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Are blood haemoglobin concentrations a reliable indicator of parasitism and individual condition in New Holland honeyeaters (Phylidonyris novaehollandiae)?

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Title: Are blood haemoglobin concentrations a reliable indicator of parasitism and individual condition in New Holland honeyeaters (*Phylidonyris novaehollandiae*)?

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Abstract

Across avian species total blood haemoglobin concentration (BHC) is the most important determinant of oxygen-carrying capacity, and most accurately reflects the potential for the bird to satisfy its oxygen requirements. This creates a close association between high BHC and high aerobic capacity, and low BHC and states of regenerative or non-regenerative anaemia. As such total BHC has been suggested to be a reliable indicator of avian health and condition. We mist netted 160 adult and 26 juvenile New Holland honeyeaters (*Phylidonyris novaehollandiae*) from ten sites across South Australia to assess the relationship between

BHC and individual health and condition traits in this species. From each bird we collected samples for blood haemoglobin estimation, inspected for the presence of external parasites (ticks), and measured basic morphometric parameters (mass, tarsus length and length of bilateral tail feathers). A relationship could not be demonstrated between BHC and tick intensity, body condition or tail feather asymmetry in adult or juvenile birds. Whilst the measurement of BHC may provide a reliable insight into individual health and condition in some avian species our results highlight the need to validate this relationship within species and populations prior to its use in avian health and condition assessments.

Keywords: avian, *Ixodes*, symmetry, haemoglobin, body condition, parasites, tick, New Holland honeyeater

Word count: Including references, figure captions, abstract, titles, etc – 4824

Introduction

The One Health concept takes a holistic approach to health, recognising the critical links between human, animal and environmental health. The primary aim of One Health is to attain optimal health for humans, animals and the environment through a collaborative effort from multiple disciplines. In recent years we have seen an increase in the number of zoonotic disease outbreaks (Harper & Armelagos, 2010; Jones et al., 2013; Morse, 1995), with several topical examples, avian influenza infection in China (Li et al., 2014) and *Salmonella* infection in the U.S. (Centre for Disease Control and Prevention, U.S Department of Health & Human

Services), having strong avian links. This has placed an increasing emphasis on the need for easy to obtain, reliable and widely applicable measurements of avian health and condition.

One physiological measure that can indicate avian health and condition is blood haemoglobin concentrations (BHC) (Minias, 2015). Similar to that in other vertebrates the primary function of blood haemoglobin in avian species is to transport oxygen from the respiratory organs to bodily tissues to permit aerobic respiration and synthesise energy for functions of the bird via metabolic processes. Across avian species the amount of oxygen supplied to the tissues per unit time (measured by the amount of haemoglobin per total surface area of erythrocytes) is consistent (Kostelecka-Myrcha, 1997). As a consequence, the ability of a bird to satisfy its oxygen requirements is most accurately reflected by the oxygen carrying capacity of its blood, total BHC. This creates a close association between high BHC and high aerobic capacity, and low BHC and states of regenerative or non-regenerative anaemia in avian species (Minias, 2015).

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Regenerative anaemia refers to the production and release of new erythrocytes from the bone marrow and can be brought on by haemorrhage and haemolysis (Tyler & Cowell, 1996). Within avian species this likely explains the observed relationship between BHC and parasitism (Dudaniec, Kleindorfer, & Fessl, 2006; Norte et al., 2013). In contrast, non-regenerative anaemia refers to a situation when the increased response of bone marrow is inadequate when compared to the increased need for new erythrocytes. This can occur due to disease, nutritional stress or starvation (Tyler & Cowell, 1996), and likely accounts for the observed relationships between BHC and many morphological characteristics, such as body condition indices (Crossin, Phillips, Wynne-Edwards, & Williams, 2013; Lobato, Braga, Belo, & Antonini, 2011; Minias, Kaczmarek, Włodarczyk, & Janiszewski, 2013), asymmetrical moult (Minias, Kaczmarek, Wlodarczyk, & Janiszewski, 2013), and wing morphology (Minias, Włodarczyk, Piasecka, Kaczmarek, & Janiszewski, 2014), physiological parameters, including blood glucose concentrations (Minias, 2014) and plasma concentrations of proteins (Minias et al., 2014), and fitness related traits, for example timing and size of eggs laid (Minias, 2014) and brood size (Minias, Włodarczyk, & Janiszewski, 2015).

