



Effects of non-random juvenile mortality on small, inbred populations

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ABSTRACT

An individual's ability to produce surviving descendants defines its evolutionary fitness, and loss of family lineages (i.e. having no surviving descendants or relatives) diminishes allelic diversity within closed populations. This high variance in individual reproductive success is difficult to detect and measure, so potential demographic impacts are poorly understood. We identify the frequency and demographic impacts of lineage loss from non-random juvenile mortality of brood mates in critically endangered wild orange-bellied parrots *Neophema chrysogaster* over 22 years. We posit that non-random juvenile mortality is the mechanism that underpins lineage loss and reproductive skew in animals with few opportunities to breed in their lives. Only ~8% of parrot mothers bred more than once and non-random juvenile mortality was more prevalent than expected by chance. Nine of ten wild maternal lineages died out in the wild population over only three years. Using population viability analysis and genetic data, we show non-random juvenile mortality results in lower observed heterozygosity than random mortality scenarios. Failure to account for diminished population genetic diversity when family lineages die out may result in worse conservation outcomes. High individual variance in reproductive success can result in rapid loss of most families from a population if there is no conservation intervention. We identify warning signs and management approaches to address this threat. Looking for and correcting lineage loss early may be an important practical step for conserving population genetic diversity of such species.

1. Introduction

Understanding the persistence of family lineages within populations can provide insights into how populations (and their genetic substructure) changes between generations and affects broader demographic processes (Jobling, 2001; Miller et al., 2007). Lineage loss – when a family lineage produces no surviving descendants – by definition diminishes allelic diversity in closed populations (Gompper et al., 1997). This high variance in individual reproductive success (i.e. reproductive skew) toward only a few individuals/family lineages has been identified as a serious conservation problem for rare species in captive breeding and reintroduction programs (Garnier et al., 2001; Jamieson and Lacy, 2012; Miller et al., 2018). By worsening inbreeding (Kelly, 2001) lineage loss may lower population viability (Kelly and Durant, 2000). Thus, loss of lineages arises at the individual level via variance in fecundity (Pettorelli and Durant, 2007) and this cumulatively affects population level allelic diversity (Gompper et al., 1997). Although lineage loss occurs

with natural selection in large populations, at small population sizes it can act as a stochastic process affecting population genetic structure and extinction risk (Gompper et al., 1997). Lineage loss may occur rapidly, with more than 80% of lineages lost in some mammals and birds over only two decades (Gompper et al., 1997). In some small wildlife populations, most individuals may be derived from only one (Kennedy et al., 2014) or few families (Gompper et al., 1997; Hedrick et al., 2019; Pettorelli and Durant, 2007). Thus, census population size (N) may be much higher than effective population size (Ne) (Nunney and Elam, 1994). In context of species conservation, this phenomenon can be frustrating if attempts to maximise population genetic diversity are undone by reproductive skews toward only a fraction of founding lineages (McLennan et al., 2018). Although reproductive skew can arise from several mechanisms (Miller et al., 2009), lineage loss may be a useful way to monitor individual fitness whilst gaining insight into underlying demographic and genetic processes of small, inbred populations. However, lineage loss may be under-reported in wildlife populations because

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collecting the required genetic data to detect and accurately measure it can be difficult. Consequently, the demographic impacts of this phenomenon remain poorly studied.

Population viability analysis (PVA) is an important tool for modelling the demographic responses of animal populations to different stressors (Beissinger and McCullough, 2002; Morris and Doak, 2002). PVAs use demographic information to model population size, genetic diversity and extinction risk over time. PVAs allow users to quantify the importance of a range of demographic parameters on simulated populations by building different scenarios (Manlik et al., 2018; McCarthy et al., 1995). Although many different aspects of life history can be built into demographic models (e.g. inbreeding and other genetic options, harvest, population supplementation), lineage loss is rarely (if ever) directly factored in to PVAs. Given the potential impact of lineage loss as a stochastic process affecting genetic diversity and population size, this is a surprising knowledge gap, and may have important ramifications for model performance and applicability to species conservation. Freely available software to implement PVA, such as the program VORTEX (Lacy, 2000; Lacy and Pollak, 2020), incorporate great flexibility in the types of ecological processes that can be modelled. Programs like VORTEX also offer users the ability to manually code additional variables that are not part of the standard array of options. Customising generalised PVA software is important for producing models that reflect the reality of a species life history, making them better for guiding biological conservation (Chaudhary and Oli, 2020). The flexibility of VORTEX presents an opportunity to incorporate lineage loss into an easily replicable modelling framework so its impacts can be studied.

