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Adaptive Organ Size Variation in *Nanorana quadranus* Across Altitudinal and Climatic Gradients: Contradicting Hesse's Rule and Digestion Theory

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Abstract Understanding phenotypic variation in the morphological and physiological characteristics of amphibians and the underlying evolutionary mechanisms is a central topic of broad significance in evolutionary biology. To explore adaptive variations in the relative sizes of key organs (heart, kidneys, lungs, and digestive tract), we surveyed 11 populations of *Nanorana quadranus* distributed in the Qinling-Daba Mountains at altitudes ranging from 586 to 1 702 m. Our results revealed distinct adaptive responses of *N. quadranus* organs to heterogeneous environmental pressures. Specifically, the relative heart size decreased with altitude, contradicting Hesse's rule, whereas digestive tract length showed no significant geographic correlation, challenging the simple extrapolation of digestion theory. These findings indicate that oxygen availability is unlikely to be the primary driver of cardiac variation in this species, and that local ecological variables may play a more important role than macrogeographic position alone in shaping digestive morphology. Further correlation analysis demonstrated that both relative heart size and relative kidney size were positively associated with the mean temperature of the coldest quarter. Collectively, our results provide novel insights into the adaptive evolution of amphibians in heterogeneous environments and highlight the regulatory roles of climate change and environmental gradients in shaping organ size variation.

Keywords digestion theory, geographic and environmental gradients, Hesse's rule, *Nanorana quadranus*, organ size

1. Introduction

Phenotypic variation or phenotype plasticity denotes an organism's adaptive capacity to alter its morphological and physiological traits in response to sustained selection pressures induced by environmental fluctuations (Giacomini *et al*., 2021; Hinds *et al*., 2022; Huang *et al*., 2020; Stearns, 1989). Extensive empirical research has confirmed that diverse organ systems undergo substantial adaptive adjustments in response to environmental changes. These adjustments are manifested as multidimensional re-modelling, encompassing changes in organ volume, mass, and functional properties (Gangenova *et al*., 2020; Huang *et al*., 2021; Liang *et al*., 2022; Liao and Lu, 2011). Consequently, dissecting the intrinsic mechanisms governing such organ-specific changes represents a fundamental prerequisite for evaluating the contribution of phenotypic plasticity to the biological survival strategies and environmental adaptation of organs. For instance, animals inhabiting high-altitude regions frequently face the dual challenges of low temperatures and hypoxia (Soobramoney *et al*., 2005; Zhong *et al*., 2017). To counteract these stresses, organisms employ phenotypic plasticity, like modifying the relative sizes of animal organs. Under elevated temperatures, organs tend to undergo structural simplification to reduce

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metabolic burden.

Two predominant theories account for geographic variation in organ size across populations: Hesse's rule and the optimal digestion theory. Hesse's rule posits that the relative heart size generally increases with elevation, a pattern driven by fluctuations in oxygen availability and shifts in energy demands shaped by environmental gradients (McEntire *et al.*, 2021). The optimal digestion theory proposes that the morphological traits of an animal's digestive tract (including the length and surface area) are intrinsically connected to its evolutionary adaptation strategies, which serve to enhance the efficiency of nutrient and energy acquisition from ingested food (Penry and Jumars, 1987).

Although Hesse's rule and the optimal digestion theory have been empirically validated across diverse animal taxa (Chen and Levy, 2023; Hammond *et al.*, 2001; Ping *et al.*, 2020), extant research predominantly concentrates on endothermic animals (Liao *et al.*, 2016; Ma *et al.*, 2016; McWilliams and Karasov, 2001; Soobramoney *et al.*, 2005). In contrast, supporting evidence for amphibians remains scarce. The heterogeneous organ growth patterns of amphibians hold dual research significance: physiologically, organ developmental status acts as a crucial biomarker for evaluating individual physiological conditions (Chen and Levy, 2023; Gotthard *et al.*, 1995); environmentally, it serves as a robust indicator of population adaptation strategies to environmental fluctuations. Systematically elucidating the relationship between amphibian organ size and environmental factors will not only enhance validation systems for organ plasticity but also establish scientific foundations for predicting amphibian physiological adaptation mechanisms under climate change scenarios.