The New Holland honeyeater (*Phylidonyris novaehollandiae*) is a small passerine, endemic to southern and eastern Australia. This species has a well-documented life history and is one of the few model species for host-parasite interactions within Australia. This species favours habitats with a dense shrub layer. Adult birds are primarily nectivorous and supplement their diet on manna, insects, lerp and honeydew (Paton, 1982a). Movement patterns vary between populations and regions but are suggested to be largely dependent upon resource availability (Higgins et al., 2001). Breeding behaviour has been documented year round but predominantly occurs between July and October (Higgins et al., 2001). Adults moult annually, commencing following the spring breeding season and taking in excess of 100 days to replace all flight feathers (Paton, 1982b). Within parts of their range New Holland honeyeaters are commonly parasitised by the first two life stages (larvae and nymph) of the three host tick, *Ixodes hirsti* (Oorebeek, Sharrad, & Kleindorfer, 2009). Ticks are found around the head of infected birds as they are removed from other parts of the body through preening (Oorebeek and Kleindorfer 2009). Tick intensities range greatly and are similar within both the adult and juvenile cohorts; although tick prevalence is significantly greater in juvenile birds (Kleindorfer et al. 2006). Despite the New Holland honeyeater having been the subject of extensive studies the relationships between BHC and individual health or condition remain unstudied.

This study assessed the relationships between BHC and individual health and condition traits within juvenile and adult New Holland honeyeaters. Birds were mist netted, blood collected for haemoglobin estimation, inspected for tick infestations, and basic morphometrics measured (mass, tarsus length and length of bilateral tail feathers) to test the following hypotheses; 1) BHC reflect tick intensity; 2) BHC reflect body condition; and 3) BHC reflect tail feather asymmetry.

Methods

Study sites

Birds were sampled from their natural populations across ten study sites in South Australia. Six sites on the Fleurieu Peninsula; (1) Sandy Creek Conservation Park (34°36'S, 138°51'E); (2) Scott Conservation Park (35°24'S, 138°44'E); (3) Aldinga Scrub Conservation Park (35°17'S, 138°27'E); (4) Scott Creek Conservation Park (35°5'S, 138°40E); (5) Newland Head Conservation Park (35°37'S, 138°29'E); (6) Cox Scrub Conservation Park (35°19'S, 138°44'E); three sites on Kangaroo Island; (7) Flinders Chase National Park (35°56'S, 136°44'E); (8) Parndana Conservation Park (35°45'S, 137°19'E); (9) Pelican Lagoon Conservation Park (35°45'S, 137°37'E); and one site on Yorke Peninsula (10) Innes Conservation Park (35°13'S, 136°53'E). Study sites chosen included all of coastal mallee-heath, shrubland and woodland habitats (Schlotfeldt & Kleindorfer, 2006; Waudby & Petit, 2007).

Physiological and morphological measurements

We mist netted birds between the months of September and October 2005-2008. Blood samples (0.01ml) were collected by jugular venipuncture using a 0.5 ml syringe (29G 1/2″, 0.33 mm × 12.7 mm) (Campbell, 1995). Each sample was immediately placed into a microcuvette and a haemoglobin measurement (g/dL) obtained using a portable haemoglobinometre (HemoCue HB 201, HemoCue AB) (Dudaniec et al., 2006). Length measurements using a standard 30 cm ruler were taken to the nearest 1 mm for mature lateral tail feathers from the follicle to the feather tip, and tail feather asymmetry assessed as the difference in length between the left and right feathers. All birds were weighed to the nearest 0.1 g and their tarsus measured to the nearest 0.1 mm, using digital scales and callipers respectively. We used these measures in a linear regression analyses to determine the standardized residuals to represent each individual's average body condition, as done in other studies (Husak, 2006). The sex of birds was genetically determined (Myers, 2011), and their age class based on the presence of adult or juvenile characteristics (including colour of gape and irides) (Disney, 1966).

