

LETTER

Integrating biobanking minimises inbreeding and produces significant cost benefits for a threatened frog captive breeding programme

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Abstract

Captive breeding is an integral part of global conservation efforts despite high costs and adverse genetic effects associated with unavoidably small population sizes. Supplementing captive-bred populations with biobanked founder sperm to restore genetic diversity offers a solution to colony size, costs and inbreeding, yet is rarely done, partly due to a lack of concrete examples or awareness amongst the conservation community of the huge potential benefits. We present a model system of the cost and genetic benefits achieved by incorporating biobanking into captive breeding of Oregon spotted frogs (*Rana pretiosa*). Backcrossing with frozen sperm every generation resulted in very large reductions in required programme expenditure compared to traditional captive breeding. This model supports the view that integration of biobanking into captive breeding would make longstanding and previously unachievable genetic diversity retention targets feasible (90% source population heterozygosity for a minimum of 100 years) at much reduced costs. This study suggests that the credibility of captive breeding as a conservation strategy would be enhanced by integrating genome storage and assisted breeding to produce far larger numbers of animals of higher genetic quality. This innovation would justify increased public and agency support for captive breeding.

KEYWORDS

assisted reproductive technologies, biobanking, captive breeding, cost benefits, cryopreservation, ex-situ conservation, genetic diversity, heterozygosity, inbreeding

1 | INTRODUCTION

Captive breeding and assisted reproductive technologies (ARTs) are recognised as important tools in the conservation of wildlife, particularly amphibians (Bishop et al.,

2012; Browne et al., 2019; Clulow & Clulow, 2016; Clulow, Upton, Trudeau, & Clulow, 2019; Della-Togna et al., 2020; Kouba et al., 2012). Since the mass extinction event that began several decades ago with the emerging infectious

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chytrid disease (Bower, Lips, Schwarzkopf, Georges, & Clulow, 2017; Scheele et al., 2019), amphibians have become increasingly reliant on captive management with estimates of more than 900 species requiring *ex-situ* insurance populations, of which 211 require urgent rescue via *ex-situ* management (Zippel et al., 2011). These programmes aim to secure populations and species against extinction and are a source of animals for research and translocations. However, amphibians are under-represented in global captive programmes and it is estimated that current global capacity can sustain *ex-situ* amphibian populations for no more than 50 species (Bishop et al., 2012; Clulow, Trudeau, & Kouba, 2014; Gagliardo et al., 2008; Murphy & Gratwicke, 2017; Zippel et al., 2011).

There are considerable costs associated with captive insurance populations, including significant economic costs and logistical difficulties (Mawson & Lambert, 2017), deleterious genetic effects of sustained captivity including inbreeding depression (Frankham, Ballou, & Briscoe, 2010), loss of fitness and translocation value (Robert, 2009) and adaptation to captivity (Frankham, 2008).

While it is not feasible to completely prevent erosion of genetic diversity in captive populations, a commonly suggested target that meets a balance between logistical achievability, economic costs and conservation outcomes is to maintain 90% (or more) of initial genetic variation (heterozygosity; H_t/H_o) for 200 years (Soulé, Gilpin, Conway, & Foose, 1986), later changed to 90% for 100 years (Frankham et al., 2010, p. 351). It is unlikely, however, that many captive assurance populations for conservation purposes meet this target due to the high costs of maintaining populations with enough individuals to avoid genetic erosion through inbreeding depression.

The issues of cost and genetic deterioration have provided an impetus to develop novel tools for managing economics and problems with inbreeding in captive populations. Biobanks (frozen living cell repositories of germ cells, embryos and somatic tissues for use in conservation genetic management) in conjunction with ARTs to utilise this stored genetic material have been proposed as a strategy to reduce holding requirements, labour and other costs needed to run captive breeding programmes (Ananjeva et al., 2017; Clulow & Clulow, 2016; Holt, Bennett, Volobouev, & Watwon, 1996; Silla & Byrne, 2019). Recent advances in successful biobanking and ARTs for amphibians over the past decade has resulted in the ability to hormonally induce gamete release (Clulow et al., 2018), freeze and store sperm long-term (Browne et al., 2019; Clulow & Clulow, 2016; Clulow et al., 2014; Kouba et al., 2013; Kouba, Vance, & Willis, 2009) and routinely perform *in vitro* fertilisations (IVF) (Clulow & Clulow, 2016; Clulow et al., 2014; Kouba et al., 2009). These advances have now paved the way

for routinely incorporating cryopreserved sperm from founder captive animals (or wild animals temporarily collected) into the management of captive amphibian populations to reduce costs and the need for large numbers of animals to maintain heterozygosity targets (Beaulerc, Johnson, & White, 2010; Dreitz, 2006; Gagliardo et al., 2008; Griffiths & Pavajeau, 2008; Kouba et al., 2011; Murphy & Gratwicke, 2017). Yet despite this, there are almost no examples of the use of biobanking technologies to achieve these outcomes in animal conservation, despite a growing infrastructure network for wildlife biobanks themselves (Comizzoli, 2015; Monfort, 2014). To date, there have been no cases of biobanking programmes leading to measurable *in situ* conservation outcomes for amphibian species and the technology thus remains a neglected tool for amphibian conservation despite being readily available (Clulow et al., 2019; Monfort, 2014).

