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The reproductive biology of the Ethiopian hedgehog, *Paraechinus aethiopicus*, from central Saudi Arabia: The role of rainfall and temperature

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ABSTRACT

We set out to test whether the Ethiopian hedgehog, *Paraechinus aethiopicus* is a seasonal or aseasonal breeder and assess which environmental cues bring about both reproductive recrudescence and its subsequent regression. In this study, the body mass, morphometry of the reproductive tract the histology of the reproductive organs and the hormone concentrations of males and females were studied over 12 consecutive months in a wild population of the Ethiopian hedgehog from central Saudi Arabia. Using these data we investigated the potential proximate environmental cues that may trigger the onset of reproduction. Temperature is important for the initial activation of the males by bringing about an increase in testosterone concentration. In female hedgehogs the first rains trigger the onset of reproductive activation with ovulation. In turn increased temperature brings about the final activation of the males with increased testes size and seminiferous tubule diameter. Pregnancies were first recorded in early spring and summer coinciding with a rise in plasma progesterone concentration that peaked in spring. The Ethiopian hedgehog was found to exhibit marked seasonal reproduction. This study strengthens the findings that changes in rainfall and temperature in the Middle East are crucial cues for the onset of reproduction in small mammals.

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1. Introduction

Small desert mammals inhabiting these arid regions need to regulate their energy expenditure and water turnover (Schwimmer and Haim, 2009). Deserts are hostile environments with marked daily and seasonal temperatures as well as sporadic rainfall patterns that results in poor primary productivity as a consequence hedgehogs need to cue into times of the year when survival of offspring will be maximal (Schmidt-Nielsen, 1964).

Reproduction in small mammals may be regulated by a number of proximate factors such as photoperiod, temperature, rainfall and in turn availability of food resources (Bronson and Heideman, 1994; Wube et al., 2009). Photoperiod is a common proximate cue utilised by organisms to time reproduction as the changes are constant from year to year (Prendergast et al., 2001; Bronson, 2009). Photoperiod is the cue of choice in organisms inhabiting

high latitudes where seasonal variability in climatic conditions and food is predictable. However, in more arid regions different proximate factors may be operational such as rainfall, temperature or food availability (El-Bakry et al., 1998; Shanas and Haim, 2004).

The pattern of rainfall and subsequent availability of water in desert environments has been found to be a very important cue for the onset of reproductive activity (Sicard et al., 1993; Bukovetzky et al., 2012; Khammar and Brudieus, 1987). Reproduction in desert areas is precarious because of the scarcity and unpredictability of rainfall, the extreme temperatures and the associated lack of food resources. Rainfall may be used as an indicator of primary production in arid environments (Nelson and Desjardins, 1987). As a general rule reproduction usually only arises in deserts when food resources and water coincide with favourable temperatures that in turn promote optimal conditions for mating, pregnancy and the subsequent production of young that maximises their survival (Bronson, 1988).

To date there has been relatively few detailed studies investigating reproduction over an entire calendar year in small mammals from Saudi Arabia. Henry and Dubost (2012), studying Cheeseman's

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gerbil, *Gerbillus cheesmani*, and Sarli et al. (2015, 2016), studying the Baluchistan gerbil, *Gerbillus nanus*, and the Arabian spiny mouse, *Acomys dimidiatus*, from south western Saudi Arabia, found these murids to cue their reproduction to rainfall. In fact, both the males and females synchronise their reproduction to the short spring rains, rapidly producing offspring that can benefit from the subsequent primary productivity.

The Ethiopian hedgehog, *Paraechinus aethiopicus*, is a small terrestrial nocturnal mammal ranging in body mass from between 280 and 500 g with a spiny integument and occurs in the semi-arid and arid deserts of North Africa and the Middle East. In Africa, it is distributed from Mauritania, Western Sahara and Morocco in the west through to Egypt, Sudan, Eritrea and Ethiopia in the east. In the Middle East it has been recorded from Syria, Jordan, Iraq and marginally in Iran, and from most of the Arabian Peninsula except for Hijaz/Asser mountains (Harrison and Bates, 1991; He et al., 2012). The Ethiopian hedgehog, is adapted to the harsh and dry conditions of the desert (Essghaier et al., 2015). It is found in wadis, around oases and sandy plains where there is vegetation, they are even known to frequent the coast (Harrison and Bates, 1991).

The hedgehog feeds on insects and other invertebrates, and may also consume vegetation and vertebrates. Even though they have a wide distribution there is currently a dearth of information pertaining to the reproductive biology of the hedgehog, apart from basic information on time of mating, courtship and numbers of young produced (Al-Musfir and Yamaguchi, 2008; Yamaguchi et al., 2013).

