



Morphometric and physiological development confirm skull ossification level as a reliable indicator of age in a passerine bird, the Noisy Miner

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Abstract

In birds, several physiological characters have been proposed and tested as possible criteria of age. Among them, skull development is likely to provide a valuable reflection of other physiological developments, however the relationship between skull ossification and other physiological and morphometric traits has rarely been quantified within species. Here, we investigated suitability of skull ossification as an indicator of age in Noisy Miners (*Manorina melanocephala*), an Australian passerine bird. We compared skull ossification with other age indicators including the bursa of Fabricius, gonadal development, and a range of commonly used body size measures that included wing length and tarsus length. We carried out morphological measurements on 1251 individual Noisy Miner carcasses that had been collected during a culling experiment in November, December and May 2015–2016 from north-eastern NSW, Australia. The probability of a bursa of Fabricius being present in an individual bird changed with skull ossification level, with a significant reduction in the size of the bursa of Fabricius correlating with the degree of skull ossification. In male Noisy Miners, birds that were at a later stage of skull ossification had significantly larger testis size than those with a lower stages of skull ossification. In females, ovary size increased significantly as skull ossification progressed. In both sexes, skull ossification level was also correlated with body mass and skeletal measures. Given this, the level of skull ossification in Noisy Miners accurately correlates with other age indicators, and can justifiably be used as a non-invasive aging method in the field for live birds.

Keywords Aging · Age indicators · Skull ossification · Noisy Miner

Zusammenfassung

Die morphometrische und physiologische Entwicklung bestätigt den Verknöcherungsgrad des Schädels als zuverlässigen Indikator für das Alter bei einem Sperlingsvogel, dem Weißstirn-Schwatzvogel (*Manorina melanocephala*).

Bei Vögeln wurden bislang einige physiologische Eigenschaften für eine mögliche Altersbestimmung vorgeschlagen und getestet. Von diesen ist die Entwicklung des Schädels wahrscheinlich gut verwertbar für andere physiologische Entwicklungen, aber der Zusammenhang zwischen Schädelverknöcherung und anderen physiologischen und morphometrischen Merkmalen ist nur selten innerhalb von Arten quantifiziert worden. In unserer Studie wurde beim Weißstirn-Schwatzvogel (*Manorina melanocephala*), einem australischen Sperlingsvogel, die Eignung der Schädelverknöcherung als Altersindikator untersucht. Wir verglichen die Schädelverknöcherung mit anderen Altersindikatoren, darunter die Bursa Fabricii, die Gonadenentwicklung

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und eine Reihe gängiger Körpermaße wie z.B. Flügel- und Fußwurzellängen. Wir führten morphologische Messungen an 1251 einzelnen, toten Weißstirn-Schwatzvögeln durch, die während einer Keulung im November, Dezember und Mai 2015–2016 im nordöstlichen NSW, Australien, gesammelt worden waren. Die Wahrscheinlichkeit, eine Bursa Fabricii bei einem einzelnen Vogel vorzufinden, änderte sich mit dem Grad der Verknöcherung des Schädels, wobei die Größe der Drüse mit dem Grad der Verknöcherung des Schädels deutlich abnahm. Bei den männlichen Vögeln in einem späteren Stadium der Schädelverknöcherung waren die Hoden deutlich größer als bei Vögeln mit weniger starker Schädelverknöcherung. Bei den weiblichen Tieren nahm die Größe der Eierstöcke mit fortschreitender Verknöcherung des Schädels deutlich zu, und bei beiden Geschlechtern korrelierte der Grad der Schädelverknöcherung auch mit der Körpermasse und den Knochenmaßen. Somit korreliert das Ausmaß der Schädelverknöcherung bei Weißstirn-Schwatzvögeln sehr genau mit anderen Altersindikatoren und kann mit Berechtigung als nicht-invasive Methode für eine Altersbestimmung an freilebenden Vögeln verwendet werden.

Introduction

Accurately aging individuals, in conjunction with key information such as population sex ratio, provides a foundation for the establishment of a range of conservation and management considerations. They include harvest regulations and strategies, evaluation and development of protocols to monitor a population's demographic structure, health and viability, impacts related to ecosystem health, and also provides an understanding of the behavioural ecology of a species (Schroeder and Robb 2005; Roach and Carey 2014; Civil et al. 2019). In birds, ecological studies focusing on understanding population processes often require knowledge about the age of individuals (Sun 2001), with these studies using this information to aid species conservation, invasive species management, survivorship, demography, and the impacts of environmental stressors on avian populations (Brook et al. 2003; Sun et al. 2011; King et al. 2013; Shao et al. 2015; Diller et al. 2016).

Despite its importance, accurate age determination can be difficult for many bird species that undergo little or no plumage change across different life stages (Broughton 1994). It is common for species to have a distinct plumage for their first year of life as an immature but, differentiation among older age classes is much rarer and more difficult to detect (Schroeder and Robb 2005). In small passerine birds, several characters have been proposed and tested as possible criteria of age. Among them, one common method is "skulling", that is, checking the extent of development of skull ossification and degree of pneumatization "(development of air-filled cavity)". This method was developed and has been refined by various investigators for a range of taxa (Miller 1946; Norris 1961; Baird 1964; Yunick 1981; Pyle et al. 1987).

Pneumatization is a good candidate character for assessing the state of maturity in songbirds as they generally complete pneumatization at four to eight months of age (Serventy et al. 1967). The time required for this process varies with species, from a few months in most, to up to a year in others (Mueller and Weise 1996). Given this, prior to utilizing skull ossification to age individuals, the developmental

timeline observed in a given species needs to be confirmed. Apart from skull ossifications as an indicator of age, the other main criteria for aging passerine birds can be categorised into either physiological development or morphometric traits (Ciriaco 2003).

Hormones produced from the hypophysis gland help to control growth, energy management and all functions of the sex organs in birds, so are linked to breeding maturity. In the Bell Miner (*Manorina melanophrys*), Poiani and Fisher (1994) found that plasma levels of androgens increased with age. Thus, the stages of gonad development for both sexes can provide an estimate of a given bird's age. These hormonal changes can also control the involution of the bursa of Fabricius (an immunosuppressive organ in birds) (Asmundson et al. 1937; Kirkpatrick and Andrews 1944), leading to an inverse relationship between sexual gonad development and bursa of Fabricius size (Kirkpatrick 1944; Davis 1947; Lewin 1963). For example, assessments of the relationship between bursa of Fabricius and testis size have shown these variables to be a reliable indicator of age and a means of distinguishing adults from juveniles particularly in gallinaeous species (Jolly 1913; Greenwood 1929; Gower 1939; Payne 1971) and in the Laysan (*Phoebastria immutabilis*) and Black-footed Albatross (*P. nigripes*) (Broughton 1994). Since the size of a bursa of Fabricius within an individual is correlated with age, it has also long been used in wildlife management settings to separate birds raised in the previous 12 months from older, breeding-age birds (e.g., Davis 1947; Henny et al. 1981). This is because the activity of this organ depends on the developmental stage of the animal (Greenwood 1929; Jolly 1913; Payne 1971). The involution of this organ occurs immediately after the bursa of Fabricius reaches its maximum development (Jolly 1915; Riddle 1928). The decrease in size of this organ is near linear for most species and once involution has commenced, the size of bursa of Fabricius should thus be a reliable index of early age (Siegel-Causey 1990). Furthermore, it has been assumed that the bursa of Fabricius involutes before the skull becomes ossified (Davis 1947).

