THE GENETIC MANAGEMENT OF SOUTHERN CORROBOREE FROGS: WORKSHOP REPORT AND PLAN

REPORT

This document records the main topics and findings of a one-day meeting of Southern Corroboree Frog Recovery Team members held in Canberra, September 24th, 2013. The meeting was directed towards developing a genetic management strategy for the captive population of frogs managed at Taronga and Melbourne Zoos, Healesville Sanctuary and the Amphibian Research Centre. The meeting was organised by the Recovery Team Chair and the Captive Program Coordinator and was facilitated by the IUCN SSC Conservation Breeding Specialist Group.

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A contribution of the IUCN/SSC Conservation Breeding Specialist Group and Taronga Conservation Society Australia

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INTRODUCTION

On September 24th, 2013, 12 contributors from 7 organisations met in Canberra to formulate a strategy for the genetic management of captive southern corroboree frogs (*Pseudophryne corroboree*), as part of the recovery effort for the species. The workshop was facilitated by the IUCN SSC Conservation Breeding Specialist Group.

The southern corroboree frog is restricted to the sub-alpine zone of the Snowy Mountains Region of Kosciuszko National Park in Australia. The species has been in continual decline for almost 30 years and in 2013, for the first time, no signs of breeding were observed outside captivity. Extinction in the wild is expected within the next 2-3 years. The primary threat is chytridiomycosis, which is caused by infection with the Amphibian Chytrid Fungus.

The current hope for the species is that sustained exposure to the chytrid fungus will eventually result in the emergence of resistance in wild populations, or that research will identify other mechanisms by which management can facilitate the establishment of self-sustaining populations. Recovery team efforts are therefore focussed on re-establishing and sustaining a genetically diverse and abundant population of the species within its natural range, using a range of techniques which include an efficient and well-targeted program of captive breeding and release.

Despite the daunting prognosis, this recovery program stands out as an excellent model of innovative, committed, science-based collaboration between the field, captive and academic communities.

At present then, the release program is open-ended. A fifty-year time-frame was used to guide discussions.

WORKSHOP INTRODUCTION

David Hunter, the Chair of the Recovery Team, gave a brief welcoming address. Participants introduced themselves and Gerry Marantelli joined the meeting remotely by telephone. Michael McFadden, Species Coordinator for the captive program, provided background on the current status of the program and on the context for the workshop.

The following desirable outcomes for the meeting were presented and agreed:

By the end of this meeting we will:

- understand what we want from the genetic management of corroboree frogs;
- understand the obstacles to achieving what we want;
- have explored the alternative strategies available;
- have agreed a long-term strategy or strategies for genetic management of corroboree frogs;
- have agreed the next steps that need to be taken, for each institution, to implement this strategy.

PRESENTATIONS

C. Lees gave a brief presentation on the genetic and demographic management of captive populations: the underlying philosophy and theory; the steps involved in setting up and managing for long-term viability and gene diversity retention; the challenges experienced during the course of most programs; and the pros and cons of various strategy options for genetic management. The presentation also included information drawn from a variety of other recovery-related documents outlining previously recommended goals for captive management of southern corroboree frogs. This formed the basis for subsequent discussion.

GOALS, TARGETS AND STRATEGIES

After discussing the information presented at length, the group stepped through a series of questions and confirmed the following 50-year program goals, targets and strategies for captive management:

- Maximise retention of wild source gene diversity (at least 95% for 50 years);
- Manage the rate of accumulation of inbreeding below detrimental levels (F<0.125 for 50 years);
- Maintain the ability to produce 2000+ viable, non-inbred (or minimally inbred) eggs for release each year (this will require breeding from at least 100 females each year);
- Without compromising other goals, organise management in a way that complements other measures to minimise the potential for chytrid and other diseases to spread in the population (design to minimise inter-institutional moves);
- Maintain within available carrying capacity and resources (MZ=100 adult spaces; TZ = 200 adult spaces; HS = 100 adult spaces; ARC = to be confirmed, but may be able to meet any shortfall between available capacity and that required to meet goals).
- Manage through a Maximal Avoidance of Inbreeding (MAI) strategy. [This was confirmed to be the best fit for the species' biology and its efficient management in captivity. Work done prior to the meeting had illustrated the ability of a scheme such as this to meet and potentially exceed the goals set and this was presented to participants during the meeting].

FOUNDER ALLOCATION

With the goals, targets and strategies agreed, participants worked on optimising allocation of the program's founder base. All founders likely to be available to the captive program have been captured and are currently residing at participating institutions. Participants worked to re-allocate individuals across institutions to maximise the number of MAI groups formed, thereby increasing the number of generations over which inbreeding could be avoided completely and improving opportunities for gene diversity retention.

Two working groups were formed for this, one to look at Melbourne Zoo and Healesville Sanctuary holdings and the other to consider Taronga Zoo's holdings The Amphibian Research Centre holds a large number of founders, but the remote access to the meeting precluded their involvement in this exercise; this may be done at a later date. Each group went through the following steps.

- Founders were grouped by source location and year, and then by sex.
- A card was made for each unit, containing the following information:
    - Number of individuals.
    - Sex of the frogs in each group.
    - Location and year of capture.

Participants arranged these cards into clusters of either four or eight MAI groups using the following rules:

- Within each group, males must be unrelated to females but males can be inter-related, as can females.
- Animals in one group must be unrelated to the animals in all other groups in that cluster.
- As far as possible, even sex-ratios, even number of genetic lines and even number of animals, in each group.

Due to the inter-relatedness of some of the "founders", which had been harvested from the same clutch, it proved difficult to construct either eight or even four MAI groups in some cases, without either leaving founders without a group, or including related males and females within an MAI group cluster. It was agreed that the priority was to ensure that each “founder” has an opportunity to contribute to the wild gene pool and that the inbreeding targets would be relaxed in some instances to accommodate this.

The DRAFT result produced one cluster of four MAI groups at Healesville Sanctuary, the same at Melbourne Zoo, and two clusters of four at Taronga Zoo. The draft founder allocation scheme constructed will require some movements from Taronga Zoo to Melbourne and Healesville. Further work is required to complete this scheme, including some checking of records and the longer-term allocation of immature animals, which were not considered in this exercise. M. McFadden, as the Species Coordinator, was charged with completing this exercise.

IMPLEMENTATION CHALLENGES

Participants stepped through a generation in the life of the proposed MAI scheme to consider the practicalities of implementation, including breeding tank set-up and supporting capacity at each stage of the scheme. Key areas of difficulty were identified as:

- When, how many and which individuals to hold back for recruitment into the captive breeding population?
- How best to deal with the issue of overlapping generations (MAI schemes are easier to apply where generations are non-overlapping)?
- Can we extend generation length?
- What information could/should be captured each year?
- How will success be measured?
- What are the next steps for each institution?
- What other actions need to be completed?

