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POSTMORTEM FINDINGS IN EIGHT SPECIES OF CAPTIVE CAECILIAN (AMPHIBIA: GYMNOPHIONA) OVER A TEN-YEAR PERIOD

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Abstract: Between July 2007 and June 2017 there were 86 deaths in the populations of eight caecilian species at the Zoological Society of London (ZSL) London Zoo. The mortality rate (deaths per animal-year at risk) ranged from 0.03 in the Congo caecilian (*Herpele squalostoma*) to 0.85 in Kaup's caecilian (*Potomotyphlus kaupii*). Among the 73 individuals examined post mortem, no cause of death or primary diagnosis could be established in 35 cases, but of the others the most common cause of death was dermatitis (22 cases). When all significant pathological findings were considered, skin lesions of varying types were again the commonest (56 cases), particularly among the aquatic species: *Typhlonectes compressicauda* (18 out of 21 cases), *T. natans* (8/10) and *P. kaupii* (12/14). Other common findings were poor gut-fill (35 cases), kidney and gastrointestinal lesions (10 cases each), generalized congestion (8 cases) and poor body condition (6 cases). This review adds to the growing body of knowledge regarding the presentations and causes of disease in captive caecilians.

Key words: Caecilian, dermatitis, disease, pathology, review.

INTRODUCTION

Among major vertebrate taxa, caecilians are one of the least known and understood groups at the present time;[43] their tropical distribution and often soil-dwelling habits mean that caecilians are not regularly encountered in routine herpetological surveys.[13] However, this is changing, and new species are being discovered frequently, with 18% of the 212 currently recognized species being described in the last decade[10] and a new family discovered recently.[19] Fresh insights have been gained into caecilians' unique anatomy, reproductive biology, physiology, and evolution[8,17,32,33,39,42] and there is increased focus on their conservation.[13,16,21,24] The captive requirements of amphibians are often complex and difficult to fulfill[36] and relatively few species of caecilian have been kept in zoological living collections. Further research is needed to establish husbandry requirements and increase our veterinary knowledge so that different species can be maintained in conservation breeding initiatives. Captive husbandry has also been identified as an effective way of acquiring new knowledge on caecilian behavior and reproductive biology[20,44] including data that can help to inform conservation assessments and action plans.

The Zoological Society of London (ZSL) London Zoo has maintained a number of wild-caught caecilian species in captivity, along with species that are more commonly kept and bred in zoos and private collections, such as *Typhlonectes compressicauda* and *T. natans*, in order to undertake applied, conservation, and husbandry-related research. These research activities are in partnership with The Natural History Museum, London, which has been at the forefront of caecilian research this century.

The first major health survey of captive caecilians covered only five species.[27] Since then there have been a number of other publications concerning caecilian health and disease.[2,5,7,12,22,30,31,34] Here we review the postmortem findings of caecilians that died at ZSL London Zoo over a 10-yr period, with the aim of increasing our

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understanding of causes of death and prevalence of pathological lesions in species that have been investigated previously, along with species that are underreported. Our aim is to use this information to improve husbandry techniques, such as basic housing and feeding, and optimize health with the ultimate aim of establishing successful breeding and research programs.

MATERIALS AND METHODS

Details of the caecilians maintained in the collection at ZSL London Zoo were obtained from the Zoological Information Management System (ZIMS), the annual inventories, and the keepers' records. Numbers of animals present at the start of the study, importations, births, deaths, and exports were all recorded with the dates on which they occurred. Mortality rates (deaths per animal-year) for each species were calculated as incidence rates.[40] Thus deaths were the disease incidents of interest and the populations at risk were all the animals present in the collection during the 10-yr study period, based on the amount of time individuals were in the collection and determined by the number of animals multiplied by the number of years they were present (calculated from the census data as animal-months [to the nearest month] and then divided by 12). For fossorial (primarily soil-dwelling) species that were only counted infrequently (in order to reduce disruption of animals and the substrates in which their burrow systems are constructed), minimum and maximum figures were obtained by assuming that deaths of individuals occurred on the day after the last census when recorded as alive, or the day before they were recorded as dead or missing, assumed dead.

