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Digestive performance in five Mediterranean lizard species: effects of temperature and insularity

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Abstract Temperature sensitivity of digestive processes has important ramifications for digestive performance in ectothermic vertebrates. We conducted a comparative analysis of temperature effects on digestive processes [gut passage times (GPTs) and apparent digestive efficiencies (ADEs)] in five lacertid lizards occurring in insular (*Podarcis erhardii, P. gaigeae*), and mainland (*P. muralis, P. peloponnesiaca, Lacerta graeca*) Mediterranean environments. GPTs were negatively correlated to temperature with mainland taxa having 10–20% longer GPTs than island taxa. In contrast to previous studies that estimate ADEs using bomb calorimetry, we compare ADEs by analyzing discrete efficiencies for lipids, sugars and proteins at three temperature regimes (20, 25, and 30°C); each of these categories produces different results. ADEs for lipids and sugars showed a monotonic increase with temperature whereas ADEs for proteins decreased with temperature. Island taxa had consistently higher ADEs than their mainland counterparts for lipids and for proteins but not for sugars. They are characterized by superior energy acquisition abilities despite significantly shorter GPTs. Their increased digestive performance relative to the mainland species appears to allow them to maximize energy acquisition in unproductive island environments where food availability is spatially and seasonally clustered.

Keywords Evolutionary physiology · Islands · Lacertidae · Phylogeny · Thermal environments

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Introduction

The profound effects of temperature on ectotherm physiology have been widely recognized and thermal optima have been demonstrated in reptiles for a variety of physiological functions such as digestion (Huey 1982; Stevenson et al. 1985). In general, increased body temperature (T_b) increases both metabolic rate as well as the rate of various digestive processes (Scoczylas 1978; Berne and Levy 1996; Toledo et al. 2002; Wang et al. 2003). Gut passage time (GPT: elapsed time from ingestion to initial appearance of marked food item in the feces), is strongly temperature dependent and decreases with increasing body temperatures (Beaupre et al. 1993; Du et al. 2000).

Digestive efficiency is an important link between the gut physiology and the ecological aspects of energy budgeting (Bedford and Christian 2000). It represents the integration of many factors such as gastrointestinal motility, enzymatic activity and nutrient substrate concentrations (Harlow et al. 1976). While it is defined as the relative percentage of ingested energy absorbed

through the gut, it is quantified as food energy minus faecal energy divided by food energy (Johnson and Lillywhite 1979; McConnachie and Alexander 2004). However, this term is somewhat misleading as faeces in addition to undigested food, also contain non-food materials such as nitrogenous wastes, intestinal cells, secreted proteins and bacteria. For that reason, the term apparent digestive efficiency (ADE) is preferred (Beaupre et al. 1993). Although ADE appears to be temperature and food quality dependent (Van Marken Lichtenbelt 1992), the effect of body temperature on the digestive efficiency of lizards is not well understood (McKinon and Alexander 1999). Whereas in some species digestive performance is directly temperature-dependent (Harwood 1979; Troyer 1987), others are only slightly (Waldschmidt et al. 1986; Ji et al. 1996), or not at all influenced (Karasov and Diamond 1985; Christian 1986; Zimmerman and Tracy 1989). Digestive efficiencies range in lizards from 30% for some herbivorous species (Ruppert 1980) to over 90% in some carnivorous or insectivorous species (Johnson and Lillywhite 1979). In lacertids specifically, ADEs between 83 and 90% have been reported (Ji et al. 1993, 1996).

Bomb calorimetry has been used widely for determining the energy content of ingested food and faeces. It provides valuable information concerning energy efficiencies for total organic material and has helped outline overall digestive performance in many species. Despite its advantages and wide application, this method also has some limitations (Witz and Lawrence 1993) and does not lend itself to calculating partial absorption efficiencies for individual major nutrients groups (proteins, lipids, and sugars). To our knowledge, only two studies in insectivorous lizards, those on *Cnemidophorus sexlineatus* of Witz and Lawrence (1993) and on *P. peloponnesiaca* and *Lacerta graeca* (Pafilis and Valakos 2004), analyze individual nutrient absorption efficiencies for sugars, proteins and lipids (ADE_{SUGARS}, $ADE_{PROTEINS}$, and ADE_{LIPIDS}). Here, following the same approach, we provide a more nuanced understanding of the saurian digestion process, and use these results to conduct interspecies comparisons. However because of the different methodology used, partial ADEs presented here should be compared only with caution to the results obtained from bomb calorimetry studies.

The overall ability of a lizard to extract energy from its prey items across a range of thermal environments depends mainly on GPTs as well as the corresponding kinetic behavior of digestive enzymes (Andrews and Asato 1977; Beaupre et al. 1993) while other digestive processes such as gastric acid secretion, activity of luminal transport proteins and gastrointestinal motility are involved as well. The rate with which food passes through the gastrointestinal tract (i.e., GPT) can have either a positive or a negative effect on the overall ability of an organism to absorb energy (Harwood 1979; Hume 1989). While high food passage rates help clear the intestine so that new food items can be processed, they may also compromise the ability of the gastrointestinal tract to digest and absorb nutrients (McConnachie and Alexander 2004). Species-specific GPTs should therefore reflect not only overall food availability and digestibility but also of the ability of digestive enzymes to process nutrients at a given temperature (Scoczylas 1978; Hume 2005). For example, rapid GPTs would be expected in areas where food is easily digestible, yet seasonally and spatially clustered.

In the present study, we compare the digestion process in five species of Mediterranean lacertid lizards. We present data on the effects of temperature on GPTs and on the digestive efficiencies of the three separate main prey components. The purposes of this study are to evaluate the effect of temperature on the digestive efficiencies and GPTs in the examined species and to examine whether there exist interspecific differences in GPTs and in ADEs of sugars, lipids and proteins. Furthermore, we try to assess whether any observed differences are best explained by the phylogenetic history of the species or by environmental characteristics such as insularity.

