Comparative Biochemistry and Physiology, Part A 189 (2015) 102–106

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Embryonic water uptake during pregnancy is stage- and fecundity-dependent in the snake *Vipera aspis*

Olivier Lourdais [a,b,*], Sophie Lorioux [a,b], Andréaz Dupoué [a], Christian Wright [b], Dale F. DeNardo [b]

[a] Centre d'Etudes Biologiques de Chizé, CNRS, 79360 Villiers en Bois, France
[b] School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form 15 May 2015
Accepted 29 July 2015
Available online 6 August 2015

Keywords:
Water balance
Viviparity
Pregnancy
Osmolality
Fecundity

ABSTRACT

Water is a crucial resource that can profoundly impact the biology of terrestrial organisms. Early life stages are particularly sensitive to hydric constraints because water uptake is an important component of embryonic development. While amniotic eggs constitute a key innovation to terrestrial life, many vertebrates are viviparous wherein the mother must be the source of water for her developing embryos. Since most viviparous squamates are lecithotrophic (i.e., energy is supplied to the offspring as yolk deposited into pre-ovulated follicles), water is the predominant resource allocated from the mother to the offspring during development. Contrary to energy that can be stored (e.g., as fat reserves), water typically cannot be acquired in advance. Therefore, the embryos' need for water can impose significant constraints on the pregnant female. We detailed water flux during pregnancy in a viviparous snake, the aspic viper (*Vipera aspis*). We found that embryonic water uptake occurred mostly during the second half of pregnancy—a period dominated by somatic growth. We also found that, somewhat unexpectedly, changes in female plasma osmolality were negatively related to fecundity. This latter result suggests that water consumption by the female is especially important for large litter sizes, and thus may suggest an important sensitivity of reproductive females to environmental water availability.

1. Introduction

Variation in environmental resources can have profound influences on physiological traits, tradeoffs, and life history traits (O'Connor et al., 2006). Water is a critical resource that can exhibit important seasonal fluctuations and is known to affect terrestrial organisms' physiology (Bradshaw, 1997), growth (Lorenzon et al., 2001), and survival (Shine and Brown, 2008; McKechnie and Wolf, 2010). Embryonic life is particularly sensitive to water availability, and eggs are sensitive to dehydration (Du, 2004; Stein and Badyaev, 2011). For instance, water supply is critical for the conversion of stored energy reserves (yolk) to embryonic mass (Vleck, 1991; Thompson and Speake, 2003; Belinsky et al., 2004). Therefore, water limitation can profoundly alter embryonic development and affect offspring quality or result in embryonic death (Brown and Shine, 2005; Lourdais et al., 2007). Such negative impacts have favored the emergence of multiple adaptations, including the selection of an appropriate nesting site and/or parental care of the eggs to minimize water loss (Shine, 2004a; Shine and Brown, 2008; Stahlschmidt and DeNardo, 2010). The eggshell's structure can also be modified either to minimize water loss (hard-shell eggs are laid in a desiccating atmosphere) or, conversely, favor water uptake (parchment shell eggs laid in a humid environment or substrate) (Deeming and Ferguson, 1991; Shine and Thompson, 2006).

While most terrestrial organisms are oviparous, viviparity has emerged on multiple occasions in amphibians, non-avian reptiles, and mammals (Blackburn, 2000; Shine, 2004b). These repeated transitions are associated with a diversity of embryonic nutrition strategies that have attracted considerable interest (Blackburn, 2006, 1999. Despite the focus on energy allocation, a viviparous female must also be the source of water for embryonic development (Thompson, 2007), and this facet of maternal resource provisioning has multiple implications to consider (Oftedal, 2002). For instance, contrary to energy that can be stored (as body fat) to prepare for energy investment during reproduction (Lourdais et al., 2002b), water typically cannot be accumulated in advance. Therefore, water availability in the environment must match the timing of the reproductive requirement for water. In many environments, water may well be more constraining than energy during reproduction because gestation often occurs during dry summer months (Lourdais et al, 2004a; Le Gaillard et al., 2012). Water limitation may alter reproductive success either by inducing embryonic mortality or by affecting offspring quality (Dauphin-Villemant and Xavier, 1986; Ross and Desai, 2005). Importantly, water demand during reproduction should be directly related to reproductive effort since an increase in the number of developing embryos increases water demand. Therefore, increased fecundity should either increase female water acquisition from the environment or cause greater hydric deficit in the female.

* Corresponding author at: CEBC-CNRS UMR 7372, 79 360 Villiers en Bois, France. Tel.: + 33 5 49 09 96 16; fax: + 33 5 49 09 65 26.
E-mail address: Lourdais@cebc.cnrs.fr (O. Lourdais).

http://dx.doi.org/10.1016/j.cbpa.2015.07.019
1095-6433/

Despite the likely importance of water in influencing female behavior, physiology, and fecundity, maternal water flux remains largely overlooked. Squamate reptiles are unique among vertebrates for the vast number of times that viviparity has independently evolved (>100, Blackburn, 2000; Stewart and Thompson, 2000). While some species show complex placentotrophy, most lizards and snakes are lecithotrophic where yolk reserves support the energy demands during embryonic requirement (Stewart and Thompson, 2000; Thompson and Speake, 2003; Van Dyke et al., 2014). Therefore, maternal resource demand during pregnancy is primarily focused on water. This situation offers a simple context to address the implication of water allocation independent of nutritional aspects. A previous study in the common lizard, *Zootoca vivipara*, suggested that water uptake is greatest during late embryonic stages when most somatic growth occurs (Dauphin-Villemant and Xavier, 1986). The same study also revealed an important impact of experimental water deprivation on reproductive success. To improve our understanding of water allocation, it is also critical to consider the impact of pregnancy and fecundity on female water balance.

