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Supplementary Material: *Reproduction, Fertility and Development*

Supplementary Material

Integrating biobanking could produce significant cost benefits and minimise inbreeding for Australian amphibian captive breeding programs

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Supporting Information S1: Cost categories and colony size information for the captive breeding of Orange-bellied frogs (*Geocrinia vitellina*) and White-bellied frogs (*Geocrinia alba*)

Here we present the cost categories and costings data used to model theoretical populations of Orange-bellied frogs (*Geocrinia vitellina*) and White-bellied frogs (*Geocrinia alba*) in this study. The itemized costs presented here allowed the estimation of a scalable dollar value per individual animal for each species using known colony sizes for the financial year from which costs were sourced (**Table S1; Table S2; Table S3**). All costs are based on the 2017/2018 breeding seasons of *G. vitellina* and *G. alba* as part of Perth Zoo's Native Species Breeding Program (NSBP). The costs presented here were obtained through a combination of face-to-face interviewing, access to financial, logistical and operations reporting, annual budgets, as well as estimates for some costs which were not readily available and more difficult to itemize and separate from the operating costs of the whole zoo (particularly relevant for utilities costs). The costs presented here are in 2018 nominal Australian dollars ($AU).

Where costs would have been spread across multiple taxa, particularly for labour costs, in the interests of modelling reflective program costs per individual species we have assigned the full costs for labour for the NSBP to individual species (to accurately reflect what a consolidated effort to captive breed one species would look like). For other costs which are not assigned to one particular species in the NSBP we have divided costs for general NSBP expenditure across the relevant species (since there were five species managed as part of the program at the time of data collection we have assigned equal one fifth costs for some general NSBP expenditure to each of the case study species referred to here) – in addition the same approach was taken for amphibian specific infrastructure where housing was shared by both species (*G. vitellina* and *G. alba*). Veterinary costs were omitted due to the bulk of these costs not being perceived as relevant to our theoretical banked and non-banked populations (i.e. chytrid quarantine costs, etc.) – these costs may be relevant to future iterations of this modelling and for real-world program design under different costings and logistics (i.e. founder collection from the wild, etc.).

For captive colony numbers, only individuals surviving past metamorphosis were included within counts (tadpoles were omitted from modelling). The numbers used for modelling were based on animals collected and housed during 2017/2018.

Table S1

Cost Categories for *G. vitellina*	**Fixed or Variable**	**Cost (2017/18 $AU)**
Facilities - set-up - recurring maintenance	Variable	$31,613.60 $240.50
Founder Collection (field costs for collection of egg clutches, including vehicles, accommodation, food) ** ** Does not include variable labour costs which are captured in costs for Husbandry Staff below.	Fixed	$3,000
Minor Equipment and Consumables** ** includes laboratory expenses	Variable	$602
Labour (Husbandry Staff salaries and Director Animal Health and Research*) **10% of Director of Animal Health and Research Salary modelled as proportionate time spent on NSBP.	Variable	$116,401.14
Utilities (electricity and water)	Variable	$11,538.16
Food	Variable	$604.17
Management (research and administration)	Fixed	$778.28
Total		**$164,777.85**

Table S2

Cost Categories *G. alba*	**Fixed or Variable**	**Cost (2017/18 $AU)**
Facilities - set-up - recurring maintenance	Variable	$31,613.60 $240.50
Founder Collection (field costs for collection of egg clutches, including vehicles, accommodation, food) ** ** Does not include variable labour costs which are captured in costs for Husbandry Staff below.	Fixed	$3,000
Minor Equipment and Consumables** ** includes laboratory expenses	Variable	$602
Labour (Husbandry Staff salaries and Director Animal Health and Research*) **10% of Director of Animal Health and Research Salary modelled as proportionate time spent on NSBP.	Variable	$116,401.14
Utilities (electricity and water)	Variable	$11,538.16
Food	Variable	$604.17
Management (research and administration)	Fixed	$778.28
Total		**$164,777.85**

Table S3

Species	**Captive colony 2017/18**
Orange-bellied frog (*Geocrinia vitellina*)	n = 56
White-bellied frog (*Geocrinia alba*)	n = 92

Supporting Information S2: Cost and genetic modelling for theoretical captive populations of Orange-bellied frogs (*Geocrinia vitellina*) and White-bellied frogs (*Geocrinia alba*) under alternate N_e/N ratios

