Using sexual dimorphism in morphometric traits to sex Eastern Yellow Robins *Eopsaltria australis*

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The identification of the sex of individuals is important for the investigation of gender roles in the behavioural ecology of species and sex-specific differences in demography. We investigated the extent of sexual size dimorphism in the Eastern Yellow Robin (*Eopsaltria australis*), a species with no sex differences in plumage, and the utility of morphometric traits to sex adult and juvenile birds. Using a sample of adult birds sexed on the basis of sex-specific breeding behaviour, we found strong sexual dimorphism in all morphometric traits measured, namely total head length, wing chord, body mass, tarsus length and tail length, with males being larger than females. Total head length was the most reliable measure to use to sex adult Eastern Yellow Robins, showing the greatest repeatability and no overlap in the measurements of males and females. Total head length averaged (± 1 s.d.) 40.1 ± 1.0 mm among adult males and 36.7 ± 0.4 mm among adult females. Juvenile birds captured within the first month of fledging showed no subsequent change in body mass and tarsus length between juvenile and older ages, and a two-way plot of body mass and tarsus length provides a reliable method for sexing juvenile birds.

INTRODUCTION

The identification of the sex of individuals is important for the investigation of gender roles in the behavioural ecology of species (e.g. Gowaty 1993; Koenig and Dickinson 2004; McNamara and Wolf 2015; Remeš *et al*. 2015) and sex-specific differences in demography (Székely *et al*. 2014) that are critical for understanding breeding systems and population dynamics. The assignment of sex may be done on the basis of sex differences in plumage or morphometric traits in sexually dimorphic species (Owens and Hartley 1998). Alternatively, the assignment of sex must rely on a DNA test, such as the chromo-helicase domain 1 gene (*CHD-1*) sex determination test (Griffiths *et al*. 1998).

The Eastern Yellow Robin (*Eopsaltria australis)* is a common, territorial resident in the woodlands and forests of eastern Australia, where it has been used as a model for examining the influences of habitat fragmentation on bird demography (Zanette 2000, 2001; Zanette and Jenkins 2000; Berry 2001; Debus 2006a, b; Debus and Ford 2012; Amos *et al*. 2014). It has a relatively complex behavioural ecology, being a facultative co-operative breeder in which the breeding pair may be assisted by one or more helpers that are typically male but may also be female (Marchant 1987; P. Lloyd unpublished data). Sexes cannot be distinguished on the basis of plumage colours (Higgins and Peter 2002). Two subspecies are recognised:

1. the nominate *E. a. australis*, distributed from the uppermost reaches of the Murray-Darling Basin in south-central Queensland south through New South Wales (NSW), Victoria and south-eastern South Australia, and

2. *E. a. chrysorrhoa*, distributed from the Clarence River drainage in north-eastern NSW north to the Atherton Tablelands in north-eastern Queensland, extending inland to the western slopes of the Great Divide (Higgins and Peter 2002).

We combined observations of sex-specific breeding behaviour with morphometric measurements to investigate the extent of sexual size dimorphism in a population of *E. a. chrysorrhoa* and the utility of morphometric traits to sex both adult and juvenile birds.

METHODS

The study was conducted as part of a longer-term study of the breeding biology and survival of the Eastern Yellow Robin and other bird species at a rural property near Imbil, south-eastern Queensland (26°29'S, 152°45'E; 80–160 m asl.). The vegetation at the site comprises a mosaic of notophyll vine forest with tall eucalypt emergents (dominated by *Corymbia intermedia, Eucalyptus siderophloia* and *E. grandis*), a plantation of Gympie Messmate (*Eucalyptus cloeziana*) and adjoining livestock grazing paddocks with scattered trees. Birds were captured at monthly intervals using mist-nets as part of a constant effort netting programme, supplemented by captures using spring traps baited with a mealworm. Nets and traps were checked every 10-20 minutes; captured birds were banded and measured, after which they were released. The positions of all captures were recorded using a hand-held GPS. Birds were banded with a numbered metal band (ABBBS) (September 2007 to November 2015) and a unique combination of three colour bands (from June 2012). Morphological measures collected from each bird included: total head length (back of head to tip of bill) and tarsus length (measured with Vernier callipers, ±1 mm precision), wing chord and tail length (measured with a ruler, ±1 mm precision), and body mass (measured with a Pesola™ spring scale or a portable digital scale with ±0.1 g precision). Body measurements of birds were taken by various bird banders, including both experienced and trainee operatives. During the breeding season (July to December), birds were also checked for the presence of a brood patch or a cloacal protuberance. Birds were aged as juveniles if they had a brown juvenile plumage prior to undergoing a post-

