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Genetic management and population modelling of translocated fauna: Dibbler (*Parantechinus apicalis*)

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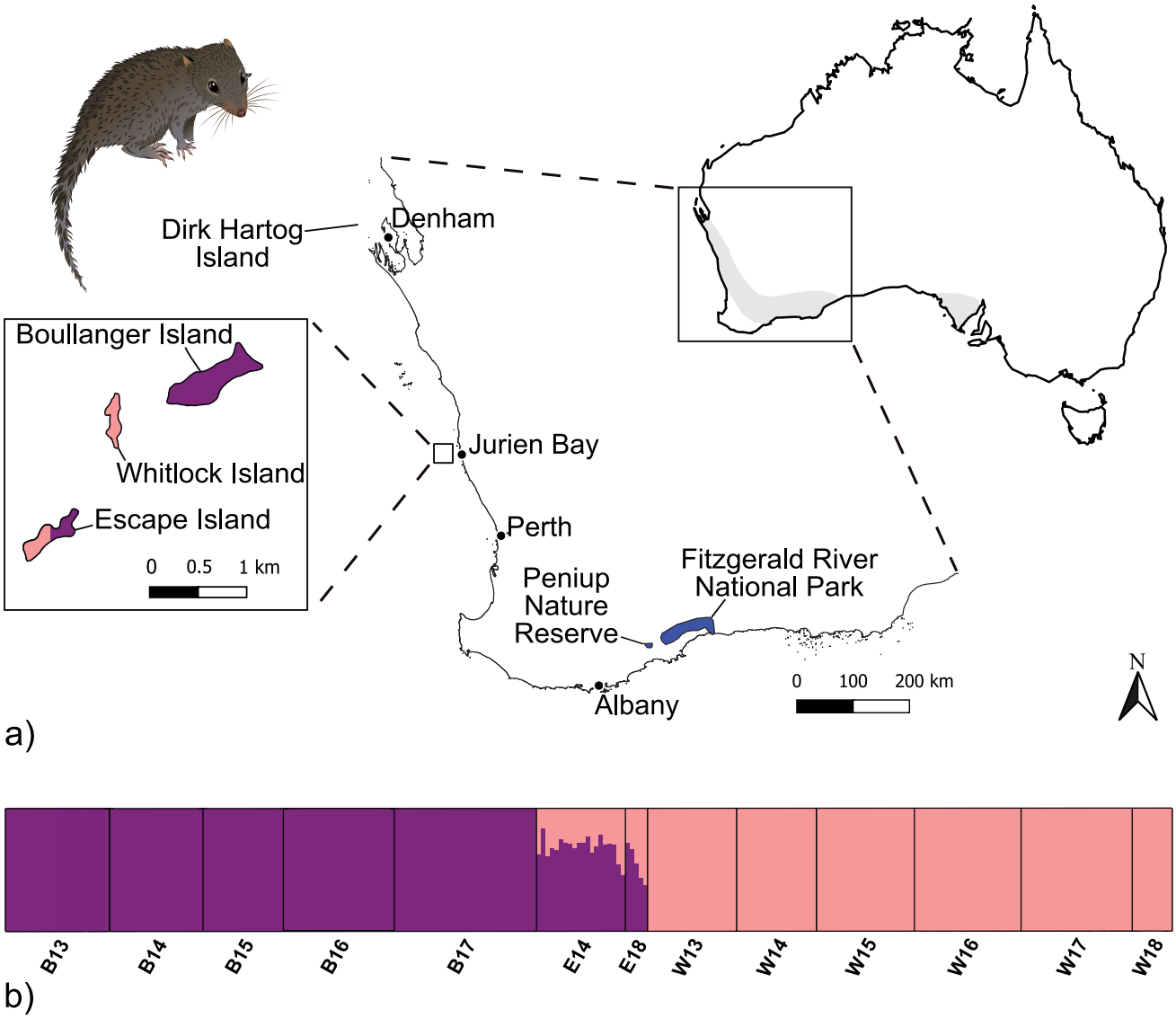

Figure 1. a) Current distribution of island dibblers in Jurien Bay and historic distribution of dibblers in Australia (inset). Dirk Hartog Island, the location of the new translocation, is also shown. b) STRUCTURE analysis showing the number of genetic clusters (K = 2) within island dibblers and the level of admixture in the Escape Island population. Black lines separate islands and collection years. B = Boullanger, W = Whitlock, E = Escape

Executive summary

With many threatened Australian species experiencing increased fragmentation and elevated extinction risk, translocations are increasingly being used in conservation management. Maintaining genetic diversity is a key component to successful translocation programs, meaning there are certain criteria for founder populations. However, gaining access to ideal founder populations may not be possible if a species is restricted to offshore islands, as island populations are often isolated with no immigration, and constrained effective population sizes in small populations lead to low genetic diversity. Captive breeding has played critical roles in conservation management of threatened species when remnant source populations are of limited or uncertain size, and mitigates the inherent risk of extinction due to over-harvesting for wild-to-wild translocations. The dibbler (*Parantechinus apicalis*) is a small (40 – 125 g) dasyurid marsupial once widely distributed in Australia, but its range has declined dramatically since European colonisation to the southwest of Australia, where it occurs naturally on mainland Australia in the Fitzgerald River National Park (~3000 km^2), and on two small islands, Boullanger (ca. 84 km^2) and Whitlock (ca. 33 km^2) Islands in Jurien Bay off the mid-west coast of Western Australia. The island populations are therefore at particular risk of extinction. Accordingly, island dibblers are a key component of a major ecological restoration project to a large (620 km^2) feral-free offshore island, Dirk Hartog Island (DHI) and are being sourced from the nearby Jurien Bay islands after being captivity-bred at Perth Zoo to enhance founder numbers. However, due to low population sizes there is a risk of over-harvesting leading to irreversible population declines.

In this study we describe the remnant genetic diversity in island populations, including the established translocated population on Escape Island, from 2013 to 2018 using 14 microsatellite markers. We then included this data in population viability analysis models developed to optimise the sensitive trade-offs involved in translocating dibblers from Jurien Bay to DHI via the Perth Zoo captive breeding population. Key conflicting objectives are maximising the retention of genetic diversity in the breeding population while minimising the detrimental impacts of harvesting on the source populations. Importantly we were able to look at the impact of various factors that may influence the establishment and growth of new dibbler populations.

Current genetic diversity in island dibblers is low overall, particularly in Whitlock Island (He = 0.02) with only one polymorphic locus out of 14, and highest in the admixed Escape Island population (He = 0.36). All measures of genetic diversity have declined over time since 2013 in Boullanger and Whitlock Islands, and for all but allelic richness in Escape Island. We confirm that there remain two distinct genetic clusters in Jurien Bay, described by Boullanger Island and Whitlock Island, and that in 2014 there is a greater Boullanger Island representation in Escape Island than Whitlock Island suggesting uneven mating success in the admixed population. Modelling predicts that survival probabilities drop below 0.2 and heterozygosities below 0.05 after 100 years for both Whitlock and Escape Islands. Interestingly there is a strong regression between survival probability and probability of reproduction-impacting drought (R^2 = 0.97, $p<0.001$), and survival probability and carrying capacity (R^2 = 0.86, $p<0.001$). An optimal translocation scenario includes founding the captive population with six mate pairs from Whitlock Island and 14 from either Escape or Boullanger, and then Dirk Hartog Island with at least 80 animals from the captive population.

With empirical data showing reductions in genetic diversity and modelling projecting further declines into the future, as well as a steady decline in population survival for all islands, here we have provided evidence to support a rescue of the Jurien Bay island populations. Moving animals to DHI, with an estimated carry capacity of 10,000, via Perth Zoo will facilitate population growth, resilience to environmental stochasticity and retention of genetic diversity. Our models provide guidelines for numbers and origins of individuals for the current and future translocation programs. We recommend ongoing monitoring of both source (inbreeding depression) and translocated (mating bias) populations.

Introduction

The dibbler (*Parantechinus apicalis*) is a small (40 – 125 g) dasyurid marsupial once widely distributed in Australia (Figure 1), but has declined dramatically due to introduced predators such as foxes (*Vulpes vulpes*) and feral cats (*Felis catus*), inappropriate fire regimes, habitat degradation due to dieback (*Phytophthora cinnamomi*) disease, and competition with house mice (*Mus musculus*) (Friend 2003). Dibblers are now restricted to the southwest of Australia (Miller et al. 2003; Mills and Bencini 2000; Mills et al. 2004; Woolley 1991) occurring naturally on mainland Australia in the Fitzgerald River National Park (~3000 km^2), and on two small islands, Boullanger and Whitlock Islands off the mid-west coast of Western Australia (Figure 1). The species is listed as Endangered under Australia's environmental legislation, the Environment Protection and Biodiversity Conservation (EPBC) Act 1999, and on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Burbridge and Woinarski 2016).

To prevent further population declines, five translocations have been implemented since 1998 using captive bred dibblers sourced from island and mainland populations (Friend 2017; Morris et al. 2015). Only three have been successful, including a translocation to Escape Island in 1998 using 88 captive-bred dibblers sourced from Boullanger and Whitlock Islands (Moro 2003). In 2001, another successful translocation to Peniup Nature Reserve was achieved using 235 captive-bred dibblers sourced from mainland populations (Friend 2017; Moro 2003; Morris et al. 2015), and in 2015, 80 captive-bred mainland dibblers were introduced to Gunton Island off the south coast of Western Australia. Although in its early stages, recent monitoring suggests the Gunton Island population has successfully established (Tony Friend, pers. comm.). However, despite these efforts, fewer than 1000 individuals, including those in translocated populations, remain in the wild (Burbridge and Woinarski 2016).

The reintroduction of dibblers to Dirk Hartog Island, a large island (62,000 ha) off the coast of Western Australia (Figure 1), is a key component of a major ecological restoration project '*Return to 1616*' led by the Western Australian Department of Biodiversity, Conservation and Attractions (DBCA) (Morris et al. 2017). Following eradication of feral cats and goats, 11 mammal and one bird species are being successively translocated to Dirk Hartog Island between 2018 and 2030, with the aim of restoring former faunal biodiversity and ecosystem processes. Dibblers are being sourced from the nearby Jurien Bay islands but are first being captivity-bred at Perth Zoo to enhance founder numbers. However, as the Jurien Bay island populations are small (10-30 adults) and subject to large fluctuations, they are vulnerable to extirpation (Tony Friend, pers. comm. Thavornkanlapachai 2016), especially if harvesting pressure is too high. Further, it is currently unknown how many founders are required to establish a population on Dirk Hartog Island.