In species that have only one opportunity to breed in their lives, if brood-mates experience non-random mortality during the first year of life (i.e. if one individual dies, then its siblings are also more likely to die), then breeders may fail to produce descendants. This non-random juvenile mortality may be a mechanism through which lineage loss and reproductive skew arises. We use a critically endangered bird, the orange-bellied parrot *Neophema chrysogaster*, as a case study to explore what happens to small populations when juvenile mortality is non-random among brood-mates. The orange-bellied parrot is one of the most endangered birds in the world (Stojanovic et al., 2018) and the survival of juveniles over their first year of life has more than halved in recent decades (Stojanovic et al., 2020b). Although they can live up to 11 years, few orange-bellied parrots live long enough to breed repeatedly (Stojanovic et al., 2020b). In this species, one failed breeding attempt usually means that an individual produces no living descendants. These life history traits make orange-bellied parrots an interesting and simplified model for exploring the effects of non-random juvenile mortality (and thus lineage loss) in a wild population. Using 22 years (1995 to 2017) of field data from the wild orange-bellied parrot population, we aimed to (i) quantify how often individuals fail to produce descendants due to non-random juvenile mortality of brood mates, (ii) identify extant maternal lineages and quantify how many surviving descendants mothers produced, and (iii) evaluate the demographic impacts of non-random juvenile mortality of brood mates using PVAs.

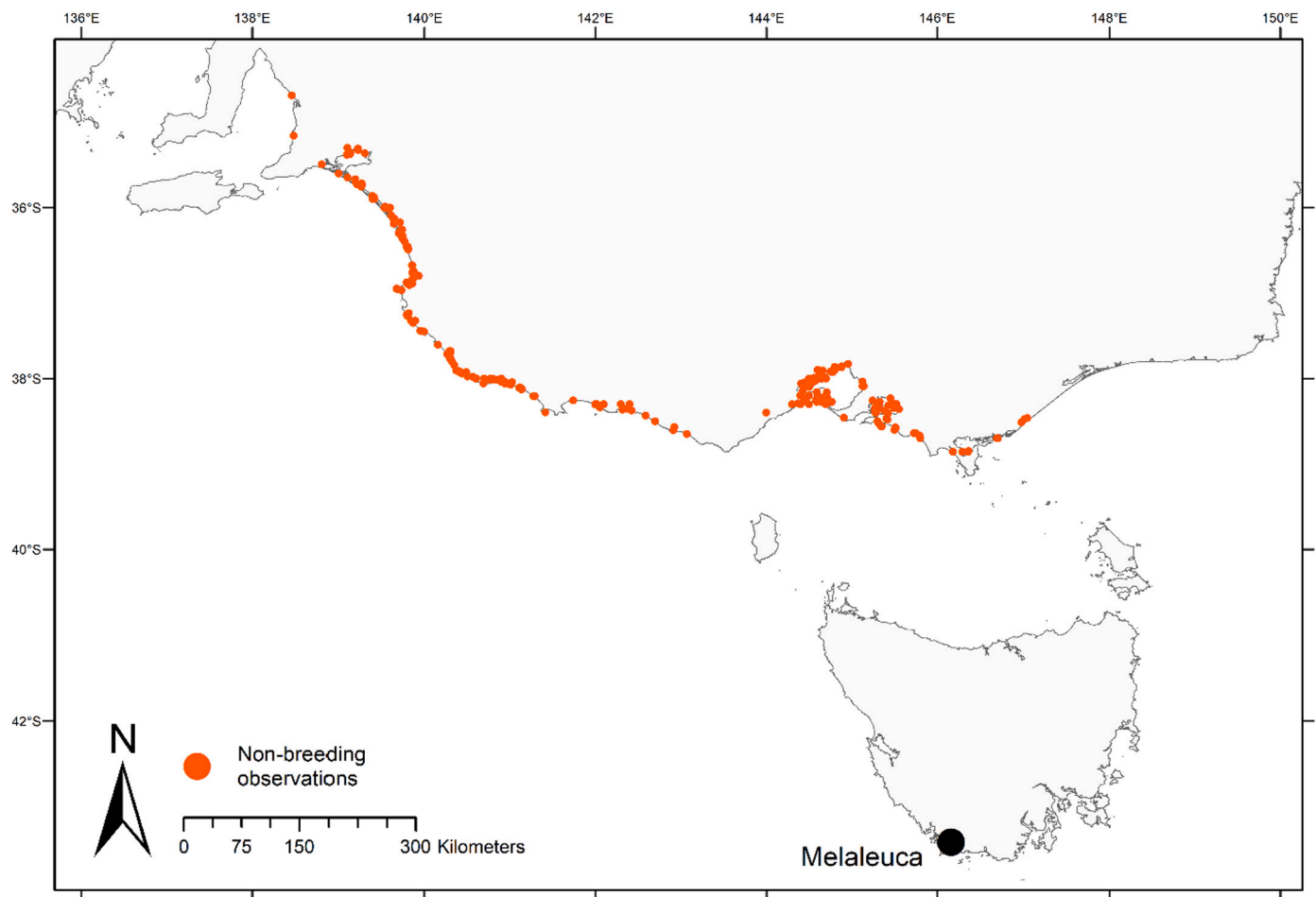


Fig. 1. Orange-bellied parrots have only one extant breeding population at Melaleuca, in southwestern Tasmania, Australia. But during winter the species migrates northward along the west coast of Tasmania before dispersing along the coastline of southern Australia. Orange points indicate observations of orange-bellied parrots in their winter range from 1950 to 2017 from the Atlas of Living Australia (www.ala.org.au), filtered for spatial accuracy and veracity.

