

Substrate preference in the fossorial caecilian *Microcaecila unicolor* (Amphibia: Gymnophiona, Siphonopidae)

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INTRODUCTION

Amphibians are the most threatened major group of vertebrates; 42 % of assessed species are threatened with extinction (IUCN et al., 2019). When the threats cannot be reversed or controlled in the short term, conservation breeding programmes are often considered essential (Gascon et al., 2007; Griffiths & Pavajeau, 2008). However, amphibians are not always easy to maintain and breed in captivity (Tapley et al., 2015). Although natural conditions in which animals may be encountered in the wild may not always be optimal, it is considered best practice to incorporate field data into captive management practices (Michaels et al., 2014a) and where these are lacking, an evidence-based approach to husbandry practices should be pursued as far as possible (Arbuckle, 2013).

Caecilians epitomise the complexities of maintaining poorly known amphibian taxa in captivity. More than 55 % of assessed caecilian species are listed as Data Deficient by IUCN (IUCN, 2019) and only six species are currently maintained by zoos and aquaria globally (Species 360, 2020) but failure to thrive or breed is not uncommon (Flach et al., 2020). Consequently, advances in husbandry are difficult to achieve. There are at least eleven published accounts of caecilian ex-situ management, but these are biased towards aquatic typhlonectid caecilians. Following Amphibian Ark's Conservation Needs Assessment process (Johnson et al., 2018), several species of caecilian have now been assessed as requiring ex-situ management for conservation purposes. These include six of the eight species of caecilian that are endemic to the Seychelles (e.g. Maddock, 2018). If these and other similar programmes are to be realised, it is imperative that advances are made in caecilian captive husbandry.

Empirical data on even the most basic husbandry parameters are lacking for most species of caecilian, including the substrate used to maintain them. We used a simple choice chamber to compare two commonly used substrate types. All methods used in this study were non-invasive and did not require a UK Home Office Licence and were compliant with the BHS Ethics Policy (British Herpetological Society, 2017). No adverse effects of this husbandry intervention were foreseeable.

METHODS

Five *Microcaecila unicolor* of unknown sex and age were collected from the Kaw mountains at Camp Patawa between 2008 and 2010 by two of the authors (DG & MW). These were transferred to ZSL London Zoo in 2013 as part of a collaborative project with the Natural History Museum's Herpetology Research Group aimed at refining methods for caecilian husbandry, developing and validating field methods, and discovering aspects of life history and behaviour. Prior to our study, specimens were housed individually in a climate-controlled facility. Room temperature ranged from 24–27 °C (night minimum–day maximum). All enclosures (56 x 56 x 35 cm) were glass and custom-made with slanted bottoms to create a humidity gradient. Ten percent of the glass lid consisted of a fine mesh for ventilation. Specimens were provided with a 15 cm deep layer of Megazorb (Northern Crop Driers (UK) Ltd.) substrate, a waste product from the paper making industry containing unbleached, wood derived cellulosic fibre and inorganic pigment (Kaolin and calcium carbonate), which is sold for equine husbandry (Tapley et al., 2104). Megazorb was soaked in reverse osmosis water mixed with tap water to an alkalinity of 15-20 mg/L and a pH of 7.5 and dosed with tap water conditioner (Tetra products), for 24 h until saturated and then drained of excess water in a cotton pillowcase.

On the 27 March 2017, five *M. unicolor* were weighed and moved into five individual choice chambers 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 of the substrate. On one side of the chamber we used Megazorb (as described above) and on the other we added moistened topsoil that had been steam treated by the manufacturer and came from a single batch. Substrates were sprinkled into the choice chambers to a depth of 15 cm by hand and were not compacted. A pH test of each substrate (K181 pH Soil Testing Kit, Bosmere © UK) showed that both were pH 7.5. An identical choice chamber (without caecilians) included a humidity and temperature data logger (Lascar (UK) EL-USB-2-LCD) in each of the substrate types, recording at five minute