Table S4. Genetic and cost analysis for hypothetical captive colonies of Orange-bellied frogs (*Geocrinia vitellina*) and White-bellied frogs (*Geocrinia alba*) designed to meet different genetic retention targets under various genetic backcross scenarios using published N_e/N values (N_e/N 1.17 for *Geocrinia vitellina* and N_e/N 1.21 for *Geocrinia alba*) *

Backcross scenario	N_e	N	F_t no backcross	F_t backcross	H_t/H_O after 100 years	Cost ($) Year 1	Cost ($) Year 2	Total captive colony cost ($) after 100 years	Back-cross cost ($) (labour and set-up)	Total program cost ($) after 100 years
G. vitellina										
90% heterozygosity retention with no backcross	120	103	0.0991	n.d	0.9009	$306,382	$254,888	$121,424,879	n.d	$121,424,879
90% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	4	0.5000	0.1000	0.9000	$29,798	$10,564	$5,035,091	$880,006	$5,915,098
95% heterozygosity retention with no backcross	244	209	0.0500	n.d	0.9500	$617,694	$513,100	$244,437,890	n.d	$244,437,890
95% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	9	0.5000	0.0500	0.9500	$44,696	$22,744	$10,837,592	$906,278	$11,743,870
99% heterozygosity retention with no backcross	1245	1064	0.0100	n.d	0.9900	$3,128,755	$2,595,849	$1,236,665,478	n.d	$1,236,665,478
99% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	43	0.5000	0.0100	0.9900	$146,001	$105,567	$50,294,596	$1,084,926	$51,379,522
G. alba										
90% heterozygosity retention with no backcross	120	99	0.0991	n.d	0.9009	$180,860	$150,777	$71,826,113	n.d	$71,826,113
90% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	4	0.5000	0.1000	0.9000	$25,201	$6,752	$3,218,657	$880,006	$4,098,663
95% heterozygosity retention with no backcross	244	202	0.0500	n.d	0.9500	$364,992	$303,501	$144,584,424	n.d	$144,584,424
95% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	8	0.5000	0.0500	0.9500	$32,522	$12,683	$6,044,222	$901,023	$6,945,246
99% heterozygosity retention with no backcross	1245	1029	0.0100	n.d	0.9900	$1,843,408	$1,529,744	$728,770,085	n.d	$728,770,085
99% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	41	0.5000	0.0100	0.9900	$92,924	$61,614	$29,355,137	$1,074,418	$30,429,555

* 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 yrs" provide program costs without backcrossing. "Total program cost after 100-years" include captive colony costs and expenditure for backcross events. 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. Colony numbers (N) were derived using the published N_e/N values of 1.17 and 1.21 for *G. vitellina* and *G. alba* respectively (Driscoll 1999). These values are demographic estimates derived from wild populations and are likely not representative of captive populations of *G. vitellina* and *G. alba* but do represent one extreme of population size using publically available species-specific values. Inbreeding coefficients (F_t) and heterozygosity (H_t/H_O) are values at 100 years. All dollar amounts shown are in Australian currency (AU$), starting in the year 2019. n.d = not determined.

