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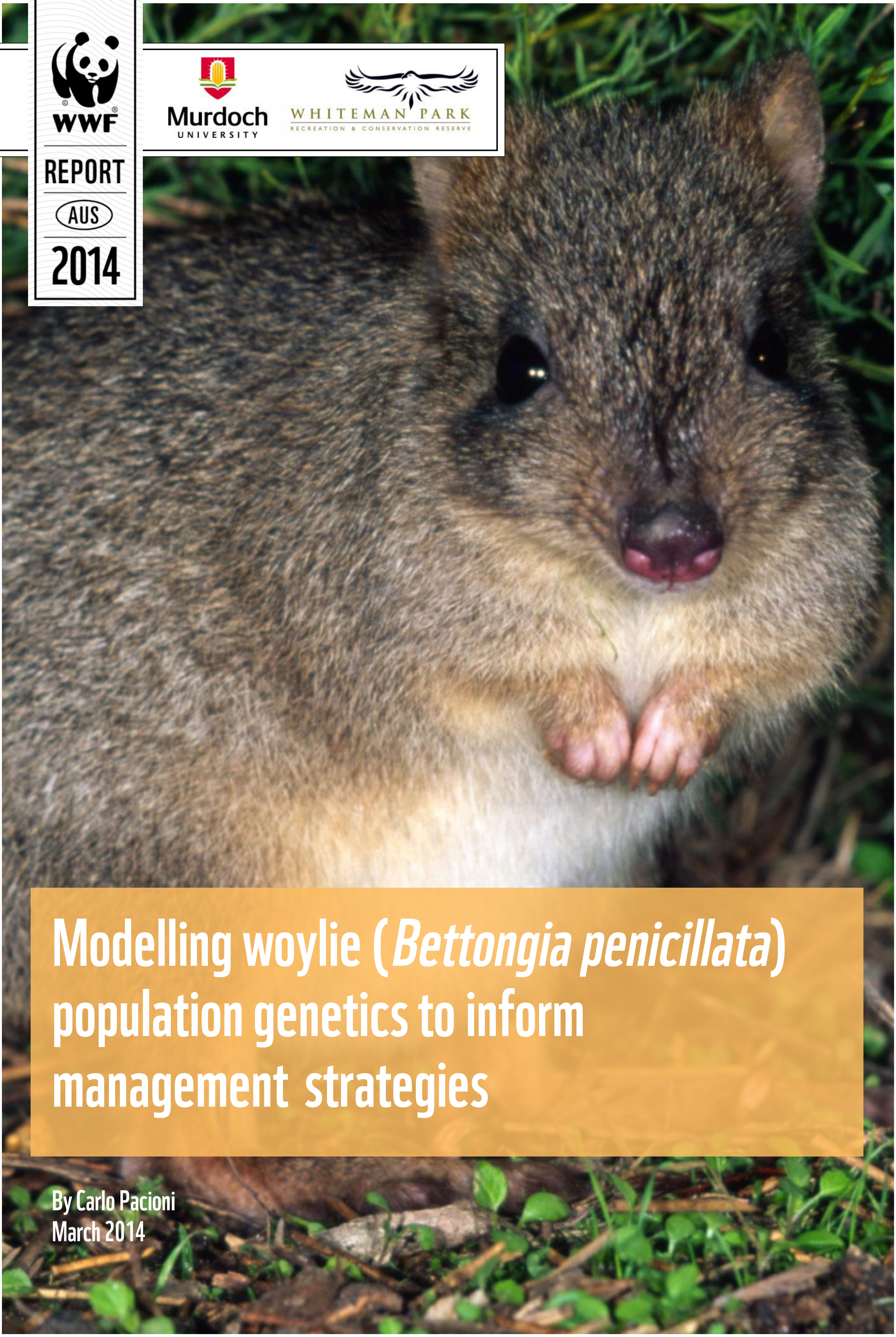

Modelling woylie (*Bettongia penicillata*) population genetics to inform management strategies

By Carlo Pacioni
March 2014

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Summary

The aim of this study was to assist the development of management guidelines for the woylie (*Bettongia penicillata*) designed to 'maximise the woylie genetic diversity at a species level', as prescribed by the Woylie Recovery Team (WRT). To meet this main conservation objective the WRT suggested the use of translocations between populations, and the establishment of an insurance population at Perup Sanctuary (PS). The aim for the insurance population at Perup Sanctuary is to capture, as much as possible, the genetic material currently present in other woylie populations.

A modelling framework was successfully developed to explore population and genetic dynamics associated with different management actions. Among the possible actions, a subset of 11 scenarios was investigated. The main results of this analysis include:

- An ongoing management strategy that involves the release of woylies from PS to the Kingston population and the supplementation of the former with woylies sourced from the Kanyana breeding program (Tutanning stock), Dryandra and Kingston appears to be the most promising management option among the ones tested. These results are in line with previous studies (e.g. Pacioni 2010; Pacioni *et al.* 2013) that indicated, when considering discrete populations, that a population of 1,000-3,000 individuals is needed to ensure long-term conservation. Any discrete population with a smaller size will incur substantial genetic loss in the investigated timeframes (20 years) and, therefore, require some level of active management.

- Some of the tested management strategies did not generate the expected genetic improvement in the recipient populations (see below for details). The survival of translocated animals is likely to be one of the most limiting factors in the success of the genetic augmentation achieved via translocation. Because adequate data for survival rate for translocated animals were not available at the time of analysis, values for this parameter were chosen arbitrarily and further research is recommended.

- If carried out regularly (e.g. yearly), the translocation of a few (1-4) individuals is adequate to improve the genetic profile of relatively small populations (less than 1,000 individuals). However, the same strategy only partially improves the genetic diversity of large (~3,000 individuals) and high density populations within the

investigated timeframe (20 years). While it remains to be tested, it is possible that this strategy may be adequate over a longer timeframe, or a larger number of individuals may need to be involved in the genetic augmentation of larger populations.

- Adaptive management of the populations (e.g. monitoring the genetic profiles of the populations involved in the translocation program at established intervals) is recommended to verify that the progress is on target or to adjust management responses accordingly.

The modelling framework that was developed includes 11 populations with at least one representative of each genetic stock (including South Australian stock). It is anticipated that this modelling framework will provide a useful platform where additional management options can be tested as the need arises.

Lastly, the following points were identified as main priorities for future modelling work:

- Sensitivity analysis for different supplement survival rates and functions
- Optimization (quantity and frequency) of supplementation strategy for either small or large populations as well as with or without presence of feral predators.
- Optimization of harvest strategy for PS (time, quantity and frequency)
- Investigate the optimal number of founders to be used to establish new populations.
- Inclusion of Dryandra as recipient population

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Acronyms and abbreviations

See also **Table 2: Details of modelled scenarios** and **Appendix II: Details of scenarios modelled in preliminary analyses** for abbreviations of scenario titles.

~	Approximately
AWC	Australian Wildlife Conservancy
Batalling	Batalling State Forest
DPaW	Department of Parks and Wildlife, Western Australia
DRY	Dryandra Woodland Nature Conservation Reserve
Dryandra	Dryandra Woodland Nature Conservation Reserve
KAN	Kanyana Wildlife Rehabilitation Centre
Kanyana	Kanyana Wildlife Rehabilitation Centre
KAR	Karakamia Sanctuary (run by AWC)
Karakamia	Karakamia Sanctuary (run by AWC)
KING	Kingston Forest (north-western compartment of the Upper Warren region)
Kingston	Kingston Forest (north-western compartment of the Upper Warren region)
NAR	Native Animal Rescue
NR	Nature Reserve
PER	Perup Nature Reserve (eastern compartment of the Upper Warren region)
Perup	Perup Nature Reserve (eastern compartment of the Upper Warren region)
PS	Perup Sanctuary
PVA	Population Viability Analysis
StPI	St Peter Island, South Australia.
VORTEX	Population Viability Analysis software
WMP	Whiteman Park
WRT	Woylie Recovery Team
WWF	WWF-Australia, also trading as World Wide Fund for Nature Australia

Background information and simulation details

The aim of this study was to assist the development of management guidelines for the woylie (*Bettongia penicillata*) designed to 'maximise the woylie genetic diversity at a species level', as prescribed by the Woylie Recovery Team (WRT). To meet this main conservation objective the WRT suggested the use of translocations, and the establishment of an insurance population at Perup Sanctuary (PS) that will be representative of the species' genetic diversity. The analyses conducted for this work were completed in June 2013. This study also attempted to include comparisons with data that became available after this date in order to make this report as informative and current as possible.

Description of the populations

Following recommendations from Perth Zoo, PS founders were selected to proportionally reflect Kingston Forest and Perup Nature Reserve population sizes. Trapping at these sites was conducted between November and December 2010, and 42 woylies (plus 18 pouch young) were released into the 420 ha predator-free Perup Sanctuary in December 2010. The sampling regime was designed to take advantage of the genetic spatial structure at Kingston Forest and Perup NR detected by Pacioni (2010) and, therefore, the PS founders were believed to be unrelated to each other.

Two additional colonies were established by sourcing animals in a similar fashion: one at Perth Zoo and one at Native Animal Rescue (NAR), a wildlife care facility in Malaga. Perth Zoo also received the last representatives from the Tutanning Nature Reserve woylie population. Upon the termination of the Perth Zoo program (August 2012), woylies from Upper Warren[1] previously housed in the zoo facilities were moved to Whiteman Park (WMP). Tutanning stock woylies were moved to another wildlife care facility, Kanyana Wildlife Rehabilitation Centre (Kanyana) to start a breeding program.

