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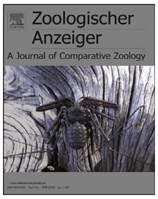

Research paper

Effects of pesticides on eggs and hatchlings of the Italian wall lizard (*Podarcis siculus*) exposed via maternal route

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ABSTRACT

In oviparous species, pesticides may affect embryo survival via maternal transfer or contaminant absorption from the soil, thus representing an important cause of population decline. Maternal transfer is a source of contamination during vitellogenesis and oviductal egg retention. Currently, there is still limited evidence of the potential risk of embryonic exposure routes in reptiles. We investigated whether different agricultural treatments affect embryo, egg, and hatchling development and survival in the Italian wall lizard. We kept gravid females from conventional and control fields in captivity until oviposition and, after eggs being incubated in a pesticide-free environment, offspring morphology and performance (running speed) were assessed. Our results showed that the size of the mother positively influences the number of the eggs and offspring, as well as hatching body condition. Bigger females from conventional treatments tended to lay more eggs. Moreover, at equal female body conditions, eggs and hatchlings from conventional treatments had worse quality (i.e., smaller size and lower body condition) than those from control areas. No effect of treatment was observed on hatchling locomotor performance. In conclusion, our study provided new insights of the direct and indirect effects of field management (i.e., pesticide exposure) on females' reproductive success through the alteration of female's behaviour, which in turn may affect offspring development and health.

1. Introduction

The continuous and rapid increase of industrialization and agriculture is among the primary threats to wildlife (van der Werf 1996). Together with habitat destruction, water and soil pollution due to the release of many pesticides in agricultural practise are the main causes of the decline of amphibians and reptiles worldwide (Gibbons et al. 2000). Field and experimental studies demonstrated that the effects of exposure to chemicals can be more severe during early life stages, which seem to be the most vulnerable (Kleinow et al. 1999; Stark et al. 2004) resulting in reduced offspring number, alterations in the genotype, and abnormal phenotype (Stark & Banks 2003).

The main routes of chemicals exposure in the embryos of reptiles are the maternal transfer and the eggs' absorption of contaminants from the surrounding environment (Gardner and Oberdorster, 2016). In early vitellogenesis, pollutant molecules (e.g., organochlorines) can be easily transported from the mother to the embryos during the female production of large quantities of lipoproteins, which are fundamental for embryonic development (Rauschenberger et al. 2004; Guirlet et al. 2010). Maternal transfer of such compounds represents an important vulnerability for developing offspring and, ultimately, for the parental fitness (Gardner & Oberdöster 2016). In addition, as other many animal groups, reptiles deposit eggs in soil, leaf litter or other rubbles where embryos can be potentially contaminated via gasses and water exchange during incubation (Marco et al. 2004a, b; Redick-Harris, 2006; Gardner & Oberdöster 2016). Unlike avian eggs with a thick calcareous outer layer, many reptiles, as lizards and snakes,

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lay soft and porous shelled eggs which allow water absorption from the environment to properly develop and increase embryo mass, especially in the early developmental stages (Packard & Packard 1980; Ackerman et al. 1985). Many factors can contribute and influence the transfer of contaminants to eggs as the physico-chemical features of the nest substrate and pore water, characteristics of the eggshell, and contaminants properties (Gardner & Oberdöster 2016). Thus, normal physiological dynamics during embryo development, including regulation of gene expression, may be disrupted by pollutants capable of permeating the shell (Trinchella et al. 2009). To date, embryonic exposure routes are still poorly known and often disregarded in reptile ecotoxicology (e.g., maternal transfer of chemicals and their bioaccumulation in eggs: Burger 1992; Burger & Gibbons 1998; Rauschenberger et al. 2007; effects of soil contaminants: Marco et al. 2004a, 2004b; Redick-Harris 2006; Trinchella et al. 2009), most studies being focused on turtles and crocodiles.

