Nestling growth and body condition of critically endangered orange-bellied parrots *Neophema chrysogaster*.

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Running Head: Nestling growth of orange-bellied parrots

Abstract

Intervening when bird nestlings are performing poorly relative to the population mean may be a management priority if individuals are of high conservation value. Assessing body condition may enable identification of potential problems before they cause mortality. We aimed to provide a tool for conservation managers to identify underperforming nestlings in a severely threatened bird population. We develop models of nestling growth and empirically quantify nestling body condition of critically endangered Orange-bellied Parrots *Neophema chrysogaster,* which have declined to only a single population in southwestern Tasmania, Australia. Using census data on growth of nestlings born over four years into the contemporary wild population, we test whether a body condition index is influenced by sex, hatch order, year of birth, brood size, whether one or both parents were captive bred, and fledging date. The best model of body condition in Orange-bellied Parrot nestlings included additive effects of year of birth and hatch order. Nestling body condition was lowest in 2013, where first hatched nestlings were 2.5 g lighter than those born in 2016, and > 4.2 g lighter than in 2017/18. Nestlings that hatched either first or in the middle of the brood were respectively 4.8 g and 3.8 g heavier than last-hatched birds. Our body condition index provides a repeatable, rapid and cheap way to assess body condition of wild orange-bellied parrot nestlings. This represents a step toward accurate evaluation of management actions aimed at improving reproductive outcomes for this species, and provides a framework for developing hypotheses to test using an empirical and measurable index of individual quality.

Key Words: nestling growth; body condition; Orange-bellied Parrot *Neophema chrysogaster*, disease

Introduction

In small populations, understanding and correcting the factors that contribute to lifetime fitness is crucial for conservation management. This may be particularly important for threatened species, where the recruitment of relatively few individuals can affect the viability of the whole small population (Elliott *et al.* 2001; Sutherland 2002; Weimerskirch *et al.* 1997). Nestling birds are highly sensitive to the conditions in which they are raised. Nestlings in good habitats tend to have better body condition and fitness than ones in poor habitats (Saino *et al.* 2018; Schmidt *et al.* 2012; Wilkin *et al.* 2009). Further, first hatched nestlings can have higher body condition than later hatched siblings (Keith Bowers *et al.* 2011). Large brood sizes where sibling competition is intense can reduce body condition of individual nestlings (Mitchell *et al.* 2011; Saino *et al.* 2018). Timing of nesting can also predict brood-level body condition, with late nests typically exhibiting poorer condition than early ones (Naef-Daenzer *et al.* 2001). When local environmental conditions are poor, birds may rear fewer, lower quality offspring (Bowers *et al.* 2017; Renton 2002) and this trait can link environmental degradation with demographic process (Rioux Paquette *et al.* 2014; Saunders 1986). Conditions experienced during early life can have carry over effects on other life history stages (Harrison *et al.* 2011; Saino *et al.* 2018), so identifying when a nestling is underweight may be a high management priority. Developing detailed individual-level approaches for assessing body condition can thus facilitate conservation intervention (Stevenson and Woods Jr 2006), typically with the aim of understanding animal health and demographic processes (Masello and Quillfeldt 2002; Saunders 1986).

