



## Body size affects digestive performance in a Mediterranean lizard

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Effective digestion is decisive for survival. In nature, where most animals feed sporadically, high digestive performance guarantees they will gain the most out of their infrequent meals. Larger body size implies higher energy requirements and digestion should function properly to provide this extra energy. Comparing Skyros wall lizards (*Podarcis gaigeae*) from Skyros Island to large (“giant”) lizards from a nearby islet, we tested the hypothesis that digestion in large individuals is more efficient than in small individuals. We anticipated that giant lizards would have higher gut passage time (GPT), longer gastrointestinal (GI) tracts and higher apparent digestive efficiencies (ADE) for lipids, sugars and proteins. These predictions were only partially verified. Giant lizards indeed had longer (than expected based on body length) GI tract and longer GPTs but achieved higher ADE only for proteins, while ADEs for lipids and sugars did not differ from the normal-sized lizards. We postulated that the observed deviations from the typical digestive pattern are explained by cannibalism being more prominent on the islet. Giant lizards regularly consume tail fragments of their conspecifics and even entire juveniles. To break down their hard-to-digest vertebrate prey, they need to extend GPT and thus they have developed a longer GI tract. Also, to fuel tail regeneration they have to raise ADEproteins. It seems that larger size, through the evolution of longer GI tract, enables giant lizards to take advantage of tails lost in agonistic encounters as a valuable food source.

*Key words:* body size, cannibalism, digestion, gigantism, islands, reptiles

### INTRODUCTION

Digestion plays a paramount role in animal physiology. Constant energy inflow and untrammelled nutrient acquisition are fundamental prerequisites for successful survival. Efficient digestion assures that animals make the most out of their food and supports continuous supply of energy and building blocks (Stevens & Hume, 2004). Digestive performance depends on important features such as gut passage time (GPT, the rate with which food passes through animal’s body), apparent digestive efficiency (ADE, the relative percentage of ingested nutrients absorbed in digestion) and the morphology of the gastrointestinal tract. The digestive system dynamically responds to internal and external changes (Barton & Houston, 1994; Karasov & Martinez Del Rio, 2007). This plasticity is more striking in ectotherms such as reptiles, which have to adjust their digestion to the continuously changing body temperature (Beaupre et al., 1993).

Although reptilian digestive plasticity has only been studied rather sporadically (Vervust et al., 2010), impressive adaptations have been reported. Reptiles may change ADEs (McKinnon & Alexander, 1999),

accelerate or retard GPT (Van Damme et al., 1991), increase or decrease the mass of internal organs (Starck & Beese, 2002; Naya et al., 2009) and even develop new microstructures to better process food (Herrel et al., 2008). These dramatic shifts are the result of changes in temperature, food availability, food quality and age (McConnachie & Alexander, 2004; Durtsche, 2006; Naya et al., 2011). To satisfy their energy requirements reptiles have to maximise digestion. The higher energy needs are (relative to the food supply), the more effective digestion should be.

Body size is an important parameter in reptilian life shaping numerous aspects of the overall biology such as life history, feeding ecology, locomotor performance, metabolism and thermoregulation (Herrel et al., 2004; Labra et al., 2007; Harlow et al., 2010; Clemente et al., 2012; Meiri, 2010). Large reptiles use more energy than small ones (Congdon et al., 1982), however at a declining rate with increasing body mass (Andrews & Pough, 1985; Nagy, 2005). The higher energy needs of large-bodied animals led us to hypothesise that body size would influence digestion so as to ensure smooth whole-animal performance.

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In order to assess the effect of body size on digestion we studied two populations of the Skyros wall lizard (*Podarcis gaigeae*) endemic to Skyros Island (Aegean Sea, Greece) and the surrounding islets. On one islet, Mesa Diavates, an extreme case of gigantism has been recorded: lizards from this population are 40% longer and almost 250% heavier than their Skyros kin and, thanks to their size, able to eat their conspecifics (Pafilis et al., 2009). According to the “island rule” small-bodied species develop larger sizes on islands (Van Valen, 1973), although the generality of the rule has been challenged (Novosolov et al., 2013; Itescu et al., 2014). Exceptional cases of intraspecific gigantism, such as that of the Mesa Diavates population, lend themselves to test the impact of body size on various aspects of ecophysiology in a comparative frame.