Population viability analyses (PVA) is widely used in planning and evaluating conservation actions for threatened populations (Akcakaya and Sjogren-Gulve 2000; Beissinger and McCullough 2002; Lindenmayer et al. 1993; Pacioni et al. 2019), and can assist with managing finely balanced trade-offs when planning translocations (e.g. White et al. 2020). In this study we use PVA to explore management options and their subsequent impact on genetic diversity for the island dibbler. We first quantify changes in the genetic variation in the Jurien Bay island populations from 2013 to 2018, and then develop a PVA model to assess the implications of harvesting island dibblers for a captive breeding program. Finally, we determine the best scenario for translocating dibblers to Dirk Hartog Island that maximises survival of both source and translocated populations, and the retention of genetic diversity.

Context

In Australia, translocations are widely used to improve the status of threatened mammal species by increasing the number of self-sustaining populations (Burbidge and Abbott 2017; Fischer and Lindenmayer 2000), and safeguarding species on predator-free havens on islands (Legge et al. 2018; Morris et al. 2015; Seddon et al. 2015). Maintaining genetic diversity is a key component to successful translocation programs (Fischer and Lindenmayer 2000; Weeks et al. 2015), and IUCN/SSC guidelines (IUCN/SSC 2013) recommend that individuals selected for translocations should provide adequate genetic diversity, and come from geographically close origins and comparable habitats to the intended destination. Further, it has been suggested that founders should capture 90–95% of genetic diversity of source populations (Allendorf and Ryman 2002; Gilpin and Soule 1986; IUCN/SSC 2013), and should be genetically compatible if they are mixed from multiple sources (Frankham et al. 2011).

Gaining access to ideal founder populations may not be possible if a species is restricted to offshore islands (IUCN 2012; Willoughby et al. 2015). Island populations are often isolated with no immigration, and constrained effective population sizes in small populations lead to low genetic diversity. Using island sources as founder populations in translocations may compound effects such as population bottlenecks in the translocated population. Depending on the number of individuals harvested (which determines the narrowness of the bottleneck), there can be increased inbreeding, loss of evolutionary potential and rapid genetic divergences from the source population(s) (Allendorf 1986; Frankham 2005; Weeks et al. 2015).

There can also be a negative impact on the source populations (Easton et al. 2019), which is particularly relevant for species whose remnant ranges are restricted to just a few locations. Harvesting too many individuals from a population can alter its population subdivision, reduce genetic variation, induce selective genetic changes and lead to irreversible population decline (Allendorf et al. 2008).

Captive breeding has played critical roles in conservation management of threatened species when remnant source populations are of limited or uncertain size, and mitigates the inherent risk of extinction due to over-harvesting for wild-to-wild translocations (e.g., the Californian condor (Snyder and Snyder 2000) and the western swamp tortoise (Kuchling et al. 2018)). While captive breeding programs provide relatively benign environments that allow populations to thrive and persist over time, issues associated with captive breeding include high cost, the possibility of individuals adapting to captivity and being unsuited to release, and the potential for disease outbreaks (Hogg 2013; Snyder et al. 1996). Nevertheless, captive breeding programs play an important role in conservation management of many species, but their implementation should be carefully designed (Hogg 2013).

Methodology

Study species

Dibblers have a predominantly insectivorous diet, a crepuscular nature and inhabit areas of dense unburnt vegetation (Friend 2003; Miller et al. 2003; Moro 2003). Female dibblers have an annual oestrous period, breed in autumn and carry a single brood of up to eight pouch young (Dickman and Braithwaite 1992; Lambert and Mills 2006; Mills et al. 2012; Woolley 1991). On Boullanger and Whitlock Islands, males die after the first mating season in some years (Mills and Bencini 2000). This occurs more often on Boullanger Island than Whitlock Island (Mills and Bencini 2000), but was not observed during the initial release of dibblers on Escape Island (Moro 2003). In mainland populations, males survive well into their second year (Friend 2003), and no facultative male die-off has been observed in captivity (Dickman and Braithwaite 1992; Mills and Bencini 2000; Mills et al. 2012; Woolley 1991). While facultative male die-off appears to be a consequence of an extreme mating strategy in response to highly seasonal and limited breeding periods (Fisher et al. 2013), reduced availability of food and nutrients may cause this phenomenon more frequently on Boullanger Island (Stewart 2006).

Study sites and tissue collection

This study focuses on three Jurien Bay island populations: two parental - Boullanger Island (26 ha) and Whitlock Island (5.4 ha), and one translocated - Escape Island (10.5 ha) (Figure 1a). Tissue samples (ear notches) from each island (Boullanger Island, N = 119; Whitlock Island, N = 118; Escape Island, N = 25) were collected as part of routine monitoring between 2013 and 2018. Samples were stored at room temperature in solutions of DMSO or 90% alcohol.

DNA extractions and genotyping

DNA was extracted using a salting out method (Sunnucks and Hales 1996) in 340 µL TNES buffer, 10 µL proteinase-K, and 3 µL RNase. DNA concentrations were measured from 20 samples chosen at random using a Qubit Fluorometer, and DNA quality was checked by running gel electrophoresis. Polymerase chain reaction (PCR) was completed using a QIAGEN Multiplex PCR PlusKit, with a one in ten dilution of all DNA samples and 14 microsatellite primer pairs (reaction details are provided in Table S1). PCR amplification was done using Eppendorf Mastercycler X50 and Eppendorf Mastercycler nexus thermocyclers, with the following cycling conditions: 95 °C for 15 minutes; a total of 30 cycles of 94 °C for 30 seconds, various annealing temperatures for different primers for 90 seconds (Table S1), 72 °C for 60 seconds; and concluded with 60 °C for 30 minutes followed by 25 °C for 60 seconds. PCR products were prepared in HiDi Formamide and GeneScan 500 LIZ size standard before being analysed with an ABI 3730 sequencer by the Western Australian State Agricultural Biotechnology Centre (SABC).

Scoring of genotypes was completed using the software GeneMapper 5 (Applied Biosystems). For validation and standardisation of genotypes, samples from Boullanger Island (n=5), Whitlock Island (n=5), and Escape Island (n=6) populations from 2012 or earlier were compared to an earlier study (Thavornkanlapachai 2016). Current genotypes were compared to original genotypes for these samples and allele calls were standardised across all loci for temporal comparisons.

Genetic analyses

To assess if null alleles were present within the populations, all loci were analysed with MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004). The genetic diversity of each population was assessed, including allele frequencies at each locus, allelic richness (an estimate of allele number per locus corrected for sample size), observed (H_O) and expected (H_E) heterozygosity. Deviations from Hardy-Weinberg Equilibrium were assessed by calculating inbreeding coefficients (F_{IS}) for each population. Positive F_{IS} values represent a deficit in heterozygosity while negative F_{IS} values represent an excess of heterozygosity. These metrics were analysed using FSTAT v2.9.3.2 (Goudet 1995) and GENALEX v.6.503 (Peakall and Smouse 2012; Peakall and Smouse 2006). Differences in allelic richness and heterozygosity values between populations were tested with Wilcoxon's rank sum test in R v3.5.1 statistical package (Team 2018), with samples paired by locus.

To measure genetic distances between populations, pairwise F_{ST} values were calculated using FSTAT v2.9.3.2 (Goudet 1995) and pairwise Jost's D_{est} values were calculated in GENALEX v.6.503 (Peakall and Smouse 2012; Peakall and Smouse 2006). Pairwise F_{ST} values measure genetic fixation or the amount of genetic drift between populations, whereas pairwise Jost's D_{est} measure allelic differentiation. For both, values can range from zero (low divergence) to one (high divergence). Clustering analysis using the program STRUCTURE v2.3.4 (Pritchard et al. 2000) was completed to visualise the genetic composition of the translocated population on Escape Island. As Escape Island was translocated 20 generations prior to our most recent cohort, we assumed allele frequencies were uncorrelated in our models but compared models that both included and excluded admixture. The number of clusters (K) was set from one to ten, and ten replicates were run per K tested, over 100,000 steps of the Monte Carlo Markov chain after a burn-in length of 10,000 steps. To confirm the best value for K, ΔK was estimated in STRUCTURE HARVESTER (Earl and Vonholdt 2012), where the largest ΔK value indicated the K value which was the best fit (Evanno et al. 2005).

Estimates of effective population size (Ne), defined as the size of an ideal population that will show an equal rate of genetic drift as the observed population, were generated in NeEstimator v2.1 (Do et al. 2014). A linkage disequilibrium model with random mating was selected, and the lowest allele frequency was set to 0.05 (Waples 2006). To detect the probability of a recent bottleneck event occurring within populations, data were analysed in BOTTLENECK v.1.2.02 (Cornuet and Luikart 1996), using the two-phase model (TPM), with the probability of the stepwise mutation model (SMM) set to 95% and variance set to 12, as recommended by (Piry et al. 1999). A one-tailed Wilcoxon sign rank test was used to determine excess heterozygotes.

To assess the number of heterozygous loci within each individual, a potential indicator of an individual's fitness, individual heterozygosity was measured as the proportion of heterozygous loci in an individual using in the software GENALEX v.6.503 (Peakall and Smouse 2012; Peakall and Smouse 2006). To determine if pairs of individuals had alleles identical by descent (IBD), Queller and Goodnight's (1989) pairwise relatedness (r) was estimated in GENALEX v.6.503 (Peakall and Smouse 2012; Peakall and Smouse 2006). Within-group means and 95% confidence intervals for each population were calculated using 999 permutations and 1000 bootstraps, respectively. Differences within populations across years were tested with Wilcoxon's rank sum test in R Core v3.5.1 (Team 2018).

Population viability analysis and sensitivity analysis

Population viability analysis was conducted in VORTEX v.10.3.2 (Lacy and Pollak 2014). VORTEX is an individual-based PVA program that utilises species life history traits and stochastic environmental factors to predict outputs such as the probability of extinction, population growth rate and genetic diversity (Lacy 1993). A baseline PVA model for island dibblers (Thavornkanlapachai 2016) was refined by consulting experts (Tony Friend, pers. comm.) and literature (Lambert and Mills 2006; Mills and Bencini 2000; Mills et al. 2004; Moro 2003). A summary of demographic parameters used in models is provided in Table 1 and further justification in Table S2. Drought was incorporated as a catastrophic event as it drives population dynamics of other dasyurid species, such as the agile antechinus and the brush-tailed phascogale (Parrott et al. 2007; Rhind and Bradley 2002). Meteorological data indicate that the Jurien Bay area has periods of low rainfall at an average frequency of approximately eight years (Meteorology 2020). Thus, catastrophes at eight-year intervals that reduced reproduction and survival by 70% were implemented (Table 1, Parrott et al. 2007; Rhind and Bradley 2002). Population projections were for 100 years, and all models ran 500 replicates.

