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RESEARCH ARTICLE

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Water availability and temperature induce changes in oxidative status during pregnancy in a viviparous lizard

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Abstract

1. Reproduction involves considerable reorganization in an organism's physiology that incurs potential toxicity for cells (e.g., oxidative stress) and decrease in fitness. This framework has been the cornerstone of the so-called 'oxidative cost of reproduction', a theory that remains controversial and relatively overlooked in non-model ectotherms.
2. Here, we used two complementary approaches in natural and controlled conditions to test whether altered access to climate conditions (water and temperature resources) alters oxidative status and mediates reproductive trade-offs in viviparous populations of the common lizard (*Zootoca vivipara*).
3. First, we examined whether access to free-standing water and differences in ambient temperature across 12 natural populations could be related to variation in oxidative status, reproductive effort and reproductive success. Second, we determined whether an experimental restriction to water triggers higher oxidative cost of reproduction and correlates with fitness measures (reproductive success, future survival rate and probability of future reproduction).
4. Pregnant females exhibited higher sensitivity than males to natural or experimental limitations in temperature and water access. That is, in restricted environments, pregnant females with higher reproductive effort exhibited stronger oxidative damage despite enhanced non-enzymatic antioxidant capacity.
5. Enhanced antioxidant defensive capacity in pregnant females was positively correlated with higher reproductive success, whereas elevated oxidative damage negatively correlated with offspring annual survival.
6. Altogether, our results revealed a context-dependent oxidative cost of reproduction that was concomitant with a conflict in water demand from offspring. These new insights should be critical for understanding ectotherm responses to heat waves and summer droughts that are increasing in frequency and duration.

KEYWORDS
antioxidant capacity, ectotherm, fitness, pregnancy, reactive oxygen metabolites, temperature, water

1 | INTRODUCTION

A central tenet to life-history theory is the importance of trade-offs in resource allocation within and between individuals in shaping reproductive strategies (Harshman & Zera, 2007; Kölliker et al., 2015; Stearns, 1992). Mobilization and allocation of resources to reproduction are linked with a myriad of physiological effects that are correlated with increased metabolic rate and hormonal signals (Harshman & Zera, 2007). In a wide diversity of species, an upstream burst in metabolic activity during reproduction can induce downstream toxicity in cells and physiological stress (Bonier, Moore, & Robertson, 2011; Dupoué et al., 2016; Harshman & Zera, 2007). For example, enhanced mitochondrial activity during reproduction inevitably generates metabolic by-products (reactive oxygen species) that can exceed the defensive barrier of antioxidant capacity and expose cells to oxidative stress (Costantini, 2014; Dowling & Simmons, 2009). Given that oxidative stress in cells can damage the whole organism, it is commonly viewed as a cost of high reproductive investment, referred hereafter as the 'oxidative cost of reproduction' (Alonso-Alvarez et al., 2004; Costantini, 2008; Metcalfe & Alonso-Alvarez, 2010; Stier, Reichert, Massemin, Bize, & Criscuolo, 2012; Viblanc et al., 2018).

Despite increasing evidence supporting the occurrence of an 'oxidative cost of reproduction', examples showing no change in oxidative status of breeding individuals (Garratt et al., 2011) or even a reduction in oxidative damage during reproduction (Oldakowski et al., 2012) have challenged this theory. In evaluating the equivocal evidence, two recent reviews concluded that characterization of the oxidative mediation of reproductive trade-offs may be more efficient in resource restricted environments (Metcalfe & Monaghan, 2013; Speakman & Garratt, 2014). This implies that relationships between reproductive effort and oxidative status may be exacerbated in altered and resource limiting environments (Cote, Meylan, Clobert, & Voituron, 2010; Isaksson, Sheldon, & Uller, 2011).

An interesting aspect of reproduction in ectothermic vertebrates is that most species are lecithotrophic, meaning that embryonic development depends on an energy resource (yolk) produced before ovulation (Van Dyke, Griffith, & Thompson, 2014). In squamate reptiles, this mode of provisioning to the developing embryo implies a temporal dissociation in resource allocation during reproduction. The major allocation of energy to reproduction in females occurs during vitellogenesis, which may involve higher oxidative damage and lower antioxidant defences (Webb, Iverson, Knapp, DeNardo, & French, 2019). Post-ovulation and up to oviposition (oviparous species) or parturition (viviparous species), maternal reproductive effort is then oriented onto regulation of body temperature through behavioural means and water/oxygen supply, so that embryos can convert yolk into somatic tissues (Lourdais, Lorioux, Dupoué, Wright, & DeNardo, 2015; Vleck, 1991). Therefore, constraints in access to temperature or water resources can have profound effects on mothers and offspring and influence a female reproductive success (Le Galliard, Massot, Baron, & Clobert, 2012; Lorenzon, Clobert, Oppliger, & John-Alder, 1999; Lorioux et al., 2013; Marquis, Massot, & Le Galliard, 2008). Notably, given the dual requirement of mother and embryos for free water, restriction of water during pregnancy in viviparous species may exacerbate costs of reproduction (Lourdais et al., 2017) and intergenerational conflicts (Dupoué et al., 2015; Dupoué, Le Galliard, et al., 2018). Understanding the proximate mechanism of reproductive trade-offs induced by oxidative status remains elusive [but see (Stier et al., 2017)]. Given the lack of data, there is a critical need to examine the relationships between oxidative status and fitness outcomes, such as long-term survival or future reproduction (Speakman et al., 2015).