2. Methods

2.1. Study system and data

Orange-bellied parrots now suffer extremely diminished genetic diversity, but the effects of inbreeding depression in this species have not been fully documented (Morrison et al., 2020a; Morrison et al., 2020b). Orange-bellied parrots breed in their first year of life – most juveniles fledge in Jan/Feb, and begin breeding after returning from their first migration in Nov/Dec of the same year they are born (~11 months of age). They breed at one location in southwestern Tasmania (Stojanovic et al., 2018), and migrate across coastal areas of the southeastern Australian mainland during winter (Fig. 1). The species has been subject to conservation management since 1979 (Department of Environment, 2016) and most of the intensive conservation action for the species has been in Tasmania (Stojanovic et al., 2020b). The population depends on nest boxes, where nestlings are individually color-ringed each breeding season. Supplementary food is provided and observations of ringed birds at feeders forms the basis of the species' monitoring program (Troy and Lawrence, 2021). The sex ratio of survivors returning from migration to the breeding ground between 2010 and 2020 was on average 1.9 ± 1.4 SD (range: 0.6 to 5.3) males per female, but this bias was corrected from 2013 onward (Troy and Lawrence, 2021). This was achieved by releasing captive bred females each spring to make the adult sex ratio as close as possible to parity and ensure the number of breeding pairs was maximized (Troy and Lawrence, 2021). Because of these management actions, the species may have avoided the negative impacts of sex ratio bias, and so we do not analyze such effects in this study. In other small parrots, male sex ratio bias correspond to high rates of extra-pair paternity (Heinsohn et al., 2018). We do not know if this is widely the case in orange-bellied parrots and it is not clear what proportion of males bred in any year, thus we do not attempt to account for paternity. We focus instead on maternal lineages and assume brood-mates were either full or half siblings (brood parasitism has not been recorded). Between 1995 and 2013 mothers were not individually identified, so matching individuals to family lineages was not possible. After 2013, individual mothers were identified at most nests. In 2013 the mothers in the wild population were not full sisters, but whether there were more distant kin relationships between mothers is not known. We accounted for potential misidentification errors in annual monitoring data (Isaac et al., 2014) by scoring individual birds as 'dead' if they were observed fewer than five times, unless they were (i) verified by >1 observer, or (ii) seen by the same person >3 times (Stojanovic et al., 2020b). We collated survival data for 797 birds (131 females, 116 males and 550 unknown sex) hatched between 1995 and 2017. We excluded individuals (i) whose siblings were unknown, (ii) with no siblings (to explicitly test for non-random mortality among siblings) and (iii) from broods of six (there were only four broods of six in the sample, i.e. too few for analysis). This left 605 individuals (67 females, 58 males and 480 unknown sex) for analysis. There were 180 broods in total, comprising two ($n = 44$), three ($n = 58$), four ($n = 47$) or five ($n = 31$) maternal siblings. All individuals in the sample entered the population as nestlings ringed in boxes (typically within a fortnight of fledging). Because birds needed to survive long enough to be ringed, we do not account for nests that failed early in the nesting phase (as eggs or young chicks), and thus we potentially underestimate lineage loss, but only 2% of our sample died between ringing and fledging. We recorded whether an individual lived/died during its first full migration and used our recent estimate of juvenile mortality rates (Stojanovic et al., 2020b) in this study. Life expectancy in our sample was mean 1.8 years (± 1.5 standard deviation). Based on wild mortality rates (Stojanovic et al., 2020b), we estimate (in the PVA step below) that only 20% of female orange-bellied parrots had a chance to breed in their lives, and only 8% of all females bred in more than one year. Causes of juvenile mortality were unknown. We created a unique identifier for each brood because nest boxes were occupied repeatedly over the study by different mothers.

2.2. Quantifying rates of non-random juvenile mortality

To evaluate if juvenile mortality occurred non-randomly, we modified the approach of Pettorelli and Durant (2007). We created a binary variable for non-random juvenile mortality where 0 = at least one chick per brood lived and 1 = the whole brood died. Maternal identity was unknown for most broods in our sample, so we could not quantify individual lifetime reproductive success. Consequently, we pooled the historical broods and fitted a log-linear model. We used the observed frequency of non-random juvenile mortality across brood sizes of one to five chicks as the response variable. To evaluate whether the observed frequency of non-random juvenile mortality exceeded levels expected due to chance, we included the (log transformed) expected rate (based on a random distribution) as an offset term in the model, and specified a Poisson error distribution with a log link. We calculated the rate of non-random juvenile mortality by taking the exponent of the coefficient of the model intercept – this is the ratio of the observed/expected number of cases where all siblings in a brood died in their first year of life. This analysis was conducted in R version 4.0.3 (R Development Core Team, 2021). In addition to the above, other exploratory analyses are available in the supplementary materials as an R Markdown file, which provides more information on why we did not model the effects of year.