The Qinling-Daba Mountains traverse central China. This region exhibits pronounced spatial heterogeneity in temperature and annual precipitation, and harbours a rich diversity of vertebrates. This high habitat diversity and species richness provide a valuable natural experimental platform for investigating how organ phenotypic plasticity in animals responds to heterogeneous environmental factors (Hu and Jiang, 2018; Wang *et al.*, 2009). Among the resident fauna, the amphibian *N. quadranus* stands out for its distinctive distribution profile. It occurs in Gansu, Sichuan, Chongqing, Shaanxi, Hubei, and Hunan, occupying mountain streams at altitudes of 335–1 830 m with a latitudinal range of 24° N to 34° N (Wang *et al.*, 2009).

This study aims to investigate patterns of organ size variation in *N. quadranus* along altitudinal gradients (586–1 751 m) and across heterogeneous environmental conditions (such as temperature and precipitation), with a focus on key organs, including the heart, kidneys, lungs, and digestive tract, thereby providing novel insights into the ecological adaptation mechanisms of amphibians.

2. Materials and Methods

2.1. Study area and sample collection Field surveys were conducted at 11 sampling sites across the Qinling-Daba Mountains using the line transect method, with a total of 27 transects (Figure 1). The latitude, longitude, and altitude of each site were recorded using a GPS positioning system (Two Steps Outdoor Assistant; Shenzhen 2bulu Information Technology Co., Ltd., Shenzhen, China) (Wang *et al.*, 2014). The sampling sites spanned a broad range of geographic conditions, with significant variation in spatial coordinates and elevation (Table 1).

Transects were surveyed at night, during which individuals were actively searched for along each line using a 12-V flashlight. To minimize capture bias and ensure sample representativeness, standardized search procedures were applied consistently across sites. Individuals of both sexes and multiple age classes were targeted to maximize sample diversity. In total, 253 individuals of *N. quadranus* were collected, including 126 males and 127 females. All specimens were kept alive and transported to the laboratory for subsequent morphological and physiological analyses. The animal study was reviewed and approved by the Animal Ethics Committee at China West Normal University.

2.2. Body index measurement Upon arrival at the laboratory, all 253 individuals were anesthetized with a benzocaine solution prior to processing (Fu *et al.*, 2022). Body mass was measured using a portable electronic balance accurate to 0.1 mg, and snout-vent length (SVL) was measured using an electronic vernier caliper with an accuracy to 0.01 mm (Yu *et al.*, 2018; Yue *et al.*, 2020). Subsequently, the samples were fixed in 4% phosphate-buffered formalin for 8 weeks (Liu *et al.*, 2018). Previous studies have demonstrated that fixation duration within this range has no significant effect on frog organ size; therefore, storage time was not included as a covariate in subsequent analyses (Zhong *et al.*, 2017).

2.3. Visceral anatomy and measurement After fixation, specimens were thoroughly rinsed with distilled water to remove residual formaldehyde and positioned ventral side up on a dissection tray. The body wall was opened along the midline using surgical scissors to expose the visceral organs. The heart, lungs, kidneys, and digestive tract were carefully dissected and removed.

Each organ was gently blotted with filter paper to remove surface moisture and weighed immediately using an electronic

