

Research article

A retrospective study of causes of mortality in captive Sumatran laughingthrush *Garrulax bicolor* from European and Indonesian zoologic institutions (2005-2021)

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Abstract

The Sumatran laughingthrush *Garrulax bicolor* is an endangered passerine endemic to Sumatra. To combat further population decline, a European Association of Zoos and Aquaria European Endangered Species Breeding Programme was established in 2011. The aim of this review was to investigate the causes of death of Sumatran laughingthrushes in captivity, allowing the development of preventative methods to reduce mortality, improve breeding rates and the long-term viability of the captive population. The deaths of 310 Sumatran laughingthrushes were reviewed from institutions participating in the breeding programme between 2005-2021. Infectious diseases were the most common causes of death, with aspergillosis the most prevalent infectious aetiology. Aspergillosis was significantly more likely in parent reared birds ($P=0.027$) and the juvenile age class ($P=0.028$), but significantly less likely in the Indonesian institution C ($P=0.043$). Trauma was the second most common cause of death, and intraspecific aggression the most prevalent traumatic aetiology for both adults and juveniles. Few conclusions could be drawn from the neonatal data due to lack of necropsy information. Systemic *Isospora* spp. infection was a rare cause of death, but there was insufficient data to assess its subclinical prevalence. Institutions should attempt to perform more necropsy procedures for all age classes and screen all deceased birds for systemic *Isospora* spp. infection. This review achieved its aim of investigating the causes of death in captive SLTs and suggested routes for further research.

Introduction

The Sumatran laughingthrush *Garrulax bicolor* (SLT), a passerine endemic to Sumatra, Indonesia, is now only found in a few submontane and montane forests on the island (Shepherd and Gomez 2018). It is classified as endangered on the IUCN Red List of Threatened Species due to habitat destruction and trapping for the caged bird trade (BirdLife International 2016; Bušina et al. 2018; Heinrich et al. 2021; Shepherd 2007; Shepherd et al. 2016). To prevent further decline of this species, a European Association of Zoos and Aquaria (EAZA) Ex-situ Programme (EEP) was established in 2011 (Heinrich et al. 2021). There are also ex-situ breeding programmes in Indonesia which are part

of the EEP. The North of England Zoological Society, Chester Zoo, published EAZA best practice husbandry guidelines for SLTs in 2017 (Owen 2017).

Captive passerines are susceptible to various viral, bacterial, fungal and parasitic diseases, many of which are associated with immunosuppression (Schmidt 2024; Smith 2015; Trupkiewicz et al. 2018). Like most birds, passerines are susceptible to strains of poxvirus and avian paramyxovirus, as well as several Gammapolyomavirus species and circoviruses (Dorrestein 2003; Schmidt 2024; Smith 2015). Secondary bacterial infections are common in passerines with *Salmonella* spp. and *Yersinia pseudotuberculosis* reportedly causing high mortality (Dorrestein 2003; Schmidt 2024; Smith 2015). Other

Enterobacteriaceae, *Escherichia coli* and *Enterobacter* spp., can cause significant infections (Dorrestein 2003; Nemeth et al. 2016; Schmidt 2024; Smith 2015). Fungal infections include aspergillosis and candidiasis. Aspergillosis is a common cause of respiratory disease in captive passerines and has been reported in captive blue-crowned laughingthrushes *Pterorhinus coutoisi* (BCLT), loggerhead shrike *Lanius ludovicianus* and wild stitchbirds *Notiomystis cincta* (Nemeth et al. 2016; O'Connor et al. 2022; Perrott and Armstrong 2011; Schmidt 2024; Smith 2015). Clinical disease may occur due to several factors, such as high environmental spore exposure, damp conditions or host immunosuppression (Schmidt 2024; Smith 2015). Whilst inhalation of spores is the main route of transmission, aspergillosis can also be transmitted via ingestion of contaminated food (Samanta and Bandyopadhyay 2017). Outdoor housed passerines may be exposed to nematodes, including *Syngamus trachea*, and cestode infestations (Schmidt 2024; Smith 2015). Systemic *Isospora* spp. infection is an important protozoal disease of captive passerines, especially in neonate and juvenile age classes (Terio and Adkesson 2018). Systemic *Isospora* spp. infection has been reported in BCLT and Bali starlings *Leucopsar rothschildi* in multiple institutions, causing splenomegaly and hepatomegaly on necropsy, but few antemortem signs (Adkesson et al. 2005; Barbón et al. 2019; Flach et al. 2022; Landolfi et al. 2020; Mohr et al. 2017; Partington et al. 1989; Smith 2015; Terio and Adkesson 2018; Trupkiewicz et al. 2018). In live birds, whole blood and buffy coat smears can be used as a screening tool, whilst impression smears of liver, spleen and lung are suggested for diagnosis at necropsy (Samanta and Bandyopadhyay 2017; Terio and Adkesson 2018). Another protozoal disease, *Plasmodium* spp., the cause of avian malaria, has high mortality and morbidity especially in canaries *Serinus canaria* (Dorrestein 2003; Schmidt 2024; Smith 2015). Trauma is also a common cause of death for wild and captive passerines, often through collision or predation, and has been identified as a leading cause of mortality in BCLT (Flach et al. 2022; O'Connor et al. 2022; Smith 2015; Trupkiewicz et al. 2018). Due to high fat diets and sedentary lifestyles, captive passerines are prone to obesity, and hence hepatic lipidosis. An incorrect diet, especially being insufficient in calcium and or vitamin D, is a commonly reported problem in captive passerines, leading to osteodystrophy, egg binding and osteomalacia (Schmidt 2024). Neoplasia is an infrequent diagnosis in passerines (Garner 2006; Schmidt 2024; Smith 2015; Trupkiewicz et al. 2018).