We compared “normal-sized” *P. gaigeae* from Skyros Island with the islet giants focusing on major components of digestion: apparent digestive efficiency for lipids, sugars and proteins, gut passage time and length of the gastrointestinal tract. We made two predictions: first, that giant lizards would achieve higher ADEs for all nutrients because larger size entails higher requirements in both energy and nutrients, and second, that giants would have longer GI tracts and consequently longer GPTs. Large body size in *P. gaigeae* giant lizards has been shown to affect reproductive traits (larger clutches comprising larger eggs, Pafilis et al., 2011) and thermoregulation (selection of higher selected temperatures, Sagonas et al., 2013). We also anticipated that body size would influence digestion.

## MATERIALS AND METHODS

### Study System

*Podarcis gaigeae*, an endemic species to the Skyros Archipelago (N 38°51', E 24°33'), is a small, insectivorous lacertid lizard (male snout vent length: 60 mm, weight: 6.6±1.3 g - animals on the satellite islets are larger) that is widely distributed in all biotopes of the main island (Valakos et al., 2008). Especially striking is the population on the islet Mesa Diavates (hereinafter Diavates; distance from Skyros: 1.4 km, area: 0.019 km<sup>2</sup>, estimated divergence time 8,700 years – Runemark et al., 2012) where lizards are much larger (male mean SVL: 85.3

mm, mean weight: 14.2±2.1 g, Pafilis et al., 2009). The particularities of Diavates are not limited in body size: the islet does not suffer from the typical food scarcity of Mediterranean islets (Castilla et al., 2008). Indeed, due to sea-derived energy and nutrients provided by seabirds, the islet hosts lush vegetation and abundant invertebrate fauna (Polis & Hurd, 1996; Pafilis et al., 2013), enabling to support one of the highest densities of lizards in the Mediterranean (around 850 lizards per hectare, Pafilis et al., 2009).

During autumn 2012 and 2013 we noosed 68 adult males (43 from Skyros and 25 from Diavates) and transferred them to the laboratory facilities of the University of Athens. We worked exclusively with males to avoid differences attributable to sex, since sexual dimorphism is common in *Podarcis* lizards (Kaliontzopoulou et al., 2015). Lizards were housed individually in plastic terraria (20 x 25 x 15 cm) that contained stones as hiding places on a substrate of sand. Lizards were fed mealworms (*Tenebrio molitor*) every other day and had access to water *ad libitum*. Room temperature was kept a constant 25°C and natural light was provided through windows. Extra heat was provided through a 60 W incandescent heating lamp kept on for 8 h each day. Temperature in terraria ranged between 25 and 39°C during the day.

### Apparent Digestive Efficiency

We evaluated the ADE for each nutrient separately (Pafilis et al., 2007; Vervust et al., 2010). Lizards were placed in individual terraria with paper-covered floor and kept at a constant temperature of 30°C. Pairs of mealworms were weighed with a digital scale (i500 Backlit Display, My Weight, accurate to 0.01 g) and matched for mass. One mealworm was force-fed to each lizard every other day while the other one was stored at -80°C for later biochemical analyses. Faeces (after removal of the urate material) were collected every twelve hours and were also stored for biochemical analyses.

To extract total lipids we homogenised a sample of 30–40 mg of tissue (mealworm and fecal material, separately) with 1.5 ml of a 2:1 mixture of chloroform and absolute methanol. The homogenate was then centrifuged at 3,000 rpm for 10 min in 4<sup>o</sup> C. We discarded the pellet and used the supernatant for the determination of lipid concentration with the use of a

**Table 1.** Statistics of snout to vent length (SVL) and gastrointestinal track length (GI) of field-collected lizards and museum specimens examined. Numbers in cells represent mean±SD; range; number of examined lizards (in parenthesis).