Materials and methods

Study animals

This study was conducted in five species of lacertid lizards (*Podarcis erhardii, P. gaigeae, P. muralis, P. peloponnesiaca* and *L. graeca*), found in the NE quadrant of the Mediterranean Basin (for collecting locations, see Fig. 1). Four of the species belong to the genus *Podarcis* while *L. graeca* belongs to the closely related *Archaeolacerta* lineage. All taxa are morphologically similar and almost exclusively carnivorous, subsisting on a diet of spiders, coleopterans, insect larvae, gastropods and ants (Maragou et al. 1997; Valakos et al. 1997; Adamopoulou et al. 1999). All species have similar temperature preferences (Pafilis 2003).

Podarcis gaigeae is a small-bodied (SVL 65 mm, Body mass 4.6 ± 0.61 g, T_{sel} 33.77°C), Aegean Sea endemic, restricted to the Skyros island group (Gruber 1986a). *P. erhardii* (SVL 70 mm, Body mass 7.58 ± 1.4 g, T_{sel} 33.9°C) has a distribution restricted to

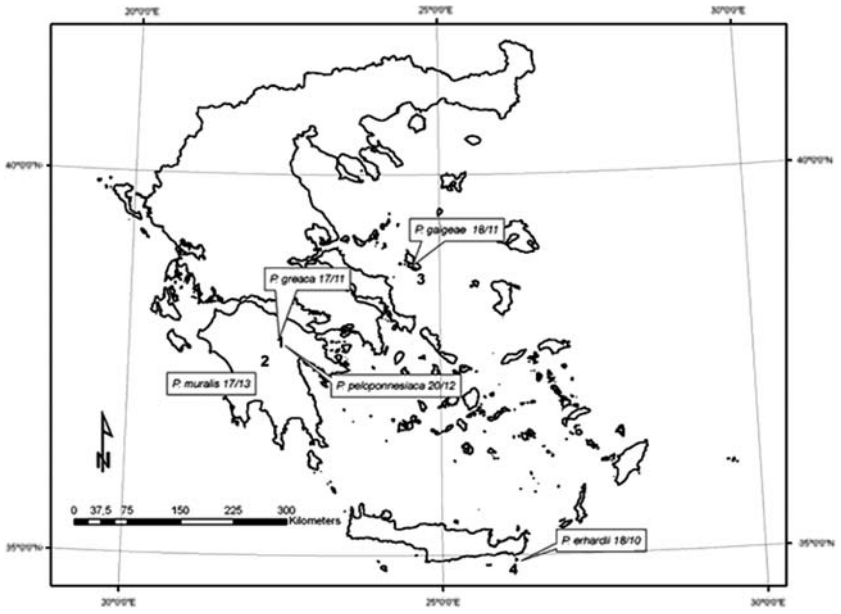

Fig. 1 Map of Greece showing the geographic origin of the lizard included in this study (*1* Stymfalia Lake, Peloponnese, *2* Mainalo Mountain, Peloponnese, *3* Skyros Island, *4* Islets around east Crete). Species identities are followed by the sample size (male/female) that was used

the southern end of the Balkan Peninsula, many central Aegean islands, as well as Crete (Gruber 1986b). Both species are the only small lacertids on the respective study islands and occur throughout all island habitats. In contrast, *P. muralis* (SVL 70 mm, Body mass 7.31 ± 1.45 g, T_{sel} 33.75°C), has a broader distribution on the Balkan Peninsula as well as Central and West Europe (Gruschwitz and Böhme 1986). In southern Greece, the species is restricted to higher elevations (above 600 m a.s.l.). Lastly, *P. peloponnesiaca* (SVL 75 mm, Body mass 9.23 ± 2.1 g, T_{sel} 33.9°C) and *L. graeca* (SVL 85 mm, Body mass 8.81 ± 1.98 g, T_{sel} 33.2°C) are narrow-range endemics with broadly overlapping ranges on the Peloponnese Peninsula in southern Greece (Böhme 1986; Bringsoe 1986).

All animals were captured in the field during their non-reproductive period. Captive animals of all species were maintained in the laboratory facilities of the Biology Department at the University of Athens. Lizards were initially housed in groups of 5–6 individuals in glass terraria (40 cm × 40 cm × 100 cm) on a sand substrate; bark pieces and stones were placed in each terrarium for cover and to facilitate thermoregulation. Light was provided by fluorescent lamps operating at a 12hL–12hD photoperiod. Each terrarium was also equipped with a 60 W incandescent heating lamp kept on for 8 h each day in coincidence with the fluorescent light period. During the heat cycle temperatures in each terrarium ranged between 28 and 38°C while for the remaining 16 h temperatures dropped to a stable 25°C as maintained by the air conditioning system. Lizards were provided with ad libitum live food [*Tenebrio molitor* larvae (mealworms)] and water.

After an acclimatization period of 20 days in the lab, each of the individual lizards was transferred to an individual clear plastic terrarium (approx. 8 cm × 8 cm × 20 cm) in three separate rooms kept at constant temperatures (20, 25, and 30°C, respectively). Fluorescent bulbs provided a 12hL–12hD photoperiod. An upper temperature environment of 30°C was selected because it approximates the mean preferred temperatures of the taxa examined, while the lower (20°C) thermal regime was chosen because it has been shown to be low enough to slow down digestion (Scoczylas 1978), while being still commonly experienced by the lizards during the spring and fall seasons. We finally selected 25°C to represent intermediate conditions. Lizards remained in the terraria for a further 2-week period to acclimatize to the new photothermal conditions. Immediately prior to the initiation of the passage time measurements, but not before the digestibility trials, food was withheld from the animals for 3–4 days, until no faeces were found in the terrarium. Lizards were assigned randomly to different temperature environments.