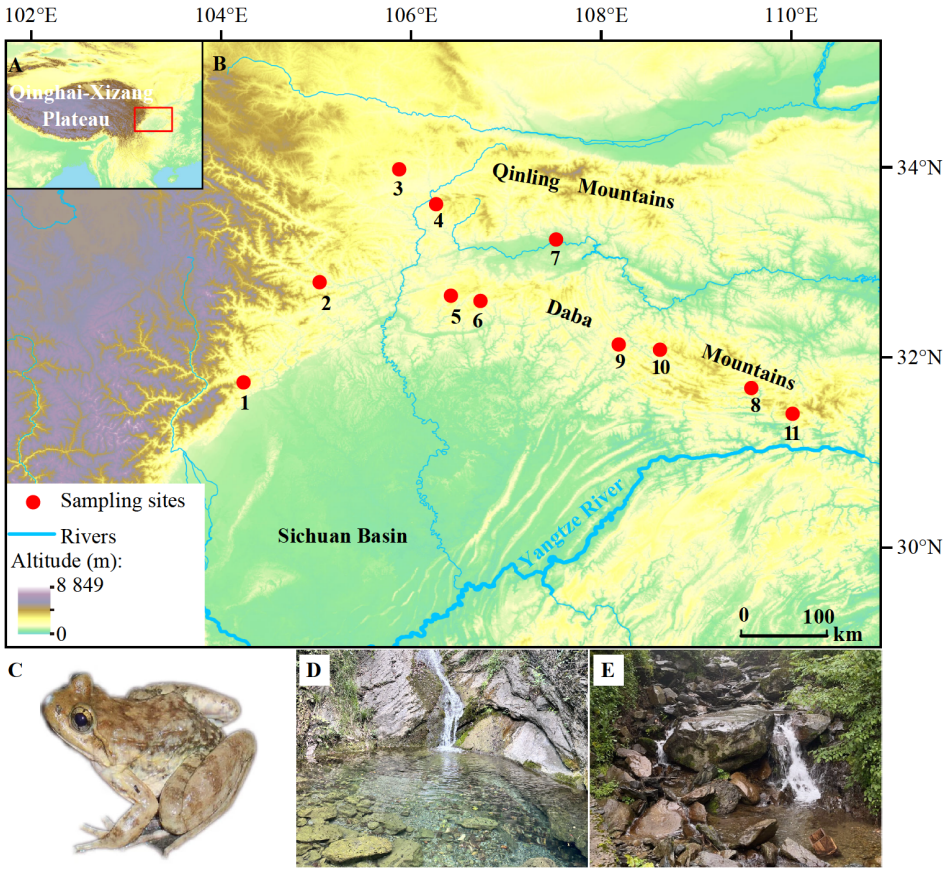


Figure 1 Distribution of the eleven sampling sites for *N. quadranus*. Sampling site codes A, B correspond to those in Table 1. *N. quadranus* (C), with its typical mountain stream habitat (D, E).

Table 1 Details of the sampling sites in this study.

Code of site	County	Longitude (E)	Latitude (N)	Altitude (m)	Samples (sex)	Collection date
1	Anxian	104.26019°	31.70631°	1 326	29 (20M, 9F)	2021/5/27
2	Wenxian	106.47413°	32.61786°	966	15 (9M, 6F)	2021/5/26
3	Huixian	105.87405°	34.00862°	1 480	26 (9M, 17F)	2021/5/25
4	Lueyang	106.29211°	33.59311°	1 569	9 (1M, 8F)	2021/5/24–25
5	Wangcang	105.10308°	32.70327°	820	16 (11M, 5F)	2021/4/13–16
6	Nanjiang	106.69415°	32.56149°	1 436	17 (11M, 6F)	2020/7/28, 2021/5/20
7	Yangxian	107.54584°	33.22274°	1 340	27 (8M, 19F)	2021/5/23
8	Wanyuan	109.99492°	31.38783°	1 249	32 (16M, 16F)	2020/7/28, 2021/5/27
9	Chengkou	108.62701°	32.08601°	1 751	20 (10M, 10F)	2021/5/26
10	Wuxi	106.42873°	31.19792°	586	32 (17M, 15F)	2021/5/25
11	Wushan	108.19166°	32.13177°	1 312	30 (14M, 16F)	2021/5/24

M: male; F: female.

balance with a precision of 0.1 mg to obtain wet mass. Subsequently, the organs were transferred to corresponding micro-centrifuge tubes and subjected to drying in a thermostatic desiccator at 60 °C for a duration of 48 h, or until the weights stabilized. Finally, the dry mass of each organ was measured using the same electronic balance, with three repeated measurements averaged for each organ (Zhao *et al.*, 2019).

To ensure the accurate measurement of the length of the digestive tract and prevent any elongation or distortion, the

digestive tract was secured to a foam board using pins to preserve its natural curvature. A caliper was placed alongside the digestive tract as a reference, and images were captured using a digital camera. Digestive tract length (from the esophagus to the vent) was subsequently quantified using TPSdig software with a precision of 0.01 mm (Zhu *et al.*, 2022).

Population-level data for body size, digestive tract length, and organ dry mass are presented as mean ± standard error in Tables S3 and S4 of the Supplementary Materials. To ensure measurement consistency, all dissections and measurements were conducted by a single researcher.

2.4. Environmental factors collection To assess the influence of environmental conditions on relative organ size (heart, kidneys, lungs, and digestive tract), we extracted 19 bioclimatic variables from the WorldClim database (https://worldclim.org/) for ecological analysis (Table S1). All climatic layers had a spatial resolution of 1 km × 1 km. Environmental data corresponding to each sampling site were extracted using ArcGIS 9.2 (ESRI, Redlands, CA, USA) (Table S2) (Sun *et al.*, 2025).

2.5. Data analysis To fulfill the assumption of normal distribution, all continuous variables (i.e., organ size measurements) were multiplied by 1 000 and $\log_{10}$-transformed prior to analysis.