The aim of this mortality review was to improve our understanding of causes of death of SLTs in captivity. This would enable development of preventative measures to help decrease mortality rates and progress captive breeding efforts, resulting in a more viable conservation breeding population. A further aim was to assess the prevalence of systemic isosporosis in captive SLTs, as systemic isosporosis is a commonly reported cause of mortality in captive passerines.

Materials and Methods

The 27 institutions involved in the SLT EEP were invited to participate by providing access to their Zoological Information Management System (ZIMS) data as well as taxon and necropsy reports between 2005 and 2021. For each bird, the ZIMS husbandry records were examined along with medical records and necropsy reports when available and summarised in an excel table. Demographic data including age, sex, body condition score, whether captive bred or wild caught, rearing status, country of residency and institution were recorded. Pathological data were summarised including gross and histopathologic lesions, ancillary testing, primary and secondary causes of death, body system and aetiology. Age classes were defined as neonate (<15 days, nestling), juvenile (15 days–1 year, fledgling to maturity) and adult

(> 1 year, sexual maturity) (Owen 2017). The data was graded for completeness as: demographic data only, demographic and gross necropsy data, referred to as gross data, and demographic, gross necropsy and histopathology data or other ancillary testing, referred to as histopathology data.

The cause of death for each bird was determined from the necropsy reports when available, with the full necropsies being reviewed by both a veterinary pathologist and experienced zoo veterinarian as well as the first author. The data were stratified by age class, with the neonatal age class analysed separately to the juveniles and adults due to differences in data quality. The causes of death for all age classes were summarised as infectious, non-infectious, trauma and undetermined. Within these broad categories data was further summarised by aetiology. Mortality rates (deaths per animal-year) were calculated for each institution as incidence rates from the method utilised by Flach et al. (2020). This involved calculating the number of birds in each institution for the first year included in this study (2005) from taxon reports generated on ZIMS and then recording births, deaths, importations and exportations. The disease incidents of interest were deaths, and the population at risk were all the birds present during the 15-year study period for each institution, recorded as number of animal-year (Flach et al. 2020). Chi-squared tests were used on categorical data and Fisher's exact tests were used when data involved low numbers. Logistic regression was used to calculate odds ratios (OR) and significance for risk factors identified for fungal disease. The dependent variable was fungal disease as the cause of death and predictors rearing status, age class and institution. Neonatal data was excluded from statistical testing due to insufficient necropsy results. Statistical significance was set at $P < 0.05$. Data was recorded in Microsoft Excel (Version 16.86 (24060216), Microsoft Corporation) and statistical analysis performed using RStudio Statistical software (Version 2023.12.1.402, RStudio, Boston, MA, USA).

Results

Fifteen of the 27 institutions invited participated, 55.6%, however, one institution had no recorded deaths. The 14 institutions providing data were in seven different countries. Thirteen institutions were European, distributed in the United Kingdom and the Channel Islands ($n=6$), Germany ($n=3$), France ($n=2$), Belgium ($n=1$) and the Netherlands ($n=1$). One institution was in Indonesia, but not located in Sumatra. Between March 2005 and January 2021 339 SLTs in the EEP died, meaning 91.4% (310/339) of all deaths were included in this study; missing data are due to lack of institutional participation.

Neonatal mortality accounted for 56.1% (174/310) of the cases, and necropsies were performed on 19.5% (34/174) of neonatal birds (Table 1). The mean age for neonatal death was four days. Cause of neonatal death was undetermined in 80.5% (140/174) of cases (Figure 1), primarily due to lack of necropsy examination (91.4%, 128/140). Trauma (8.0%, 14/174), infectious (5.7%, 10/174) and non-infectious (5.7%, 10/174) causes of death had similar prevalence in the neonatal age group (Figure 1). The primary aetiology was parental aggression or neglect (7.5%, 13/174) after unknown (no cause of death determined and no necropsy report combined) (80.5%, 140/174). Only 5.2% of neonates received histological exam and none were screened for systemic *Isospora* spp. infection by PCR. Subsequent results have neonatal data removed, unless specified, due to insufficient necropsy results. Data from three birds of unknown age were also excluded from age class analysis.

