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Perfluorooctanoic acid exposure impact a trade-off between self-maintenance and reproduction in lizards (*Eremias argus*) in a gender-dependent manner

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ABSTRACT

The trade-off between self-maintenance and reproduction has been explored wildly in reptiles. However, the effects of exogenous pollutants on the life history traits of reptiles have not been paid attention to. In the current study, lizards (*Eremias argus*), living in the soil polluted by perfluorooctanoic acid (PFOA) were selected as the main focus. Bodyweight, survival rate, clutch characteristics and biochemical analysis (immune response, lipid accumulation, sex steroid secretion, antioxidant level, and metabolomics) were investigated and the results revealed that lizards' life-history trade-offs are gender-dependent: females were more inclined to choose a "Conservative" life-history strategy. After 60 days of exposure to PFOA, larger body weight, higher survival rate, stronger immune response, and lighter egg mass in females suggested that their trade-offs are more biased towards self-maintenance. Whereas, the "Risk" strategy would more popular among males: reduced body weight and survival rate, and suffering from oxidative damage indicated that males made little investment in self-maintenance.

1. Introduction

As a class of emerging anthropogenic surfactants, perfluoroalkylated substances (PFASs) are being increasingly used in daily life and industrial production, such as clothing, cosmetics, coating, and furniture (Jantzen et al., 2017; Meng et al., 2018). However, owing to their chemical and biological inertia, they widely exist in the environment and organisms, even in the polar bears living in the Arctic (Hoover et al., 2017; Jantzen et al., 2017; Pedersen et al., 2016; Zhao et al., 2012). As a class of endocrine disruptors (EDCs), PFASs have attracted great attentions of scientists due to their widespread application and detection (Pignotti et al., 2017). Perfluorooctanoic acid (PFOA) is one of the most abundant PFASs in the environment, which is an organic acid consisting of an eight-carbon chain and a carboxylic group (Jantzen et al., 2017; Li et al., 2017). Soil pollution of PFOA and its influences on the ecology have raised increasing concern. The high level of PFOA was observed in agricultural soils in America (Renner, 2009; Xiao et al., 2015) and in the soil of China's coastal areas, PFOA content was up to 47.7 ng/g (Meng et al., 2018). In addition, previous studies have reported enzymatic activities and microorganisms have been changed and the antioxidant system of earthworm has been damaged in PFOA-contaminated soils (Yuan et al., 2017; Zhang et al., 2013). Additionally, the endocrine disruption effect of PFOA on humans and animals cannot be ignored: in utero exposure to PFOA, male offspring's hormones and semen qualities were changed. In a study on Japanese medaka, fecundity were significantly reduced after PFOA treatment. Exposure of rainbowfish resulted in altered thyroid hormone levels and the increased activities of antioxidase (Kang et al., 2019; Li et al., 2017; Miranda et al., 2020).

As an essential soil surface animal, lizards form a significant

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connection between invertebrates and higher predators (Simoniello et al., 2014). Due to relatively small home ranges, lizards are more vulnerable to soil pollutants (Mingo et al., 2017). Moreover, the resources that lizards obtain from the environment need to be allocated to a lot of competing life-history demands like immunity, growth and, reproduction (Durso and French, 2018). On the one hand, individuals need to ensure their own survival. On the other hand, achieving reproductive success is also crucial. Therefore, this reality necessarily results in trade-offs of investment of resources among these life-history traits (Richard et al., 2012). Trade-offs between self-maintenance and reproduction have been widely reported. Dieting and training could affect the spawning and the immune function of *Anolis* lizards has been proven (Husak et al., 2016). Durso and French (2018) found that artificial predation pressure can alter the proportion of protein in side-blotched lizards' healing wounds and eggs (Durso and French, 2018). Different mating strategies could influence litter quality and maternal immune response in common lizard (Richard et al., 2012). However, few studies have clarified how environmental contaminants affect lizards' life-history traits trade-offs.

Eremias argus is a kind of common lizard widely distributed in China, Mongolia, and Korean. *E. argus*, a kind of squamata, is sensitive to exogenous pollutants and it is easy to raise in the laboratory (Dugarov et al., 2017). Based on the above characteristics, *E. argus* was selected as the test organism for this study. Male and female were exposed to soil contaminated with PFOA for 60 days. The purpose of this study was to explore whether PFOA exposure results in a trade-off between lizard's self-maintenance and reproduction. Self-maintenance involves many aspects, including growth and development, wound healing, immunity, antioxidation and other characteristics that are conducive to individual survival and enables individual to maintain a good physical condition (Dupoue et al., 2018; Durso and French, 2018; Richard et al., 2012). On the other hand, reproduction involves a series of investments in reproductive organs or offspring. Females mainly invest in eggs, and egg weight and protein content are important indicators of female's reproductive investment, while male testes mass and index are commonly used to evaluate male's reproductive investment (Husak et al., 2016). In addition, we explored important biomarkers linking self-maintenance and reproduction and tried to clarify possible causes of observed trade-off. Consequently, the body weight, survival rate, immune response, gonad antioxidant level, and metabolomics were analyzed for self-maintenance investment, while clutch characteristics (egg length and mass), testes mass and index, and sex hormone level were determined for reproduction investment. Moreover, vitellogenin (VTG) content (both in female's ovary and egg) and energy mobilization (lipid accumulation and corticosterone levels) are measured as bonds between self-sustainment and reproduction.

2. Material and methods

2.1. Animal husbandry

Mature *E. argus* were collected from the wild (Inner Mongolia Province, China) and housed in transparent plastic incubators (47 × 32 × 23 cm) containing a shelter, 5 cm of soil and sand. Opportunities for thermoregulation provided by a 25 W light bulb hanging above the plastic. Lizards were housed at 25 ± 2 °C. The light was sequentially on for 14 h/day between 06.00 and 20.00 to create a 10–14h dark-light cycle and humidity is controlled between 40 and 50%. Female (except for pregnant females) and male lizards were selected according to their snout-vent length (SVL) and bodyweight (SVL: 4.9–5.8 cm and 4.8–5.9 cm for male and female, respectively; bodyweight: 3.16–5.01 g and 3.12–4.31 g for male and female, respectively)for this study. All *E. argus* were acclimatized to the laboratory for two weeks before the study.

