



The combined effects of atrazine and warming on environmental adaptability in lizards (*Eremias argus*) from the perspective of a life-history traits trade-off: Gender differences in trade-off strategies may reverse mortality risk

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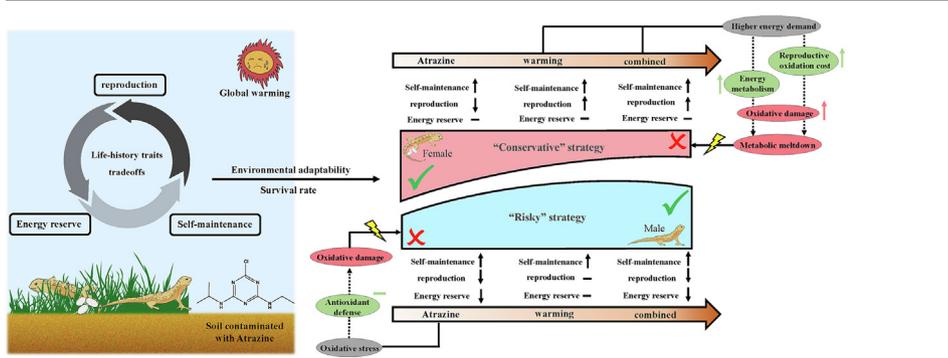
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HIGHLIGHTS

- Warming and atrazine cause life-history traits trade-off in breeding season lizards.
- The life-history traits trade-offs have difference between males and females.
- Male lizards are more sensitive to atrazine exposure.
- Pregnant females show higher sensitivity to high temperature.
- Different trade-off strategies lead to winners and losers under environment stress.

GRAPHICAL ABSTRACT



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ABSTRACT

Life-history theory suggests that organisms must distribute a limited share of their energetic resources among competing life-history trait demands. Therefore, the trade-off strategies individuals develop for particular life-history traits in a given environment may profoundly impact their environmental adaptability. In this study, lizards (*Eremias argus*) were exposed to single and combined atrazine (4.0 mg·kg⁻¹ and 20.0 mg·kg⁻¹) and different temperatures (25 °C and 30 °C) for 8 weeks during the breeding season. The effects of atrazine and warming on the adaptability of lizards were explored by examining changes in trade-offs via several key life history traits (i.e., reproduction, self-maintenance, energy reserves, and locomotion). The results show that after atrazine exposure at 25 °C, both female and male lizards tended to allocate energy to self-maintenance by reducing energy allocation to reproductive process. The lower energy reserves of males are considered a “risky” life-history strategy and the observed higher mortality may be related to atrazine-induced oxidative damage. The retention of energy reserves by females not only ensured their current survival but also facilitated survival and reproduction in subsequent stages, which can be regarded as a “conservative” strategy. However, under high temperature and/or combined atrazine exposure, the “risky” strategy of males caused them to consume more energy reserves to invest in self-maintenance, which ensured their immediate survival, and profited from more rapid degradation of atrazine. In contrast, the “conservative” strategy of females could not meet their higher reproductive and self-maintenance demands under high temperatures, and the elevated reproductive oxidative and metabolic costs led to individual mortality. Gender differences in life-history trade-off strategies can directly lead to “winners” and “losers” from environmental stress within a species.

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1. Introduction

Reptiles are the second largest category of terrestrial vertebrates after birds. Reptiles occupy an important position in the food chain and play a vital role in maintaining the natural ecological balance (Chang et al., 2022; Pincheira-Donoso et al., 2013). However, the species and population trends of terrestrial reptiles decline worldwide (Chang et al., 2022; Wang et al., 2021a). Global climate change and environmental pollution are major causes of population decline in this taxon (Araujo et al., 2006; Wang et al., 2020; Weir et al., 2010). In addition, other causes are the loss and degradation of habitat, diseases and parasitism, as well as the introduction of invasive species (Chang et al., 2022; Gibbons et al., 2000; Mendonca et al., 2022). With the continued increase of global temperatures and environmental pollution from human agricultural activities, the survival and adaptation of these terrestrial reptiles require further attention.

Life-history traits such as survival, growth/development, and reproduction are interrelated characteristics that are tightly connected to individual fitness (Kubicka and Kratochvil, 2009; Zera and Harshman, 2001). Life-history theory predicts that investing acquired energetic resources into a particular trait denies those same resources from being allocated to other traits, making life-history trade-offs necessary (Husak et al., 2016). Cases of such trade-offs have been widely reported in many species, including birds (Fragueira et al., 2021), fish (Einum and Fleming, 2000), amphibians (DeVore et al., 2021; Liao et al., 2014), insects (Corbel and Carazo, 2022; Godfrey and Rypstra, 2018), and reptiles (Caracalas et al., 2021; Durso and French, 2018; Husak et al., 2016; Recknagel and Elmer, 2019; Webb et al., 2019). Actually, because of resource and time scarcity caused by environmental pressures, in nature, organisms often cannot simultaneously maximize all their life-history traits (Agatz et al., 2013; Dupoue et al., 2018a; Zhang et al., 2020b). Therefore, the adaptability of individuals to their environment depends on the trade-off strategies they employ for different life-history traits in a given environment.

In many species, warming can alter the environmental adaptations of individuals by affecting the trade-offs of the life-history traits (Fragueira et al., 2021; Rutschmann et al., 2016). For example, in many ectothermic species, higher ambient temperatures accelerate embryonic development, which lead to accelerated reproduction (Kimmit et al., 2022; Potter and Felmy, 2022). This requires individuals to allocate more resources to reproduction. In addition, climate warming can also constrain activity patterns (Sinervo et al., 2010) and has been shown to significantly increase maintenance costs (Dillon et al., 2010; Dupoue et al., 2022) and impose the risk of heat stress (Huey and Kingsolver, 2019). Therefore, the effects of climate warming on life-history traits are critical to determine overall fitness. As ectotherms, reptiles are particularly sensitive to temperature stress (Chang et al., 2022; Nie et al., 2022). Several models predict an increase of the reptile mortality risk in the future because of expected climate warming, highlighting the importance of the impact of climate warming on animal populations (Sinervo et al., 2010). Therefore, research that links heat stress with individual life-history traits trade-offs is urgently required to gain insights into the potential fitness consequences of climate warming on species, especially reptiles.

Besides global warming, environmental pollution is a further important driver that threatens biological adaptability (Debecker and Stoks, 2019). On the one hand, the direct toxicity of contaminants on organisms (e.g., neurotoxicity and endocrine disruption) may affect the adaptation of individuals by interfering with their potential life-history trade-offs (especially growth or reproduction vs. self-maintenance trade-off) (Godfrey and Rypstra, 2018; Zhang et al., 2020b); on the other hand, contaminants may also indirectly affect both the energy balance and allocation patterns of organisms at the top trophic levels through the food chain by affecting the bottom trophic level of the ecosystem (Wang et al., 2021a; Wang et al., 2021b). This also affects the adaptation of species above this bottom trophic level. Terrestrial reptiles are particularly susceptible to contaminants because of their limited range and direct contact with soil (Mendonca et al., 2022; Rich and Talent, 2009). However, reptile

pesticide-risk assessment data are often replaced by data on birds because of a lack of guidelines for assessing their ecotoxicity (Mingo et al., 2016; Weir et al., 2010). Considering that these two taxa (i.e., birds and reptiles) have their own specific life history traits, it is necessary to explore the effects of pollutants on reptile adaptations.

The triazine herbicide atrazine (ATR) is considered a potential environmental endocrine disruptor, which has been the focus of regulatory attention because it can commonly be detected in the environment (Wang et al., 2018; Yang et al., 2021). The controversy over the use of ATR endures. Although ATR has been banned in the European Union and its use is restricted in other countries, it is still used in large quantities worldwide (Qin et al., 2015; Stradtman and Freeman, 2021). Currently, the most studied vertebrates that are affected by ATR are fish (Blahova et al., 2020; Dionne et al., 2021; Wang et al., 2018), amphibians (Hayes et al., 2010) and birds (Qin et al., 2015). These studies concentrated on showing that ATR exposure has adverse effects on various biological processes including morphology, growth, and sexual differentiation (Yang et al., 2021). Furthermore, ATR exposure may lead to dysfunctions in the endocrine system, development, reproduction, and immune system (Galbiati et al., 2021; Liu et al., 2017). In fact, among vertebrate species, reptilians are particularly sensitive to endocrine disruption (Boggs et al., 2011; Galoppo et al., 2020). Therefore, experiments are needed to examine the effects of ATR on reptile adaptations, especially considering that global warming and contaminants may also interact with each other (Nie et al., 2022).