The goal of this study was to investigate whether variation in temperature and limitations in water availability amplify the oxidative cost of reproduction and correlate with delayed fitness costs in the viviparous form of the common lizard (*Zootoca vivipara*). We first investigated how oxidative status relates to reproduction and environmental conditions in natural populations using a 'cross-sectional study'. We compared variation in oxidative status among pregnant females and adult males from 12 natural populations distributed across a gradient of air temperature and water availability (permanent in humid peat bogs vs. periodic in dry meadows, Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017; Dupoué, Rutschmann, et al., 2018). We subsequently examined the effects of water restriction on oxidative status in pregnant females and adult males, and reproductive effort in females using an 'experimental study'. We induced a two-week period of water restriction during pregnancy, a protocol known to trigger hydric costs of reproduction and mother–offspring conflict for water (Dupoué, Le Galliard, et al., 2018). An important aspect of the studied populations is that all adult females engage in reproduction so that we cannot quantify the cost of pregnancy (Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017). That is, reproductive female *Z. vivipara* commit energy to a litter prior to fertilization. Thus, all females carry eggs through the breeding season. However, a fraction of females carry unfertilized eggs and do not give birth to viable offspring.

In each study, we compared how pregnant females and males responded to environmental conditions, which is a valid but not sufficient approach to quantify oxidative cost of reproduction (Olsson, Healey, Perrin, Wilson, & Tobler, 2012). Therefore, we examined how resource allocation trade-offs shape the relationships between oxidative status and reproductive effort (litter mass) of females, given that it may depend on both environmental conditions and individual morphotype [linear, reticulated or both (Lepetz, Massot, Chaine, & Clobert, 2009)]. We hypothesized oxidative cost of reproduction to occur in this species and to depend on environmental conditions. We predicted females to display higher changes in oxidative status than males, and we expected female responses to be influenced by the level of reproductive effort. We also expected these relationships to be amplified in colder habitats or when water access was scarce. In the experimental study, we investigated the delayed effects of maternal changes in oxidative status on fitness estimates including reproductive success, future survival of mother and offspring and females' future reproduction. Given the species high affinity for hydric conditions, we also predicted that limited access to a water resource would increase the oxidative cost of reproduction and mediate the intergenerational conflicts previously illustrated (Dupoué, Le Galliard, et al., 2018).

2 | MATERIALS AND METHODS

2.1 | Study species

The European common Lizard, *Zootoca vivipara*, is a small (adult snout-vent length, SVL ~50–75 mm), widespread species in the family Lacertidae. The species is typically found in cold and mesic habitats (marsh, peat bogs) across northern Eurasia. In this species, both parity modes exist with oviparous (egg-laying) and viviparous (live-bearing) populations (Heulin, Osenegg-Leconte, & Michel, 1997). These two forms are geographically dissociated in most of the distribution range, and even in areas of syntopy, they are genetically distinct and reproductively isolated from each other (Cornetti et al., 2015). At our study sites, *Z. vivipara* is strictly viviparous and contains individuals of the main three dorsal morphotypes. Parturition occurs from late June through July with females giving birth to an average of 6 neonates (range 1–12) that are immediately autonomous.

2.2 | Cross-sectional study

2.2.1 | Capture and husbandry

In June 2017, we caught 159 adult pregnant females and 103 adult males from 12 populations distributed throughout the Massif Central mountain range (see Table S1). Lizards were captured by hand. We obtained blood samples (ca. 60 µl whole blood) from the post-orbital sinus within 3 min post-capture (Meylan, Dufty, & Clobert, 2003). Samples were kept fresh in a cooler on ice and brought back to a field laboratory the same day. In the laboratory, blood samples were centrifuged for 5 min at 13,500 x *g*; plasma and blood cells were separated and kept frozen at −28°C in airtight tubes until subsequent analyses.

Adult females were transferred to the laboratory and housed in individual terraria (18 × 12 × 12 cm) with sterilized soil, a shelter, and basking opportunities until parturition. Each lizard was provided a 20–30°C thermal gradient for 6 hr per day (09:00–12:00 and 14:00–17:00) using a 25 W incandescent light bulb placed over one end of each terrarium. We also provided water three times per day at 09:00, 13:00 and 17:00 to mimic natural conditions found in habitats with permanent access to water. We fed lizards with 2 crickets (*Acheta domesticus*) every two days. We maintained females in these conditions until parturition. After the birth of the neonates, we measured litter mass as an estimator of reproductive effort (Dupoué & Lourdais, 2014). We released each female together with offspring at the exact capture location within three days after parturition.

