AMPHIBIAN POPULATION MANAGEMENT GUIDELINES

These guidelines are subject to further refinement.
Please check with the Amphibian Ark (www.amphibianark.org) for any updates.

Created at an amphibian population management workshop held on
10-11 December 2007 at San Diego Zoo, San Diego, California, U.S.A
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Amphibian Ark (AArk)
Association of Zoos and Aquariums (AZA)
Australasian Regional Association of Zoological Parks and Aquaria (ARAZPA)
AZA Population Management Center, Lincoln Park Zoo
Chicago Zoological Society - Brookfield Zoo
Conservation Breeding Specialists Group (CBSG) of the IUCN Species Survival Commission
Fresno Chaffee Zoo
Zoological Society of London (ZSL), London Zoo
Minnesota Zoological Garden
Sandfire Dragon Ranch
Toledo Zoological Gardens
World Association of Zoos and Aquariums (WAZA)
Zoological Society of San Diego

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INTRODUCTION

Compiled by Brandie Smith, Association of Zoos and Aquariums

The maintenance of genetic variation within a population increases the probability of both its long- and short-term survival and that of the comprising individuals. As the basis for evolution, genetic variation allows populations to adapt to changing environments (Allendorf 1986; Lewontin 1974; Selander 1983) and many studies have shown its benefits to individual fitness (Hedrick et al. 1986; Allendorf and Leary 1986; Ralls et al. 1995; Lacy et al. 1993; Wildt et al. 1987). Small populations are especially susceptible to loss of genetic variation through the process of genetic drift (Nei et al. 1975). This random fluctuation in allele frequencies can greatly impact the genetic composition of small populations, hastening their demise.

The science of population management has been greatly advanced through programs developed for captive populations (Ballou and Lacy 1995; Lacy et al. 1995; Ballou and Foose 1996). Professionally managed zoos and aquariums maintain populations of animals for display, conservation, research, and education purposes (Hutchins & Conway 1995). Because these populations are small and widely dispersed, they are managed cooperatively through captive breeding programs such as the Association of Zoos and Aquariums (AZA) Species Survival Plan (SSP®) and Population Management Plan (PMP), the Australasian Regional Association of Zoological Parks and Aquaria (ARAZPA) Australasian Species Management Program (ASMP), and the European Association of Zoos and Aquaria (EAZA) Endangered Species Programme (EEP). Through these programs, specific breeding recommendations are made to help maintain sustainable populations that are genetically diverse and demographically stable.

The goal of captive genetic management is to stop evolution. More specifically, management is intended to minimize changes in a population's gene pool to retain as many of the genetic characteristics of the population's original founders as is possible (Ballou and Lacy 1995). Founders are individuals that are assumed to be unrelated and that have living descendents. It is currently feasible to slow the loss of genetic diversity in pedigreed populations through intense management. The genetic constitution of the entire population can be examined from information found in the pedigree, animal-by-animal breeding recommendations can be made, and the effects of long-term management evaluated.

The current strategy used worldwide by cooperative captive breeding programs to minimize loss of genetic diversity pairs individuals according to a mean kinship (MK) value (Ballou and Lacy 1995). Under this strategy, an individual's genetic importance can be assessed based on the number and degree of relatives that the individual has in the population. Individuals with the lowest mean kinship are priority breeders. Mean kinship has proven to be the best strategy at maintaining genetic diversity in pedigreed populations, tested against alternatives in both a computer simulation (Ballou and Lacy 1995) and on living organisms (Montgomery et al. 1997). Mean kinship is only effective when the entire pedigree is known and pairings can be controlled. This strategy is practical for many species in captivity including elephants, komodo dragons, and vultures, but impractical for species with insufficient information or those where we have less control of pairings. For these species, recommendations are more lenient by attempting to minimize inbreeding and prevent fixation of alleles in subpopulations.

The class Amphibia includes three orders – anurans (frogs and toads), caecilians, and caudates (salamanders and newts) – and covers over 6000 species which exhibit a wide range of natural histories and reproductive strategies. Although some amphibians follow a reproductive model that allows individual identification, known parentage, and controlled pairings, many more do not. In addition, behavioral considerations are very important in maintaining captive amphibian populations and specific environmental cues may be needed to achieve reproduction in captive breeding programs (Pramuk and Gagliardo 2008). Consequently many species of amphibians in captivity do not fit the mean kinship model and a diverse range of specific management techniques must be implemented to maximize the maintenance of genetic diversity. These techniques are the topic of these 'Amphibian Population Management Guidelines.'

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DATA MANAGEMENT FOR AMPHIBIAN POPULATIONS

Compiled by Sarah Long and Kristine Schad, AZA Population Management Center

Management of amphibian populations in zoos depends on databases called "studbooks." A studbook is a record of the chronological history of a single managed species. It is compiled from institutional data based on all known information about each individual in the population, including its relationships to other individuals and dates of birth and death. Studbooks provide the data for demographic and genetic analyses, which in turn help ensure a population's survival in zoos & aquariums. Studbooks tracking individuals can be created easily using SPARKS or PopLink software (see below).

DEMOGRAPHY

Definition: Demography is the science of how a population's size, structure, and distribution have changed in the past and how they might be expected to change in the future.
Goals: To achieve and maintain desired population sizes, stable age structures, and biologically appropriate sex distributions.
Implementation: Determining and recommending the number of births or hatches that will help the population achieve its demographic goals.
Information required: In order to plan the appropriate number of births or hatches, we need to know information about the animals' reproductive capabilities (e.g., age at first and last reproduction, litter/clutch size, interbirth interval, probabilities of breeding at various ages, etc.) and mortality rates (probability of dying at different ages, lifespan, etc.).
Raw data required: Reproductive and mortality data are essentially derived from four critical pieces of information—birth dates, parentage or other monitoring of reproductive performance, death dates, and sex.

POPULATION GENETICS

Definition: Population genetics is the study of how a population's genetic structure, more specifically, how the frequencies of alleles (variants of genes), are distributed within and between populations, as well as how these distributions change over time.
Goal: To preserve gene diversity and avoid inbreeding.
Implementation: Gene diversity is maintained and inbreeding is avoided through the careful selection of breeding individuals or groups.
Information Required: In order to determine which males, females, or groups should be reproducing, we need to know their pedigrees. Pedigrees give us information about the comparative genetic value of each animal or group (how unique or common their alleles might be) and their relatedness to each other. If individual or group parentage is unknown or uncertain, then the other variables become important as clues for how animals may be related (e.g., source, location, and date of birth or immigration, etc.).
Raw data required: Pedigree and relatedness information is based on parentage data that are traced through the generations from the living animals back to the wild born/hatched founders.