The residual method was employed to control for the effects of overall body size on organ dimensions. Specifically, residuals were calculated from linear regressions of log-transformed organ size (mass or length) against log-transformed SVL. These residuals were used as indices of relative organ size (Muñoz-Muñoz *et al.*, 2021). Body condition index (BCI) was calculated as the residual from a regression of log-transformed body mass on log-transformed standardized body length for all individuals (Huang *et al.*, 2020).

To test for differences in relative organ size among populations, generalized linear models (GLMs) were constructed with relative organ size as the dependent variable, population as the independent variable, and BCI included as a covariate (Deng *et al.*, 2023). This approach controlled for individual variation in body condition and improved the robustness of population-level comparisons.

To examine the effects of geographical gradients and climatic factors on relative organ size, multicollinearity among the 19 environmental variables was first assessed using Pearson correlation coefficients (Figure S1). Variables exhibiting strong correlations ($|r| > 0.8$) were excluded. Variance inflation factors (VIFs) were then calculated for the remaining variables, and those with VIF > 10 were removed to further reduce multicollinearity. Ultimately, five variables were retained for analysis, all with VIF values < 10, including mean diurnal temperature range (VIF = 1.093), temperature seasonality (VIF = 1.037), mean temperature of the coldest quarter (VIF = 1.420), and precipitation of the wettest quarter (VIF = 1.365).

To examine the influence of geographical gradients, a linear mixed model (LMM) was initially constructed, with relative organ size as the dependent variable. Sex and population were considered as random effects, while environmental variables (altitude, latitude, longitude, mean daily range of temperature change, isothermality, temperature seasonality, mean temperature of coldest quarter, and precipitation of wettest quarter) were included as fixed effects. However, models incorporating sex as a random effect yielded zero variance estimates, indicating a negligible contribution of sex to geographic variation in organ size. Similarly, when population was treated as a random effect, no significant correlations were detected among relative organ size, BCI, and environmental variables (Table S3).

Consequently, final analyses were conducted using GLMs, with relative organ size as the dependent variable, environmental variables as predictors, and population included as a covariate (Table 2). Additionally, population-level mean organ sizes were calculated, and multiple regression analyses were performed to examine relationships between average organ size and geographic gradients (altitude and latitude) across populations, thereby clarifying population-specific patterns (Zhu *et al.*, 2022).

All statistical analyses were performed in R 4.4.1.

3. Results

3.1. Influence of body condition on relative organ size Linear regression analyses revealed that body condition had a significant effect on the relative sizes of all examined organs, including the heart, lungs, kidneys, and digestive tract. Moreover, the relative dimensions of these organs exhibited a consistent increasing trend with improving body condition (Table 3).

3.2. Variation in organ size among groups After controlling for body condition, GLM analyses revealed highly significant differences in relative organ size across all groups (Table 4). The correlation between target variables and all examined organs (heart, kidneys, lungs, digestive tract) was statistically significant ($p < 0.001$). Among these, the lungs exhibited the highest effect strength ($\beta = 1.395$) and model explanatory power ($R^2 = 0.160$), while the digestive tract

Table 2 Effects of environmental factors on body condition and relative organ size of *N. quadranus* assessed by generalized linear models.

