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# A novel holistic framework for genetic-based captive-breeding and reintroduction programs

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**Abstract:** *Research in reintroduction biology has provided a greater understanding of the often limited success of species reintroductions and highlighted the need for scientifically rigorous approaches in reintroduction programs. We examined the recent genetic-based captive-breeding and reintroduction literature to showcase the underuse of the genetic data gathered. We devised a framework that takes full advantage of the genetic data through assessment of the genetic makeup of populations before (past component of the framework), during (present component), and after (future component) captive-breeding and reintroduction events to understand their conservation potential and maximize their success. We empirically applied our framework to two small fishes: Yarra pygmy perch (*Nannoperca obscura*) and southern pygmy perch (*Nannoperca australis*). Each of these species has a locally adapted and geographically isolated lineage that is endemic to the highly threatened lower Murray–Darling Basin in Australia. These two populations were rescued during Australia's recent decade-long Millennium Drought, when their persistence became entirely dependent on captive-breeding and subsequent reintroduction efforts. Using historical demographic analyses, we found differences and similarities between the species in the genetic impacts of past natural and anthropogenic events that occurred in situ, such as European settlement (past component). Subsequently, successful maintenance of genetic diversity in captivity—despite skewed brooder contribution to offspring—was achieved through carefully managed genetic-based breeding (present component). Finally, genetic monitoring revealed the survival and recruitment of released captive-bred offspring in the wild (future component). Our holistic framework often requires no additional data collection to that typically gathered in genetic-based breeding programs, is applicable to a wide range of species, advances the genetic considerations of reintroduction programs, and is expected to improve with the use of next-generation sequencing technology.*

**Keywords:** conservation genetics, extinction, fish, genetic diversity, management, restoration genomics

Un Marco de Referencia Holístico Novedoso para Programas de Reproducción en Cautiverio Basada en Genética y de Reintroducción

**Resumen:** *Investigaciones sobre biología de la reintroducción han proporcionado un mejor entendimiento del, a menudo, éxito limitado de las reintroducciones de especies y han resaltado la necesidad de aproximaciones rigorosas científicamente en los programas de reintroducción. Examinamos la literatura reciente sobre reproducción en cautiverio basada en genética y reintroducción para exhibir la subutilización de los datos genéticos. Diseñamos un marco de referencia que obtiene la mayor ventaja de los datos genéticos mediante la evaluación de la composición genética de las poblaciones antes (componente pasado del marco de referencia), durante (componente presente), y después (componente futuro) de eventos de reproducción en cautiverio y de reintroducción para entender su potencial de conservación y maximizar su éxito. Aplicamos nuestro marco de referencia empíricamente con dos especies de peces pequeños:* Nannoperca obscura

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*y* N. australis*. Cada especie tiene un linaje adaptado localmente y aislado geográficamente endémico de la muy amenazada Cuenca Baja Murray-Darling, Australia. Las dos poblaciones fueron rescatadas durante la reciente Sequía del Milenio que duró diez años en Australia, cuando su persistencia se volvió totalmente dependiente de esfuerzos de reproducción en cautiverio y subsecuente reintroducción. Mediante análisis demográficos históricos, encontramos diferencias y similitudes entre las especies en los impactos genéticos de eventos naturales y antropogénicos pasados que ocurrieron in situ, como el asentamiento europeo (componente pasado). Subsecuentemente, el mantenimiento exitoso de la diversidad genética en cautiverio – no obstante la contribución de reproductores sesgada – fue posible por el manejo cuidadoso de la reproducción basada en genética (componente presente). Finalmente, el monitoreo genético reveló la supervivencia y reclutamiento de crías obtenidas en cautiverio liberadas en el medio silvestre. Nuestro marco de referencia holístico a menudo no requiere de datos adicionales a los obtenidos típicamente en programas de reproducción basados en genética, es aplicable a un rango amplio de especies, es un avance en las consideraciones genéticas de los programas de reintroducción, y mejorará con el uso de tecnología de secuenciación de última generación.*

**Palabras Clave:** conservación genética, diversidad genética, extinción, genética genômica de restauración, manejo, peces

## Introduction

Reintroduction programs are often a final attempt at maintaining or restoring biodiversity in the wild when all other avenues have failed or are considered too late. Their aim is to reintroduce extinct populations or species, or supplement declining populations, by releasing captive-bred individuals or translocating wild individuals. Research in reintroduction biology has provided a greater understanding of the limited success of previous species reintroductions and led to a call for a scientifically rigorous framework (Seddon et al. 2007). Reintroduction programs now often attempt to incorporate scientific knowledge that identifies and can be used to mitigate the original cause of population decline and that informs release techniques to maximize survival. As human-driven environmental changes, such as habitat loss and climate change, further threaten global biodiversity, reintroductions will likely become an increasingly attractive management option.