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RECORD KEEPING FOR INDIVIDUAL MANAGEMENT

- Individually identify, mark, and record the different founders and their descendants, if possible.
    - Use any feasible method – transponders, implanted tags, photographs of unique markings, separate enclosures (both for individuals and groups), etc.
- Parentage of individuals
    - Record sire and dam whenever possible
- Sex of each individual, if possible
- Birth/hatch date, Location, and Origin
    - If wild caught, record date, site location, possible relationship to other wild caught individuals (i.e., several amphibians captured from same water source), and date animal entered captivity
    - If zoo/aquarium born, record parents and their wild caught locations
- Locations and transfers of individuals (i.e., moving to a new enclosure, mixing with a new individual/group, transferring to a new institution)
- Enclosure composition (i.e., who housed with whom, in breeding situation or not)
- Death date, Location, and Cause
    - Note if death was due to various natural causes vs. managed cull

RECORD KEEPING FOR GROUP MANAGEMENT

INITIAL DATA ENTRY

- Identify, mark, and record the different founders or founder groups with unique identifiers.
    - Use any feasible method –label separate enclosures, etc.
- Track enclosures, locations
- Origin or parentage of group (founders from the wild, split from another group, combinations of other groups). Be as specific as possible to track group pedigree and genetic composition.
    - If wild caught, record date, site location, possible relationship to other wild caught individuals (i.e., several amphibians captured from same water source), and date animals entered captivity.
- Group composition (who is housed with whom)
- Generation number (e.g., founder, F1, F2, etc.)

ONGOING DATA COLLECTION

- Take regular census counts (weekly, monthly, or as often as is feasible) and record dates associated with these counts to identify:
    - Number in each life stage (i.e. eggs/clutches, metamorphs, adults)
    - Number of each sex (if possible)
    - Number of deaths
        - Cause of death (various natural causes vs. managed cull)
- Record any events and dates associated with them
    - Transfer of groups (new enclosure, location information)
    - Splitting groups (record ID & location of new subgroups)
    - Merges of groups (record ID & location of new combined group)
    - Reproductive or developmental events

Ballou J.D. and Foose T.J. 1996. Demographic and genetic management of captive populations. In Kleiman D.G., Lumpkin S., Allen M., Harris H., Thompson K. (eds.) *Wild Mammals in Captivity*. Chicago, IL: University of Chicago Press. p. 263-283.

Population Group Management Workshop; 2002 May 16-18; Seattle, Washington. Association of Zoos and Aquariums; 2002.

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SOFTWARE

SPARKS (Single Population Analysis and Record Keeping Software, ISIS) is a DOS-based computer program designed to be used in the management and analysis of studbook databases. A studbook is an electronic record of the history of a captive population. It includes information on every individual in a population, including pedigrees and dates of birth, death and transfers between institutions. The studbook traces the entire history of each individual in a population; these collective histories describe the population's genetic and demographic identity.

SPARKS software is available to all members of ISIS (International Species Information System) on the ISIS software installation CD.

PopLink is a Windows-based computer program designed to be used in the management and analysis of studbook databases. Similarly to SPARKS, PopLink can help maintain, analyze and export the data for a captive population that are relevant to its genetic and demographic management. PopLink can import and export a studbook from/to SPARKS, the current software used to manage studbook datasets. Studbook keepers can use PopLink to track and maintain all the data relevant to an individual species within zoos. Population biologists can use PopLink to store analytical data, the version of the studbook used in the genetic and demographic analyses that management decisions are based on. PopLink includes many tools that assist with the development and maintenance of the analytical data necessary for management. PopLink was developed by Lincoln Park Zoo.

PopLink is shareware that is distributed free of charge by Lincoln Park Zoo from www.lpzoo.org/poplink. Any questions or comments can be directed to software@lpzoo.org**.**

PM2000 software provides a suite of tools for genetic and demographic analysis and management of pedigreed animal populations (a studbook). PM2000 combines the capability of the MS-DOS programs GENES (written by Robert Lacy, Chicago Zoological Society), DEMOG (written by Laurie Bingaman-Lackey and Jon Ballou, National Zoological Park), and CAPACITY (written by Jon Ballou), as well as adding some new features. PM2000 was developed by JP Pollak (Cornell University), Bob Lacy, and Jon Ballou.

PM2000 is shareware that can be obtained from http://www.vortex9.org/pm2000install.zip

Other population management tools are currently being developed, by Zoological Society of London, Chicago Zoological Society, National Zoo (Washington) and likely elsewhere. It is expected that these additional tools will be available soon to help with management of amphibians, especially those species for which pedigrees cannot be accurately tracked and managed at an individual level

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GENETIC MANAGEMENT OF AMPHIBIAN POPULATIONS

Compiled by Sarah Long, AZA Population Management Center

Generally, high levels of gene diversity are associated with GREATER/HIGHER values of the following:

- Number of founders (founders = unrelated individuals who help establish a population) (See Appendix A, Figures 1, 2, & 3)
- Proportion of breeding individuals (# breeding individuals / total # individuals) (See Appendix A, Figures 4, 5a, & 5b)
- Population growth rate (See Appendix A, Figures 6 & 7)
- Population size (starting size and target size) (See Appendix A, Figure 4)
- Number of offspring that survive to reproduce

BASIC GUIDELINES FOR GOOD GENETIC MANAGEMENT:

FOUNDERS

- Start the population with at least 20 founders, ideally with an equal sex ratio (i.e., 10.10). (Note: throughout this document, the notation x.y will be used to signify x number of males and y number of females.) (See Appendix A, Fig. 1, 2, & 3)
    - This means at least 20 individuals (or groups of individuals) that are unrelated and that will successfully reproduce. Realize that many more than this number may have to be captured to ensure that 20 actually survive and successfully reproduce.
    - Collection of founders should be targeted towards obtaining as many unique lineages as possible (e.g., collect from different locations and, if possible, different sites at each location to reduce the probability of collecting related animals).

BREEDING – HOW MANY?

- Produce an equal number of offspring from each founder to equalize family sizes within the space available for the taxon. (See Appendix A, Figures 4, 5a, & 5b)
    - Produce at least 5 offspring per founder.
    - Keep numbers of offspring equal across founders – based on the amount of space available, divide spaces for offspring equally for each founder.

POPULATION GROWTH – HOW QUICKLY SHOULD THE POPULATION GROW?

- Species with short generation times (reproductive lifespan < 5 years) will need to have as many individuals produce as many offspring as fast as possible. A larger target population size is also beneficial (both to avoid demographic crisis and better retain genetic diversity). (See Appendix A, Figures 6 & 7)

ENVIRONMENT

- Environmental conditions should encourage reproduction and minimize unintentional selection in the highly altered zoo environment. However, conditions should also not be so narrow and rigid as to encourage unintentional selection to specific captive conditions. Variation in the captive environment will help maintain genetic variation and allow for testing of possible improvements in husbandry.

BREEDING – WHICH INDIVIDUALS/GROUPS?

- Once founders have successfully reproduced, keep these same pairs/groups together; do not mix and match unnecessarily. If potential founder pairs fail to breed successfully, then try other pairings and any other available manipulations to try to propagate their genes.
- Prioritize breeding the parental generation before the offspring.
    - Parents are always more genetically valuable than their offspring.
    - However, attempt to breed the 2nd generation before the founders die to test husbandry methods.
    - Descendants can be bred with founders when there are no other options.
- Prioritize underrepresented lineages (those with fewer descendants) for breeding and pair animals with similar genetic value:
    - If lineages are unequal, breed the smaller, underrepresented family lines with other underrepresented family lines.
    - If space allows, breed overrepresented family lines with other overrepresented family lines.

**Note: if individuals can be marked and individual pedigrees tracked, then breeding those with the lowest mean kinship will achieve several of the above goals.