Routine gross postmortem examinations were carried out on all caecilians that were submitted (Fig. 1); others were not examined because carcasses were not found (despite looking through the substrate—likely decomposed and/or eaten by small invertebrates or conspecifics), were too autolyzed for meaningful examination or were required for studies by the Natural History Museum. Where the age category of the individual was uncertain, total length was compared with published ranges for the species.[38] Body condition was assessed by viewing and palpating the perispinal musculature along the body, and hydration by the appearance and feel of the skin, subcutaneous tissues, and viscera. In most cases, swabs from the coelomic cavity, skin, and any organs showing abnormalities were submitted for bacterial culture, and fecal samples were submitted for

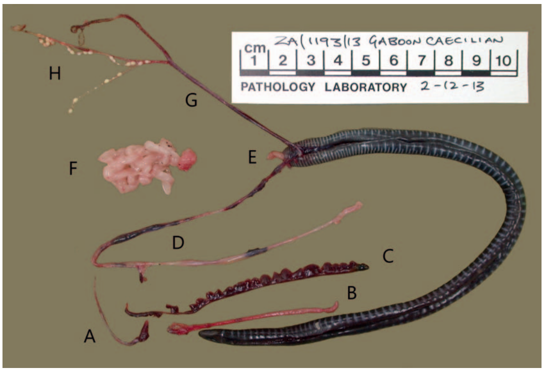

Figure 1. Caecilian dissection. Adult female *Geotrypetes seraphini* showing viscera (**A** = heart, **B** = lung, **C** = liver with gall bladder, **D** = alimentary tract with spleen, **G** = kidneys and oviducts, **H** = ovaries), coelomic fat bodies (**F**) and prolapsed cloaca (**E**).

parasitological examination and bacterial culture. Samples of a range of tissues were fixed in 10% buffered formalin and subsequently trimmed and submitted for routine histopathological processing and staining with hematoxylin and eosin. Over the 10-yr period, sections were examined by three different histopathologist co-authors. Samples of skin, liver, and occasionally other tissues were frozen. Skin samples were subsequently tested for the presence of chytrid fungus. DNA was extracted and subjected to quantitative real-time polymerase chain reaction (qPCR) diagnostic assay, using *Batrachochytrium dendrobatidis* (Bd) primers specific to the ITS-1/5.8S region of ribosomal gene.[4] PCR inhibition was reduced by modifying the protocol to include bovine serum albumin (BSA).[11] Since 2013 primers have also been available for *B. salamandrivorans* (Bsal) so samples from the years 2013–2017 were tested for both species simultaneously using a duplex qPCR.[3] Liver samples were tested by real-time qPCR using ranavirus primers.[28]

Fecal samples were diluted in physiological saline and examined by direct microscopy for the presence of parasites and their oocysts and eggs. Tissue swabs and feces were inoculated onto Columbia blood agar (CBA) plates with 5% horse blood and incubated at 37°C in aerobic conditions, with daily examination for the presence of bacterial colonies. Colonies of interest were subcultured and provisionally identified on the basis of: a) colonial characteristics, b) microscopic examination of a Gram's stained preparation, and c) biochemical tests, using the API 20NE commercial test kits and online software (bioMérieux UK Limited, Basingstoke, Hampshire

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Table 1. Numbers of caecilians maintained at the Zoological Society of London, London Zoo during the 10-yr period of July 2007–June 2017.

Species	No. present on 1 July 2007	No. imported (year)	No. born (year)	No. exported (year)	No. died (No. examined post mortem)	No. present on 30 June 2017	No. animal-years	Mortality rate (deaths per animal-year)
Typhlonectes compressicauda	10	13 (2013) 3 (2014)	0	1 (2015)	22 (21)	3	48.1	0.46
Typhlonectes natans	6	6 (2013) 2 (2014) 1 (2016)	1 (2012)	3 (2013)	10 (10)	3	38.8	0.26
Geotrypetes seraphini	0	10 (2008) 18 (2012) 1 (2016)	4 (2009) 1 (2013)	1 (2016)	25 (17)	8	76.8–81.1[a]	0.31–0.33[a]
Herpele squalostoma	0	3 (2008) 5 (2014)	0	0	1 (1)	7	39.3	0.03
Microcaecilia unicolor	0	5 (2013) 2 (2016)	0	1 (2016)	1 (1)	5	18.7	0.05
Rhinatrema bivittatum	0	8 (2013) 2 (2016)	0	1 (2016)	7 (7)	2	8.9	0.79
Potomotyphlus kaupii	0	9 (2013)	5 (2015)	0	14 (14)	0	16.4	0.85
Idiocranium species	0	4 (2014) 3 (2016)	0	1 (2016)	6 (2)	0	7.2–8.1[a]	0.74–0.83[a]
Total	16	95	11	8	86 (73)	28		

[a] Animals missing recorded as dead either on the day of discovery (maximum animal-years, minimum mortality rate) or on the day after the previous census (minimum animal-years, maximum mortality rate).

