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Bone marrow fat content analysis confirms starvation as a cause of death for Australasian bitterns (*Botaurus poiciloptilus*) in New Zealand

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ABSTRACT

Wildlife managers conserving threatened species rely on information about causes of population declines. Finding carcasses to record cause of death, sickness or injury in a timely manner is challenging. We used fat content in bone marrow from deceased Australasian bitterns (*Botaurus poiciloptilus*) to determine whether birds had starved by comparing results among 34 bitterns where the cause of death was assigned via independent autopsies. Fat content was near zero in the eight specimens that died of starvation. Many other specimens had depleted fat content, 17 of which were recorded as dying from impact trauma. This evaluation provided confidence to interpret our database of 126 Australasian bittern mortality records evaluated via autopsy or observation. The most common causes of death were impact trauma (56.3%; mainly vehicles and powerlines) and starvation (17.5%) with a further 5.8% having characteristics of both starvation and impact trauma raising the issue of whether starvation contributed to the deaths of individuals where impact trauma was the primary cause of death or vice versa. Although this dataset is biased by specimens more easily observed by the public, the occurrence of starved/near starved bitterns is of conservation concern and indicates wetland food supplies are likely under stress.

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Introduction

Wildlife managers tasked with conserving threatened species rely heavily on accurate information about the causes of population declines. Information on causes of death and factors reducing survival provides a useful indicator of which pressures require management to recover declining populations. However, determining the cause of death can be problematic with rare and cryptic species, as sick, injured, or deceased individuals are difficult to find in a timely manner. Of carcasses that are discovered, many are too decomposed

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to determine the cause of death. However, determining the cause of death can provide valuable information on potential threats that need to be managed in threatened species.

The Australasian bittern (*Botaurus poiciloptilus*; matuku hūrepo) is a rare, cryptic species that is critically endangered and largely confined to wetlands in Australia and New Zealand (Herring et al. 2021; Robertson et al. 2021). It is threatened by a complex array of pressures including habitat loss, degradation of feeding and breeding habitats, and predation (Williams 2024). Food supplies of Australasian bitterns are thought to be limited in New Zealand given 90% of wetlands have been lost, degradation in water quality is commonplace and native fish habitats have been degraded by sedimentation and competition with exotic fish (McDowall 1984; Ryan 1991; Rowe 2007; Ausseil et al. 2011; Dunn et al. 2018). In addition, autopsies of dead birds handed to authorities indicated that starvation may be a regular cause of death (Stuart Hunter, Massey University, pers. comm.). Starvation is a persistent pressure for several animal populations (Newton 1980; Martin 1987; McCue 2010; Bowler et al. 2019). However, detecting starvation, and measuring its role as a factor limiting survival, as well as contributing to population declines, is challenging.

Bone marrow fat is the final fat reserve to be mobilised for energy by a calorie-deprived animal during a state of emaciation, and provides an objective measurement of starvation (Raglus et al. 2019). Ecologists have used bone marrow fat assessments to investigate causes of mass mortality events, causes of starvation, and energy loss when fasting in seabirds, waterfowl and owls (e.g. Thouzeau et al. 1997; Baduini et al. 2001; Moore and Battley 2003). The aim of this study was to apply bone marrow fat content analysis to Australasian bittern carcasses handed into the Zoology and Ecology Department, Massey University, to determine if the method correctly identified birds already assessed as starved via autopsy, and thus, whether routine analysis of carcasses would shed light on the role of starvation as a cause of decline. In addition, we evaluated the data of potential causes of death in a larger sample of birds that we collated from autopsied carcasses from Massey University's and Department of Conservation (DOC) databases (n = 126) to determine the likely importance of starvation in contributing to their deaths.

Methods

Sampling Australasian bittern carcasses

We sampled as many Australasian bittern carcasses as possible that came into the Massey University Ecology department or Tāwharau Ora/School of Veterinary Science between 2017 and 2020. We were able to obtain 34 samples within this time, one of which was an older sample from 2015. Of these 34 samples, fifteen came from Bay of Plenty (Ad ♀ = 2, Juv ♀ = 3, SubAd ♀ = 2, Ad ♂ = 3, Juv ♂ = 3, Ad unk = 2); five from Waikato (Ad ♀ = 1, SubAd ♀ = 1, Ad ♂ = 1, SubAd ♂ = 1, Ad unk = 1); three from Manawatu (Ad ♂ = 1, Juv ♂ = 1, Unk unk = 1); two were from Hawkes bay (both Ad ♂); four from Canterbury (Juv ♀ = 1, Juv unk = 2, Unk unk = 1) and three from the West Coast (Ad ♀ = 1, SubAd ♀ = 1, Ad ♂ = 1). For an additional two samples, sex and location were unknown (Juv unk = 1, Unk unk = 1).