Juvenile birds accounted for 23.2% (72/310) of cases, and necropsies were performed on 54.1% (39/72) of juvenile birds (Table 1). The leading cause of death for juvenile birds was

Table 1. The level of data collected for each age class

Age class	Demographic data	Gross data	Histopathology data	Total
Neonate	140 (80.5%)	25 (14.4%)	9 (5.2%)	174
Juvenile	33 (45.8%)	14 (19.4%)	25 (34.7%)	72
Adult	23 (37.7%)	16 (26.2%)	22 (36.1%)	61
Unknown age	3 (100%)	0	0	3
Total	199	55	56	310

infectious disease (40.3%, 29/72) (Figure 1) and the primary infectious aetiology was fungal disease, 58.6% (17/29) (Table 2). Of those fungal cases, 94.1% (16/17) were due to *Aspergillus* spp. infection. The second most common cause of death in juvenile birds was trauma (9.7%, 7/72). The most prevalent traumatic aetiology in this age class was self-inflicted wounds or collision, 42.9% (3/7) (Table 2). Compared to adult birds that underwent necropsy, significantly more juveniles died from infectious causes ($P=0.008$). There was no significant difference for non-infectious, trauma and undetermined causes of death between adult and juveniles that underwent necropsy.

Adult birds represent 19.6% (61/310) of cases and necropsies were performed on 62.3% (38/61) of adult birds (Table 1). The main cause of death in adults was infectious disease (26.2%, 16/61) (Figure 1). Bacterial infections were the primary infectious aetiology, 43.8% (7/16), followed by fungal infections, 25% (4/16) (Table 2). Of those fungal infections 50% (2/4) were due to *Aspergillus* spp. Trauma was the next most prevalent cause of death in adult birds (24.6%, 15/61), with the majority due to intraspecific aggression (53.3%, 8/15) (Table 2).

Infectious causes of death in adult and juvenile birds combined accounted for 33.8% (45/133) of cases in these two age classes, and 46.7%, (21/45) of these were due to fungal disease (Table 2). Bacterial disease accounted for 26.7% (12/45) of combined adult and juvenile cases (Table 2). No specific bacteria were dominant, although *Proteus* spp. ($n=2$), *Klebsiella pneumoniae* ($n=1$), *Enterococcus* spp. ($n=1$) and non-haemolytic *Escherichia coli* ($n=1$) were identified (Table 2). Protozoal infection as a primary cause of death accounted for 13.3% (6/45) of infectious adult and juvenile cases. Systemic *Isospora* spp. infection was the most prevalent protozoal infection (5/6 cases, 83.3%). In cases of systemic *Isospora* spp. infection, body condition varied from good to emaciated, but consistent gross postmortem findings included marked diffuse hepatomegaly and splenomegaly, with a granular appearance to the spleen, and diffuse congestion of the intestines and lungs. In cases without histology, diagnosis was by observation of parasites in splenic or hepatic lymphocytes in impression smears. The one case with histology described severe mixed infiltrates of lymphocytes, plasma cells and macrophages throughout many organs, but especially affecting the liver, lung,

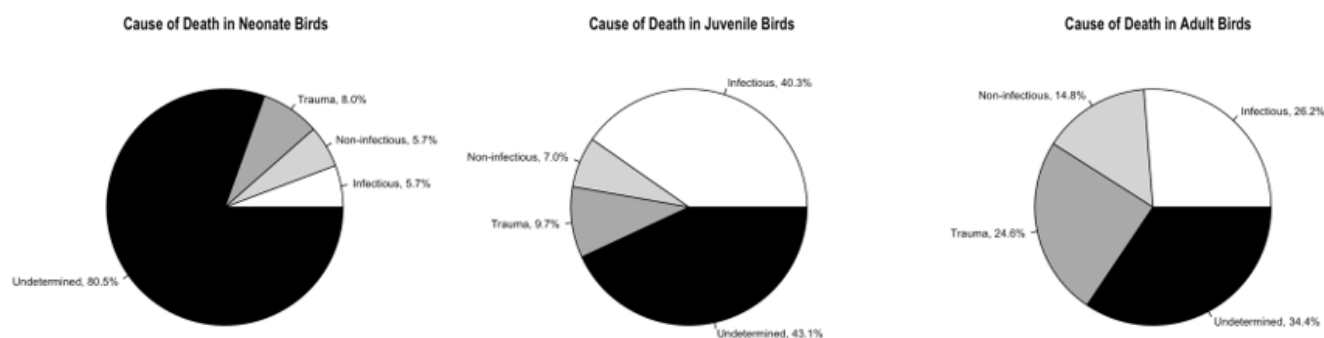
**Figure 1.** Cause of death for each age class

