The value of *ex situ* collections for global biodiversity conservation in the wild

by

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Declaration

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Summary

Despite the best efforts of conservation practitioners global biodiversity is continuing to decline. The role of zoos and aquariums in conserving global biodiversity *ex situ* has become increasingly important as more species become threatened with extinction. As *ex situ* conservation resources are limited, evidence-based decision making is required to identify, and prioritise, the management actions necessary to increase the potential of *ex situ* conservation efforts. The efficacy of *ex situ* conservation efforts is currently hindered by 1) entrenched taxonomic biases in collection planning and the prioritisation of large, charismatic vertebrates, 2) the unsustainability of *ex situ* populations due to limited space availability and management practices, and 3) limited considerations of the potential for *ex situ* collections to conserve and reintroduce genetic variation into populations using biological samples and advanced reproductive technologies. In this thesis I explore the multifaceted contribution of *ex situ* collections to global biodiversity conservation. I focus on the importance of standardised, globally shared *ex situ* records, and their potential to inform collection planning, population sustainability and genetic conservation decision-making.

In Chapter 2 I addressed the taxonomic bias in collection planning and assessed the importance of large, charismatic vertebrates in driving both visitor attendance and fundraising for conservation in the wild. Using data from >450 zoological collections globally I identified a net positive effect of large charismatic vertebrates on both visitor attendance, and subsequently conservation fundraising. I also revealed that numerous other factors, such as species richness and species uniqueness, play equally important roles. I suggest that the taxonomic bias in collection planning is potentially an effective conservation strategy, but encourage a more creative approach to collection planning and an assessment of the conservation potential of traditionally non-charismatic species. In Chapter 3 I investigated the potential of globally shared zoological records to provide the management insights necessary to increase the sustainability of *ex situ* populations, utilising 753 *ex situ* flamingo (*Phoenicopteridae*) populations as a case study. I both confirm and contradict existing management guidelines, highlighting the potential of globally shared zoological records, and provide species-specific management recommendations to promote the sustainability of *ex situ* flamingo populations.

In Chapters 4 and 5 I highlighted the role of *ex situ* collections in conserving the genetic diversity of living populations in gene banks, such as the San Diego Zoo Frozen Zoo®, and the potential of *ex situ* samples to contribute to future genetic rescue and de-extinction efforts. In Chapter 4 I revealed that 5.1% of all threatened amphibian, bird, mammal, and reptile species are represented within the San Diego Zoo Frozen Zoo® and that further sampling from within the global zoo and aquarium community could increase this representation to 16.5%. I provide future sampling suggestions based on both sampling opportunities and existing conservation priorities. In Chapter 5 I addressed the taxonomic bias in de-extinction research and prioritised for de-extinction investigation the 122 species of recently extinct plants, incorporating both the feasibility and the probability of reintroduction success. I show that *ex situ* samples, such as herbarium specimens, are currently available for nearly all recently extinct plant species, each with the potential to provide seeds capable of germination. These studies highlight the importance of *ex situ* samples in conserving genetic diversity and their potential to reintroduce genetic variation into existing populations, enhancing ecosystem health and stability.

This thesis illustrates the important conservation and management insights that can be derived from globally shared *ex situ* records, providing the recommendations necessary to increase the efficacy of global *ex situ* conservation efforts. The current rate of biodiversity loss suggests that the importance of *ex situ* conservation is only going to increase, however the limited resources available mean that evidence-based decision-making is necessary to ensure conservation opportunities are not overlooked and that existing strategies are effective in achieving their goals. This thesis demonstrates the multi-faceted contribution of *ex situ* collections to global biodiversity conservation and highlights their future potential, conserving not only individual species, but also ecosystems and the services vital to sustaining human civilisation.

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1. General Introduction

1.1 Biodiversity Conservation

Habitat loss and fragmentation, global climate change, introduced species and diseases, pollution, overexploitation and an exponentially increasing human population are all putting pressure on wildlife populations and resulting in range contractions, population declines and increased extinction rates (Butchart *et al.* 2010; Ceballos *et al.* 2015; Ceballos *et al.* 2017; Ehrlich 1988; IPBES 2019). The current rate of extinction being observed is up 100 times higher than the predicted background extinction rate and has led to the belief that we have now entered the sixth mass extinction event of our planet's history, with more species now threatened with extinction due to anthropogenic activity than ever before (Barnosky *et al.* 2011; Ceballos *et al.* 2015; IPBES 2019). This global loss of biodiversity is expected to have negative cascading consequences on not only ecosystem functioning and services, but also on the services vital to sustaining human civilisation (Ceballos *et al.* 2017; Ceballos *et al.* 2020; Díaz *et al.* 2018; IPBES 2019). Despite various attempts to conserve global biodiversity, no reduction in the rate of biodiversity loss has been observed, with predicted accelerations in extinction rates almost guaranteed (Butchart *et al.* 2010; Ceballos *et al.* 2020). As a result, there has been an urgent call to protect and conserve extant global biodiversity, in order to both further prevent species' extinctions and ensure sustainable human health and well-being (Conde *et al.* 2011; Ellis 2013; Turner *et al.* 2012).

The preservation of natural communities and populations of species within their natural habitat, termed *in situ* conservation, has always been the primary goal of conservation, protecting not only individual species, but also their habitat, surrounding species and even entire ecosystems (Balmford *et al.* 1995; Snyder *et al.* 1996). This has resulted in notable conservation successes, including that of grey whales (*Eschrichtius robustus*) in the Eastern Pacific, mountain gorillas (*Gorilla beringei beringei*) in the Virunga Mountains and Southern sea otters (*Enhydra lutris nereis*) along the California coast (Hughes *et al.* 2019; Reilly 1992; Robbins *et al.* 2011). Despite these successes and the fact that the costs associated with effective *in situ* conservation are persistently lower than those associated with *ex situ* conservation, the current areas set aside for *in situ* conservation are minimal and ensuring their long-term protection is extremely difficult (Alibhai and Jewell 1994; DeFries *et al.* 2005; Golden Kroner *et al.* 2019). The validity of our reliance on *in situ* conservation is further undermined due to the vulnerability of wild populations in the face of climate change as climatic niche conditions shift (Pritchard *et al.* 2012;

Stralberg *et al.* 2020). As a result, it has been acknowledged that for wildlife to persist some level of human intervention and management will likely be required, increasingly blurring the distinction between *in situ* and *ex situ* conservation (Dickie *et al.* 2007; Keulartz 2015).

Ex situ conservation consists of the preservation of species outside of their native habitat (AZA 2016b). This is primarily undertaken by the 10,000-12,000 zoos and aquariums globally, of which approximately 1,000 are organised and accredited as part of internationally recognised organisations, such as the European Association of Zoos and Aquaria (EAZA), the Association of Zoos and Aquariums (AZA, North America) and the World Association of Zoos and Aquariums (WAZA) (Kelly 1997). Similarly, *ex situ* plant conservation is undertaken by the approximately 3,500 botanic gardens, of which 350 are also involved in global seed banking efforts (O'Donnell and Sharrock 2017). Collectively these institutions represent an almost unparalleled conservation resource, providing the practical experience, scientific knowledge, financial resources and physical capacity to breed and maintain thousands of diverse species (Oberwemmer *et al.* 2011).

1.2 *Ex Situ* Conservation

Although zoos and aquariums were not established to conserve wildlife, the conservation value and potential of *ex situ* management has long been known (Conway 2003; IUDZG/IUCN SSC 1993). The captive breeding and subsequent reintroduction of species back into their native habitat was originally termed the 'Ark Concept' (Durrell 1976; Soulé *et al.* 1986) and has been achieved for several species, including the California condor (*Gymnogyps californianus*) and Arabian oryx *(Oryx leucoryx*) (Chemnick *et al.* 2000; Stanley Price 1989). Embracing their conservation potential has resulted in zoos and aquariums playing a fundamental role in the recovery of 28% of all vertebrate species whose International Union for Conservation of Nature (IUCN) Red List threat statuses have improved (Hoffmann *et al.* 2010). Furthermore, for the species currently listed as "*Extinct in the Wild*" under the IUCN Red List, such as Père David's deer (*Elaphurus davidianus*) and the Alagoas curassow (*Mitu mitu*), captive breeding remains the only option for continued species persistence. Unfortunately, the observed successes have been somewhat opportunistic and the hope of establishing wild populations with captive-bred animals has shown limited success (Beck *et al.* 1994; Brichieri-Colombi *et al.* 2019; Jule *et al.* 2008). Additionally, the recent revelation that the majority of *ex situ* population management programmes are not meeting sustainability criteria, and are thus not viable long-term, has caused serious concern among population managers and conservation practitioners (Lees and

Wilcken 2009; Powell *et al.* 2019). Despite these limitations, it is widely agreed that *ex situ* breeding and management can be used as an effective conservation tool to conserve species, particularly when integrated with *in situ* conservation efforts (Bowkett 2009; Byers *et al.* 2013; IUCN SSC 2014). Zoos and aquariums have the capacity to directly contribute to *in situ* conservation efforts through the generation of financial contributions, provision of animals for reintroduction purposes, veterinary expertise and conservation education (Brichieri-Colombi *et al.* 2019; Coonan *et al.* 2010; Gusset and Dick 2010; Smith *et al.* 2007).

Although accredited zoos and aquariums are conservation-orientated organisations, they must also fulfil several other objectives, including education, research and entertainment (Frost 2010; Roe *et al.* 2014). To fulfil these multiple roles, zoo collections must attract recreational visitors, which can place competing demands on the composition of collections as public preferences do not always align with conservation priorities (Turley 1999). In reality, the public attend zoos and aquariums on a recreational basis and expect to see large, charismatic vertebrates, particularly mammals (Carr 2016; Frynta *et al.* 2010; Gunnthorsdottir 2001; Marešová and Frynta 2008; Moss and Esson 2010; Puan and Zakaria 2007; Ward *et al.* 1998). This creates tension between economic viability and conservation credibility, with institutions fulfilling some roles at the expense of others (Fa *et al.* 2014; Pritchard *et al.* 2012; Whitworth 2012). If zoos and aquariums are to persist and make meaningful contributions to biodiversity conservation, they must adapt to both visitor preferences and conservation requirements, being tactical with their living collections to best serve their own priorities and objectives (Kelly 1997; Whitworth 2012).

1.3 *Ex Situ* Collection Planning

Until relatively recently personal preference, availability, and competition between institutions determined the species found within collections (Hancocks 2001). However, over the last four decades zoos and aquariums have shifted away from institution-level management of species towards co-operative breeding and population management programmes across multiple institutions and regions, ensuring the persistence of self-sustaining *ex situ* populations (Ballou and Traylor-Holzer 2011; Che-Castaldo *et al.* 2019). Zoos and aquariums can only maintain a tiny fraction of all the species on the planet due to the limited amount of space available to them, and this ultimately means the inclusion of one species in a collection occurs at the exclusion of at least one other (Balmford *et al.* 1996; Conway 1986; Conway 1987; Foose 1983). If the species most in need of conservation efforts were also the most preferred by visitors, then decisions regarding the composition of zoo and aquarium collections would be relatively simple (Palmer *et al.* 2018). In reality, this is rarely the case, and species compete for inclusion both within taxonomic groups (e.g. *Ursidae*) and across taxonomic groups (e.g. *Canidae*, *Felidae*) (Long *et al.* 2011; McCann and Powell 2019). The global zoo and aquarium community has sufficient resources to maintain viable populations of only 1,000 to 2,000 species and how institutions decide to fill these spaces has become a contentious issue and one which is only set to intensify as the number of threatened species rises (Balmford *et al.* 1996; Traylor-Holzer *et al.* 2019).

Perceived visitor preferences have fuelled the belief that large vertebrates, particularly mammals, are necessary in order to attract visitors, and this has resulted in the biased composition of modern collections (Balmford *et al.* 1996; Conde *et al.* 2011; Conde *et al.* 2013; Fa *et al.* 2011; Fa *et al.* 2014; Frynta *et al.* 2013; Hancocks 2001; Kawata 2013; Pritchard *et al.* 2012). This strong taxonomic bias is criticised as such species are often costlier to maintain, demonstrate poor *ex situ* reproductive success, require exponentially greater enclosure sizes and raise significant ethical and welfare issues (Balmford *et al.* 1996; Young 2015). As a result, it has been recommended that zoos and aquariums shift their focus away from large vertebrates towards smaller-bodied species (preferably amphibians, invertebrates and fish), threatened species, native species and consolidate their conservation efforts on fewer species overall (Fa *et al.* 2014; Keulartz 2015; Palmer *et al.* 2018). However, as the global zoo and aquarium community has a limited capacity, there are concerns that the removal of popular species could result in reduced visitor attendance, lowering economic viability and consequently *in situ* conservation investment (Carr 2016; Kawata 2013; Ward *et al.* 1998; Whitworth 2012).

Conversely, the ‘flagship’ approach of using large vertebrates in collections to stimulate public education and *in situ* conservation fundraising, i.e. “*Strategic Collection Planning*” (Hutchins *et al.* 1995), is potentially an effective conservation strategy, helping to conserve other species and habitats (Bowkett 2014). This recognises the current bias towards large mammals and considers it a legitimate and potentially highly effective conservation strategy, owing to their ability to act as flagship species (Hutchins and Wemmer 1991; Leader-Williams and Dublin 2000). The global zoo and aquarium community spends approximately $350 million annually on *in situ* wildlife conservation activities and represents the third largest conservation organisation contributor globally (Gusset and Dick 2011). Although the majority of institutions contribute less than 5% of their operating income to *in situ* conservation activities, these contributions significantly impact global wildlife conservation efforts, with greater funding from *ex situ* institutions showing a positive relationship with *in situ* project success and viability (Bettinger and Quinn 2000; Fa *et al.* 2011; Gusset and Dick 2010; Mace *et al.* 2007). A doubling in the number of *in situ* projects supported by AZA member institutions from 325 in 1992 to 650 in 1999 suggests that *ex situ* institutions are becoming increasingly involved in *in situ* conservation efforts, and if this continues zoos and aquariums could become the primary non-governmental *in situ* conservation organisations (Conway 2003).

Nearly all *ex situ* conservation activities are funded by paying visitors and the popularity of the species within institutional collections is positively correlated with visitor attendance (Whitworth 2012). While national population size and gross national income have both been correlated with international zoo attendance (Davey 2007), Whitworth (2012) failed to find any significant relationship between socio-demographic variables and institutional attendance in the UK, suggesting that an institution's species composition is more important in determining attendance than socio-economic variables. The evidence for what determines popularity is contested and often inconsistent. Although several studies have shown a clear preference among the public for large, charismatic vertebrates, particularly mammals (Carr 2016; Moss and Esson 2010), this is contradicted by findings of body mass not being an important factor in determining species popularity (Ward 2000; Whitworth 2012). Threat status and nativeness have been shown to be important for determining species popularity (Carr 2016; Roe *et al.* 2014), but have also been contradicted (Whitworth 2012). These issues are not easily resolved, and the divide will only expand as animal welfare standards improve, resulting in increased enclosures sizes (Long *et al.* 2011).

If zoos and aquariums are to become undisputed conservation organisations it is imperative that institutions utilise their limited space and resources appropriately and develop rational and systematic criteria for the identification of species to be selected for *ex situ* conservation efforts and management (Balmford *et al.* 1996; Hutchins *et al.* 1995). The argument that there are immovable visitor preferences and that zoos must exhibit large vertebrates in order to attract visitors and remain economically viable has yet to be systematically evaluated (Bowkett 2014; Fa *et al.* 2014). Work to date has yet to firmly link collection composition to attendance worldwide, with studies often being limited by the species assessed and being institution or country specific, while information on correlates of conservation outcomes, such as *in situ* contributions is almost non-existent, inhibiting informed collection planning decisions and policy formation (Carr 2016; Whitworth 2012). Globally studies have only assessed the role of socio-economic variables. Although informative, these bivariate relationships fail to capture the complexity of direct and indirect drivers of visitor attendance and have failed to account for the composition of individual collections and how they relate to institutional visitor attendance. This necessitates an integrated assessment of how collection composition and socio-economic variables affect both institutional visitor attendance and *in situ* conservation contributions. If by housing large vertebrates zoos and aquariums can contribute more to *in situ* conservation, protecting not only individual species but their extended habitats, then their presence in collections at the exclusion of other, smaller-bodied species may represent an optimal conservation strategy.

1.4 *Ex Situ* Population Sustainability

Zoos and aquariums require a diverse selection of species within their collections to achieve their goals and objectives, from threatened species where zoos and aquariums play a direct role in their conservation and recovery through captive breeding programmes, to large, charismatic species which increase visitor attendance and generate funds for *in situ* conservation (Powell *et al.* 2019). Regardless of the individual species maintained, the ability of zoos and aquariums to fulfil these goals relies on the long-term sustainability of *ex situ* populations. *Ex situ* population management originally aimed to maintain 90% of the genetic variation within a population over a 200 year period (Soulé *et al.* 1986), however this was subsequently revised to retain 90% of genetic variation over a 100 year period to reflect the economic resources and species holding capacities of zoological institutions (Foose *et al.* 1995). Despite these revisions, Lees and Wilcken (2009), and subsequent regional analyses, have all revealed that the population sizes necessary to maintain long-term genetically and demographically sustainable populations are currently not being met (Hibbard *et al.* 2011; Leus *et al.* 2011; Long *et al.* 2011).

The majority of managed *ex situ* populations contain less than 100 individuals and if zoos and aquariums are to increase their managed *ex situ* population sizes to those deemed necessary for self-sustainability by Lees and Wilcken (2009), they would have to create an additional >100,000 spaces within their collections (Powell *et al.* 2019). In reality, the sustainability of *ex situ* populations is substantially hindered by small population sizes, low genetic diversity, limited reproductive success, inadequate population management and husbandry, limited *ex situ* species holding capacity, poor fulfilment of breeding and transfer recommendations and logistical constraints (Che-Castaldo *et al.* 2019; Macek 2014; Wilson *et al.* 2019). Unless drastic changes to collection planning and management practices are implemented, the vast majority of *ex situ* populations will not persist in the long-term, even for the most charismatic of species (Kaufman 2012; Powell *et al.* 2019).

Despite their popularity with the public and their almost ubiquity across zoological collections, the sustainability of *ex situ* flamingo (*Phoenicopteridae*) populations has been a particular concern among population managers, primarily due to their demonstrably poor rates of reproductive success (Brown and King 2005; King 2000; Shannon 2000; Stevens *et al.* 1992; Whitfield 2002). This has created a deficit in the number of captive flamingos, with all species still relying on the periodic importation of wild-caught individuals and institutions consistently stating that they would like to hold more flamingos than are currently available (King and Bračko 2014). As a result, increased knowledge surrounding the basic reproductive biology of all flamingo species and improved management practices are necessary if *ex situ* flamingo populations are to become self-sustaining (Johnson and Cézilly 2008; King 1994; Sandri *et al.* 2018). Flock size has already been identified as a key determinant of reproductive success, with larger flocks demonstrating higher reproductive output (Farrell *et al.* 2000; King 2008; King and Bračko 2014; Pickering *et al.* 1992; Sandri *et al.* 2018; Stevens and Pickett 1994; Stevens 1991). In a bid to increase population sizes and sustainability it has been universally recommended that flamingos be housed in minimum flock sizes of 20 birds and ideally in flocks of >40 birds to achieve a reasonable chance of reproductive success (Brown and King 2005). Although practical, these guidelines are generated from a very limited body of knowledge and fail to consider, or are unable to separate, the effects of species-specific differences in reproductive behaviour, flock sex ratio and environmental conditions, making it almost impossible to draw general conclusions from the research to date (Pickering *et al.* 1992; Rose *et al.* 2014; Stevens and Pickett 1994; Stevens 1991). As a result, many questions remain unanswered and the universal implementation of management decisions may be premature, potentially hindering the sustainable development of *ex situ* flamingo populations.

In order to successfully manage *ex situ* flamingo populations and ensure their long-term sustainability, a species-specific identification of the optimal flock size and composition necessary for reliable reproductive success is urgently needed, with the subsequent tailoring of management recommendations accordingly (King and Bračko 2014; Rose *et al.* 2016; Sandri *et al.* 2018; Stevens and Pickett 1994). This requires longitudinal, multi-institutional and multi-species studies to provide a more complete understanding of the factors involved in enhancing the reproductive success of *ex situ* flamingo populations (Rose *et al.* 2014). Globally shared zoological records, such as those currently contained within Species360's Zoological Information Management System (ZIMS), represent a unique resource to investigate the relationship between flock size and structure on reproductive success on a global scale, spanning latitudinal and climatic gradients. ZIMS is the largest real-time database of comprehensive and standardised zoological information encompassing more than 1,100 zoological collections globally, including detailed *ex situ* records for more than 22,000 species and 10 million individual animals, living and historic (Species360 2019; ZIMS 2019). A preliminary analysis of 167 Species360 member institutions in 2013 showed that for Chilean flamingos (*Phoenicopterus chilensis*), flocks consisting of over 100 birds had the highest probability of reproductive success (Teare 2014). This single year study demonstrates the conservation potential and management information that can be generated from globally shared zoological records and provides a foundation for further research endeavours. If utilised appropriately, globally shared zoological records have the potential to fundamentally alter *ex situ* population management and conservation for thousands of species, filling significant demographic knowledge gaps which can further support *in situ* conservation efforts (Conde *et al.* 2019).

1.5 *Ex Situ* Contributions to Biobanking

With the increasing realisation that zoos and aquariums are unable to maintain genetically and demographically sustainable living *ex situ* populations, there is increasing relevance and importance to the emerging role of zoos and aquariums to contribute to the cryogenic preservation of biological material in gene banks (termed 'biobanking') to preserve genetic variation and ensure population sustainability (Ballou 1992; Hobbs *et al.* 2018; Powell *et al.* 2019). A gene bank is a collection of cryopreserved biological material, which can consist of living cell cultures, gametes, tissues samples, embryos, feathers etc., established to conserve the genetic diversity within a population of plants or animals (Hobbs *et al.* 2018; Wildt 1997). Cryopreserved living cells in particular represent a unique and expandable resource that can be continually thawed, grown and a portion re-banked for future use (Ryder and Onuma 2018). When utilised in conjunction with cell-based human intervention and assisted reproductive technologies, cryopreserved living cells and gametes have the potential to preserve and reintroduce genetic variation into future populations, extending the reproductive lifetime of individuals almost indefinitely, maintaining a species' adaptability potential and allowing more species to be maintained *ex situ* (Ballou 1992; Critser and Russell 2000; Hobbs *et al.* 2018; Praxedes *et al.* 2018). Furthermore, the potential to introduce genetic variation into wild populations using the same methodology and genetic rescue theory provides one method to help restore *in situ* population health and viability (Staerk *et al.* 2018; Whiteley *et al.* 2015). Therefore, the long-term cryopreservation of living cell cultures represents a unique and almost unparalleled resource for future *ex situ* population management and global biodiversity conservation (Ballou 1992).

The cryopreservation of living cell cultures and germplasm can be used to restore genetic diversity and population health of small and fragmented *in situ* populations, which often suffer from low genetic diversity and the resulting negative effects of inbreeding, reducing their adaptability potential (CBSG 2015; CPSG 2016; Ryder and Onuma 2018; Whiteley *et al.* 2015). This is referred to as genetic rescue and has been proven to be successful across various taxa (Johnson *et al.* 2010; Pimm *et al.* 2006; Sunquist and Sunquist 2001; Whiteley *et al.* 2015), including the black-footed ferret (*Mustela nigripes*), where cryopreserved gametes were used in conjunction with assisted reproductive technologies to reintroduce lost genetic variation, via viable offspring, into the extant population, increasing the population's genetic diversity and ultimately contributing to the species' recovery (Howard *et al.* 2016). Similarly, living somatic cell cultures can be used to conduct somatic cell nuclear transfer (SCNT) or 'cloning' of threatened species, as already demonstrated in the endangered gaur (*Bos gaurus*) and banteng (*Bos javanicus*) (Hobbs *et al.* 2018; Peterson 2016; Ryder and Onuma 2018; Secher *et al.* 2017). Combined with the utilisation of gestational and ova surrogates, SCNT can in theory be used to resurrect species from extinction, providing living somatic cell cultures also exist. In 2003, SCNT was conducted using a cryopreserved living cell culture from the last living Pyrenean ibex (*Capra pyrenaica pyrenaica*) which had died in 2000, although the individual did not survive the immediate postpartum period, it's birth marked the first de-extinction event in history and highlighted the power of cryopreserved living cell cultures and biobanking in biodiversity conservation (Folch *et al.* 2009). This method of conservation shows exceptional promise if planned and used appropriately, and is particularly useful where conventional means of species recovery, such as live animal transfers, are not practical or possible.

Despite the conservation potential of biobanking, its continued development is hindered by a lack of standardised information surrounding the species currently represented within global gene bank collections, the selection and prioritisation of species for future genetic sample collection and the availability of samples for future collection; issues which have been raised by conservation practitioners at both the 2015 and 2016 IUCN Conservation Planning Specialist Group (IUCN CPSG) Annual Meetings (CBSG 2015; Clarke 2009; CPSG 2016; Hobbs *et al.* 2018; Ryder *et al.* 2000; Staerk *et al.* 2018). To date, sample collection has been predominantly opportunistic and the only coordinated attempt to characterise the biodiversity represented within existing collections has been an assessment of 67 "*Critically Endangered*" or "*Extinct in the Wild*" species within five biobanking facilities (Hobbs *et al.* 2018; Ryder and Onuma 2018). Although the function and objectives of individual gene banks may vary, if biobanking is to be considered a viable and respected conservation tool moving forward it is imperative that the way in which species are selected for sampling follow a clear and transparent methodology to ensure that important conservation opportunities are not being overlooked (Hobbs *et al.* 2018).

This lack of existing gene bank information and ad hoc approach to species sample collection warrants a holistic approach to the prioritisation of species for future sample collection, incorporating the probability of species extinction, the probability of sample acquisition and the probability of successful implementation in the future. Existing prioritisation schemes have been put forward as one method to identify species with heightened probabilities of extinction for *ex situ* conservation efforts (da Silva *et al.* 2019), and the incorporation of a species' IUCN Red List status and its representation under various other conservation prioritisation schemes, such as the Convention on International Trade of Endangered Species of Fauna and Flora (CITES), could provide one method to identify priority species. Similarly, with their diverse living collections and ease of access, zoos and aquariums have a unique opportunity to contribute to biobanking and the preservation of genetic diversity (Benirschke 1984; Praxedes *et al.* 2018). Approximately 15% of all threatened terrestrial vertebrate species are currently managed *ex situ* and the availability of globally shared zoological records in ZIMS could provide one mechanism of identifying suitable sample collection opportunities from within the global zoo and aquarium community (Conde *et al.* 2011). Although not a replacement for conventional conservation practices, biobanking has the potential to radically alter species conservation, through the addition of genetic diversity into existing populations or potentially even the reversal of species extinctions. However, in order to achieve these goals, suitable samples must be obtained and maintained in perpetuity, and for species which are never sampled, these opportunities will never be realised.

1.6 *Ex Situ* Contributions to De-Extinction

Despite the best efforts of *in situ* and *ex situ* conservation practitioners, the rate of human-induced species extinctions has increased in frequency over the last century, with predicted future accelerations in extinction rates almost guaranteed (Butchart *et al.* 2010; Ceballos *et al.* 2015; Ceballos *et al.* 2020). However, contemporary developments in ancient DNA sequencing, advanced reproductive technologies and genome engineering, such as CRISPR-Cas9, mean that extinction may not necessarily be forever (Doudna and Charpentier 2014; Folch *et al.* 2009). The resurrection of once-extinct species, termed 'de-extinction', and subsequent reintroduction into the wild, has the potential to not only reverse global biodiversity loss, but also to restore ecosystem health and stability, providing resilience to future disturbances (Cardinale *et al.* 2012; Shapiro 2017).

Currently, de-extinction can be achieved through three fundamental pathways: back-breeding, cloning and genome engineering, each of which rely on the availability of suitable *ex situ* samples, genomic information and extant close relatives (Shapiro 2015a). This can be seen from the resurrection of the Pyrenean ibex (*Capra pyrenaica pyrenaica*) through cloning (SCNT), which was only made possible due to the availability of cryopreserved *ex situ* living cell cultures and extant close relatives to act as gestational and ova surrogates (Folch *et al.* 2009). Unfortunately, the vast majority of extinct species do not have cryopreserved living cell cultures available and therefore SCNT is not an option, highlighting the importance of collecting living cell cultures of currently threatened species before they go extinct (Shapiro 2017). Similarly, back-breeding, the selective breeding of extant individuals to resurrect specific ancestral traits within the living population, relies upon both the availability of extant relatives and the persistence of target ancestral traits in the living population (Shapiro 2017). Although this has produced ecological proxies of extinct species, such as Heck cattle which today fill the ecological niche of the extinct aurochs (*Bos taurus primigenius*), back-breeding is typically seen as a ‘phenotypic’ de-extinction rather than a true de-extinction, restoring extinct phenotypes and ecological roles regardless of genetic considerations (Heck 1951; Shapiro 2015a). In contrast, genome engineering alters the genome of an extant species, in cells *in vitro*, to incorporate and express genes from an extinct species. Although genome engineering does not require living cell cultures, it still requires at least partial genomic information from the extinct species and extant close relatives for genome altering and surrogate purposes (Shapiro 2015b). Despite the vast genome alterations necessary, genome engineering projects are currently underway to resurrect both the woolly mammoth (*Mammuthus primigenius*) and passenger pigeon (*Ectopistes migratorius*) using their extant close relatives the Asian elephant (*Elephas maximus*) and band-tailed pigeon (*Patagioenas fasciata*) (Callaway 2015; Shapiro 2015a).

Similar to the existing taxonomic biases in conservation management and ecological research, charismatic vertebrates such as the woolly mammoth and thylacine (*Thylacinus cynocephalus*) have been the focus of nearly all de-extinction discussion and research to date, with almost no consideration for the potential benefits of resurrecting extinct plant species (IUCN SSC 2016; Jones 2014; Martinelli *et al.* 2014; McCauley *et al.* 2017). Considering the reduced legal, ethical and welfare considerations when considering de-extinction in plants compared to vertebrates and the relative ease of plant cloning, it is surprising that plants have been excluded from this discussion, particularly given their role in ecosystem functioning and their importance in ensuring food security (Martinelli *et al.* 2014; Sherkow and Greely 2013; Turner 2017). In fact, plants are almost conspicuous in their absence from the discussion surrounding de-extinction, with the American chestnut (*Castanea dentata*) being the sole representative of the Kingdom mentioned in the literature, a species which never actually became extinct (Jones 2014; Martinelli *et al.* 2014; McCauley *et al.* 2017; Ogden 2014; Shapiro 2015a). In contrast to vertebrates, the resurrection of extinct plant species can be much more straightforward if suitable seed and herbarium specimens exist, as shown by Godefroid *et al.* (2011) who used 144-year-old herbarium seed samples to germinate the locally extinct *Bupleurum tenuissimum*. Similar to animals, *ex situ* plant conservation is undertaken by the approximately 3,500 botanic gardens, of which 350 are also involved in global seed banking efforts (O'Donnell and Sharrock 2017). Collectively, these institutions contain an immense repository of not only cryopreserved viable seed samples, but also herbarium specimens capable of producing high quality DNA sequences and seeds capable of germination, representing an untapped resource for plant de-extinction efforts (Abeli *et al.* 2020; Godefroid *et al.* 2011). Although vertebrate de-extinction has helped to capture the imagination and attention of the general public, this taxonomic bias and disregard for the potential benefits of plant de-extinction has also created a vertebrate-centric view of de-extinction and the technology required to achieve it, resulting in missed opportunities to reverse global biodiversity loss and restore lost ecosystem services.

Addressing this taxonomic bias, and the prioritisation of plant species for de-extinction research, is critical to the development of de-extinction as a legitimate conservation tool, ensuring that opportunities for de-extinction are not being overlooked and that resources are being allocated appropriately. This requires an integrated assessment of intrinsic and extrinsic species values, the probability of project success, the availability of suitable *ex situ* samples and the associated costs involved. Over 387 million plant specimens are preserved in the approximately 3,000 herbaria globally, all of which have the potential to provide not only DNA sequences for genome engineering, but also seed samples capable of germination, however an assessment of the extinct plant species represented within herbaria and seed banks has yet to be conducted (Abeli *et al*. 2020). In addition, the IUCN Red List of Threatened Species provides species-specific threat and habitat assessments for not only threatened species, but also recently extinct species (IUCN 2018). When combined with known *ex situ* sample availability, this provides a more holistic approach to de-extinction candidate selection, avoiding the selection of species with limited possibilities of successful de-extinction and subsequent reintroduction. De-extinction is similar to genetic rescue in that it cannot replace conventional conservation practices, particularly for long-extinct species with limited or no samples available. However, for species which have recently gone extinct and for species which are likely to become extinct in the near future, de-extinction offers the only potential mechanism to resurrect species and restore their ecosystem functions, reversing global biodiversity loss and enhancing ecosystem stability (Seddon *et al.* 2014; Shapiro 2017).

1.7 Thesis structure

This thesis explores the multi-faceted contribution of *ex situ* collections to global biodiversity conservation, both *in situ* and *ex situ*. Utilising globally shared zoological and botanical records I investigate the current contributions of *ex situ* collections to biodiversity conservation and highlight their future potential in four broad areas:

1) **Collection composition**: an integrated assessment of the collection composition and socio-economic factors influencing both institutional attendance and institutional *in situ* contributions for over 450 zoological institutions globally. This allowed the identification of the institutional and species characteristics associated with increased *in situ* contributions, providing guidance for collection planners moving forward to increase institutional conservation investment.
2) **Population sustainability:** an investigation into the influence of flock size and structure on reproductive success in captive flamingo flocks globally, while also accounting for climatic variability and temporal trends in institutional flock sizes and reproductive output. I provide species-specific management recommendations to help improve the sustainability of *ex situ* flamingo populations.
3) **Biobanking:** an assessment of the current representation of species within the Frozen Zoo® collection of living cell cultures housed at the San Diego Zoo Institute for Conservation Research. This allowed me to identify gaps within the collection and prioritise species for future genetic sample collection, highlighting their congruency with global wildlife conservation prioritisation schemes and identifying opportunities for sample collection from within the global zoo and aquarium community.
4) **De-extinction**: a conservation project efficiency approach to the prioritisation of recently extinct plants for future de-extinction research efforts, incorporating intrinsic and extrinsic species values, while also considering the probability of project success, through an identification of suitable herbarium and *ex situ* samples for potential future de-extinction efforts, and key habitat and threat information.