Here, we aim to rectify this situation and create awareness among conservation practitioners of the massive potential to save funding, reduce captive animals required and thus conserve more species with existing resources by incorporating biobanked sperm into captive populations using readily available ARTs. We aimed to do this by modelling the economic costs and genetic diversity benefits under various biobanking scenarios for an existing conservation programme for the Oregon spotted frog (*Rana pretiosa*), where robust data exist for the costs associated with captive populations. We modelled our target parameters (economic cost and number of animals required to maintain heterozygosity; H_t/H_o) under a scenario of no incorporation of cryostored founder sperm, and a scenario of back-crossing every generation with cryostored founder sperm using existing ARTs. We outline the stark cost-savings that are possible and the greatly reduced number of animals required to achieve the 90% heterozygosity, 100-year benchmark, and explore whether we can exceed this benchmark with 95% and 99% heterozygosity retention, and estimate the costs for these ambitious new potential targets.

2 | METHODS

2.1 | Study species

The Oregon spotted frog (*R. pretiosa*) has been lost from more than 90% of its historical range due to continued habitat loss and fragmentation (leading to genetic isolation of populations), disease (chytridiomycosis) and introduced predators (Blouin, Phillipsen, & Monsen, 2010; Pearl, Adams, Bury, & McCreary, 2004; Pearl, Bowerman, Adams, & Chelgren, 2009). The species is listed as 'vulnerable' by the International Union for Conservation of

Nature (Hammerson & Pearl, 2004), and ‘endangered’ under Canada’s Species at Risk Act (SARA, 2002) and the US Endangered Species Act (USFWS, 1973). We have selected this species as a case study due to the availability of effective population size (N_e) data (Phillipsen, Bowerman, & Blouin, 2010) and captive breeding costs (Kissel, Palen, & Govindarajulu, 2017).

2.2 | Models

We modelled various 100-year captive breeding cost scenarios in which the size of the live captive colony reflected those required to maintain different levels of the source population heterozygosity or broadly accepted effective population sizes (N_e). Scenarios included avoiding inbreeding depression in the short-term (N_e 100), retaining evolutionary potential in perpetuity (N_e 1000) and retaining single locus genetic diversity (N_e 100,000–1,000,000) (Frankham et al., 2010; Frankham, Bradshaw, & Brook, 2014; Lande & Barrowdough, 1987). We determined the required colony sizes (N) for the different objectives, based on two different N_e/N ratios, the instantaneous N_e/N ratio of 0.086 (Phillipsen et al., 2010) and the average N_e/N for captive populations of 0.3 (Mace, 1986), on the expectation that the ratio for *R. pretiosa* in captivity would lie within this range. We also modelled how costs of different theoretical genetic retention goals including 90% of source population heterozygosity (Soulé et al., 1986), as well as more ambitious targets proposed here of 95% and 99% of source population heterozygosity would be reduced by incorporating biobanking strategies in which founder sperm were cryopreserved and reintroduced (via IVF) at every generational interval (3-year intervals for *R. pretiosa*) up to 100 years after the colony was established.

We derived a genetic backcrossing model to determine the census size (N) of the captive colony required to maintain various levels of genetic diversity (with heterozygosity values derived from changes in inbreeding coefficients) under two scenarios (non-backcrossed populations representing the minimum number of individuals required to meet genetic retention targets without genetic intervention and populations with backcrossing every generation at 3-year intervals). For backcrossed populations, the N_e required to achieve each particular genetic goal was determined by random substitution of values into iterative genetic models until 100-year H_t/H_0 met desired genetic retention targets. Derived N values were substituted into an economic costing model based on costs for the 2012/13 breeding season of the *R. pretiosa* captive breeding programme at the Greater Vancouver Zoo, provided in Kissel et al. (2017). This was used to estimate an economic *ex-situ* holding cost per individual animal and 100-year cap-

tive colony costs for each derived colony size (N) with or without backcrossing.

2.3 | Cost modelling

Year one economic *ex-situ* holding cost per individual (C) is given by the following equation (for detailed cost categories and modelling procedures, see Supporting Information):

$$C = ((I + E + L + U + V + F) / N) + M \quad (1)$$

where all costs are in nominal Canadian dollars (November 2019), and where I represents facilities and infrastructure, E , minor equipment and consumables, L , labour costs, U , utilities, V , veterinary costs, F , food costs, N , captive colony size, and M , fixed management costs.

Year one programme costs for any conventional non-backcrossed population (P_c) and programme costs when genetic retention goals are met using backcrossing (P_{bc}) are given in Equations (2) and (3), respectively, and were incorporated into iterative processes in Microsoft® Excel 16.27 to model 100-year accumulating costs based on appropriate varying recurrent costs and assumed inflation rates:

$$P_c = C * N \quad (2)$$

$$P_{bc} = (C + B) * N \quad (3)$$

where C is year one economic *ex-situ* holding cost (as given in Equation 1); B , biobanking costs (basic infrastructure and labour for IVF and cryopreservation, see Supporting Information) and N represents number of adult frogs in the colony.