Table 2. Tally of causes of death in neonate, juvenile and adult Sumatran laughingthrush by age class and aetiology

Cause of death	Number of neonate SLTs	Number of juvenile SLTs	Number of adult SLTs	Total
Undetermined total	140	32	20	192
Infectious				
Infectious fungal				
<i>Aspergillus</i> spp.	2	16	2	
Unspecified		1	2	
Total	2	17	4	
Infectious parasitic				
Systemic <i>Isospora</i> spp. infection		5		
Helminths		1	2	
<i>Plasmodium</i> spp.		1		
Unspecified			3	
Total		7	5	
Infectious bacterial				
<i>E. coli</i> and <i>Enterococcus</i> spp.			1	
<i>Proteus</i> spp.	1	1		
<i>Proteus</i> and <i>Klebsiella</i> spp.		1		
<i>Pseudomonas aeruginosa</i>	2			
Unspecified	3	3	6	
Unknown	2			
Total	8	5	7	
Total infectious	10	29	16	55
Trauma				
Intraspecific aggression		2	8	
Predation			4	
Self-inflicted/collision		3	1	
Husbandry accident	1	1	1	
Parental aggression	11	1		
Unknown	2		1	
Total trauma	14	7	15	36
Non-infectious				
Hepatic lipidoses			3	
Husbandry accident		1	1	
Metabolic bone disease	1	1	1	
Cardiac arrest			1	
Emaciation		1		
Egg binding			1	
Foreign body				
Haemorrhage			1	
Hypocalcaemia			1	
Limb deformity			1	
GI obstruction	7			
Navel hernia	1			
Parental rejection	1			
Total non-infectious	10	4	10	24
Total deaths	174	72	61	307

spleen, small intestinal lamina propria and caecal lymphoid tissue. In this case parasitic bodies were observed in hepatocytes. Systemic *Isospora* spp. infection caused 1.6% (5/310) of overall

mortality. All five cases were juvenile birds with ages ranging from 53 days to 138 days. There were protozoal cases in 26.7% (4/15) of the institutions, but there were only one or two cases at

each. Parasitic infections involving helminths as a primary cause of death were rare (6.7%, 3/45) and helminth species were not identified in the postmortem reports.

Aspergillosis was the most prevalent aetiology for infectious causes of death. In cases of fungal disease, body condition score varied from thin to good. The majority of cases describe classic gross and histological granulomatous fungal pneumonia and/or airsacculitis, some with comorbidities. However, there were also two cases with tracheitis or pneumonia only detectable by histopathology. Three cases describe syringeal granulomas. Two unusual cases of fungal encephalitis were also noted. One with presumed multisystemic spread, and one hypothesised to have spread from the nasal passages forming a large cerebral granuloma.

Of the 21 fungal cases in adults and juveniles, 18 were due to *Aspergillus* spp., and 16 of those 18 were in juveniles, as previously mentioned. Fifteen of the aspergillosis cases were parent reared birds. Risk factors for death due to fungal disease of rearing status, breeding season, age class and continent were assessed using Fisher's exact or chi squared as appropriate. Breeding season was not a significant risk factor ($P=0.740$). Age class ($P=0.002$) and continent ($P=0.042$) were both significant and rearing status reached a p value of 0.163, so a logistic regression model was used to assess these three risk factors further. The logistic regression model used age class and rearing status as above, but individual institution rather than continent as there was only one institution in the Asia category. This model showed fungal disease was significantly more likely in the juvenile age class ($P=0.028$, OR 6.71) and parent reared birds ($P=0.027$, OR 12.77), but was significantly less likely in the Indonesian institution C ($P=0.043$, OR 19.09) compared to all other institutions.

Including data from neonate birds, 69 birds were hand reared and 189 parent reared. Rearing status was unknown for 52 birds. In juvenile and adult parent reared birds, infectious causes of death (36.8%, 25/68) were the most frequently diagnosed, then trauma 13.2% (9/68) and non-infectious causes 13.2% (9/68). In juvenile and adult hand reared birds, infectious causes of death (27.8%, 10/36) were also the most prevalent, followed by trauma (22.2%, 8/36) and non-infectious (11.1%, 4/36). Although rearing status was a significant risk, specifically relating to fungal disease, there was no significant difference between parent reared and hand reared birds for other disease categories.

There were 22 cases of trauma in adult and juvenile birds. Intraspecific aggression was the most common aetiology (45.5%, 10/22) (Table 2). There were four cases of self-inflicted wounds or collision trauma occurring at different institutions. Four cases of predation occurred at the same institution (Table 2). Only three of the trauma cases had histology data included. Accidental trauma, collisions, or intraspecific trauma occurred in all institutions.