Response variable	Environmental factors	β	SE	t	p
Heart	Altitude	0.000	0.000	−2.587	< 0.05
	Longitude	−0.457	0.096	4.753	< 0.05
	Latitude	−0.787	0.180	−4.367	< 0.05
	Log(bio2)	41.054	9.433	4.352	< 0.05
	Log(bio3)	−59.079	13.045	−4.529	< 0.05
	Log(bio4)	0.906	5.526	0.164	0.870
	bio11	−0.209	0.053	3.970	< 0.05
	Log(bio16)	−0.987	1.188	−0.831	0.407
Kidney	Altitude	0.000	0.000	−1.090	0.277
	Longitude	−0.480	0.157	−3.060	< 0.05
	Latitude	−0.930	0.294	−3.162	< 0.05
	Log(bio2)	40.329	15.370	2.624	< 0.05
	Log(bio3)	−56.969	21.249	−2.681	< 0.05
	Log(bio4)	6.015	9.047	0.665	0.507
	bio11	−0.192	0.086	2.241	0.026
	Log(bio16)	−2.159	1.910	−1.130	0.260
Lung	Altitude	0.000	0.000	−0.441	0.660
	Longitude	−0.503	0.151	−3.343	< 0.05
	Latitude	−0.909	0.284	3.200	< 0.05
	Log(bio2)	43.546	14.690	2.964	< 0.05
	Log(bio3)	−65.628	20.338	−3.227	< 0.05
	Log(bio4)	−0.722	8.595	−0.084	0.933
	bio11	−0.154	0.083	−1.860	0.064
	Log(bio16)	−2.826	1.844	−1.533	0.127
Digestive tract	Altitude	0.000	0.000	−0.538	0.591
	Longitude	0.079	0.068	1.162	0.246
	Latitude	0.010	0.127	0.081	0.936
	Log(bio2)	5.219	6.691	0.780	0.436
	Log(bio3)	−8.346	9.247	−0.903	0.368
	Log(bio4)	−12.611	3.913	−3.223	< 0.05
	bio11	0.003	0.037	0.086	0.932
	Log(bio16)	−1.561	0.831	−1.879	0.061
Body condition	Altitude	0.000	0.000	−2.172	< 0.05
	Longitude	−0.009	0.003	−3.387	< 0.05
	Latitude	0.002	0.006	0.319	0.750
	Log(bio2)	14.367	4.011	3.582	< 0.05
	Log(bio3)	−21.595	5.532	−3.904	< 0.05
	Log(bio4)	−9.162	2.252	−4.069	< 0.05
	bio11	0.012	0.022	0.547	0.585
	Log(bio16)	−1.334	0.494	−2.698	< 0.05

Table 3 Regression analysis of body condition index (BCI) and relative organ size in *N. quadranus*.

Regression variables	β	t	p	R^2
Heart	0.833	6.060	< 0.001	0.130
Kidney	1.314	5.802	< 0.001	0.122
Lung	1.395	6.739	< 0.001	0.160
Digestive tract	0.271	2.697	< 0.001	0.025

Table 4 Variations in relative organ size among *N. quadranus* populations after body condition index correction via generalized linear models.

Organ	Heart	Kidney	Lung	Digestive tract
Population				
F	6.7969	3.5726	4.5336	5.6828
p	< 0.001	< 0.001	< 0.001	< 0.001
Body condition index				
F	54.3566	43.5731	29.5281	15.755
p	<0.001	< 0.001	< 0.001	< 0.001

showed the lowest effect strength (β = 0.271) and model explanatory power (R^2 = 0.025), with pronounced differences observed across different organs.

3.3. Correlations between relative organ size and environmental factors Altitude was significantly associated only with relative heart size, which decreased with increasing elevation (heart: t = −2.587, p < 0.05; Figure 2). No significant relationships with altitude were detected for the kidneys, lungs, or digestive tract (kidneys: t = −1.090, p = 0.277; lungs: t = −0.441, p = 0.660; digestive tract: t = −0.538, p = 0.591).

Longitude was significantly correlated with the relative size of the heart, kidneys, and lungs, but not with digestive tract length. Specifically, the relative heart and kidneys increased with longitude (heart: t = 4.753, p < 0.05; kidneys: t = 3.060, p < 0.05), whereas the relative size of the lungs decreased with longitude (lungs: t = −3.343, p < 0.05) (Figure 3A). Relative digestive tract length did not show a significant correlation with longitude (digestive tract: t = 1.162, p = 0.246).

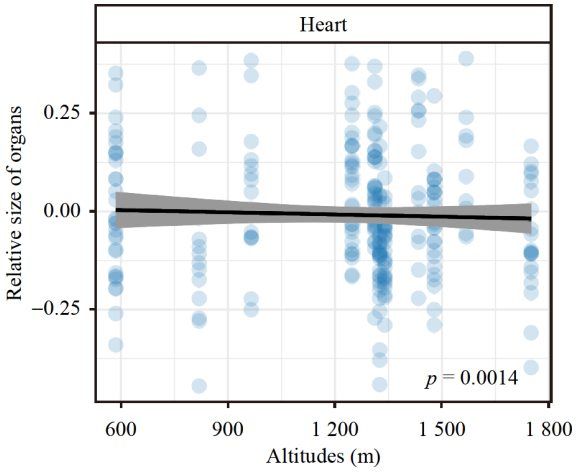


Figure 2 Changes in the correlation relative heart size with altitude.