Sensitivity analyses, defined as the evaluation of how changes to life-history traits affect population growth or long-term viability (Beissinger and McCullough 2002; McCarthy et al. 1995), were implemented on the baseline model. Each parameter was tested sequentially while keeping all other parameters constant and included mortality rates for juveniles and adults (0% to 100%, in increments of 5%), population carrying capacity (0 to 300, in increments of 20), founder number (10 to 100 in increments of 10) and frequency of droughts (0% to 20% probability in increments of 2.5%).

Table 1. Demographic parameters for the dibbler population on Boullanger Island used for population viability analysis.

Parameter	Value
Reproductive system	Polygynous[a]
Inbreeding depression	NA
Age of first offspring	1 (10 months)[b]
Maximum age of reproduction	3[b]
Maximum lifespan	3[b]
Maximum no. of broods per year	1[a]
Maximum no. of progeny per brood	8[a]
Sex ratio at birth (% in males)	49.7[c]
% Adult females producing	90[d]
Mean (± SD) no. of progeny per brood	7.4 ± 0.1[e]
Mortality (± SD)	
0-1 years of age	59% ± 10[f]
>1 years of age	♂ : 35% (with 8-year facultative semelparity) ± 10[g] ♀ : 35% ± 10[c]
Catastrophe	1
Frequency of catastrophe (%)	12.5[g]
Severity (proportion of normal values)	
Reproduction	0.3[g,h]
Survival	0.3[g,h]
Initial population size	84[c,f]
Population carrying capacity (± SD)	100 ± 13[c]
Years modelled	100
No. of iterations	500

NA denotes not applicable. Superscripts denote sources of data. Justifications of demographic parameters for all modelled populations are presented in Table S2. [a]Lambert and Mills, 2006; [b]Mills and Bencini, 2000; [c]Tony Friend, pers. comm.; [d]Moro, 2003; [e]Mills et al. 2004; [f]Calculated – see Table S2; [g]Parrott et al., 2007; [h]Rhind and Bradley, 2002

Validation of baseline model

After sensitivity testing, a best-performing (i.e. demographically realistic) baseline model was used to estimate current population sizes, genetic diversity, and allele frequencies based on census data available for 2012 (Thavornkanlapachai 2016). Initial population sizes were set to 68, 29, and 26 for Boullanger, Whitlock, and Escape Islands respectively. Simulations were run for six years (500 replicates), and predictions from the model for 2018 were compared to empirical data collected in 2017/2018. This allowed for evaluation of how well the optimised baseline model captured short term (and by extrapolation, long term) viability and genetic diversity within the island populations.

Optimal harvesting models and translocation scenarios

One captive breeding population and one new population on Dirk Hartog Island were simulated, to reflect current management actions. As Perth Zoo can accommodate ten dibbler breeding pairs (Cathy Lambert, pers. comm.), 11 scenarios were simulated to determine the ideal harvest design to provide ten males and ten females for captive breeding, without detrimentally affecting the populations on Boullanger and Whitlock Islands. These scenarios ranged from harvesting ten males and ten females from one island only, to harvesting five males and females from each island. A further 11 scenarios were simulated as above, using Escape and Whitlock Islands as source populations.

To model a translocated population on Dirk Hartog Island, the same parameters were used for the translocated population established on Escape Island, but with a much larger estimated carrying capacity of 10,000 based on island size. As female dibblers can produce a maximum of eight offspring per brood, and the captive breeding program will run for two years, up to 160 dibblers could be available for translocation to Dirk Hartog Island released over two consecutive years (Saul Cowen, pers. comm.). Allowing for some captive mortality and retention of adults for a second breeding season, release groups of 30, 40, 50, 60 and 70 juveniles per year were modelled to determine the impact of varying founder size on survival probability and genetic diversity.

Modelling the current captive-breeding and release program

In 2018, nine dibblers from Whitlock Island (five females, four males) and five dibblers from Escape Island (two females, three males) were opportunistically captured for Perth Zoo's latest captive breeding program. As seven female dibblers can produce up to 112 dibblers over two years, we modelled release groups of 20 (to account for the possibility of fewer dibblers being born in captivity than expected), 30, 40, and 50 juveniles per year at Dirk Hartog Island, and compare this scenario with the translocation scenarios we devised above. We made the same assumptions regarding captive mortality and retention of breeding pairs.

Findings

The percentage of polymorphic loci in each population was 7.1% (1/14) in Whitlock Island, to 64.3% in Boullanger Island (9/14) and 85.7% (12/14) in Escape Island. Since 2013, two loci had become monomorphic in two populations (locus Pa2D4 in the Boullanger Island population, and locus 4.4.10 in the Whitlock Island population). Analysis of markers in MICROCHECKER confirmed that no loci contained null alleles.

Overall, the Escape Island population had the highest allelic and genetic diversity (Table 2 and Figure 2). Across all populations, expected heterozygosity (H_E) was consistently lower than observed heterozygosity (H_O). Pairwise comparisons indicated that allelic richness, H_O and H_E values in the Whitlock Island population were significantly lower than both Boullanger and Escape Island populations (Wilcoxon's rank sum test; $P < 0.05$). In contrast, there were no significant differences in allelic richness, H_O or H_E values between Boullanger and Escape Island populations. Inbreeding coefficients (F_{IS}) ranged between -0.14 to 0.11, but no F_{IS} value was significantly different from zero after correction for multiple comparisons. Mean values of individual heterozygosity were greatest in Escape Island, lowest in Whitlock Island and declined over time in all three populations (Figure 2b and Figure S1). Overall, mean values of Queller and Goodnight's pairwise relatedness (r) across multiple years in the Whitlock Island population were similar to a full-sib relationship (r = 0.83 – 0.92), which was substantially higher than Boullanger Island (r = -0.02 – 0.12) and Escape Island (r = -0.05 – 0.24) (Figure 2d). The greatest temporal change in relatedness was a reduction in the Escape Island population from 2014 (r = 0.236, approximately half-sib relatedness) to 2018 ($r \approx 0$; see Figure 2).

Baby dibblers. Image: Perth Zoo

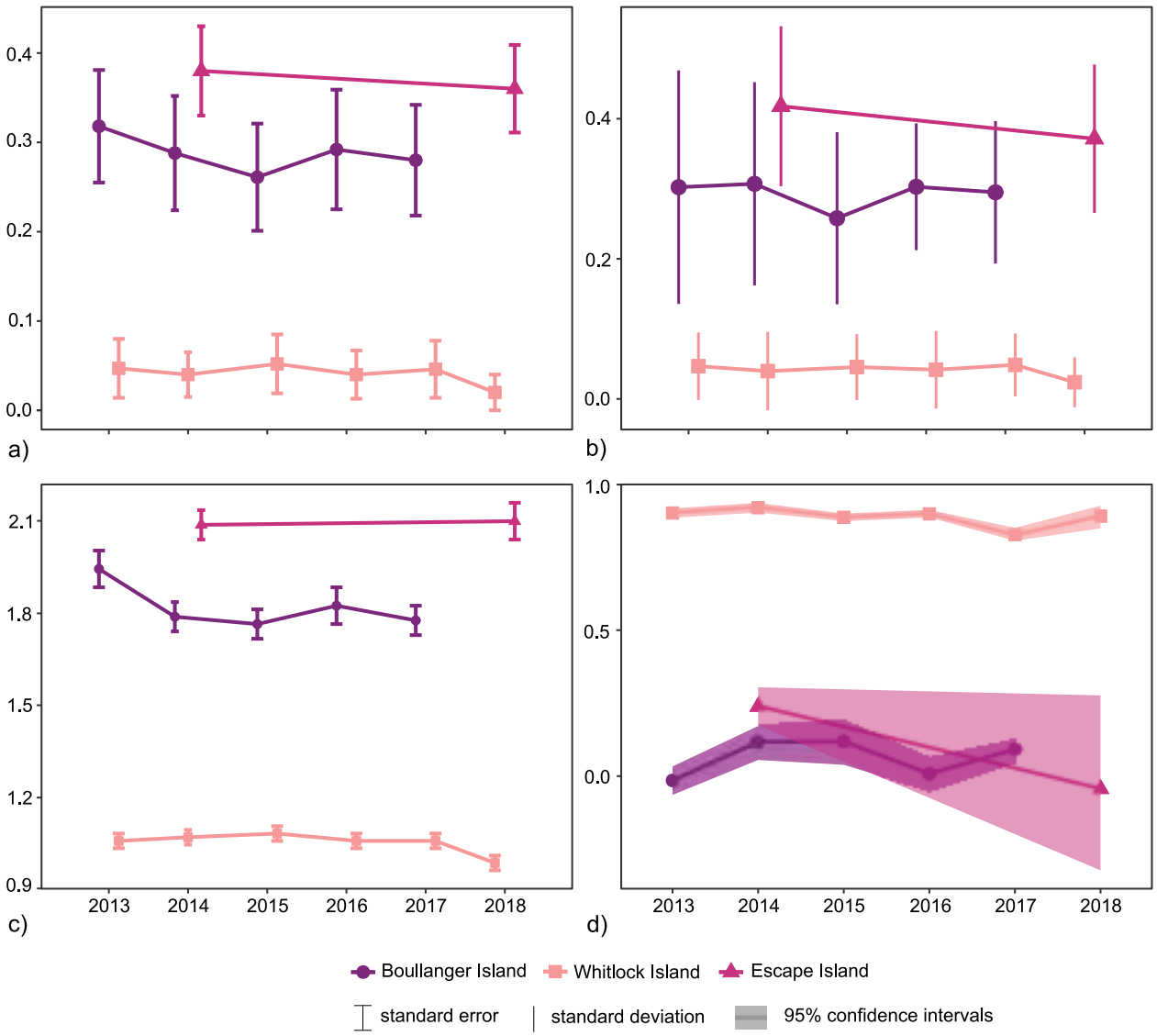

Figure 2. Changes in genetic diversity in island dibbler populations over time. a) expected heterozygosity, b) individual heterozygosity, c) allelic richness, d) relatedness

Table 2. Genetic diversity in dibblers on Boullanger, Whitlock, and Escape Islands. N: number of individuals with genotypes; NA: mean number of alleles per locus, N_{AR}: allelic richness, H_O: observed heterozygosity; H_E: is expected heterozygosity; F_{IS}: inbreeding coefficient. Standard errors for means are presented in brackets.