1.7.1 Chapter 2: A system wide approach to managing zoo collections for visitor attendance and *in situ* conservation

Zoo collections reflect a compromise between conservation, science, education and entertainment objectives. The persistent belief that large, charismatic vertebrates are necessary to attract visitors may lead to a trade-off between maximizing visitor attendance and conservation objectives due to the resource and welfare requirements needed to maintain them. Conversely, the 'flagship' approach suggests that a focus on large mammals could be an effective conservation strategy, as a driver of fundraising for *in situ* conservation projects. Here I used a global dataset of over 450 zoo collections to develop a model of how zoo species composition and socio-economic factors influence visitor attendance and *in situ* conservation project contributions, both directly and indirectly, to test for the first time whether zoos must exhibit large vertebrates to attract visitors and fund *in situ* conservation. I show that zoos with many animals, large animals, with high mammal species richness and which are dissimilar to other zoos achieve higher numbers of visitors and contribute to more *in situ* conservation projects; however, a strong trade-off between number of animals and mean species body mass indicates that alternative composition strategies, such as many small animals, may also be effective. My results demonstrate the key role of large vertebrates in promoting both visitor attendance and *in situ* conservation investment, but also highlight that they are only one part of a complex system of determinants. This evidence-based work can help guide future collection planning processes, increasing both institutional visitor attendance and *in situ* conservation contributions, helping to limit global biodiversity loss.

1.7.2 Chapter 3: Flock size and structure influence reproductive success in four species of flamingo in 753 captive populations worldwide

Consistent with the majority of managed *ex situ* populations, *ex situ* flamingo (*Phoenicopteridae*) populations are not meeting necessary sustainability criteria and are thus not viable long-term, stemming from their poor reproductive success. Previous work has identified both flock size and environmental suitability as key determinants of *ex situ* reproductive success, but has failed to consider species-specific differences in reproductive behaviour and is often limited by the number of institutions, species and geographic regions assessed, hindering the identification of species-specific requirements and the associated management recommendations necessary to improve the sustainability of *ex situ* flamingo populations. Here I combined current and historic zoological records from the Zoological Information Management System with high resolution global climatic data to model under a common framework how flock size and structure influence reproductive success in *ex situ* flamingo populations between 1990 and 2018. I assess four of the six extant species of flamingo (*Phoenicopterus chilensis*, *Phoeniconaias minor, Phoenicopterus roseus* and *Phoenicopterus ruber*), while simultaneously investigating temporal trends in institutional flock sizes and the reproductive seasonality of *ex situ* flamingo populations. I find that flock size has a strong positive effect on the probability of reproduction and the predicted number of chicks per flock in all species of flamingo, with flock sizes of 69 – 127 necessary to achieve a 50% probability of reproduction. I further show that a balanced sex ratio and the introduction of new individuals to a flock both increase reproductive success. Contrary to expectations, I reveal that climatic variables play a limited role in determining *ex situ* flamingo reproductive success. For the first time, I provide species-specific management recommendations to increase the reproductive success of global *ex situ* flamingo populations, highlighting the importance of both individual institutions and regional associations. This family-specific analysis demonstrates the management information and conservation potential of globally shared zoological records and I encourage the continued sharing and standardisation of records to promote both *ex situ* population sustainability and biodiversity conservation.

1.7.3 Chapter 4: Maximising the potential for living cell banks to contribute to global conservation priorities

The lack of information surrounding the species currently represented within global gene bank collections and the inconsistent selection of species for future sample collection both significantly hinder the development of biobanking as an effective conservation tool, likely resulting in missed conservation opportunities and enhancing extinction risks. Here I investigated the representation of amphibians, birds, mammals and reptiles within the Frozen Zoo® collection of living cell cultures housed at the San Diego Zoo Institute for Conservation Research and their congruency with global wildlife conservation prioritisation schemes, including the IUCN Red List, CITES and the Alliance for Zero Extinction (AZE). I identified gaps within the collection and prioritised for genetic sample collection the 5,799 unsampled "*Threatened*" species under the IUCN Red List, assessing their probability of extinction through their overlap with other conservation prioritisation schemes, and the probability of sample acquisition from within the global zoo and aquarium community. I find that 965 species and 5.1% of all "*Threatened*" amphibians, birds, mammals and reptiles are currently represented within the San Diego Zoo Frozen Zoo® and that sampling from within existing zoo and aquarium collections can increase this representation to 16.5%. I identify three species for priority sample collection which are listed under every prioritisation scheme and which are also present within the global zoo and aquarium community, including the Crested Ibis (*Nipponia nippon*), and five species listed in every prioritisation scheme, but not found within the global zoo and aquarium community, including the Christmas Frigatebird (*Fregata andrewsi*). Priority species based on subsets of prioritisation schemes are also provided. I reveal the potential of zoos and aquariums to contribute to global biobanking efforts and emphasise the need for increased global collaboration and encourage the formation of a global database of gene banks. I highlight the difficulties in obtaining living cell cultures *in situ* and the associated concerns over intellectual property rights and genetic resource sovereignty, encouraging the establishment of new gene banks in biodiversity rich countries.

1.7.4 Chapter 5: A conservation project efficiency approach to the prioritisation of plants for de-extinction research

The current taxonomic bias in de-extinction research towards charismatic vertebrates, such as the woolly mammoth (*Mammuthus primigenius*) and great auk (*Pinguinus impennis*), has resulted in a biased view of de-extinction as a whole and the technology required to achieve it. The conspicuous absence of plants form the de-extinction literature is surprising given the relative ease of plant cloning, their role in ecosystem functioning, their agricultural importance and the reduced ethical and welfare concerns associated with them compared to vertebrate species. Here I implemented an estimated conservation project efficiency ranking framework to prioritise for de-extinction research the 122 species of plant listed as "*Extinct*" under the IUCN Red List. This framework incorporated intrinsic and extrinsic species values (such as economic, medicinal and scientific value), while also considering indicators for the probability of project success and associated costs through an identification of available herbarium specimens, *ex situ* samples, extant relatives and key habitat and threat information. I find that nearly all extinct species have known herbarium specimens (114/122 species) and extant relatives (117/122 species), however none are represented within any of the seed or germplasm banks assessed. In addition, I reveal that 19/122 species have already been rediscovered, however only six species have had their IUCN Red List status updated to reflect this new information. Four of the top five ranking species have been rediscovered (*Melicope paniculata*, *Melicope cruciata, Astragalus nitidiflorus* and *Madhuca insignis*), leaving *Delissea subcordata* as the highest-ranking candidate species for de-extinction research. Although last recorded in 1934, this Hawaiian lobelioid has numerous herbarium specimens, published DNA sequences and extant genus-specific relatives available, increasing the probability of project success and decreasing estimated project costs. I highlight a lack of available quantitative data for extinct plant species, particularly with regards to their ecological role, interspecific interactions, historical threats and current habitat suitability. I strongly encourage seed and tissue collection from rediscovered species and more detailed IUCN Red List assessments for all species.

1.7.5 Chapter 6: Discussion

The final chapter of this thesis draws general conclusions from the work presented within the thesis, discusses the overall consequences and implications for global *ex situ* management and conservation efforts and the importance and potential of globally shared zoological and botanical records. I highlight the need for improved record sharing between non-Species360 member institutions and outline future research avenues to enhance the conservation potential of the *ex situ* community.

1.8 Additional work

In addition to the chapters presented within this thesis, the PhD process also provided me with the opportunity to collaborate with several other researchers and contribute to additional inter-disciplinary research projects. Publications resulting from these contributions and collaborations are listed below:

1.8.1 Helminth Infections of European badgers (*Meles meles*) in Ireland

I led the data analysis and provided feedback on drafts for two collaborative projects led by Rachel Louise Byrne then of Trinity College Dublin, and currently Liverpool School of Tropical Medicine. These projects discuss the diagnostic techniques and helminth parasite communities of European badgers (*Meles meles*) in Ireland.

Byrne, R. L., Fogarty, U., Mooney, A., Harris, E., Good, M., Marples, N. M., and Holland, C. V. (2020). The helminth parasite community of European badgers (*Meles meles*) in Ireland. *Journal of Helminthology* **94**. doi:10.1017/S0022149X19000051

Byrne, R. L., Fogarty, U., Mooney, A., Marples, N. M., and Holland, C. V. (2018). A comparison of helminth infections as assessed through coprological analysis and adult worm burdens in a wild host. *International Journal for Parasitology: Parasites and Wildlife* **7**. doi:10.1016/j.ijppaw.2018.11.003

1.8.2 Mammalian Demographic Responses to Climate Change

I was involved in the data collection and provided feedback on drafts of a project investigating the demographic responses of mammals to predicted changes in climate. This manuscript is currently under review.

Paniw, M. *et al.* (Under Review). Global analysis reveals complex demographic responses of mammals to climate change.

2. A system wide approach to managing zoo collections for visitor attendance and *in situ* conservation

Authors: Andrew Mooney, Dalia A. Conde, Kevin Healy and Yvonne M. Buckley

Author contributions: All authors developed the concept of the manuscript. A.M., D.A.C. and K.H. collected the data. A.M. undertook the analysis and drafted the text in consultation with Y.M.B. A.M. produced the tables and figures. All authors contributed to the writing of the manuscript.

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2.1 Abstract

Zoos contribute substantial resources to *in situ* conservation projects in natural habitats using revenue from visitor attendance, as well as other sources. I use a global dataset of over 450 zoos to develop a model of how zoo composition and socio-economic factors directly and indirectly influence visitor attendance and *in situ* project activity. I find that zoos with many animals, large animals, high species richness (particularly of mammals), and which are dissimilar to other zoos achieve higher numbers of visitors and contribute to more *in situ* conservation projects. However, the model strongly supports a trade-off between number of animals and body mass indicating that alternative composition strategies, such as having many small animals, may also be effective. The evidence-base presented here can be used to help guide collection planning processes and increase the *in situ* contributions from zoos, helping to reduce global biodiversity loss.

2.2 Introduction

Modern zoos contribute to the recovery and conservation of threatened species through *ex situ* breeding within institutions (Conde *et al.* 2011) and through substantial contributions to *in situ* conservation projects in natural habitats (Gusset and Dick 2011). In order to fulfil their multiple roles, zoo collections must attract recreational visitors (Turley 1999) and perceived visitor preferences have fuelled the belief that large vertebrates, particularly mammals, are necessary in order to attract visitors (Martin *et al.* 2014). However, compared to smaller species, large animals are often costlier to maintain, prove more difficult to breed in captivity, require larger enclosure sizes (Balmford *et al.* 1996) and raise ethical and welfare issues (Young 2015). As the global zoo community has a limited capacity (IUDZG/IUCN SSC 1993), zoos have been encouraged through conservation objectives to shift their focus towards smaller-bodied species (particularly amphibians, invertebrates and fish), native species, threatened species and specialise on fewer species (Keulartz 2015; Palmer *et al.* 2018). However, this compositional shift could result in reduced visitor attendance, lowering economic return and consequently *in situ* conservation investment (Carr 2016; Kawata 2013).

The global zoo and aquarium community fulfils several objectives, including conservation, education, research and entertainment (Turley 1999). These multiple roles can place competing demands on the composition of zoo collections as public preferences do not always align with conservation priorities. Collectively, the global zoo and aquarium community attracts more than 700 million visitors every year and invests more than $350 million in wildlife conservation *in situ*, representing the third largest conservation organisation contributor globally (Gusset and Dick 2011). These *in situ* conservation activities are primarily funded by paying visitors, in conjunction with other sources, and the popularity of institutional collections (in terms of the species within the collection) is positively correlated with attendance (Whitworth 2012). There is evidence for the flagship approach of using popular, large vertebrates in zoo collections to drive public education and *in situ* conservation fundraising (Hutchins *et al.* 1995), helping to protect other species and habitats (Bowkett 2014; Conway 2011). However to my knowledge, work to date has yet to unequivocally link collection species composition to attendance worldwide, with most studies limited by the range of species, institutions and countries assessed (Carr 2016; Whitworth 2012). Socio-economic variables also drive attendance (Davey 2007) but the relative influence of socio-economic and collection composition variables on attendance has not been assessed. While the direct effects of various factors on attendance have been the focus of previous studies, such approaches fail to capture the complexity of potential indirect drivers of, and trade-offs for, visitor attendance. A framework linking the direct and indirect effects of collection composition variables on conservation outcomes, such as *in situ* contributions, would allow for more informed collection planning decisions and policy formation.

I test whether collection composition and socio-economic variables affect both institutional attendance (458 zoos worldwide) and *in situ* contributions (subset of 119 zoos). I use Structural Equation Modelling (SEM) to test the determinants of both visitor attendance and *in situ* conservation contributions as part of a system of species and zoo characteristics and broader socio-economic variables (Table 2.1). I use vertebrate composition data from Species360 member institutions, in conjunction with attendance information from the International Zoo Yearbook and *in situ* project contribution reports from the Association of Zoos and Aquariums (AZA) (see 2.3 Methods). I find that zoos with many animals, large animals, high species richness (particularly of mammals), and which are dissimilar to other zoos achieve higher numbers of visitors and contribute to more *in situ* conservation projects. However, the model strongly supports a trade-off between number of animals and body mass indicating that alternative composition strategies, such as having many small animals, may also be effective.

Table 2.1 | Description of the variables used within the Structural Equation Models. *Weighted for species abundance per institution.

Variable	**Description**
Attendance	Annual institution attendance (2015)
Species Richness	Total number of species per institution (2017)
Total Animals	Total number of individual animals per institution (2017)
Mammal Species Richness	Total number of mammalian species per institution (2017)
Institution Area	Institutional area in hectares (ha) (2015)
Threatened Species Proportion*	The proportion of IUCN Red List of Threatened Species "*Threatened*"' species (CR, EN, VU) per institution (2017)
Mean Species Body Mass*	The mean species body mass (g) per institution (2017)
Diversity	Brillouin index measure of within collection diversity (alpha diversity) (2017)
Dissimilarity	The mean Raup-Crick Dissimilarity Index per institution, measuring compositional dissimilarity between collections (2017)
GDP	Gross Domestic Product (US$) (2015)
National Population Size	National population size for each country (2015)
10 km Population	Estimated population count within a 10 km radius of the institution (2015)
In Situ Contributions	The annual number of field conservation programmes in which individual AZA member institutions contribute to in some capacity (2015)

2.3 Methods

2.3.1 *A priori* Meta-Model

Structural Equation Modelling (SEM) integrates multivariate relationships, testing both direct and indirect effects within a system (Fan *et al.* 2016). SEM requires a strong theoretical and empirical knowledge of the study system to guide model specification and modification (Fan *et al.* 2016). I conducted a literature review of the relationships between institutional attendance, zoo species composition and *in situ* contributions. Based on this prior theoretical knowledge and proposed causal relationships I developed a hypothetical *a priori* meta-model (Grace *et al.* 2010; Grace *et al.* 2016; Appendix Figure A.1). This meta-model represents general relationships between multiple variables, while omitting statistical details (Grace *et al.* 2010). A thorough description of both the prior theoretical knowledge and proposed causal relationships used to generate the *a priori* meta-model depicted in Appendix Figure A.1 are explained in Appendix Note A.1. This hypothesised causal diagram was combined with available data to test the effects of species body mass on institutional attendance in the context of institutional compositional characteristics and socio-economic variables.

2.3.2 Data

Annual attendance figures and institutional area were obtained from the International Zoo Yearbook (IZY/ZSL 2016). In the absence of available revenue data, I use visitor attendance as a proxy of income to potentially fund *in situ* activities. Institutional vertebrate species holdings (mammalian, avian, reptilian and amphibian) were obtained from Species360 (Species360 2019). Species360 is an international non-profit organisation that hosts and develops the Zoological Information Management System (ZIMS), the largest database of comprehensive and standardised information on more than 1,100 zoo and aquarium collections globally. IZY and Species360 member institutions were cross-referenced and Theme Parks, Aquariums and Conservation/Science Centres removed to prevent potential biases, resulting in a sample size of 458 institutions in 58 countries (Appendix Figure A.2). Safari parks and similar drive-through animal parks were treated the same as other institutions.

Both the IZY and ZIMS databases are based on submitted records from individual institutions. While these databases have not been subjected to editorial verification, potentially permitting differences in attendance calculations (e.g. exclusion of annual pass holders) or failure to update species holdings, they represent the only global databases of zoo attendance figures (IZY/ZSL 2016) and collection composition records (ZIMS). As a result, ZIMS is used by the IUCN, Convention on International Trade in Endangered Species (CITES), the Wildlife Trade Monitoring Network (TRAFFIC), United States Fish and Wildlife Service (USFWS) and Department for Environment, Food and Rural Affairs (DEFRA) (Species360 2019).

Taxonomy and the status on the IUCN Red List of Threatened Species were standardised for the 4,822 vertebrate species present using the *'taxize'* package (version 0.9.5; Chamberlain and Szöcs 2013) in the statistical programme R (version 3.4.3; R Core Team 2017). Species richness, number of animals, taxonomic and IUCN Red List status representation, and both alpha and beta diversity indices were calculated using data from ZIMS species holdings (see Table 2.1 for variables list).

Species body mass was obtained from the Species Knowledge Index (Conde *et al.* 2019), which standardises data across 22 different global demographic databases. Species-level body mass information was available for 4,214 species. Body mass for the remaining 608 species was inferred at the Genus, Family or Order level using the same datasets. This allowed the mean species body mass of each institution to be calculated as shown in Equation 2.1.

$$\bar{M} = \frac{\sum_{i=1}^{n} x_i m_i}{\sum_{i=1}^{n} x_i} \qquad \text{Equation 2.1}$$

Where $\bar{M}$ is the mean abundance weighted species body mass per institution, x_i is the number of individuals of species i, m_i is the body mass of species i where i goes from 1 to n species per institution.

To assess socio-economic factors, I used gross domestic product (GDP) and national population size for each country (World Bank 2017). Institutional GPS co-ordinates were used to calculate total population sizes within 10-kilometer radii in ArcGIS using estimated global population counts (CIESIN 2017).

In order to assess the *in situ* contributions of individual institutions the Association of Zoos and Aquariums (AZA) Annual Report on Conservation and Science was consulted (AZA 2016a). This provided the number of field conservation programmes in which AZA member institutions were involved in 2015. When cross-referenced with IZY and Species360 members, this provided a sample size of 119 institutions across four countries for which I could analyse *in situ* contributions. The number of projects, as a measure of *in situ* conservation contributions, does not provide further resolution on the form the contribution takes (e.g. financial, expertise, resources, animals, training etc.). However, a separate analysis of the relationship between the number of *in situ* projects supported and the total *in situ* financial investment per institution was conducted on anonymised data from 83 individual British and Irish Association of Zoos and Aquariums (BIAZA) institutions. These data show a clear positive relationship between the number of *in situ* projects supported and total *in situ* financial expenditure. As this data-set was anonymised, I was unable to include it in my integrated model; however, these data are shown in Appendix Figure A.5 and support my assumption that the number of *in situ* projects is a meaningful proxy for the total *in situ* financial investment per institution.

2.3.3 Analyses

Two distinct SEM frameworks were tested, the Attendance model and the *In Situ* model. The Attendance model tested the relationship between visitor attendance and all the various specified variables for 458 institutions globally. This model did not include any *in situ* contribution data. The *In Situ* model tested the relationship between visitor attendance, *in situ* contributions and all the various specified variables for a subset of 119 institutions in North America for which *in situ* contribution data were available. The results of the Attendance model were used to guide the development of the Attendance linked pathways in the *In Situ* model as the larger sample size of the Attendance model had higher power. The results of the Attendance model are combined with the results of the *In Situ* Model in Figure 2.3, with a yellow box delineating the boundary of the two models. Only the additional *in situ* pathways of the *In Situ* model are reported, as all other relationships were derived from the Attendance model due to its higher statistical power.

All analyses were carried out using the R programme (version 3.4.3) and the packages '*lavaan*' (version 0.5-23.1097; Rosseel 2012) and '*lavaan.survey'* (version 1.1.3.1; Oberski 2014) for structural equation modelling. All variables were both mean centred and expressed in units of standard deviation to allow direct comparisons of effect sizes between variables.

2.3.4 Attendance Model

I combined prior theoretical knowledge and proposed causal relationships to create the *a priori* SEM meta-model (Appendix Figure A.1 and Appendix Note A.1). The meta-model captured all evidence-based relationships that I found in my literature review and all plausible and suspected predictors of attendance that I hypothesised. This model was then refined to create the final model depicted in Figure 2.3 using the approach described in Grace *et al.* (2015) and similar to that implemented in Grace *et al.* (2016). In summary, the *a priori* meta-model was modified through addition and deletion of pathways using model-data fit procedures to produce a range of plausible alternative models which were compared using AICc values. All modifications to the model, with pathways removed or inserted, were based on quantitative recommendations, theoretical intuition, and model-data fit. Model-data fit was assessed using a combination of absolute fit indices (e.g. Standardised Root Mean Square Residual) and incremental fit indices (e.g. Comparative Fit Index), to account for the differential sensitivity of fit indices to data distribution, model size and sample size (Hu and Bentler 1999). Modification indices were used to guide the addition of suspected pathways, with a standard cut-off level for the chi-square test criterion of 3.84 (Burnham and Anderson 2002). Highest value modification indices were considered first, however as modification indices do not take into account whether or not relationships make theoretical sense, intuitive theoretical relationships were also considered. Following the addition of these pathways, p-values were then used to identify potentially unsupported pathways, with a threshold of 0.05. Highest p-values were considered first for removal. Overall model selection from the pool of competing models was achieved using AICc values (Burnham and Anderson 2004), with a threshold of more than two AICc units lower than the nearest competing model being considered sufficient for model selection. The AICc values of competing models are shown in Appendix Table A.1. The final selected Attendance model was validated, using four random subsets of the existing data (n = 200 each time), to ensure parameter estimates were similar when using different datasets from the same sample (Fan *et al.* 2016). Institutions were included within countries in the model.

2.3.5 *In Situ* Model

Due to the lower sample size in the *In Situ* model, which only covered four countries, I did not include GDP and Country as variables. I started with the most complete model to predict both *in situ* contributions and attendance. Initial attendance links were based on the results of the best Attendance model. Model-data fit and model selection were assessed in the same manner as for the Attendance model.

Tests of mediation were performed on mediated pathways to ensure both direct and indirect effects of variables were justified in both models. Values for both Absolute Fit Indices and Incremental Fit indices were supportive of good model fit (Appendix Table A.2). All standardised path coefficients, total effect sizes, significance values and proposed interpretations of causal pathways for both models are shown in Table 2.2. See Figure 2.1 for bivariate relationships between attendance, *in situ* contributions and their strongest predictors. These models incorporate species abundance per institution, however models using species presence-absence only were also assessed and provided overall similar results and conclusions, with qualitative differences found in only four links per model. An updated meta-model reinforces many previously supported relationships, such as those between species body mass, species richness and the number of animals present (Appendix Figure A.3).

2.3.6 Species Presence-Absence SEM Frameworks

The Attendance and *In Situ* model results reflecting species presence-absence only are shown in Appendix Figure A.4. Chi-squared statistics, fit indices, standardised path coefficients and proposed interpretations for both the Attendance and *In Situ* models reflecting species presence-absence are also presented (Appendix Tables A.3 and A.4). Residual covariances for both the Attendance and *In Situ* models are shown in Appendix Table A.5 (species abundance models) and Appendix Table A.6 (species presence-absence models).

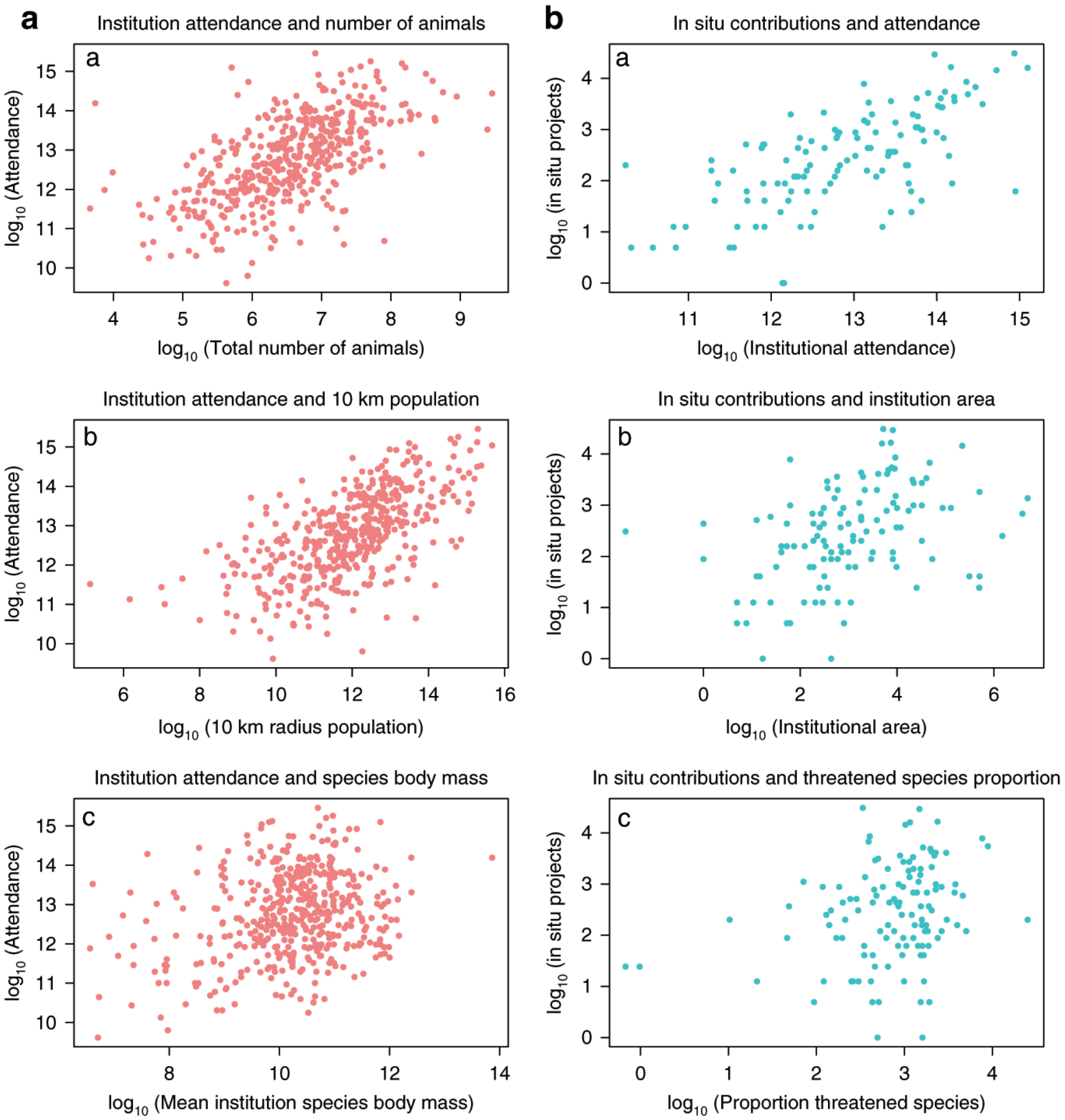

Figure 2.1 | Bivariate relationships between institutional attendance, *in situ* contributions and their strongest predictors. A (left panel, n = 458), log10 transformed bivariate plots of institutional attendance and total number of animals, 10 km radius population and mean species body mass (a-c). B (right panel, n = 119), log10 transformed bivariate plots of institutional *in situ* contributions and attendance, institutional area and the proportion of threatened species present per institution (a-c). All variables are adjusted for species abundance per institution.

2.4 Results

2.4.1 Total effects of composition and socio-economic variables

I found that zoos with high attendance contribute to more *in situ* conservation projects (Figure 2.2). Zoo area and the proportion of threatened species are also positively correlated with *in situ* conservation projects, albeit these effects are weaker than attendance (Figure 2.2). Collection composition variables (total no. of animals, total species richness, mammal species richness, compositional dissimilarity and species body mass) are more important in determining attendance than socio-economic variables (population density and gross domestic product [GDP]).

2.4.2 Direct and indirect effects of variables

The total effects of each variable (Figure 2.2) are composed of direct and indirect effects (shown in Figure 2.3); for example the strong direct effect of body mass on attendance is weakened in the total effect of body mass on attendance due to negative effects of body mass on species richness, total number of animals and dissimilarity. Of the collection composition variables, the total number of animals had the largest direct positive effect on attendance, followed by abundance weighted mean species body mass, with compositional dissimilarity and mammal species richness having smaller direct positive effects (Figure 2.3).

Consistent with previous findings (Davey 2007), I found that human population size and GDP had positive direct effects on institutional attendance, however GDP also had a negative indirect effect on attendance via a negative effect on total number of animals (Figure 2.3). Contrary to expectations (Whitworth 2012), threatened species representation had no direct or indirect effects on attendance. Species richness had a strong positive indirect effect on attendance mediated by total number of animals, but species richness had a smaller direct negative effect on attendance. Mammal species richness alone had a direct positive effect on attendance as well as multiple indirect positive effects through the total number of animals. Mammal species richness also had a small negative effect on attendance mediated by species richness. However, the total effect of mammal species richness on attendance was greater than that of overall species richness (Figure 2.2 and Table 2.2), suggesting mammals are more important in driving visitor attendance than other taxonomic groups.

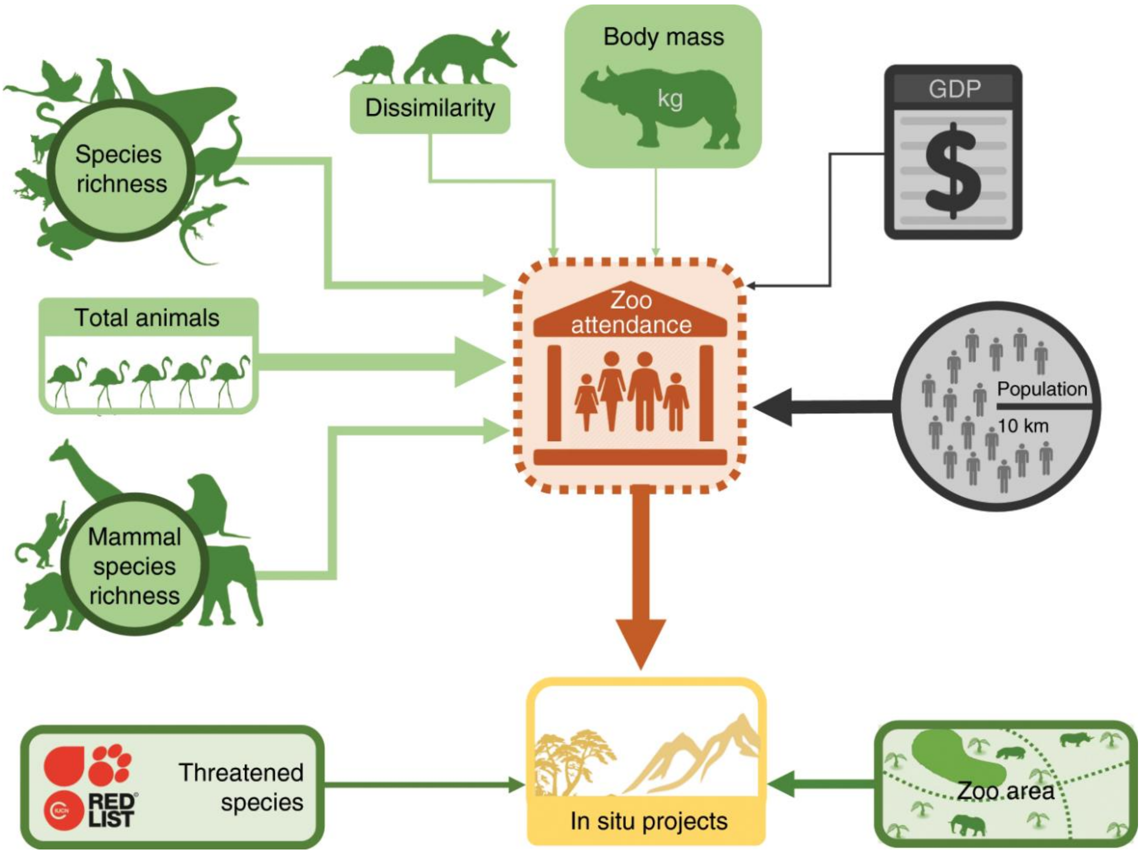

Figure 2.2 | Total effects of institutional variables and socio-economic variables on visitor attendance and *in situ* contributions. This simplified version of the SEM framework shows the total effects of explanatory variables on attendance and *in situ* contributions as arrows with line width representing the standardised relative effect sizes. All total effects were positive. Grey boxes represent socio-economic variables and green boxes represent institutional variables.

