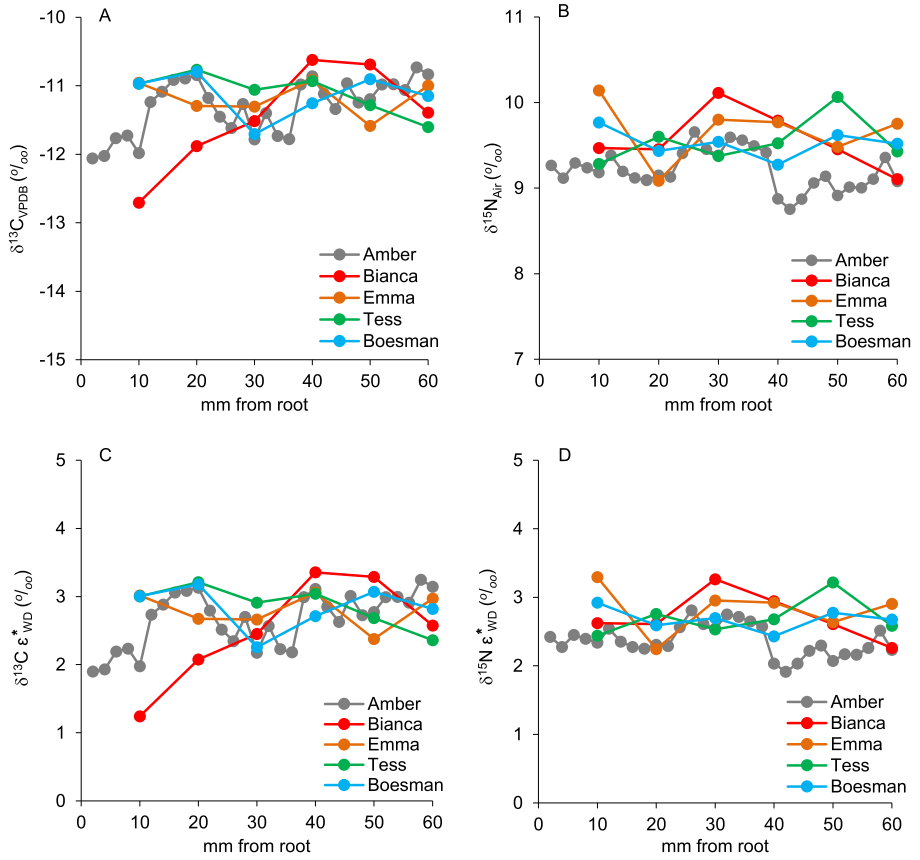

FIGURE 7 $\delta^{13}C$ and $\delta^{15}N$ values of the analysed whisker segments (A, B) used to calculate ε^{*}_{WD} (C, D) for the five lions [Color figure can be viewed at wileyonlinelibrary.com]

values. The bootstrapped means (± *SD*) across all individuals were 2.7 ± 0.12 ‰ for $\delta^{13}C$ values and 2.5 ± 0.08 ‰ for $\delta^{15}N$ values (Table 6).

4 | DISCUSSION

4.1 | Whisker growth rates

The two analytical approaches used to estimate whisker growth rates, i.e. Δlength/Δtime metric and MELMs, showed similar results. Within-individual growth rates revealed that whisker growth was faster towards the whisker tip (first 109 days of the experiment) and slower towards the root (last 76 days) (Table 4). This was probably because the thin whisker tip has smaller nuclear bodies of hair cells; therefore, little assimilated material is deposited towards the tip, taking fewer days to grow than the thick basal section or whisker root.[77] Whisker growth rates have been reported to vary with species, age, sex, reproductive status and food deprivation.[45,51] In this study, whisker growth rates of the adult male leopard (Diesel) and sub-adult female lions (Bianca, Emma and Tess) were similar. Moreover, the felids did not undergo nutritional stress during the experiment as they were maintained daily on diets that met their caloric requirements by the

TABLE 6 Individual and bootstrapped mean $\delta^{13}C$ and $\delta^{15}N$ ε^{*}_{WD} values for the five lions

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Individual	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Amber	2.7	3.1	2.2	2.4	2.9	1.9
Bianca	2.5	3.0	2.0	2.7	3.3	2.2
Emma	2.8	3.3	2.3	2.8	3.4	2.3
Tess	2.9	3.3	2.4	2.7	3.2	2.2
Boesman	2.8	3.3	2.4	2.7	3.2	2.2
Bootstrapped mean ± *SD*: $\delta^{13}C$ = 2.7 ± 0.12, $\delta^{15}N$ = 2.5 ± 0.08						

Zoo staff. During the experiment, Emma fell pregnant twice; her first litter was born on 23 May and the second on 8 October 2014 (the gestation period for the lion is 110 days[78]). Consequently, her whisker growth was expected to be slower than that of the other felids because nutrients would have been devoted to foetus growth.[79] However, this was not the case as her whisker growth was similar to that of individuals that were not mated. Thus, the aforementioned factors seem to not have affected the measured felid whisker growth rates.