Various morphometric characters have been used in relation to age determination in birds, including body mass and skeletal measures such as tarsus length, wing length, head and/or bill size (Mueller and Weise 1996). Body mass increases after hatching and can be used for detecting age of nestlings and fledglings. Other important morphometric measures include the length of the wing chord, which can be reliably used to age and even sex individuals in sexually dimorphic Brown Falcon (*Falco berigora*) nestlings (McDonald 2003). There are some limitations in the use of mass and skeletal measures for aging birds. For example, these measurements are only useful while the bird is growing but, once adult size is reached, the measure is no longer informative.

It is believed that when aging birds, more accurate results can be obtained by a multi-character method, taking into account both physiological and morphometric developments (Siegel-Causey 1989). This is challenging however, as some of the physiological methods require dissection and/or are difficult to undertake in field situations. In addition, many morphometric measurements are species-specific and can vary widely from among species. Therefore, a more universal method that can incorporate both physiological and morphometric developments is required. Here, we examined the relationship between available age indicators (including bursa of Fabricius development, testis/ovary condition and body size measures including wing, tail, tarsus and head-bill length) with the degree of skull ossification, to investigate if skull development can be used as a non-invasive but reliable indicator of age in the Noisy Miner (*Manorina melanocephala*), an Australian passerine species that is of considerable interest due to its impact on other species of conservation significance. The species is a common honeyeater that lives in large, complex colonies containing both kin and non-kin year-round (Higgins et al. 2001) and is highly aggressive and excludes other species from areas it occupies, having an overall negative impact on the biodiversity of native birds, particularly small native avifauna (Davitt et al. 2018). It is an altricial species which displays no clear plumage-related indicators of age unlike other species in the genus (Clarke and Heathcote 1988). This provides an appropriate model species to explore aging of birds based on morphometric and physiological developments and it is important to understand the age composition of colonies for management practices (Etezadifar et al. 2022).

The range of the Noisy Miner is characterised by both population expansion and density increases, with accompanying negative impacts on ecological communities (Maron 2007). This is because Noisy Miners both benefit from human-induced landscape changes in the eucalypt-dominated habitats of this region, such as increased habitat fragmentation (Barati et al. 2016), but also their complex mobbing system that aggressively excludes competitors

from areas that they occupy (Arnold 2000). Removal via direct culling has been considered as the preferred management and control practice for this species (Melton et al 2021). Despite this, the species is known to demonstrate rapid recolonisation of culled areas, and the dynamics of recolonisation in this obligately social species are not well known. Understanding the age group of stable colonies and newly recolonised populations will be beneficial for future population management decisions. Given this, it is important to understand the age composition of resident birds before culling and also of new populations. The objectives of this study were to test whether skull ossification is the best practical and reliable indicator for aging Noisy Miners in the field based on the following key points. We expected the growth of body measurements like head-bill length, tail length, tarsus length, wing chord length to be positively correlated with gonad size and negatively correlated with bursa of Fabricius growth as internal indicators of age. We predict that because gonads size likely increases with age, and bursa of Fabricius size typically decreases with age, then there should be a negative correlation between these measures as individuals grow and obtain adult status (Jolly 1913; Greenwood 1929; Gower 1939; Kirkpatrick 1944; Davis 1947; Lewin 1963; Payne 1971; Broughton 1994).

We also tested how body mass differed in birds with various skull ossification levels. We expected that, as the skull of individual birds develops from incomplete through to full ossification (Pyle et al. 1987), body mass would increase with the individual's growing age.

We also tested if skull ossification was associated with the above internal physiological measures and external morphometric characteristics. We predicted that with higher level of skull ossification, other external age indicators would be longer, and internal age indicators such as the bursa of Fabricius would be smaller, while gonadal size would concurrently increase. We tested if there was a relationship between bursa of Fabricius size and skull ossification stages in birds for which a bursa of Fabricius was present.

Methods

Study areas and sample sources

Samples used in the study were collected from multiple sites during a wider Noisy Miner removal experiment that investigated the restoration of woodland bird communities (see Davitt et al. 2018 for details). Briefly, this experiment was conducted in open eucalypt woodland remnants in the New England Tablelands bioregion of New South Wales during 2015–2016. This study removed entire colonies of Noisy Miners from six different sites.

Noisy Miner colonies were removed from each treatment site in three separate removal periods, with removals occurring during November and December 2015 (spring/summer), and also again in May 2016 (autumn). On each removal occasion, a trained, licensed shooter supervised by an experienced ornithologist attempted to remove all Noisy Miners from treatment sites, using a shotgun loaded with bird shot, as the miners responded to playback of their “chur” alarm call (Holt et al. 2017). Carcasses were stored frozen at $-20\text{ }^{\circ}\text{C}$.

The number of culled samples varied among sites with an average around 100 birds per site and per cull, resulting in 2340 bird carcasses being available for analysis. Of all available samples, 1251 carcasses were used in this study. Only birds that had fully developed flight feathers, and thus would have already fledged and have been free-flying, were used in the analysis.

We collected the following body measurements: tarsus length, wing chord length, tail and head-bill length. These measurements were selected as they are considered to be repeatable measures in the field, and have been successfully used in studies examining body condition and sexual dimorphism across a range of species (e.g., Iko et al. 2004; Shephard et al. 2004; Jakubas and Jakubas 2011; Ura et al. 2016).

We used a digital scale (Scout Pro SP401, $d=0.1\text{ g}$, OHAUS Corporation, USA) to measure body mass of each carcass. Wing and tail were measured with a steel butted and un-butted ruler ($\pm 1\text{ mm}$) respectively, while tarsus and head to bill were measured with digital callipers to the nearest millimetre (KINCROME, Digital Vernier calliper, K11100, Kincome, Australia; accurate to $\pm 0.01\text{ mm}$).