The analysis of reproductive success (i.e., offspring number and quality) across pesticide treatments, besides providing direct effects of pesticide via maternal transfer or egg-shell absorption, may also indirectly estimate the stress experienced by adult females before and after reproduction (Gardner & Oberdöster 2016). Indeed, toxicants effects may occur later in life by lowering the energy stores that are available for reproduction through increased overall stress on the individual, decreased feeding activity, or altered metabolic pathways (Gardner & Oberdöster 2016). The ultimate effect of pesticide can be therefore measured as smaller clutch size and/or lower egg quality, or even reproduction delay or suppression (Gardner & Oberdöster 2016). Thus, when suboptimal conditions for reproduction occur, females may be forced to choose between reducing the number of offspring and reducing the per capita energetic investment in the normal offspring number (e.g., a few large offspring or many small offspring) (Gardner & Oberdöster 2016). However, direct and indirect effects of pollutants must be disentangled. For instance, in female garter snakes living in contaminated habitats, lower body condition, metabolic and reproductive rates were due to reduced food availability, rather than to pollution per se (Sasaki et al. 2016). In those cases, body condition indices have been commonly used as indicators of stress and reproductive success (Jakob et al. 1996; Stevenson & Woods 2006; Lazić et al. 2017; Megía-Palma et al. 2020). The decrease in body condition can have important consequences for the individual's fitness, including ability to compete for breeding opportunities and fecundity, and ultimately affect survival capacity or reproduction chance, thus influencing population structure and dynamics (Sibly & Hone 2002).

As result of their immense diversity, reptiles provide novel opportunities for evaluating the process of maternal transfer of pollutants. In this background, we decided to delve into maternal route of pesticides exposition using as model species the oviparous Italian wall lizard, *Podarcis siculus* (Rafinesque-Schmaltz 1810). This generalist lizard is widely present in urbanized and cultivated areas of the Mediterranean and temperate climate regions of Italy. It is characterized by seasonal reproductive cycle (Corti et al. 2010) with females producing 1–3 clutches per year from May to July with an average of 2–8 eggs per clutch (Angelini et al. 1982; Henle 1988; Capula et al. 1993). Eggs are preferentially buried in sandy or crumbly substrates, at the end of 10–20 cm deep tunnels (Fretey 1975). In nature, incubation varies between 6 and 11 weeks depending on incubation temperature, which does not affect the sex-ratio (Van Damme et al. 1992). Although the reproduction of *P. siculus* has been broadly studied (Corti et al. 2010), few data are available on eggs and hatchlings (Gubanyi 2003; Biaggini & Corti 2019). Many fitness-related traits such as growth, locomotor performance, and behaviour of hatchlings may be potentially affected by the exposure to adverse environmental conditions experienced at the embryo stage (Boone et al. 2001). Among the performance traits widely used to assess individual quality in reptiles, sprint speed is relatively easy to measure and thus the most studied aspect of reptiles' locomotor abilities (Garland & Losos 1994; Van Damme & Vanhooydonck 2001; Žagar et al. 2017). However, only few studies evaluated the effects of pollutant exposure on lizard speed performances (Marco et al. 2004a; 2004b; DuRant et al. 2007; Holem et al. 2008; Amaral et al. 2012). The main goal of this study is to determine whether the exposure of adult female lizards to pesticides (i.e., those inhabiting conventional fields) could influence offspring development (i) directly by transferring pollutants to the embryos during vitellogenesis, or (ii) indirectly by determining higher morphological stress (i.e. lower body condition) that in turn affects eggs (i.e., smaller clutch and eggs size), and hatchlings (i.e., survivorship and performance). New-born lizards with low body condition, morphological abnormalities and low locomotor performances could be good indicators of an overall reduced quality of hatchlings (Sinervo & DeNardo 1996; Fuentes et al. 2020).