Results of this study concur with those of Ibrahim and Wright[51] who found no significant differences between the growth rates of male and female rats and mice. However, male fur seals were shown to have longer whiskers and therefore exhibited higher whisker growth rates than females.[49] Other studies reported different whisker growth rates amongst species and age groups. For instance, Hirons et al[45] showed that Steller sea lions and habor seals (*Phoca vitulina*) had different whisker growth characteristics. Their study, and that of Rea et al,[50] also revealed that juvenile Steller sea lion whisker growth rates were twice those of adults.

The mean felid whisker growth rates of 0.74 mm day^{-1} (estimated from whisker lengths grown over 185 days) and 0.65 mm day^{-1} (estimated from whisker lengths grown over 76 days) were within the range of values for laboratory mice (0.3–1.0 mm day^{-1}), laboratory rats (0.6–1.5 mm day^{-1}), harbor seals (0.075–0.78 mm day^{-1}) and Steller sea lions (0.44–0.87 mm day^{-1}).[47,50,51] Both whisker growth rates were much higher than those for most pinniped species. For instance, whiskers of Steller sea lions were reported to grow at 0.05–0.07 mm day^{-1},[45] leopard seals (*Hydrurga leptonyx*) at 0.08–0.1 mm day^{-1},[38] Antarctic fur seals (*Arctocephalus gazelle*) at 0.13 mm day^{-1},[40] and gray seals (*Halichoerus grypus*) at 0.24 mm day^{-1}.[48] The felid whisker growth rates of this study were also higher than the measured whisker growth rates of sea otters[44] and Eurasian badgers,[36] i.e. 0.21 mm day^{-1} and 0.43 mm day^{-1}, respectively. However, the mean felid whisker growth rate of 0.65 mm day^{-1} was similar to that of stoats (0.60 mm day^{-1}).[52]

The estimation of whisker growth rates in this study was restricted by a number of factors. Typical of whisker growth studies, the sample available for the experiment was small and unbalanced (i.e. three lionesses, one male lion and one male leopard). The unavailability of Boesman's full whisker growth trajectory further reduced the sample size and made it impossible to ascertain the whisker growth rate of male lions. The scenario of finding broken whiskers during collection seems to be realistic and should be expected as a similar setback was also encountered in previous studies (e.g.[39]). Although the position of Boesman's second and third giraffe meat feeding bouts along the unbroken part of the whisker suggests a slower assimilation rate (and possibly whisker growth rate) than for the smaller-bodied lionesses and the leopard (Figure 2), such a hypothesis could not be tested in this study due to the small sample size. The sample size of this study may also have limited the accurate evaluation of the influence of species, sex, age and reproduction on the measured whisker growth rates.

The second limitation was the indiscernibility of the first feeding bout contained towards the whisker tips. There is no tangible explanation for the uncertainty observed for the thinner region of whiskers. However, it seems likely that the relative 'thinness' of the distal parts of a whisker represent an accelerated growth period, with tissue accretion not occurring in as well-defined time steps as in the thicker, more slower-growing whisker regions. Stricker et al[56] experienced a similar challenge of increased uncertainties towards the whisker tips of both pup and adult seals. However, such an error had a smaller effect on adult whiskers as the lengths necessary to achieve mass requirements and acquire comparable information were smaller. Future studies using SIA of whiskers should therefore consider sectioning whiskers by weight, rather than length, as this might provide more information on whisker growth.

Thirdly, the fourth feeding bout revealed that whiskers were still in the anagen (growing) phase when they were removed at the end of the experiment. This implies that the mean felid whisker growth rate presented in this study was calculated using incomplete growth trajectories of individuals as whiskers had not reached their asymptotic length, potentially affecting the measured growth rates. However, the lengths of the sampled felid whiskers ranged from 128 to 140 mm, which is close to the maximum length (i.e. 150 mm) encountered both in the field and at the Zoo to date. It is interesting that the $\delta^{13}C$ values for the fourth feeding bout were much lower than those for whisker segments of the other feeding bouts. The higher lipid content found in the root material than in keratin might have influenced the low $\delta^{13}C$ values of the root whisker segments since lipids are depleted in ^{13}C relative to proteins,[80,81] although most lipids were removed during pre-treatment of the samples.