Methods

Gut passage times were estimated by quantifying the period of time between consumption and defecation of a marked mealworm. Mealworms were marked by introducing into their abdomen a small piece

($3 \times 2 \times 0.1$ mm^3) of plastic material (PVC) as an indigestible marker (Van Damme et al. 1991). These mealworms were then force-fed to the lizards. Once a lizard consumed a mealworm, it was returned to its individual terrarium. Faeces production was monitored at 2-h intervals throughout day and night, and faeces produced from each individual were collected immediately. Faeces were searched for the presence of a plastic marker, and time of collection was recorded. Faecal material was placed in liquid nitrogen immediately after collection and was stored at –80°C until later biochemical analysis.

Mealworms originating from an in-house breeding colony were weighed to the nearest 0.1 mg and matched for mass to create pairs of similarly sized food items. The first worm of each pair was then force-fed to the lizard every second day while the second one was used for a biochemical analysis, which was conducted separately for each individual lizard. Because force-feeding may potentially affect a lizard's digestive performance (Harwood 1979), we followed the same exact handling protocol as to eliminate any possible biases. Furthermore, to avoid any potential effects of meal mass on study outcomes, we attempted to adjust mealworm size to lizard mass, and there was no indication that meal size had any confounding effects on the variables measured in this study. We stopped the experiment when we had accumulated enough faeces to conduct the necessary biochemical analyses (Table 1). Urate material was removed from each faex before the biochemical content analysis. Initial concentrations of lipids, sugars and proteins were then used to calculate partial ADEs. These individual ADEs for lipids, sugars and proteins (denoted here as $U_{L/S/P}$), were computed according to the following equation:

$$U_{L/S/P} = (I - E/I) \times 100,$$

where I = amount (lipids, sugars or proteins) ingested, and E = amount (lipids, sugars or proteins) remaining in the faecal material after enteric absorption was completed.

Extraction of total lipids was performed from a homogenized sample (30–40 mg) using 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°C. The pellet formed was discarded and the supernatant was used for the determination of total lipid concentration, using diluted of phosphovaniline according to the method described by Alexis and Papaparaskeva-Papoutsoglou (1986). As a standard a mixture of olive and corn oil was used (2:1 v/v).

Table 1 Apparent digestive efficiencies (ADEs) of lipids, sugars and proteins at three experiment temperatures: means ± standard deviation, number of trials, and min–max

Species	ADE_{LIPIDS}			ADE_{SUGARS}			$ADE_{PROTEINS}$		
	20°C	25°C	30°C	20°C	25°C	30°C	20°C	25°C	30°C
L. graeca	72.8 ± 0.2, 49, 72.3–73.5	72.9 ± 0.4, 45, 72.6–75.2	75.1 ± 0.6, 44, 73.0–77.6	72.2 ± 3.4, 29, 71.1–89.6	79.6 ± 0.6, 26, 78.3–80.8	78.3 ± 0.5, 23, 76.7–79.1	65.3 ± 1.8, 49, 62.7–72.9	62.7 ± 1.3, 45, 56.9–66.2	50.6 ± 1.3, 44, 46.7–53.6
P. peloponnesiaca	74.5 ± 1.6, 50, 72.8–81.4	79.9 ± 1.7, 59, 76.2–84.1	84.4 ± 1.4, 59, 77.2–86.7	71.5 ± 0.5, 31, 70.4–72.8	77.4–0.3, 41, 76.9–77.9	80.2 ± 0.5, 34, 79.0–81.4	69.2 ± 1.0, 50, 66.0–71.5	60.0 ± 1.6, 58, 55.8–65.5	46.1 ± 3.9, 59, 31.8–54.8
P.muralis	73.0 ± 3.6, 48, 72.4–79.7	80.2 ± 0.3, 51, 79.7–81.2	85.1 ± 0.24, 45, 84.6–85.6	70.2 ± 0.5, 32, 69.3–71.1	78.1 ± 0.3, 32, 77.4–78.8	81.0 ± 0.3, 32, 80.5–81.7	70.1 ± 1.5, 48, 68.6–75.2	56.8 ± 0.8, 51, 52.9–58.9	48.3 ± 1.8, 47, 44.9–52.8
P.erhardii	80.2 ± 0.4, 47, 79.5–81.5	84.2 ± 0.4, 51, 83.4–86.2	85.1 ± 0.3, 45, 84.7–85–7	71.7 ± 0.5, 30, 69.8–72.6	74.5 ± 0.3, 33, 73.9–75.2	79.8 ± 0.6, 28, 78.6–80.7	64.0 ± 1.1, 47, 61.7–67.7	63.1 ± 1.9, 51, 59.8–73.2	61.8 ± 0.8, 45, 59.2–63.5
P. gaigeae	79.3 ± 1.0, 47, 78.5–85.8	80.2 ± 2.0, 51, 78.8–94.2	83.7–1.1, 48, 82.3–89.7	71.7 ± 0.3, 32, 70.8–72.5	76.6 ± 0.6, 32, 75.1–77.4	77.4 ± 0.3, 27, 76.8–78.0	66.2 ± 2.0, 47, 57.9–71–5	64.9 ± 1.0, 51, 61.2–67.0.5	63.3 ± 1.7, 47, 54.3–65.5

Total sugar amounts in food and faeces were determined using the method described by Dubois et al. (1956). Faeces or mealworms (150 mg) were homogenized with H_2O at a 1:10 w/v ratio and then boiled for 30 min. Twenty microliters of this sample were then diluted (1: 500 v/v) in H_2O and incubated with 1 ml phenol (5% w/v) and 5 ml of 95% H_2SO_4. The sample was then incubated for 10 min at room temperature, and then for 40 min at 30°C. The absorbance was then read at 490 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech) and glucose content was estimated against a known glucose standard.