2.2. Experimental design and sample collection

E.argus were randomly separated into four groups (33 males and 33 females per group): Control group, 0.05 mg/kg PFOA group (L), 0.5 mg/kg PFOA group (M), and 5 mg/kg PFOA group (H) for 60 days. PFOA (Purity: 99%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). PFOA concentration in the L group was based on environmental concentration (Meng et al., 2018) and PFOA doses in the other two groups were 10 times (M) and 100 times (H) higher than those in L, respectively. PFOA concentration unit is mg/kg soil weight (mg: the weight of PFOA, kg: the weight of soil). The mixing processes of PFOA and soil are as follows: first, 99% PFOA was fully dissolved in double distilled water and gradually diluted into 10 ml of high, medium and low concentration PFOA aqueous solution, corresponding to the H, M, and L treatment groups, respectively. Then, 10 ml of PFOA aqueous solution was evenly dripped into the soil and double distilled water was added in the same way in the control group soil. Last, the soil was mixed by a mechanical shaker until the particles disappear. All lizards (4 groups) were separated into 12 plastic incubators, 3 incubators for each group with 3 replicates (11 males and 11 females per incubators). Lizards had access to sufficient mealworms (*Tenebrio molitor*), and each individual consumed a mealworm (about 100 mg) per day on average. Lizards were allowed to ingest calcium powder every other week and ad libitum access to a water bowl every day. In addition, lizards' excreta was cleaned once a week. At the end of the exposure, all lizards were weighed and sacrificed by freezing anesthesia. Brain, liver and gonad tissues were collected and weighed. Blood samples were collected into centrifuge tubes coated with sodium heparin in order to get plasma. All samples were stored at −20 °C until analysis. Animal experiments were approved by ethical committee for Laboratory Animals Care and Use of Research Center for China Agricultural University.

2.3. Physiological and biological analyses

2.3.1. Physical condition, females' reproduction output and male's testes index

The weights of lizards were measured every other week and the number of dead animals was counted every day. Females' clutch characteristics (egg length and egg mass) were monitored regularly twice a day. Eggs are collected immediately after being produced by females and stored at −20 °C for subsequent analysis. Testes from males were weighted and testes index was calculated as the weights of testes/male lizard's body mass.

2.3.2. Assay of enzyme activity, hormone and protein content

Biochemical indicators in lizards' plasma, liver, gonads and, brain were measured. In plasma, luteinizing hormone (LH), sex steroids testosterone (T) and estradiol (E_2), corticosterone (CORT), and immunoglobulin M (IgM) were investigated using assay kits obtained from Shanghai Enzyme-linked Biotechnology Co., Ltd. In liver tissue, total cholesterol (T-CHO) and triglyceride (TG) in the liver were measured using commercially available assay kits (Nanjing Jian Cheng Bioengineering Institute). In gonads, the antioxidant enzymes activities and oxidative stress markers contents including catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) were determined by commercially available assay kits (Nanjing Jian Cheng Bioengineering Institute). Moreover, the content of vitellogenin (VTG) was measured in ovaries and eggs, respectively by assay kits obtained from Shanghai Enzyme-linked Biotechnology Co., Ltd. In brain tissue, the level of gonadotropin-releasing hormone (GnRH) was investigated using an assay kit produced by Elabscience Biotechnology Co., Ltd. The validation of the commercial kits and the method of sample extraction are listed in Table S1.

2.3.3. Metabolomics analysis

To elucidate changes in metabolites in females, lizard's liver (50 mg) was placed into a 1.5 ml EP tube with 800 μL of cold water, 200 μL of cold methanol and grinding ball, homogenized for 3 min. Then, the samples were centrifuged for 10 min at 10,000 rpm at 4 °C after left on ice for 10 min. The supernatant was transferred to another EP tube. The above process was repeated twice and the supernatants from both extractions were combined. Then, supernatants were dried with nitrogen and reconstituted in 550 μL of 100 mM sodium phosphate buffer (pH 7.4) containing D2O (a lock signal) and 1.08 mM TSP-d4 (a chemical shift reference). Finally, the supernatants were transferred into 5-mm NMR tubes. The detailed description method of ^{1}H NMR spectra and metabolomics data analysis were shown in supporting information.

2.4. Data analysis

Using SPSS v20.0 (IBM, USA), student t-test (bodyweight), log-rank test (survival rate), and one-way ANOVA with post hoc Duncan (all other data) and were performed to determine significance. Data were normally distributed and homogeneity of variance was confirmed by Levene's test. Graphical plottings were realized by GraphPad Prism v6.0 (GraphPad Software, Inc. USA) and values were presented as means with standard deviation (means ± SD).

3. Results

3.1. Investment in self-maintenance

3.1.1. Wt change and survival

Body weights of lizards in the Control, L, M, and H groups were continuously monitored during 60 days (Fig. 1a and b). For females (Fig. 1a), there were the same trends of lizard's body weight changes in the four groups: as time went by, females' weights increased at the beginning of the experiment, peaked at the 28th day, and then decreased until the end of exposure. Besides, no obvious difference between the three treatment groups and the control group was observed. The reason why females' weight rises first and then drop is this experiment period covers the whole process of lizard from pregnancy to laying the first clutch. With regard to males (Fig. 1b), compared with the control group, lizards' weights in the L group were distinctly declined at 14th day (4.11 ± 0.60g and 3.77 ± 0.68 for the Control and the L group, respectively). At 28th day, there were significant decreases of weights in the L and H groups (Control: 4.30 ± 0.44g, L group: 3.98 ± 0.62g, and H: 4.01 ± 0.59) and after that lizards' weights decreased dramatically in all three treatment groups (Control: 4.27 ± 0.48g, L group: 3.57 ± 0.60g, M group: 3.54 ± 0.45g, and H: 3.43 ± 0.70). The difference between males and females was observed in weight change. The females treated with PFOA were basically the same as the control group, while the males' weights decreased significantly compared with the control.

Lizards' survival rates during the experiment period were analyzed (Fig. 1c and d). For females (Fig. 1c), the lowest survival rate was observed in the H (94%) group, followed closely by the M and L groups (97%), yet, the survival rate in the control group was 100%, although there was no significant difference among control and three PFOA-dosed groups. For males (Fig. 1d) compared to the