Eremias argus (an oviparous lizard species) is a lizard species native to China. These lizards are acutely vulnerable to climate warming and pesticides exposure because of their small body size, high metabolism, diurnal activity habits, and habitats that overlap with agricultural areas (Nie et al., 2022). Moreover, they breed from April to June each year, which coincides with the warm season. Periods of extreme energetic demand (such as the breeding season) inevitably require energy trade-offs among numerous competing traits. The lizards not only need to ensure their survival and reproduction success, but also reserve energy for life activities in subsequent stages (such as after breeding or hibernation). In addition, locomotor performance is crucial for basic activities (e.g., foraging) and good locomotor performance improves the success rate of reproduction. However, locomotor performance also requires a significant investment of energy resources and is traded off with other life-history traits. Especially when the lizards are subjected to environmental stresses such as elevated temperature and pesticides, energy trade-offs among life-history traits can be expected to be more intense. Therefore, *E. argus* provides an ideal system for studying these trade-offs. The purposes of this work were (1) to examine the effects of warming and ATR exposure on the trade-offs between fundamental life-history traits of *E. argus* by quantifying the indicators related to lizard reproduction, self-maintenance, energy reserve and locomotor performance (Table 1); (2) to correlate changes in life-history traits trade-offs under different stressors with environmentally relevant adaptations (environment-related survival rate); (3) to elucidate the regulation mechanisms of the life-history trade-offs in lizards under different environmental stresses from two perspectives of energy metabolism and hormone levels. The results of this study improve the understanding of the efforts organisms make to adapt to environmental stressors and the mechanism of environmental adaptation. This research not only provides basic information needed for the conservation and management of lizard populations but also presents new perspectives for studying the population dynamics and life history of lizards.

2. Material and methods

2.1. Chemicals

ATR (BR, 97 %) was purchased from Yuanye Biotechnology Company (Shanghai, China). High-performance liquid chromatography (HPLC) grade solvents were obtained from Fisher Scientific, and the other analytical reagent were supplied by Sinopharm Chemical Reagent Co., Ltd.

Table 1
Specific indicators of lizards in their key life-history traits.

Life history traits		Indicators
Reproduction	Male	Testes mass and testes coefficient
	Female	Number of clutches, egg mass, egg-TP, and egg-VTG
Self-maintenance	Immunity	Immunoglobulin M (IgM)
	Thermal defense	Heat shock protein 70 (Hsp 70)
	Detoxification	Glutathione S-transferase (GST)
Energy reserve	Antioxidant defense	Total antioxidant capacity (T-AOC)
		Adipose tissue mass and coefficient, triglyceride (TG)
Locomotor performance		Maximum sprint speed ($\text{cm}\cdot\text{s}^{-1}$)

Note: All biochemical indicators were measured with commercially available kits, following the manufacturer's instructions. The details of kits are listed in the supporting information (Table S4).

2.2. Lizard husbandry and maintenance

180 mature lizards (90 males, bodyweight: 3.60–4.50 g, 90 females, bodyweight: 3.40–5.00 g) with similar sizes were selected from an *E. argus* population that was artificially raised in a pollution-free environment at China Agricultural University. Before the experiment, lizards were domesticated under laboratory conditions for two weeks (light: dark cycle:14:10 h, temperature: 25 ± 1 °C, humidity: 40–50 %), during which they could freely obtain food and water. All experiments and protocols followed ethical guidelines set by the Animal Research and Ethics Committee of China Agricultural University for the care and use of laboratory animals.

2.3. Experimental design

After acclimation, lizards were weighed again and randomly divided into six treatment groups (15 females and 15 males per group): Control group (25 °C, 0 $\text{mg}\cdot\text{kg}^{-1}$), low concentration ATR treatment group (25 °C, 4.0 $\text{mg}\cdot\text{kg}^{-1}$), high concentration ATR treatment group (25 °C, 20.0 $\text{mg}\cdot\text{kg}^{-1}$), high temperature treatment group (30 °C, 0 $\text{mg}\cdot\text{kg}^{-1}$), high temperature with low concentration ATR treatment group (30 °C, 4.0 $\text{mg}\cdot\text{kg}^{-1}$), and high temperature with high concentration ATR treatment group (30 °C, 20.0 $\text{mg}\cdot\text{kg}^{-1}$), hereafter referred to C, L, H, CT, LT, HT treatments, respectively. The exposure concentration was set according to the calculation result of the predicted environmental concentration (PEC) of ATR in soil (3.90 $\text{mg}\cdot\text{kg}^{-1}$) (Table S1); the low concentration was 4.0 $\text{mg}\cdot\text{kg}^{-1}$, and the high concentration was 20.0 $\text{mg}\cdot\text{kg}^{-1}$ ($5 \times$ PEC). The detailed PEC calculation process and the steps of ATR soil contamination are presented in the supporting information. Controlled temperature heating pads were used to keep the temperature of different heat treatments at 25 ± 1 °C and 30 ± 1 °C, respectively. In each treatment, 15 females and 15 males were divided into 5 replicates, and 3 females and 3 males per replicate were placed together in a feeding box ($32 \times 22 \times 15$ cm) containing 2 kg of soil. During the experiment, all lizards were fed the equal amount of mealworms every two days (approximately 2.0 g per box) and excrement was cleaned once a week. The exposure experiment lasted for 8 weeks. One week before the end of the experiment, the running speed of lizards was tested to evaluate locomotor performance, and thermal preference trials were carried out to evaluate the resting metabolic rate (RMR). At the end of exposure, lizards were sacrificed by rapid decapitation after freezing with anesthesia as described previously (Amaral et al., 2012; Wang et al., 2020). Blood, liver, testes, and adipose tissue were collected, and weighed to calculate organ coefficients according to Eq. (1). All samples were stored at -20 °C until further analysis.

$$\text{OC} = \frac{\text{organ mass(g)}}{\text{body mass(g)}} \times 100\%; \quad (1)$$

where organ mass and body mass are the mean organ mass and mean body mass of the three females (or males) in each replicate.

2.4. Soil ATR content validation and degradation analysis

Soil sample were collected in all treatments at 0, 3, 7, 14, 21, 28, 42, and 56 days of the experiment. The content of ATR in soil was measured by HPLC-mass spectrometry (HPLC-MS/MS) and the ATR degradation of ATR during the experiment was evaluated. Specific methods and instrumental parameters are provided in the supporting information, and the fortified recoveries and linear range of ATR in soil are listed in Table S2.

2.5. Biological analyses

2.5.1. Physical condition and survival rate

The body weight of lizards in each treatment was measured at the beginning and end of the experiment, and the relative growth rate (RGR) of the body weight was calculated by Eq. (2). During the experiment, the dead individuals were recorded in each treatment, and the survival rate was calculated.

$$\text{RGR} = \frac{W_e - W_0}{W_0} \times 100\%; \quad (2)$$

where W_0 and W_e represent the weight (g) of the lizard at the beginning and end of the experiment, respectively.

2.5.2. Distribution of life-history traits

Based on the main energy-consuming life activities of lizards during the breeding season, the distribution of several key life-history traits was evaluated: reproduction, survival (self-maintenance), growth (energy reserves), and activity (locomotor performance). Specific indicators were listed in Table 1.

Reproduction involves a series of investments in reproductive organs and/or offspring. Females mainly invest in eggs, while male testes mass and coefficients are commonly used to evaluate male's reproductive investment. During ATR exposure, the feeding boxes were checked twice each day to observe whether any female had spawned, and clutch characteristics such as the spawning date, number of clutches and the clutch size were recorded. The collected eggs were stored at -20 °C. At the end of the experiment, the content of total protein (egg-TP) and vitellogenin (egg-VTG) in eggs was determined. Testes from males were weighted and testes coefficients were calculated according to Eq. (1).

Survival involves many aspects, including wound healing, immunity, detoxification and antioxidation metabolism, and other characteristics related to self-maintenance. Therefore, indicators related to self-maintenance were used to represent the investment of lizards into their survival. Immunoglobulin M (IgM), which is found in all jawed vertebrates is the first immunoglobulin produced in response to infection and is very effective in activating complement (Han et al., 2020). The content of IgM in lizard plasma was determined to assess lizard immunity. The expression of heat shock protein (Hsp) is considered a protective mechanism in response to thermal stress, as it enhances the heat tolerance of reptiles (Zhang et al., 2020a). The content of Hsp70 in lizard plasma was determined to assess thermal defense investments. Glutathione S-transferase (GST) is a phase II biotransformation enzyme that is critical for detoxification and can transform a variety of endogenous and heterogeneous compounds, including herbicides and pesticides (Blahova et al., 2020). GST activity in the liver was used to assess the investment in lizard detoxification. Total antioxidant capacity (T-AOC) refers to the total level of antioxidants composed of various antioxidant substances and antioxidant enzymes that protect cells and the entire body from oxidative stress damage. The T-AOC content in lizard plasma was measured as an index for antioxidant defense traits.