juvenile moult at 1-2 months after fledging, immatures if they had one or more retained juvenile feathers in the wing coverts after the post-juvenile moult, and as adults if they had no retained juvenile feathers.

Territory boundaries and the composition of territorial groups were established through regular re-sighting of colour-banded birds whose location was recorded on a hand-held GPS. A sample of colour-banded birds was sexed largely on the basis of nesting behaviours recorded during the 2014 and 2015 breeding seasons; active nests were observed continuously for 1–2 hours using a Swarovski® spotting telescope to record the identities and behaviours of all birds attending nests. Individuals that were observed building nests, incubating eggs or brooding nestlings were characterised as female, as only females undertake these parental care behaviours in this species (Zanette *et al.* 2000). Furthermore, all individuals possessing a fully-developed brood patch were characterised as female. Breeding males were distinguished from helpers by their banding history relative to that of any helpers present i.e. breeding males were older birds and most helpers were first banded as juvenile or immature birds on the territories of breeding males.

Measurements from juvenile and immature birds were excluded from the data set for analysis. Multiple measures of each morphometric trait obtained from recaptures of particular individuals were averaged for each individual prior to analysis. Mean measurements of males and females were compared using two-tailed Welch's unequal variances t-tests. Means are expressed ± 1 standard deviation (s.d.). Repeatability (*r*), a measure of the consistency of multiple measurements on the same individual, was calculated as the proportion of variation in a trait that is due to differences among individuals not to differences within an individual, using the equation $r = S^2A/(S^2 + S^2A)$, where S2A is the between-group variance and S^2 is the within-group variance (Lessels and Boag 1987).