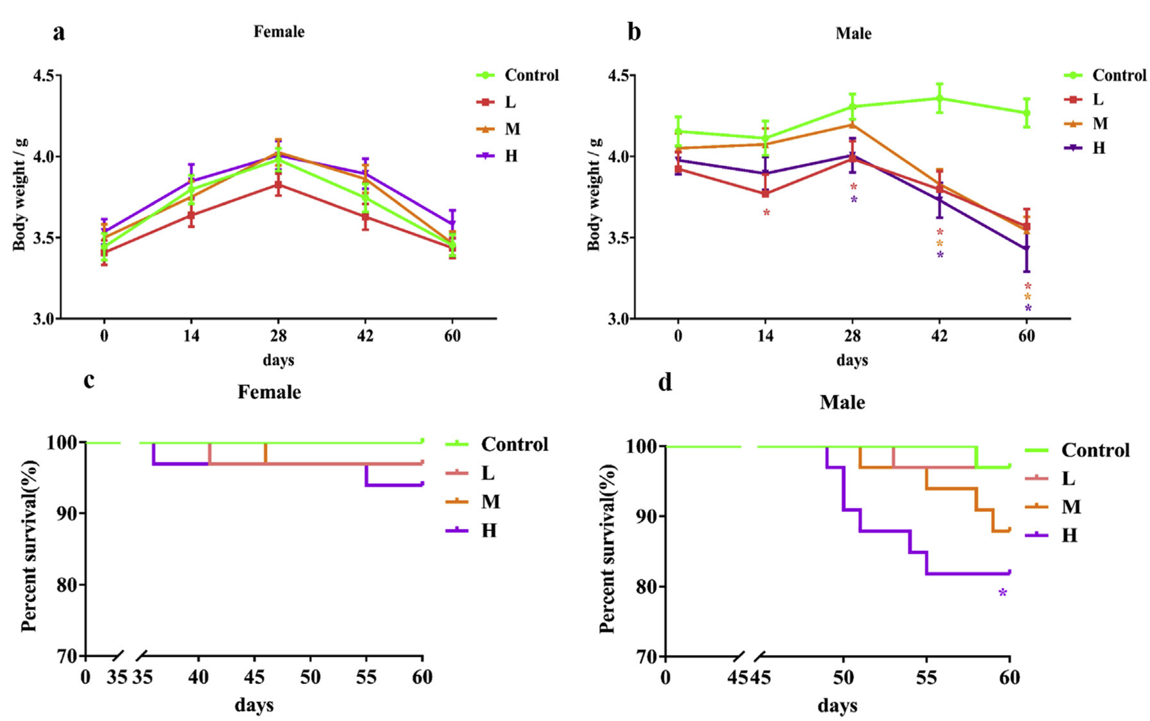

Fig. 1. Body weight for female (a) and male (b) during the 60 days in control, L, M, and H group. Bars indicate standard deviation (SD). * represents a significant difference compared to control at each sampling time. Different colored asterisks correspond to different colored lines. Survival rate for female (c) and male (d) during the 60 days in control, L, M, and H group. (L: 0.05 mg/kg group; M: 0.5 mg/kg group, and H: 5 mg/kg group).

control, survival rates showed a dose-dependent decrease in the three treatment groups. Lizard in the H group had the lowest survival rate (82%) and it was statistically distinct compared to the control (97%). Survival rates in the L and M groups are 97% and 88%, respectively, although there was no significant difference from the control group. Results of body weight and survival rate indicated that the effect of PFOA pollution on lizards was gender-specific. Similar to the result of body weight, the survival rates in the three PFOA-dosed groups were consistent with that in the control group in females. Whereas, in the H group in males, the survival rate was significantly reduced compared to the control group.

3.1.2. Antioxidant system and immune response

As an antioxidant metalloenzyme, superoxide dismutase (SOD) activity in lizard's gonad was determined in this study (Fig. 2a). In ovaries, SOD activities were decreased in the three PFOA-dosed groups compared to the control (Control: 164.29 ± 14.99, L: 110.14 ± 15.90, M: 70.24 ± 12.37, and H: 96.23 ± 12.75). On the contrary to the ovary, SOD activities in testes were increased in a dose-dependent manner (Control: 94.88 ± 15.04, L: 146.01 ± 24.34, M: 217.79 ± 15.90, and H: 222.23 ± 38.18). Moreover, Catalase (CAT) activities were also measured in lizard's gonad in both genders (Fig. 2b). CAT activities increased at low concentration of PFOA and decreased at high concentration in ovaries in the treatment groups (Control: 35.09 ± 2.02, L: 44.26 ± 3.76, M: 30.21 ± 2.55, and H: 28.50 ± 3.03). In testes, CAT activities showed elevations in three PFOA-dosed groups compared to the control (Control: 20.25 ± 3.42, L: 35.67 ± 3.30, M: 39.73 ± 2.72, and H: 40.41 ± 5.56). 8-hydroxydeoxyguanosine (8-OHdG) (Fig. 2c) and malondialdehyde (MDA) (Fig. 2d) are markers of DNA oxidative damage and end products of lipid oxidation, respectively. In ovaries, there was no significant change in the contents of two oxidation products compared to the control. Unlike females, PFOA exposure caused a dose-dependent increase in the 8-OHdG and MDA contents in testes suggesting that testes sufferer from oxidative stress (8-OHdG: Control: 3.75 ± 0.69, L: 5.32 ± 0.85, M: 9.14 ± 1.32, and H: 12.60 ± 3.11; MDA: Control: 10.74 ± 2.28, L: 17.67 ± 2.60, M: 23.31 ± 1.58, and H: 33.26 ± 3.21).