2.4 | Genetic modelling

We provide a summary of the approach and equations used for genetic modelling. For detailed procedures and model assumptions and parameters, see Supporting Information.

The predicted rate of loss of heterozygosity was derived from the relationship between the inbreeding coefficient (F_t) and heterozygosity (H) in Equation (4) (Frankham et al., 2010). This allowed determination of residual heterozygosity (H_t/H_0) at each generation for which F_t was estimated. F_0 for founders was assumed to be 0:

$$H_t/H_0 = 1 - F_t \quad (4)$$

The increase in inbreeding between generations in the captive colony without backcrossing was determined from

the relationship in Equation (5) (Frankham et al., 2010, p. 271), which determines F_t (inbreeding coefficient, generation t) from N_e (effective population size) and F_{t-1} (inbreeding coefficient, generation $t-1$). This allows sequential determination of F_t for any generation (t) up to the number required to reach 100-year heterozygosity benchmarks (33 generations for *R. pretiosa* assuming generation interval of three years):

$$1 - F_t = (1 - 1/[2N_e]) * (1 - F_{t-1}) \quad (5)$$

We employed an iterative process, using Equation (6) to determine the effect of recurrent backcrossing (each generation) using frozen founder sperm on the rate of inbreeding for the hypothetical populations modelled in each of the three backcross scenarios:

$$F_t = \left[1 - (1/2)^t\right] / (2N_e) \quad (6)$$

where N_e represents the effective number of founder males and t is the number of backcross generations (for detailed derivation of Equation 6, see Supporting Information).

3 | RESULTS

Maintenance of genetic viability of hypothetical captive colonies of *R. pretiosa* using increasingly rigorous criteria would require increased colony census numbers and therefore higher setup and total programme costs (Table 1). The values here are those obtained assuming a N_e/N ratio of 0.086, while those for a ratio of 0.3 are given in the Supporting Information (Table S4). Avoiding inbreeding depression in the short-term ($N_e = 100$; $N = > 1163$ live individuals) would require Year 1 costs of more than CAD\$1.8 million followed by CAD\$340 million in total 100-year programme costs (Table 1). Costs for more ambitious and optimal genetic retention objectives (retaining evolutionary potential and single locus genetic diversity) would increase rapidly and exponentially. Census colony number requirements range from 10,000 to more than 11 million live individuals, with total 100-year programme costs of more than CAD\$3.4 billion and CAD\$3.4 trillion, respectively (Table 1).

A captive population of *R. pretiosa* designed to retain 90% of source population heterozygosity (< 10% loss in heterozygosity after 100 years) without genetic intervention ($N_e = 157$; $N = > 1800$) would require significant Year 1 start-up costs of more than CAD\$2.8 million, followed by total 100-year programme costs of more than

CAD\$537 million (average annual expenditure of > CAD\$5.37 million; Table 1). This population would meet suggested 90% targets with a residual H_t/H_0 of 0.900 after the captive period (Table 1; Figure 1). More ambitious targets of 95% ($N_e = 322$; $N = > 3700$) and 99% ($N_e = 1642$; $N = > 19,000$) retention would require significantly increased investment of more than CAD\$1 billion and CAD\$5.5 billion across 100 years to maintain 0.9500 and 0.9900 residual H_t/H_0 , respectively (Table 1; Figure 1).

Using the biobanked frozen founder sperm to backcross females in every generation to founder males in hypothetical captive populations designed to meet the above genetic retention targets would significantly reduce required live *R. pretiosa* and therefore programme costs. Backcrossing every generation at 3-year intervals into a colony designed to retain 90% heterozygosity ($N = 58$ founder males; same number of live females per generation) would require CAD\$1.1 million in biobanking specific investment across 100 years, and would allow a reduction of 1768 individuals to a constant live census colony of only 58 female *R. pretiosa* per generation, reducing Year 1 costs to CAD\$121,000 and reducing total forecast 100-year programme costs to only CAD\$20.9 million (average annual investment of CAD\$3,600 per individual), a 96% reduction in cost compared to the conventional no backcrossing programme (Table 1). Incorporating biobanking into populations designed to meet 95% and 99% genetic retention targets would result in higher costs and colony sizes ($N = 116$ and 581 founder males, respectively) but still far less than for non-backcrossed populations. Maintaining 95% of initial source population heterozygosity ($N = 116$ founder males) by backcrossing every generation would require CAD\$1.8 million in biobanking specific investment, but would allow a reduction in live census colony numbers of 3628 individual *R. pretiosa* to a constant live census colony of 116 individuals (live females), with total forecast 100-year programme costs of more than CAD\$38.8 million (Table 1). Backcrossing every generation into a population designed to meet a 99% genetic retention target ($N = 581$ founder males) would require CAD\$8.2 million in biobanking specific investment, but would allow a reduction in required census captive colony numbers of over 18,512 individual *R. pretiosa* to 581 individual live females, with total forecast 100-year programme costs of more than CAD\$181 million (Table 1).