RG22 6HY, United Kingdom). Skin swabs were additionally cultured on Sabouraud's medium to determine if there were any fungal isolates.

Postmortem reports for all caecilians that died between 1 July 2007 and 30 June 2017 were reviewed and, where necessary and possible, additional diagnostic tests performed. The aim was to establish and compare: a) primary cause of death, or reason for euthanasia, b) all relevant pathological diagnoses, and c) microbiological and other findings. Because of their high frequency, skin lesions were further investigated and reviewed.

RESULTS

At the start of the review, 1 July 2007, there were 16 individuals of two aquatic caecilian species in the collection: 6 *Typhlonectes natans* and 10 *T. compressicauda*. Two terrestrial species, *Geotrypetes seraphini* and *Herpele squalostoma*, were acquired in 2008, followed in 2013 by a third aquatic species, *Potomotyphlus kaupii* (the alternative spelling *Potamotyphlus* is also in use), and two terrestrial species, *Rhinatrema bivittatum* (terrestrial, but with aquatic or subaquatic larvae) and *Microcaecilia unicolor*. The eighth and final caecilian, an undescribed *Idiocranium* species that is terrestrial, was imported in 2014 (Table 1). In total, 95 animals were imported and 11 were born so that, with the 16 original animals, 122 individuals were present at some point during the 10-yr period. Eight animals were exported and 86 died or went missing (assumed dead) of which 73 were submitted for postmortem examination, leaving 28 individuals in the collection at the end of the review period.

The population size and mortality rate for each species are shown in Table 1 and reveal three groupings in terms of mortality rate: very low (0.03–0.05 deaths per animal-year, *H. squalostoma* and *M. unicolor*), medium (0.26–0.46 deaths per animal-year, both *Typhlonectes* species and *G. seraphini*) and very high (0.74–0.85 deaths per animal-year, *R. bivittatum*, *P. kaupii,* and the *Idiocranium* species), with almost 30 times difference between the mortality rate of the hardiest species (0.03 deaths per animal-year) and the most susceptible (0.85 deaths per animal-year). The reason for this difference in mortality rate is unclear, but it is worth noting that the species in the lowest mortality rate category are generally considered to be more dedicated burrowers that, of the terrestrial species, spend the least time on the surface. The range of age-categories, plus the body weights, body lengths, and body conditions

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Table 2. Numbers and characteristics of caecilian species examined post mortem at the Zoological Society of London, London Zoo, July 2007–June 2017.

Species	No. examined: newly hatched/ juvenile/adult	No. examined: male/female/ unknown	Adult body weight (g) Mean (min–max)	*n*	Adult total length (cm) Mean (min-max)	*n*	Adults' body condition: very thin/ slightly thin/ normal/fat
Typhlonectes compressicauda	0/0/21	10/9/2	51.1 (18.5–110.9)	21	35.7 (30.2 – 42.0)	19	0/5/14/0
Typhlonectes natans	1/0/9	4/4/2	92.9 (14.1–202.0)	8	51.1 (36.4 – 59.5)	8	2/1/7/0
Geotrypetes seraphini	0/3/14	3/8/6	22.8 (5.7–56.0)	13	24.5 (18.5 – 32.0)	13	0/3/9/1
Herpele squalostoma	0/0/1	1/0/0	12.4	1	27.2	1	0/0/1/0
Microcaecilia unicolor	0/0/1	0/0/1	14.9	1	33.0	1	0/0/1/0
Rhinatrema bivittatum	0/4/3	0/1/6	6.8 (1.7–14.1)	3	22.2 (20.8 – 24.6)	3	1/0/2/0
Potomotyphlus kaupii	0/5/9	2/3/9	54.6 (23.4–113.7)	7	48.7 (33.0 – 54.2)	8	0/1/6/0
Idiocranium species	0/0/2	2/0/0	3.5 (2.7–4.2)	2	16.5 (16.2 – 16.8)	2	0/0/2/0
Total	1/12/60	22/25/26					3/10/42/1

of adult individuals are given in Table 2. The exact age of the majority of individuals was not known so age could not be taken into consideration when comparing mortality rates, and neither could the length of time individuals were maintained at other collections prior to importation.