2.2.2 | Population characteristics

We characterized environmental conditions relevant to water balance regulation (Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017) and physiological stress levels (Dupoué, Rutschmann, Le Galliard, Clobert, et al., 2017; Dupoué, Rutschmann, et al., 2018) for each population. We first considered the presence of permanent versus periodic water sources (i.e. in peat bog vs. meadow type habitats) available to the lizards, because this determines the availability of free-standing water during the summer (breeding) season (Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017). All studied populations have natural spatiotemporal heterogeneity in vegetation structure and microclimate, but our water access index is a reliable and integrative description of hydric conditions (Dupoué et al., 2018; Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017). We also recorded air temperature using three data loggers (Thermochron iButtons, Maxim Integrated Products, Sunnyvale, CA, USA, ± 0.0625°C) per population to measure thermal conditions (Dupoué, Rutschmann, Le Galliard, Clobert, et al., 2017; Dupoué et al., 2018). Loggers were placed at microhabitats where we found most lizards, that is within vegetation at ground level and completely shaded to avoid the effect of radiation. For all populations, we extracted the daily minimum and maximum temperatures (T_{min} and T_{max}, respectively) within the same time frame from 25 June to 21 July (Table S1).

2.3 | Experimental study

2.3.1 | Capture and husbandry

In May and June 2016, we caught 99 adult females and 58 adult males from 24 outdoor enclosures (10 × 10 m) at the Centre de Recherche en Ecologie Expérimentale et Prédictive (Saint-Pierre-lès-Nemours, France, 48°17′11.42N, 2°40′46.00E), as previously described (Josserand et al., 2017). At capture, each lizard was identified with a unique toe-clip code, measured (snout-vent length: SVL ± 0.5 mm) and weighed (body mass: BM ± 1 mg). Lizards were individually housed in terraria (25 × 15 × 16 cm) under husbandry conditions as extensively detailed previously (Dupoué, Le Galliard, et al., 2018). They were maintained in a temperature gradient (17–38°C) for 6 hr per day, standardized mass of food (400 ± 20 mg of *Acheta domesticus*), a water bowl and three sprays of water per day with similar timing and water quantity as in the cross-sectional study (see above). Adult males were released a few days after the experiments. Pregnant females were maintained in captivity until parturition to measure reproductive effort. Immediately after parturition, the neonates were separated from their mother, given a unique mark by toe clipping and measured.

We estimated offspring and mother annual survival rate, and the subsequent reproductive effort of a female in the next breeding season by recapturing individuals after a year between May and June 2017. On the day of capture, lizards were identified by their unique toe-clip code, measured, weighed and then housed in terraria. We estimated lizard survival with a high degree of reliability thanks to a recapture rate close to 1 (Le Galliard, Fitze, Ferrière, & Clobert, 2005). Pregnant females were further monitored as described above until parturition to assess their next reproductive effort.

2.3.2 | Experimental design

We randomly assigned adult males and females to one of two hydric treatments following a previously established procedure to contrast both air humidity and access to free-standing water (Dupoué, Le Galliard, et al., 2018; Lorenzon et al., 1999). In the control treatment, lizards were maintained in the same conditions as described above (females: *n* = 56, males: *n* = 32). In the water-restricted treatment, we removed the water bowl and reduced the misting frequency to once per day occurring at 09:00 during 17 days (females: *n* = 43, males: *n* = 26). The control treatment mimics habitats with permanent access to water (e.g. peat bog, marsh), whereas the water-restricted treatment reflects drier conditions (e.g. heath, dry meadows) in which, during summer, morning dew is the only source of free water. Female reproductive timing (parturition date – experimentation dates) did not differ between treatments (t_{98} = −1.0, *p* = .320), and exposure to hydric treatments occurred on average from 32 to 15 days before parturition depending on inter-individual differences in parturition dates. After the period of water restriction, all individuals were returned to the control water conditions, having ad libitum access to water in a water bowl and being misted three times per day.

Lizards were bled at the beginning and the end of the water restriction period using the same protocol as in the field study to assess changes in hydration state and plasma oxidative status (Table 1). Plasma was separated from the packed red blood cells, aliquoted into two 10 µl samples and kept frozen at −28°C in airtight tubes until analyses were performed. One plasma sample was used to measure plasma osmolality, the best indicator of hydration state in vertebrates with a non-functioning salt gland, using a protocol previously described (Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017). The other sample was used to assess oxidative status as described below.

2.4 | Oxidative status assays

We assessed two markers of oxidative status within 9 months after blood sampling, and colorimetric assays were conducted on separate

TABLE 1 Physiological responses following the exposure to water restriction

	Females		Males		Statistical effect of			
	Control	Water-restricted	Control	Water-restricted	Initial value	Sex	Treatment	Sex* Treatment
ΔBM (g)	18.77 ± 0.96	−1.51 ± 1.62	0.67 ± 0.62	−6.77 ± 1.12	*p* = .068	*p* < .001	*p* < .001	*p* < .001
Δosmo (mOsm/kg)	1.98 ± 4.54	56.17 ± 6.62	−6.46 ± 5.36	30.90 ± 8.50	*p* < .001	*p* = .022	*p* < .001	*n.s.*
ΔROMs (mgH_2O_2/dl)	0.72 ± 0.15	1.32 ± 0.26	0.77 ± 0.23	1.64 ± 0.28	*n.s.*	*n.s.*	*p* < .001	*n.s.*
ΔOXY (µmol HClO/ml)	−36.97 ± 10.98	30.40 ± 16.41	−70.23 ± 11.50	5.96 ± 13.27	*p* < .001	*p* = .037	*p* < .001	*n.s.*