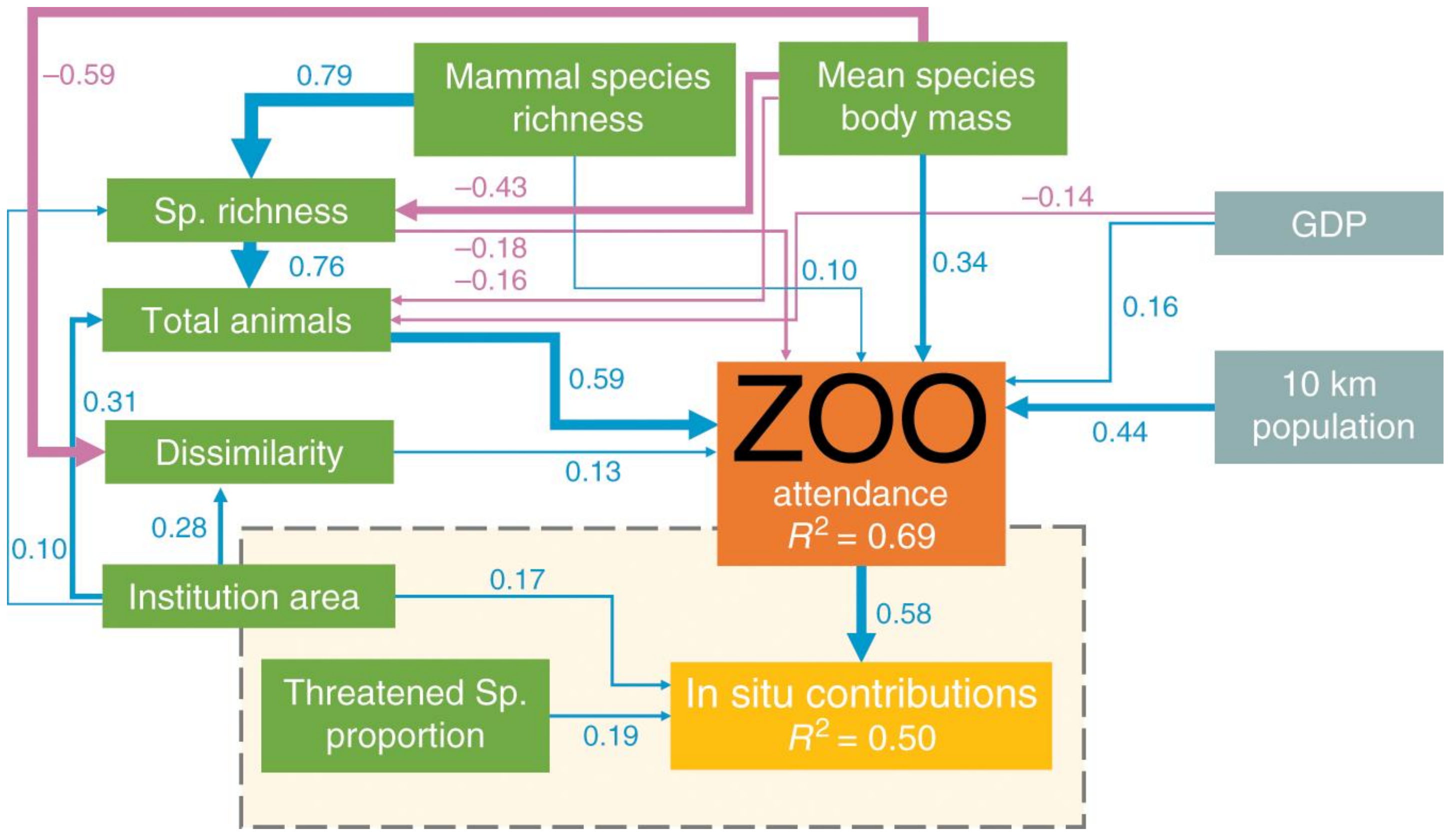

Figure 2.3 | The SEM framework showing direct and indirect connections between institution attendance (n = 458), *in situ* contributions (n = 119) and various institutional and socio-economic variables. Path coefficients shown are standardised. The yellow box indicates the additional pathways included for the 119 institutions for which *in situ* investment data were available. Blue arrows represent positive effects and pink arrows represent negative effects. Line width represents relative effect sizes. Grey boxes represent socio-economic variables and green boxes represent institutional variables.

Table 2.2 | Direct and total standardised effect sizes and proposed interpretations for both the Attendance and *In Situ* models. Also provided are R^2 values, standard errors and p-values. Relationships are ranked according to direct effect size magnitude. Model results presented reflect abundance adjusted models. Only the *in situ* component of the *In Situ* model is reported as all other pathways were analogous to the Attendance model.

	P-Value	**Direct Effect (SE)**	**Total Effect**	**Interpretation**
			Attendance Model	
Attendance ($R^2 = 0.689$)				
Attendance ~ Total Animals	< 0.001	0.587 (0.041)	0.587	Attendance is positively correlated with total number of animals in an institution
Attendance ~ 10km Population	< 0.001	0.444 (0.034)	0.444	Attendance is positively correlated with the local population size (10 km radius) surrounding an institution
Attendance ~ Body Mass	< 0.001	0.340 (0.030)	0.062	Attendance is positively correlated with mean species body mass for an institution
Attendance ~ GDP	< 0.001	0.163 (0.027)	0.083	Attendance is positively correlated with national GDP
Attendance ~ Dissimilarity	< 0.001	0.125 (0.031)	0.125	Attendance is positively correlated with collection dissimilarity
Attendance ~ Mammal Species Richness	0.021	0.102 (0.044)	0.309	Attendance has a small, but positive correlation with number of mammal species present in an institution
Attendance ~ Species Richness	0.004	-0.184 (0.064)	0.262	Attendance is directly negatively correlated with institutional species richness

	P-Value	Direct Effect (SE)	Total Effect	Interpretation
Attendance Model				
Total Animals ($R^2 = 0.783$)				
Total Animals ~ Species Richness	< 0.001	0.759 (0.050)	0.759	The total number of animals in an institution is positively correlated with institutional species richness
Total Animals ~ Institution Area	< 0.001	0.309 (0.045)	0.382	The total number of animals in an institution is positively correlated with institutional area
Total Animals ~ GDP	0.047	-0.136 (0.069)	-0.136	The total number of animals in an institution is negatively correlated with national GDP
Total Animals ~ Body Mass	< 0.001	-0.157 (0.036)	-0.483	The total number of animals in an institution is negatively correlated with the mean species body mass of an institution
Species Richness ($R^2 = 0.678$)				
Sp. Richness ~ Mammal Species Richness	< 0.001	0.790 (0.067)	0.790	Institutional species richness is strongly positively correlated with institutional mammal species richness
Sp. Richness ~ Institution Area	0.017	0.096 (0.040)	0.096	Institutional species richness is positively correlated with institutional area
Sp. Richness ~ Body Mass	< 0.001	-0.429 (0.043)	-0.429	Institutional species richness is negatively correlated with the mean species body mass of an institution

	P-Value	Direct Effect (SE)	Total Effect	Interpretation
Attendance Model				
Dissimilarity ($R^2 = 0.257$)				
Dissimilarity ~ Institution Area	< 0.001	0.277 (0.051)	0.277	Collection composition dissimilarity is positively correlated with institutional area
Dissimilarity ~ Body Mass	< 0.001	-0.593 (0.077)	-0.593	Collection composition dissimilarity is negatively correlated with the mean species body mass of an institution
***In Situ* Model**				
In Situ Contributions ($R^2 = 0.496$)				
In Situ ~ Attendance	< 0.001	0.583 (0.074)	0.583	Institutional *in situ* contributions are positively correlated with institutional attendance
In Situ ~ Threatened Species Proportion	0.004	0.189 (0.066)	0.189	Institutional *in situ* contributions are positively correlated with the proportion of threatened species in an institution
In Situ ~ Institution Area	0.015	0.169 (0.069)	0.320	Institutional *in situ* contributions are positively correlated with institutional area

2.5 Discussion

No support was found for linking species body mass directly with *in situ* project activity. This suggests that *in situ* activity does not directly rely on the presence of large vertebrates, instead the effect of body mass is mediated by institutional attendance. I conclude that the absence of large vertebrates from collections may not necessarily result in reduced *in situ* project activity, presuming institutional attendance can be maintained in their absence through an increase in collection dissimilarity, species richness and/or total number of animals.

Additional compositional options may also be considered to increase the *in situ* contributions of institutions. The direct link between the proportion of threatened species present and institutional *in situ* contributions suggests that greater institutional investment in threatened species *ex situ* is positively correlated with higher *in situ* conservation activity. This may be through the integration of species-specific *in situ* and *ex situ* conservation actions as encouraged in the IUCN Species Survival Commission (SSC) "*Guidelines on the Use of Ex situ Management for Species Conservation*" and the contemporary "*One Plan*" approach to species conservation suggested by the IUCN SSC Conservation Planning Specialist Group (Byers *et al.* 2013; IUCN SSC 2014). Interestingly, the proportion of threatened species was not an important factor in driving attendance, which may contradict evidence of perceived species popularity (Whitworth 2012). Although a greater focus on threatened species *ex situ* could result in greater *in situ* conservation, this may not influence visitor attendance, which is more important in determining overall *in situ* contributions.

The positive effects that total number of animals, mammal species richness and mean species body mass all have on attendance, together with the direct correlation between attendance and *in situ* project activity, supports the flagship approach of exhibiting large vertebrates. This indicates that institutions with numerous large-bodied species, and in particular mammals, are more likely to achieve higher annual attendance and contribute to a greater number of *in situ* conservation projects. This provides the first indication, to my knowledge, that the flagship approach of using popular, large vertebrates in zoo collections to drive public education and *in situ* conservation fundraising is being utilised effectively to significantly increase the *in situ* conservation contributions of zoos globally. The flagship approach potentially results in increased global wildlife conservation, as greater financial *in situ* contributions in particular, have been shown to increase project success and viability (Gusset and Dick 2010).

Achieving a collection composed of numerous large-bodied species may encounter significant hurdles as demonstrated by the support for trade-offs revealed between increasing mean species body mass and both the total number of animals and collection dissimilarity. While the strong direct effect of mean species body mass on attendance provides support for the inclusion of large-bodied species, the trade-offs encountered with increasing mean species body mass present an alternative strategy - exhibiting numerous, unique, smaller-bodied species. These alternative correlative pathways influencing attendance and *in situ* project activity demonstrate that several alternative collection compositions can result in high attendance and *in situ* contributions, potentially resulting in the future diversification of collection planning strategies. My results indicate the need to consider multiple direct and indirect drivers of attendance to enable the detection of trade-offs and avoid collection planning and policy formation that do not take the full complexity of the system into account.

Increased concerns over the welfare of large vertebrates under human care can cause significant decreases in attendance (Wright *et al.* 2015), highlighting the importance of acquisition, welfare and management considerations. My results indicate that ethical, management and welfare considerations may conflict with simplistic attendance maximization strategies. For example, although collection dissimilarity is positively correlated with attendance; population management and conservation breeding recommendations encourage institutions to consolidate their collections to enhance management efficacy, resulting in higher uniformity of collections (Conde *et al.* 2011). In addition, the recommendation to replace large vertebrates with numerous, unique, smaller-bodied species fails to address the serious challenges to the establishment of *ex situ* populations for species not presently maintained (Hutchins *et al.* 1995). These issues are not easily resolved, and trade-offs will become more common as animal welfare standards and enclosure sizes increase (Keulartz 2015).

The utilisation of animals under human care to educate the public and increase *in situ* conservation contributions is in line with The World Zoo and Aquarium Conservation Strategy, which states explicitly that animals held in zoos should "*play a conservation role that benefits wild counterparts*" (Barongi *et al.* 2015). This reflects the flagship and the "*One Plan*" conservation approaches, both of which ultimately contribute to Target 12 (conservation of species) of the United Nations Convention on Biological Diversity Aichi Biodiversity Targets (SCBD 2010). Historically, personal preferences, availability, and competition between institutions were the main determinants of collection composition (Hutchins *et al.* 1995). Today collection composition decisions are largely shaped by individual institutions in consultation with both regional and international associations, for example the Taxon Advisory Groups of regional associations, such as the AZA (Hutchins *et al.* 1995; Kawata 2013). The evidence presented here may be used to help guide policy-makers and collection planners to promote not only direct conservation, but also visitor attendance and *in situ* contributions.

My findings support the continued exhibition of popular, large-bodied species to drive attendance and *in situ* conservation activity, but not exclusively so, in agreement with previous recommendations (Hutchins *et al.* 1995; Whitworth 2012). The exhibition of large numbers of animals in collections that are dissimilar to other zoos is a viable alternative strategy. Each institution must make value-driven decisions regarding their collection composition in order to fulfil their institution-specific goals (Palmer *et al.* 2018) and to ensure the genetic and demographic sustainability of the species within the global zoo network. However, consideration of public preferences and expectations of collection composition can result in greater attendance and increased *in situ* conservation contributions.

3. Flock size and structure influence reproductive success in four species of flamingo in 753 captive populations worldwide

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Author contributions: A.M., A.T. and D.A.C. developed the concept of the manuscript. A.M., J.S. and S.S. collected the data. A.M. undertook the analysis and drafted the text in consultation with all authors. A.M. produced the tables and figures. All authors contributed to the writing of the manuscript.

3.1 Abstract

As global wildlife populations continue to decline, the health and sustainability of *ex situ* populations in zoos and aquariums has become increasingly important. The majority of managed *ex situ* populations are not meeting sustainability criteria and are therefore not viable in the long-term, as demonstrated by the low rates of reproductive success seen in *ex situ* flamingo (*Phoenicopteridae*) populations. Both flock size and environmental suitability have been shown to be important determinants of *ex situ* flamingo reproductive success; however previous work is limited by the number of species, institutions and geographic areas assessed and has failed to consider species-specific differences in reproductive behaviour. The identification of species-specific requirements and associated management recommendations is necessary to improve *ex situ* flamingo population sustainability. Here I combined current and historic globally shared zoological records for four of the six extant species of flamingo (*Phoenicopterus chilensis*, *Phoeniconaias minor*, *Phoenicopterus roseus,* and *Phoenicopterus ruber*) to analyse how flock size, structure and climatic variables have influenced reproductive success in *ex situ* flamingo populations at 753 zoological institutions between 1990 and 2018. I show that flock size has a strong non-linear relationship with reproductive success for all species, with flock sizes of 69 – 127 birds necessary to achieve a 50% probability of reproduction. In addition, I show that a balanced sex ratio and the introduction of new individuals to a flock both increase reproductive success, while climatic variables play a limited role in determining *ex situ* flamingo reproductive success. I provide species-specific management recommendations to increase the reproductive success of global *ex situ* flamingo populations and encourage greater collaboration between individual institutions and regional associations. My analyses demonstrate the conservation potential and management information available from globally shared zoological data and I strongly encourage the continued sharing of standardised zoological records.

3.2 Introduction

Wildlife populations are declining worldwide resulting in heightened extinction risks (Butchart *et al.* 2010; Ceballos *et al.* 2015; Ceballos *et al.* 2017; Ehrlich 1988; IPBES 2019; WWF 2018). As a result, the conservation of remaining populations is seen as a priority, and the *ex situ* preservation and management of populations in zoological collections is one approach that has shown success in preventing species extinctions (AZA 2016b; Chemnick *et al.* 2000; Pritchard *et al.* 2012; Stanley Price 1989). The *ex situ* management of populations is predominantly undertaken by the global zoo and aquarium community, which consists of ~1,000 institutions organised as part of internationally recognised organisations, such as the European Association of Zoos and Aquaria (EAZA) and the Association of Zoos and Aquariums (AZA, North America) (Kelly 1997). Collectively, the global zoo and aquarium community manages 15% of all threatened terrestrial vertebrates (Conde *et al.* 2011). However, the effectiveness of *ex situ* species conservation relies on the ability of zoos and aquariums to maintain genetically and demographically sustainable populations. The sustainability of *ex situ* populations is hindered by small population sizes, low genetic diversity, poor population growth rates, limited reproductive success, inadequate population management, limited *ex situ* species holding capacity and logistical constraints (Che-Castaldo *et al.* 2019; Macek 2014). The majority of co-operatively managed *ex situ* populations are not meeting sustainability criteria and will not persist in the long-term, unless changes are made to collection planning and management practices (Hibbard *et al.* 2011; Lees and Wilcken 2009; Leus *et al.* 2011; Long *et al.* 2011; Powell *et al.* 2019).

Flamingos (*Phoenicopteridae*) have been kept in captivity since the Roman period and today are one of the most commonly represented avian species in zoological collections globally, with an estimated two thirds of all EAZA institutions containing at least one flamingo species (King 2000; King and Bračko 2014; Ogilvie and Ogilvie 1986; Perry 2005; Rose *et al.* 2014). Despite the popularity of flamingo exhibits and their prevalence across collections, zoos globally have been unable to maintain self-sustaining *ex situ* populations for any flamingo species, with all still relying on the periodic importation of wild-caught individuals (Brown and King 2005; King 2000; Shannon 2000; Stevens *et al.* 1992). This is primarily due to poor rates of reproductive success and high egg loss, resulting in the number of deaths consistently exceeding the number of hatchings (King 1994; Stevens *et al.* 1992). This is particularly evident in *Phoeniconaias minor, Phoenicoparrus andinus,* and *Phoenicoparrus jamesi* populations, which have shown considerably poorer *ex situ* reproductive success when compared to *Phoenicopterus chilensis,* *Phoenicopterus roseus,* and *Phoenicopterus ruber* populations (Figure 3.1) (Brown and King 2005; Conway 1965; Pickering 1992; Whitfield 2002). This has created an overall deficit in the number of captive flamingos (King and Bračko 2014). Population declines combined with increasing difficulties in sourcing and importing wild-caught individuals means that the sustainability of *ex situ* flamingo populations has become a concern among population managers (Brown and King 2005; King 1994; Stevens *et al.* 1992). As a result, improved management practices and increased knowledge surrounding the basic reproductive biology of all flamingo species are necessary if populations are to become self-sustaining (Johnson and Cézilly 2008; King 1994; Sandri *et al.* 2018).

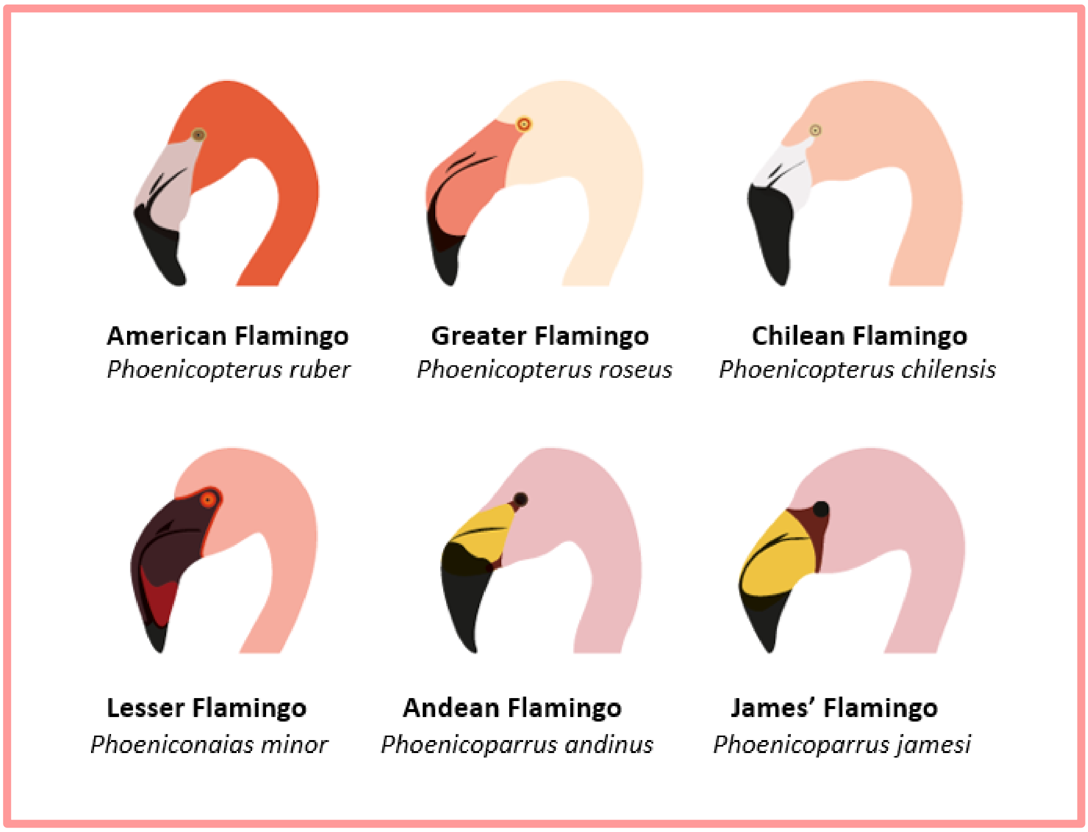


Figure 3.1 | A graphical representation of the six extant species of flamingo. These images are used to identify species throughout this body of work. Image adapted with permission from Krienitz *et al.* (2016). Although *ex situ* populations exist for all six extant flamingo species, the greater flamingo (*Phoenicopterus roseus*), Chilean flamingo (*Phoenicopterus chilensis*), and American flamingo (*Phoenicopterus ruber*), are the most widely represented species (93% of all EAZA flamingos with a combined 8,837 individuals), followed by the lesser Flamingo (*Phoeniconaias minor*; 6%). In comparison, the Andean flamingo (*Phoenicoparrus andinus*) and James' flamingo (*Phoenicoparrus jamesi*) have negligible *ex situ* populations (King and Bračko 2014).

In the wild, flamingos inhabit traditionally inhospitable environments, occupying large shallow lakes and lagoons, where their occurrence is directly tied to the presence of food resources. Their range extends from India, across southern Europe, and down to southern Africa (*Phoeniconaias minor* and *Phoenicopterus roseus*), to the Caribbean and the southern half of South America (*Phoenicopterus chilensis* and *Phoenicopterus ruber*). Both *Phoenicoparrus andinus* and *Phoenicoparrus jamesi* show reduced geographic ranges and are restricted to the high-altitude Andean plateaus of Peru, Chile, Bolivia, and northwest Argentina (Brown and King 2005). Flamingos are not truly migratory, but are instead considered nomadic, moving to find resources when environmental conditions become unsuitable (Rose *et al.* 2014). All species are obligate colonials and occur in flocks numbering in the thousands and even tens of thousands, however reproduction has been recorded in flocks of just tens of individuals (Sprunt 1975; Stevens and Pickett 1994). Although no flamingo species is considered endangered (IUCN statuses include "*Least Concern"* [*Phoenicopterus roseus* and *Phoenicopterus ruber*], "*Near Threatened*" [*Phoenicopterus chilensis*, *Phoenicoparrus jamesi* and *Phoeniconaias minor*] and "*Vulnerable*" [*Phoenicoparrus andinus*]), the availability of suitable breeding and feeding sites is declining globally, predominantly as a result of anthropogenic activities (Brown and King 2005). Given their environmental sensitivity and ecological specialism, flamingos are particularly vulnerable to even subtle environmental changes, however their ability to adapt when suitable conditions appear is advantageous (Brown and King 2005). Reproduction in the wild is typically erratic and is often delayed until suitable environmental conditions, such as temperature and rainfall, are present (Berry 1975; Bucher 1992; Bucher and Curto 2012; Cézilly *et al.* 1996; Vargas *et al.* 2008). This can result in numerous years where no reproduction is observed, even in well-established colonies and localities (Allen 1956; Brown 1958; Rooth 1965). The few long-term studies of wild flamingos have shown reduced mate fidelity when compared to captive flamingos, however older individuals, in excess of 50 years, appear capable of reproducing successfully both in the wild and captivity (Bennett 1987; King 1994; Pickering 1992; Pradel *et al.* 2012; Shannon 1985; Stevens *et al.* 1992; Studer-Thiersch 1975; Studer-Thiersch 1998; Wilkinson 1989)

Multiple factors appear important in determining reproductive success in captive flamingos, ranging from flock composition (including flock size, sex ratio, and age structure) and management, to enclosure design, diet, and environmental suitability (Pickering *et al.* 1992). A clear positive relationship exists between flock size and reproductive success in captive flamingos (Farrell *et al.* 2000; Pickering *et al.* 1992; Sandri *et al.* 2018; Stevens 1991; Stevens and Pickett 1994). Larger flocks reproduce more frequently and also rear a greater number of chicks compared to smaller non-reproductive flocks (Campbell and Lack 1985; Pickering *et al.* 1992). The importance of flock size in determining reproductive success across all species is "*the most important factor for optimizing breeding*" as stated by King (2008). Although flamingos can reproduce in very small flocks given suitable conditions, consistent reproduction is only achieved above minimum flock sizes of 20-40 birds, depending on the species (Pickering *et al.* 1992). Experiments have demonstrated increases in reproductive success following an increase in flock size (Stevens 1991). Additionally, the introduction of new individuals into an established flock can increase reproductive success in subsequent years beyond the benefit incurred from an increase in flock size alone (Farrell *et al.* 2000; Rose *et al.* 2014; Stevens and Pickett 1994). Flock managers have experimented with the separation and reintroduction of flocks prior to the commencement of the breeding season, however, results to date are inconclusive (Shannon 2000). Evidence also exists that an even sex ratio increases both the probability of a flock reproducing and the degree of breeding success observed (King 2008; Stevens 1991). Although it is assumed that flocks reflect an even sex ratio, many captive flamingos are not sexed (King 2008). Uneven sex ratios not only promote the formation of atypical partnerships (same-sex and triad partnerships), but a male-skewed sex ratio is also associated with colony unrest, higher rates of egg breakage and significantly lower reproductive success (King 2006; King 2008; Wilkinson 1989).

Beyond the requirements of flock composition, weather conditions, and particularly sufficient rainfall, play an important role in determining if and when reproduction will occur (Pickering 1992; Stevens 1991). Captive flamingos only breed if there has been sufficient rainfall, even in relatively constant captive environments, although species-specific variations and sensitivities have been recorded (Farrell *et al.* 2000; Ogilvie and Ogilvie 1986; Palmes 1981; Pickering *et al.* 1992; Studer-Thiersch 2000). This sensitivity likely stems from the fact that rainfall is often unpredictable and variable in natural flamingo habitats, yet rainfall provides the conditions necessary for nest building and the rapid proliferation of small food organisms (Stevens and Pickett 1994). Therefore, flamingos must be adaptive and respond quickly to changes in rainfall (Studer-Thiersch 2000). Similarly, both high temperatures and prolonged photoperiod appear to stimulate reproduction in captive flamingos, even in flocks which are housed indoors (Duplaix-Hall and Kear 1975; King 2008; Murton and Kear 1978). Conversely, prolonged periods of rainfall, cold, and cloud cover can inhibit reproductive activity (King 2008). While suitable climatic conditions play an important role in the synchrony of reproductive events, they act in conjunction with flock dynamics and socially facilitated behaviours, such as courtship displays, to provide finer-scale synchrony and determine reproductive success (Clayton 1978). Ultimately, no single factor is essential for reproduction to occur, rather the effects of individual factors are cumulative once a threshold level of requirements is met (King 2008).

The Flamingo Husbandry Guidelines (jointly developed by the AZA and EAZA Taxon Advisory Groups (TAGs), in collaboration with Wildfowl and Wetlands Trust in Slimbridge) represent best management practices for all species of flamingo and provide recommendations to increase the probability of reproduction in captive flocks (Brown and King 2005). They recommend that a minimum flock size of 20 birds be maintained for welfare purposes, and >40 birds to achieve a reasonable chance of reproductive success (Brown and King 2005). These recommendations have been incorporated into global flock management practices, with some institutions attempting to mimic such conditions through the use of mirrors within enclosures to artificially increase flock sizes, however, results to date are not clear (King and Bračko 2014; Whitfield 2002). The EAZA Ciconiiformes and Phoenicopteriformes TAG has implemented 11 strategies based on the Flamingo Husbandry Guidelines aimed at managing the current captive flamingo deficit through improvements in conditions and breeding success (King and Bračko 2014). These strategies encourage zoos to house only a single flamingo species, maintain an even sex ratio, and to increase flock sizes to >40 individuals, while simultaneously addressing management issues such as known health problems, predation, pinioning, and logistical constraints (King and Bračko 2014). Collectively, these guidelines represent the most promising solution to the current *ex situ* flamingo sustainability crisis and their recommendations have been applied to the global *ex situ* management of all flamingo species, but particularly *Phoenicopterus chilensis, Phoeniconaias minor, Phoenicopterus roseus,* and *Phoenicopterus ruber*. The *ex situ* populations of *Phoenicoparrus andinus* and *Phoenicoparrus jamesi* are minimal, and combined with their poor reproductive success, these species are not considered a priority for future *ex situ* management. Although *Phoeniconaias minor* shows demonstrably poor reproductive success and small population sizes, attempts to discourage institutions from the continued breeding of this species have not been well received (King and Bračko 2014).

The implementation of revised management practices resulted in an improvement in overall flamingo reproductive success between 2005 and 2010 (King and Bračko 2014). Despite these improvements, *ex situ* populations are still unable to meet flock size recommendations and the *ex situ* populations of all flamingo species remain unsustainable (Rose *et al.* 2014). The recommendations of the Flamingo Husbandry Guidelines are based on evidence from a limited number of studies, often investigating a single geographic region, a single institutional flock, and/or are species-specific (Rose *et al.* 2014; Pickering *et al.* 1992; Stevens and Pickett 1994;

Stevens 1991). Although the relationship between flock size and reproductive success is clear and consistent, the justification for the exact management recommendations is lacking, with the recommended flock sizes being described as “*somewhat arbitrary*” (King and Bračko 2014). For example, an analysis of *Phoenicopterus chilensis* at 167 zoological institutions in 2013 showed that flocks consisting of over 100 birds had the highest probability of reproducing (Teare 2014). In addition, many studies fail to consider or are unable to separate the effects of species-specific differences in reproductive behaviour, flock sex ratio and environmental conditions, making it difficult to draw general conclusions from previous studies, potentially hindering the sustainable development of *ex situ* flamingo populations (Rose *et al.* 2014; Stevens 1991). As a result, many questions remain unanswered, and the universal implementation of management recommendations may be ineffective. A species-specific confirmation of the optimal flock size and composition necessary for reliable reproductive success is urgently required (King 1994; Sandri *et al.* 2018; Stevens and Pickett 1994). This necessitates temporal, multi-institutional, and multi-species studies to provide a better understanding of the factors involved in promoting captive flamingo reproduction and the long-term sustainability of *ex situ* flamingo populations (Rose *et al.* 2014). Ultimately species-specific requirements must be identified, and management recommendations tailored accordingly, converting theoretical knowledge into management practice (King and Bračko 2014; Rose *et al.* 2016).

The globally shared records currently contained within the Zoological Information Management System (ZIMS), operated by Species360, provide a unique opportunity to investigate the relationship between flock size and reproductive success on a global scale. ZIMS is the largest real-time database of comprehensive and standardised information spanning more than 1,100 zoological collections globally, providing the number of institutions currently managing each species and both their current and historic population sizes (Species360 2019; ZIMS 2019). For four flamingo species (*Phoenicopterus chilensis, Phoeniconaias minor, Phoenicopterus roseus,* and *Phoenicopterus ruber*), held in 753 zoological collections globally, I test how flock size and structure (proportion of reproductive females and proportion of new individuals) influence reproductive success over the period 1990 – 2018. I also test whether captive reproductive success is influenced by latitudinal and climatic gradients, by incorporating measures of both temperature and precipitation. I determine how flamingo flock sizes have changed over 1990 – 2018 and test the seasonality of *ex situ* flamingo reproduction. This is the most comprehensive assessment, to my knowledge, of the determinants of reproductive success in captive flamingos under a common modelling framework, providing the opportunity to assess the recommendations of the Flamingo Husbandry Guidelines and unravel potential species-specific differences in reproductive behaviour. Flamingos represent an ideal candidate species for understanding the role of flock size on reproductive success as they are currently not under any form of contraception or management that would discourage breeding, particularly due to their continued demand within collections (King and Bračko 2014). The methods developed here can easily be applied to other species, such as the boat-billed heron (*Cochlearius cochlearius*), which are also proving difficult to breed in captivity. Results from this study will have direct population management implications and could be directly incorporated into global flock management practices, improving the sustainability of *ex situ* flamingo populations.

3.3 Methods

In order to investigate how flock size and structure influence reproductive success in captive flamingos I utilised current and historic globally shared zoological records from current and past ZIMS member institutions. I combined demographic data with high resolution global climatic data within the same statistical modelling framework to gain a more complete view of the determinants of reproductive success in captive flamingo populations, while also revealing temporal trends in institutional flock sizes and the reproductive seasonality of captive flamingos.

3.3.1 Flamingo Data

Complete flamingo species holdings data across 753 zoological collections globally were obtained from ZIMS in April of 2019 (ZIMS 2019). These data are based on submitted records from individual institutions and although failure to input data correctly or update species holdings accurately may result in errors, ZIMS represents the only global database of zoo collection composition records and as result is used by the IUCN, CITES, TRAFFIC, USFWS and DEFRA (Species360 2019). Data were screened for suspect cases (such as impossible longevity) using the '*BaSTA.ZIMS*' R package and removed as necessary (version 1.0.1; Colchero 2018). Due to data quality and availability, analyses were restricted to the period 1990 - 2018 and to the species *Phoenicopterus chilensis*, *Phoeniconaias minor, Phoenicopterus roseus* and *Phoenicopterus ruber*. *Phoenicoparrus andinus* and *Phoenicoparrus jamesi* were excluded from analyses due to their negligible population sizes and low priority for future *ex situ* management.

The total number of births in each institution per year was calculated for each species. This allowed the seasonality of *ex situ* flamingo reproduction to be quantified for each species. Unfortunately historical flock sizes were not readily available from ZIMS and the flock size for each institution between 1990 and 2018 was calculated by integrating current flock size data with known birth, death and historic movement data from ZIMS. This allowed temporal trends in flock size to be quantified and compared between species. A subset of calculated flock sizes for *Phoeniconaias minor* were verified against individual institution ZIMS inventory reports to ensure data validity. This revealed a strong linear relationship between the calculated flock size and the flock sizes recorded in the ZIMS inventory reports (Appendix Figure B.1), suggesting that my measure of flock size in a year is a meaningful proxy for the actual flock size reported by the institution in that year. The total number of males, females and unsexed individuals were subsequently calculated for each institutional flock for each year. To test the effects of sex ratio, I calculated the proportion of reproductive females (>2 years of age) in each flock in each year.

As many individuals remain unsexed, this represents a conservative value for the proportion of reproductive females per flock. In order to assess how the introduction of new individuals into a flock influences reproductive success in the subsequent year I calculated the proportion of the flock in year *t* made up of individuals added in year *t-1*. This incorporates the time lag in reproductive success associated with the introduction of new individuals and separates the benefit of adding new individuals from the benefit of an increase in flock size alone (Stevens and Pickett 1994). A complete list of all variables calculated can be found in Table 3.1.

Table 3.1 | A complete list of the explanatory variables used in this analysis. Note: Mean Annual Temperature (MAT) is provided by WorldClim as °C multiplied by 10, and similarly mean annual variation in temperature as MAT standard deviation multiplied by 100. Both were divided (by 10 and 100 respectively) prior to the modelling procedure to avoid confusion in the units used.

Variable	**Description**
Year	Current year (*t*)
Births	Number of births in year *t*
Flock Size	Flock size in year *t*
Proportion of Additions	The proportion of the flock in year *t* made up of additions from year *t-1*
Proportion of Reproductive Females	The proportion of the flock made up of potentially reproductively active females (>2 years of age) in year *t*
MAP	Mean annual precipitation (mm)
MAT	Mean annual temperature (°C)
MAP Var	Mean annual variation in precipitation (MAP coefficient of variation)
MAT Var	Mean annual variation in temperature (MAT standard deviation)

3.3.2 Climatic Data

The influence of climatic variables was assessed using data provided by WorldClim. The WorldClim database averages 19 different climatic variables derived from monthly temperature and rainfall values at a 1-km spatial resolution for the period 1970-2000 (Hijmans *et al.* 2005). These include variables such as mean annual temperature, mean annual precipitation and seasonality metrics that have been widely used in species distribution modelling (Booth *et al.* 2014). WorldClim data were extracted for the co-ordinates of individual institutions (latitude and longitude), using the '*raster*' R package (version 2.6-7), to investigate the influence of climatic variables on reproductive success in *ex situ* flamingo populations (Hijmans *et al.* 2005). Specifically, I calculate the mean annual temperature (MAT) and mean annual precipitation (MAP) for each institution. Given the sensitivity of flamingos to subtle environmental changes I also included measures of variation in both mean annual temperature (MAT standard deviation) and mean annual precipitation (MAP coefficient of variation). Co-ordinate details were only available for current Species360 member institutions, therefore climatic information could not be calculated for previous member institutions for which historical data were available (institutions which were once Species360 members, but have since left). This reduced the sample size from 753 institutions to 353 institutions for analyses including climate variables.