Total protein levels were determined using the Biuret method (Layne 1957). Briefly, the pellet of centrifugation obtained from the lipid analysis (see above) was dissolved with 0.5 ml of 0.1 N NaOH and incubated at 37°C for 30 min while being vortexed occasionally. Fifty microliters of each sample were diluted with 950 ml of H_2O; this was subsequently added to a volume of 4 ml of Biuret Reagent. We incubated this mixture for 30 min at room temperature and then read the absorbance at 550 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech). The standard used was bovine serum albumin (0.5–10 mg/ml).

One-way ANOVAs were used when testing for differences in larvae nutrient contents. One-way ANCOVAs with log-transformed fresh body mass as the covariate were used to test for the effects of temperature, species identity and island/mainland origin on GPTs and three different ADEs. Relationships among the different physiological variables were examined using Pearson correlations. An initial analysis of all relevant physiological variables did not detect any differences between male and female lizards, we have subsequently combined data from both sexes for the analyses. All tests were two-tailed; we set $\alpha = 0.05$. Statistical analyses were conducted using SPSS-11.0 (SPSS Inc. 1989–2002). Statistical analysis followed Zar (1984). Principal components analysis (PCA) was applied to analyze the structure of interrelationships among the ADE and GPT values. The factor scores of the PCA were then used to complete a perceptual mapping of the species.

Because conventional statistical methods assume that a star phylogeny produced the species analyzed, whereas this is rarely the case in nature, such methods tend to produce inflated Type I errors (Garland Jr et al. 1993; Brashares et al. 2000). Consequently, we repeated all conventional analyses in a phylogenetically informed context using the appropriate statistical program (PDAP version 6.0, Garland Jr et al. 2002). For this analysis, we constructed a phylogenetic tree of the five lizard species (Fig. 2) based on molecular information as provided in the published literature (Harris et al. 1998; Chondropoulos et al. 2000; Poulakakis et al. 2003, 2005).

Results

Gut passage times

Gut passage times for all species decreased significantly at higher temperature regimes (Fig. 3), while GPT differed significantly between species (ANCOVA, $P < 0.05$). Significant differences in GPTs were found among the examined species at all three experimental temperatures (ANCOVA, 20°C: $F_{4,51} = 1{,}119.05$, $P < 0.05$; 25°C: $F_{4,47} = 28.37$, $P < 0.05$; 30°C: $F_{4,51} = 12.32$, $P < 0.05$). Tukey post-hoc tests indicated the existence of two distinct groups, which were the same in all three temperatures ($P < 0.05$). The first group consisted of the island taxa *P. erhardii* and *P. gaigeae* and was characterized by faster GPTs than the second group consisting of the three mainland taxa (*P. peloponnesiaca, P. muralis and L. graeca*). Repeating the analysis while taking the phylogenetic relationships of the species into account, detected the same differences between mainland and island species

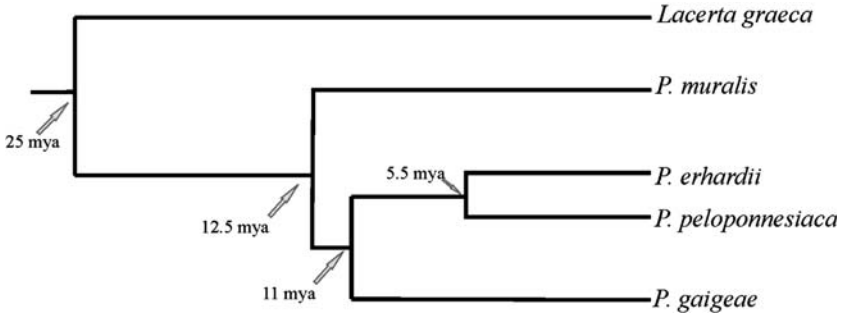

Fig. 2 Phylogenetic tree of the species used in this study. Tree topology is based on molecular data. Divergence times (*numbers at nodes* given in million years ago, *mya*) are calibrated using geological events (see the text for details)

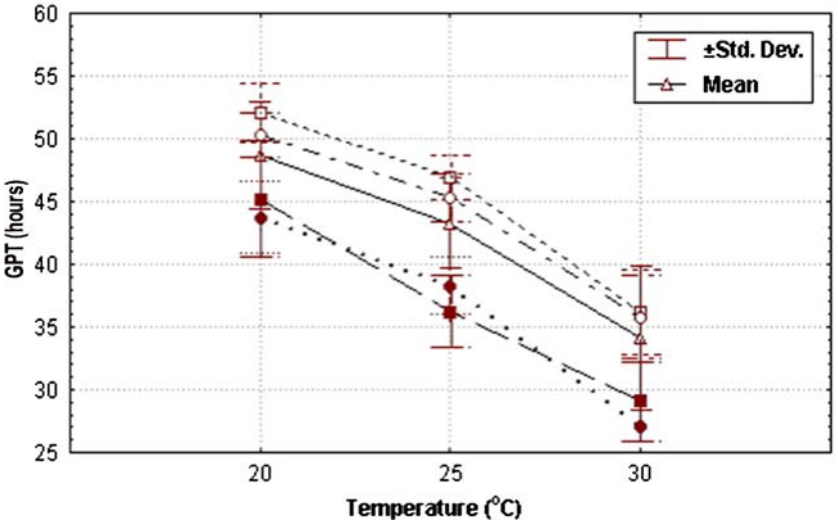

Fig. 3 Gut passage times (*GPT*s) of the species examined at three experimental temperatures. *Symbols* represent species means, *bars* SD, open circle *P. muralis*, filled circle *P. erhardii*, filled square *P. gaigeae*, open square *P. peloponnesiaca*, open triangle *L. graeca*

(ANOVA, 20°C: actual $F_{1,3} = 8.74$, 95% boundary = 6.12 therefore $P < 0.05$; 25°C: actual $F_{1,3} = 15.06$, 95% boundary = 7.45 therefore $P < 0.05$; 30°C: actual $F_{1,3} = 30.42$, 95% boundary = 5.06 therefore $P < 0.05$).