CULLING

- Cull surplus offspring from the population when necessary in order to equalize founder lineages & to stay within target population size.
    - "Culling" is used here to refer to any method of permanently removing individuals from the primary breeding population, such as:
        - Transfer to any nonbreeding population (for research, display, etc.)
        - Release back into native environment when appropriate
        - Euthanasia (when animals are euthanized, biomaterials should be preserved, for example by depositing in the frozen zoo at San Diego Zoo, California, U.S.A. or Frozen Ark, University of Nottingham, UK)
    - To avoid selection prejudices:
        - The number of individuals/groups to be culled should be based on equalizing family size.
        - The selection of which individuals/groups to be culled from a family lineage should be <u>randomly</u> chosen (e.g. do not target for culling only the fast-developing tadpoles, the slowest swimmers, the ugliest specimens, etc.).
        - If individuals and pedigrees are tracked, then culling those animals with the highest mean kinships will achieve the two goals above
        - If culling is necessary, it should be done at the earliest life stage possible without compromising the stability and survival of the population.
    - Culling should follow appropriate disposition policies (government, institution, association, etc.).

SPECIAL CONSIDERATIONS FOR POPULATIONS MANAGED AS GROUPS - Basically, the same rules as described above apply to groups, but some special considerations are worth mentioning:

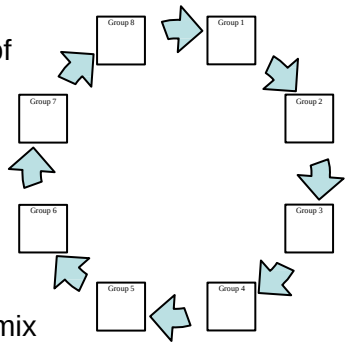
GROUP SIZE

- Keep group sizes as small as is effective for the biology of the species while meeting the husbandry needs for captive management
- Keep as many groups as space and reproductive biology allows.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group after they breed to allow other individuals to breed.

GROUP BREEDING STRATEGIES

There are several strategies to retain gene diversity in populations of group-living animals:

A. Once reproduction occurs, systematically transfer individuals among groups in a "round robin" manner (see figure). We recommend one of these methods:
   - Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
   - Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
   - Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.



OR

B. Keep each unique founder group together indefinitely and allow them to interbreed (and inbreed) without mixing with other groups. This method does not imply maintaining the entire population of a species as a single group (i.e. do not put all your eggs in one basket). Rather, this method assumes some initial subdivision into many smaller groups, to safeguard against catastrophic events, and then moving forward with isolated group breeding. This strategy can maintain founder lineages in each group, but also involves the risks of rapid genetic loss, if some groups are lost, and quick, possibly deleterious, inbreeding. The population should be monitored for signs of inbreeding depression, so transfers to reverse inbreeding can be implemented if inbreeding is threatening success. Transfers may be necessary eventually if inbreeding depression develops.

OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

Ballou J.D. and Foose T.J. 1996. Demographic and genetic management of captive populations. In Kleiman D.G., Lumpkin S., Allen M., Harris H., Thompson K. (eds.) *Wild Mammals in Captivity*. Chicago, IL: University of Chicago Press. p. 263-283.

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DEMOGRAPHIC CONSIDERATIONS FOR MANAGEMENT OF AMPHIBIAN POPULATIONS

Compiled by Lisa Faust, Alexander Center for Applied Population Biology, Lincoln Park Zoo

Establishing stable and viable populations of amphibians in captivity is initially dependent on working out the husbandry techniques to ensure survival and reproduction of wild-caught individuals. Once those techniques are established, the decision of how large a population should be maintained to ensure long-term viability should be considered, ideally for each individual population by an experienced population biologist. It is difficult to generalize about a single magic number that is a minimum viable population size from a demographic perspective because the demographic patterns of amphibian species in the wild fall into a wide range of life histories and the goals for a captive population may vary. Amphibian populations can be very "fast" species with high fecundity, high mortality, and large fluctuations in population size over time or "slow" species with lower fecundity and mortality which have more stable population sizes over time, or may lie somewhere in between the two life history extremes (for a good summary, see Green 2000).

However, demographic theory and some pre-existing research on amphibians can provide some minimal starting guidance on important demographic considerations for captive amphibian management. If a population's size is too small, it becomes susceptible to stochasticity, which is variability in survival and reproduction that can be due to demographic or environmental processes, which can result in further declines in population size or extinction. A general rule of thumb is that populations may be less susceptible to demographic stochasticity if they are at least 100 individuals (Morris and Doak 2002).

In addition, the total population size is not the only important determinant of a population's viability, as different life stages can be more or less important to long-term persistence. Biek et al. (2002) looked at several amphibian populations with a range in demography (although most would still be considered "fast" species) to determine the importance of different life stages to the population's long-term growth rate. They found that post-metamorphic vital rates were more critical to long-term growth than pre-metamorphic vital rates. This indicates that, once basic husbandry techniques have been worked out, improving survival rates in post-metamorphic stages is the most important objective for increasing the size of a captive population.

An additional important consideration for management of demographic risks includes protecting populations from catastrophic loss by essentially "not putting all your eggs in one basket". When setting up a new captive population, it would be extremely risky to setup the entire population in a single tank because of the risks from common catastrophes such as electrical failures, disease outbreaks, issues with water contamination, food problems, etc. These risks can be mitigated by spreading the population across multiple tanks in the same area, in multiple locations across an institution, or at multiple institutions.

Ultimately, once husbandry techniques have been perfected for new taxa, managers and population biologists should fully evaluate its population biology. A target population size can be set based on genetic goals, and then demographic management tactics can be planned to meet that target size goal based on the population's survival and fecundity rates. Amphibians can vary hugely with respect to fecundity, although husbandry considerations and high mortality of early life stages will narrow the range that reach metamorphosis. From a population management perspective, the important consideration for population growth is the number of progeny per brood that survive to breeding age. Culling of individuals from large broods to reduce numbers to a desired or manageable level should generally aim to leave equal numbers per brood, or should use mean kinship strategies to determine which ones to cull.

Biek R., Funk W.C., Maxell B.A., and Mills L.S. 2002. What is Missing in Amphibian Decline Research Insights from Ecological Sensitivity Analysis. Conservation Biology 16(3): 728-734.

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Morris W.F. and Doak D.F. 2002. Quantitative Conservation Biology. Sinauer Associates Inc. Sunderland, MA. 479 pp.

