Comparative Biochemistry and Physiology, Part A 264 (2022) 111100

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Research Article

Scaling at different ontogenetic stages: Gastrointestinal tract contents of a marsupial foregut fermenter, the western grey kangaroo *Macropus fuliginosus melanops*

Adam J. Munn [a], Edward P. Snelling [b,c,d], David A. Taggart [b], Marcus Clauss [e,*]

[a] Laboratory for Ecological and Applied Physiology, Otford, NSW 2508, Australia
[b] Department of Ecology and Environmental Science, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia
[c] Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[d] Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[e] Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

ARTICLE INFO

Edited by: Michael Hedrick

Keywords:
Marsupial
Digestion
Gastrointestinal tract
Development
Ontogeny
Allometry

ABSTRACT

Prominent ontogenetic changes of the gastrointestinal tract (GIT) should occur in mammals whose neonatal diet of milk differs from that of adults, and especially in herbivores (as vegetation is particularly distinct from milk), and even more so in foregut fermenters, whose forestomach only becomes functionally relevant with vegetation intake. Due to the protracted lactation in marsupials, ontogenetic differences can be particularly well investigated in this group. Here, we report body mass (BM) scaling relationships of wet GIT content mass in 28 in-pouch young (50 g to 3 kg) and 15 adult (16–70 kg) western grey kangaroos *Macropus fuliginosus melanops*. Apart from the small intestinal contents, in-pouch young and adults did not differ in the scaling exponents ('slope' in log-log plots) but did differ in the scaling factor ('intercept'), with an implied substantial increase in wet GIT content mass during the out-of-pouch juvenile period. In contrast to forestomach contents, caecum contents were elevated in juveniles still in the pouch, suggestive of fermentative digestion of milk and intestinal secretion residues, particularly in the caecum. The substantial increase in GIT contents (from less than 1 to 10–20% of BM) was associated mainly with the increase in forestomach contents (from 25 to 80% of total GIT contents) and a concomitant decrease in small intestine contents (from 50 to 8%), emphasizing the shifting relevance of auto-enzymatic and allo-enzymatic (microbial) digestion. There was a concomitant increase in the contents-to-tissue ratio of the fermentation chambers (forestomach and caecum), but this ratio generally did not change for the small intestine. Our study not only documents significant ontogenetic changes in digestive morpho-physiology, but also exemplifies the usefulness of intraspecific allometric analyses for quantifying these changes.

1. Introduction

Scaling relationships are widely used to investigate how the sizes of various body parts or the rates and durations of various physiological and life-history processes vary proportionately with body mass (Calder, 1996; Sibly et al., 2012; Prothero, 2015). Interspecific scaling relationships with data from mature animals are often used to derive or corroborate general rules, for example a geometric scaling of body length with body mass (Silva, 1998). Yet, even if such rules are commonly accepted based on comparative data, it does not automatically follow that ontogenetic scaling must replicate the same pattern. Ontogenetic scaling patterns are often used to compare the developmental trajectories between species (e.g. Weston, 2003). If the development to maturity only comprised a simultaneous, proportional increase of all body parts from initial to mature size, then the rules governing interspecific scaling should apply ontogenetically as well. However, such a development is rather unlikely, because different functional requirements of organs at different life stages often lead to heterochrony, i.e. different body parts develop at different rates (Huxley, 1932; Gould, 1966). For example, sexual organs may not be needed during juvenile stages and therefore mature later than organs important for juvenile survival. In many organisms, the organs responsible for

* Corresponding author.
E-mail addresses: a.munn@unswalumni.com (A.J. Munn), edward.snelling@up.ac.za (E.P. Snelling), david.taggart@adelaide.edu.au (D.A. Taggart), mclauss@vetclinics.uzh.ch (M. Clauss).

https://doi.org/10.1016/j.cbpa.2021.111100
Received 29 September 2021; Received in revised form 25 October 2021; Accepted 26 October 2021
Available online 30 October 2021

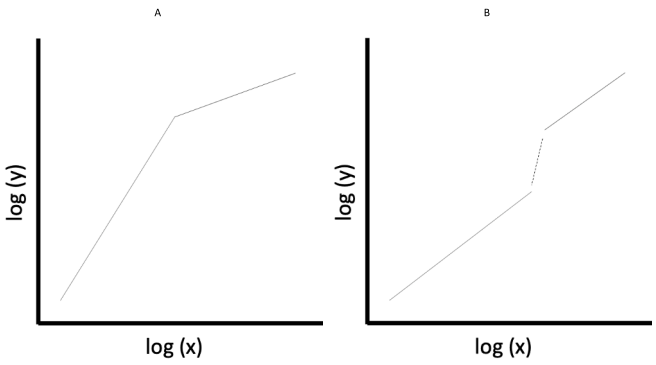

Fig. 1. Schematic multiphasic scaling relationships. (A) Biphasic growth with a breakpoint; (B) Multiphasic growth with a (presumed) steep growth between the two stages of similar growth at different levels of magnitude.

nutrient supply undergo distinct changes during ontogeny, which may be investigated using a scaling approach.

Because of heterochrony, a simple scaling function might not be adequate to describe a developmental pattern. A heterochronic development of several organs should necessarily translate into different stages in the growth of a specific organ, with a lower body mass scaling exponent during slower growth and a higher body mass scaling exponent at higher growth. Thus, so-called 'biphasic' growth patterns have been described (Huxley, 1932; Gould, 1966), for example with respect to the mammalian brain, where the general assumption is that of a rapid growth phase followed by a slow growth phase (Halley, 2016; Tsuboi et al., 2018). Regardless of the debate of how the data patterns should be interpreted (for brain growth Packard, 2019; Tsuboi, 2019; Packard, 2021) (and for a general approach Glazier, 2021), there is no *a priori* flaw in the hypothesis of multiphasic growth. Arguably, the number of different growth stages detected may well depend on the size and type of sample (e.g. whether many different embryological stages were sampled). For example, brain growth of rats and cats, as collated by Halley (2016) and re-analyzed by Packard (2021), visually appears to comprise more than two growth stages.

The use of logarithmic data transformation for the investigation of ontogenetic scaling has been repeatedly criticised and justified (reviewed by Glazier, 2021). In samples that reflect a data series where a steep slope in log-log plots abruptly changes to a shallow slope (Fig. 1A), as in many of the brain datasets mentioned above, a theoretical alternative description of the phenomenon using a single equation derived from untransformed data is feasible (Packard, 2019, 2021) even though the resulting biological interpretation is unclear (Tsuboi, 2019). For intraspecific patterns that display two parallel lines in log-log plots for different body size groups (with an implied very steep growth stage in between; Fig. 1B), however, the use of a single allometry with common scaling factors and intercepts for all measurements, while theoretically feasible, appears unsuitable, and the heuristic advantage of using log-transformed data is evident. Such a pattern has been observed in the ontogenetic scaling of the digestive tract of western grey kangaroos *Macropus fuliginosus melanops* (Munn et al., 2021). Here, we used data on the wet content mass of the same specimens to further illustrate varying patterns of ontogenetic scaling, and the relevance of clearly describing the sample set with respect to expected ontogenetic and life-history stages of the species.

The gastrointestinal tract (GIT) of a macropod is an ideal model for the investigation of scaling patterns for several reasons (Tyndale-Biscoe and Janssens, 1988). As marsupials, western grey kangaroos have a very large body mass range after birth – from pouch-bound, suckling young (1 g to 2 kg) to temporarily pouch-leaving, suckling and vegetation-consuming young (2 to 5 kg), to permanently out-of-pouch suckling and vegetation-consuming young (5 to 10 kg), to weaned juveniles (10 to 12 kg) and finally mature adults (up to 35 kg in females and 70 kg in males) (Munn et al., 2021). As mature-stage herbivores, kangaroos undergo a dietary shift from an animal-based diet – i.e. milk, typically considered high-quality and easily digestible by the animal's own enzymes mainly in the small intestine – to plant matter, including cell walls ('fibre') that generally reduces overall digestibility and requires fermentative digestion by microbes in a 'fermentation chamber' (Stevens and Hume, 1998). In macropods, fermentative digestion takes place in the voluminous forestomach as well as in the caecum and proximal colon (Munn et al., 2006; Munn and Dawson, 2006; Munn et al., 2009). The distal colon is mainly considered important for water reabsorption (e.g. Osawa and Woodall, 1992). In adult mammals, there is a clear difference in the relative GIT content mass between the trophic groups, with carnivores having less than one-half of the herbivores' dry matter gut fill (De Cuyper et al., 2020). Based on these considerations, we hypothesise two different scaling relationships for the suckling and the mature kangaroo stages for total digestive tract content mass – not necessarily in terms of the scaling exponent (the slope in the log-log plot), but in terms of the overall magnitude (the 'intercept'). To our knowledge, no scaling of wet GIT content mass for mammalian carnivores exists in the literature; for herbivores, wet gut content mass scaling is typically considered to be linear (or isometric, at a scaling exponent of about 1.0) (Parra, 1978; Clauss et al., 2007).

