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Originally published at:
To chew or not to chew: faecal particle size in herbivorous reptiles and mammals

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Abstract

A major difference between reptile and mammalian herbivores is that the former do not masticate their food. Actually, food particle size reduction by chewing is usually considered one of the adaptations facilitating the higher metabolic rates of mammals. However, quantitative comparisons of ingesta particle size between the clades have, to our knowledge, not been performed so far. We measured mean faecal particle size (MPS) in 79 captive individuals of 14 reptile herbivore species (tortoises, lizards and Corucia zebrata) by wet sieving and compared the results to a mammalian dataset. MPS increased with body mass in both clades, but at a significantly higher level in reptiles. Limited evidence in free-ranging and captive individuals of Testudo hermanni indicate that in reptiles, the ability to crop food and the food particle size significantly influence faecal particle size. The opportunistic observation of a drastic particle size difference between stomach and intestinal contents corroborates findings that in reptiles, in contrast to terrestrial mammals, significant ingesta particle size reduction does occur in the gastrointestinal tract, most likely due to microbial action during very long ingesta retention. Whether behavioural adaptations to controlling ingesta particle size, such as deliberate small bite sizes, are adaptive strategies in reptiles remains to be investigated.

Key Words: herbivory, lizards, tortoises, retention time, mastication, digestion, fermentation
Introduction

The digestion of food particles in the gastrointestinal tract of herbivores depends on several factors like the chemical composition of the food, in particular the fibre fraction (Hummel and others, '06) and the retention time in the digestive tract (because fermentation by gut microbes is a time-dependent process, Stevens and Hume, '98). The size of the ingesta particles is a further fundamental factor that influences digestion. Smaller particles are fermented at higher rates by symbiotic microbes (e.g. Bjorndal and others, '90), and therefore, retention time in the gut and the efficiency of particle size reduction (i.e., chewing efficiency) can compensate for each other (Clauss and others, '09). In mammalian herbivores, different adaptations to reduce ingesta particle size have led to a large variety in chewing efficiency (Fritz and others, '09).

Similar to other vertebrate taxa, those reptiles that are herbivorous have comparatively larger body sizes (Pough, '73; Troyer, '91; King, '96; Bjorndal, '97), namely all tortoises, *Chelonia mydas*, about 30 species of *Iguanidae*, 17 species of *Agamidae* and *Corucia zebrata* (Iverson, '80). Herbivorous reptiles tend to be folivorous and mostly eat fully developed leaves. In contrast to herbivore mammals or birds, reptiles do not chew their food; it is bitten off and swallowed without any further mechanical disintegration (Hotton, '55; Ostrom, '63; Sokol, '67; Edmund, '69; Throckmorton, '76; Guard, '80; Bjorndal and Bolten, '92; Herrel and De Vree, '99; Herrel and others, '99; Herrel and others, '04). Consequently, particularly long ingesta retention times are needed to achieve degrees of fibre digestion comparable to that of mammals (Karasov and others, '86). For example, passage times of tortoises reach up to 200-300 hours (Bjorndal, '87; Bjorndal, '89; Barboza, '95; Hatt and others, '02), whereas most herbivorous mammals have mean ingesta retention times of around 48 hours (Clauss and others, '07). Herbivorous reptiles are all hindgut fermenters with long intestines, a
fermentation chamber in the area of the caecum and proximal colon, and (with the exception of tortoises) special anatomical structures usually interpreted as adaptations for delaying ingesta passage and thus enhancing digestive efficiency (Iverson, '80; Stevens and Hume, '98; Cooper and Vitt, '02); additionally, intestinal stasis has been hypothesized as a particular adaptation in herbivorous reptiles (Guard, '80), and antiperistaltic movements of the large intestine have been described in tortoises that will also contribute to long ingesta retention (Hukuhara and others, '75). In this respect, studies from Kim et al. ('65) indicate significant differences of gut motility between reptiles and mammals. All these adaptations support the concept that reptiles compensate for the absence of a chewing mechanism by increased ingesta retention.