To categorise each skull according to its ossification level, we followed Pyle (1987). After removing the crown feathers, we peeled back the skin with a scalpel and recorded the degree of ossification under a table lamp (Brilliant LTG. Max 60 w. Australia). Each skull was classified as either “un-ossified”, “semi-ossified” and “completely ossified”. The repeatability measurement for skull ossification category was completed for 50 randomly selected samples that were scored independently on two separate days; in all cases the results were consistent with each of the 50 birds placed in the same category on both days.

To identify the condition of the bursa of Fabricius in each sample, we followed the dissection of vertebrates’ guide (De Iuliis and Pulnera 2011). The presence or absence and the length and width of bursa of Fabricius was recorded. Also, the presence or absence and length and width of left testis and ovary were measured using digital callipers at this stage to the nearest millimetre.

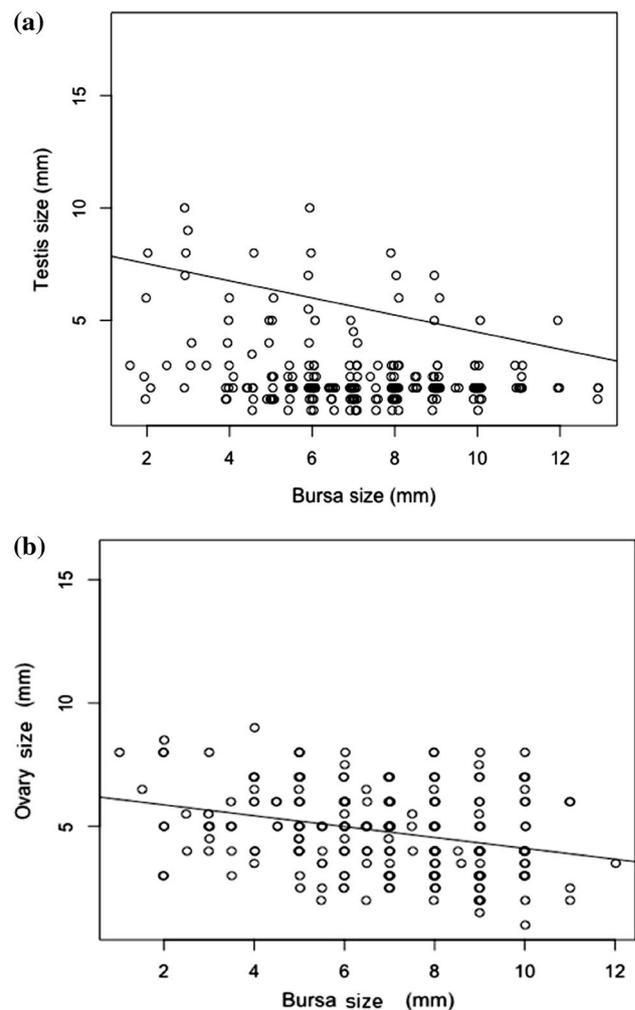


Fig. 1 **a** The negative correlation between testis size (measured as its length) and bursa of Fabricius size in male Noisy Miners in which both bursa of Fabricius and testis were present. Dots show testis and bursa sizes and the solid line represents the line of best fit ($R^2=-0.26$, $t=-4.2375$, $df=244$, $p<0.001$, $N=246$). **b** The correlation between ovary size and bursa of Fabricius size in female Noisy Miners. Ovary and bursa of Fabricius sizes were negatively correlated ($R^2=-0.30$, $t=4.6472$, $df=225$, $p<0.001$, $N=227$). Dots show ovary length and width and the solid line represents the line of best fit

Statistical analysis

As outlined earlier, subjects sampled were obtained after being culled by firearm, thus it is likely that some carcasses may have lost weight via fluid or tissue loss. Therefore, we initially investigated if there were significant weight differences in body mass of birds from live-trapped birds versus sampled carcasses herein for each sex using an ANOVA. In this test the type of body weight (culled vs live-trapped) was an independent predictor and body mass the dependent variable.

Table 1 ANOVA and Tukey post hoc test results examining the variation in morphometric measurements among birds with different between skull ossification levels (un-ossified, semi-ossified and completely ossified)

Trait	N	F	P-value	Tukey post hoc test
Tarsus length	1135	$F_{2,1134}=2.35$	$p > 0.1$	–
Wing chord length	1135	$F_{2,1134}=50.84$	$p < 0.001$	Skull 1–0: $p = 0.009$ Skull 2–0: $p < 0.00001$ Skull 2–1: $p < 0.00001$
Head-bill length	1135	$F_{2,1109}=31.19$	$p < 0.001$	Skull 1–0: $p = 0.0003$ Skull 2–0: $p < 0.00001$ Skull 2–1: $p = 0.0002$
Tail length	1119	$F_{2,1118}=5.90$	$p > 0.05$	–

Post hoc tests were applied if there were significant differences for the main effect

As multiple measurements were made for testis, ovary and bursa of Fabricius (length and width for each), we investigated if the length and width of testis, ovary or bursa of Fabricius, respectively, were correlated such that using either the length or width in analyses along was sufficient. These tests were carried out using a Pearson's product-moment correlation test.

Similarly, for both sexes with a measurable bursa of Fabricius, we tested if the relative size of the testis or ovary were correlated with bursa of Fabricius using a Pearson's product-moment correlation. The prediction was that as gonad size increases with age, and bursa of Fabricius size tends to decrease, then there should be a negative correlation between these measures. Presence/absence of bursa of Fabricius in relation to skull ossification levels was then tested using a 2 by 3 contingency table. Further, we examined if skull development was representative of the development of other physiological and morphological traits. We used multiple ANOVA tests with skull development level as a predicting factor (with three levels), and all other physiological (bursa of Fabricius size, testis size and ovary size) and morphological (body mass, tarsus length, head-bill length, wing chord length and tail length) characteristics as dependent variables. All statistics were performed in the R environment, version 1.2.5033 (R Core Team 2019).

Results

Are length and width of the testis, ovary or bursa of Fabricius correlated?

The bursa of Fabricius and either the left testis or ovary of focal individuals were measured via a length and width measure. To see if these were correlated for a given individual, we conducted a series of Pearson's correlations to determine if a single measure for each could be used

without loss of information. The measurements of testis length and width were indeed significantly correlated for male miners (Pearson's product-moment correlation, $R^2 = 0.96$, $t = 99.07$, $df = 648$, $p < 0.001$, $N = 650$). The measurements of the length and width of ovary were also significantly correlated within females (Pearson's product-moment correlation, $R^2 = 0.86$, $t = 39.28$, $df = 531$, $N = 533$). Similarly, there was a significant positive correlation between bursa of Fabricius length and width at the individual level (Pearson's product-moment correlation, $R^2 = 0.95$, $t = 70.005$, $df = 510$, $p < 0.001$, $N = 512$). Given this correlation for all three measures, only the length measures are used for testis, ovary and bursa of Fabricius size in subsequent analyses for simplicity.