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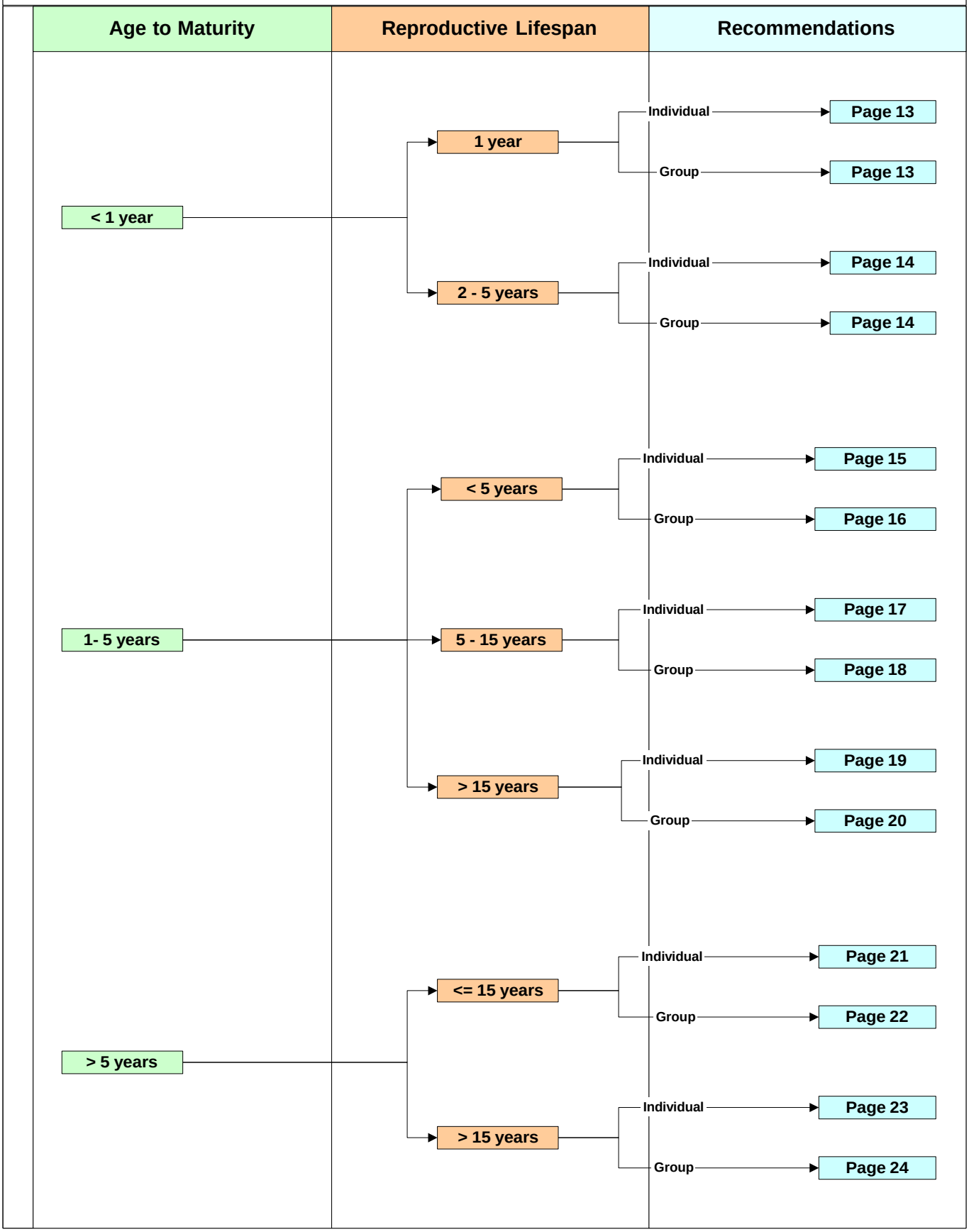
Decision Tree



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Age to Maturity	Reproductive Lifespan
< 1 year	1 year

Example Species: ***Acris crepitans***

Population Management Issue: These species will lose genetic diversity very fast; so many founders and large population sizes will be needed.

INDIVIDUAL MANAGEMENT

- Individual Management may not be feasible for these types of species. However, if you choose to manage individually use the group management recommendations below to ensure long-term viability of the population.

GROUP MANAGEMENT

How many founders to collect?

- You want 50.50 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (i.e., if you expect 50% of the collected animals to survive and reproduce, you should collect 100.100 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 50.50 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 6 months and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
10	635
15	950
25	1590
40	2540
55	3490
70	4430
85	5480
100	6330

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible due to short lifespan (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:

A. Once reproduction occurs, systematically transfer individuals among groups in a "round robin" manner. We recommend one or more of these methods:
   - Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
   - Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
   - Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
< 1 year	2 - 5 years

Example Species: *Eleutherodactylus, Nectophrynoides*, some *Hyperoliidae*

Population Management Issue: These species will lose genetic diversity fast. When managed in groups, larger population sizes are needed to ensure an adequate effective population size.

INDIVIDUAL MANAGEMENT

- Individual Management may not be feasible for these types of species. However, if you choose to manage individually use the group management recommendations below to ensure long-term viability of the population.

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 2 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	400
40	635
55	875
70	1110
85	1350
100	1585

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible due to short lifespan (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:

A. Once reproduction occurs, systematically transfer individuals among groups in a “round robin” manner. We recommend one or more of these methods:
   - Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
   - Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
   - Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
1 - 5 years	< 5 years

Example Species: some *Hylidae*, some *Hyperoliidae*, *Scaphiophryne*

Population Management Issue: These species have short reproductive life spans, so breeding opportunities can be lost if delayed. With relatively short generation time, large population sizes will still be needed.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 3 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	135
40	215
55	290
70	370
85	450
100	530

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005).
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

Age to Maturity	Reproductive Lifespan
1 - 5 years	< 5 years

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 3 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	265
40	425
55	590
70	740
85	900
100	1060

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:
A. Once reproduction occurs, systematically transfer individuals among groups in a “round robin” manner. We recommend one or more of these methods:

- Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
- Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

OR

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
1 - 5 years	5 - 15 years

Example Species: ***Dendrobatidae*, *Typhlonectes*, *Tylototriton*/*Echinotriton*, *Theloderma*, *Cynops*, *Leptodactylus*, *Ceratobatrachus*, *Mantella*, *Atelopus***

Population Management Issue: These species have life histories that often start to approximate those of typical larger vertebrates, and therefore population management strategies can often be more like that used for most birds and mammals. However, although genetic management becomes easier, there may be more of a risk of demographic failure for species maintained at smaller numbers.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 6 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	70*
40	110
55	150
70	190
85	225
100	265

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005).
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
1 - 5 years	5 - 15 years

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 6 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	140
40	225
55	300
70	370
85	450
100	530

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:
A. Once reproduction occurs, systematically transfer individuals among groups in a “round robin” manner. We recommend one or more of these methods:

- Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
- Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

OR

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
1 - 5 years	> 15 years

Example Species: *Salamandra*, some *Ambystoma*

Population Management Issue: These species have life histories very much like those of the larger vertebrates. Population management would benefit from moving toward individual management, rather than group management, whenever feasible.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 7 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	60*
40	95*
55	125
70	160
85	195
100	230

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005).
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

Age to Maturity	Reproductive Lifespan
1 - 5 years	> 15 years

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 7 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	115
40	185
55	250
70	320
85	390
100	455

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:
A. Once reproduction occurs, systematically transfer individuals among groups in a “round robin” manner. We recommend one or more of these methods:

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- Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

OR

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
> 5 years	**≤ 15 years**

Example Species:

Population Management Issue: Very slow population growth when fecundity is low, and the possibility for replacements of the population with predominantly the progeny of one or a few pairings when fecundity is high, means that each paring and each individual is important to population success.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 5 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	80*
40	130
55	175
70	225
85	270
100	320

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005).
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

Age to Maturity	Reproductive Lifespan
> 5 years	**≤ 15 years**

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 5 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	160
40	255
55	350
70	445
85	540
100	635

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

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- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

OR

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Age to Maturity	Reproductive Lifespan
> 5 years	**> 15 years**

