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RESEARCH ARTICLE

Convergent digestive adaptation to resource limitation in an insular lizard across a microgeographic archipelago

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Abstract

1. Island systems provide valuable natural laboratories for investigating how ecological constraints drive physiological evolution. Although dietary shifts are known to influence morphological divergence in insular lizards, their consequences for digestive physiology remain insufficiently understood.
2. Using 10 wild populations of the Skyros wall lizard (*Podarcis gaigeae*) across the Skyros Archipelago, we tested whether fine-scale variation in habitat and prey availability predicts divergence in digestive performance. We quantified arthropod abundance, gastrointestinal morphology, gut passage time, digestive enzyme activity and apparent digestive efficiency (ADE) under standardized laboratory conditions.
3. Islet populations consistently exhibited slower gut passage, longer gastrointestinal tracts, higher protease and lipase activities, and greater ADE relative to populations on the main island of Skyros, indicating enhanced digestive performance in resource-limited environments.
4. AIC_C-based model selection showed that prey availability and habitat type, rather than geographic distance or genetic relatedness, best explained patterns of digestive divergence among populations.
5. These results demonstrate repeated and independent adaptive responses to resource scarcity and highlight digestive plasticity as a key mechanism facilitating persistence in insular habitats.

KEYWORDS
adaptive response, digestive physiology, Greece, island ecosystems, *Podarcis gaigeae*, resource limitation, Skyros

1 | INTRODUCTION

Understanding how organisms adjust to spatially variable environments is central to evolutionary ecology. Ecological differences among populations can generate divergent selection, leading to phenotypic and physiological differentiation that influences fitness, energy allocation and life-history trajectories (Coltman et al., 2000; Endler, 1986; Pafilis et al., 2011; Wanamaker et al., 2020). While large-scale environmental gradients frequently drive such divergence (Futuyma & Moreno, 1988; Schluter, 2000; Zhang et al., 2019), adaptive evolution may also arise across small geographic distances when ecological heterogeneity is pronounced and persistent (Futuyma & Moreno, 1988). But such evidence is scarce or missing due to weaker selection pressures, high habitat connectivity or low environmental heterogeneity among populations (Ebert, 1994).

Islands provide powerful natural laboratories for examining these dynamics (MacArthur & Wilson, 1967; Whittaker et al., 2017). Their discrete boundaries, simplified food webs and resource constraints often promote ecological release, niche expansion and rapid evolutionary change of island populations (Buckley & Jetz, 2007; Novosolov et al., 2013; Taverne et al., 2019). In particular, population density and prey limitation are recurring features of Mediterranean island ecosystems, especially for insectivorous lizards whose energetic demands can exceed local arthropod abundance (Pérez-Mellado & Corti, 1993; Van Damme, 1999). Such conditions may favour shifts in trophic niche, foraging strategy and digestive biology.

Digestive physiology plays a critical, but often overlooked, role in these adaptive responses. Efficient nutrient acquisition depends on a suite of coordinated traits, including gastrointestinal morphology, enzymatic activity, gut passage time and nutrient assimilation (Hume, 2005; Karasov & Martinez Del Rio, 2007). Several hypotheses predict how these traits may (genetically) evolve or plastically respond to dietary constraints. The flexible gut morphology hypothesis proposes that animals increase gut size or remodel gut morphology to prolong digestion and enhance absorption under low-quality or scarce diets (Herrel et al., 2008; Sagonas et al., 2015; Sibly, 1981; Starck, 2005; Vervust et al., 2010). The nutrient balancing hypothesis suggests that digestive enzyme activity should increase when specific nutrients are limiting, improving extraction efficiency (German et al., 2010; Westoby, 1974). Together, these frameworks imply that organisms inhabiting food-poor environments may evolve physiological strategies that compensate for energetic shortfalls.

Mediterranean lacertid lizards exhibit remarkable dietary flexibility in response to food scarcity, including facultative omnivory, herbivory and frugivory on islands (Brock et al., 2014; Pérez-Mellado & Corti, 1993; Van Damme, 1999; Vervust et al., 2010). This trophic shift has been linked to modifications in head morphology, digestive anatomy, feeding performance and body size, traits that frequently evolve in insular lizard populations (Herrel et al., 2008; Meiri, 2008; Runemark et al., 2015; Sagonas et al., 2015). However, most research compares mainland and island populations separated by broad geographic, ecological and evolutionary distances. Whether similar digestive adaptations emerge repeatedly at microgeographic scales where populations remain genetically connected but ecologically distinct remains largely unexplored.

The Skyros Archipelago in the Aegean Sea provides an ideal setting to address this question. The endemic Skyros wall lizard (*Podarcis gaigeae*) inhabits both the main island of Skyros and several nearby satellite islets that differ substantially in vegetation structure, prey availability and habitat productivity (Pafilis et al., 2013; Runemark et al., 2015). Genetic evidence shows that islet populations were isolated recently and sequentially, and are more closely related to adjacent mainland populations than to each other (Runemark, 2012; Runemark et al., 2012), creating replicated natural experiments in ecological divergence. Previous work indicates that islet populations experience chronic food limitation and occasionally supplement their diets with plant material and seeds (Brock et al., 2014; Runemark et al., 2015), suggesting strong selective pressures on digestive performance. Here, we integrate ecological, morphological and physiological data from 10 populations across the archipelago to test whether digestive traits diverge predictably in response to resource availability. Specifically, we ask whether differences in prey abundance and habitat type explain variation in gut passage time, digestive enzyme activities, gastrointestinal tract morphology and apparent digestive efficiency (ADE). If digestive traits are shaped by local ecological conditions, we expect repeated shifts towards slower passage time, increased enzyme activity and enhanced nutrient assimilation in populations inhabiting resource-limited environments. Alternatively, if gene flow or developmental constraints limit adaptation, digestive traits should vary idiosyncratically among populations. By linking fine-scale ecological variation to organismal physiology, this study provides new insight into how environmental pressures drive convergent physiological responses and underscores the importance of digestive flexibility for persistence in insular ecosystems.

2 | MATERIALS AND METHODS

2.1 | Study system

During May 2022, we visited five mainland sites (Atsitsa [AT], Nyfi [NF], Palamari [PL], Molos [MO] and Agios Fokas [AF]) on Skyros Island (N 38°51′, E 24°33′) (Central Aegean Sea, Greece) and five surrounding islets (Lakonisi [LK], Mesa [MD] and Exo Diavate [ED], Atsitsa Islet [AI] and Agios Ermolaros [AE]) (Figure 1). Two hundred and twenty-four adult *Podarcis gaigeae* lizards were captured in the field (see Table 1 for sample size) under the permit ΥΠΕΝ/ΔΔΔ/16809/663 and subsequently transported to the animal facilities of the University of Thessaloniki and individually housed in plastic terraria (20 cm x 25 cm x 15 cm) containing a sandy substrate and artificial shelters. The room temperature was kept at 25°C, with a controlled photoperiod (12 light: 12 dark) provided by fluorescent bulbs. Additional incandescent lamps (60 W) and UVB lamps were placed in a standardized position above each terrarium, allowing lizards to thermoregulate behaviourally for 12 h/d. Although