The 73 caecilians that were examined post mortem were submitted throughout the year, although 30 (41%) were submitted in the months of November and December (Table 3). Over the 10 yr there were increased submissions in 2013 and 14, but this correlated with the increased numbers of species and individuals maintained in the collection. The precise cause of death could not be established in 35 cases (open diagnosis, Table 4), 13 of which were uncertain due to the degree of autolysis. Dermatitis, often of unknown etiology, but presenting as significant damage to the epidermis and/or dermis, was deemed responsible for death in 22 cases and was the major cause of death of *T. compressicauda* and *P. kaupii*. Dermatitis was also the main feature of three cases of chytridiomycosis in *G. seraphini*. Six individuals, including four *G. seraphini*, escaped from their vivaria and were subsequently found dead, and in varying degrees of desiccation. Two *G. seraphini* died under general anesthesia for the investigation of constipation with associated cloacitis associated with ingestion of coir (coconut fiber derivative) substrate, while each of the five remaining cases had unique diagnoses.

In addition to the predominant causes of death there was a wide range of other gross and histopathological lesions (Table 5), the most prevalent being skin lesions (56 cases; 77% of the total). Poor gut-fill (assessed subjectively as inadequate ingesta in the alimentary tract) was another common finding (35 cases; 48%), but only

Table 3. Seasonality of caecilian submissions for postmortem examination at the Zoological Society of London, London Zoo, July 2007–June 2017.

Month	*Typhlonectes compressicauda*	*Typhlonectes natans*	*Geotrypetes seraphini*	*Herpele squalostoma*	*Microcaecilia unicolor*	*Rhinatrema bivittatum*	*Potomotyphlus kaupii*	*Idiocranium* species	Total
Jan	3	1	1	0	0	0	0	0	5
Feb	1	0	0	0	1	0	0	0	2
Mar	0	0	0	0	0	0	2	0	2
Apr	5	0	0	0	0	0	1	0	6
May	2	2	0	1	0	0	0	0	5
Jun	0	2	0	0	0	0	4	0	6
Jul	2	0	0	0	0	0	3	0	5
Aug	0	0	2	0	0	0	1	0	3
Sep	0	0	1	0	0	1	0	1	3
Oct	1	0	3	0	0	2	0	0	6
Nov	2	1	2	0	0	4	3	1	13
Dec	5	4	8	0	0	0	0	0	17
Total	21	10	17	1	1	7	14	2	73

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Table 4. Causes of death in caecilians at the Zoological Society of London, London Zoo, July 2007–June 2017.

Main finding	*Typhlonectes compressicauda*	*Typhlonectes natans*	*Geotrypetes seraphini*	*Herpele squalostoma*	*Microcaecilia unicolor*	*Rhinatrema bivittatum*	*Potomotyphlus kaupii*	*Idiocranium* species	Total
Open (autolyzed)	8 (2)	8 (2)	5 (3)	0	1 (1)	6 (4)	5 (1)	2	35 (13)
Dermatitis	12	1	1	0	0	1	7	0	22
Exposure–escaped tank	1	0	4	0	0	0	1	0	6
Chytridiomycosis	0	0	3	0	0	0	0	0	3
Death under anaesthesia	0	0	2	0	0	0	0	0	2
Anasarca	0	0	1	0	0	0	0	0	1
Trauma	0	0	0	0	0	0	1	0	1
Stomatitis	0	1	0	0	0	0	0	0	1
Pneumonia	0	0	1	0	0	0	0	0	1
Renal failure	0	0	0	1	0	0	0	0	1
Total	21	10	17	1	1	7	14	2	73

6 carcasses (8%) were assessed as being in poor body condition and 7 (10%) were dehydrated. Generalized congestion and/or vascular distension (as recorded in the gross findings, but probably the same) was reported in 8 cases (11%) and edematous signs (ascites and/or anasarca) in 6 (8%), while tissue nodules and/or granulomas and mineralization were detected in 6 (8%) and 4 (5%) cases respectively. The commonest location of lesions were the kidneys and gastrointestinal tract (10 cases each; 14%), cloaca and lungs (6 each; 8%), liver and spleen (4 each; 5%). Suspected perispinal bruising was observed in three aquatic animals (2 *T. natans* and 1 *P. kaupii*) but was not confirmed histologically. The consistent position, dorsal to the gall bladder, indicated likely muscle staining with bile pigment post mortem rather than bruising.