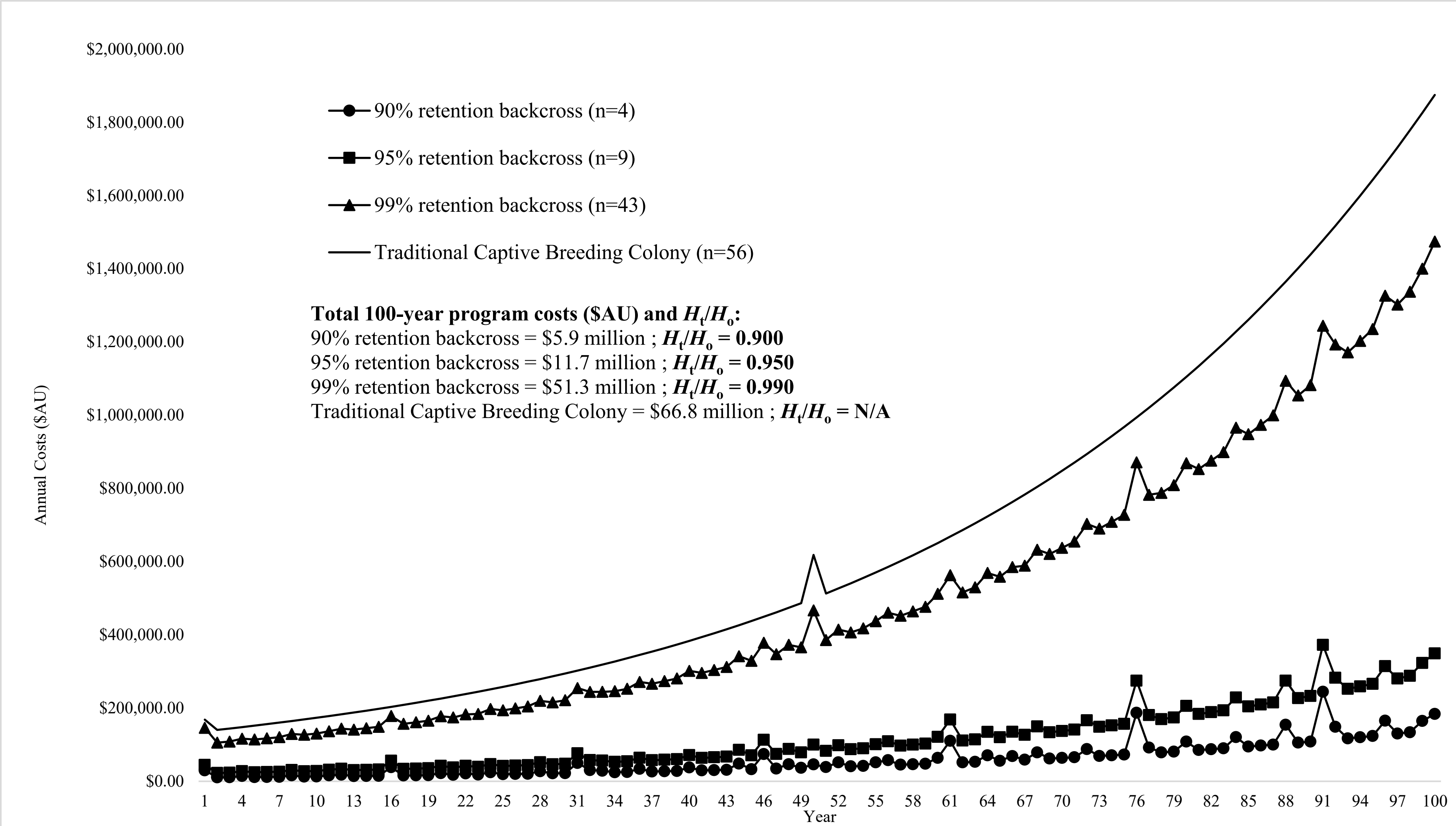

Fig. S1. Projections of 100-year annual costs for hypothetical captive Orange-bellied frog (*Geocrinia vitellina*) populations designed to meet different genetic retention targets by incorporating biobanking technology. Projected 100-year total program costs are shown for modelled populations of *G. vitellina* designed to retain 90%, 95% and 99% of source population heterozygosity by recurrent genetic backcross with frozen founder sperm (4-year intervals). Projected costs for backcrossed populations represent colonies under an assumed species-specific N_e/N ratio of 1.17. Projected 100-year annual program costs are also shown for the actual *G. vitellina* captive population size held at Perth Zoo as at 2017/18 for comparison. N = colony size for all modelled populations and the current captive holdings held for wild population head-starting. All dollar amounts are shown in Australian currency (AU$), starting in the year 2019.