There is still a great need to improve the science behind reintroduction programs. Restoration genetics will play an ever-increasing role in reintroduction programs and presents several unique opportunities (Frankham 2010), as already envisioned for translocations (Weeks et al. 2011). Unlike several other disciplines, genetics has the potential to provide information without direct observation. This includes information about past changes in population size due to natural and anthropogenic impacts, suitability of likely source populations for captive-breeding or translocations, inbreeding and relatedness of captively bred individuals, and identity of reintroduced individuals. We propose that genetic data sets typically gathered in captive-breeding programs contain this untapped information that can be used to improve restoration efforts.

We showcase this for the captive-breeding and reintroduction of two endangered freshwater fishes: Yarra pygmy perch (YPP) (*Nannoperca obscura*) and southern pygmy perch (SPP) (*N. australis*). These small fishes occur in southeastern Australia, including the Murray–Darling Basin (MDB) (Hammer et al. 2010; Unmack et al. 2013). The MDB is characterized by naturally variable flow and episodes of drought and flood and supplies much of Australia's irrigation water for agriculture (Kingsford et al. 2011). The MDB is currently influenced by numerous anthropogenic impacts that threaten aquatic biodiversity, such as regulation of river flow, water abstraction, and habitat degradation (Kingsford et al. 2011).

A prolonged drought known as the Millenium Drought occurred from 1997 to 2010 in southeastern Australia (Van Dijk et al. 2013), the severity of which was exacerbated in the MDB due to water abstraction for irrigation. The impacts of the drought were especially notable from 2007 to 2010 in the terminal lakes of the system (Kingsford et al. 2011; Wedderburn et al. 2012). Small-bodied fishes had a high risk of extinction in the terminal lakes because they are poor dispersers with low potential for recolonization and rely on habitat that completely dried out (Brauer et al. 2013; Hammer et al. 2013). In response, a partnership was formed between governmental and nongovernmental organizations and other stakeholders. They implemented extensive in situ and ex situ conservation actions, including habitat monitoring, fish rescue, captive-breeding, and release of fish upon the return of favorable conditions (Hammer et al. 2013). The approximately 200 originally rescued YPP likely represent the entire remaining gene pool for the species in the basin because they are an evolutionarily significant unit (ESU) endemic to the lower MDB and were assumed extirpated from their only known area of occupancy (approximately 35 km$^2$) (Hammer et al. 2010; Wedderburn et al. 2012; Brauer et al. 2013). The 68 originally rescued SPP represent a management unit (MU) that is genetically and environmentally divergent from other MUs of the MDB (Hammer 2001). Hence, the future of these locally