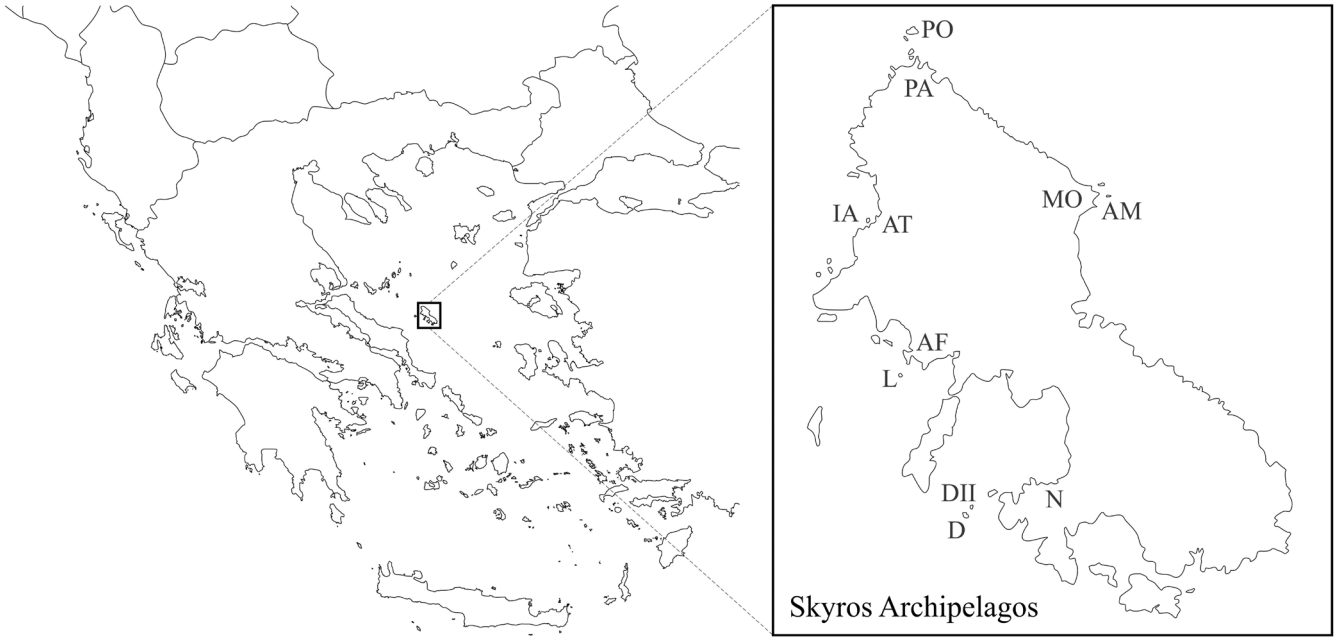

FIGURE 1 Map of Greece focusing on the Skyros Island and its surrounding islets. The letter codes denote sampling localities. PL refers to Palamari; MO to Molos; AT to Atsitsa; AF to Agios Fokas; NF to Nyfi; IA to Islet of Atsitsa; LK to Lakonisi; MD to Mesa Diavates; and ED to Exo Diavates.

we did not employ data loggers within each terrarium and body temperature was not monitored continuously, all units were maintained under identical conditions and heating setup; therefore, we expect thermal regimes to have been highly similar across terraria. Furthermore, given that lizards may differ in their thermoregulatory behaviour, we incorporated each individual's preferred body temperature (T_{pref}), obtained from a separate thermal gradient experiment (Reppa et al., 2023), as a covariate in the analyses to account for potential thermal effects. All lizards were allowed to acclimatize for a 2-week period prior to the experiment, were fed mealworms (*Tenebrio molitor*) coated with mineral powder (TerraVit Powder, JBL GmbH & Co. KG) every other day (husbandry feeding schedule), and had access to water ad libitum. Each lizard was sexed, and then we recorded snout–vent length (SVL, in mm) with a digital calliper (Silverline 380,244, accurate to 0.01 mm) and mass using a digital scale (i500 Backlit Display, My Weight, accurate to 0.1 g).

Podarcis gaigeae is a small-bodied insectivorous lacertid lizard (SVL around 60 mm, body mass 6.58 ± 1.3 g) that lives in most habitats on Skyros archipelago (Valakos et al., 2008). The four islets, Atsitsa Island, Lakonisi, Mesa Diavates and Agios Ermolaos, surrounding Skyros Island share similar vegetation that comprises sparse phrygana shrubs and nitrophilous plants and are characterized by a decrease of arthropod availability as a consequence of the low vegetation coverage (Pafilis et al., 2013) (Figure S1). However, Exo Diavates stands out as an exception, as it is covered by dense herbaceous vegetation dominated by nitrophilous species. This lush vegetation is fuelled by marine subsidies that, in turn, support a rich invertebrate fauna (Pafilis et al., 2011), increasing resource availability and habitat conditions for the local lizard population. The Skyros habitats, on the other hand, show remarkable differences in terms of vegetation type and density as well as prey availability (Pafilis et al., 2013; Runemark et al., 2015). Palamari (PA) and Molos (MO) are semi-dunal biotopes where arthropod abundance and vegetation are scarce, Nyfi (NF) and Agios Fokas (AF) hold dense phrygana shrubs and maquis vegetation with high prey availability, while Atsitsa (AT) habitat is a dense pine forest with reduced invertebrate abundance (Pafilis et al., 2013) (Figure S1).

2.2 | Ethics approval

All experiments, procedures and animal housing were approved by the Ethical Committee of the Aristotle University of Thessaloniki (59618/2022) and the Greek Ministry of Environment and Energy (ΥΠΕΝ/ΔΔΔ/16809/663 assigned to K. Sagonas). Experiments and procedures were conducted in accordance with national legislation and adhered to the ASASB/ABS Guidelines for the use of animals in behavioural research. Lizards were transferred at the Animal Facility to cloth bags to minimize the stress associated with handling, housed individually and checked daily while in captivity to monitor their welfare.

2.3 | Invertebrate prey availability

To quantify prey availability for islet and mainland populations, pitfall traps were deployed in all sites, in the same microhabitats where lizards were captured to ensure that prey availability data reflected the conditions experienced by the lizards and to complement the datasets of 2007 (Runemark et al., 2015) and 2013 (Pafilis et al., 2013).

TABLE 1 Values for snout–vent length (mm), body mass (g), gut passage time (h), apparent digestive efficiency for proteins (AED_{Pr}) and lipids (ADE_{Lip}), enzymatic activity of proteases and lipases ($\mu moles \times gr^{-1} \times min^{-1}$) are given for the 10 *P. gaigeae* populations.