Additionally, the GIT comprises different functional units (Stevens and Hume, 1995), which facilitates the depiction of the change in the proportions of its individual components (Munn et al., 2021). During the transition from lactivore to herbivore, the sites of enzymatic digestion, the glandular stomach and the small intestine, are not expected to change their relationship to body mass, but they should decrease as a proportion of the total GIT at the expense of the main fermentation chamber. Such a shift has been described in a hindgut fermenter, the horse (Smyth, 1988), and in several foregut fermenters, including domestic ruminants (Wardrop and Coombe, 1960; Godfrey, 1961b; Short, 1964; Ruckebusch et al., 1983) and other artiodactyls like peccaries (Langer, 1978) and hippopotamids (Langer, 1976; Langer, 1988), in colobine primates and sloths (Langer, 1988), in muroid rodents (Maddock and Perrin, 1983) and in macropods (Langer, 1979; Munn et al., 2021). We expect such a shift in the dimensions of the forestomach sections to be also reflected in the contents of the respective sections, as demonstrated in domestic cattle (Godfrey, 1961a). Notably, such a shift will not be evident in an ontogenetic sample of specimens that are already completely weaned, as recently recorded for red kangaroos *Osphranter rufus* by Dawson et al. (2021).

Based on geometry (Calder, 1996) and the empirical relationships derived for herbivorous mammals mentioned above, we expect an isometric, linear mass-mass scaling, i.e. with a scaling exponent that includes 1.0 in the 95% confidence interval (95%CI), within each of the life stages. Steeper exponents that do not include linearity in the 95%CI are termed 'hyperallometric', and are expected for overall models that include both juvenile and adult kangaroos. Shallower exponents that do not include linearity in the 95%CI are termed 'hypoallometric'. The general expectation therefore also is that when expressed as a % of body mass, gut contents should be constant (i.e. the body mass scaling should include 0 in the 95%CI of the exponent). By contrast, no specific exponent was predicted for the scaling of GIT section contents when expressed as % of total GIT contents; the only expectation was a generally negative scaling for GIT sections of auto-enzymatic digestion (the acid hindstomach and the small intestine) when assessed across life stages, and a corresponding positive scaling for GIT sections of fermentative digestion (the forestomach and the hindgut).

2. Methods

2.1. Animal and gut contents sampling

The animal sample has been described previously in scaling studies on the macropod heart (Snelling et al., 2015a; Snelling et al., 2015b),

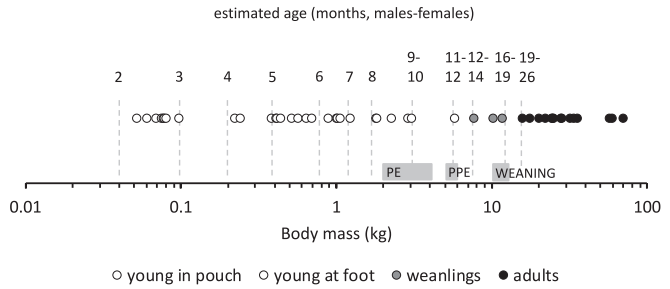

Fig. 2. Body mass distribution and age and life stage interpretation of the sample of western grey kangaroo *Macropus fuliginosus melanops* in the present study. The grey bars denote the body mass range of occasional pouch exit (PE), permanent pouch exit (PPE) and weaning (from Munn et al., 2021); age estimates derived from growth curves for *M. f. melanops* from Poole et al. (1982) and Poole et al. (1985).

muscle-tendon configuration of the hindlimb (Snelling et al., 2017), femoral bone perfusion (Hu et al., 2018) and GIT tissue mass (Munn et al., 2021). Animals were obtained from a planned management cull. A licensed marksman shot all animals according to Australian legal requirements and code of practice (www.environment.gov.au). Approval to take organ and tissue samples was granted by the University of Adelaide Animal Ethics Committee (S-2011-223). The animals represented 27 females and 20 males, and were selected so that a comprehensive range of developmental life-history stages and body masses was obtained, covering approximately 5 orders of magnitude, from the smallest (52 g) to the largest animal (70.5 kg) (Fig. 2).

Each carcass was weighed by spring or platform balance (Mettler-Toledo AE163, Mettler-Toledo, Switzerland; Sartorius QS16, Sartorius, Germany; or PCE-CS 300, PCE Instruments, UK; 2.0, 5.0, 20 or 100 kg capacity; at time of collection). In larger animals (at about >7 kg intact body mass), the abdominal cavity was opened ventrally and the entire GIT was ligated anteriorly at the oesophagus and caudally at the rectum before removal and stored frozen at −20 °C within 3 h of collection. For smaller animals (at about <7 kg intact body mass), the entire carcass was stored frozen at −20 °C within 3 h of collection. Each carcass or entire GIT was thawed prior to dissection. Once excised and thawed, GITs were ligated at the junction of each major section, preventing mixing between compartments. After removal of mesentery, connective tissue and fat, the individual sections were weighed full and again after emptying, rinsing and dry-blotting. Wet content mass was calculated by subtraction. The original data is given as Table S1.

2.2. Statistical analyses

Body mass data used in this study represents full and intact body mass (i.e. including the GIT and its contents). The sampled individuals were put into the four categories of in-pouch young ($n = 28$, 50 g to 3 kg body mass), young-at-foot ($n = 1$, 5.8 kg), weanlings ($n = 3$, 7.7 to 11.8 kg) and adults ($n = 15$, 15.9 to 70.5 kg) (Fig. 2). While all individuals were depicted in plots, general and group-specific scaling was assessed only for in-pouch juveniles and adults, and additionally for the group of adults and weanlings combined. Due to the sample size, females and males were not assessed separately. Linear models on log-transformed data as log(GIT content mass) = log(a) + b log(body mass) were performed in R v 3.3.2 (R_Core_Team, 2015) with the 'nlme' package (Pinheiro et al., 2011); model estimates are given with their 95%CI. Models were performed on the whole dataset, and additionally with the inclusion of group (joey-in-pouch or adult) as a cofactor and the group × body mass interaction, using the small sample corrected Akaike's information criterion (AICc) to compare model performance, considering models that differed by more than 2 (ΔAICc >2) as providing a different fit to the data (Burnham et al., 2011). In the case of a non-significant interaction, the model was repeated without it. Additionally, the individual scaling equations of in-pouch juveniles, adults, and adults plus weanlings were calculated separately. The significance level was set to 0.05. Data are displayed in log-log plots, with plots of the untransformed data given in the Online Supplement.

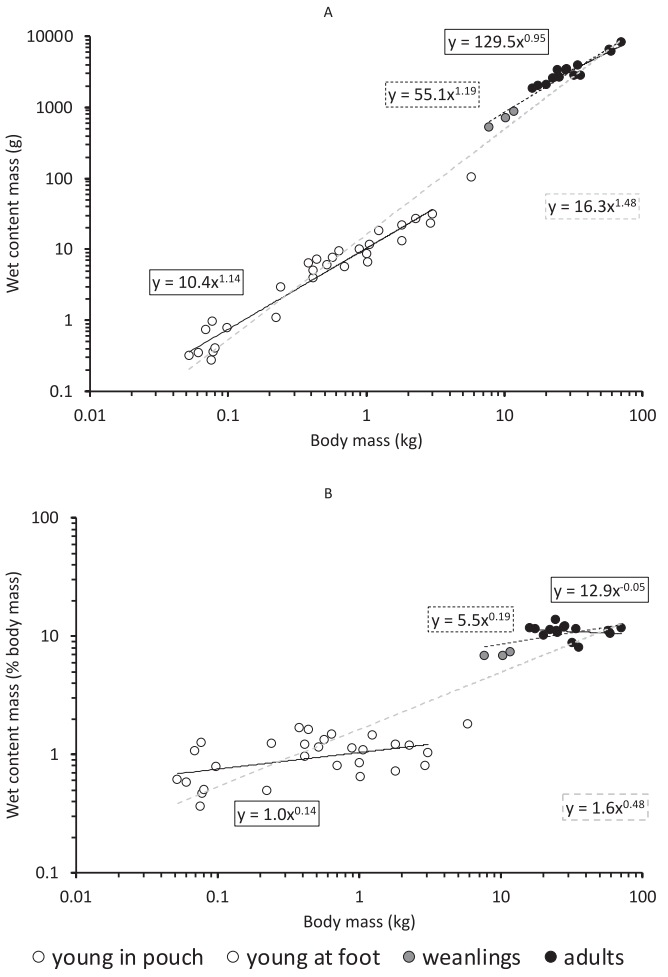

Fig. 3. Scaling of (A) absolute or (B) relative total gastrointestinal wet content mass with body mass in western grey kangaroo *Macropus fuliginosus melanops*. Regression lines denote the in-pouch and the adult samples (black lines), the adult animals together with the weanlings (black interrupted line) and the in-pouch together with the adult animals (grey interrupted line). For statistics, see Table 1.

3. Results

3.1. Total GIT content mass

The total GIT wet content mass scaled hyperallometrically for the entire sample, but the data were poorly reflected by the allometric model with one common intercept for all specimens (Fig. 3A), and therefore the model including a different intercept for the groups was much better supported (Table 1). The group × body mass interaction was not statistically significant ($P = 0.346$). In adults, content mass scaling included linearity (isometry) in the 95%CI of the exponent. Including the weanlings along with the adults led to hyperallometric scaling at an exponent whose 95%CI was above 1. Plotting the data as % of body mass made it even more evident that the two groups could not be reflected by a single allometric scaling, and that the young-at-foot (out of the pouch but still returning to suckle) and the weanlings should be considered intermediate stages between the in-pouch young and adults (Fig. 3B).