Population	N (± se)	NA (± se)	N_{AR} (± se)	H_O (± se)	H_E (± se)	F_{IS} (± se)
Boullanger Island						
2013	22.7 (0.1)	2.00 (0.21)	1.87 (0.05)	0.31 (0.06)	0.32 (0.06)	0.04 (0.02)
2014	20.8 (0.1)	1.86 (0.21)	1.74 (0.04)	0.31 (0.08)	0.29 (0.06)	-0.05 (0.03)
2015	18.0 (0.0)	1.86 (0.21)	1.72 (0.04)	0.26 (0.07)	0.26 (0.06)	0.06 (0.02)
2016	25.0 (0.0)	1.86 (0.21)	1.77 (0.05)	0.30 (0.29)	0.29 (0.07)	-0.02 (0.02)
2017	31.8 (0.1)	1.86 (0.21)	1.73 (0.04)	0.29 (0.06)	0.28 (0.06)	-0.07 (0.02)
Whitlock Island						
2013	19.8 (0.2)	1.14 (0.10)	1.13 (0.02)	0.05 (0.03)	0.05 (0.03)	0.00 (0.11)
2014	18.0 (0.0)	1.21 (0.11)	1.14 (0.02)	0.04 (0.02)	0.04 (0.03)	0.01 (0.04)
2015	21.8 (0.2)	1.21 (0.11)	1.15 (0.02)	0.05 (0.03)	0.05 (0.03)	0.11 (0.15)
2016	23.9 (0.1)	1.14 (0.10)	1.13 (0.02)	0.04 (0.03)	0.04 (0.03)	-0.03 (0.10)
2017	25.0 (0.0)	1.14 (0.10)	1.13 (0.02)	0.05 (0.03)	0.05 (0.03)	-0.06 (0.06)
2018	9.0 (0.0)	1.07 (0.07)	1.07 (0.02)	0.02 (0.02)	0.02 (0.02)	-0.14 (0.00)
Escape Island						
2014	20.0 (0.0)	2.07 (0.20)	1.99 (0.04)	0.42 (0.06)	0.38 (0.05)	-0.08 (0.02)
2018	5.0 (0.0)	2.00 (0.18)	2.00 (0.05)	0.37 (0.06)	0.36 (0.05)	0.04 (0.03)

Pairwise F_{ST} and pairwise Jost's D_{est} values indicated that all populations were significantly differentiated from each other ($P < 0.01$) (Table 3). The Escape Island population had a smaller genetic distance to Boullanger Island, relative to its genetic distance to Whitlock Island (Table 3). Clustering and ΔK analysis showed that the island populations form two distinct genetic groups (K = 2), and models that included admixture showed better convergence and greater absolute probabilities. Dibblers from the 2014 Escape Island population reflected the expected admixture given their founders but had greater genomic contribution from Boullanger Island (69%). By 2018, representation of the two parental populations was more even in the Escape Island population (55% Boullanger Island versus 45% Whitlock Island), although the sample size was low (n = 5) (Figure 1b).

Boullanger and Escape Island populations showed significant deviation from the mutation-drift equilibrium (Wilcoxon's one-tailed test; $P < 0.05$), suggesting that a bottleneck event has occurred recently within these populations (Table 4). No significant bottleneck event was detected in the Whitlock Island population (Table 4), although it is not possible to generate a reliable probability based on only one polymorphic locus with a one-tailed Wilcoxon sign rank test (or any tests run in BOTTLENECK Piry et al. 1999). Estimates of effective population size (Ne) were low for Boullanger and Escape Islands but could not be determined for most Whitlock Island populations (Table 4). The upper confidence intervals of infinity in Whitlock and Escape Island populations (Table 4) were likely due to low power resulting from low sample size (Escape Island 2018), small number of polymorphic markers (Whitlock Island all years) or a combination of both (Whitlock Island 2018) (Waples and Do 2010).

Table 3. Pairwise distance values for dibblers from Boullanger, Whitlock, and Escape Islands. Pairwise F_{ST} are above diagonal and pairwise Jost's D_{est} below the diagonal. Values significantly greater than zero (P < 0.01) after correction for multiple comparisons are shown in bold.

		Boullanger Island					Whitlock Island						Escape Island	
Population	Year	2013	2014	2015	2016	2017	2013	2014	2015	2016	2017	2018	2014	2018
Boullanger Island	2013		0.02	0.02	0.00	**0.04**	**0.58**	**0.58**	**0.59**	**0.61**	**0.61**	**0.55**	**0.23**	**0.27**
	2014	0.01		0.02	0.00	0.02	**0.58**	**0.58**	**0.59**	**0.61**	**0.61**	**0.56**	**0.28**	**0.30**
	2015	0.01	0.01		-0.01	0.00	**0.60**	**0.61**	**0.61**	**0.64**	**0.64**	**0.58**	**0.27**	**0.31**
	2016	0.00	0.00	0.00		0.02	**0.57**	**0.58**	**0.58**	**0.60**	**0.60**	**0.55**	**0.27**	**0.30**
	2017	**0.02**	0.01	0.00	0.01		**0.53**	**0.54**	**0.54**	**0.56**	**0.56**	**0.52**	**0.27**	**0.29**
Whitlock Island	2013	**0.33**	**0.29**	**0.28**	**0.30**	**0.26**		-0.01	-0.01	-0.01	0.03	0.09	**0.44**	**0.47**
	2014	**0.34**	**0.30**	**0.29**	**0.31**	**0.28**	0.00		-0.01	-0.02	0.03	0.04	**0.44**	**0.48**
	2015	**0.34**	**0.29**	**0.28**	**0.31**	**0.27**	0.00	0.00		-0.02	-0.01	0.03	**0.45**	**0.48**
	2016	**0.34**	**0.30**	**0.29**	**0.32**	**0.28**	0.00	0.00	0.00		0.00	0.02	**0.47**	**0.52**
	2017	**0.34**	**0.30**	**0.29**	**0.32**	**0.28**	0.00	0.00	0.00	0.00		0.02	**0.47**	**0.52**
	2018	**0.36**	**0.32**	**0.31**	**0.34**	**0.30**	0.00	0.00	0.00	0.00	0.00		**0.40**	**0.42**
Escape Island	2014	**0.17**	**0.20**	**0.19**	**0.19**	**0.18**	**0.22**	**0.22**	**0.22**	**0.23**	**0.23**	**0.24**		-0.02
	2018	**0.19**	**0.20**	**0.20**	**0.20**	**0.18**	**0.12**	**0.12**	**0.13**	**0.13**	**0.13**	**0.14**	-0.02	

Table 4. Effective population size (Ne) estimates and results of testing for bottleneck events in populations of dibblers from Boullanger, Whitlock, and Escape Islands. N: sample size

Population	N	Ne (95% CI*)	Bottleneck?
Boullanger Island			
2013	23	6.6 (1.7 – 50.9)	Y
2014	21	7.6 (1.9 – 65.3)	Y
2015	18	2.7 (1.6 – 7.9)	N
2016	25	3.1 (1.4 – 13.7)	Y
2017	32	3.9 (2.4 – 10.9)	Y
Whitlock Island			
2013	20	∞ (0.0 – ∞)	N
2014	18	∞ (0.0 – ∞)	N
2015	22	22.6 (0.0 – ∞)	N
2016	24	0.6 (0.1 – 2.0)	N
2017	25	∞ (1.7 – ∞)	N
2018	9	∞ (∞ – ∞)	N
Escape Island			
2014	20	17.6 (3.4 – ∞)	Y
2018	5	17.8 (1.5 – ∞)	N

*95% CI values were estimated by jackknife re-sampling.

Population viability analysis

The baseline model predicted that for those populations that survive 100 years, Boullanger Island's population size remained relatively stable, Whitlock Island's population decreased (from 33 to 26) and Escape Island's population increased (from 21 to 33) (Figure 3). However, extinction rates for all three islands were high and increased over time. Population simulations predicted the average time to extinction for Boullanger, Whitlock and Escape Islands were 50, 34 and 35 years, respectively. The probability of survival after 100 years was 48% for the Boullanger Island population, 13% for the Escape Island population and 6% for the Whitlock Island population. Declines in the Boullanger Island survival probabilities over time were more gradual compared to Whitlock and Escape Islands (Figure 3). Genetic diversity was also projected to decline over time, again more gradually in Boullanger Island, and after 100 years all populations were estimated to have very low gene diversity (< 0.1; Figure 3), high observed homozygosity (> 0.9), and reduced numbers of alleles per locus (< 2).

19 day old dibbler fetuses. Image: Perth Zoo

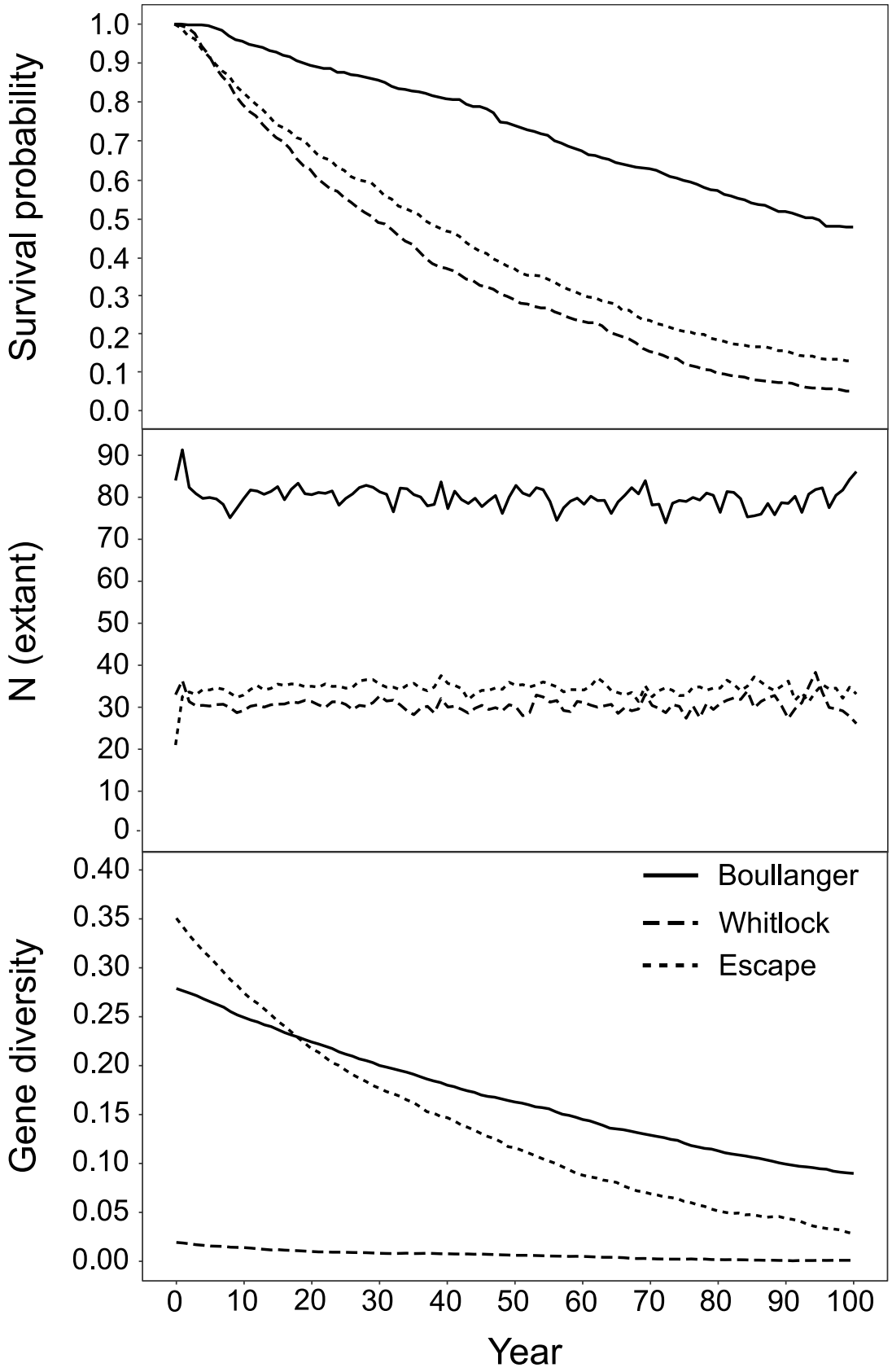

Figure 3. One hundred-year projections of survival probability, population size and gene diversity for the dibbler populations on Boullanger (solid line), Whitlock (large dashes), and Escape (small dashes) Islands.