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[1] Note that the data provided from Perth Zoo at the time this model was developed indicated that only animals from Perup were moved to WMP, so the model presented here includes only Perup genetic stock in the simulated translocation between Perth Zoo and WMP. In fact, one of the 5 animals introduced to WMP was actually from Kingston. However, the author believes this results in only a minimal underestimation of the genetic profile of this population in the model. To avoid confusion, in the rest of the document these animals are referred to as Perup stock.

NAR only received wild animals from Perup and paired them with woylies from a wildlife carer in Roleystone. NAR is managing its woylie colony avoiding back crossing to limit genetic inbreeding.

In addition to the woylies from Perup NR, WMP sourced animals from Chidlow Marsupial Hospital and a wildlife carer in Wellard (Dryandra Woodland stock) (Pacioni 2012). The founders used to establish the WMP colony were selected based on their genotypes to maximise the genetic diversity of the colony and minimise inbreeding (Pacioni 2012). WMP plans to expand the reserve by adding 108 ha (giving a total of ~160 ha with an estimated carrying capacity of 200 woylies) within the next six months.

The north-western compartment of the Upper Warren region is referred to as Kingston (Kingston Forest), and the eastern compartment as Perup, following Pacioni *et al* (2011).

The other woylie populations included in this study are described elsewhere (Orell 2004; Pacioni *et al.* 2011; Pacioni *et al.* 2013; Wayne *et al.* 2013).

Modelling details

For the purpose of this study 'population' is defined as a (discrete) site where woylies are detectable. The term 'genetic stock' is used to indicate the genetic profile of a population. It should be noted that, while some populations may belong to the same genetic stock (e.g. Dryandra Woodland and the Australian Wildlife Conservancy's Karakamia Sanctuary), minimal differences in their genetic profiles may still be present, but these are of little significance and related to small differences in allele frequencies. Baseline genetic data were sourced from Pacioni *et al* (2011; 2013) and Pacioni (2012).

Nine neutral loci with different levels of polymorphism were monitored during the simulations. Expected heterozygosity and mean number of alleles were used as indices of genetic diversity. Nei's (1987) genetic distance D was used to calculate the genetic differentiation between populations.

Incomplete genetic data were available for NAR. It was known that 50% of the animals were sourced from Perup, but there were no data for the rest of the animals (although it is suspected that these may be from the Dryandra stock). Therefore, only the eight founders from Perup were included in the models as founders of the population.

Because the establishment of WMP and PS was prior to the Kanyana breeding program, short simulations (50 iterations) were used to generate the (average) population size and genetic make up of these populations at the time when the breeding program started (August 2012). Demographic data obtained from trapping data were used to set the initial population size and age classes of these populations. In the case of WMP, the animals received from NAR were added three months after the establishment of the WMP population (WMP unpublished records). Values obtained from these short simulations were then used to initialise[2] the PS and WMP populations for the final modelling presented here.

While potentially all woylie populations could have a role in the management of the species, based on the indications of the Woylie Recovery Team and further discussions with Dr Wayne and Dr Page, a limited subset of the populations were included in the simulated scenarios. An initial population size for the newly established population at Kanyana was created reflecting (as much as possible) the age and gender reported from trapping data. For the other (large) populations (i.e. Karakamia Sanctuary, Dryandra Woodland, Kingston Forest, Perup Nature Reserve, Batalling Forest, and St Peter Island) a stable demographic distribution among age classes was used. Carrying capacity and initial population sizes for the latter populations (Table 1) were selected in such a way that the mean population sizes (N) would reflect estimations from Wayne *et al.* (2013).

A virtual population was included in the simulation. This population, named 'Species', represents the species-wide genetic stock and was generated by merging the genetic profiles of the following populations: Perup, Kingston, Dryandra, Batalling, WMP, Karakamia, and St Peter Island (StPI). The initial population size was equal to 658 (i.e. the sum of genotyped woylies in the above mentioned studies), with a carrying capacity of 10,000 (considered large enough to limit genetic drift over the time frame investigated in this study) and with similar parameters to the wild populations (i.e. density dependent reproduction, density dependent feral predator predation and baiting program). By doing so, it was possible to calculate, at the end of each simulation, the genetic distance of each population from ‘Species’ (the virtual population representative of the actual species' genetic stock), hence providing an indirect measure of the success of each potential management option.

It is important to note that, while the 'Species' population is large, its actual population size is finite (Table 1) and, therefore, will be subject to genetic drift. The modelled genetic drift that is present in this population is by no means intended to represent the expected genetic

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[2] 'Initialise' refers to the initial values used to start the simulations. These may include initial population size, sex ratio, allele frequencies etc.

drift at a species level. The only purpose of this virtual population is to serve as a point of reference from which to evaluate whether the results of each scenario are close to the desired target level of genetic diversity.

The baseline PVA model developed in Pacioni (2010) was modified as follows and executed in VORTEX v10 (Lacy and Pollak 2013). Seasonal reproduction was modelled for Karakamia, WMP and StPI using the formula:

$$\left\{\frac{\left[89.3-(89.3-57)\times\left(\frac{N}{K}\right)^{16}\right]\times\left(\frac{\mathrm{N}}{0.1+\mathrm{N}}\right)-4}{2}+4\right\}+\frac{\left[89.3-(89.3-57)\times\left(\frac{N}{K}\right)^{16}\right]\times\left(\frac{\mathrm{N}}{0.1+\mathrm{N}}\right)-4}{2}\times\frac{sin(\pi\times Y)}{2}$$

where 83.9 and 57 represent the breeding rate at low and high density, respectively (see Pacioni 2010 for details on how these values are obtained and how the density dependent breeding rate is implemented in the PVA model); N = population size; K = carrying capacity; 4 is the maximum percentage of breeding females in the non-breeding season (Ward *et al.* 2008); sin =sine and Y = time-unit of the simulation. Continuous breeding was assumed in all the other populations. In all sites, reproduction was modelled as density dependent except for the Kanyana breeding program and NAR, where a fixed 90% reproduction rate was used.

Movements between populations were modelled using the modified 'DN dispersal' function in VORTEX and only the movements from the Kanyana breeding program to other populations were modelled with the functions 'Harvest' & 'Supplementation'. As opposed to previous simulations where movements between populations were modelled with a dispersal rate (see Appendix II), the option 'DN' now allows for a fixed (i.e. not conditioned to other variable) and absolute (i.e. not a rate) number of individuals to be moved (if animals are available).

Probability of dispersers' survival was modelled with a density dependent function where a 95% of survival was assumed when $\frac{N}{K}=0$ and it was proportionally decreased depending on the density of the recipient population - i.e. survival = $95\times(1-\frac{N}{K})$. It should be noted that, while this function is likely to be adequate for a population at high density (Van Weenen 1996), it probably generates an overestimated mortality rate for populations at low density. In fact, WMP data on the survival of actual 'supplements' (animals already introduced from other populations to supplement the resident population - Rafferty, unpublished data) suggest that survival (at current WMP density) is possibly substantially higher than that modelled. However, given that insufficient data are available to model this parameter, a conservative approach was preferred. For animals supplemented from Kanyana into PS, a fixed rate of 90% survival was assumed based on expert opinions, because PS is a predator-free (fenced), resource-rich environment; the animals to be moved will be young, healthy individuals; and only a small number will be introduced, hence reducing the likely effects of increased density (see page 26 Limitations of the Study).

In previous analyses (see Appendix II), Karakamia was used as both a source and recipient population. However, the Australian Wildlife Conservancy has not, to date, given approval to receive additional animals within the fenced sanctuary, so the focus of successive work (and this report) was on the use of other populations. Preliminary results mentioned above have been validated and are broadly reliable (see Appendix II).

The Batalling State Forest population was not used as source or recipient population in this model given the current management concerns, e.g. the likelihood of high mortality due to feral predators in this unfenced area. For the same reason, the Dryandra population was not included as potential recipient population. These populations were, nevertheless, integrated in the modelling framework to enable future modifications of the model should the inclusion of these populations as either source or recipient become a feasible option.

Natural migration rates from Kingston to Perup and *vice versa* were quantified as equal to 2.5% and 0.5% in a previous study (Pacioni *et al.* 2011). When applying these migration rates to the population size estimated after the decline by Wayne *et al.* (2013), no movements were detected within the Upper Warren region. Hence, to simplify the models, no natural migration between the two populations within the Upper Warren region was modelled.

Table 1. *Initial population size (N_i), mean population size (scenarios with no supplementations) and carrying capacity (K) of the modelled populations.*

Site	**Initial population size (N_i)**	**Mean population size (SE) (at the end of the simulation)**	**Carrying capacity (K)[b]**
Perup Sanctuary	42+18[a]	868 (14)	900
Whiteman Park	32	187 (2)	200 (70 the first 12 months)
Karakamia Sanctuary	565	571 (4)	600
Kanyana	6	0	20
Native Animal Rescue	8	0	20
Dryandra Woodland	2,700	3191 (61)	6,000
Kingston Forest	3,000	3200 (62)	6,000
Perup NR	5,000	5344 (104)	10,000
Batalling Forest	242	256 (5)	500
St Peter Island	3,000	2818 (18)	3,000
Species	658	5327 (104)	10,000

[a] pouch young

[b] the carrying capacity is somewhat artificial. This is because most functions are density dependent and, therefore, carrying capacity was selected to obtained the estimated population sizes (Wayne *et al.* 2013) at equilibrium (in the baseline model with no management).