Nutritional composition of mealworms

All lizards were fed mealworms originating from the same in-houses source. A nutritional analysis of these larvae revealed the following content: 15.8% lipids, 4.2% sugars, 19.9% proteins and 60.1% water. Lipid, sugar and protein concentrations of the larvae fed to the each lizard species were identical with no statistically significant differences detected (ANCOVA, $P > 0.05$).

ADE for nutrients

Statistically significant among-species differences were found in the lipid content of the faeces for all three temperatures (e.g., 30°C ANCOVA, $F_{4,236} = 47.99$, $P < 0.05$). ADE for lipids (ADE_{LIPIDS}, Table 1) was positively related to temperature in all species (ANCOVA, $P < 0.05$). ADE_{LIPIDS} also differed significantly among species at all experimental temperatures (ANCOVA, 20°C: $F_{4,236} = 636.60$, $P < 0.05$; 25°C: $F_{4,252} = 437.87$, $P < 0.05$; 30°C: $F_{4,236} = 935.62$, $P < 0.05$) but Tukey post hoc tests did not identify any consistent patterns of grouping among the examined taxa. *L. graeca* had lower ADE_{LIPIDS} values at all temperatures compared to the other species. Furthermore, the island species *P. erhardii* and *P. gaigeae* achieved higher ADE_{LIPIDS} values than the mainland taxa at both 20 and 25°C, though this pattern broke down at 30°C. These mainland-island differences were supported by phylogenetically corrected ANCOVAs for 20°C (actual $F_{1,3} = 63.73$, 95% boundary = 6.52, $P < 0.05$) but not for 25°C or 30°C (25°C: actual $F_{1,3} = 1.20$, 95% boundary = 6.48 therefore $P > 0.05$; 30°C: actual $F_{1,3} = 0.17$, 95% boundary = 7.19, $P > 0.05$).

At the upper end of the temperature range, we found clearly significant (25°C) or marginally non-significant (30°C) interspecies differences in the amount of remaining sugars measured in the faeces; this was not the case at 20°C (ANCOVA, 20°C: $F_{4,149} = 0.33$, $P > 0.05$, 25°C, $F_{4,159} = 10.65$, $P < 0.05$; 30°C, $F_{4,139} = 2.39$, $P = 0.053$). As in the case of lipids, ADE_{SUGARS} values increased with temperature between 20 and 30°C for all taxa (ANCOVA, $P < 0.05$) (Table 1). ADE_{SUGARS} differed significantly among species at all examined temperatures (ANCOVA, 20°C: $F_{4,149} = 6.18$, $P < 0.05$; 25°C: $F_{4,159} = 588.86$, $P < 0.05$; 30°C: $F_{4,139} = 256.21$, $P < 0.05$). Post hoc analysis (Tukey tests) of ADE_{SUGARS} indicated that at 20°C only *Podarcis muralis* ($P < 0.05$) differed significantly from all other taxa, while at 25°C and at 30°C all taxa were different from each other. In general, any between-species differences detected in ADE_{SUGARS} did not follow any consistent pattern and almost all (with the exception of a marginal difference between island and mainland species at 25°C) of these between-taxa differences in ADE_{SUGARS} were also not supported by phylogenetically informed ANCOVAs (20°C: actual $F_{1,3} = 4.12$ 95% boundary = 7.58, $P > 0.05$; 25°C: actual $F_{1,3} = 7.39$, 95% boundary = 5.71 therefore $P < 0.05$; 30°C: actual $F_{1,3} = 0.005$, 95% boundary = 5.87, $P > 0.05$).

In all examined taxa, faecal protein concentrations changed with temperature (e.g., ANCOVA, *L. graeca*: $F_{2,135} = 110.5$, $P < 0.05$). Concomitant changes were observed in $ADE_{PROTEINS}$; these also decreased with increasing temperature (Table 1, ANCOVA,

$P < 0.05$). Significant interspecific differences were detected in faecal protein concentrations, as well as in the corresponding $ADE_{PROTEINS}$ values (ANCOVA, 20°C: $F_{4,237} = 137.3$, $P < 0.05$; 25°C: $F_{4,251} = 240.1$, $P < 0.05$; 30°C: $F_{4,236} = 136.4$, $P < 0.05$). Post hoc tests (Tukey) suggested that all species were distinct from each other except the following cases: at 20°C values for *L. graeca* did not differ from those of *P. gaigeae*; at 25°C the values for *L. graeca* did not differ from *P. erhardii* and at 30°C there was a significant grouping of the two island taxa (*P. erhardii* and *P. gaigeae*).

Repeating the analyses while taking the phylogenetic history of the species into account demonstrated that while at 20 and 25°C, there is no significant differentiation between island and mainland taxa, at 30°C island species have distinctly higher $ADE_{PROTEINS}$ (ANCOVA, 20°C: actual $F_{1,3} = 2.64$, 95% boundary = 6.06, therefore $P > 0.05$; 25°C: actual $F_{1,3} = 1.5$, 95% boundary = 6.79, therefore $P > 0.05$; 30°C: actual $F_{1,3} = 26.38$, 95% boundary = 7.22, $P < 0.05$).