However, ingesta particle size has not been investigated systematically in reptiles so far. Actually, only Guard ('80) and Foley et al. ('92) described ingesta particle size in reptiles in a quantitative way. Therefore, the aim of this study was to compare faecal particle sizes of different herbivore reptiles to that of herbivore mammals. Specifically, we hypothesized that

a) reptiles have larger faecal particles than mammals of comparable body size
b) because bite size should be related to body size in reptiles (Bjorndal and Bolten, '92), faecal particle size increases in reptiles with increasing body mass
c) faecal particle size is a function of food particle size in reptiles
d) captive reptiles that receive prepared food (and therefore cannot crop plant parts on their own) have larger faecal particle sizes than their free-ranging conspecifics (which can use the physical resistance of their food plants – which are anchored in the ground by roots or on twigs – to crop smaller bites)

Additionally, we tested in a small, opportunistic sample of individuals, whether particle size does not change noticeably along the digestive tract (as reviewed for mammals in Fritz and
others, '09), or whether significant differences in particle size along the digestive tract do occur (as demonstrated in an agamid by Foley and others, '92).

**Methods**

Seventy-nine individual faecal samples of 14 different reptile species were available for this study (Table 1). All animals sampled were healthy adult individuals from different zoological institutions in Europe. Diets varied between the institutions; main components were different amounts of hay, herbs, browse, leaves, salad, vegetables or fruits. Animals receiving a diet comprising predominantly pellets or small chopped components were excluded from this study. Body mass was either known for the individuals sampled, or estimated with reference to literature data. Additionally, faecal samples from free-ranging *Testudo graeca* (n=5) and *Testudo hermanni* (n=7) were available for comparison between free-ranging and captive animals. Captive animals of these species (*T. graeca*: n=9; *T. hermanni*: n=10) were sampled on two diets: a diet in which the food items, mainly parsley and grass hay, were chopped, and a diet in which these items were offered intact. The body masses of both captive and free-ranging animals of these two species were measured by weighing. Additionally, we had the opportunity to investigate gastrointestinal contents of adult *Corucia zebrata* (n=8) and *Iguana iguana* (n=1). All individuals of *Corucia zebrata* received the same diet consisting of mainly dandelion and some additional leaves of *Ficus benjamina*. Diets had been fed over weeks preceding the death of the animals. In the iguana, ingesta between the caecocolical septa and in the sulcus that bypasses the recessi between the septa were sampled separately. All faeces and ingesta were stored frozen after sampling until analysis.

Particle size analysis was performed by wet sieving with a Retsch® AS 200 digit laboratory sieve analyser (Retsch, Haan, Germany) with six sieves of 4, 2, 1, 0.5, 0.25 and 0.125 mm mesh size (linear dimension of holes). Particles passing the finest sieve were lost. Prior to sieving, samples were thawed, weighed in on a petri dish and then suspended in a beaker
(1000 ml) with water for a minimum of 12 hours, which was stirred irregularly until the faces had disintegrated. The sample material was then poured over the sieve cascade and rinsed with one litre of water. Sieving time was 10 minutes at a vibration intensity of approximately 2 mm and a water throughput of 2 l/min. The particles of each sieve fraction were transferred onto pre-weighed petri dishes, dried at 103°C for 24 hours, and weighed after cooling to room temperature in a desiccator using an analysis balance with measuring accuracy of 1 mg (Model AE160, Mettler-Instrumente, Gießen, Germany). The geometric mean of the particle size (MPS) of each sample was calculated after fitting a suitable function to the respective sample data using TableCurve 2D v5.01 (Systat Software UK Ltd., London, UK) as previously done in mammals (Hummel and others, '08; Fritz and others, '09). An average digesta particle size was calculated for each species. It should be noted that in sieve analysis, the finest fraction is either lost (as in this study) or cannot be adequately described (because the size of the particles in this fraction is not defined; this could only be achieved quantitatively by an optical analysis).

For the comparison with mammals, mammalian data from Fritz et al. (‘09) were used, excluding ruminants. For the interclade comparison, data were ln-transformed. If no significant difference in the slopes of the body mass-particle size relationship was found between the clades, a GLM with clade (reptile/mammal) as a fixed factor and body mass as a covariate was performed to test for significant influence of body mass or clade on faecal particle size. Similarly, data for free-ranging and captive tortoises were analysed for an influence of body mass and a difference between animals of different origin. In the captive animals that were fed two different diets, faecal particle size was compared by paired t-test. Due to the low sample size, gastrointestinal contents of I. iguana are only presented without statistical evaluation; differences in particle size between the caecum and the distal colon (faeces) of C. zebrata were tested by Wilcoxon-test. The tests were performed using the SPSS
16.0 (SPSS Inc., Chicago, IL, USA) statistical software package. The significance level was set to $\alpha=0.05$.