Bacteriological swabs were taken from most cases (60, 82%); usually from the coelomic cavity, skin, and abnormal-looking organs (Table 6). In 78% of these cases a mix of bacterial species was cultured, most likely due to postmortem invasion and/or contamination. In some cases, specific bacteria predominated cultures, most commonly *Aeromonas hydrophila* (33 isolates, 55%). The fungus *Batrachochytrium dendrobatidis* was identified by PCR either ante or post mortem and two of the three cases in *G. seraphini* also showed typical histopathological lesions (described in detail elsewhere[12]). Two Bd-PCR–positive *P. kaupii* did not have characteristic histological changes, albeit they were moderately autolyzed, and were not defined as cases of chytridiomycosis. A further 46 individuals representing all eight species were Bd-PCR negative and none of the 21 caecilians tested for *B. salamandrivorans* were positive. Various unidentified fungi were observed histologically in another 10 cases. Flagellated protozoa were observed in fecal samples from 10 animals, and nematodes were identified from another 10 animals, either histologically (cross-sections of adults present, for instance *Rhabdias*-like species in lung sections, Fig. 2) or by the presence of ova in feces. In the absence of significant, widespread parasitic disease, further identification was not attempted. There was no evidence of ranavirus in any of the 20 caecilians tested.

Gross and histological descriptions of the observed skin lesions were quite variable (Table 7) but with several common features. Excess or abnormal sloughing was seen in 16 cases (29%) although it was often noted that the state of autolysis was possibly partly responsible. Red-

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Table 5. Significant findings in postmortem examinations of caecilians at the Zoological Society of London Zoo, July 2007–June 2017.

Finding	*Typhlonectes compressicauda*	*Typhlonectes natans*	*Geotrypetes seraphini*	*Herpele squalostoma*	*Microcaecilia unicolor*	*Rhinatrema bivittatum*	*Potomotyphlus kaupii*	*Idiocranium* species	Total
Poor body condition	2	2	1	0	0	1	0	0	6
Dehydration	1	0	5	1	0	0	0	0	7
Poor gut-fill	10	7	3	0	0	5	9	1	35
General congestion, vascular distension	4	1	2	0	0	1	0	0	8
Ascites/anasarca	0	1	3	0	0	1	1	0	6
Trauma	0	0	0	0	1	0	1	0	2
Lipidosis	0	0	0	0	0	0	1	1	2
Serous fat atrophy	0	0	1	0	0	0	0	0	1
Celomitis/serositis	1	0	2	0	0	0	0	0	3
Nodules/granulomas	2	3	1	0	0	0	0	0	6
Tissue mineralisation	1	1	1	1	0	0	0	0	4
Arteritis	0	0	1	0	0	0	0	0	1
Skin lesions[a]	18	8	10	1	0	6	12	1	56
Cloacitis/cloacal prolapse	1	3	2	0	0	0	0	0	6
Stomatitis	0	1	0	0	0	0	0	0	1
Rhinitis	0	0	1	0	0	0	0	0	1
Lung lesions	1	1	4	0	0	0	0	0	6
Heart lesions	0	0	1	0	0	0	0	0	1
Liver lesions	1	1	0	0	0	1	1	0	4
Kidney lesions	1	3	2	1	0	1	1	1	10
Spleen lesions	2	0	1	0	0	1	0	0	4
Gastrointestinal lesions	2	1	6	1	0	0	0	0	10
Pancreatic lesions	0	0	0	0	0	0	0	1	1

[a] Dermatitis, skin wounds etc.

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Table 6. Number of identifications of micro- and macroparasites during, or following, postmortem examinations of caecilians at the Zoological Society of London, London Zoo, July 2007–June 2017.