	Population	SVL	Mass	GI length	GPT	ADE_{Pr}	ADE_{Lip}	ADMD	Proteases	Lipases
Skyros Island ('mainland')	Agios Fokas (AF)	49.3 ± 9.92 *N* = 20	4.43 ± 2.12 *N* = 20	101.00 ± 6.69 *N* = 9	36.25 ± 2.71 *N* = 20	61.68 ± 10.43 *N* = 20	78.10 ± 5.61 *N* = 20	75.09 ± 12.32 *N* = 20	357.63 ± 7.59 *N* = 9	79.59 ± 7.59 *N* = 9
	Atsitsa (AT)	50.76 ± 8.85 *N* = 31	4.30 ± 1.68 *N* = 31	98.78 ± 10.94 *N* = 9	38.45 ± 4.40 *N* = 31	60.23 ± 14.67 *N* = 31	81.04 ± 5.11 *N* = 31	72.89 ± 10.85 *N* = 31	358.88 ± 3.69 *N* = 9	76.37 ± 5.26 *N* = 9
	Molos (MO)	57.32 ± 3.46 *N* = 22	5.07 ± 1.05 *N* = 22	96.67 ± 9.88 *N* = 9	36.86 ± 3.04 *N* = 22	66.14 ± 7.54 *N* = 22	81.44 ± 4.21 *N* = 22	73.63 ± 10.21 *N* = 22	376.84 ± 5.53 *N* = 9	91.45 ± 5.59 *N* = 9
	Nyfi (NF)	46.79 ± 6.29 *N* = 17	3.14 ± 0.93 *N* = 17	89.33 ± 9.37 *N* = 9	35.59 ± 3.14 *N* = 17	64.50 ± 8.25 *N* = 17	79.13 ± 1.68 *N* = 17	78.37 ± 11.23 *N* = 17	354.97 ± 11.19 *N* = 9	74.60 ± 5.21 *N* = 9
	Palamari (PL)	49.00 ± 6.09 *N* = 15	4.15 ± 0.91 *N* = 15	82.22 ± 9.32 *N* = 9	36.2 ± 2.46 *N* = 15	74.98 ± 8.10 *N* = 15	85.20 ± 3.81 *N* = 15	72.78 ± 13.29 *N* = 15	385.59 ± 11.64 *N* = 9	85.06 ± 7.82 *N* = 9
Islets	Agios Ermolaos (AE)	57.80 ± 6.28 *N* = 29	4.51 ± 1.51 *N* = 29	102.78 ± 5.95 *N* = 9	41.21 ± 1.88 *N* = 29	72.97 ± 9.09 *N* = 29	86.0 ± 6.16 *N* = 29	85.06 ± 3.33 *N* = 29	383.02 ± 15.65 *N* = 9	93.51 ± 6.44 *N* = 9
	Islet of Atsitsa (AI)	64.67 ± 4.75 *N* = 27	6.13 ± 1.67 *N* = 27	107.00 ± 5.05 *N* = 9	39.41 ± 3.18 *N* = 27	70.21 ± 10.71 *N* = 27	84.96 ± 4.00 *N* = 27	84.51 ± 4.65 *N* = 27	366.27 ± 12.44 *N* = 9	83.50 ± 7.23 *N* = 9
	Exo Diavates (ED)	83.22 ± 4.50 *N* = 25	14.62 ± 2.29 *N* = 25	120.23 ± 10.18 *N* = 9	41.96 ± 2.94 *N* = 25	69.01 ± 2.42 *N* = 25	83.23 ± 4.48 *N* = 25	78.24 ± 8.34 *N* = 25	361.15 ± 10.91 *N* = 9	77.55 ± 6.57 *N* = 9
	Lakonisi (LK)	67.74 ± 7.91 *N* = 19	8.39 ± 3.47 *N* = 19	103.44 ± 11.24 *N* = 9	41.84 ± 2.41 *N* = 19	72.38 ± 8.24 *N* = 19	84.96 ± 4.72 *N* = 19	84.62 ± 4.22 *N* = 19	376.46 ± 12.64 *N* = 9	84.62 ± 3.00 *N* = 9
	Mesa Diavates (MD)	60.89 ± 6.43 *N* = 19	5.13 ± 1.85 *N* = 19	99.35 ± 12.25 *N* = 9	41.16 ± 1.71 *N* = 19	75.18 ± 11.03 *N* = 19	89.01 ± 3.61 *N* = 19	85.68 ± 3.62 *N* = 19	390.51 ± 6 0.45 *N* = 9	95.83 ± 5.63 *N* = 9

Note: Data are reported as means ± standard deviation and sample size (*N*).

Invertebrate availability was quantified as the total number of individuals captured per trap per day. For taxonomic comparisons, we used the relative abundance of each prey group (proportion of individuals) across sites. Each pitfall trap consisted of plastic cups (diameter 7 cm, height 10 cm) and were placed every 15 m on two transects situated at least 15 m apart. Ten traps were placed per locality. Traps were functional for 2 months (May and June) when invertebrate abundance (Trihas & Legakis, 1991) and lizards' activity are highest. While this capture method does not take flying insects into account, we considered the sampling bias unimportant because *P. gaigeae* diet comprises mainly soil arthropods (Adamopoulou et al., 1999; Runemark et al., 2015). Invertebrate availability was compared using GLM. We further examined whether the proportions of the prey taxa (based on number of individuals) differed between populations and between Skyros and islet environments using χ^2 tests of the seven taxonomic groups that constituted more than 2% of the number of specimens across all sites.

2.4 | Gut passage time

To estimate the rate at which food passes through the gut, lizards were fasted for 4 to 5 days until no faeces were present in the terrarium. GPT was then calculated as the interval between the ingestion of a mealworm containing a red plastic marker (Van Damme et al., 1991) and the first detection of the marker in the faeces. Following consumption, lizards were returned to their terraria and allowed to thermoregulate freely. Terraria were inspected every hour, and all faecal pellets were collected and examined. Although lizards may occasionally expel a single food item in multiple pellets, this was not observed in our study; nonetheless, every sample was checked to ensure accurate detection. After GPT estimation, lizards were returned to the standard husbandry feeding schedule (mealworms every other day, as described above). Because GPT was always measured following the standardized fasting period, subsequent food intake did not influence our estimates, despite evidence from other studies that variation in feeding rate can affect passage time (Schop et al., 2019).

2.5 | Digestive efficiency

While the term 'digestive efficiency' is widely used to evaluate how animals extract nutrients and energy from their food, an innate shortfall of the method is that non-dietary materials in faeces, such as sloughed intestinal cells, microbial matter and nitrogenous wastes, are also taken into account (Johnson & Lillywhite, 1979; McKinon & Alexander, 1999). Therefore, the term 'apparent' digestive efficiency (ADE) is used as an indicator of the relative percentage of ingested nutrients absorbed through the gut (Beaupre et al., 1993; McKinon & Alexander, 1999). ADE was estimated for proteins and lipids. The ADE trials were conducted after the completion of the GPT experiment, following a 3-week period in which lizards were maintained under their standard husbandry feeding schedule. During the ADE experiment, each lizard was offered two mealworms (*Tenebrio molitor*) of similar size and mass per day, provided voluntarily in their terraria. To allow for biochemical comparisons, two 'twin' mealworms of equivalent size and mass were stored at −80°C for subsequent biochemical analyses. Food consumption was quantified by weighing the mealworms before and after the feeding trial. Lizards were allowed a maximum of 2 h to consume the food, after which any remaining mealworms were removed from the terraria. Faecal material was collected separately for each lizard, and any urate fraction was carefully removed and discarded prior to preservation. The cleaned faecal samples were then frozen immediately in liquid nitrogen and stored at −80°C until further analysis.

We determined total lipids in faeces using the method of Alexis et al. (1985). Thirty to forty milligrams of tissue was homogenized with 1.5 mL of a 2:1 mixture of chloroform and absolute methanol. The homogenate was centrifuged at 3000 rpm for 10 min in 4°C. The formed pellet was discarded, and the supernatant was used for the determination of lipid levels using a dilution of phosphovaniline against an olive oil and corn oil standard (2:1 v/v). Absorbance was read at 530 nm using a spectrophotometer (Novaspec II, Pharmacia Biotech).

We estimated total protein concentration within tissue using the classical Biuret method (Layne, 1957) compared with a bovine serum albumin standard (0.5–10 mg/mL). The pellet obtained from the lipid analysis was dissolved with 0.5 mL of 0.1 N NaOH and incubated at 37°C for 30 min. Following this, 50 µL of the dissolved sample was diluted in 950 mL H_2O, and 4 mL of Biuret Reagent was added. The mixture was incubated for 30 min at room temperature, and then, the absorbance was measured at 550 nm.

ADEs for lipids and proteins were calculated according to the following equation:

$$\mathrm{ADE}_x = \frac{100\left(I_x - E_x\right)}{I_x},$$

where I_x is the amount of the nutrient (x=proteins or lipids) ingested and E_x is the amount of the nutrient (x=proteins or lipids) remaining in the faecal material after the enteric absorption.

In addition, we calculated the apparent dry matter digestibility (ADMD) (Naya & Božinović, 2006) as a complementary measure of digestive performance ADMD was defined as follows:

$$\mathrm{ADMD} = \frac{I_{\text{mg dry food intake}} - F_{\text{mg dry excerta produced}}}{I_{\text{mg dry food intake}}},$$

where I is the dry mass of food ingested (mg) and F is the dry mass of faeces excreted (mg). Unlike ADE, which is nutrient-specific, ADMD integrates across the entire meal and thus provides a simple estimate of the proportion of ingested dry matter that was assimilated, although it does not account for differences in energy concentrations or nutrient composition.