4.2 | Whisker growth pattern

Whisker growth simulations suggested that growth pattern does not have a major effect on observed growth for a whisker length of ~50 mm (measured from the root), or the last 75 days of growth, assuming that the growth rates are similar to those found in this study. Both linear and non-linear von Bertalanffy growth simulations estimated time and whisker length indistinguishably for this segment of a whisker (Figures 6A and 6B). However, beyond this length (towards the whisker tip), simulated growth patterns begin to deviate from each other. These results imply that studies using SIA of whiskers to infer felid feeding ecology should not automatically assume that whisker growth is constant, but should consider the whisker length measured. Although the whisker growth observed in this study suggested a non-linear growth pattern (Figure 5), further confirmation of this result was limited by the sample size that was too small (n = 3) for non-linear (von Bertalanffy) model fitting. Therefore, future investigations on

whisker growth should endogenously mark whiskers for a longer period to acquire more data that will provide all the parameters required for non-linear model fitting.

Studies on rats, mice, Steller sea lions and sea otters found that the whiskers of these species followed a linear growth pattern.[44,45,51] This differs from experiments conducted on gray seals, leopard seals and Eurasian badgers that reported non-linear whisker growth in these species.[36,38,48] All these studies measured growth patterns from whiskers that had a mean length of >70 mm, except for Eurasian badgers (mean = 45 mm).

4.3 | Lion whisker-diet TDFs

The lion $\delta^{13}C$ ε^*_{WD} values estimated in this study (2.5–2.9 ‰, Table 6) fall within the range (2.7–3.5 ‰) of previously reported TDFs for herbivore hair (e.g.[30,82]), and were similar to the hair-diet TDFs for some terrestrial carnivores, i.e. the red fox (*Vulpes vulpes*) (2.6 ‰)[60] and Canada lynx (*Lynx canadensis*) (2.4 ‰).[61] The values also fall within the 2.2–3.9 ‰ range of whisker-diet TDFs reported for some marine mammalian carnivores.[31,44,55,56,62] However, the reported hair-diet $\delta^{13}C$ TDFs for some terrestrial felids were either much lower or higher than the mean lion value (2.70 ± 0.12 ‰) of this study. For example, hair-diet $\delta^{13}C$ differences reported for the African lion were 1.1 ± 0.2 ‰, mountain lion (*Puma concolor*) 4.7 ± 0.6 ‰ and bobcat (*Lynx rufus*) 5.5 ± 0.5 ‰.[61] In another study, snow leopard (*Uncia uncia*) and tiger (*Panthera tigris*) hair-diet $\delta^{13}C$ values of 6.0 ± 1.25 ‰ and 6.5 ± 0.54 ‰, respectively, were reported.[6] The estimated mean lion $\delta^{13}C$ ε^*_{WD} value was also higher than TDFs for commonly analysed mammalian tissues, i.e. muscle, liver and blood, in isotope ecology (e.g.[30,60,62]). This was possibly because whiskers (and hair) are constructed from α-keratin, which is synthesised from non-essential amino acids glycine and serine, and these are naturally enriched in ^{13}C for some animals.[83,84]

The observed mean lion $\delta^{15}N$ ε^*_{WD} value of 2.5 ± 0.08 ‰ was similar to the ^{15}N trophic level difference of 2.5 ‰ obtained from a meta-analysis.[85] The lion $\delta^{15}N$ ε^*_{WD} values (2.4–2.8 ‰, Table 6) were also similar to the whisker-diet TDFs for some pinnipeds (2.6–3.0 ‰),[31,62] but lower than the $\delta^{15}N$ whisker-diet TDFs of sea otters (5.5 ‰)[44] and the hair-diet TDFs of some felid species, i.e. bobcats, mountain lions and the African lion (3.5–4.5 ‰).[61] Furthermore, the lion $\delta^{15}N$ ε^*_{WD} values were lower than reported stepwise ^{15}N enrichment values of 3–4 ‰ across trophic levels in food webs.[27,28,86] The values obtained in this study were, however, higher than the $\delta^{15}N$ hair-diet TDFs reported for the tiger and snow leopard, i.e. −0.3 and 0.3 ‰, respectively.[6] Although $\delta^{15}N$ TDFs can be affected by nutritional stress, dietary quality and quantity,[85,87,88] these factors did not influence the observed lion $\delta^{15}N$ TDFs as the animals were well maintained by Pretoria Zoo staff and fed diets that met their caloric requirements.