There were 14 cases of non-infectious disease in adult and juvenile birds, but no dominant aetiology. Three adult or juvenile birds died due to hepatic lipidosis, two deaths were categorised as metabolic bone disease, with one case describing osteomalacia, and two deaths were due to husbandry accidents. All other causes were single individuals (Table 2).

Comorbidity was found in 18.1% (56/310) of cases, including neonatal data. In cases with identified comorbidities, infectious disease was the most common cause of death 44.6% (25/56), then neonatal death 25% (14/56), followed by non-infectious disease 14.3% (8/56), then undetermined cause of death 10.7% (6/56) and lastly trauma cases 5.4% (3/56). Birds that died due to infectious and non-infectious causes of death or as neonates were significantly more likely to have a comorbidity ($P<0.010$) than birds with a traumatic cause of death. Parasitism was the most common comorbidity, 21.4% (12/56), with 83.3% (10/12) being helminths; nine cases were unidentified gastrointestinal helminths and one

case of *Syngamus trachea*. For the primary infectious cases, 32% (8/25) of the comorbidities were parasitism and for primary trauma cases, 100% (3/3) of the comorbidities were parasitism. There was not another dominant comorbidity.

Deaths peaked during the breeding seasons in both Asia and Europe. The breeding season for Europe is April to August, with peaks in deaths in June and July, whilst in Asia it is October to February, with peaks in deaths in January, March and November (Figure 2). Even with neonatal deaths removed there are still peaks of death in the breeding seasons for each continent. Seasonality did not significantly affect cause of death.

There were 56 female and 48 male deaths. Sex was undetermined in 206 birds, of which 173 were neonates. Sex did not significantly affect cause of death, body system or aetiology. There were low levels of reproductive disease in the birds reviewed (Table 2).

The mortality rates (animal-years) have the neonatal data removed as not all institutions were breeding. Institutional adult and juvenile mortality ranged from 0.00 to 0.62 animal-years (Figure 3) with an average of 0.29 animal-years. The Indonesian institution C had the lowest mortality rate, whilst institution G had the highest mortality rate, but had low numbers of birds. Institution M had no deaths or births, whilst institution O only had neonatal deaths. Institution H had a 0% neonate mortality rate, whilst institution O had a 100% mortality rate (Table 3).

Discussion

This study highlights infectious disease and trauma as the leading causes of death of SLTs in captivity, with aspergillosis dominating in this species in western Europe. Whilst further analysis found that parent reared and juvenile birds were more susceptible to aspergillosis, the EAZA guidelines state SLTs should be parent reared where possible as the birds breed sooner, more reliably, and exhibit the full repertoire of natural behaviours (Owen 2017). It is proposed, as hand reared birds have a more sterile environment and become more habituated to humans, they may be less stressed at weaning, and more comfortable in captivity (Flach et al. 2022), possibly affecting their risk of developing fungal disease. In addition, parent reared birds are likely to be in naturalistically planted aviaries where spore levels may be higher. They may also experience intraspecific aggression at fledging, leading to stress and immunosuppression. This study found juvenile SLTs are more susceptible to aspergillosis compared to adult birds. As juveniles, they may not have a fully developed immune system, predisposing to aspergillosis (Samanta and Bandyopadhyay 2017). Inadequate housing, due to inadequate perching opportunities or lack of visual barriers, may lead to stress in juveniles, resulting in immunosuppression, and further predisposing to aspergillosis (Smith 2015). Further investigation is required to determine if husbandry changes might affect this. Consequently, all these factors may contribute to increased aspergillosis rates in parent reared juveniles.

This study found the Indonesian institution was statistically less likely to have deaths due to aspergillosis. This may be due to climate differences, such as birds experiencing warmer and less variable temperatures, resulting in less thermal or physiological stress, and thus less immunosuppression. Whilst the Indonesian institution is not in Sumatra, where this species is endemic, its altitude (800m) is within the range for the species, so the climates may be similar between these areas. Additionally, husbandry and diet will likely markedly differ in the Indonesian institution when compared to Europe, with birds receiving better UVB exposure due to access to unfiltered tropical sunlight and thus potentially having higher vitamin D levels and improved immunity. In the montane forests of Sumatra, humidity is high; however, the EAZA best practice guidelines give no recommendation for humidity