Finally, although ADE values are widely used to evaluate how animals extract nutrients and energy from their food, this approach has recognized limitations because non-dietary components of faeces are included

as above mentioned. Moreover, subsequent re-analyses have demonstrated that ADE as a ratio-based metric can inflate error and potentially yield spurious conclusions (Beaupre, 1995; Wehrle & German, 2023). For this reason, in addition to calculating ADE values for comparison with previous studies, we analysed digestive performance using an ANCOVA approach, treating nutrient excretion (mg of protein or lipid content in faeces) as the dependent variable, nutrient intake (mg) and SVL or GPT as covariates, and population as the factor. This approach avoids the statistical shortcomings of ratio-based indices, allows direct testing of treatment effects while controlling for intake, and produces results that can be more reliably compared across datasets in future meta-analyses.

2.6 | Intestinal tissue preparation

After the completion of the ADE experiments, lizards were fasted 48 h before euthanasia/dissection to allow food to pass through and avoid residual food influence on enzyme assays. Dissections were undertaken over ice to avoid enzyme and tissue degradation and were performed only on a subset of individuals ($N = 90$; 9 lizards per population). The gastrointestinal (GI) tract, spanning from the stomach to the cloaca, was cut lengthwise and rinsed with Ringer's solution (0.1 M NaCl, 1.8 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.0 mM HEPES-NaOH, pH 7.6). Care was taken to remove any remaining food residues and faecal matter, and in the rare cases where undigested food was present, samples were excluded from further analyses. This ensures that the subsequent estimation of enzymatic activities was based solely on the intestinal tissue itself, rather than residual waste or food particles. The GI tract was then measured using digital callipers (to 0.01 mm) and a precision scale (wet mass, to 0.001 g). The tract was divided into three sections based on general morphology: Section 1 (foregut/stomach), Section 2 (midgut) and Section 3 (hindgut). The pancreas was carefully excluded from all samples. Each section was weighed and measured again before being frozen in liquid nitrogen and stored at −80°C for subsequent enzyme activity analysis. Intestine tissue samples were thawed at 21 to 23°C and homogenized with 0.1 M phosphoric buffer (pH = 7.0).

Tissue blanks were used as controls to distinguish enzymatic activity from baseline substrate concentrations. Standardized intestinal enzymatic activity was calculated on the basis of absorbance and expressed as $\mu g \times g^{-1} \times min^{-1}$ for each section. Given that most of the enzymatic activity occurs in the midgut, we have limited our analysis and presentation of results to Section 2.

2.7 | Assays of digestive enzyme activities

Protease activity was estimated using the Lowry et al. (1951) method with denatured casein (4% w/v) as the substrate, a well-established approach. This method quantifies total protein, including small peptides generated during digestion. Following careful tissue preparation and the removal of digesta to minimize confounding factors, this method can also serve as an indirect measure of protease activity through protein hydrolysis.

Homogenized tissue was incubated for 30 min at 32°C, and the reaction was terminated by adding 10% (v/v) trichloroacetic acid (TCA). The sample was centrifuged at 6000 rpm for 10 min; the pellet was discarded, and the supernatant was used to determine hydrolysed protein. Folin phenol reagent was added to the sample, which was then incubated for another 30 min. Finally, the concentration of the reduced Folin reagent was measured at 750 nm using a spectrophotometer (Barnstead/Turner SP-830 Plus).

To estimate total lipase activity, we used the oxidation of glycerol to dihydroxyacetone phosphate by glycerol kinase, a method modified from Kessler and Lederer (1965). While the original method was optimized to measure lipoprotein lipase (LPL) activity in adipose tissue, skeletal muscle and plasma, its principle of quantifying glycerol release from triglyceride hydrolysis is broadly applicable for assessing lipase activity. Here, using olive oil as a substrate to reflect the hydrolytic activity of lipases, the method was modified to suit intestinal tissue homogenates. Because the gastrointestinal tract was rinsed and the pancreas was excluded, our measurements primarily reflect tissue-associated lipase activity in the intestinal mucosa (e.g. brush-border and intracellular lipases), rather than pancreatic secreted lipases in the lumen. Although this assay does not differentiate among lipase types, it provides a consistent comparative index of intestinal enzymatic potential across populations.

The reaction was initiated by incubating the homogenized tissue at 32°C for 60 min with 0.5 mL of olive oil as the substrate. To terminate the reaction, samples were incubated at 100°C for 10 min. The samples were then centrifuged at 3000 rpm for 5 min, and 0.5 mL of the supernatant was mixed with 2 mL of glycine phosphate buffer and 0.4 mL of NAD. Lipase activity was quantified based on the amount of released glycerol, determined by measuring the absorbance of NADH at 340 nm every 10 min until stabilization.

2.8 | Statistical analyses

One-way analysis of variance (ANOVA) was used to compare SVL, ADE, ADMD, GPT and enzymatic activity among populations. When significant effects were detected, the analyses were repeated using analysis of covariance (ANCOVA), with SVL included as a covariate. A MANCOVA was also performed to investigate the relationship between GPT, ADE and digestive enzyme activities. Although GPT was estimated under standardized fasting conditions (single marked mealworm) and ADE/enzyme activities were measured under daily feeding, GPT was treated as an individual-level trait reflecting intrinsic digestive performance. In this way, the analysis served to test whether individual variation in GPT covaried with variation in ADE and digestive enzyme activities. Because GPT reflects the residence time of food in the gut, it represents a direct physiological constraint on nutrient extraction. Thus, even though GPT and ADE/enzyme activity were measured under different short-term feeding regimes, they can be meaningfully compared as complementary indicators of digestive performance. We performed an ANCOVA to reduce the within-group error in GPT caused by SVL and the length of GI tract. Variation in GI tract morphology was assessed through regression analysis with

SVL on one side and GI tract length on the other. In all cases, Tukey HSD post hoc test was applied. We used PCA to investigate the interrelationships between SVL, ADE, GPT and ADMD values. Likewise, a second PCA was run to a subset of data using SVL, ADE for lipids and proteins, GPT, GI length, ADMD and enzymatic activity.

To infer whether the different aspects of digestive efficiency differ between populations in response to locally available preys or have independently changed across the landscape, we computed GLMs with invertebrate availability (diet), habitat (islet or 'mainland'), geographic distances as an index of populations' connectivity, population divergence time and genetic distances (F_{ST}; Runemark et al., 2012) as explanatory variables using the nlme R package (Pinheiro et al., 2021) in R version 4.3 (R Development Core Team, 2024). The values of the first and second principal component (PC1 and PC2) were used as dependent variables. We determined which model best explains digestive performance by comparing AIC_C of the models.

3 | RESULTS

3.1 | Invertebrate availability

From all sampling sites we collected 22,390 individuals belonging to more than 15 invertebrate taxa; larvae were considered a separate group. The most abundant orders were Coleoptera (14.42%), Hymenoptera (6.54%), Formicidae (33.46%) and Isopoda (21.92%) comprising 76.34% of the available invertebrate soil fauna. The taxonomic composition and abundance of available prey groups differed significantly between sites ($\chi^2 = 10{,}818$, df = 126, $p < 0.001$), as well as between islet and mainland environment ($\chi^2 = 1959.9$, df = 6, $p < 0.001$; Figure 2). Because the taxonomic composition of *P. gaigeae* diet may differ from prey availability (as estimated by pitfall traps) (Runemark et al., 2015) we repeated the analysis only for the most common preys found in *P. gaigeae* stomachs (i.e. Coleoptera, Hymenoptera, Formicidae, Araneae, Isopoda, Helicidae, Larvae and Orthoptera). Chi-square test revealed that the abundance of these prey groups differed strongly between islet and Skyros environment ($\chi^2 = 8.18$, df = 63, $p < 0.001$), with the populations of Palamari, Molos, Mesa Diavates, Atsitsa Islet and Agios Ermolaos having lower proportion of these taxonomic categories than the remaining populations. Notably, Exo Diavates population had one of the highest abundances and comparable to Skyros sites. Sampling site had a significant effect on total number of invertebrates as well ($F_{9,190} = 36.22$, $p < 0.001$), with Nyfi (258.45 ± 81 invertebrates per trap), Exo Diavates (252 ± 126), Atsitsa (125 ± 33) and Palamari (99 ± 33) holding the highest abundances.