TDFs may vary between individuals of the same species due to differences in diet, age and sex.[58,59] In this study, the sampled felids consumed the same diet in equal proportions; therefore, diet switching or alteration did not affect the obtained whisker-diet TDFs. Despite age and sex differences of the individual lions (Table 1), the $\delta^{13}C$ (2.5–2.9 ‰) and $\delta^{15}N$ (2.4–2.8 ‰) whisker-diet TDFs were similar amongst individual lions. Thus, these two factors seem not to have affected the accurate estimation of lion whisker-diet TDFs presented in this study. Other studies found corresponding results; for instance, the fur-diet TDFs of male and female mountain lions were similar (i.e. the $\delta^{13}C$ and $\delta^{15}N$ TDFs for the female were 4.3 and 4.4 ‰, and those of the male were 5.1 and 4.6 ‰, respectively).[61] The $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of two ringed seals (*Pusa hispida*) from different age and sex classes were also similar.[62] However, in all cases, definitive deductions were limited by the small and/or unbalanced sample.

One of the important, yet controversial, stages of isotopic discrimination experiments is sample preparation, as there are different schools of thought on how samples should be prepared for analysis. The issue of lipid-extracting dietary sources has been addressed differently among ecologists over the years. Experiments on the estimation of tissue-diet TDFs are advised to first assess the lipid content of prey sources as automatic removal of lipids might result in unreliable estimates if animals consume lipid-rich diets, since lipids are typically ^{13}C-depleted compared with other macronutrients.[55,89] Another major concern is the use of chloroform/methanol solution to extract lipids as the chemical can cause ^{15}N fractionation of 0.3–2.5 ‰.[6,90] Although a significant difference between lipid-extracted and non-lipid-extracted meat samples (chicken and beef combined) was found in this study, the difference was quite small implying that the lipid content of both beef and chicken was too small to have resulted in differences in $\delta^{13}C$ and $\delta^{15}N$ values.

Studies on isotopic tissue-diet differences are often limited by sample size as zoological institutions where most experiments are carried out can only accommodate a small number of species and individuals, especially large carnivores. In this study, the available small and unbalanced sample, i.e. five lions (four females and one male) with 30 data points from one individual (Amber) and six from the others, was used to estimate lion ε^*_{WD}. Another limitation was that the Zoo diet comprised two types of meat (chicken and beef) which were not isotopically homogeneous, although the isotope compositions of both meat types (as well as the proportions of each meat eaten by experimental individuals) were consistent over time. It is possible that some individuals assimilated more of the chicken than beef, or vice versa, which could influence the obtained values.[75] Furthermore, the meat was composed not only of muscle, but also of other tissues such as bone, skin, feathers and adipose tissue, which were also consumed by the lions. These tissues have varying isotopic signatures that all contribute to the observed whisker isotope signatures.[30,32] However, the vast majority of the diet comprised muscle, and broad similarities in estimated TDFs across individuals suggest that the above factors did not have major effects on the obtained results.

5 | CONCLUSIONS

Whiskers are increasingly used in stable-isotope-based dietary studies of free-ranging animals as the tissue provides an archive of dietary patterns recorded during the period of growth. This study contributed to resolving questions about the major limiting factors related to use of whiskers, i.e. whisker growth rates, whisker growth patterns and whisker-diet TDFs. The study showed that the whisker growth rates and whisker-diet TDFs of large-bodied felids fall within the range of values previously reported for several marine and terrestrial mammals.

However, the $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs differed from the hair values of some felid species. Age, sex and diet did not seem to influence $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs, and whisker growth differed only minimally between individuals. Further investigations need to be carried out on male lions using full growth trajectories for an understanding of their whisker growth and effects thereof. In addition, a larger and balanced sample is needed to provide more knowledge on life history influences on felid whisker growth rate. The results of this study also highlighted that whisker growth can be assumed to be linear for the proximal ~50–60 mm of whiskers longer than 128 mm, but the non-linear growth in whisker sections longer than 60 mm (measured from the root) may have substantial effects on interpreting timeframes.

The study did not directly measure the duration of whisker growth cycle; however, the conclusion that it is longer than 6 months could be reached as the whiskers were still growing. Thus, more research on the period of felid whisker growth cycle and shedding is needed. Long-term studies on turnover rates should be conducted to track the change in whisker-diet TDFs, along with any potential fluctuations in stable isotope ratios of the diet. It will be important to study a variety of felid species and individuals in the future to explore the causes of such wide variation in felid hair TDFs as this might provide more insights into isotope discrimination. Despite the outlined limitations of this study, the similarity of calculated growth rates between the three lionesses and leopard for the proximal ~50 mm of whiskers longer than 128 mm suggest that the mean felid whisker growth rate of 0.65 mm day^{-1} can be used with confidence in future dietary studies of lion and leopard. Moreover, the mean lion $\delta^{13}C$ and $\delta^{15}N$ whisker-diet TDFs of 2.7 and 2.5 ‰, respectively, are probably the best appraisal for the species thus far since they were estimated using more individuals (n = 5) sustained on a consistent chicken and beef diet over multiple years.

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