Is there a negative correlation between testis or ovary size and bursa of Fabricius size within individuals?

As expected, there was a significant negative correlation between the size of bursa of Fabricius and testis in male birds (Pearson's product-moment correlation, $R^2 = -0.26$, $t = -4.2375$, $df = 244$, $p < 0.001$, $N = 246$, Fig. 1a) and between the size of bursa of Fabricius and the ovary in females (Pearson's product-moment correlation, $R^2 = -0.30$, $t = -4.64$, $df = 225$, $p < 0.001$, $N = 227$, Fig. 1b).

Relationship between oviduct shape and presence/absence of bursa of Fabricius

A convoluted oviduct, even if not swollen, indicates that the bird has laid an egg in its lifetime (Proctor and Lynch 1998; Wyllie and Newton 1999). We therefore tested if birds with straight oviducts were more likely to have bursa of Fabricius present. Of all female birds examined ($N = 370$), 72% of birds with a straight oviduct also had a bursa of Fabricius present, whereas only 7.5% of birds with non-straight oviduct had bursa of Fabricius present. Females with straight

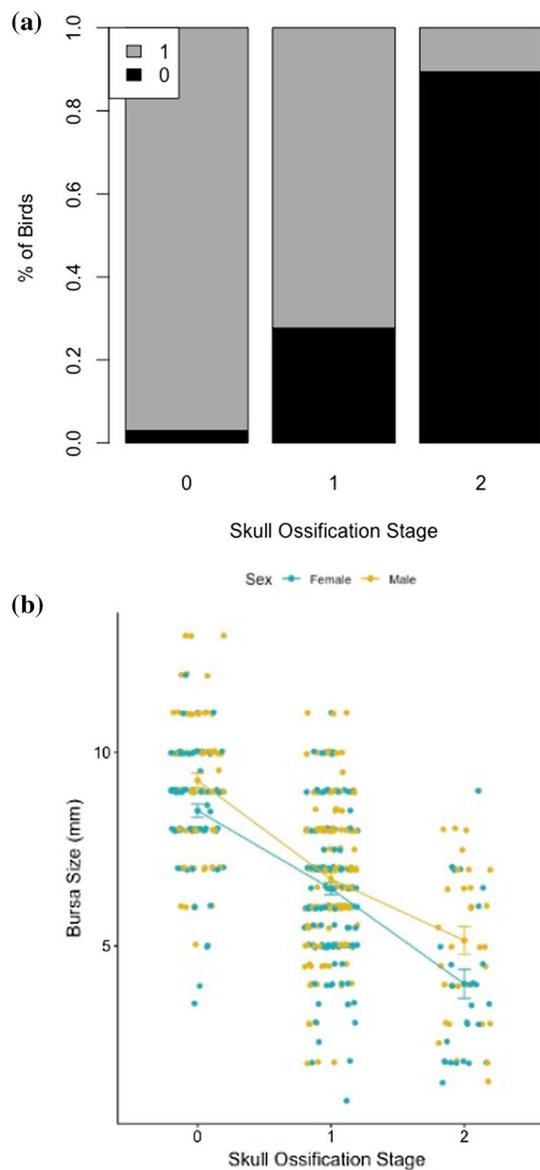


Fig. 2 **a** Percentages of birds where gonad and bursa of Fabricius present (1: shaded bars) or absent (0: solid bar) in birds of different skull ossification stages ($\chi^2=578.22$, $df=2$, $p<0.001$, $N=1137$). Skull ossification level is classified as un-ossified=0 ($N=169$), semi-ossified=1 ($N=394$) and completely ossified=2 ($N=574$). **b** Variation in the size of the bursa of Fabricius during different stages of skull ossification in birds where bursa of Fabricius was present. Skull ossification level is classified as un-ossified=0 ($N=160$), semi-ossified=1 ($N=285$) and completely ossified=2 ($N=61$). Dots show all bursa sizes measured and solid lines represent mean length for each skull ossification group. Bursa size significantly reduced in both males ($F_{1,253}=145$, $p<0.001$, $N=255$) and females ($F_{1,249}=147.3$, $p<0.001$, $N=251$) as ossification progressed

oviducts were significantly more likely to have bursa of Fabricius present ($\chi^2=137.5$, $df=1$, $p<0.001$), suggesting that the condition of oviduct and presence of bursa of Fabricius are associated.

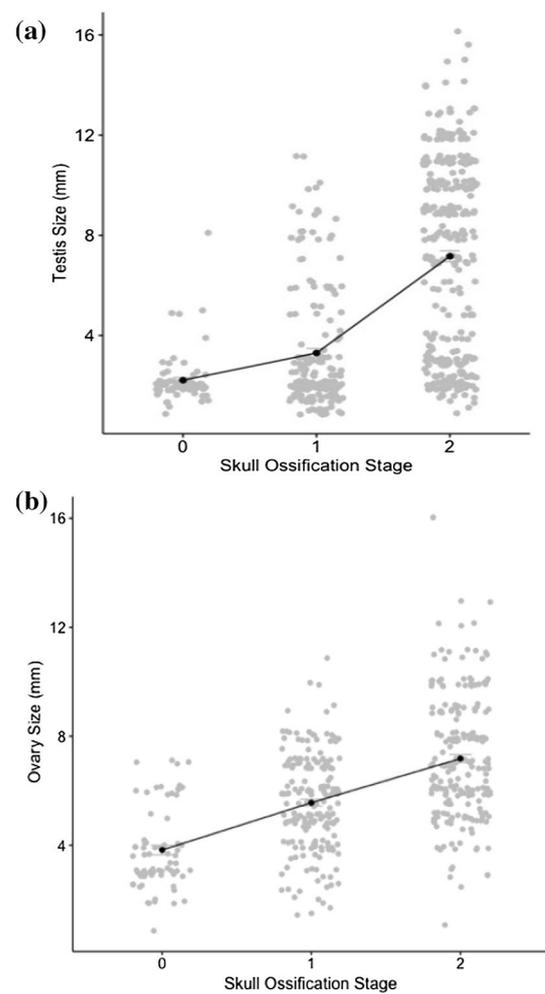


Fig. 3 **a** Variation of the testis size during different stages of skull ossification in birds where testis size could be measured, showing that size of gonad increased with the development of skull pneumatization ($F_{1,590}=225.3$, $p<0.001$, $N=612$). Skull ossification levels were classified as un-ossified=0 ($N=83$), semi-ossified=1 ($N=192$) and completely ossified=2 ($N=337$). Grey dots show all testis sizes measured and black dots represent mean length for each skull ossification group. **b** Variation of the ovary size during different stages of skull ossification in female Noisy Miners ($F_{2,479}=87.34$, $p<0.001$, $N=533$). Skull ossification levels were classified as un-ossified=0, semi-ossified=1 and completely ossified=2. Grey dots show all ovary sizes measured and black dots represent mean length for each skull ossification group