2. Materials and methods

2.1. Study area

Fieldwork activity was conducted in ten localities in central Italy (Latium, Rome and Viterbo provinces, about 507 m a.s.l.) in June 2019. The traditional agroecosystem of northern Latium hilly landscapes is represented by hazelnut orchards (Nera et al. 2020). Hazelnut management activities have been extensively adopted in the last 50 years in these areas, leading to monocultures with high tree density and a massive application of chemicals in order to satisfy the increased market demand (Biasi et al. 2010), which have been claimed to be responsible for the high pollution in the soil and waters of the area (Garnier et al. 2010). All study sites were hazelnut orchards with similar geology, climate, and they were separated by at least 2 km from each other to a maximum distance of 50 km. Five studied fields were under conventional management, exposed to a combination of fungicides and insecticide, such as tebuconazole, lambda-cyhalothrin, thiophanate-methyl and deltamethrin, which have been repeatedly applied twice a year. Additionally, five selected hazelnuts fields, with no history of chemical application for at least 10 years, were used as control (Table S1). The current and historical land and pesticide usage were obtained through consultation with the landowners.

2.2. Lizard sampling and housing

Overall, 23 pregnant females were caught by noose or hand: 13 from the five conventional areas and 10 from the five control fields (Table S1). Females gravid state was assessed in the field by recognition of the presence of mating scars in their ventral surface and by ventral palpation for detection of oviductal eggs (Galán 1997; Castilla & Bauwens 2000). Lizards were immediately transferred to the Fondazione Bioparco of Rome, where they were measured for snout-vent length (SVL) with a calliper (precision 0.01 mm) and body weight (Digital scale, DIPSE TP 2000; precision 0.1 g); we did not weight lizards after oviposition. During pregnancy body condition is affected by clutch mass due to the investment of fat bodies surplus into vitellogenesis (Carretero 2006), and individual body mass could be estimated by subtracting the clutch mass from female body mass. In our case, this approach was not applicable because lizards from control and conventional sites oviposited after different number of days from collection date (Mann–Whitney U-test, median control = 13, conventional = 7.5, U = 15, p = 0.003). Therefore, the findings on lizard body condition should be interpreted cautiously. Lizards were housed individually in outdoor 60 L plastic containers at environmental temperature and natural photoperiod. Each container was filled with 10 cm of already sterile sand, daily moistened, and furnished with two large barks to provide refuge and a terracotta dish for water. Moreover, a net covered each plastic container to prevent both the entrance of predators (e.g., birds) and lizard escape. Animals were fed *ad libitum* with living crickets (*Acheta domesticus* (Linnaeus, 1758)) of appropriate size and checked every three days for skin folds, which indicate egg laying (Van Damme et al. 1992).

Number of eggs, individual egg mass (KOBWA precision balance to the nearest 0.001 g), and egg size (estimated as egg volume using digital photographs on a metric reference through ImageJ software v1.5j8, Schneider et al. 2012) were recorded for each clutch. Then, eggs were introduced in small, marked plastic boxes (110 ml) filled with wet vermiculite (1:1, distilled water: vermiculite), that were weighted, and incubated in a thermo-insulation box at 26.6 °C (Gubanyi 2003). For each plastic box, water losses were measured periodically, and distilled water was added to keep initial weights. Eggs were checked daily for freshly emerged hatchlings. Females were released to their collection site one month after oviposition. Within 24 h from hatching, new-borns were measured for SVL with a calliper to the nearest 0.01 mm, weighted (KOBWA, ± 0.001 g precision) and checked for any abnormalities or behavioural alterations. Incubation period was calculated as the number of days between oviposition and pipping. Eggs that appeared to deteriorate were kept until all other hatchlings had emerged before being scored as dead and evaluated as unfertilized eggs or dead embryos using a bright-light candling procedure (Rauschenberger et al. 2007; McDiarmid et al. 2012).