Table 1
Results of statistical analyses (re-transformed from log-transformed data) for total gastrointestinal wet content mass according to $y = a\ BM^b\ c$, where c denotes a factor for mature animals as compared to in-pouch young.

Sample	Model		Mean (95% CI)	t	P	AICc
In-pouch & Adults	BM	a	16.3 (13.8, 19.2)	33.20	<0.001	9.84
		b	1.48 (1.41, 1.55)	42.60	<0.001	
	BM + group	a	10.3 (8.9, 11.8)	32.11	<0.001	−28.94
		b	1.13 (1.04, 1.22)	24.64	<0.001	
		c	6.9 (4.5, 10.6)	8.72	<0.001	
In-pouch	BM	a	10.4 (8.7, 12.3)	26.88	<0.001	–
		b	1.14 (1.03, 1.25)	20.33	<0.001	
Adults	BM	a	129.5 (74.7, 224.3)	17.34	<0.001	–
		b	0.95 (0.79, 1.11)	11.62	<0.001	
Adults & Weanlings	BM	a	55.1 (34.2, 89.0)	16.42	<0.001	–
		b	1.19 (1.04, 1.34)	15.92	<0.001	

3.2. Forestomach content mass

The forestomach wet content mass scaled hyperallometrically for the entire sample, but the data were again poorly reflected by the allometric model with one common intercept for all specimens (Fig. 4A) and therefore, the model including a different intercept for the groups was much better supported (Table 2). The group × body mass interaction was not significant ($P = 0.964$). In both groups, content mass scaling included linearity (isometry) in the 95%CI of the exponent. Including the weanlings with the adults again led to hyperallometric scaling at an exponent whose 95%CI was above 1.

When expressing the forestomach content mass as a % of the total GIT content mass, a positive scaling with BM resulted for the entire sample, but again, a model including a different intercept for the groups was much better supported (Table 2); the group × body mass interaction was, again, not significant ($P = 0.493$). In this model, the forestomach content did not change its percentage of the total GIT contents with body mass, but was only at a 5.5 times higher level in the adults (Fig. 4B). When analysing the groups individually, there was no scaling in the in-pouch juveniles and only a slight scaling in the adults (Table 2).

3.3. Hindstomach content mass

The hindstomach wet content mass also scaled hyperallometrically for the entire sample, but the data were again poorly reflected by the allometric model with one common intercept for all specimens (Fig. 5A) and therefore, the model including a different intercept for the groups was much better supported (Table 3). Again, the group × body mass interaction was not significant ($P = 0.346$). In both groups, content mass scaling included linearity (isometry) in the 95%CI of the exponent, but the scaling was not significant in the adults. Including the weanlings with the adults led to a significant scaling that included linearity (isometry) (Table 3).

When expressing the hindstomach content mass as a % of the total GIT content mass, a negative scaling with BM resulted for the entire sample (Fig. 5B) that was better supported than a model including a different intercept for the groups (which was not significant, Table 3); the group × body mass interaction was also not significant ($P = 0.513$). When analysing the groups individually, the scaling was never significant (Table 3).

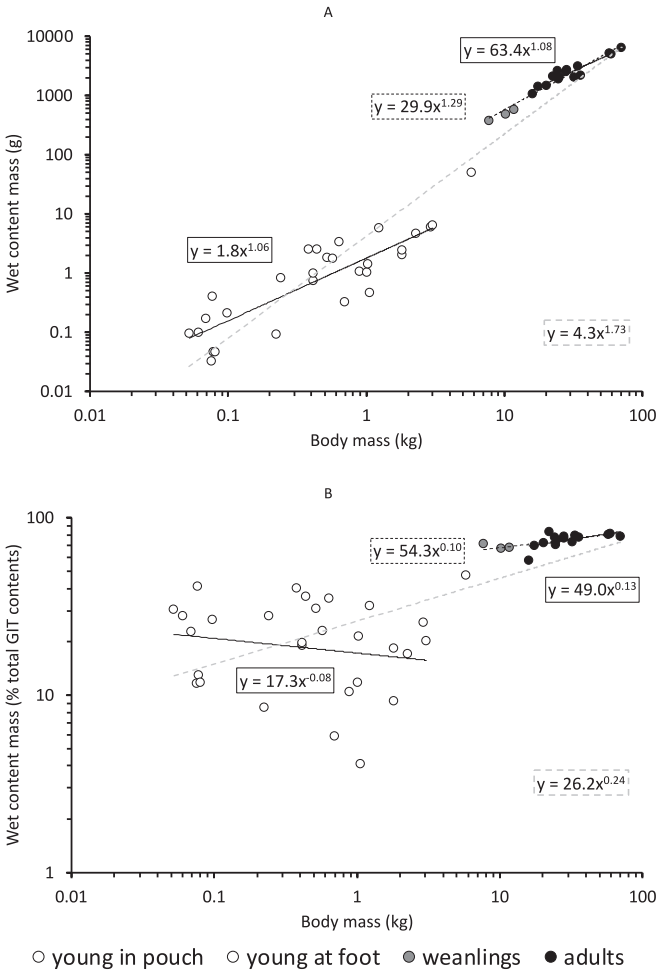

Fig. 4. Scaling of (A) absolute or (B) relative forestomach wet content mass with body mass in western grey kangaroo *Macropus fuliginosus melanops*. Regression lines denote the in-pouch and the adult samples (black lines), the adult animals together with the weanlings (black interrupted line) and the in-pouch together with the adult animals (grey interrupted line). For statistics, see Table 2.

3.4. Small intestine content mass

The small intestine wet content mass also scaled hyperallometrically for the entire sample (Table 4). The model including the interaction performed better, with significantly shallower scaling in adults than in in-pouch juveniles (Fig. 6A). In in-pouch juveniles, content mass scaling was above linearity (isometry) in the 95%CI of the exponent, and the scaling was not significant in the adults. Including the weanlings with the adults led to a significant scaling that included linearity (isometry) (Table 4).

When expressing the small intestine content mass as a % of the total GIT contents, a negative scaling with BM resulted for the entire sample (Fig. 6B), but the simple model fitted the data poorly compared to the model that included the group × body mass interaction (Table 4); the slope for the adults was significantly shallower than that of the in-pouch juveniles. When analysing the groups individually, the scaling was not significant in the in-pouch juveniles but significantly negative in the adults, regardless of whether the weanlings were included in the analysis (Table 4).

3.5. Caecum content mass

The caecum wet content mass also scaled hyperallometrically for the

Table 2
Results of statistical analyses (re-transformed from log-transformed data) for forestomach wet content mass (absolute or as % of total gastrointestinal contents) according to $y = a\ BM^b\ c$, where c denotes a factor for mature animals as compared to in-pouch young.

Sample	Model		Mean (95% CI)	t	P	AICc
Absolute mass (g)						
In-pouch & Adults	BM	a	4.3 (3.1, 5.9)	8.67	<0.001	66.24
		b	1.73 (1.59, 1.86)	24.59	<0.001	
	BM + group	a	1.8 (1.3, 2.4)	3.70	<0.001	−28.94
		b	1.06 (0.86, 1.25)	10.59	<0.001	
		c	37.6 (14.6, 96.9)	7.51	<0.001	
In-pouch	BM	a	1.8 (1.2, 2.6)	2.97	0.006	–
		b	1.06 (0.81, 1.30)	8.35	<0.001	
Adults	BM	a	63.4 (32.9, 122.1)	12.41	<0.001	–
		b	1.08 (0.89, 1.27)	11.04	<0.001	
Adults & Weanlings	BM	a	29.9 (18.1, 49.3)	13.30	<0.001	–
		b	1.29 (1.13, 1.44)	16.45	<0.001	
Relative mass (% of total gastrointestinal contents)						
In-pouch & Adults	BM	a	26.2 (21.7, 31.6)	34.09	<0.001	20.60
		b	0.24 (0.16, 0.32)	6.01	<0.001	
	BM + group	a	17.5 (14.1, 21.7)	26.02	<0.001	4.33
		b	−0.07 (−0.21, 0.09)	−1.02	0.314	
		c	5.5 (2.8, 10.5)	5.06	<0.001	
In-pouch	BM	a	17.3 (13.2, 22.6)	20.91	<0.001	–
		b	−0.08 (−0.25, 0.09)	−0.94	0.355	
Adults	BM	a	49.0 (36.2, 66.3)	25.21	<0.001	–
		b	0.13 (0.04, 0.21)	2.79	0.015	
Adults & Weanlings	BM	a	54.3 (45.0, 65.4)	42.01	<0.001	–
		b	0.10 (0.04, 0.15)	3.31	0.004	

entire sample (Table 5). The model including a difference in the intercept between the groups once again performed better, with a higher intercept in adults than in-pouch juveniles (Fig. 7A); the body mass-group interaction was not significant ($P = 0.162$). In in-pouch juveniles, content mass scaling was above linearity (isometry) in the 95%CI of the exponent, but the scaling included linearity (isometry) in the adults with or without the weanlings (Table 5).