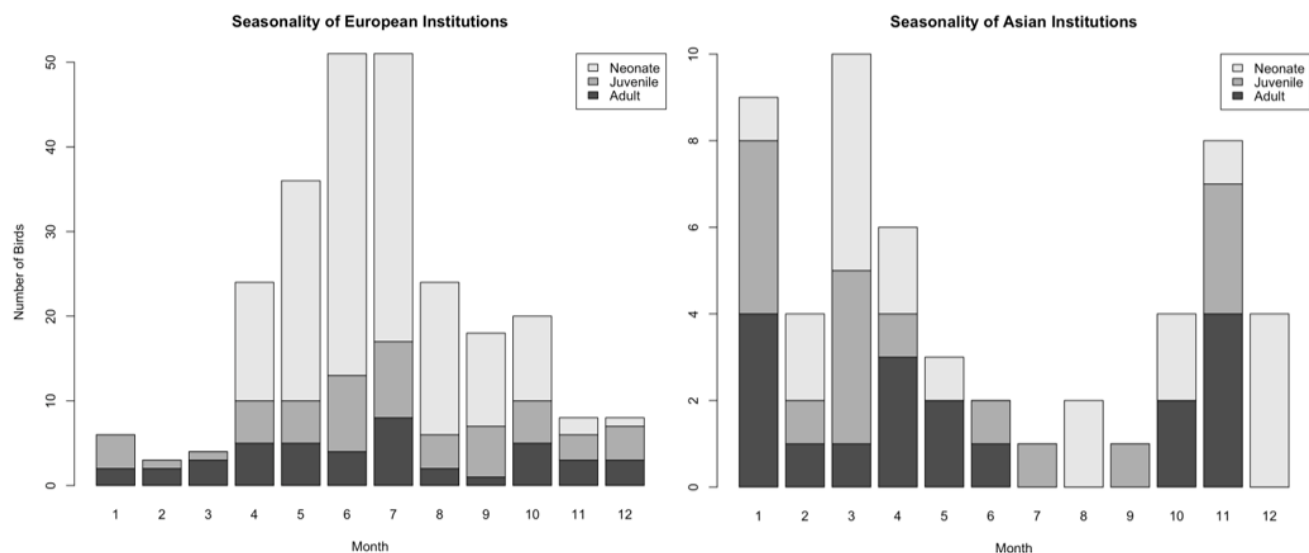


Figure 2. Seasonality of deaths in Sumatran laughingthrushes of all age classes. The typical breeding season in Europe is April (4) to August (8) and in Asia is October (10) to February (2).

of the enclosures post hatching (Owen 2017). However, as high humidity would normally predispose to aspergillus spore formation, poor ventilation in captivity may be a contributory factor (Joseph 2003; Owen 2017; Stiller 2010; Tell 2005). The EAZA best practice guidelines state that in Europe, SLTs can be kept in outdoor aviaries, as temperatures can fall to 8°C at night in Indonesian montane forests, and once acclimatized, SLTs are hardy birds and able to tolerate temperatures below freezing. The guidelines confirm SLTs should always have access to dry frost-free indoor accommodation they can be confined in, although they may still choose to roost outside (Owen 2017). However, the stress of persistent low temperatures encountered in European winters, combined with potentially poor ventilation and low humidity of heated indoor housing, may all result in immunosuppressed birds that are predisposed to aspergillosis. Consideration should be given to housing this species in indoor tropical houses with UVB provision, where heat would be more constant, humidity higher, and a more stable environment possible. *Aspergillus* spp. is an

opportunistic pathogen (Godoy and Matushima 2010; McMillan and Petrak 1989; Owen 2017; Schmidt 2024; Smith 2015; Stiller 2010; Tell 2005), and 44.4% of birds in this data set with aspergillosis had a comorbidity, highlighting the importance for preventive medicine programmes in captivity to minimise subclinical and clinical disease. Although there has been speculation about the seasonality of aspergillosis in other species such as stitchbirds (Cork et al. 1999), this study found no correlation between aspergillosis and seasonality in either continent.

One of the aims of this study was to assess the prevalence of systemic *Isospora* spp. infection in captive SLTs. Unfortunately, due to the lack of sufficient histology data in all age classes of this data set, no conclusions can be made regarding prevalence of systemic isosporosis in SLTs, or of the pathogenicity of this infection in this species. There is a low rate of mortality due to systemic *Isospora* spp. infection in this data set compared to other studies of passerines (Barbón et al. 2019; Flach et al. 2022; Mohr et al. 2017; Partington et al. 1989). One study found no deaths

Table 3. Percentages of neonates that died in breeding institutions only between March 2005 to January 2021

Institution	A	B	C	D	E	F	H	L	N	O	Total
Neonate Mortality %, (n)	52.8% (66)	31.8% (7)	27.1% (19)	57.1% (24)	48.3% (29)	28.6% (12)	0% (0)	80.0% (4)	76.9% (10)	100% (3)	174
Number of neonates	125	22	70	42	60	42	7	5	13	3	389