Bone fat analysis

From dead specimens held at Massey University, both wing and leg bones were sampled. The right-hand side tibiotarsus and ulna were dissected from the carcasses in their

entirety with all adhering tissue removed. If the bittern had sustained either a broken right tibiotarsus or ulna (i.e. potential loss of bone marrow), the left-hand side of each respective bone was taken instead. Each bone was then broken into three fragments using wire-cutting pliers to expose the bone marrow. All three fragments were placed into pre-weighed, labelled dishes lined with aluminium foil and then dried at 70°C for seven days before being cooled in a desiccator and reweighed to obtain the dry mass (± 0.001 g). After drying, each bone was placed into individual filter-paper envelopes. The envelopes containing bones were individually submerged in a sealed glass bottle of 100 ml petroleum ether (Fisher Chemical, Loughborough, United Kingdom). The petroleum ether from each bottle was replaced after five days. After 10 days, each envelope was removed and dried at 70°C for one day, cooled in a desiccator and weighed to obtain the bones' fat-free dry masses. Fat mass was calculated as the difference between the dry mass and the fat-free dry mass and percentage fat was calculated as fat mass/dry mass \times 100, rounded to nearest percentage point.

Calibration against autopsied individuals

We then examined data on symptoms and causes of death in a sample of 126 Australasian bitterns evaluated via direct observation or autopsies obtained from the Tāwharau Ora/School of Veterinary Science Pathology Register, autopsies from other vet practices, and DOC's National Bittern Database. We were interested in not only describing causes of death, but examining whether weight data from specimens may help predict the importance of reduced body fat in causes of death, and based on our bone marrow analyses, estimating the number of birds where starvation may have contributed to other known causes of death. Of 126 records of cause of death in our database, 58 were from autopsied specimens and 68 were submitted as records to the Department of Conservation's National Matuku/Bittern database. Of these records, 23 cases were excluded from analysis because they represented birds being shot legally or illegally by hunters prior to, or soon after, the species was fully protected in 1904. Records came from throughout New Zealand, from Northland to Southland and from both east and west coasts.

Results

Bone fat content analyses

We compared results among 34 bitterns that had a cause of death assigned via independent autopsies at Massey University. Of these, six (17.7%) had died of starvation, 17 (50%) from impact trauma, three (8.8%) from a combination of starvation and impact trauma, and eight (23.5%) from unknown causes. The samples represented a range of percentage bone marrow fat values from zero to 74%, and the fat content of the ulna and tibiotarsus samples were highly correlated ($R^2 = 0.77$, [Figure 1](#)). Bone marrow fat content of both the ulna and tibiotarsus was near zero in the specimens that died of starvation, whereas it varied markedly among specimens that died of impact trauma (0–58% ulna, 0–47% tibiotarsus). Three specimens had characteristics of both starvation and impact trauma, so the proximate cause of death was unknown. However, all three had