There is limited information on the reproductive biology of the desert hedgehog which is mainly confined to the mating behaviour, gestation period and weaning period from around rubbish dumps in Qatar (Yamaguchi et al., 2013). Yamaguchi et al. (2013) observed mating events to occur in the Ethiopian hedgehog mainly during late winter and into early spring (February and March), following a period of hibernation (December to January), however, they also record a second mating period in early summer (May to July). This implies that females may have the reproductive potential to breed twice a year, whereas males appear to be sexually active throughout the year. Currently, there is no reported histological or hormonal profiles to confirm the reproductive biology of the Ethiopian hedgehog from a natural environment (Yamaguchi et al., 2013).

In this study we investigated the reproductive biology of the Ethiopian hedgehog from central Saudi Arabia by collecting hedgehogs and euthanizing them on a monthly basis with an aim to measuring the testosterone in males and progesterone in females over an entire calendar year. In addition we examined the gross morphology and detailed histology from the post-mortem of the samples. We hypothesized that reproduction in the Ethiopian hedgehog would be highly dependent on rainfall. We predicted that reproductive recrudescence of the gonads and an associated elevation of the reproductive sex hormones would occur following the rains and the increase in ambient temperature, whereas the ovaries and testes would regress and the hormone concentrations would be lower during the cooler and dry months. As a consequence we predicted that the Ethiopian hedgehog would reproduce seasonally following the rains when food availability would be maximal.

2. Methods and materials

2.1. Study site and animal capture

Sampling took place in central Saudi Arabia, in and around the town of Unizah (26.0840° N, 43.9940° E) (Al Qassim Province). The capture zones were predominantly desert ecosystem comprising

annual grasses, small herbaceous plants, small trees, rocky outcrops and small wadis. Hedgehogs were captured on a monthly basis by hand at night, using a beam light while driving around from July 2014 until June 2015 (Table 1).

Collection of hedgehogs was authorised by the Saudi Wildlife Authority and the research protocol was approved by the animal ethics committee of the University of Pretoria (Ethics number EC017-16) which follows the South African National Standards for animal welfare and research. The animals were handled and euthanised humanely. The animals were used for a number of other research studies to ensure maximum use of the euthanised animals. The animal experimentation was conducted in accordance with ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

2.2. Dissection

On the day of capture the animals were weighed using Sartorius U4600P balance 1200 g Capacity, with 01 g readability. The animals were euthanised using an overdose of ether in a euthanizing chamber. Once the animal had expired, whole blood was obtained from the heart using a 3.0 cc syringe with a 25 g x 5/8" needle. Immediately after collection, the blood was transferred into a Vacutainer lithium heparin blood collection tubes and centrifuged at 3000 rpm for 15 min. Plasma was collected and stored at –20 °C for hormonal analysis.

2.3. Histology

Fresh ovaries and testes were weighed using a balance Adam Equipment PW 254 PW Series Analytical Balance, 250 g Capacity, with 0.0001 g readability. The length and width were measured (mm) using a pair of digital callipers (Mitutoyo American Corporation, Aurora, Illinois, USA) once fat and connecting tissue were removed. Testicular and ovarian volume (mm³) was calculated using gonadal length and width by using the formula for the volume of an ellipsoid ($V = 4/3 \pi ab^2$ where a represents half the maximum length and b half the maximum width (Woodall and Skinner, 1989). The gonads were initially fixed for 24 h in Bouin's fixative and subsequently subjected to a series of histological preparations, involving dehydration and embedding in wax (Drury and Wallington, 1967). Ovaries were cut into 5 µm-thick sections and testes were cut into 8 µm-thick sections with a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, N.Y., U.S.A.). Sections were mounted in consecutive order on microscope slides with gelatine and dried in an oven at 36 °C for 48 h and thereafter stained with Ehrlich's haematoxylin and counter-stained with eosin (Drury and Wallington, 1967). Testicular sections were used to measure round seminiferous tubules with a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany) and photographed at ×10 magnification with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0; Motic China Group, LTD., Xiamen, P.R. China). The programme Motic Images Plus 2.0 ML (Motic China Group, LTD., Xiamen, P.R. China) was used to measure the diameter of 100 seminiferous tubules (µm).

Ovarian sections, taken from both ovaries of each female, were examined serially for stages of follicular growth with a light microscope at ×100, ×200 and ×400 magnifications. Follicular stages were identified and classified according to Bloom and Fawcett (1964). Primordial follicles are generally observed in large numbers on the periphery of the ovary. For all other follicular stages the total number was counted throughout each ovary.

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