Title: Additive effects of temperature and water availability on pregnancy in a viviparous lizard

Running title: environmental constraints and maternal costs

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Summary statement: Dehydration during pregnancy negatively affects maternal physiology but has little effect on reproductive output. These effects are additive to temperature regimes and therefore similar in both warmer and colder environments.

Abstract: One of the greatest current threats to biodiversity is climate change. Yet, an understanding of organismal responses to fluctuations in both temperature and water availability is currently lacking, especially during fundamental life-history stages such as reproduction. To further explore how temperature and water availability impact maternal physiology and reproductive output, we used the viviparous form of the European common lizard (*Zootoca vivipara*) in a two-by-two factorial design manipulating both hydric and thermal conditions for the first time. We collected blood samples and morphological measurements during early pregnancy and post-parturition to investigate how water availability, temperature, and a combination of the two influences maternal phenology, morphology, physiology and reproductive output. We found, that dehydration during gestation negatively affects maternal physiological condition (lower mass gain, higher tail reserve mobilization) but has little effect on reproductive output. We found that these effects are mainly additive to temperature regimes with a proportional increase in maternal costs in warmer environments. Our study demonstrates the importance of considering combined effects of water and temperature when investigating organismal responses to climate changes, especially during periods crucial for species survival such as reproduction.

1. Introduction

Predicting how organisms will be impacted by global climate change is frequently viewed in the context of thermal effects on biological and ecological processes (Huey et al., 2012; Kearney et al., 2009; Nowakowski et al., 2018; Parmesan, 2006). However, it is equally important to consider the effects of water availability on biodiversity because water is a fundamental resource, rainfall events are forecasted to be less reliable resulting in reduced water availability for many species (Marvel et al., 2019; Schlaepfer et al., 2017), and predicted changes in ambient temperatures are inextricably linked to changes in precipitation (Alexander et al., 2006). For example, during periods of prolonged drought, increasing temperatures and decreasing water availability frequently covary (Rahmstorf and Coumou, 2011), which can have a pronounced effect on hydration and survival (McLaughlin et al., 2002; Riddell et al., 2019). These combined effects are especially relevant in ectotherms who rely on behavioral or physiological selection of optimal body temperatures that maximize performance, survival, and reproduction (Huey, 1982; Angilletta et al., 2002; Huey et al., 2012; Sinervo et al., 2010) but risk losing greater volumes of water when maintaining higher body temperatures (Claussen 1967; Munsey 1972). Even though dual changes in temperature and water availability are predicted across the globe, it remains largely unknown if and how fundamental life-history stages, such as reproduction, will be affected by these combined changes (Rozen-Rechels et al. 2019).

In many environments, precipitation is already unevenly distributed throughout the year or water is rare, and water availability can therefore be limited during periods of reproductive investment (Hao et al., 2018). Previous research has found that changes in rainfall patterns, independent of temperature shifts, are currently challenging reproductive strategies (Visser and Both, 2005) and can have negative impacts on entire ecosystems (McCluney et al., 2012). In juxtaposition, shifts in temperature, independent of altered rainfall patterns, can drastically reduce fitness (Deutsch et al., 2008) and unpredictable temperature regimes have been implicated in large-scale extirpation events (Valladares et al., 2014). The negative effects of fluctuating temperature and water availability may have interactive, detrimental consequences on reproductive events. The physiological impacts of maternal hydration before and after pregnancy have been previously explored (Nelson et al., 1989; Hanson et al., 1994; Gesquiere et al., 2008; Bukovetzky et al., 2012), but far less is known about the direct consequences of hydration during gestation on maternal physiology and reproductive output. Some recent studies have found that

reproductive females with limited access to water are able to tolerate dehydration (i.e., hyperosmolarity, Brusch et al., 2017; Dupoué et al., 2018a) or can utilize alternative hydric resources during gestation (e.g., bound water, Brusch et al., 2018) to buffer themselves from changes in water availability. However, these past studies focused on a single physiological challenge, water deprivation, and it is unclear how multiple, concurrent challenges, as predicted under climate change scenarios, may influence maternal physiology during reproduction. Recent work has started to emphasize potential interactions between temperature and water availability in ectotherms (Dupoué et al., 2020a; Rozen-Rechels et al., 2019). Yet, a combined, experimental approach exploring the relationship between thermo- and hydro-regulation during reproduction is currently lacking.

To further explore how temperature and water availability impact maternal physiology and reproductive output, we used the viviparous form of the European common lizard (*Zootoca vivipara*) in a two-by-two factorial design manipulating jointly hydric and thermal conditions for the first time. We investigated how water availability (± water restrictions), temperature (hot or cold daily cycle), or a combination of the two throughout gestation, influences maternal physiology and reproductive output. Unique to our experimental design relative to most thermal biology studies, we used similar water vapor deficits between temperature treatments to eliminate the possibility of increased desiccation at higher temperatures, and therefore only temperature was manipulated. The common lizard is wide-ranging, cold-adapted squamate that is sensitive to water deprivation (Lorenzon et al., 1999; Reichling, 1957). Higher temperatures may accelerate embryonic development, as it does in other viviparous lizards (Deeming, 2004; Du and Shine, 2015), while simultaneously increasing metabolic and subsequent water loss rates of gravid females and consequently negatively impacting maternal physiological condition if they are not able to compensate with greater food intake (Dillon et al., 2010; Lourdais et al., 2017). Conversely, if lower temperatures delay embryonic development, the prolonged gestational period may exacerbate the effects of water restrictions and similarly reduce maternal conditions. To ground truth our study in ecological relevance, we used contrasting water availability treatments that mimic the natural conditions of wild populations with either limited or permanent access to water (Dupoué et al. 2017; Lorenzon et al., 1999). We also selected temperature treatments that reflect either warm, favorable daily conditions for embryonic development (long access to preferred temperature without thermal stress) or cold, suboptimal daily conditions

(short access to preferred temperature), and which correspond to the thermal environments experienced by this species in their typical range excluding extremely hot or cold environments. We tested the hypothesis that contrasted temperature regimes and water availability will differentially impact aspects of maternal physiological condition and reproductive output. We used body mass and condition, tail width, plasma osmolality, oxidative stress, antioxidant capacity, and food consumption as measures to evaluate maternal physiological condition. We used gestation length to evaluate reproductive phenology. Finally, we used total litter size and mass, and individual offspring size and mass as measures for reproductive output. We made the following predictions: (1) water restrictions will negatively affect maternal traits but will not impact reproductive output; (2) high temperatures will shorten pregnancy duration but negatively impact maternal physiological condition; and (3) a combination of high temperature and water restrictions during pregnancy will negatively affect both maternal traits and reproductive output.

2. Materials and methods

2.1 Study species

Zootoca vivipara is a small-bodied lizard species (adult snout-vent length, SVL 50-75 mm) in the family Lacertidae with the widest geographic range of all terrestrial reptiles (Dely and Böhme, 1984). The species is predominately found in cooler, mesic habitats with permanent access to water across northern Eurasia and occurs in both viviparous and oviparous forms. At our study sites, all females were viviparous. As lecithotrophic viviparous species, the majority of maternal energetic investment occurs during vitellogenesis (Blackburn, 2015). After ovulation embryos remain enveloped in a thin membrane throughout gestation (Heulin, 1990; Panigel, 1956), which supports important maternal water transfers (Dauphin-Villemant et al. 1986) and also calcium, and some micronutrients to the developing embryos (Stewart, 1992; van Dyke and Beaupre, 2012). The young of viviparous females are usually born in the membranes and hatch within 1–2 hours of parturition (Lorenzon et al., 2001). Females emerge from over-wintering in late April to early May, with mating and fertilization occurring shortly thereafter (Bleu et al., 2013). Parturition occurs from late June through July and females typically give birth to 6 neonates (range 1-12) depending on their body size (Dupoué et al., 2018a; Foucart et al. 2014).