3.2 | Body size and gut length

The comparison of body size and body mass revealed significant differences between populations (ANOVA; SVL: $F_{9,214} = 63.01$, $p < 0.001$ and Mass: $F_{9,214} = 74.86$, $p < 0.001$; Table 1; Figure 3). Lizards from Exo Diavates were significantly larger and heavier than those from other populations, followed by Lakonisi and Atsitsa Islet. In contrast, Mesa Diavates, Agios Ermolaos and mainland populations were generally smaller. The smallest sizes were recorded in Palamari and Atsitsa habitats (Games Howell test). As expected, strong sexual body size dimorphism was observed in nearly all populations (all $ps < 0.05$), with males being larger than females, except for Atsitsa Islet ($t = 1.41$, df = 20.12, $p = 0.175$), Nyfi ($t = -0.09$, df = 7.16, $p = 0.932$) and Palamari ($t = -1.50$, df = 12.60, $p = 0.157$).

Podarcis gaigeae populations showed significant differences in gastrointestinal (GI) tract length, with individuals from Atsitsa Islet, Exo Diavates and Lakonisi showing longer GI tracts, while those

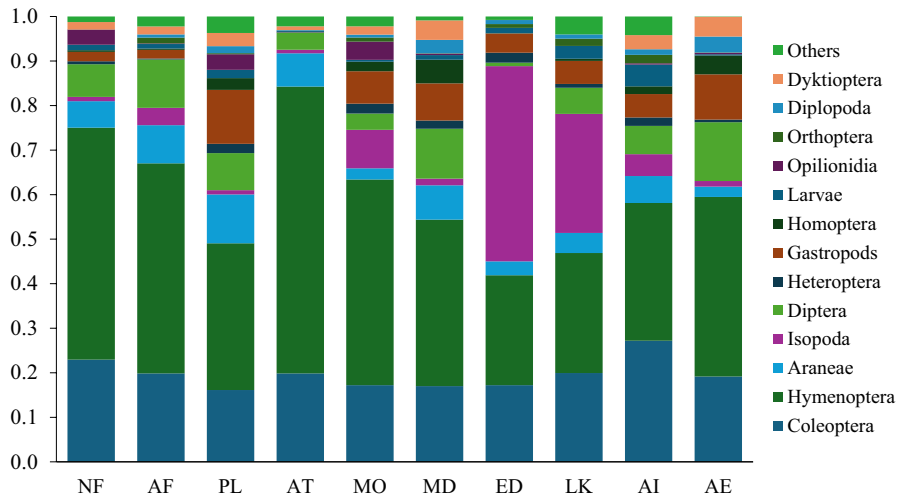

FIGURE 2 Relative representation of the different taxonomic composition of available prey for islet and mainland populations of the Skyros wall lizard. All taxonomic groups that constitute more than 1% of available prey are included; the taxonomic composition of available prey differed significantly between sites, habitats and environments. 'Mainland' Skyros populations are Nyfi (NF), Agios Fokas (AF), Palamari (PL), Atsitsa (AT) and Molos (MO), while islet populations are represented by Mesa Diavates (MD), Exo Diavates (ED), Lakonisi (LK), Islet of Atsitsa (AI) and Agios Ermolaos (AE).

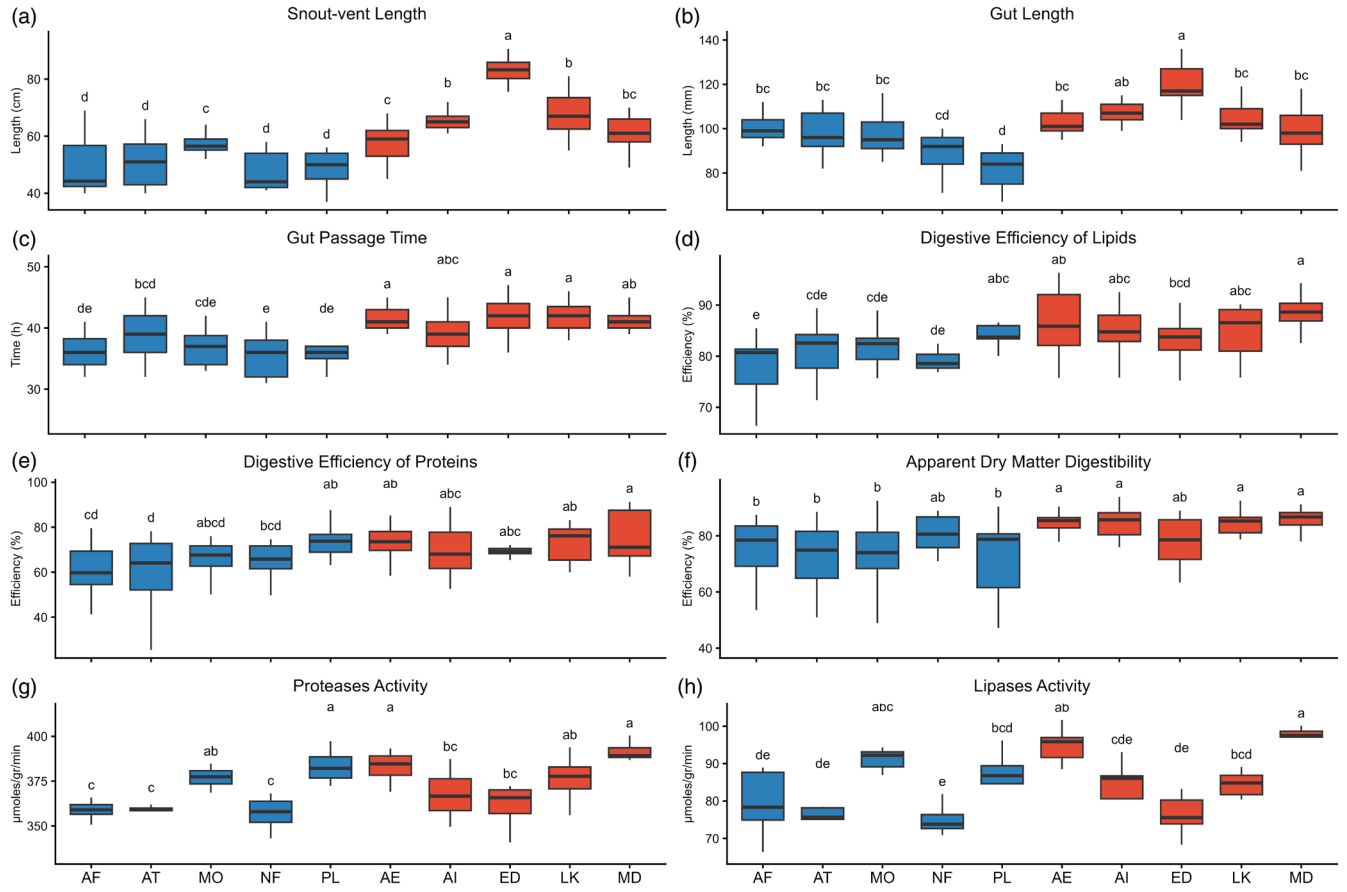

FIGURE 3 Boxplots illustrating snout–vent length (cm), gastrointestinal length, digestive performance variables. Red represents islet populations, while blue corresponds to 'mainland' Skyros populations. Horizontal lines indicate the median and vertical lines the standard deviation. The edges of the boxes limit the first and third quartiles. PL refers to Palamari; MO to Molos; AT to Atsitsa; AF to Agios Fokas; NF to Nyfi; IA to Islet of Atsitsa; LK to Lakonisi; MD to Mesa Diavates and ED to Exo Diavates.

from Palamari and Nyfi had shorter ($F_{9,80}=10.43$, $p<0.001$). As expected, gut length was positively correlated with SVL ($t=6.26$, df = 88, $p<0.001$). To account for potential size effects, the analysis was repeated using the residuals of GI tract length on SVL. Although this adjustment reduced the magnitude of differences, significant variation among populations remained ($F_{9,80}=2.53$, $p=0.013$) with islet lizards exhibiting longer guts than those of Skyros Island.