3.3.3 Modelling Procedure

Flock size between 1990 and 2018 was modelled separately for each species using generalised linear mixed effects models with year included as a fixed effect, with random effects of the intercept and slope of year for each institution. To assess reproductive success for each species, a two-step modelling approach was implemented, similar to Buckley (2015). Models were built for 1) the probability of a flock reproducing in a given year, and if reproduction occurred, 2) the predicted number of chicks produced per flock. The probability of a flock reproducing was modelled as a zero-inflated binomial generalised linear mixed effects model. Given that reproduction had occurred, the number of predicted chicks per flock was modelled as a zero-truncated Poisson generalised linear mixed effects model. For both reproduction model structures I included the institutional country and institution nested within country as random effects in order to allow for any potential national and institutional differences. Potential non-linearities between response variables and flock size were tested using quadratic and cubic relationships with flock size. Similarly, two-way interactions between flock size and both the proportion of reproductive females and proportion of additions were included to better understand the flock dynamics that encourage reproductive success. All variables were mean centered and expressed in units of standard deviation prior to modelling. In addition to this standardisation, I investigated the appropriateness of a log transformation of flock size, retaining the transformation where it improved model fit. All figures and results are presented on the original response variable scale to help aid interpretability. All analyses were carried out using the R programme (version 3.4.3; R Core Team 2017) and the '*glmmTMB*' package (version 0.2.3; Brooks *et al*. 2017).

3.3.4 Total and Climate Analyses

Latitude and longitude were only available for current Species360 member institutions and this reduced the number of institutions for which I could analyse climatic data. The data were therefore modelled first without climatic data (larger sample size including 753 current and past Species360 members, *i.e.* "Total Analysis"). A second analysis was then done on a subset of institutions for which climatic data were available (353 current Species360 members, *i.e.* "Climate Analysis"), this time including climatic variables in addition to the variables included in the Total Analysis. Total and Climate Analyses revealed quantitatively similar results, despite the reduction in sample size for the Climate Analyses. Therefore, only the additional climatic variables of the Climate Analysis are reported in the results, as all other relationships were derived from the Total Analysis due to its higher statistical power. A summary of the data available for each analysis is presented in Table 3.2, highlighting the number of current and past Species360 member institutions for each species.

3.3.5 Model Selection

Based on the *a priori* hypothesised relationships, a separate maximal model for reproduction containing all possible explanatory variables (flock size, year, proportion of additions and proportion of reproductive females) and selected two-way flock size interactions (with the proportion of additions and the proportion of reproductive females), was generated for each species. Model simplification and selection between top-performing models was conducted using AICc values, with a threshold of more than two AICc units lower than the nearest competing model being considered sufficient for model selection (Burnham and Anderson 2002). This procedure was repeated for the Climate Analysis, additively including the four climatic variables in Table 3.1 in addition to all the variables in the Total Analysis maximal model. Once again, model simplification and selection was achieved by comparing AICc values of competing top-performing models. Only the climatic relationships from the Climate Analysis are considered in the results, with all other relationships being derived from the Total Analysis due to its much larger sample size and higher statistical power. The maximal starting models for both the 'Probability of Reproduction' and 'Number of Chicks' models, showing both the Total and

Climate Analyses, are shown in Tables 3.3 and 3.4 respectively. Residual diagnostic plots investigating overdispersion and zero-inflation parameters were generated using the ‘*DHARMa*’ package in order to confirm the validity of all final models (version 0.2.6; Hartig 2019). Figures were generated using the ‘*ggplot2*’ R package (version 3.2.1; Wickham 2016).

3.4 Results

3.4.1 Flamingos in Zoos and Aquariums

As of April 2019, there were a total of 19,704 extant flamingos across the Species360 member institution network, spanning 353 institutions in 53 countries (Table 3.2; ZIMS 2019). *Phoenicopterus chilensis, Phoenicopterus roseus,* and *Phoenicopterus ruber* all have similarly large *ex situ* populations (ranging from 5,644 to 6,753 individuals), whereas *Phoeniconaias minor* has a much smaller extant *ex situ* population of 1,170 individuals. Similarly, the number of institutions housing flamingos as of April 2019 ranges from 162 to 191 for *Phoenicopterus chilensis, Phoenicopterus roseus,* and *Phoenicopterus ruber*, yet only 100 institutions house *Phoeniconaias minor*. This bias is also reflected in the total number of births for each species between 1990 and 2018. *Phoenicopterus chilensis, Phoenicopterus roseus,* and *Phoenicopterus ruber* produced between 5,291 and 5,755 chicks, across 108 to 163 institutions, whereas *Phoeniconaias minor* produced only 265 chicks across 16 institutions (Table 3.2). The sex ratio of *ex situ* flamingo populations is relatively even for *Phoenicopterus chilensis, Phoenicopterus roseus,* and *Phoenicopterus ruber*, however *Phoeniconaias minor* shows a strong male bias (55.8% of all individuals; Table 3.2). Importantly, 25.2% (4,970/19,704 birds) of individuals have not yet been sexed, and this is consistent across the four species studied. All species show remarkably similar mean flock sizes over the period 1990 to 2018 (18.0 to 19.2 birds), despite the noted difficulties in maintaining and reproducing *Phoeniconaias minor*. Summary statistics for *Phoenicoparrus andinus* and *Phoenicoparrus jamesi* are also shown for comparison (Table 3.2).

The global distribution of current institutions housing flamingos is presented on a global map showing mean annual temperature at a 1-km resolution (Figure 3.2), global distributions showing mean annual precipitation and species-specific institutional distributions can be found in Appendix B (Appendix Figures B.2 – B.6). The majority of institutions with flamingos are found in the Northern Hemisphere, in Europe and North America. Although there is some overlap with known *in situ* flamingo habitat, the majority of institutions are far from the tropical and subtropical habitats in which most *in situ* flamingo populations are situated. This is a reflection of Species360 membership, which is heavily biased towards AZA and EAZA institutions. Therefore, it is likely that considerable *ex situ* flamingo populations exist outside of the distribution shown.

Table 3.2 | Summary statistics for each flamingo species maintained within the Species360 member institution network for the period 1990 - 2018. Past institutions are those which were Species360 members in the past but have since left. This does not mean they do not currently house flamingos; however contemporary data are unavailable. Living population as of April 2019: M = male, F = female and U = undetermined. Total Births encompass the period 1990 – 2018. Note that each institution may currently house, or have previously housed, more than one flamingo species.

Species	**Living Population (April 2019)**				**Current Institutions**	**Past Institutions**	**Institutions with Births (1990 – 2018)**	**Mean Flock Size (± SD)**	**Total Births (1990 – 2018)**
	M	**F**	**U**	**Total**					
Phoenicopterus chilensis	2108	1955	1581	5644	191	213	163	19.0 (± 25.6)	5291
Phoeniconaias minor	653	266	251	1170	100	94	16	19.0 (± 28.4)	265
Phoenicopterus roseus	2457	2590	1706	6753	162	172	108	19.2 (± 26.8)	5755
Phoenicopterus ruber	2411	2250	1427	6088	172	189	130	18.0 (± 25.8)	5695
Phoenicoparrus andinus	14	19	5	38	6	7	2	5.9 (± 6.0)	6
Phoenicoparrus jamesi	5	6	0	11	8	5	1	4.8 (± 3.1)	6

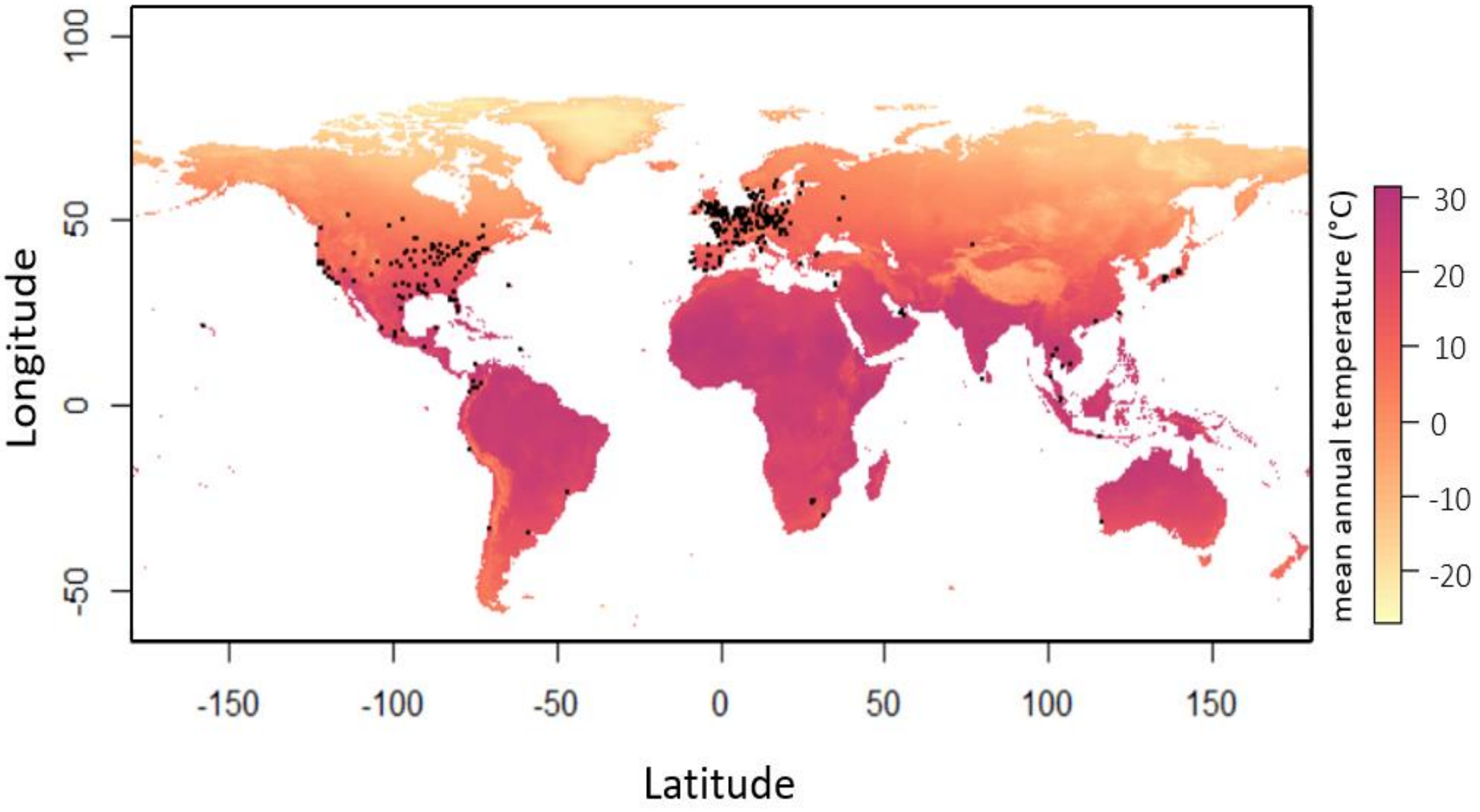


Figure 3.2 | The global distribution of current Species360 member institutions containing flamingo flocks. These are presented on a global map showing mean annual temperature (°C) at a resolution of 1 km (darker meaning higher temperatures).

3.4.2 Temporal Patterns

Overall flock sizes of *Phoenicopterus chilensis*, *Phoeniconaias minor, Phoenicopterus roseus,* and *Phoenicopterus ruber* have significantly increased over time ($p < 0.05$ for all relationships; Figure 3.3). Flock size is very consistent between these four species, with an increase in flock size from a mean of 11.33 (± 1.49 S.D.) birds in 1990 to 20.81 (± 0.60 S.D.) birds in 2018 observed across all species. No sudden change in flock size was recorded, as might be expected following the introduction of the Flamingo Husbandry Guidelines in 2005, instead I observe a steady increase in flock sizes through time. *Phoenicoparrus andinus* flocks showed a modest increase over the same period, though this was non-significant ($p = 0.365$), from a mean of 6.42 to 8.1 birds, whereas *Phoenicoparrus jamesi* was the only species to show a decrease in flock sizes (albeit non-significant, $p = 0.831$), from a mean of 4.84 to 4.75 birds.

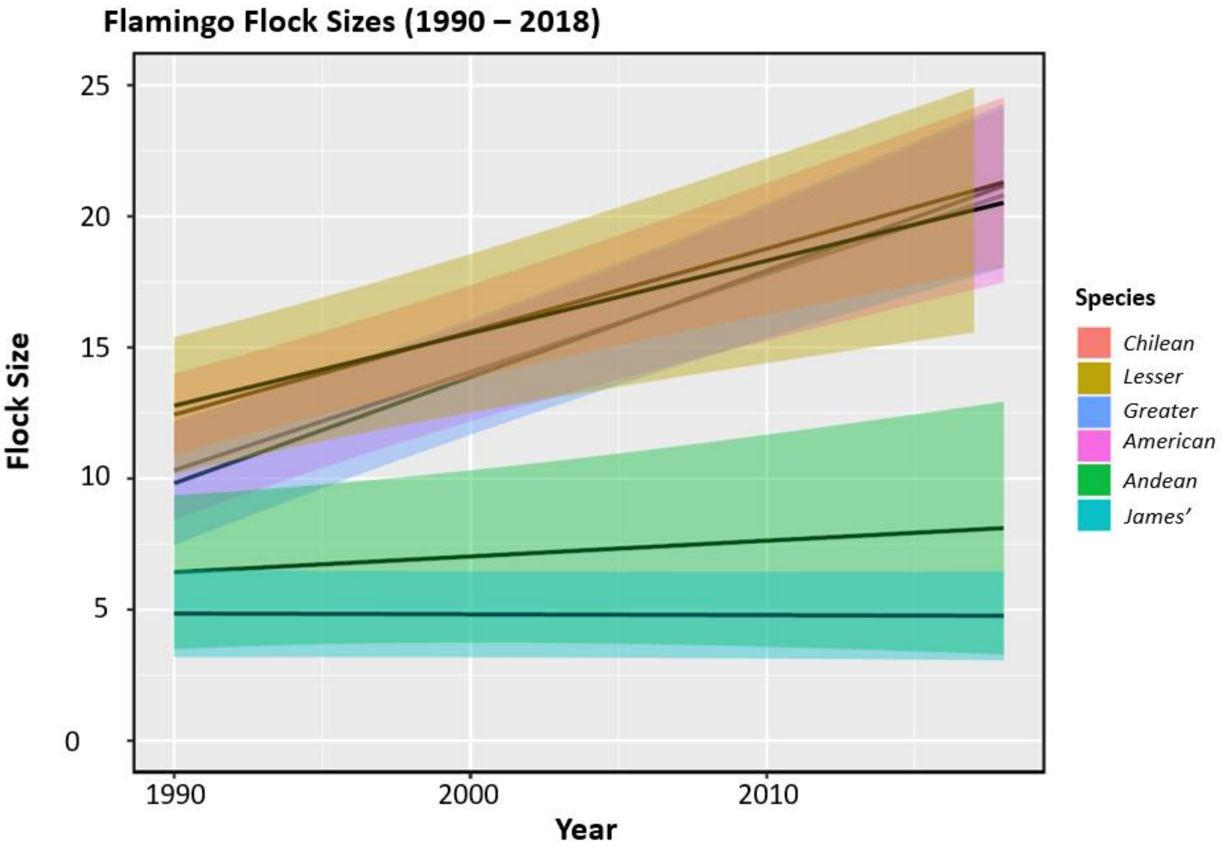


Figure 3.3 | Changes in *Phoenicopterus chilensis*, *Phoeniconaias minor*, *Phoenicopterus roseus*, and *Phoenicopterus ruber* flock sizes between 1990 and 2018. Flock sizes of *Phoenicoparrus andinus* and *Phoenicoparrus jamesi* are shown for comparison. Black lines represent predicted values and shaded areas represent 95% confidence intervals.

3.4.3 Birth Seasonality

The frequent management interventions employed by institutions in order to encourage reproduction rely on the accurate timing and synchrony of reproductive events. Therefore, it is important to understand when reproduction is most likely to occur, and plan management interventions around these predictions. I found that *Phoeniconaias minor* shows no clear pattern in annual reproductive output, with reproduction occurring in most months, however the lowest number of births occurred from September to November (Figure 3.4). In contrast, *Phoenicopterus roseus* shows a clear breeding season (May to June), though reproduction can occur year-round. Similarly, *Phoenicopterus ruber* reproduces mainly in June and July and *Phoenicopterus chilensis* reproduces from July – September, although reproduction has been observed during every month of the year for both species. European and North American institutions show similar patterns in reproductive seasonality; however these trends are reversed in the Southern Hemisphere (Africa and South America). For example, *Phoenicopterus ruber* consistently reproduces in June and July in the Northern Hemisphere, however the highest reproduction in African institutions occurs in December and January (Appendix Figure B.10). The number of institutions in the Southern Hemisphere is negligible in comparison to those in the Northern Hemisphere, so further records are needed in order to see whether seasonality patterns are consistent. The seasonality of reproduction according to geographic location is shown for each species in Appendix B (Appendix Figures B.7 – B.10).

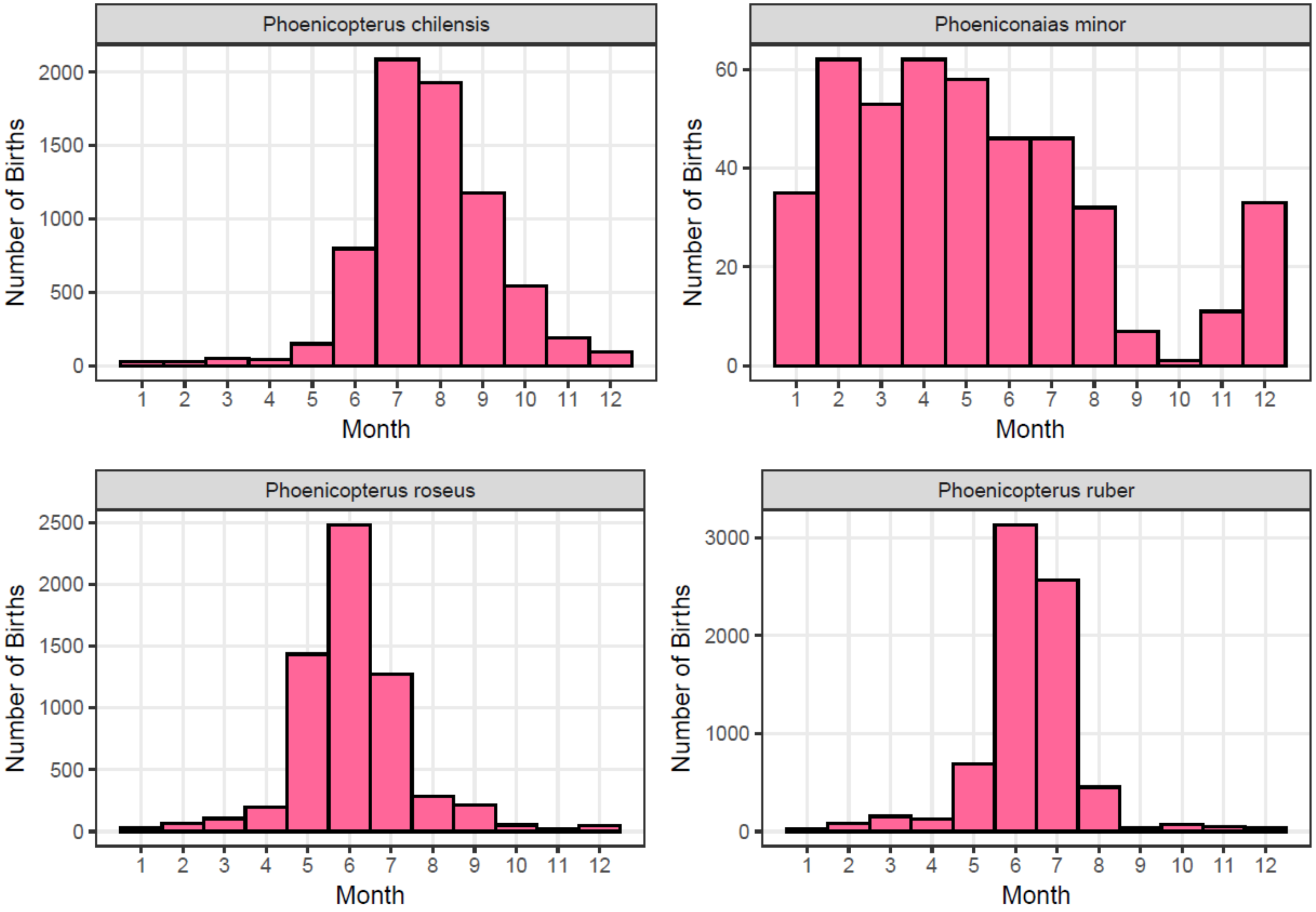


Figure 3.4 | The reproductive seasonality of *Phoenicopterus chilensis, Phoeniconaias minor, Phoenicopterus roseus* and *Phoenicopterus ruber*, showing the total number of births in each month per species. Data reflects institutions in the Northern Hemisphere only (Europe and North America). Months; 1-12 = January – December.

3.4.4 Reproductive Success

Flock size has a strong positive influence on the probability of reproduction and the predicted number of chicks per flock in all four species of flamingo analysed (Figures 3.5 – 3.8). The final species-specific 'Probability of Reproduction' and 'Number of Chicks' models (combining both the Total Analysis and Climate Analysis results) can be found in Tables 3.3 and 3.4 respectively. Results reveal that the recommended flock size of 40 individuals results in a predicted probability of reproduction of 12% for *Phoenicopterus chilensis*, 0% for *Phoeniconaias minor*, 8% for *Phoenicopterus roseus,* and 10% for *Phoenicopterus ruber*. Based on the results presented here, the flock size necessary to achieve a 50% probability of reproduction is 103 for *Phoenicopterus chilensis*, 127 for *Phoeniconaias minor*, 69 for *Phoenicopterus roseus,* and 95 for *Phoenicopterus ruber*. Even accounting for the wide confidence intervals of the analyses, this still suggests all species need over 50 individuals to achieve a 50% probability of reproduction for the most conservative estimates at the lower 5% interval. However, it should be noted that the number of institutions with flock sizes >100 individuals is limited and as a result there is considerable variation observed within the 95% confidence intervals shown, suggesting further investigation is required. Similarly, a balanced sex ratio, or one that is female biased, appears to enhance both measures of reproductive success in three of the four species (see Figure 3.9). The addition of new individuals to a flock also seems to increase the number of chicks produced per flock (*Phoenicopterus chilensis, Phoenicopterus roseus,* and *Phoenicopterus ruber;* Figure 3.10), but has little influence on the probability of reproduction (except in *Phoeniconaias minor*).

Although important, climatic variables play a limited role in determining reproductive success. Contrary to expectations, high mean annual precipitation lowers the probability of reproduction (*Phoenicopterus chilensis* and *Phoenicopterus ruber*) and the number of chicks produced (*Phoenicopterus chilensis* only). Mean annual temperature appears to have no effect on the probability of reproduction, however a positive relationship exists between the number of chicks produced and both higher (*Phoenicopterus ruber*) and more variable mean annual temperatures (*Phoeniconaias minor*). A summary of the flock structure and climatic variables which influence the probability of reproduction and the number of chicks produced in *ex situ* flamingo flocks is shown in Figure 3.11 and Table 3.5. A summary of species-specific findings is highlighted in Appendix Figure B.34. Detailed species-specific significant results considering both the probability of reproduction and the number of chicks produced, combining both the Total and Climate analyses are discussed in Appendix B (Appendix Note B.1). All final species-specific Total and Climate Analysis models can be found in Appendix Tables B.1 and B.2, with associated standardised effect sizes, standard errors and p-values shown in Appendix Tables B.3 - B.10.

Table 3.3 | The original maximal and final species-specific 'Probability of Reproduction' models. Only the climatic relationships from the Climate Analysis are shown; these are highlighted in bold. All other relationships are derived from the Total Analysis due to its greater sample size and higher explanatory power. Not all relationships are statistically significant.

Probability of Reproduction Model Selection	
Maximal Model	Probability of Reproduction ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females **+ Mean Annual Precipitation + Mean Annual Temperature + Variation in Mean Annual Precipitation + Variation in Mean Annual Temperature** + (Year|Institution Code) + (1|Country)
Species	**Final Selected Model**
Phoenicopterus ruber	Probability of Reproduction ~ log(Flock Size) + log(Flock Size)^2 + Year + Proportion of Reproductive Females **+ Mean Annual Precipitation** + (Year|Institution Code) + (1|Country)
Phoenicopterus chilensis	Probability of Reproduction ~ log(Flock Size) + log(Flock Size)^2 + Year **+ Mean Annual Precipitation + Variation in Mean Annual Temperature** + (Year|Institution Code) + (1|Country)
Phoenicopterus roseus	Probability of Reproduction ~ Flock Size + (Flock Size)^2 + Proportion of Additions + Flock Size: Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females **+ Mean Annual Precipitation + Variation in Mean Annual Precipitation + Variation in Mean Annual Temperature** + (Year|Institution Code) + (1|Country)
Phoeniconaias minor	Probability of Reproduction ~ Flock Size + Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (1|Institution Code) + (1|Country)

Table 3.4 | The original maximal and final species-specific 'Number of Chicks' models. Only the climatic relationships from the Climate Analysis are shown; these are highlighted in bold. All other relationships are derived from the Total Analysis due to its greater sample size and higher explanatory power. Not all relationships are statistically significant.

Number of Chicks Model Selection	
Maximal Model	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females **+ Mean Annual Precipitation + Mean Annual Temperature + Variation in Mean Annual Precipitation + Variation in Mean Annual Temperature** + (Year|Institution Code) + (1|Country)
Species	**Final Selected Model**
Phoenicopterus ruber	Number of Births ~ log(Flock Size) + log(Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions **+ Mean Annual Temperature** + (Year|Institution Code) + (1|Country)
Phoenicopterus chilensis	Number of Births ~ Flock Size + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females **+ Mean Annual Precipitation** + (Year|Institution Code) + (1|Country)
Phoenicopterus roseus	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Proportion of Reproductive Females + (Year|Institution Code) + (1|Country)
Phoeniconaias minor	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females **+ Variation in Mean Annual Temperature** + (1|Institution Code) + (1|Country)

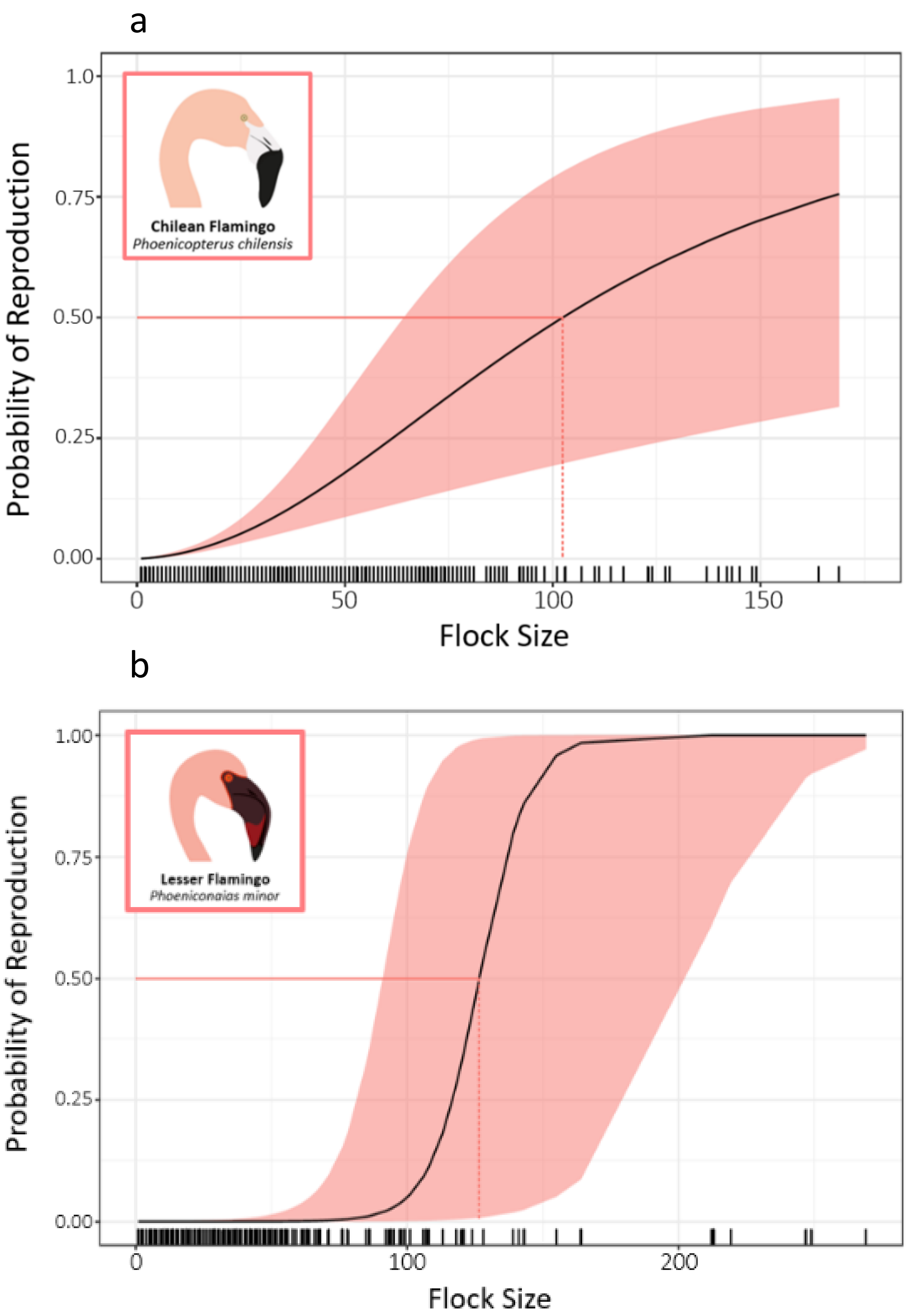


Figure 3.5 | The relationship between *ex situ* flock size and the probability of reproduction for *Phoenicopterus chilensis* (a) and *Phoeniconaias minor* (b) between 1990 and 2018. Black lines represent predicted values on the original response variable scale and shaded areas represent 95% confidence intervals. Dashed lines represent the flock size necessary to achieve a 50% probability of reproduction.

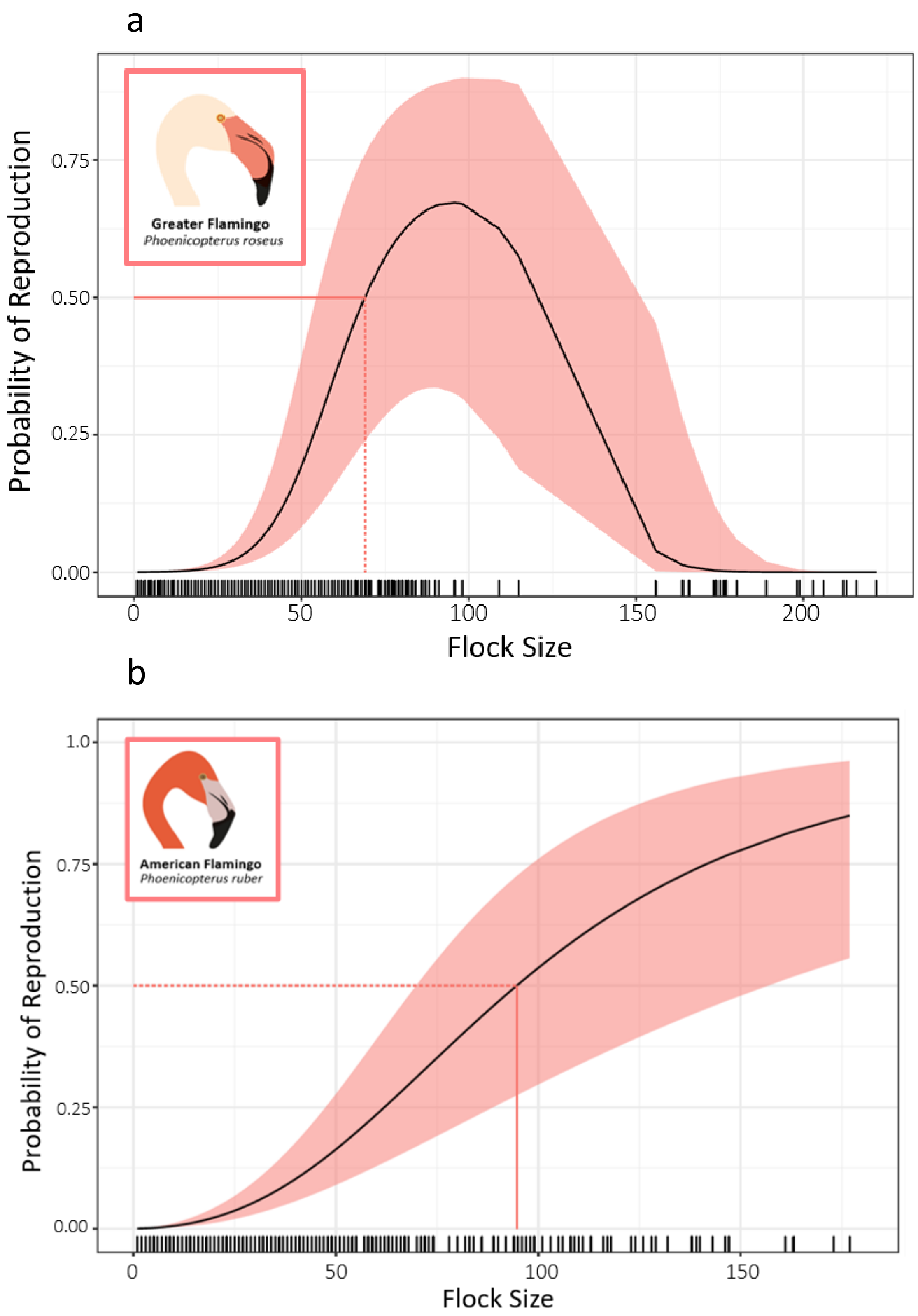


Figure 3.6 | The relationship between *ex situ* flock size and the probability of reproduction for *Phoenicopterus roseus* (a) and *Phoenicopterus ruber* (b) between 1990 and 2018. Black lines represent predicted values on the original response variable scale and shaded areas represent 95% confidence intervals. Dashed lines represent the flock size necessary to achieve a 50% probability of reproduction.