2.2 Experimental design

Females used for this study were captured in the first week of June 2018 from five different sites on the Plateau de Millevache (Limousin, France) separated by a maximum distance of 30 km (Table S1). Gravid females (N = 97) were captured by hand and reproductive status was confirmed using abdominal palpation. Non-reproductive females were immediately released at the site of capture. Within 72 h of capture, all females were moved to the Centre d'Etudes Biologiques de Chizé, France. Mass and SVL of lizards were recorded, after which all visible ectoparasites were removed with forceps and females were treated with an anti-parasite spray of Frontline (Merial Inc. Duluth, GA, USA). Females were then housed individually with an 12L:12D light regimen in opaque containers (30 × 18 × 10 cm) filled with approximately 1 cm of soil and two different shelters and provided *ad libitum* access to food (*Acheta domestica*) and water. During the 8 h light cycle, a subsurface heating element was provided below one end of each cage to create a thermal gradient (20° – 40°C). All females were held in these conditions for ca. seven days prior to the start of the experiment after which an initial blood sample and scanned image were collected (see below).

The experiment was performed from 15-June until parturition for all females so as to mimic stable and consistent differences in temperature and water availability throughout the majority of gestation. Pregnant females maintain stable body temperatures during the day (range: 29° – 33°C) by selecting appropriate micro-climates (Le Galliard et al., 2003; Dupoué et al. 2017, 2018b). We assigned lizards of similar mean body size to one of two water regimens with permanent access to water or a water restriction (hereafter referred to as wet and dry, respectively) following similar protocols used in previous studies (Dupoué et al., 2018a; Lorenzon et al. 1999). Females in the wet group (n = 49) were provided with water *ad libitum* and water was sprayed twice per day (morning and afternoon) on one wall of each container so that drops could be used for drinking up to 1 h after spraying. Females assigned to the dry group (n = 48) were only provided with water once per day (morning) by similarly spraying one wall of each container. Lizards were further divided into one of two temperature regimens, hereafter called "cold" or "hot". Each day of the experiment, females in the cold group (n = 49) were held overnight (17h00 to 9h00; 16 hours) at 15°C, during a portion of the day (11h00 to 15h00; 4 hours) at 31°C, and spent 4 hours where temperatures were either gradually increasing or decreasing to avoid abrupt changes. Females in the hot group (n = 48) were held overnight (19h00 to 07h00, 12 hours) at 22°C instead of 15°C, during a majority of the day (09h00 to

17h00, 8 hours) at 31°C, and similarly were held for the remaining four hours with temperatures either gradually increasing or decreasing (Fig. 1). Final treatment groups of the factorial design were thus hot and dry (n = 24), hot and wet (n = 24), cold and dry (n = 24), or cold and wet (n = 25). Stable temperatures were maintained by housing females of similar treatment groups in climatic chambers (Vötsch VP 600, Balingen, Germany). The relative humidity in each chamber was adjusted depending on the temperature so that the water vapor deficit remained constant around 1 kPa (equivalent to 60% RH at 20°C).

During the study, each female was provided with live crickets (400 ± 20mg) every other day which were dusted with vitamin powder containing calcium and vitamin D3. During feedings any remaining crickets were removed and counted in order to quantify the total number and ratio of crickets consumed (i.e., number of crickets consumed / crickets offered) throughout the study. Females were weighed once per week and each cage was visually inspected twice per day for parturition. After parturition, females and their litter (total mass of living and stillborn offspring) were weighed, and a final blood sample and scanned image were collected. Offspring were classified as either living or stillborn in order to quantify the proportion of stillborn (i.e., number of stillborn / total offspring) for each female. Living neonates were also individually weighed and scanned. Females and neonates were placed into separate individual containers, provided a thermal gradient (20° – 40°C), and *ad libitum* access to food and water, before being released at the maternal site of capture.

2.3 Blood sample collection and determination of plasma osmolality and oxidative status

We used 20 µL hematocrit tubes to collect a ~50 µL blood sample from the postorbital sinus of all reproductive females. Total time for capture, restraint, and blood collection did not exceed 3 min. We immediately centrifuged the blood samples at 4,000 *g* for 5 min at 20°C to separate plasma from blood cells, and then aliquoted plasma (~20 µL) into separate vials and froze at -30°C until we used them to measure plasma osmolality and oxidative status. We determined plasma osmolality using a vapor pressure osmometer (±3 mOsm kg^{-1}; model 5100C; Wescor Inc., Logan, Utah, USA). To allow for duplicate readings, plasma samples were diluted (1:1) in reptile Ringer's solution (300 mOsm kg^{-1}) following methods from Secor et al. (1994). Prior to use, we calibrated the osmometer using sealed osmolality standards (100, 290, and 1000 mOsm kg^{-1}) in accordance with factory recommendations. We ran samples in triplicate and used 290

188 mOsm kg^{-1} standards to check the osmometer for variation after every sample. If the standard
189 varied more than the limits of the osmometer (±3 mOsm kg^{-1}), the osmometer was recalibrated.
190 If it continued to vary more than the limits, the osmometer head was cleaned and the machine
191 was recalibrated before continuing duplicate analysis beginning with the last sample prior to
192 calibration/cleaning to verify correct measurement of the sample.
193 In order to examine oxidative status, we performed two plasma-based assays within five
194 months of blood collection. To evaluate the activity of organic hyperoxides (an index of
195 oxidative damage; Costantini, 2016), we measured the concentration of reactive oxidative
196 metabolites (ROMs) using d-ROMs colorimetric kits (MC003, Diacron International, Italy).
197 Antioxidant capacity (OXY) was also assessed to determine the non-enzymatic ability of diluted
198 female plasma samples (1:100) to neutralize an oxidant attack from hypochlorous acid
199 (Constantini, 2011) using OXY-absorbent test kits (MC435, Diacron International, Italy). For all
200 assays, we used a pooled plasma sample of randomly selected females (n = 8) three times in each
201 96-well plate to measure coefficients of variation in ROMs (intra-plate: 2.8%; inter-plate: 6.0%)
202 and OXY (intra-plate: 3.4%; inter-plate: 13.8%).
203
204 *2.4 Scanned images*
205 Prior to the experimental manipulation and just after parturition, the ventral surface of all
206 females was scanned at 600 dots per inch using a flatbed scanner (Hewlett-Packard Co., ScanJet
207 3670). From these images, we measured tail width at the 7th sub-caudal scales using Inkscape
208 (v.0.92.3). We also scanned each neonate just after parturition to accurately measure their SVL.
209 Animals were gently restrained by hand behind the neck and the tip of the tail during image
210 collection.
211
212 *2.5 Statistical analyses*
213 Before testing the effect of water availability and temperature on maternal traits and reproductive
214 output, we identified a set of explanatory variables to model data from reproductive females.
215 Larger females typically emerge from overwintering, ovulate, and females with earlier ovulation
216 dates give birth earlier in the year (Bauwens and Verheyen, 1985). Therefore, we included
217 parameters on relative body size (individual SVL minus mean SVL of all females) and relative
218 duration of treatment (individual time in treatment minus mean time in treatment of females

within respective treatments). We did not directly manipulate litter size or mass (i.e., reproductive effort) and therefore included a variable describing individual differences in reproductive effort (standardized residuals from a linear regression of litter mass against SVL) to control for potential allocation differences between females (Dupoué et al., 2020b). We also explored potential interactions between reproductive effort and treatments to test for different allocation trade-offs depending on temperature and water availability. However, these interactions were never significant (all $p > 0.05$) and therefore removed from all models. Starting with a full model, we used stepwise removal of insignificant variables (Arnold, 2010; Zuur et al., 2010).