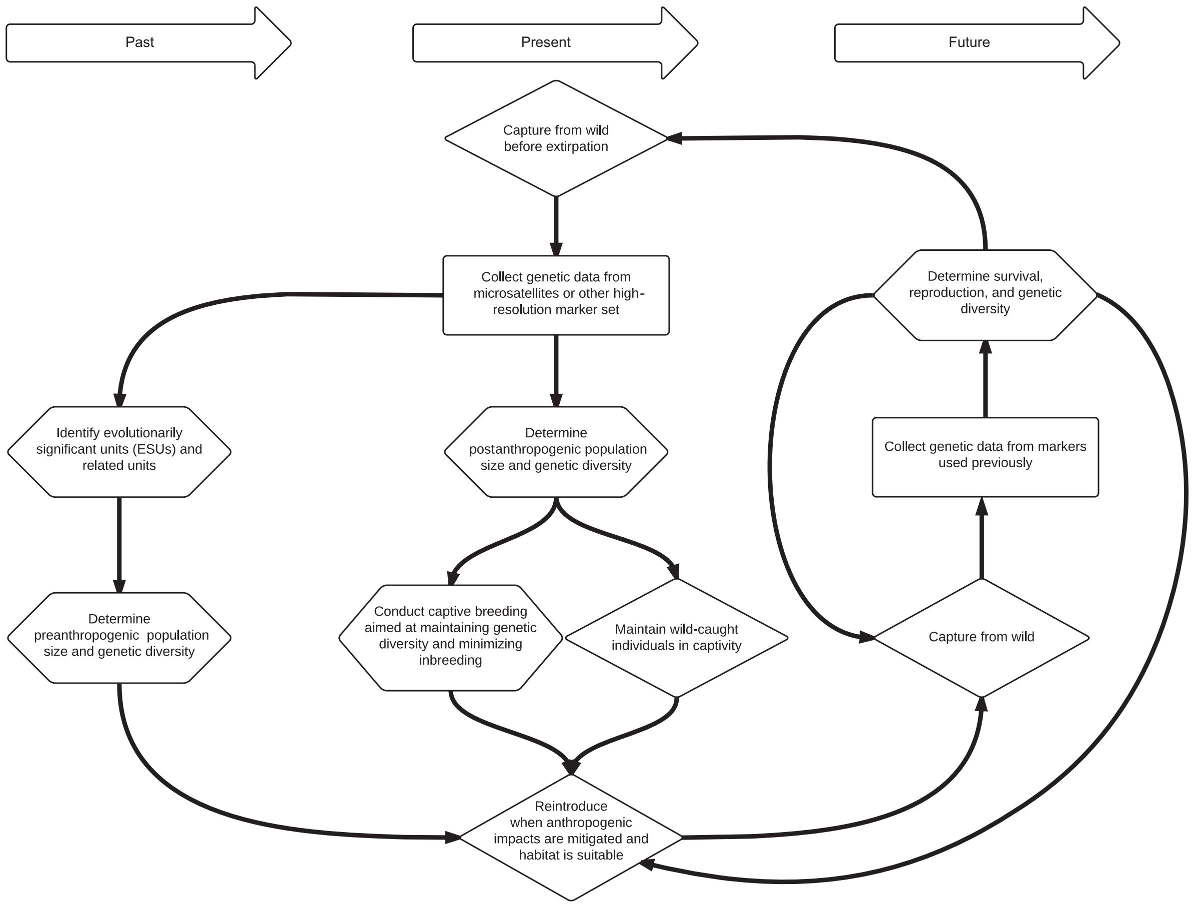

*Figure 1. Framework for a holistic approach to genetic-based captive-breeding and reintroduction programs. Rectangles are activities requiring only genetic input, hexagons are activities requiring genetic and benefiting from nongenetic input, and diamonds are activities requiring no genetic input.*

adapted and geographically isolated lineages in the wild became completely dependent on captive-breeding and reintroduction efforts.

We devised a holistic framework that examines the genetic makeup of captive-bred and reintroduced populations before (past component of the framework), during (present component), and after (future component) captive-breeding and reintroduction (Fig. 1). We also examined the recent literature on genetic-based captive-breeding and considered this literature in relation to our framework. Finally, we empirically implemented the framework with two pygmy perch species to showcase its application and performance. In the empirical implementation, we used genetic signatures of historical demographic changes to ascertain the requirements of the two species for continued persistence (past component). We implemented a genetic-based captive-breeding program that included assessing the influence of the novel breeding program and the fish mating system on the genetic composition of reared individuals (present component). We also genetically confirmed the survival and breeding of captive-bred released individuals in the wild (future component). This genetic-based framework is expected to advance the genetic considerations of reintroduction programs and can be easily adopted in other species, often without the need for additional data collection or prior detailed knowledge of species ecology.

## Methods

### Literature Search

We searched for articles on genetic-based captive-breeding published from 2011 to 2014. We used the same search parameters and criteria as Witzenberger and Hochkirch (2011) and recorded whether studies examined the genetic makeup of populations before, during, and after captive-breeding and reintroduction in accordance with our proposed holistic approach (Fig. 1). We also recorded the species or subspecies captively bred, the DNA marker or markers used, and the specific contribution of genetics to the study.

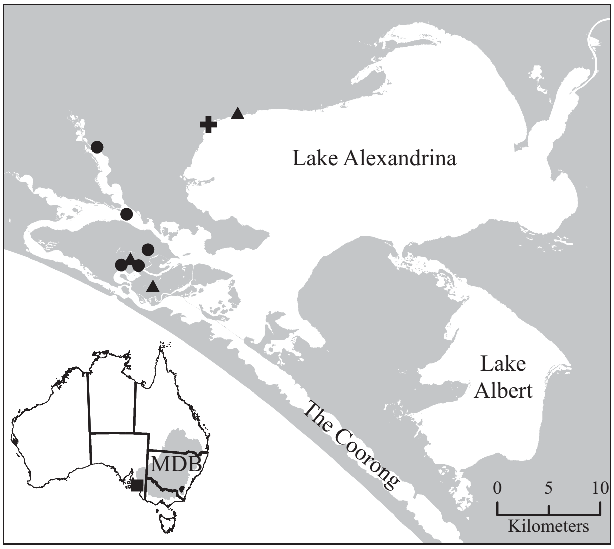

*Figure 2. Location of the Murray–Darling Basin (MDB) (gray in inset of Australia), the lower lakes region (square in inset of Australia) of the MDB, and the reintroduction sites at the lower lakes region (circle, Yarra pygmy perch; triangle, southern pygmy perch; plus sign, location of Fig. 3 water level measurements [Milang Jetty, Station A4260524]).*