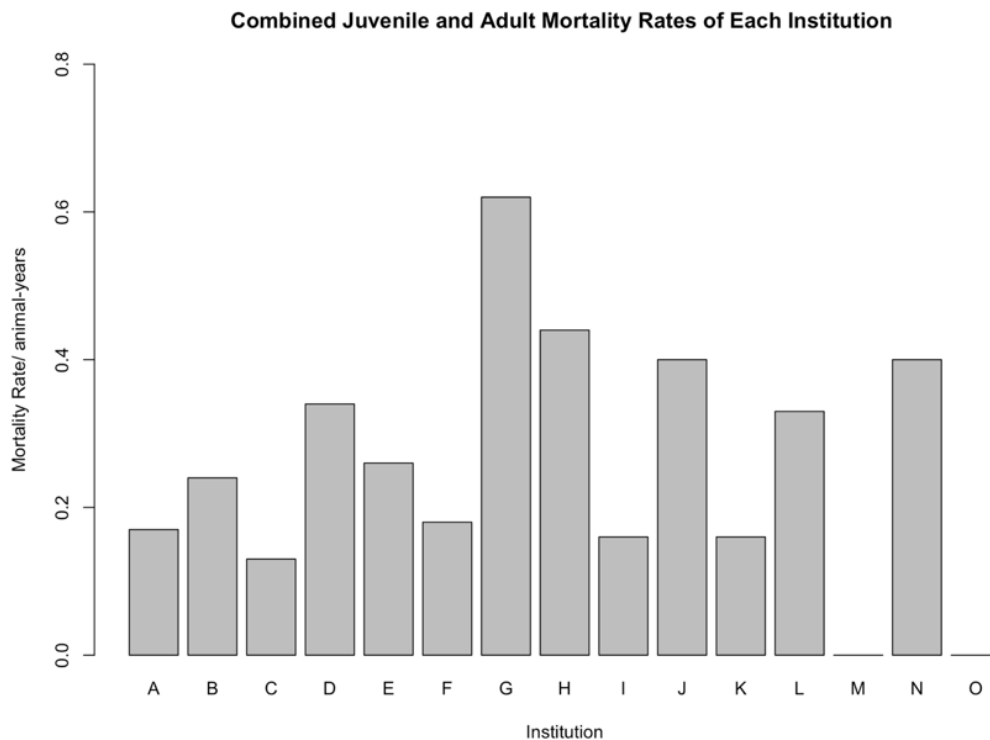


Figure 3. Juvenile and adult annual mortality rates (animal-years) for all institutions submitting data between March 2005 – January 2021, controlled for numbers of birds present at an institution per year as not all birds were present in all years. The Indonesian institution is C; A, B, D, E, K and L are in the United Kingdom and the Channel Islands; F, G and H are in Germany; I, J, M, N and O are from Belgium, France and the Netherlands, in no specific order. O had neonatal mortality only and M had no mortality.

attributed to systemic isosporosis in BCLTs under 6 weeks old, which is in direct contrast to another study in the same species which found 82% of chicks dying between zero and six days old, suggesting further research is required for this disease (Barbón et al. 2019; Mohr et al. 2017). Others have noted systemic *Isoospora* spp. infection appears to be a health concern for laughingthrushes generally (Owen 2017).

Whilst subclinical systemic *Isoospora* spp. infection can be difficult to diagnose at necropsy, it is comparatively easy in the live bird via whole blood or buffy coat smears (Adkesson et al. 2005; Giacomo et al. 1997). When systemic *Isoospora* spp. infection is the cause of death, clear gross pathological changes of hepato-splenomegaly are usually expected, as was seen in this study (Schmidt 2024). In comparison to BCLTs where 100% of the systemic *Isoospora* spp. infection cases had splenomegaly and 95% hepatomegaly (Barbón et al. 2019), in this dataset 100% had splenomegaly, but only 40% had hepatomegaly. In contrast to other passerine reviews, where higher rates of systemic *Isoospora* spp. infection have been found (Barbón et al. 2019; Flach et al. 2022; Mohr et al. 2017; O'Connor et al. 2022), deaths appear infrequent in this data set. This is possibly due to lack of necropsy screening and missing of subclinical cases. Impression smears of the spleen, liver and lung are considered the gold standard diagnostic at necropsy (Terio and Adkesson 2018). Impression smears and buffy coat smears do not require expensive equipment and should be available to all institutions and veterinarians. Improved diagnostics for systemic

Isoospora spp. infection would provide data on the true prevalence in this species.

This study did not find any deaths attributed to viral disease, despite this type of disease being frequently reported in other passerine species in the literature (Dorrestein 2003; Schmidt 2024; Smith 2015). Similarly, no cases directly attributed to *Salmonella* spp. or *Yersinia* spp. were found, although specific viral testing and bacteriological culture was not commonly undertaken, so these cases may have been missed.