It was agreed that the approach to all of these could change over time depending on circumstances, but short-term approaches to all were developed. In summary:

- In the first instance, for each MAI group, one individual from each clutch (as far as can be determined) will be held back in alternate years. This should limit growth of the adult population to within manageable limits.
- Once breeding is reliable and consistent, further thought will be given to extending generation length by retaining only the later clutches produced by MAI groups – whilst for security retaining earlier clutches until those later clutches are secure.
- Overlapping generations will be dealt with by grouping adults with their same-sex offspring at the point of strategic “single-sex rotations”.
- The practicalities of extending generation length in a way that will minimise risk of founder loss will be tested.
- Wherever possible the following information will be tracked:
    - Which MAI group each individual belongs to;
    - Number of females and males that breed each year (estimated min. and max.);
    - Adult males vs adult females vs juveniles;
    - Each individual’s generation number (F0 vs F1 vs F2);
    - Ages and age-specific mortality;
    - Ages and age-specific fecundity (also inter-birth interval to confirm whether captive females breed in alternate years – this could affect the recruitment strategy).
- The goals and targets articulated earlier will be used to measure success. In some cases it will not be possible to measure performance against goals directly. Instead, in some cases, population simulation models built from estimated parameters may be used to provide pessimistic and optimistic estimates of performance.

ACTIONS

Actions arising were as follows:

- M. McFadden to review DRAFT founder allocation and finalise recommendations for group composition and transfers to Zoos Victoria (Feb 2014).
- Healesville Sanctuary and Melbourne Zoo staff to prepare to receive animals from Taronga Zoo for the establishment and management of their agreed MAI groups (April/May 2014).
- M. McFadden, with input from the wider group, to prepare the following protocols (Jan 2014):
    - protocol for standardised data collection and reporting;
    - protocol for holding back individuals for recruitment into the adult breeding population; including a standardised approach to estimating number of clutches per nest;
    - protocol for analysing and reporting on success;
    - protocol for estimating minimum and maximum number of males and females breeding annually.
- C. Lees to provide a report on the meeting, including presentation materials (Nov. 2013).
- C. Lees to provide a draft guide for the long-term management of an MAI scheme for corroboree frogs, for further development by the group (Nov. 2013).
- C. Lees and M. McFadden to maintain regular contact until actions and protocols are finalised (ongoing).
- D. Hunter and C. Lees to look for opportunities through CBSG to elevate awareness of the work of the S. Corroboree Recovery Team as a model "One Plan Approach" style collaboration between a variety of sectors (ongoing).
- M. Evans to continue to share data on northern corroboree frogs (ongoing).
- M. McFadden and all to work through the practicalities of extending generation length with minimal risk of founder loss (long-term).
- Monitor and when possible include in the overall strategy, frogs managed in large outdoor chytrid-free areas (long-term).

IMPLEMENTATION FRAMEWORK

Michael McFadden (Taronga Zoo) is the regional zoo association's (ZAA) appointed Species Coordinator for this taxon. In this role he will coordinate and support the implementation of this strategy for and in close collaboration with, the wider recovery team.

Managers at participating institutions will be responsible for implementing site-specific program recommendations, for providing data, communicating issues, and for contributing knowledge and skills to the development of the program over time.

MEETING CLOSE

Finally, the group revisited the targeted outcomes for the workshop and agreed that all had been achieved. David Hunter closed the meeting, thanking all for their contributions.

INTRODUCTION

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The current hope for the species is that sustained exposure to the chytrid fungus will eventually result in the emergence of resistance in wild populations, or that research will identify other mechanisms by which management can facilitate the establishment of self-sustaining populations. Recovery team efforts are therefore focussed on re-establishing and sustaining a genetically diverse and abundant population of the species within its natural range, using a range of techniques which include an efficient and well-targeted program of captive breeding and release.

Despite the daunting prognosis, all involved in the workshop agreed that this recovery program stands out as an excellent model of innovative, science-based collaboration between the field, captive and academic communities.

There is no information at present that would allow managers to make informed estimates about when or whether resilience to chytrid might emerge in this species. No other end-point or exit strategy has been considered for the program. At present then, the release program is open-ended. A fifty-year time-frame was used to guide discussions.

CURRENT CAPTIVE POPULATION STATUS

The following provides a summary of the status of the captive population at the time of the workshop. This information was presented to the group at the outset of the workshop and was used throughout to inform discussions. Data gathering was done by Michael McFadden in consultation with holding institutions. Some revisions were made during workshop discussions in light of additional insight and experience with other, similar taxa.

CURRENT AND PROJECTED CARRYING CAPACITY

The population is currently held at four institutions: the Amphibian Research Centre (ARC), Taronga Zoo, Melbourne Zoo and Healesville Sanctuary. The latter two institutions belong to a single parent organisation – Zoos Victoria. A breakdown of current numbers, along with projected carrying capacity, is shown in Table 1.

Table 1: Current and projected carrying capacity for southern corroboree frogs.

Institution	Current Numbers (total frogs)	Projected Carrying Capacity (K)
Amphibian Research Centre	~400 frogs	To be confirmed
Taronga Zoo	~400 frogs	200 adults
Melbourne Zoo	~150 frogs	100 adults
Healesville Sanctuary	~50 frogs	100 adults

It was agreed that carrying capacity should be measured in terms of the number of adult breeders able to be maintained. This has implication for the number of staff and supporting tanks required to rear and hold associated offspring. Institutions need to take into account their ability to accommodate this when reporting on carrying capacity. In addition:

- Maximum capacity for breeding males is likely to be determined by the number and size of breeding tanks.
- Females can be rotated through the tanks – once each female has laid, eggs are removed and another female can be moved in. Capacity for breeding females will therefore depend on availability of holding space outside the breeding tanks.
- Juveniles being raised to replace breeding stock need to be held or in some way marked to indicate their genetic grouping. This may constrain capacity.

Note that in the near future it may be possible to hold large numbers of individuals in large outdoor, chytrid-free areas within natural range. These could provide a large volume of additional capacity for the program but are still experimental and are not considered here.

AGE STRUCTURE

The current captive population comprises a large number of wild-caught individuals (N~ 355), many of which are immature, and a smaller number of young, captive-born individuals. The latter may yet be released to the wild to extend generation time.

Figures 1 and 2 show ages and sexes for the Taronga and Melbourne Zoo animals. These were the only two data sets available at time of writing. Individuals can be sexed only at maturity. Immature animals have been apportioned 50:50 to each sex in line with currently observed sex-ratios.

Taronga holds approximately 134 adults and 280 juveniles. Melbourne holds approximately 25 adults and 136 juveniles, Healesville Sanctuary holds 53 (mostly captive-born) and a further ~400 adult frogs are held at ARC, the details of which were not able to be confirmed in advance of the meeting.