Latitude also exhibited organ-specific effects. The relative sizes of the heart and kidneys decreased with increasing latitude (heart: t = −4.367, p < 0.05; kidneys: t = −3.126, p < 0.05), while the relative size of the lungs increased with increasing latitude (lungs: t = 3.200, p < 0.05). No significant relationship was detected between latitude and relative digestive tract length (digestive tract: t = 0.081, p = 0.936) (Figure 3B).

Temperature-related variables displayed distinct association patterns across organs. The relative sizes of the heart and lungs decreased with increasing mean daily range of temperature change (heart: t = 4.352, p < 0.05; lungs: t = 2.964, p < 0.05), whereas the relative size of the kidneys increased with the mean daily change in temperature (kidneys: t = 2.264, p < 0.05). No significant association was observed between relative digestive tract length and mean daily range of temperature change (digestive tract: t = 0.780, p = 0.436; Figure S2).

With increasing isothermality, the relative size of heart, kidneys, and lungs size decreased significantly (heart: t = −4.529, p < 0.05; kidneys: t = −2.681, p < 0.05; lungs: t = −3.227, p < 0.05), whereas relative digestive tract was not significantly correlated with isothermality (digestive tract: t = −0.903, p = 0.368; Figure S3).

Temperature seasonality significantly influenced only the relative digestive tract length, which increased with seasonal increases in temperature (digestive tract: t = −3.223, p < 0.05). No significant relationship was detected between temperature seasonality and the relative sizes of heart, kidneys, or lungs