We used linear models to examine the effect of water availability (wet or dry) and temperature (hot or cold) on mass and tail width changes and changes in plasma osmolality in reproductive females throughout the experiment. We used temperature, water availability, and their interaction as fixed effects, and relative body size and reproductive effort as covariates. To model the duration that females were in the experiment and differences in litter size and mass we used models with only relative body size as a covariate. Similarly, we used linear models to compare differences in initial and final OXY and only used relative duration as a covariate. Models used to investigate differences in initial and final ROMs, included that same fixed effects and interactions but did not include any covariates. We used general linear models for ratio and proportion data (crickets consumed and number of stillborn) with temperature, water availability, and their interaction as fixed factors. For ratio of crickets consumed, we used relative body size, relative duration, and reproductive effort as covariates, and for proportion of stillborn, only relative duration was used as a covariate. For offspring data (mass and SVL), we used linear mixed-effect models with maternal treatments (temperature, water, and their interaction) as fixed factors, SVL or mass as covariates, and maternal identity as a random factor to control for non-independence among offspring from the same litter. All analyses were performed using R software (R Development Core Team, version 3.5.2) using the packages ‘nlme’, ‘lattice’, ‘car’, ‘lsmeans’, and ‘MASS’ (Fox and Weisberg, 2011; Pinheiro et al., 2018; Russell, 2016; Sarkar, 2008; Venables and Ripley, 2002). Data are presented as mean ± SEM and differences were accepted as significant at the level of $p < 0.05$.

3. Results

3.1 Impact on gestation length and female morphology

When comparing the duration that females were in their experimental treatments (time until parturition), we found a significant main effect of temperature regime ($F_{1,92} = 172.04$, $p < 0.001$), but no main effect of water availability or interaction between the two kinds of treatment ($p > 0.05$). Regardless of water availability, reproductive females exposed to hot temperatures gave birth sooner in the study compared to those exposed to cold temperatures. Comparing duration against the relative body size of females further revealed a significant interaction between relative body size and treatment ($F_{1,93} = 13.31$, $p < 0.001$). The relationship with relative body size was more pronounced in reproductive females exposed to cold ($\beta = -1.68$, SE = 0.24, $R^2_{adj} = 0.497$) compared to hot temperatures ($\beta = -0.73$, SE = 0.10, $R^2_{adj} = 0.499$; Fig. 2).

Females at the beginning of the experiment had similar body mass between treatments ($F_{3,93} = 0.16$, $p = 0.93$; Table S2). Mass change during the experiment (pre-parturition mass – initial mass) was additively influenced by water availability ($F_{1,91} = 8.39$, $p = 0.004$) and temperature regime ($F_{1,91} = 32.48$, $p < 0.001$), but we found no interaction between temperature and water availability ($F_{1,91} = 0.82$, $p = 0.366$). Females exposed to hot and wet conditions gained less mass during gestation compared to all other treatment groups (Fig. 3C). Similarly, post-partum body condition (body mass adjusted to the mean body size of females: $F_{1,91} = 186.75$, $p < 0.001$) was influenced by water availability ($F_{1,91} = 4.12$, $p = 0.045$) and temperature treatment ($F_{1,91} = 17.57$, $p < 0.001$), with females exposed to hot and dry conditions having the lowest post-parturition body condition (3.21±0.12g) and females exposed to cold and wet conditions having the highest post-parturition body condition (3.73±0.17g). We found no interaction between temperature and water availability for post-parturition body condition ($F_{1,91} < 0.01$, $p = 0.94$).

There was no initial difference in tail width among the treatment groups ($F_{3,93} = 0.11$, $p = 0.95$; Table S2). Most females had reductions in tail width during the experiment and these decreases were additively influenced by water availability ($F_{1,91} = 17.23$, $p < 0.001$) and temperature treatment ($F_{1,91} = 5.75$, $p = 0.018$), with females exposed to hot and dry conditions losing the most tail width and females exposed to cold and wet conditions losing the least tail width (Fig. 3A). We found no interaction between temperature and water availability ($F_{1,91} = 0.49$, $p = 0.486$).

3.2. Changes in blood parameters

Plasma osmolality change during the experiment was additively influenced by water availability ($F_{1,74} = 16.26$, $p < 0.001$) and temperature treatment ($F_{1,74} = 7.62$, $p = 0.007$), with females exposed to hot and dry conditions having the largest increases in plasma osmolality (308±2mOsm kg^{-1} to 323±3mOsm kg^{-1} for mean initial and final values, respectively), females in hot and wet, and in cold and dry conditions having intermediary increases in plasma osmolality (305±2mOsm kg^{-1} to 314±2mOsm kg^{-1} and 311±2mOsm kg^{-1} to 318±1mOsm kg^{-1}), and females exposed to cold and wet conditions having decreased plasma osmolality (323±3mOsm kg^{-1} to 315±3mOsm kg^{-1}, Fig. 3B). We found no interaction between temperature and water availability ($F_{1,74} = 2.04$, $p = 0.157$).

There was no initial difference in oxidative stress (ROMs; $F_{3,93} = 0.40$, $p = 0.75$) or non-enzymatic antioxidant capacity (OXY; $F_{3,93} = 1.32$, $p = 0.27$) among the treatment groups. After parturition, females in dry conditions throughout pregnancy had significantly higher ROMs ($F_{1,93} = 8.34$, $p = 0.005$), but we found no significant interaction or main effect of temperature regime ($p > 0.05$; Fig. 4A). When examining OXY values after parturition, we found a significant interaction between temperature and water availability ($F_{1,92} = 6.39$, $p = 0.013$). Within the hot treatments, females in wet conditions had higher OXY values compared to those in dry. In juxtaposition, females exposed to cold and wet treatments had slightly lower OXY values compared to those in dry (Fig. 4B).

3.3 Impact on food intake

When evaluating the ratio of crickets consumed during the same period, we found a significant main effect of water availability ($F_{1,94} = 4.16$, $p = 0.044$) and temperature regime ($F_{1,95} = 36.43$, $p < 0.001$) but no interaction between the two ($F_{1,90} = 2.76$, $p = 0.100$). A Tukey's HSD post-doc test revealed that females in the hot temperature regimes ate a greater ratio of crickets than those in the cold regimes ($p < 0.01$) regardless of water regime, and within each temperature regime, females in wet conditions consumed a higher proportion of crickets although not significantly so ($p > 0.05$; Fig. 5).