Parasite sampling

All birds caught were carefully examined for the presence of ticks. Extensive visual searches were conducted around the head of birds in particular as ticks have only been observed on this region of the body at the study sites (Kleindorfer, Lambert, & Paton, 2006; Oorebeek & Kleindorfer, 2008a, 2008b, 2009). Ticks could be easily seen by deflecting the feathers in this region. All ticks found were removed with forceps and preserved in 90% ethanol. Ticks had previously been identified as *Ixodes hirsti* using molecular techniques (Chapman, Marando, Oorebeek, & Kleindorfer, 2009).

Statistical analysis

We assigned birds to separate categories for tick intensity (two categories in total for juveniles and four for adults) and tail feather asymmetry (four categories in total for juveniles and seven for adults) according to their degree of infestation or asymmetry (Cat. 1 = 1 tick/1 mm tail feather asymmetry, Cat. 2 = 2 ticks/2 mm tail feather asymmetry, etc). Similarly, birds were assigned to one of two body condition categories, 'above average' or 'below average' if they had a body condition score of ≥ 0 or <0 respectively (these categories were chosen based on the way in which we derived body condition, standardised residuals of a linear regression analysis of mass on tarsus length). For both juvenile and adult birds we tested if the effect of our three variables of interest (tick intensity, body condition and tail feather asymmetry) on BHC varied across years and conservation parks using a two-way ANOVA. However, significant interaction effects must be interpreted with caution as for adult birds our data violated the assumption of homogeneity of variances under the two-way ANOVA design, and for both adult and juvenile birds sampling was not conducted at all parks in all years. Furthermore, cell sample sizes for juvenile birds were small, ranging from 0-10 with a mean of 2.6 (SD = 2.49). Main effects were investigated using a one-way ANOVA and Tukey pairwise comparisons post hoc testing as under the one-way ANOVA design data satisfied the assumptions of normal distribution and homogeneity of variances. All statistical tests were conducted in IBM SPSS Statistics 22.

Results

Eight of ten sites sampled for New Holland honeyeaters supported resident tick populations. From these 10 sites 186 birds were examined, 160 of which were adults and 26 juveniles.

Juveniles

Within the juvenile cohort BHC ranged from 138 to 225 g/dL, with a mean of 184 (SD = 22) g/dL. Twelve birds had tick infestations of 1 to 2 ticks, giving an overall prevalence of 46%. Tick intensity varied; 58 % had 1 tick, and 42 % had 2 ticks. For tick infested juvenile birds the median tick intensity was 1 (95 % CI = 1, 2). Fifteen birds showed some degree of tail feather asymmetry, ranging from 1 to 4 mm, with the median difference between bilateral tail feathers being 1 (95 % CI = 0, 2) mm. Sixteen birds had a body condition below average, with the mean body condition score for juvenile birds being 1.31 (SD = 0.94).

We found no significant effect of tick intensity on BHC across conservation parks (F (1, 19) = 1.60, N = 26, P = 0.22) and no significant difference in the mean BHC for juveniles with varying levels of tick intensity (F (2, 23) = 0.54, N = 26, P = 0.59) (Figure 1). The effect of tick intensity on BHC across years was unable to be calculated due to insufficient mean square values. Body condition showed a significant effect on BHC across years (F (1, 22) = 7.26, N = 26, P = 0.13) and across conservation parks (F (3, 18) = 3.44, N = 26, P = 0.04), but there was no significant difference in the mean BHC for juveniles in above and below average body condition (F (1, 24) = 2.50, N = 26, P = 0.95) (Figure 2). Tail feather asymmetry showed no significant effect on BHC across years (F (2, 18) = 0.74, N = 26, P = 0.49) or conservation parks (F (5,13) = 0.72, N = 26, P = 0.62), and there was no significant difference in the mean BHC for juveniles with varying levels of tail feather asymmetry (F (4, 21) = 0.43, N = 26, P = 0.79) (Figure 3).