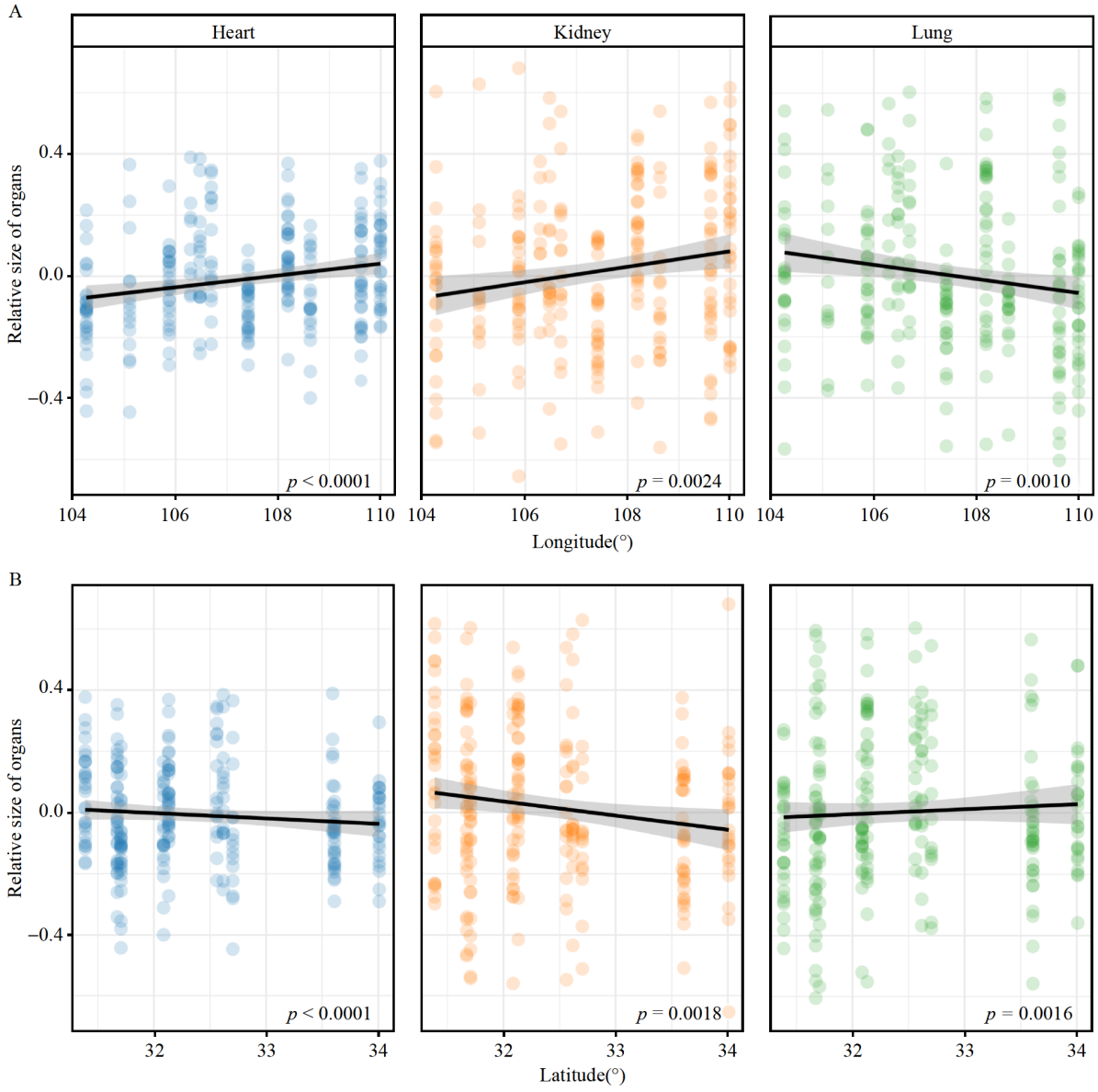


Figure 3 A: Changes in relative heart, kidneys, and lung size correlations with longitude. B: Changes in relative heart, kidney, and lung sizes in correlation with latitude.

(heart: $t = 0.164$, $p = 0.870$; kidneys: $t = 0.665$, $p = 0.507$; lungs: $t = -0.084$, $p = 0.933$; Figure S4).

Relative heart and kidney sizes were positively associated with the mean temperature of the coldest quarter (heart: $t = 3.970$, $p < 0.05$; kidneys: $t = 2.241$, $p < 0.05$) (Figure S5). No significant association was observed between the mean temperature of the coldest quarter and the relative lung size, and relative digestive tract length (lungs: $t = -1.860$, $p = 0.064$; digestive tract: $t = 0.086$, $p = 0.932$).

No significant correlation was detected between precipitation of the wettest quarter and the relative sizes of the heart, kidneys, lungs, or digestive tract (heart: $t = -0.831$, $p = 0.407$; kidneys: $t = -1.130$, $p = 0.260$; lungs: $t = -1.533$, $p = 0.127$; digestive tract: $t = 1.879$, $p = 0.061$).

4. Discussion

This study demonstrates pronounced geographic variation in the relative sizes of four major organs (the heart, lungs, kidneys, and digestive tract) across 11 populations of *N. quadranus*. By integrating morphological data with multiple environmental variables, including body condition, geographic gradients, and climatic factors, we identified organ-specific

responses to environmental heterogeneity. Notably, relative heart size was negatively correlated with altitude, which contradicts Hesse's rule. The relative length of the digestive tract was not significantly associated with the aforementioned environmental factors, providing little support for classic digestive theory. These findings suggest that traditional expectations regarding organ size adaptation may not be applicable to these populations, underscoring the complexity associated with organ size adaptation to environmental gradients and emphasizing the need for a more in-depth understanding of the ecological and evolutionary drivers of organ size variation.

Geographical variation in heart size serves as a crucial indicator of biological adaptation to environmental pressures; however, its underlying mechanisms display significant divergence across taxonomic groups. According to the classical Hesse's rule, organisms inhabiting cold, low-oxygen environments at high altitudes or latitudes are expected to evolve larger hearts to enhance oxygen transport efficiency (Soobramoney *et al*., 2005; Zhong *et al*., 2017). Although this pattern has been supported in several mammalian species, such as deer mice (*Peromyscus maniculatus*) (Pfeifer *et al*., 2018; Zhong *et al*., 2017), empirical evidence from amphibians remains inconsistent. For example, *Polypedates megacephalus* exhibits increased heart size with increasing altitude (Zhong *et al*., 2017), whereas the Andrew's toad (*Bufo andrewsi*) displays the opposite trend (Cardilini *et al*., 2016; Zhao *et al*., 2019). Consistent with the latter, our results reveal a negative association between relative heart size and either altitude or latitude in *N. quadranus*, indicating that oxygen limitation is unlikely to be the primary driver of cardiac variation in this species.

Climatic factors may instead play a dominant role in shaping cardiac morphology. Precipitation can indirectly influence organ size by altering food availability, whereas temperature directly affects metabolic demand. In contrast to the findings of positive correlations between heart size and temperature/precipitation in *Meriones meridianus* (Liao *et al*., 2010), relative heart size in *N. quadranus* decreased with increasing diurnal temperature range. We hypothesize that in environments characterized by significant diurnal temperature fluctuations, individuals may reduce metabolic maintenance costs by moderately decreasing heart size, thereby optimizing energy allocation and maintaining physiological homeostasis (Costanzo, 2018; Ma *et al*., 2016). These findings indicate that cardiac adaptation in *N. quadranus* reflects a balance between oxygen delivery and energy economy, emphasizing a multidimensional framework of phenotypic integration driven by local climatic conditions.