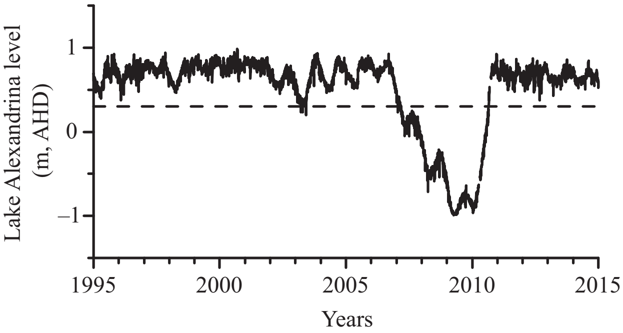

*Figure 3. Mean daily water level (Australian height datum [AHD]) in Lake Alexandrina from 1995 to 2014 (inclusive as measured at Milang Jetty, Station A4260524, plus sign in Fig. 2) (dashed line, major or total habitat loss at approximately 0.3 m AHD). Graph modified from Hammer et al.'s (2013) Fig. 3, and data from the Department of Environment, Water and Natural Resources, Government of South Australia.*

### Population Rescue, Captive Program, and Reintroduction

The YPP and SPP were rescued before habitat desiccation from the lower MDB in 2007 and 2008 (Figs. 2 & 3). In 2010, a subset of these individuals formed the basis of a genetic-based breeding program at Flinders University in Adelaide as part of a broader captive-breeding and reintroduction program. Work at Flinders University with the animal subjects was conducted under approval from the Flinders University Animal Welfare Committee (approval E313). Widespread rainfall and the subsequent return of normal regulated water levels occurred in the lower MDB in 2010 and 2011 (Fig. 3). This enabled 4900 YPP and 1350 SPP from all captive-breeding efforts and wild-born rescued individuals to be released into the wild from 2011 to 2013 (Fig. 2). Reintroduction sites within the lower MDB were chosen based on factors influencing habitat quality. Details of nongenetic components of this conservation effort are in Hammer et al. (2013).

### Genetic-Based Captive-Breeding Program

Eighty-four YPP and 65 SPP rescued from the wild were available in 2010 for implementing a genetic-based breeding program aimed at minimizing inbreeding and preserving genetic variation. The natural breeding system and environment were mimicked based on expert knowledge. Broodstock numbers and composition enabled us to establish 11 breeding groups for each species. The YPP breeding groups consisted of 3 dams and 2 sires, and SPP breeding groups consisted of 2 dams and 2 sires.

Breeding groups were selected based on analyses of microsatellite markers (16 and 17 loci genotyped for YPP and SPP, respectively) that we developed for the two species (Carvalho et al. 2011; Carvalho et al. 2012) (Supporting Information). This included avoiding the use of inbred brooders and ensuring low estimated pairwise relatedness within breeding groups. Relatedness between individuals of the same sex in a breeding group was minimized to increase the power of subsequent parentage assignment.

### Historical Population Demography of Captive Populations

The possibility of past changes in population size (pre- or post-European settlement) was examined for each species through two approaches (Supporting Information). First, we used extended Bayesian skyline plots to assess population size changes from the present to the time of the common ancestor in the genealogy. We then performed two bottleneck tests, the heterozygosity-excess test and the M-ratio test, to assess reductions in population size during the recent past. For these analyses, we used the microsatellite data set initially generated to guide the captive-breeding program.

### Pygmy Perch Mating System and Broodstock Contribution

We used the same protocols outlined above to genotype 1188 F1 offspring in the two species for assessing their mating system and parental contribution. We sought to genotype a subset of 60 randomly chosen offspring per breeding group. Offspring were allocated to the most likely parent pair and a power analysis was conducted

(Supporting Information). We used chi-square goodness-of-fit tests to assess brooder contribution to allocated offspring in each breeding group. The maintenance of genetic variation between parents and offspring was assessed by calculating number of alleles, and observed ($H_O$) and expected ($H_E$) heterozygosity. This was completed for all potential broodstock and all genotyped offspring, and for each broodstock and offspring group.

### Recaptured Offspring after Reintroduction Events

We used a genetic approach to assess survival and reproduction of reintroduced offspring. Fish were captured at the lower lakes region of the MDB on 8 occasions from November 2011 to October 2014 and released on the day of capture. Genetic samples were taken from these captures and genotyped as outlined above. Assessing survival and reproduction involved identifying whether each captured fish was a captive-bred offspring that was previously genotyped (based on identical multilocus genotypes) or was not previously genotyped (based on allocation to a parent pair) (Supporting Information). If neither of these were the case and the fish was captured from a site where only genetic-based bred fishes were released, the fish was likely a wild-born offspring.

## Results

### Literature Search

We identified 54 peer-reviewed articles on genetic-based captive-breeding programs (Supporting Information). Although several classes of DNA markers were used, the vast majority were microsatellites (93%), and none were single-nucleotide polymorphisms (SNPs). Only 13% of the studies assessed whether there were anthropogenic or natural changes in genetic diversity or demography before captive-breeding (past component of our framework). They used only traditional bottleneck tests and coalescent-based parametric models to conduct their assessments; there was no use of coalescent-based nonparametric approaches. Only 17% of the studies genetically assessed or monitored a supplemented wild population or reintroduced population after captive-breeding (future component). No studies examined the genetic makeup of populations before, during, and after captive-breeding and reintroduction.