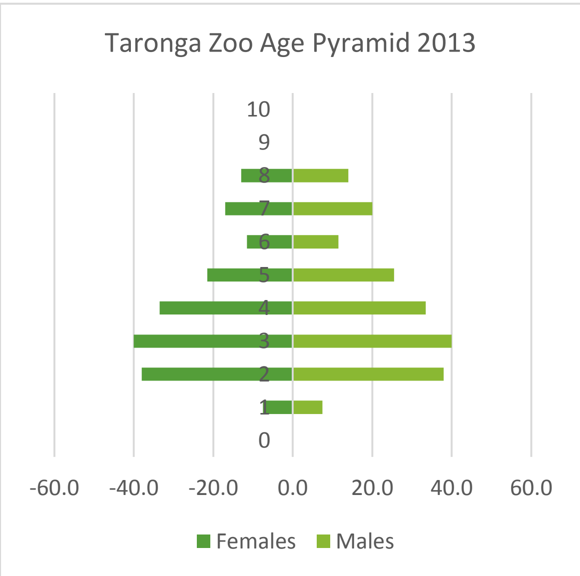

Figure 1. Age Pyramid for Taronga Zoo holdings of wild-caught southern corroboree frogs in 2013

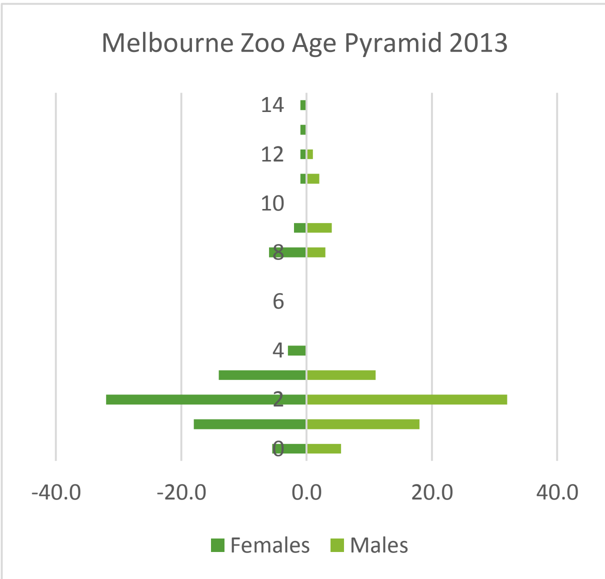

Figure 2. Age Pyramid for Melbourne Zoo holdings of wild-caught southern corroboree frogs in 2013

LIFE HISTORY CHARACTERISTICS

Experience to date suggests the following life history characteristics:

- AGE AT FIRST BREEDING: 3-4 years for both sexes.
- LONGEVITY: prior to the workshop longevity estimates were around 14-15 years. However, during workshop discussions G. Marantelli suggested that the species may live and breed to around 20-25 years, though this would be expected from only a small percentage of individuals.
- SEX-RATIO AT BIRTH: assumed to be 50:50. Individuals are not sexed until mature and ratios at adulthood have been approximately 50:50 (e.g. sex-ratio at Taronga Zoo for n=67 was 34:33).
- BREEDING SYSTEM: males build nests and females choose which nests to deposit eggs in. Multiple females (usually 1-3) may lay eggs in a single nest, such that fewer than 100% of males are in the breeding pool in any given year.

REPRODUCTIVE SUCCESS (PROPORTION OF INDIVIDUALS THAT BREED SUCCESSFULLY).

Frogs are transferred to tanks for the breeding season. At Taronga Zoo tanks typically hold 4-5 males and a roughly equivalent number of females, though the latter may be removed after a period and replaced with other females that have not yet had the chance to breed. At Melbourne Zoo, tanks are quite close together and at times females will show more interest in males calling from a neighbouring tank. When this occurs, to secure breeding, females are generally transferred to the preferred male.

At Melbourne Zoo all females bred successfully in 2012 (N~13); the number of sires is not confirmed. At Taronga Zoo it is estimated that 70-75% of females bred in 2011-2012 (N~65), with approximately 2-3 out of 5 males successfully siring offspring.

At ARC and Healesville breeding has been less successful.

[Note that at present, the difference in female body weight before and after time spent in the breeding tank is used to assess whether or not an individual has laid a clutch of eggs. The number of successful males is measured as the number of egg-laden nests. Which males have bred and with which females is only occasionally known as a result of opportunistic observation.]

MANAGEMENT OF SURPLUS

The release program can accommodate as many animals as the program can produce.

FOUNDERS

The current captive population housed at Taronga and Melbourne Zoos has been collected over many years, with living animals dating back to clutches sampled in 1999. Adults still housed at ARC were collected before that. It is unlikely that any more founders will be collected from the wild.

Founders are wild-caught individuals that are no more related than the population average. Though there are ~355 wild-caught frogs at Taronga and Melbourne Zoos, and a further ~400 at ARC, often 10-20 individuals originate from the same nest and so are likely to be closely related. Individuals collected from the same site in different years are considered to be unrelated. Those collected from the same nest in the same year are assumed to be at least half-siblings, sharing the same sire but possibly different dams. The inter-relatedness of "founders" will need to be taken into account when assessing gene diversity captured and founder retention over time.

Eggs collected most recently are considered particularly valuable as those genetic lines have had the longest exposure to chytrid.

GOALS AND TARGETS

Release of captive-bred stock is currently the primary means through which this species will be sustained at wild sites. Maintaining a high quality output from the source population over time will therefore be an important component of the recovery effort for the species. To produce this high quality output, the captive population will need to retain the following attributes:

- favourable disease status;
- high productivity;
- long-term demographic stability;
- genetic health.

Though these may be inter-related, the management of genetic health can at times run counter to efforts to secure the first three outcomes. This effect can be minimised through careful planning.

With these things in mind, goals, targets and strategies should be directed towards: optimising the genetic health of the population without compromising the demographic and disease management goals of the program; and making the most efficient use of the skills and resources available.

The group heard a presentation on the genetic and demographic management of captive populations: the underlying philosophy and theory; the steps involved in setting up and managing for long-term viability and gene diversity retention; the challenges experienced during the course of most programs; and the pros and cons of various strategy options for genetic management. The presentation also included information drawn from a variety of other recovery-related documents outlining previously recommended goals for captive management of southern corroboree frogs.

After discussing the information presented at length, the group confirmed 50-year program goals and targets for captive management. These are shown in Table 2:

Table 2: Fifty-year goals and targets for the captive population of southern corroboree frogs.

Goals	Targets
Maximise retention of wild source gene diversity	Retain at least 95% of source population for 50 years
Manage the rate of accumulation of inbreeding below detrimental levels	Maintain mean inbreeding below F=0.125
Maintain the ability to produce 2000+ viable, non-inbred (or minimally inbred) eggs annually	Breeding annually from at least 100 females (based on clutch sizes of around 30, each with at least 20 viable eggs)
Complement other disease control measures	Where compatible with other goals, minimise exchanges between facilities
Manage within available capacity and resources	(MZ=100 adult spaces; TZ = 200 adult spaces; HS = 100 adult spaces; ARC = to be confirmed but may be able to meet any shortfall between available capacity and that required to meet goals)

A standard gene diversity target used both in Australasia and internationally is retention of 90% of wild source gene diversity over the program period, based on (Soulé *et al*., 1986). This still represents a substantial loss of diversity and, wherever possible, more ambitious targets are set.

Similarly, the inbreeding target is based on a rule of thumb widely applied to captive programs and equates to the level expected as a result of a mating between half-siblings. Any overt signs of inbreeding depression in captivity are generally observed at higher inbreeding coefficients than this. However, inbreeding depression is expected to be more severe in the wild as a result of the harsher environment and, as this is a release program, it would not be prudent to relax this target until necessary.