When expressing the caecum content mass as a % of the total GIT contents, there was no significant scaling for the entire sample (Fig. 7B); the simple model basically fitted the data as well as the model including a (non-significant) difference in intercept between the groups (Table 5); the body mass-group interaction was not significant ($P = 0.424$). When analysing the groups individually, there was a positive scaling in the in-pouch juveniles, but no significant scaling in the adults, regardless of whether the weanlings were included in the analysis (Table 5).

3.6. Colon and rectum content mass

The colon and rectum wet content mass also scaled hyperallometrically for the entire sample (Table 6). The model that included a difference in the intercept between the groups performed better overall, with a higher intercept in adults than in-pouch juveniles and linear

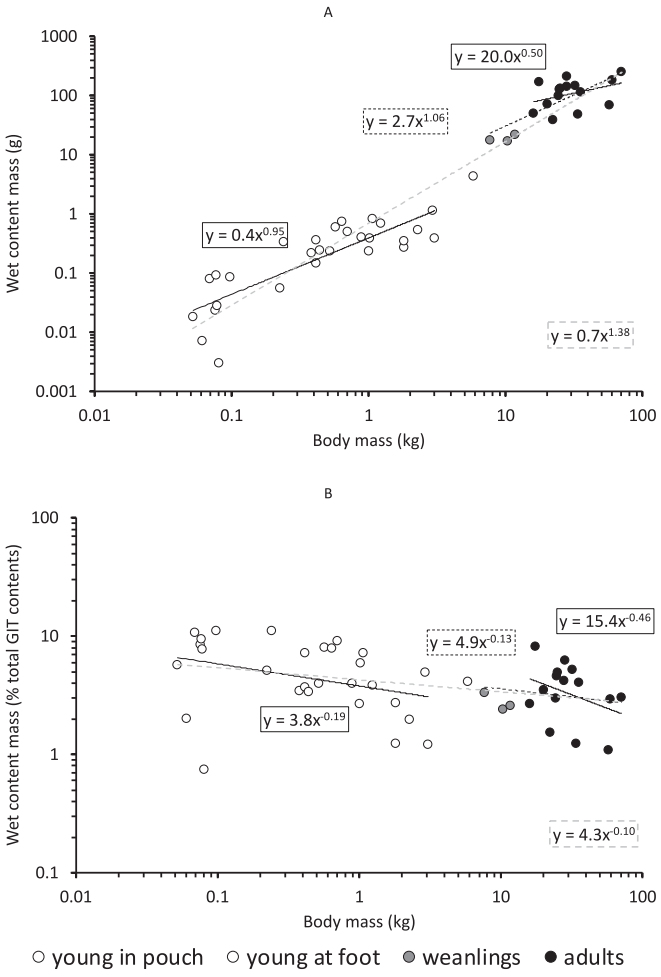

Fig. 5. Scaling of (A) absolute or (B) relative hindstomach wet content mass with body mass in western grey kangaroo *Macropus fuliginosus melanops*. Regression lines denote the in-pouch and the adult samples (black lines), the adult animals together with the weanlings (black interrupted line) and the in-pouch together with the adult animals (grey interrupted line). For statistics, see Table 3.

(isometric) scaling included in the 95% CI (Fig. 8A). The body mass-group interaction was not significant ($P = 0.740$). In all groups, content mass scaling included linearity (isometry) in the 95% CI of the exponent (Table 6).

When expressing the colon and rectum content mass as a % of the total GIT content mass, there was a negative scaling for the whole sample (Fig. 8B); the simple model basically fitted the data as well as the model including a (just non-significant) difference in intercept between the groups (Table 6); the body mass-group interaction was not significant ($P = 0.835$). When analysing the groups individually, there was no scaling in any of the groups (Table 6).

3.7. Untransformed data

Inspecting the plots showing the untransformed data did not suggest that a single allometric equation could reflect the data in a meaningful way (Online Supplement).

4. Discussion

Our ontogenetic study displays in detail the contrasting scaling of GIT wet content mass between juvenile kangaroos, that are exclusively dependent on mother's milk, and adult kangaroos, that are exclusively

Table 3
Results of statistical analyses (re-transformed from log-transformed data) for hindstomach wet content mass (absolute or as % of total gastrointestinal contents) according to $y = a\ BM^b\ c$, where *c* denotes a factor for mature animals as compared to in-pouch young.

Sample	Model		Mean (95% CI)	*t*	*P*	AICc
Absolute mass (g)						
In-pouch & Adults	BM	a	0.7 (0.5, 0.9)	−2.47	0.018	56.39
		b	1.38 (1.26, 1.51)	22.23	<0.001	
	BM + group	a	0.4 (0.3, 0.5)	−5.45	<0.001	41.87
		b	0.93 (0.71, 1.14)	8.34	<0.001	
		c	12.0 (4.2, 34.3)	4.63	<0.001	
In-pouch	BM	a	0.4 (0.3, 0.6)	−4.71	<0.001	–
		b	0.95 (0.70, 1.20)	7.45	<0.001	
Adults	BM	a	20.0 (2.21, 83.6)	2.64	0.020	–
		b	0.50 (−0.15, 1.14)	1.50	0.158	
Adults & Weanlings	BM	a	2.7 (0.6, 12.7)	1.26	0.227	–
		b	1.06 (0.58, 1.53)	4.36	<0.001	
Relative mass (% of total gastrointestinal contents)						
In-pouch & Adults	BM	a	4.2 (3.5, 5.2)	14.11	<0.001	26.38
		b	−0.10 (−0.18, −0.02)	−2.32	0.025	
	BM + group	a	3.7 (2.8, 5.0)	8.89	<0.001	28.29
		b	−0.20 (−0.39, −0.02)	−2.16	0.037	
		c	1.7 (0.7, 4.2)	1.23	0.227	
In-pouch	BM	a	3.8 (2.8, 5.2)	8.31	<0.001	–
		b	−0.19 (−0.39, 0.02)	−1.81	0.081	
Adults	BM	a	15.4 (1.61, 51.6)	2.34	0.036	–
		b	−0.46 (−1.12, 0.21)	−1.34	0.203	
Adults & Weanlings	BM	a	4.9 (1.2, 20.2)	2.19	0.044	–
		b	−0.13 (−0.57, 0.30)	−0.61	0.553	

Table 4
Results of statistical analyses (re-transformed from log-transformed data) for small intestine wet content mass (absolute or as % of total gastrointestinal contents) according to $y = a\ BM^b\ c\ int$, where *c* denotes a factor for mature animals as compared to in-pouch young, and *int* the body mass-group (young or adult) interaction.

Sample	Model		Mean (95% CI)	*t*	*P*	AICc
Absolute mass (g)						
In-pouch & Adults	BM	a	5.4 (4.7, 6.2)	24.87	<0.001	−7.57
		b	1.16 (1.10, 1.21)	40.48	<0.001	
	BM × group	a	5.3 (4.4, 6.3)	18.61	<0.001	−10.80
		b	1.15 (1.04, 1.27)	19.96	<0.001	
		c	15.8 (3.2, 77.1)	3.41	0.002	
		int.	0.2 (0.1, 0.5)	−3.28	0.002	
In-pouch	BM	a	5.3 (4.5, 6.3)	19.53	<0.001	–
		b	1.15 (1.04, 1.26)	20.95	<0.001	
Adults	BM	a	83.7 (15.1, 464.6)	5.06	<0.001	–
		b	0.36 (−0.14, 0.86)	1.41	0.181	
Adults & Weanlings	BM	a	23.7 (7.6, 73.8)	5.46	<0.001	–
		b	0.72 (0.37, 1.06)	4.03	0.001	
Relative mass (% of total gastrointestinal contents)						
In-pouch & Adults	BM	a	33.3 (28.7, 38.6)	46.51	<0.001	0.98
		b	−0.33 (−0.39, −0.27)	−10.38	<0.001	
	BM × group	a	51.1 (45.3, 57.6)	64.20	<0.001	−40.46
		b	0.01 (−0.06, 0.09)	0.32	0.750	
		c	1.3 (0.4, 3.7)	0.43	0.673	
		int.	0.2 (0.1, 0.5)	−3.65	<0.001	
In-pouch	BM	a	51.1 (46.6, 56.1)	82.85	<0.001	–
		b	0.01 (−0.05, 0.07)	0.41	0.682	
Adults	BM	a	64.6 (15.2, 274.3)	5.65	<0.001	–
		b	−0.59 (−1.01, −0.17)	−2.74	0.017	
Adults & Weanlings	BM	a	43.0 (17.9, 103.3)	8.41	<0.001	–
		b	−0.47 (−0.74, −0.21)	−3.47	0.003	

herbivorous. In particular, the expected difference in gut fill between the two life stages is evident. These data patterns have well-known implications for analytical approaches for the derivation of allometric relationships, as well as for understanding basic digestive physiology. Before addressing these issues, some constraints of the present study are examined.