3.3 | Gut passage time

There were highly significant differences in the time food passes through the gut between populations ($F_{9,214}=14.90$, $p<0.001$) and habitats ($t=9.85$, df = 192.45, $p<0.001$) (Table 1; Figure 3). The post hoc Tukey HSD test indicated the formation of two main groups that partially match the two different environments, that is islets and Skyros habitats. In particular, the first group comprised the four islet populations Lakonisi, Exo and Mesa Diavates and Agios Ermolaos, and was characterized by 10% slower passage times, whereas the second group included the Skyros populations with faster food passage (~37 h vs. 41 h respectively). Notably, Atsitsa population and the islet of Atsitsa demonstrated an intermediate time of food passage through the gut. Given the effects of SVL on GPT (Pearson test; $t=13.94$, df = 222, $p<0.001$, $r=0.68$) and GI length on GPT ($t=4.21$, df = 88, $p<0.001$, $r=0.41$), we repeated the analysis considering lizard's body and gut length. As before, the comparison revealed significant differences (ANCOVA; SVL: $F_{9,213}=13.74$, $p<0.001$ and GI: $F_{9,79}=4.47$, $p<0.001$), with Agios Ermolaos, Atsitsa Islet and Exo Diavates (but not Mesa Diavates) demonstrating slower food gut passage time. The latter suggests that SVL alone is not enough to explain the variation in GPT observed between *P. gaigeae* populations.

3.4 | Digestive performance between populations

Analysis of variance showed that lizards from Agios Ermolaos, Atsitsa Islet, Mesa Diavates and Exo Diavates achieved significantly higher ADE for lipids ($F_{9,214}=10.79$, $p<0.001$) and proteins ($F_{9,214}=6.78$, $p<0.001$), followed by the populations of Palamari and Lakonisi, while the remaining mainland populations showed the lower efficiency (Table 1; Figure 3). These results also held true when the time food passed through the gut (lipids: $F_{9,213}=8.71$, $p<0.001$ and proteins: $F_{9,213}=5.80$, $p<0.001$), SVL (lipids: $F_{9,213}=9.36$, $p<0.001$ and proteins: $F_{9,213}=5.87$, $p<0.001$) and GI tract length (lipids:

$F_{9,79}=6.96$, $p<0.001$ and proteins: $F_{9,79}=4.19$, $p<0.001$) were considered as covariates. Likewise, habitat had a significant effect on the data, with islet populations achieving higher efficiency in protein and lipid digestion compared with their mainland kin for both proteins and lipids (both $ps<0.001$). However, this pattern was not universal, as the population from Palamari (mainland) showed values comparable to islets, whereas Exo Diavates (islet) exhibited lower protein ADE than the rest of the islet populations. Apparent dry matter digestibility (ADMD) also showed significant differences between populations ($F_{9,214}=8.83$, $p<0.001$), with islet populations achieving on average higher digestibility than mainland populations. When size traits were taken into account, the differences remained (all $ps<0.001$). Likewise, the ANCOVA approach for nutrient absorption and SVL as a covariate showed that islet populations had significantly higher absorption for proteins ($F_{11,212}=22.92$, $p<0.001$) and lipids ($F_{11,212}=39.47$, $p<0.001$) than mainland ones. Similar results were detected when lizards' T_{pref} were considered as a covariate (results not shown).

The comparison of proteases ($F_{9,80}=13.91$, $p<0.001$) and lipases ($F_{9,80}=12.93$, $p<0.001$) activities in the midgut revealed that lizards from Agios Ermolaos, Islet of Atsitsa, Mesa Diavates, Lakonisi, Molos and Palamari had significantly higher activities than Exo Diavates, Nyfi, Agios Fokas and Atsitsa conspecifics. The differences remained when SVL, GPT or gut length were considered (all $ps<0.001$). Finally, multivariate analysis for GPT, ADE and enzyme activities showed similar results, with islet populations having higher digestive performance than Skyros populations ($F_{9,80}=4.68$, $p<0.001$), but notable variation also existed among populations occupying the same habitat type.

3.5 | Principal component analysis

PCA analysis for ADE for proteins and lipids as well as GPT yielded two main axes that together can explain 75.56% of data variation. ADE for lipids (37.38%) loaded strongly on the first principal component (PC), while ADE for proteins (48.45%) and GPT (51.53%) comprised the second component (Figure 4). In the corresponding plot, islanders separated from their mainland counterparts along the