Correlation analyses

GPTs and ADE values were correlated to each other at all three experimental temperatures using both conventional as well as phylogenetically informed methods (Table 2). A significant relationship of GPTs on ADE_{LIPIDS} was detected at 20°C (but not at 25 or 30°C). GPTs were also clearly correlated with $ADE_{PROTEINS}$ at all temperatures (positively at 20°C and negatively at 25 and 30°C). No clear relationship between GPTs and ADE_{SUGARS} could be detected at any temperature. After accounting for the phylogenetic relationships of the species, the same overall results were obtained, except at 25°C where we detected a significant correlation between GPTs and ADE_{LIPIDS} and ADE_{SUGARS} (Table 2).

Principal component analysis

The results of the PCA analysis for 30°C are shown in Table 3a, b as well as in Fig. 4. Components 1 and 2 accounted for 94.5% of the total variation (Table 3a). GPT and $ADE_{PROTEINS}$ loaded heavily on Component 1, while ADE_{LIPIDS} and ADE_{SUGARS} comprised Component 2 (Table 3b). In the corresponding ordination plot (Fig. 4), *L. graeca* separated from all *Podarcis* taxa along PC2 while the island taxa *P. gaigeae* and *P. erhardii* appear isolated from the rest of the species along PC1. Repetition of these analyses at 20 and 25°C provided analogous results and is omitted here for brevity's sake.

Table 3 Principal component analysis of GPT values and ADE values at 30°C

Component	Eigenvalue	% Variance	Cumulative %
(a) Eigenvalues, percent of variance and cumulative percentage			
1	2.3690	59.225	59.225
2	1.4103	35.257	94.483
3	0.2116	5.292	99.775
4	0.0090	0.225	100.000

	Component			
	1	2	3	4
(b) Component matrix (Eigenvectors)				
GPT	0.3729	0.0456	0.2310	0.3503
ADE_{LIPIDS}	0.0001	0.6613	0.3155	0.0229
ADE_{SUGARS}	0.2232	0.2659	0.4512	0.0595
$ADE_{PROTEINS}$	0.4036	0.0270	0.0022	0.5670

Discussion

Gut passage times

The strongest and perhaps least surprising result of this study is that GPTs are unambiguously temperature dependent. Despite slightly different baseline values, GPTs in all taxa decreased with rising temperature (Fig. 3). These responses are in agreement with previous studies (see Table 4 and references therein) which have demonstrated three patterns of temperature dependence in lizard food evacuation times: (1) GPTs decrease with increasing temperature, (2) GPTs

Table 2 Correlation coefficients of GPT values versus ADE values

	GPT					
	20°C		25°C		30°C	
ADE_{LIPIDS}	–0.896*	–0.973*	–0.344	–0.869*	–0.206	–0.291
ADE_{SUGARS}	–0.077	–0.113	0.593	0.923*	0.450	0.461
$ADE_{PROTEINS}$	0.822*	0.975*	–0.811*	–0.936*	–0.969*	–0.986*

In the first column, we present the Pearson correlation coefficients; the second column contains the results of the independent contrast correlation analyses

*Denotes significance at $P < 0.05$

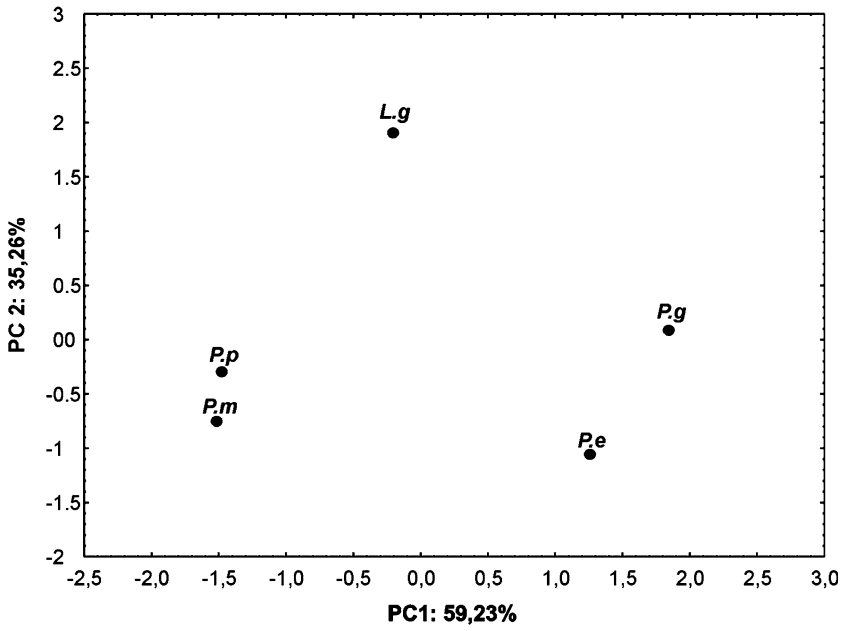

Fig. 4 Principal component analysis ordination plot of the examined species at 30°C. Lg: *Lacerta graeca*, Pe: *P. erhardii*, Pp: *P. peloponnesiaca*, Pm: *P. muralis*, Pg: *P. gaigeae*

Table 4 Previously published data on lizard gut passage times (GPTs) with corresponding references