Considering that the size of adult lizards basically remains unchanged, changes of energy reserve are used to assess the growth distribution of lizards. Adipose tissue from lizards was weighted and adipose coefficients were calculated according to Eq. (1). In addition, triglyceride (TG) content in lizard liver samples was also determined.

Locomotor performance of lizards was tested to evaluate their ability to be active. All lizards in each treatment group, except for those involved in the thermal preference trials (see Section 2.5.3), participated in this test. The maximum sprint speed ($\text{cm}\cdot\text{s}^{-1}$) of lizards was recorded and quantified using a Go pro motion camera according to a previously used method (Nie et al., 2022). The specific methods are provided in the supporting information.

2.5.3. Assessment of liver status and energetics

Liver mass and coefficient were used to assess the size of lizard livers. Livers were weighted and liver coefficients were calculated according to Eq. (1). Enzymatic activities of superoxide dismutase (SOD) and catalase (CAT), as well as levels of malondialdehyde (MDA) were used as proxies for oxidative damage. Lizards can optimize energy distribution by regulating their metabolic rate. The metabolic rate is determined by a combination of body mass, body temperature and activity state, which is an important indicator to assess individual energetic changes. Therefore, one week before the end of the experiment, 12 lizards (6 males and 6 females) were selected from each treatment. After the body mass of lizards was recorded, their mean body temperature was measured according to thermal preference trials as previously reported (Wang et al., 2021a). The RMR ($\text{J}\cdot\text{h}^{-1}$) of lizards was estimated according to Eq. (3) (Ma et al., 2022). Detailed data are presented in Table S3. Moreover, the activities of isocitrate dehydrogenase (IDH) and lactate dehydrogenase (LDH) in the lizard liver were measured as representatives of aerobic and anaerobic metabolism, respectively. Na^+/K^+ -ATPase activity and glucose content in liver samples were also determined. All biochemical indicators were measured with commercial kits, and details are presented in the supporting information.

$$\text{RMR} = \exp(-10 + 0.51 \cdot \log(M_b) + 0.12 \cdot T_b); \quad (3)$$

where M_b is the mass of the lizard (g) and T_b is its average body temperature ($^{\circ}\text{C}$);

2.5.4. Hormone assay

The levels of hormones in the plasma of lizards, including corticosterone (CORT), Luteinizing hormone (LH), testosterone (T), estradiol (E2) and progesterone (Pg), were determined using commercially available ELISA kits. Specific operations followed the manufacturer's instructions. The validation of commercial kits and the method of sample extraction are listed in Table S4.

2.6. Data analysis

All biomarker analyses were derived from five biological replicates (including 5 females and 5 males) sampled in each treatment. Two-way analysis of variance (ANOVA) was used to identify the effects of main factors (ATR, Thermal, and $\text{ATR} \times \text{Thermal}$) on each biomarker, followed by Tukey post-hoc test for multiple comparisons of significant differences among treatments. Before the statistical analysis, data were tested for normality and homoscedasticity using the Kolmogorov-Smirnov test and Levene median test, respectively. All data are reported as the mean \pm standard deviation (SD), and significance levels were set at $p < 0.05$ (*). Additionally, principal component analysis (PCA) was performed separately in female and male lizards to evaluate distribution and correlation patterns based on the major physiological biomarker endpoints measured in different treatments. All data analyses were conducted in SPSS 26.0, and the results were plotted by Graph Pad Prism 8 (Graph Pad Software, USA). PCA analysis was conducted in Origin 2021.

3. Results

3.1. Weight change and survival

Both "ATR" and "Thermal" were major factors in the weight change of male and female lizards (Table S5). For male lizards (Fig. 1A), only the

mean body weight of the control group increased by 2.96 % during the experiment; in all other treatments, the lizards showed a significant weight loss compared to the control group. For females (Fig. 1C), body weight loss was observed in all treatments, which was most likely due to egg laying by females during the breeding season; nevertheless, it was still observed that females in ATR or/and high temperature treatments showed a higher body weight loss, which was especially pronounced in the ATR treatment group at 30 $^{\circ}\text{C}$ (LT and HT group).

The results of survival rate of lizards are shown in Fig. 1B and D. In males (Fig. 1B), the survival rate at 25 $^{\circ}\text{C}$ decreased according to the ATR dose, but the differences were not significant. Survival rates were 100 %, 86.7 %, and 73.3 % for C, L and H groups, respectively. At 30 $^{\circ}\text{C}$, all male lizards survived, and only one lizard died in the HT group. For females (Fig. 1D), at 25 $^{\circ}\text{C}$, the survival rates in groups C, L, and H were 93.3 %, 93.3 % and 86.7 %, respectively. However, the survival rate was significantly reduced at 30 $^{\circ}\text{C}$, and the survival rates of CT, LT, and HT were 80.0 %, 73.3 % and 66.7 %, respectively. In addition, two-way ANOVA also showed that the "Thermal" significantly affected the survival rate of female (Table S5).

3.2. Distribution of life-history traits

3.2.1. Reproduction

In male lizards, the results showed that the testes mass was significantly affected by "ATR" (Table S5). Regardless of the temperature, the male testes mass exposed to ATR was significantly lower compared to the groups without ATR exposure (Fig. 2A), and this effect remained when the testes mass was converted into the testes coefficient (Fig. 2C).

For females, at 25 $^{\circ}\text{C}$, compared with the C group, the egg mass (Fig. 2B), egg-TP (Fig. 2D) and the number of clutches (Fig. 2B) in L and H groups decreased at different degrees, and the spawning date was significantly delayed (Fig. S2A). At 30 $^{\circ}\text{C}$, the lizards in LT and HT groups had significantly lower egg mass (Fig. 2B) and egg-TP (Fig. 2D) compared to the C group, but compared to the CT group, the difference was not significant. Interestingly, compared with each treatment group at 25 $^{\circ}\text{C}$, at 30 $^{\circ}\text{C}$, the lizards laid their eggs earlier (Fig. S2A) and had relatively more clutches (Fig. 2B). In addition, compared to the C group, the egg-VTG content was significantly increased in the CT group (Fig. S2B) and the VTG/TP ratio was relatively high (Fig. S2C).

3.2.2. Self-maintenance

The results of IgM levels in lizard plasma are shown in Fig. 2E, F. At both 25 $^{\circ}\text{C}$ or 30 $^{\circ}\text{C}$, compared with the treatment without ATR, the IgM content decreased in both female and male lizards exposed to ATR (L, H vs. C; LT, HT vs. CT). For same dose treatments at different temperatures, male lizards' IgM levels at 30 $^{\circ}\text{C}$ were higher than at 25 $^{\circ}\text{C}$ in each treatment. The content of IgM in the HT group (vs. H groups) was significantly increased (by 16.6 %), while in females, IgM levels at 30 $^{\circ}\text{C}$ was lower than at 25 $^{\circ}\text{C}$, and the IgM content in the HT group was significantly lower (by 17.6 %) compared with the H group.

The plasma levels of Hsp70 showed that ATR induced male and female lizards to increase the level of Hsp70 at 25 $^{\circ}\text{C}$, but the difference was not significant (Fig. 2G, H). Similarly, high temperature also significantly upregulated Hsp70 levels in lizard plasma (CT vs. C: increase in males of 50.9 % and females increase of 28.8 %). However, under combined exposure to high temperature and ATR, plasma Hsp70 levels decreased in an ATR dose-dependent manner in both male and female lizards (Fig. 2G, H).

Fig. 2I and J show the GST activity in the livers of male and female lizards, respectively. The results indicate that compared with the C group, the GST activity of male and female lizards in L and H groups increased at 25 $^{\circ}\text{C}$, but not significantly (Fig. 2I and J). At 30 $^{\circ}\text{C}$, increased GST activity was observed in male lizards of LT and HT groups (vs. CT group), while this was not observed in females.