Example Species: *Cryptobranchus*, *Andrias*

Population Management Issue: Genetic diversity can be maintained with relatively small populations, but these small populations may be vulnerable to demographic collapse or loss due to local environmental catastrophes hitting one out of only a few facilities.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 10 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	45*
40	65*
55	90*
70	115
85	140
100	160

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005)
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

Age to Maturity	Reproductive Lifespan
> 5 years	**> 15 years**

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 10 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	80*
40	130
55	180
70	225
85	270
100	320

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

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Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
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- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

OR

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

ADDITIONAL CONSIDERATIONS

- Manipulating husbandry to minimize adaptation to captivity
- Avoid intentional and unintentional selection
- Husbandry learning curve—when bringing in a new species, possibly start with both pairs and groups to figure out best way to get them to breed
- Multiple paternity in individually-managed populations
- Husbandry research
- Holding capacity
- Number of holding institutions involved in each species
- Duration of captive program
- Disease concerns
- Enforcement of recommendations
- Training for those involved in amphibian captive management at their institutions
- Data entry issues—most software was not built for amphibian life history
- Ever-changing amphibian taxonomy
- 'Rules of Thumb' that should be researched more fully
    - High Priority
        - Develop methods to sex amphibians
        - Group management modeling
        - Effectiveness and Implications for possibility of selection for resistance or immunity to the *Batrachochytrium dendrobatidis* (Bd) chytrid fungus
    - Intermediate Priority
        - Demographic stability and fluctuations because captive demography may be very different from wild
        - Prevalence of different reproductive issues: multiple paternity, parthenogenesis, sperm storage, etc.
    - Lower Priority
        - Need to develop a tissue bank to preserve specimens/genetic material
        - Modeling of Ne/N guidelines per tank
        - Natural history research
        - Re-create Griffith's salmon study, but with amphibians—inadvertent selection for captive adaptations

APPENDIX A
Explanations for Population Management Recommendations

Compiled by Kevin Willis, Minnesota Zoo

Recommendation Question1: How many founders to collect?

Figure 1: The probability that a sample of N animals will contain at least one individual of each sex. This assumes that the source population has a 50%/50% male/female sex ratio. Any group with a size of 5 animals or larger has a 90% chance of including at least one of each sex. This assumes random sampling of individuals and no sexually dimorphic behaviors. The equation is P =1-0.5^(N-1).

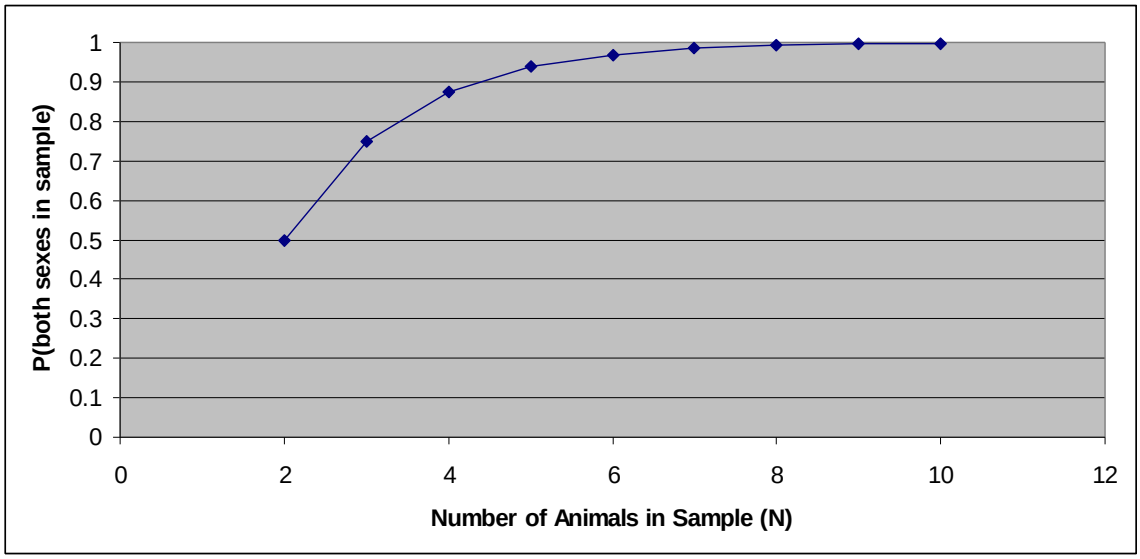
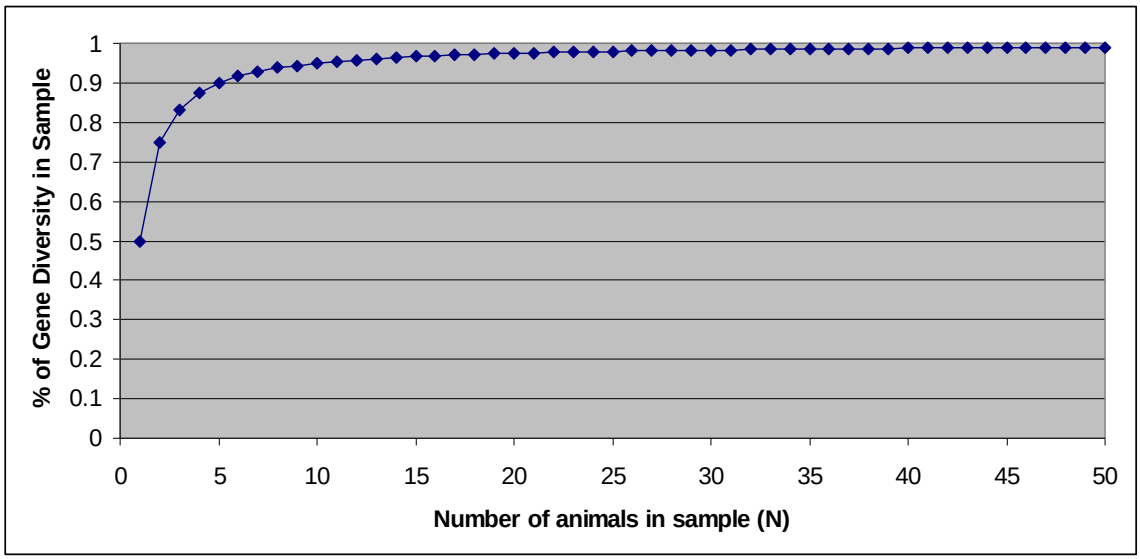
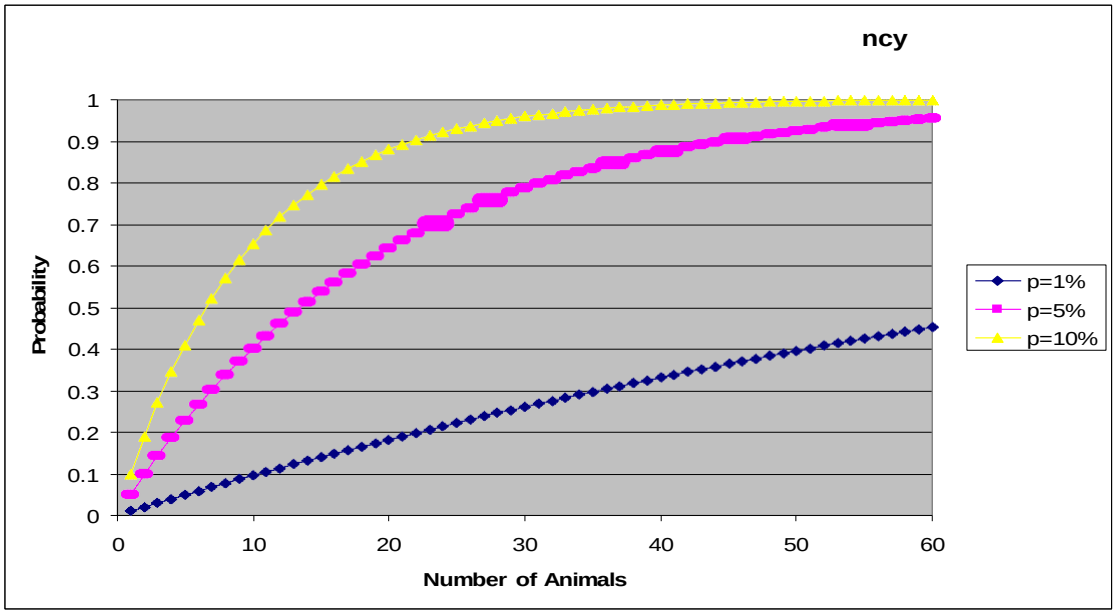