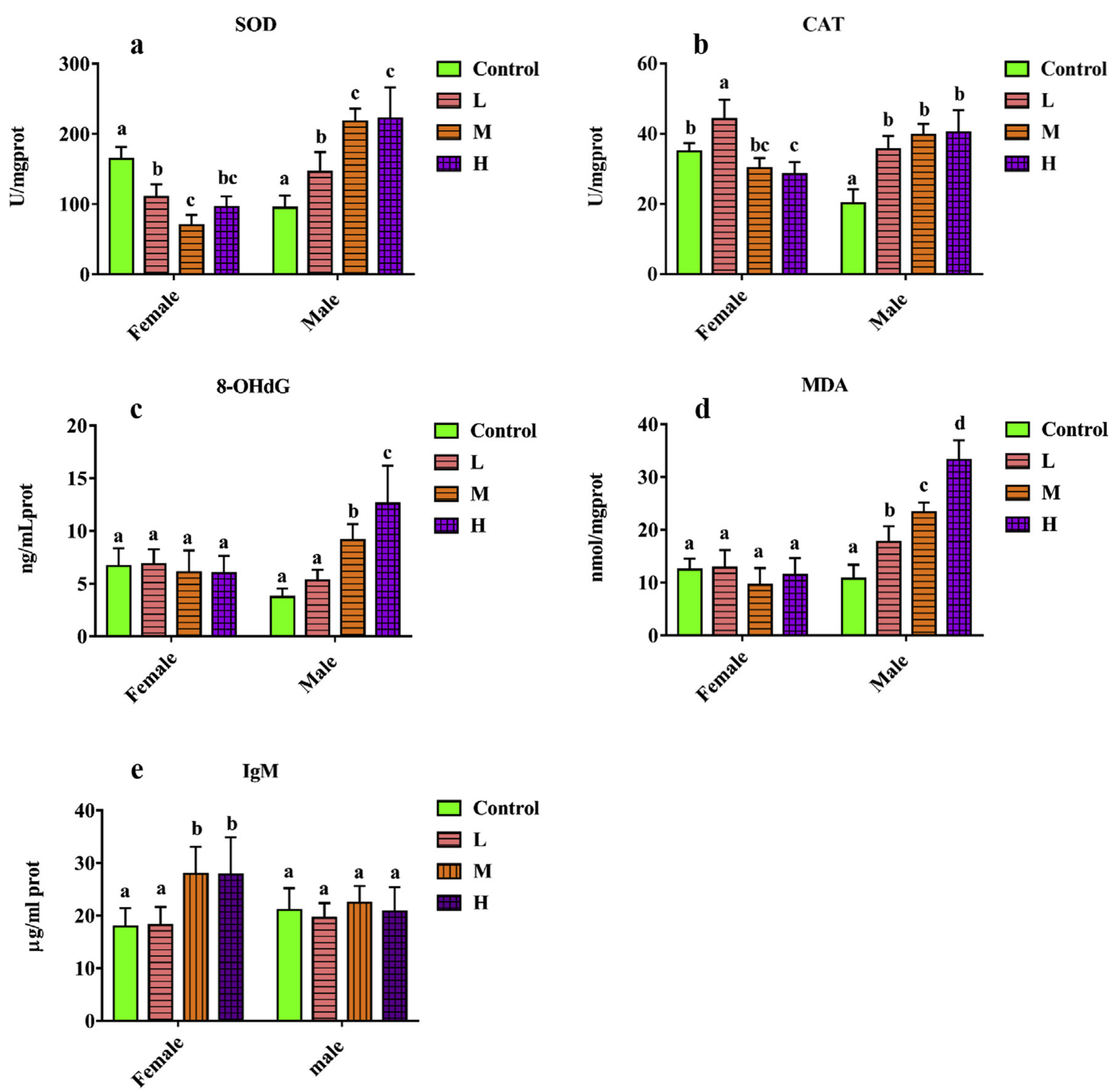

Fig. 2. The antioxidant enzymes activities and oxidative stress markers contents including superoxide dismutase (SOD) (a), catalase (CAT) (b),malondialdehyde (MDA) (c) and 8-hydroxydeoxyguanosine (8-OHdG) (d) in gonads (n = 3), immunoglobulin M (IgM) (e) content in plasma (n = 6) for female and male after 60 days PFOA exposure. Bars indicate standard deviation (SD). * represents a significant difference compared to control. (L: 0.05 mg/kg group; M: 0.5 mg/kg group, and H: 5 mg/kg group).

The immune response is an important part of self-maintenance in life-history traits. Therefore, immunoglobulin M (IgM) were measured in plasma in both sexes (Fig. 2e). For female, the levels of lizard's plasma IgM showed an obvious elevation in the M and H groups (Control: 17.96 ± 3.15, M: 27.94 ± 4.70, and H: 27.82 ± 6.46). However, there is no significant difference among each group in males. The above results show that females invest more in self-maintenance than males. Indeed, females maintain their own survival and growth by activating the immune response and antioxidant system.

3.2. Metabolomics study in females

$_{1}$H NMR-based metabolomics analysis was conducted to evaluate metabolic changes of female lizard's liver in the H group compared with control (Fig. 3). The resulting PCA score plot is shown in Fig. 3a. The score plot showed obvious separation and was divided into two main groups (Control and H groups) indicated that exposure to high dose PFOA was responsible for the metabolites variation in female lizards. A PLS-DA permutation model was generated (Fig. 3b) with a well goodness of fit ($R^2Y = 0.942$ and $Q^2 = 0.767$). Generally speaking, there are 425 variables with VIP >1.0 and 183 buckets with $P < 0.05$ by *t*-test. Thus, 16 metabolites that play an important role in the differences in the metabolic profile were identified on account of the VIP and P values (Fig. 3c). The results of metabolomics analysis indicated that after PFOA exposure, great changes have taken place in levels of substances participated in carbohydrate and energy metabolism (alanine, acetate, pyruvate, succinate, β-glucose, α-glucose, α-mannose, α-arabinose, α-xylose, and GTP), amino acid metabolism (glutamine), lipid metabolism and osmoregulation (lipid, choline, hypoxanthine, and trimethylamine N-oxide (TMAO)). Compared to the control, almost identified metabolites in the H group were up-regulated apart from lipid and methylmalonate.

3.3. Investment in reproduction

3.3.1. Female's clutch characteristics and male's gonad index

Clutch characteristics of females including egg length and egg mass were showed in Fig. 4a and b, respectively. Egg lengths in three PFOA-dosed groups were lower than those in the control, yet there is no significance. Regarding changes in egg mass, they