Figure 1____

Figure 2____

Adults

Within the adult cohort BHC ranged from 123 to 235 g/dL, with a mean of 188 (SD = 20) g/dL. Seventeen birds had tick infestations of 1 to 4 ticks, giving an overall prevalence of 11 %. Tick intensity varied; 47 % had 1 tick, 35 % had 2 ticks, 6% had 3 ticks, and 12 % had 4 ticks. For tick infested adult birds the median tick intensity was 2 (95 % CI = 1, 2). One hundred and twenty five birds showed some degree of tail feather asymmetry, ranging from 1 to 7 mm, with the median difference between bilateral tail feathers being 1 (95 % CI = 1, 1) mm. Sixteen birds had a body condition below average, with the mean body condition score for adult birds being 1.67 (SD = 1.32).

We found no significant effect of tick intensity on BHC across years ($F (3, 149) = 0.51$, $N = 160$, $P = 0.68$) or conservation parks ($F (6, 140) = 0.85$, $N = 160$, $P = 0.53$), and no significant difference in the mean BHC for adult birds with varying levels of tick intensity ($F(4, 155) = 0.39$, $P = 0.82$) (Figure 1). Body condition showed no significant effect on BHC across years ($F (3, 152) = 0.49$, $N = 160$, $P = 0.69$) or conservation parks ($F (8, 141) = 0.62$, $N = 160$, $P = 0.76$), and there was no significant difference in the mean BHC for adults in above and below average body condition ($F (1, 158) = 1.39$, $N = 160$, $P = 0.24$) (Figure 2). Tail feather asymmetry showed no significant effect on BHC across years ($F (11, 138) = 1.05$, $N = 160$, $P = 0.41$) but did show a significant effect on BHC across conservation parks ($F (20, 123) = 2.00$, $N = 160$, $P = 0.01$), and a significant difference in the mean BHC for adult birds with varying levels of tail feather asymmetry ($F (7, 152) = 2.17$, $N = 160$, $P = 0.04$) (Figure 3). Tukey pairwise comparisions tests showed no significant difference in the mean BHC for adult birds across all combinations of tail feather asymmetry (Table 1).

Figure 3____

Table 1____

Discussion

We found no significant difference in mean BHC for adult or juvenile birds across all levels of tick intensity. Tick infestations in birds have previously been suggested to have both detrimental effects (Norte et al., 2013) and no effect (Williams & Hair, 1976) on BHC. Contrasting results are unlikely to be explained by differences in the parasitising tick life stage, as the majority of tick infestations in birds are by immature life stages (larvae and nymphs). Difference in tick intensity between studies is the most probable cause of contrasting relationships.

We recorded a maximum tick intensity for juvenile and adult birds of 2 and 4 ticks respectively, substantially lower than those at which significant relationships between BHC and tick infestation have been found (Norte et al., 2013). Other studies reporting significant relationships between BHC and other parasites have likewise documented higher average parasite loads (Dudaniec et al., 2006; Krams et al., 2013; O'Brien, Morrison, & Johnson, 2001). Low levels of parasite infestation may be insufficient to cause a noticeable reduction in BHC in birds due to their ability to rapidly produce red blood cells (Campbell, 1995; Sturkie, 2012). Whilst immature red blood cells are only capable of synthesising a fraction of the amount of haemoglobin when compared to mature cells (O'Brien et al., 2001), this may be enough to mask the effects of low level tick infestation on BHC.