Figure 2: The average percent of the gene diversity of the source population captured in a sample of N randomly selected animals. This assumes the population is both homogeneous (i.e., no subpopulational structuring) and in Hardy-Weinberg equilibrium. Any number of founders larger than 20 will allow you to start with 97.5% potential gene diversity. The equation is GD = 1-1/(2N).



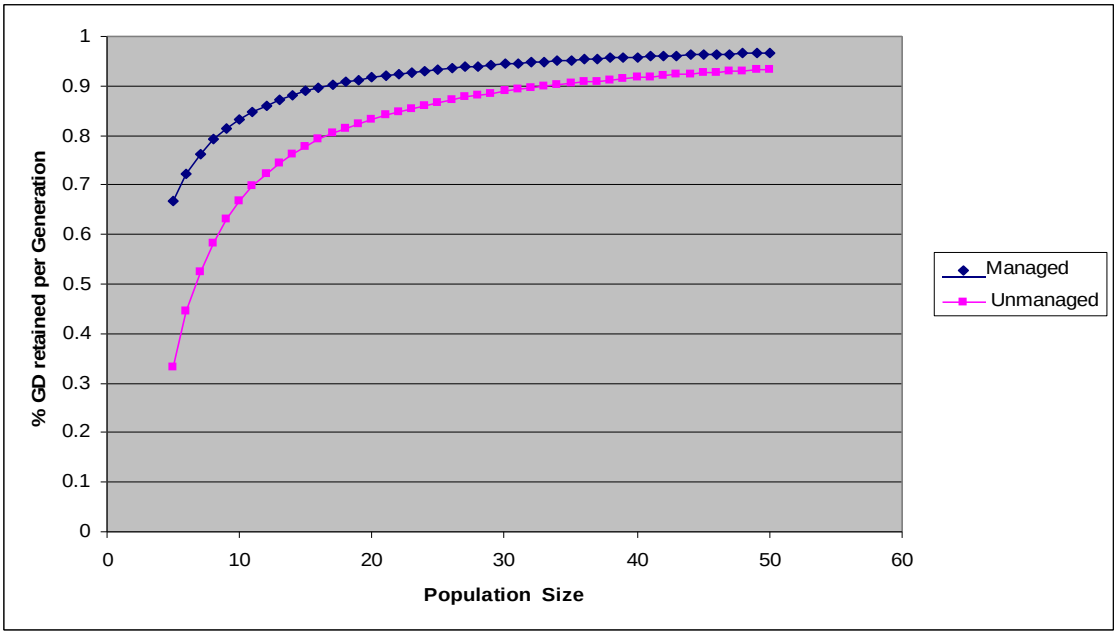
****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Figure 3: Probability of obtaining an allele of a given frequency. In addition to gene diversity, the probability that alleles are collected in a sample of N individuals is also of interest. This is a little more complex as the frequency of the allele is also a factor. In this figure the probability of obtaining an allele of frequency p in a sample of N randomly selected individuals is given for the values of p and samples sizes of 1 to 60 animals. The equation is 1 – (1-p)^N.



Recommendation Question 2: What is the target population size?

Figure 4: Gene diversity is lost on average each generation by an amount inversely proportional to the effective population size of the population. The average rate of loss is 1/[2Ne] of the remaining gene diversity per generation, where Ne is the effective breeding population size, and factors such as the number of animals that produce offspring influence the relationship between the total population size and the effective population size. Shown here is a relationship between the population size (N) and the minimal rate of loss (in which Ne = 2 * N), a typical rate of loss (with Ne = 0.3 * N) for an intensively managed population, and a typical rate of loss (with Ne = 0.15 *N) for an unmanaged (group or random-breeding) population.



Please Note: Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Figure 5a: The number of generations until gene diversity drops below 90% for a population of size N with rates of loss as defined in figure 4. Gene diversity is lost with each generation. The general equation is G=log(0.75)/log(1-1/(0.3*2N)) for an intensively managed population and unmanaged (group or random-breeding) population.