Skull development stage as an indication of individual bird age

When the presence/absence of bursa of Fabricius was tested in birds with various (Table 1) skull ossification levels, a significant departure from a random distribution was found; more birds with an ossified skull lacked a bursa of Fabricius than expected by chance, and fewer birds lacked the bursa of Fabricius when they had also yet to develop full skull ossification ($\chi^2=578.22$, $df=2$, $p<0.001$, Fig. 2a). Given

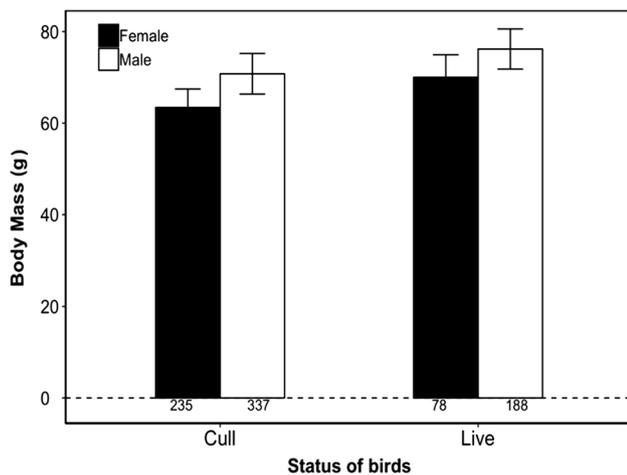


Fig. 4 Comparisons of the body mass of both sexes between the carcasses of culled birds and live trapped individuals ($t = -13.47$, $df = 390.46$, $p < 0.001$, live males $N = 188$, Culled males $N = 337$) ($t = -10.695$, $df = 113.34$, $p < 0.001$, live females $N = 78$, culled females $N = 235$) from previous studies in the same region (Barati et al. 2021)

this, the probability of a bursa of Fabricius being present in an individual bird changes with a putative measure of focal bird age, skull ossification level.

There was a significant reduction in the bursa of Fabricius size as skull ossification developed, with birds with fully developed skulls having the smallest bursa of Fabricius size ($F_{1,509} = 287.9$, $p < 0.001$, $N = 512$, Fig. 2b). Similarly, there were also significant changes in the testis size with increasing skull ossification (Fig. 3a, $F_{1,590} = 225.3$, $p < 0.001$, $N = 650$), and birds that were at later stage of skull ossification had significantly larger testis sizes than those with earlier stages of skull ossification. For females, there was also a significant difference between the ovary size of the birds at different skull ossification stages (ANOVA test, $F_{2,479} = 87.34$, $p < 0.001$, $N = 533$, Fig. 3b) as ovary size increased with developing skull ossification from level 0 (un-ossified) to level 2 (fully ossified) (Fig. 3b).

Development of skull with increasing body size

Body mass of adult male birds that had been culled by firearm were significantly lighter (between 4.5 and 6 g) than the body mass of adult males that had been live-trapped in similar areas (Barati et al. 2021) (Welch Two Sample t -test $t = -13.47$, $df = 390.46$, $p < 0.001$, $N_{\text{Live}} = 188$, $N_{\text{Shot}} = 337$ Figure 4). The mass loss followed similar patterns for adult female birds (Welch Two Sample t -test, $t = -10.695$, $df = 113.34$, $p < 0.001$, $N_{\text{Live}} = 78$, $N_{\text{Shot}} = 235$. Figure 4). Given this, the mass loss of birds culled, likely due to fluid loss, was found to be consistent among male and female

birds, but nonetheless the mass of culled birds should not be directly compared to that of free-living individuals.

As expected, there was a significant difference between body mass of birds for the three different skull ossification status in both male ($F_{2,609} = 30.8$, $p < 0.001$, $N = 711$, Fig. 5) and female ($F_{2,516} = 39.88$, $p < 0.001$, $N = 518$, Fig. 5) birds. Tukey post hoc tests showed that all three categories were significantly different from each other in both sexes (all $p < 0.01$). We also tested whether birds with different skull ossification levels varied in the other morphometric traits. There was not a statistically significant difference for the tarsus length among birds of different skull ossification levels in male ($F_{2,608} = 0.26$, $p > 0.1$, $N = 611$, Fig. 5) and female birds ($F_{2,515} = 0.52$, $p > 0.1$, $N = 518$, Fig. 5). However, wing chord length varied significantly among birds of different skull ossification levels in both male ($F_{2,608} = 31.5$, $p < 0.0001$, $N = 611$), and female miners ($F_{2,515} = 0.52$, $p < 0.0001$, $N = 518$, Fig. 5). ANOVA and subsequent post hoc tests showed that all three categories were significantly different in both sexes (all $p < 0.01$). Further, head-bill length varied significantly among birds of different skull ossification levels in both sexes (male: $F_{2,595} = 25.58$, $p < 0.0001$, $N = 598$, females: $F_{2,505} = 10.82$, $p < 0.0001$, $N = 508$, Fig. 5). Post hoc tests showed that all three categories were also significantly different in both males and females ($p < 0.01$). Finally, tail length was not significantly different among birds with three skull ossification levels in both male ($F_{2,604} = 11.8$, $p > 0.1$, $N = 607$) and females ($F_{2,504} = 11.8$, $p > 0.1$, $N = 507$).

Discussion

Development of skull as an indicator of age in the Noisy Miner

Overall, the results confirmed that skull ossification stage was significantly associated with physiological traits including larger gonads and a smaller bursa of Fabricius size, as well as positive correlations with morphometric measurements including body mass, head-bill and wing chord length. Thus, skull ossification can justifiably be used as an indication of bird age in the field for live birds, or when carcasses are available for dissection.