Trauma was the second most common cause of death, with intraspecific aggression the most common traumatic aetiology. Aggression is reported to be rare in pairs of laughingthrushes, and no fatalities have been reported to the EEP between female/male pairings (Owen 2017). However, direct aggression through aviary mesh has been reported, so pairs cannot be housed directly next to each other. From this data set we do not know the housing condition of birds dying of intraspecific aggression, so are unable to determine if any particular social situation is responsible for this high prevalence. The frequency of intraspecific aggression warrants further investigation into housing conditions in future cases. The best practice guidelines advise not to house SLTs with other birds unless essential as they may eat their eggs or chicks (Owen 2017). Interspecific aggression was not noted in this data set, suggesting guideline compliance. All institutions had a case of accidental trauma/collisions or intraspecific trauma, suggesting all institutions should ensure they are adhering to the best

practice guidelines for aviary size, predator and vermin barriers, appropriate substrates, plants, materials, as well as minimising disturbances and reducing stress during keeper entry. This will help prevent accidental trauma and reduce stress in the birds (Owen 2017). Intraspecific aggression is an unusual aetiology in a passerine species and may reflect an inherent characteristic of the species previously not acknowledged.

Neonatal deaths accounted for 56.1% (174/310) of deaths in the study. As 80.5% (140/174) of cases of neonatal death received no necropsy, it is difficult to draw meaningful conclusions from these data. Parental aggression or neglect was the main primary aetiology diagnosed. As only one of these cases had a histology report, it is problematic to confirm if parental aggression was the true cause of death in all cases. There may have been underlying conditions that caused the parent birds to display typical passerine behaviour of pecking the moribund neonate for stimulation. On gross necropsy, this pecking appears as bruising or trauma to the skin which can be construed as a trauma case. High neonatal mortality rates have been reported in BCLT, half of which were suggested to be due to *Isoospora* spp. (Mohr et al. 2017). Due to the difficulties with autolysis of neonates at necropsy, the use of PCR to diagnose infection in this age class should be considered (Landolfi et al. 2020; Mohr et al. 2017). Institutions should perform thorough necropsy and histopathology on deceased neonatal SLTs to investigate neonatal deaths further and improve breeding success (Owen 2017).

The low number of non-infectious disease cases in this data set suggest the nutritional needs of the birds are being met. The low rate of non-infectious disease in hand reared birds is understandable as they are provided a complete nutritional balance. It also suggests good techniques are employed in hand rearing. Neoplasia was not prevalent in this species, which is in line with data for other passerines (Garner 2006; Schmidt 2024; Smith 2015; Trupkiewicz et al. 2018).

Between March 2005 and January 2021, 339 SLTs participating in the EEP died and 91.4% (310/339) of all deaths were submitted to this study. The mortality rates show there is considerable variation in mortality across institutions (Figure 3), suggesting husbandry practices and housing are not standardised. Neonatal mortality also ranges from 0% to 100% across institutions. These results may be due to differing levels of SLT management experience across institutions, as well as low numbers of birds in some institutions. Further information, beyond the scope of this study, is required from each institution regarding their specific husbandry practices to establish if any specific management practice is perhaps detrimental or beneficial resulting in the variable mortality rates.

Due to the use of data from different institutions over a 15-year period, this study is limited by variation in record keeping, husbandry management, facilities and resources available. Multiple individuals undertook necropsy exams, resulting in inevitable inconsistencies in technique, interpretation and conclusions. To minimise the effect of this the full necropsies were reviewed by both a veterinary pathologist and experienced zoo veterinarian as well as the first author. As there is data from multiple institutions, the conclusions drawn can be applied to more than one institution. Although there are data from different continents, there are only data for one institution in Asia and thus any conclusions drawn regarding Asia, and comparisons between the two continents, are only applicable to that institution. Whilst the total sample size for this study is sufficient, the lack of histopathology for many animals reduces the reliability of the cases. Despite the logistic regression being underpowered based on positive numbers, the authors decided it was the best statistical test to use to enable use of institution, rather than continent as a factor as there was only one Asian institution.

Conclusions

Infectious causes, primarily fungal disease, were the leading cause of death in SLT, with aspergillosis the most common aetiology. Further investigation is needed into why the Indonesian institution was less likely to have fungal disease. If it is related to the climate, attempts to create a similar environment to Sumatra might be useful in reducing cases of fungal disease in European institutions. Juvenile birds were significantly more likely to have aspergillosis. It is hypothesised this may be due to immunosuppression during the juvenile stage, supported by the finding that infectious disease had an increased risk of comorbidity. Institutions should attempt more necropsy procedures and histopathology on deceased SLTs, especially neonates, due to the insufficient number of necropsy results available to draw conclusions for this age class. Institutions should also be encouraged to screen for systemic *Isoospora* spp. infection to assess prevalence in SLTs. Trauma was the second most common cause of death. Further research should be undertaken to ascertain the predisposing factors around intraspecific aggression so appropriate recommendations can be made to prevent its occurrence, including further research into specific institutional husbandry practices. This review has provided more information on SLT mortality, and suggested future research avenues.

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