"ATR" was the main effect of lizard T-AOC in both males and females (Table S5). Regardless of temperature, T-AOC increased with increasing doses of ATR in both male and female lizards. In females, T-AOC levels

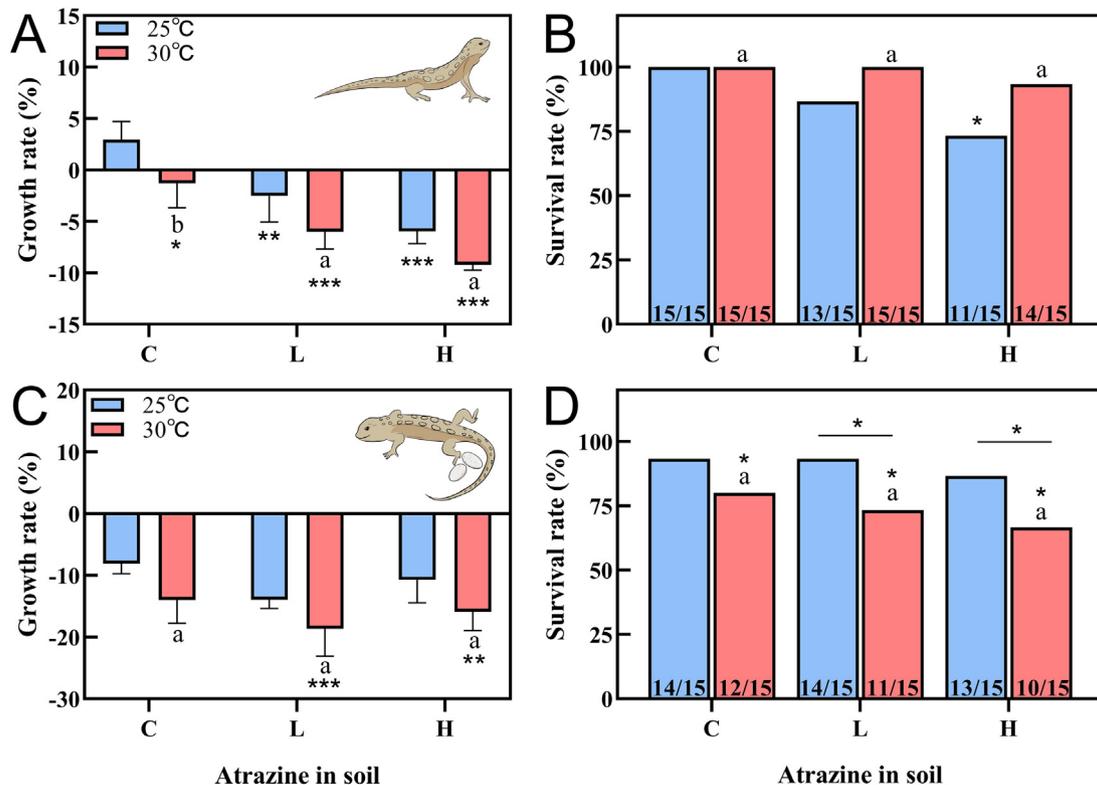


Fig. 1. Effects of atrazine and temperature on body weight (A male, C female) and survival rate (B male, D female) of lizards. The numbers presented at the bottom of the bars in C and D represented the number of individuals that survived until the end of the experiment and the total sample size in each treatment. The bars represent the mean \pm standard deviation of the original data. *, **, *** represent significant differences between different treatments and the control group at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. *, **, *** with a horizontal line beneath indicate significant differences between different temperature treatments at the same ATR concentration at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Lower-case letters indicate significant differences between ATR treatment groups at different doses under heat treatment (30 °C).

were significantly higher in H group than in C group, and levels were significantly increased in LT and HT groups compared with CT group (Fig. 2N); however, no significant differences were observed in male lizards (Fig. 2K). Additionally, “Thermal” also significantly affected the plasma T-AOC of female and male lizards (Table S5). The T-AOC content of lizards exposed to different doses of ATR at 30 °C was significantly higher than that of lizards at the same dose at 25 °C (CT vs. C: increased by 40.0 % for males and 59.7 % for females; LT vs. L: increased by 80.7 % for males and 94.7 % for females; HT vs. H: increased by 56.3 % for males and 31.5 % for females).

3.2.3. Energy reserve

The results of adipose tissue mass in male lizards (Fig. 2M) showed that the adipose tissue mass of lizards in H group was significantly lower compared to C group at 25 °C; at 30 °C, compared with C or CT group, significant decreases were observed in LT and HT groups. For females, the adipose tissue mass showed no significant difference in all treatment groups. After converting adipose tissue mass into adipose tissue coefficient, the change of adipose tissue coefficient (Fig. 2O and P) was similar to that of adipose tissue mass. Compared with different treatments at 25 °C (C, L, and H groups), the TG levels in liver of male lizards in different treatments exposed to 30 °C (CT, LT, and HT groups) were significantly lower by 32.0 %, 29.7 %, and 36.7 %, respectively (Fig. 2Q). In female lizards, a significant reduction in TG levels compared to C group was observed in LT and HT groups.

3.2.4. Locomotion performance

Two-way ANOVA showed that “ATR” was the main effect of the maximum sprint speed in male lizards, while females were significantly influenced by both “ATR” and “Thermal” (Table S5). In male lizards (Fig. 2S),

the maximum sprint speed was significantly reduced in L, H, LT, and HT groups compared to C group, and significant differences were also observed in LT and HT groups compared to CT group. In females (Fig. 2T), at 25 °C, a significant difference was only observed between H group and C group; while at 30 °C, significant decreases were found in both LT and HT groups compared to C group.

3.3. Liver status and energetics assessment

Liver mass (Fig. 3A and F) and liver coefficient (Fig. 3B and G) were counted separately for female and male lizards to evaluate the status of lizard livers. “ATR”, “Thermal” and “ATR-Thermal interaction” had no significant effects on the two indicators of male lizards (Table S5), and no significant difference was found among treatment groups (Fig. 3A and B). However, “Thermal” was a major effect on the liver mass and coefficient in females. Compared with C group, the liver mass of female lizards in L and H groups showed no significant difference, while it increased significantly in CT and HT groups (Fig. 3F). The results of the female liver coefficient showed a significant increase in CT, LT, and HT groups compared with C group. Compared with C, L, and H groups at 25 °C, the liver coefficient of CT, LT, and HT groups at 30 °C increased significantly by 35.7 %, 26.1 % and 27.2 %, respectively (Fig. 3G).

Enzymatic activities of SOD and CAT, as well as levels of MDA were used as proxies for antioxidant defense capacities and oxidative damage, respectively. In male lizards, the SOD activity was strongly affected by ATR exposure (Table S5). At 25 °C, compared with C group, SOD activities of lizards in H and LT groups were significantly increased; at 30 °C, SOD activities in LT and HT groups were significantly increased compared with CT group (Fig. 3C). CAT activities were strongly induced by high temperature (Table S5), which increased significantly in different treatments at 30 °C