The deterministic growth rate (r) of the Boullanger Island population was high and comparable to that of the Escape Island population (0.52 and 0.48 respectively), while the Whitlock Island population showed the lowest r value at 0.40. Linear regression on sensitivity testing showed that the frequency of droughts has the greatest impact on the survival of new dibbler populations while carrying capacity also has a strong effect. Founder size did not have a strong impact on survival. Sensitivity analyses are summarised in Figure 4.

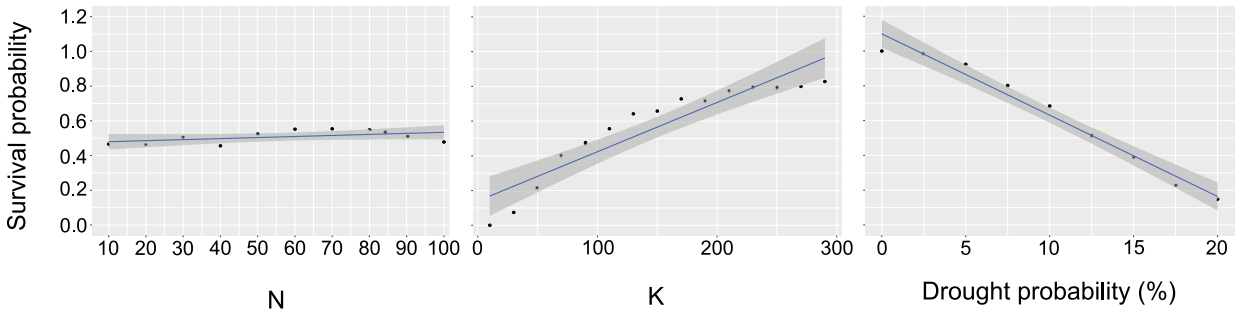

Figure 4. Regression analysis on the impact of three demographic parameters on survival probability in island dibbler populations. *Survival probability=0.0006 ×N+0.4737,R^2=0.24,p=0.126; survival probability=0.0028×K+0.1397,R^2= 0.86,p<0.001; survival probability=-0.0468 ×drought probability+1.0989,R^2=0.97,p<0.001; Standard errors are shown (ribbons)*

Models of recent population dynamics

PVA models parameterised with population size estimates and allele frequencies from year 2012 predicted similar values to the observed 2017/2018 estimates of population size, gene diversity, and number of alleles for all three island populations (Tables 2 and 5). Concordant with the observed values, the Whitlock Island population had the lowest predicted gene diversity values, whereas Escape Island had the highest. The 6-year probability of survival was 0.98 in Boullanger Island, 0.91 in Escape Island and 0.90 in Whitlock Island.

Table 5. Population parameters for island dibblers in 2018 estimated by population viability analysis. H_E: expected heterozygosity.

Island	Population size (SE)	Number of alleles (SE)	H_E (SE)	Inbreeding[1] (SE)
Boullanger	80.2 (1.5)	1.87 (0.00)	0.30 (0.00)	0.70 (0.00)
Whitlock	31.4 (0.8)	1.17 (0.00)	0.05 (0.00)	0.95 (0.00)
Escape	35.1 (0.8)	1.90 (0.00)	0.32 (0.00)	0.66 (0.00)

[1]Inbreeding calculated as 1 – observed heterozygosity (H_O)

Models of harvesting and translocation scenarios

The best scenario for establishing a captive breeding program of ten breeding pairs from the parental populations was to harvest 14 dibblers (7 males, 7 females) from Boullanger Island or Escape Island, and six dibblers (3 males, 3 females) from Whitlock Island (scenario 8, Table 6). This scenario maintained a high probability of survival in both source populations in the years immediately following harvest and produced the highest genetic diversity for the captive bred population. While harvesting more than 14 individuals from Escape Island slightly increased genetic diversity in the captive population, survival of the Escape Island population drops sharply when more than 14 individuals are harvested.

Based on these translocation scenarios, harvesting 80 individuals from the captive breeding program over two years (40 per year) should be the lower threshold to found a population on Dirk Hartog Island when Boullanger and Whitlock Islands are the source populations, due to slight increases in gene diversity and a survival probability over 90% (Table 7). When Escape and Whitlock Islands are the source populations, harvesting a minimum of 80 individuals from the captive breeding program over two years should also be the lower threshold (Table 7). Initially genetic diversity in Dirk Hartog Island is higher than both source populations if Boullanger and Whitlock Islands are mixed; if Escape and Whitlock Islands are mixed initial genetic diversity in Dirk Hartog Island is only higher than Whitlock Island. However, after 100 years, genetic diversity on Dirk Hartog Island is substantially higher (0.26) than 100-year projections for all source populations (0.00 to 0.09, Figure 3).

Table 6. Impact of 11 harvesting scenarios for a captive breeding program on source populations. An equal sex ratio is assumed at harvest. Gene diversity is equivalent to expected heterozygosity. Numbers to the left of the forward slash represent translocations using Boullanger Island, whereas numbers to the right represent translocations using Escape Island.

Population	Scenario	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9	SC10	SC11
	# adults harvested	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
Boullanger Island / Escape Island	P(survival) one year post-harvest	1.00 / 0.98	1.00 / 0.95	1.00 / 0.93	1.00 / 0.89	1.00 / 0.88	1.00 / 0.88	0.99 / 0.87	0.98 / 0.88	0.97 / 0.80	0.96 / 0.83	0.95 / 0.75
	P(survival) two years post-harvest	1.00 / 0.97	1.00 / 0.92	1.00 / 0.91	1.00 / 0.88	0.99 / 0.87	0.99 / 0.87	0.99 / 0.85	0.97 / 0.87	0.97 / 0.79	0.95 / 0.81	0.95 / 0.74
	# adults harvested	**20**	**18**	**16**	**14**	**12**	**10**	**8**	**6**	**4**	**2**	**0**
Whitlock Island	P(survival) one year post-harvest	0.89 / 0.83	0.86 / 0.86	0.87 / 0.87	0.88 / 0.88	0.87 / 0.90	0.90 / 0.90	0.91 / 0.90	0.94 / 0.95	0.97 / 0.94	0.98 / 0.99	1.00 / 1.00
	P(survival) two years post-harvest	0.88 / 0.83	0.85 / 0.85	0.87 / 0.86	0.87 / 0.87	0.87 / 0.89	0.89 / 0.88	0.90 / 0.89	0.91 / 0.94	0.96 / 0.92	0.95 / 0.97	0.99 / 0.98
Captive Breeding	Gene diversity one year post-founding	0.044 / 0.019	0.115 / 0.079	0.171 / 0.131	0.216 / 0.172	0.251 / 0.210	0.276 / 0.240	0.294 / 0.269	0.302 / 0.294	0.301 / 0.311	0.292 / 0.329	0.271 / 0.340

Table 7. Impact of founder size on reintroduced population of dibblers from captive breeding after 100 years, using optimal harvest scenarios from Table 6 (Scenario 8). $N_{founder}$ is the number of translocated individuals; N_{ext} is the number of individuals expected in a surviving population after 100 years; gene diversity is equivalent to expected heterozygosity (H_E).

Source populations	$N_{founder}$	60	80	100	120	140
Boullanger and Whitlock Islands	N_{ext}	7577	7665	7834	7692	7710
	P(survival)	0.89	0.91	0.94	0.93	0.94
	Gene Diversity	0.26	0.27	0.27	0.27	0.27
	Inbreeding[1]	0.74	0.73	0.73	0.73	0.73
Escape and Whitlock Islands	N_{ext}	7778	7729	8005	7936	7547
	P(survival)	0.89	0.89	0.87	0.89	0.90
	Gene Diversity	0.25	0.26	0.26	0.26	0.26
	Inbreeding[1]	0.75	0.74	0.74	0.74	0.74

[1]Inbreeding is calculated as 1 – H_O.

Modelling the current captive-breeding and release program

A model that simulated the recent harvest of island populations for captive breeding (nine individuals from Whitlock Island and five individuals from Escape Island) showed a minimum of 80 dibblers should be harvested from the captive breeding program over two years (40 per year) to found a population on Dirk Hartog Island (Table 8), as survival probability exceeds 80%. However, this projected probability of survival is lower than for the recommended scenarios across all founder sizes (Table 7). With 80 founders, at year one post-translocation genetic diversity of the Dirk Hartog Island population is projected to be initially higher (0.17) than the genetic diversity of the Whitlock Island source population (0.02) and not higher than the Boullanger (0.27) or Escape Island (0.32) source populations but will be higher than all source populations after 100 years, likely due to a large population on Dirk Hartog Island sustained by its greater carrying capacity. 100-year projections show that expected heterozygosity would be 38.4% lower for the Dirk Hartog Island population relative to the optimal scenario modelled (Table 7).

Table 8. Projected population parameters for Dirk Hartog Island after 100 years based on four sizes of a founder population from the current captive breeding program. $N_{founder}$ is the number of translocated individuals; N_{ext} is the number of individuals expected in a surviving population after 100 years; gene diversity is equivalent to expected heterozygosity (H_E).

$N_{founder}$	40	60	80	100
N_{ext}	7229	7768	7457	7787
P(survival)	0.77	0.79	0.81	0.81
Gene Diversity	0.16	0.16	0.16	0.17
Inbreeding[1]	0.84	0.84	0.84	0.83

[1]Inbreeding is calculated as 1 – H_O.