4.1. Constraints of the study

The data presented do not reveal growth rates of the animals, because the ages of the animals were unknown. Therefore, while we can describe the pattern of growth for a certain body mass range, we cannot deduce whether this body mass range is actually passed through expeditiously or slowly, and therefore, we cannot weigh whether the transitionary growth period between two 'static' growth stages is brief or long. Given this constraint, it may be semantically more accurate to refer to a 'transition period' rather than a mathematical 'break point' that suggests a singular, abrupt change in scaling pattern. In Fig. 2, we attempt to illustrate the periods involved by re-calculating the estimated ages of the animals based on growth curves given for this species in the literature. These estimates indicate that the in-pouch phase, visually apparent as a plateau in several of our graphs, occurs at about 10 months of age, of a similar or even slightly shorter duration than the transition period between a young-at-foot and an adult animal.

Our dataset only contains four specimens in the transition stage from a purely milk-dependent juvenile to a purely vegetation-consuming adult. Although an even sampling of life stages is preferred, out-of-pouch young during their transitory period from permanent pouch exit to weaning are less common compared to adults (which may also being carrying in-pouch young). It is especially within the body mass range across this transitional life stage, and the corresponding 10–16 months of development, that distinct changes in the contents of the total GIT, forestomach, hindstomach, small intestine and colon/rectum must be located, as evidenced by the corresponding lack of transitions in our dataset. It was only for the contents of the caecum that the development towards the magnitude of weaned and adult animals was already reflected in the pouched-young life stage (Fig. 7). Thus, our grasp of this transition period, which must be marked by distinctive changes in digestive anatomy and physiology, remains limited. The visual patterns of the three weanling animals in Fig. 4 seem to suggest that this transition period might also be characterised by several 'stable phases' that would necessarily have to be interrupted by growth bursts.

The inclusion or exclusion of the three animals designated as 'weanlings' illustrates the problem associated with ascribing a specimen to an age category. The scaling of gut contents changed notably

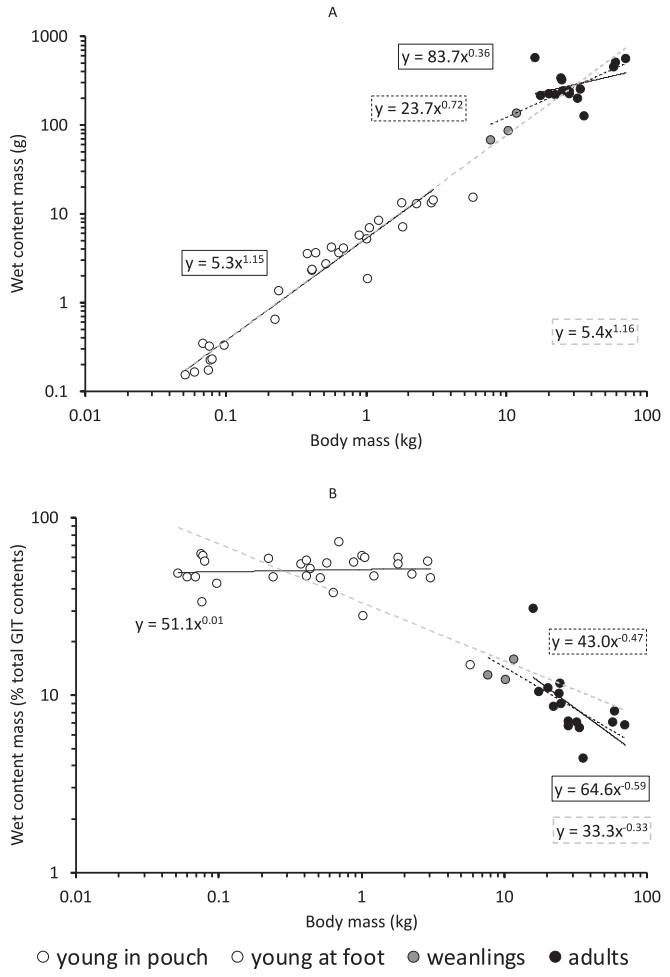

Fig. 6. Scaling of (A) absolute or (B) relative small intestine wet content mass with body mass in western grey kangaroo *Macropus fuliginosus melanops*. Regression lines denote the in-pouch and the adult samples (black lines), the adult animals together with the weanlings (black interrupted line) and the in-pouch together with the adult animals (grey interrupted line). For statistics, see Table 4.

depending on whether the animals designated as 'weanlings' were included in, or excluded from, the allometry of the adult animals. Including these three animals led to hyperallometric scaling of total GIT and forestomach content mass, and to a significant scaling of hindstomach and small intestine content mass. These effects did not occur when these three animals were excluded from the analysis. Only for the caecum and colon contents did the inclusion of weanlings make little qualitative difference to the overall scaling pattern. In the absence of a denser ontogenetic sample during the weaning period, we cannot invoke more than plausibility and the visual pattern of the present study as well as the transient position of these animals' total GIT and forestomach tissue mass (Fig. 4 in Munn et al., 2021) as a reason to consider these three animals as an intermediate stage in terms of digestive tract development.

4.2. Allometric approaches

The recent debate on the appropriateness of log-transformation for the investigation of ontogenetic development shall not be reiterated here; we refer to the current review by Glazier (2021). In accord with this review, a comparison of the visual patterns in log-log space (Figs. 3–8) with those in the Online Supplement, yield the view that without transformation, important stages in the data cannot be adequately evaluated.

Table 5
Results of statistical analyses (re-transformed from log-transformed data) for caecum wet content mass (absolute or as % of total gastrointestinal contents) according to $y = a\ BM^b\ c$, where c denotes a factor for mature animals as compared to in-pouch young.

Sample	Model		Mean (95% CI)	t	P	AICc
Absolute mass (g)						
In-pouch & Adults	BM	a	0.5 (0.4, 0.6)	−7.15	<0.001	22.39
		b	1.55 (1.47, 1.64)	37.82		
	BM + group	a	0.4 (0.3, 0.5)	−7.21	<0.001	19.36
		b	1.36 (1.19, 1.52)	16.19	<0.001	
		c	2.9 (1.3, 6.5)	2.66	0.011	
In-pouch	BM	a	0.4 (0.3, 0.5)	−6.23	<0.001	–
		b	1.39 (1.20, 1.57)	14.41	<0.001	
Adults	BM	a	5.8 (1.2, 27.6)	2.19	0.047	–
		b	0.88 (0.42, 1.33)	3.76	0.002	
Adults & Weanlings	BM	a	3.9 (1.4, 10.3)	2.70	0.016	–
		b	0.99 (0.69, 1.29)	6.48	<0.001	
Relative mass (% of total gastrointestinal contents)						
In-pouch & Adults	BM	a	3.1 (2.5, 3.7)	10.86	<0.001	26.44
		b	0.07 (−0.01, 0.16)	1.64	0.108	
	BM + group	a	3.7 (2.8, 5.0)	9.12	<0.001	26.27
		b	0.23 (0.05, 0.41)	2.49	0.017	
		c	0.4 (0.2, 1.0)	−1.93	0.061	
In-pouch	BM	a	3.8 (2.7, 5.3)	8.02	<0.001	–
		b	0.25 (0.04, 0.46)	2.29	0.030	
Adults	BM	a	4.5 (0.8, 23.9)	1.74	0.105	–
		b	−0.07 (−0.56, 0.42)	−0.29	0.773	
Adults & Weanlings	BM	a	7.0 (2.4, 20.3)	3.59	0.003	–
		b	−0.20 (−0.52, 0.13)	−1.20	0.249	

As expected, models that included a difference in the intercept for the two growth stages with parallel slopes in log-log space typically showed a better data fit by AICc than an allometry with a common intercept for the two growth stages. This observation cautions against the uncritical inclusion of specimens in the derivation of any allometric analysis. Arguably, other mathematical approaches than one or several simple scaling functions could be applied to the data. In particular, the pattern of two parallel lines in log-log space might call for a sigmoidal function, like an expanded Gompertz model. However, in the absence of a larger number of species for which parameter settings of such models could be compared, the value of such a fitting exercise in terms of biological insight would be slight. The scaling functions used here allow a comparison with existing knowledge considering the scaling of gut contents and the identification of the body mass range of the transitional stage.