Findings from this study confirm that the size of the bursa of Fabricius is negatively correlated with gonadal development in both sexes of the Noisy Miner. These results are consistent with other finding from examining other avian species (Kirkpatrick 1944; Davis 1947; Lewin 1963). The gonads of a bird presumably mature in response to the suitable external stimuli of the environment and, in some species, this seems to control the involution of the bursa (Asmundson et al. 1937; Kirkpatrick and Andrews 1944). At least among Galliformes, testosterone and progesterone have been

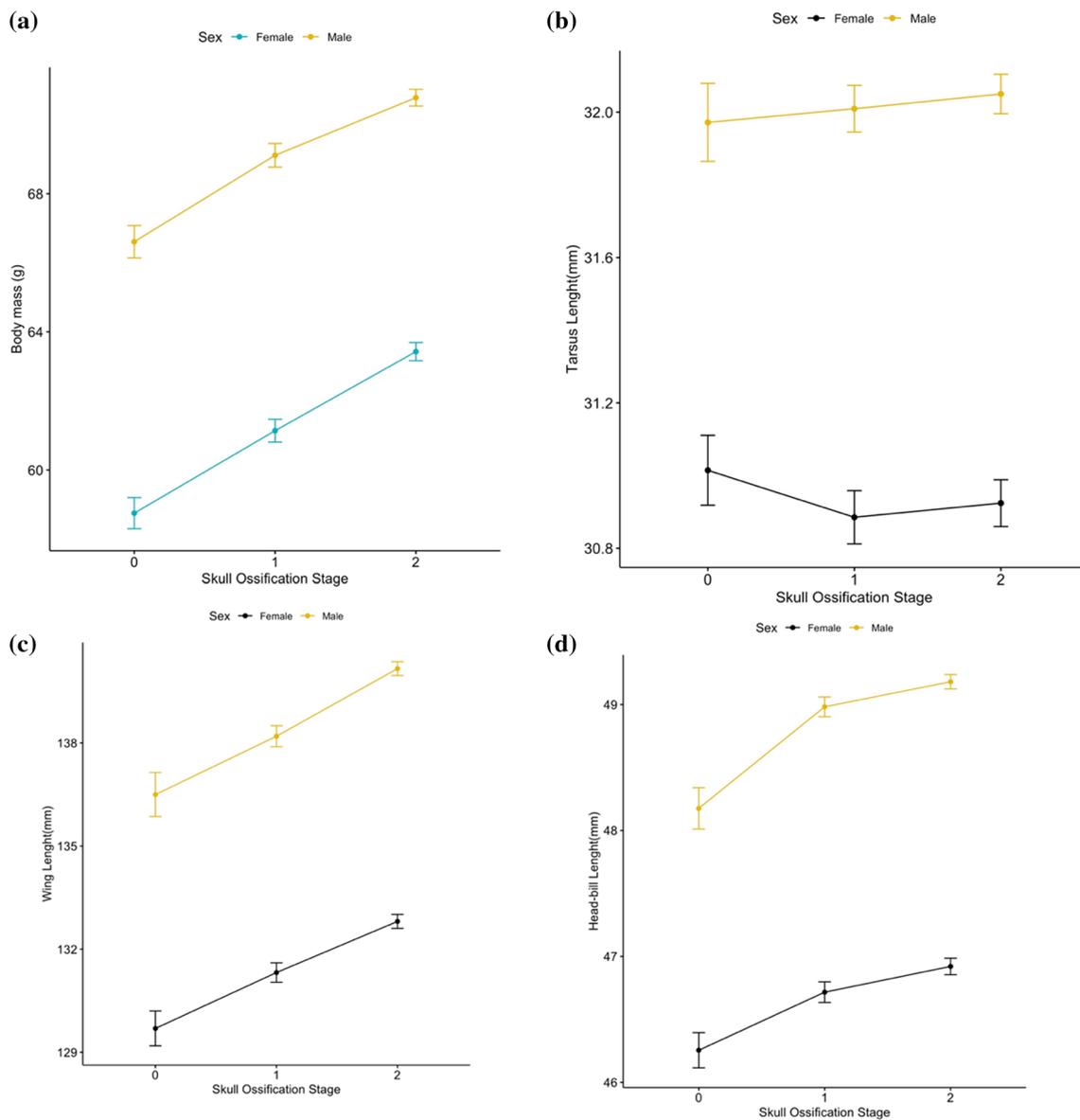


Fig. 5 **a** Variations in body mass with skull ossification levels in Noisy Miner (skull ossification levels were classified as un-ossified=0 ($N=164$), semi-ossified=1 ($N=395$) and completely ossified=2 ($N=572$)). Body mass significantly increased with skull ossification levels in both males ($F_{2,609}=30.8$, $p<0.001$, $N=613$) and female ($F_{2,516}=39.88$, $p<0.001$, $N=518$) Noisy Miners analysed separately. **b** Variation in tarsus length for birds with different skull ossification levels separated for male and female Noisy Miners. Skull ossification levels were classified as un-ossified=0 ($N=163$), semi-ossified=1 ($N=395$) and completely ossified=2 ($N=571$). Tarsus length did not vary significantly among birds with different skull ossification levels in both males ($F_{2,608}=0.26$, $p>0.1$, $N=611$) and females ($F_{2,515}=0.52$, $p>0.1$, $N=518$). **c** Variation in wing chord

length of male and female Noisy Miners with different skull ossification levels. Skull ossification levels were classified as un-ossified=0 ($N=163$), semi-ossified=1 ($N=395$) and completely ossified=2 ($N=571$). Wing chord length varied significantly among birds with different skull ossification levels in both males ($F_{2,608}=31.5$, $p<0.0001$, $N=611$) and females ($F_{2,515}=0.52$, $p<0.0001$, $N=518$). **d** Variation in head-bill length of male and female Noisy Miners with different skull ossification levels. Skull ossification levels were classified as un-ossified=0 ($N=140$), semi-ossified=1 ($N=395$) and completely ossified=2 ($N=571$). Head-bill length varied significantly among birds with different skull ossification levels in both males ($F_{2,595}=25.58$, $p<0.0001$, $N=598$, Fig. 2.21) and females ($F_{2,505}=10.82$, $p<0.0001$, $N=508$, Fig. 2.21)

demonstrated experimentally both to inhibit bursal development and stimulate bursa involution (e.g., Vujic et al. 1983; Mase and Oishi 1991). These findings may explain the inverse relationship between gonadal development and

bursa size reported for a number of bird species (Kirkpatrick 1944; Davis 1947; Lewin 1963; this study).

In Noisy Miners, bursa of Fabricius size was positively correlated with skull ossification which confirms the

hypothesis outlined in the introduction of this study. We also detected a negative correlation between testis and bursa size, but the relationship was not very strong ($R^2 = -0.26$), potentially due to only a few adult males having large testis. However, in this species where not all individuals breed each year given the cooperative breeding social system (Higgins et al. 2001; Barati et al. 2018a, 2018b), an adult might have small or large gonads throughout its adult life depending on when in the year it is sampled. Thus, the level of skull ossification may indicate physiological development such as bursa of Fabricius state in Noisy Miners, but may not always be a reflection of the state and size of the gonads.