Note: Table reports the mean (± *SE*) of changes in body mass (ΔBM) and plasma osmolality (Δosmo) as indexes of changes in hydration state, and the changes in oxidative status including reactive oxygen metabolites (ΔROMs) and non-enzymatic antioxidant capacity (ΔOXY). Table also reports the statistical differences between water-restricted and control individuals within each sex. See text for details.

days (experimental study: 6th–19th January 2017, cross-sectional study: 12th–23rd March 2018). In both the experimental and cross-sectional studies, we balanced both sexes on each plate, respectively, as well as populations and treatment. First, we measured the ROMs in plasma (4 µl) as an index of oxidative damage. The d-ROMs colorimetric kit (MC003, Diacron International, Italy) measures the plasma concentration of ROMs, a reliable index of oxidative damage, related to the activity of organic hydroperoxides (Costantini, 2016). Samples were read at 540nm after 75min of incubation at 37°C. We used the same plasma sample to measure the antioxidant capacity (5 µl of plasma diluted 1:100) using the OXY-absorbent test (MC435, Diacron International, Italy). This kit measures the plasma non-enzymatic antioxidant capacity to neutralize a massive oxidant attack from hypochlorous acid (Costantini, 2011). After 10min of incubation at 37°C, samples are read at 540nm. In the experimental study, we used rabbit plasma measured at least eight times in each plate (n = 9 plates in total) to assess coefficients of variation in ROMs (intra-plate: 2.7%; inter-plate: 4.9%) and OXY (intra-plate: 10.9%; inter-plate: 12.9%). In the cross-sectional study, we used a pool of lizard plasma measured at least 6 times in each plate (n = 4 plates in total) to assess coefficients of variation in ROMs (intra-plate: 18.7%; inter-plate: 20%) and OXY (intra-plate: 7.8%; inter-plate: 11.2%). Variations in ROMs of lizards from the cross-sectional study were relatively high so that associated results should be interpreted cautiously.

2.5 | Statistical analyses

We analysed the variation of oxidative status using R software (R Development Core Team, version 3.2.0, http://cran.r-project.org/).

In the cross-sectional study, we analysed the natural variation in ROMs and OXY using linear mixed models [package *lme4* (Bates, Maechler, Bolker, & Walker, 2015)] including the fixed effects of sex and morphotypes (linear, reticulated or both), while accounting for population and assay plate as random effects. We lacked data on reproductive effort for 3 populations, because the females were captured, bled and released the same day. We further investigated the fixed effects of environmental temperatures (T_{min} and T_{max}) and water access (permanent or periodic) within each sex. We compared mixed-effect linear models in which population identity was included as a random effect to account for repeated sampling within the same population (Tables S2 and S3). To determine whether oxidative status co-varies with main or additive effects of temperature and/or water conditions, we used the Akaike information criterion corrected for small sample size (AICc, package AICcmodavg, Mazerolle 2017) to compare a range of models. Our set of models included a model with only the intercept (i.e. null model) that was compared with models testing the effects of temperature (linear or nonlinear) and water access, either alone or in addition (Table S2). We used a log transformation of temperature covariates to test nonlinear relationships since this procedure provides better results than using quadratic term (Dupoué, Rutschmann, Le Galliard, Clobert, et al., 2017). We used a similar approach to investigate the effects of environmental conditions and of female morphotype (linear, reticulated or both) on reproductive effort with litter mass as the response variable (Table S3).

In the experimental study, we used linear mixed models to test whether the changes in ROMs (ΔROMs) and changes in OXY (ΔOXY) following hydric treatment were impacted by the fixed effects of initial values of ROMs and OXY (at the onset of hydric treatment), morphotypes, sex, hydric treatment, and the interaction between sex and treatment, while accounting for assay plate as random factor. Given that initial and final sampling sessions were measured in two different plates for each individual, and because we analysed the changes (ΔROM and ΔOXY) with initial value as linear covariate, we included the plate of the final sample as random term. We used this approach (i.e. analyse of physiological change) because we measured oxidative status only twice and repeated measurements are best modelled by a single measurement of intra-individual change in response variables (Dupoué, Le Galliard, et al., 2018). Second, it allows us to obtain an individual measure to further investigate the correlations with reproductive effort and fitness estimates. In pregnant females, we used similar model constructions to test the correlation between ΔROMs or ΔOXY, reproductive effort and reproductive timing. We used logistic regression with a logit link and binomial error term to analyse variation in reproductive success on the day of parturition (number of viable neonates vs. number of stillborn, aborted or undeveloped eggs). We analysed the effects of hydric treatment, ΔROMs or ΔOXY and their interaction on long-term fitness estimates with mixed-effects models (logistic for annual survival rates and linear for next reproductive effort) with enclosure into which the lizard was released as a random factor.

In both studies, preliminary investigations of the data showed that ROMs were positively correlated with OXY depending either on sex in the cross-sectional study (Figure S1a, b) or on hydric treatment in the experimental one (Figure S1c, d). Therefore, results are presented hereafter for each marker of oxidative status separately.