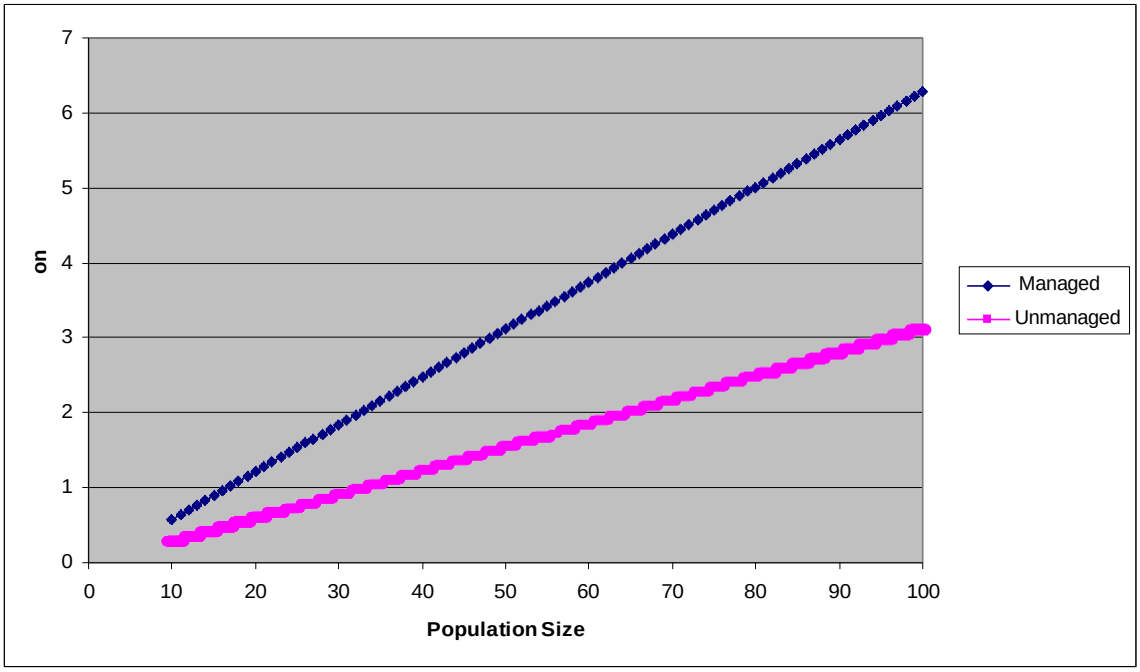
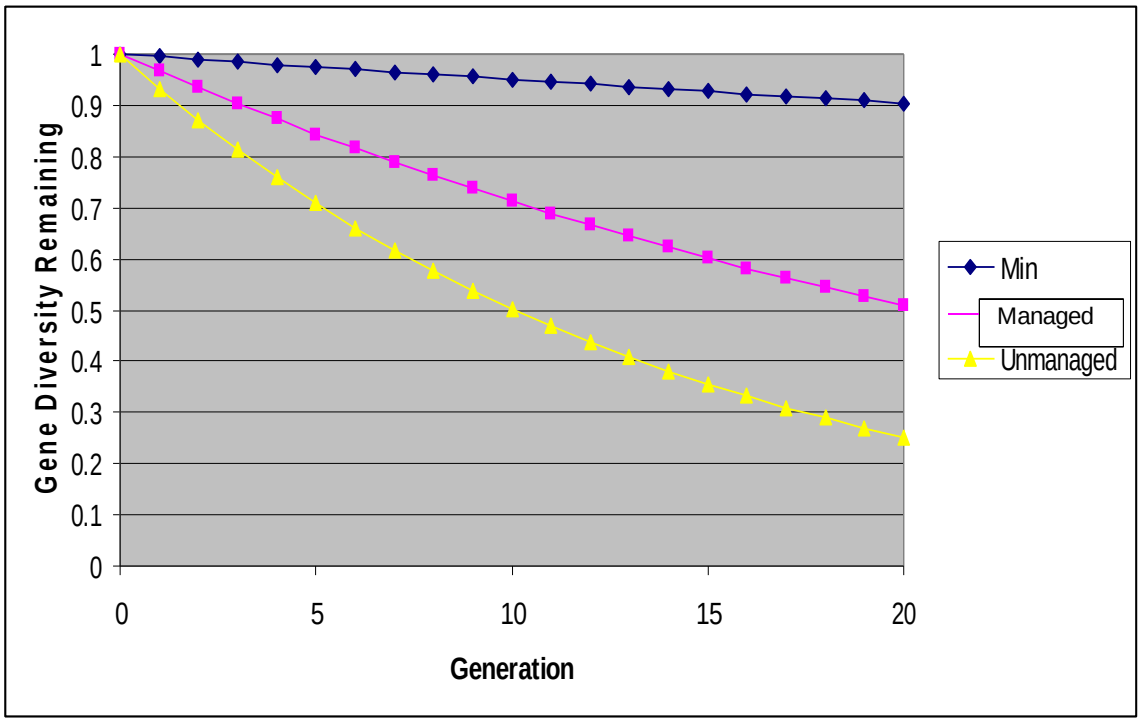


Figure 5b: Proportion of gene diversity remaining for each generation for 20 generations based on a population of 50 animals with rates of loss of gene diversity as defined in figure 4. Gene diversity is lost with each generation and gene diversity will be lost faster with less intensive management (as with groups). The general equation is GDt = (1-1/[4N])^t for an intensively managed population and unmanaged (group or random-breeding) population.
Note that this is an alternate view of the data in Figure 5a, but illustrates the same basic principle.



****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

Recommendation Question 3: How quickly should you grow the population to the target size?

Figure 6: Gene diversity remaining for each generation following the founding population. The remaining gene diversity in generation t for a population of initial size X and target size N which grow at different rates. The formula is GD(t+1) = GDt*(1-1/2N)^(year/G);
where G = the generation interval, GDt is gene diversity at time t, GD(t+1) is not GD times (t+1) but rather GD at time t+1, and N is the effective population size. The graph is for populations with an effective size of 50, all starting with GD=1, and values of G = 2, 4 and 8.

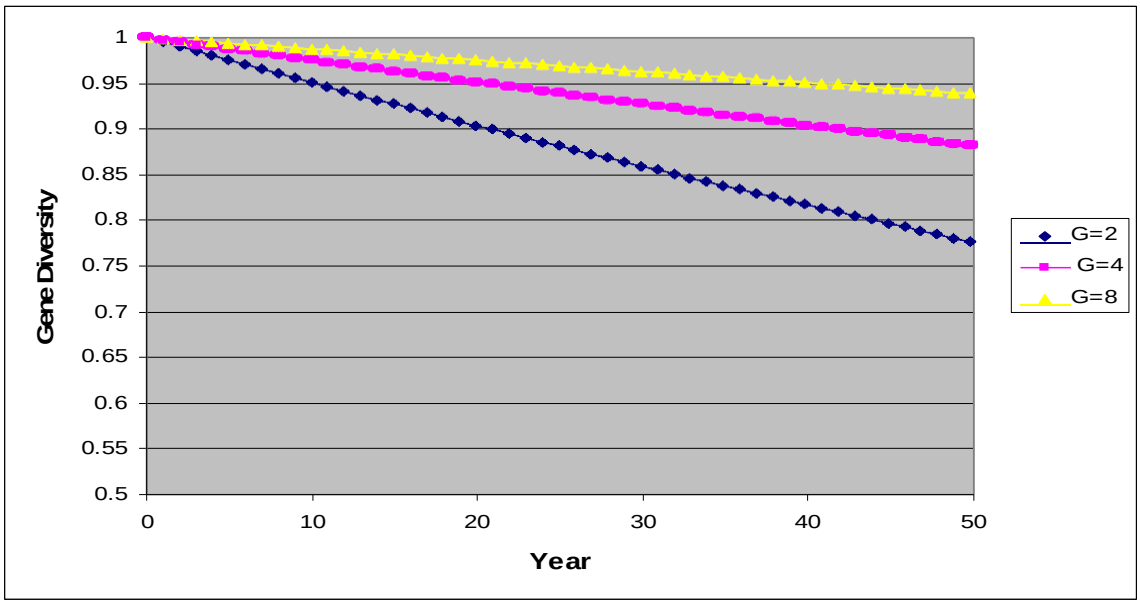
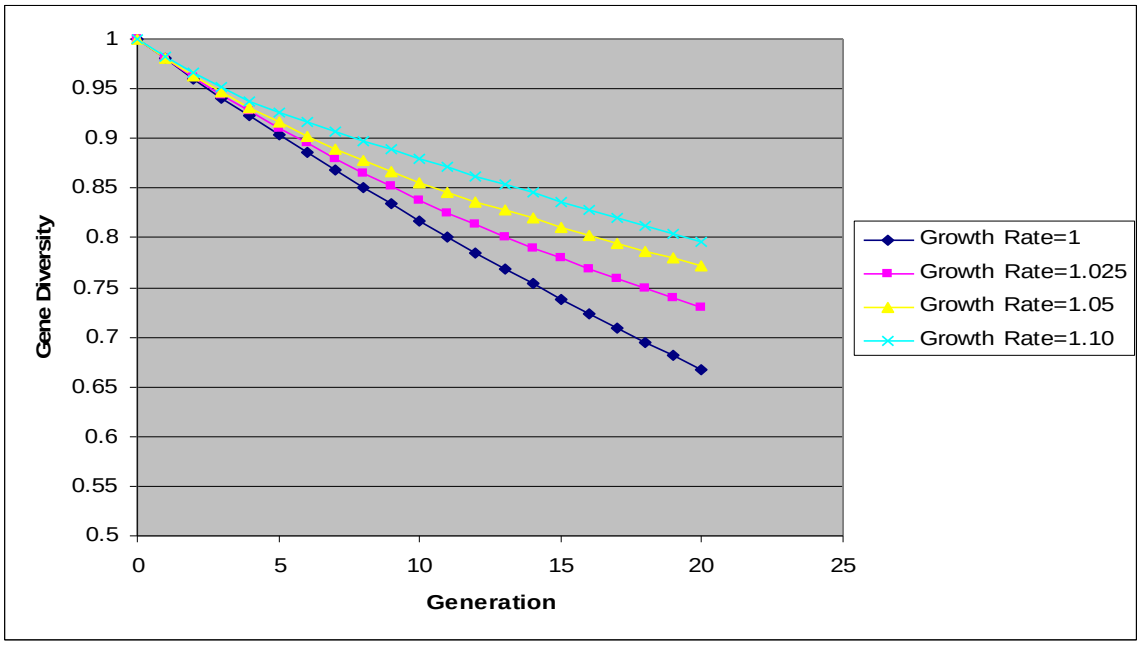