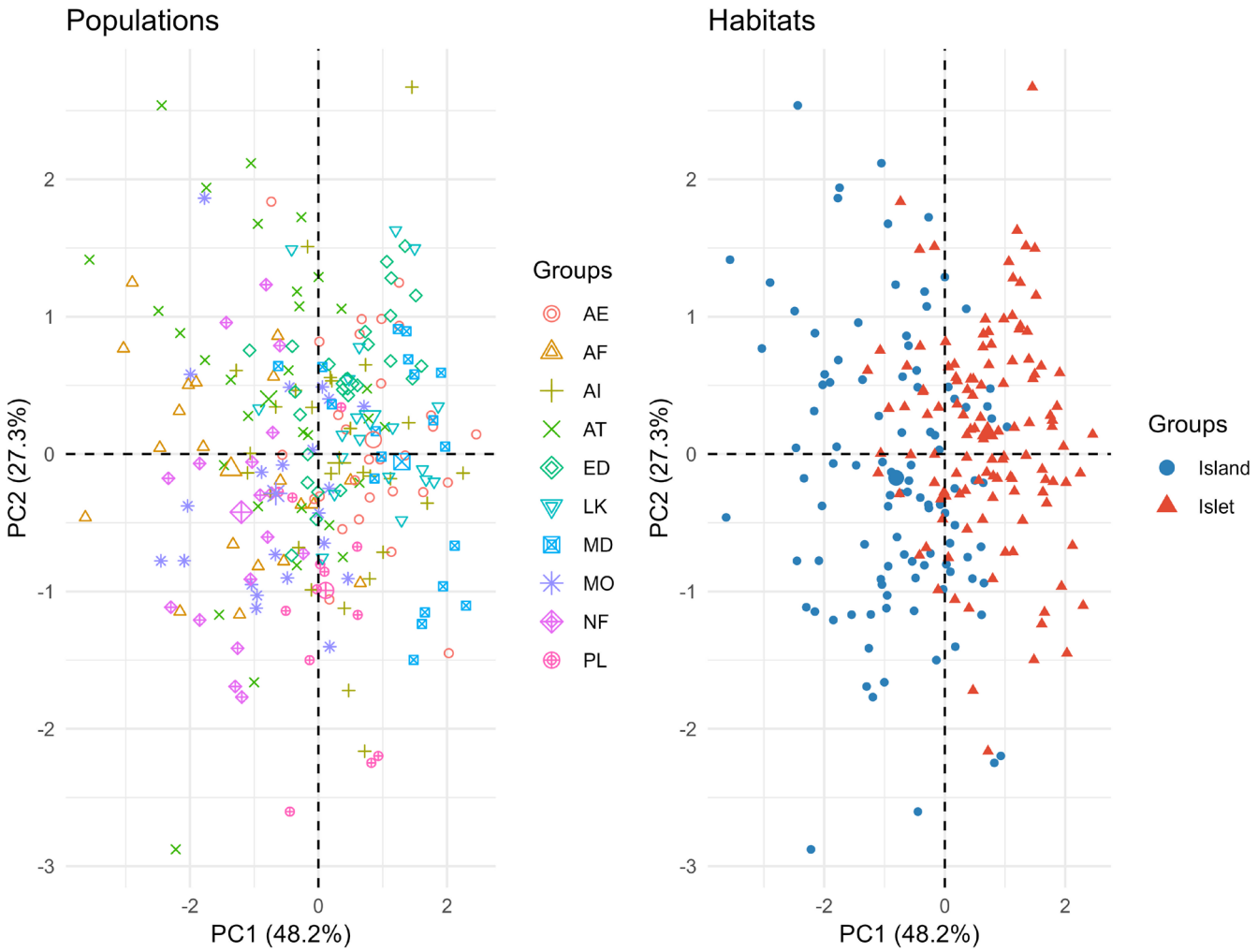

FIGURE 4 Principal component analysis (PCA) plots summarizing the differences in digestion among *P. gaigeae* populations in Skyros Archipelagos. Apparent digestive efficiency (ADE) for lipids load strongly on PC1, while ADE for proteins and GPT comprise PC2. PL refers to Palamari; MO to Molos; AT to Atsitsa; AF to Agios Fokas; NF to Nyfi; IA to Islet of Atsitsa; LK to Lakonisi; MD to Mesa Diavates; and ED to Exo Diavates.

PC1. While insular and mainland lizards got isolated along the PC2, we did not observe clear differences between islet populations or between mainland populations. Similar results were obtained when proteases and lipases' enzymatic activity was taken into account, with islanders together with Molos and Palamari lizards clustering together along the PC1 (38.93%; enzyme activity for proteases and lipases loaded on PC1).

3.6 | Links between differences in prey availability and digestive efficiency

There was a significant negative correlation between the different indices of digestive performance (e.g. ADE for protein and lipids, ADMD, GPT and enzymatic activity) with invertebrate abundance for the most common prey items often found in *P. gaigeae* faeces (Runemark et al., 2015) (Spearman test; all $ps < 0.001$). On the other hand, we found no correlation between prey availability or the different metrics of digestion with population or geological divergence times (all $ps > 0.05$). Using AIC_C, that is better suited for small sample sizes and penalizes the inclusion of more explanatory factors higher than AIC, we found that a combined model with both invertebrate availability of the most frequent prey items and habitat type can better explain the observed differences in PC1 between populations ($AIC_C = 28.10$), rather than other models, with 93.0% support (ΔAIC differences are higher than 2 that is required to declare one model as having a significantly better fit than another) (Table 2). For PC2 we obtained similar results.

TABLE 2 Akaike's second-order information criterion (AIC_C) results for the 10 first candidate models.

Model parameters	Model ID	*k*	ΔAIC_C	WAIC
Invertebrate Abundance + Habitat	1	4	0	0.941
Invertebrate Abundance + Habitat + Populations Divergent Time	2	5	7.68	0.02
Invertebrate Abundance + Habitat + Geographic Distances	3	5	8.77	0.012
Invertebrate Abundance + Habitat + Genetic distances	4	5	8.83	0.011
Invertebrate Abundance + Populations Divergent time	5	4	10.49	0.005
Habitat	6	3	11.27	0.003
Genetic Distances + Geographic Distances	7	5	11.35	0.003
Invertebrate Abundance + Genetic distances	8	4	13.92	0.001
Habitat + Geographic distance	9	4	14.14	0.001
Invertebrate Abundance + Geographic distance + Populations Divergent Times	10	4	14.55	0.001

4 | DISCUSSION

Insularity is known to promote rapid morphological and body size evolution among animals, often due to reduced interspecific competition (Itescu et al., 2018; Meiri, 2008) and the exploitation of novel food resources (Runemark et al., 2015). In addition to these well-documented morphological changes, island environments may also shape physiological traits associated with resource acquisition and energy use. Previous work on the Skyros wall lizard has shown that digestive performance can vary with body size differences between mainland and giant islet populations (Pafilis et al., 2016). Building on this work, we examine whether ecological variation among populations, particularly differences in prey availability and habitat structure, is associated with divergence in digestive physiological traits across the archipelago. Our results indicate that digestive physiology and performance vary markedly among populations even across relatively short ecological timescales and small geographical scales. In accordance with the flexible gut morphology hypothesis and the nutrient balancing hypothesis, we found that islet lizards living in low-food-availability environments exhibited longer gut passage times, upregulated digestive enzyme activity and tended to lengthen their guts. These changes likely increased nutrient absorption and contribute to the higher apparent digestive efficiencies we observed, suggesting adaptive responses to resource scarcity and dietary shift. Given the documented evolutionary structuring of *P. gaigeae* populations across the Skyros archipelago (Runemark et al., 2010, 2012), the repeated ecological associations observed here are consistent with parallel responses to local environmental conditions.

Dietary shifts are recognized as major ecological drivers of digestive system evolution. In our study, we were able to link the efficiency of digestive physiology metrics, including gut passage time, gastrointestinal tract length and enzyme activity with invertebrate prey abundance and habitat type. As anticipated, digestive efficiency, gut passage time and activity of digestive enzymes were negatively correlated with prey abundance, with islet lizards generally exhibiting higher values than those from the main island. This pattern is consistent with the idea that enhanced digestive efficiency is favoured under conditions of food limitation, whereas in prey-rich environments, the benefits of maximizing nutrient extraction may be reduced, as longer gut passage times can limit feeding throughput and require individuals to remain inactive while processing food. In ectotherms such as lizards, prolonged digestion may also require extended basking periods to maintain optimal body temperatures for digestive processes, potentially constraining time available for other activities, such as foraging or predator avoidance (Wehrle & German, 2023). The observed divergence in physiological traits may reflect phenotypic plasticity in response to islet conditions or parallel or convergent trait evolution driven by similar selective environments (Cerca, 2023; Schluter, 2000). Runemark et al. (2012), applying isolation-with-migration models between pairs of islet and main island populations in Skyros Archipelago, revealed a significant correlation between genetic divergence times and estimated geological divergence times, which ranged from 5700 to 18,000 years.

This supports a scenario in which surrounding islets were sequentially isolated from Skyros (Runemark et al., 2012). Genetic distance analyses using microsatellites further showed that *P. gaigeae* islet populations are more closely related to the geographically nearest populations on Skyros than to each other. Contrary to these results, the comparison of digestive efficiency among populations revealed an opposite pattern: Islet populations exhibit more similar physiology to each other than to their genetically closer mainland counterparts. According to AIC_C model selection, which favours simpler models, prey availability and habitat type rather than populations divergence times and genetic distances are the most significant factors explaining the observed differences in the physiology and morphology of the digestive tract between populations for all selected models. Altogether, our data indicate that digestive traits at Skyros wall lizard have evolved in parallel to adapt to low invertebrate prey availability.