Specimens	Trait	Skyros Island	Diavates islet
Field	SVL (mm)	61.82±2.79; 56.25–67.34; (43)	83.21±4.50; 75.56–90.60; (25)
	Weight (g)	6.01±0.50; 4.96–6.75; (43)	14.62±2.89; 10.23–18.45; (25)
Museum	SVL (mm)	60.31±3.49; 54.3–66.24; (20)	82.62±4.52; 72.65–88.75; (20)
	GI (mm)	93.47±5.28; 84.81–101.09; (20)	123.50±8.76; 105.50–136.83; (20)

**Table 2.** Statistics of apparent digestive efficiency (ADE %) and gut passage time (GPT) in lizards caught in the field. Numbers in cells represent mean±SD; range; number of examined lizards (in parenthesis)

Trait	Skyros Island	Diavates islet
ADE lipids	84.54±1.79; 81.75–89.36; (43)	85.11±2.20; 81.28–89.44; (25)
ADE sugars	76.40±1.26; 74.36–78.50; (42)	76.12±2.29; 71.58–79.87; (23)
ADE proteins	62.83±3.44; 55.39–67.66; (43)	70.01±2.42; 63.76–73.11; (25)
GPT (h)	35.53±2.99; 31–41; (30)	42.04±2.97; 36–47; (24)

dilution of phosphovaniline according to the Alexis et al. (1985) method. A mixture of olive oil and corn oil (2:1 v/v) was used as a standard.

Total sugars were estimated according to the Dubois et al. (1956) method. A homogenate of 150 mg of tissue with dd H<sub>2</sub>O (at 1:10 w/v ratio) was boiled for 30 min. We took 20 µl of this sample, diluted them in dd H<sub>2</sub>O (1: 500 v/v) and then incubated them with 1 ml phenol (5% w/v) and 5 ml of 95% H<sub>2</sub>SO<sub>4</sub> for 10 min at 20°C, and then for 40 min at 30°C. We read the absorbance at 490 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech). Glucose content was estimated against a known glucose standard.

To estimate the final concentration of total proteins we used the classical Biuret method (Layne, 1957). The pellet of centrifugation obtained from the lipid analysis was dissolved with 0.5 ml of 0.1 N NaOH, incubated in a water bath at 37°C for 30 min and vortexed. We then diluted 50 µl with 950 µl of dd H<sub>2</sub>O and added them to a volume of 4 ml of Biuret Reagent. The mixture was incubated for 30 min at room temperature. The absorbance was read at 550 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech). Bovine serum albumin (0.5–10 mg/ml) was used as a standard.

Concentrations of lipids, sugars and proteins in mealworms and faeces were used to calculate ADEs for each lizard, according to the following equation:

$$ADE_x = 100(I_x - E_x) / I_x,$$

where  $I_x$  stands for the amount of each nutrient ingested, and  $E_x$  is the amount of the nutrient that remained in the faeces after the enteric absorption was completed (x=proteins, lipids or sugars).

### Gut passage time and GI tract length

Food was withheld from the lizards for three days prior to the experiment. We measured the time food remained in the GI tract by recording the period between consumption and defecation of a marker (PVC) that was previously inserted into a mealworm (Van Damme et al., 1991). The marked mealworms were force-fed to lizards that were then returned to their terraria. We inspected terraria every hour for the presence of the marker and recorded collection time.

In order to measure the length of the GI tract we dissected 40 museum specimens (20 from Skyros and

20 from Diavates, all males) from the Herpetological Collection of the Natural History Museum of Crete.

### Statistical Analysis

To examine the normality and heteroscedasticity of our data set we applied the Kolmogorov-Smirnov and Lilliefors normality tests and Levene's test correspondingly. When residuals were heteroscedastic, we used permutation tests since they are more powerful than non-parametric tests. We performed randomisation t-tests with 9999 replications to analyse differences in body length, body weight, gut passage time and GI tract length between the two populations using the permute package in R v. 3.0.0 (R Development Core Team, 2013). Similarly, we performed a randomisation t-test to examine for differences in SVL between museum specimens and lizards collected in the field. To assess possible effects of SVL, environment (Diavates or Skyros) and their interaction on GPT we used generalised linear models (GLM) by applying 9999 permutations and Bray-Curtis dissimilarity with environment as fixed factor. To perform this analysis we used the vegan package (Oksanen et al. 2013) in R. Permutation multivariate ANOVA with 9999 iterations was used to analyse the digestive efficiency between populations using  $ADE_{\text{proteins}}$ ,  $ADE_{\text{lipids}}$  and  $ADE_{\text{sugars}}$  as dependent variables and environment as a fixed factor. To explain the variation of the digestive efficiency between populations we conducted a permutation GLM using GPT, population and their interaction as explanatory variable.