Despite this individual variation, overall testis size was found to be positively correlated with skull ossification level in male Noisy Miners. Several studies have documented age-related variation in testis size, with older adults generally having larger testes than younger adults (Selander and Hauser 1965; Morton et al. 1990; Deviche et al. 2000; Graves 2004; Laskemoen et al. 2008). Generally, the results show that the growth of gonads have a positive relationship with the degree of skull ossification. Positive correlation of the gonads with body size has also been shown to exist in some species (Cartar 1985; Mailler 1988; Moller 1991). However, this is not always a simple relationship due to the effect of potential confounding factors such as environmental traits on gonadal sizes in adult birds.

Some Noisy Miners exhibiting no cranial ossification had involute bursa (0.4%), and some of them with bursa exhibited complete ossification (4.83%). However, most Noisy Miners with complete ossification were shown to have very small or an involute bursa of Fabricius. Given this, the organ probably demonstrates involuting before the skull becomes completely pneumatized in this species. Results from the current study on the bursa size and its relation to skull ossification level were consistent with previous reports on Noisy Miners (Vickers 2017). Among the morphometric traits assessed, body mass, head-bill and wing chord length were significantly associated with skull pneumatization. Thus, skull ossification can represent development in these morphometric traits.

Conclusion

Assessing skull ossification level can reveal the age class of Noisy Miners in terms of adult/juvenile based on its correlation with a range of other measures in this study. Finding the exact age of individuals beyond these classes though is complicated (Davis 1947). In this study, skull ossification levels in Noisy Miners were found to be correlated with both physiological and morphological traits and thus represents both these types of development. Given this, and for ease of measuring for the Noisy Miner, skull

ossification level can justifiably be used as an indication of aging in the laboratory condition and museums when carcasses are available. It is also possible to inspect the extent of ossification of live birds in the field by wetting and parting the crown feathers (Mueller and Weise 1996), so this method is also a useful tool when working with live birds. Although aging of birds using skull ossification index is a reliable method and in the most cases can have an accurate outcome, we recommend using it along with other aging criteria such as body mass to control for potential errors.

Finally, the limitation with aging based on skull ossification can be related to the timing of skull ossification. If skull ossification occurs substantially before birds reach their first year of age, young birds would have fully ossified skulls and thus potentially be erroneously classified as adults using our methodology. We have not seen this in the Noisy Miner, but because these relationships are species-specific this possibility needs to be tested with known age individuals.

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Author contributions The participants in this study have consented to the contents, submission of the manuscript for publication and availability of the data.

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Availability of data and materials The datasets generated during and/or analysed during the current study are available at University of New England library (RUNE). Data available on request due to privacy/ethical restrictions: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Conflict of interest The authors declare no conflict of interest. The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was carried out in accordance with the approved (AEC19-060) guidelines and regulations of University of New England, Animal Ethics Committee.

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References

- Arnold KE (2000) Group mobbing behaviour and nest defence in a cooperatively breeding Australian bird. *Ethology* 106:385–393
- Asmundson VS, Gunnand CA, Klose AA (1937) Some responses of the immature female fowl to injections of mare gonadotropic hormone and oestrin. *Poult Sci* 16:194–206
- Baird J (1964) Ageing birds by skull ossification. *EBBA News* 27:162–163
- Barati A, Andrew RL, McDonald PG (2021) Nestling sex does not influence provisioning effort of parents or helpers in a cooperative bird with a male-biased helper contingent. *Behav Ecol Sociobiol* 75:94. <https://doi.org/10.1007/s00265-021-03038-9>
- Barati A, Andrew RL, Gorrell JC, McDonald PG (2018a) Extra-pair paternity is not driven by inbreeding avoidance and does not affect provisioning rates in a cooperatively breeding bird, the Noisy Miner (*Manorina melanocephala*). *Behav Ecol* 29:244–252
- Barati A, Andrew RL, McDonald PG (2018b) Genetic relatedness and sex predict helper provisioning effort in the cooperatively breeding Noisy Miner. *Behav Ecol* 29:1380–1389
- Barati A, Etezadifar F, McDonald PG (2016) Fragmentation in eucalypt woodlands promotes nest-tree occupancy by a despotic species, the Noisy Miner (*Manorina melanocephala*). *Austral Ecol* 41:897–905
- Broughton JM (1994) Size of the bursa of Fabricius in relation to gonad size and age in Laysan and Black-Footed Albatrosses. *The Condor* 96:203–207
- Brook BW, Sodhi NS, Soh MCK, Haw Chuan L (2003) Abundance and projected control of invasive house crows in Singapore. *J Wildl Manag* 67:808
- Cartar RV (1985) Testis size in sandpipers. *Naturewissenschaften* 72:157–158
- Clarke MF, Heathcote CF (1988) Methods for sexing and ageing the bell miner (*Manorina melanophrys*). *Emu Austral Ornithol* 88:118
- Ciriaco E (2003) Age-related changes in the avian primary lymphoid organs (thymus and bursa of fabricius). *Microsc Res Tech* 62:482–487
- Civil MA, Cheney B, Quick NJ, Islas-Villanueva V, Graves JA, Janik VM, Thompson PM, Hammond PS (2019) Variations in age- and sex-specific survival rates help explain population trend in a discrete marine mammal population. *Ecol Evol* 9:49
- Davis DE (1947) Size of bursa of fabricius compared with ossification of skull and maturity of gonads. *J Wildl Manag* 3:224–251
- De Iuliis G, Pulera D (2011) The dissection of vertebrates. Second edition. Ontario, Canada.
- Deviche P, Wingfield JC, Sharp PJ (2000) Year-class differences in the reproductive system, plasma prolactin and corticosterone concentrations, and onset of prebasic molt in male dark-eyed juncos (*Junco hyemalis*) during the breeding period. *Gen Comp Endocrinol* 118:425–435
- Diller LV, Hamm KA, Early DA, Lamphear DW, Dugger KM, Yackulic CB, Schwarz CJ, Carlson PC, McDonald TL (2016) Demographic response of northern spotted owls to barred owl removal. *J Wildl Manag* 80:691–707
- Davitt G, Kimberly Maute K, Major RE, McDonald PG, Maron M (2018) Short-term response of a declining woodland bird assemblage to the removal of a despotic competitor. *Ecol Evol* 8:4771–4780
- Etezadifar F, Vickers JAT, French K, McDonald PG, Barati A, Andrew RL, Major RE (2022) Repeated experimental removals unveil sex and age-specific dispersal strategies in a social passerine bird. *Wildl Res.* <https://doi.org/10.1071/WR21170>
- Gower WC (1939) The use of the bursa of Fabricius as an indication of age in game birds. *J Wildl Manag* 11:244–251
- Graves GR (2004) Testicular volume and asymmetry are age-dependent in black-throated blue warblers (*Dendroica caerulescens*). *Auk* 121:473–485
- Greenwood AW (1929) Some observation on the thymus gland in the fowls. *Proceed Royal Soc* 50:26–37
- Henny CJ, Carter J, Carter B (1981) A review of Bufflehead sex and age criteria with notes on weights. *Wildfowl* 32:117–122
- Higgins PJ, Peter JM, Steele WK (2001) Handbook of Australian. Oxford University Press, Melbourne, New Zealand and Antarctic birds
- Holt J, Barati A, McDonald PG (2017) The complex acoustic repertoire of a highly social species, the noisy miner, *Manorina melanocephala*. *Emu* 117:19–30
- Iko WM, Dinsmore SJ, Knopf FL (2004) Evaluating the use of morphometric measurements from museum specimens for sex determination in mountain plovers (*Charadrius montanus*). *West North Am Nat* 64:492–496
- Jakubas KW, Jakubas D (2011) Predicting the sex of the sedge warbler (*Acrocephalus schoenobaenus*) by discriminant analysis. *Ornis Fenn* 88:90–97
- Jolly J (1913) L'involution physiologique de la bourse de Fabricius et ses relations avec l'apparitions de la maturite` sexuelle. *Comptes Rendus De La Soc De Biol* 75:638–648
- Jolly T (1915) La bourse de Fabricius et les organes limpo-epitheliaux. *Arch D'anatomie Microsc* 16:363–547
- King RS, Trutwin JJ, Hunter TS, Varner DM (2013) Effects of environmental stressors on nest success of introduced birds. *J Wildl Manag* 77:842–854
- Kirkpatrick CM, Andrews FN (1944) The influence of the sex hormones on the bursa of Fabricius and the pelvis of ring-necked pheasants. *Endocrinology* 34:340–345
- Kirkpatrick CM (1944) The bursa of Fabricius in Ring-necked Pheasants. *J Wildl Manag* 8:118–129
- Laskemoen T, Fossøy F, Rudolfson G, Lifjeld JT (2008) Age-related variation in primary sexual characters in a passerine with male age-related fertilization success, the bluethroat (*Luscinia svecica*). *J Avian Biol* 39:322–328
- Lewin V (1963) Reproduction and development of young in a population of California Quail. *Condor* 65:249–278
- Mailler AP (1988) Ejaculate quality, testes size and sperm competition in primates. *J Hum Evol* 17:479–488
- Maron M (2007) Threshold effect of eucalypt density on an aggressive avian competitor. *Biol Cons* 136:100–107
- Mase Y, Oishi T (1991) Effects of castration and testosterone treatment on the development and involution of the bursa of