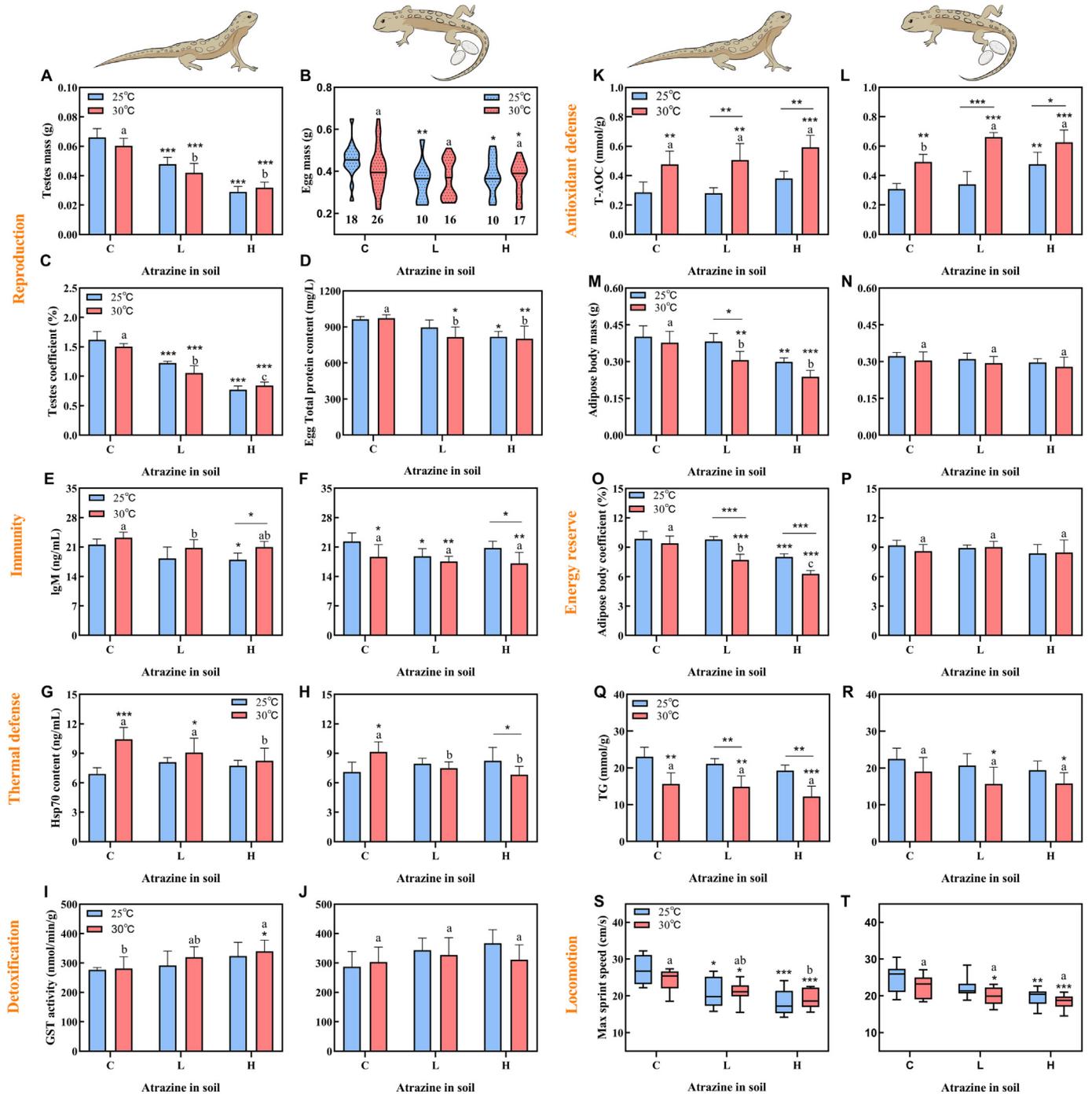


Fig. 2. Effects of atrazine and temperature on the distribution of key life-history traits in lizards. The numbers in Fig. B represented the total number of clutches laid by females collected in each treatment during the experiment. The first and third columns show indicators for males, and the second and fourth columns show indicators for females. The bars represent the mean \pm standard deviation of the original data. *, **, *** represent significant differences between different treatments and the control group at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. *, **, *** with a horizontal line beneath represent significant differences between different temperature treatments at the same ATR concentration at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Lower-case letters indicate the significant difference between ATR treatment groups at different doses under heat treatment (30 °C).

compared to 25 °C (Fig. 3D). The MDA levels of lizards increased in response to ATR exposure, and increases were significant in H group and HT group compared with C group and CT group, respectively. Moreover, compared with the same concentration of ATR at 25 °C, the MDA content of each treatment group was lower at 30 °C, but the difference was not significant (Fig. 3E). In female lizards, the SOD activity was induced by high temperature (Table S5), which was higher in each treatment group at 30 °C than at 25 °C, and significant differences were observed between H

group and HT group (Fig. 3H). The CAT activity levels were influenced by ATR and the interaction of ATR and thermal (Table S5). CAT activity significantly increased in the L group at 25 °C and the CT group at 30 °C (vs. C group), but decreased in LT and HT groups, and significant differences were observed in the HT group (Fig. 3I). The results of MDA content in female livers showed that MDA content increased at different degrees in other groups compared to C group, and significant increase were observed in L, H, LT, and HT groups (Fig. 3J).

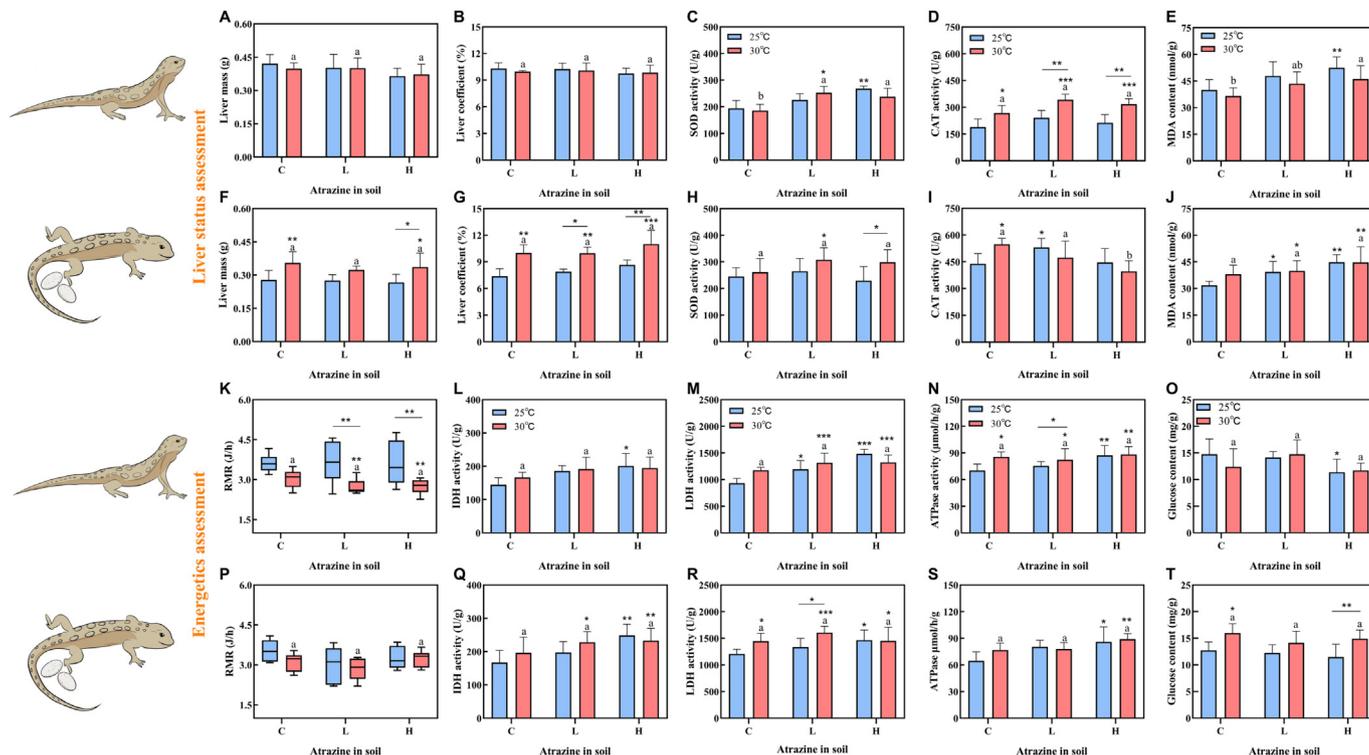


Fig. 3. Effects of atrazine and temperature on liver damage and energetics biomarkers in lizards. Panels A–E and P–T show data for males, and panels F–J and K–O show data for females. The bars represent the mean ± standard deviation of the original data. *, **, *** represent significant differences between different treatments and the control group at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. *, **, *** with a horizontal line beneath represent significant differences between different temperature treatments at the same ATR concentration at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Lower-case letters indicate significant differences between atrazine treatment groups at different doses under heat treatment (30 °C).

The RMR of lizards was quantified based on their body mass and body temperature (Table S3). The RMR of males was significantly affected by thermal effects (Table S5), and in the combined treatment LT and HT groups, RMR was significantly reduced compared to C group. The RMR of lizards in the LT and HT groups was significantly reduced by 25.5 % and 23.9 % compared to L and H groups, respectively (Fig. 3K). However, no significant change of RMR was observed in females (Fig. 3P).

The liver IDH activity of male and female lizards was significantly affected by ATR exposure (Table S5). Compared with C group, the IDH activity of male lizards in H group was significantly increased (Fig. 3L), and female lizards in the H, LT, and HT groups also had significantly higher IDH activity levels (Fig. 3Q). The LDH activity in male lizards was significantly affected by ATR exposure and the interaction of ATR with thermal effects, whereas in females, thermal effects were the prominent factor affecting LDH activity (Table S5). Compared with C group, the LDH activity of male lizards in L, H, LT, and HT groups increased significantly (Fig. 3M), as well as that of females in H, CT, LT, and HT groups (Fig. 3R). Na^+/K^+ -ATPase is an enzyme that plays a significant role in the transport of Na^+ and K^+ across the cell membrane, whole body ion regulation, and cellular energy balance in animals. In male lizards, Na^+/K^+ -ATPase activities in H, CT, LT, and HT groups were significantly higher than in C group (Fig. 3N). In females, significant differences were observed only in H and HT groups compared with C group (Fig. 3S). Finally, the glucose level was analyzed as a sensitive indicator of stress and an indicator that energy reserves are being intensively utilized. The glucose levels in the liver of male lizards were mainly affected by ATR exposure, and were significantly lower in H group than in C group (Fig. 3O). In females, the glucose content was strongly affected by the thermal effect. High temperature treatment significantly increased glucose levels in lizard livers, with increase of 20.3 %, 15.6 %, and 30.3 % in CT, LT, and HT groups at 30 °C compared to C, L, and H groups at 25 °C, respectively (Fig. 3T).