4.3. Digestive morpho-physiology

During the stages of exclusive herbivory (in specimens designated 'adult'), the western grey kangaroo showed the expected close-to-linear (isometric) scaling for total GIT, forestomach, hindstomach, caecum and colon content masses. It was only for the small intestine content mass that no significant scaling (with an exponent of zero included in the 95% CI; Table 4) was observed within the adult kangaroos. A linear (isometric) scaling of gut content mass or volume has been described in several adult mammalian herbivores, most notably the ruminants (Ramzinski and Weckerly, 2007; Weckerly, 2010), and is generally interpreted as indicating a geometrically proportional relationship. The fact that this scaling is steeper than that usually expected for energy

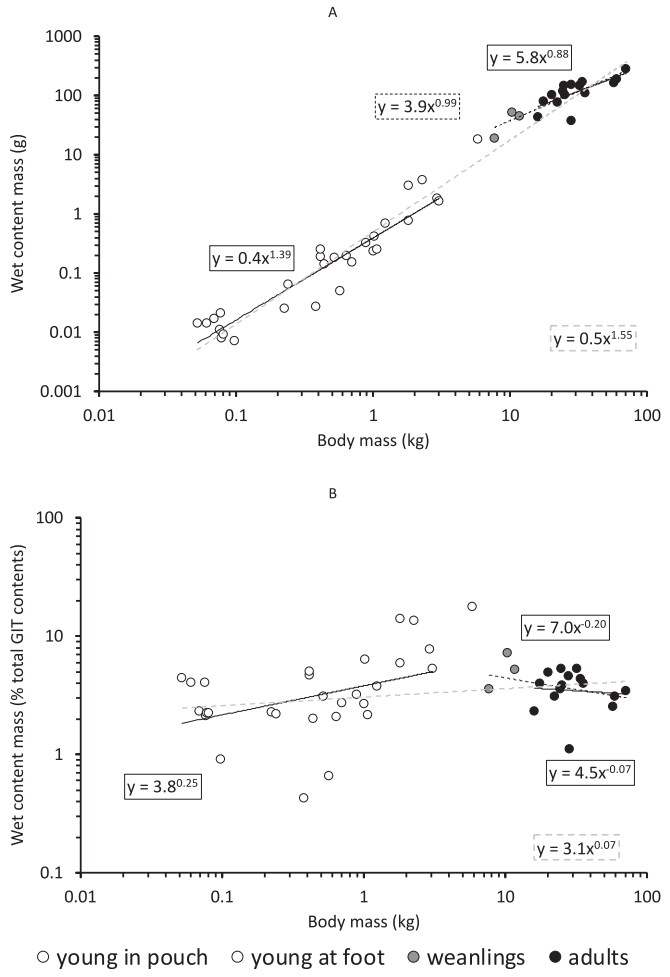

Fig. 7. Scaling of (A) absolute or (B) relative caecum wet content mass with body mass in western grey kangaroo *Macropus fuliginosus melanops*. Regression lines denote the in-pouch and the adult samples (black lines), the adult animals together with the weanlings (black interrupted line) and the in-pouch together with the adult animals (grey interrupted line). For statistics, see Table 5.

Table 6
Results of statistical analyses (re-transformed from log-transformed data) for colon and rectum wet content mass (absolute or as % of total gastrointestinal contents) according to $y = a\ BM^b\ c$, where c denotes a factor for mature animals as compared to in-pouch young.

Sample	Model		Mean (95% CI)	*t*	*P*	AICc
Absolute mass (g)						
In-pouch & Adults	BM	a	2.1 (1.7, 2.5)	7.84	<0.001	18.23
		b	1.35 (1.28, 1.43)	34.63	<0.001	
	BM + group	a	1.6 (1.2, 2.0)	3.71	<0.001	13.53
		b	1.14 (0.99, 1.30)	14.68	<0.001	
		c	3.1 (1.5, 6.6)	3.04	0.004	
In-pouch	BM	a	1.6 (1.2, 2.1)	3.11	0.005	–
		b	1.15 (0.96, 1.34)	11.99	<0.001	
Adults	BM	a	7.1 (2.6, 19.4)	3.83	0.002	–
		b	1.04 (0.74, 1.33)	6.94	<0.001	
Adults & Weanlings	BM	a	5.4 (3.0, 9.8)	5.51	<0.001	–
		b	1.12 (0.93, 1.30)	11.91	<0.001	
Relative mass (% of total gastrointestinal contents)						
In-pouch & Adults	BM	a	12.8 (10.6, 15.3)	27.49	<0.001	17.87
		b	−0.13 (−0.21, −0.05)	−3.35	0.002	
	BM + group	a	15.4 (11.9, 19.8)	21.03	<0.001	17.72
		b	0.01 (−0.15, 0.18)	0.18	0.861	
		c	0.5 (0.2, 1.0)	−1.98	0.054	
In-pouch	BM	a	15.3 (11.3, 20.8)	17.40	<0.001	–
		b	0.01 (−0.19, 0.21)	0.10	0.921	
Adults	BM	a	5.5 (1.9, 16.1)	3.11	0.008	–
		b	0.09 (−0.23, 0.40)	0.53	0.603	
Adults & Weanlings	BM	a	9.8 (5.0, 19.2)	6.59	<0.001	–
		b	−0.08 (−0.28, 0.13)	−0.71	0.488	

requirement rates opens the possibility that larger individuals might ingest disproportionately more of a given food, or might digest food whose digestibility is constrained by the retention time to a greater extent (reviewed in Clauss et al., 2013).

In in-pouch juvenile kangaroos, isometric linear scaling was seen for the forestomach and hindstomach, but larger specimens had disproportionately more contents in the small intestine and the caecum, leading to an overall hyperallometric scaling of total GIT content mass in this group. With respect to the small intestine, this might indicate a slightly increasing use of milk during pouch time, similar to a slightly hyperallometric small intestine tissue scaling (Munn et al., 2021). The finding for the caecum appears peculiar by comparison. We have already reported the early increase in caecum tissue mass in pouched young (Munn et al., 2021), which is not paralleled by the hyperallometric scaling of contents mass, but actually precedes it. The most parsimonious explanation is that certain components of milk are microbially fermented in the caecum, initially increasing tissue growth and subsequently facilitating higher content volumes. The fact that this does not occur in the forestomach, the main site of microbial fermentation post-weaning, is not surprising. During the lactivorous suckling stage, the ventricular or gastric groove (Janssens and Ternouth, 1987; Langer, 1988; Langer, 1994) is thought to direct milk to the acid hindstomach, bypassing fermentation in the forestomach of foregut fermenters. Hence, any substrates in milk that are not completely degraded during auto-enzymatic digestion thus present opportunity for microbial fermentation in the caecum. Additionally, remains of acid hindstomach and small intestinal secretions will also be available for the caecal microbiome. Thus, the (possibly gradual) increase in caecum volume may anticipate the (possibly more distinct) increase in forestomach volume once solid food is ingested. Detailed analyses of juvenile caecal content would be required to further investigate this phenomenon.

During the transition from an in-pouch juvenile to an adult macropod, western grey kangaroos undergo a marked change in the distribution of GIT content mass (Fig. 9), with an increase of forestomach contents from 25 to 80% of total content mass, and a reduction of small intestine contents from 50 to 8%. According to our data, this change occurs between 3 and 15 kg, which corresponds to a 10–16-month period (Fig. 2). For the stomach alone, this represents an increase of the forestomach part from 78 to 96% of the stomach contents, which resembles the distribution of the dry matter in the forestomach of the eastern grey kangaroo *Macropus giganteus* (Langer et al. 1980), and the proportional volume estimates for juvenile (156 days) and adult pademelon *Thylogale thetis* (Langer, 1979). However, this author describes an even earlier shift of volume proportions in the pademelon, with the majority of stomach volume represented by the forestomach compartments in the earliest joey stages. This observation cautions against making assumptions about the directionality of ontogenetic changes without having investigated the full ontogenetic body mass range.

During the in-pouch stage in kangaroos, the forestomach mucosa changes from a uniform cell type that can secrete acid and pepsin to a mucosa of cardiac gastric glands (Griffiths and Barton, 1966; Waite

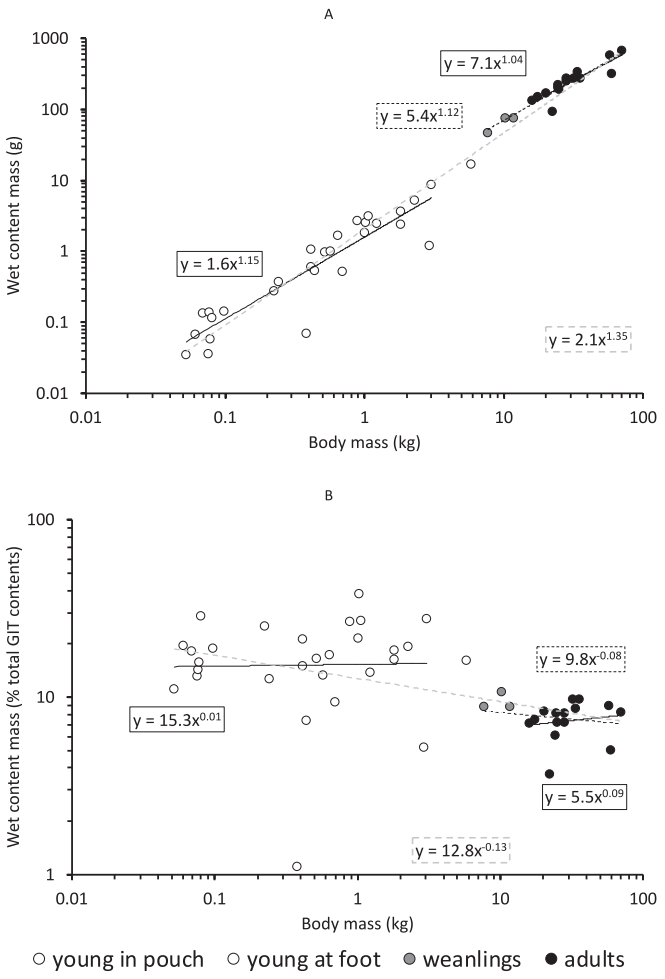

Fig. 8. Scaling of (A) absolute or (B) relative colon and rectum wet content mass with body mass in western grey kangaroo *Macropus fuliginosus melanops*. Regression lines denote the in-pouch and the adult samples (black lines), the adult animals together with the weanlings (black interrupted line) and the in-pouch together with the adult animals (grey interrupted line). For statistics, see Table 6.