intervals for the duration of the 40 day study. To control for potential positional effects, each choice chamber was rotated 180° every three days. During the study, ambient temperature ranged between 23–26 °C (night minimum–day maximum) and photoperiod was 10L:14D. Caecilians were offered food three times a week; two live *Eisenia* earthworms were placed in each side of the choice chamber at each feeding event. We recorded the position of each caecilian once, daily between 09:00 and 16:00 h; the choice chambers were gently lifted, and the location of the caecilian determined as part of the body was visible through the side or base of the container. The experiment ended after 40 days, on the 05 May 2017, the *M. unicolor* were weighed again at the end of the study. We used Social Science Statistics, (2020) for all statistical analyses. The presence of each individual caecilian was scored daily with a 0 (present in topsoil) or 1 (present in Megazorb), following methods used for *Agalychnis* frogs by Michaels et al. (2014b). Mass changes in each caecilian were calculated as the difference between initial and final masses. Mean choice scores over the 40 day period were calculated for each individual caecilian and these were analysed using a one-sample t-test (two-tailed) against a test mean of 0.5 (i.e. the mean expected if there is no substrate preference), following methods used by Michaels et al. (2014b).

RESULTS AND DISCUSSION

All five *M. unicolor* were recorded much more frequently in the Megazorb substrate (93 % of the 200 daily observations across all individuals). The mean (\pm standard deviation) preference score was 0.945 ± 0.051 , which was significantly different from a mean of 0.5 ($t = 17.454$, $df = 4$, $p = 0.000032$ two-tailed). The mean temperature of topsoil (25.4 ± 0.437 °C) was not significantly different from the mean temperature of Megazorb (25.2 ± 0.753 °C) ($t = -0.987485$, $df = 4$, $p = 0.37931$ two-tailed). Humidity of the two substrates was also not significantly different. The air in both substrate types was supersaturated with water vapour, the mean relative humidity exceeding 100 % 105.9 ± 1.85 % and 104.0 ± 1.26 % in topsoil and Megazorb respectively ($t = 1.533475$, $df = 4$, $p = 0.19994$ two-tailed). We did not record any null observations and caecilian burrows were observed in both substrate types in every enclosure even though the caecilian may not have been observed in one or other of the substrates during daily checks. Caecilians were generally secretive and never observed feeding. All individuals decreased in mass over the course of the study (mean percentage mass decrease 16.4 % [12.2–21.3 %]), however we were unable to quantify how well specimens were feeding during the study because this would have resulted in unnecessary disturbance and a significant deviation from our standard husbandry practices with this species (Table 1).

Our results show that *M. unicolor* has a statistically significant preference for Megazorb as a diurnal resting site. Although substrate preference might differ among caecilian species, our findings are congruent with previous research showing that another burrowing caecilian (*Geotrypetes seraphini*) exhibited a strong preference for the Megazorb substrate over another substrate (coir) under almost identical experimental conditions (Tapley et al., 2014). To

Table 1. The mass of five *M. unicolor* at the beginning and the end of the substrate choice test and mean daily substrate choice score based on scoring 0 (present in topsoil) or 1 (present in Megazorb)

Choice chamber #	Mass at start of test (g)	Mass at end of test (g)	Mass change during test (g)	Mean choice score
1	29.6	23.8	-5.8	0.975
2	35.4	30.4	-5	1
3	32.7	28.7	-4	0.95
4	29.1	22.9	-6.2	0.85
5	25.7	21.8	-3.9	0.95

ensure data from the current study were comparable with the previous study, observations were not made between 16:00 and 09:00 h. Coir is a relatively powdery substrate and does not retain burrow structures; Tapley et al. (2014) suggested that this could have explained the preference of Megazorb over coir because burrows are energetically costly to construct (Ducey et al., 1993). In the current study with *M. unicolor*, topsoil did retain burrow structures and none of the recorded environmental factors differed between topsoil and Megazorb, so it is unclear why there should be a preference for Megazorb over topsoil. However, given that immediately before the start of the test all the caecilians had been housed in Megazorb, the preference could have resulted from learned behaviour and/or neophobia. All the caecilians lost mass during the test and we suspect this results from increased activity associated with being moved to a new enclosure rather than both, or one of, the substrates being inappropriate.

Despite the limited number of substrates that have been tested to date, the current results and those of Tapley et al. (2014), which together involve two species in two families of caecilians, suggest that Megazorb is a justified choice of substrate for the maintenance of terrestrial caecilians. However, it would be preferable for husbandry practitioners to have access to microhabitat data from the wild prior to the implementation of any conservation breeding programme for caecilians and for these substrate conditions to be replicated in captivity. The provision of multiple substrate types in zones within an enclosure may also be considered in order to provide the animals with a choice.

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