3.4 Impact on reproductive output

We did not detect any significant differences in litter size ($F_{3,93} = 0.49$, $p = 0.688$), total litter mass ($F_{3,92} = 0.49$, $p = 0.688$), individual offspring mass ($F_{3,92} = 1.08$, $p = 0.363$) or snout-vent length ($F_{3,92} = 0.35$, $p = 0.786$) among the treatment groups. When comparing the proportion of stillborn offspring among treatment groups, we found a significant effect of temperature ($F_{1,95} = 6.69$, $p = 0.011$), where females exposed to cold temperatures had three times more stillborn compared to females exposed to hot temperatures, irrespective of water regime ($p < 0.05$; Table 1).

4. Discussion

Understanding how animals cope with both altered temperature regimes and restricted resources, such as water, will enable us to better predict how they might be affected by anticipated climate change scenarios. This is especially important because in many environments climate warming induces concurrent changes in mean temperature and water availability as well as in the occurrence of extreme weather events (Fischer and Knutti, 2015; Mann et al., 2017; Rahmstorf and Coumou, 2011). Ours is the first study to explore the independent and combined effects of water availability and thermal conditions during gestation in a vertebrate. Our results regarding water availability are mostly consistent with previous work in squamate reptiles: mild dehydration during gestation negatively affects maternal physiology but has little effect on reproductive output (Dupoué et al., 2015, Lourdais et al., 2015). For all traits except anti-oxidant capacity of females, we also found that these effects are additive to temperature regimes and therefore similar in both warmer and colder environments despite very significant effects of thermal conditions on the length of gestation and maternal physiology. Overall, our study adds to a growing body of evidence that water is a fundamental currency used during reproduction. Our study also emphasizes the importance of considering multiple, concurrent physiological challenges when investigating organismal responses to climate changes, especially during fundamental life-history stages such as reproduction.

We found that temperature but not water availability affected gestation length, with higher temperatures resulting in a shorter period of pregnancy (Fig. 2). In both temperature regimes smaller females had a longer duration of pregnancy, although this size-dependent gestation length was far more pronounced in the cold treatments. Maternal body size is often an important factor influencing reproductive output and timing (Blueweiss et al., 1978; Díaz et al.,

2012; Iverson et al., 1997; Kiefer et al., 2008), including in this species (Bauwens and Verheyen, 1985). Higher temperatures in our study appear to have accelerated gestation and partially outweighed the size-dependent timing of pregnancy suggesting that small females caught-up for initial differences in the timing of reproduction when provided with warmer environmental conditions. These results are in accordance with previous research showing that higher maternal temperatures result in shorter gestation length and faster embryonic development both in oviparous and viviparous squamates (Dubey and Shine, 2011; Foucart et al., 2018; Lorioux et al., 2012, 2013a). They also confirm comparative findings among wild populations showing that climate warming induces phenological acceleration (e.g., shorter gestation time and lower variance in laying date) and significant demographic changes (Massot et al., 2017; Rutschmann et al., 2016).

Previous field and laboratory studies further suggested that increased gestational temperatures can also impact reproductive output, offspring phenotypes, and future fitness (Chamaille-Jammes et al., 2006; Lorioux et al. 2013b; Lourdais et al 2004; Ma et al., 2014; Marquis et al., 2008). Although we did not investigate effects on future reproduction and survival, we detected no significant differences in total litter size and mass, and offspring metrics between any of the experimental groups but found that females had a poorer embryonic success in the cold treatment. In ectotherms, embryonic metabolism is temperature-dependent and mothers often behaviorally thermoregulate to maintain optimal temperatures for embryonic development (Farmer, 2003; Gillooly et al., 2002; Lorioux et al. 2013a; Lourdais et al., 2013; Mettouris et al., 2017). Extreme temperatures are often associated with decreased reproductive success in ectotherms because they compromise behavioral thermoregulation and may increase physiological stress and decrease performance when they cannot be avoided behaviorally (Clusella-Trullas et al., 2011; Sinervo et al., 2010; Wang et al., 2016). In our study, females were not allowed to thermoregulate but had longer access time to preferred temperature (31°C) in the hot than in the cold treatment. The hot treatment temperature (31°C) was within the range of preferred temperature during gestation and was not meant to induce thermal stress or heat-related embryonic mortality (Le Galliard et al., 2003). Thus, embryonic success was maximized in hot conditions probably because gravid females were given better opportunities to maintain their preferred body temperatures during the prolonged daytime period (Foucart et al., 2018; Le Galliard et al., 2010; Le Henanff et al., 2013; Lorioux et al., 2012, 2013a). While females in the

cold treatment were provided access to preferred temperatures for 4 hr each day, that window does not appear to have been large enough to effectively maintain embryonic development in these cold-adapted lizards, resulting in a greater proportion of stillborn as seen in wild populations (Le Galliard et al. 2010).

Higher temperatures and dry conditions resulted in additively higher tail width loss (Fig. 3A) and lower total body mass change during gestation (Fig. 3C). Tail reserves are a very important site for storing accumulated resources in many species of lizards (reviewed in Bateman and Fleming, 2009). However, our study is the first to demonstrate the negative impact of dehydration, enhanced by higher temperatures, on tail morphology. The tails of *Z. vivipara* are composed of both lipids and proteins (Avery, 1974), and increased muscle catabolism at this site may release bound water to offset physiologically damaging levels of dehydration (Brusch et al. 2018). Additionally, altered tail reserves likely explain lower body mass after parturition in females exposed to hot and dry treatments. A decreased maternal body condition after reproduction has been shown to challenge future survival due to increased starvation or predation risks if food is not readily available or if weaker mothers are less able to avoid predation (Bleu et al., 2013). These females also had higher plasma osmolality (Fig. 3B) suggesting that both high temperatures and water shortage challenge hydric state during pregnancy and lead to dehydration. Surprisingly, we did not detect an interactive effect. That is, gravid females exposed to hot and dry conditions did not have significantly higher plasma osmolality compared to females who were exposed to cold and wet or hot and wet conditions (Fig. 3B). Females may have increased muscle catabolism to release bound water and support some of the water demands of reproduction (Brusch et al., 2018) as females in dry conditions had higher tail width loss compared to females in wet (Fig. 3A). Alternatively, females may have behaviorally adjusted their behavior to maximize drinking when water was available in the morning as has been shown in this species (Rozen-Rechels et al., 2020).

In addition to being more dehydrated, females in dry conditions throughout pregnancy also had higher markers of oxidative damage after parturition, regardless of temperature treatment (Fig. 4A). Unlike all the other dependent variables measured in our study, we found a significant interaction between temperature and water availability on non-enzymatic antioxidant levels. Dehydrated females in the hot treatment had the lowest non-enzymatic antioxidant levels (Fig. 4B) which, coupled with high levels of oxidative damage, equates overall to the highest

levels of oxidative stress. These results suggest that the production of potentially damaging oxygen species is linked to hydric resources and it appears that females were able to protect themselves with increased antioxidant production, as shown previously (Dupoué et al., 2020a; Stier et al., 2017). These results further complement recent comparative findings between wild populations, showing that warmer climates may be associated with lower OXY levels in pregnant *Z. vivipara* (Dupoué et al., 2020b), although as demonstrated here, thermal impacts were conditioned by water resources. Importantly, the magnitude of alterations in oxidative status during pregnancy can have serious and delayed consequences on offspring mortality rate, which will need further examinations (Dupoué et al., 2020a). Our study did not explicitly measure such reproductive trade-offs however, and females in the hot treatments were exposed to water restrictions for ~3 weeks, half that of females from the cold treatments. It is worth noting that kinetic changes in oxidative stress are not stable during gravidity (Kouyoumdjian et al., 2019; Speakman and Garratt, 2014), so this may partly explain observed differences post-parturition. Additionally, antioxidants serve a myriad of functions and their production is frequently temporarily mismatched with the creation of reactive oxygen species over acute timescales (Costantini, 2019; Halliwell and Gutteridge, 2015). Females in the hot treatments may not have had enough time or hydric resources to balance their oxidative status and a mechanistic understanding of these results is beyond the scope of this study but deserves further exploration.

