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Effects of mild wintering conditions on body mass and corticosterone levels in a temperate reptile, the aspic viper (*Vipera aspis*)

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ABSTRACT

Temperate ectotherms are expected to benefit from climate change (e.g., increased activity time), but the impacts of climate warming during the winter have mostly been overlooked. Milder winters are expected to decrease body condition upon emergence, and thus to affect crucial life-history traits, such as survival and reproduction. Mild winter temperature could also trigger a state of chronic physiological stress due to inadequate thermal conditions that preclude both dormancy and activity. We tested these hypotheses on a typical temperate ectothermic vertebrate, the aspic viper (*Vipera aspis*). We simulated different wintering conditions for three groups of aspic vipers (cold: ~6 °C, mild: ~14 °C and no wintering: ~24 °C) during a one month long period. We found that mild wintering conditions induced a marked decrease in body condition, and provoked an alteration of some hormonal mechanisms involved in emergence. Such effects are likely to bear ultimate consequences on reproduction, and thus population persistence. We emphasize that future studies should incorporate the critical, albeit neglected, winter season when assessing the potential impacts of global changes on ectotherms.

1. Introduction

Climate change is now clearly established and is predicted to accelerate in the next century (IPCC core writing team, 2014), while it is considered as one of the main driver of biodiversity loss (Sala et al., 2000; Sinervo et al., 2010; Bellard et al., 2012). Predictions regarding the impact of global warming on biodiversity have become a central theme in ecology (see Pereira et al., 2010 for a review) and one essential prerequisite is to understand the proximate mechanisms by which species will respond to environmental changes (Dillon et al., 2010; Pereira et al., 2010; Huey et al., 2012; Seebacher and Franklin, 2012). Notably, clarification of metabolic adaptations and physiological tolerance are essential to better understand macroecological responses to climate change such as shift in species distribution (Chown et al., 2003; Bernardo and Spotila, 2006; Bernardo et al., 2007; Dillon et al., 2010).

Due to their direct dependency on environmental temperature, ectotherms are expected to strongly respond to thermal constraints mediated by climate change (Deutsch et al., 2008; Huey et al., 2012, see Lillywhite, 2013 for a review). Most ectotherms lack the ability to produce significant metabolic heat and their body temperature is driven by ambient conditions. As a consequence, any change in environmental temperature will affect most aspects of their life cycle through body temperature changes (Angilletta, 2009). Accordingly, impacts of global warming have been shown to influence rates of embryonic development, growth rates, reproduction, activity, maintenance costs, and survival rate (Chamaille-Jammes et al., 2006; Parmesan, 2007; Pörtner and Knust, 2007; Sinervo et al., 2010; Neuheimer et al., 2011; Bestion et al., 2015; Stahlschmidt et al., 2015). Ultimately, climate warming is thought to induce effects on distribution and population persistence (see Parmesan, 2006 for a review). Recent studies have suggested that tropical ectotherms are particularly at risk through increased maintenance costs, overheating and disproportionately reduced activity times (Deutsch et al., 2008; Dillon et al., 2010); while climate change should increase the potential activity time of temperate ectotherms due to latitudinal gradient in thermal breadth and may even enhance their fitness (Parmesan et al., 1999; Buckley et al., 2011, but see Bestion et al., 2015).

Although the hypothesis that temperate ectothermic vertebrates are expected to benefit from climate warming is supported by empirical data (Chamaille-Jammes et al., 2006; Clarke and Zani, 2012; Huang et al., 2013; Stahlschmidt et al., 2015, but see Bestion et al., 2015), very few studies have focused on the impacts mediated by climate change during the overlooked, yet critical, winter season (Zani, 2008; Williams et al., 2015). Winter is a key season for terrestrial ectothermic vertebrates, as it may represent more than half of their time budget and have long-lasting consequences on other life-history stages (Williams et al., 2015). During this season, low temperatures impede normal activity, cellular metabolism and thus major physiological functions (e.g. locomotion, digestion). Therefore, ectotherms stay inactive for several months, secluded in thermally buffered den (Ultsch, 1989). This period is an important facet of ectotherms life history for at least three reasons. First, the selection of appropriate shelter is critical to

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avoid low temperatures and associated mortality risks. Second, for most of these species, reproduction occurs immediately following emergence; which suggests that the changes in neurophysiology that characterize reproduction are likely to occur and thus to be triggered during winter "dormancy" (Lutterschmidt and Mason, 2009). Finally, during this period food intake is absent and specific mechanisms are triggered to optimise energy conservation (Patterson and Davies, 1978; Hailey and Loveridge, 1997; Tattersall and Ultsch, 2008). In turn, individuals can mobilize these body reserves to fuel both emergence and early reproductive mechanisms upon emergence (Chen et al., 2013) despite reduced feeding opportunities.