**Results**

In general, when compared to sieve analyses from mammals (e.g. ruminants, Clauss and others, '02), a high proportion of retained particles remained on the sieves with the larger pore sizes in reptiles (Table 1). Accordingly, reptiles had larger calculated mean faecal particle sizes than mammals (Fig. 1). Standard deviations of mean particle size measurements were of a considerable magnitude in several reptile species (Table 1). The General Linear Model indicated a highly significant influence of body mass on faecal particle size ($p<0.001$) as well as a highly significant difference between the two clades, mammals and reptiles ($p<0.001$). The overall model, including clades as a fixed factor and body mass as a covariate, had a high explanatory power ($r^2=0.71$).

Although numerical differences between means were as expected in *T. graeca* (Table 1), General Linear Models indicated no significant influence of body mass or origin on mean faecal particle size in this species (Fig. 2a; free-range vs. chopped food: body mass $p=0.803$, origin $p=0.248$; free-range vs. whole food: body mass $p=0.556$, origin $p=0.179$). Mean faecal particle size also did not differ between animals that received chopped or whole food (paired t-test, $p=0.249$).

General Linear Models always indicated a significant influence of body mass on mean faecal particle size in *T. hermanni* (Fig. 2b); differences between free-ranging animals and animals receiving chopped food were not significant, but those between free-ranging animals and animals receiving whole food were (free-range vs. chopped food: body mass $p=0.002$, origin $p=0.272$; free-range vs. whole food: body mass $p<0.001$, origin $p=0.011$). The difference in mean faecal particle size between animals that received chopped or whole food was also highly significant (paired t-test, $p<0.001$).
There were no ingesta in the stomachs of the dissected *C. zebrata*. Mean particle size in the caecum (9.30 ± 6.71 mm) and colon (11.65 ± 4.02 mm) did not differ significantly (p=0.686). In the iguana, there was a drastic difference in particle size between the stomach and the distal digestive tract (Fig. 3). Additionally, ingesta between the caecocolical septa had a lower particle size than ingesta in the colon and rectum.

**Discussion**

This study confirms that reptiles, in general, have larger ingesta particles than mammals; the difference is of such a scale that although particle size increases with body mass in both clades, reptiles generally have larger mean faecal particles even than mammals that exceed them in body size by magnitudes. Additionally, mean particle size measurements in reptiles (Table 1) often had considerably larger standard deviations as compared to values in mammals (Fritz and others, '09); this is an indication that variation in diets fed and food particle size have a larger effect in reptiles, whereas the chewing process itself in mammals has an equalizing effect on faecal particle size. This study also provides limited evidence that cropping and bite size could be of importance in reptile digestive physiology, and that differences in particle size must probably be expected along the reptile gastrointestinal tract even in the absence of particular grinding mechanisms.

The limitations of this study must be recognized. Given the influence of the diet on faecal particle size, in particular differences between free-ranging and captive animals as documented for some mammals by Hummel et al. ('08) and for one of the two tortoise species in this study (Fig. 2b), it would be best to perform an investigation such as this one on faeces from free-ranging animals only – a major logistical challenge. The reptile species of this study received diets that consisted mainly of vegetables and fruits, leaves, grass, herbs, hay and straw in tortoises; in contrast to mammals, pelleted feeds are fed less frequently to captive reptiles and were not fed to the animals in this study. The recording of the different
proportions of feeds ingested, which necessitates – in reptiles – intake trials of at least seven consecutive days per animal, was beyond the scope of this study. Therefore, the data from this study must be used with caution, and comparisons between individual species should not be made. However, for the large-scale comparison between clades intended here, data gained from captive animals is appropriate.