Body condition is typically calculated as an index of body mass corrected for body size, which in nestling birds, increases with age (Labocha and Hayes 2012). Such estimates can provide a reasonable index of individual condition if they are interpreted cautiously (Schamber *et al.* 2009; Stevenson and Woods Jr 2006). In this study we develop a nestling body condition index for critically endangered Orange-bellied Parrots *Neophema chrysogaster* that provides a way to assess individual condition corrected for age. This species may be the most endangered parrot in the world, and in 2016 only two wild-born females bred in the last wild population (Stojanovic *et al.* 2018a). Orange-bellied Parrots are extinct across most of their historical breeding range and persist only at one breeding location in south western Tasmania, Australia (Stojanovic *et al.* 2018a). Between 2010 and 2019 the population also exhibited a male-biased adult sex-ratio and releases of captive born birds (which began in 2013) have been female-biased to address this issue (Troy and Hehn 2019). Given the species chronic population decline and tiny contemporary population size, every wild parrot is of high conservation value, so maximising individual survival is crucial. To date, evaluation of nestling condition has relied primarily on qualitative assessment of body condition, meaning that interventions (e.g. fostering, veterinary support) are likely to be delivered after nestlings exhibit clear visual signals that they are unwell (e.g. lethargy, emaciation). A quantitative body condition index may enable conservation managers to identify problems earlier and may lower mortality rates if problems can be corrected before they escalate. We use census data from the last wild population of Orange-bellied Parrots over four years to evaluate the impact of environmental factors on nestling body condition. Our aim is to develop an empirical means of evaluating the impact of future management actions targeted at improving conditions in the breeding grounds for Orange-bellied Parrots.

Methods

Study species, site and management

The last known breeding site of the Orange-bellied Parrot is on the Melaleuca plains, south-western Tasmania, Australia (Lat: 43°25'16.54", Long: 146° 9'44.14"). The weather was similar over the four breeding seasons (Nov – Mar) when we collected data (2013, 2016 – 2018). Over the study monthly mean rainfall ranged from 55 – 82 mL and monthly mean temperatures ranged from 13.6 – 18.7° C (data sourced from the Bureau of Meteorology website for weather station 094041). The species is a natal site philopatric migrant, breeding during the Austral summer in forest adjacent to buttongrass *Gymnoschoenus sphaerocephalus* dominated moorlands (Higgins 1999). In Tasmania, the herbs and forbs that grow in moorlands after fire are the historically preferred foods. Food has been scarce at Melaleuca due to prolonged lack of fire (Stojanovic *et al.* 2018a), and breeding birds rear their nestlings primarily on supplementary food. This is in the form of a seed mix comprising red millet *Eleusine coracana*, Japanese millet *Echinochloa esculenta*, white millet *Panicum miliaceum*, grey sunflower *Helianthus annuus* and quinoa *Chenopodium quinoa* (Troy and Hehn 2019). Seed is provided *ad libatum* as part of a larger program focussed on delivering conservation action for the species (Department of Environment 2016). We consider that nestlings are unlikely to have experienced variation in food abundance due to *ad libatum* feeding, irrespective of whether supplementary feeding affects reproductive parameters differently to natural foods (Harrison *et al.* 2010). Consequently we do not consider the effects of supplementary feeding in our analysis (due to lack of a control group where supplementary feeding did not occur).

Data collection

We collected data at nest boxes (for details see Stojanovic *et al.* 2019) checked between January and March and represent a near census of all nestling Orange-bellied Parrots born into the contemporary wild population (3 nestlings fledged before being measured). We present data on 106 Orange-bellied Parrot nestlings (45 males, 54 females, 7 unknown, Table 1). Apart from in 2016, nestlings were removed from nests once to record morphometric data (wing chord – to the nearest mm with a wing ruler, mass – to one decimal place in grams using electronic scales), brood size and to collect blood for sexing and disease screening via brachial venepuncture. In 2016 nestlings were measured approximately every third day (from day 4 after hatching until fledging) to collect data for models of nestling growth. Sex was assigned to nestlings using molecular techniques (using blood collected using brachial venepuncture) or based on visual observations after they reached adulthood (Troy and Gales 2016). There were 36 first-hatched nestlings, 39 middle and 31 last hatched nestlings. Mean fledging date was January 30th (range: January 15th – March 24th). Captive-born mothers reared 73 nestlings, and wild-born mothers reared 30 (3 nestlings were reared by a mother of unknown provenance). Nestlings from 2016 that were measured repeatedly for growth models (i.e. all known nestlings including progeny of wild and captive born females) were handled on average 5.7 times (± 1.2 sd) between 1 and 34 days of age. Too few data were available to develop and compare separate growth models for progeny of wild vs. captive born parents.