The model only allows the movement of young animals (< 3 yrs) for supplementation purposes. No constraints on the sex ratio were applied, so the sex ratio of the supplemented animals is a reflection of the sex ratio of the source populations.

Preliminary analyses confirmed that, for the purpose of genetic augmentation, the removal of the offspring from the founders of the Kanyana colony (Tutanning stock) was preferable to back crossing offspring with the founders and subsequently moving a proportion of the resulting Kanyana colony. Therefore, the latter option was not included in further models.

Eleven scenarios (Table 2), over the next 20 years, were modelled starting in August 2012 unless otherwise specified.

Table 2. *Details of modelled scenarios.*

Scenario	Abbreviation	Description
1	NoSuppl	No populations received any supplementation.
2	KAN2PS	All offspring from Kanyana (Tutanning genetic stock) go to PS.
3	PS100outPER_KAN2PS	As 2 but 100 individuals are removed from PS into **Perup** before the first supplementation
4	PS100outKING_KAN2PS	As 2 but 100 individuals are removed from PS into **Kingston** before the first supplementation
5	PS100outPER_KANDRY2PS	As 3 with the inclusion of a supplementation of 40 individuals from Dryandra into PS
6	PS100outPER_KANDRYKING2PS	As 3 with the inclusion of a supplementation of 40 individuals from Kingston into PS
7	OG_PS100outPER_KANDRYKING2PS	Ongoing management option: as 6, but (100) animals are removed every nine* months from PS and released into **Perup**. These translocations are followed by a release of 40 individuals from Dryandra and 40 from Kingston into PS
8	OG_PS100outKING_KANDRYKING2PS	Ongoing management option: as 6, but (100) animals are removed every nine* months from PS and released into **Kingston**. These translocations are followed by a release of 40 individuals from Dryandra and 40 from Kingston into PS
9	NAR2WMP	Four (juvenile) woylies are released into WMP from NAR every six months.
10	WMP2StPI	As 9 with the addition of 10 woylies moved to StPI once a year
11	WMP_D2StPI	As 9 with the addition of 20 woylies moved to StPI once a year

* The selection of nine month interval is arbitrary, mainly due to the ease of implementing such intervals in the model and because it seemed an approximately realistic interval. This was chosen purely for demonstration purposes and is not intended as a prescription for management practice.

Results & Discussion

For simplicity, results were grouped by population. Only those scenarios that were relevant to each population were reported in each section - i.e. not all scenarios are reported in each section and figures (please note that the vertical axis is not consistent between figures).

Perup Sanctuary

Simulations suggest that PS will reach approximately 50% of its carrying capacity by the end of 2013 and 100% by mid-2015. These projections were generated running 500 iterations from the establishment of PS (December 2010) to August 2032 (Figure 1). The results implied that the mean probability of survival of supplements from wild populations at the beginning of the translocation program into PS is equal to 71% and will progressively decrease to less than 10% by mid 2015 (See also Appendix I). Based on data that became available after the analysis was conducted, this is probably a conservative value (at least for fenced areas). In fact, 83% of the founders were still alive in the surveys conducted between April and July 2013. (For animals sourced from Kanyana and introduced to PS later, the survival rate for the model was fixed at 90% - see Modelling Details.) The demographic projections are very close to actual trapping data that indicated a population size of 250-280 in April 2013 (Wayne unpublished data) when simulated data indicates 264 (SE=2) in March 2013.

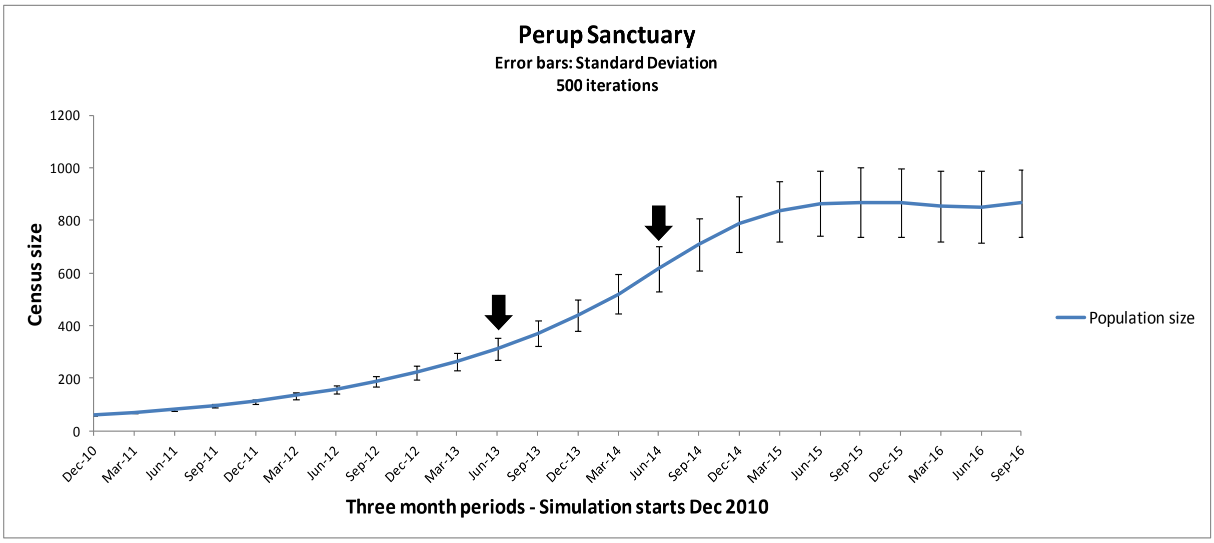

Figure 1. Demographic projections of Perup Sanctuary from establishment to 2016. Arrows indicate June 2013 and June 2014.

The inclusion of Dryandra and Kingston as source populations for the supplementation program of PS is a substantial improvement compared to the exclusive use of offspring from the Kanyana breeding program (Figures 2 and 3). Removing 100 individuals from PS before releasing 'supplements' does not seem to improve the genetic outcome at PS. It actually may cause, in the short term, a small reduction in the mean number of alleles. However, this option provides a number of additional benefits for other populations (see below), especially within the ongoing management scenario. Furthermore, the detrimental effect can be easily mitigated by removing fewer animals (a smaller number of animals can be used initially in the case of the ongoing management option as there is evidence that there is no negative effect of removing individuals after PS reaches carrying capacity. On the contrary, taking a larger number of animals from PS will positively influence the outcome for the recipient populations - see below). Based on these considerations, removing animals from PS before releasing supplements into PS remains a valid management action. The inclusion of Kingston in the supplementation program of PS (scenario PS100outPER_KANDRYKING2PS, see Table 2 for details) results in an improvement in PS's genetic profile as well as a reduction of the genetic distance between PS and 'Species' (Figure 4). Perup was not considered as a potential source population to supplement PS because PS is already considered a good representation of the Perup genetic profile.

The genetic improvement of PS is maximised if an ongoing management program is carried out (Figures 2 and 3). The ongoing management strategy will have the benefit of maximising the genetic diversity of the PS population as well as reducing the rate of the genetic drift. Clearly, most of the improvement occurs in the first few years and then, once most of the alleles are shared between the source and receipient populations, the ongoing supplementation option 'only' couteracts the genetic drift. The benefits of such an effect are not substantial in the timeframe modelled in this study, but most likely they will be relevent in the longer term. It may be possible that the frequency and number of animals to be moved into PS could be 'optimised' in order to maximise the benefit-cost ratio. The genetic improvement could be more substantial than that reported here should the survival of supplements be higher than the modelled rate.

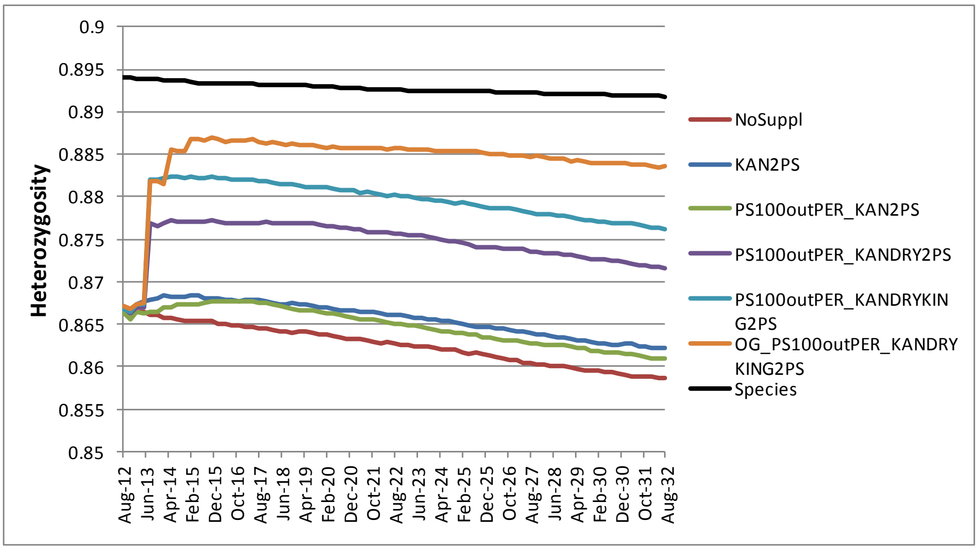

Figure 2. Perup Sanctuary mean expected heterozygosity trends over time. See Table 2 for abbreviations of scenarios in the legend.