2.3. Observed maternal lineage loss in the wild after 2013

We also directly measured the extent of maternal lineage loss after 2013 when the identity of most mothers was known. During this time period, release of captive-bred birds to the wild occurred annually. We quantified the number of eggs laid, and the number of surviving daughters and granddaughters produced by all known wild-born mothers. Since captive-bred birds were not routinely released before 2013 (Department of Environment, 2016), wild parrots born before then were treated as founders and represented the last surviving wild maternal lineages of orange-bellied parrots (for this purpose we define 'wild' as having no known captive-born maternal ancestry). We optimistically assume the founding mothers were unrelated, thus likely overestimating the number of surviving lineages at the start of this period.

2.4. Population viability analysis – background

Non-random juvenile mortality should diminish the genetic diversity of a population with each generation as families (i.e. the genetic sub-structure of a population) die out and the population becomes increasingly reproductively skewed to surviving lineages. We used VORTEX 10.5.5.0 (Lacy et al., 2020) to model the impact of non-random juvenile mortality on orange-bellied parrots using detailed life history data from long term field studies, and recent empirical genetic data (Morrison et al., 2020b). The demographic values used in all of our simulations (and the justification for why these values were selected) are provided in Table 1. We provide a full list of the PVA scenarios, including their shared and differing parameters, in Table 2.

We defined two pairs of scenarios, where within each pair the rate of juvenile mortality was set to either 49% or 80% (see below and Table 1 for further detail). Within scenario pairs, we only varied the way that juvenile mortality was encoded in VORTEX, i.e. either random or non-random among siblings within a brood (using a state variable – see below). We outline the settings for each pair of models in Table 2.

VORTEX assumes by default that at the start of simulations all founders are unrelated. This poses two problems in context of our study: (i) simulations may not have enough time to accumulate the negative effects of inbreeding depression if founders are unrelated, and (ii) all surviving orange-bellied parrots likely have at least some level of kinship. Given these considerations, we used contemporary genetic data on wild orange-bellied parrots (Morrison et al., 2020a; Morrison et al., 2020b) to set more realistic starting parameters. In those studies, 165

Table 1

Demographic parameters common to all population viability analyses (PVA) of orange-bellied parrots. The main components reflect the key tabs in the program VORTEX where the PVA was implemented.

Main component	Demographic parameters	Values used	Justification
Inbreeding depression	Lethal equivalents	No inbreeding depression (i.e. option unchecked).	Excluded because observed mortality rates in the contemporary wild population (Stojanovic et al., 2018) already account for potential lethal effects of inbreeding on juvenile mortality rates.
Carrying capacity	Carrying capacity	1000 ± 0 SD	Optimistic assumption to remove carrying capacity limits.
	Mating system	Monogamy	Social monogamy within a breeding season (Higgins, 1999).
Reproductive system	Age range of first offspring and maximum age of reproduction – both sexes	First offspring = 1 year Maximum age = 11 years	Breeds at 1 year old after completing a migration (Higgins, 1999) and optimistic assumption that all birds that survive migration attempt to breed.
	Maximum lifespan	11	Longest-lived wild individual (Stojanovic et al., 2020b).
	Maximum number of broods per year	1	Short breeding season and only one recorded case of double-brooding in the wild (Stojanovic et al., 2018).
	Maximum number of progeny per year	6	Historical (Higgins, 1999) and contemporary sources (Stojanovic et al., 2020a).
	Sex ratio at birth (% males)	50%	Unpublished data from the contemporary population.
Reproductive rates	Percentage adult females breeding	=MIN(1:100/F) * 100	Provision of 100 artificial nesting sites (Stojanovic et al., 2018).
	Distribution of broods per year	100% have 1 brood	Evidence from the field (Troy and Lawrence, 2021).
	Number of offspring per female per brood	Mean of 3.5 ± 1 SD	Recent and historical data (Higgins, 1999; Stojanovic et al., 2020a).
Mortality rates	Adult mortality rate	42.6 ± 2 SD	22 years of field data (Stojanovic et al., 2020b).
	Juvenile mortality rates	80% ± 20 SD (worst case) 49% ± 20 SD (best case)	22 years of field data, either historical (best case scenario = data from 1995) or contemporary (worst case scenario = data from 2017) (Stojanovic et al., 2020b).
Mate monopolization	% males in the breeding pool	100%	Management efforts to rectify adult sex ratio biases (Troy and Lawrence, 2021).
Initial population size	Initial population size	120	Historical population sizes (Stojanovic et al., 2020b).
	Specified age distribution, based on 60 individuals per sex	Age 1 = 43, age 2 = 12, age 3 = 4, age 5 = 1	Automated calculations within VORTEX and set manually for each scenario.
Genetics	Genetic Management – Kin relationships at the start of the simulations	0.022	Starting kinships were calculated using COANCESTRY (Wang, 2011) using genetic data from earlier studies of the wild population (Morrison et al., 2020a; Morrison et al., 2020b).

parrots were sequenced using DArTseq, a reduced representation sequencing method (Kilian et al., 2012; Sansaloni et al., 2010) with the *PstI* and *SphI* enzymes. Sample collection, DNA extraction and DArT sequencing is described in detail elsewhere (Morrison et al., 2020b). We used these genetic data to undertake a molecular estimate of kinship and inbreeding among wild orange-bellied parrots as a more realistic starting point for our analysis than an assumption that they were unrelated. Using COANCESTRY (Wang, 2011) as described in detail elsewhere (Hogg et al., 2019), we used TrioML to estimate pairwise relatedness and individual inbreeding for all parrots in the sample (see Table 1).