3 | RESULTS

3.1 | Cross-sectional study

In natural populations, pregnant females and males had similar levels of ROMs (respectively: 5.77 ± 0.24 and 5.78 ± 0.26 mgH_2O_2/dl; $t_{249.0}$ = 0.2, p = .855). However, females exhibited higher levels of OXY than males (256.1 ± 4.4 and 240.3 ± 3.9 µmol HClO/ml; $t_{247.4}$ = 2.2, p = .032, respectively). In both sexes, morphotypes did not differ in ROMs and OXY (all p > .677). In pregnant females, litter mass positively correlated with both ROMs ($t_{129.2}$ = 2.0, p = .045; Figure 1a) and OXY ($t_{127.8}$ = 4.1, p < .001; Figure 1b), independent of the population water access (interaction terms, all p > .324). Litter success was positively correlated with litter mass (z = 6.2, p < .001). It was not correlated with levels of ROMs (z = 0.1, p = .899; Figure 1c), but it was influenced by an interaction between access to water and

OXY ($z = -2.1$, $p = .033$). That is, litter success positively correlated with OXY in populations with permanent access to water ($z = 3.2$, $p = .002$; Figure 1d), whereas no correlation occurred in populations with periodic access to water ($z = -0.8$, $p = .410$; Figure 1d).

Model selection showed that oxidative status markers were more sensitive to environmental conditions in females than in males (Table S2). That is, T_{max} negatively correlated with concentration of ROMs in pregnant females (T_{max}: $t_{159.0} = 5.7$, $p < .001$, $\log(T_{max})$: $t_{159.0} = -5.9$, $p < .001$; Figure S2a) but not in males (Table S2). Moreover, OXY had a negative correlation with T_{max} ($t_{44.1} = -5.3$, $p < .001$; Figure S2b), and was stronger when pregnant females faced a permanent access to free-standing water than a periodic source ($t_{156.3} = 3.5$, $p < .001$). In males, OXY was negatively correlated with T_{max} (T_{max}: $t_{10.6} = 3.1$, $p = .011$, $\log(T_{max})$: $t_{10.7} = -3.2$, $p = .009$) and was independent from access to water (Table S2). In pregnant females, T_{max} also negatively influenced litter mass ($t_{10.2} = -2.8$, $p = .020$; Figure S2c) independently of the access to free-standing water (Table S3).

3.2 | Experimental study

At the onset of experiments, pregnant females had lower initial levels of ROMs and OXY than males (respectively: ROMs: 2.76 ± 0.06 and 3.12 ± 0.09 mgH_2O_2/dl; $t_{145.1} = 4.0$, $p < .001$; OXY: 208.8 ± 8.2 and 229.8 ± 9.2 µmol HClO/ml; $t_{146.0} = 2.6$, $p = .009$), irrespective of treatment affiliation (all $p > .236$). Following the two-week period of water restriction, both males and pregnant females exhibited similar and significant dehydration levels that were positively correlated with the increase in ΔROMs and ΔOXY compared with control individuals (Table 1). The pattern was unrelated to their morphotypes (all $p > .070$). Changes in oxidative status were positively correlated with changes in plasma osmolality only in pregnant females (Figure S3).

In pregnant females, litter mass positively correlated with ΔROMs ($t_{80.0} = 2.6$, $p = .012$; Figure 2a) and ΔOXY ($t_{82.4} = 2.0$, $p = .044$; Figure 2b) in water-restricted females, whereas no relation occurred in the control (all $p > .421$; Figure 2a,b), and regardless of female reproductive timing (all $p > .098$). Litter success was positively correlated with litter mass ($z = 3.0$, $p = .003$). It was also either impacted by the interaction between hydric treatment and ΔROMs ($z = 2.1$, $p = .037$) or hydric treatment and ΔOXY ($z = -2.1$, $p = .037$). First, litter success tended to decrease with increasing ΔROMs in control females ($z = -1.7$, $p = .086$; Figure 3a), whereas no relation occurred in water-restricted females ($z = 1.2$, $p = .234$; Figure 3a). Second, litter success was positively correlated with ΔOXY in water-restricted females ($z = 2.0$, $p = .045$; Figure 3b) but not in controls ($z = -1.0$, $p = .306$; Figure 3b). Female annual survival rate and next reproductive effort were uncorrelated with ΔROMs or ΔOXY (all $p > .265$). Offspring annual survival rate was negatively correlated with maternal ΔROMs ($z = -2.2$, $p = .030$; Figure 3c) in both maternal water treatments (interactive terms: $z = 0.0$, $p = .977$) and was not correlated with maternal ΔOXY ($z = -0.6$, $p = .533$; Figure 3d).