Reductions in live captive colony numbers increase rates of inbreeding significantly in non-backcrossed populations (Table 1). In populations designed to retain 90%, 95% and 99% heterozygosity, the rate of inbreeding (F_t) is decreased by 80%, 90% and 98%, respectively, when backcrossing every generation (Table 1; Figure 1).

TABLE 1 Genetic and cost analysis for hypothetical captive colonies of Oregon spotted frogs (*Rana pretiosa*) designed to meet different genetic retention targets under various genetic backcross scenarios

<i>Hypothetical captive population</i>	N_e	N	F_1 no backcross	F_1 backcross	H_t/H_0 after 100 years	Cost (\$) Year 1	Cost (\$) Year 2	Total captive colony cost (\$) after 100 years	Backcross cost (\$) (labour and setup)	Total programme cost (\$) after 100 years
Genetic Viability										
Avoid inbreeding depression	100	1163	n.d.	n.d.	n.d.	\$1,819,865	\$1,116,852	\$343,172,052	n.d.	\$343,172,052
Maintain evolutionary potential	1000	11,628	n.d.	n.d.	n.d.	\$18,115,848	\$11,084,070	\$3,405,868,283	n.d.	\$3,405,868,283
Retain single locus genetic diversity (minimum and maximum)	100,000 1,000,000	1,162,791 11,627,907	n.d. n.d.	n.d. n.d.	n.d. n.d.	\$1,810,673,975 \$18,106,656,949	\$1,107,477,977 \$11,074,695,311	\$340,302,453,706 \$3,402,998,684,820	n.d. n.d.	\$340,302,453,706 \$3,402,998,684,820
Backcross scenarios										
90% heterozygosity retention with no backcross	157	1826	0.0999	n.d.	0.9000	\$2,851,943	\$1,748,109	\$537,142,813	n.d.	\$537,142,813
90% heterozygosity retention by backcrossing every generation (3-year intervals)	n.d.	58	0.5000	0.1000	0.9000	\$121,157	\$64,757	\$19,887,450	\$1,104,713	\$20,992,164
95% heterozygosity retention with no backcross	322	3744	0.0485	n.d.	0.9500	\$5,839,540	\$3,575,433	\$1,098,637,123	n.d.	\$1,098,637,123
95% heterozygosity retention by backcrossing every generation (3-year intervals)	n.d.	116	0.5000	0.0500	0.9500	\$219,114	\$120,131	\$36,902,429	\$1,897,635	\$38,800,065
99% heterozygosity retention with no backcross	1642	19,093	0.0100	n.d.	0.9900	\$29,740,315	\$18,194,018	\$5,590,591,595	n.d.	\$5,590,591,595
99% heterozygosity retention by backcrossing every generation (3-year intervals)	n.d.	581	0.5000	0.0100	0.9900	\$1,002,900	\$563,118	\$173,022,262	\$8,254,678	\$181,276,940

Note: Year 1 (start-up) costs, as well as Year 2 costs are costs in Years 1 and 2 of 100 years of colony life. 'Backcross costs' are the estimates of costs of genetic backcross events (generation of offspring from cryopreserved founder sperm) for each backcross scenario based on number of offspring to be generated. 'Total captive colony costs after 100 years' are programme costs without backcrossing. 'Total programme cost after 100 years' include captive colony costs and expenditure for backcross events. Captive colony sizes modelled include baseline estimates for populations to avoid inbreeding depression, retain evolutionary potential, and retain single locus genetic diversity (without backcrossing; Frankham et al., 2014). Backcross scenarios tested: 90%, 95% and 99% heterozygosity retention with no backcross and backcross every generation. Effective population size (N_e) and colony numbers (N) are shown for all hypothetical colonies. Inbreeding coefficients (F_1) and heterozygosity (H_t/H_0) are values at 100 years. All dollar amounts shown are in Canadian currency (CAD\$, 2019). n.d. indicates not determined.