RESULTS AND DISCUSSION

Two-way plots of morphometric traits among 21 males and 18 females sexed on the basis of nesting behaviours suggested that adult Eastern Yellow Robins exhibited strong sexual dimorphism in morphometric traits (Fig 1). Females were significantly smaller than males in all traits (Table 1). There was limited overlap in the measurements of males and females for
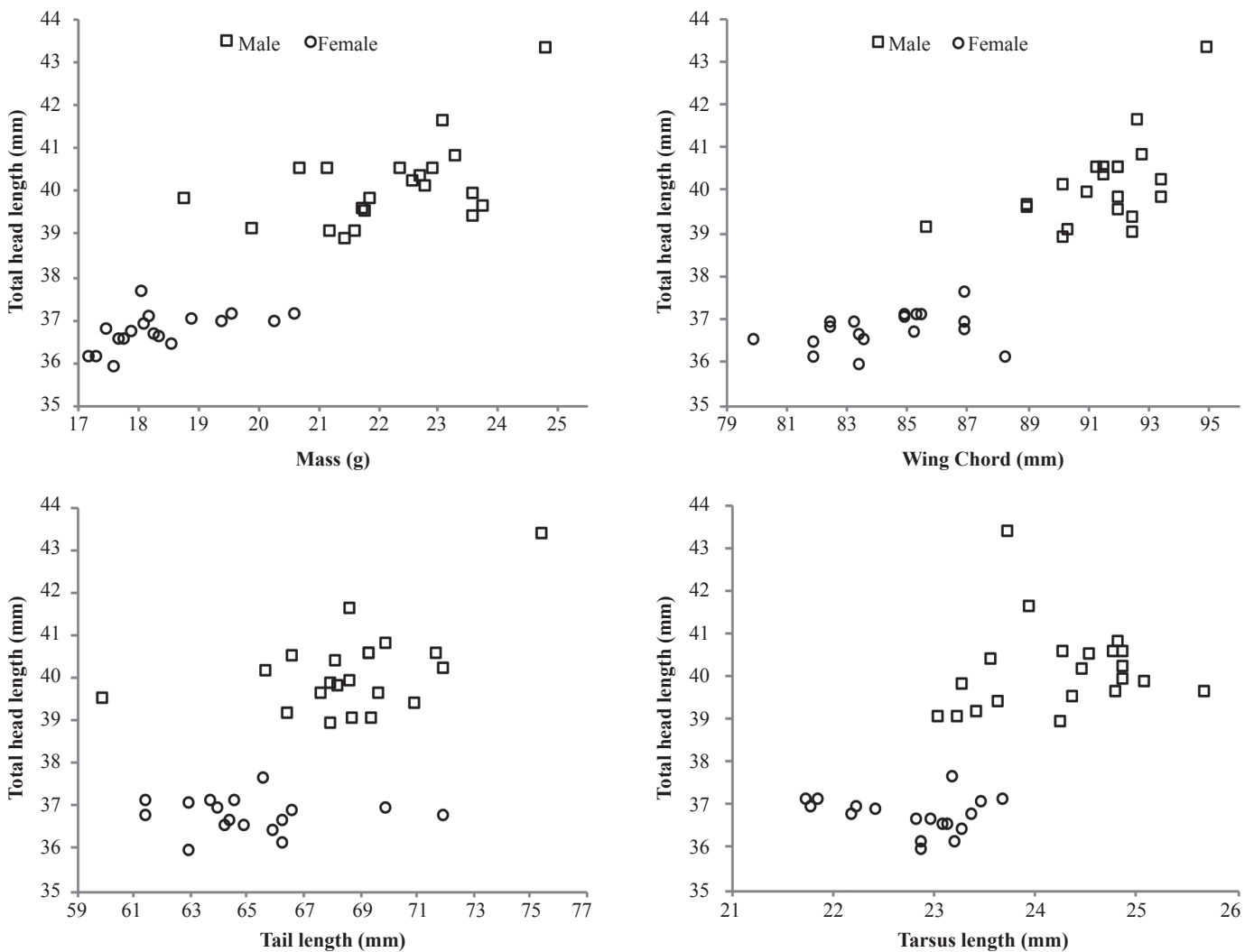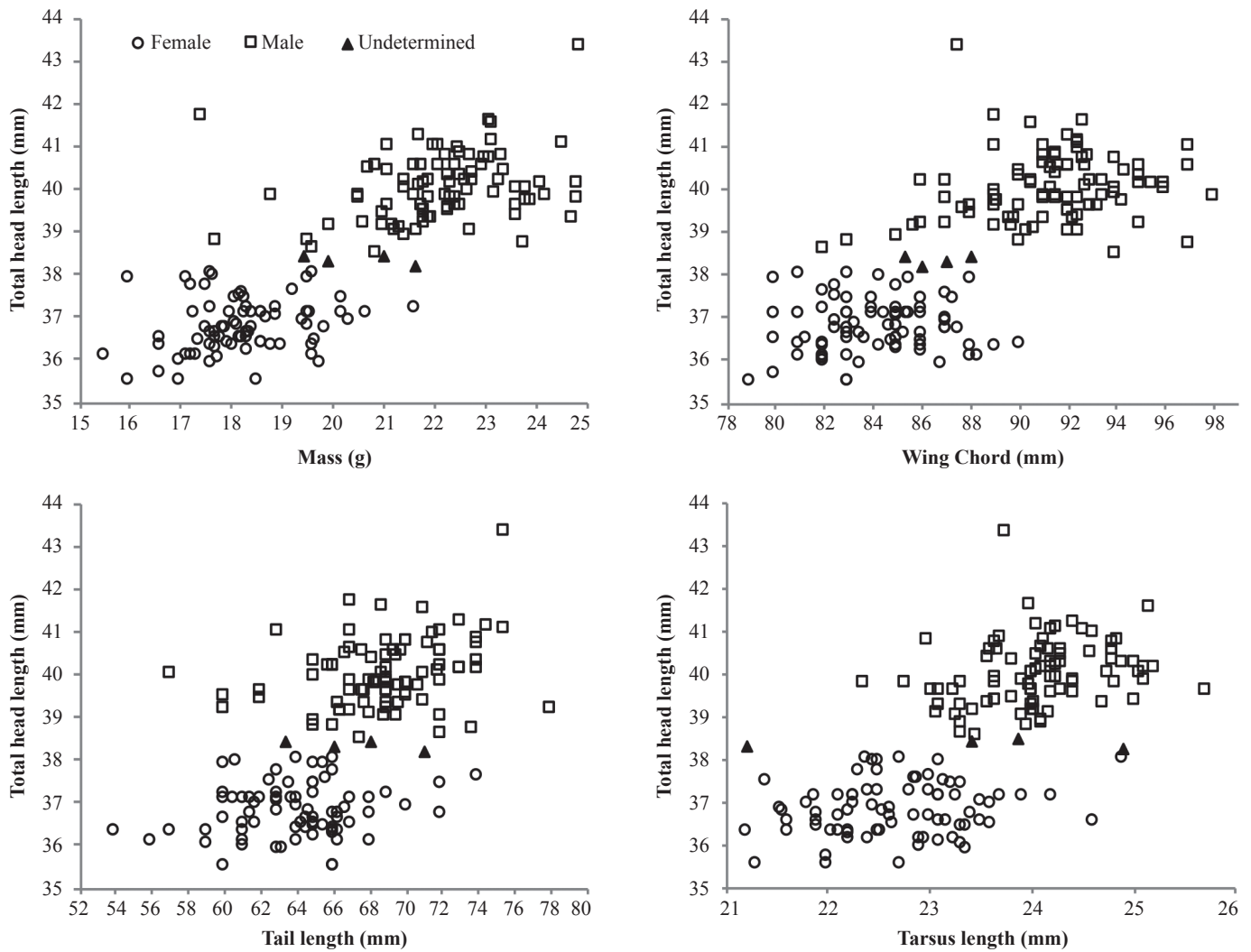