Blood haemoglobin concentrations did not reflect the body condition of adult or juvenile birds. This was in contrast to that expected based on previous studies on Great Tits (Parus major) in central Sweden (Dufva, 1996), nestling Welcome Swallows in south-eastern

Australia (Lill, Rajchl, Yachou-Wos, & Johnstone, 2013), and Bar-tailed Godwits in the Netherlands (Dufva, 1996; Lill et al., 2013; Piersma, Everaarts, & Jukema, 1996). A relationship between BHC and body condition is commonly seen in migratory birds when fuel stores are accumulated prior to departure (Minias, Kaczmarek, Włodarczyk, et al., 2013). This increases the oxygen-carrying capacity of their blood to meet the high metabolic requirements of long distance migrations. A similar relationship may also arise during periods of starvation when the production of red blood cells is suppressed (McCue, 2010). New Holland honeyeaters most commonly remain in areas with a constant supply of nectar and are said to be resident or sedentary, with some populations documented to be nomadic (Higgins et al., 2001). Not having the need to accumulate fuel stores or increase BHC for extended periods of flight, and having a constant supply of nectar supplemented by other food resources, may explain the lack of relationship between BHC and body condition in this species. Avian species in which BHC may better reflect indices of body condition are likely those that suffer greater levels of dietary or metabolic stress from variable and unpredictable resource availability, for example arid zone and migratory birds.

Furthermore, individuals with a below average body condition relative to the population mean are not necessarily in poor condition. If the entire population is in exceptional body condition then birds below the population average may still be in reasonable body condition, and still well above the stage at which the production of red blood cells is suppressed due to nutrient deficiency.

No significant difference was found in the mean BHC at all levels of tail feather asymmetry for juvenile birds. In contrast, the mean BHC across all levels of tail feather asymmetry for adult birds was not equal. Further investigation using pairwise comparisons found no significant difference in mean BHC for all combinations of tail feather asymmetry, indicating that BHC and tail feather asymmetry were not significantly related within the adult cohort. Significant relationships between BHC and plumage asymmetry have previously been recorded during moult (Minias, 2015). The moulting period involves extensive vascularisation of growing quills. This is accompanied by a substantial increase in water consumption during this period, which likely increases blood plasma volume and consequently decreases BHC as total erythrocyte count remains constant (Chilgren & deGraw, 1977). New Holland honeyeaters moult annually, commencing following the spring breeding season (end of October/start of November) and taking approximately 130 days (Paton, 1982b). Our sampling period (September October) coincided with the breeding season to capture maximum tick densities. This sampling period is prior to the commencement of moult for the majority of birds, and likely explains the lack of relationship between BHC and tail feather asymmetry found in this study.

Blood haemoglobin concentrations have previously been identified to be related to a large number of ecological, morphological, physiological and fitness related traits, and as a result have been suggested to be reliable indicators of avian health and condition. However, this study has found no relationship between BHC and tick intensity, body condition or tail feather asymmetry in adult or juvenile New Holland honeyeaters. We recognise that confounding variables, such as the year and location of sampling, may obscure variation in relation to the variables of interest (tick intensity, body condition and tail feather asymmetry) although consider this unlikely based on the interaction terms calculated. Whilst the measurement of BHC may provide a reliable insight into individual health and condition in some avian species our results highlight the need to validate this relationship within species and populations prior to its use in avian health and condition assessments. Further investigation into other morphological, physiological and fitness related traits that display a stronger association with BHC may also prove useful in avian health and condition assessments, such characteristics may include faecal parasite loads, body mass or fat loads, or wing feather asymmetry. Finally, one must ask the question, if strong relationships were to exist between BHC and morphological, physiological or fitness related traits, but there is nothing we can do to reduce the associated risks, are BHC worth testing? In many cases it may not be possible to manage or mitigate population health or condition risks, whilst in others the eradication of parasites or increasing of food resources, as seen for New Zealand forest birds, to ensure population persistence is possible (Armstrong et al., 2002). This illustrates that solutions to possible avian population health and condition risks must be considered prior to the commencement of population health and condition assessments to safeguard scarce and valuable conservation funds.