When free-standing water is limited, some organisms are able to rely almost entirely on metabolic (as a by-product of metabolism) or dietary (that present in food) water (Karasov 1983; Nagy and Gruchacz, 1994; Ostrowski et al. 2002). Because our experimental manipulations began after vitellogenesis, which is considered the main energy allocation period in this species (Foucart et al. 2014), and because metabolism is relatively low in squamate reptiles (Andrews and Pough, 1985), metabolic water was likely negligible (Shoemaker and Nagy, 1977). Additionally, temperature and metabolism are tightly linked in ectotherms (Dillon et al., 2010), but females in the hot treatments, regardless of water availability, had no significant differences in plasma osmolality changes (Fig. 3B), which further supports that metabolic water was negligible in our study. This still leaves the possibility that females in our experiment were able to partially compensate the hydric demands of reproduction through increased food consumption and dietary intake of water. Females in hot treatments ate a greater ratio of crickets (Fig. 5). If dietary water was an important component of hydration, we might expect lizards consuming a

greater ratio of crickets to have decreased plasma osmolality, which was not the case in our study. Because we did not detect a significant difference in plasma osmolality change between females in hot and wet treatments and cold and dry treatments, it is difficult to conclude if females were gaining any hydric benefit from food consumption. Overall, these results may suggest that free-standing water is the most important source during pregnancy and essential for maintaining maternal hydration. Especially considering that previous research has found no net osmotic advantage from eating in non-reproductive squamates (Lillywhite, 2017; Murphy and DeNardo, 2019; Wright et al., 2013). Higher temperatures still had an important effect on food consumption, and females with *ad libitum* access to water consumed marginally higher ratios of crickets, indicating a much more complex relationship between the temperature, water availability, and food consumption that merits future investigation.

Previous studies examining limited hydric resources during periods of reproductive investment have similarly found that mothers are burdened with the majority of water imbalance in favor of embryonic hydration (Brusch et al., 2018; Dupoué et al., 2015, 2018a). While developing embryos often depend on maintaining water balance for survival (Cagle et al., 1993, Warner and Andrews, 2002), water imbalance is not necessarily physiologically detrimental in all cases. For example, immune enhancement as the result of dehydration has been documented in multiple taxa (Brusch and DeNardo, 2017; Hoang, 2001; Moeller et al., 2013) including mothers and embryos during conflicts for limited hydric resources (Brusch and DeNardo, 2019; Brusch et al., 2017). Future studies should focus on the interplay between osmolality and physiological functions in both mothers and embryos, especially considering that previous explorations of more cryptic physiological traits have elucidated that both mothers and embryos are impacted by dehydration (Brusch et al., 2019).

Warmer and drier environmental conditions may challenge reproductive modes in several ways and these lines of research deserve further attention. Future studies should continue to consider the interaction between temperature and water availability and further explore the impacts they have on maternal physiology, reproductive output, and offspring physiology. In particular, developmental temperatures and hydric conditions outside optimal ranges, which may become more frequent under expected climate change scenarios, may lead to increased embryonic mortality (Gibbons et al., 1983; Madsen et al., 2006; Packard et al. 1977; Semlitsch 1987; Sperry and Weatherhead, 2008). Therefore, considering both hydro- and thermoregulation

under joint conditions of thermal and hydric stress will be essential for understanding ectothermic responses to predicted shifts in temperature and water availability (Rozen-Rechels et al., 2019; Smith et al., 2019; Wake and Vredenburg, 2008).

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Table 1 – Details of reproductive output from female *Zootoca vivipara* held in either hot and dry (HD), hot and wet (HW), cold and dry (CD), or cold and wet (CW) conditions throughout pregnancy (mean ± SEM).

Treatment	Litter mass (g)	Offspring mass (mg)	Offspring SVL (mm)	Proportion stillborn
HD	1.15 ± 0.09	17.33 ± 0.17	19.51 ± 0.08	0.03 ± 0.02
HW	1.10 ± 0.09	17.02 ± 0.18	19.63 ± 0.09	0.07 ± 0.03
CD	1.17 ± 0.09	16.93 ± 0.19	19.50 ± 0.11	0.11 ± 0.03
CW	1.09 ± 0.09	17.38 ± 0.18	19.59 ± 0.08	0.19 ± 0.05

Litter mass represents the total mass of living and stillborn offspring from each female. Offspring mass and snout-vent-length (SVL) is only for living neonates from each female. Proportion of stillborn represents the number of stillborn divided by the total litter size (living and stillborn).

Figure legends

Figure 1 – Hourly temperature regimens of reproductive female *Zootoca vivipara* held in climatic chambers. Lizards were exposed to stable (±0.1°C) hot (closed triangles; n = 48) or cold (open circles, n = 49) temperature treatments during pregnancy.

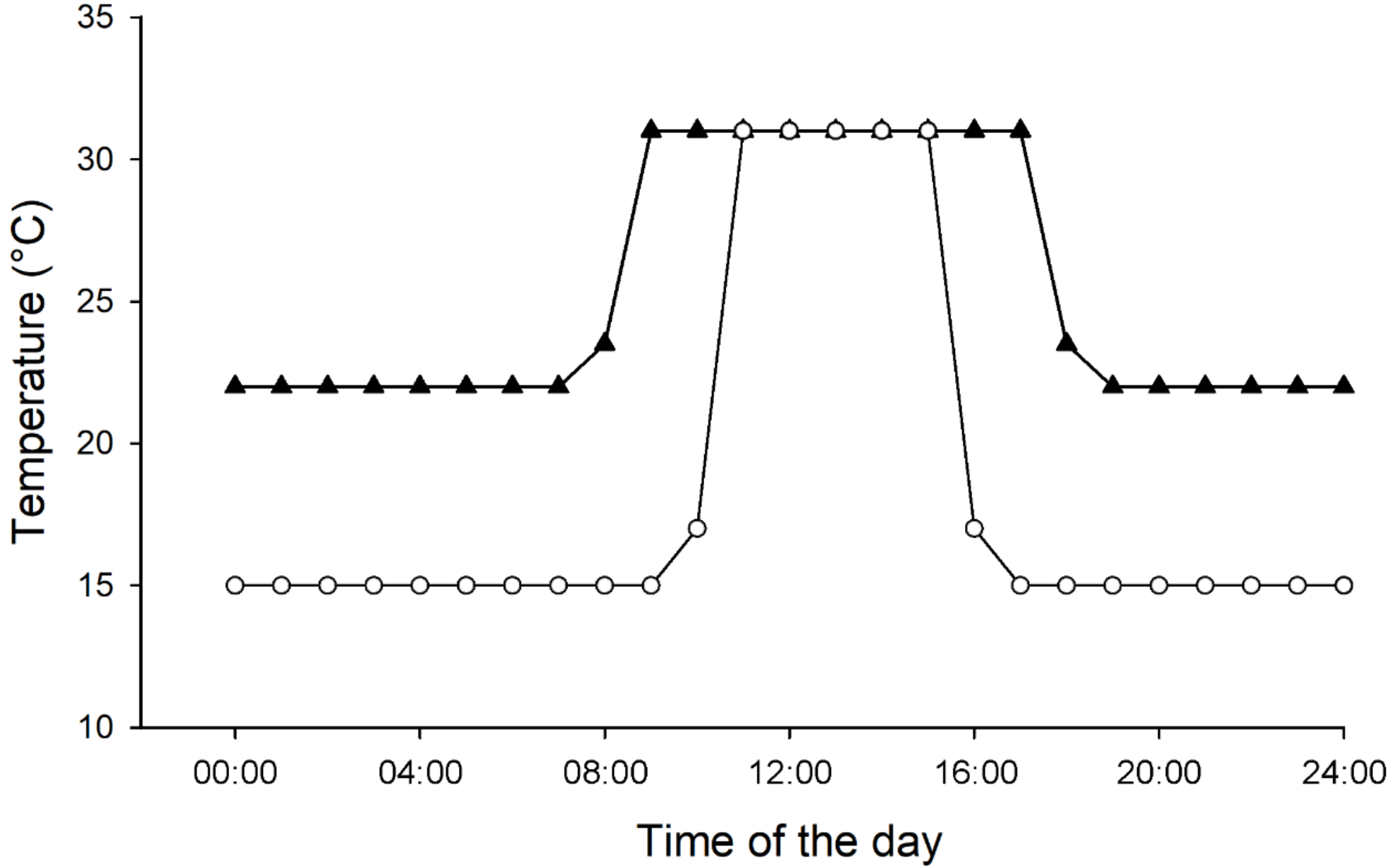
Figure 2 – Relationships between body size and gestation length in temperature treatments. Duration of experimental regimen (number of days from beginning of the experiment to parturition) and snout-vent length (mm) measured in reproductive female *Zootoca vivipara* exposed to hot (open circles, dashed line; n = 48) or cold (closed circles, solid line; n = 49) temperature regimes during pregnancy. A line of best fit is included for each significant relationship ($p < 0.05$).