2.3. Locomotor performance

Hatchling locomotor abilities were measured by hand-chasing juveniles on horizontal surface 1 m-long trail on a cork substrate (Van Damme et al. 1997). The track was built in a cardboard box 120 × 15 cm with 30 cm high vertical walls. Before any test, hatchlings were kept for at least 1 h in a terrarium exposed to an infrared light of 150 W to let them thermoregulate and attain their preferred body temperature (Veríssimo & Carretero 2009). Each hatchling was tested three times to estimate individual repeatability, allowing a rest of 10 min between each trial. All running tests were recorded with a digital camera (Canon SX620 HS) at a filming speed of 30 frames per second (fps). Records in which animals completely stopped or turned around during the race were not considered. The position of the lizard across each trial was digitalized using Tracker video analysis software (v5.1.3, Brown & Cox 2009). The maximum performing capacity (MPC), a good representative measure to estimate the physiological limits of the lizards, was calculated as the highest speed recorded among the three trials. For each run, we also estimated the mean maximum speed (MMS) as the speed average across the whole trial for any interval of two sequential frames (Gomes et al. 2017). Since offspring lizards showed very high variability in their performances among trials, we decided to not analyse the MMS. MPC was implemented using Signal package (Signal developers, 2013) for R, version 3.6.3 (R Core Team 2020). At the end of the experiment, offspring were released to the area where the mothers had been collected.

2.4. Statistical analysis

Body condition (BCI) of individual lizards was assessed by calculating the Residual Index, based on the residuals from a regression of ln-transformed body mass against ln-transformed SVL (Brown 1996). To detect differences between females from different treatment managements, a two-way nested ANOVA was conducted using BCI as dependent variable, treatment (conventional and control) and population ID (nested within treatment) as the independent variables.

Spearman rank correlation was performed to analyse the relationships among the clutch size, egg volume (EV), egg mass (EM), hatchling SVL (H-SVL), weight (HW), and female snout-vent length (F-SVL). These analyses were performed for each treatment (control and conventional) separately and False Discovery Rate was adopted to correct alpha value (Benjamini & Hochberg 1995).

Mann–Whitney U-test was used to compare the number of undeveloped eggs (infertile/dead embryos) between treatments. Difference among characteristics of eggs and hatchlings from dissimilar treatments were investigated by general lineal model nested ANCOVAs considering EV, EM, and hatchling body condition (H–BCI) as dependent variable, treatment (conventional and control) and population ID as the factors, and F-SVL as covariate. As for the clutch size and total number of hatchlings, we applied a Generalized linear model (Poisson error distribution and Log link function) with the same factors and covariate specified above. ANCOVA analysis was also performed to determine any possible differences of new-borns maximum performing capacity (MPC) between treatments and populations, considering H-SVL as a covariate. All analyses were performed in STATISTICA v.8.0 (StatSoft Inc, 2007). The assumptions of all analyses were previously verified, and data were logarithmically transformed when necessary.

3. Results

Egg-laying took place on average within 16 days (range 6–35, SE = 2.73) of captivity for control site and 8 days (range 4–16, SE = 1.08) for conventional sites. Overall, 109 eggs (first clutch) were laid. Incubation lasted 36 days on average (range 29–39; SE = 0.57) in both conventional and control sites and a total of 98 new-borns hatched with no external developmental alterations; no hatchlings died during the study. The number of undeveloped eggs (infertile: control = 1, conventional = 3; dead embryos: control = 3; conventional = 4) per clutch did not differ between treatment (Mann–Whitney U-test, median control = 0, conventional = 0, U = 57.50, p = 0.64). Means (±SE) of female SVL (F-SVL), clutch size, egg and hatchling features are reported in Table S2.

No difference of female body condition was detected between treatments ($F_{1,13} = 0.01$, $p = 0.92$). A positive and significant correlation between clutch size and female SVL was found in females from conventional areas ($R_{Spearman} = 0.50$, $p = 0.01$), but not in females from control ones ($R_{Spearman} = 0.52$, $p = 0.12$). Maternal

Table 1
Spearman R coefficient among eggs volume (EV) and mass (EM), hatchling weight (HW) and body length (H-SVL), and female body size (F-SVL) in *Podarcis siculus*. **a** = control fields; **b** = conventional fields. * = significance after False Discovery Rate ($p \leq 0.015$).