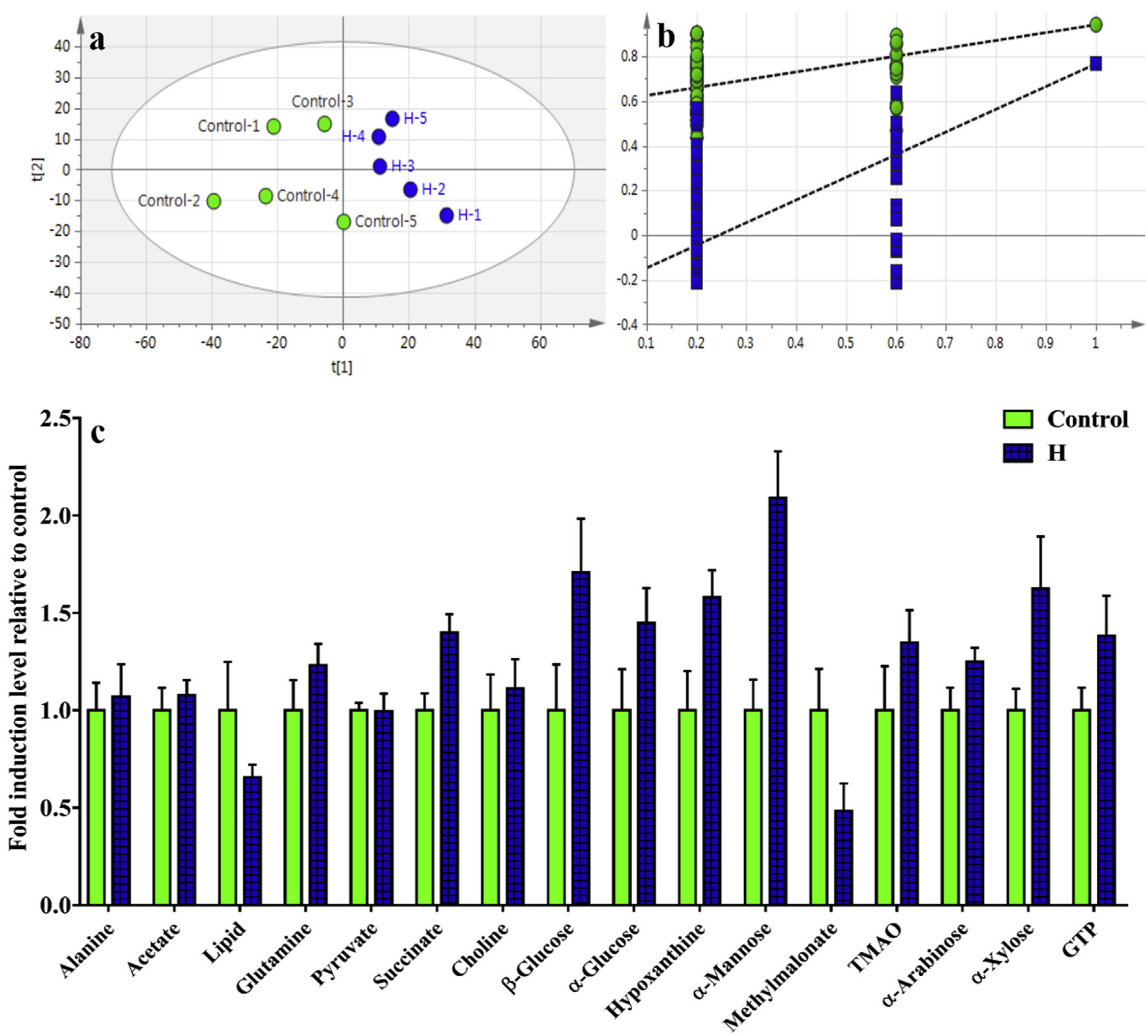

Fig. 3. Resulting PCA score plot (a) and PLS-DA permutation model (b) of $_{1}$H NMR-based metabolomics analysis between control and H group in female's liver. n = 5 (H: 5 mg/kg group).

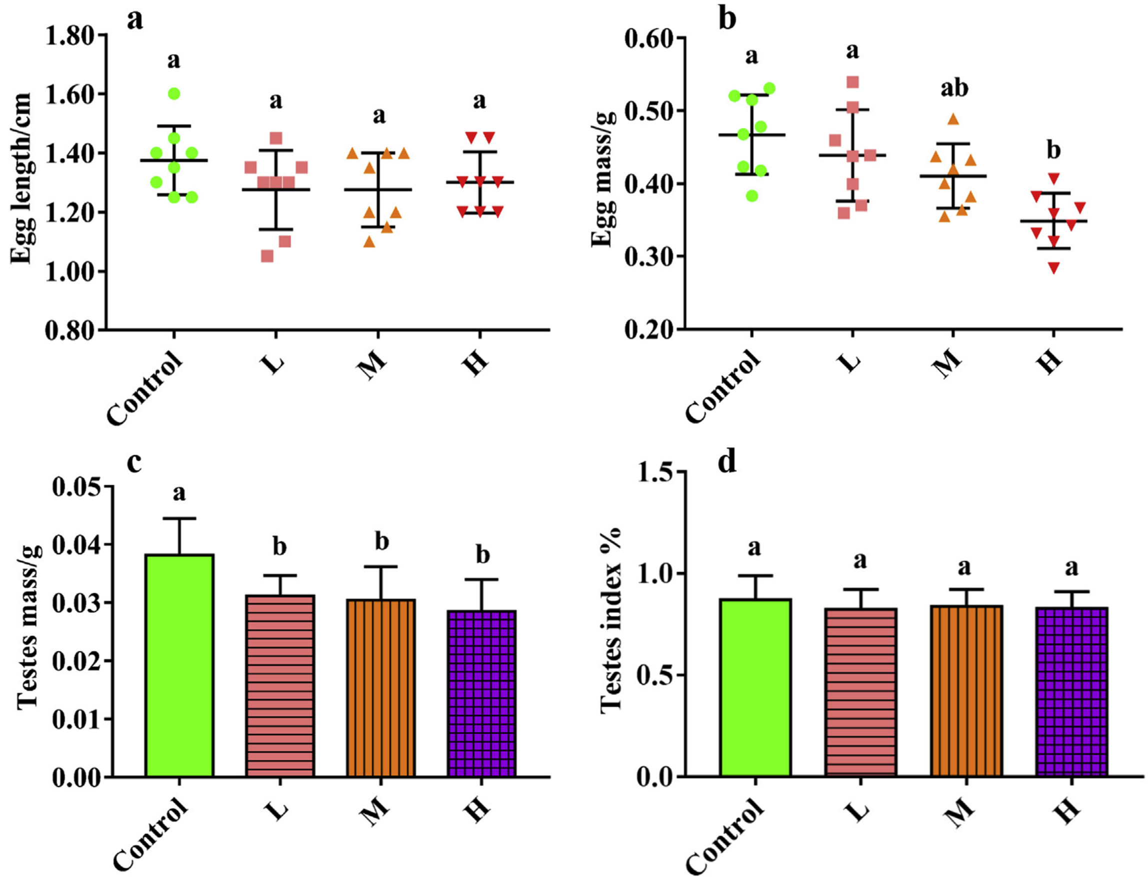

Fig. 4. Egg length (a), egg mass (b), testes mass (c) and testes index (d) during the 60 days in control, L, M, and H group. Bars indicate standard deviation (SD). * represents a significant difference compared to control. n = 9 (L: 0.05 mg/kg group; M: 0.5 mg/kg group, and H: 5 mg/kg group).

displayed a strong decline in three treatment groups in a dose-dependent manner compared to the control (Control: 0.47 ± 0.05, L: 0.44 ± 0.06, M: 0.41 ± 0.04, and H: 0.35 ± 0.04), suggesting that the individual's investment in reproduction gradually decreased with the increase of exposure dose of PFOA. For males, testes mass and index served as measures of investment in reproduction (Husak et al., 2016). Male testes masses (Fig. 4c) were significantly reduced by PFOA exposure (Control: 0.038 ± 0.006, L: 0.031 ± 0.003, M: 0.030 ± 0.005, and H: 0.029 ± 0.005). While testes indexes (Fig. 4d testes mass/lizard's body mass) have not shifted noticeably in any treatment group and this may ascribe to the reduced body mass.