The difference in ingesta particle size, and the correlated absence of a mastication or grinding mechanism in reptiles, is considered as one of the very important physiological differences between the clades (Reilly and others, '01). Because the intake of soil or stones has sporadically been observed in both free-ranging and captive reptiles (Moodie, '12; Sokol, '71), it has been speculated that grinding in the stomach, similar to an avian gizzard, could support mechanical disruption of food (McArthur and others, '04). However, the general difference between mammals and reptiles in this study does not suggest that grinding plays a major role in the processing of ingesta in reptiles. Even though a large difference in particle size was observed between the stomach and the lower digestive tract in the *I. iguana* in this study, corresponding to similar results in *Uromastyx aegypticus* by Foley et al. ('92), this does not support the concept of ingesta grinding in the stomach. Firstly, no stones were found in the stomach content, which is consistent with observations of Rand et al. ('90) who did not find stones or sand in the digestive tract of 31 free-ranging *I. iguana*. Secondly, in the faeces of *I. iguana* (and other reptiles), ingested plant parts, such as leaves, can be recovered nearly intact (Fig. 4; see also Bjorndal, ’79; Iverson, ’82). Electron microscopy applied to leaves from the faeces of gopher turtles (*Gopherus polyphemus*) indicated that the remaining structure are intact skeletons of cutin and xylem bundles (Bjorndal and others, ’90). These structures are also excreted mostly intact in mammalian faeces, but because they are chewed upon, the original leaf-shape is no longer recognizable (Habers and others, ’91). In reptiles, the mesophyll and phloem between the xylem skeleton are digested off (Bjorndal and others, ’90).

When analysing stomach and rectum contents by wet sieving as in this study, the skeleton leaf
is reduced drastically in weight. As the skeleton leaf has the same size but not the same weight than an intact or not fully digested leaf, this will lead to a lower calculated mean particle size in the total faeces, as tmean particle size is derived from the weights of the remnants on the different sieves (see Methods).

Such a digestion of intact plant matter in the course of bacterial digestion and fermentation, linked with a distinct reduction in particle size or particle volume, usually does not occur to a similar extent in terrestrial, herbivorous mammals (reviewed by Fritz and others, '09). However, in a herbivorous marine mammal, the dugong (*Dugong dugon*), ingesta particle size decreases continuously along the digestive tract (Lanyon and Sanson, '06), which is probably due to a combination of the absence of gravity-defying structural fibres in marine plants (like lignin or cellulose as found in terrestrial plants, which have to keep themselves erect) and extremely long ingesta retention times in this species (Lanyon and Marsh, '95). Even in ruminants, particle size reduction by microbial digestion alone can be substantial if, in *in situ* experiments, incubation times are artificially prolonged (e.g. Nocek and Kohn, '88). For feeds often fed to captive reptiles, such as herbs and salads, Hummel et al. ('09) demonstrated in an in vitro system that, depending on the content of structural fibre components, particle size can be drastically reduced with long incubation times. Apparently, the long ingesta retention times observed in reptiles (see Introduction) facilitate such a digestive particle size reduction. An interesting question is if prolonged exposure to low pH in the stomach and ensuing acid fibre hydrolysis as observed in herbivorous fish (Lobel, '81) adds to the digestive particle size reduction in reptiles.

The fact that at very long ingesta retention times, ingesta particle size can be reduced by microbial action only, explains the two opposing strategies observed in small hindgut fermenting mammals and the hindgut fermenting reptiles: in mammals with their higher metabolism, a prevention of any constraint on food intake is important, and hence larger food particles that represent difficult-to-digest bulk are often expelled selectively from the hindgut.
A common finding in such herbivores, therefore, is a higher proportion of small particles in the caecum as opposed to the faeces (Laplace and Lebas, '77; Foley and Cork, '92; Vispo and Hume, '95). In herbivorous reptiles, with their lower metabolism, a lower food intake and consecutively longer retention times occur, and hence even a selective retention of larger food particles, as reported by Guard ('80) and Barboza ('95), is feasible. Accordingly, no increased proportion of small particles in the caecum are usually found in reptiles (cf. Foley and others, '92 and the comparison of caecum and colon contents in the C. zebrata of this study).