Before finalizing our parameter selection (Table 1), we undertook a sensitivity test to explore the effects of variation in different parameters which (i) lacked any field data to inform settings, or (ii) were known to vary widely based on field data. We used model one as the base and ran 100 samples for 100 iterations, with a Latin hypercube sampling approach. We included the following variables in the sensitivity test: juvenile mortality (range: 30–80%), adult mortality (range: 33–50%), initial population size (range: 5–305), carrying capacity (range: 500–1500), kin relationships at the start of the simulation (range: 0–0.5), proportion of females that bred each year (range: 50–100%), and brood size (range: 1.5–5.5). For each variable in the sensitivity test we included extreme values of each parameter to explicitly identify the impacts of the full scope of parameter variation on simulated population sizes. We used linear models and model averaging to quantify the effects of each uncertain parameter on the following response variables each in turn: mean population size, observed heterozygosity and stochastic growth rates of simulations. We present the full results in the supplementary materials but provide a brief summary here of the direction of the relationships between the most impactful variables. We found that population sizes were most affected by juvenile mortality rates (negative relationship), brood sizes (positive relationship) and the proportion of females breeding (positive relationship). Observed heterozygosity was most affected by juvenile and adult mortality rates (both had negative relationships) and proportion of females breeding (positive relationship). Stochastic growth rates were most affected by juvenile and adult

mortality rates (both had negative relationships) and brood size (positive relationship). Based on the sensitivity test we selected optimistic values for uncertain parameters for which there was no data from the wild population in our subsequent PVAs, and for the remaining variables we set parameters based on recent studies of the wild population (Table 1).

2.5. Comparison of PVA scenarios

We ran 1000 simulations for each scenario and ran them over 50 years. We compared overall (N.all) population sizes and gene diversity (observed heterozygosity) between modelled scenarios using a Welch two sample *t*-test in R (R Development Core Team, 2021). These *t*-tests were performed on the raw results of the VORTEX simulations from each scenario, and we considered effects significant at $p < 0.01$.

2.6. Encoding non-random juvenile mortality in VORTEX

Annual juvenile mortality is usually encoded in VORTEX as a mean percentage of individuals that die (with standard deviation for environmental variation). For models one and three (Table 2), we followed this usual approach and set juvenile mortality to 49% and 80% respectively with a standard deviation of 20. These mortality rates represent the lowest and highest rates of mortality observed in the wild population (Stojanovic et al., 2020b).

In contrast, there is no inbuilt function to account for non-random juvenile survival of brood-mates in VORTEX. To overcome this, we made an individual state variable (IS1: FamilySurvival) that caused non-independence of juvenile survival within broods. For the 80% mortality scenario (model four, see Table 2), FamilySurvival was initialized for each mother so that each year her entire brood had their survival sampled from a distribution with mean of 20%, and variation among dams (families) between 0 and 40%. First year mortality for each member of a given mothers' brood thus became 1-FamilySurvival. This was achieved in VORTEX with the function

Table 2

Results of population viability analysis in VORTEX. Pair = pairs of models based on juvenile mortality rates; ID = the model ID; Juvenile mortality rate = the rate at which first-year mortality was set; mortality schedule = whether or not juvenile mortality was included in the standard way in VORTEX (i.e. a randomly drawn value for each individual) or as a state function that caused mortality of brood mates to be non-random; stoch.r = population stochastic growth rate; N.extant = mean final size of the extant populations; N.all = mean final size across both extant and extinct populations; GD = gene diversity, i.e. observed heterozygosity; SD = standard deviation.

Pair	ID	Juvenile mortality rate	Mortality schedule	Stoch.r	SD (r)	N.extant	SD (N.extant)	N.all	SD (N.all)	GD	SD (GD)
1	1	49%	Random	0.02	0.27	420.61	110.51	420.61	110.51	0.95	0.01
	2	49%	Non-random	0.02	0.25	412.81	107.79	412.81	107.79	0.94	0.01
2	3	80%	Random	-0.20	0.40	19.25	15.86	0.08	1.5	0.68	0.22
	4	80%	Non-random	-0.18	0.33	16.5	22.41	0.08	1.62	0.61	0.08

$$\text{FamilySurvival} = \text{MIN}(20 + (20 * \text{NRAND}) ; 40).$$

This has the property whereby survival values for individuals are drawn from a normal distribution that is symmetrically truncated to ensure values are selected between 0 and 40%. For the 49% mortality scenario (model two, see Table 2), we used the formula $\text{FamilySurvival} = 51 + 20 * \text{NRAND}$ because truncation was not an issue. We did not repeat this process for fathers because of the uncertainty about paternity. For models that included the state variables (models two and four), we also included a standard deviation of 20 due to environmental variation (to match the random mortality scenarios). For all models, individuals that survived their first year of life reverted from whatever their juvenile mortality rate was to the constant adult survival rate (Table 1).