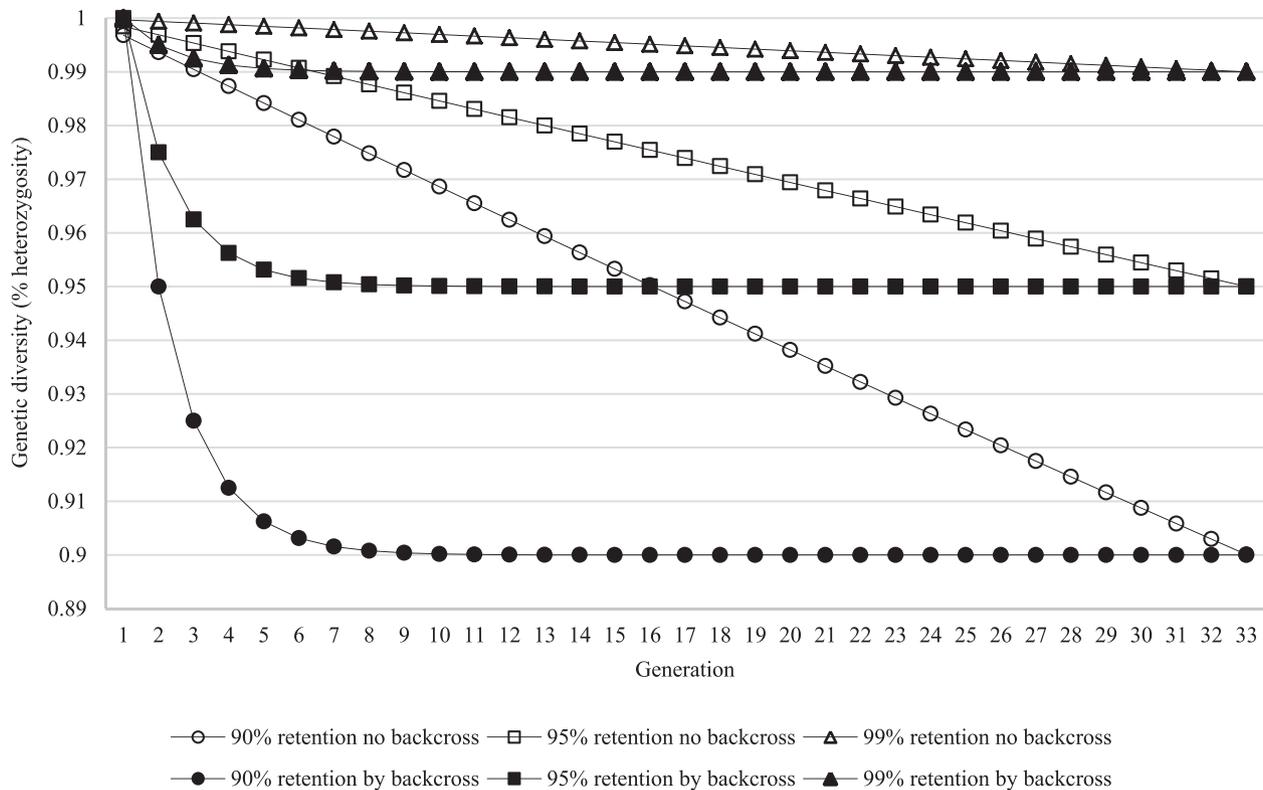


FIGURE 1 Genetic retention (% initial heterozygosity, converted to decimal value, y-axis) across 100 years (33 generations) in hypothetical captive populations of *Ranapretiosa* designed to meet different genetic retention goals (90%, 95% and 99% of source population heterozygosity) without backcrossing or with backcrossing live females to founder males using cryopreserved founder sperm each generation (3-year intervals)

4 | DISCUSSION

We present powerful theoretical support for the proposition that biobanking technology should be considered as a complementary tool in captive breeding programmes. The use of such technology would address the two most common criticisms of captive breeding: cost and loss of wild-type genetic diversity and fitness. We showed this with models integrating biobanking scenarios into estimates of costs and inbreeding in the captive management of a colony of *R. pretiosa*. These models demonstrated huge reductions in costs and required sizes of the live colony while achieving reductions in the rate of inbreeding sufficient to achieve and exceed the suggested standard for captive genetic diversity (< 10% loss in heterozygosity after 100 years). Use of biobanked males will also reduce genetic adaptation to captivity. Such adaptation increases with generations of reproduction in captivity and the half of the genome contributed by biobanked males in the current generation is not subjected to such adaptation, while the male contributions in prior generations are subjected to less adaptation than in colonies maintained without biobanking of males (Frankham, 2008). Such genetic adaptation to captivity may substantially reduce reproductive

fitness when captive populations are returned to the wild (Frankham, 2008).

The models applied here demonstrate extremes of population size and cost that would be necessary to achieve theoretical targets for the retention of source population genetic diversity in the absence of using biobanked sperm to restore source population heterozygosity. Clearly, the numbers involved and costs would preclude such approaches, but they serve to demonstrate how challenging it is to maintain source population heterozygosity under captive or small population conditions. This is an important question given the growing number of species that now only survive as *ex-situ* populations. Should the opportunity arise to reintroduce such populations to the wild, the approach that we advocate here would improve the likelihood of reintroductions occurring from genotypes that are less inbred, less domesticated and have cost less to maintain prior to release.

Required census numbers (>1800) estimated in this study to retain 90% heterozygosity for captive *R. pretiosa* are likely to be similar for other amphibian species of similar N_e/N ratio if such approaches were used in the absence of back-crossing. Given the limited resources available for most captive programmes, it is more likely that much

smaller captive colonies would be maintained, and the rate of inbreeding between generations would be much higher as a result than the target levels here, falling far short of maintaining 90% of heterozygosity. Given that amphibians are amongst the simplest and least costly vertebrate taxa to manage in captivity (Conde et al., 2015), the levels of heterozygosity modelled here would be even less likely to be achieved in captive breeding programmes across other more challenging vertebrate taxa. Not surprisingly, goals for the retention of heterozygosity are rarely achieved globally (Lees & Wilcken, 2009), and even high profile and well-resourced captive breeding programmes, such as giant pandas, struggle with issues of inbreeding and small census colony sizes (Shen et al., 2009; Wei et al., 2012, 2018).