To test whether the relationship between SVL and GI tract length differ from the expected 1:1 isometric slope we used the SMATR package (Warton et al., 2012) and regressed GI tract length on SVL. Differences between the regression slopes of GI tract length and SVL among population were also examined based on the equation of Zar (2010). All tests were performed in R v. 3.0.0 (R Development Core Team, 2013).

## RESULTS

Lizards from Diavates are significantly longer (randomisation t-test;  $p < 0.001$ ) and heavier (randomisation t-test;  $p < 0.001$ ) than those from Skyros (Table 1). No differences in SVL were found between field-caught lizards and museum specimens (randomisation t-test; Skyros:  $p = 0.963$  and Diavates:  $p = 0.779$ ; Table 1).

Diavates achieved higher digestive efficiency compared to the Skyros population ( $F=34.99$ ,  $p<0.001$ ). The variable that contributes the most and causes this difference is  $ADE_{\text{proteins}}$  (62%;  $F_{1,66}=76.92$ ,  $p<0.001$ ), while  $ADE_{\text{lipids}}$  ( $F_{1,66}=1.32$ ,  $p=0.253$ ) and  $ADE_{\text{sugars}}$  ( $F_{1,63}=0.64$ ,  $p=0.423$ ) had no significant effects, contributing approximately 19% each (Table 2). The regression model using GPT and its interactions with environment showed that food retention time had small effect on  $ADE_{\text{proteins}}$  ( $F_{1,50}=36.193$ ,  $p<0.001$ ). There was no significant interaction effect in the variation of  $ADE_{\text{proteins}}$  ( $F_{1,50}=0.203$ ,  $p=0.686$ ).

Diavates lizards achieved longer food retention times compared to their Skyros kin (randomisation t-test;  $p<0.001$ ; Table 2). When SVL and its interaction with environment were used as an explanatory variable to the initial model the differences remained (based on 9999 iterations; SVL:  $p=0.004$  and SVL  $\times$  ENV:  $p=0.034$ ), with SVL having small effects on GPT (AIC<sub>c</sub> values were 286.3 for SVL and 275.7 for population).

Snout-vent length and GI tract length of museum specimens are given in Table 1. Diavates lizards are longer than the Skyros ones (randomisation t-test;  $p<0.001$ ) and have longer GI tracts (randomisation t-test;  $p<0.001$ ). In both populations SVL and GI tract length are positively correlated (Skyros:  $r=0.56$ ,  $p=0.009$  and Diavates:  $r=0.79$ ,  $p<0.001$ ) (Fig. 1). The relationship between SVL and GI tract in the case of Diavates lizards shows evidence of positive allometry (slope= $1.531\pm 2.845$ ,  $t=1.90$ ,  $df=18$ ,  $p=0.037$ ), while lizards from Skyros seem to follow isometry (slope= $0.851\pm 2.967$ ,  $t=0.51$ ,  $df=18$ ,  $p=0.309$ ) (Fig. 1). The regression slopes between populations were significantly different ( $t=2.044$ ,  $df=36$ ,  $p=0.048$ ).

## DISCUSSION

Body size shapes the performance of numerous functions in reptiles (Meiri, 2008). We found that it also affects digestion, at least to a certain extent. Our working hypotheses were only partially verified. Diavates giants did digest proteins more efficiently but shared the same ADEs for lipids and sugars with their Skyros normal-sized peers. On the other hand, giant lizards indeed had a longer GI tract and a concomitant more prolonged GPT.

Lipid absorption occurred in similar rates in both populations (Table 2). Previous research revealed that ADE values for lipids differed considerably in lacertid lizards among species and populations (Pafilis et al., 2007; Vervust et al., 2010). These differences were largely attributed to differences in the quality and quantity of food. According to Pafilis et al. (2007), island species are able to rapidly extract lipids from their food; this high  $ADE_{\text{lipids}}$  guarantees optimal use of the limited resources of the islands. Vervust et al. (2010) studied two *P. siculus* populations with huge differences in the consumption of plant material (61% vs. 6% during summer; Herrel et al., 2008); thus plant-eaters had to absorb as many lipids as they could from their primarily plant diets.