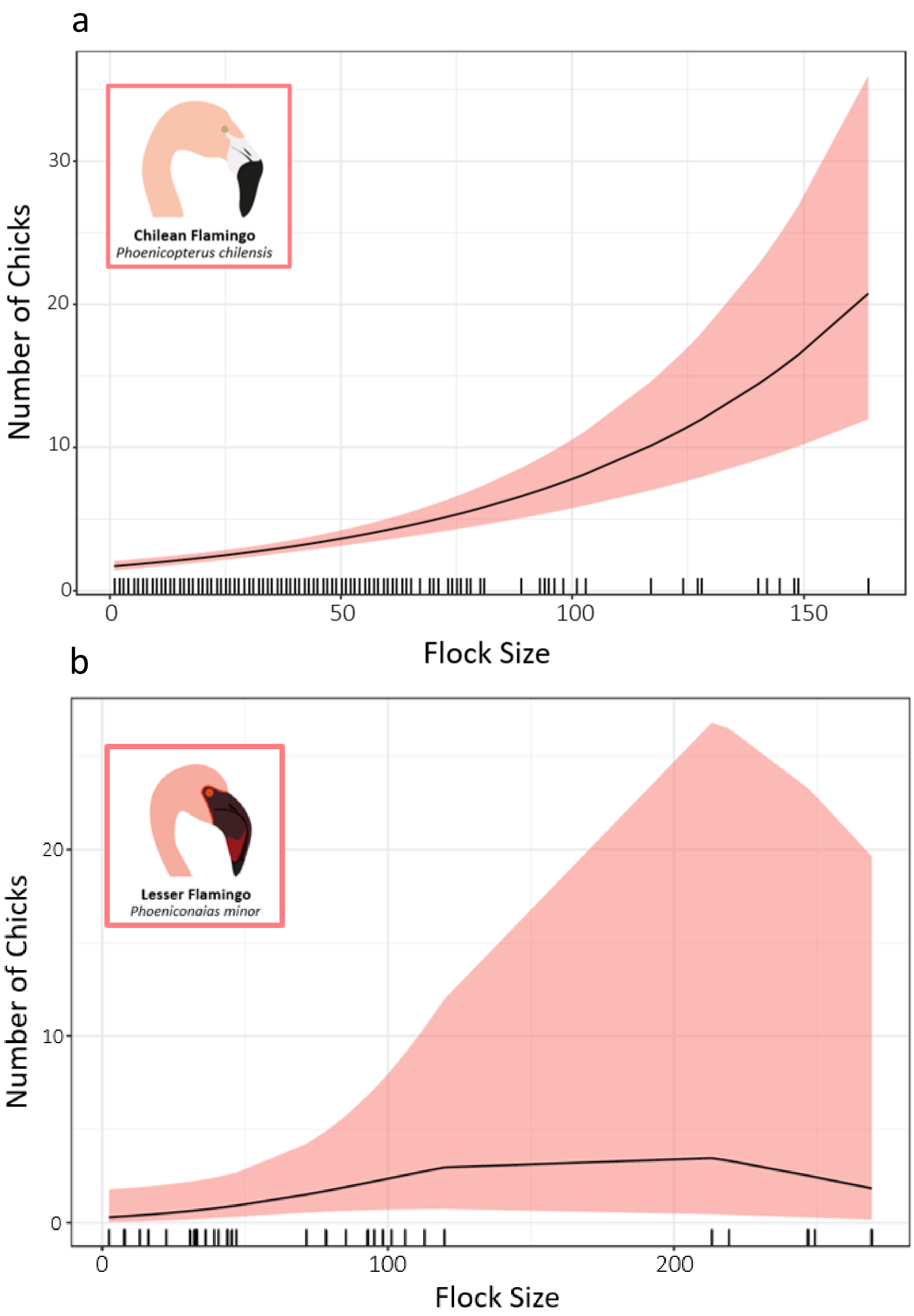


Figure 3.7 | The relationship between *ex situ* flock size and the number of chicks produced for *Phoenicopterus chilensis* (a) and *Phoeniconaias minor* (b) between 1990 and 2018. Black lines represent predicted values on the original response variable scale and shaded areas represent 95% confidence intervals.

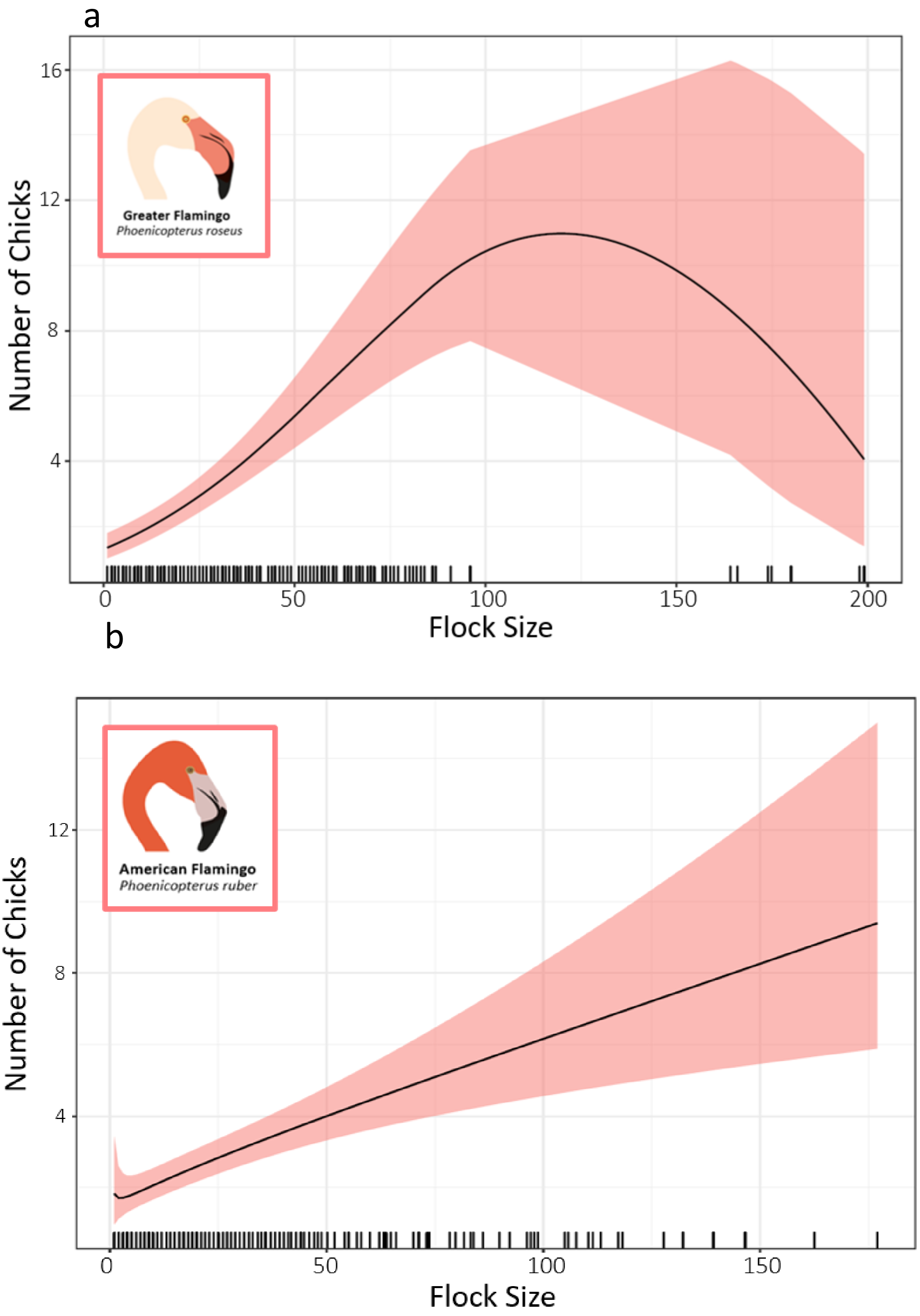


Figure 3.8 | The relationship between *ex situ* flock size and the number of chicks produced for *Phoenicopterus roseus* (a) and *Phoenicopterus ruber* (b) between 1990 and 2018. Black lines represent predicted values on the original response variable scale and shaded areas represent 95% confidence intervals.

Table 3.5 | A summary of the flock structure and climatic variables which influence both the probability of reproduction and the number of chicks produced in *ex situ* flamingo flocks. Prop Repro = the proportion of the flock made up of reproductively active females (>2 years of age) in year *t*. New Individuals = the proportion of the flock in year *t* made up of additions from year *t-1*. MAP = Mean annual precipitation (mm), MAT = mean annual temperature (°C), MAP Var = variation in mean annual precipitation (MAP coefficient of variation), MAT Var = variation in mean annual temperature (MAT standard deviation). Black ticks represent positive relationships and red ticks represent negative relationships. Positive relationships may represent Flock Size interactions. Dashes represent relationships for which no statistical support was found.

Species	Flock Size	Prop Repro	New Individuals	MAP	MAT	MAP Var	MAT Var
	Probability of Reproduction						
Phoenicopterus chilensis	✓	-	-	✓	-	-	✓
Phoeniconaias minor	✓	✓	✓	-	-	-	-
Phoenicopterus roseus	✓	✓	-	-	-	-	-
Phoenicopterus ruber	✓	✓	-	✓	-	-	-
Total Species	**4**	**3**	**1**	**2**	**0**	**0**	**1**
	Number of Chicks						
Phoenicopterus chilensis	✓	✓	✓	✓	-	-	-
Phoeniconaias minor	✓	✓	-	-	-	-	✓
Phoenicopterus roseus	✓	✓	✓	-	-	-	-
Phoenicopterus ruber	✓	-	✓	-	✓	-	-
Total Species	**4**	**3**	**3**	**1**	**1**	**0**	**1**

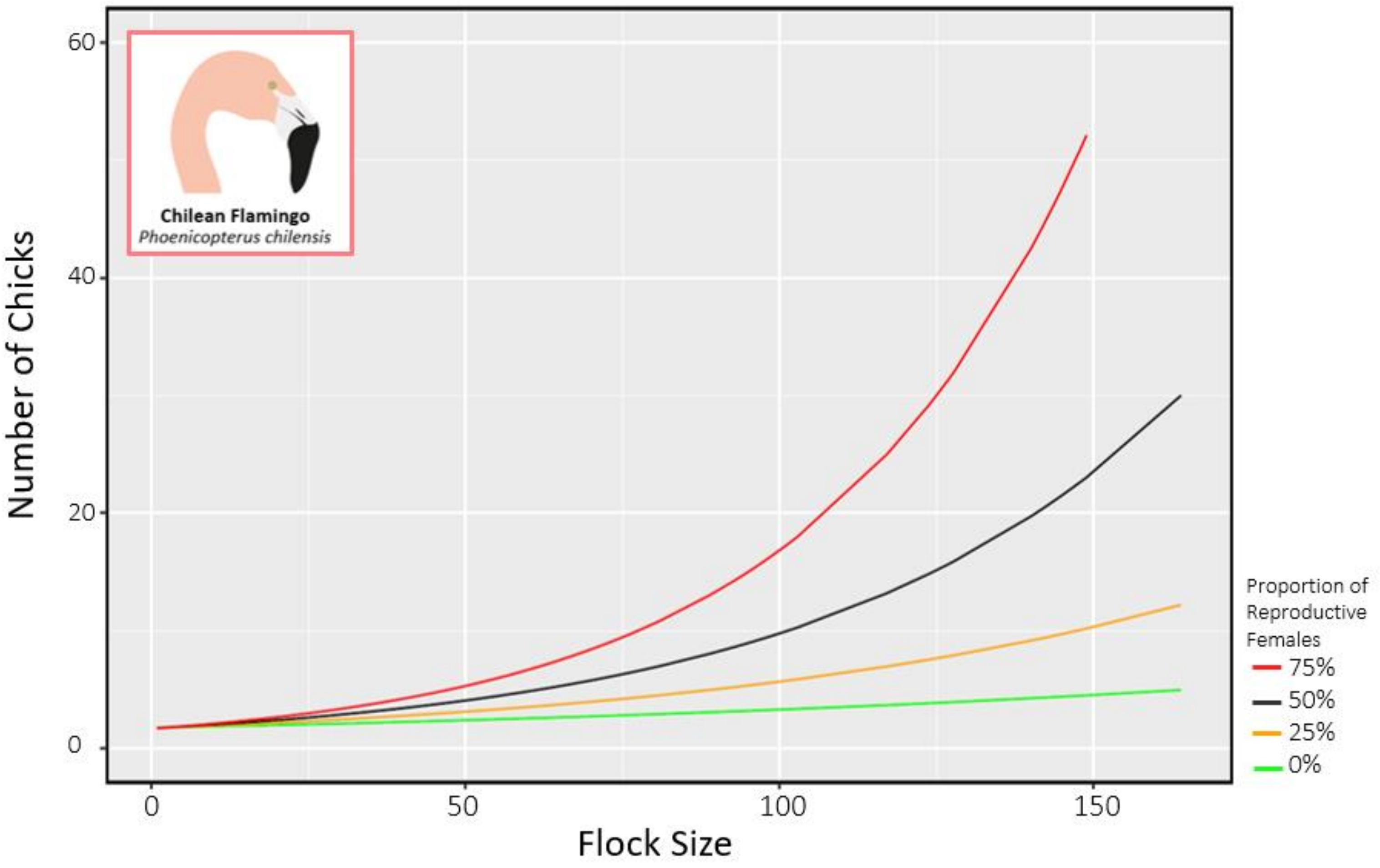


Figure 3.9 | The relationship between *ex situ* flock size and the number of chicks produced for *Phoenicopterus chilensis* at varying proportions of reproductive females per flock between 1990 and 2018. Coloured lines represent predicted values on the original response variable scale.

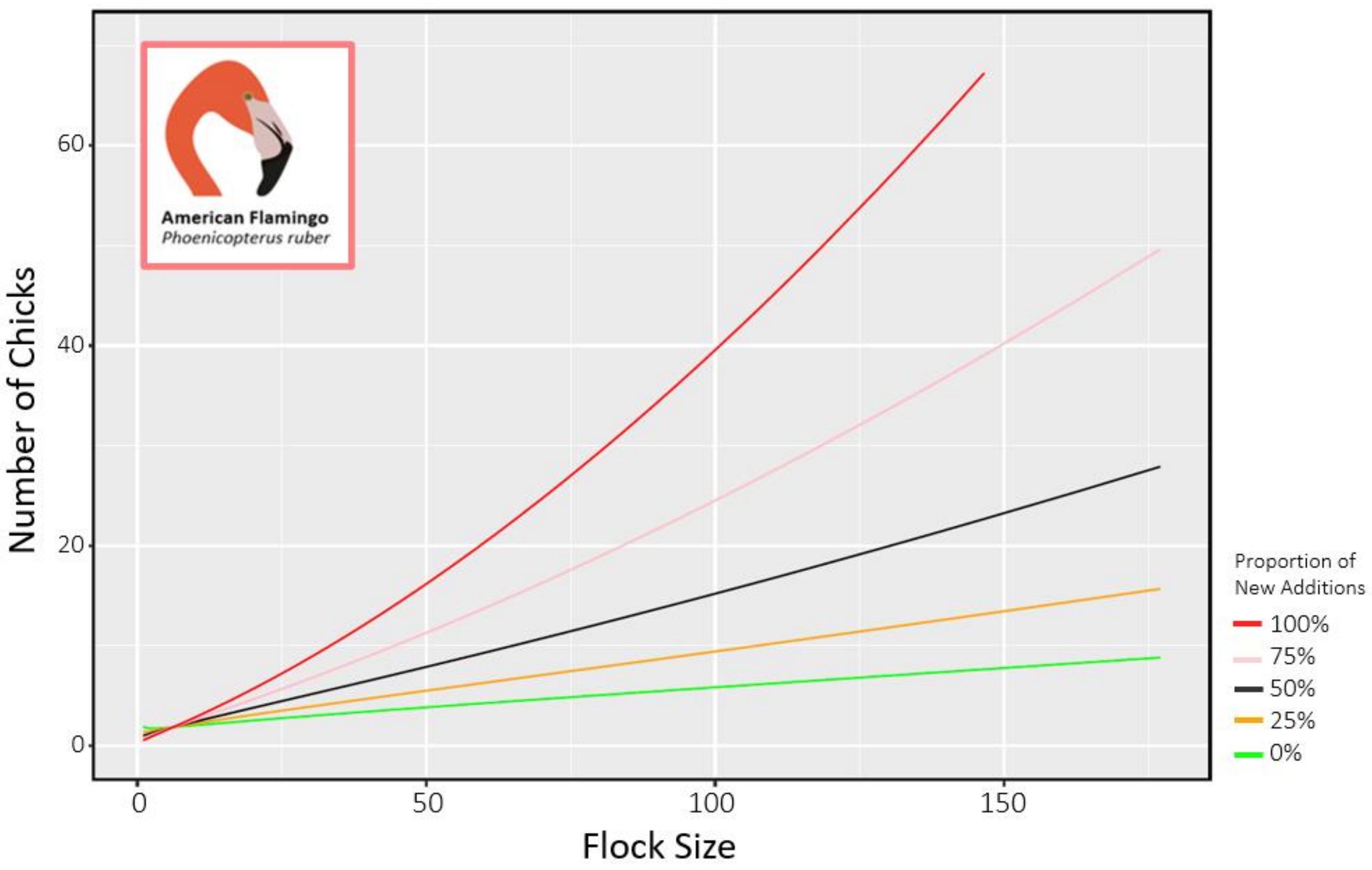
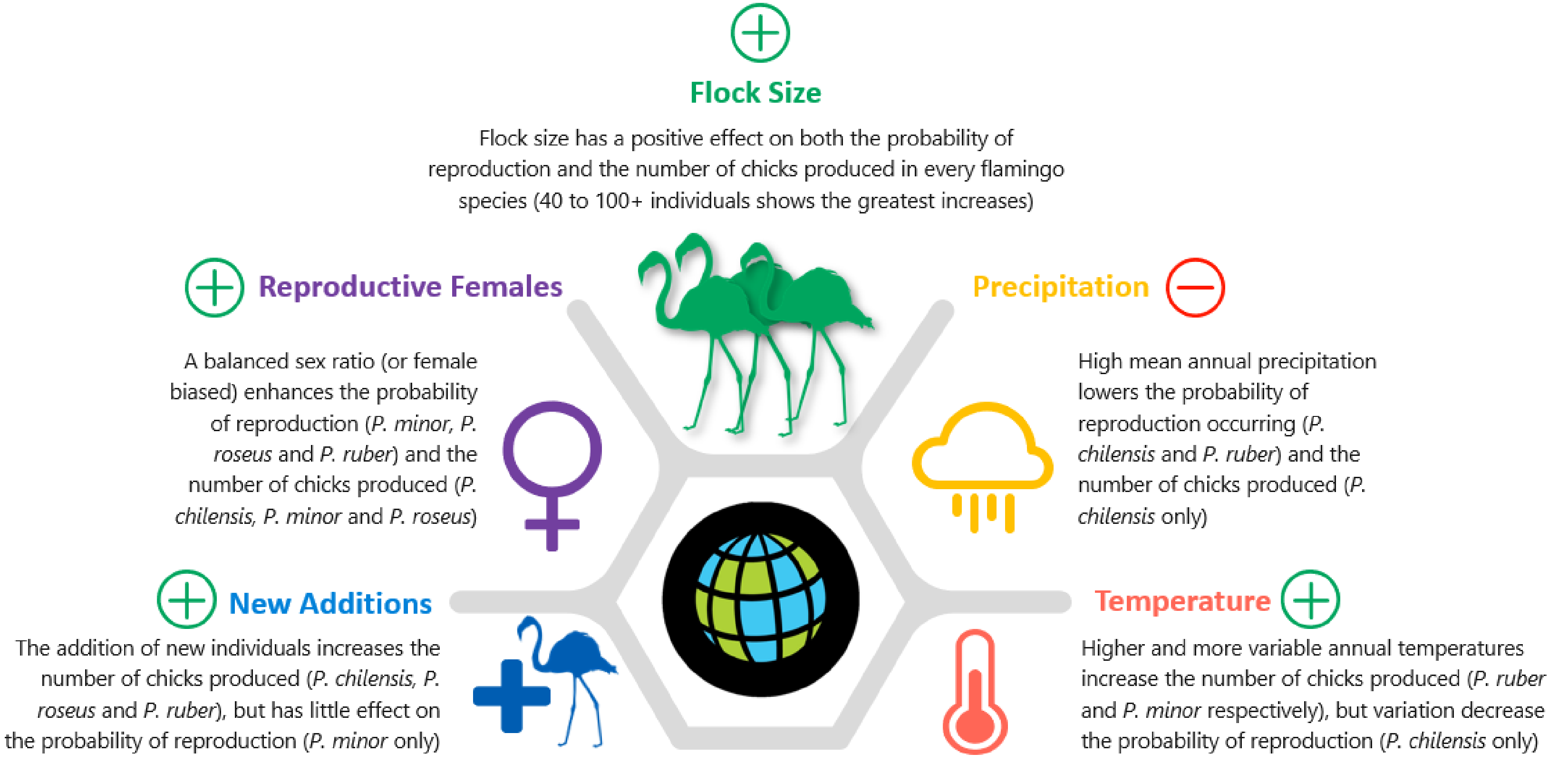


Figure 3.10 | The relationship between *ex situ* flock size and the number of chicks produced for *Phoenicopterus ruber* at varying proportions of new additions per flock between 1990 and 2018. Coloured lines represent predicted values on the original response variable scale.



Flock Size ⊕

Flock size has a positive effect on both the probability of reproduction and the number of chicks produced in every flamingo species (40 to 100+ individuals shows the greatest increases)

Reproductive Females ⊕

A balanced sex ratio (or female biased) enhances the probability of reproduction (*P. minor, P. roseus* and *P. ruber*) and the number of chicks produced (*P. chilensis, P. minor* and *P. roseus*)

Precipitation ⊖

High mean annual precipitation lowers the probability of reproduction occurring (*P. chilensis* and *P. ruber*) and the number of chicks produced (*P. chilensis* only)

New Additions ⊕

The addition of new individuals increases the number of chicks produced (*P. chilensis, P. roseus* and *P. ruber*), but has little effect on the probability of reproduction (*P. minor* only)

Temperature ⊕

Higher and more variable annual temperatures increase the number of chicks produced (*P. ruber* and *P. minor* respectively), but variation decrease the probability of reproduction (*P. chilensis* only)

Figure 3.11 | A summary of the flock structure and climatic variables which influence both the probability of reproduction and the number of chicks produced in *ex situ* flamingo flocks of *Phoenicopterus chilensis* (*P. chilensis*), *Phoeniconaias minor* (*P. minor*), *Phoenicopterus roseus* (*P. roseus*) and *Phoenicopterus ruber* (*P. ruber*). Green circles denote positive relationships, whereas red circles denote negative relationships. Flamingo silhouette image provided by Phylopic under Public Domain license.

3.5 Discussion

Here I used globally shared zoological records to investigate temporal trends in *ex situ* flamingo flock size and reproductive output, identifying the optimal flock size and structure necessary to enhance reproductive success. The overall increase in both flock size and the number of chicks per flock between 1990 and 2018 suggest that *ex situ* flamingo management is moving in a positive direction. I confirm here, for four flamingo species commonly kept in zoos and aquariums, the positive effect of flock size on both the probability of a flock reproducing and the number of chicks produced. However, flock size guidelines are not consistent among flamingo species and species-specific differences must be considered and incorporated into management recommendations. While I found that reproductive success is indeed enhanced in flocks of 40+ individuals, reinforcing the flock size recommendations of the 2005 Flamingo Husbandry Guidelines (Brown and King 2005), a flock size of just 40 individuals results in a probability of reproduction of just 0 - 12% for the four species. Rather than viewing 40 individuals as the target, this is an absolute minimum flock size if reproductive success is the institutional-specific goal of *ex situ* management, and I suggest that significantly larger flocks should be maintained wherever possible to achieve reproductive success. Flock sizes of between 69 and 103 birds appear necessary to achieve a 50% probability of reproduction in any year, however for *Phoeniconaias minor* the required flock size increases to 127 birds, with a negligible probability of reproduction in flocks of less than 100 individuals of this species. An increase in flock size will not only increase the reproductive success of captive flamingos, but as shown in Chapter 2, an increase in the number of institutional animals also increases both visitor numbers and consequently *in situ* conservation investment (Mooney *et al.* 2020).

I confirm that both an even sex ratio and the introduction of new individuals positively influence reproductive success in all species, consistent with previous studies (King 2008; Stevens 1991; Stevens and Pickett 1994), however the influence of both sex ratio and flock additions can vary depending on the flock size context. While I found that climatic variables in general play a limited role in determining captive reproductive success, high annual rainfall is negatively associated with reproductive success, consistent with King (2008). I encourage a more detailed assessment incorporating seasonality and photoperiod to potentially reveal more nuanced relationships not identified here (Wilson *et al.* 2019).

Despite the majority of contemporary flocks still being well below the previously recommended minimum flock size of 40 birds, important synergistic interactions of flock size with both the proportion of reproductive females and the proportion of new individuals per flock provide an important and underappreciated mechanism to mitigate the effects of small flock sizes and encourage reproduction through management practice. Although the effect is greatest at larger flock sizes, the introduction of new individuals into small *Phoenicopterus ruber* flocks can increase reproductive success the following year, compared to similarly sized flocks with no new individuals. This relationship is reversed in *Phoenicopterus chilensis*, whereby the greatest positive effect of adding new individuals is at smaller flock sizes. Based on this evidence I recommend that institutions regularly move individuals to help encourage reproduction, particularly in smaller flocks. Although genetic management is not currently considered an effective or practical management solution for flamingos, the regular movement of individuals may simultaneously increase reproductive success while limiting any inbreeding depression and genetic diversity loss in *ex situ* populations (King and Bračko 2014). However, I also believe that further research is warranted on the possibility of separating and reintroducing existing flocks prior to the breeding season to encourage reproduction, particularly where physical transfer of individuals is not possible or practical (Shannon 2000; Stevens and Pickett 1994). Reproductive success can also be enhanced in smaller flocks of both *Phoenicopterus chilensis* and *Phoeniconaias minor* through an increase in the proportion of reproductive females present. This is particularly important in flocks of *Phoeniconaias minor*, where an overall male bias exists and minimum flocks of 100 birds are required for reliable reproduction (Table 3.2; Figure 3.5b). Here, an increase in the proportion of reproductive females to achieve an even sex ratio can decrease the flock sizes necessary to achieve reliable reproduction to approximately 50 birds while simultaneously addressing known welfare concerns (Appendix Figure B.20).

The presence of unattached males is known to be disruptive to colony dynamics and can result in higher rates of egg breakage (King 2006; King 2008). Therefore, an approach towards an even sex ratio should also be considered a priority from an animal welfare perspective. Similarly, the extremely high prevalence of monogamy and long-term pair bonds in captive flamingos, despite their rarity in the wild, is likely a direct result of limited mate choice (Johnson and Cézilly 2008; Rose and Croft 2020; Rose *et al.* 2014). An increase in both flock size and the periodic introduction of new individuals could potentially provide a tool to promote wild type behaviour in captive settings. Increasing flock size not only provides greater opportunities for mate choice, but also allows for enhanced social stimulation in the form of synchronised group displays, pair formation, and nesting - key elements of flamingo behavioural welfare (Rose *et al.* 2014; Stevens 1991). Despite being a colonial species, captive flamingos have been shown to have preferred dyadic relationships, spanning several years (Rose and Croft 2020). This suggests that breaking up existing social groups may prove disruptive; however, it may also facilitate new pair formation, increasing social interactions, and outbreeding opportunities (Shannon 2000). Further work is required in this area and greater consideration needs to be given to the social dynamics within flocks and how management practices influence these dynamics (Shannon 2000).

Although comprehensive, this study is unable to capture important determinants of reproductive success, such as institutional management practices, enclosure design, diet, and wing condition (Sandri *et al.* 2018). For example, it has previously been noted that the movement of flamingos indoors at night negatively influences reproductive success (King 2008). Similarly, the pervasive problem of foot lesions in captive flamingos is a noted cause of high mortality and is likely to influence reproductive success, despite not being observed in the feet of wild flamingos (King and Bračko 2014; Nielsen *et al.* 2010). The wing condition of male birds (full-winged, wing-clipped, or pinioned) is arguably the main cause of male infertility, with up to 75% of traditionally pinioned males unable to successfully copulate (King 2008). Pinioned males appear unable to correctly balance on the back of females during copulation, a reproductive obstacle not seen in wild populations, and as a result the maintenance of full-winged birds has been encouraged to increase reproductive success (Duplaix-Hall and Kear 1975; Pickering 1992). Although flamingos are not currently under any known contraceptive or management practice that would prevent reproduction, deliberate institutional interventions to stop reproduction in very large flocks have been known to occur and may explain why my results show decreases in reproductive success at very large flocks (approximately 150+ individuals) (Rose *pers. comms.*). Similarly, these results may reflect limited space availability and population density issues, warranting further investigation. While these management issues are not in the scope of the current study, they are dealt with in greater detail as part of the Flamingo Husbandry Guidelines and the 11 EAZA Ciconiiformes and Phoenicopteriformes TAG strategies (Brown and King 2005; King and Bračko 2014).

With the implementation of the recommendations presented here, in conjunction with the further research outlined above, I believe that the sustainability of *ex situ* flamingo populations can be further enhanced. Doing so will rely on the co-operation and cohesive management of flamingo populations across all stakeholders and the continued sharing of global zoological records through standardised databases, such as ZIMS. The relationships and patterns revealed here were only possible through the availability of long-term globally standardised animal records, however I expect that a large number of institutions containing substantial flamingo populations are not considered in this study due to a lack of data recording and/or shared reporting. The implementation of standardised animal record sharing is critical to understand the current status and sustainability of all *ex situ* populations globally, not just flamingos. A lack of standardised record sharing can place *ex situ* conservation breeding programmes at risk of failure and result in the extinction of species, as demonstrated by the recent extinction of the Catarina pupfish (*Megupsilon aporus*) in 2014 (da Silva *et al.* 2019; Valdés González *et al.* 2020). The limited number of Species360 member institutions in the Southern Hemisphere points to a clear divide that needs to be bridged if the equitable sharing of knowledge and the promotion of *ex situ* population sustainability is to be realised. The ability for current Species360 member institutions to sponsor the membership fee of other institutions provides one mechanism for economically disadvantaged institutions to share their data in such a manner, while simultaneously allowing existing institutions to increase their conservation investment.

Ultimately the implementation of the recommendations will fall upon regional and institutional population managers. Fortunately, the extreme popularity of flamingos with all stakeholders has made it easier to gain momentum for management improvements compared to other avian species, with the majority of institutions willing to make necessary management changes in order to increase population sustainability (King and Bračko 2014). However, this popularity has also made it clear that certain institutions are reluctant to relocate their flamingos to other institutions (King and Bračko 2014). In addition, limited space, lack of standardised husbandry practices, the need for the importation of new individuals, and for improved fulfilment of breeding and transfer recommendations in managed populations all pose significant problems for currently managed *ex situ* populations (Wilson *et al.* 2019). Nearly 40% of all AZA cooperatively managed colonial bird breeding programmes state that they require additional space if population sustainability goals are to be met (Wilson *et al.* 2019). If all flamingo populations reach the minimum recommended population sizes and reproductive rates necessary, population sustainability will only continue if these high reproductive rates are maintained, which will only occur if significantly more space is made available. This will necessitate international collaboration and communication between zoological organisations and may involve the incorporation of non-zoological facilities, such as sanctuaries and non-accredited institutions, to provide additional species holding capacity in order to ensure population growth rates can be maintained (Wilson *et al.* 2019). Although seemingly simple, the recommendation to periodically move individuals between flocks may also encounter significant obstacles, with 33% of all co-operatively managed AZA populations having reported issues with the fulfilment of breeding and transfer recommendations between institutions (Wilson *et al.*

2019). The joint development of the Flamingo Husbandry Guidelines between AZA, EAZA and WWT Slimbridge is a promising sign that these goals can be realised, and that inter-regional management is possible. This shift from regional to global management, although logistically challenging, can provide one solution to these issues. Global species management can be achieved through the suggestion of the WAZA Committee for Population Management to manage all *ex situ* populations of a species under a single Global Species Management Plan, as already implemented for babirusa (*Babyrousa sp*.) (Macek 2014; WAZA 2020a).

The sustainable management of *ex situ* flamingo populations is only one component of the global effort to conserve flamingos. Through this thorough exploration of the population dynamics and climatic conditions underlying captive flamingo reproduction I hope to add to the global body of knowledge on species-specific flamingo biology and ecology. This is in line with the integrated approach to species conservation encouraged by the IUCN CPSG; their "*One Plan Approach*" promotes the exchange of knowledge and collaboration between all parties involved in the conservation of a species (Byers *et al.* 2013). Given their ubiquity and popularity across zoological collections, I suggest that flamingos could also benefit from an Integrated Collection Assessment and Planning (ICAP) workshop, a collaborative process developed by the IUCN CPSG in collaboration with regional zoo and aquarium associations (Traylor-Holzer *et al.* 2019). This would bring together members of the *in situ* and *ex situ* communities to help apply the IUCN SSC Guidelines on the Use of *Ex Situ* Management for Species Conservation to the issue of regional and global *ex situ* flamingo management. Such a workshop could provide guidance to institutions on conservation and education messaging priorities, in addition to identifying opportunities for the integration of *in situ* and *ex situ* conservation efforts (Traylor-Holzer *et al.* 2019). Given ongoing biodiversity loss, connecting the power of globally shared *ex situ* records and management expertise with *in situ* conservation practitioners is critical to the identification of the most efficient conservation actions and potential management strategies necessary to ensure that both *in situ* and *ex situ* populations remain sustainable long into the future.

4. Maximising the potential for living cell banks to contribute to global conservation priorities

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Author contributions: All authors developed the concept of the manuscript. A.M., O.R. and M.H. collected the data. A.M. undertook the analysis and drafted the text in consultation with J.S., D.A.C and Y.M.B. A.M. produced the tables and figures. All authors contributed to the writing of the MS.