- Fabricius and the thymus in the Japanese Quail. *Gen Comp Endocrinol* 84:426–433
- McDonald PG (2003) Nestling growth and development in the brown falcon, *Falco berigora*: an improved ageing formula and field-based method of sex determination. *Wildl Res* 30:411–418
- Melton C, Reside A, Simmonds J, McDonald P, Major R, Crates R, Catterall C, Clarke M, Grey M, Davitt G, Ingwersen D, Robinson D, Maron M (2021) Evaluating the evidence of culling a native species for conservation benefits. *Conserv Sci Pract*. <https://doi.org/10.1002/csp2.549>
- Miller AH (1946) A method for determining the age of live passerine birds. *Bird Banding* 17:33–35
- Moller AP (1991) Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *Am Nat* 137:882–906
- Mueller WP, Weise CM (1996) An assessment of age determination methods for captured passerine birds. *Field Stn Bull* 29:21–27
- Norris RA (1961) A modification of the miller method of ageing live passerine birds. *Bird Banding* 32:55–57
- Payne LN (1971) The lymphoid system in physiology and biochemistry of the domestic fowl. *Acad Lond N Y* 2:985–1037
- Poiani A, Fletcher T (1994) Plasma levels of androgens and gonadal development of breeders and helpers in the bell miner (*Manorina melanophrys*). *Behav Ecol Sociobiol* 34:31–41
- Proctor NS, Lynch PJ (1998) *Manual of Ornithology: Avian Structure and Function*. Yale University Press, New Haven, Connecticut
- Pyle P, Howell SNG, Yunick RP, DeSante DF (1987) *Identification Guide to North American Passerines*. California, Slate Creek, p 278
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Riddle O (1928) Studies on the physiology of reproduction in birds. Growth of the gonads and bursa of Fabricii in doves, with data for body growth and age at maturity. *Am J Physiol* 86:248–265
- Roach DA, Carey JR (2014) Population biology of aging in the wild. *Annu Rev Ecol Syst* 45:421–443
- Schroeder MA, Robb LA (2005) *Criteria for gender and age Techniques for wildlife investigations and management*, 6th edn. The Wildlife Society, Maryland, USA, pp 303–338
- Selander RK, Hauser RJ (1965) Gonadal and behavioral cycles in the great-tailed grackle. *The Condor* 67:157–182
- Serventy DL, Nicholls CA, Farner DS (1967) Pneumatization of the cranium of the zebra finch (*taeniopygia castanotis*). *Ibis* 109:570–578
- Shao M, Chen B, Cui P, Dai N, Chen H (2015) Sex ratios and age structure of several waterfowl species wintering at Poyang Lake, China. *Pak J Zool* 48:839–844
- Shephard J, Catterall CP, Hughes J (2004) Discrimination of sex in the white-bellied sea-eagle (*Haliaeetus leucogaster*) using genetic and morphometric techniques. *Emu Austral Ornithol* 104:83–87
- Siegel-Causey D (1989) Cranial Pneumatization in the Phalacrocoracidae. *Wilson Bull* 101:108–112
- Siegel-Causey D (1990) On use of size of the Bursa of Fabricius as an index of age and development. *J Field Ornithol* 61:441–444
- Sun RY (2001) *Principle of animal ecology*. Beijing.
- Sun Y, Bridgman CL, Wu H, Lee C, Liu M, Chiang P, Chen C (2011) Sex ratio and survival of mandarin ducks in the Tachia river of central Taiwan. *Waterbirds* 34:509–513
- Ura T, Azuma N, Hayama S, Higashi S (2016) Sexual dimorphism of Latham's snipe (*Gallinago hardwickii*). *Emu Austral Ornithol* 105:259–262
- Vickers JAT (2017) Demographic shifts in Noisy Miner (*Manorina melanocephala*) populations following removal. Honours Dissertation, University of Wollongong.
- Vujic D, Milicevic Z, Micic M, Isakovic K, Jankovic BD (1983) Atrophy of the bursa of Fabricius caused by neonatal gonadectomy. *Period Biol* 85:107–109
- Wyllie I, Newton I (1999) The proportions of female Sparrowhawks and Kestrels which bred in their first year of life. *Ibis* 141:504–506
- Yunick RP (1981) Further observations on skull pneumatisation. *North Ame Bird Bander* 6:40–43

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