### Genetic-Based Captive-Breeding Program

There were high values for inbreeding in six YPP and one SPP relative to the other brooders, so these seven individuals were excluded from consideration as brooders. The relatedness estimates of the remaining brooders were between −0.944 and 1.000 for YPP and −0.557 and 0.546 for SPP, so we gave preference to low values within each breeding group when choosing final brooder combinations (Supporting Information).

### Historical Population Demography of Captive Populations

The skyline plots (Fig. 4) indicated differences between the historical demographics of the two species. The effective population size before European settlement was greatest for SPP. There was no evidence of changes in population size for YPP, but we found evidence of a demographic reduction in population size within the last several hundred generations for SPP.

The heterozygosity-excess test showed no evidence ($p > 0.05$) of a recent genetic bottleneck in either species, regardless of the test parameters. The M-ratio test showed evidence of a bottleneck under only one and 12 out of 52 test parameter sets for YPP and SPP, respectively, indicating that it was not robust to parameter changes and therefore was an unreliable test of a bottleneck (Supporting Information).

### Pygmy Perch Mating System and Broodstock Contribution

The genetic-based captive-breeding program produced approximately 2600 and 1400 offspring for YPP and SPP, respectively. The power analysis showed that the proportions of offspring correctly allocated to parent pairs among breeding groups were 0.938 to 1.000 for YPP and 1.000 for SPP. During parentage analysis, only 7 (out of 578) offspring in YPP were unallocated, and no offspring (out of 591) were unallocated in SPP (Supporting Information).

There was significant evidence of a skewed contribution of brooders to offspring for both species. However, the genetic-based choice of brooders ensured no or only minimal loss of genetic variation in all offspring compared with the potential broodstock (Supporting Information). The combined genotyped offspring maintained, at very similar allele frequencies, all 43 alleles present in the wild-caught YPP and 102 of the 106 alleles in SPP. The offspring also had greater heterozygosity than the wild-caught broodstock (YPP [captive-bred offspring *vs.* wild-caught broodstock]: $H_O = 0.445$ *vs.* 0.397, $H_E = 0.436$ *vs.* 0.395; SPP: $H_O = 0.702$ *vs.* 0.643, $H_E = 0.667$ *vs.* 0.653).

### Recaptured Offspring after Reintroduction Events

Reintroductions used equal numbers of offspring from each breeding group in the genetic-based captive-breeding program. Approximately 2200 YPP offspring and 1000 SPP offspring were released in spring 2011 and autumn 2012 at sites in the lower MDB where favorable habitat had returned (Fig. 2).

Thirteen YPP and 71 SPP were captured after reintroduction. The latter included large gravid females captured in 2014. Eleven captured SPP were offspring born