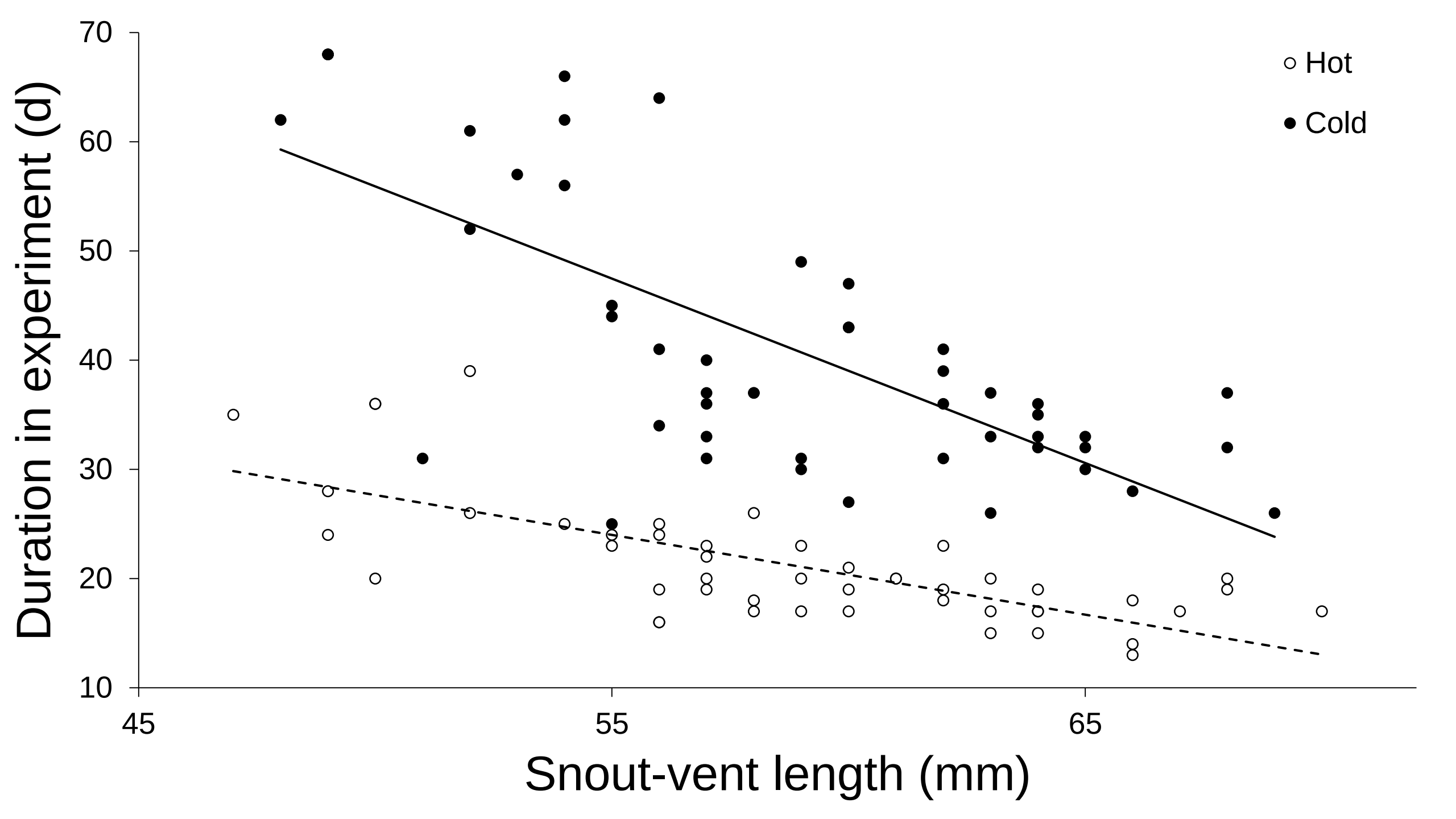
Figure 3 – Average morphological and physiological changes in reproductive female *Zootoca vivipara*. Change in tail width (A), plasma osmolality (B), and body mass (C) measured from beginning of the experiment to parturition for lizards held in either hot and dry (HD; n = 24), hot and wet (HW; n = 24), cold and dry (CD; n = 24), or cold and wet (CW; n = 25) conditions throughout pregnancy. Error bars represent ±1 SEM. Different letters indicate significant differences among groups ($p < 0.05$; Tukey's HSD post-hoc test).