The increased risk of disease transfer as a result of moves between institutions was discussed. In general, participants agreed that because transferring animals between institutions is likely to be necessary for meeting gene diversity and inbreeding targets, it should not be ruled out. Instead, the risk of transfers should be assessed and managed alongside other disease risks within the program. However, it was also agreed that it would be sensible, if possible, to design the program to minimise the rate of movements between institutions, provided that this would not prejudice success in other areas.

It was agreed that carrying capacity should be reported in terms of the number of breeding adults that can be accommodated, rather than the total number of frogs. For a given number of adult breeders there will be associated resource implications in terms of breeding, rearing, juvenile holding and staffing. Institutions will need to calculate adult holding capacity based on institution-specific circumstances.

MAXIMAL AVOIDANCE OF INBREEDING STRATEGY

Three broad strategies for genetic management were discussed: "**Random" breeding**, in which the population is separated into relatively large, anonymous groups and population models are used to calculate optimal levels of transfer between groups to reduce inbreeding and improve genetic outcomes. This can be relatively simple and inexpensive once established. The downside to this approach is the number of assumptions that must be made about population dynamics and the difficulty of measuring and therefore of managing, performance over time. Experience with northern corroboree frogs suggests that at low levels of within-group intervention, genetically effective size may be small compared with census size. This could prejudice ability to reach program goals in southern corroboree frogs.

Maximal Avoidance of Inbreeding (MAI) (Princée, 1995) is a strategy for minimising the rate of accumulation of inbreeding over time and, for populations that breed evenly and consistently, minimising the erosion of gene diversity. It operates by separating the population into a number of even groups and managing a "round-robin" sequence of exchanges between groups, each generation, of only one sex. MAI has the advantage of achieving better results than "Random" breeding whilst still allowing for group-wise breeding, incomplete parentage data, and incomplete data on individual age and generation.

For reliably breeding species, better gene diversity outcomes are possible using a strategy of **managing by mean kinship** (Ballou & Lacy, 1995). However, this is generally more costly, requires pair-wise breeding (which has not yet been successfully demonstrated in this species), and the tracking of more information about individual animals, including parentage, than is currently possible.

Participants agreed that of the strategy options discussed, a customised MAI scheme would be the best fit for the species' biology and for the preferred system of management in captivity. Table 3 estimates the potential for an MAI scheme to meet and potentially exceed the program targets set.

Table 3. Potential performance of an 8-group MAI scheme in meeting program targets

Goals	Targets and Constraints	**1 x 8-Group MAI Scheme**; Est. **74 founders**; **7.7 adults per group**
Maximise retention of wild source gene diversity	Retain at least 95% for 50 years	355 captured from 37 nests. Assuming conservatively: 74 founders, 50-70% breed, GD captured = 98.65%, total 800 animals, T=7 and Ne/N=0.3: Expect >95% GD at 50 years ~ 97.20%
Manage the rate of accumulation of inbreeding	Maintain population average below 0.125 for 50 years	For 8-Group MAI F=0.125 at generation 11 (50 years+)
Maintain the ability to produce 2000+ viable eggs annually	Breeding annually from at least 100 females (based on clutch sizes of around 30, each with at least 20 viable eggs)	~100 - 150 females breeding each year (assuming 50-75% breeding rate)
Minimise disease spread	Minimise exchanges between facilities (where no other targets are compromised)	Post set-up, no movements between TZ and Zoos Vic for 50 years.
Manage within available capacity and resources	TZ (200 adults); MZ (100 adults); HS (100 adults); ARC (to be confirmed)	Requires ≈400 adult spaces plus associated rearing and juvenile space.

This rough estimate of what could be achieved with an eight-group MAI scheme, beginning from a conservative estimate of the founder-base available, indicates that under reasonable conditions it should be possible to meet and in some cases exceed program targets with the resources and husbandry expertise currently available.

Further details of the mechanics of an MAI scheme are provided in Appendix III.

FOUNDER DISTRIBUTION

All founders likely to be available to the captive program have been captured and are currently residing at participating institutions. Participants worked to re-allocate individuals across institutions to maximise the number of MAI groups formed, thereby increasing the number of generations over which inbreeding could be avoided completely and improving opportunities for gene diversity retention.

Two working groups were formed for this, one to look at Melbourne Zoo and Healesville Sanctuary holdings and the other to consider Taronga Zoo's holdings. The Amphibian Research Centre holds a large number of founders, but the remote access to the meeting precluded their involvement in this exercise; this may be done at a later date. Each group went through the following steps.

- Founders were grouped by source location and year, and then by sex.
- A card was made for each unit, containing the following information:
    - number of individuals;

    - sex of the frogs within each group;
    - location and year of capture.
- Participants arranged these cards into clusters of either four or eight MAI groups using the following rules:
    - within each group, males must be unrelated to females but males can be inter-related, as can females;
    - animals in one group must be unrelated to the animals in all other groups in that cluster.

Due to the inter-relatedness of some of the "founders", which had been harvested from the same clutch (see Appendix II), it proved difficult to construct the required complement of either four or eight groups in some cases, without leaving animals out. It was agreed that: THE PRIORITY IS TO ENSURE THAT EACH FOUNDER HAS AN OPPORTUNITY TO CONTRIBUTE TO THE WILD GENE POOL AND THAT THE INBREEDING TARGETS WOULD BE RELAXED IN SOME INSTANCES TO ACCOMMODATE THIS.

The DRAFT result produced one cluster of four MAI groups at Healesville Sanctuary, the same at Melbourne Zoo, and two clusters of four at Taronga Zoo. The draft founder allocation scheme constructed will require some movements from Taronga Zoo to Melbourne and Healesville. Further work is required to complete this scheme, including some checking of records and the longer-term allocation of immature animals, which were not considered in this exercise. M. McFadden, as the Species Coordinator, was charged with completing this work.

MAI IMPLEMENTATION

Implementation of MAI schemes can be challenging as the biological characteristics of species rarely fit the theoretical ideals for which MAI was designed. In addition, gaps in knowledge about the species and its long-term performance in captivity complicate forward-planning. The group worked through the practical implementation of the proposed MAI scheme, step-by-step, over a generation. The following challenges were identified for further consideration:

- When, how many and which individuals to hold back for recruitment into the captive breeding population: holding back too many would lead to capacity problems whereas retaining too few could de-stabilise the population's demographic performance.
- How best to deal with the issue of overlapping generations: MAI schemes are easier to apply where generations are non-overlapping. Inadequate consideration of this in species with overlapping generations could lead to unplanned inbreeding, or periods between single-sex rotations that are either too long or too short.
- What is the best way to extend generation length? Extending generation length should reduce loss of gene diversity, inbreeding accumulation and the rate of captive adaptation but some strategies for achieving it will increase the risk of loss of founder lines.
- What information could/should be captured each year? This generally requires trading-off the benefits of acquiring more information against the cost of greater vigilance and reporting time.
- How will success be measured? In programs managed by mean kinship there are software tools that assist this task. Some customisation and innovation is required in programs managed through MAI.
- What are the next steps for each institution? It is important that this is clear as unlike mean kinship management, which is able to react annually to shifting program characteristics, MAI is a long-term scheme which, though more flexible in some areas can collapse prematurely if the strategy is not applied uniformly across the program.

