Towards evidence-based husbandry for caecilian amphibians: Substrate preference in *Geotrypetes seraphini* (Amphibia: Gymnophiona: Dermophiidae)

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ABSTRACT - Maintaining caecilians in captivity provides opportunities to study life-history, behaviour and reproductive biology and to investigate and to develop treatment protocols for amphibian chytridiomycosis. Few species of caecilians are maintained in captivity and little has been published on their husbandry. We present data on substrate preference in a group of eight Central African *Geotrypetes seraphini* (Duméril, 1859). Two substrates were trialled; coir and Megazorb (a waste product from the paper making industry). *G. seraphini* showed a strong preference for the Megazorb. We anticipate this finding will improve the captive management of this and perhaps also other species of fossorial caecilians, and stimulate evidence-based husbandry practices.

INTRODUCTION

The paucity of information on caecilian ecology and general neglect of their conservation needs should be of concern in light of global amphibian declines (Alford & Richards 1999; Stuart et al., 2004; Gower & Wilkinson, 2005). Conservation breeding programmes are becoming more important for the long-term survival of many amphibian species (Gascon, 2007; Griffiths & Pavajeau, 2008). The requirements of captive amphibians are complex (Antwis et al., 2014; Antwis & Browne, 2009; Ogilvy et al., 2012; Verschooren et al., 2011) and further research is needed to ensure that this lack of knowledge does not undermine future conservation breeding initiatives. Maintaining caecilians in captivity provides opportunities to investigate behaviour and reproductive biology (Kouete et al., 2012; Wilkinson et al., 2013), to develop treatment protocols for amphibian chytridiomycosis (Wake, 1994; O' Reilly, 1996) and to establish husbandry requirements.

Whenever possible, husbandry should be informed by field data (Tapley & Acosta, 2010; Michaels & Preziosi, 2013) but these are often unavailable, especially for understudied taxa such as caecilians. Folklore husbandry, i.e. methods or supposed best practices established without evaluation and often justified for unknown reasons (Arbuckle, 2009), is obviously less desirable than integrating existing ecological and biological information into evidence-based husbandry plans that attempt to mimic good conditions in nature (Arbuckle, 2013). It should be noted that the natural conditions in which animals are encountered in the wild may not always be optimal.

Few species of caecilians are maintained in captivity

(Gower & Wilkinson, 2005) and little has been published on the captive husbandry of terrestrial caecilians (Wake, 1994; O' Reilly, 1996). A basic parameter in terrestrial caecilian husbandry is substrate, but data on tolerances and preferences in the wild or in captivity are mostly lacking. Terrestrial caecilians are reported from a wide range of soil pH (Gundappa et al., 1981; Wake, 1994; Kupfer et al., 2005). In the laboratory, burrowing capabilities of four species of terrestrial caecilians were limited by soil compaction, and they showed preferences for burrowing in the least compacted soil available and for utilising existing, rather than constructing new burrows (Ducey et al., 1993). More data are required on the habitats that are preferred or tolerated by caecilians and it is likely that substrate preference will differ between caecilian species.

Geotrypetes seraphini is a widely distributed caecilian, found from Guinea to Angola (Scholz et al., 2010). It is likely to be surface active on occasion given that it has been collected in pitfall traps (Wollenberg & Measey, 2009) and appears to be fairly regularly collected from the wild for the pet trade (Gower & Wilkinson, 2005). It is maintained by several zoological collections including Zoological Society of London, London Zoo. In December 2013, two G. seraphini at ZSL London Zoo were observed with inflammation around the vent and a marked swelling in the last 2 cm of the body that palpation indicated was due to a solid mass in both individuals. Specimens were anesthetised in an aqueous solution of tricaine methanesulfonate (MS-222) for further examination. In both cases, compacted coir substrate formed a solid mass at the end of the gastrointestinal tract, one specimen died during the procedure and the second died the day after. Post mortem examination did not determine whether or not there was an issue in the function of the hindgut or cloaca resulting in the mass of substrate, or whether the substrate was the primary cause of the compaction. There were no other remarkable pathological findings. Coir had been used as a substrate for this species for several years at ZSL London Zoo without problem, but it was decided to investigate an alternative substrate. Here we present experimental evidence for a clear substrate preference in captive *G. seraphini*.

METHODS

Historic and current husbandry

Geotrypetes seraphini have been in the herpetology living collection at ZSL London Zoo for five years and have bred on two occasions. Initially, animals were maintained in groups in various sized plastic boxes containing moist coir at ambient room temperatures (18-28°C) in an off-show area and fed *ad libitum* on annelid worms (*Lumbricus terrestris* and *Dendrobanea* species) three times per week supplemented irregularly with 3rd instar live and dead crickets (*Gryllus bimaculatus* and *G. assimilis*) and bloodworm (*Chironomus* species) in shallow water-filled dishes.

In March 2013 a dedicated caecilian breeding facility was initiated at ZSL London Zoo as part of a collaborative project with The Natural History Museum's Herpetology Research Group aimed at developing methods for caecilian husbandry and revealing life-history and behaviour. The facility currently comprises two climatically-controlled rooms and houses seven species of caecilian.

Substrate type

Megazorb (Northern Crop Driers (UK) Ltd.) was selected as a potential substrate for *G. seraphini* after communications with other keepers of fossorial caecilians who used a similar product, Carefresh® (product of U.S.A./Absorption Corp in WA, www.absroptioncorp. com) because of its availability, ease of maintenance and because it is sterile and meets laboratory standards (Danté Fenolio & Dennis Parmley, pers. comm). Although Carefresh® was available in the UK, the manufacturers were unable to confirm that the product was unbleached and therefore potentially harmful to caecilians. Megazorb is a waste product of the paper industry that contains unbleached wood-derived cellulosic fibre and inorganic pigment (kaolin and calcium carbonate).