4 | DISCUSSION

Oxidative stress is often considered as a causal mechanism underlying the trade-off between current reproductive investment

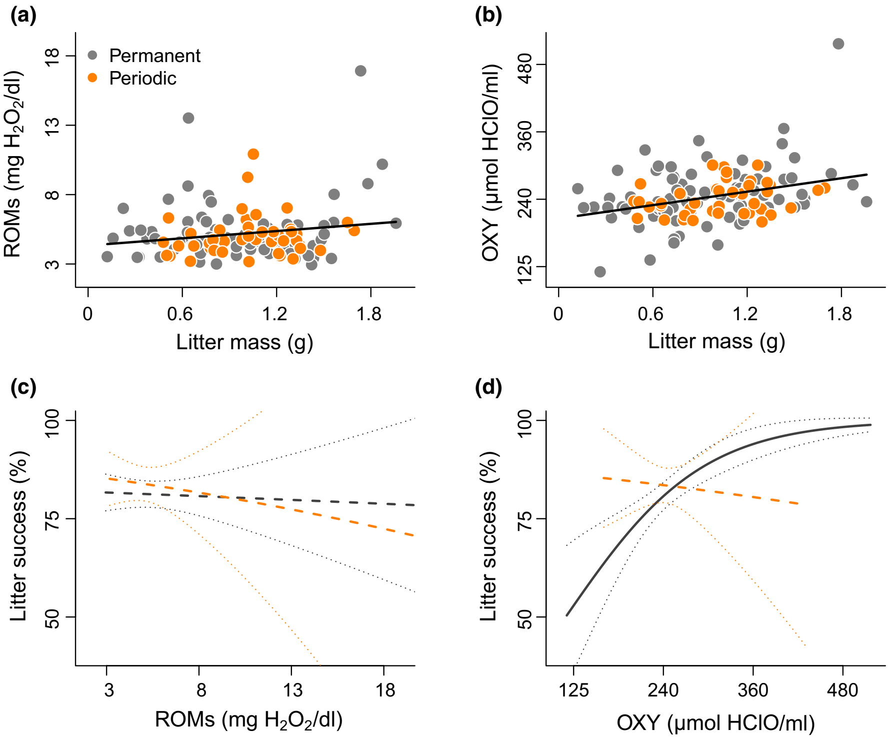

FIGURE 1 Correlations between two markers of oxidative status and reproductive effort (litter mass) and reproductive success (viable offspring vs. nonviable offspring) in pregnant females from natural populations with either permanent (grey points) or periodic (orange points) access to free-standing water. The regression lines of selected models are illustrated when relationships are significant (solid lines) or non-significant (dashed lines). Litter mass was positively correlated with both (a) the level of oxidative damage (ROMs) and (b) the non-enzymatic antioxidant capacity (OXY). ROMs were not correlated with litter success (see c), while OXY positively influenced litter success in populations with permanent access to free-standing water (see d)

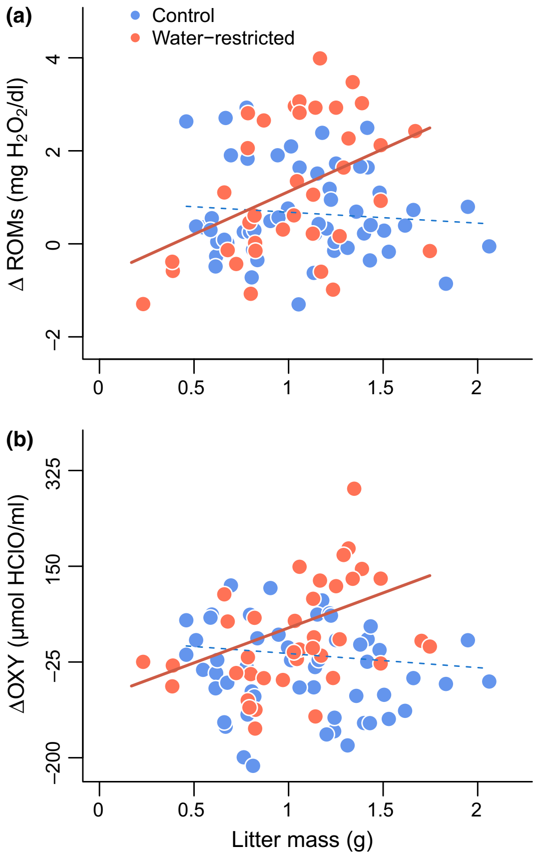

FIGURE 2 Relationships between reproductive effort and changes in maternal oxidative status in pregnant females following a two-week period of water restriction or a control rearing. The regression lines of selected models are illustrated when relationships are significant (solid lines) or non-significant (dashed lines). Both (a) the changes in reactive oxygen metabolites (ΔROMs) and (b) the changes in non-enzymatic antioxidant capacity (ΔOXY) were positively correlated with litter mass in water-restricted females (red circles and lines), whereas no correlation was found in control females (blue circles and lines)

and future survival or reproductive investment (Costantini, 2008; Metcalfe & Monaghan, 2013; Speakman & Garratt, 2014). In this study, we investigated whether an increased investment in reproduction altered oxidative status when lizards had limited access to water and temperature. Our analyses showed that pregnant females from colder environments with periodic water availability had higher non-enzymatic antioxidant levels in relation to reproductive effort. The enhanced defensive capacity was also positively correlated with reproductive success. However, pregnant females displayed higher oxidative damage as a consequence of restricted access to water, concomitant with the conflicting demands imposed by their offspring. Our results highlight that reproductive trade-offs can be modulated by oxidative status under resource limitations.