It was agreed that the approach to all of these could change over time depending on circumstances but short-term approaches to all were developed as follows:

RECRUITING FOR THE BREEDING POPULATION

In the first instance, within each MAI group, one individual from each clutch (as far as can be determined) will be held back in alternate years.

This should limit growth of the adult population to within manageable limits, according to population models built for and demonstrated at the workshop. If breeding or mortality rates fall outside the expected range this can be revisited.

Once breeding is reliable and consistent, further thought will be given to extending generation length (see below).

OVERLAPPING GENERATIONS.

In the case of non-overlapping generations, when adults die they are replaced by their offspring. At this point in the MAI scheme, males are placed in a newly constituted group NOT with their siblings but with the female offspring of another group, predetermined by the scheme.

Overlapping generations will be dealt with by grouping adults with their same-sex offspring at the point of strategic “single-sex rotations”, which will occur at roughly 5-7 year intervals initially. This may have an impact on the ability to measure success but should have no other adverse consequences.

EXTENDING GENERATION LENGTH

Extending generation length should reduce loss of gene diversity, inbreeding accumulation and the rate of captive adaptation. The strategy proposed for achieving such an extension in this program is to release 100% of each founder’s earlier clutches and recruit only from later ones.

There is a risk to delaying recruitment in this way, which is that founders (and subsequent descendant representatives) could die before replacing themselves. We do not currently have good estimates of age-specific mortality on which to base an analysis of this risk. To manage this risk it may be possible to hold on to earlier litters until later litters have been secured, at which point they can be released. The practicalities of this will be explored over the coming seasons.

At present, generation length (defined as the average age at breeding) is likely to be in the region of 7-8 years. Given the new estimates of longevity and breeding potential discussed at the workshop it may be possible to extend generation length beyond 10 years.

INFORMATION TO BE TRACKED

Wherever possible the following information will be tracked:

- Which MAI group each individual belongs to;
- Number of females and males that breed each year (estimated min. and max.);
- Adult males vs adult females vs juveniles;
- Each individual’s generation number (F0 vs F1 vs F2);
- Ages and age-specific mortality;
- Ages and age-specific fecundity (also inter-birth interval to confirm whether captive females breed in alternate years).

MEASURING SUCCESS

Performance will be assessed against the goals and targets articulated earlier. In some cases it will not be possible to measure performance against goals directly. Instead, in some cases, population simulation models built from estimated parameters may be used to provide pessimistic and optimistic estimates of success.

Protocols for the above activities will be drawn up by the captive program coordinator, Michael McFadden with input from other group members.

AGREED ACTIONS

Actions were summarised during the final session of the day. Most of the action relevant to the captive management strategy will fall to the captive program coordinator, M. McFadden. However, assistance will be required from others in a number of areas.

The following actions were agreed during the meeting:

Action	Responsibility	Deadline
Review DRAFT founder allocation and finalise recommendations for group composition and transfers to Zoos Victoria	M. McFadden	February 2014
Prepare to receive animals from Taronga Zoo and for the establishment and management of agreed MAI groups.	Healesville Sanctuary and Melbourne Zoo staff	April-May 2014
Prepare protocols for: **1) Standardised data collection and reporting.** **2) Holding back individuals for recruitment into the adult breeding population (including standardised method for estimating number of clutches per nest).** **3) Analysing and reporting on success.** **4) Estimating minimum and maximum numbers of males and females breeding annually.**	M. McFadden with input from the wider group	January 2014
Provide a meeting report including presentation materials.	C. Lees with input from others.	December 2013
Provide a draft protocol for the long-term management of an MAI scheme for corroboree frogs, for further development by the group.	C. Lees	December 2013
Look for opportunities through CBSG to elevate awareness of the work of the S. Corroboree Recovery Team as a model OPA-style collaboration between a variety of sectors.	D. Hunter and C. Lees	Ongoing
Continue to share relevant data and experience on n. corroboree frog management to assist both programs.	M. Evans	Ongoing
Explore the limits of extending generation length without risk of founder loss (by releasing each MAI group's earlier clutches only after later ones have been produced, taking care to account for the potential impact of any interim adult attrition).	M. McFadden to lead, all to input.	Ongoing until a protocol is agreed.

Action	Responsibility	Deadline
Monitor and when possible include in the overall strategy, frogs managed in large outdoor chytrid-free areas	M. McFadden & D. Hunter	Ongoing until strategy amendment agreed

IMPLEMENTATION AND NEXT STEPS

Michael McFadden (Taronga Zoo) is the regional zoo association's (ZAA) appointed Species Coordinator for this taxon. In this role he will coordinate and support the implementation of this strategy for and in close collaboration with, the wider recovery team.

Managers at participating institutions will lead on implementing site-specific program recommendations, providing institutional data, communicating issues, and will contribute knowledge and skills to the development of the program over time.

It was agreed that it would be helpful if CBSG could remain in contact with the program until it is running smoothly. C. Lees will continue to liaise with M. McFadden on this.

REFERENCES

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APPENDIX I: WORKSHOP AGENDA

PURPOSE OF THE WORKSHOP:

By the end of this workshop we will:

- Understand what we want from the genetic management of Corroboree frogs;
- Understand the obstacles to achieving what we want;
- Have explored the alternative strategies available;
- Have agreed a long-term strategy or strategies for genetic management of Corroboree frogs;
- Have agreed the next steps that need to be taken, for each institution, to implement this strategy.

PARTICIPANTS

Rupert Baker (Healesville Sanctuary)
Mason Hill (Healesville Sanctuary)
Deon Gilbert (Melbourne Zoo
Peter Harlow (Taronga Zoo)
David Hunter (Recovery Team Chair, NSW Office of Environment and Heritage)
Caroline Lees (CBSG Australasia)
Gerry Marantelli (Amphibian Research Centre)
Michael McFadden (Taronga Zoo)
Murray Evans (Conservation Planning and Research, ACT Government)
Meaghan O'Connor (Tidbinbilla Nature Reserve)
Raelene Hobbs(Melbourne Zoo)
Chris Banks (Zoos Victoria)
Lee Berger (James Cook University)

AGENDA

9.00	Welcome and introduction to the workshop (Michael M.)
9.15	Participant introductions (All)
9.30	Genetic management in captivity: overview (Caroline L)
10.00	Genetic management strategies: overview and recommended approach (Caroline L)
10.45	BREAK
11.00	Distributing founder lineages – group exercise (All)
12.00	LUNCH
12.45	Strategy implementation – site-specific implications – group discussion (All)
14.45	Next steps and concluding remarks
15.00	Close of meeting

APPENDIX II. FOUNDER CLUTCHES

Eggs collected from the same site but in different years are considered unrelated. Those collected from the same site in the same year are considered full-siblings for the purpose of inbreeding management but could be half-siblings as multiple females may lay eggs in the nest of a single male.