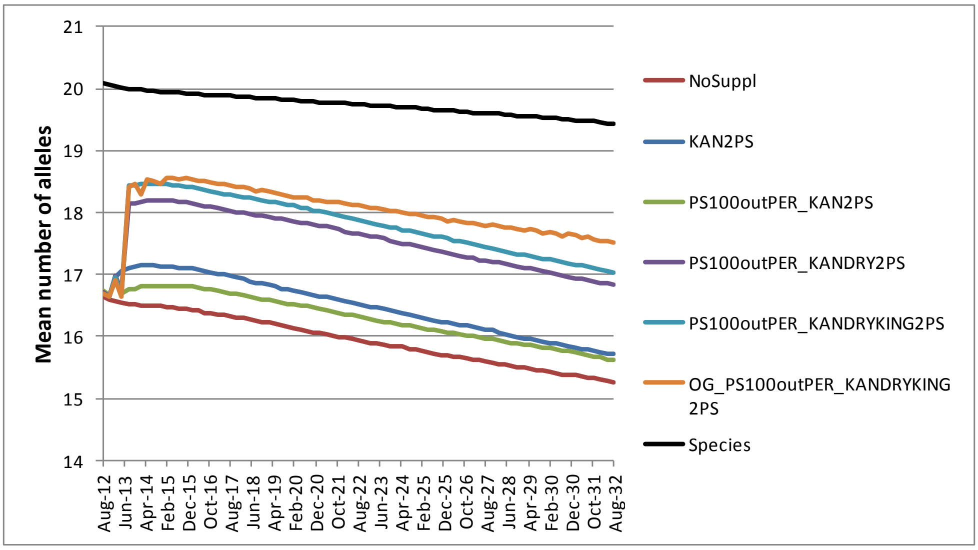

Figure 3. Perup Sanctuary mean number of alleles over time. See Table 2 for abbreviations of scenarios in the legend.

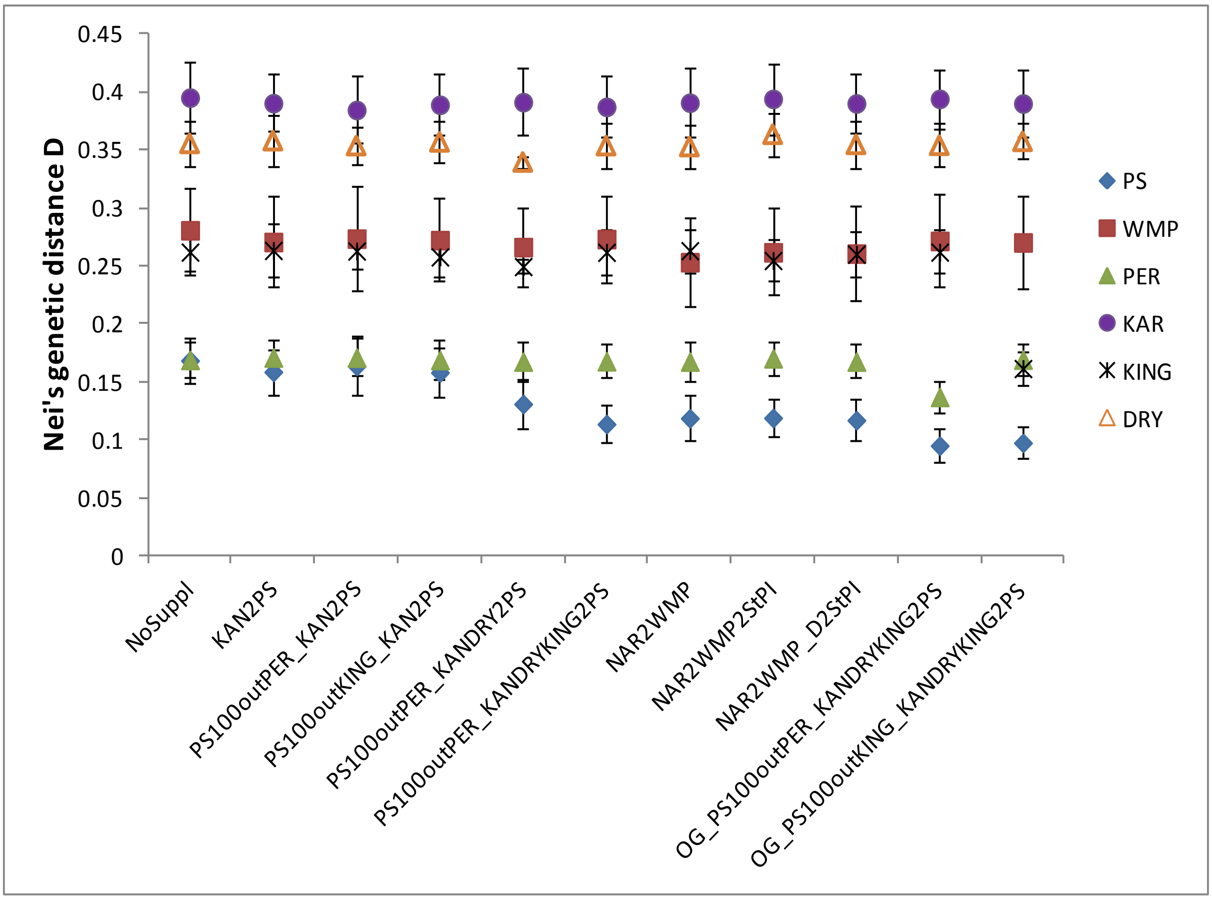

Figure 4. Mean Nei's (1987) genetic distance D between the modelled populations and the 'Species'. On the horizontal axis: modelled scenarios (see Table 2 for abbreviation).

PS = Perup Sanctuary; WMP = Whiteman Park; PER = Perup; KAR = Karakamia; KING = Kingston; DRY = Dryandra.

Perup Nature Reserve

There is no long-term genetic improvement from the one-off release of 100 woylies from PS into Perup NR. The only scenario that produces a detectable positive outcome over the modelled 20 years is the 'ongoing management option' (see Table 2). As explained above, optimising the frequency and number of animals moved between populations in the ongoing option was not attempted, but it could be investigated further to maximise the benefit-cost ratio.

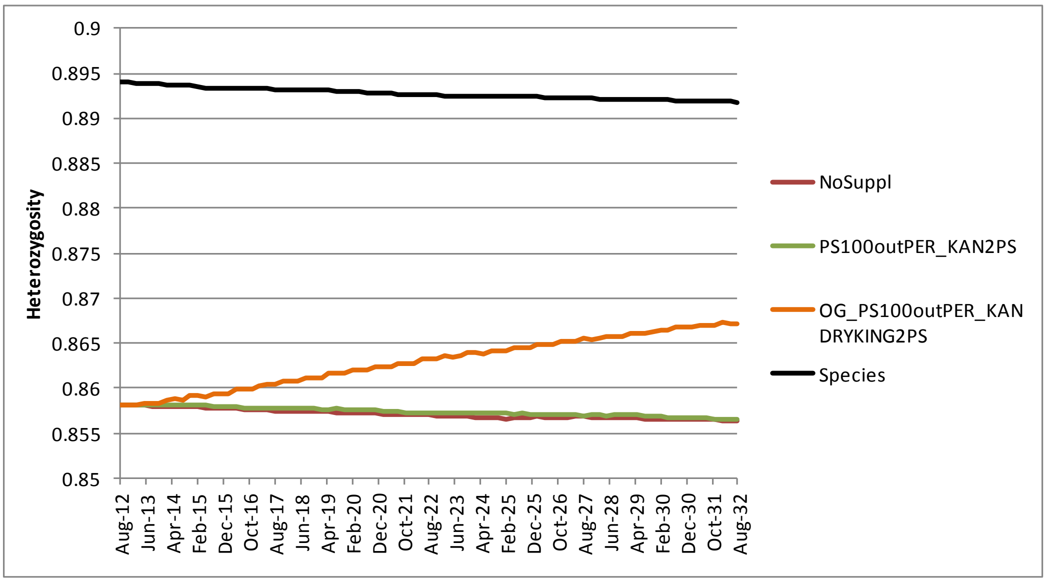

Figure 5. Perup mean expected heterozygosity trends over time. See Table 2 for abbreviations of scenarios in the legend.

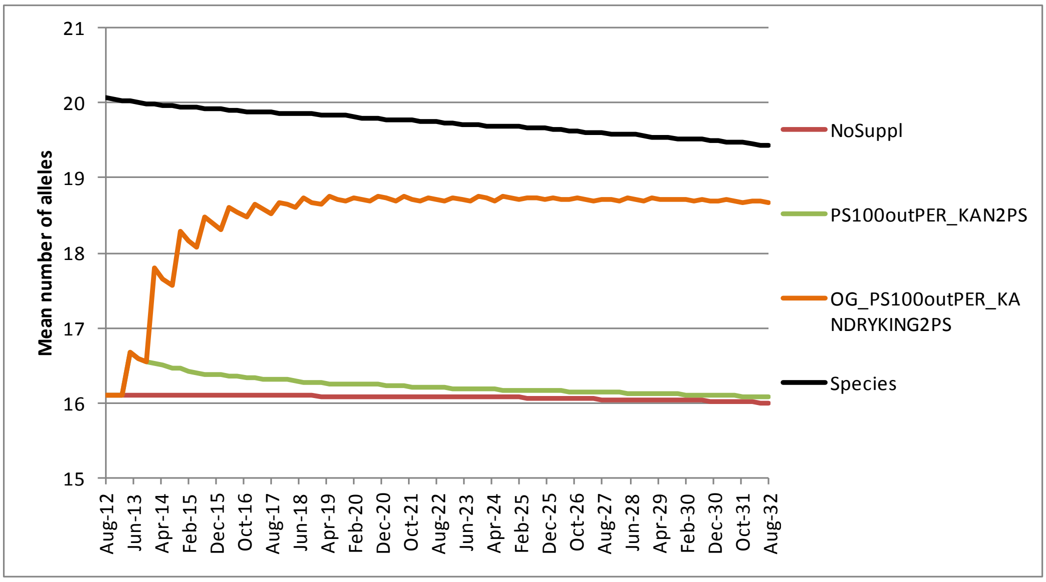

Figure 6. Perup mean number of alleles over time. See Table 2 for abbreviations of scenarios in the legend.