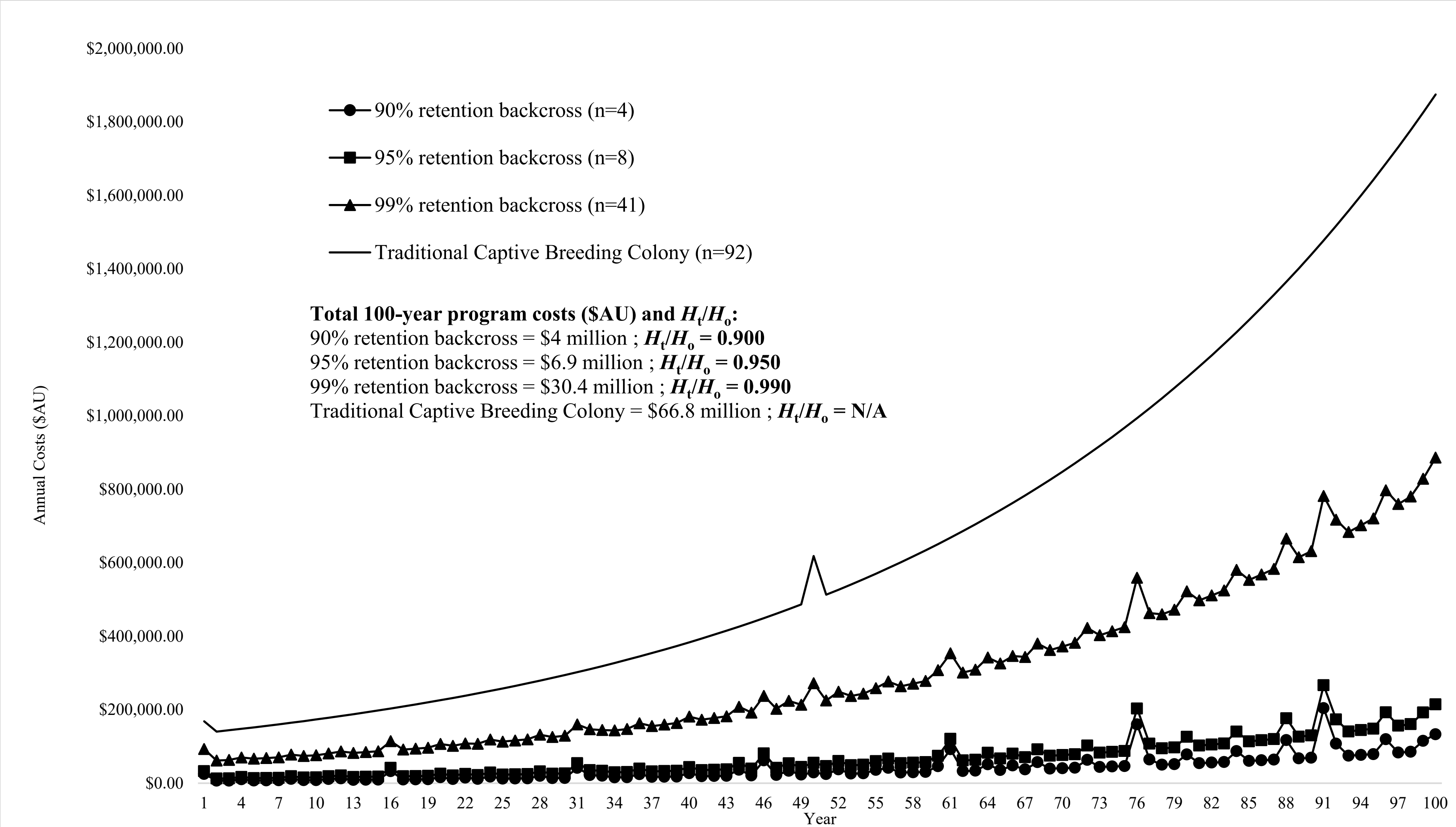

Fig. S2. Projections of 100-year annual costs for hypothetical captive White-bellied frogs (*Geocrinia alba*) populations designed to meet different genetic retention targets by incorporating biobanking technology. Projected 100-year total program costs are shown for modelled populations of *G. alba* designed to retain 90%, 95% and 99% of source population heterozygosity by recurrent genetic backcross with frozen founder sperm (4-year intervals). Projected costs for backcrossed populations represent colonies under an assumed species-specific N_e/N ratio of 1.21. Projected 100-year annual program costs are also shown for the actual *G. alba* captive population size held at Perth Zoo as at 2017/18 for comparison. N = colony size for all modelled populations and the current captive holdings held for wild population head-starting. All dollar amounts are shown in Australian currency (AU$), , starting in the year 2019.