4.1 Abstract

As biodiversity continues to decline globally, the role of gene banks in preserving genetic diversity (biobanking) has become increasingly important. Although biobanking represents a powerful conservation tool, a lack of standardised information surrounding the species currently represented within global gene bank collections and the inconsistent selection of species for future sample collection both hinder the conservation potential of biobanking, resulting in missed conservation opportunities and enhancing extinction risks. I investigate the representation of amphibian, bird, mammal and reptile species within the San Diego Zoo Frozen Zoo® living cell collection and subsequently implement a qualitative framework for the identification and prioritisation of threatened species for future sampling efforts, considering their congruency with global conservation prioritisation schemes (including the IUCN Red List, CITES, AZE, EDGE and Climate Change Vulnerability), and the probability of sample acquisition from within the global zoo and aquarium community. I show that 965 species, including 5.1% of all IUCN Red List "*Threatened*" amphibians, birds, mammals and reptiles, are currently represented within the collection and that sampling from within existing zoo and aquarium collections can increase representation to 16.5% (1,006 species). I identify three species for priority sample collection listed under every prioritisation scheme and which are also represented within the global zoo and aquarium community, including the whooping crane (*Grus americana*), crested ibis (*Nipponia nippon*) and Siberian crane (*Leucogeranus leucogeranus*), while also providing species prioritisations based on subsets of prioritisation schemes and sampling opportunities. I highlight a lack of available quantitative data, difficulties in obtaining *in situ* samples and concerns over genetic resource sovereignty, and encourage the formation of a global biobanking database and the establishment of new gene banks in biodiversity rich countries.

4.2 Introduction

The current rate of extinction has led to suggestions that we have now entered the sixth mass extinction event of our planet's history (Barnosky *et al.* 2011; Wake and Vredenburg 2008). Over the last century alone, the rate of vertebrate species extinctions has been estimated to be 100 times higher than the predicted background extinction rate (Ceballos *et al.* 2015). Beyond these documented species extinctions, drastic population declines and range contractions have been recorded for numerous extant species, even those currently of least conservation concern (Ceballos *et al.* 2017; Ceballos *et al.* 2020). Severe population declines and fragmentation are associated with a decrease in genetic diversity and increased vulnerability to demographic stochasticity (Ballou 1992). Loss of genetic diversity negatively affects both the long- and short-term fitness of a population, further enhancing the species' extinction risk through reductions in reproductive potential, disease resistance, environmental stress tolerance and survival (Allendorf and Leary 1986; Allentoft and O'Brien 2010; Ballou 1992; Keller and Waller 2002; Spielman *et al.* 2004; Wildt *et al.* 1987). As a result, there has been an urgent call to conserve extant global biodiversity, and particularly genetic diversity, in order to ensure population health and persistence (Reed and Frankham 2003).

Ex situ conservation provides an important tool for addressing the conservation of biodiversity; it consists of the preservation of species outside of their natural habitat (AZA 2016b). *Ex situ* conservation is primarily undertaken by the approximately 1,000 zoos and aquariums globally which are organised as part of internationally recognised organisations, such as the World Association of Zoos and Aquariums (WAZA) (Kelly 1997). However, zoos and aquariums can only maintain a tiny fraction of all extant species due to their limited carrying capacity and minimum population sizes required to ensure population sustainability (Conway 1986; Conway 2011; Foose 1983; Keulartz 2015). While zoos and aquariums currently manage 15% of all threatened terrestrial vertebrates (Conde *et al.* 2011), the majority of *ex situ* population management programmes are not meeting sustainability criteria (Lees and Wilcken 2009). In reality, the intensive demographic and genetic management afforded by *ex situ* conservation are currently hindered by small population sizes, poor population growth rates and incomplete pedigree records (Lees and Wilcken 2009; Staerk *et al.* 2018). Like small *in situ* populations, small *ex situ* populations are also vulnerable to demographic stochasticity and suffer from genetic diversity loss and inbreeding complications (Ballou 1992). It has therefore been estimated that zoos and aquariums only have the potential to manage sufficiently large *ex situ* populations of 100 mammal species if they are to achieve the demographic and genetic goals of *ex situ* conservation

(Conway 1987). However, the conservation of genetic variation through the cryogenic preservation of biological materials (termed ‘biobanking’) contributes additional genetic resources which can contribute to *ex situ* and *in situ* population sustainability and conservation. Zoos and aquariums have the ability to supplement the genetic diversity of their living collections through the use of gene banks, and their living collections can also provide an important source of samples for gene banks (Ballou 1992; Hobbs *et al.* 2018).

A genome resource (gene) bank is a collection of cryopreserved material (e.g. viable cell cultures, gametes, tissues samples, whole organisms and embryos, serum, plasma, red blood cells, feathers, hair) established to conserve the genetic diversity of a population of plants or animals (Hobbs *et al.* 2018; Wildt 1997). Cryopreservation at extremely low temperatures ensures that the structural and functional integrity of cells is maintained when returned to physiological temperatures, and, if stored appropriately, these cells can remain in a state of suspended animation for decades and possibly even centuries (Hobbs *et al.* 2018). Long-term cryopreservation of genetic material and living cells means that potentially high levels of genetic diversity can be maintained almost indefinitely, representing a unique resource for scientific study, captive population management and biodiversity conservation (Ballou 1992). Living cell cultures in particular represent an expandable resource that can be continually thawed, grown and a portion banked again for future use (Ryder and Onuma 2018).

When utilised in conjunction with assisted reproductive technologies, cryopreserved living cells have the potential to preserve and reintroduce genetic variation into future populations over long periods of time, maintaining a species’ adaptability potential (Critser and Russell 2000; Hobbs *et al.* 2018). Cryopreservation extends the reproductive lifetime of an individual almost indefinitely and removes limiting factors such as gender and age, ultimately permitting more species to be maintained *ex situ* (Ballou 1992; Praxedes *et al.* 2018). Similarly, the potential to introduce genetic diversity into wild populations, using cryopreserved living cell cultures and genetic rescue theory, provides one mechanism to reverse the effects of inbreeding depression, restore population health and potentially even reverse species extinctions (Folch *et al.* 2009; Staerk *et al.* 2018; Whiteley *et al.* 2015). Therefore, the long-term cryopreservation of genetic material represents a unique and powerful resource for scientific study, captive population management and *in situ* biodiversity conservation (Ballou 1992).

Although biobanking represents a potentially powerful tool for conservation, important composition and acquisition challenges associated with biobanking efforts remain: (1) a lack of knowledge surrounding the biodiversity currently represented within gene banks globally, (2) how to select and prioritise species for genetic sample collection in the future and (3) the availability of suitable samples for collection (CBSG 2015; Clarke 2009; CPSG 2016; Hobbs *et al.* 2018; Ryder *et al.* 2000; Staerk *et al.* 2018). The only coordinated attempt to characterise biodiversity within existing collections has been an assessment of the IUCN "*Critically Endangered*" and "*Extinct in the Wild*" species represented within five biobanking facilities (Ryder and Onuma 2018). In addition, the lack of a consistent species selection plan has been an issue raised by conservation practitioners at both the 2015 and 2016 Annual Meetings of the International Union for Conservation of Nature's Conservation Planning Specialist Group (IUCN CPSG) (CBSG 2015; CPSG 2016). To date, biobanking sample collection has been mainly opportunistic, and large-bodied species, with which we share emotional connections, are being shown default prioritisation preference and important conservation opportunities for other species are likely being missed, enhancing extinction risks (Hobbs *et al.* 2018). The prioritisation of management actions for conservation, such as the prioritisation of samples for acquisition, depends on the benefits provided from sample acquisition, the successful use of biobanked samples to improve conservation outcomes and the costs entailed (see Joseph *et al.* 2009).

The utilisation of existing prioritisation schemes has been suggested as one way to identify priority species for sample collection and *ex situ* conservation efforts (da Silva *et al.* 2019). Collectively, the IUCN Red List is recognised as one of the most widely used methods for species conservation prioritisation, providing an indicator for the probability of extinction and conservation status for wildlife populations globally (Adriaens *et al.* 2015; Hoffmann *et al.* 2008). Over 70,000 animal species have already been assessed under the IUCN Red List, with 19.4% currently considered "*Threatened*" with extinction (IUCN 2019). The IUCN Red List status is the conventional criteria considered for conservation action, and genetic sample collection of IUCN Red List "*Threatened*" species and those on the brink of extinction is deemed an urgent priority while the species in question still exists (CPSG 2016; Ryder and Onuma 2018). While prioritising species with the highest probability of extinction may preserve representatives of at-risk global biodiversity, it has been shown that the majority of "*Data Deficient*" species (amphibians and mammals) are also threatened with extinction and occupy smaller geographical ranges, compared with data sufficient species (Bland *et al.* 2015; Howard and Bickford 2014). Therefore, although comprehensive and invaluable, there are further criteria that should be considered beyond the IUCN Red List to assess the probability of extinction when prioritising species for conservation actions.

The IUCN Red List can be incorporated into alternative prioritisation schemes to consider other important criteria and species characteristics, providing more detailed recommendations for species conservation prioritisation. The Alliance for Zero Extinction (AZE) identifies species which are currently listed as *“Threatened”* under the IUCN Red List, and which are also restricted to a single geographic location (Ricketts *et al.* 2005). For example, the Christmas frigatebird (*Fregata andrewsi*) and Abbott's booby (*Papasula abbotti*) are both *“Threatened”* under the IUCN Red List and all their known populations are confined to Christmas Island (AZE 2018). These species represent a uniquely threatened group of animals, beyond their IUCN status alone, and are therefore considered high priorities for living cell cryopreservation (Ryder and Onuma 2018). Similarly, evolutionarily unique species have been put forward as potential priorities for genetic sampling (Ryder and Onuma 2018), and the ‘Evolutionary Distinctiveness and Global Endangerment’ (EDGE) score provides one method of reconciling those species which are both *“Threatened”* under the IUCN Red List and which are also phylogenetically distinct (Isaac *et al.* 2007; Isaac *et al.* 2012). The overexploitation of natural populations through the unsustainable global trade in wildlife has been recognised as one of the most prominent threats to global biodiversity, threatening the persistence of natural populations and enhancing the probability of extinction (Engler and Parry-Jones 2007; Roe 2008; Rosen and Smith 2010; Wyler and Sheikh 2008). Although the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) tries to regulate the legal trade in wildlife, the species currently covered under CITES represent possible priorities for genetic sample collection, owing to their enhanced probability of extinction beyond that assessed by the IUCN Red List. Each of these prioritisation schemes highlight a uniquely threatened and vulnerable group of species that can be considered collectively or individually as priorities for future genetic sample collection, depending on the institutional-specific goals and priorities of individual gene banks.

Although the prioritisation of species at higher risk of extinction will likely lead to large conservation benefits, due to their currently small population sizes, this also results in the selection of species with limited genetic diversity available and which require large resource investment, ultimately providing limited prospects of meaningful recovery (Hobbs *et al.* 2018). In reality, genetic sample collection should not be taken at the time of extinction, but rather prior to it, when sufficient extant genetic diversity still exists. This would ensure the highest probability of successful implementation of cryopreserved samples and therefore recovery success (Ryder and Onuma 2018). By prioritising species with sufficiently large populations and genetic diversity remaining, and which are most likely to benefit from genetic rescue, we can maximise the reduction in extinction probability. Balancing the current probability of extinction with the probability of both sample acquisition and successful implementation in the future, will ultimately provide the most cost-effective manner in which to prioritise species for sample selection. Although the most appropriate methodology to identify such species remains unclear, the threat level of the species is likely to be an important consideration. For example, Foden *et al.* (2008) highlight that up to 71% of species currently listed as "*Least Concern*" under the IUCN Red List are susceptible to elevated extinction risks due to climate change. These species are typically of low conservation concern, yet they represent species which are susceptible to rapid population declines if climate change projections are realised (Keith *et al.* 2014). In addition, species already sampled, species yet to be sampled, keystone species, ecosystem engineers, pollinators, scavengers, species of economic value and species of scientific value have all been put forward as potential priorities for genetic sampling for various reasons (CPSG 2016; Holt *et al.* 1996; Hobbs *et al.* 2018; Houck 2016; Ryder *et al.* 2000). Although the goal and function of individual gene banks may vary, globally limited resources mean that the way in which we select species for sampling must follow a clear and transparent methodology. This is imperative if biobanking is to be considered a respected and viable method of conservation moving forward and to ensure that the conservation potential of cryopreserved genetic samples can be fully realised.

Facilities containing cryopreserved genetic samples can be found in Africa, Asia, Australia, Europe and North America, many in co-operation with local zoos and aquariums (Clarke 2009; Hobbs *et al.* 2018; Ryder and Onuma 2018). Given their diverse living collections, ease of access, global distribution, veterinary expertise, and financial resources, zoos and aquariums have a unique opportunity, if not obligation, to contribute to biobanking and the preservation of genetic materials for scientific study and conservation (Benirschke 1984; Praxedes *et al.* 2018). The Frozen Zoo® collection housed at San Diego Zoo Institute for Conservation Research is by far the largest and most diverse gene bank collection of wildlife living cell cultures globally (SDZG 2020). Established in 1975, this collection now contains an estimated 10,000 living cell cultures, representing nearly 1,200 taxa, including a cell culture of the extinct po'ouli (*Melamprosops phaeosoma*), a bird native to Maui (Peterson 2016). In order to increase the probability of sample acquisition, reduce acquisition costs and maximise the genetic diversity of acquired samples, we should utilise the sampling opportunities provided by the global zoo and aquarium community to prioritise species for sample collection. Similarly, the interception and confiscation of animals involved in the legal and illegal wildlife trade presents a non-traditional, but invaluable, opportunity to increase the probability of acquisition and acquire a diverse range of samples, particularly from species currently protected under CITES. In the absence of such sampling opportunities, *in situ* sample collection can also be conducted in collaboration with zoos and aquariums, many of which are involved in the *in situ* conservation of species (Dickie *et al.* 2007).

Here I address the contemporary composition and acquisition challenges associated with global biobanking efforts by analysing for the first time the current representation of species (amphibians, birds, mammals and reptiles) within the Frozen Zoo® collection of living cell cultures housed at the San Diego Zoo Institute for Conservation Research. I compile indicators of current and future probability of extinction of the biobanked samples through the alignment of the collection with multiple global wildlife conservation prioritisation schemes (IUCN, CITES, AZE, EDGE and Climate Change Vulnerability). To guide future acquisition strategies, I use results of my composition survey to identify gaps within the current collection and prioritise for genetic sample collection the 6,122 species currently listed as *"Threatened"* (CR, EN, VU) or "*Extinct in the Wild*" (EW) under the IUCN Red List, assessing overlap with other conservation prioritisation schemes. A critical component of a prioritisation scheme for sample acquisition is the identification of resource-effective opportunities for sample collection. I identify opportunities for sample acquisition from the global zoo and aquarium community and confiscations or recovery of illegally traded CITES listed species. Recommendations from this assessment can be integrated into the global management of *ex situ* populations, prompting veterinary sample collection from certain species in the case of death or anaesthesia events. Failure to accurately quantify the biodiversity currently represented within biobanks and appropriately prioritise species for future genetic sample collection not only hinders the development of global biobanking efforts, but likely results in missed sample acquisition and conservation opportunities, increasing both extinction risks and global biodiversity loss.

4.3 Methods

4.3.1 Species Prioritisation for Genetic Sampling

A biobanking efficiency framework was developed for characterising the features of the San Diego Zoo Frozen Zoo® living cell collection based on the project efficiency metric developed by Joseph *et al.* (2009). However, due to a lack of quantitative data, formal prioritisation was not attempted, and a qualitative assessment is provided. Following the characterisation of the existing San Diego Zoo Frozen Zoo® living cell collection, according to the biobanking efficiency components, I identified gaps within the current collection and assessed for sample acquisition the 6,122 species (amphibians, birds, mammals and reptiles) currently listed as *"Threatened"* (CR, EN, VU) or "*Extinct in the Wild*" (EW) under the IUCN Red List (IUCN 2019). I assessed the probability of sample acquisition through the identification of opportunities for sample collection from the global zoo and aquarium community, as represented by Species360 members and potential illegal trade confiscations of CITES listed species.

4.3.2 Biobanking Efficiency Framework

Biobanking efficiency is outlined in Equation 4.1:

$$E_i = \frac{W_i B_i S_i}{C_i}, \quad \text{Equation 4.1}$$

where *E* is the biobanking efficiency score for the biobanked species *i*, *W* is the species weight which here represents phylogenetic distinctiveness, *B* is the conservation benefit of biobanking, *S* is the probability of project success and *C* is the cost of biobanking and associated sample implementation efforts. I have assigned indicators for each component of this biobanking efficiency framework which act to increase or decrease efficiency.

Species evolutionary distinctiveness can be used to increase the weighting, *W*, for species which are evolutionarily more distinct and for which biobanking may be particularly important due to a lack of close living relatives from which genetic information could be inferred. The Evolutionarily Distinct and Globally Endangered (EDGE) prioritisation scheme combines evolutionary distinctiveness of a species with global endangerment using IUCN Red List criteria (Isaac *et al.* 2007; Isaac *et al.* 2012). Evolutionary Distinctiveness is one possible measure of *W* in Equation 4.1. I consider that the EDGE prioritisation, which includes risk of extinction through the use of the IUCN Red List criteria for *"Threatened"* species, to indicate species of higher priority weighting if evolutionary distinctiveness is desired.

The conservation benefit, *B*, of biobanking is the difference in probability of persistence of a species with and without biobanking ($X_0 – X_i$), where X_i is the probability of extinction with biobanking and X_0 is the probability of extinction without biobanking. Given the breadth of global biodiversity with the potential to be sampled, I draw upon various wildlife conservation prioritisation schemes (IUCN Red List, CITES, AZE, and Climate Change Vulnerability) to indicate the probability of extinction, X_0. I assume that biobanking will reduce the threat of extinction to the value X_i.

The probability of project success, *S*, can loosely be defined in this context as the probability of successful introduction of genetic variation from biobanks into existing *in situ* and/or *ex situ* populations, enhancing population health and sustainability. The probability of project success, *S*, is highly dependent on species characteristics, the nature of the biobanking technology used and the probability of successful implementation of genetic rescue, which itself depends on *in situ* and/or *ex situ* breeding populations being available for management. The probability of biobanking success can therefore be indicated if an *ex situ* population currently exists and if it is being actively managed to ensure genetic and demographic population sustainability (*i.e.* the presence of a regionally managed *ex situ* studbook for the species). Biobanking technology, through the maintenance and use of living cell cultures, is assumed to be higher for better known groups of species, *i.e.* mammals > birds > reptiles > amphibians.

The cost of a project, *C*, is decreased (and therefore efficiency, *E*, increased) if samples are easily and cheaply attainable and the biobanking technology is well developed. I assume that samples from species which are represented within zoos and aquariums will be more easily and cheaply obtained. I also assume that samples from confiscations of CITES listed species will also be more easily obtained than species in their natural habitats.

4.3.3 Frozen Zoo® | San Diego Zoo Institute for Conservation Research

To establish the representation of species within the Frozen Zoo® collection housed at the San Diego Zoo Institute for Conservation (San Diego Zoo Frozen Zoo® hereafter), I analysed the 9,830 living cell culture accessions (representing 1,001 taxa) deposited as of April 2019. I excluded 2.6% records (26 taxa out of 1,001) which referred to hybrid individuals, 0.8% of records (8 out of 1,001 taxa) which referred to genus only level individuals and 0.2% of records (2 taxa out of 1,001) which referred to fish samples. This left only amphibian, bird, mammal and reptile samples to be assessed. I characterise, for the first time, the conservation value of the species represented within the San Diego Zoo Frozen Zoo® collection through their probability of extinction and their congruency with global wildlife conservation prioritisation schemes (IUCN, CITES, AZE, EDGE and Climate Change Vulnerability) as outlined below.

4.3.4 Conservation Priorities

Each of the prioritisation schemes outlined below highlights a uniquely threatened and vulnerable group of species, with a heightened probability of extinction, which are considered current priorities for conservation intervention. Prioritisation of such threatened species will increase the conservation benefit, *B*, of potential biobanking efforts, increasing the probability of persistence of a species. These prioritisation schemes can be considered individually or collectively to identify priorities for future genetic sample collection. Here I highlight the representation of species under each prioritisation scheme within the living cell collection of the San Diego Zoo Frozen Zoo®. As many species are included under more than one prioritisation scheme, I also assess the overlap of currently represented species between different prioritisation schemes. Similarly, when prioritising IUCN Red List *"Threatened"* and "*Extinct in the Wild*" species not currently represented within the San Diego Zoo Frozen Zoo®, I highlight the species under each prioritisation scheme individually and also their overlap between different prioritisation schemes to identify priorities for future sample collection. Considering these prioritisation schemes collectively helps to identify the most at-risk and uniquely vulnerable species with heightened extinction risks beyond that assessed by individual prioritisation schemes. If sampled, such species can greatly increase the conservation benefit, *B*, of cryopreserved genetic samples, reducing extinction risks and increasing the probability of persistence of a species.

4.3.5 IUCN Red List

The International Union for Conservation of Nature's (IUCN) Red List is the most comprehensive resource summarising the conservation status and extinction risk for plant and animal species globally (Rodrigues *et al.* 2006). Species with no known extant individuals are categorised as "*Extinct*" (EX). Species which only survive under *ex situ* management are categorized as "*Extinct in the Wild*" (EW). Species categorized as "*Critically Endangered*" (CR), "*Endangered*" (EN) and "*Vulnerable*" (VU) are collectively referred to as *"Threatened"*, and they represent species with elevated risks of extinction and endangerment respectively. Species which do not qualify for a *"Threatened"* status, typically widespread and abundant species with lower extinction risks, are categorised as "*Near Threatened*" (NT) and "*Least Concern*" (LC). In addition, species which lack sufficient data to make an adequate risk assessment are categorized as "*Data Deficient*" (DD) and species yet to be evaluated are categorised as "*Not Evaluated*" (NE) (IUCN 2019). Although the 34 animal species currently categorised as "*Extinct in the Wild*" are not considered *"Threatened"*, they represent species which are extremely vulnerable to extinction, as can be seen by the recent extinctions of the Pinta Island tortoise (*Chelonoidis abingdonii*) and the Catarina pupfish (*Megupsilon aporus*), species which had both been classified as EW prior to their extinctions (Trask *et al.* 2020). Here I analyse and summarise the IUCN Red List status, and therefore probability of extinction, of all species currently represented within the living cell cultures of the San Diego Zoo Frozen Zoo® and their overlap with other conservation prioritisation schemes.

4.3.6 CITES

The global legal trade in wildlife is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), an agreement between 183 Parties (State governments or regional economic integration organisations) with the aim of ensuring that the international trade in wildlife does not threaten the ability of a species to persist in the wild (CITES 1973). There are approximately 5,800 animal species listed on one of the three CITES Appendices, protecting them from overexploitation as a result of the international wildlife trade (CITES 2019). Species listed in Appendix I are those highly threatened with extinction, and, as a result, trade in such species is only permitted in unique circumstances. Appendix II species are those which are not necessarily threatened with extinction, but which have the potential to become threatened if their trade is not strictly controlled. Appendix III species are again not necessarily currently threatened with extinction, but where one country has requested other CITES Parties to assist in controlling the trade of the species (CITES 1973). Owing to the international trade in wildlife, these species possess a heightened probability of extinction beyond their IUCN Red List assessments. Here I analyse the number of CITES listed species represented within the living cell cultures of the San Diego Zoo Frozen Zoo® and their overlap with other prioritisation schemes, identifying gaps for future priority sample collection. Additionally, the availability of intercepted and confiscated CITES listed species from the illegal wildlife trade represents a unique opportunity to obtain samples and reduce the cost of a project, *C*, therefore increasing overall biobanking efficiency.

4.3.7 Alliance for Zero Extinction (AZE)

The Alliance for Zero Extinction (AZE) works with governments, multilateral institutions and conservation-oriented organisations to identify and conserve global sites of biodiversity conservation importance and species at a heightened risk of extinction due to restricted geographic ranges. The AZE identifies species which are currently listed as "*Critically Endangered*" (CR) or "*Endangered*" (EN) under the IUCN Red List, and which are also restricted to a single geographic site globally, making them particularly vulnerable to extinction beyond their IUCN Red List status (Ricketts *et al.* 2005). AZE currently recognises 1,483 threatened species (representing amphibians, birds, mammals, reptiles, fish, crustaceans and selected plants), which are distributed among 853 sites globally (AZE 2018). Here I assess the representation of AZE listed amphibian, bird, mammal and reptile species within the living cell cultures of the San Diego Zoo Frozen Zoo® and identify gaps for future sample collection.

4.3.8 Evolutionary Distinct and Globally Endangered (EDGE)

The evolutionary distinctiveness of a species is one potential way to prioritise species for conservation actions, and this can be done using a species' EDGE score. EDGE scores are a combination of the amount of unique evolutionary history a species represents (Evolutionary Distinctiveness/ED) in addition to its current conservation status (Global Endangerment/GE) (Isaac *et al.* 2007; Isaac *et al.* 2012). In order to qualify as EDGE, a species must be phylogenetically distinct (ED score above the median), and also be assessed as "*Critically Endangered*" (CR), "*Endangered*" (EN) or "*Vulnerable*" (VU) under the IUCN Red List. Currently EDGE lists exist for amphibians, birds, corals, mammals, reptiles and elasmobranchs, with approximately 13% of all amphibians currently being considered EDGE (EDGE 2019). Here I assess how many species of amphibians, birds, mammals and reptiles, which have been identified as EDGE (both evolutionarily distinct and also globally endangered), are represented within the living cell cultures of the San Diego Zoo Frozen Zoo®, and I identify gaps for future priority sample collection.

4.3.9 Climate Change Vulnerability (CCV)

The role of climate change in increasing extinction rates and biodiversity loss has already been confirmed, therefore species which are highly vulnerable to climate change can be considered of high conservation priority (Gardali *et al.* 2012; Stork 2010; Williams *et al.* 2003). The IUCN trait-based assessment of species' vulnerability to climate change is one method of measuring this vulnerability and prioritising candidate species for conservation action (Foden *et al.* 2013). These assessments incorporate individual species' sensitivity (ability to persist *in situ*), adaptive capacity (measured as dispersal ability and evolvability) and their predicted exposure to projected climatic changes (projected changes in temperature and precipitation). Trait-based climate change vulnerability assessments have currently been completed for all amphibian, bird and coral species, with all species assessed as high or low vulnerability to climate change (Foden *et al.* 2013). Here I assess the number of amphibian and bird species currently listed as highly vulnerable to climate change by Foden *et al.* (2013) which are also represented within the living cell cultures of the San Diego Zoo Frozen Zoo® and their overlap with other prioritisation schemes, identifying gaps for future sample collection. Given the potential of gene banking for preserving vast amounts of extant genetic material, I also identify species which are currently not listed as *"Threatened"* under the IUCN Red List, but which are highly vulnerable to future climate change. These species represent new conservation priorities, as they will likely become threatened in the future if current climate change projections are realized. However, contemporary sampling of such species while sufficient genetic variation remains will likely result in an increased probability of successful implementation of cryopreserved samples, increasing the probability of project success, *S*, and therefore reducing future extinction probabilities (Foden *et al.* 2013).

4.3.10 Sample Collection Opportunities from Zoos and Aquariums

Given the influence and importance of zoo and aquarium collections in biobanking efforts to date, I used vertebrate composition data from the global zoo and aquarium community (as represented by Species360 member institutions) to identify opportunities for future genetic sample collection. Species360 is an international non-profit organisation that hosts and develops the Zoological Information Management System (ZIMS), the largest real-time database of comprehensive and standardised information spanning more than 1,100 zoological collections globally (Species360 2019; ZIMS 2019). This reveals which species are currently being managed *ex situ*, the number of institutions currently managing them and their current population sizes, increasing the probability of sample acquisition and reducing project costs, *C*, helping to inform species prioritisation. I particularly highlight the species being managed *ex situ* to ensure genetic and demographic sustainability via managed studbooks in the European Association of Zoos and Aquaria (EAZA) and the Association of Zoos and Aquariums (AZA, North America). The availability of genetically-characterised, managed populations will increase the probability of project success, *S*, subsequently increasing overall biobanking efficiency, *E*. I also emphasise the potential of zoos and aquariums to contribute to biobanking for the species currently categorised as "*Extinct in the Wild*". These species only persist *ex situ* and therefore zoos and aquariums represent the only option for potential sample collection in the future. Although my focus is on Species360 member institutions, it is imperative to stress that other zoos and aquariums which are not Species360 members also contain valuable living collections, however the lack of standardised data sharing for these collections results in my inability to obtain sufficient data to include these institutions.

4.3.11 Taxonomic Standardisation

Taxonomy for the San Diego Zoo Frozen Zoo® collection was standardised to the species level using the currently accepted scientific name according to the IUCN (genus and epithet). I retrieved the currently accepted IUCN name using the '*taxize*' R package (version 0.9.5; Chamberlain and Szöcs 2013), which also assesses synonyms. Manual searches were done for species not automatically standardised and in the absence of a current IUCN name or synonym I used the currently accepted name according to the Catalogue of Life (Roskov *et al.* 2019). Taxonomy was standardised across each prioritisation scheme and data source in the same manner as conducted for the San Diego Zoo Frozen Zoo® collection. The San Diego Zoo Frozen Zoo® collection, and several of the prioritisation schemes (IUCN Red List, CITES), operate at the subspecies level, whereas other schemes work at the species level (AZE, EDGE, Climate Change Vulnerability). In order to ensure consistency between data sources, all subspecies were reduced to species. Figures were generated using the '*ggplot2*' R package (version 3.2.1; Wickham 2016). Animal silhouette images used throughout are provided by Phylopic under Public Domain license (http://phylopic.org/). Venn diagrams were generated using the Bioinformatics and Evolutionary Genomics web tool (2020).

4.4 Results

4.4.1 San Diego Zoo Frozen Zoo® Collection

The San Diego Zoo Frozen Zoo® living cell collection comprises 9,750 samples representing 965 species of the four studied taxonomic classes, with a mean number of 10.1 (± 20.14 S.D.) samples per species (Table 4.1; Figure 4.1). Mammals are the most represented class with 8,021 samples representing 511 species. Mammals also possess the largest sample sizes per species, with a mean of 15.7 (± 25.80 S.D.) samples. Amphibians and reptiles are the least represented classes, with only 24 and 120 species and mean sample sizes of 3.71 (± 5.31 S.D.) and 2.85 (± 2.95 S.D.) respectively (Table 4.1; Figure 4.1).

4.4.2 IUCN Red List

Of the 965 species currently held in the San Diego Zoo Frozen Zoo®, 311 (32.2%) are currently listed as *"Threatened"* under the IUCN Red List (Table 4.1; Figure 4.2). These 311 species represent 5.1% (311 out of 6,110 species) of all *"Threatened"* amphibian, bird, mammal and reptile species under the IUCN Red List. Mammals are the most represented class, with 185 *"Threatened"* species currently being found in the San Diego Zoo Frozen Zoo®, representing 15.2% of all *"Threatened"* mammals (185 out of 1,219 species). Amphibians are the least represented class, with only 1.1% of IUCN *"Threatened"* species having been already sampled (9 out of 2,092 species). Of the remaining species; 538 are listed as "*Least Concern*", 75 as "*Near Threatened*", 9 species are listed as "*Data Deficient*" and 25 have yet to be assessed (Table 4.2; Figure 4.2). Importantly, 50% of the "*Extinct in the Wild*" species (6 out of 12 species) have already been sampled, including all EW mammal species (Table 4.2).

Table 4.1 | The representation of amphibian, bird, mammal and reptile species within the San Diego Zoo Frozen Zoo® (SDZG FZ) and a comparison with the number of total and “*Threatened*” species listed under the IUCN Red List.

	San Diego Zoo Frozen Zoo®				**IUCN Red List**	
	Total Species	Threatened Species (% of Total Species)	Total Samples	$\bar{x}$ Samples per Species (± S.D.)	Total Species (% in SDZG FZ)	Threatened Species (% in SDZG FZ)
Amphibia	24	9 (37.5%)	89	3.71 (± 5.31)	6722 (0.4%)	2092 (1.1%)
Aves	310	70 (22.6%)	1298	4.12 (± 7.16)	11126 (2.8%)	1492 (4.7%)
Mammalia	511	185 (36.2%)	8021	15.7 (± 25.80)	5692 (9.0%)	1219 (15.2%)
Reptilia	120	47 (39.2%)	342	2.85 (± 2.95)	7217 (1.7%)	1307 (3.6%)
Total	965	311 (32.2%)	9750	10.1 (± 20.14)	30757 (3.1%)	6110 (5.1%)

Table 4.2 | The IUCN Red List status of the species present within the San Diego Zoo Frozen Zoo® living cell collection. Percentages represent the percentage of total IUCN Red List species within that particular category that are currently found within the San Diego Zoo Frozen Zoo®.