In contrast to mammals, in which chewing itself (and associated adaptations such as dental design or particle sorting mechanisms) determines ingesta particle size (Fritz and others, '09), the size of the ingested particles in reptiles should only depend from the dimensions of the ingestive apparatus, plant morphology, and the effect of food cropping. Direct investigations on an influence of ingestive morphology in reptiles are missing, but in birds, beak morphology was demonstrated to correlate with bite size in duck and geese (Cope and others, '95; Durant and others, '03; Van der Graaf and others, '06). Plant morphology itself may influence ingesta particle size if morphological structures of the plant, e.g. leaf size, are distinctively smaller than bite size (Hummel and Clauss, '10). The effect of food cropping has, to our knowledge, hardly been investigated. Results from our study are equivocal (Fig. 2). In theory, even an animal without chewing adaptations can regulate ingesta particle size by either selecting small particle food, or by biting off small pieces from a plant, as demonstrated by Björndal and Bolton (1992) for freshwater turtles. The latter, however, is often not possible in captivity where food, such as hay, is offered in loose form; this means that the animal lacks a resistance against which it can pull and pluck off a piece of the forage. The data in the T. hermanni support this theory: the ingesta particles of free-ranging (i.e. by necessity actively cropping) tortoises was similar to captive specimens fed chopped food; in
contrast, captive specimens fed whole parsley and hay had larger faecal particles. In faeces of captive tortoises, long parts of hay stems or leaves are regularly found (Fritz, pers. obs.). Bjorndal and Bolten ('92) describe digestive advantages of smaller bite size in juvenile vs. adult turtles. Whether the deliberate plucking off of small plant parts is a strategy used by free-ranging herbivorous reptiles remains to be investigated.

To conclude, our results show that the evolution of a masticatory apparatus and chewing behaviour in mammals has led to a dramatic ingesta particle size reduction. This facilitates a faster fermentation of the ingested plant material (Bjorndal and others, '90), which makes faster ingesta passage feasible (Clauss and others, '09), and thus allows a high food throughput necessary to fuel the metabolic demands of endothermy (Crompton and Parker, '78; Karasov and others, '86; Reilly and others, '01).

Acknowledgements

This work was supported by DFG grant CL 182/3-1 and is contribution no. 60 of the DFG Research Unit 533. The Biology of Sauropod Dinosaurs. We thank the zoological gardens of Berlin-Friedrichsfelde, Cologne, Wuppertal, Munich, Zurich, Vienna, and the Al Wabra Wildlife Preservation, Wolfgang Wegehaupt and Geraldine Kopsch for access to samples, and Sandra Mosimann for the care of the captive tortoises. J. Fritz was partly supported by the Karl-Heinz-Kurtze-Foundation.

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Table 1. Mean (± SD) body mass (BM), percentage (dry mass) of all retained particles retained on a particular sieve (linear pore size), and mean faecal particle size (MPS) in reptiles used in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>BM</th>
<th>0.125 mm</th>
<th>0.25 mm</th>
<th>0.5 mm</th>
<th>1 mm</th>
<th>2 mm</th>
<th>4 mm</th>
<th>MPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Iguana iguana</td>
<td>8</td>
<td>2.711 ± 0.432</td>
<td>12.887 ± 15.132</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>12.887 ± 15.132</td>
</tr>
<tr>
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<td>17.663 ± 8.408</td>
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<tr>
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<td>102.500* ± 86.125</td>
<td>32.194 ± 14.899</td>
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<tr>
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<td>5</td>
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<td>59.739 ± 12.230</td>
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<td></td>
<td></td>
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<td>3</td>
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<td>5.869 ± 3.748</td>
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<td></td>
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</tr>
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</table>

*estimated body mass
Figure 1. Mean faecal particle size (average value per species) in nonruminant herbivorous mammals (Fritz and others, '09) and herbivorous reptiles (this study, data from table 1) across the body size range.
Figure 2. Mean faecal particle size in individuals of a) *Testudo graeca* and b) *Testudo hermanni* from the wild, or from captivity fed chopped and whole food.
Figure 3. Mean particle size in the ingesta of different sections of the gastrointestinal tract of an individual *Iguana iguana.*
Figure 4. Leaf recovered from the faeces of an *Iguana iguana*. Note that the shape of the leaf and the vascular bundles are nearly intact.