A special remark should be made for Exo Diavates as this site deviates in several ways from the other islets of the study and in some variables even resembles the strategies and traits of the Skyros mainland populations. As mentioned above, marine subsidies (Kolb et al., 2010; Wait et al., 2005) in the form of nitrogen-rich guano, fish scraps or even the dead bodies of seabirds that nest on the islet enrich the soil and enhance primary productivity, supporting greater plant diversity and higher invertebrate abundance (Pafilis et al., 2011, 2013). Stomach content analysis of the Exo Diavates population also revealed the frequent presence of lizard body parts (Adamopoulou et al., 1999; Runemark et al., 2015). Indeed, these lizards practice cannibalism to a great extent (Cooper et al., 2014; Pafilis et al., 2009). This extraordinary dietary strategy, together with the increased availability of arthropod prey, likely influences digestive performance. Similar differences in digestive traits between the Exo Diavates population and mainland Skyros populations have previously been reported by Pafilis et al. (2016), further supporting the ecological interpretation emerging from our AICc model selection.

In agreement with previous studies documenting morphological and physiological changes to diverse diets (Donihue et al., 2023; Sagonas et al., 2015; Vervust et al., 2010), lizards from islet populations exhibited approximately a 10% increase in gut transit time and a 12% longer gastrointestinal (GI) tract compared with Skyros populations (Figure 3). This elongation of the GI tract together with the negative relationship between GI tract length and invertebrate abundance that was recorded, likely benefits islet lizards by slowing food passage rates and thereby providing additional time for digestive enzymes to act and maximizing the energy absorption (McConnachie & Alexander, 2004). The adaptive modulation hypothesis (Karasov, 1992; Karasov & Martinez Del Rio, 2007) predicts that digestive enzyme activity should scale with the availability of dietary substrates, reducing the energetic costs of producing unnecessary enzymes. Correlation analysis in our study revealed a negative relationship between protease and lipase activity and invertebrate prey abundance. Rather than downregulating enzyme production under food scarcity, *P. gaigeae* populations appeared to upregulate digestive enzyme activities, a pattern more consistent with the nutrient balancing hypothesis and a compensatory modulation strategy aimed at maximizing nutrient extraction from limited resources. This interpretation is in line with numerous studies in mammals, fish and birds showing that digestive enzyme activity exhibits plasticity in response to diet composition and feeding frequency (German et al., 2010, 2015; Horn et al., 2006; Oguchi et al., 2022; Perry et al., 2007; Schondube et al., 2001). In *P. gaigeae*, modulation of digestive enzymes activities thus appears to represent an adaptive or plastic response to reduced prey availability and increased dietary reliance on plant material (Pafilis et al., 2013; Wehrle et al., 2020). As reviewed by Karasov et al. (2011) and Leigh et al. (2018), a shift to a lower-protein or plant-rich diet, along with low feeding frequency, can trigger enhanced digestive enzyme activity to efficiently break down available food into absorbable nutrients during gut transit. While there are no records of leaf-eating in *P. gaigeae*, the term herbivory or plant consumption can encompass different feeding strategies, including frugivory and granivory. Fruits typically contain relatively low structural fibre and high concentrations of simple carbohydrates, allowing relatively rapid digestion. However, plant material may still require longer retention times than a strictly insectivorous diet to ensure efficient nutrient assimilation, particularly when lipids or complex carbohydrates are present, which may favour increased gut passage times and digestive tract capacity (Levey & MartÍnez del Rio, 2001; Wehrle & German, 2023).

Apparent digestive efficiency and dry matter digestibility depend on the combined effects of gut passage time, enzymatic activities and body size (Karasov & Martinez Del Rio, 2007; Mcnab, 2002; Naya & Božinović, 2006; Pafilis et al., 2007). In this study, we found that protein and lipid digestive efficiency, as well as digestibility values, were higher in islet populations compared with their mainland counterparts. This finding aligns with the established positive relationship between ADE, gut passage time and enzymatic activity. Conversely, body size appeared to have a minor influence on the digestion of *P. gaigeae* across the landscape.

Notably, not all populations from islets or the mainland exhibited similar patterns, with Exo Diavates, in line with the study of Pafilis et al. (2016), serving as a key exception as above mentioned. Another example of this deviation was also evident in lipid digestion, where lizards from Palamari and Molos displayed similar increases (and high correlation) to those seen in islet populations (Figure 3). Lipids serve as a primary energy reservoir due to their high-density energy storage capacity, making them an efficient resource for sustaining physiological demands over time. Animals that frequently experience periods of limited food availability often accumulate significant fat reserves as an adaptive strategy. Lizards living on the unpredictable Mediterranean islands, where food availability is spatially and seasonally clustered (Pafilis et al., 2007; Pérez-Mellado & Corti, 1993), store lipids in their tails (Doughty et al., 2003). Our results strongly support this adaptation, as lizards inhabiting small islets or resource-limited habitats, such as open dunes, where arthropod abundance is lower than in forests and phrygana (Buchholz, 2009; Frazier & Schriever, 2022), were found to digest lipids more efficiently.

Altogether, this suggests that specific habitat characteristics may have shaped adaptations in the digestive tract of *P. gaigeae*.

5 | CONCLUSIONS

Overall, our findings suggest that dietary niche, rather than insularity per se, is the primary driver of digestive divergence in the Skyros wall lizard. Other biotic and abiotic factors not examined in this study, however, such as ambient habitat temperature, may also contribute to the variation detected in digestive physiology (Pafilis et al., 2007). While previous studies have demonstrated that food scarcity can drive divergence in digestive efficiency and gut morphology (Karasov & Douglas, 2013; Sagonas et al., 2015; Vervust et al., 2010), these have often focused on large geographical scales and populations that diverged long ago. Our study complements this body of knowledge by highlighting how dietary niche specialization drives digestive adaptations at a fine scale, emphasizing the role of localized environmental pressures in shaping digestive traits. Such findings may also have significant implications for conservation, emphasizing the importance of preserving both species and their ecological contexts to maintain their capacity to respond to environmental change. We note, however, that the number of islets included in this study remains relatively limited due to the geographical structure of the archipelago, and therefore, our inference is based on a small number of replicated island-islet systems. Furthermore, given the absence of genetic data or experimental approaches in the present study, we cannot determine whether the observed divergence in digestive traits among populations reflects phenotypic plasticity, genetically based adaptation or a combination of both. Future work incorporating common-garden experiments or population genomic analyses will be necessary to disentangle the relative contributions of plasticity, genetic adaptation and diet in shaping physiological divergence among populations.

AUTHOR CONTRIBUTIONS

Kostas Sagonas, Panayiotis Pafilis and Efstratios D. Valakos conceived the idea and designed methodology. Kostas Sagonas, Panayiotis Pafilis and Aikaterini Reppa, Kyriaki Papageorgiou, Giannis Tounias and Panayiota Santikou were involved in data collection. Kostas Sagonas and Foteini Paraskevopoulou conducted data analysis and led the writing of the manuscript with support from Panayiotis Pafilis, Aristeidis Parmakelis and Efstratios D. Valakos. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used for the analyses are available in the Zenodo repository https://doi.org/10.5281/zenodo.19404714 (Reppa et al., 2026).

STATEMENT ON INCLUSION

Our study was conducted by a team of researchers based at several institutions across Greece. All authors were actively involved in shaping the research questions and designing the study, ensuring that diverse perspectives from across our collaborating institutions were incorporated from the outset. Whenever relevant, we cited scientific literature produced by researchers in Greece. We also engaged with local communities and stakeholders on the islands to communicate our research activities and findings throughout the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Representative photographs of the study sites on Skyros Island (Molos, Palamari, Nyfi, Agios Fokas and Atsitsa) and surrounding islets (Exo and Mesa Diavates, Lakonisi, Atsitsa Islet and Agios Ermolaos) used in this study. The images illustrate habitat characteristics across sites, highlighting variation in vegetation structure, substrate type and habitat openness. These photographs provide visual context for the environmental conditions experienced by *Podarcis gaigeae* populations sampled in this study.

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