Figure 1. *Two-way plots of total head length against other morphometric traits among adult Eastern Yellow Robins sexed on the basis of nesting behaviours (n = 21 males, 18 females).*

Table 1

Comparison of morphometric traits (mean ± 1 s.d.) between adult male and female Eastern Yellow Robins using Welch's unequal variances *t*-test, and repeatability (*r*) for each trait.

Morphometric trait	Male (*n* = 21)	Female (*n* = 18)	*t*	*P*	*r* (*n* = 49)
Total head length (mm)	40.1 ± 1.0	36.7 ± 0.4	14.1	<0.001	0.89
Wing chord (mm)	91.4 ± 2.0	84.4 ± 2.2	10.56	<0.001	0.84
Mass (g)	22.2 ± 1.4	18.4 ± 1.0	9.85	<0.001	0.78
Tarsus length (mm)	24.3 ± 0.7	22.8 ± 0.6	6.93	<0.001	0.66
Tail length (mm)	68.7 ± 2.9	65.2 ± 2.9	3.86	<0.001	0.31

Figure 2. *Two-way plots of total head length against other morphometric traits among adult Eastern Yellow Robins sexed on the basis of total head length (see text for categories used).*

all traits except total head length, for which there was no overlap (Fig 1). The range in total head length was 35.9–37.6 mm among females and 38.9–43.3 mm among males, numerically slightly larger than the ranges of 36.1-37.3 mm for females and 37.6-41.4 mm for males for *E. a. chrysorrhoa* recorded in Australian Bird Study Association (2016).

The repeatability of trait measurements, calculated from data for 49 adult individuals with three or more repeat measurements, was greatest for total head length (*r* = 0.89, meaning that 89% of the variation in total head length is due to differences among individuals and 11% is due to variation within individuals), and least for tail length (*r* = 0.31, Table 1). Repeatability of the measurements of different traits may be related to: (1) temporal variation in the trait e.g. diurnal and seasonal variation in body mass (Cresswell 1998; Rozman *et al*. 2003) or moult and wear affecting feather length; (2) differences between measuring instruments when multiple instruments are used; (3) human

error in measuring, recording or transcribing, or (4) differences in the way that the trait is measured by different banders. The large difference in the repeatability of wing chord ($r = 0.84$) and tail length ($r = 0.31$), two measures influenced by feather length, suggests that the banders measured wing chord more consistently than tail length, most likely due to greater human error in measuring tail length and/or differences in the way that the trait is measured by different banders. Similarly, of the two structural traits that are not expected to change once a bird reaches adulthood, total head length was measured more consistently ($r = 0.89$) than tarsus length ($r = 0.66$).