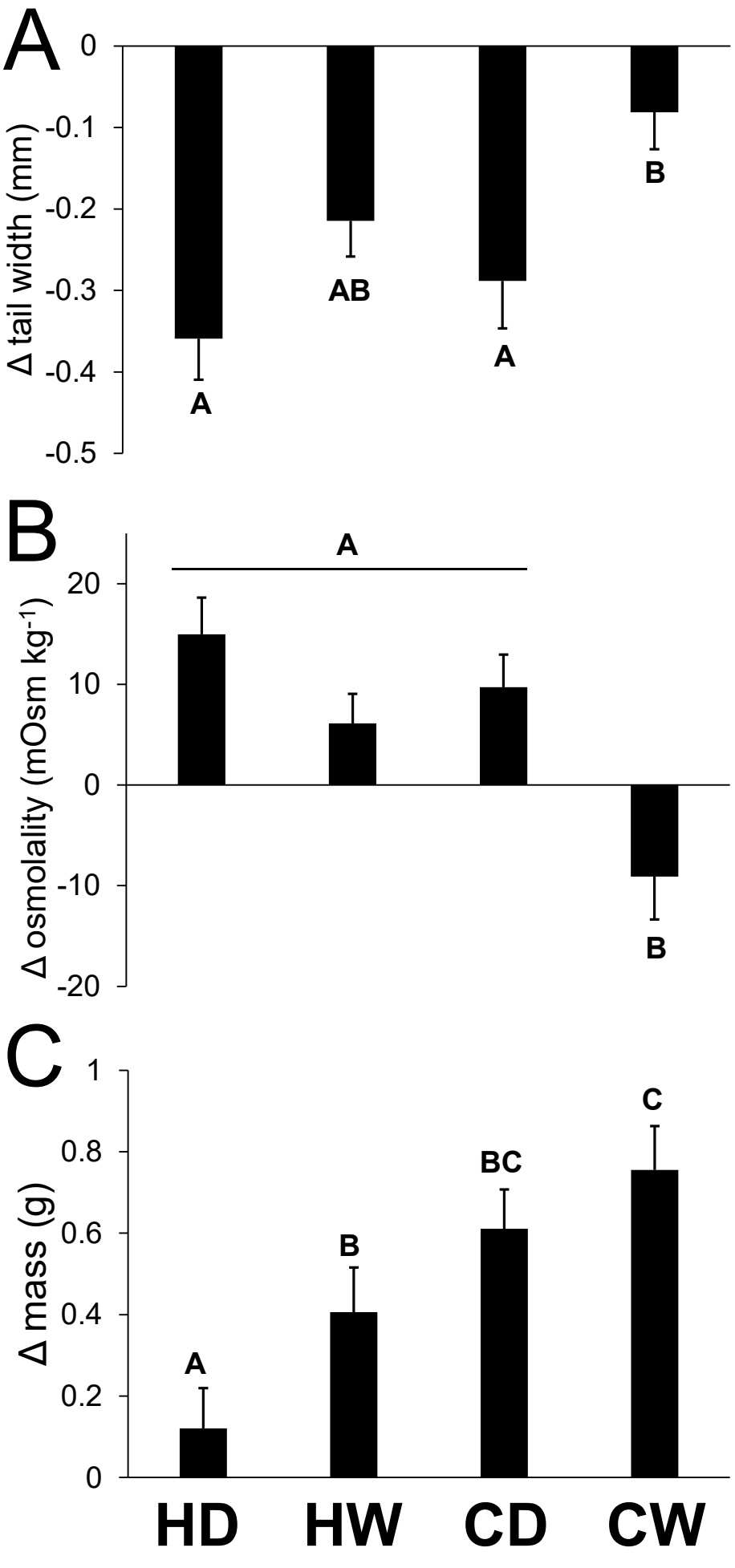
Figure 4 – Average markers of oxidative status in post-reproductive female *Zootoca vivipara.* Oxidative damage [ROMs; A] and non-enzymatic antioxidant capacity [OXY; B]) measured in lizards held in either hot and dry (HD; n = 24), hot and wet (HW; n = 24), cold and dry (CD; n = 24), or cold and wet (CW; n = 25) conditions throughout pregnancy. Error bars represent ±1 SEM. Oxidative damage was significantly higher ($p < 0.05$) in females held without water regardless of temperature. Different letters indicate significant differences among groups (Tukey's HSD post-hoc test). There was a significant temperature by water availability interaction effect ($p < 0.05$) on non-enzymatic antioxidant capacity.

Figure 5 – Average ratio of crickets consumed by reproductive female *Zootoca vivipara.* Lizards were held in either hot and dry (HD; n = 24), hot and wet (HW; n = 24), cold and dry (CD; n = 24), or cold and wet (CW; n = 25) conditions throughout pregnancy. Error bars

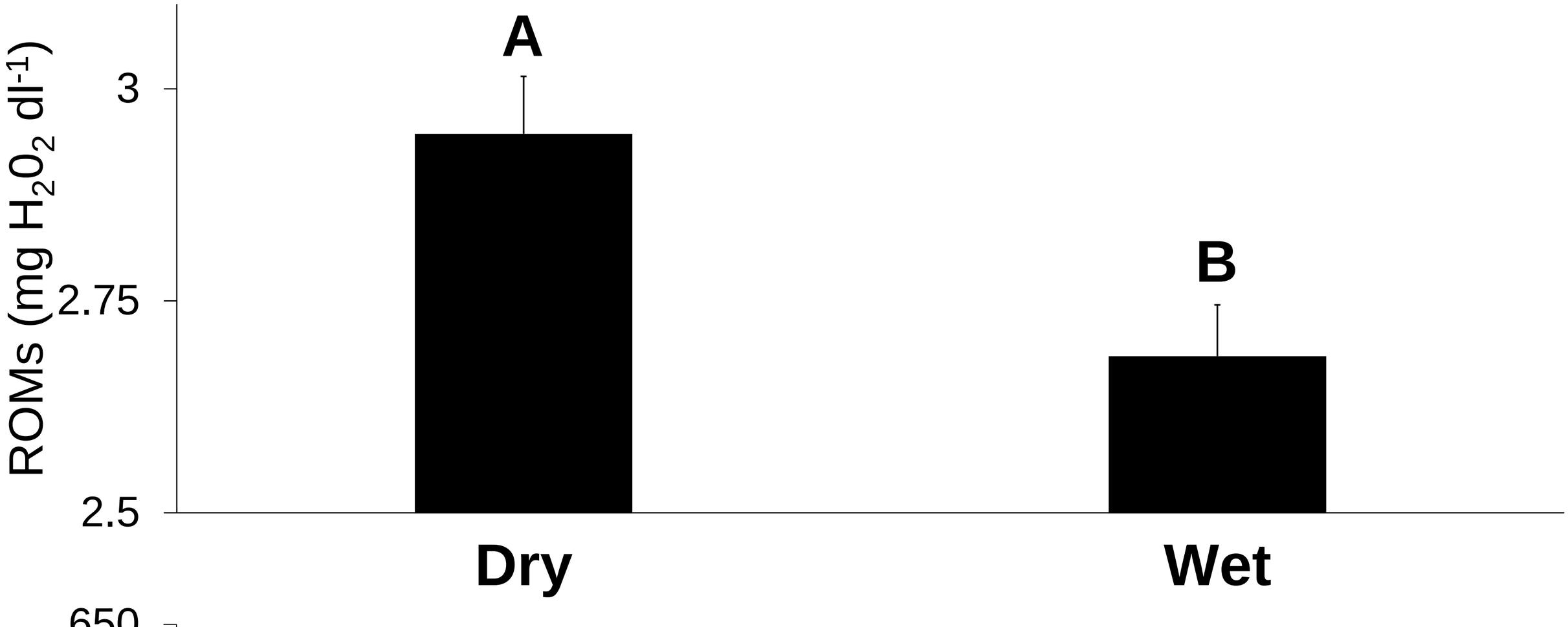
represent ±1 SEM. Different letters indicate significant differences among groups ($p < 0.05$; Tukey's HSD post-hoc test).