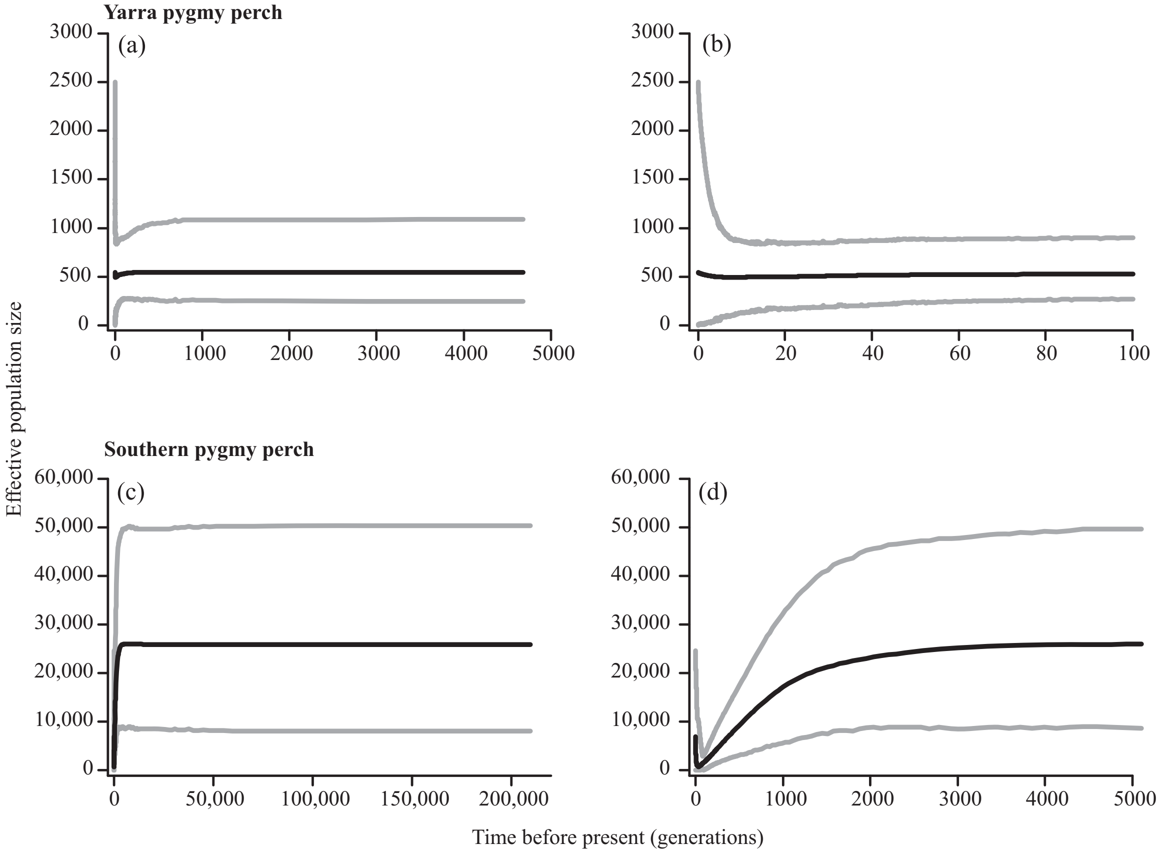

*Figure 4. Estimated changes in effective population size of Yarra pygmy perch (a and b) and southern pygmy perch (c and d) over time: (a and c) complete plots and (b and d) a section of the plots from the present to the recent past (black lines, median effective population size; gray lines, upper and lower bounds of the 95% highest posterior density interval).*

and genotyped in captivity, and eight were offspring born and not genotyped in captivity (with one caught twice). The remaining 52 individuals were assigned as likely wild-born offspring of the genetic-based captive-bred fish (Supporting Information). For YPP, the power to identify captured individuals was low, which is consistent with their low genetic variation. So, eight of the 13 captured YPP were classified as possible offspring from the genetic-based breeding program based on no mismatching loci according to Mendelian inheritance to a parent pair. Therefore, there was evidence of survival and wild spawning of offspring from the genetic-based breeding program.

## Discussion

Our genetic-based framework links the past, present, and future in captive-breeding and reintroduction programs (Fig. 1). Each of these aspects has been used to inform previous captive-breeding and reintroduction programs (Supporting Information), but it is novel to use them with forethought in a cohesive framework. We successfully implemented the framework in endangered populations of two species of pygmy perches from the lower MDB.

### Past Component of the Framework

The past component of the framework is the investigation of the history of imperiled populations. There are two key aspects of population history that can inform captive-breeding and reintroduction. First, the course of evolution for different populations within a species can be used to define ESUs and related units (Moritz 1994). Limited resources may then be allocated toward conserving populations or lineages that represent different evolutionary trajectories within a species. Specifically, identification of these units aids in the choice of sites from which individuals will be captively bred. Population structure and assignment analyses for wild and captive populations are widespread in genetic-based captive-breeding studies (Supporting Information). However, these provide information only about the current population structure. There has been little effort to conduct phylogeographic analyses, which are needed to define the historical legacy of conservation units (Moritz 1994). The captive individuals of the YPP and SPP represented the entire remaining gene pools for the region and consisted of an ESU and an MU, respectively, which are locally adapted and geographically isolated (Hammer 2001; Hammer et al. 2010; Wedderburn et al. 2012; Brauer et al. 2013). These lineages would have become extinct without captive-breeding.

The second key aspect is natural and anthropogenic past change (or lack thereof) in the demographics and consequently genetic diversity of the target population. The history of a population shapes its capacity to persist during captive-breeding and reintroduction. Persistence may be more likely if the population exists in stressful environments and undergoes changes in abundance naturally (Bouzat 2010) or has a small abundance naturally and so individuals are adapted to low population densities (Lankau & Strauss 2011). Persistence may be less likely if the population has a high abundance naturally (Lankau & Strauss 2011) or the anthropogenic collapse in population size caused inbreeding depression or the loss of genetic diversity and evolutionary potential (Frankham 2005). captive-breeding programs aim to minimize inbreeding and loss of genetic diversity, typically under the assumption that founders are outbred and accurately represent the genetic diversity of the natural population (see "Present Component of the Framework" below). This is surprising given that the assumption often does not hold due to the conditions that necessitated captive-breeding (Frankham 2005). Population history needs to be inferred and appropriate management actions taken, such as carefully considered injection of genetic diversity from other populations (Weeks et al. 2011). Such analyses are rarely conducted in captive-breeding and reintroduction programs (Supporting Information).