We used a viviparous snake, the aspic viper, *Vipera aspis*, to describe water flux during pregnancy. We used high-resolution ultrasonography to monitor embryonic volume and estimate water intake during development. We also monitored relevant maternal traits including body mass and plasma osmolality over pregnancy. Our main hypothesis was that embryonic water requirements should influence maternal water intake and water balance.

We tested the following predictions:

(1) Water uptake by the embryos should mainly occur during the second half of gestation when embryonic somatic growth occurs.

(2) Maternal mass change during pregnancy should reflect water intake and be closely related to the number of developing embryos.

2. Material and methods

2.1. Study species and maintenance

The aspic viper, *V. aspis*, is a small viviparous snake of the western Palearctic region. This species is a typical capital breeder and thus accumulates energy needed for reproduction over an extended period before engaging in a reproductive effort (Bonnet et al., 2002; Lourdais et al., 2002b). Pregnancy is a long process, lasting up to three months, and it is associated with an increase in thermal preference and precision, which necessitates an increase in thermoregulatory activities (Saint Girons, 1952; Naulleau, 1979; Ladyman et al., 2003) and modified escape tactics (Lorioux et al., 2013a). Reproduction in females is also associated with reduced movement and food intake (Saint Girons, 1952). The study species is lecithotrophic, and it has been shown that food intake after ovulation has no influence on reproductive output (Lourdais et al., 2002a)

2.2. Experimental design

We collected 42 pregnant females (12 in 2009 and 30 in 2010) in neighboring districts (Loire Atlantique, Maine et Loire, Vendée,) of west-central France. Reproductive status (late stages of vitellogenesis) was first determined by manual palpation of the abdomen and then confirmed in the laboratory with high-resolution ultrasonography (SonoSite microMaxx, Inc., Bothell, WA, USA). Body mass (±1 g) and snout–vent length (±5 mm) were recorded, and scale clipping was used to identify individuals.

Female vipers were housed in cages (100 cm × 30 cm × 35 cm) that had a thermal gradient (18 °C–41 °C) created by placing a 75 W incandescent light bulb over one side of the cage. The light was on for 6 h per day enabling basking from 10:00 to 16:00 h, but forcing body temperatures to drop to room temperature (18 °C) at night. Each cage contained a main shelter (half cylinder of polyvinyl chloride (PVC) pipe, diameter = 15 cm, length = 37 cm, with two 3-cm circular openings in the sides) located at one end of the cage opposite from the basking zone and three secondary shelters (half cylinder PVC pipe, diameter = 15 cm, length = 25 cm) scattered throughout the cage to facilitate concealed movements to the basking zone.

Females were randomly assigned to one of the cages, some housing two females and some housing three as a result of space limitations. Importantly, no agonistic behavior was observed in co-housed individuals. Females were provided water *ad libitum*, but were not fed until parturition, since they typically do not eat during gestation (Lourdais et al., 2002a). Importantly, a pre-ovulation ecdysis occurs and provides a reliable indicator of the onset of gestation in this species (Lorioux et al., 2013b).

2.3. Variables measured

2.3.1. Embryonic volume

We monitored the change in volume of the embryonic unit (i.e., embryo, yolk, and extra-embryonic membranes) to estimate embryonic water uptake during pregnancy. Using high-resolution ultrasonography (see Lorioux et al., 2013b), we determined total volume at three different stages of embryonic development (Hubert and Dufaure, 1968): "ovulation" (−1.9 ± 0.7 days before ecdysis), mid-pregnancy (29.8 ± 0.9 days after ecdysis; 37.6 ± 1.2% of developmental duration), and late pregnancy (55.5 ± 1.3 days after ecdysis; 70.6 ± 1.5% of developmental duration). For each female, we collected sagittal view images of the most cranial and most caudal embryonic units and measured their heights (H), lengths (L), and general shape (see Maritz and Douglas, 1994). We estimated embryonic volume (cm^3) following the method described in Maritz and Douglas (1994), which considers embryonic unit L, H, and a coefficient associated with its form (F_{emb}): Embryonic volume $= \pi \times L \times H^2 \times F_{emb}$. For analysis, we averaged the volume of the two embryonic units measured at each stage for each female. For one female, the initial volume was not recorded and this individual was removed from the analysis.

2.3.2. Body mass (BM)

Change in BM has been well established as an estimator of water loss in squamate reptiles that are food-restricted (DeNardo et al., 2004; Lillywhite et al., 2008a,2008b; Dupoué et al., 2014). BM was collected during early and late pregnancy stages and also after parturition. We calculated BM changes (g) between early and late pregnancy stages for all individuals.

2.3.3. Osmolality

We also measured changes in female plasma osmolality (Osmo), as this parameter is an effective indicator of hydration state (Peterson, 2002). For safety reasons, each snake was encouraged to enter its head and upper body into a clear plastic tube, and then blood was collected via cardiocentesis (Saint Girons et al., 1993). This variable was only measured in the 12 pregnant females collected in 2009, which had substantial variation in litter size (1 to 11). We collected blood samples (100 μL) using a 1 mL heparinized syringe and a 27-gauge needle. Blood samples were collected at early, mid, and late pregnancy and after parturition. Plasma osmolality ($mOsm.kg^{-1}$) was measured from 10 μL triplicates (intra-individual variation <1%) as described in (Wright et al., 2013).

2.3.4. Litter traits

For each female, we recorded litter size (number of undeveloped ova, stillborn, and neonates), fit litter size (number of neonates only), litter mass (mass of undeveloped ova, stillborn, and neonates), and fit litter mass (mass of neonates only) (see details in methods of Lourdais et al., 2002a).