Kingston Forest

The Kingston population will benefit from both a one-off and an ongoing release of 100 woylies from PS into Kingston, mainly due to its genetic distance from the source population. Even the one-off option will introduce a large amount of highly diverse genetic material and its effect will be still detectable after 20 years. The model predicts that over 20 years the 'ongoing management option' will generate a genetic profile of similar genetic diversity to Perup despite the lower starting values (Figures 7 and 8).

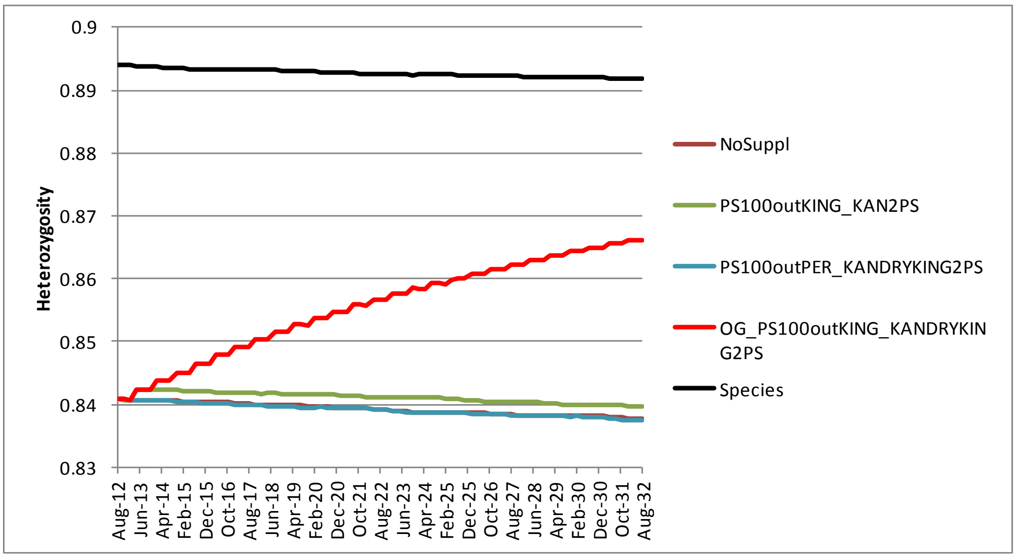

Figure 7. Kingston mean expected heterozygosity trends over time. See Table 2 for abbreviations of scenarios in the legend.

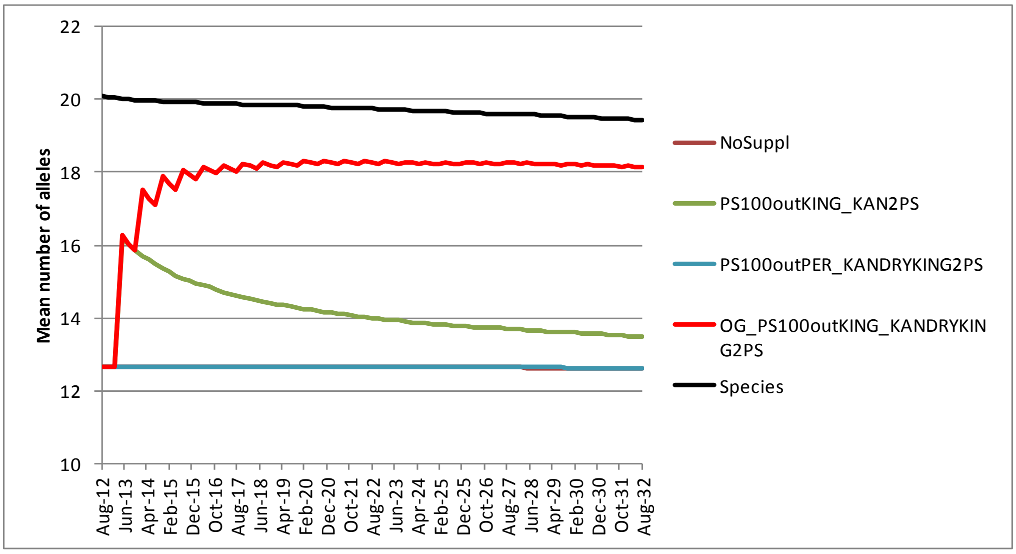

Figure 8. Kingston mean number of alleles over time. See Table 2 for abbreviations of scenarios in the legend.

Whiteman Park

WMP seems to receive very little improvement from the planned supplementations from NAR (Figures 9 and 10). This is probably a result of NAR's genotypes being well represented in WMP's founders. An additional reason for this somewhat unexpected result may be the limited survival of the supplements. In fact, supplements are successfully released almost exclusively during the first five years (time-unit=20. See Appendix I). Preliminary data from WMP (Rafferty unpublished data) indicates that, at least so far, the survival of supplements that have been introduced to the low density enclosure at WMP[3] has been in reality much higher than the survival rates modelled here but, for consistency, the same function was maintained.

Sourcing animals from WMP to supplement St Peter Island (StPI) has a slightly negative effect on the genetic profile of the WMP population (Figures 9 and 10), but not to such an extent to recommend the exclusion of this management action. The selection of WMP as a possible source population for the genetic 'rescue' of the South Australian populations was based only on logistical reasons: WMP is close to the airport, with staff on ground permanently and is a population with high genetic diversity. However, this scenario was developed only as a demonstration of the potential benefit of introducing new genetic material into the South Australian populations. Similar beneficial effects (see below) will be obtained also with other source populations if these were of comparable genetic diversity.

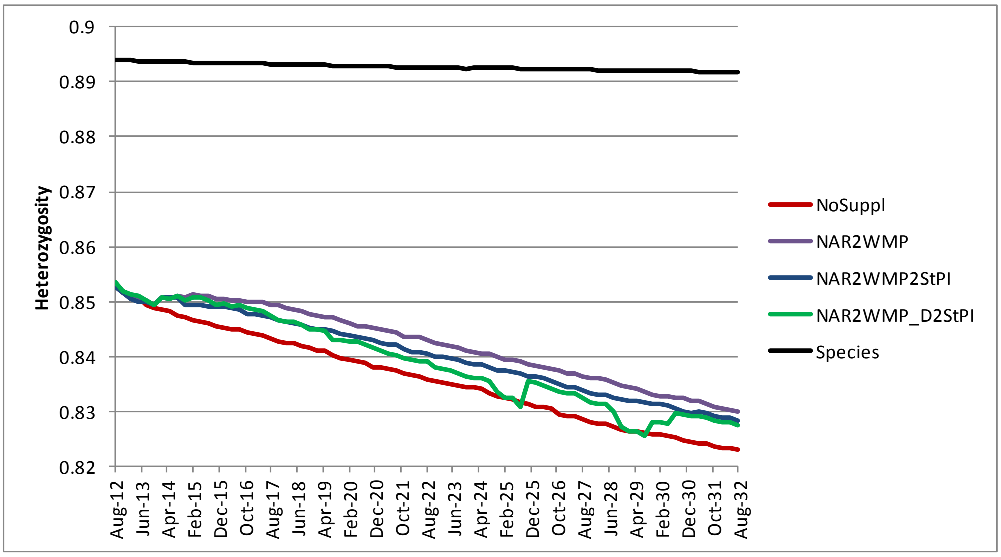

Figure 9. Whiteman Park mean expected heterozygosity trends over time. See Table 2 for abbreviations of scenarios in the legend.

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[3] Note that mortality of supplements into WMP has also been observed when woylies were released into the high-density enclosure.

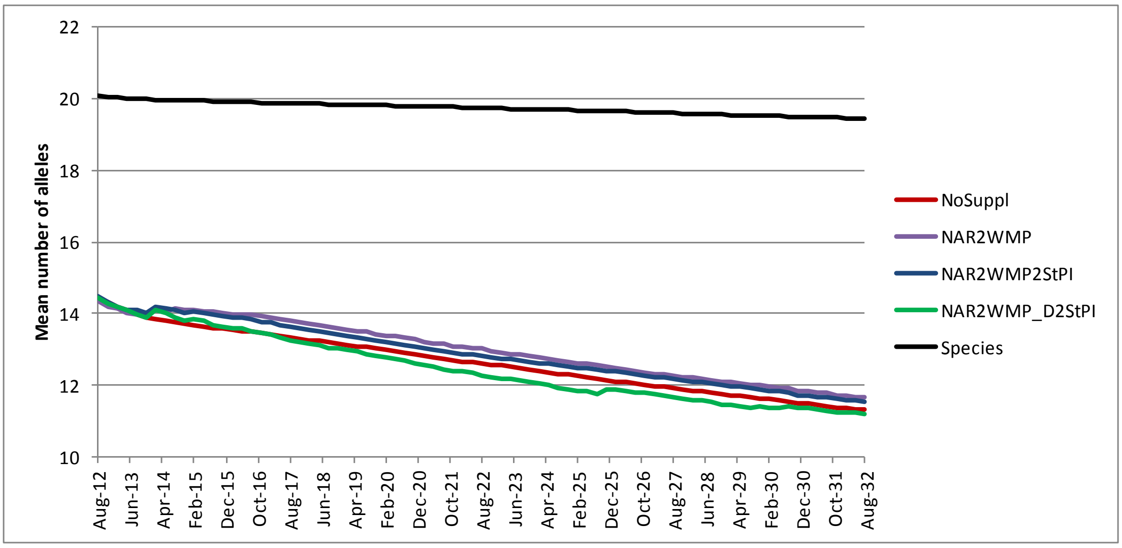

Figure 10. Whiteman Park mean number of alleles over time. See Table 2 for abbreviations of scenarios in the legend.