3.3.2. Sex steroid levels regulated by the hypothalamus-pituitary-gonad (HPG) axis

The sex hormone level of vertebrates is strictly regulated by the hypothalamus-pituitary-gonadal (HPG) axis. Therefore, gonadotropin-releasing hormone (GnRH) in lizard's brain, luteinizing hormone (LH), and sex steroids such as estradiol (E2) and testosterone (T) in plasma were measured (Fig. 5). GnRH is a neurohormone released by the hypothalamus that promotes LH secretion. In the present study, GnRH levels (Fig. 5a) rose in females in the L and H groups but fell in males in all treatment groups (For female: Control: 3.14 ± 0.26, L: 5.39 ± 0.99, and H: 5.31 ± 1.19; for male: Control: 3.10 ± 0.33, L: 2.49 ± 0.33, M: 1.86 ± 0.22, and H: 2.19 ± 0.15). LH, a kind of glycoprotein gonadotropin, could stimulate the conversion of cholesterol into sex hormones. The results (Fig. 5b) showed that LH levels decreased distinctly in both sexes except for males in the H group (For female: Control: 0.35 ± 0.02, L: 0.32 ± 0.01, M: 0.31 ± 0.02, and H: 0.30 ± 0.02; for male: Control: 0.38 ± 0.03, L: 0.29 ± 0.03, and M: 0.32 ± 0.04). As a consequence, levels of sex steroid E2 (Fig. 5c) were dramatically suppressed compared with control (Control: 4.12 ± 0.41, L: 2.78 ± 0.58, and M: 2.52 ± 0.51). For males, level of sex steroid T (Fig. 5d) showed a downward trend, although not statistically significant.

3.4. The relationship between self-maintenance and reproduction

3.4.1. Vitellogenin content in female's ovary and egg

Vitellogenin content (VTG) is not only the precursor of vitellin but also has an antioxidant effect in the lizard. Our result (Fig. 6) showed that no significant change of VTG contents was found in all treatment groups. However, after PFOA treatment, the VTG contents in the eggs were obviously decreased (Control: 2.34 ± 0.22, L: 2.06 ± 0.08, M: 2.10 ± 0.09, and H: 2.02 ± 0.10).

3.4.2. Energy mobilization

Lipid content is one of the key factors for individual energy reserve and reproductive success. Thus, the contents of total cholesterol (T-CHO) and triglyceride (TG) in lizard's liver were determined (Fig. 7). These results showed that the influences of different concentrations of PFOA on T-CHO levels were dose-dependent. With the increased dose of PFOA exposure, T-CHO (Fig. 7a) contents in females in three treatments were declined compared to the control (Control: 20.91 ± 1.99, L: 19.81 ± 1.37, M: 17.39 ± 0.92, and H: 16.60 ± 1.02). While it is almost precisely reverse in males:T-CHO contents showed an obvious increase in the trend in both M and H groups (Control: 5.00 ± 0.41, M: 13.76 ± 1.63,

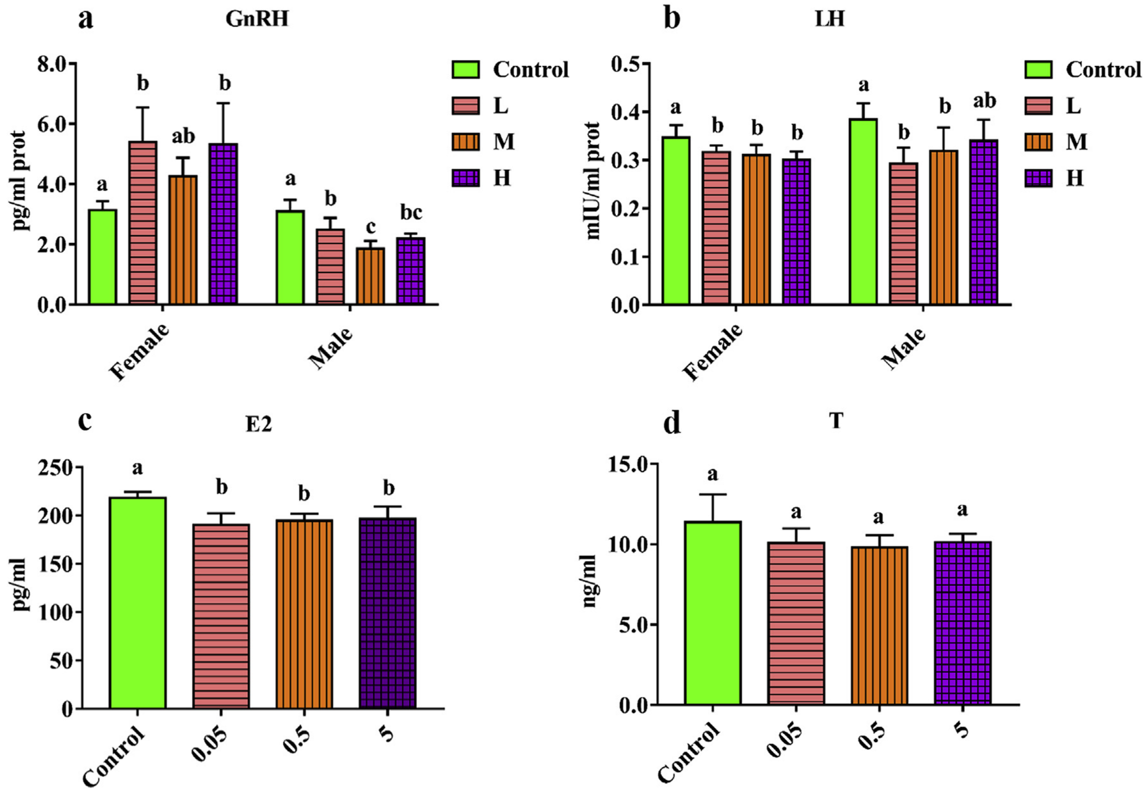

Fig. 5. Levels of gonadotropin-releasing hormone (GnRH) (a) in brain, luteinizing hormone (LH) (b), and sex steroids estradiol (E_2) (c) and testosterone (T) (d) in plasma for female and male after 60 days PFOA exposure. Bars indicate standard deviation (SD). * represents a significant difference compared to control. n = 6. (L: 0.05 mg/kg group; M: 0.5 mg/kg group, and H: 5 mg/kg group).