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Table 1: Tukey pairwise comparisons for the blood haemoglobin concentrations (g/dL) of adult New Holland honeyeaters (*Phylidonyris novaehollandiae*) across all levels of tail feather asymmetry (difference in length (mm) between bilateral tail feathers).

Diff. = difference, SE = standard error, CI = confidence interval

(I) Tail Asymmetry	**(J) Tail Asymmetry**	**Mean Diff. (I-J)**	**SE**	**P**	**Lower 95 % CI**	**Upper 95 % CI**
0 / Symmetrical	1 / 1 mm	6.94	4.10	0.690	-5.64	19.53
	2 / 2 mm	15.51	5.06	0.051	-0.03	31.06
	3 / 3mm	1.74	7.30	1.000	-20.70	24.18
	4 / 4 mm	21.03	8.09	0.164	-3.83	45.89
	5 / 5 mm	-0.97	8.09	1.000	-25.83	23.89
	6 / 6 mm	7.89	8.09	0.977	-16.97	32.74
	7 / 7 mm	12.49	10.31	0.927	-19.19	44.18

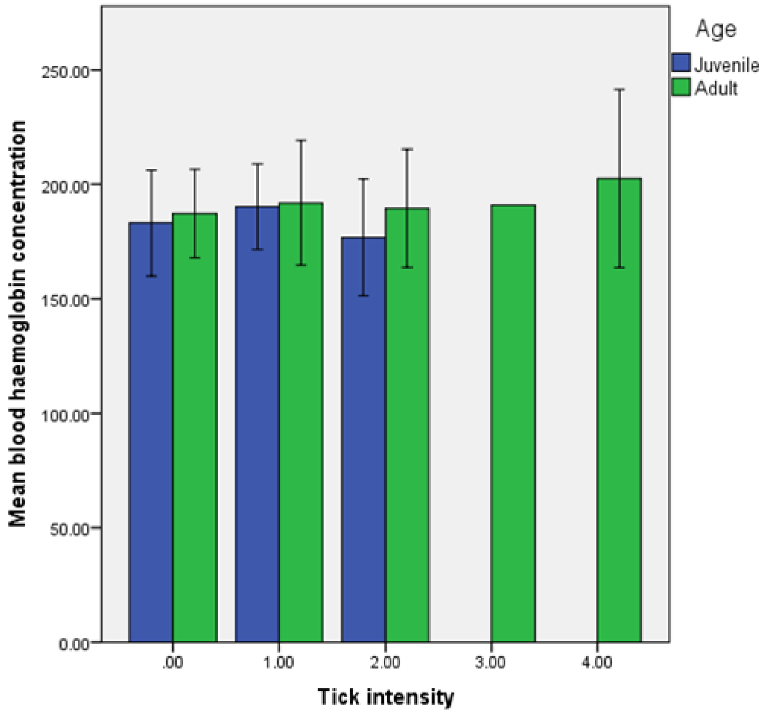

Figure1: Mean blood haemoglobin concentration (g/dL) for each level of tick intensity (number of ticks on bird) for juvenile and adult New Holland honeyeaters (*Phylidonyris novaehollandiae*). Error bars represent +/- 1 standard deviation.

Birds were sampled from their natural populations across ten study sites in South Australia.