	EM (g)	H-SVL	HW	F-SVL
a				
EV (mm^3)	0.325	0.205	−0.127	0.268
EM (g)		−0.120	−0.031	−0.470*
H-SVL (mm)			0.500*	0.100
HW (g)				−0.148
b				
EV (mm^3)	0.369*	−0.040	−0.120	−0.210
EM (g)		0.199	0.098	−0.404*
H-SVL (mm)			0.492*	0.162
HW (g)				0.202

SVL, both in control and in conventional treatments, was negatively correlated with egg mass, while no significant correlation emerged among eggs and hatchling sizes (Table 1). All females laid at least one clutch, and only four of them (two from conventional and two from control treatments) were able to lay a further clutch (Table S1) without new copulation with a male. No statistical analysis for the second clutch were performed due to insufficient sample size.

No effect of treatment on total number of eggs (GLZ, F-SVL Wald = 3.87, d. f = 1,1, p = 0.050; treatment Wald = 0.03, d. f = 1,1, p = 0.87) and hatchlings (GLZ, F-SVL Wald = 5.79, d. f = 1,1, p = 0.02; treatment Wald = 0.04, d. f = 1,1, p = 0.84) was observed. However, both eggs characteristics (EV and EM) were significantly larger in control areas than in conventional ones (Table 2). Offspring from control areas showed larger body condition than those from conventional sites (Table 2). Treatment did not affect offspring maximum performance capacity: MPC (ANCOVA: H-SVL $F_{1,87} = 0.05$, p = 0.83; treatment $F_{1,87} = 3.95$, p = 0.15).

4. Discussion

The understanding of how agricultural practices affect the environment and biodiversity has become an urgent issue for the need to propose ecologically and toxicologically effective strategies aimed at decreasing the contaminants impact on wildlife (Blaustein et al. 2011). Non target species are usually impacted by pesticide exposure through different transfer routes (Amaral et al. 2012). Our study provides new insights of the effects of field management (i.e., pesticide exposure) on female reproductive success. Indeed, although reproduction occurs in both exposed and not exposed females with similar body condition, the eggs and hatchlings from areas subjected to conventional treatments showed lower quality (i.e., smaller size and lower body condition) than those from control areas, with expected repercussions in the mid-term survival of juveniles (i.e., during hibernation; Sinervo & DeNardo 1996). Besides a direct impact of pesticide on offspring via maternal transfer, our findings could further suggest a potential indirect effect of pollutants through the alteration of female's behaviour (i.e. reduced feeding activity; Sasaki et al. 2016) and performances (i.e. lower energy allocation to reproduction; Shine 1980), which in turn may affect offspring development and health. To our knowledge, the effects of pesticide exposure on offspring via maternal transfer of contaminants and parental stress have not been adequately investigated in reptiles yet. Experimental studies on lizard have mainly focused on pesticides influence on eggs and offspring by the absorption of contaminants from the soil trough the eggshell (Marco et al. 2004a; 2004b; 2005; Schaumburg et al. 2015; Trajcheska et al. 2016).