Table 1. Summary of data on broods and nestlings of wild Orange-bellied Parrots presented in this study. † indicates the total count over the study. ‡ indicates the mean over the study.

	2013	**2016**	**2017**	**2018**	**Over all**
No. broods monitored	4	9	12	10	35†
No. nestlings measured	15	24	33	34	106†
$\bar{x}$ brood size	4.2	3.1	2.9	4	3.55‡

Hatching order was assigned to nestlings using wing chord (longest wing corresponding to the first hatched nestling). During 2016, later hatched nestlings never overtook an older sibling in wing chord, so we assumed this measure was a reliable indicator of hatch order. We also assigned each nestling a brood position, first, middle (2nd to up to xth depending on brood size), or last hatched, because sibling competition may vary depending on hatch order relative to brood size (Magrath *et al.* 2003). Fledge date of each nestling was estimated using the formula for growth of the wing chord (below) to estimate the age of nestlings on the day they were measured. Based on a sample of 28 nestlings whose hatch date was known in 2018 (determined using video monitoring inside nest boxes), the mean ± sd discrepancy between the predicted and true fledge date was 1.0 ± 3.2 days for first hatched, 1.9 ± 3.2 days for middle hatched and 6.4 ± 4.4 days for last hatched nestlings. Provenance (wild versus captive-born) of the mother of each nestling was determined by the uniquely numbered leg rings of all mothers (provenance of the mother may affect offspring quality, Willoughby and Christie 2018). We recorded whether nestlings were reared in a nest box located in one of two clusters, either near (< 500 m) or far (>1.5 km) from supplementary food at Melaleuca. For each nestling, we also recorded the following factors based on their known impacts on nestling body condition both in other species and Orange-bellied Parrots: (i) year of birth, (ii) fledging date, (iii) brood size (as an index of sibling competition), (iv) hatching order, and (v) the occurrence of a disease outbreak (the species is considered highly vulnerable to epidemics, which have intermittently afflicted the wild and captive populations Peters *et al.* 2014).

Analytical approach

Body mass provides a reasonable index of body condition in birds (Labocha and Hayes 2012), but in nestlings is confounded with age. To account for this, we follow Saunders (1986) and develop growth curves for wing chord as a means of estimating age, and body mass as a means of estimating condition. We used data collected from all known-age nestlings born in the 2016 cohort to model growth. We fitted the logistic formula

y = phi1/(1+exp(-(phi2+phi3*x)))

where y = wing chord (mm) or body mass (g), x = age (days), phi1= curve asymptote, phi2 = curve inflection point, and phi3 = curve gradient. Using the formula for wing chord based on known age nestlings, we estimated the age of each nestling on the day it was measured, and calculated a body condition index adapted from Stojanovic et al. (2018b). This was the difference between body mass on the day a nestling was measured, and predicted mean mass of an average 2016 nestling of the same age. This approach provides a relative body condition index of nestling Orange-bellied Parrots. A nestling in average condition relative to the 2016 mean would return a body condition index score of 0, whereas better than average nestlings return positive values, and poorer than average nestlings return negative values.

Using the body condition index of each nestling as the response variable, we fitted a saturated linear mixed model with the following fixed effects (i) sex, (ii) hatch order, (iii) brood position, (iv) fledge date (expressed as Julian date), (v) brood size and (vi) provenance of the mother, (vii) distance to supplementary food (near/far), and (viii) year. A disease outbreak only occurred in 2016. Thus 'disease status' was confounded with year and so was excluded from analysis. We included a unique nesting attempt identifier as a random effect (to account for the inclusion of siblings in the sample). We used backward selection to derive the most parsimonious model based on ΔAIC. All analyses were conducted in R (R Development Core Team 2019). Linear mixed models were implemented using 'lme4 1.1-13' (Bates *et al.* 2015). The research was conducted with approval from the Australian National University Animal Ethics Committee (A2016/48) and the Tasmanian Department of Primary Industries, Parks Water and Environment (TFA17037).