Species	Temperature (°C)	GPT (hours or days*)	Reference
Lacertidae			
Takydromus sexlineatus	24/26/30	52.1/51.5/27.4	Zhang and Ji (2004)
Takydromus wolteri	26/30	50/38	Chen et al. (2003)
Takydromus septentrionalis	26/30	48/35	Ji et al. (1996)
Lacerta vivipara	20/25/30	18.8/13.5/11.2	Van Damme et al. (1991)
Lacerta vivipara	20/30	21/11.2	Avery (1971)
Podarcis muralis	n.a.	33–40	Taddei (1951)
Podarcis sicula	n.a.	33–40	Taddei (1951)
Lacerta viridis	n.a.	32–45	Taddei (1951)
Other families			
Eumeces elegans	22/26/32	74/50/45	Du et al. (2000)
Uta stansburiana	22	4.6*	Waldschmidt et al. (1986)
Cordylus melanotus	20/25/30/32	7*/5.5*/4*/3.9*	McConnachie and Alexander (2004)

Values marked with * are in days

decrease with increasing temperature but this decrease levels off at high temperatures, and (3) GPTs decrease with increasing temperature at lower temperatures and then increase again at higher temperatures (Du et al. 2000; Chen et al. 2003). Although our results appear to follow the first pattern, we cannot conclude this with certainty, given that most of the upper-end levelling-off of GPTs occurs above 30°C (see, e.g., Zhang and Ji 2004, Fig. 3), a temperature region not explored in this study.

A comparison of food passage rates reveals that the five species fall into two distinct categories, with the mainland taxa (*P. muralis*, *P. peloponnesiaca* and *L. graeca)*, exhibiting 10–20% longer GPTs than the island taxa (*P. gaigeae* and *P. erhardii)*. These differences persist across all temperature regimes and are robust to the application of phylogenetically informed statistical methods. Island species are hence able to process food significantly faster; this may allow them to void the gastrointestinal tract more quickly, making space for new food items.

Apparent digestive efficiency

The majority of previously published studies estimate ADE using bomb calorimetry. This method, although simple and precise, has the disadvantage of not distinguishing between major nutrient components of food, and probably overestimates ADEs (Witz and Lawrence 1993). For lizards, only one study has addressed in detail this issue by estimating separate nutrient absorption efficiencies for lipids, sugars and proteins (Witz and Lawrence 1993).

Lipids are slightly soluble in water and tend to separate out into an oily phase in stomach. Afterwards they are emulsified in duodenum and form micelles, which then absorbed by the intestinal brush border (Berne and Levy 1996). In this analysis, we demonstrate that lipid absorption in the lizard gut is a temperature-dependent process, with ADE_{LIPIDS} values increasing at higher temperatures. Dietary lipids can affect whole animal physiology processes (Simandle et al. 2001). Past research has shown that ADE values are influenced both by the rate at which food passes through the gut, the degree of enzymatic activity (e.g., of lipases, Harwood 1979; Beaupre et al. 1993), as well as the ability of the organism to emulsify (thanks to bile acids which form micelles) and absorb lipids. In this case, however, the temperature-dependent rise in ADE_{LIPIDS}, cannot be attributed to increases in the period of time food stays in the gastrointestinal tract, given that GPTs have been shown to decrease at higher temperature regimes. Instead, any changes in ADE_{LIPIDS} can be best explained by shifts in the second set of processes (enzymatic breakdown, emulsification, and absorption). Such changes are likely to underlie both the comparatively poor ability of *L. graeca* to absorb lipids and the very high ADE_{LIPIDS} of *P. erhardii* and *P. gaigeae* (which occur despite fairly rapid GPTs).

Sugars are broken down by a sequence of enzymes that are activated in pancreas and duodenum. The ADE_{SUGARS} values we report vary little among the species examined and are in general agreement with the few values published previously (Witz and Lawrence 1993; Pafilis and Valakos 2004). Because an important fraction of the sugars contained in insect prey comes in the form of indigestible nitrogenous polysaccharides such as chitin (Neville 1975) ADE_{SUGARS} values lay well below the expected 100%. The absence of chitinases from the gastrointestinal tract of reptiles renders these polysaccharides inaccessible to lizards and results in comparatively low ADE_{SUGARS} values (Witz and Lawrence 1993).

Sugar absorption in the gastrointestinal tract is a temperature-sensitive process and occurs mainly in duodenum and upper jejunum (Berne and Levy 1996). The uniform increases in ADE_{SUGARS} at higher temperatures, are probably best explained by the higher activity of sugar-digesting enzymes in warmer environments, with the optimum temperature for amylase in reptiles being over 40°C (Scoczylas 1978 and references therein). Nevertheless, enzymatic activity seems not to be influenced in the same manner by rising temperature in all species. Species like *P. muralis*, which are poor sugar digesters at 20°C, outperform all others at 30°C. Overall, however, there is no clear pattern in ADE_{SUGARS} among the taxa studied here, and island species in particular do not differ in any obvious manner from their mainland counterparts.

The third branch of digestive efficiency, $ADE_{PROTEINS}$, follows a different pattern than the previous two. First, overall levels of $ADE_{PROTEINS}$, range between 46–70% for the species examined and are thus distinctly lower than both ADE_{LIPIDS}, and ADE_{SUGARS}. Second, absorption of proteins decreases at higher temperatures resulting in a concomitant drop in $ADE_{PROTEINS}$. Digestion of proteins begins in the stomach and is crucially dependent on the duration of time the food remains there. Chemical decomposition, which is initiated by the secretion of acid gastric juices containing proteolytic enzymes (Scoczylas 1978) requires time, especially at low temperatures. The more time is allowed to complete the digestion of proteins, the higher the resulting $ADE_{PROTEINS}$. As GPTs become considerably shorter at higher temperatures, it is thought that not enough time remains for complete protein digestion and absorption; this would then explain the reduced $ADE_{PROTEINS}$ values observed at 25 and 30°C (da Diefenbach 1975a, b).