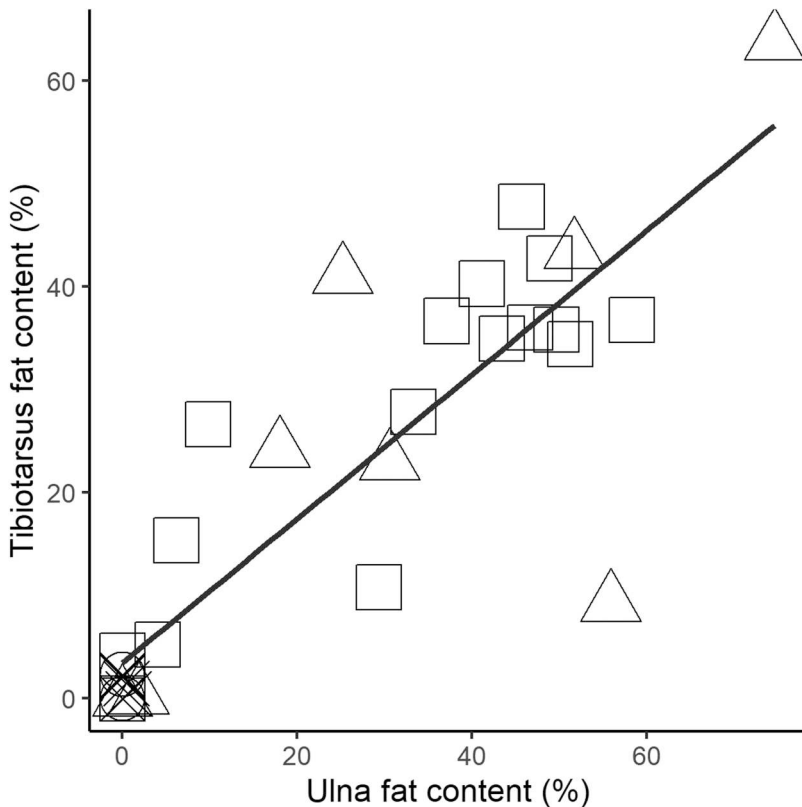


Figure 1. Scatter plot comparing bone marrow fat content in ulna (wing) and tibiotarsus (leg) bone samples from 33 Australasian bittern. The graph excludes one bird for which a tibiotarsus sample was unavailable. The line represents the linear regression equation $y = 0.7x + 3.4509$ and $R^2 = 0.77$. Causes of death from autopsy results: X = starvation, Square = impact trauma, triangle = unknown, Circle = impact trauma and starvation.

near-zero bone fat contents. Overall, birds that died of impact trauma and unknown causes had a continuum of bone marrow fat levels, including some with low fat levels (Figure 1).

Australasian bitterns are sexually dimorphic in mass, with recently caught wild adult males averaging 1792g (\pm SD 265, range 1191–2250 g, $n = 23$), and females (1067 g; $n = 1$) (Unpubl. data). Autopsied specimens were not always weighed because they were too damaged, i.e. if decomposed or limbs and organs were lost due to trauma. Average mass of autopsied individuals where starvation contributed to the cause of death was 643 g (\pm 296, range 307–1271, $n = 11$; males = 752 g (\pm 328, range 385–271, $n = 6$); females 480 g (\pm 158, range 307–669, $n = 4$)). In comparison, mass of birds that died of impact trauma averaged 971 g (\pm 369, range 416–1670, $n = 15$). This pattern was consistent across age and sex groups, albeit with a low sample size (Figure 2). However, the relationship between mass and bone marrow fat content, though linear, was highly variable ($R^2 = 0.37$; Figure 3). Some birds with relatively high weights had very low bone marrow fat content (Figure 3). The relationship between tibiotarsus fat content and specimen weights was similar, but slightly more variable ($R^2 = 0.25$).