Choice chamber experiment

On the 2nd January 2014, eight *G. seraphini* (wild-caught from Cameroon) were weighed and moved into eight individual choice chambers (Fig. 1) constructed using 360 mm x 200 mm x 200 mm faunariums (Exoterra, Rolf C. Hagen (UK) Ltd., Castleford, UK). A solid 150 mm acrylic sheet secured with aquatic grade silicone, incompletely divided each enclosure equally such that caecilians could only move between substrates by moving

over the surface. Humid coir or moist Megazorb (washed in tap water) were added on different sides of the choice chamber to a depth of 150 mm after squeezing out excess moisture (Fig. 1). Neither substrate was sterilised but all handling of substrate and caecilians was while wearing powder free nitrile gloves.

Ambient temperature ranged between $20-27^{\circ}$ C (night minimum/day maximum) following discussions with Marcel Tala Kouete, a researcher who has worked in the field with *G seraphini*. Photoperiod was 10L:14D for the duration of the study.

At the start of the trial all individuals were weighed and four individuals were placed in the coir and four in the Megazorb. An identical ninth choice chamber included only a humidity and temperature data logger (Lascar (UK) EL-USB-2-LCD) in each of the substrate types, recording every five minutes for the duration of the study. Choice chambers were rotated every three days by 180° to control for potential positional effects (e.g. due to different lighting). Caecilians were fed three times a week with two live *Dendrobanea* worms or two pre-killed *G. assimilis* placed in each side of the choice chamber at each feeding event. If the substrate started to dry out visibly, aged tap water was added and the top 2 cm of the soil was turned by hand. The pH of each of the substrates was recorded using a K181 pH Soil Testing Kit (Bosmere © UK).

The position of each caecilian was recorded once every day between 09:00 and 16:00 hrs by gently lifting the choice chamber. Location could be established mostly without disturbing animals because parts could usually be seen through the clear base and/or sides of the chamber. Otherwise the position of each caecilian had to be established by sifting through the substrate in the choice chamber by hand. A control was not deemed appropriate in this study because health issues had been observed in animals that had been provided coir as a substrate. Because Megazorb had not previously been used as a substrate for caecilians it was not considered appropriate to house them on this substrate alone. The experiment ended after 39 days, on the ninth of February 2014, the G. seraphini were weighed and two separate groups transferred to enclosures with a Megazorb substrate.



Figure 1. Choice chambers used to assess substrate preference in *G. seraphini*, with Megazorb to the front of each chamber and coir to the rear.



RESULTS

Figure 2. The number of daily records for each caecilian in each substrate type.

Specimen	Mass (g) 02.01.14	Mass (g) 09.02.14
1	10.3	11.7
2	8.5	8.1
3	14.2	14.2
4	16.2	19.2
5	16.5	20.2
6	21.6	23.9
7	10.6	10.4
8	9.6	10.2

Table 1. Body masses of each caecilian at the beginning and the end of the choice chamber trials.

Substrate	Mean temperature (C)	Mean humidity (%rh)	pН
Coir	22.7 (+/- 1.5)	98.8 (+/- 2.8)	7.5
Megazorb	22.8 (+/- 1.4)	100.3(+/-3.8)	7.5

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All individuals were recorded much more frequently (91% of the 312 daily observations) in the Megazorb (Fig. 2). Burrows were seen in both types of substrate in all individual choice chambers, even for individual 2, which was never observed in the coir during the daily inspections. Caecilians were generally secretive and never observed feeding. Five out of eight individuals became heavier, the mass of one individual did not change and two individuals lost weight over the 39 day period (Table 1). Temperature, humidity and pH were very similar in both substrates (Table 2).

DISCUSSION

We made no observations at night when *G. seraphini* is expected to be most active, but our results indicate a clear

preference for Megazorb over coir as a diurnal resting site in our captive G. seraphini (Fig 2). Temperature, humidity and pH were similar in both portions of the chamber and do not explain the preference between the two substrates (Table 2) although substrate humidity could not be completely standardised in this study because the different substrates appeared to dry out at different rates (the surface layer of coir seemed to dry out more rapidly than Megazorb). Rotating the choice chambers had no impact on the temperature or the humidity recorded by the data loggers. Burrows appeared to be more clearly defined in the Megazorb and were perhaps more stable in this substrate due to the larger particle size than the coir, and substrate preference might be explained by caecilians selecting substrates in which they did not have to frequently construct new burrows, which is energetically costly (Ducey et al., 1993). Coir is somewhat powdery and G. seraphini in this substrate often had small coir particles attached to their skin (BT, pers. obs.).

We compared only two substrates for one species. Other substrates should be evaluated and substrate preference might vary with the species in question. Neither of the tested substrates are natural for *G. seraphini* which, in Cameroon have been collected by digging in (mostly wet) soil, sometimes under logs and occasionally under leaf litter (MW & DJG, pers. obs). Further research evaluating substrate preference choice incorporating leaf litter and other refugia would be beneficial. Some caecilians may be epigeic at least some of the time (Gower et al., 2004) and refugia may be as important as substrate type for these taxa. This study demonstrates that improved (and evidence-based) husbandry for caecilians can be progressed through simple experiments. It is hoped that this study will encourage similar research for other caecilian species.

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