Discussion

Current genetic variation within island dibbler populations

Low genetic variation is common in island populations of many mammal species (Eldridge et al. 1999; Frankham 1997), in line with the results of this study. Genetic drift is expected to have a large impact on the genetic diversity of island dibblers, as smaller populations are more prone to drift than larger ones (Frankham 1997; Wright 1931). Escape Island now has the highest genetic diversity due to the population being admixed from Boullanger and Whitlock Islands (Moro 2003). Like other studies ((Kennington et al. 2012; Olson et al. 2013; White et al. 2018), this endorses the use of multiple source populations to achieve higher genetic diversity in translocated populations. Furthermore, the higher individual heterozygosity observed in Escape Island dibblers relative to dibblers on other islands, suggests this mixed population may have higher fitness (Di Fonzo et al. 2011; Foerster et al. 2003; Pujolar et al. 2005).

Despite relatively high heterozygosity, genetic diversity has declined in the Escape Island population since its establishment in 1998. The loss of genetic diversity is not unexpected as it is isolated with no gene flow. Drift decreases heterozygosity at the rate of 1/2Ne (Wright 1931), and if the population size remains small for multiple generations it is more likely to suffer large losses of heterozygosity (Weeks et al. 2015). Interestingly, the 2014 population on Escape Island had greater genomic representation from Boullanger Island than Whitlock Island, but representation was more even by 2018 (although the 2018 Escape Island population had a small sample size of 5). Genetic distances (F_{ST} and Jost's D_{est}) were also greater between Whitlock and Escape Islands than between Boullanger and Escape Islands in 2014. This representation bias in the Escape Island population towards Boullanger Island was also seen in early cohorts, but representation became more even by 2012 (Thavornkanlapachai 2016). This trend was explained by an unequal male mating success in captivity, as males from Boullanger Island are 20% larger than males from Whitlock Island (Mills et al. 2004; Thavornkanlapachai 2016), and heavier and younger males were observed to have higher mating success in captivity (Lambert and Mills 2006; Wolfe et al. 2000). With time on Escape Island representation became more equal suggesting factors other than body size are important for male mating success in the wild (Thavornkanlapachai 2016). However, our observation of a bias towards Boullanger Island by 2014 suggests a mating bias may be being maintained in the wild. As biased mating leads to a decrease in heterozygosity, an increase in inbreeding and a reduction in Ne (Hedrick et al. 2019), and has been reported in the wild in another small island marsupial (Thavornkanlapachai et al. 2019), confirmation of these observations would be relevant for future translocations.

Effective population size (Ne) estimates for island dibblers were small or undefined with evidence that two of the three island populations have undergone recent bottleneck events. However, facultative male die-off on Boullanger Island may cause an overestimation of recent bottlenecking events and underestimates of Ne. Therefore, the reduction in estimated Ne for Boullanger Island between 2012 and 2017 may be misleading. Infinite estimates of Ne generally suggest very large population sizes (Wang 2005; Waples and Do 2010), however this cannot be the case for Whitlock Island given the carrying capacity is 42. As more than five polymorphic loci are required to accurately predict Ne values of 100 using the linkage disequilibrium method (Waples and Do 2010), infinite estimates for Whitlock Island likely reflect the low power from the low number of polymorphic loci, for example there were only two polymorphic loci in the 2017 cohort. Overall, the Ne values for all island populations are much smaller than Ne values recommended to avoid inbreeding depression ($Ne \geq 100$ Frankham et al. 2014). While small Ne estimates do not mean populations will go extinct (see Eldridge et al. 1999), they suggest populations are more prone to the effects of genetic drift and loss of heterozygosity, and are therefore susceptible to inbreeding depression and the loss of adaptive potential (Frankham 2005).

Whitlock Island dibblers were found on average to be highly related to each other ($r > 0.8$), implying high levels of inbreeding and a deficit in heterozygotes. In contrast, negative inbreeding coefficients (F_{IS}) in the recently sampled Whitlock Island populations revealed an unexpectedly high heterozygosity, which can indicate a population has recently passed through a bottleneck (Luikart and Cornuet 1998). However, our tests did not support a bottleneck in Whitlock Island, and earlier studies have shown this population to have positive F_{IS} values (Mills et al. 2004; Thavornkanlapachai 2016). These contradictory results are likely due to the low power associated with few polymorphic loci available for this population (e.g. three loci in 2016 and one locus in 2018) in conjunction with the small sample size in 2018 (n = 9). Future studies with more informative genetic markers, such as single nucleotide polymorphisms (SNPs), will provide greater statistical power and can better resolve whether inbreeding is occurring.

Long term viability of current and future populations of island dibblers

Small isolated populations are susceptible to reductions in survival and gene diversity, especially in the absence of immigration (i.e. gene flow) (Frankham 1997; Lacy 1997; Lavery et al. 2013; Sigg 2006). They are also particularly susceptible to stochastic environmental factors, compared to larger populations or those reared in benign environments such as captive breeding facilities (Burgman et al. 1988; Frankham 1998). Here, we show that the survival probabilities and genetic diversities of dibbler populations on the Jurien Bay Islands are predicted to decline over time, and the smaller populations (Whitlock and Escape Islands) will likely expire within 50 years. These observations are consistent with observed downward population trajectories (Tony Friend, pers. comm.) and the reduction of genetic diversity in the last six years reported here.

Island dibbler populations appear to be particularly sensitive to the frequency of drought and carrying capacity. Although the exponential deterministic growth rates are relatively high, so too are the estimated rates of juvenile and adult mortality. High juvenile mortality is typical of mammals that mature early and produce large numbers of offspring after a short gestation (Promislow and Harvey 1990). If stochastic events such as drought occur more frequently, temporarily reducing reproduction and increasing mortality, and population recovery is constrained by limited carrying capacities, population sizes may fall below extinction thresholds. Concerningly, drought events will likely become increasingly severe as periods of low rainfall become more frequent in the future (CSIRO 2018; Harris et al. 2018). To compound matters, due to its extensive sand-based habitat, Boullanger Island is projected to erode as sea levels rise further reducing its carrying capacity (Tony Friend, pers. comm.). In contrast, the carrying capacities for Whitlock and Escape Islands are not expected to change over the next century, but the small size of these islands limits population growth, and so could substantially constrain long-term viability (e.g. Morita and Yokota 2002).

With an estimated carrying capacity of 10,000 on Dirk Hartog Island, the inclusion of island dibblers in the ecological restoration initiative will be critical for their long-term viability. As our evidence shows neutral genetic variation in island dibblers is declining, genetic management will be required to prevent further loss. Our PVA modelling indicates admixing island populations in a captive breeding program will maximise the genetic diversity and subsequent adaptive potential of the introduced population on Dirk Hartog Island. If the population establishes successfully, the ideal founder population (Scenario 8) will be able to capture higher genetic diversity relative to single source populations, and the predicted growth of the Dirk Hartog Island population to a census size above 6000 will facilitate the retention of this diversity.

Applications

The benefits of using PVA to optimise translocation designs have been demonstrated in many other threatened vertebrates, including banded hare-wallabies (White et al. 2020), woylies (Pacioni et al. 2019), golden bandicoots (Ottewell et al. 2014), pygmy rabbits (Zeoli et al. 2008) and frogs (Easton et al. 2019). The good agreement between historical empirical data and our simulations gives confidence that our models can predict demographic and genetic changes relatively accurately. We have therefore provided a management tool that can help predict outcomes of conservation actions, even in the absence of further empirical data. Furthermore, our sensitivity analyses show that the viability of dibbler populations are strongly influenced by carrying capacity and the frequency of drought. Consequently, these factors should be prioritised in the conservation management of Jurien Bay island dibblers, and which a move to Dirk Hartog Island will go some way to address.

The current captive breeding program implemented at Perth Zoo should result in higher genetic diversity in the new Dirk Hartog Island population, relative to the founder populations, and reach survival probabilities of 0.8 if 80 individuals or more can be released over two years. Harvesting these numbers would not be feasible without a captive breeding program due to i) the very low current census sizes of the Jurien Bay island populations, and ii) the logistical resources and time required to trap this number of dibblers in the wild. However, the program would benefit from replacing three Whitlock Island dibblers with nine Escape Island dibblers. This would increase genetic diversity in the translocated population by 53% and would reduce the threat of overharvesting on Whitlock Island. Due to downward population trajectories and the sensitivity of population viability on Whitlock Island to population crashes, we strongly advocate that harvesting is avoided during drought-driven periodic declines.

Impact

With empirical data showing reductions in genetic diversity and modelling projecting further declines into the future, as well as a steady decline in population survival for all islands, here we have provided evidence to support a rescue of the Jurien Bay island populations. Moving animals to Dirk Hartog Island, with an estimated carrying capacity of 10,000, via Perth Zoo will facilitate population growth, resilience to environmental stochasticity and retention of genetic diversity while minimising the impact on the fragile source populations. Our models will provide guidelines for numbers and origins of individuals for the current and future translocation programs.

Broader implications

Since European settlement in the early 1800s Australia has lost much of its' faunal diversity, and a further 106 mammalian species are at risk of extinction (Woinarski et al. 2015). With many threatened Australian species experiencing increased fragmentation and subsequently elevated extinction risk, devising strategies to maximize genetic diversity is increasingly being considered for long-term management. Dibblers are a prime example of these vulnerable species, as we predict that Jurien Bay island populations will decline over the next century. The use of PVA has provided important insights into the genetic management of the Jurien Bay island populations, as well as a framework for planning translocations to Dirk Hartog Island via a captive breeding program which, if successful, will provide a critical insurance population where relictual genetic variation can be maintained. Our approach can provide a guideline for the conservation management of other at-risk species.

Future research priorities

Future research priorities of island dibblers should include ongoing monitoring. For example, it would be particularly relevant to assess the potential for non-random mating in admixed translocated populations. Due to further reductions in survival and reproductive output inbreeding depression could have on the island populations, monitoring life history parameters associated with fecundity and longevity in source populations would also be informative, especially in the remnant Jurien Bay island populations.