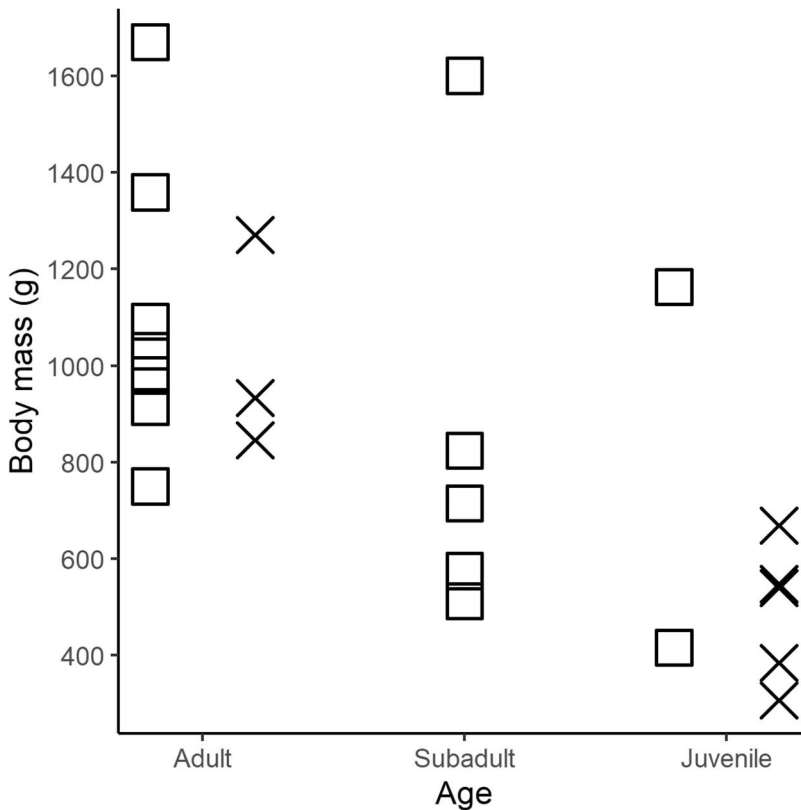


Figure 2. Weights of different age-classes of Australasian bittern that died of impact trauma ($n = 14$) or starvation ($n = 8$). X = starvation, Square = impact trauma.

Cause of death in Australasian bitterns sampled

Cause of death was recorded in 95 contemporary cases (Table 1). The most common causes of death were impact trauma (56.3%) and starvation (17.5%) with a further 5.8% having characteristics of both starvation and impact trauma.

A range of causes were responsible for impact trauma, with collisions with vehicles being the most frequent cause (27.2% of impact trauma records). However, this is likely an underestimate because several birds were assumed to have been run over by vehicles or trains because of their being found in close proximity to roads or track (8.6%). In addition, collisions with other structures were also a factor, with 19% of cases being found dead below windows or under powerlines. Powerlines were also implicated in electrocuting or entangling birds. Overall, powerlines featured as contributing to the cause of death in 9.7% of cases (Table 1). Birds that died from impact trauma generally had multiple broken bones and bruising. In the case of the six birds that cause of death was attributed to both impact trauma and starvation, the pathologist commented that all were emaciated (weights varied from 517–846 g) and may have lived with injuries for some time. One bird had old fractures in the wing and five had fractures in the legs (one of which had apparently healed). The pathologist generally commented that birds classed as starved were generally emaciated. They had very low weights and had much

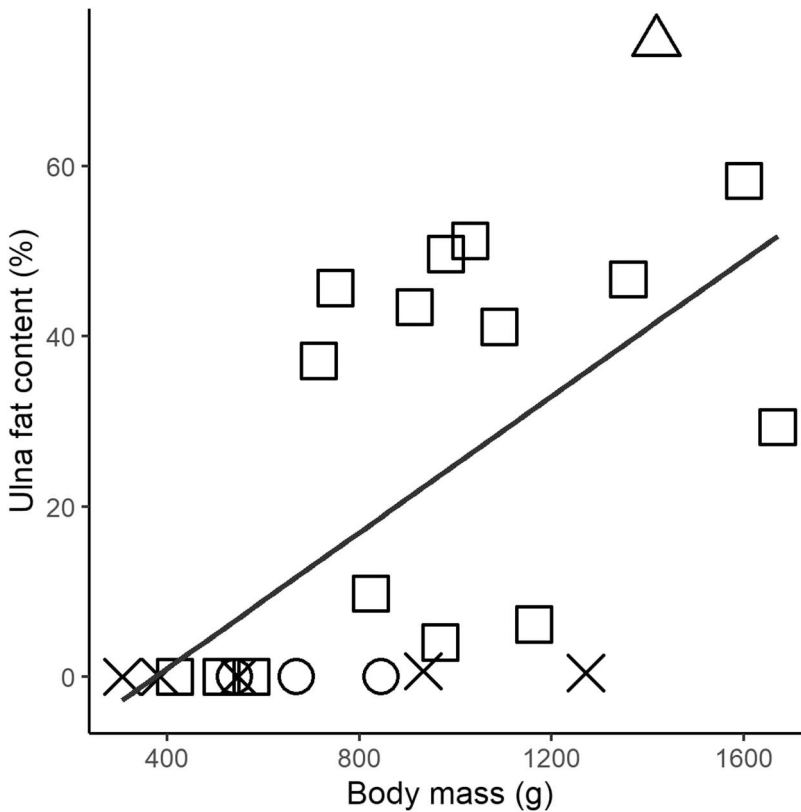


Figure 3. The relationship between ulna fat content (%) and the weights of Australasian bittern specimens ($n = 24$ where weights were known). The line represents the linear regression equation $y = 0.04x - 15.075$ and $R^2 = 0.38$. X = starvation, Square = impact trauma, triangle = unknown, Circle = impact trauma and starvation. The relationship between tibiotarsus fat content (%) and specimen weights was similar ($n = 23$ where weights were known), but with a regression equation of $y = 0.0254x - 4.0473$ and $R^2 = 0.2458$.

reduced pectoral muscle mass and minimal if any body fat reserves and empty digestive tracts.