Results

We present the growth models for wing length and body mass of 24 nestlings repeatedly measured in 2016 in Figure 1. For body mass the asymptote of the curve was 51.2 g with a gradient of 0.27, and for wing chord the asymptote was 105 mm and gradient was 0.16 (Figure 1). Data were sparse for nestlings > 30 days old because they began to fledge from that age.

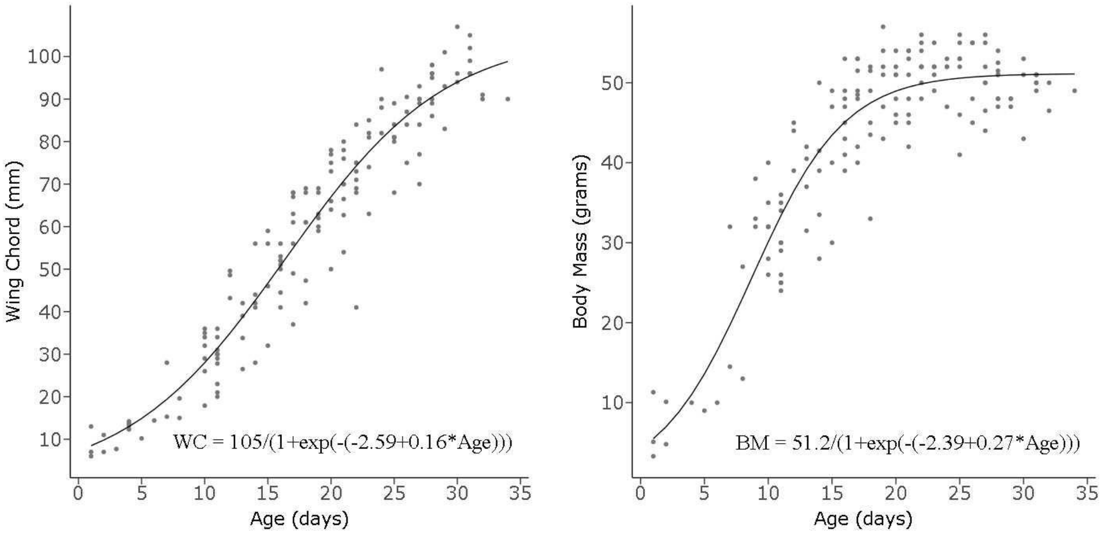

Figure 1. Models of growth of wing chord (WC; left) and body mass (BM; right) of nestling Orange-bellied Parrots from the 2016 cohort. Points show raw data and lines are the models of best fit. We show the formula for each model on the graphs.

For our analysis of body condition of all wild nestlings born over the study period, we present a list of all single term models for comparison against the preferred model in Table 2. The best model of body condition of wild nestling orange-bellied parrots after backward selection contained effects of both year and brood position (we provide model estimates and confidence intervals in Figure 2). Based on this model, body condition of first and middle hatched nestlings were comparable, while last hatched nestlings had the worst condition in each brood. Nestling body condition was lowest in 2013, where first hatched nestlings were 2.5 g lighter than those born in 2016, and > 4.2 g lighter than in 2017/18. Fledge date, provenance of the mother, distance to supplementary food, nestling sex and brood size did not explain the patterns observed in the body condition data (Table 2).

Table 2. Single term models of body condition of nestling orange bellied parrots including the eight covariates measured, ranked by AIC. The preferred model (indicated by *) was produced by backward selection from a saturated model including the eight main fixed effects.