Table S5. Genetic and cost analysis for hypothetical captive colonies of White-bellied frogs (*Geocrinia alba*) and Orange-bellied frogs (*Geocrinia vitellina*) designed to meet different genetic retention targets under various genetic backcross scenarios using an N_e/N of 0.141; representing a median value between a range of temporal N_e/N estimates published for various amphibians (n = 5) (Frankham *et al.* 2019) **and the red spotted newt (*Notophthalmus viridescens*) (N_e/N 0.073)** (Frankham 1995)*

Backcross scenario	*N_e*	*N*	*F_t no backcross*	*F_t backcross*	*H_t/H_O after 100 years*	*Cost ($) Year 1*	*Cost ($) Year 2*	*Total captive colony cost ($) after 100 years*	*Back-cross cost ($) (labour and set-up)*	*Total program cost ($) after 100 years*
G. vitellina										
90% heterozygosity retention with no backcross	120	851	0.0991	n.d	0.9009	$2,503,192	$2,076,989	$989,478,956	n.d	$989,478,956
90% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	35	0.5000	0.1000	0.9000	$122,165	$86,079	$41,010,595	$1,042,891	$42,053,486
95% heterozygosity retention with no backcross	244	1730	0.0500	n.d	0.9500	$5,084,739	$4,218,201	$2,009,558,548	n.d	$2,009,558,548
95% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	71	0.5000	0.0500	0.9500	$229,430	$173,774	$82,788,599	$1,232,049	$84,020,647
99% heterozygosity retention with no backcross	1245	8830	0.0100	n.d	0.9900	$25,936,819	$21,513,546	$10,249,109,285	n.d	$10,249,109,285
99% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	355	0.5000	0.0100	0.9900	$1,075,630	$865,588	$412,370,628	$2,724,290	$415,094,918
G. alba										
90% heterozygosity retention with no backcross	120	851	0.0991	n.d	0.9009	$1,525,200	$1,265,813	$603,032,422	n.d	$603,032,422
90% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	35	0.5000	0.1000	0.9000	$81,942	$52,717	$25,116,789	$1,042,891	$26,159,681
95% heterozygosity retention with no backcross	244	1730	0.0500	n.d	0.9500	$3,096,576	$2,569,160	$1,223,950,434	n.d	$1,223,950,434
95% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	71	0.5000	0.0500	0.9500	$147,835	$106,097	$50,546,878	$1,232,049	$51,778,927
99% heterozygosity retention with no backcross	1245	8830	0.0100	n.d	0.9900	$15,789,147	$13,096,761	$6,239,329,143	n.d	$6,239,329,143
99% heterozygosity retention by backcrossing every generation (4-year intervals)	n.d	355	0.5000	0.0100	0.9900	$667,655	$527,201	$251,162,027	$2,724,290	$253,886,317

* 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 yrs" provide program costs without backcrossing. "Total program cost after 100-years" include captive colony costs and expenditure for backcross events. 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. Colony numbers (N) were derived using the median value (N_e/N 0.141) from a range of comprehensive temporal published N_e/N estimates for a range of amphibian species (n = 6) appropriate for alternate species modelling or estimation (**Table 1**). Whilst these values are comprehensive N_e/N estimates they are derived from wild populations and likely do not accurately represent captive populations of *G. vitellina* and *G. alba*, although do represent one reliable extreme of population size for this modelling. We assume that the true N_e/N values for theoretical captive populations of *G. vitellina* and *G. alba* would lie somewhere between the median value presented here (N_e/N 0.141) and the wild derived N_e/N values modelled above (**Table S4**). Inbreeding coefficients (F_t) and heterozygosity (H_t/H_O) are values at 100 years. All dollar amounts shown are in Australian currency (AU$), starting in the year 2019. n.d = not determined.