In contrast, the benefits of introducing biobanking to the programme, modelled for an extreme scenario of backcrossing every generation to achieve 90% to 99% source heterozygosity, are stark in comparison. The benefits are clear in terms of economic feasibility and genetic benefits of heterozygosity, and probable substantially higher genetic fitness. The differences in all aspects of the economic costs are clear in comparing biobanked to non-banked strategies for *R. pretiosa*, for example, start-up costs of CAD\$2.8 million are required, followed by 100-year programme costs of more than CAD\$537 million to retain 90% heterozygosity in non-banked scenarios versus start-up costs of only CAD\$121,000 and 100-year programme costs of around CAD\$20 million with backcrossing every generation (a 26-fold reduction in overall costs). Even taking into account the costs of cryostorage, and the labour costs of retrieving frozen samples and generating live offspring by IVF, the economic benefits of backcrossing to founders are clear (5% of total 100-year programme costs; CAD\$14,000 in Year 1 setup and around CAD\$7.4k in Year 1 labour (freezing of founder sperm), followed by > CAD\$785,000 in accumulated labour costs spread across the total life of the programme). In building our case, we have been highly conservative in opting to use the published N_e/N ratio of 0.086 derived from a wild population (Phillipsen et al., 2010). Captive populations often have a lower N_e/N ratio; average around 0.3 (Mace, 1986). Application of that value to the captive *R. pretiosa* model (Table S4) results in a similar proportionate benefit.

Recent advances in cryobiology could allow this technology to be incorporated across existing amphibian captive breeding programmes with minimal added costs (Clulow et al., 2019). Effective assisted reproduction and biobanking protocols may not exist for many threatened amphibian species due to a lack of species-specific knowledge in the fundamental reproductive sciences. However, likely timeframes of 3 to 10 years (depending on the maximum reproductive life of females of various species) would

be frequently available to refine species-specific protocols before the F_1 generation which was required in many programmes.

Unfortunately, biobanking has few examples of application or *in situ* outcomes in formal recovery programmes. The flagship example of the black-footed ferret (*Mustela nigripes*) is an exception. In that case, starting with only 18 founders, a captive breeding programme was established, and sperm were frozen despite the fact that the ARTs to use the cryopreserved sperm were not yet developed. However, after a 20-year period of captive breeding, suitable ARTs were established and cryopreserved sperm was incorporated back into the population via artificial insemination to add lost heterozygosity back to the increasingly inbred captive colony. This increased the overall genetic diversity of the 8000 animal captive population and at last published report 3000 individuals were translocated to the wild (Holt, 2016; Howard, Lynch, Santymire, Marinari, & Wildt, 2016; Howard, Marinari, & Wildt, 2003; Monfort, 2014). Interestingly, in that case, alleles were not added back until 20 years after the programme was established (after multiple generations without backcrossing to founders), yet the benefits to heterozygosity were apparent, even in the absence of continuous backcrossing as modelled here.

We have demonstrated the theoretical economic and genetic benefits of utilising biobanking in *ex-situ* conservation programmes, however, proof of concept examples are still absent for amphibian species in practice. Life-history traits (fecundity, iteroparity and fast life histories (Bloxam & Tonge, 1995); advances in sperm-cryopreservation, cryobiology and assisted reproduction, and low *ex-situ* holding costs (Conde et al., 2015) make amphibians ideal for demonstrating the value of the biobanking approach in practice. We have presented the Oregon spotted frog as a model of cost-benefit across programme economic costs against genetic benefits, but there are a large range of amphibian species that could be candidates for this approach in practice (Clulow & Clulow, 2016; Clulow et al., 2014, 2019). The integration of biobanking technology into captive breeding programmes should be tested widely in practice because the potential conservation benefits are enormous.

Modern zoos and similar institutions maintain captive colonies for conservation of threatened species as a stated part of their mission, and social licence to operate. Directors and boards of institutions should consider the benefit to their credibility from adopting the approach modelled in this paper: maintenance of animals that are fitter and more like those from wild populations (better quality animals for later reintroductions) and drastically reduced costs that allow institutions to hold multiples of species. An order of magnitude increase in efficiency and productivity of captive breeding would be a gain not lost on the broader

community, governments or on the agencies that fund these programmes.

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AUTHOR CONTRIBUTIONS

LGH, JC, DF, SC and RU conducted the scientific literature review. RU provided data. LGH, DF and JC performed the modelling. LGH lead the writing and all authors provided ideas and substantial edits to the paper.