Our data suggest that total head length is the most reliable measure to use to sex adult Eastern Yellow Robins, which accords with the observations of Rogers *et al.* (1990). To test this, we developed the rule that any individual with an average total head length less than or equal to 38.0 mm is assigned female gender, whilst any individual with an average total head length greater than or equal to 38.5 mm is assigned male gender, and any individual with an average total head length greater than 38.0 mm but less than 38.5 mm is assigned undetermined gender. Applying these rules to 170 adult individuals captured at the study site since 2007 resulted in 88 individuals being assigned male gender, 77 individuals being assigned female gender and four individuals (2.4%) being assigned undetermined gender (Fig 2). The strongly bimodal distribution of total head length in the adult population (Fig 3) supports the use of total head length to sex adults. Rogers *et al.* (1990) developed a similar rule to sex adult *E. a. australis*, categorising birds as female if total head length was less than 36.4 mm and as male if it was greater than 37.7 mm; this rule sexed birds correctly 80.6% of the time and incorrectly 0.4% of the time, with 19.0% of birds having an intermediate total head length and being assigned undetermined gender. The limited overlap between males and females in the relationship between total head length and tarsus length (Fig 2), two structural measures of body size, suggests that a two-way plot of these two traits provides the best approach to sexing adult Eastern Yellow Robins.

To investigate the potential use of morphometric traits to sex juvenile birds, we calculated the absolute change in the measurement of traits of nine birds that had been measured first
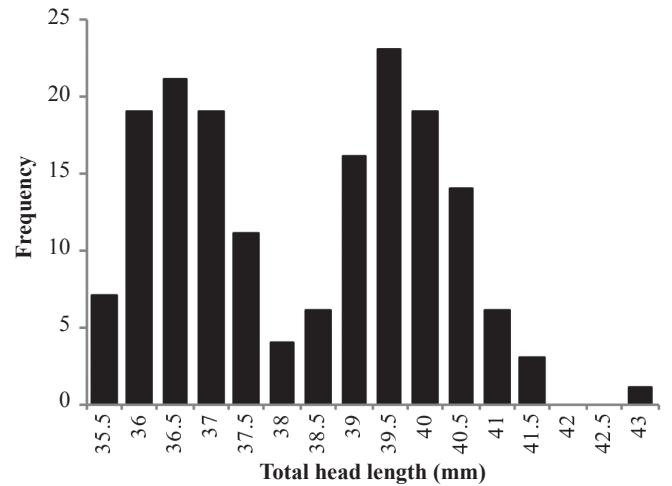

Figure 3. *Frequency distribution of total head length (grouped in 0.5 mm intervals) among adult Eastern Yellow Robins.*