Multiple methods and potentially multiple genetic marker types can be used to infer past demography across different time scales (Garrick et al. 2015). Past demography can be inferred by the distinct patterns it leaves in gene genealogies of extant populations. We particularly recommend the use of the Bayesian skyline approach because of its wide applicability. Bayesian skylines are often implemented using DNA sequence data to infer deep histories. Here, we used the microsatellite data from captive-breeding so we could also infer recent histories. Bayesian skylines are not constrained by simple, parametric population models that are often biologically unrealistic, such as linear or exponential changes in effective population size (Ho & Shapiro 2011). They also require only one temporal sample of individuals to trace past population size changes but can incorporate multiple temporal samples to improve accuracy. We also implemented traditional bottleneck tests, specifically the heterozygosity-excess test and the M-ratio test. We advocate their use where a demographic decline is thought to have occurred recently and as long as users are aware of their limitations (Peery et al. 2012). Population decreases should continue to be monitored visually because they are not expected to be genetically detectable when the change is relatively small or has so far occurred for only a few generations.

We used genetic data from the captive-breeding program of the two pygmy perch species to infer each of their demographic histories and make associated conservation and management recommendations. Both species showed stable effective population sizes before European settlement, up to around 200 years ago. This indicates that evolutionarily based responses to the naturally dynamic hydrology may have buffered large changes in effective population size. One such response described for pygmy perches is reproductive bet-hedging (Morrongiello et al. 2012). The YPP showed a naturally smaller effective population size than SPP and therefore may have an intrinsically higher extinction risk. Only SPP decreased in effective population size and genetic diversity during European settlement. This is consistent with known details of their population decline; SPP had a much larger distribution in the MDB that became fragmented after European settlement compared with the narrow range of YPP in the lower MDB (Cadwallader 1978; Hammer et al. 2009). This suggests that managers need to reestablish a more natural environment for the SPP population to return to its natural size and to prevent further loss of genetic diversity. We found no evidence of a decrease in genetic diversity in either species due to the recent drought crisis. This result was expected because genetic evidence of bottlenecks require longer lasting demographic collapses. The breeding programs therefore had the potential to maintain the natural long-term genetic diversity of YPP but only the reduced predrought genetic diversity of SPP. The SPP genetic diversity may be augmented by translocating individuals from elsewhere in the MDB.

## Present Component of the Framework

The present component of the framework is the captive maintenance and reintroduction of the target populations. Arguably, the most widely recognized contribution of genetics to captive-breeding and reintroduction programs is minimizing inbreeding and maximizing the maintenance of genetic diversity in captivity (Supporting Information). We found that many captive-breeding programs are conducted under the assumption that founding breeders are unrelated and not inbred. The danger in this assumption is that there may be high relatedness and inbreeding in these individuals due to anthropogenically induced population collapses in the wild. This can result in further inbreeding in the captive-breeding program and individuals that are not representative of the ancestral population contributing to the next generation. An alternative is to estimate relatedness of the potential founders (Doyle et al. 2001; Russello & Amato 2004), as we did here. Blouin (2003) provides a guide to DNA-based estimates of relatedness and assignment to relationship categories. Inbreeding in founder individuals may also be genetically estimated, as we did for pygmy perches, in order to exclude inbred individuals from consideration as founders.

If this is not possible, the assumption that founders are unrelated often has minimal impact on later generations of captive-breeding programs that implement a mean kinship (MK) strategy (Rudnick & Lacy 2008). The MK strategy has arisen as a standard way to minimize the loss of genetic diversity in long-term captive populations by breeding in pairs the individuals that have the lowest MK (Ballou & Lacy 1995). The MK may be unambiguously calculated from pedigree information, but when pedigrees are incomplete, individuals may be genetically assigned to relationship categories or their relatedness estimated (e.g., Ivy et al. 2009).

It is important to consider whether the species breeding system may inhibit implementing the MK strategy, such as when breeding naturally occurs in groups rather than pairs and when extrapair copulations occur that likely require genetic analyses to confirm parentage. In such situations, a strategy that considers breeding groups is required (e.g., Wang 2004). For pygmy perches, breeding groups were formed to mimic natural breeding in a way that maximized the power of subsequent parentage assignment by minimizing relatedness between individuals of the same sex. The parentage assignment showed a highly skewed contribution of brooders to offspring, which decreases effective population size and suggests the possibility of sexual selection in these species. Sexual selection is corroborated in both species based on nuptial color in males during the breeding season. In SPP, the nuptial color correlates with body size and condition (Morrongiello et al. 2010), and size is positively correlated with male dominance (Mitchell 1976). Any future captive-breeding of the species will require parentage analyses and multiple breeding groups to monitor and maximize the maintenance of genetic diversity.