Figure 7: Gene diversity is lost every generation and decreases more quickly with lower population growth rates. In this example, each population starts with an effective size of 25 individuals and grows to a maximum effective size of 50 individuals. Each line represents a different growth rate per generation. The growth rate is multiplied by the number of effective individuals in this generation to determine the number of effective individuals in the subsequent generation and the rate of loss of gene diversity follows the standard drift equation.



****Please Note:** Throughout this document, the notation x.y will be used to signify x number of males and y number of females.

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APPENDIX C
Applying Molecular Genetics to Captive Amphibian Populations

Andrea S. Putnam and Jamie A. Ivy, San Diego Zoo Global (March 2017)

The genetic management of captive amphibian populations can be hindered by ambiguities surrounding unknown pedigrees, species identification, and potential hybridization. In some of these cases, molecular (DNA) data can help resolve management issues. While the cost of generating molecular data on both small and genome-wide scales has become increasingly affordable, care must be taken to ensure the correct DNA markers and analyses are applied to management questions. **To avoid common pitfalls and ensure successful outcomes, we strongly recommend contacting a population advisor with experience in advising captive populations prior to collaborating with a research group when undertaking molecular data collection.** For each type of management question listed below, a brief description of best practices regarding the extent of population sampling and the type of molecular data that should be collected are described:

Species Identification – Ideally, the taxonomy of the species of interest (and closely related species) will have been assessed across the species' geographic range and published in a peer-reviewed journal using multiple types of data (allele frequency, genetic distance, morphology, e.g.). Individuals within a captive population can then be analyzed and compared using the same approach as the published research, taking advantage of reference data already generated and available. Sequence data from nuclear or mitochondrial DNA, or a panel of single nucleotide polymorphisms (SNPs) are the most common types of molecular data used to identify individuals at the species level. For species that do not already have well defined taxonomy at the molecular level, extensive laboratory work on wild-caught or museum specimens may be necessary to establish a benchmark for captive-population screening. In other words, diagnostic molecular differences between species must first be clearly identified before species assignment can be made for individuals of uncertain taxonomy.

Citations:

Meyer CP, Paulay G. DNA barcoding: error rates based on comprehensive sampling. *PLoS biol* 3.12 (2005): e422.

Pante E, Abdelkrim J, Viricel A, Gey D, *et al*. Use of RAD sequencing for delimiting species. *Heredity* 114.5 (2015): 450-459.

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Hybridization – Similar to species identification, detecting hybrids is most feasible when the taxonomy of both potential parental species or subspecies has been resolved at the molecular level. Diagnostic molecular differences between taxonomic units of interest (species or subspecies) must be clearly identified before hybridization can be investigated. Detection of ancestral hybridization, or hybridization deep in a captive population's pedigree, may be particularly challenging to ascertain if few diagnostic differences between taxonomic units of interest can be established. Again, a panel of SNPs or sequence data are typically the genetic data of choice. Mitochondrial data are sometimes employed, although this type of data is limiting because it only allows for the identification of hybridization within the maternal portion of a genome.

Citations:

Hohenlohe PA, Day MD, Amish SJ, *et al*. Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. *Molecular Ecology*. 22 (2013):3002–3013.

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Ito H, Langenhorst T, Ogden R, Inoue-Murayama M. Population genetic diversity and hybrid detection in captive zebras. *Scientific reports* 5 (2015):13171.

Parentage -- To identify the parents of an individual, it is important to obtain genetic samples from all potential parents and the individual in question; it will typically be impossible to rule out as a parent any individual not sampled. In parentage analyses, microsatellite data are frequently used and usually acceptable. Microsatellite markers are often species-specific, although microsatellites may be shared across a genus, and developing new microsatellite markers for a species can be labor intensive. For species where there is very little genetic diversity (e.g., a captive population with few founders and a deep pedigree), it may be necessary to use SNP data instead of microsatellites to resolve parentage.

Citations:

Hauser L,Baird M, Hilborn R, Seeb LW, Seeb JE. An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (*Oncorhynchus nerka*) population. *Molecular Ecology Resources* 11 (2011):150–161.

Jones AG, Small CM, Paczolt KA, Ratterman NL. A practical guide to methods of parentage analysis. *Molecular Ecology Resources* 10 (2010):6–30.

Kinship - Molecular data can be used to resolve kinships among animals when pedigrees are unknown. The biggest challenge in resolving unknown ancestry, however, is adequately sampling individuals within the population and using the right genomic tools for analysis. Sampling the entire living population is optimal because current analysis methodologies only allow for kinships to be estimated between sampled animals; if not all animals are sampled, there will still be holes in the living population's kinship matrix (analogous to unknown parentage causing holes in pedigrees). In some cases data from only a subset of a population may be utilized, but only with close advisement from a population advisor. SNP data collection through reduced-representation genome sequencing (RAD) or a SNP-chip is the molecular marker of choice for kinship estimation, as microsatellites produce inaccurate estimates with high sampling variances. Once SNPs have been identified, careful consideration must be taken in how researchers estimate relationships among animals. The methods typically used to estimate relatedness in wild populations often cannot be applied to established captive populations. This is because captive populations are often inbred, not randomly mating, and spread across several institutions. Currently, the use of similarity indices such as allele-sharing values are considered the least-biased estimator of kinship in captive populations (see Discussion section of Ivy, Putnam *et al*. 2016 for additional information). Populations that are most likely to benefit from using genomic tools to resolve unknown ancestry are ones where the outcome will have a large effect on the management of the population. Populations where founders are still living and/or the current pedigree is tracked are examples of populations that may benefit the most. If unknown parentage will continue to be perpetuated in a population, molecular data also must continually be used to resolve kinships.

Citations:

Ivy JA, Miller A, Lacy RC, DeWoody JA. Methods and prospects for using molecular data in captive breeding programs: an empirical example using parma wallabies (*Macropus parma*). *Journal of Heredity* 100.4 (2009): 441-454.

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