	DD	LC	NT	VU	EN	CR	EW	EX	NA	Total
Amphibia	0	14	0	2 (0.3%)	4 (0.4%)	3 (0.5%)	1 (50%)	0	0	24
Aves	0	210	27	37 (4.6%)	18 (3.8%)	15 (6.7%)	3 (60%)	0	0	310
Mammalia	7	259	44	74 (13.8%)	73 (15.1%)	38 (18.9%)	2 (100%)	1	13	511
Reptilia	2	55	4	21 (4.2%)	9 (1.7%)	17 (5.9%)	0 (0%)	0	12	120
Total	9 (0.3%)	538 (2.9%)	75 (3.5%)	134 (5.4%)	104 (4.4%)	73 (5.8%)	6 (50%)	1 (0.3%)	25	965

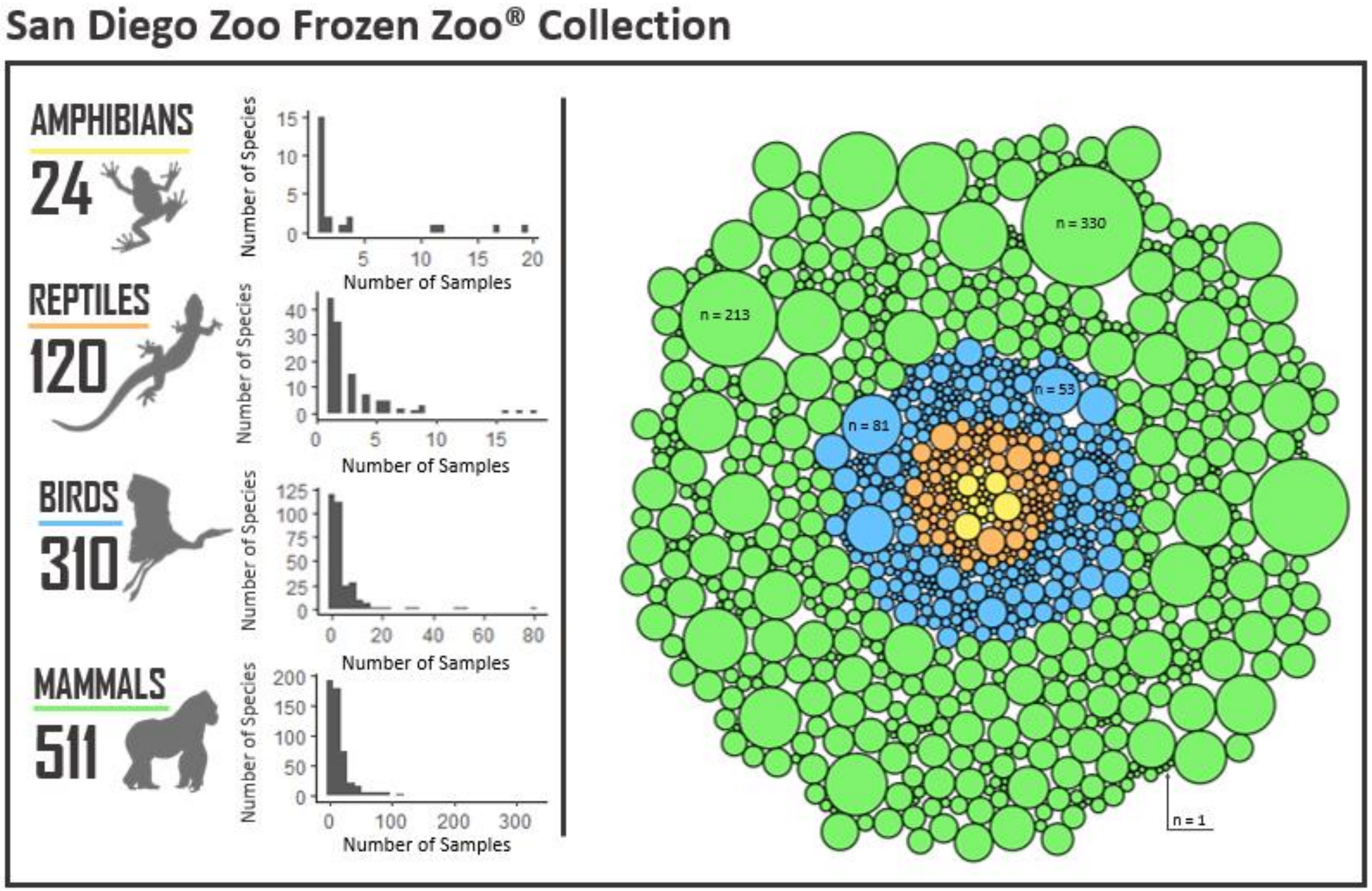

Figure 4.1 | The total number of amphibian, bird, mammal and reptile species within the San Diego Zoo Frozen Zoo®. Also shown are the number of samples per species within each class. The sample size of each species (n = 965) is also represented by individual bubbles on the right. The size of the bubble is proportional to the number of samples of that species within the San Diego Zoo Frozen Zoo® (see annotated bubbles for reference), with values ranging from 1 – 330 samples (mean: 10.1, SD: 20.14, yellow = amphibians, orange = reptiles, blue = birds and green = mammals). Animal silhouette images are provided by Phylopic under Public Domain license (http://phylopic.org/).

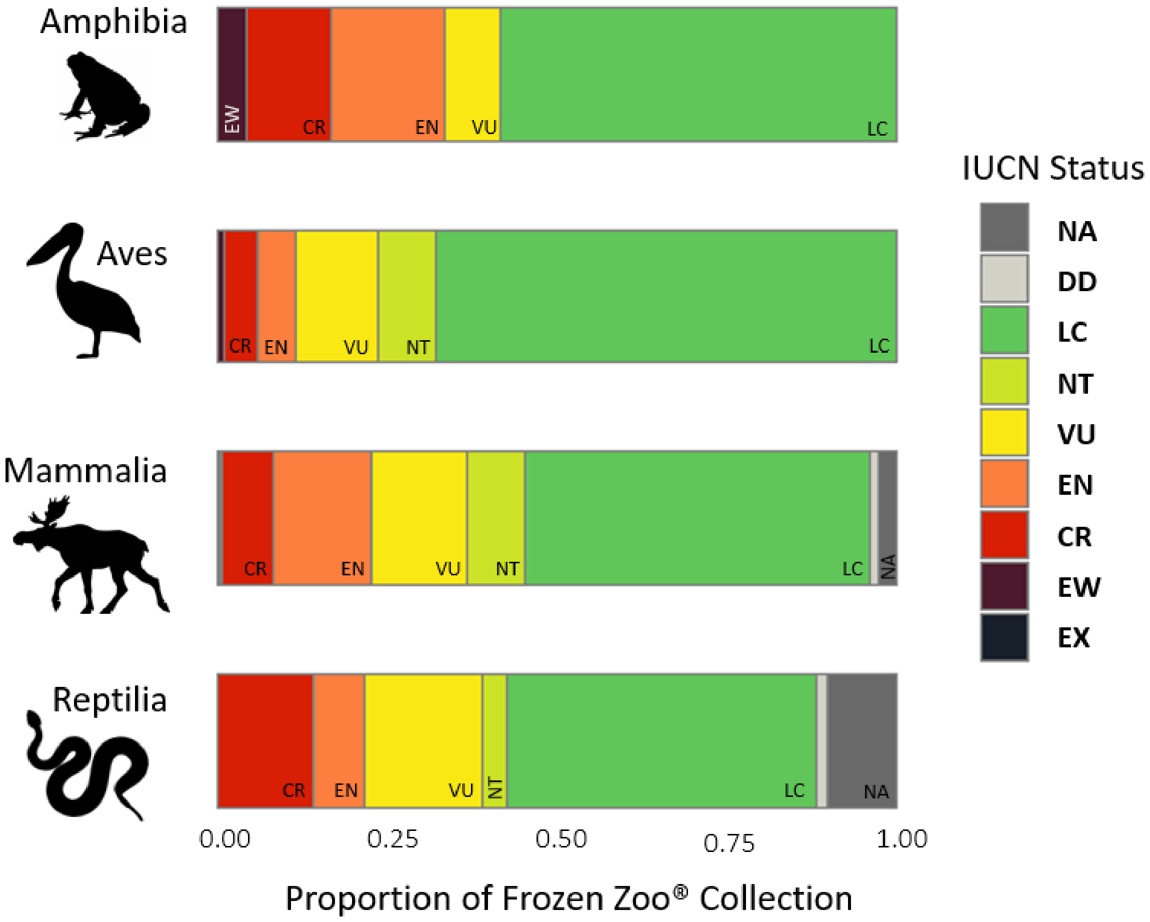

Figure 4.2 | The proportion of amphibian, bird, mammal and reptile species within the San Diego Zoo Frozen Zoo® according to their IUCN Red List status. EX = Extinct, EW = Extinct in the Wild, CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, LC = Least Concern, DD = Data Deficient, NA = Not Assessed. Animal silhouette images are provided by Phylopic under Public Domain license (http://phylopic.org/).

4.4.3 AZE | CITES | CCV | EDGE

Of the species listed in CITES, 13.9% (469 species) are present within the San Diego Zoo Frozen Zoo® (Table 4.3). Similarly, when assessing AZE, Climate Change Vulnerability and EDGE, 26 (2.3%), 92 (2.5%) and 163 (5.7%) species have all been sampled, respectively. The highest representation is the number of CITES listed mammals that are also within the San Diego Zoo Frozen Zoo® (279 species, representing 32.2% of all CITES listed mammals). Amphibians consistently show the lowest representation within the San Diego Zoo Frozen Zoo® across the prioritisation schemes (0 - 3.7%). Of course, each of these prioritisation schemes have the potential to overlap, and this overlap is shown in Figure 4.3. One species, the Spix's macaw (*Cyanopsitta spixii*), is included in each prioritisation scheme and has already been sampled. However, the Climate Change Vulnerability assessment does not include mammals or reptiles. Therefore, there are an additional five species which have already been sampled and which have been assessed as *"Threatened"* under the IUCN Red List, in addition to being included on the AZE, CITES and EDGE prioritisation schemes. These are the addax (*Addax nasomaculatus*), Chinese alligator (*Alligator sinensis*), Roti snake-necked turtle (*Chelodina mccordi*), golden-crowned sifaka (*Propithecus tattersalli*) and vaquita (*Phocoena sinus*). There are 387 species within the San Diego Zoo Frozen Zoo® that have not been included under any prioritisation scheme and which are not currently listed as *"Threatened"* or "*Extinct in the Wild*" under the IUCN Red List. These include the aardvark (*Orycteropus afer*) and the greater flamingo (*Phoenicopterus roseus*).

Table 4.3 | The total number of amphibian, bird, mammal and reptile species within the San Diego Zoo Frozen Zoo® that are also considered of high conservation priority by the AZE, Climate Change Vulnerability assessment, CITES and EDGE. Percentages reflect the percentage of species within that particular category that are currently found within the San Diego Zoo Frozen Zoo®. The Climate Change Vulnerability Assessment does not include mammals or reptiles.

	Prioritisation Scheme			
	AZE	**Climate Change**	**CITES**	**EDGE**
Amphibia	3 (0.5%)	6 (0.4%)	6 (3.7%)	0 (0%)
Aves	9 (4.5%)	86 (3.7%)	108 (7.4%)	38 (5.4%)
Mammalia	8 (3.6%)	NA	279 (32.2%)	106 (18.7%)
Reptilia	6 (10.9%)	NA	76 (8.6%)	19 (3.3%)
Total	26 (2.3%)	92 (2.5%)	469 (13.9%)	163 (5.7%)

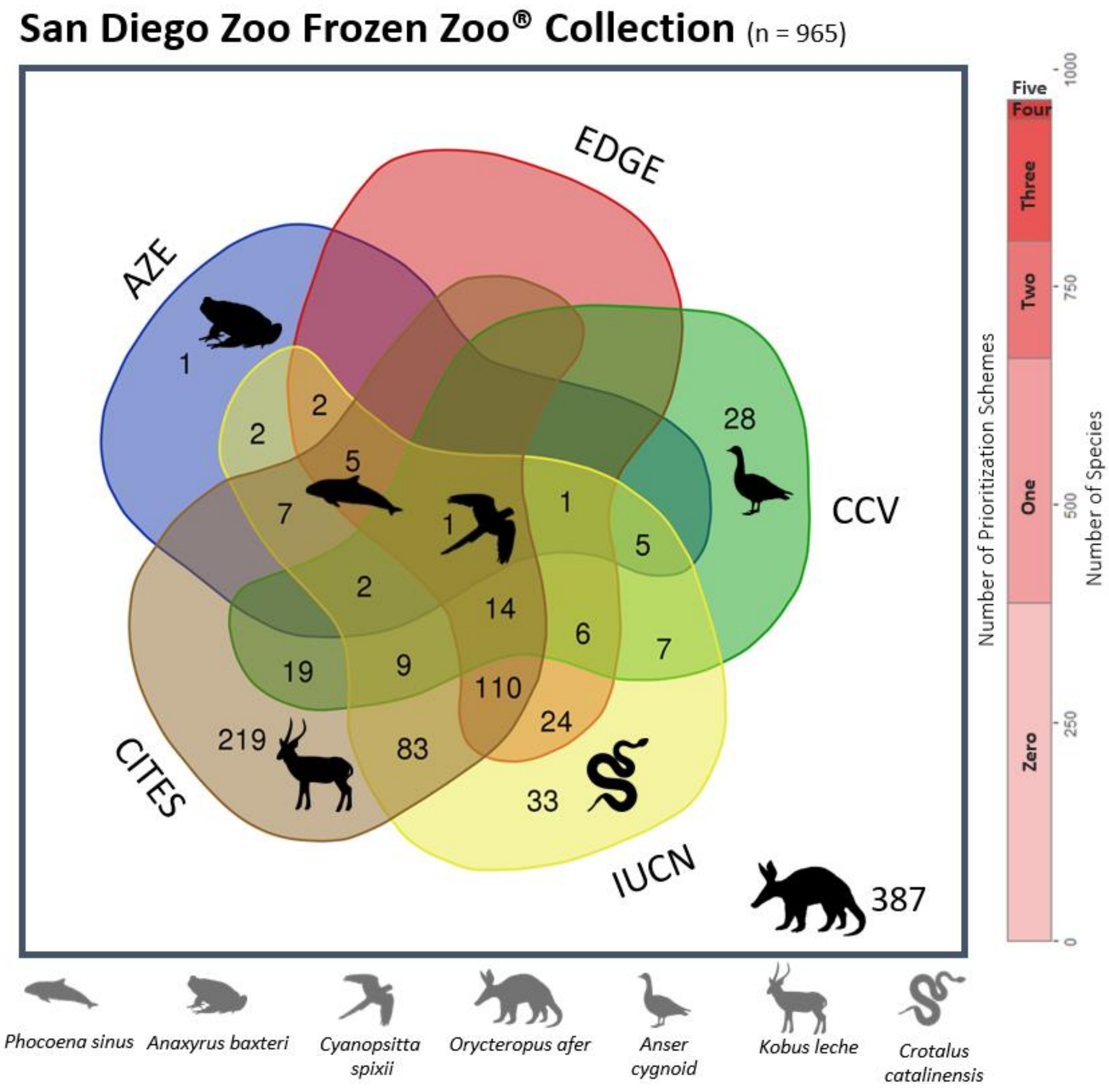

Figure 4.3 | The number of amphibian, bird, mammal and reptile species currently represented within the San Diego Zoo Frozen Zoo® and their representation under one or more prioritisation schemes, indicated by numbers within overlapping areas. AZE = Alliance for Zero Extinction, EDGE = Evolutionary Distinct and Globally Endangered, CCV = Climate Change Vulnerability, IUCN = IUCN Red List "*Threatened*" (CR, EN and VU) species, CITES = Convention on International Trade of Endangered Species of Fauna and Flora. The number of species and the number of prioritisation schemes they appear under is shown in the bar on the right. A limited number of representative species within different subsets are shown as silhouettes, such as the Spix's macaw (*Cyanopsitta spixii*). Animal silhouette images are provided by Phylopic under Public Domain license (http://phylopic.org/). Both AZE and EDGE prioritisation schemes select species already listed as "*Critically Endangered*" (CR), "*Endangered*" (EN) or "*Vulnerable*" (VU) under the IUCN Red List and are therefore not independent of it.

4.4.4 Future Sample Collection Opportunities

There are 5,799 *"Threatened"* species under the IUCN Red List which do not yet appear in the San Diego Zoo Frozen Zoo® and these represent an initial list for potential prioritisation for future sample collection. When looking at opportunities for sample collection, I identify 695 *"Threatened"* species yet to be sampled which are also found in at least one Species360 member zoo or aquarium (Figure 4.4). These species are found in a mean number of 14.5 (± 32.1 S.D.) institutions with mean population sizes of 135.1 (± 521.9 S.D.) individuals (Table 4.4). Combined, this means we can increase the representation of *"Threatened"* species to 1,006 out of 6,110 species, if all identified species in zoos and aquariums are sampled (16.5% of all *"Threatened"* amphibian, bird, mammal and reptile species). Reptiles show the greatest opportunity to increase *"Threatened"* species representation, with 217 candidate species being found in zoos and aquariums (representing 17.2% of all non-sampled *"Threatened"* reptiles). Although amphibians have the lowest number of candidate *"Threatened"* species in zoos and aquariums to be sampled (112, 5.38% of non-sampled *"Threatened"* amphibian species), their notably large *ex situ* population sizes of 323.1 (± 1,121.7 S.D.) represent a unique opportunity to collect multiple samples, reducing project costs, *C*, and increasing overall project efficiency, *E*.

Table 4.4 | The numbers of "*Threatened*" amphibian, bird, mammal and reptile species according to the IUCN Red List that are currently not represented within the San Diego Zoo Frozen Zoo® (SDZG FZ) and their presence within the global Zoo and Aquarium community, as represented by Species360 members (ZIMS). Also shown are mean (± S.D.) *ex situ* population sizes per species and the mean (± S.D.) number of institutions at which the species is currently maintained.

	Threatened Species not in SDZG FZ	Threatened Species not in SDZG FZ (in ZIMS)	x̄ ZIMS Population Size per Species (± S.D.)	x̄ Number of ZIMS Institutions per Species (± S.D.)
Amphibia	2083	112 (5.38%)	323.1 (± 1121.7)	7.5 (± 27)
Aves	1422	213 (14.99%)	122.3 (± 368.1)	21.2 (± 39.4)
Mammalia	1034	153 (14.7%)	63.8 (± 154.2)	7.6 (± 12.1)
Reptilia	1260	217 (17.22%)	101 (± 236)	16.6 (± 34.8)
Total	**5799**	**695 (11.98%)**	**135.1 (± 521.9)**	**14.5 (± 32.1)**

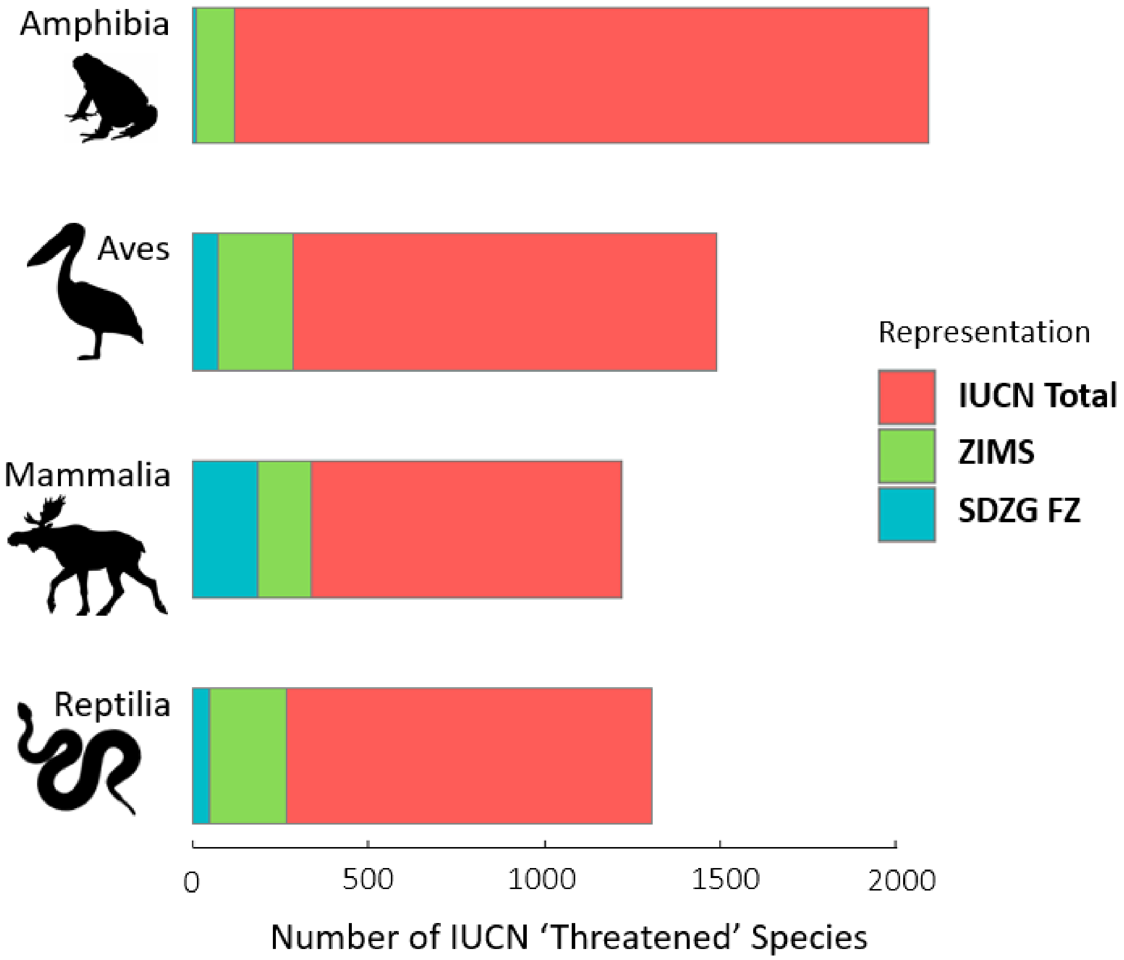

Figure 4.4 | The number of amphibian, bird, mammal and reptile species currently listed as "*Threatened*" under the IUCN Red List and their representation within the San Diego Zoo Frozen Zoo® and the global zoo and aquarium community, as represented by Species360 members (ZIMS). Species not currently represented within the San Diego Zoo Frozen Zoo®, but which are found in the global zoo and aquarium community represent opportunities for future sample collection (green). Animal silhouette images are provided by Phylopic under Public Domain license (http://phylopic.org/).

4.4.5 Priorities for Future Sampling

Although the unsampled 5,799 *"Threatened"* and "*Extinct in the Wild*" IUCN Red List species provide an initial framework for species prioritisation, the limited resources and sample opportunities available require a more nuanced approach, considering multiple criteria and stakeholder interests. Here I cross-reference the 5,799 unsampled *"Threatened"* IUCN Red List species with the other prioritisation schemes and identify opportunities for sample collection within the global zoo and aquarium network, enhancing the probability of sample acquisition. I find three species which are listed in every prioritisation scheme and which are also present within the global zoo and aquarium network (Table 4.5; Figure 4.5). These are the whooping crane (*Grus americana*), crested ibis (*Nipponia nippon*) and Siberian crane (*Leucogeranus leucogeranus*). Given that the Climate Change Vulnerability assessment does not include mammals or reptiles, I identify a further nine species which are listed by AZE, CITES, EDGE and which are also found in at least one zoo or aquarium, including the Marañón poison frog (*Excidobates mysteriosus*) and the Togian Islands babirusa (*Babyrousa togeanensis*). As a result of the clear conservation concern for these species and the relative ease of sample availability (substantially reducing project costs, *C*), I believe that genetic sampling has the potential to provide large species-specific conservation benefits, *B*. Therefore, these species represent high priorities for future sample collection with potentially high biobanking efficiency scores, *E*.

Given the demographic and genetic management made by possible by *ex situ* management, I highlight the 127 unsampled *"Threatened"* IUCN Red List species currently being managed by studbooks as part of either the European Association of Zoos and Aquaria (EAZA) or the Association of Zoos and Aquariums (AZA, North America) (Table 4.5). Several of these species, such as the western crowned pigeon (*Goura cristata*) and Humboldt penguin (*Spheniscus humboldti*) are also prioritised as part of the Climate Change Vulnerability assessment, CITES and EDGE. These species have the benefit of being actively managed to maintain genetic diversity and would therefore possess a higher probability of project success, *S*, if sampled. This is in addition to their existing lower project costs, *C*, and large conservation benefits, *B*, resulting in such species potentially yielding the highest biobanking efficiency scores. As a result, special consideration should be given to the collection of samples from these species in the future.

Table 4.5 | The prioritisation of amphibian, bird, mammal and reptile species for global biobanking efforts. Prioritisation is based on their current IUCN Red List Status, other global conservation prioritisation schemes and representation within the global zoo and aquarium community, as represented by Species360 members (ZIMS).

IUCN Status	Prioritisation Scheme / Sample Availability	Number of Species	Reasoning
'EW'	ZIMS	5	'EW' species where known *ex situ* samples are available
	Non-ZIMS	1	'EW' species where no known *ex situ* samples are available
"Threatened" **(CR, EN, VU)**	ZIMS/CCV/EDGE/CITES/AZE	3	Species included in all prioritisation schemes where known *ex situ* samples are available
	ZIMS/EDGE/CITES/AZE	9	Species included in all prioritisation schemes, except CCV (CCV excludes mammals and reptiles) and where known *ex situ* samples are available
	CCV/EDGE/CITES/AZE	5	Species included in all prioritisation schemes where no known *ex situ* samples are available
	EDGE/CITES/AZE	42	Species included in all prioritisation schemes, except CCV (CCV excludes mammals and reptiles) and where no known *ex situ* samples are available
	EAZA/AZA	127	Special consideration should be given to species which are being actively managed *ex situ* as part of international studbooks

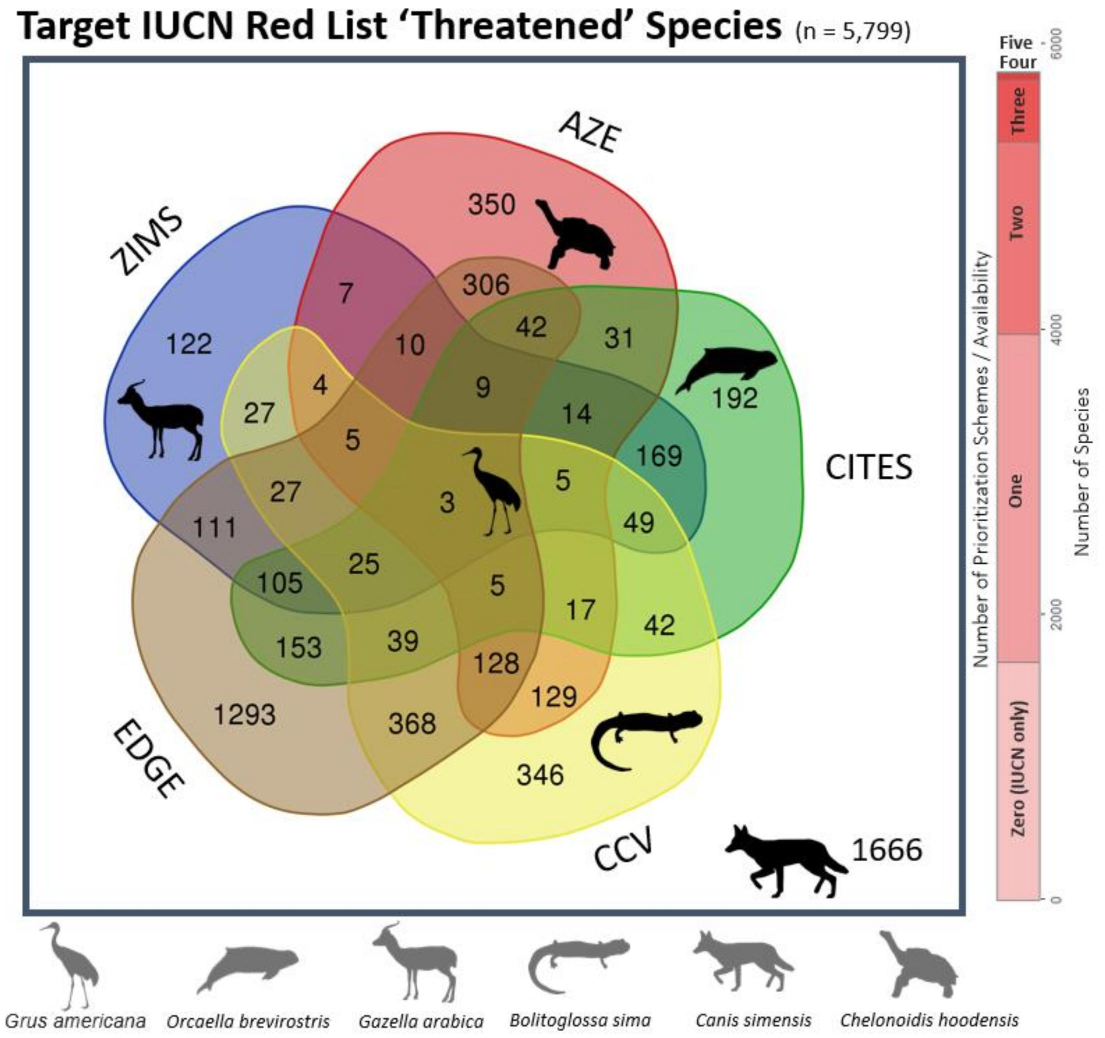

Figure 4.5 | The number of amphibian, bird, mammal and reptile species currently listed as "*Threatened*" under the IUCN Red List, but not currently represented within the San Diego Zoo Frozen Zoo®, and their representation under one or more prioritisation schemes (AZE = Alliance for Zero Extinction, EDGE = Evolutionary Distinct and Globally Endangered, CCV = Climate Change Vulnerability, CITES = Convention on International Trade of Endangered Species of Fauna and Flora). Also shown is their presence within the global zoo and aquarium community, as represented by Species360 members (ZIMS). The total number of unsampled IUCN Red List "*Threatened*" species appears in the bar on the right, highlighting the number of other prioritisation schemes they appear under and their presence within the global zoo and aquarium community. Animal silhouette images are provided by Phylopic under Public Domain license (http://phylopic.org/). Both AZE and EDGE prioritisation schemes select species already listed as "*Critically Endangered*" (CR), "*Endangered*" (EN) or "*Vulnerable*" (VU) under the IUCN Red List and are therefore not independent of it.

Although zoos and aquariums play important roles, sample collection is not, and should not, be limited to *ex situ* institutions, and therefore I identify five species which are listed in every prioritisation scheme, but not currently maintained in a Species360 member institution, such as the Christmas frigatebird (*Fregata andrewsi*). Again, when excluding the Climate Change Vulnerability assessment, I identify a further 42 species listed by AZE, CITES and EDGE which are not found in Species360 institutions, such as the Yangtze giant softshell turtle (*Rafetus swinhoei*). Although absence of these species from *ex situ* institutions may substantially reduce the probability of project success, *S*, and increase project costs, *C*, their absence should not exclude them from biobanking efforts. In fact, it implies that these species are more vulnerable to failures in *in situ* conservation efforts, increasing the potential conservation benefits, *B*, if sampled. Despite the logistical and financial implications of sampling species *in situ*, I believe these species are clear priorities for future sample collection. Similarly, although amphibian cells are the most difficult taxonomic group to grow, with prolonged culture times, both increasing projects costs, *C*, and decreasing the probability of project success, *S*, this should not exclude them from future biobanking efforts (Kouba *et al.* 2013; Zimkus *et al.* 2018).

My focus here has been on the species currently listed as *"Threatened"* under the IUCN Red List, however species which are predicted to become *"Threatened"* in the near future should also be given consideration. If predicted climate change projections are realised, numerous species have the potential to suffer rapid population declines and become *"Threatened"*. Knowledge of such species is invaluable for planning biobanking efforts while high levels of extant genetic diversity remains, increasing the probability of future biobanking project success, *S*. I identify 2,349 species of amphibian and bird which are not currently represented in the San Diego Zoo Frozen Zoo® or listed as *"Threatened"* under the IUCN Red List, but which have been assessed by Foden *et al.* (2013) as highly vulnerable to climate change (Table 4.6). Of these 2,349 species, 347 are currently found in at least one Species360 member zoo or aquarium (Table 4.6; Figure 4.6). Examples include the griffon vulture (*Gyps fulvus*) and the Sira poison frog (*Ranitomeya sirensis*), both of which are currently listed as "*Least Concern*" under the IUCN Red List but classified as highly vulnerable to climate change by Foden *et al.* (2013).

Table 4.6| The total number of amphibians and birds assessed by Foden *et al.* (2013) as ‘high’ and ‘low’ vulnerability to climate change and their representation within the San Diego Zoo Frozen Zoo® (SDZG FZ). Also shown are the number of ‘high’ and low’ vulnerability species not represented in the San Diego Zoo Frozen Zoo® and their representation in the global zoo and aquarium community, as represented by Species360 members (ZIMS). These are shown as either “*Threatened”* (CR, EN and VU) or “*Non-Threatened*” based on the current IUCN Red List status of the species.

	Climate Change Vulnerability Assessment				
	Amphibia		**Aves**		
	High	**Low**	**High**	**Low**	**Total (High)**
Total Species Assessed	1,360	4,804	2,300	7,454	3,660
Species in the Frozen Zoo®	6	18	86	224	92
Species not represented in the Frozen Zoo®					
Total	1,354	4,786	2,214	7,230	3,568
IUCN Threatened	623	1,210	596	634	1,219
IUCN Non-Threatened	731	3,576	1,618	6,596	2,349
Species present in zoos and aquariums (ZIMS)					
Species in ZIMS	75	396	417	1,737	492
IUCN Threatened	30	82	115	98	145
IUCN Non-Threatened	45	314	302	1,639	347

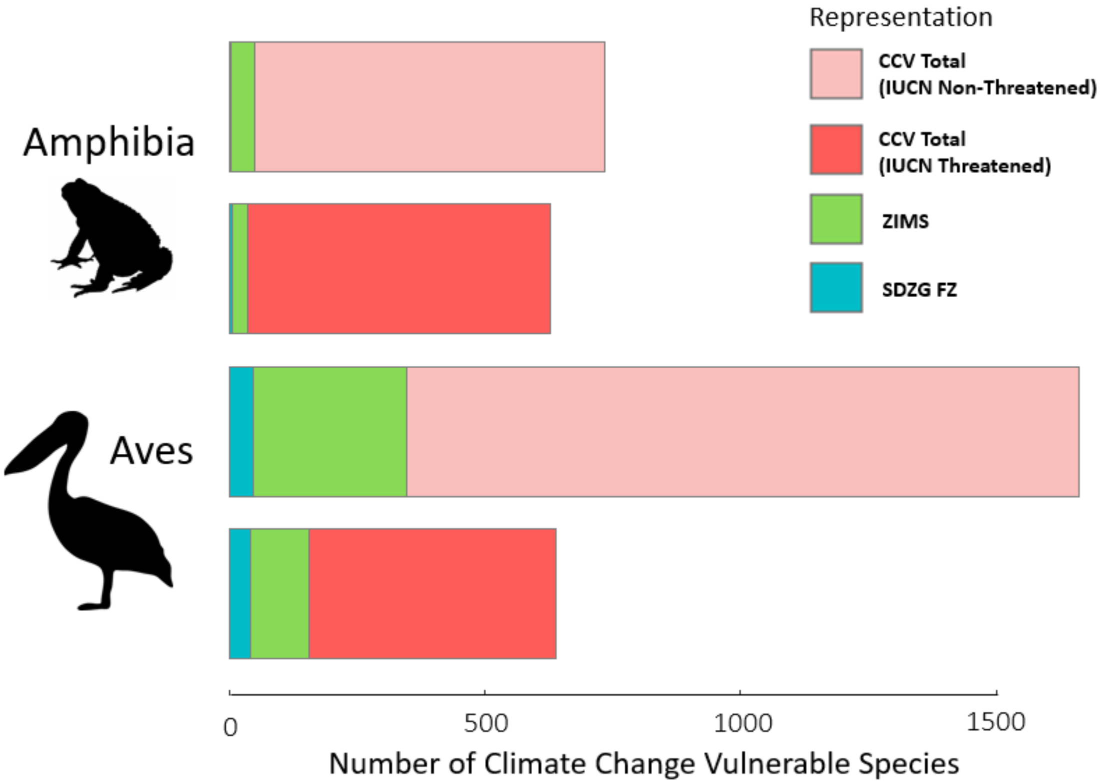

Figure 4.6 | The total number of amphibian and bird species which have been classified as highly vulnerable to climate change (CCV) by Foden *et al.* (2013) and their representation within the San Diego Zoo Frozen Zoo® (SDZG FZ) and the global zoo and aquarium community, as represented by Species360 members (ZIMS). These are shown as either "*Threatened*" (CR, EN and VU) or "*Non-Threatened*" based on the current IUCN Red List status of the species. Species not currently represented within the San Diego Zoo Frozen Zoo®, but which are found in the global zoo and aquarium community represent opportunities for future sample collection (green). Animal silhouette images are provided by Phylopic under Public Domain license (http://phylopic.org/).