St Peter Island

An improvement in the mean number of alleles was detectable even with the small number of animals (see Appendix I) being moved to StPI. However, there was no substantial increase in the mean expected heterozygosity (Figure 11 and 12). This is probably because more time is necessary for the new alleles to mix in the population. This scenario was primarily developed to test the level of effort that should be required to carry out a 'genetic rescue' of this and similar populations. The two largest South Australian populations (StPI and Wedge Island) present a challenge because their remoteness makes them more difficult to manage, but the populations have been stable since their establishment (Delroy *et al.* 1986; Wayne *et al.* 2013). Their poor genetic profiles (Pacioni *et al.* 2013) call for active management to improve their conservation value, but at the same time, the high density of these populations limits the number of animals that can be released into them (at least with the survival rate used in this study). In fact, it has been demonstrated that with a similar number of supplements, there is a substantial improvement of the genetic profile of smaller populations such as Karakamia (see Appendix II).

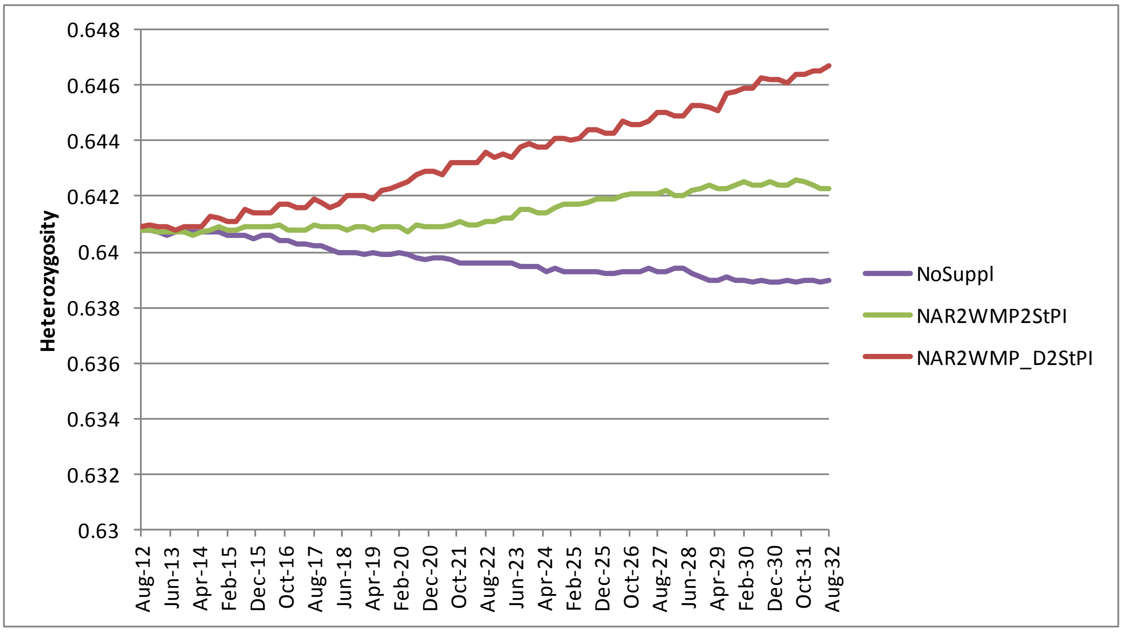

Figure 11. St Peter Island mean expected heterozygosity trends over time. See Table 2 for abbreviations of scenarios in the legend.

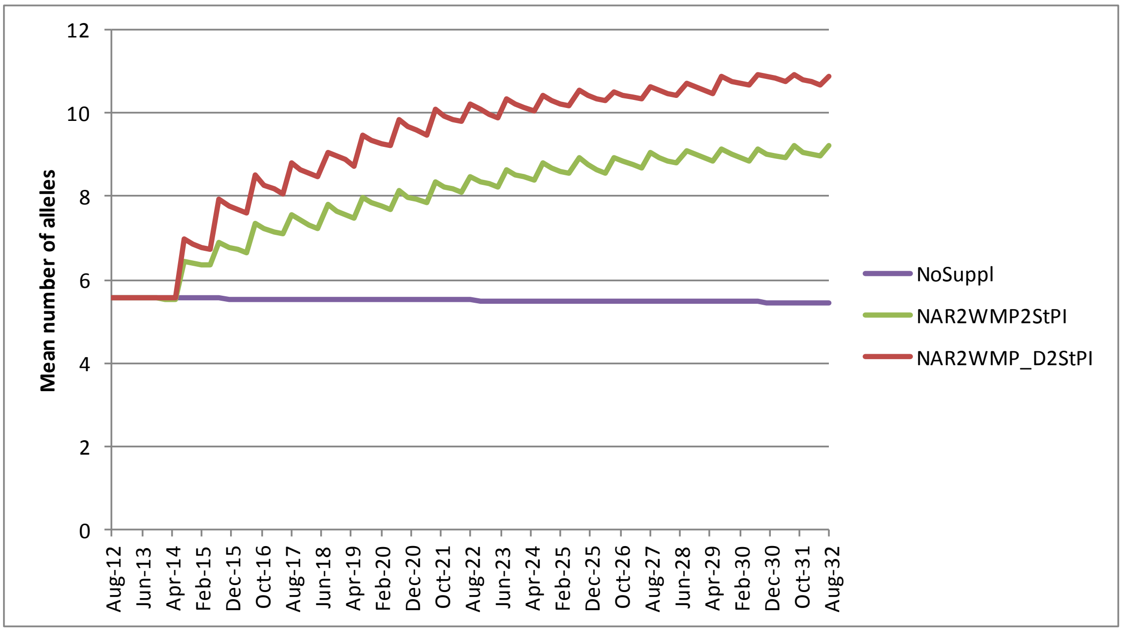

Figure 12. St Peter Island mean number of alleles over time. See Table 2 for abbreviations of scenarios in the legend.

General recommendations

This study indicates that ongoing management (i.e. regular genetic input into PS and regular supplementation of other populations with animals from PS) is the most beneficial strategy of those modelled for the conservation and recovery of the woylie. Such a strategy maximises the genetic diversity of the PS population; produces the minimum genetic distance from PS to the 'species' profile; and, if Kingston is used as the primary recipient population for animals removed from PS, it also has the benefit of dramatically improving Kingston's genetic profile. Further analysis may be able to optimise the number of animals that need to be moved and/or the frequency of the translocations. The strategy could be further optimised once more data on the mortality of supplements becomes available. A 'combined' scenario where animals from PS are released into both Kingston and Perup was not formally investigated, but it is expected that the outcome would be somewhere in between the two scenarios presented here and potentially an option to be considered.

By extension, the inclusion of other large populations such as Dryandra (as recipient) and Batalling in the ongoing management scenario would be beneficial to ensure the conservation of the species' entire genetic diversity. In fact, the results presented here may be potentially transferred to (non-modelled) alternative scenarios: for example, the genetic improvement of a wild population would be comparable to the modelled results if the survival of supplements, the genetic distance between source and recipient populations and the proportion of supplements and population size are similar.

This study clearly demonstrates the negative effect on genetic diversity caused by limited population sizes. While in the modelled timeframe of 20 years the heterozygosity loss in PS is only ~1% (without supplementation), in the longer term the population size of PS is probably the main limitation of its value as an insurance population. If PS is to remain as an effective insurance population for anything greater than 20 years, then it will require ongoing management to counteract the loss of genetic material due to genetic drift. Similarly, the higher rate of genetic loss in WMP (~3% with no supplementation) is a direct consequence of its smaller population size.

While this study focuses on the use of translocations as a tool for mitigating this limitation, other strategies may be equally valuable. For example, management strategies supporting larger population sizes (e.g. more effective predator control, understanding and managing the causes of woylie decline, and improving habitat quality and therefore carrying capacity) may complement translocations and potentially be more sustainable in the longer term. Simulation studies and empirical evidence indicated that a population size of at least 1,000-3,000 individuals is needed to ensure persistence of each discrete population and adequate genetic diversity (Pacioni 2010; Pacioni *et al.* 2013).

The use of an adaptive management framework is recommended. This can be achieved by monitoring the genetic profiles (in terms of heterozygosity and mean number of alleles) of the populations involved in the translocation program at established intervals (e.g. every 5 to 10 generations) to verify that the progress is on target or to adjust management responses accordingly; assuming that the overall objective (to maintain maximum genetic diversity at the species level) does not change.

StPI and Wedge Island in South Australia were not included as a source in any scenarios because the distances and the costs involved are substantial, and as a result these islands are currently not considered feasible source populations for translocations. However, this option should not be dismissed outright as the South Australian populations have mitochondrial haplotypes and a few private alleles that are not found in Western Australia (Pacioni *et al.* 2011; Pacioni *et al.* 2013), but were historically present (Pacioni unpublished data). If the logistical difficulties of accessing and moving these individuals can be overcome, there may be value in modelling the possible benefit of using StPI or Wedge Island as source populations for supplementary translocations to mainland populations.