ETHICS STATEMENT

The authors conducted no data collection or scientific inquiry which required ethics considerations. Our manuscript complies with proper ethical scientific standards.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Ananjeva, N. B., Uteshev, V. K., Orlov, N. L., Ryabov, S. A., Gakhova, E. N., Kaurova, S. A., ... Browne, R. K. (2017). Comparison of the modern reproductive technologies for amphibians and reptiles. *Russian Journal of Herpetology*, *24*(4), 275–290.
- Beauclerc, K. B., Johnson, B., & White, B. N. (2010). Genetic rescue of an inbred captive population of the critically endangered Puerto Rican crested toad (*Peltophryne lemur*) by mixing lineages. *Conservation Genetics*, *11*(1), 21–32.
- Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Moreno, J. G. (2012). The amphibian extinction crisis—what will it take to put the action into the Amphibian Conservation Action Plan? *SAPI EN. S. Surveys and Perspectives Integrating Environment and Society*, *5*(2), 97–111.
- Blouin, M. S., Phillipsen, I. C., & Monsen, K. J. (2010). Population structure and conservation genetics of the Oregon spotted frog, *Rana pretiosa*. *Conservation Genetics*, *11*(6), 2179–2194.
- Bloxam, Q. M. C., & Tonge, S. J. (1995). Amphibians: Suitable candidates for breeding-release programmes. *Biodiversity & Conservation*, *4*(6), 636–644.
- Bower, D. S., Lips, K. R., Schwarzkopf, L., Georges, A., & Clulow, S. (2017). Amphibians on the brink. *Science*, *357*(6350), 454–455.
- Browne, R. K., Silla, A. J., Upton, R., Della-Togna, G., Marcece-Greaves, R., Shishova, N. V., ... Mansour, N. (2019). Sperm collection and storage for the sustainable management of amphibian biodiversity. *Theriogenology*, *133*, 187–200.
- Clulow, J., & Clulow, S. (2016). Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: Bringing the ARTs up to speed. *Reproduction, Fertility and Development*, *28*(8), 1116–1132.
- Clulow, J., Pomeroy, M., Herbert, D., Upton, R., Calatayud, N., Clulow, S., ... Trudeau, V. L. (2018). Differential success in obtaining gametes between male and female Australian temperate frogs by hormonal induction: A review. *General and Comparative Endocrinology*, *265*, 141–148.
- Clulow, J., Trudeau, V. L., & Kouba, A. J. (2014). Amphibian declines in the twenty-first century: Why we need assisted reproductive technologies. In *Reproductive Sciences in Animal Conservation* (pp. 275–316). Cham: Springer.
- Clulow, J., Upton, R., Trudeau, V. L., & Clulow, S. (2019). Amphibian Assisted Reproductive Technologies: Moving from Technology to Application. In *Reproductive Sciences in Animal Conservation* (pp. 413–463). Cham: Springer.
- Comizzoli, P. (2015). Biobanking efforts and new advances in male fertility preservation for rare and endangered species. *Asian Journal of Andrology*, *17*(4), 640–645.
- Conde, D. A., Colchero, F., Güneralp, B., Gusset, M., Skolnik, B., Parr, M., ... Flesness, N. (2015). Opportunities and costs for preventing vertebrate extinctions. *Current Biology*, *25*(6), R219–R221.
- Della-Togna, G., Howell, L. G., Clulow, J., Langhorne, C. J., Marcece-Greaves, R., & Calatayud, N. E. (2020). Evaluating amphibian biobanking and reproduction for captive breeding programs according to the Amphibian Conservation Action Plan objectives. *Theriogenology*, *150*(1), 412–431.
- Dreitz, V. J. (2006). Issues in species recovery: An example based on the Wyoming toad. *BioScience*, *56*(9), 765–771.
- Frankham, R. (2008). Genetic adaptation to captivity in species conservation programs. *Molecular Ecology*, *17*(1), 325–333.
- Frankham, R., Ballou, J. D., & Briscoe, D. A. (2010). *Introduction to Conservation Genetics* (2nd edition). Cambridge, UK: Cambridge University Press.
- Frankham, R., Bradshaw, C. J. A., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, *170*, 56–63.
- Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., & Zipfel, K. (2008). The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook*, *42*(1), 125–135.
- Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, *22*(4), 852–861.