3. Results

3.1. Quantifying rates of non-random juvenile mortality

We found evidence that non-random juvenile mortality of brood mates occurred more frequently than expected by chance. The ratio of observed to expected non-random juvenile mortality was 1.37, meaning that individual orange-bellied parrots had a significantly elevated risk of dying if their siblings died during in the first year of life (d.f. = 3, z = 2.33, p = 0.019, Fig. 2). Further exploratory analyses are presented as R Markdown in Supplementary materials.

3.2. Observed maternal lineage loss in the wild after 2013

Between 2013 and 2016 when all mothers in the wild population were known, nine of the ten founding wild maternal lineages died out. Ten individual first generation wild orange-bellied parrot mothers attempted to breed during this period, producing 56 eggs and 36 nestlings. These wild mothers bred only once in their lives with one exception – a female (silver silver L) who was the first recorded individual to ever breed twice in the same year, producing seven offspring in total (Stojanovic et al., 2018). Silver silver L was herself a second generation wild born individual, hatching in 2015 to an unidentified mother. Of the seven offspring reared by silver silver L (who died before the 2017 breeding season), only one daughter (red red D) survived. Thus red red D represented the last surviving wild maternal lineage of orange-bellied parrots at the end of the study period. Interestingly, red red D survived at least until the 2021 breeding season, which at 6 years of age, makes her the oldest breeding mother in the contemporary wild population.

Over this same time, 29 first generation captive bred orange-bellied parrots collectively produced 127 eggs and 75 nestlings. On average captive born mothers had only 1.2 opportunities to breed (5 bred in two successive years when they were recaptured and housed in captivity over winter to prevent mortality during migration/winter). Only one surviving daughter of a captive bred mother recruited into the wild breeding population by 2016.

Thus, regardless of some repeated breeding by individual captive and wild born mothers in the contemporary population, only one maternal

family lineage managed to survive over two generations.

3.3. Population viability models

The full results of the sensitivity test are presented in the supplementary materials. Table 2 outlines the overall means and standard deviations of population stochastic growth rate, size and observed heterozygosity for each scenario. The means and standard errors for observed heterozygosity for each model over the 50 year simulation are shown in Fig. 3. Overall the PVAs showed that regardless of the severity of juvenile mortality rates, stochastic growth rates and population sizes did not differ between models with random and non-random mortality of brood mates at the juvenile stage. Scenarios with non-random juvenile mortality resulted in significantly lower overall observed heterozygosity regardless of whether juvenile mortality rates were low (t = -13.26, d.f. = 1956.4, p < 0.0001) or high (t = -4.0146, d.f. = 3.7935, p = 0.02) (Table 2) – this effect emerged quickly regardless of the severity of mortality rates (Fig. 3). No simulations went extinct in pair one, but in pair two all but eight simulations went extinct within 20 years (there were only four surviving simulations each in models three and four). The rapid extinction of most simulations in pair two explains the increasing uncertainty of estimates after 20 years observable in models three and four in Fig. 3.

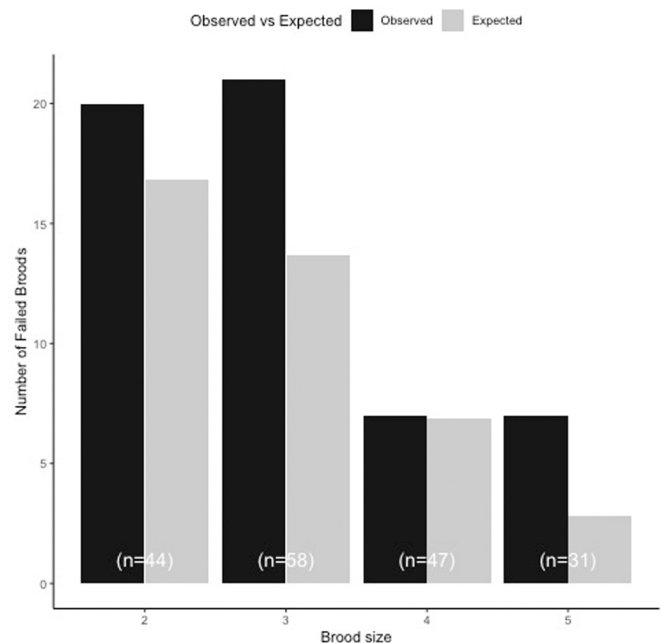


Fig. 2. Histogram of observed (black) versus expected (grey) frequency of the occurrence of failed broods (i.e. where the whole brood died in their first year of life) in orange-bellied parrots. Total number of broods recorded in each size class presented in the figure, and the data are separated by brood size classes.