Obviously, the ratio of resident to received animals ('supplements') will influence the level of genetic augmentation. This is clearly demonstrated in Scenarios 10 and 11 for StPI where 0.5-2 animals/year are released (see Appendix I) but the population shows only limited improvement in heterozygosity. Given the costs and difficulties involved with interstate translocations, it would be sensible to establish a release protocol that ensures adequate survival of translocated animals before attempting the genetic rescue of these high-density populations. The introduction of a few (1-4) individuals may be an adequate strategy for populations of limited size (i.e <1,000) and it may be successful for populations of similar size to StPI (approximately 3,000 individuals) in longer timeframes than those modelled here (although the latter hypothesis has not been explicitly tested). In reality if an interstate translocation is attempted, given the expense of such an exercise it is likely that larger numbers of animals would be translocated on fewer occasions, or perhaps only once. To this end, considerations should be given to possible additional factors that could positively influence the survival of the supplements (e.g. season, removal of a proportion of residential individuals, sex-ratio of supplements, etc.).

WMP is limited by its small carrying capacity; however, as a source population it presents the benefits of being recently established, conveniently located near Perth, at low density (allowing modification of its genetic profile) and having a relatively high genetic diversity (which is comparable to Perup and Kingston). While the WMP population is not a top priority population for woylie conservation in its own right; if other non-wild populations (i.e. non naturally occurring populations) are considered unsuitable sources of supplements for translocation trials, WMP would appear to be the obvious alternative source, at least in the short term.

Limitations of the study

The model assumes that animals that have survived the first three months after the translocations will have equal probability to survive and reproduce as the pre-existing resident animals. This may not be necessarily accurate as reproductive success and survival of translocated individuals may be lower than existing residents, even in the long term. However, the mortality of supplements in the first three months post-translocation was modelled to be fairly high (Appendix I) as a conservative approach that broadly reflected what had previously been reported in translocations into high density populations (Van Weenen 1996). It should be noted that no sensitivity tests have been carried out around the 'supplements' survival parameter and it is expected that different values will substantially influence the outcome of the translocations. As data become available on this key population parameter, the simulations may be refined. While exploring the results generated by this study, it appeared that an exponential density dependent function could be a valid alternative for modelling the survival of translocated individuals to the method used in this study (See also Appendix III) - i.e. survival $=95 \times \left(1 - \left(\frac{N}{K}\right)^{\mathrm{a}}\right)$. The collection of field data on survival rates of supplements introduced to populations of different densities will be extremely valuable for testing this hypothesis.

The use of short simulations to initialise PS and WMP populations comes with the disadvantage of reducing the stochasticity of the results generated by the model as each parameter is (re)initialised with a mean value (rather than a range of values). Additionally, genetic diversity is somewhat overestimated as all alleles that do not have a probability of retention equal to 0% will be included in the initial population (though the ones with low probability of retention most likely will have low frequencies and will therefore be quickly removed due to genetic drift). These contraindications are not expected to have an impact on the results of this study.

The recommendations made in this report are based only on genetic criteria and other factors may make them difficult to implement. For example, risks associated with disease transmission, and differences in predation levels between the unfenced populations were not taken into account. These, as well as the available financial and human resources of the responsible agencies, should also be considered when making management decisions.

To conclude, this study emphasised the importance and value of population modelling for the management of endangered species. A reduction in genetic variability can impact the evolutionary potential (Frankham 1996; Frankham *et al.* 1999) and reduce the fitness of populations (Ralls *et al.* 1988; Eldridge *et al.* 1999; O'Grady *et al.* 2006). Consequently, genetic studies have been recommended to assess the appropriateness of translocations as an effective conservation option (Stockwell *et al.* 1996; IUCN 1998; Moritz 1999). In this context, PVA modelling allowed the evaluation of different management actions, facilitating the decision-making process of otherwise very complex management decisions. Moreover, this study may assist in defining and quantifying the success of the planned translocation program.

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Appendix I

Mean number of successfully released (survived) woylie from --> into. Scenario codes in parentheses, where applicable.

Time-unit[a]	KAN --> PS (KAN2PS)	DRY & KING --> PS (KANDRYKING2PS)[b]	PS --> PER[c]	PS --> KING[c]	NAR --> WMP[d]	WMP-->StPI (WMP2StPI)[e]	WMP-->StPI (WMP_D2StPI)[f]
0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
2	2	0	0	0	0.1	0	0
3	2.4	0	38.3	48.2	0	0	0
4	2	62.1	0	0	0.4	0	0
5	2.3	0	0	0	0	0	0
6	2.6	0	43.2	42.6	2.5	0	0
7	1.8	52.1	0	0	0	0	0
8	1.9	0	0	0	1.6	0.9	2.5
9	1.8	0	44.1	48.7	0	0	0
10	1.5	41.4	0	0	1	0	0
11	1.7	0	0	0	0	0	0
12	1.6	0	46.7	47.1	0.6	0.4	2.5
13	1.5	13.3	0	0	0	0	0
14	1.1	0	0	0	0.4	0	0
15	0.9	0	51.8	49.3	0	0	0
16	0.8	8.6	0	0	0.1	1.6	1.4
17	0.9	0	0	0	0	0	0
18	0.7	0	41.9	43.6	0	0	0
19	0.3	4.6	0	0	0	0	0
20	0.5	0	0	0	0	0.7	1.5
21	0.1	0	44.4	37.7	0	0	0
22	0.6	5.7	0	0	0	0	0
23	0.6	0	0	0	0	0	0
24	0.2	0	46.7	49.1	0	1	1.6
25	0.4	2.8	0	0	0	0	0
26	0	0	0	0	0	0	0
27	0.1	0	49.1	43.3	0	0	0
28	0.1	5.9	0	0	0.2	1	1.7
29	0.1	0	0	0	0	0	0
30	0	0	48	48.7	0	0	0
31	0	5.6	0	0	0	0	0
32	0	0	0	0	0	0.4	1.3
33	0	0	45.4	48.8	0	0	0
34	0	14	0	0	0.1	0	0
35	0	0	0	0	0	0	0
36	0	0	45.5	48.2	0	1.3	3
37	0	9	0	0	0	0	0
38	0	0	0	0	0	0	0
39	0	0	47.1	48.9	0	0	0
40	0	6.7	0	0	0.2	0.2	1.8

Time-unit[a]	KAN --> PS (KAN2PS)	DRY & KING --> PS (KANDRYKING2PS)[b]	PS --> PER[c]	PS --> KING[c]	NAR --> WMP[d]	WMP-->StPI (WMP2StPI)[e]	WMP-->StPI (WMP_D2StPI)[f]
41	0	0	0	0	0	0	0
42	0	0	47.1	45.5	0	0	0
43	0	6.4	0	0	0	0	0
44	0	0	0	0	0	0.4	1.2
45	0	0	46.4	46.3	0	0	0
46	0	7.2	0	0	0	0	0
47	0	0	0	0	0	0	0
48	0	0	45.2	43.3	0	1	1.7
49	0	7.4	0	0	0	0	0
50	0	0	0	0	0	0	0
51	0	0	39.5	43.1	0	0	0
52	0	8.8	0	0	0	0.4	2.9
53	0	0	0	0	0	0	0
54	0	0	42	44.2	0	0	0
55	0	5.3	0	0	0	0	0
56	0	0	0	0	0	0.6	2.5
57	0	0	39	41.1	0	0	0
58	0	3	0	0	0	0	0
59	0	0	0	0	0	0	0
60	0	0	46.8	48.6	0	1.4	2
61	0	3.5	0	0	0	0	0
62	0	0	0	0	0	0	0
63	0	0	49.1	43.2	0	0	0
64	0	5.2	0	0	0.1	1.2	1.5
65	0	0	0	0	0	0	0
66	0	0	48.1	50.7	0	0	0
67	0	9.4	0	0	0	0	0
68	0	0	0	0	0	0.3	2.4
69	0	0	43.3	48.1	0	0	0
70	0	7	0	0	0	0	0
71	0	0	0	0	0	0	0
72	0	0	48	46.5	0.2	0.9	2.1
73	0	8.2	0	0	0	0	0
74	0	0	0	0	0	0	0
75	0	0	44.4	42.6	0	0	0
76	0	5.3	0	0	0.1	0.9	2.4
77	0	0	0	0	0	0	0
78	0	0	38.2	39.7	0	0	0
79	0	10.3	0	0	0	0	0
80	0	0	0	0	0.1	0.6	1.3

[a] Time-unit = three month period

[b] 80 individuals (40 Dryandra and 40 Kingston) are sourced every three time-units, starting from time-unit 4.

[c] 100 individuals are removed from PS every three time-units, starting from time-unit 3.

[d] Four individuals (if available) are removed from NAR every two time-units, starting from time-unit 3.

[e] 10 individuals are removed from WMP every four time-units, starting from time-unit 8.

[f] 20 individuals are removed from WMP every four time-units, starting from time-unit 8.