A further area of research that is worth considering is the crossing of Jurien Bay island dibblers with dibblers from the mainland (e.g. Fitzgerald River National Park or Peniup Nature Reserve), which could potentially increase genetic diversity and population viability of the island dibblers. Mainland population sizes are larger and more genetically diverse than those on the Jurien Bay islands (Friend 2003; Mills et al. 2004; Thavornkanlapachai 2016), and should therefore be more robust to harvesting. Using them to supplement island dibblers could lead to a genetic rescue effect, which would increase genetic diversity, fitness and evolutionary potential, ultimately reducing the risk of extinction (Akesson et al. 2016; Frankham 2015; Heber et al. 2013; Hedrick and Fredrickson 2010; Weeks et al. 2017). However, several studies have found that crossing different populations or subspecies results in pre- and post-zygotic reproductive barriers, thereby reducing the overall fecundity and viability of species (Ramsey et al. 2003; Skoracka 2008; Vines and Schluter 2006). Outbreeding depression, where hybrid offspring are maladapted to environments and consequently suffer reductions in fitness could also occur (Edmands 1999; Frankham et al. 2011; Marr et al. 2002; Tymchuk et al. 2007). For example, the Jurien Bay Islands have been separated from the mainland for over 6500 years (Keighery et al. 2002), and presumably a greater time still has passed since gene flow occurred between the Jurien Bay dibbler populations and those 600 km away on the south coast of Western Australia. What's more, the two regions have different climates and habitats. Consequently, island dibblers have begun to diverge from mainland dibblers - mainland dibblers are significantly larger in body size and weight than island dibblers (Mills et al. 2004; Woolley 1991) meaning non-random mating could potentially occur if mainland and island dibblers were to inter-breed (Lambert and Mills 2006; Thavornkanlapachai 2016), and island dibblers have a shorter breeding season and gestation period relative to mainland dibblers, as well as mate and produce offspring later in the year (Friend 2003; Mills and Bencini 2000; Mills et al. 2012). In addition, it is also possible adaptation to different local habitats may have occurred. Hence, while there is an obvious rationale to mixing island and mainland dibblers to address low genetic diversity, we recommend the use of *ex situ* husbandry to determine the success of island x mainland crosses before this becomes a recommended management option. In particular, it will be critical to determine if hybrid females can produce viable, fecund offspring that show no evidence of outbreeding depression.

Data sets

Genotype data from 14 microsatellites and population viability analysis models are available upon request.

Recommendations

We recommend:

- Based on modelling projections, and without any other intervention, the movement of animals from Whitlock and Escape Islands is required for the survival of these populations
- To maximise genetic diversity of new island dibbler populations, island source populations should be mixed
- Mixing 14 individuals from Boullanger and 6 from Whitlock achieves an optimal balance of genetic diversity in the captive population and survival in the source populations
- A founder size of $\geq$ 80 for new populations is needed to achieve survival probabilities of $\geq$ 0.8, and captive breeding is required to facilitate this number
- A carrying capacity of at least 200 is required at new sites to achieve reasonable ($\geq$ 0.8) probability of survival
- Ongoing monitoring of island dibbler populations is required - to assess whether there is mating bias in admixed translocated populations, and inbreeding depression in parental populations
- Updating genetic analysis of island populations using more informative markers (SNPs) for more reliable estimates (esp. Whitlock Island)
- Escape Island could be used as a surrogate for Boullanger and Whitlock Islands in future translocations, but some supplementation from both parental populations may be necessary to retain all relictual genetic variation
- Monitoring data is incorporated into PVA at regular intervals to evaluate translocation outcomes against model predictions

Conclusion

In summary, this study has demonstrated how using both PVA models and genetic information can generate recommendations for ongoing and future conservation actions for threatened species such as the dibbler. The PVA model developed in this study, complemented with more informative genetic markers such as SNPs, should be used to evaluate the viability of the translocated population on Dirk Hartog Island, and to better inform ongoing management of the species. Expanding the modelling developed here to encompass all extant dibbler populations (i.e., island and mainland) will also be useful for predicting the long-term consequences of admixture between the more distantly related source populations. With many threatened Australian species experiencing increased fragmentation and subsequently elevated extinction risk, devising strategies to maximize genetic diversity is increasingly being considered for long-term management.

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Ethics statement

All samples for this study were collected by state government conservation agencies as part of routine monitoring processes and under appropriate approval from animal ethics committees.

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Appendices

Table S1. The 14 microsatellites used to characterise genetic diversity within island populations of dibblers.

Locus	Origin (species)	Repeat	Size (bp)	Multiplex	Anneal Temp (oC)	Primer sequence (5'-3')	Reference
Aa4A	*Antechinus agilis*	NA	165-167	1	46	F: TTTGATCCTCAGAGACTTGAT R: CCAAATCTACGTAAAATATCC	Banks et al. (2005)
DG1A1	*Dasyurus geoffroii*	$(AC)_{20}$	197-227	3	54	F: ATTTGCTTCTTGCTCCCTACAGC R: TTTCACTCCTTCTGAGTTTATCACC	Spencer et al. (2007)
3.1.2	*Dasyurus* spp.	$(CA)_{18}$	143-169	2	52	F: AGGAAACTTCACAAGTGTCGA R: ATTAATGACTCATCTGTTGTTGG	Firestone (1999)
3.3.1	*Dasyurus* spp.	$(CA)_{20}$	91-145	3	54	F: CAGCCCTTGAGTCTTGAGATT R: CATACCACCCCAGGAGTTTC	Firestone (1999)
3.3.2	*Dasyurus* spp.	$(CA)_{21}$	108-148	1	46	F: AATAGCAGAGACTCGATCC R: AGCCTTTATTACCTGGGAAG	Firestone (1999)
4.4.2	*Dasyurus* spp.	$(CA)_{19}$	70-110	1	56	F: GAAATCCAAGCTCATTTTAG R: AATCAACTCTGGAATGCATC	Firestone (1999)
4.4.10	*Dasyurus* spp.	$(CA)_{29}$	179-217	3	54	F: AATGCTAGATTTCACTCCC R: CCTCACATTTCTGGAACTG	Firestone (1999)
Pa1B10	*Parantechinus ap-icalis*	$(GAAA)_{46}$	277	4	58	F: AAGGAGGGATGGAGGAGGAA R: CAGTGTTCGAATGACATTGGCTAC	Mills and Spencer (2003)
Pa2A12	*P. apicalis*	$(GT)_{21}$	131	2	56	F: ATCCTGGAGAAGAGAAGACCTGC R: GTGGCTTATTCCATGCTTGTAGG	Mills and Spencer (2003)
Pa2B10	*P. apicalis*	$(GT)_{23}$	213	4	58	F: GAGAAAAAATATGCACAAGCACC R: AAGGAGAAAAAGTTAATACCATCCC	Mills and Spencer (2003)
Pa2D4	*P. apicalis*	NA	193-197	2	56	F: CAATCTGTCAATAACCTTCCCCC R: TGGAGGACCTCCAGAAAGTTAGC	Mills and Spencer (2003)
Pa4B3	*P. apicalis*	$(GT)_{15}$	121	4	58	F: GAAGGACAACATTCCCGATTGT R: CCTACCCTAATTGCAAATCCTTTC	Mills and Spencer (2003)
Pa7A1	*P. apicalis*	$(GAA)_{85}$	300	2	56	F: CTCCACCTCTCTAGACATGACCC R: TTTACTTGCTTTGTACTAGAGGCC	Mills and Spencer (2003)
Sh6e	*Sarcophilus laniarius*	$(CA)_6$ $(A)_2(CA)_{18}$	203-217	3	54	F: GATTCTAGAAGGGATAGCAAGC R: GACACTCCATAGAAATGCACTG	Jones et al. (2003)

Table S2. Demographic and life history traits of dibblers from Boullanger (BO), Whitlock (WH), and Escape (ES) Islands, as well as the proposed captive breeding program (CB) and Dirk Hartog (DH) Island populations, used in population viability analysis within the software VORTEX (Lacy and Pollak 2014). NA denotes not applicable. A description for each of the parameters are detailed below the table.

Parameter	*Parantechinus apicalis*
Scenario Settings	
Number of iterations	500
Number of years (timesteps)	100
Duration of each year in days	365
Extinction definition	Only one sex remains
Number of populations	BO = 1, WH = 1, ES = 1, CB = 1, DH = 1
Species Description	
Inbreeding depression - Lethal equivalents - Percent due to recessive lethal alleles	 NA NA
EV correlation between reproduction and sur-vival	0.5 (default)
EV correlation among populations	0.5 (default)
Dispersal	NA (closed populations)
Reproductive Systems	
Reproductive system	Polygynous
Age of first offspring	1
Maximum age of reproduction	3
Maximum lifespan	3
Maximum number of broods per year	1
Maximum number of progeny per brood	8
Sex ratio at birth – in % males	49.7
Reproductive Rates	
% adult females breeding	90
SD in % breeding due to EV	10
Distribution of number of offspring per female per brood - Mean - Standard Dev	 BO = 7.4, WH = 6.2, ES & DH = 7.0, CB = 7.12 BO = 0.1, WH = 0.23, ES & DH = 1.1, CB = 1.17
Mortality rates	
Mortality of females as % - Mortality from age 0-1 (± SD) - Annual mortality after age 1 (± SD)	 BO, WH, ES, DH = 59 ± 10, CB = 29.4 ± 10 35 ± 10
Mortality of males as % - Mortality from age 0-1 (± SD) - Annual mortality after age 1 (± SD)	 BO, WH, ES, DH = 59 ± 10, CB = 29.4 ± 10 BO = (((Y%8)<1)*65)+35 ± 10, WH, ES, CB, DH = 35 ± 10
Catastrophes	
Number of types of catastrophes	1
Frequency and extent of occurrence - Frequency %	 BO, WH, ES, DH = 12.5, CB = 0
Severity (proportion of normal values) - Reproduction - Survival	 BO, WH, ES, DH = 0.3, CB = 1 BO, WH, ES, DH = 0.3, CB = 1

Parameter	*Parantechinus apicalis*
Mate Monopolisation	
% Males in breeding pool	100
Initial Population Size	
Initial Population Size	BO = 84, WH = 33, ES = 21, CB & DH = 0
Distribution	Use stable age distribution
Carrying Capacity	
Carrying Capacity (K)	BO = 100, WH = 42, ES = 45, CB = 80, DH = 10000
SD in K due to EV	BO = 13, WH = 12, ES = 12, CB = 0, DH = 1000
Genetics	
Number of neutral loci to be modelled	15
Loci included in summary statistics	Additional loci only

Scenario Settings: Though dibblers reach sexual maturity by the age of 10 months, they are seasonal breeders and will only breed once every year. This is why the duration of each year in days is set to 365 (one calendar year).

Species Description: It is uncertain how inbreeding affects dibblers, or whether inbreeding does affect populations. It was decided that with such small population numbers, if inbreeding were to have any deleterious effects, they would have been purged from populations. Therefore, inbreeding was not selected in the model, making the lethal equivalent and the percentage due to lethal alleles not applicable.

Reproductive Systems: As dibblers become sexually mature by the age of 10 months (Mills and Bencini 2000), the age of first offspring is set to 1 year. Values for sex ratio at birth in Boullanger and Whitlock Islands were provided by Tony Friend (pers. comm.), the value stated in this table is the mean of these two values.

Reproductive Rates: The percentage of females breeding was obtained from (Moro 2003). As there were no available data on the environmental variation of female breeding, we have assumed a value of 10% to be the standard deviation. The distribution of number of offspring per brood for Boullanger and Whitlock Islands were obtained from (Mills et al. 2004); for Escape Island from (Moro 2003); for captive breeding from (Lambert and Mills 2006). As Dirk Hartog Island will be a new translocated population, current data is unknown. We have assumed that Dirk Hartog Island will be similar to Escape Island, an established translocated population that was formed from captive breeding Boullanger and Whitlock Island dibblers.