The two markers of oxidative status were positively correlated depending either on sex (cross-sectional study) or when lizards were in challenging physiological state (i.e. cumulative constraints of pregnancy and water restriction). Positive associations between ROMs and OXY also depended on context in endotherms, suggesting that oxidative stress is amplified in restricting environments (Récapet, Arrivé, Doligez, & Bize, 2019). We found that pregnant female lizards had higher non-enzymatic antioxidant capacity than males and experienced higher changes in oxidative status when resources are scarce. A common pattern in oviparous reptiles is for breeding females to exhibit higher sensitivity than male reptiles (Costantini, 2018). Here, we provided evidence that a viviparous species may also experience a potential extra-cost of reproduction in females (Costantini, 2018). In *Z. vivipara*, all adult females ovulate during reproductive season and invest in egg production even in the absence of mating opportunity (Bleu, Le Galliard, Meylan, Massot, & Fitze, 2011), so non-reproductive females were lacking in our study. This lack of a true control precludes our ability to conclude whether or not there is an oxidative cost of reproduction. However, sexual differences and positive correlations with reproductive effort demonstrated *a minima* oxidative cost of reproduction.

Antioxidant capacity was positively correlated with reproductive effort in pregnant females. Boosted defensive activity against oxidation during reproduction has been previously documented in ectotherms (Meylan, Haussy, & Voituron, 2010; Olsson et al., 2012) and recently considered as an underlying mechanism of oxidative shielding (Blount, Vitikainen, Stott, & Cant, 2016). That is, reproductive females may increase antioxidant capacity prior to gestation to prevent harmful action of oxidative damage to either them or their embryos, which is documented in endotherms (Blount et al., 2016; Viblanc et al., 2018; Vitikainen et al., 2016). However in both our cross-sectional and experimental analyses, an increase in antioxidants was obviously not sufficient to fully shield the increase in oxidative damage. Based on our combined approach, our results followed the prediction that temperature and water may modulate such changes in oxidative status during reproduction (Stier et al., 2017). As an heliothermic ectotherm with affinities for relatively wet habitats, the common lizard has a strong dependence on basking opportunities and water availability for individual maintenance and annual reproduction (Bleu et al., 2013; Lorenzon et al., 1999; Marquis et al., 2008). Oxidative status changes at the individual level were positively associated with the rate of dehydration measured by changes in plasma osmolality, especially in pregnant females compared with males (Figure S3). Thus, lower plasma water content in dehydrated individuals might have contributed to increase oxidative marker concentration per plasma volume, especially in pregnant females. In other words, changes in oxidative markers might be due to a 'concentration' effect caused by dehydration and not solely to an increase in the production and release of oxidative markers in the blood plasma. Irrespective of underlying mechanisms, the end result was a higher concentration of ROMs or OXY per plasma volume in dehydrated lizards, with associated consequences for oxidative balance.

In addition, we found that fitness could be correlated with markers of oxidative status contingent on water availability. The boost in non-enzymatic antioxidant capacity in water-restricted females was positively related to offspring developmental success, although no relation was evident in control females. Positive associations between

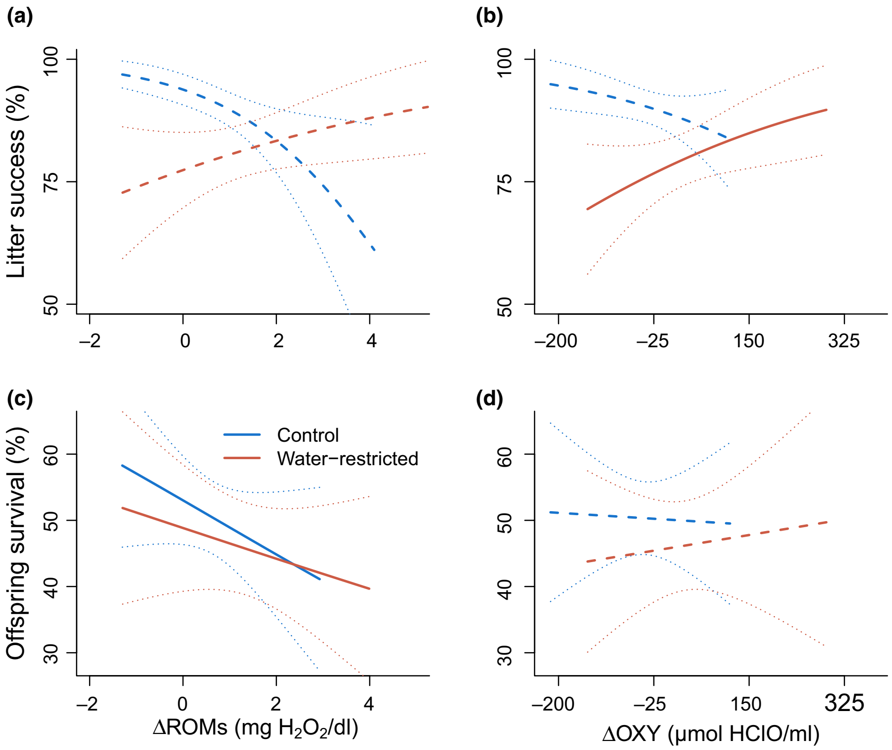

FIGURE 3 Relationships between the change in maternal oxidative status following a two-week period of water restriction vs. a control rearing and fitness estimates, including litter success (the proportion of viable neonates) and the annual survival rate of the offspring. The regression lines of selected models are illustrated when relationships are significant (solid lines) or non-significant (dashed lines). Changes in maternal reactive oxygen metabolites (ΔROMs) did not correlate with reproductive success in control (blue lines, see a) nor in water-restricted females (red lines, see a). Maternal increase in non-enzymatic antioxidant capacity (ΔOXY) positively correlated with reproductive success in water-restricted females (solid red line, see b). Increase in maternal ΔROMs, but not ΔOXY, negatively impacted offspring annual survival (see c and d)