4. Discussion

Non-random mortality of brood mates post-fledging occurs more often in orange-bellied parrots than expected by chance, and this can contribute to rapidly eroding genetic diversity. Even the optimistic scenario (pair one) showed significant negative impacts of non-random juvenile mortality on observed heterozygosity. However, the optimistic scenario was based on mortality rates from 1995, and thus does not reflect the modern population. Nevertheless, the optimistic scenario

suggests that if efforts to recover the species are successful (and juvenile mortality rates can be reduced to historical levels), then the population may be able to sustain non-random juvenile mortality despite declining genetic diversity. In contrast, pair two was based on the contemporary wild population – these high rates of juvenile mortality led to rapid extinction of most simulations regardless of the mortality schedule. Before the high mortality simulations became extinct, we found that non-random juvenile mortality again resulted in lower observed heterozygosity (Fig. 3). Our study provides new insights into how non-

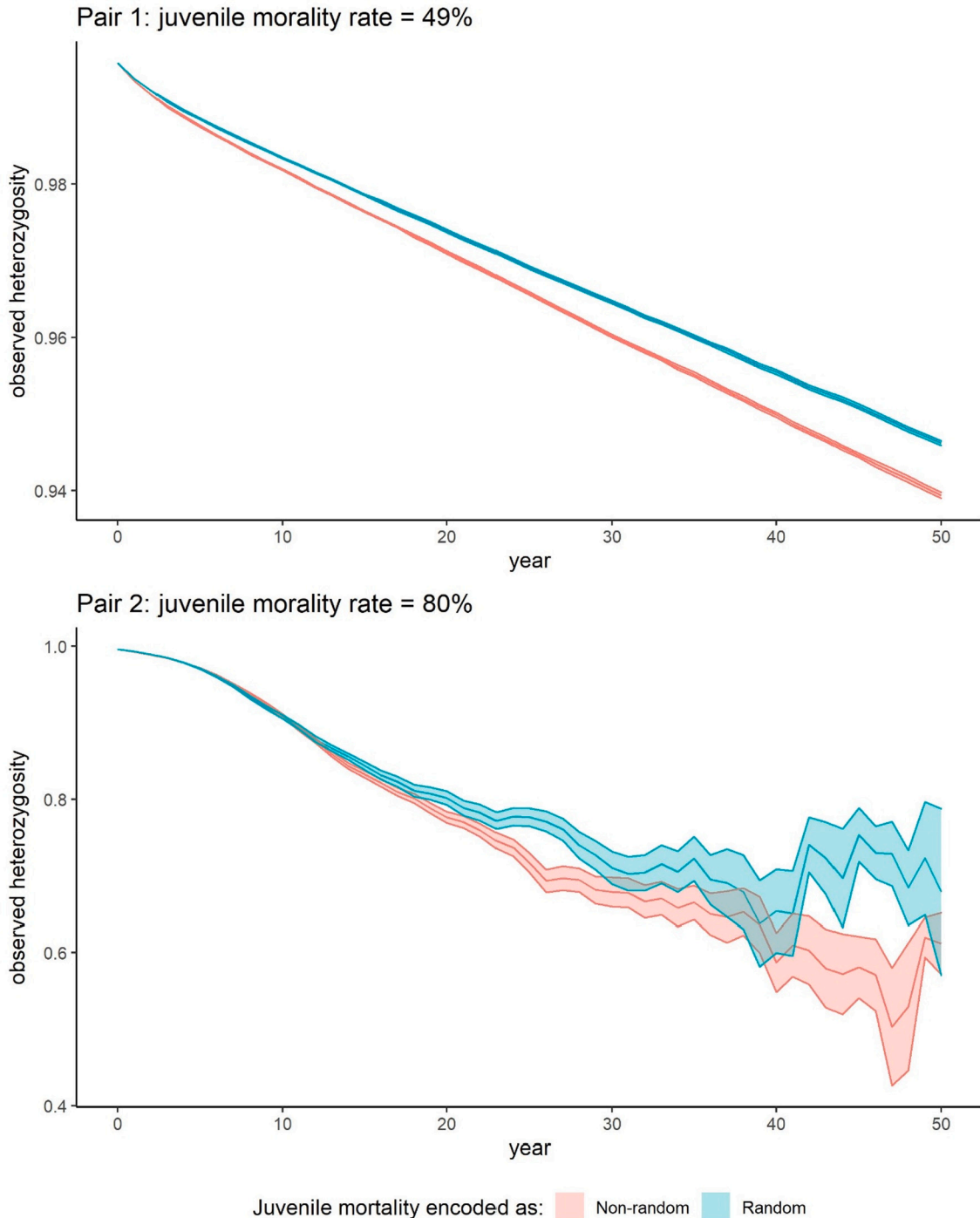


Fig. 3. Mean observed heterozygosity over time (ribbons indicate standard errors) for PVA scenarios that contrasted effects of random (blue) and non-random (red) mortality of orange-bellied parrot brood mates. We varied the severity of juvenile mortality rates between scenarios. Non-random mortality of brood mates significantly reduced observed heterozygosity of the population. In pair two most simulations went extinct within 20 years, which is why the uncertainty of estimates increased after this time.

random juvenile mortality may have hastened the species' decline to its current state. Although we did not find significant differences in population sizes between random and non-random mortality scenarios, the loss of even a few individuals can have important genetic ramifications for conservation of a tiny and declining population. For example in the case of orange-bellied parrots, in 2016 silver silver L was only wild female to produce a surviving lineage. The precarious state of the orange-bellied population illustrates how the death of even a handful of individuals could be a conservation problem. Monitoring which individuals live or die in small populations can provide important insights into underlying population genetic processes affecting a species and better inform conservation efforts.