3.4. Endocrine disturbance and hormonal changes

In male lizards, compared with C group, the T content in H, LT and HT groups decreased significantly (Fig. 4B), and a significant reduction of Pg levels was observed in L, CT, and LT groups (Fig. 4D). In females, compared to C group, LH levels were significantly lower in L, CT, and LT groups, while the E2 and Pg contents were significantly increased in the CT and LT groups, respectively. In addition to these observations, no major differences were detected for these sex hormones between the other treatment groups.

CORT levels were measured in the plasma of male and female lizards (Fig. 4E and J). At 25 °C, CORT levels in male lizards increased with ATR dose, and a significant difference was observed in H group compared to C group (Fig. 4E); however, this difference was not observed in females (Fig. 4J). At 30 °C, male and female lizard CORT levels decreased in a dose-dependent manner with ATR. Compared with CT group, CORT levels were significantly lower in male lizards of LT and HT groups (Fig. 4E); in females, similar changes were observed in HT group (Fig. 4J). In addition, in males, the CORT content in CT group was significantly higher compared to C group, but in HT group was significantly lower compared to H group (Fig. 4E).

3.5. Principal component analysis

In male lizards, the main measured physiological biomarkers are represented in the PCA, with the first two principal components explaining 52.2 % of the total variance of the data (Fig. 5A). The first axis (PC 1), which explains about 30.2 % of the variance of the data, separates the treatments without ATR (C and T groups) from ATR treatments (H, LT, and HT groups), except for L group. Although only explaining 22.0 % of the total variance, the second axis (PC 2) separates the treatments at 30 °C (CT, LT,

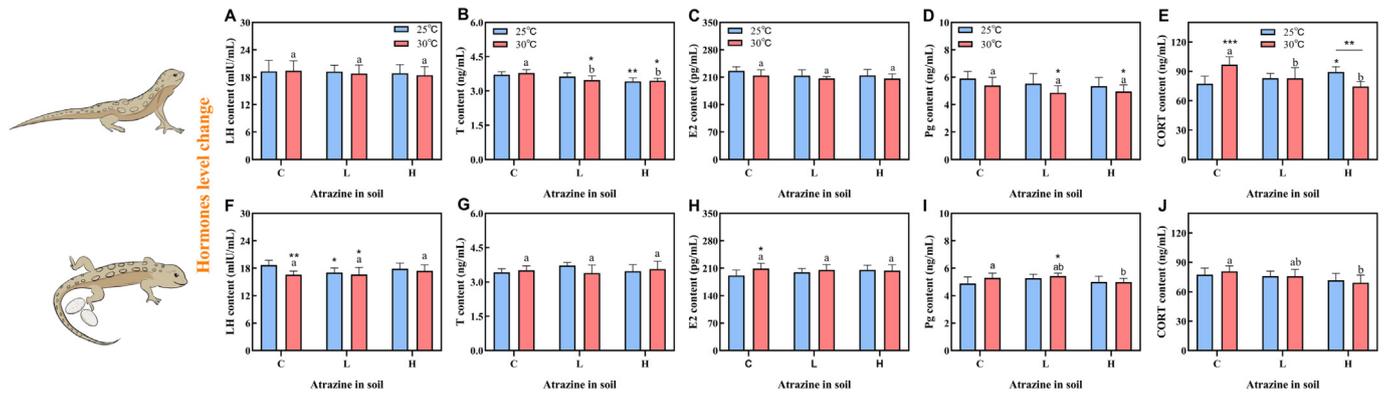


Fig. 4. Effects of atrazine and temperature treatment on hormone levels in lizards. Panels A–E show data for males, and panels F–J show data for females. The bars represent the mean ± standard deviation of the original data. *, **, *** represent significant differences between different treatments and the control group at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. *, **, *** with a horizontal line beneath represent significant differences between different temperature treatments at the same ATR concentration at levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Lower-case letters indicate the significant difference between ATR treatment groups at different doses under heat treatment (30 °C).

and HT groups) from treatments at 25 °C (C, L, and H groups). PCA clearly showed that in male lizards, high-concentration ATR treatment (H group) combined with ATR and high temperature treatments (LT and HT groups) were positively correlated with energy metabolism (IDH, LDH, and $\text{Na}^+ / \text{K}^+ \text{-ATP-ase}$), oxidative stress (SOD, CAT, MDA, and T-AOC), detoxification (GST) and thermal defense (HSP70) parameters; however, they were negatively correlated with immune (IgM) and energy reserve (TG and glucose) parameters. Additionally, the PCA results emphasize that the level of oxidative damage (represented by MDA content) caused by ATR exposure in male lizards was closely related to SOD activity.

In female lizards, 44.4 % of the total data variance could be explained by the first two principal components (Fig. 5B). Treatments at 25 °C (C, L, and H groups) and treatments at 30 °C (CT, LT, and HT groups) tended to separate along PC 1, which explained about 27.5 % of the data variance. The result of PCA indicates that the combined high temperature and ATR treatments (LT and HT groups) were the prominent factor affecting energy metabolism (IDH, LDH, and $\text{Na}^+ / \text{K}^+ \text{-ATP-ase}$) and oxidative stress (SOD, MDA, and T-AOC) parameters in female lizards, and negatively correlated to immune (IgM) parameters. Particularly, in female lizards, the level of oxidative damage induced by combined ATR and high temperature exposure correlated strongly with parameters of the energy metabolism.

4. Discussion

The survival rate is considered to be a direct fitness trait and thus, has been widely employed in evaluations of the vulnerability of organisms to environmental stressors (Liu et al., 2022). In the present study, survival rates of breeding lizards exposed to single or combined ATR and high temperature treatments showed gender differences depending on different stressors. At 25 °C, female lizards had a higher survival rate than males after exposure to ATR; the opposite was found for high temperature or combined stressors, where the survival rate of male lizards was higher than that of females (Fig. 1B and D). This suggests that gender differences in survival rates under different stresses may be related to the differences in life-history traits trade-offs between females and males. Therefore, the reasons for the observed gender differences in trade-off strategies were further explored from the individual (body weight changes and metabolic rate), organ (organ mass and coefficients), and physiological (energy metabolism, oxidative stress and hormonal changes) levels. The results clarify the possible mechanism of how environmental pressures driving life-history traits trade-offs, and highlight that different life-history traits trade-off strategies are essential for understanding the environmental adaptability of animals.

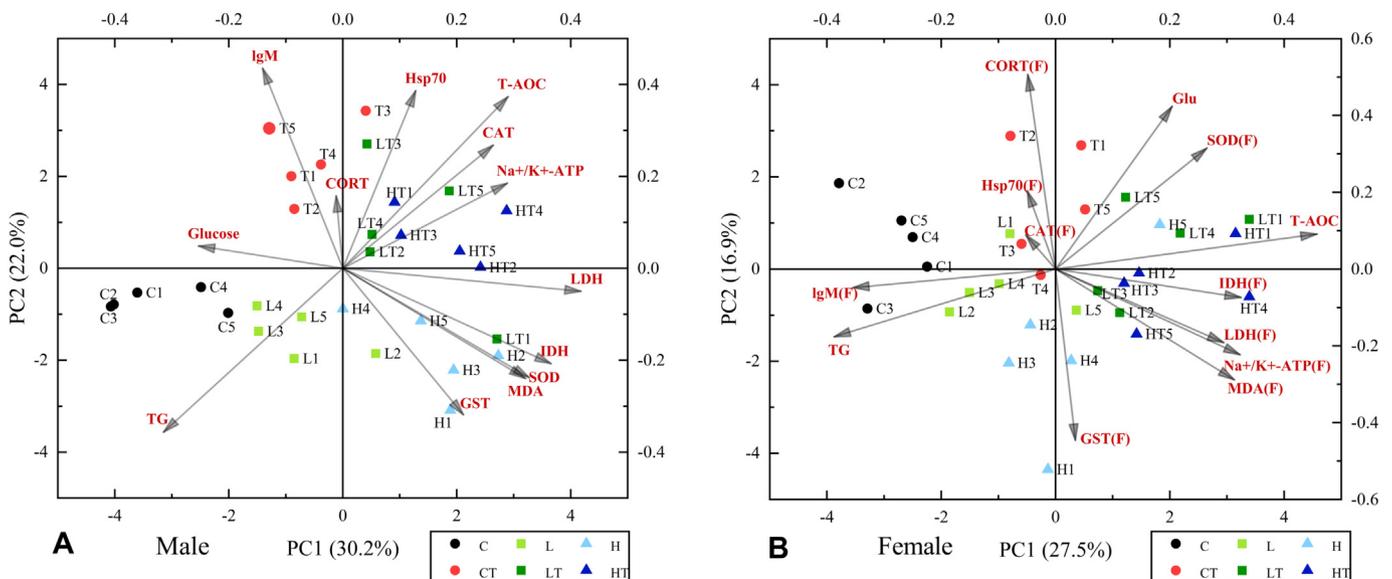


Fig. 5. Principal component analysis (PCA) integrating the major physiological biomarker endpoints measured across different treatments in male (A) and female (B) lizards.