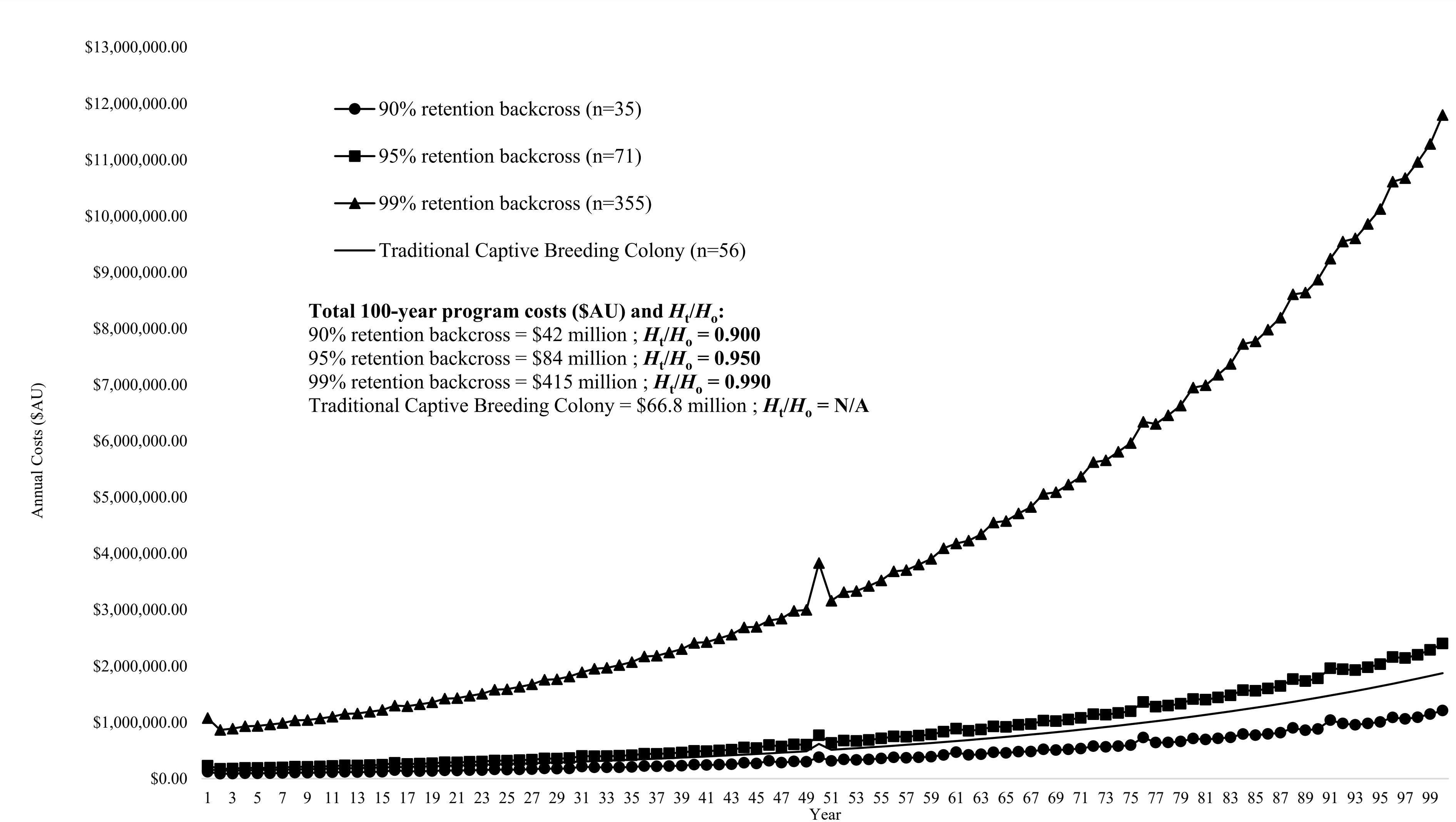

Fig. S3. Projections of 100-year annual costs for hypothetical captive Orange-bellied frog (*Geocrinia vitellina*) populations designed to meet different genetic retention targets by incorporating biobanking technology. Projected 100-year total program costs are shown for modelled populations of *G. vitellina* designed to retain 90%, 95% and 99% of source population heterozygosity by recurrent genetic backcross with frozen founder sperm (4-year intervals). Projected costs for backcrossed populations represent colonies under an assumed N_e/N ratio of 0.141. Projected 100-year annual program costs are also shown for the actual *G. vitellina* captive population size held at Perth Zoo as at 2017/18 for comparison. N = colony size for all modelled populations and the current captive holdings held for wild population head-starting. All dollar amounts are shown in Australian currency (AU$), starting in the year 2019.