Reproductive success depends on number of offspring but also on the rate of correctly developed embryos and hatchling growth (Sparling et al. 2010). Bigger *Podarcis* lizard females are known to lay more eggs, and the larger the clutch the smaller and lighter the eggs (Biaggini & Corti 2017, 2019). In our study, the size of the mother positively influences the number of the eggs and offspring, as well as hatching body condition. Bigger females from conventional treatments tended to lay more eggs, thus adopting a strategy that prioritises offspring number rather than their size to maximize the reproductive efficiency. According to terminal investment hypothesis, when the likelihood of an organism to reproduce in the future declines, it tends to invest a higher amount of energy into present reproduction (Clutton-Brock 1984). Here, no correlation was found between the size of the mother and the average size of hatchlings (Amaral et al. 2012). In general, the observed clutch size was comparable to that recorded by Capula et al. (1993) for the same species (6.00 ± 1.52 eggs). The egg mass recorded in our populations was larger than reported by In den Bosch & Bout (1998) (0.4 ± 0.05 g) and Biaggini & Corti (2019) (0.369 ± 0.072 g). The incubation period was also slightly higher in our lizards than that observed by Gubanyi (2003) (about 31 days), and by Biaggini & Corti (2019) (32.53 ± 1.02 days). The hatchling weight was comparable to In den Bosch and Bout (1998) (0.5 ± 0.06 g).

Table 2
Nested ANCOVAs testing the effects of treatment (conventional vs. control fields) and population (nested in treatment effect) on eggs volume (EV) and mass (EM), and hatchling body condition (H–BCI). Covariate: F-SVL = female log SVL. Significant effects are marked in bold.

	EV		EM		H–BCI	
	F	p	F	p	F	p
F-SVL	0.544	0.462	0.348	0.556	8.056	**0.006**
Population (Treatment)	3.833	**<0.001**	2.787	**0.008**	2.626	**0.013**
Treatment	9.404	**0.002**	30.798	**<0.001**	4.385	**0.039**

Regarding the difference between control and conventional sites, at equal body condition, females from conventional areas laid lighter and smaller eggs. Moreover, although no morphological malformations were found, hatchlings from control areas showed a better body condition than those from conventional fields. Reproduction phase induces a great stress in females (Shine 1980), and adverse behavioural effects due to chemical application may diminish reproductive success through prezygotic (e.g., reduced abilities to attract mates, or impaired timing and type of breeding behaviour) or postzygotic (e.g., egg attendance, hiding, carrying, and offspring; Gross & Shine 1981) mechanisms (Sparling et al. 2010). Considering that the pesticides released in the study fields are lipophilic compounds, they could be transferred to the eggs at each spawn (Sparling et al. 2010). Tebuconazole and the pyrethroid insecticides were found highly hazardous for new-borns' developmental rates in amphibians (Berrill et al. 1993; Bernabò et al. 2016) and zebrafish (Li et al. 2019). Lipophilic contaminants in the eggs from the first clutch probably derived from lipids stored in females during the previous season through dietary intake from polluted areas (Bishop et al. 1994; Carretero 2006). In reptiles, different studies on alligators and turtles demonstrated the importance of maternal transfer of lipophilic pollutants (Bishop et al. 1994; Nagle et al. 2001; Rauschenberger et al. 2007), resulting in high contaminants burdens in the eggs and being responsible for a wide range of developmental abnormalities (i.e., deformed tail, missing limb or developmental asynchrony, skull not fused and abnormal gonadal morphology) (Bell et al. 2006; Van Meter et al. 2006). In this study, we failed to detect any malformations, probably because (i) the pesticides in our system do not have the potential to alter offspring quality and survivorship; or (ii) the maternal pollutant burden alone was not strong enough to induce morphological abnormalities. Since both maternal and egg environmental absorption routes are likely to act with potential synergic effects, it will be interesting to analyse and compare both routes simultaneously in future studies to address whether soil contamination could affect in some way the maternal transfer outcomes. Moreover, further data will be necessary to support our results.

We could not test any effect of treatments on the second oviposition due to the very small dataset, and because lizards were captive fed. Indeed, in lacertid lizards, the first clutch is mainly constructed from stored reserves, but later clutches depend on current energy intake (Braña et al. 1991). Although there are not ecotoxicological studies on female's investment in second clutches, it is known that offspring from later clutches are often at a disadvantage in respect to their earlier siblings because of lack of time to secure energy stores needed for overwintering and of poor competitive abilities compared with the larger offspring from earlier clutches. Furthermore, Braña & Ji (2000) showed how in *Podarcis muralis* (Laurenti, 1768) eggs from first clutches were larger than those from subsequent clutches, likely because total energy investment was largely reduced in later clutches, so that any increase in egg size would represent a reduction of fecundity. Thus, we can hypothesize that apparently healthy females living in a stress environment (i.e., polluted areas) are subjected to even higher conflicting energy demands, such as those for reproduction, foraging, and basking activities with consequently poor reproduction investment and offspring fitness success.