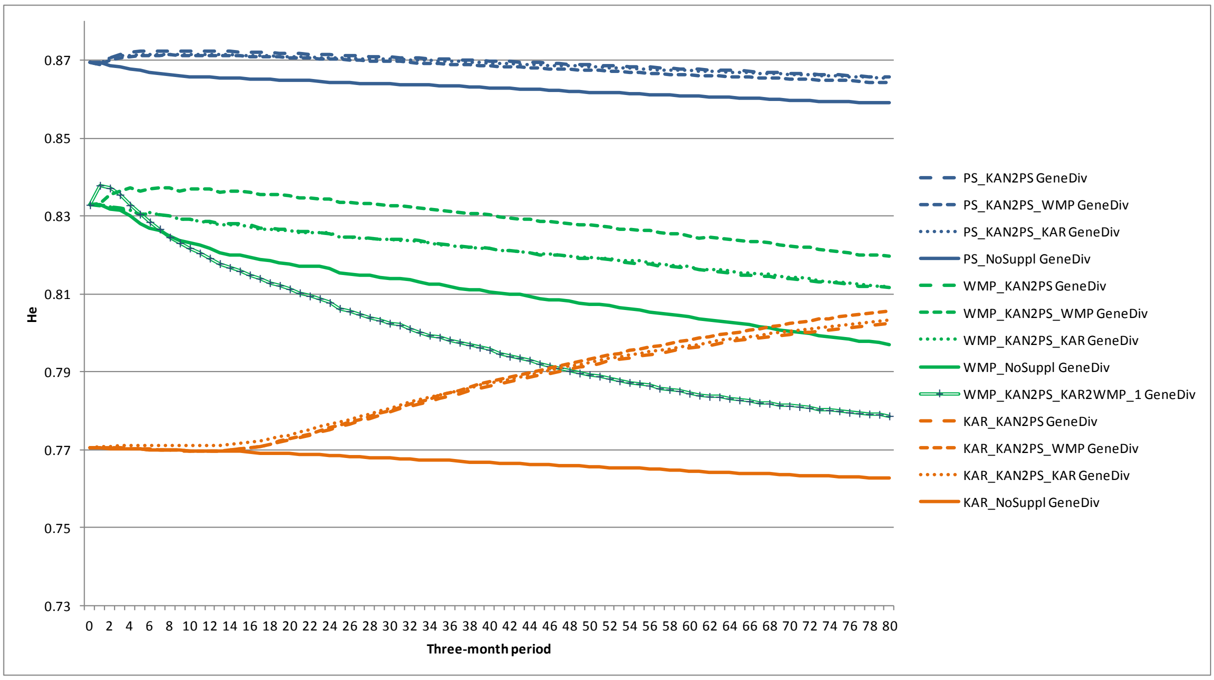
Appendix II

Details of scenarios modelled in preliminary analyses.

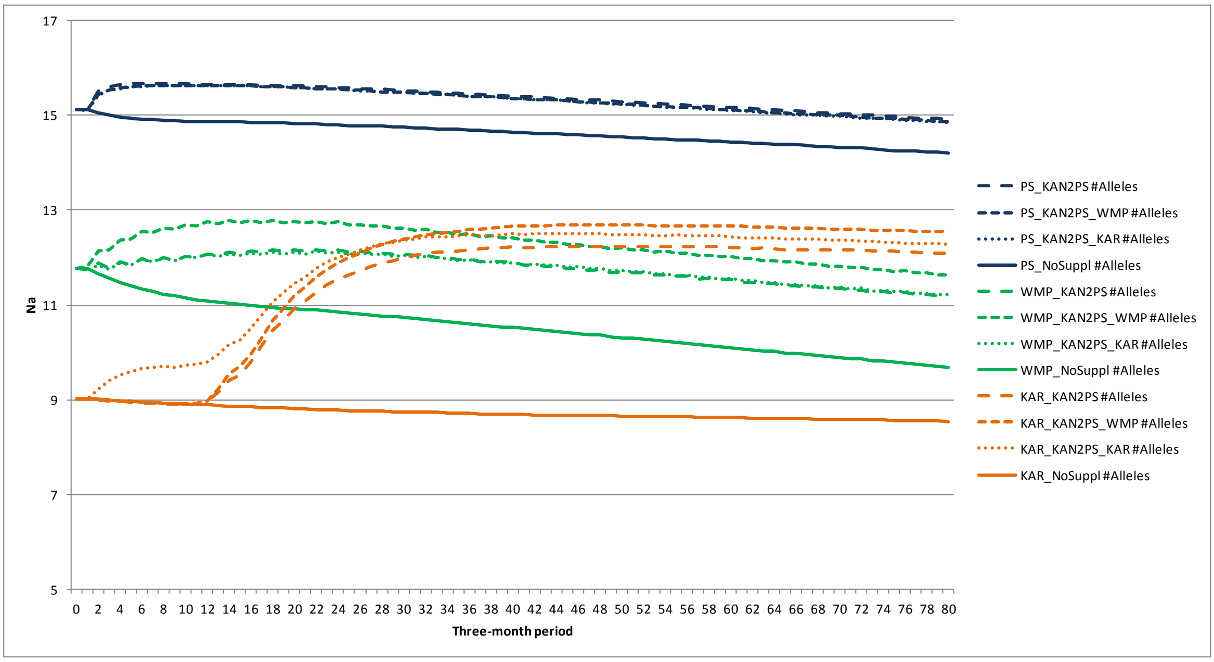
Scenario	Abbreviation	Description
1	NoSuppl	No populations received any supplementation.
2	KAN2PS	All offspring from Kanyana (Tutanning genetic stock) go to PS.
3	KAN2PS_WMP	2 females and 1 male from Kanyana go to PS; if anything else is available it goes go to WMP
4	KAN2PS_KAR	2 females and 1 male from Kanyana go to PS; if anything else is available it goes go to KAR
5	KAN2PS_KAR2WMP_1	As scenario 2 but dispersal from KAN to WMP is 1% every quarter

NOTE: Movements between WMP and KAR were modelled as dispersal where a proportion of the total number of individuals in a population moves between the two sites (0.2% KAR to WMP every 6 months; 1% WMP to KAR every quarter after the first year).

These scenarios refer to preliminary simulations that were performed before the final PVA model was developed. Therefore, there are some differences in the model used for these preliminary simulations and the model used to generate the data presented in this report. For example, the preliminary model initialised WMP and PS in such a way that the populations were modelled as slightly smaller than the actual populations, resulting in a slight overestimation in the genetic improvement due to the addition of new animals in PS and WMP in the preliminary analyses. Also, the genetic diversity of the founders of PS was slightly overestimated in the preliminary analyses.

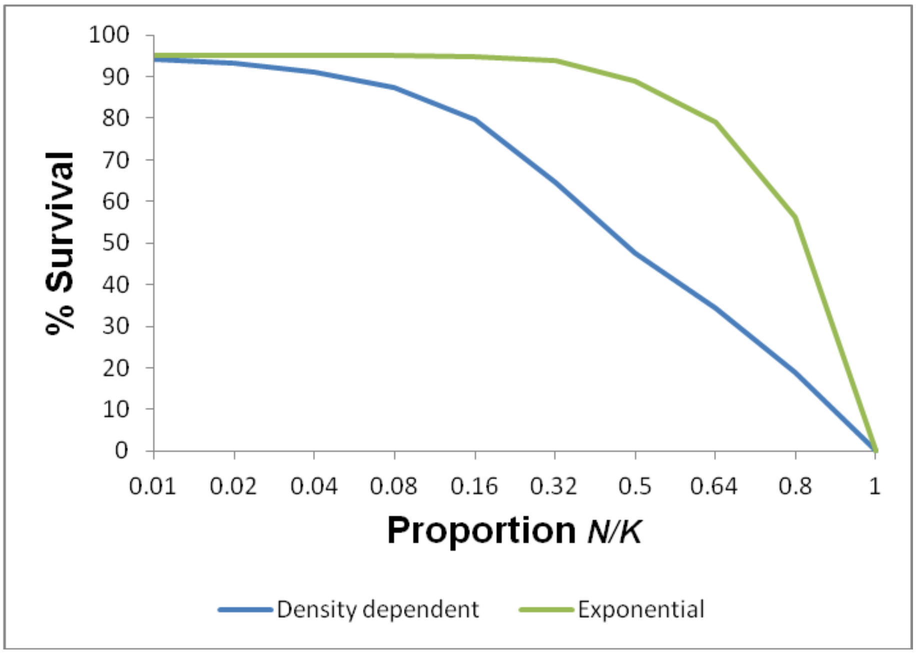


Heterozygosity trends over time in preliminary analyses.



Average number of alleles over time in preliminary analyses.

Appendix III



Graphical representation of differences between a simple density dependent function *versus* an exponential function (a=4), the latter being possibly a valid alternative to model survival of translocated animals.

About the author

Carlo Pacioni DVM, MVS, PhD

Carlo Pacioni holds a Research Associate position at Helix Molecular Solution where he is actively involved in applied research for wildlife conservation and vertebrate pest management. He is currently working on a number of projects, including population modelling of wild dogs in Western Australia and an assessment of the economic impact of starlings (*Sturnus vulgaris*). He also holds a position as adjunct Research Associate at Murdoch University, Western Australia, where he is involved in various conservation genetic and epidemiological projects.

As part of his doctoral thesis (Murdoch University, 2011), Carlo investigated several aspects of woylie ecology using molecular based approaches, and conducted population viability analysis and health and disease investigations in wild woylie populations. He had previously completed the Masters in Conservation Medicine at the same university.

Before starting his post-graduate studies in Australia in 2005, Carlo completed a degree in Veterinary science at the University of Perugia (Italy) in 2000; volunteered in different Wildlife Rescue Centres in Spain, Argentina and Australia; completed additional veterinary training in Spain and Germany in internal medicine, neurology, surgery, radiology and exotic animal medicine; and held a position as veterinarian at the Sáenz Peña Zoo, Argentina.

(c) SABRINA TROCINI

Dr Carlo Pacioni taking an ear tissue sample from a woylie for DNA analysis.

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