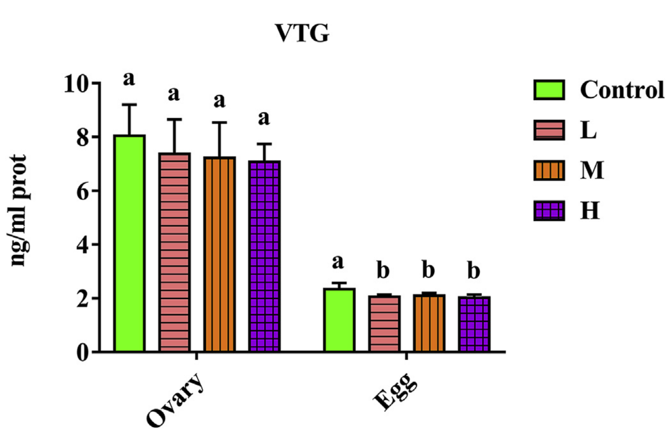

Fig. 6. Vitellogenin (VTG) content in ovary and egg after 60 days PFOA exposure. Bars indicate standard deviation (SD). * represents a significant difference compared to control. n = 3 (L: 0.05 mg/kg group; M: 0.5 mg/kg group, and H: 5 mg/kg group).

and H: 16.11 ± 1.26). TG levels (Fig. 7b) showed notably reduction in females in the L and H groups (Control: 12.90 ± 1.54, L: 6.21 ± 1.59, and H: 8.60 ± 0.96). In contrast to females, an elevation of TG levels was observed in males in the H group (Control: 5.76 ± 0.37 and H: 13.61 ± 1.85).

Corticosterone (CORT) hormone is important for energy mobilization, lipid accumulation as well as regulation of the immune system (Love et al., 2016; Zheng et al., 2017). In this study, CORT plasma levels (Fig. 7c) showed the same tendency with T-CHO. Compared with the control group, a dose-dependent increase of CORT level was observed in females (Control: 52.90 ± 2.58, L: 49.25 ± 2.43, M: 45.26 ± 1.98, and H: 44.98 ± 1.93), whereas it showed increased in males in a dose-dependent manner after PFOA exposure (Control: 43.26 ± 1.31, L: 46.21 ± 1.48, M: 51.33 ± 1.90, and H: 52.05 ± 1.68).

4. Discussion

During times of extreme energetic demand like breeding season inevitably leads to a sequential allocation of resources among numerous competing traits in animals in order to obtain an optimal life-history strategy (Cornelius et al., 2019; Gray et al., 2018). In recent years, more and more attention has been focused on trade-offs among life-history characteristics of organisms (Gray et al., 2018; Kulaszewicz et al., 2017b; Ruiz, 2010; Tien et al., 2009). Similarly, reports of trade-offs in lizards have also gradually increased which have been mainly concentrated on factors such as food and water restriction, exercise and thermal effect (Dupoué et al., 2017; Husak et al., 2016; Rutschmann et al., 2016). However, lizards are important soil surface animals that are susceptible to soil contaminants (Chang et al., 2018; Chen et al., 2017). Hence, the influence of exogenous pollutants on lizards' life history characteristics is not to be overlooked. PFOA, one of the Persistent organic pollutants, is widely detected in soil (Lechner and Knapp, 2011; Xiang et al., 2018). Previous reports have pointed out the toxicity mechanism of PFOA in mammals, amphibians, and fish but studies on reptile are very limited (Lee et al., 2017; Li et al., 2017; Tang et al., 2018; Zhang et al., 2019). Besides, these studies focused only on the PFOA enrichment in sea turtles, do not take into account the impacts of PFOA on animal's life-history characteristics (Guerranti et al., 2013; Keller et al., 2005; Keller et al., 2012).

In the present study, we probed into the trade-off between self-

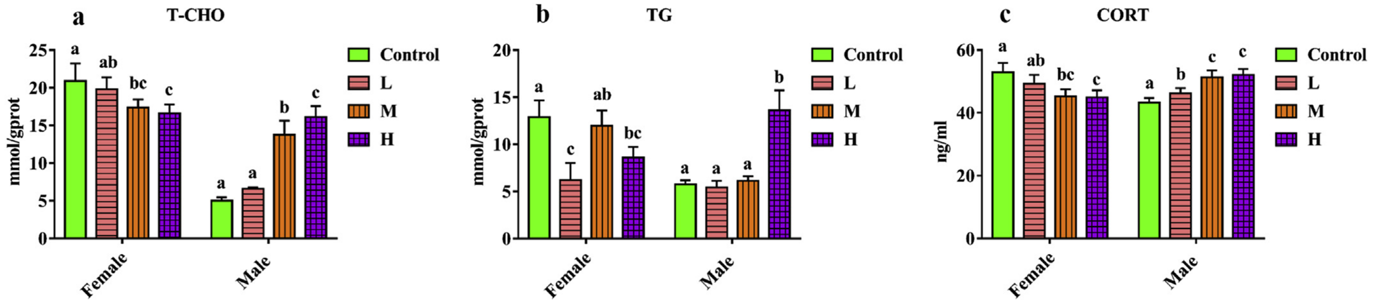

Fig. 7. Total cholesterol (T-CHO) (a), triglyceride (TG) (b) content in liver, and plasma corticosterone (CORT) (c) levels for female and male after 60 days PFOA exposure. Bars indicate standard deviation (SD). * represents a significant difference compared to control. n = 3 (L: 0.05 mg/kg group; M: 0.5 mg/kg group, and H: 5 mg/kg group).

maintenance and reproduction in *E. argus* after treatment with PFOA. This is the first study on the influences of contaminant on the life-history traits in lizards. Our results indicated that after PFOA exposure, the investment of resources in life-history characteristics by both sexes is vastly different. Females tend to choose a “conservative” life history strategy but not in males, where the “risky” strategy would be favored by them. According to previous study, different morphs of lizards may represent the two opposite extremes of the trade-off between a “risky-strategy” and “conservative strategy”. On the one side, individuals with strong immunity and stamina, and low reproductive competitiveness are considered to follow “conservative strategy”. Because this strategy is beneficial to lizard’s own health and longer survival. On the other side, lizards with low immunity and stamina, and high reproductive competitiveness are supposed to “risky strategy”, which may impact the survival and fitness of lizards (Sacchi et al., 2017).