Why are some broods more likely to fail than others? The answer to this question remains unclear, but several possibilities could be further explored. We have previously shown that in captive orange-bellied parrots, heavy mothers produce heavier offspring, and likewise, some maternal lineages consistently produce heavy- or light-weight descendants (Stojanovic et al., 2019). Similar patterns exist in other species (Ronget et al., 2018), and early life conditions can carry over to affect later life history stages (Fayet et al., 2016; Saino et al., 2018), including juvenile survival (Merrill et al., 2021) and even future generations (Burton and Metcalfe, 2014). We have previously shown that wild orange-bellied parrot nestlings experience variance in their body condition between years and among hatch orders (Stojanovic et al., 2020a). Whether there are maternal (or paternal) effects on the quality and subsequent survival of offspring is not known, but needs to be studied. Alternatively, genetic factors may explain some non-random juvenile mortality. Deleterious mutations of the mitochondrial genome can be maternally inherited in some species, and these mutations may not be subject to selection if they affect one sex worse than the other (Gemmell et al., 2004). This, and other genetic explanations for non-random juvenile mortality could be tested using genomic techniques such as wild pedigree reconstruction (Grueber and Jamieson, 2008). Pedigrees would also shed more light on the true extent of lineage loss in this species by providing confident assignment of paternity (which we ignore in this study). Other chronically inbred parrot species have purged deleterious alleles from their genomes and suffer relatively few negative effects of diminished population genetic diversity (Dussex et al., 2021), but whether this might also be the case for orange-bellied parrots is unknown. Thus, fully understanding the mechanisms that lead to non-random juvenile mortality will require an interdisciplinary approach incorporating both environmental and genetic data.

Our results also revealed that orange-bellied parrots lost most of their contemporary wild maternal lineages within only a three year period. This short time frame may be too rapid for effective conservation intervention. Practitioners should instead look for early warning signs that non-random juvenile mortality (and thus lineage loss) is occurring. In the case of orange-bellied parrots, their diminished genetic diversity can now only be corrected with major interventions such as hybridisation to related species (Hogg et al., 2021). This would be ethically and logistically complicated – it is more feasible to look for lineage loss before opportunities for effective interventions are missed. As well as being challenging to detect in the real world, failing to account for non-random juvenile mortality in demographic models may hinder conservation of small populations. This source of modelling error may produce overly optimistic outcomes, especially for small, inbred populations. Our approach is a new and straightforward way to encode non-random juvenile mortality into a widely used PVA software (Chaudhary and Oli, 2020). This approach is highly relevant for other conservation practitioners. For example, avoiding reproductive skew in reintroduced or captive populations is critically important for the maintenance of their genetic diversity (Jamieson, 2011; Miller et al., 2009). Accounting for non-random juvenile mortality rates will improve the relevance of PVAs aimed at evaluating reintroduction and captive breeding strategies for species with individually variable breeding success. In our case, the high mortality rate and short life span of orange-bellied parrots meant that

non-random juvenile mortality of brood mates was equivalent to lineage loss for most mothers. In species that have multiple opportunities to breed over their lives, the impacts we observed may be dampened unless there is a relationship between survival of all progeny of a given mother rather than just within the same brood.

Family lineages are an important proxy for population genetic substructure, and being aware of how lineages persist, mix and die out is valuable information (Vonholdt et al., 2008). But most threatened species lack any genetic data (Brandies et al., 2019; Pierson et al., 2016), hindering direct monitoring of pedigrees. Even though we could not identify the mothers of most broods before 2013, we nevertheless detected non-random juvenile mortality using data on individual mortality and by identifying brood-mates. Threatened species management programs commonly collect this type of information. Warning signs for unidentified but problematic lineage loss may include small population size, small brood/litter sizes and highly variable individual reproductive success. Targeted assistance for lineages at risk (e.g. nest protection, fostering and other reproductive manipulations) could be implemented in situ for wild populations, especially when reproductive skew is suspected. Alternatively, targeted collection of a few individuals from rare lineages for captive breeding and future release may be effective. Targeting rare families (which may be a reasonable proxy for under-represented alleles) may preserve genetic diversity in circumstances where genomic data are unavailable. Indeed, this is the premise of the mean kinship management strategy, which targets the least represented lineages for breeding to equalise family sizes and thus maximise N_e (Caballero and Toro, 2000). As the global biodiversity crisis escalates, it is increasingly essential to protect small populations against further diminishment of population size and genetic diversity. Looking for and, if possible, correcting lineage loss may be an important practical step for improving the conservation of small, inbred populations.

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Ethics

The research utilised data collected by the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE) during their implementation of the Orange-bellied Parrot Tasmanian Program.

CRedit authorship contribution statement

DS conceived the study, analysed the data and drafted the manuscript. DS, TN, RL, CH, KAF and RH analysed the data and reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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