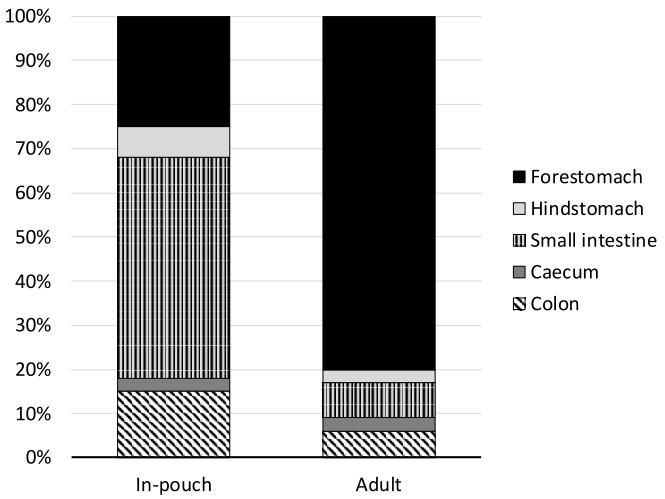

Fig. 9. Interpretative summary of the changes in the distribution of gut contents in the western grey kangaroo *Macropus fuliginosus melanops*. The change between the two extremes takes place in a 10–16-month period between 3 and 15 kg.

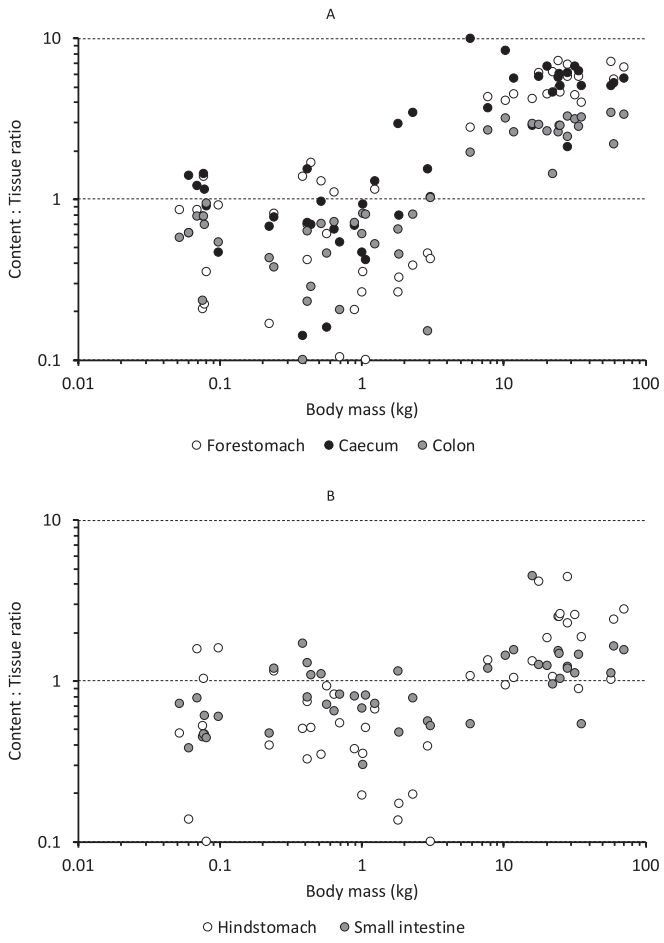

Fig. 10. The development of the gastrointestinal (GIT) wet content mass:tissue mass ratio (g/g) in western grey kangaroo *Macropus fuliginosus melanops* for (A) the GIT sections in which microbial fermentation of plant material occurs and (B) the GIT sections of auto-enzymatic digestion. Data combined from the present study and Munn et al. (2021).

et al., 2005; Kwek et al., 2009) that primarily secrete mucus, and tissue capable of auto-enzymatic activity shifts from the entire foregut complex to being restricted to the hindstomach only. The extent to which this is an adaptation for milk digestion in the greater forestomach complex prior to restriction of acidic digestion in the hindstomach remains speculative. On the one hand, the presence of a gastric groove, and especially the absence of notable forestomach contents in early joeys in the present study, indicates that the uniform cell type of the kangaroo forestomach early in life may not be an adaptation for milk digestion in the whole forestomach complex, but may simply be a feature of macropod forestomach developmental program. On the other hand, it has been questioned whether the gastric groove is active in in-pouch kangaroo young (Hume, 1982), and milk in the forestomach of pouch young has been reported (Janssens and Ternouth, 1987; Kwek et al., 2009). To our knowledge, the question remains unresolved.

Another feature of the developmental program of the kangaroo foregut is that the stomach mucosa reaches a state comparable to that of adults in in-pouch young well before vegetation is ingested in bulk (Griffiths and Barton, 1966; Waite et al., 2005); this process is possibly supported by the change in milk composition during pouch life (Kwek et al., 2009).

Our data demonstrate a momentous shift in GIT composition and structure in a developing macropod, with gut content mass changing from less than 1% in in-pouch young to between 10 and 20% of body mass in adults, representing a ten- to twenty-fold increase. This change greatly surpasses that of the GIT tissue mass, which changes from 1 to

2% of contents-free body mass to about 2.5–3.5% across development in this species (i.e. 'only' a two-fold increase) (Munn et al., 2021). Combining the data on total GIT content mass and total GIT tissue mass, there is a change in the contents-to-tissue ratio from the in-pouch young (mean ± SD: 0.65 ± 0.19), to young-at-foot (1.72), weanlings (3.07 ± 0.12) and then adults (3.98 ± 0.58). Inspecting this ratio for the individual GIT sections suggests a more or less parallel increase in the main sections where microbial fermentation of plant material occurs – the forestomach (in-pouch vs. adult: 0.63 ± 0.45 vs. 5.60 ± 1.10) and the caecum (1.24 ± 1.34 vs. 5.28 ± 1.29) (Fig. 10A). By contrast, the increase is less pronounced in the major site of auto-enzymatic digestion, the small intestine (0.75 ± 0.32 vs. 1.46 ± 0.88) (Fig. 10B). The hindstomach (0.55 ± 0.40 vs. 2.29 ± 1.04) and the colon (0.56 ± 0.25 vs. 2.82 ± 0.52) show an intermediate difference. In particular for the fermentation sites, this is suggestive of the expansibility of gut tissue that is supported by, but not necessarily requires, the haustration evident in the macropod forestomach (Langer and Takács, 2004). Such expansibility of the kangaroo foregut may represent a key difference between the kangaroos and ruminants (Munn et al., 2008), and has notable implications for kangaroo ecology (Munn and Dawson, 2006).

In conclusion, macropod marsupials undergo conspicuous shifts in their digestive morpho-physiology – justifying the comparison by Janssens and Ternouth (1987) who likened it to the metamorphosis of a tadpole into a frog. Among the unresolved questions about this transition from a milk to vegetation diet is whether the forestomach is initially involved in the digestion of milk, and the detailed documentation of developmental changes in young kangaroos at the out-of-pouch but still suckling stage.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

We acknowledge the humane marksmanship of the conservation cull, and we thank the marksman for allowing access to carcasses. We are indebted to volunteers who assisted in the field and in the laboratory. We thank John Snelling from the University of Adelaide for the donation of a utility vehicle to collect carcasses. This research was supported by the Australian Research Council (DP120102081).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2021.111100.