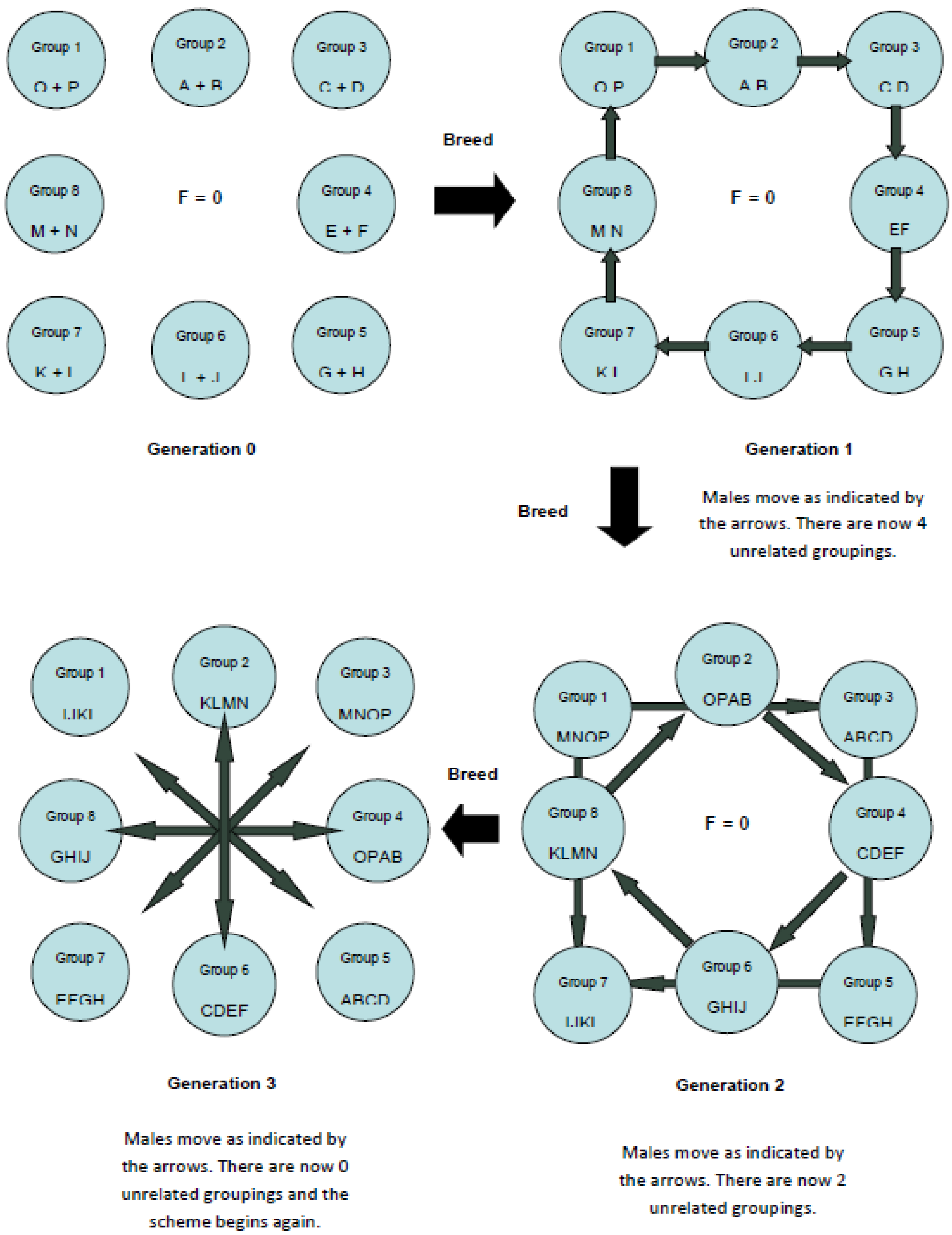
Founder Site	Number (M.F.U.)	Total	Year	Location
Jugumba	0.1	1	1999	MZ
Ogilvies Quarry	0.1	1	2000	MZ
Mt. Dargals	1.1	2	2001	MZ
Snakey Plain	1.2	3	2002	MZ
Jugumba Fire Trail	2.4	6	2003	MZ
Jugumba	13.14	27	2004	TZ
Ogilvies Quarry	6.3	9	2005	MZ
Far Dargals A	1.1	2	2006	TZ
Far Dargals B	1.1	2	2006	TZ
Manjar	6.6	12	2006	TZ
Dargals Saddle A	3.1	4	2006	TZ
Dargals Saddle B	0.4	4	2006	TZ
Snakey Plain	6.7	13	2006	TZ
Site A	0.0.11	11	2007	TZ
Hell Hole	0.0.12	12	2007	TZ
Upper Cool Plains A	12.16	28	2008	TZ
Upper Cool Plains B	0.0.8	8	2008	TZ
Seldom's Bog	0.0.11	11	2008	TZ
Manjar	0.0.16	16	2009	TZ
Upper Ogilvies	0.0.11	11	2009	TZ
Upper Jugumba	0.0.12	12	2009	TZ
Far Dargals	0.0.17	17	2009	TZ
Dargals	0.0.4	4	2009	TZ
Hell Hole	0.0.5	5	2009	TZ
Misc	0.0.2	2	2009	TZ
Manjar	0.0.9	9	2010	TZ
Dargals Flat	0.0.18	18	2010	TZ
Snakey Plain	0.0.11	11	2010	TZ
Hell Hole	0.0.14	14	2011	MZ
Snakey Plain	0.0.15	15	2011	MZ
Snakey Plain	0.0.21	21	2011	TZ
Relly's Bog	0.0.7	7	2011	TZ
Manjar A	0.0.12	12	2011	TZ
Manjar B	0.0.12	12	2011	TZ
Hell Hole A	0.0.6	6	2011	TZ
Hell Hole B	0.0.5	5	2011	TZ
Far Dargals	0.0.2	2	2011	TZ

APPENDIX III. MAI WITH 8 GROUPS

The following provides draft protocols for the year-by-year management of an MAI scheme for corroboree frogs. This document should be modified by practitioners to increase its utility.

PROGRAM FLOW

Each letter (A – P) indicates a unique founder line.



TERMS USED

MAI Group – a grouping of founders whose collective genetic lines will be managed as though they were a single animal.

Breeding Tank – the physical space where animals are placed to breed

Holding tanks – where juveniles and adults are held outside the breeding season

Rearing tanks – where eggs are reared to metamorph stage

OPTIMISATION

Within the resource constraints available, the MAI cycle described below will produce optimal results for the release program where:

- from the founder generation onwards, all animals produce prolifically and equally AND
- the individuals held back for recruitment into the breeding population are of sufficient number and genetically representative of the previous generation AND
- the number of generations occurring within the 50-year program window is minimised by extending generation time in captivity (by holding back offspring from each individual's later clutches OR by starting breeding at a later age, provided that this does not result in individuals being lost from the population prior to contributing offspring)

Optimisation of the proposed scheme will therefore rely upon:

- first-class husbandry and the resulting ability to manipulate breeding and mortality rates
- sound observation and records keeping so that the level of adherence of the program to the proposed scheme and targets can be tracked and modified where necessary.