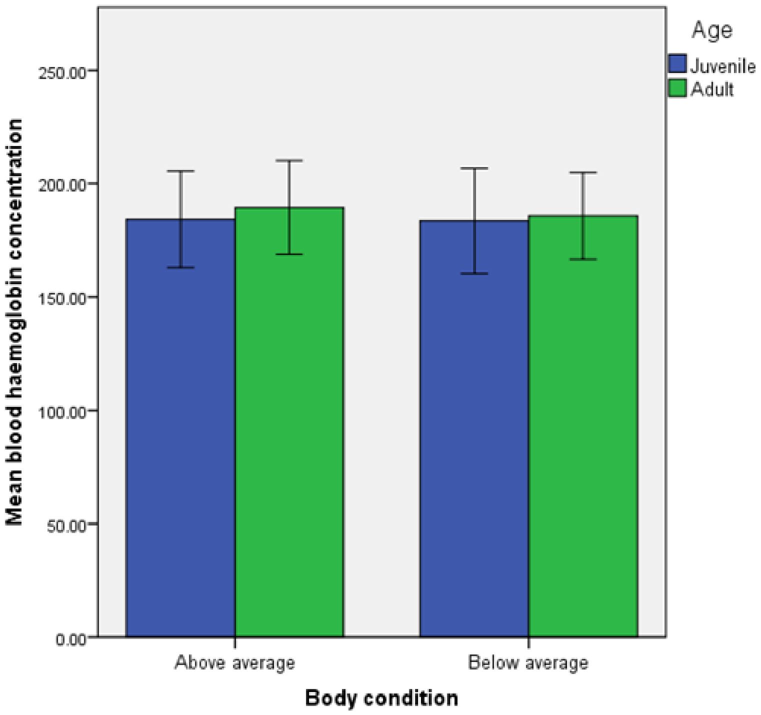

Figure 2: Relationship between mean blood haemoglobin concentration (g/dL) and body condition (a score of relative body mass in relation to body size) for juvenile and adult New Holland honeyeaters (*Phylidonyris novaehollandiae*). Error bars represent +/- 1 standard deviation.

Birds were sampled from their natural populations across ten study sites in South Australia.

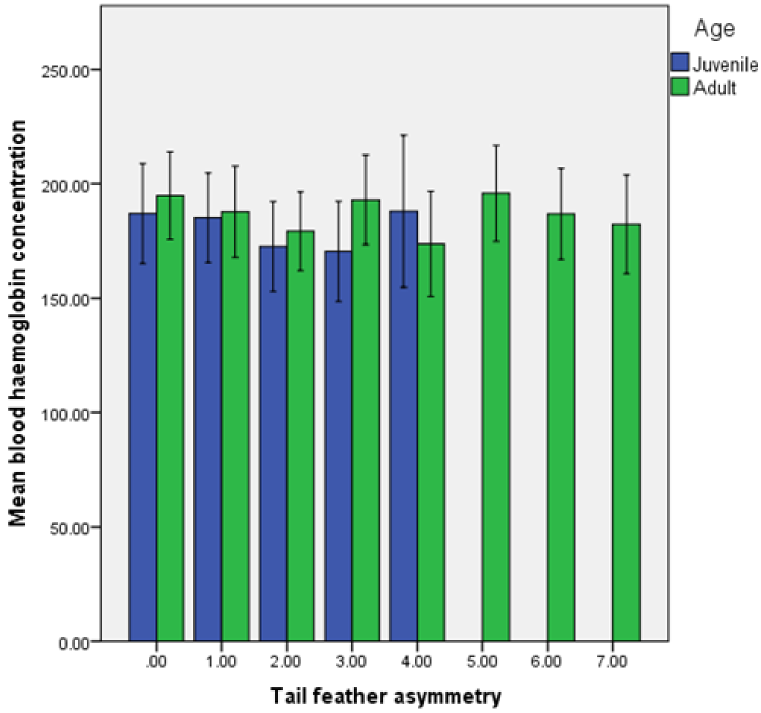

Figure 3: Mean blood haemoglobin concentration (g/dL) for each level of tail feather asymmetry (difference in length (mm) between bilateral tail feathers) for juvenile and adult New Holland honeyeaters (*Phylidonyris novaehollandiae*). Error bars represent +/- 1 standard deviation.

Birds were sampled from their natural populations across ten study sites in South Australia.