We think that the lack of variation in lipid absorption is explained by the similar food abundances. Giant and small lizards had access to the same insect resources

(Adamopoulou et al., 1999). Diavates harbours high food availability thanks to marine subsidies that support lush vegetation and robust invertebrate communities (Pafilis et al., 2013). Skyros has the typical Aegean vegetation with phrygana and maquis dominating the landscape, fuelling high invertebrate abundances (Trihas & Legakis, 1991). Food supplies are alike in both Skyros and Diavates and hence there is no efficient cause for differentiation in  $ADE_{\text{lipids}}$ , unlike in the two aforementioned cases (Pafilis et al., 2007; Vervust et al., 2010).

Lizards from Diavates likewise assimilated sugars in the same rate as the Skyros ones (Table 2). Pafilis et al. (2007) reported a similar value for the normal-sized *P. gaigeae* ( $77.4\pm 0.3\%$ ). However, authors in the latter study did not find a clear pattern regarding  $ADE_{\text{sugars}}$ : though lipid and protein absorption indicated a distinct grouping of insular species (including *P. gaigeae*) on one side and mainland species on the other, sugar absorption did not conform to this scheme (Pafilis et al., 2007). Since sugars are the most direct source of energy, the pathway for their hydrolysis should be prioritised to ensure uninterrupted energy flow (Berne & Levy, 1996; Hoffman, 2014). This should explain the lack of differences in  $ADE_{\text{sugars}}$  between the two populations.

Contrary to  $ADE_{\text{lipids}}$  and  $ADE_{\text{sugars}}$ , protein absorption differed considerably between the two populations with giant lizards achieving higher values than the Skyros ones ( $70.01\pm 2.42\%$  vs.  $62.83\pm 3.44\%$  respectively). Digestion of all nutrients occurs in the stomach and this applies more in the case of proteins where digestion depends directly on the period food remains therein. All in all, the more time is allowed to complete the digestion of proteins, the higher the resulting  $ADE_{\text{proteins}}$  (Scoczylas, 1978). GPT was longer in Diavates lizards (Table 2), thus food stays longer in their GI track and digestive efficiency for proteins rises. However, since temperature might affect digestion (McKinnon & Alexander, 1999; Pafilis et al., 2007), we cannot exclude a possible impact of the different selected temperatures (Sagonas et al., 2013) on  $ADE_{\text{proteins}}$ .

We anticipated that Diavates lizards would have longer GI tracts because of their larger body. Indeed, giant lizards had significantly longer GI tract than their Skyros kin ( $123.50\pm 8.76$  mm vs.  $93.47\pm 5.28$  mm respectively). However the longer GI tract was not merely a by-product of the larger body size. Giant lizards seem to have developed a longer GI tract that increases more rapidly with increasing SVL (Fig. 1). An identical pattern was observed in the above-mentioned *P. siculus* populations where plant-eating lizards had longer stomachs and intestines (Vervust et al., 2010). The question, then, arises: what is the incentive of Diavates lizards to invest in a much longer GI tract?

Digestive morphology has been reported to adapt properly to suit the particular feeding habits of lizards (Karasov & Diamond, 1983; Dearing, 1993; Carretero, 1997; Karasov et al., 2011). The aforementioned plant-eating *P. siculus* population developed longer GI tract and even cecal valves to increase GPT (and thus improve digestive efficiency) in response to a clear shift towards herbivory (Herrel et al., 2008; Vervust et al., 2010). An



increase in the length and surface area of the GI tract is favored in herbivorous lizards since it allows for maximal absorption of nutrients (Herrel et al., 2004; Herrel, 2007). We postulated that the longer GI tract in *Diavates* and the advantages it confers are related to an extreme feeding behaviour, cannibalism.