INITIAL SEEDING OF MAI GROUPS

- MAI groups that are to be included in the genetic management plan should number a power of 2 (e.g. 4, 8, 16, 32 etc)
- The total number of MAI groups managed at each site will be constrained by the availability of breeding tanks, auxiliary holding and rearing tanks and resources, staff time, space, number of founders and by any recommended minimum breeding group size
- It is assumed that the sites involved will seed between 4 and 16 "tanks"
- MAI groups should be seeded with wild-caught individuals in the first instance.
- Each group should contain roughly the same number of individuals and an even sex-ratio (which in reality may be achieved by the strategic rotation of males and females through the tanks.
- Males in the group should be unrelated to females.
- Individuals in any one group should be unrelated to those in every other group.
- Where groups of wild-caught animals are related (i.e. captured from the same nest), one of the following should be considered:
- Where only one sex is involved, keeping those individuals together but rotating them through the breeding tank such that only one is present at any one time
- Where both sexes are involved, moving one of the sexes to another institution for incorporation into one of their MAI groups and treating the other sex as above OR,
    - Keeping both sexes and allocating their time in the breeding tank so that only one representative is present at any one time OR

    - Using one representative in the relevant MAI group and using the others for breeding via a "wild-card" tank which is not part of the MAI scheme but which nevertheless produces young for release to the wild.
- Each MAI group should contain a spread of age-classes.

FIRST 5-7 YEARS

During the first 5-7 years, founders currently in the juvenile age-classes will continue to become available as they move into the reproductive age-classes.

These can be either added to existing MAI groups, used to replace individuals that die (as part of existing MAI groups) or used to seed new sets of MAI groups. The latter would be expected to produce better genetic results overall (as properly managed it could extend the period over which inbreeding can be avoided and allow closer management of individual founder lines) though if breeding success is highly differentiated amongst the groups the latter may be required.

MAI CYCLE

Once a set of MAI groups is established:

Each group is assigned a number – in this example from 1-8.

To simplify tracking and because no ability to differentiate between lines within groups is assumed from this point forward, each group is assigned two letters of the alphabet – one delineating all of the female genetic lines and one all the male ones (e.g. AB)

YEAR 1

SEED BREEDING TANKS

At the start of the breeding season, each of the 8 breeding tanks is seeded with a number of males.

ROTATION

Where tanks space is limited, females that are part of the group may be swapped in and out during the season to give a larger number the opportunity to breed.

[Males are not switched within-season but may take turns in the tank between seasons. If possible, those males in the MAI Group that did not get an opportunity to breed in this year will be prioritised for breeding opportunities in subsequent years.]

RE-HOUSE POST-SEASON

At the end of the breeding season, adults are returned to male and female holding pens. All individuals at TZ and MZ at least have dorsal and ventral digital photographs allowing individual identification.

Eggs are collected, moved to rearing pens and raised to hatching ready for release to the wild.

RELEVANT TARGETS ARE:

TOTAL OF 100 CLUTCHES, GENERATING 2000 VIABLE EGGS FOR RELEASE

RETAIN FOR RECRUITMENT

Prior to release, a subset of hatchlings is retained for recruitment into the captive breeding population. ASSUMING THAT THE MAI GROUPS BEGIN AT CAPACITY the aim of this is to ensure that each individual in the MAI Group replaces itself in the next generation. Due to attrition between the ages of 0-5 this will require that more than 1 hatchling is held back for each individual in the MAI Group.

Hatchlings will be held back from each MAI Group in alternate years. Assuming that each individual has a breeding life of at least 5 years, and assuming that they breed each year, there could therefore be 2-3 individuals held back for each breeder. However, due to asymmetrical breeding success within MAI Groups this may also result in the same lines being collected several times. Attempts will be made to reduce this problem by tracking which males are on which nests, and which females in the tank breed successfully in any given year (by tracking body weight change).

In the year when an MAI group is sampled for recruitment, attempts will be made to hold back one egg from each clutch laid. As each nest may contain multiple clutches, and as these egg masses may be indistinguishable, a rough guide will be used to sampling. That is, the number of eggs sampled from any given nest will correspond to the number of clutches thought to be present, using the following guide:

- 1-30 eggs = 1 clutch
- 31-60 eggs = 2 clutches
- 61-90 eggs + 3 clutches etc.

NOTE THAT THE NUMBER HELD BACK FROM EACH CLUTCH/MAI GROUP SHOULD BE REVISED AS MORE DATA BECOME AVAILABLE ON AGE-SPECIFIC MORTALITY RATES.

Note that in the first 10 years of the program, if capacity is limited, offspring of the younger founders should take precedent as these founders have had more chytrid exposure.

RELEASE

All hatchlings not required for recruitment or for other recovery-related purposes are released.

END OF YEAR 1 HOLDINGS INVENTORY

For each MAI Group (example given for Group 1)

Holding 1: F0 Females (A)

Holding 2: F0 Males (B)

Holding 3: F1 offspring (AB) – aged 0

Total of 8 identifiable separate genetic lines (though in reality there should be more than this).

Range of inbreeding coefficients expected in each MAI Group = F=0

YEAR 2

Check that there are sufficient F0 adults in each MAI Group to make up a breeding tank (at least 5.5)

If not, recruit from other MAI Groups any surplus founders thought not to have bred in previous years, or recruit from recently matured juveniles.

Seed breeding tanks

Where possible, seed with males that did not get an opportunity to breed in Year 1.

ROTATION

Where tanks space is limited, females that are part of the group may be swapped in and out during the season to give a larger number the opportunity to breed.

RE-HOUSE POST-SEASON

At the end of the breeding season, adults are returned to male and female holding pens. Eggs are collected, moved to rearing pens and raised to hatching ready for release to the wild.

RELEVANT TARGETS ARE:

TOTAL OF 100 CLUTCHES, GENERATING 2000 VIABLE EGGS FOR RELEASE

RETAIN FOR RECRUITMENT

From Tanks 5-8:

Hold back at least one hatchling from each nest.

Where a nest is thought to contain multiple clutches, add an extra hatchling for each additional clutch. See Guide from Year 1:

RELEASE

All hatchlings not required for recruitment or for other recovery-related purposes are released.

END OF YEAR 2 HOLDINGS INVENTORY

For each MAI Group (example given for Group 1)

Holding 1: F0 Females (A)

Holding 2: F0 Males (B)

Holding 3: F1 offspring (AB) – aged 0

F1 offspring (AB) – aged 1

Total of 8 identifiable separate genetic lines (though in reality there should be more than this).

Range of inbreeding coefficients expected in each MAI Group = F=0

YEARS 3, 4, 5, 6

Repeat pattern, recruiting from MAI Groups 1-4 in years 3 & 5 and from MAI Groups 3-8 in years 4 & 6.