Not all lizards examined here absorb proteins in the same manner, with the mainland taxa (*P. muralis, P. peloponnesiaca* and *L. graeca*) differing clearly from their island counterparts (*P. gaigeae, P. erhardii*). Whereas $ADE_{PROTEINS}$ decreased for both groups at 30°C relative to 20°C, these decreases were much more pronounced in the mainland group (approx. 30%) compared to the island group (approx. 5%). This difference is especially noteworthy considering that island taxa area also characterized by shorter GPTs, a trait that—as mentioned earlier—has a negative impact on the ability of an organism to digest and absorb protein. The superior ability of island species to extract protein at higher temperatures, despite short GPTs, is probably best explained by either enhanced activity of digestive enzymes or by improved absorption in the upper part of the examined temperature range (Pafilis, unpublished data). Digestive physiology has been reported to adjust properly to suit a food scarce environment (McConnachie and Alexander 2004) as islands.

The majority of previous studies examining digestive processes in reptiles have used bomb calorimetry and have concluded that total ADE is relatively insensitive to temperature (Waldschmidt et al. 1986; Van Damme et al. 1991; Du et al. 2000; Chen et al. 2003; McConnachie and Alexander 2004; Zhang and Ji 2004). This finding could be considered unexpected given that GPTs—which have been shown to effect ADE—as well as digestive enzyme activities are clearly influenced by temperature (Hungate 1966).

Within a certain thermal range, however, increasing body temperature can increase the effectiveness of digestive enzymes while simultaneously reducing exposure time of food to enzymatic action because of decreasing GPTs (Harwood 1979). Hence, the overall stability of total ADE against rising temperatures had been interpreted by some as the cancelling-out of gains due to higher enzymatic activities by losses due to faster GPTs (Chen et al. 2003; McConnachie and Alexander 2004; Zhang and Ji 2004). By separately examining ADE_{LIPIDS}, ADE_{SUGARS}, and $ADE_{PROTEINS}$, the three components comprising total ADE, we show instead that the relative constancy of total ADE against rising temperatures can also be seen as the result of a shift from a digestive process with high $ADE_{PROTEINS}$ to a process with high ADE_{LIPIDS} and ADE_{SUGARS}. Our results support the idea that at higher temperatures, a larger fraction of the needs of an animal are derived from sugars and lipids as opposed to proteins. Through appropriate thermoregulation, it is therefore possible—as shown by Simandle et al. (2001)—for a lizard to selectively enhance its supply in certain food categories in order to cover specific needs.

ADEs reported for proteins, lipids and sugars are lower compared to the values calculated from previous studies in temperatures of 20 and 25°C (in 30°C ADEs suit perfectly to literature data). Many reasons may explain this fact, such as methodological differences (bomb calorimetry versus separate analysis), different thermal regimes (studies were not carried out under identical temperature conditions), different laboratory diet (mealworms did not consist the provided food always) and different digestive adaptations of each species correspond to varying environmental conditions.

Ecological significance of island environments

The present analysis of lacertid digestive physiology, whether conducted in a phylogenetic context or not, has repeatedly highlighted the distinctiveness of the island subgroup (*P. erhardii* and *P. gaigeae*) in comparison to the rest of species (*P. muralis*, *P. peloponnesiaca* and *L. graeca*). This first group is distinguished by: (1) 12–20% shorter GPTs; (2) consistently higher ADE_{LIPIDS}, which at 20°C lie approx. 8% above the rest; (3) higher $ADE_{PROTEINS}$ at 25 and 30°C (exceeding at 30°C by almost 30%, the corresponding values of the mainland taxa). These differences are also evident in the PCA, which at all temperatures consistently places the island taxa apart from the rest of the species (Fig. 4). The physiological similarities between *P. erhardii* and *P. gaigeae* and their differences to the rest of the group are remarkable considering that these two species are not each other's closest relatives, but are rather nested within a cluster of mainland taxa (see Fig. 2). Based on the differences described previously, these island species are characterized by exceptional abilities to extract nutrients from their food both at low (for lipids), as well as high (for proteins) temperatures. This is particularly noteworthy because they simultaneously also possess much faster GPTs than their mainland counterparts, yet this is a trait that normally undercuts efficient absorption. As a result of these characteristics, island endemics appear to be in a superior position to extract rapidly and efficiently nutrients from their food. This is in contrast to conventional understanding of digestive trade-offs, as current theory suggests that organisms can either maximize rate of energy intake by passing digesta rapidly through the gastrointestinal tract, or maximize digestive efficiency through long food retention times (Barton and Houston 1993, 1994). The first strategy is optimal in environments with high food abundance whereas the second strategy performs best under conditions of limited food availability. The fact that the island lacertids have high efficiencies despite short GPTs suggests that they may be able to maximize ADE via shifts in other key elements of digestion such as increased gut surface area or length, elevated enzyme activities and/or improved nutrient uptake rates, although more research is clearly needed. Indeed, studies from other island reptiles or species that have irregular feeding habits reveal a variety of morphological adaptations. These include higher intestinal capacity in the tropical lizard *Cnemidophorus murinus* (Dearing 1993) as well as increased gut surfaces in the insular lacertid *P. pityusensis* (Carretero 1997). While some of these adaptations like the enlarged intestines and compartmentalized colons characteristic of many iguanas (Iverson 1980) are permanent, several snake species are able to extensively reorganize their gastrointestinal tract in response to meals (Starck and Beese 2001, 2002).

On one hand, this high nutrient absorption efficiency ensures optimal use of limited resources especially at the relatively high ambient temperatures prevailing on the islands. On the other hand, because peak insect occurrence is brief and limited to well-defined periods in spring and fall, and because prey is generally encountered in distinct clusters (e.g., invertebrate mating or foraging aggregations) (Perez-Mellado and Corti 1993; Maragou et al. 1996), rapid GPTs allow for maximum acquisition of energy.

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