35
30
25
20
15
10
Temperature (°C)
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of the day

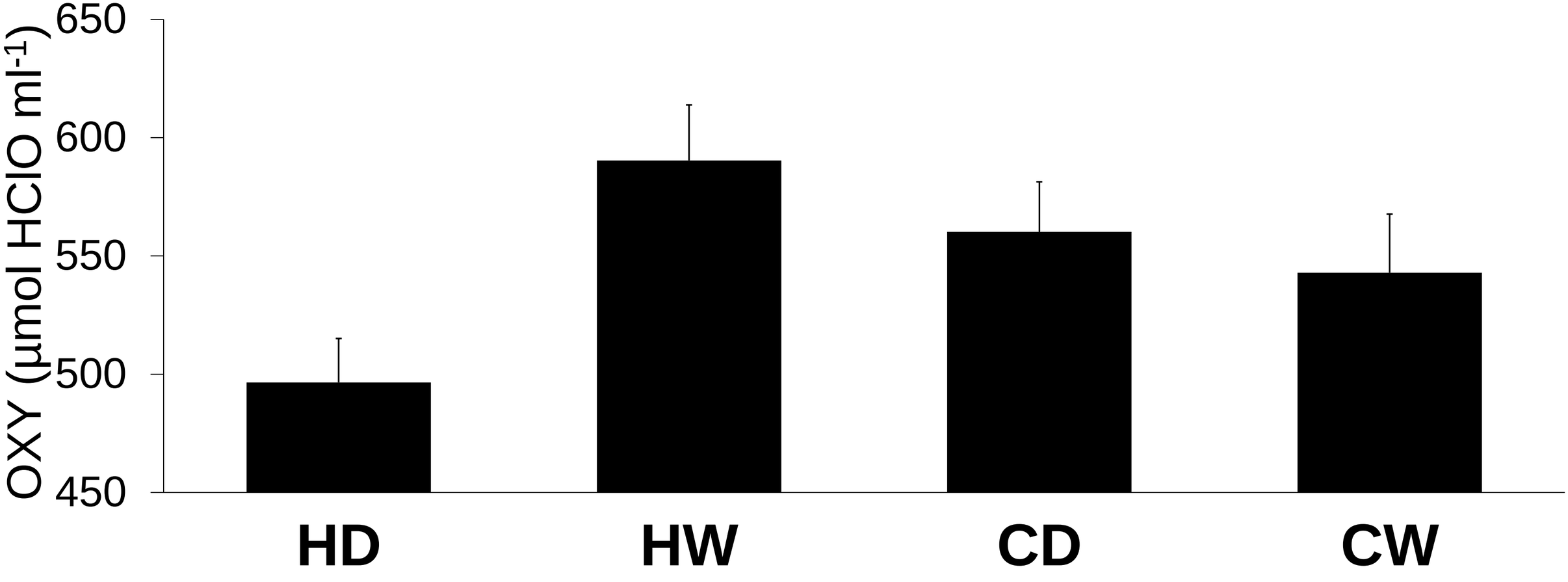

Duration in experiment (d)
70
60
50
40
30
20
10
45
55
65
Snout-vent length (mm)
Hot
Cold

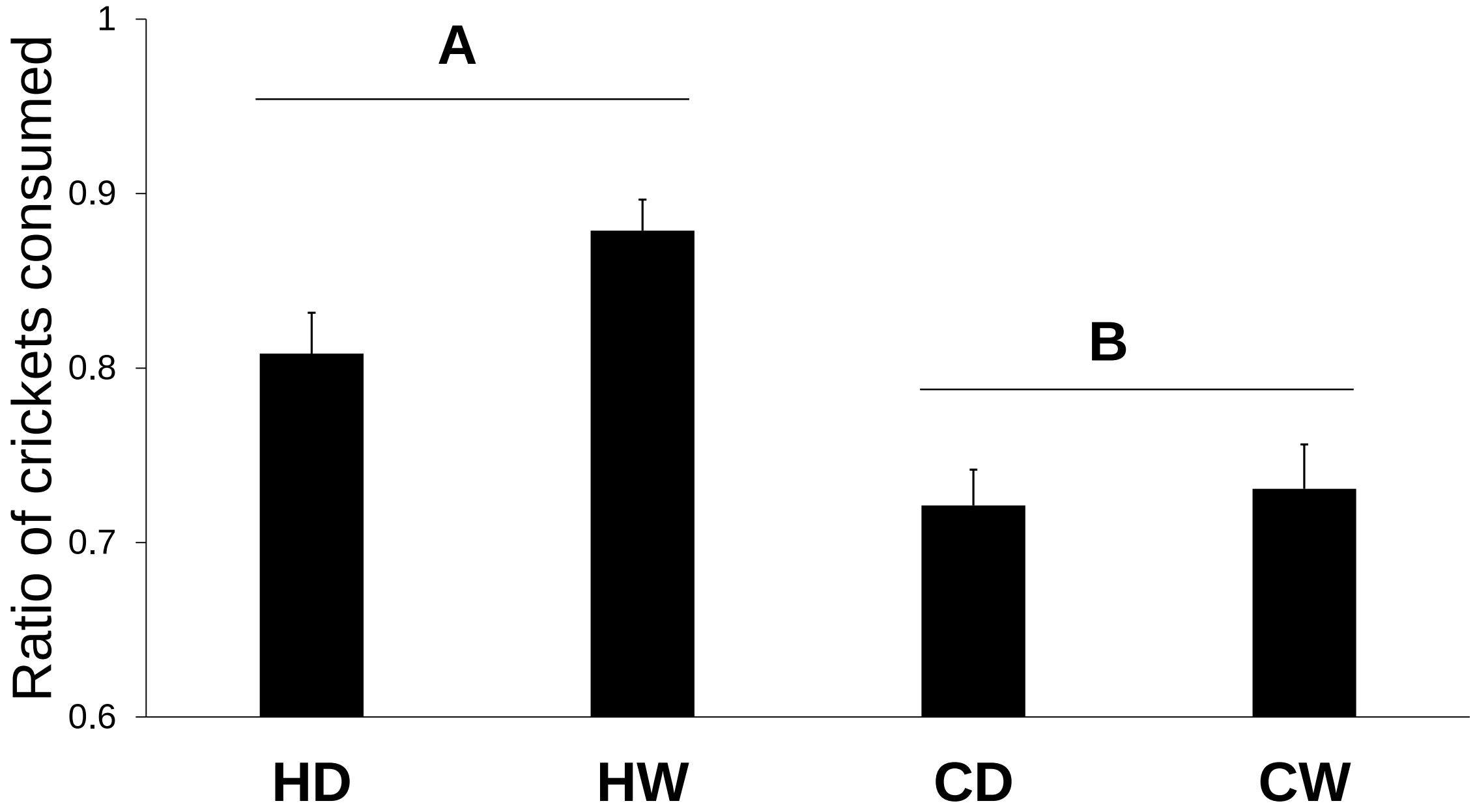

A
0
-0.1
-0.2
-0.3
-0.4
-0.5
Δ tail width (mm)
A
AB
A
B
B
A
20
10
0
-10
-20
Δ osmolality (mOsm kg-1)
B
C
1
0.8
0.6
0.4
0.2
0
Δ mass (g)
A
B
BC
C
HD
HW
CD
CW

A


ROMs (mg H_2O_2 dl^{-1})
3
2.75
2.5
A
B
Dry
Wet

B


OXY (µmol HClO ml^{-1})
650
600
550
500
450
HD
HW
CD
CW


Ratio of crickets consumed
1
0.9
0.8
0.7
0.6
A
B
HD
HW
CD
CW