END OF YEAR 6 HOLDINGS INVENTORY

For each MAI Group (example given for Group 1)

Holding 1: F0 Females (A)

Holding 2: F0 Males (B)

Holding 3: F1 offspring (AB) – aged 0

F1 offspring (AB) – aged 1

F1 offspring (AB) – aged 2

F1 offspring (AB) – aged 3

F1 offspring (AB) – aged 4

F1 offspring (AB) – aged 5

It should be possible to distinguish these cohorts through individual pattern markings.

Range of inbreeding coefficients expected in each MAI Group = F=0

Year 7 (OR BEFORE IF MORTALITY IS HIGHER THAN EXPECTED)

By Year 7, and possible before, the number of F0 adults in each MAI Group should be starting to decline and those founders that bred successfully should be represented amongst the juveniles held back for recruitment to the breeding population.

If both the breeding adults and the juvenile age-classes are surviving well, it may be possible to extend this phase for another couple of years. The risks to consider are:

- The risk of allowing the adult breeding population to decline below the level required to generate the target 2000 viable eggs for release
- The risk of losing the genetic contribution of the 4-5 year-old animals as a result of pre-breeding attrition.

Where these risks are considered too high, move males as described below.

SEED BREEDING TANKS

For each MAI group, move remaining founder males plus all F1 males to a new MAI group using the formula below:

MAI GROUP 1 → MAI GROUP 2

MAI GROUP 2 → MAI GROUP 3

MAI GROUP 3 → MAI GROUP 4

MAI GROUP 4 → MAI GROUP 5

MAI GROUP 5 → MAI GROUP 6

MAI GROUP 6 → MAI GROUP 7

MAI GROUP 7 → MAI GROUP 8

MAI GROUP 8 → MAI GROUP 1

Leave the females in each group where they are, augmenting the breeding pool as needed, with females bred within that MAI Group.

As usual, where possible, seed tanks with males that did not get an opportunity to breed in previous years (unless considered non-breeding) though giving greater weight to any remaining founders.

ROTATION

Where tanks space is limited, females that are part of the group may be swapped in and out during the season to give a larger number the opportunity to breed.

RE-HOUSE POST-SEASON

At the end of the breeding season, adults are returned to male and female holding pens. Eggs are collected, moved to rearing pens and raised to hatching ready for release to the wild.

RELEVANT TARGETS ARE:

TOTAL OF 100 NESTS, GENERATING 2000 VIABLE EGGS FOR RELEASE

RETAIN FOR RECRUITMENT

From Tanks 1-4:

Hold back at least one hatchling from each nest

Where a nest is thought to contain multiple clutches, add an extra hatchling for each additional clutch. See Guide from Year 1:

RELEASE

All hatchlings not required for recruitment or for other recovery-related purposes are released.

END OF YEAR 7 HOLDINGS INVENTORY

For each MAI Group (example given for Group 1)

Holding 1: F0 Females (A)+F1 Females (OP)

Holding 2: F0 Males (B)+F1 Males (AB)

Holding 3: F2 offspring (OPAB) – aged 0

F1 offspring (AB) – aged 1

F1 offspring (AB) – aged 2

F1 offspring (AB) – aged 3

F1 offspring (AB) – aged 4

F1 offspring (AB) – aged 5

Inbreeding coefficient expected in each MAI Group = F=0.00

YEARS 8-12

These years should see the end of the F0 generation (by year 10) and a maturing to adulthood of the first F2 cohorts. Continue as for previous years

END OF YEAR 12 HOLDINGS INVENTORY

For each MAI Group (example given for Group 1)

Holding 1: F1 Females (OP)

Holding 2: F1 Males (AB)

Holding 3: F2 offspring (OPAB) – aged 0

F2 offspring (OPAB) – aged 1

F2 offspring (OPAB) – aged 2

F2 offspring (OPAB) – aged 3

F2 offspring (OPAB) – aged 4

F2 offspring (OPAB) – aged 5

YEAR 13

By Year 13, and possible before, the number of F1 adults in each MAI Group should be starting to decline and those F1s that bred successfully should be represented amongst the juveniles held back for recruitment to the breeding population.

If both the breeding adults and the juvenile age-classes are surviving well, it may be possible to extend this phase for another couple of years. The risks to consider are:

- The risk of allowing the adult breeding population to decline below the level required to generate the target 2000 viable eggs for release
- The risk of losing the genetic contribution of the 4-5 year-old animals as a result of pre-breeding attrition.

Where these risks are considered too high, move males as described below.

SEED BREEDING TANKS

Move all males in each MAI Group to a new target MAI Group as indicated below:

MAI GROUP 1 → MAI GROUP 3

MAI GROUP 2 → MAI GROUP 4

MAI GROUP 3 → MAI GROUP 5

MAI GROUP 4 → MAI GROUP 6

MAI GROUP 5 → MAI GROUP 7

MAI GROUP 6 → MAI GROUP 8

MAI GROUP 7 → MAI GROUP 1

MAI GROUP 8 → MAI GROUP 2

Leave females where they are, augment the breeding pool as needed, with females bred within that MAI Group.

As usual, where possible, seed breeding tanks with males that did not get an opportunity to breed in previous years (unless considered non-breeding) though giving greater weight to any remaining F1s.

ROTATION

Females may be swapped in and out of the tank to give a larger number the opportunity to breed.

RE-HOUSE POST-SEASON

At the end of the breeding season, adults are returned to male and female holding pens. Eggs are collected, moved to rearing pens and raised to hatching ready for release to the wild.

RELEVANT TARGETS ARE:

TOTAL OF 100 NESTS, GENERATING 2000 VIABLE EGGS FOR RELEASE

RETAIN FOR RECRUITMENT

From Tanks 1-4:

Hold back at least one hatchling from each nest

Where a nest is thought to contain multiple clutches, add an extra hatchling for each additional clutch. See Guide from Year 1:

RELEASE

All hatchlings not required for recruitment or for other recovery-related purposes.

END OF YEAR 13 HOLDINGS INVENTORY

For each MAI Group (example given for Group 1)

Holding 1: F1 Females (OP)+F2 Females (OPAB)

Holding 2: F1 Males (AB)+F2 Males (OPAB)

Holding 3: F3 offspring (KLMNOPAB) – aged 0

F2 offspring (OPAB) – aged 1

F2 offspring (OPAB) – aged 2

F2 offspring (OPAB) – aged 3

F2 offspring (OPAB) – aged 4

F2 offspring (OPAB) – aged 5

Inbreeding coefficient expected in each MAI Group = F=0.00

YEARS 14-17

Repeat pattern.

YEARS 18-23

Complete once final migration of males using the following guide:

MAI GROUP 1	→	MAI GROUP 5
MAI GROUP 2	→	MAI GROUP 6
MAI GROUP 3	→	MAI GROUP 7
MAI GROUP 4	→	MAI GROUP 8
MAI GROUP 5	→	MAI GROUP 1
MAI GROUP 6	→	MAI GROUP 2
MAI GROUP 7	→	MAI GROUP 3
MAI GROUP 8	→	MAI GROUP 4

Once this final phase is complete, inbreeding can no longer be avoided, though the rate of accumulation can be minimised by repeating the pattern.