References

Burnham, K.P., Anderson, D.R., Huyvaert, K.P., 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behav. Ecol. Sociobiol. 65, 23–35.
Calder, W.A., 1996. Size, Function and Life History. Havard University Press, Cambridge, MA.
Clauss, M., Schwarm, A., Ortmann, S., Streich, W.J., Hummel, J., 2007. A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. Comp. Biochem. Physiol. A 148, 249–265.
Clauss, M., Steuer, P., Müller, D.W.H., Codron, D., Hummel, J., 2013. Herbivory and body size: allometries of diet quality and gastrointestinal physiology, and implications for herbivore ecology and dinosaur gigantism. PLoS One 8, e68714.
Dawson, T.J., Norton, M.A., Rodoreda, S., Abbott, S.K., McLeod, S.R., 2021. The burden of size and growth for the juveniles of large mammalian herbivores: structural and functional constraints in the feeding biology of juveniles relative to adults in red kangaroos, *Osphranter rufus*. Ecol. Evol. 11, 9062–9078.
De Cuyper, A., Meloro, C., Abraham, A.J., Müller, D.W.H., Codron, D., Janssens, G.P.J., Clauss, M., 2020. The uneven weight distribution between predators and prey: comparing gut fill between terrestrial herbivores and carnivores. Comp. Biochem. Physiol. A 243, 110683.
Glazier, D.S., 2021. Biological scaling analyses are more than statistical line fitting. J. Exp. Biol. 224 jeb241059.
Godfrey, N.W., 1961a. The functional development of the calf II. Development of rumen function in the calf. J. Agric. Sci. 57, 177–183.
Godfrey, N.W., 1961b. The functional development of the calf I. Growth of the stomach of the calf. J. Agric. Sci. 57, 173–175.
Gould, S.J., 1966. Allometry and size in ontogeny and phylogeny. Biol. Rev. 41, 587–640.
Griffiths, M., Barton, A.A., 1966. The ontongeny of the stomach in the pouch young of the red kangaroo. Wildl. Res. 11, 169–185.
Halley, A.C., 2016. Prenatal brain-body allometry in mammals. Brain Behav. Evol. 88, 14–24.
Hu, Q., Nelson, T.J., Snelling, E.P., Seymour, R.S., 2018. Femoral bone perfusion through the nutrient foramen during growth and locomotor development of western grey kangaroos (*Macropus fuliginosus*). J. Exp. Biol. 221 jeb168625.
Hume, I.D., 1982. Digestive Physiology and Nutrition of Marsupials. Cambridge University Press, Cambridge.
Huxley, J.S., 1932. Problems of Relative Growth. Methuen, London, UK.
Janssens, P.A., Ternouth, J.H., 1987. The transition from milk to forage diets. In: Hacker, J.B., Ternouth, J.H. (Eds.), The Nutrition of Herbivores. Academic Press, Sydney, pp. 281–305.
Kwek, J.H., De Iongh, R., Digby, M.R., Renfree, M.B., Nicholas, K.R., Familari, M., 2009. Cross-fostering of the tammar wallaby (*Macropus eugenii*) pouch young accelerates fore-stomach maturation. Mech. Dev. 126, 449–463.
Langer, P., 1976. Functional anatomy of the stomach of *Hippopotamus amphibius*. S. Afr. J. Sci. 72, 12–16.
Langer, P., 1978. Anatomy of the stomach of the collared peccary. Z. Säugetierkd. 43, 42–59.
Langer, P., 1979. Functional anatomy and ontogenetic development of the stomach in the macropodine species *Thylogale stigmatica* and *Thylogale thetis*. Zoomorphol. 93, 137–151.
Langer, P., 1988. The Mammalian Herbivore Stomach. Gustav Fischer Verlag, Stuttgart/New York.
Langer, P., 1994. Weaning time and bypass structures in the forestomachs of Marsupialia and Eutheria. In: Chivers, D.J., Langer, P. (Eds.), The Digestive System of Mammals: Food, Form and Function. Cambridge University Press, Cambridge.
Langer, P., Takács, A., 2004. Why are taeniae, haustra, and semilunar folds differentiated in the gastrointestinal tract of mammals, including man? J. Morphol. 259, 308–315.
Langer, P., Dellow, D.W., Hume, I.D., 1980. Stomach structure and function in three species of macropodine marsupials. Aust. J. Zool. 28, 1–18.
Maddock, A.H., Perrin, M.R., 1983. Development of the gastric morphology and fornical bacterial/epithelial association in the white-tailed rat *Mystromys albicaudatus*. Afr. Zool. 18, 115–127.
Munn, A.J., Dawson, T.J., 2006. Forage fibre digestion, rates of feed passage and gut fill in juvenile and adult red kangaroos (*Macropus rufus*): why body size matters. J. Exp. Biol. 209, 1535–1547.
Munn, A.J., Banks, P., Hume, I.D., 2006. Digestive plasticity of the small intestine and the fermentative hindgut in a marsupial herbivore, the tammar wallaby (*Macropus eugenii*). Aust. J. Zool. 54, 287–291.
Munn, A.J., Streich, W.J., Hummel, J., Clauss, M., 2008. Modelling digestive constraints in non-ruminant and ruminant foregut-fermenting mammals. Comp. Biochem. Physiol. A 151, 78–84.
Munn, A.J., Clissold, F., Tarszisz, E., Kimpton, K., Dickman, C.R., Hume, I.D., 2009. Hindgut plasticity in wallabies fed hay either unchopped or ground and pelleted: fiber is not the only factor. Physiol. Biochem. Zool. 82, 270–279.
Munn, A.J., Snelling, E.P., Taggart, D.A., Seymour, R.S., 2021. Ontogenetic scaling of the gastrointestinal tract of a marsupial foregut fermenter, the western grey kangaroo *Macropus fuliginosus melanops*. J. Comp. Physiol. B. 191, 371–383.
Osawa, R., Woodall, P.F., 1992. A comparative study of macroscopic and microscopic dimensions of the intestines in five macropods. II. Relationship with feeding habits and fibre content of the diet. Aust. J. Zool. 40, 99–113.
Packard, G.C., 2019. The fallacy of biphasic growth allometry for the vertebrate brain. Biol. J. Linn. Soc. 128, 1057–1067.
Packard, G.C., 2021. Allometric growth in mass by the brain of mammals. Anat. Rec. 304, 1551–1561.
Parra, R., 1978. Comparison of foregut and hindgut fermentation in herbivores. In: Montgomery, G.G. (Ed.), The Ecology of Arboreal Folivores. Smithsonian Institution Press, Washington DC, pp. 205–229.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Development Core Team, 2011. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3, pp. 1–102. Available at. https://cran.r-project.org/web/packages/nlme/.
Poole, W., Carpenter, S., Wood, J., 1982. Growth of grey kangaroos and the reliability of age determination from body measurements II. The western grey kangaroos, *Macropus fuliginosus fuliginosus*, *M. f. melanops* and *M. f. ocydromus*. Wildl. Res. 9, 203–212.
Poole, W., Carpenter, S., Wood, J., 1985. Growth of grey kangaroos and the reliability of age determination from body measurements II. The western grey kangaroos, *Macropus fuliginosus fuliginosus*, *M. f. melanops* and *M. f. ocydromus*: a correction. Wildl. Res. 12, 561–562.
Prothero, J.W., 2015. The Design of Mammals. A Scaling Approach. Cambridge University Press, Cambridge.
R_Core_Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. http://www.R-project.org/.
Ramzinski, D.M., Weckerly, F.W., 2007. Scaling relationship between body weight and fermentation gut capacity in Axis deer. J. Mammal. 88, 415–420.
Ruckebusch, Y., Dardillat, C., Guilloteau, P., 1983. Development of digestive functions in the newborn ruminant. Ann. Rech. Vét. 14, 360–374.

Short, H.L., 1964. Postnatal stomach development of white-tailed deer. J. Wildl. Manag. 28, 445–458.
Sibly, R.M., Brown, J.H., Kodric-Brown, A., 2012. Metabolic Ecology. A Scaling Approach. Wiley-Blackwell, Chichester, UK.
Silva, M., 1998. Allometric scaling of body length: elastic or geometric similarity in mammalian design. J. Mammal. 79, 20–32.
Smyth, G.B., 1988. Effects of age, sex, and post mortem interval on intestinal lengths of horses during development. Equine Vet. J. 20, 104–108.
Snelling, E.P., Taggart, D.A., Maloney, S.K., Farrell, A.P., Leigh, C.M., Waterhouse, L., Williams, R., Seymour, R.S., 2015a. Scaling of left ventricle cardiomyocyte ultrastructure across development in the kangaroo *Macropus fuliginosus*. J. Exp. Biol. 218, 1767–1776.
Snelling, E.P., Taggart, D.A., Maloney, S.K., Farrell, A.P., Seymour, R.S., 2015b. Biphasic allometry of cardiac growth in the developing kangaroo *Macropus fuliginosus*. Physiol. Biochem. Zool. 88, 216–225.
Snelling, E.P., Biewener, A.A., Hu, Q., Taggart, D.A., Fuller, A., Mitchell, D., Maloney, S. K., Seymour, R.S., 2017. Scaling of the ankle extensor muscle-tendon units and the biomechanical implications for bipedal hopping locomotion in the post-pouch kangaroo *Macropus fuliginosus*. J. Anat. 231, 921–930.
Stevens, C.E., Hume, I.D., 1995. Comparative Physiology of the Vertebrate Digestive System. Cambridge University Press, New York.
Stevens, C.E., Hume, I.D., 1998. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol. Rev. 78, 393–427.
Tsuboi, M., 2019. Biological interpretations of the biphasic model of ontogenetic brain–body allometry: a reply to Packard. Biol. J. Linn. Soc. 128, 1068–1075.
Tsuboi, M., van der Bijl, W., Kopperud, B.T., Erritzøe, J., Voje, K.L., Kotrschal, A., Yopak, K.E., Collin, S.P., Iwaniuk, A.N., Kolm, N., 2018. Breakdown of brain–body allometry and the encephalization of birds and mammals. Nat. Ecol. Evol. 2, 1492–1500.
Tyndale-Biscoe, C.H., Janssens, P.A., 1988. The Developing Marsupial: Models for Biomedical Research. Springer, Berlin.
Waite, R., Giraud, A., Old, J., Howlett, M., Shaw, G., Nicholas, K., Familari, M., 2005. Cross-fostering in *Macropus eugenii* leads to increased weight but not accelerated gastrointestinal maturation. J. Exp. Zool. A 303, 331–344.
Wardrop, I.D., Coombe, J.B., 1960. The post-natal growth of the visceral organs of the lamb I. The growth of the visceral organs of the grazing lamb from birth to sixteen weeks of age. J. Agric. Sci. 54, 140–143.
Weckerly, F.W., 2010. Allometric scaling of rumen-reticulum capacity in white-tailed deer. J. Zool. 280, 41–48.
Weston, E.M., 2003. Evolution of ontogeny in the hippopotamus skull: using allometry to dissect developmental change. Biol. J. Linn. Soc. 80, 625–638.