The high population density in *Diavates* triggers harsh intraspecific competition, which is expressed as attacks against conspecifics and infanticide as mentioned above (Pafilis et al., 2009). Intraspecific aggression has been linked to tail loss with subsequent post-autotomy consumption of shed tails (Pafilis et al., 2008; Bateman & Fleming, 2009) to compensate for the energetic cost of caudal autotomy that can be particularly severe (Pafilis et al., 2005). Stomach content analyses revealed that adult *P. gaigeae* consume parts of their age-peers (tails and limbs) or entire juveniles (Adamopoulou et al., 1999). Cannibalistic propensities are much higher in *Diavates* than in *Skyros* (21.4% vs. 1.2% respectively, Pafilis et al. 2009). *Diavates* males were far more aggressive to juveniles and other males than lizards from *Skyros* in staged encounters (male-male assaults: 75% in *Diavates*, 12% in *Skyros*, male-juvenile assaults: 69% in *Diavates*, 17% in *Skyros*; Cooper et al., 2014). Cannibalism is relatively common among lacertids inhabiting small islets (Castilla & Van Damme, 1996; Dappen, 2011). Its extraordinary frequency in *Diavates* highlights cannibalism as an important driving factor for morphological and physiological changes.

Eating a conspecific (or part of it) besides its obvious advantages in elimination of rivals and reduction of intraspecific competition, offers an energetically rich meal (Elgar & Crespi, 1992). Though nutritious, this lizard-meal is hard to digest (compared to the typical invertebrate prey of lacertids) because of its size and composition (scales and bones). Larger meals require longer GPTs (Secor & Diamond, 1997, 2000). Snakes are known to increase the mass of their internal organs after feeding to lengthen GPT and improve digestive efficiency (Starck & Beese, 2001, 2002). *Diavates* giant cannibals seem to undergo selection for longer GI tracts (and thus extended GPTs) so as to facilitate the breakdown of the vertebrate prey into smaller food particles and ameliorate nutrient assimilation. However, we shall point out that other modifications in the alimentary system (e.g., cecal valves) might contribute to the higher GPT. Further studies are required to resolve this issue.

There is another benefit from the extended stay of the food in the GI tract. Thanks to the longer GPT, giant lizards are able to maximise  $ADE_{\text{proteins}}$ , a time-dependent procedure. Proteins are widely used as building material in the construction of tissues. Tail regeneration in lizards is an extreme case of tissue reconstruction (Bellairs & Bryant, 1985; Higham et al., 2013). Regenerated tails may have the same or even higher protein concentration compared to the original ones (Simou et al., 2008; Boozalis et al., 2012). The very common intraspecific attacks in *Diavates* are reflected to the high percentage of regenerated tails observed in the field (88.4% vs. 32.16% in *Skyros*, Pafilis et al., 2009). We think that giant lizards, in order to deal with the high probability of tail loss and

concomitant regeneration, opt to create and maintain a steady channel of protein fuelling. Hence they developed a longer GI tract that increases GPT and eventually supports high  $ADE_{\text{proteins}}$ . It is noteworthy to add that lizards are a prey richer in proteins than insects (Zuffi et al., 2010). When tails are lost in intraspecific encounters, regeneration follows and the need for proteins increases. Thus the protein-rich conspecific prey is preferable to the typical insectivorous diet.

Our findings suggest that larger lizards altered certain parameters of their digestive performance and diverge from their normal-sized conspecifics. *Diavates* giants used the possibilities offered by the larger body to resolve their idiosyncratic problems arose from cannibalism. Mediterranean islet lizards that live on a small modicum and employ extreme feeding behaviours to survive (Castilla & Herrel, 2009; Brock et al., 2014), offer a unique system to study shifts in important digestive features. Future research could shed more light on the nature of our results, e.g., clarifying the underlying mechanism and the use of nutrients by lizards. Studies on lizard-eating lizards (such as the pygopodid *Lialis burtonis*) could clarify the impact of vertebrate prey on the length of GI tract and duration of GPT. Measurements of digestive efficiency (especially  $ADE_{\text{proteins}}$ ) during tail regeneration could support (or refute) our hypothesis regarding the high protein assimilation. Also, feeding lizards with isotopically labelled meals during tail reconstruction would provide direct evidence of how proteins are used. Further research focusing on these parameters would yield interesting insights about the evolution of digestion.

## ACKNOWLEDGEMENTS

We owe thanks to K. Antonopoulos of *Skyros* for sea transportation and his valuable help during the years. The study was supported by the Latsis Foundation Research Projects 2010.

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Accepted: 2 September 2015