Other causes of death included predation by introduced mammalian predators (1.9%), disease or illness (4.9%; a range of factors were respiratory diseases, cancer and renal failure) and shooting (1%). The pathologists commented that birds with unknown causes of death were generally heavy and in good body condition with no obvious signs of trauma or underlying disease. All eight carcasses that were able to be tested for lead contained only trace levels of lead (mean = 0.050 ± 0.048 mg/kg, range 0.001–0.13 mg/kg) in their livers.

Discussion

Evaluation of the technique

Measuring bone marrow content was successful at identifying Australasian bittern that were starving, and likely a contributor to, or cause, of death, with all starved bitterns

Table 1. Causes of death in 103 Australasian bittern, 1996–2023 (from autopsies and visual observations). The sample excludes 23 historical records of bitterns being shot (prior to the species being fully protected; O'Donnell and Robertson 2016).

Cause of death	Specific cause	<i>n</i>
Illness		5
Entanglement	Powerlines	1
	Tree branches/roots	3
Starvation		18
Starvation and impact trauma		6
Impact trauma	Unknown trauma	24
	Powerlines	8
	Vehicle	23
	Windows	3
Shooting		1
Electrocution	Powerlines	1
Predation	Cat	1
	Mustelid	1
Unknown		8
Total		103

having near zero fat levels. However, some birds that died of impact trauma and unknown causes also had low-to-medium fat levels. Thus, in both groups, starvation may also have contributed to the cause of death, although we do not know if there are thresholds along our scale where starvation becomes a significant contribution. However, bone marrow fat deposits are generally only mobilised after most other body fat has disappeared, so once bone marrow fat content declines, this indicates severe starvation (Baduini et al. 2001; Guglielmo and Burns 2001). For example, Thouzeau et al. (1997) indicated that the lipid mass in the skeletons of barn owls (*Tyto alba*) was depleted rapidly (by c. 80%) only when birds were already under severe stress, further supporting the notion that many of the specimens sampled in this study were experiencing some symptoms of starvation regardless of the immediate cause of death.

Identifying whether there are thresholds for determining if starvation is a significant factor in the body condition of birds requires further study. Both Hutchinson and Owen (1984) and Moore and Battley (2003) found in waterfowl that birds with 20–40% bone marrow fat were not dying of starvation, but those that had fat contents of 0–16% did starve. Whether birds with intermediate levels were under stress and may have ultimately died was unknown. However, if c. 20% fat content is a meaningful threshold, then 18 bittern may have been starving (53%), including birds that were classified as dying from impact trauma or unknown cause of death (c.f. Figure 3). In addition, the sample of birds that had characteristics of both starvation and impact trauma, indicates that sometimes the cause of death may be related to both factors. Whether starved birds become lethargic and less alert, and thus less able to avoid vehicle collisions, or if the opposite is true – birds that are injured may not be able to feed effectively and ultimately starve – is unknown. Both are plausible hypotheses that warrant further investigation.

Our results also show that measuring the weights of dead specimens alone does not necessarily help in interpreting the cause of death. Although very light birds appeared to be starving, some heavier birds were also likely under stress based on the low bone marrow fat content in some specimens. Nevertheless, the method was especially useful when incomplete or severely degraded carcasses with only bones and feathers were

found and were the only remaining forensic evidence that could be used to determine the cause of death.

Causes of death

Our evaluation of the relationship between bone marrow fat content and starvation gave us the confidence to validate our database of Australasian bittern mortality records, particularly in relation to starvation. However, the bone marrow results also caused us to suspect that some records of death caused by impact trauma may also be confounded by starvation because some specimens also had low, or near-zero, bone marrow fat levels.