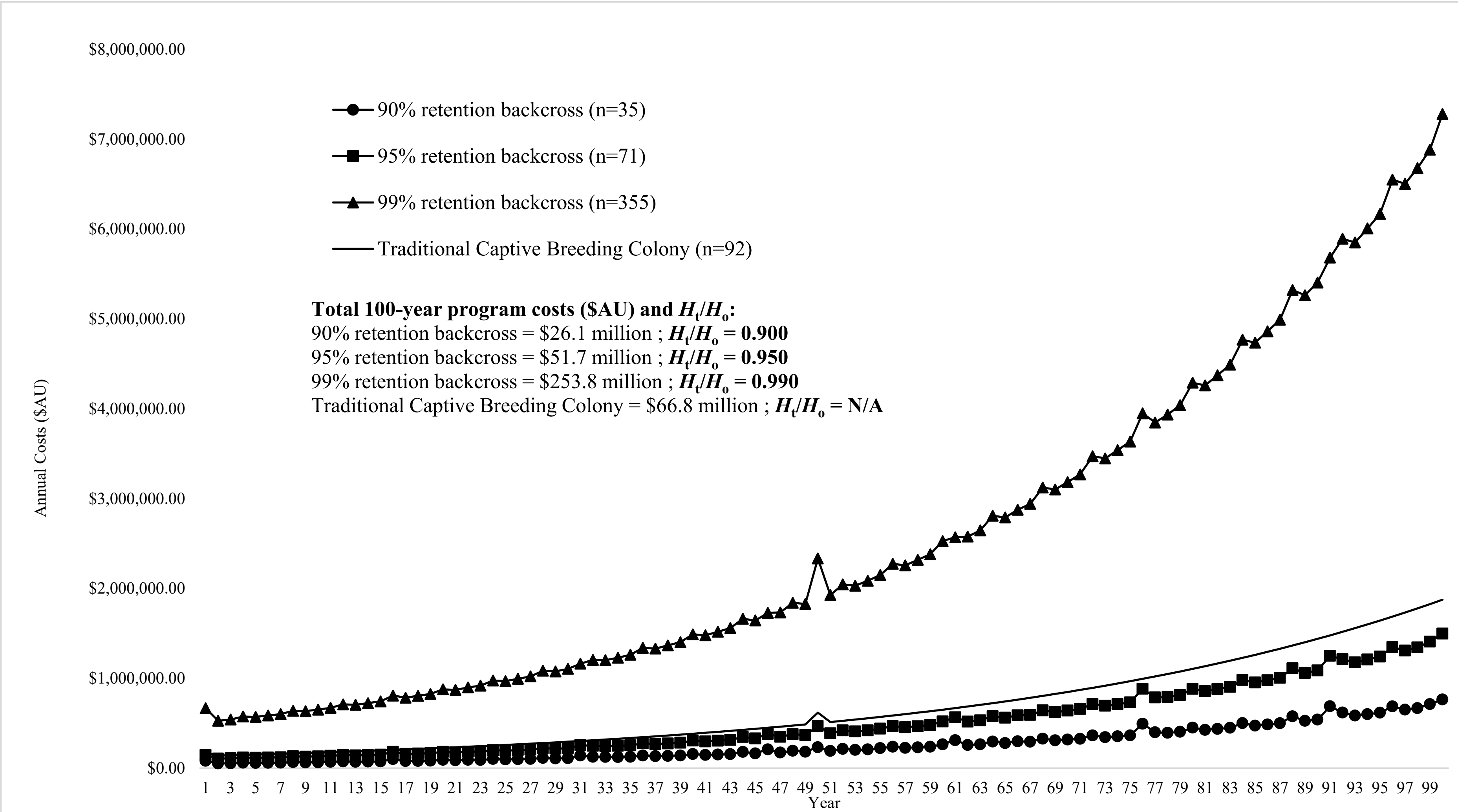

Fig. S4. Projections of 100-year annual costs for hypothetical captive White-bellied frogs (*Geocrinia alba*) populations designed to meet different genetic retention targets by incorporating biobanking technology. Projected 100-year total program costs are shown for modelled populations of *G. alba* designed to retain 90%, 95% and 99% of source population heterozygosity by recurrent genetic backcross with frozen founder sperm (4-year intervals). Projected costs for backcrossed populations represent colonies under an assumed N_e/N ratio of 0.141. Projected 100-year annual program costs are also shown for the actual *G. alba* captive population size held at Perth Zoo as at 2017/18 for comparison. N = colony size for all modelled populations and the current captive holdings held for wild population head-starting. All dollar amounts are shown in Australian currency (AU$), , starting in the year 2019.

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