4.1. Effects of atrazine exposure at room temperature on life-history traits of lizards

The present study showed that after exposure to ATR at room temperature, overall, both female and male lizards reduced their allocation of energy to reproductive processes, as evidenced by delayed egg laying (Fig. S2A), reduced clutch numbers (Fig. 2B), lower egg weight and total protein content within eggs (Fig. 2B and D) in females, as well as significantly reduced testis mass and coefficient (Fig. 2A and C) in males. In terms of self-maintenance, in addition to immunosuppression (Fig. 2E and F), the activity of the detoxifying enzyme GST (Fig. 2I and G), T-AOC (Fig. 2K and L), and antioxidant enzyme system (Fig. 3C, D, H, and I) were activated to varying degrees both in female and male lizards. Moreover, poor locomotion performance was observed both in female and male lizards exposed to ATR at 25 °C (Fig. 2S and T). This suggests that alterations of these life-history traits in lizards are associated with the toxic effects of ATR. As reported previously, ATR can affect the secretion of downstream sex hormones by altering pituitary hormone levels, ultimately leading to reproductive disorders (Qin et al., 2015; Wirbisky and Freeman, 2015). In the present study, the T and Pg levels of male lizards decreased after exposure to ATR (Fig. 4B and D), and both the LH and Pg levels changed in female lizards (Fig. 4F and I). In fact, abnormal levels of steroid hormones usually affect spermatogenesis or interfere with the ovulation cycle (Zhang et al., 2020a). Thus, changes in these hormones may be responsible for the reduced allocation to reproductive processes in lizards. Additionally, ATR profoundly affects oxidative stress markers and detoxifying enzymes, and has a broad inhibitory effect on innate immunity, humoral immunity and cellular immunity (Singh et al., 2018; Wang et al., 2018). In summary, ATR has been shown to have endocrine-disrupting effects. It not only affects endocrine and reproductive organs but also the immune system via multiple mechanisms, leading to mitochondrial dysfunction and oxidative stress. Long-term exposure to ATR will harm the health of lizards.

Notably, the only life-history traits that differed between females and males after exposure to ATR at room temperature could be found in energy reserves. The adipose tissue mass and coefficient of male lizards decreased significantly (Fig. 2M and O), whereas females tended to maintain their energy reserves (Fig. 2N and P). The role of stored energy is most likely substantial. A previous study highlighted the importance of stored energy to fuel reproduction, and further reported that higher fat body scores were related to relatively higher investment in self-maintenance (Durso and French, 2018). Moreover, larger energy stores are associated with higher survival and reproductive rates, with implications for fitness (Denryter et al., 2022). Therefore, preserving stored energy whenever possible is an adaptive strategy for female lizards, which not only ensures their immediate survival but may also enable them to achieve higher reproductive success rates in the future.

In this study, the reasons why male lizards had a lower survival rate than females after exposure to ATR at room temperature were analyzed. Firstly, males are more sensitive to ATR exposure. On the one hand, evidence indicates that compared with males, females often have stronger antioxidant defenses and may be more resistant to oxidative stress due to the ability of estrogen to act as an electron donor (Vina et al., 2006). In the present study, PCA analysis clearly showed that ATR treatment, especially at high concentrations (i.e., H group), significantly induced oxidative damage (represented by MDA content) in male lizards. The level of oxidative damage was highly correlated with antioxidant enzyme activity (SOD) (Fig. 5A), which was not observed in females (Fig. 5B). On the other hand, several previous studies found that the detoxification and pollutant metabolism of is better in females than in males, resulting in greater accumulation of contaminants in males (Li et al., 2022; Wen et al., 2015). Moreover, a study on the effect of L-Glutofosinate-ammonium on lizard reproduction showed that it accumulated much more in eggs than in testes and ovaries (Zhang et al., 2020a). Thus, the ATR accumulated in female lizards may be excreted with eggs, which improves female survival. Together, these results demonstrate that males are more sensitive to ATR

exposure, consistent with the results of a recent study (Guedegba et al., 2022).

Secondly, the immunosuppressive response induced by ATR (Fig. 2E) may also be an important reason for the decline of the survival rate of males, because having to deal with parasites and diseases has severe consequences on the fitness individuals may achieve (Sacchi et al., 2017). Finally, the possible reason for the observed decline of the survival rate is that males face higher energy costs when allocating life-history traits. The relatively high RMR of male lizards exposed to ATR at room temperature indicated that they spend additional energy on thermoregulation and energy metabolism to resist the negative effects of ATR exposure (Nie et al., 2022; Wang et al., 2021a). The lower energy reserves and more apparent weight loss in males confirm the hypothesis that the distribution life-history traits are costly in males. Notably, prior studies reported that ATR exposure affects the appetite of animals, which can lead to poor physical condition of individuals as a result of reduced food intake (Belanger et al., 2016; Blahova et al., 2020). Unfortunately, the actual food intake of lizards was not specifically counted during the experiment, further research is needed to determine the effect of ATR exposure on the energy cost of life history trade-offs in lizards.

4.2. Effects of high temperature and/or combined atrazine exposure on life-history traits in lizards

In this study, male lizards exposed to ATR at high temperature survived better than male lizards that were exposed to ATR at room temperature. This is contrary to many studies claimed that pollutants such as pesticides become more toxic under climate warming (Nie et al., 2022; Noyes et al., 2009). In fact, climate warming mainly influences the environmental behavior of pesticides by affecting their metabolism, degradation, and migration, thereby altering the extent of their biological hazards (Delcour et al., 2015; Zhang et al., 2020a). In the present study, it was observed that at a high temperature, ATR degraded faster in soil (Fig. S1), and male lizards exposed to ATR at high temperature had relatively lower levels of oxidative damage compared with males exposed to ATR at room temperature (Fig. 3E). This result suggests that the high temperature mitigated the negative effects of ATR exposure on male lizards by accelerating the degradation rate of ATR. In addition, more apparent energy mobilization (Fig. 3L–O), better energy consumption (Fig. 2M and O), and higher energy investment in self-maintenance (Fig. 2E, G, I, and K) in males further indicate that males make great efforts when faced with high temperature and/or combined ATR exposure. Particularly, male lizards conserve energy by reducing RMR (Fig. 3K). In conclusion, these results explain the higher survival rate of male lizards at high temperatures.