Parasite(s)	*Typhlonectes compressicauda*	*Typhlonectes natans*	*Geotrypetes seraphini*	*Herpele squalostoma*	*Microcaecilia unicolor*	*Rhinatrema bivittatum*	*Potomotyphlus kaupii*	*Idiocranium* species	Total
Bacteria, mixed isolate/No. tested	18/19	10/10	9/15	1/1	0/0	3/4	6/10	0/1	47/60
Aeromonas hydrophila[a]	11	6	5	1	0	1	9	0	33
A. sobria[a]	2	1	0	0	0	0	0	0	3
Pseudomonas fluorescens[a]	4	0	0	0	0	0	0	0	4
P. aeruginosa[a]	0	0	1	0	0	0	0	0	1
Vibrio alginolyticus[a]	2	2	0	0	0	0	2	0	6
Citrobacter freundii[a]	1	0	2	0	0	0	0	0	3
Escherichia coli[a]	0	3	0	1	0	0	0	0	4
Acid-fast bacilli	3	0	0	0	0	0	2	0	5
Fungi, unidentified	4	0	1	0	0	1	4	0	10
Batrachochytrium dendrobatidis	0	0	3	0	0	0	2	0	5
Achlorophyllous algae	0	0	0	0	0	0	2	0	2
Flagellated protozoa	1	0	9	0	0	0	0	0	10
Cryptosporidium-like bodies	2	0	0	0	0	0	0	0	2
Coccidia ± oocysts	0	0	2	1	0	0	0	0	3
Nematodes	0	2	7	1	0	1	1	0	12
Cestodes	2	0	0	0	0	0	0	0	2
Mites	2	0	0	0	0	0	0	0	2

[a] Predominant species cultured.

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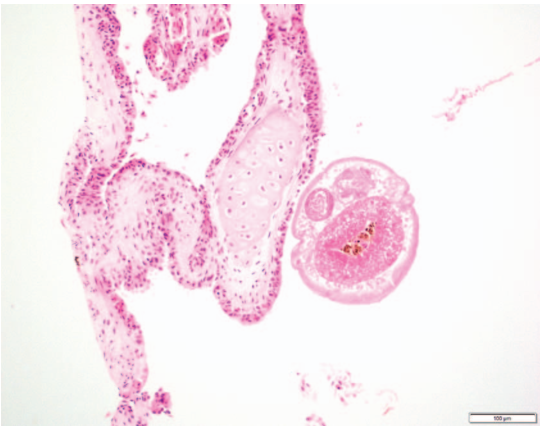

Figure 2. Lung, *Geotrypetes seraphini*. Cross-section of *Rhabdias*-like nematode. H&E. Scale bar: 100 μm.

dening or hyperemia of the skin was recorded in 10 cases (18%) and was particularly prevalent in *P. kaupii* (8 of 12 cases with skin lesions: 67%) whereas the 20 cases with skin erosion and ulceration (with various similar descriptions, such as ulcerative dermatitis and erosive dermatopathy, 36%) were recorded in all except one of the seven species that had skin lesions (Fig. 3). There was no obvious age or sex bias (19 males, 20 females, 16 small individuals for which sex not determined) and most cases (49, 89%) were in normal or slightly low body condition, with only 5 appearing to be thin or emaciated (one individual's condition was not recorded). Bacterial culture was carried out on 30 skin swabs from these cases,

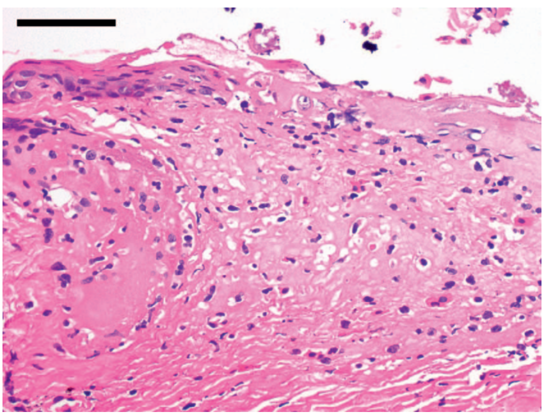

Figure 3. Skin, *Potomotyphlus kaupii*. Full thickness epidermal ulceration exposing inflamed dermis with edema and infiltrates of neutrophils and macrophages extending into the subjacent subcutis. Marginal epidermis is irregularly hyperplastic and vacuolated. H&E. Scale bar: 100 μm.

Table 7. Finding in caecilians with skin lesions (except wounds) at the Zoological Society of London, London Zoo, July 2007–June 2017.