Fixed effect	**df**	**AIC**	**ΔAIC**
brood position + year*	8	593.434	0
brood position	5	598.591	5.157
hatch order	7	603.8958	10.4618
Year	6	613.8527	20.4187
fledge date	4	614.0306	20.5966
mother provenance	5	614.4577	21.0237
distance to supplementary food	4	615.2818	21.8478
Sex	5	616.0664	22.6324
Null	3	616.7801	23.3461
brood size	7	619.5292	26.0952

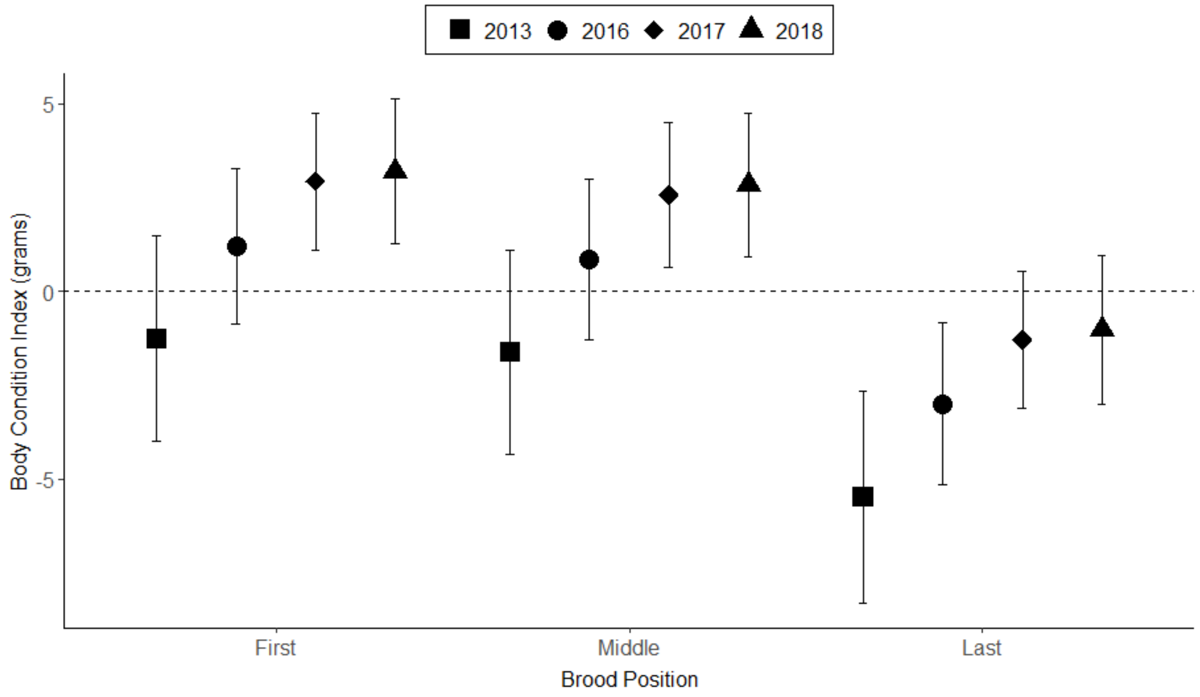

Figure 2. Model estimates and confidence intervals from the preferred model showing the effect of year of birth and brood position (first, middle or last hatched) on our nestling body condition index for nestling Orange-bellied Parrots.

Discussion

The body condition of Orange-bellied Parrot nestlings depended on the year of their birth and the order in which they hatched (with body condition index declining from first to last-hatched). We found no evidence of effects of sibling competition, sex, fledge date or distance to supplementary food on the body condition index. Interestingly, although having captive-bred parents can have important implications in other species (Araki *et al.* 2007; Willoughby and Christie 2018), we found no effect of maternal provenance on nestling body condition in Orange-bellied Parrots, but our sample size for wild mothers was small.