We advocate for captive-breeding to occur for as short a time as possible, with individuals released as soon as there is habitat of sufficient quality for reintroduction. We suggest this because genetic diversity typically decreases and inbreeding increases as captive-breeding continues (Rudnick & Lacy 2008) and because fitness in the wild can be reduced in as little as one generation of captive-breeding and can be cumulative across captive-bred generations (Araki et al. 2007; Frankham 2008). In pygmy perches, short-term holding and subsequent release of wild-caught fish to restored habitat was impossible due to the drought crisis lasting longer than the average reproductive life span of the fish. The least time possible in captivity—one generation—was therefore completed in environments simulating natural habitat, which provided the best chance of limiting fitness loss. If long-term captive-breeding is required, strategies should be implemented that aim to avoid adaptation to captivity (Williams & Hoffman 2009) and sustain rather than simply minimize the loss of genetic diversity (Lacy 2013).

### Future Component of the Framework

The future component of the framework is biological and genetic monitoring to assess recovery in the wild. Monitoring is especially important at the early stages of recovery (Armstrong & Seddon 2008) and when the maintenance of a population is a balance between ecosystem and anthropogenic interests (Hobbs et al. 2009). Monitoring should include genetic comparisons between the reintroduced population, the captive-bred population (present component), and the preanthropogenic-impact population (past component). This is rarely done (Supporting Information).

There is a paucity of studies that apply genetic approaches to reintroductions, whether reintroductions are from captive-bred or translocated individuals, even though the genetic theory is comparable to that used extensively in conservation genetics for assessing small populations. Genetic approaches can be used to monitor many aspects of reintroduced populations: survival, recruitment, abundance, genetic diversity, relatedness or relationship categories, inbreeding, inbreeding depression, and population connectivity (Schwartz et al. 2007; Groombridge et al. 2012; Jamieson & Lacy 2012; Keller et al. 2012).

For the pygmy perches, we found genetic evidence that released fish were not only surviving, but also reproducing in the wild. We suggest that recapture rates are low-to-moderate given the large spatial scale of the lakes ($> 600$ km$^2$) and that populations are still well short of predrought abundance and likely long-term sustainable levels. Continued monitoring and adaptive management (e.g., regulation of environmental water flow) are needed to ensure the success of the reintroductions, especially because climate change is predicted to cause decreases in rainfall in the MDB (Kingsford et al. 2011).

### From Genetic- to Genomic-Based Captive-Breeding and Reintroduction

Collecting data at thousands of DNA markers is becoming cheaper and easier due to next-generation sequencing technology. These genomic data sets have a higher information content than genetic data sets and so are expected to increase substantially the performance of the analyses we propose (Allendorf et al. 2010). For example, the microsatellite-based relatedness estimates do not capture the full variation in true relatedness (Van de Casteele et al. 2001). Their accuracy and precision depend on the number of loci, number of alleles per locus, frequency of those alleles, and relatedness composition of the data set (e.g., Blouin et al. 1996; Van de Casteele et al. 2001).

This issue can be partly mitigated by using the estimator that best captures the true relatedness of simulated individuals (e.g., Russello & Amato 2004; Ivy et al. 2009). However, these concerns are becoming obsolete with the greater power of thousands of SNPs (Allendorf et al. 2010) and the development of relatedness estimators specific to these SNP data sets (e.g., Lipatov et al. 2015).

Genome-wide data sets can also provide information about adaptive (functional or ecologically relevant) genetic diversity in the past, present, and future because they are more likely to contain markers associated with ecologically relevant traits (Allendorf et al. 2010). For example, fitness may be reduced during captive-breeding, but this cannot be detected using neutral genetic variation. The reduction in fitness can be inhibited in our framework by using next-generation sequencing to identify genomic regions associated with key phenotypic traits (e.g., inbreeding depression) and employing these adaptive markers along with neutral markers to select breeders in captive populations (Allendorf et al. 2010). Studies targeting a small number of functional genes, such as the major histocompatibility complex, have already proved useful in genetic-based captive-breeding (Sommer 2005) (Supplementary Information).

### Final Remarks

Frameworks such as ours are rarely applied but are a vital step towards more effective restoration programs. Most genetic-based programs focus on what is immediately perceivable and perhaps logistically easier to study (i.e., the present captive-bred population). We showed how genetic data sets typically gathered in such programs can also be used to track naturally and anthropogenically caused changes in the genetic composition of the target population across time. For the endangered pygmy perches, we found species-specific attributes in historical demography despite the two species existing in the same ecological system; maintenance of genetic diversity during captive-breeding despite unequal contribution of brooders to offspring; and survival and reproduction of captive-bred individuals reintroduced in the wild.

The methods used within the past, present, and future components were chosen with the intent of being widely applicable. Differences in the known biology and ecology of the species, its current demographic state, the anthropogenic impact that led to the need for captive-breeding, and the resources available, mean each program will need to be considered on a case-by-case basis when deciding what course to take during the application of the framework. We have exemplified this by presenting the course taken for the pygmy perches.

On a broader level, we argue for the full potential of genetics and genomics to be realized and implemented in captive-breeding and reintroduction programs so that there is increased success at protecting biodiversity.

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## Supporting Information

Additional information describing the methods and results is available online (Appendix S1). The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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