as juveniles in brown plumage (i.e. within the first month of fledging) and again at older ages between two and 33 months thereafter. There was no discernible change in body mass (-0.2 ± 0.7 g) and tarsus length (-0.1 ± 0.5 mm) between the first month after fledging and older ages, whereas total head length, wing length and tail length increased by an average of 3.4 ± 4.3 mm, 3.3 ± 3.7 mm and 7.8 ± 12.8 mm, respectively, as the bill and feathers grew to full length. This suggests that only a relationship between body mass and tarsus length could be used to sex juvenile birds in brown plumage. The two-way plot of these two traits in the adult population sexed on the basis of total head length (Fig 4) suggests that whilst there is some overlap between males and females, a large proportion of juvenile birds could be sexed on the relationship between these two traits. This is supported by the two-way plot of body mass and tarsus length of juvenile birds in the first month post-fledging (Fig 4), with the juveniles sexed on the basis of total head length once they became adults. In our banding data set, tarsus length had relatively low repeatability despite evidence that this structural trait does not change with age post-fledging. To improve repeatability (i.e. reduce the variance in measurements of the same individual) and the utility of tarsus length to sex birds,
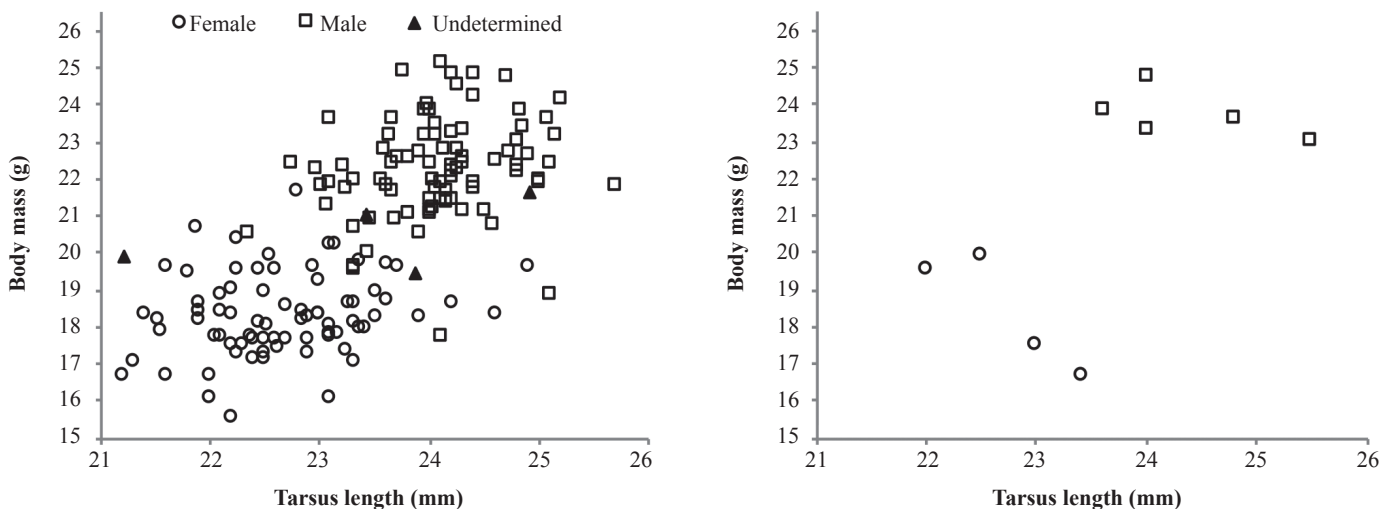

Figure 4. *Two-way plot of tarsus length and body mass among adult Eastern Yellow Robins sexed on the basis of total head length (left) and among juveniles in the first month after fledging sexed on the basis of total head length as adults (right).*

measurement of tarsus length should either be undertaken by the same bander on all occasions or should be confirmed through comparison of the measurements obtained by two different banders on each capture occasion.

The body size of bird species varies geographically, with individuals of the same species typically being larger at higher latitudes and in cooler areas (James 1970; Blackburn *et al*. 1999; Ashton 2002). The Eastern Yellow Robin is widely distributed across eastern Australia and therefore its body size is expected to vary geographically in response to climatic variation across its range. The measurements of average total head length, wing chord and tail length of the nominate subspecies *E. a. australis* reported in Rogers *et al*. (1990) are all smaller than the corresponding averages for *E. a. chrysorrhoa* reported here, confirming that the two subspecies differ in size. Sexual size dimorphism results from differential selection among males and females and is generally explained as an evolutionary response to either sexual selection or, less commonly, intersexual competition (Shine 1989; Dale *et al*. 2007). We would not expect the selective pressures on sexual size dimorphism in the Eastern Yellow Robin to vary geographically. Therefore, whilst the absolute measures of morphometric traits of male and female Eastern Yellow Robins may vary across the range of the species, it should be possible to reliably sex both adult and juvenile birds anywhere in the species' range on the basis of sexual size dimorphism.

ACKNOWLEDGEMENTS

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