	Typhlonectes compressicauda	*Typhlonectes natans*	*Geotrypetes seraphini*	*Herpele squalostoma*	*Microcaecilia unicolor*	*Rhinatrema bivittatum*	*Potomotyphlus kaupii*	*Idiocranium* species	Total
Number affected	18	8	9	1	0	6	12	1	55
Lesions described									
Excess-abnormal sloughing	5	3	1	1	0	3	3	0	16
Dry skin	0	0	0	0	0	1	0	1	2
Skin hyperemia	0	0	0	1	0	1	8	0	10
Skin erosion/ulceration, erosive dermatopathy	6	3	4	0	0	2	4	1	20
Epidermal degeneration/necrosis	1	0	3	0	0	0	0	0	4
Epidermal serocellular crusts	1	0	0	0	0	0	0	0	1
Epidermal nodules	1	0	0	0	0	0	0	0	1
Epidermal hyperplasia	2	1	3	0	0	0	1	0	7
Epidermal hyperkeratosis	0	0	3	0	0	0	0	0	3
"Dermatitis" (unclassified)	1	1	1	0	0	0	0	0	3
Multifocal white lesions	1	0	0	0	0	0	0	0	1

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from which all except one yielded mixed species. *Aeromonas hydrophila* was again the most common species identified (22 isolates, 73%). Acid-fast bacilli (suspect *Mycobacterium* species) were observed in 5 of 25 skin scrapings stained with Ziehl–Neelsen's stain but were only present in scant or moderate numbers. Mixed bacteria were usually seen in histological sections of affected skin, often with fungi. No significant fungi were cultured from skin swabs but, as already noted, chytridiomycosis was diagnosed in three caecilians. Three cases (one *T. natans* and two *G. seraphini*) had histological evidence of nematode infiltration (and in one case also evidence from fecal testing), two *T. compressicauda* had mites in skin sections examined histologically, and two *P. kaupii* had evidence of achlorophyllous algae, although these were not *Prototheca* species (N. Roschanski, Berlin Free University, pers. comm.). Nuclear inclusions were noted in epithelial cells of a single *T. compressicauda*, suggestive of viral involvement, but no viral particles were detected on electron microscopy (D. Everest, Animal and Plant Health Agency, pers. comm.).

DISCUSSION

This review of findings from necropsies of a range of caecilian species at ZSL London Zoo adds to the scant information on causes of mortality in captive populations of these fascinating and underreported animals, including the first findings from several species not included in previous publications. It is noticeable that some species, in particular *P. kaupii*, *R. bivitattum,* and the *Idiocranium* species had a much higher mortality rate than others, resulting in elimination of the entire *P. kaupii* and *Idiocranium* populations within the study period. This did not appear to be due to the populations being older than other species because both consisted of smaller (presumably younger) and larger (seemingly adult) animals. However, exact ages of individuals were generally not known so the mortality rates were based exclusively on time spent in the collection at ZSL London Zoo. Unfortunately, the cause of death could not be established in many cases, but this was not unexpected given the inevitable delays in finding dead individuals and the resulting autolysis, and because of the paucity of background information on caecilian health.

The high prevalence of dermatitis and other skin lesions among these cases was entirely expected; dermatitis and other skin lesions have been frequently documented in mortality reviews of other captive amphibian populations.[9,27,29] Dermatitis was considered the cause of death in 22 individuals, but was probably a major factor in many more, because damage to the skin reduces animals' ability to maintain water and electrolyte balance. Dermatitis affected the three aquatic species mainly but was also seen in several *G. seraphini* and *R. bivatattum*. The exact etiology of these skin lesions is often unknown, but poor water quality is always assumed to be an important predisposing factor in aquatic species and was probably involved in many of the cases recorded here, although they were managed in accordance with parameters recorded at collection sites or from parameters reported in the literature[1,35] and there were no direct links to abnormal water parameters. Similarly, substrate quality could affect the terrestrial species and dry skin was noted in two cases despite substrates never being allowed to dry out. However, anything compromising the epithelial integrity is likely to allow entry of opportunistic bacteria and fungi, many of which were recorded on bacterial culture and/or histopathology. Examples included the nematodes, mites, and achlorophyllous algae seen in some individuals, although it should be noted that the algae were not the known pathogenic *Prototheca* species. Many environmental *Mycobacterium* species may be associated with skin lesions in amphibians but were only present in low numbers in 20% of skin smears. However, alternative acid-fast staining methods, such as Fite–Faraco, may prove more sensitive and could be included in future screening of smears and histological sections. The chytrid fungus *Batrachochytrium dendrobatidis* was responsible directly for skin lesions and mortality in three *G. seraphini*[12] but was also detected by PCR in two *P. kaupii* that had no characteristic histological lesions. The infection can be treated successfully,[31] hence antemortem testing is strongly recommended. Suspicious intranuclear inclusions were noted in the skin of one *T. compressicauda* but these were not found to have viral bodies on transmission electron microscopy, a finding that matches a case of whole-body edema and mineralized fat in a *Typhlonectes* species.[34]