The deposition of second clutches without previous copulation was an interesting result. By estimating the time spanning from the day of capture to the day of the last fertile clutch, we can assess an average female sperm storage of 25 days (range: 20–36; SE = 3.84). In lizards, the ability to store sperm has been proved in some species (e.g. *Calotes versicolor* (Daudin 1802): Shanbhag, 2003; *Anolis sagrei*, Duméril & Bibron, 1837: Calsbeek et al., 2007; *Ctenophorus pictus* (Peters 1866): Olsson et al., 2007) including lacertids (Zotos et al. 2012) as (i) necessary reproductive choice when the mating season does not match the time of egg production (Uller & Olsson 2008); (ii) the need to avoid repeated mating to reduce risk of predation (Shanbhag 2003); (iii) facilitation of female choice and sperm competition (Olsson & Madsen 1998). Since in our system mating and eggs production occurred within the same season, and females probably copulated multiple times (≥2 Carretero et al. 2006), the first two hypotheses may be more unlikely. On the other hand, sperm competition has been confirmed in the genus *Podarcis* (Carretero et al. 2006). To our knowledge, there is only a previous study on the congeneric lizard *P. muralis* in urban areas showing female inability to store sperm for medium or long-term and lay fertile eggs (Pellitteri-Rosa et al. 2012). Further comparative studies on sperm storage in *P. siculus* populations living in different environments (natural, urban, and rural areas) are needed to understand if sperm storage does constitute a selective advantage for such species living in stressed environment and verify the consistency of our results.

Locomotor performance has an important biological relevance in lizards and can determine the success in crucial events as prey capture (Webb 1986), predator evasion (Huey & Dunham 1987; Vanhooydonck & Van Damme 2003), and social dominance (Garland et al. 1990). In our study, no difference in locomotor performance was found in hatchlings from different environments, although hatchling from control areas showed higher body condition. Although there are no other studies in lizards using the locomotor abilities as indicator of pollutant effect by maternal transfer to offspring, our results matched previous locomotor performance assays performed using lizards exposed to different pesticides (Holem et al. 2006; 2008; DuRant et al. 2007; Amaral et al. 2012), or new-borns hatched from eggs subjected to perturbation of soil quality (Marco et al. 2004a). By contrast, Marco et al. (2004b) demonstrated decreased locomotor ability and consequently increase of energetic cost of locomotion following egg incubation in arsenic contaminated substrate. Further studies will be necessary to disentangle whether performance assays on hatchlings represent a sufficiently sensitive tool to evaluate the consequences of pesticide exposure by both maternal transfer or/and eggs absorption.

In conclusion, our study suggests that the reduced values of eggs and hatchlings fitness could be the result of pollution-induced changes in the biotic and abiotic environments, in addition to previously reported directs effects of pollution on eggs (Marco et al. 2004a, b). Further investigations are needed to fully clarify this point through an integrated examination of exposure to different chemicals, a consistent measure of their direct and indirect effects, and the use of non-destructive biomarkers (i.e., locomotor performance, growth rate and morphology abnormalities). Moreover, experimental designs both in laboratory and in the field should be expected to better unravel how pesticide application may affect lizard reproductive output and offspring survival.

Author contributions

GS contributed to the study conception and design. Data collection was made by GS. Captive breeding was performed by GS and DM. Data analysis was performed by GS and VG. The first draft of the manuscript was written by GS, VG, LV, and MAC. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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.jcz.2021.06.001.

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