Mortality Rates: The value for mortality from age 0-1 years was estimated from juvenile mortality in captive-bred populations (Lambert and Mills 2006). The value presented for island populations was the captive-bred mortality multiplied by two. The estimated annual mortality rate of dibblers after age 1 was obtained from (Moro 2003). Facultative male die-off only occurs on Boullanger Island (Dickman and Braithwaite 1992; Mills and Bencini 2000; Wolfe et al. 2000), and is modelled using the formula "=(((Y%8)<1)*65)+35", which states that every 8th year, mortality of males will equal 100. As the frequency of facultative male die-off has not been quantified, we have assumed that the phenomenon occurs every eight years, based upon trends of low rainfall periods in the Jurien Bay region (Meteorology 2020). Because Dirk Hartog Island will be a new translocated population, current data is unknown. We have assumed that Dirk Hartog Island will be similar to the translocated population on Escape Island, in that there will be no facultative male die-off. As there is no available data regarding the environmental variation of mortality for dibblers, we have assumed the standard deviation of 10% for mortality in each age-group.

Catastrophes: Based on meteorological data of the Jurien Bay region (Meteorology 2020), trends show low rainfall periods occurring on average every eight years. It is unknown how severe the impact of droughts are on dibblers, though from studies on related species agile antechinus (Parrott et al. 2007) and brush-tailed phascogale (Rhind and Bradley 2002), survival and reproduction post-drought was on average 30%.

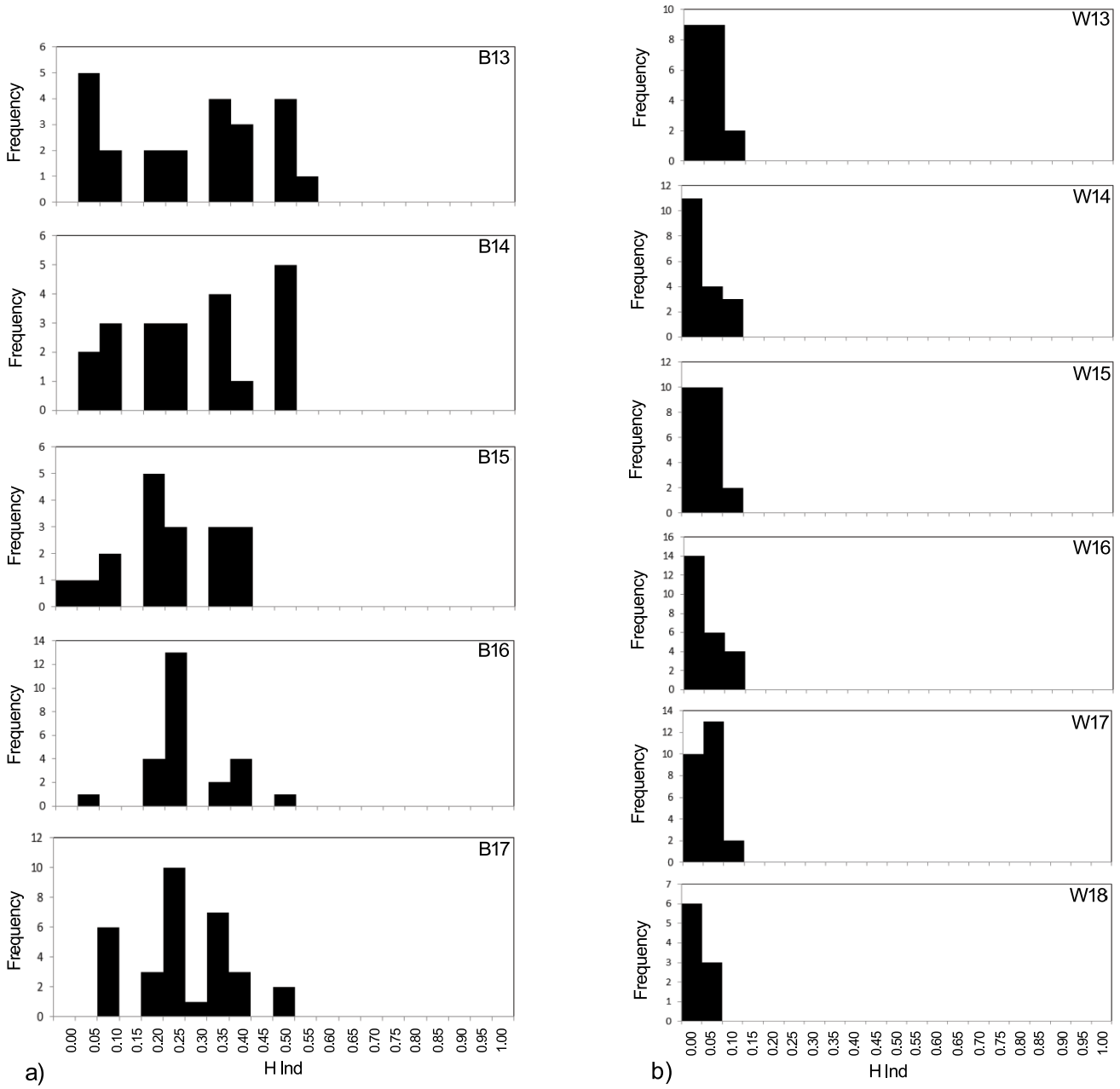
Mate Monopolisation: As dibblers are polygynous, and with small island sizes, it is assumed that all sexually active males will breed in the breeding season.

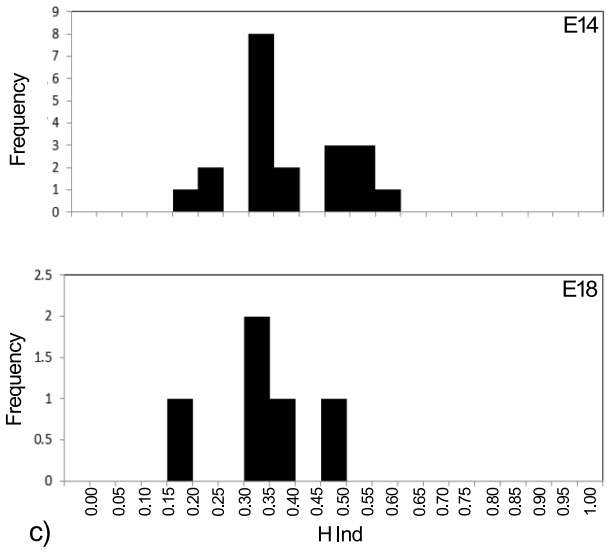
Initial Population Size: Current estimates of population sizes is calculated from the most recent monitoring event in June 2018, where dibblers on Boullanger and Whitlock Islands were trapped (Tony Friend, pers. comm.). Boullanger: assumed recent facultative male die-off event has occurred; five females with pouch young were trapped, with population size estimates to be twice this number. Assuming ten females produce 74 pouch young (adult males died post-mating), initial population size would be 84. Whitlock: trapped 22 individuals, population estimated to be 1.5 times this number. As it is unknown whether females with pouch young were trapped, initial population was set to 33. Escape: has not been monitored in recent years, but estimated to have around 20 individuals. Captive breeding population starts at zero in year 1, before being supplemented with ten mating pairs (20 individuals) in year 2. Dirk Hartog Island population starts at zero in year 1, and will be supplemented with a minimum of 60 individuals from a captive breeding population over years 3 and 4.

Carrying Capacity: The K for Whitlock Island was calculated from multiplying the largest known density of dibblers (Mills and Bencini 2000) with the size of the island. The K for Boullanger and Escape Islands were estimated based upon expert opinion (Tony Friend, pers. comm.). The SD in K for Boullanger and Whitlock Islands were calculated from the smallest known densities of dibblers (Mills and Bencini 2000) with the size of the islands. The SD in K for Escape Island mirrors Whitlock, based upon expert opinion (Tony Friend, pers. comm.) that Escape and Whitlock Islands are similar. The K for captive breeding was provided by Cathy Lambert (pers. comm.) and does not have variation, as Perth Zoo can only hold eight mating pairs and 64 pouch young per annum. The K for Dirk Hartog Island is an estimate provided by experts (Saul Cowen, pers. comm.). The SD in K for Dirk Hartog Island is unknown, and is assumed to be 10% of the estimated K.

Genetics: Allele frequencies at 14 microsatellite loci were chosen to model genetic diversity. These were the same loci initially chosen by (Thavornkanlapachai 2016). Because VORTEX models its own neutral loci, the number listed is 15 to account for the additional loci.

Figure S1. Frequency distributions of individual heterozygosity across time cohorts of a) Boullanger Island, b) Whitlock Island and c) Escape Island Jurien Bay island dibblers. BXX: Boullanger Island and year; WXX: Whitlock Island and year; EXX: Escape Island and year.





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Scientist Tony Friend with a dibbler. Image: Emma Massenbauer, DBCA

Dibbler. Image: Perth Zoo

Further information:
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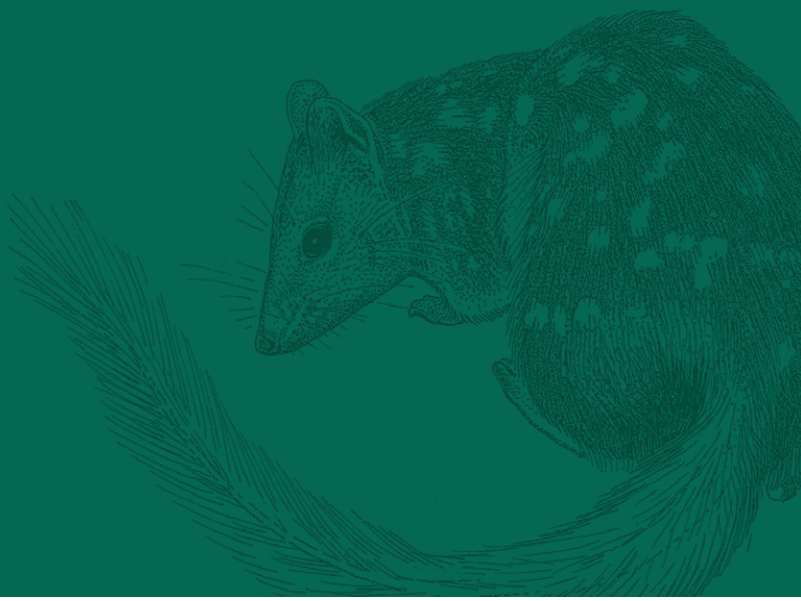

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