- Hammerson, G., & Pearl, C. A. (2004). *Rana pretiosa*. *The IUCN Red List of Threatened Species 2004*: e.T19179A8848383. <https://doi.org/10.2305/IUCN.UK.2004.RLTS.T19179A8848383.en>.
- Holt, W. V. (2016). The black-footed ferret recovery program: A strong advocate for establishing semen banking programs as support tools for small population welfare. *Animal Conservation*, 19(2), 116–117. <https://doi.org/10.1111/acv.12270>
- Holt, W. V., Bennett, P. M., Volobouev, V., & Watwon, P. F. (1996). Genetic resource banks in wildlife conservation. *Journal of Zoology*, 238(3), 531–544.
- Howard, J. G., Lynch, C., Santymire, R. M., Marinari, P. E., & Wildt, D. E. (2016). Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret. *Animal Conservation*, 19(2), 102–111. <https://doi.org/10.1111/acv.12229>
- Howard, J. G., Marinari, P. E., & Wildt, D. E. (2003). Black-footed ferret: Model for assisted reproductive technologies contributing to in situ conservation. In *Conservation biology series* (pp. 249–266) Cambridge, UK: Cambridge University Press.
- Kissel, A. M., Palen, W. J., & Govindarajulu, P. (2017). A decision-theory approach to cost-effective population supplementation for imperiled species. *Ecological Economics*, 142, 194–202.
- Kouba, A. J., Lloyd, R. E., Houck, M. L., Silla, A. J., Calatayud, N., Trudeau, V. L., ... Vance, C. (2013). Emerging trends for biobanking amphibian genetic resources: The hope, reality and challenges for the next decade. *Biological Conservation*, 164, 10–21.
- Kouba, A. J., Vance, C., Calatayud, N., Rowilson, T., Langhorne, C., & Willard, S. (2012). Assisted reproductive technologies (ART) for amphibians. *Amphibian Husbandry Resource Guide, (2nd edition)* 2, 60–118.
- Kouba, A. J., Vance, C. K., & Willis, E. L. (2009). Artificial fertilization for amphibian conservation: Current knowledge and future considerations. *Theriogenology*, 71(1), 214–227.
- Kouba, A. J., Willis, E., Vance, C., Hasenstab, S., Reichling, S., Krebs, J., ... Germano, J. (2011). Development of assisted reproduction technologies for the endangered Mississippi gopher frog (*Rana sevosa*) and sperm transfer for in-vitro fertilization. *Reproduction, Fertility and Development*, 24(1), 170.
- Lande, R., & Barrowdough, G. (1987). Effective population size, genetic variation, and their use in population management. In *Viable populations for conservation*. Cambridge, UK: Cambridge University Press.
- Lees, C. M., & Wilcken, J. (2009). Sustaining the Ark: The challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook*, 43(1), 6–18.
- Mace, G. (1986). Genetic management of small populations. *International Zoo Yearbook*, 24(1), 167–174.
- Mawson, P. R., & Lambert, C. (2017). Challenges of operating a multi-species breeding-for-release facility at Perth Zoo, Australia. *International Zoo Yearbook*, 51(1), 165–174.
- Monfort, S. L. (2014). “Mayday Mayday Mayday”, the millennium ark is sinking! In *Reproductive Sciences in Animal Conservation* (pp. 15–31). Cham: Springer.
- Murphy, J. B., & Gratwicke, B. (2017). History of captive management and conservation amphibian programs mostly in zoos and aquariums. Part I-Anurans. *Herpetological Review*, 48(1), 241–260.
- Pearl, C. A., Adams, M. J., Bury, R. B., & McCreary, B. (2004). Asymmetrical effects of introduced bullfrogs (*Rana catesbeiana*) on native ranid frogs in Oregon. *Copeia*, 2004(1), 11–20.
- Pearl, C. A., Bowerman, J., Adams, M. J., & Chelgren, N. D. (2009). Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon spotted frogs (*Rana pretiosa*). *EcoHealth*, 6(2), 209–218.
- Phillipsen, I. C., Bowerman, J., & Blouin, M. (2010). Effective number of breeding adults in Oregon spotted frogs (*Rana pretiosa*): Genetic estimates at two life stages. *Conservation Genetics*, 11(3), 737–745.
- Robert, A. (2009). Captive breeding genetics and reintroduction success. *Biological Conservation*, 142(12), 2915–2922.
- SARA (2002). Canada’s Species at Risk Act (SARA). Retrieved from <https://laws-lois.justice.gc.ca/eng/acts/S-15.3/FullText.html>
- Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., ... Catenazzi, A. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, 363(6434), 1459–1463.
- Shen, F., Zhang, Z., He, W. E. I., Yue, B., Zhang, A., Zhang, L., ... Watanabe, T. (2009). Microsatellite variability reveals the necessity for genetic input from wild giant pandas (*Ailuropoda melanoleuca*) into the captive population. *Molecular Ecology*, 18(6), 1061–1070.
- Silla, A. J., & Byrne, P. G. (2019). The role of reproductive technologies in amphibian conservation breeding programs. *Annual Review of Animal Biosciences*, 7, 499–519.
- Soulé, M., Gilpin, M., Conway, W., & Foose, T. (1986). The millenium ark: How long a voyage, how many staterooms, how many passengers? *Zoo Biology*, 5(2), 101–113.
- USFWS, doi (1973). *Endangered Species Act of 1973, as amended through the 108th Congress*. Washington, DC, USA: Department of the Interior, US Fish and Wildlife Service.
- Wei, F., Costanza, R., Dai, Q., Stoeckl, N., Gu, X., Farber, S., ... Swaisgood, R. (2018). The value of ecosystem services from giant panda reserves. *Current Biology*, 28(13), 2174–2180.
- Wei, F., Hu, Y., Zhu, L., Bruford, M. W., Zhan, X., & Zhang, L. (2012). Black and white and read all over: The past, present and future of giant panda genetics. *Molecular Ecology*, 21(23), 5660–5674.
- Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., ... Townsend, E. (2011). The Amphibian Ark: A global community for ex situ conservation of amphibians. *Herpetological Conservation and Biology*, 6(3), 340–352.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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