Collisions and starvation are clearly important causes of mortality in our sample of Australasian bittern and live birds that are injured are regularly handed into wildlife centres and attempts to rehabilitate them made (Williams and Brady 2014). However, the true contribution of collisions to mortality in Australasian bitterns at a population level is still unclear because some causes of death are cryptic and likely under reported, especially compared to collisions with anthropogenic structures and vehicles. For example, it is unlikely that birds that die of natural causes (e.g. illness, old age, or preyed upon in the wild) would be found regularly because birds are very hard to find even when alive and usually inhabit difficult to penetrate habitats. For example, recent monitoring of a small sample of nests showed that birds were preyed upon by Australasian harriers (*Circus approximans*, Williams 2024), a cause not detected in the current study. In another study, involving the deployment of trail cameras on artificial bittern nests, pukeko (*Porphyrio melanotos*), stoats (*Mustela erminea*), ferrets (*M. furo*) and brushtail possums (*Trichosurus vulpecula*), as well as harriers, were found preying on eggs (Williams 2024). Radio-tracking of wild Australasian bitterns in 2012–2013 also confirmed that starvation is a common cause of death. Seven of 17 birds monitored for > 12 months died; of these mortalities, five starved (29.4%, $n = 5$), one was hit by a car (5.9%) and one died of unknown causes (unpubl. data).

Lead is a toxic metal and high levels could contribute to erratic behaviour that may contribute to road deaths if birds do not avoid vehicles (Fallon et al. 2017). Although lead was detected in 8% of specimens, the levels found were very low ($0.13 < \text{mg/kg}$ of liver), so it is unlikely to have contributed to their deaths. Levels below a threshold of 6 mg/kg dry weight are generally considered as evidence of low levels of exposure with limited health implications (Franson 1996; Pain et al. 2019).

Managing the causes of starvation

Restoring food supplies generally is likely required to contribute to the recovery of this threatened species. Although Australasian bittern feed on a variety of food types, they appear to feed primarily on small to medium sized fish, as do other *Botaurus* bittern species overseas, where restoration of fish-food supplies is a focal conservation action (Gilbert et al. 2003; Noble et al. 2004; Polak 2007; Williams 2024). The distribution and abundance of indigenous fish populations have both declined markedly in New Zealand, and many are threatened with extinction (Allibone et al. 2010; Dunn et al. 2018). The relative importance of specific fish species in the diet of Australasian bitterns is unknown, yet it is vital information if restoring fish stocks becomes a focus of

conservation management. The status and importance of different fisheries and their spatial variation across the range of Australasian bittern is also unknown. What is known is that Australasian bittern mainly occupy lowland coastal wetlands (O'Donnell and Robertson 2016) where most of the pressure on fish stocks is increasing. In an analysis of the national freshwater fish database, Joy (2009) recorded a significant reduction in biotic integrity over the previous c.40 years, especially in lowland sites.

There is a clear need to investigate any potential factors that could affect food availability and prey accessibility. Australasian bitterns are opportunistic feeders consuming a diverse range of prey suggesting they could exhibit a certain level of resilience to starvation when food sources are unpredictable (Marchant and Higgins 1990; Williams 2024). Nevertheless, their restricted adaptability in foraging methods and particular requirements regarding prey size might hinder the species' capacity to find food in compromised wetland habitats (Kushlan 1976; Williams 2024). The substantial size of Australasian bitterns indicates that they need significant amounts of prey to maintain their populations. While they do consume smaller prey, such as insects, non-native mosquito fish (*Gambusia affinis*) and frog tadpoles, these are probably insufficient to support a long-term Australasian bittern population alone unless they can be readily captured in large numbers (Williams 2024).

Understanding the contribution of starvation

Results indicate that starvation is likely an important contributor to mortality in Australasian bittern, and understanding its contribution to reducing population viability in this critically threatened species remains a priority. However, habitat loss and food limitation alone do not account for the threat status of Australasian bittern (O'Donnell and Robertson 2016; Williams 2024). More than many threatened species in New Zealand, the Australasian bittern faces threats from a complicated mix of causes, such as loss and degradation of habitat due to various human activities, a decline in food sources, poor water quality, and inconsistent water level management, all of which lead to starvation and reduced survival rates. Moreover, excessive predation by birds of prey and non-native mammals, the encroachment of weeds that alter and diminish habitat, incidents of collisions with vehicles and other infrastructure, as well as disturbances caused by humans are also regarded as threats to bittern populations (Williams 2024).

Understanding the demography of starvation remains a critical gap in our knowledge, including its relative contribution to overall mortality in Australasian bitterns. The sample of starving bitterns in this study included both adult and juvenile bitterns, both sexes and both wild and rehabilitated birds. However, because the collection of samples was serendipitous, the demography of the sample of birds assessed may have been biased in some way.

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