The lower survival rate of females exposed to high temperature and/or combined ATR can be explained according to the following two aspects. Firstly, high temperatures increase the reproductive output of females, thereby exacerbating their reproductive cost. The high temperature itself can accelerate the embryonic development of pregnant females, leading to earlier and more frequent spawning (Fig. S2B and Fig. 2B). More frequent spawning has been identified as a key factor in the summer mortality of many species, as an upstream burst in metabolic activity during reproduction inevitably induces physiological stress downstream, such as oxidative stress (Dupoue et al., 2020; Travers et al., 2009; Webb et al., 2019). Additionally, the reproductive process can alter the thermal resistance of lizards, and after-spawning, animals tend to be more sensitive to high temperatures. In part, this can also be attributed to a decrease in production of HSPs and a reduced immune response (Travers et al., 2009; Zhang et al., 2020a). In this study, stronger immunosuppression (Fig. 2F), lower Hsp70 levels (Fig. 2H), and more intense oxidative stress (Fig. 2L and Fig. 3J) were observed in females in LT and HT groups (compared to T group). Thus, although females will reduce their input into the reproductive process by decreasing egg mass and the total protein content within eggs under high temperature and/or combined ATR exposure (Fig. 2B and D), more frequent spawning still makes reproduction costly. Secondly, high temperature and/or combined ATR exposures impose additional metabolic costs

on pregnant female lizards. Under combined stressors, females face higher energy demands for self-maintenance in addition to reproduction. However, in the present study, female lizards exposed to high temperature and/or combined ATR did not consume more of their fat reserves to compensate for higher energy requirements as males, which compensated for higher energy consumption by modulating the size of their livers (Fig. 3F and G). As the major site of metabolism, studies have shown that larger livers often increase the metabolic rate of the whole-organism, as well as resource assimilation and catabolism by enhancing cell size and cell number, mitochondrial density, enzyme concentration and fuel storage (Sun et al., 2022). Indeed, the relatively higher RMR (Fig. 3P), more significant changes in energy metabolic parameters (Fig. 3Q–S), and higher glucose levels in the liver (Fig. 3T) in female lizards exposed to high temperature and/or combined ATR further indicate that females had to exert greater efforts in energy mobilization. However, this imposes additional metabolic costs, as PCA clearly showed that combined heat and ATR exposure (i.e., HT group) induced oxidative damage in female lizards, which is closely related to energy metabolic parameters (Fig. 5B). In summary, female lizards cannot meet their higher energy requirements for reproduction and self-maintenance simultaneously when exposed to high temperatures and/or combined ATR. Ultimately, this may lead to individual mortality by causing “metabolic meltdown”.

4.3. Gender differences in life history traits trade-offs and “winners” versus “losers” under environmental stressors

Because the allocation of resources to different life-history traits typically differs between males and females, sex-specific differences in life history trade-offs can also be expected. The research of Zhang et al. (2020b) on the effects of pollutants on the life-history trade-offs in lizards showed that the life history trade-off of lizards exposed to perfluorooctanoic acid (which has endocrine disrupting effects) during the breeding season depends on gender. Female lizards tend to allocate energy to self-maintenance by reducing their investment in reproductive processes, whereas males allocate little to self-maintenance. The authors suggest that female lizards exposed to contaminants are inclined to choose a “conservative” life history strategy, while a “risky” strategy is more prevalent among males. Here, the gender differences in life history trade-off strategies are extended to general environmental stressors (i.e., pollutants, high temperatures, and/or their combination). The gender difference in energy reserves is incorporated into life history trade-off strategies. The retention of fat stores in females can be regarded as a “conservative” life history strategy, while the depletion of energy reserves in males can be regarded as a “risky” strategy. The gender differences in life-history traits trade-off strategies will directly lead to environmental stress “winners” and “losers” within the species. Under environmental stressors, “winning” individuals tend to be those with the lowest mortality during exposure and/or the highest ability to reproduce afterwards (Fragueira et al., 2021). Therefore, for females, the “conservative” strategy of retaining fat reserves as much as possible is conducive to their survival and reproduction in the subsequent stage (e.g., hibernation) (Yu et al., 2022). This enables them to increase their reproductive fitness when exposed to ATR at room temperature. However, although male lizards consume more energy reserves to resist the negative effects of ATR at room temperature, their high mortality still means that they are the “losers”. Surprisingly, this “win-lose” situation is reversed at high temperatures. Under high temperature and/or combined ATR exposure, the “conservative” strategy of female lizards cannot meet their higher energy demand, thus, higher mortality means that they are the “losers”; however, because of the faster degradation of ATR under high temperature, the “risky” strategy of males to mobilize more energy consumption has instead ensured their current survival and means that they are the “winners”. It is worth noting that the depletion of fat reserves in male lizards may adversely affect their survival activities at subsequent stages (e.g., hibernation), which may result in maladaptation (Nie et al., 2022). In conclusion, these results emphasize the importance of life history trade-off strategies to understand environmental adaptation in animals.

However, whether individuals surviving under different environmental stresses in this study will continue to maintain their “winning” advantage in the future still needs to be explored by long-term experiments, especially experiments that span life-history stages.

4.4. Possible mechanisms for driving life-history traits trade-offs and future directions

Although the regulatory mechanisms for the trade-off between biological life-history traits under environmental stresses are still largely uncertain, these mechanisms may be related to the metabolic rate of organisms (Liu et al., 2022) and the regulation of endocrine hormones (De La Torre and Lopez-Martinez, 2023; Zera and Harshman, 2001). On the one hand, metabolic rates have been found to be related to energetic balances and trade-offs between competing life-history traits (such as immunity and growth), and these rates might affect survival by determining the energetic balance and the pace of life (Liu et al., 2022). Therefore, downregulating metabolic rates under a warming climate help to conserve energy, as well as mitigate the pressure of higher body temperatures imposed on the individual, as warming negatively impact survival (Dillon et al., 2010; Norin and Metcalfe, 2019). Such a strategy provides an advantage for lizards under climate warming (like the male lizards in this study at high temperatures). As one of the most energetically costly life-history traits under the breeding season, reproduction might aggravate the negative effects of high temperatures and further depress survival (Dupoue et al., 2018a; Webb et al., 2019) if lizards maintain a high metabolic rate (like the female lizards in this study at high temperatures). Future research can study the regulating effects of the energetic state (modified by metabolic rates) on the trade-offs between life-history traits.

On the other hand, endocrine hormone regulation has been identified as the mechanism that links life-history traits. Environmental stress usually activates the hypothalamus-pituitary-adrenal (HPA) axis of vertebrates to release glucocorticoids (GCs) to modulate daily and seasonal rhythms (Dupoue et al., 2018b). The GC stress response is considered an important feature of energy mobilization. In this study, the CORT level was significantly higher in male lizards exposed to either ATR or high temperature (Fig. 4E). This may be related to the observed decrease of energy reserves in male lizards (Fig. 2M). Because the CORT can stimulate the energy turnover from adipose and hepatic tissues and help individuals to optimize energy distribution (Sapolsky et al., 2000; Wang et al., 2021a). However, the continuous stress imposed by GCs under long-term environmental pressure may suppress reproductive and immune responses with consequences for individual health (Caizergues et al., 2022). Under combined stressors, male lizards strategically reduced their CORT levels, which may help to alleviate the negative effects of continuous stress exposure and promote individual survival. In female lizards, the non-significant decrease of CORT levels under environmental pressures may be caused by the reproductive process inhibiting the hypothalamus-pituitary-adrenal axis. The “conservative” strategy that tends to store energy may also be related to lower CORT levels. Furthermore, in this study, sex hormones level in lizards also changed after exposure to environmental stresses. For example, the levels of T and Pg in male lizards decreased significantly, and in females, LH, E₂, and Pg contents changed abnormally (Fig. 4). Several studies have also shown that changes in sex hormones levels may promote or inhibit individual reproductive efforts, which in turn will be traded-off against other life-history traits (Finch and Rose, 1995; Zhang et al., 2020a). Thus, hormones are notable candidates as mediators of life history trade-offs by regulating multiple antagonistic processes. In the future, further research on endocrine regulation during trade-offs at the hormone receptor level and on hormonal control of gene expression will help to improve the understanding of animal adaptations that are mediated by life history trade-offs.

5. Conclusion

This work explored the effects of atrazine and warming on the environmental adaptation (environment-related survival rate) of lizards in the

breeding season from the perspective of life-history traits trade-off. The results suggested that there were gender differences in the life-history trade-off strategies of lizards under environmental stress, and this difference may lead to mortality risk reversal. Overall, male lizards were more sensitive to atrazine exposure, while pregnant females showed higher sensitivity to high temperatures. Additionally, we also found that atrazine and warming may regulate life-history traits trade-off by affecting energy metabolism and hormone levels in lizards, thereby altering their environmental adaptations. Our results emphasize that life-history traits trade-off strategies are essential for understanding the environmental adaptability of animals. Further research on how environmental stress drives life-history traits trade-off in lizards and elucidating the reasons for gender differences in trade-off strategies will improve our understanding of environmental adaptations in reptiles such as lizards. Our study of breeding season lizards may not fully reflect the effects of atrazine and warming on lizard adaptations. Future studies across different life-history stages or life stages will help us to comprehensively understand the effects of environmental stress on the adaptations of lizards from the different age-classes at the population level.

CRedit authorship contribution statement

Yufan Nie: Conceptualization, Methodology, Investigation, Writing – original draft. **Zikang Wang:** Investigation. **Simin Yu:** Investigation. **Luyao Zhang:** Investigation. **Rui Liu:** Investigation. **Yuping Liu:** Investigation. **Wentao Zhu:** Supervision. **Zhiqiang Zhou:** Supervision. **Jinling Diao:** Supervision, Funding acquisition, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.163078>.

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