Kidneys play a critical role in adaptation to hypoxic and thermally stressful environments by regulating blood pressure and osmoregulation (Gerich, 2010). Classical expectations predict larger kidneys in animals inhabiting high-altitude or high-latitude regions to cope with environmental stress. However, our results reveal a significant negative correlation between relative kidney size and latitude in *N. quadranus*. A similar pattern is observed in *Bufo gargarizans*, in which kidney size decreases with increasing altitude despite enhanced water reabsorption capacity (Ping *et al*., 2020). This pattern suggests that, under cold and arid conditions, organisms may evolve more efficient renal function rather than increasing organ size. Additionally, energy limitation may constrain investment in energetically costly organs, favoring allocation toward functions such as immunity and reproduction. Developmental constraints may further contribute to reduced kidney size, as shortened growth periods in cold environments can limit organ development, as observed in high-latitude populations of *Rana temporaria* (Morrison and Hero, 2003).

In amphibians, lung morphology is a critical determinant of respiratory performance, directly mediating the ability to cope with spatial and temporal variation in environmental oxygen availability and metabolic demand. In cold and high-latitude regions, increasing lung volume can enhance gas exchange efficiency and compensate for reduced oxygen availability (Gao *et al*., 2019; Niu *et al*., 2024). Our findings corroborate this pattern, with relative lung size increasing with latitude. In contrast, amphibians inhabiting warm, humid, low-latitude regions tend to exhibit smaller lungs, as ambient conditions provide sufficient oxygen to meet metabolic demands (Hutchison, 2008). Furthermore, diurnal temperature variation significantly affects lung size. In environments characterized by notable diurnal temperature fluctuations, amphibians may decrease their lung volume to minimize energy expenditure and water loss (Hardy *et al*., 2022). This response may reflect an adaptive strategy to limit energy expenditure and water loss while maintaining adequate respiratory efficiency.

The digestive tract, as the primary organ for nutrient acquisition and energy assimilation, is known to exhibit considerable morphological plasticity in response to dietary changes and varying environmental conditions (Krasnov *et al*., 2022; Mai *et al*., 2019). Environmental gradients in temperature and precipitation induced by altitude or latitude can indirectly influence digestive morphology by altering food availability, habitat structure, and biological rhythms (Paola *et al*., 2007). Classical digestive theory predicts that herbivores exhibit longer digestive tracts than omnivores or carnivores (Hansson, 1985; Korn, 1992). However, consistent with findings in *Duttaphrynus melanostictus* (Deng *et al*., 2023), we

detected no significant correlation between digestive tract length and broad geographic gradients in *N. quadranus*. These results challenge a simple geographic extrapolation of digestive theory and suggest that digestive morphology in this species is shaped more strongly by local ecological variables than by macrogeographic position alone.

Elevated temperatures can increase metabolic rates and food intake, potentially selecting for longer digestive tract to enhance nutrient assimilation efficiency (Marklein *et al*., 2020). Additionally, seasonal fluctuations in resources may drive structural elongation of the digestive tract, increasing its capacity for nutrient utilization during specific temporal periods (Beale *et al*., 2023). Consequently, while the macrogeographical gradient itself does not directly elucidate the variation in digestive tract morphology of *N. quadranus*, localized ecological factors such as temperature and precipitation likely play a critical role in shaping digestive strategies.

5. Conclusions

Our research has revealed several significant patterns that connect organ size in amphibians with environmental factors. This study further investigates how environmental changes impact organ dimensions, underscoring the adaptive variations observed among different organs in responding to ecological pressures. Phenotypic variation in organs results from complex interactions among environmental conditions, genetic factors, developmental constraints, and sexual selection. In widely distributed species like *N. quadranus*, population-level genetic differentiation may also influence organ size variation. Our findings indicate that organ size variation cannot be fully explained by macrogeographic gradients alone, emphasizing the need for integrative frameworks that consider ecological, genetic, and developmental aspects. Future studies on related taxa across steeper geographic gradients or extreme environments may enhance our understanding of how environmental severity shapes organ size evolution in amphibians.

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Supplementary information Supplementary information is available at https://doi.org/10.3724/ahr.2095-0357.2025.0034

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