Disease may lower nestling condition (Peters *et al.* 2014; Troy and Kuechler 2018), but how this affected our results is not clear. In 2016 an outbreak of *Pseudomonas aeruginosa* affected some individuals in the population, arising from consumption of contaminated seed (Stojanovic *et al.* 2018a). Unfortunately, it is not clear whether all nestlings in the 2016 population were exposed to *Pseudomonas*, or whether sub lethal exposure resulted in weight loss. Thus, it is not possible to directly measure the effects of this disease outbreak on individual body condition with the data we presented. Future studies could use our body condition index to evaluate impacts on nestlings where detailed veterinary data are available. No disease outbreak was detected in 2013, so we consider that either the small sample size (Table 1) or some other unmeasured factor contributed to the unusually low masses we recorded.

Hatching order is important in determining nestling body condition in birds (Keith Bowers *et al.* 2011; Magrath *et al.* 2003), and our results are evidence of this trait in Orange-bellied Parrots. The difference in modelled estimates of our body condition index between the first/second hatched and last-hatched nestlings over the study period (Figure 2) suggests that late hatched nestlings are substantially disadvantaged. In other species, this disadvantage can carry over and influence survival in later life history stages (Martínez-Padilla *et al.* 2017; Schmidt *et al.* 2012), but the small contemporary population size of orange-bellied parrots hinders testing of this possibility. Our results suggest late-hatched nestlings may receive the greatest benefit from “head-starting” (i.e. holding juveniles in captivity over winter before releasing them at the breeding ground the following spring). This management strategy is currently being trialled to reduce the high migration-associated mortality affecting this cohort (Troy and Kuechler 2018). The ongoing implementation and potential benefits of head starting is being evaluated against the risk of loss of wild behaviours, reduced survival or reproductive outputs, and other potential maladaptive consequences.

Our approach to estimating body condition provides an empirical and objective means of detecting if nestling Orange-bellied Parrots are underperforming relative to average condition for their age. This approach has applications for managing both the wild and captive populations of this species (Department of Environment 2016). Using simple measures of wing chord and mass, our body condition index provides a repeatable, rapid and cheap way to assess condition of Orange-bellied Parrot nestlings. This method has been proposed in other endangered parrots (Saunders 1986). This represents an important step toward accurate evaluation of management actions aimed at improving reproductive outcomes for this species, and provides a framework for hypotheses testing. For example, Stojanovic et al. (2018a) suggest that controlled burning of moorland could increase natural food abundance in the breeding grounds, which may benefit nestlings. Our body condition index may provide a way to test this prediction on recent management efforts to implement ecological burning (unpublished data, D.S.) at the study site. Our study also shows that the age of nestlings whose hatch date is unknown can be estimated using wing chord. However, this approach is less accurate for last hatched nestlings.

Our results are similar to those of other parrots that show variation in nestling quality among years (Masello and Quillfeldt 2004; Renton 2002) and hatch orders (Masello and Quillfeldt 2002; Waltman and Beissinger 1992). Given that no disease was detected in 2013, inter-annual variation in nestling condition was only partly explained by disease outbreaks. Temperature during development affects growth rates of other parrots (Larson *et al.* 2015), and this, like other unmeasured factors (parental experience, food quality) may also explain some component of inter-annual variation in body condition. For example Stojanovic et al. (2018a) note that during our study period natural foods are rare due to infrequent burning of the study site, and it is not known whether nestlings reared on seed or natural foods would differ when evaluated using our body condition index.

Given the sensitivity of nestlings to conditions during early life, our study shows how easily collected data may be used to understand the impacts of a range of intrinsic and extrinsic factors on nestling body condition. For threatened species, this kind of information may be critical to identifying ways to alleviate stress in early life and avoid carry over effects on later life history stages (Burton and Metcalfe 2014; Harrison *et al.* 2013). Identifying when nestlings are performing poorly relative to the population is often a management priority in small populations where each individual is of high conservation value. Our study provides a tool for rapidly assessing body condition with easily collected data that may be used to identify problems early enough to enable intervention and reduce avoidable mortality.

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Data Availability Statement

Data are stored at the Tasmanian Government Orange-bellied Parrot Tasmanian Program.

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