Several animals escaped from their enclosures and died of exposure and dehydration/desiccation, a fate noted previously for captive caecilians.[7,26,27] Cloacal inflammation and prolapse may be linked to direct trauma, irritation, and infection and hence linked to dermatitis, as seen in the two aquatic *Typhlonectes* species. In contrast, the two cases in *G. seraphini* were associated with

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intestinal impaction (partial or complete) with ingested coir substrate. The choice of suitable substrate for terrestrial-fossorial species is therefore of obvious importance in caecilian husbandry.[37]

Edematous conditions, whether involving only subcutaneous and lymph spaces (lymphedema), or the coelomic cavity (hydrocoelom), or both (anasarca) are very well recognized in anurans[6] and have also been reported in caecilians.[7,26,27,34] Fluid accumulation can be due to many different causes, but generalized infections (especially mycobacteriosis and ranavirus infection), plus skin, kidney, and gastrointestinal diseases are often cited. Four of the six cases in our study were examined histologically and three had renal pathology while the fourth had an acute skin ulcer.

Most of the founding stock were collected from the wild as adults (the study included no larvae of *Rhinatrema bivittatum* and no especially small, and presumably young, individuals of the viviparous or direct-developing species) and imported by the Natural History Museum. Many were held for several years at the museum and then at ZSL London Zoo, so mortality in these individuals may have been, at least in part, age-related although, as with many aspects of caecilian natural history, there is a general lack of data on the longevity of the vast majority of species. In contrast, deaths of multiple individuals in an enclosure succumbing with the same signs of disease, and disappearances of several individuals in a short period of time, were likely due to outbreaks of disease and/or environmental stress and not linked to old age.

Although there is variation in survival in current captive conditions, we have no evidence that caecilians are particularly delicate. Here we have cited inappropriate water and substrate quality or chemistry as possible causes of ill health, and discussed concern for skin wounds as sites of dangerous infection. However, it should be noted that at least some caecilian species can be found in less than pristine habitats in nature. For example, *T. natans* have been observed in eutrophic waters possibly contaminated by the oil industry in Venezuela,[13] *T. compressicauda* in water affected by road and sewage run-off (Gower and Wilkinson, pers. comm., P. Gaucher, pers. comm.), and *G. seraphini* and *H. squalostoma* occur widely and sometimes commonly in human-modified landscapes in Cameroon (Gower and Wilkinson, pers. comm.), albeit with no comparative mortality rates between these and unaffected habitats. In addition, soil-dwelling caecilians have been known to recover rapidly from injuries to the skin,[23,25] and healing or healed wounds are not uncommon on wild-caught animals.[41] Caecilians in captivity in poor health may be more likely to be negatively affected by suboptimal environmental conditions, but also these suboptimal conditions may predispose to disease. There is also likely to be variation in robustness among species. However, all of these questions, like many aspects of caecilian natural history, remain unstudied. On the positive side, it should be noted that there were many individuals showing signs of disease that were successfully treated and therefore do not form part of this review. Common treatments included rehydration therapy, temporary housing in relatively sterile enclosures, and courses of antibiotic, antifungal, and anti-inflammatory drugs.

Caecilians remain relatively unknown; 14% of caecilian species have never been assessed by the International Union for the Conservation of Nature (IUCN), and of the assessed species, 54% are Data Deficient.[14] Many caecilians are highly threatened, and some species are considered global priorities for amphibian conservation on the basis of threat and evolutionary history,[15] with some species being recommended for ex situ conservation management following Amphibian Ark's Conservation Needs Assessment process.[18] Maintaining this particular group of animals at ZSL London Zoo has resulted in a number of important outputs, including the documentation of the first case of lethal chytridiomycosis in this amphibian order,[12] the development of effective treatment protocols for caecilians with chytridiomycosis,[31] and the refinement of husbandry techniques[37] including improvements to vivaria to prevent escapes and replacement of coir by more suitable substrate material. Many questions remain, such as the exact cause of death in many cases, the relative contributions of infectious agents, nutrition, and environmental conditions in pathogenesis, the apparent seasonal peak of mortality in November and December, and the wide variation in mortality rate between species. Therefore, it is vital that more work is undertaken to understand the causes of disease in species held in captivity and it is hoped that this review will add to the growing body of knowledge.

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