4.1. “Conservative” life history strategy in female

In this study, a “conservative” strategy is used to describe investing more resources in self-maintenance (the maintenance both in weight and survival rate, activation in immune response and antioxidant system) rather than the current reproductive success (decreased egg mass and E2 level). Compared with the control group, the body weight and survival rate of females did not change significantly after exposure to PFOA, whereas the egg mass decreased in a dose-dependent manner. The reduction of investment in reproduction may attribute to the following three reasons: first, endocrine disruption of PFOA affect the secretions of hormones. The development of sexual behaviors almost depends on the sex hormone. E2, as a vital sex steroid is regulated by pituitary gonadotropins LH and the lower LH content leads to the dramatically suppressed E2 secretion (Pandey et al., 2017). A lot of reports describes that E2 participates in ovarian follicular development, oviductal growth and vitellogenesis (Al-Amri et al., 2012; Jones, 1975). Thus, the declined level of E2 was not conducive to the development of germ cells and the synthesis of nutrients. Moreover, the lower VTG content in eggs after PFOA exposure was observed. Indeed, VTG, circulating vitellin precursor, is directly related to clutch mass in a reptile (Garstka et al., 1982). As a consequence, the egg mass was decreased after PFOA treated. Lastly, the lipid accumulation such as T-CHO and TG in female was inhibited in PFOA treatment groups. Kulaszewicz et al. have shown that the breeding season is one of the most energetically demanding stages for birds (Kulaszewicz et al., 2017a). Similarly, reproduction activity is also energetically expensive in a lizard (Husak et al., 2016). As an important energy storage material, the decline of lipid would have a negative impact on reproduction.

More interestingly, after PFOA exposure, enhanced immune response in female lizards was observed. However, the immune function that is important for survival is also energy-intensive (Richard et al., 2012). Exactly, the lower lipid levels in this study were probably due to females investing a lot of resources into the immune system in order to ensure their own survival. Previous research supports our results: in common wall lizard, immune-challenged individuals resulted in a major burst of energy consumption consequently, and they cannot simultaneously guarantee a high immune response and increase lipid reserves (Sacchi et al., 2017). In addition, another aspect of self-maintenance was reflected in the investment in the ovarian antioxidant system. Our results showed that the stimulation induced by PFOA exposure may disrupt the dynamic balance of production and elimination of reactive oxygen species (ROS), leading to changes in the CAT and SOD activities, which are the ROS scavengers. It was the positive response of antioxidant enzymes that kept the ovaries from suffering oxidative damage. MDA and 8-OHdG were two important oxidation markers. Compared with the control group, there was no obvious influence on the content of MDA and 8-OHdG suggesting that female individuals protect their ovaries from oxidative damage. On the other hand, the antioxidant capacity of VTG needs to be taken into account, because VTG may also repress ROS contents in lizards (Olsson et al., 2012). Reduced VTG content assigned to eggs may indicate that VTG was involved in the antioxidant function in the females’ ovaries in order to ensure their own health. Finally, the results of metabolomics analysis further supported the above views. In addition to lipid and methylmalonate contents, other metabolite levels were significantly increased in females. The up-regulation of these substances participated in carbohydrate and energy metabolism and osmoregulation revealed the fact that when females were challenged to exogenous pollutant PFOA, instead of reducing investment in their own physiology, the females tended to push the costs onto their eggs which may bound to lower quality offspring. However, this “Conservative” life history strategy is not useless, which ensures the female’s good body condition and may allow for better reproductive success in the future.

4.2. “Risky” life history strategy in male

Based on our results, the trade-off in males didn’t bias toward self-maintenance after PFOA exposure, which is mainly reflected in lower survival rates and notably reduced body weight in the three PFOA-dosed groups. Moreover, the increase of MDA and 8-OHdG contents suggested that testes suffered from severe oxidative stress, which may directly result in the reduction of testes mass. Also, PFOA exposure did not induce an immune response in males. Males did not invest in two energy-consuming functions (immunity and antioxidation), so lipid accumulation was observed. We believe this strategy of giving up self-maintenance investment is a "risky" life history strategy, which can't guarantee good physical condition leading to serious challenges to individual' s survival.

Interestingly, CORT, a kind of glucocorticoid, is involved in the regulation of energy, which increased in a concentration-dependent manner in males in three treatment groups. In another study of male lizard (*common lizard*) with higher CORT levels showed greater interest in females, as demonstrated by their behaviors: higher chasing frequency, tongue extrusions, and approaches. As a consequence, these males launched more mating attempts (Gonzalez-Jimena and Fitze, 2012), Therefore, the elevation of CORT in male *E. argus* treated with PFOA in this study may suggest that life-history trade-offs are more prone to reproduction. However, the reductions both in testes mass and T level (no significance) in PFOA treatment groups were observed and this result did not support male's investment in reproduction. In summary, we couldn't come to a clear conclusion about male's reproductive investment. If males invest resources neither in self-maintenance nor in reproduction, then an interesting question will be asked: where does the male's energy go? Indeed, the trade-off of life-history trait is not only limited to self-maintenance and reproduction but also exists among other competing traits like locomotor performance and thermoregulation (Husak et al., 2016; Sacchi et al., 2017).

4.3. Gender difference in life-history traits trade-off

This study showed that different genders may represent different life history strategies in the *E. argus*. It is worth pondering over the reasons for this gender difference in life-history traits trade-off: first, endocrine disruption of PFOA is sex-dependent. A study on Japanese medaka suggested that the impacts of PFOA were different for each sex (Kang et al., 2019). Indeed, in our results, compared to the control group, sex hormone levels regulated by the HPG axis are various between the sexes after exposure to PFOA. Second, van den Heuvel et al. (1991) found that the metabolic rate of PFOA in female rat is faster than that in male, which results in the half-life of PFOA in female is much shorter than that in male. The long-term accumulation of PFOA may lead to the poor fitness of male than female. In this study, this is evidenced by higher mortality, lower body weight, and poor immunity in males after exposure to PFOA compared to females (Heuvel et al., 1991). Lastly, during the breeding season, compared with males, lower body temperature in females is considered to be favorable for embryo development, and the consequently decreased thermoregulation costs respect to males might invest more resources to self-maintenance like an immune response (Sacchi et al., 2017; Schwarzkopf and Andrews, 2012).

5. Conclusion

Our results illustrated that by pursuing different strategies, males and females might take different measures to adapt to the living environment polluted by PFOA. Females tended to adopt a "Conservative" life-history strategy, which is mainly reflected in higher survival rate, body weight change basically consistent with the control group, and significantly reduced egg mass. For males, they preferred "Risky" strategy which means males made little investment in self-maintenance such as declined survival rate and body weight as well as suffering from oxidative damage. In addition, life history features in lizard include more than just self-maintenance and reproduction, locomotor performance traits including running, sprinting and jumping, also play an important role in life-history trade-offs (Husak et al., 2016; Husak et al., 2017), which should be taken into account in the future study.

Author statement

I declare that this work does not create a conflict of interest with any other organization or individual.

Declaration of competing interest

I declare that this work does not create a conflict of interest with any other organization or individual.

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Appendix A. Supplementary data

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