maternal antioxidant capacity and reproductive success are consistent with results from other vertebrates (Møller, Karadas, & Mousseau, 2008; Ogilvy, Preziosi, & Fidgett, 2012). In natural populations, we also found a positive correlation between female antioxidant levels and litter success, though surprisingly, it occurred in populations with permanent access to water and therefore only in optimal water environments. This unexpected difference between the laboratory and the field study might be explained by differences in the frequency of exposure to water restriction. Females from the experimental study faced a sudden and acute water restriction, whereas those from natural populations with periodic water access were facing chronic water restriction periods before pregnancy and throughout the duration of the breeding season. They were therefore likely to acclimatize and/or adapt their physiology to current local conditions (Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017). Further experimental translocations and common garden experiments will therefore help depicting how natural constraints may shape reproductive investment, oxidative status and breeding success (Kouyoumdjian et al., 2019).

In the long run, the lower annual survival rate of offspring was negatively associated with increased maternal oxidative damage, suggesting deleterious maternal effects derived from oxidative stress on offspring. To the best of our knowledge, survival costs in offspring resulting from higher maternal oxidative stress have rarely been demonstrated (Costantini, 2014). Moreover, this relationship was independent of water conditions suggesting a general trend. It is worth noting that these lizards were maintained in outdoor enclosures with lower risk of attacks by predators, and hence, we may have underestimated the impacts on offspring survival costs. In this species however, annual offspring survival is determined by trophic competition, individual quality, parasitism and climate conditions (Le Galliard, Marquis, & Massot, 2010), whereas the impact of avian predation was not evident (Le Galliard, Paquet, & Mugabo, 2015). To confirm our results, a long-term monitoring programme following individuals in natural populations will provide an opportunity to estimate annual variation in survivorship and other components of fitness (Bleu et al., 2013; Clobert et al., 2000). How maternal changes in oxidative damage during reproduction decreased subsequent survival offspring later in life remains an open question. Deleterious maternal effects are possibly caused by transplacental transfer of pro-oxidant molecules and higher foetus oxidative stress (Rossner et al., 2009). In addition, increased maternal ROMs could have indirectly altered the embryo development (e.g. lower growth and immunity). These hypotheses imply that large molecules (lipids, amino acids, carbohydrates) carrying oxidative damage would cross the placental structures. Although the transport of organic nutrients remains poorly understood in many viviparous reptiles, such maternal provisioning is known to occur (Van Dyke & Beaupre, 2012; Van Dyke, Brandley, & Thompson, 2014). From an evolutionary perspective, these differences in fitness responses between mother and offspring could represent a functional basis of the intergenerational conflict in resource allocation (Kölliker et al., 2015). Although the mechanisms are unknown, we hypothesize that modified oxidative status was an important mediator of intergenerational conflicts for water resource.

5 | CONCLUSIONS

The understanding of proximate factors mediating the reproductive trade-offs is a central topic in evolutionary ecology. For this purpose, a body of evidence suggests that the imbalance between oxidative damage and antioxidant capacities is one mediator shaping cost of reproduction (Costantini, 2008; Metcalfe & Monaghan, 2013; Speakman & Garratt, 2014). Our study supports this assumption but only when environment becomes resource limiting. Aside from this, our results provide new evidence of a potential role of oxidative status in mediating intergenerational conflicts for water. Altogether, these results demonstrate the importance of considering oxidative status to understand animal responses to changes in temperature and water resources. This may be critical for the understanding and prediction of population trends in population exposed to various levels of global warming and extreme climatic events such as heatwaves or summer droughts.

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CONFLICT OF INTEREST

The authors declare having no conflicts of interest.

AUTHORS' CONTRIBUTIONS

A.D. conceived the ideas, designed the methodology, analysed the data and led the writing of the manuscript together with S.M. and J.-F.L.G. All authors participated in data collection and contributed critically to the drafts of the manuscript and gave final approval for publication.

ETHICAL APPROVAL

Cross-sectional study was performed in accordance with laws relative to capture, transport and experiments on *Zootoca vivipara* (DREAL Languedoc Roussillon permit #2013-274–0002, DREAL Midi-Pyrénées permit #81-2013–05, and DREAL Auvergne, permit 2013/DREAL/259). Experimental study was made following acceptance of independent ethic committee (APAFIS#5108-20 16040811272391 v4).

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DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository, https://doi.org/10.5061/dryad.73n5tb2sn (Dupoué et al., 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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