In cold and temperate areas, current climatic alterations, and especially the increase of surface temperature occurs partly through the elevation of the annual minima (DeGaetano, 1996; Easterling et al., 1997; Easterling et al., 2000), thereby producing overall warmer winter, and thus milder overwintering conditions (Zani, 2008; Williams et al., 2015). Mild winters are expected to greatly influence the responses of terrestrial ectotherms to climate change (Williams et al., 2015). Notably, patterns of resource utilization are likely to be affected by warmer winter temperature through elevated metabolic rates, while resource acquisition is limited. Therefore, milder winters are expected to decrease body condition upon emergence, which, in turn would affect crucial life-history traits, such as winter and spring survival and reproduction (Irwin and Lee, 2000; Zani, 2008; Sorvari et al., 2011; Zani et al., 2012). Independently from energetic considerations, inappropriate overwintering conditions (out of an appropriate thermal range) may also affect reproduction through the disruption of the neurophysiological mechanisms that trigger reproduction upon emergence (Lutterschmidt, 2012). Eventually, non-optimal overwintering temperature may cause a state of chronic stress where an individual cannot be active because temperature is too low, but can neither be dormant because temperature is too high. Corticosterone (CORT), both involved in energy use and response to stress in terrestrial ectotherm, has previously been shown to increase at non-optimal body temperatures (Li et al., 2011; Dupoué et al., 2013; Telemeco and Addis, 2014, but see Sykes and Klukowski, 2009). Importantly, high circulating levels of CORT are known to negatively interact with animal long-term survival (Breuner et al., 2008; Goutte et al., 2010) or reproduction (reviewed in Bonier et al., 2009). Taken together these elements suggest that mild wintering conditions due to current climate change could negatively affect temperate ectothermic vertebrates, through altered patterns of energy conservation and direct (neurophysiological mechanisms, chronic stress) and indirect (through reduced body conditions) effects of suboptimal temperatures on animal fitness.

In this study, we examined the influence of overwintering temperature on patterns of mass loss and baseline CORT upon emergence in a typical temperate ectothermic vertebrate, the aspic viper (*Vipera aspis*). We experimentally simulated different wintering conditions for three groups of aspic vipers (cold: ~6 °C, mild: ~14 °C and no wintering: ~24 °C) during a one month long period. Specifically, we expected body mass loss during dormancy to be positively related to temperature. Upon emergence, we also predicted baseline CORT to be abnormally high in the mild wintering temperature group because of chronic stress.

2. Materials and methods

2.1. Study species and husbandry

The aspic viper (*V. aspis*) is a typical small sized (~50 cm) temperate western palearctic snake species. Individuals are active from early March to the end of October, and rely on sit-and-wait foraging mode to accumulate vast amounts of energy before reproduction (capital breeding), resulting in low reproductive frequency (Naulleau and Bonnet, 1996; Bonnet et al., 1999). From October to late February individuals seclude in thermally buffered underground refugia where they attain a relatively low and constant body temperature (~5–7 °C) (Duguy, 1962).

In this study, we used adult, captive-born snakes from a colony raised at the Centre d'Etudes Biologiques de Chizé (CEBC) since 2009. Individuals were maintained in plastic boxes (30 × 16 × 10 cm) containing a shelter and a water bowl with water ad libitum. A thermal gradient (18–40 °C) was created by a heating cable located at one side of the cage. Snakes were fed with mice once every two weeks.

2.2. Experimental design

We exposed non reproductive snakes to ecologically relevant ambient temperature (T_a) simulating different wintering conditions. Vipers were moved to a transparent plastic box (35 × 25 × 12.5 cm) and transferred in a climatic chamber (Vötsch Industrietechnik,VP 600, Balingen, Germany). The cold treatment ($T_a = 5.7 \pm 1.7$ °C) was designed to mimic the temperature in typical winter den (Duguy, 1962; Shine and Bonnet, 2009). The control treatment ($T_a = 24.0 \pm 0.5$ °C) corresponded to an optimal temperature selected by active snakes that are not engaged in any specific activity (e.g., digestion, skin sloughing or reproduction, Ladyman et al., 2003). Finally, we subjected a group of vipers to a mild winter treatment ($T_a = 13.9 \pm 0.4$ °C). This temperature was selected to simulate warmer winter conditions due to elevation of annual minima.

Snakes were randomly assigned within sexes to a treatment (Table 1). At the onset of experiment, females were heavier and had lower CORT levels than males (both $p < 0.02$, Table 1), but within sexes, snake body mass and CORT levels were similar between treatments (both $p > 0.7$, Table 1).

The experiment was conducted between December and January 2013 consistent with natural biological cycle of snakes (see above). However, in order to limit putatively deleterious effects (i.e., strong body mass loss in the control group), we limited the duration of the experiment to one month (35 ± 2 days). Two weeks before exposure to wintering treatment, all snakes were acclimatized to the control treatment (no wintering, $T_a = 25$ °C). Temperature of each climatic chamber was recorded using miniature temperature data logger (Thermochron iButtons, Dallas Semiconductor, Dallas, TX, USA), to control for potential deviations. Snakes were kept unfed during the experiment to avoid potential effect of contrasted feeding activity between treatments on body mass and CORT levels. In order to mimic the conditions of a typical winter den, drinking water was not available.

2.3. Physiological parameters

2.3.1. Body mass

We measured the change of body mass over exposure to wintering treatment as an index of change in snake body reserve (Lourdais et al., 2002a, 2002b). Initial and final body masses were measured at the onset and the end of the exposure to wintering treatment respectively.

2.3.2. Baseline CORT

We measured the changes in baseline CORT level as an indicator of putative chronic stress (Wingfield et al, 1998; Sapolsky et al., 2000; Landys et al., 2006). Blood samples were collected in a random order at the onset and the end of the treatment. Within less than 4 min (mean ± SE: 3.35 ± 0.12 min), we collected blood samples (100 μl) via cardiocentesis using a 1 ml syringe with a 27-gauge heparinized needle. CORT levels were not related to handling time ($r^2 = 0.0008$, $p = 0.78$, $n = 98$), and were therefore considered to be representative of "baseline CORT" levels. Immediately after collection, blood from each sample was placed into a 0.675 ml microcentrifuge tube and centrifuged for 3 min at 3000 rpm. The plasma was separated, collected and stored at −28 °C. Plasma CORT concentrations were then determined at the CEBC by following a well-established radioimmunoassay protocol