4.4.6 Extinct in the Wild

Of the 12 amphibian, bird, mammal and reptile species currently listed as "*Extinct in the Wild*" under the IUCN Red List, six (50%) are already represented within the San Diego Zoo Frozen Zoo® (Table 4.7). These consist of the Wyoming toad (*Anaxyrus baxteri*), Hawaiian crow (*Corvus hawaiiensis*), Guam rail (*Hypotaenidia owstoni*), Guam kingfisher (*Todiramphus cinnamominus*), Père David's deer (*Elaphurus davidianus*) and scimitar-horned oryx (*Oryx dammah*). In addition to the species already sampled, there are five more EW species in at least one Species360 member zoo or aquarium yet to be sampled. These are the Alagoas curassow (*Mitu mitu*), black softshell turtle (*Nilssonia nigricans*), Socorro dove (*Zenaida graysoni*), Christmas Island chained gecko (*Lepidodactylus listeri*) and Kihansi spray toad (*Nectophrynoides asperginis*), and can be found in a mean number of 8.6 (± 14.8 S.D.) institutions globally, with mean population sizes of 1,577 (± 3,360.8 S.D.) (Table 4.7). If all are sampled, these species could increase the representation of EW species in the San Diego Zoo Frozen Zoo® to 91.7% (11 out of 12 species) (Table 4.7). Although all EW mammals are represented in the San Diego Zoo Frozen Zoo®, none of the three EW reptiles have been sampled, however, two of these species can be found in the world's zoos and aquariums, each in a single institution with population sizes of 43.5 (± 60.1 S.D.) individuals. Although not considered *"Threatened"* under the IUCN Red List, these species represent a uniquely vulnerable group of species, owing to their sole reliance on continued *ex situ* management to prevent extinction (Trask *et al.* 2020). As a result, I believe these species represent a high priority for future sample collection.

Table 4.7 | The number of “*Extinct in the Wild*” (EW) amphibian, bird, mammal and reptile species according to the IUCN and their representation within the San Diego Zoo Frozen Zoo® (SDZG FZ) and the global zoo and aquarium community, as represented by Species360 members (ZIMS). Also shown are mean (± S.D.) population sizes per unsampled species and the mean (± S.D.) number of institutions at which the species are currently maintained.

	IUCN ‘EW’ Species	IUCN ‘EW’ Species in SDZG FZ (% of IUCN Total)	IUCN ‘EW’ Species not in SDZG FZ (in ZIMS) (% of IUCN Total if sampled)	$\bar{x}$ ZIMS Population Size per Species (± S.D.)	$\bar{x}$ Number of ZIMS Institutions per Species (± S.D.)
Amphibia	2	1 (50%)	1 (100%)	7588 (± 0)	4 (± 0)
Aves	5	3 (60%)	2 (100%)	105 (± 86.3)	18.5 (± 23.3)
Mammalia	2	2 (100%)	NA	NA	NA
Reptilia	3	0 (0%)	2 (66.7%)	43.5 (± 60.1)	1 (± 0)
Total	12	6 (50%)	5 (91.7%)	1577 (± 3360.8)	8.6 (± 14.8)

4.5 Discussion

As extinction rates continue to rise, the role of *ex situ* conservation and gene banking efforts will only become more important in safeguarding species and genetic diversity. However, the full potential of biobanking has yet to be completely realised and appreciated. Here I show that 965 species and 5.1% of all *"Threatened"* amphibian, bird, mammal and reptile species are already represented within the living cell collection of the San Diego Zoo Frozen Zoo®, and that sampling of species from within the global zoo and aquarium community can increase this representation to 16.5%. This immense biodiversity repository represents an unparalleled resource for future scientific research and biodiversity conservation. Although the San Diego Zoo Frozen Zoo® living cell collection is by far the largest and most diverse collection of its kind globally, other gene banks also contain limited living cell collections, such as the National Zoological Gardens of South Africa Biobank and the Kunming Cell Bank in China, however data regarding their collections are not publicly available (Ryder and Onuma 2018). Consequently, the number of species reported here represents an overall underestimation of the total number of living cell cultures biobanked globally. Of particular note is the limited presence of reptiles, and especially amphibians, within the San Diego Zoo Frozen Zoo®, despite the chytrid fungus threatening global amphibian populations (Weldon *et al.* 2004). Although this partly reveals historical biases in sample collection, the optimal conditions for successful cell growth in preparation for cryogenic storage also vary across taxonomic groups, particularly with respect to temperature (Benirschke 1984; Houck *et al.* 2017; Ryder and Onuma 2018). Despite the optimisation of sample collection and preparation techniques for mammals, the successful culturing of amphibian cells is currently hindered by chromosomal variation and prolonged population doubling periods (Kouba *et al.* 2013; Okumoto 2001; Zimkus *et al.* 2018). Therefore, further work is needed to identify the optimal conditions under which to successfully and reliably grow cells of various taxonomic groups, increasing the probability of project success, *S*. Furthermore, there has been considerable interest in the establishment of gene banks and the optimisation of sampling methods for aquatic species and invertebrates in the future (CPSG 2016; Holt *et al.* 1996).

The aligning of existing and future living cell collections with global prioritisation schemes has the potential to increase the conservation benefit, *B*, of cryopreserved samples and reduce extinction probabilities. In addition to the 5.1% of IUCN Red List *"Threatened"* amphibians, birds, mammals and reptiles represented, 50% of the "*Extinct in the Wild*" species and 13.9% of CITES listed species are also represented. Although the representation of AZE and EDGE species may seem comparably lower (2.3% and 5.7% respectively), it is important to highlight the difficulties in obtaining viable samples from species with such limited *in situ* geographic ranges and population sizes. In reality, obtaining *in situ* samples is an extremely difficult process, due not only to difficulties in finding appropriate samples, but also prolonged and expensive national and international permitting processes, which are designed to reduce the risk of disease transmission and protect threatened species (Ryder and Onuma 2018). The establishment of the Nagoya Protocol on Access and Benefit Sharing in 2010, highlights concerns over intellectual property rights, genetic resource sovereignty and the equitable sharing of potential benefits arising from the utilisation of genetic resources. Although this supplementary agreement to the 1992 Convention on Biological Diversity (CBD) aims to both conserve and sustainably use biodiversity, it can pose significant challenges for future *in situ* sample collection and substantially reduce the probability of sample acquisition; simultaneously decreasing the probability of project success, S, and increasing project costs, C (CBD 1992; Ryder *et al.* 2000; Watanabe 2015). Consequently, the samples available from zoos and aquariums are increasingly valuable in future biobanking efforts (Staerk *et al.* 2018). Not only can zoos and aquariums increase the representation of "*Extinct in the Wild*" species from 50% to 91.7%, but they can also provide access to an additional 695 *"Threatened"* species from which samples can be taken, many of which are AZE, Climate Change vulnerable, CITES and/or EDGE listed (Figure 4.5). Furthermore, 127 of these species are already being actively managed as part of AZA/EAZA management programmes to maintain genetic diversity and demographic sustainability in their living populations, providing access to multiple genetically characterised samples per species, increasing the probability of project success, S.

None of the species found within the San Diego Zoo Frozen Zoo® contain more than the minimum of 500 individuals considered necessary for a genetically sustainable population (Frankham *et al.* 2002). With a mean of only 10.1 (± 20.14 S.D.) samples per species, and many species with a single representative sample, the question arises of whether we should focus on increasing existing species sample sizes in the future or sampling new species. For the purpose of genetic rescue, there is little value in sampling single individuals and individuals with insufficient extant genetic diversity to sustain viable populations (Hobbs *et al.* 2018). For example, the extinct po'ouli is represented by a single male individual, with no further prospects of increasing the genetic variation sampled (Ryder and Onuma 2018). Although genetic rescue may not be possible, as the sole representative sample of an entire species, this sample is invaluable in scientific studies and for any potential future de-extinction efforts. Even when sampling large extant populations, the goal of conserving genetic diversity is not always clear-cut. For instance, the survival of only two female northern white rhinos (*Ceratotherium simum* *cottoni*) means that extinction is almost an inevitable outcome for this subspecies and sampling may seem of little conservation value. In contrast, the genetically distinct southern white rhino (*Ceratotherium simum simum*) is a conservation success story and has seen a population rise from as low as 20-50 in the early 1900's to approximately 18,000 today (Emslie 2020). However, a recent genome-wide assessment of genetic diversity and inbreeding levels within nine cryopreserved northern white rhino samples revealed similarly high levels of heterozygosity and even lower levels of inbreeding when compared to the entire extant southern white rhino population (Tunstall *et al.* 2018). Questions surrounding future sample collection should therefore not only focus on which species to sample, but also which individuals are the most genetically diverse and appropriate for future genetic rescue attempts, incorporating chromosomal analyses to identify potential abnormalities (Mastromonaco *et al.* 2012; Staerk *et al.* 2018). Further work is clearly warranted on identifying these individuals and defining how many individuals are required to meet genetic and conservation objectives (Holt *et al.* 1996). Active liaising with studbook keepers and pedigree analysis software can help resolve some of these issues for *ex situ* populations with management programmes, though this will require extensive global collaboration, co-operation and investment.

Although the work of the San Diego Zoo Frozen Zoo® is impressive, international co-ordination and the establishment of further gene bank collections is necessary and should be undertaken in the near future, particularly in biodiversity rich countries with high numbers of threatened species (Ryder *et al.* 2000). This regional biobank establishment circumvents some of the impediments and concerns regarding access and benefit sharing, sovereignty issues and international transport of samples, while simultaneously reducing project costs substantially (Clarke 2009). Local specialists will also have the expertise and knowledge necessary to identify the most appropriate sample collection opportunities and the long-term storage capabilities in their region (Clarke 2009). The lack of any resource for the identification of which species have already been sampled, and where they are currently held, is also a prominent concern among stakeholders (Ryder and Onuma 2018). To prevent unnecessary duplicate sampling and to co-ordinate future sample selection, it is imperative that a global database of gene bank repositories be established and co-ordinated between institutions (CBSG 2015; Ryder and Onuma 2018; Ryder *et al.* 2000). Furthermore, a clear commitment must also be made on behalf of each institution to ensure the perpetuity of their collection, and in the unlikely event of catastrophic failure, duplicate collections should also be established, as has already been undertaken by the San Diego Zoo Frozen Zoo® (Benirschke 1984; Ryder *et al.* 2000).

The European Association of Zoos and Aquaria (EAZA) has taken initial steps to tackle these problems through the establishment of the EAZA Biobank, consisting of four gene banking hubs dedicated to the curation, long-term maintenance and registration of samples from the European and Middle Eastern zoo and aquarium community (EAZA 2020). All current EAZA member institutions (>400 institutions in 48 countries) are encouraged to contribute samples to their regional gene bank hub, regardless of the threat status of the species (Staerk *et al.* 2018). International co-ordination provides access to a much wider range of species and number of individuals than a single institutional collection, in addition to the provision of standardised storage procedures and facilities for institutions without the means to establish their own gene bank collections (CBSG 2015). The EAZA biobanking hubs represent an advancement toward the routine and systematic collection of samples, and it has been suggested that sampling for biobanking be incorporated into the regional membership association accreditation process for zoos and aquariums (CPSG 2016). Ultimately, the ambitions for gene banking to substantially contribute to biodiversity conservation will only be realised through national, regional and global co-ordination to increase biobanking efforts. The World Association of Zoos and Aquariums (WAZA) has realised the potential of the global zoo and aquarium community to contribute and co-ordinate this endeavour, and during their 2018 annual conference approved the "*Bangkok Resolution on Viable Cell Culture Cryobanking for Species Conservation*" (Resolution 73.2), which encourages all WAZA members to become involved in the effort to bank living cell cultures where possible.

Ultimately, the goal of global gene banking efforts will be to add genetic diversity into existing *in situ* and/or *ex situ* populations, enhancing population health and sustainability. The three-way exchange of genetic material between gene banks, zoos and aquariums and wild populations would allow for all populations of a species to be managed collectively, reducing extinction risks and costs (Ballou 1992). The introduction of cryopreserved material from gene banks can complement conventional and existing *in situ* and *ex situ* conservation efforts, especially in small and fragmented populations (Ryder and Onuma 2018). Similarly, existing *in situ* conservation projects can potentially be used as a source for future gene bank samples (CPSG 2016). This global management of a species, incorporating genetic knowledge, *in situ* sample collection and *ex situ* management is in line with the "*One Plan*" approach to species conservation, as encouraged by the IUCN CPSG, which considers all populations of a species, both inside and outside their natural range, as one single metapopulation (Byers *et al.* 2013; Staerk *et al.* 2018). However, these ambitions are all contingent on technological advancements and the prioritisation of *in situ* conservation activities.

Although the qualitative assessment presented here represents an initial species prioritisation, formal prioritisation is necessary to reveal those species with the highest biobanking efficiency scores, E (Equation 4.1). Unfortunately, a lack of available quantitative data, particularly with regards to measuring the conservation benefit, B, of biobanking and the probability of project success, S, means that formal prioritisation of species for future sampling efforts was not possible. Despite genetic rescue having been proven in practice through the translocation of live animals, the ability of biobanked living cells to achieve the same result has yet to be proven outside of model organisms (Ryder and Onuma 2018; Whiteley *et al.* 2015). The limited successes in assisted reproductive technologies that have been observed have also come at extremely high financial costs and are therefore unlikely to be replicated for many other species in the near future (Hobbs *et al.* 2018). In reality, the routine application of assisted reproductive technologies in wildlife conservation has yet to be realised, even under *ex situ* conditions, and their role in conserving threatened species in their natural habitats is almost non-existent (Holt *et al.* 1996). Therefore, the predicted conservation benefits of biobanking are primarily theoretical in nature and contingent on the continued development of assisted reproductive technologies and knowledge surrounding the reproductive physiology of the species concerned (Ballou 1992). Even if such technologies were to advance to sufficiently high levels that would permit routine use in both *in situ* and *ex situ* wildlife conservation settings, the overarching species-specific concerns and threats must also be adequately addressed if species persistence is to be achieved. Even in cases where successful genetic rescue has already been achieved, continued intensive management is required if suitable habitat and adequate gene flow between populations are not provided (Whiteley *et al.* 2015). A particularly striking example of the necessity for suitable habitat and adequate gene flow can be seen in the case of the Florida panther (*Puma concolor coryi*). This subspecies has undergone successful genetic rescue involving the translocation of live individuals, but habitat saturation, continued habitat loss, small population sizes and persistent inbreeding all mean that the original population is likely to return to high levels of inbreeding in the near future, ultimately requiring further genetic rescue attempts, and translocations, until suitable habitat is designated and gene flow can be restored (Bijlsma *et al.* 2010; Johnson *et al.* 2010; Liberg *et al.* 2005; Whiteley *et al.* 2015).

Long-term biodiversity conservation is therefore unlikely to be achieved solely through genetic rescue, particularly if sufficient habitat is not made available and threats adequately addressed. However, short-term fitness benefits and population increases can be used as part of an integrated species-specific conservation and management plan to restore genetic diversity and temporarily add viability and resilience to populations, until suitable habitat can be guaranteed

(Hobbs *et al.* 2018; Staerk *et al.* 2018). The utilisation of cryopreserved living cells for biodiversity conservation is ultimately only one part of the conservation toolbox and is not to be considered as a substitute or replacement for conserving living animals themselves and their natural habitats (Clarke 2009; Holt *et al.* 1996). The role of gene banks should be seen as complimentary to traditional and existing conservation activities. Without the continued maintenance of living animal populations, suitable habitat allocation and reductions in species-specific threats, cryopreserved genetic material can yield practically no conservation benefit (Hobbs *et al.* 2018; Holt *et al.* 1996).

Although only two infertile female northern white rhinos remain in the world, San Diego Zoo Global has made a commitment to rescue this subspecies from extinction (SDZG 2020). The San Diego Zoo Frozen Zoo® contains living cell cultures from 12 individual Northern White Rhinos (including both surviving females), which have been collected over the last 35 years, and these all have the potential to generate gametes which can be used to create northern white rhino embryos. It should be noted that three of these 12 samples have a chromosomal rearrangement reducing their normal 2n=82 diploid number to 81, which can reduce fertility (Houck *et al.* 1994). Through artificial insemination, *in vitro* fertilization, and then embryo transfer into southern white rhino surrogates, San Diego Zoo Global is aiming to save the northern white rhino from otherwise imminent extinction. This incredibly ambitious conservation project, if successful, would undeniably guarantee the role of biobanking and assisted reproductive technologies in biodiversity conservation moving forward. For species on the cusp of extinction, such as the Javan rhino (*Rhinoceros sondaicus*), vaquita (*Phocoena sinus*) and the saola (*Pseudoryx nghetinhensis*), biobanking may be the only way to ensure their survival in the future. Although only one part of the conservation toolbox, gene banking has the potential to fundamentally alter how we conserve species, be it by adding genetic diversity to existing populations, saving species on the brink of extinction, or potentially even bringing species back from extinction. However, for species without any living cell culture, these opportunities are not possible, and for recently extinct species which were never sampled, such as the Yangtze river dolphin (*Lipotes vexillifer*) and the Christmas Island pipistrelle (*Pipistrellus murrayi*), these possibilities will never be realised. These poignant extinctions only emphasise the crucial role of gene banks, such as the San Diego Zoo Frozen Zoo®, and the philosophy of American Historian, Daniel J. Boorstin, and subsequently the Frozen Zoo® founder, Dr. Kurt Benirschke: "*You must collect things for reasons you don't yet understand*".

5. A conservation project efficiency approach to the prioritisation of plants for de-extinction research

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Author contributions: All authors developed the concept of the manuscript. A.M. collected the data. A.M. undertook the analysis and drafted the text in consultation with Y.M.B. A.M. produced the tables and figures. All authors contributed to the writing of the manuscript.

5.1 Abstract

Despite the best efforts of both *in situ* and *ex situ* conservation practitioners, human-induced species extinctions are increasing in frequency. However, recent technological advancements now mean that a suite of genetic rescue tools can be deployed to reverse global biodiversity loss by increasing population viability and even result in de-extinction for some species. Despite their diversity and extensive use by humans, plants are conspicuous in their absence from the de-extinction discussion, with charismatic vertebrates such as the woolly mammoth (*Mammuthus primigenius*) and thylacine (*Thylacinus cynocephalus*) being the focus of much work to date. This is surprising considering the relative ease of plant cloning, the central role of plants in ecosystem functioning, their importance in ensuring food security and the reduced legal, ethical and welfare concerns associated with plants compared to charismatic vertebrates. Here I prioritise for de-extinction research the 122 species of plant listed as "*Extinct*" under the IUCN Red List.

I implement a de-extinction project efficiency ranking framework to incorporate species values (such as economic, medicinal and scientific value), while also considering indicators for the probability of project success and associated costs through an identification of suitable herbarium and *ex situ* samples, extant relatives and key habitat and threat information. Nineteen of the 122 "*Extinct*" listed species have been rediscovered in the wild, however the Red List status of only six species has been updated to reflect this new information. Nearly all species have known herbarium specimens (114/122) and congeneric relatives (117/122), however none are represented within any of the *ex situ* seed banks assessed. Of the top five scoring species, four have been rediscovered (*Melicope paniculata*, *M. cruciata*, Rutaceae; *Astragalus nitidiflorus,* Fabaceae and *Madhuca insignis,* Sapotaceae), leaving the Hawaiian lobelioid *Delissea subcordata* (Campanulaceae) as the highest-ranking species for de-extinction research. Although not recorded since 1934, this species has numerous herbarium specimens, published DNA sequences and extant genus-specific relatives, increasing the probability of project success. I encourage more detailed IUCN Red List assessments, seed and tissue collection for newly rediscovered species, and highlight a lack of available quantitative data for extinct plant species, particularly with regards to their ecological role and interspecific interactions, inhibiting informed de-extinction decision-making.

5.2 Introduction

Despite the best efforts of both *in situ* and *ex situ* conservation practitioners, the frequency of human-induced species extinctions is increasing, with the rate of vertebrate species extinctions over the last century being 100 times higher than the predicted background rate of extinctions (Barnosky *et al.* 2011; Ceballos *et al.* 2015; IPBES 2019). Although plants have historically suffered fewer mass extinction events compared to animals, it is estimated that 20% of plant species are currently threatened with extinction (Brummitt *et al.* 2015; Cascales-Miñana and Cleal 2014). This acceleration in extinction rates has resulted in the belief that we have now entered the sixth mass extinction event of our planet's history, with predicted negative cascading consequences on both ecosystem functioning and the services required to sustain human life (Ceballos *et al.* 2017; Ceballos *et al.* 2020; Díaz *et al.* 2018). However, recent developments in genome engineering techniques and ancient DNA sequencing mean that de-extinction, the process of bringing an extinct species back to life, is a legitimate possibility to resurrect once-extinct species, reversing global biodiversity loss and restoring ecosystem health and stability (Seddon *et al.* 2014; Shapiro 2017).

De-extinction can be achieved through three main pathways: back-breeding, cloning and genome engineering (Shapiro 2015a). Back-breeding is the term used to describe the selective breeding of extant species to resurrect specific ancestral traits within the living population (Shapiro 2017). This method of de-extinction is only possible where the extinct species has closely related extant relatives and where the target ancestral traits still persist within the living population (IUCN SSC 2016; Shapiro 2017). The breeding of individuals is based on their phenotype and as a result, back-breeding is referred to as a 'phenotypic' de-extinction rather than a true de-extinction, restoring an extinct species' phenotype and ecological role rather than focussing on genetic similarity (Shapiro 2015a). Back-breeding projects have already tried to recreate the extinct aurochs (*Bos taurus primigenius*), a large wild bovid that once inhabited Asia, Europe, and North Africa, but which became extinct in 1627 due to anthropogenic activities. In the 1920's and 1930's Heinz and Lutz Heck tried to recreate the aurochs by selectively breeding domestic cattle breeds with the desired ancestral traits, ultimately creating what are referred to as Heck cattle today (Heck 1951). Similar projects are underway to recreate the quagga (*Equus quagga quagga*), a subspecies of the common zebra that became extinct in 1883 (Martinelli *et al.* 2014).

Conversely, cloning, or somatic cell nuclear transfer (SCNT) can be used to create an exact genetic copy of an organism, by injecting a nucleus from an adult somatic cell into an enucleated donor egg cell (Wilmut *et al.* 2002). Subsequent cellular reprogramming by the host egg cell into a pluripotent stem cell allows the cell to develop into an embryo in the same way following a normal fertilisation event, with the resulting organism having an identical nuclear genome sequence to the somatic cell donor (Shapiro 2017). Unfortunately, cloning in animals generally requires intact living cells and close extant relatives to act as gestational surrogates, however, clones from less well-preserved cells have also been created (Loi *et al.* 2001). Cloning is generally easier in plants through a host of plant cell tissue culture approaches, often but not exclusively, utilising meristem cells (Murashige and Skoog 1962; Oo *et al.* 2018). Although the efficiency of cloning techniques are notably poor in wildlife, the preservation of a living cell culture from the last living Pyrenean Ibex (*Capra pyrenaica pyrenaica*) in 2000, allowed scientists to clone the individual in 2003 using gestational and ova surrogates, making it the first individual in history to be resurrected from extinction (Folch *et al.* 2009). Unfortunately, the vast majority of extinct species do not have well-preserved somatic cells, and for other taxonomic groups, such as birds, reptiles and monotremes, SCNT is not yet possible (Shapiro 2017). The potential of cloning for de-extinction efforts is ultimately contingent on the availability of living somatic cells, highlighting the importance of biobanking efforts in preserving representatives of currently extant threatened species prior to their extinction. In plants it is also possible to use cell culture techniques to derive whole viable plants from haploid material, doubling it to make double haploids which are essentially equivalent to highly inbred diploid lines (Seymoura *et al.* 2012). Such plant tissue culture methods have a long history in *in vitro ex situ* conservation (Shukla *et al.* 2012).

Finally, genome engineering can be used to alter the genome of an extant species, in cells *in vitro*, to incorporate and express genes from an extinct species, where the resulting cells can be used for cloning approaches, such as SCNT (Shapiro 2017). Although genome engineering does not require living somatic cells from extinct species, it does require at least partial knowledge of their genome and extant close relatives for genome altering and surrogate purposes (Shapiro 2015b). For long-extinct species with no close living relatives, such as the New Zealand Moa (*Dinornithiformes*), ancient DNA sequencing will likely never correctly assemble their full genome (Shapiro 2017). However, active genome engineering projects are currently underway for the woolly mammoth (*Mammuthus primigenius*), which diverged from the Asian elephant (*Elephas maximus*) only 5 million years ago (Callaway 2015). This has resulted in an estimated 1.4 million fixed nucleotide differences between the two species (Lynch *et al.* 2015), of which only 62 have been incorporated into the Asian elephant genome to date (Shapiro 2017). However, the consequences of large-scale genome editing on genome stability are still unknown (Shapiro 2017).

As demonstrated by the examples above, the majority of the discussion and investment in de-extinction research has focussed on large, charismatic vertebrates (mammals and birds), with almost no acknowledgement of the potential for the de-extinction of plant species (IUCN SSC 2016; Jones 2014; Martinelli *et al.* 2014; McCauley *et al.* 2017). This taxonomic bias mirrors the existing biases in conservation management and ecological research, hindering our knowledge of which species have even gone extinct (Iacona *et al.* 2017; IUCN SSC 2016). Although both Seddon *et al.* (2014) and Iacona *et al.* (2017) modified conservation prioritisation methods to prioritise species for de-extinction, their work did not extend to plants and several of the criteria they employ are not directly applicable to plants, reinforcing entrenched taxonomic biases and preventing non-charismatic species from being included in the conversation (Turner 2017). As noted by Turner (2017), large, charismatic vertebrates are also the species which raise the most serious animal welfare and ethical concerns, a critically important consideration in the selection of de-extinction candidates. Considering the relative ease of plant cloning, their role in ecosystem functioning, their importance in ensuring food security and our ability to extract medicinal compounds from their tissues, it is surprising that plants have received such little attention in this context (Martinelli *et al.* 2014; Sherkow and Greely 2013). In addition, plants represent a promising route to advance our de-extinction understanding and technologies, while simultaneously avoiding the legal, ethical and welfare requirements of vertebrate de-extinction.

In fact, the only mention of plants as potential de-extinction candidates has been the American chestnut (*Castanea dentata*), a keystone tree species native to eastern North America which was nearly eradicated by an introduced fungal pathogen (*Cryphonectria parasitica*) (Jones 2014; Martinelli *et al.* 2014; McCauley *et al.* 2017; Newhouse *et al.* 2014; Ogden 2014; Shapiro 2015a). After failed back-breeding attempts between the American chestnut and the fungal-resistant Chinese chestnut (*Castanea mollissima*), genome engineering and gene transfer techniques have subsequently created the transgenic American chestnut referred to as 'Darling4', which displays an intermediate resistance to the fungal pathogen (Martinelli *et al.* 2014; Newhouse *et al.* 2014). Although successful, the inclusion of this species in the de-extinction literature is misplaced, as the American chestnut never actually became extinct.

In contrast to vertebrates, the creation of new individuals of an extinct species in plants can be much more straightforward, providing the right samples exist. For example, using a 30,000-year-old piece of immature ovarian tissue from *Silene stenophylla* preserved in the permafrost, Yashina *et al.* (2012) were able to undertake *in vitro* tissue culture and clonal micropropagation to successfully generate whole fertile plants. The prospect of finding suitably preserved extinct plant tissues, capable of germination, is therefore a realistic possibility that could result in the first true plant de-extinction (Abeli *et al.* 2020; Legendre *et al.* 2014; Martinelli *et al.* 2014). Similarly, the preservation of seeds in *ex situ* seed and germplasm banks globally can be used to generate whole fertile plants. There are currently over 1,750 seed and germplasm banks globally, including more than 350 seed banking botanic gardens (Hay and Probert 2013; O'Donnell and Sharrock 2017). Collectively these facilities contain in excess of 4.6 million accessions (Hay and Probert 2013). Although most are dedicated to the preservation of domestic crop diversity, several also contain wild plant material (Abeli *et al.* 2020). The Millennium Seed Bank (MSB) partnership, founded and operated by the Royal Botanic Gardens, Kew, UK (RBG Kew), has conserved seeds from nearly 40,000 species of seed-bearing wild vascular plant and represents the largest *ex situ* conservation programme in the world (Abeli *et al.* 2020).

Furthermore, the more than 387 million specimens preserved in the approximately 3,000 herbaria globally can not only be used to provide morphological and historical occurrence information, but also for genome sequencing (Abeli *et al.* 2020). In addition, although not under optimal storage conditions, herbarium specimens have also been able to provide viable seeds capable of germination, as shown by Godefroid *et al.* (2011) who used seeds from 144-year-old herbarium specimens to resurrect the locally extinct *Bupleurum tenuissimum* in Belgium. Unfortunately, although relatively straightforward, the efficacy of herbarium seed germination is demonstrably low, with only eight out of 2,672 seeds germinating for Godefroid *et al.* (2011), with none of those producing viable seedlings themselves (Abeli *et al.* 2020). Despite these limitations, the availability of viable *ex situ* samples and herbarium specimens can provide an unparalleled resource for future plant de-extinction efforts and help guide candidate species selection.

There are numerous intrinsic and extrinsic species values which should be considered during any formal species prioritisation to assess the value of the species' de-extinction, the probability of project success and the associated costs involved (Iacona *et al.* 2017). For example, species which were of economic, agricultural, medicinal or scientific significance are much more likely to gain financial support for any de-extinction efforts compared to species of little human-interest or species which were once considered pests (Seddon *et al.* 2014).

Even if a species is of great human interest, the possibility of de-extinction is still reliant on the availability of suitable *ex situ* samples and specimens, a critical fact overlooked by de-extinction prioritisation schemes to date (Iacona *et al.* 2017; Seddon *et al.* 2014). However, Jones (2014) does highlight the importance of de-extinction feasibility and suggests the prioritisation of species with shorter generation times, simpler genomes and subspecies, such as the Bali tiger (*Panthera tigris balica*), to reduce costs and increase the probability of success. The prioritisation of ecologically unique species is also considered an effective way to help restore lost ecosystem functions, however this may only be possible for recently extinct species due to a lack of historical data surrounding the biology and ecology of extinct species and the extent of environmental changes since the species' extinction (IUCN SSC 2016; Jones 2014; McCauley *et al.* 2017). Similarly, a sound knowledge and understanding of the original threats posed to the extinct species and current habitat availability are imperative if the de-extinction is to be successful, as, if those threats have not been resolved and if no habitat is currently available, there is no reason to expect that the species will not go extinct again once reintroduced back into the wild (IUCN SSC 2016; Jones 2014; Seddon *et al.* 2014; Wood *et al.* 2017). Therefore, regardless of the de-extinction pathway selected, any prioritisation of candidate species for de-extinction research should incorporate human-perceived values, sample availability and both habitat and threat information.

It is generally agreed that the goal of de-extinction projects should be to create functionally equivalent proxies of extinct species, capable of restoring lost ecological processes and restore ecosystem functions, and not to generate a limited number of individuals to be maintained in captivity (Iacona *et al.* 2017; Seddon *et al.* 2014; Shapiro 2017; Wood *et al.* 2017). This has been termed 'ecological enrichment' and will require the creation of numerous genetically distinct individuals to establish a self-sustaining *in situ* population capable of adapting in response to environmental change and that is not at risk of future extinction (Iacona *et al.* 2017; Steeves *et al.* 2017). Unfortunately, this is likely to be impossible for many vertebrate species due to the limited genetic diversity available for extinct species and the costs associated with advanced genome engineering techniques. However, for plants with numerous preserved *ex situ* seed samples and herbarium specimens, this obstacle could be overcome, allowing for the restoration of taxonomic and functional diversity within ecosystems, providing not only ecosystem services, but also resilience to future disturbances (Cardinale *et al.* 2012).

Here, I address the taxonomic bias in de-extinction research and develop a de-extinction efficiency ranking framework to prioritise for de-extinction research efforts the 122 species of plants listed as "*Extinc*t" under the IUCN Red List (IUCN 2018). I consider intrinsic and extrinsic species values, such as ecological, economic, medicinal and taxonomic values, while also considering the estimated probability of project success and associated costs, through the identification of suitable *ex situ* samples and herbarium specimens, and key habitat and threat information. I combine and integrate data from multiple sources, including the IUCN Red List of Threatened Species, the RBG Kew, the William and Lynda Steere Herbarium at the New York Botanical Garden, the Svalbard Global Seed Vault, the Smithsonian Botany Collection and the Global Biodiversity Information Facility (GBIF). Recommendations from this prioritisation can be used to guide plant de-extinction candidate selection, prompting more detailed feasibility and risk analyses. Although an emerging field, failure to include plant species in the conversation surrounding de-extinction and overlooking the possible benefits posed by extinct plant species, hinders the development of de-extinction technology and the credibility of de-extinction as an effective conservation tool, likely resulting in missed opportunities to restore ecosystem functioning and prevent further ecosystem disturbance.

5.3 Methods

5.3.1 Species Prioritisation for De-Extinction Research

An estimated de-extinction efficiency ranking framework was developed to prioritise for de-extinction research efforts the 122 species of plants listed as "*Extinct*" under the IUCN Red List as of October 2018 (IUCN 2018). This ranking framework was adapted from the conservation project efficiency metric developed by Joseph *et al.* (2009). This framework incorporates intrinsic and extrinsic species values, such as medicinal value, while also considering the ranked estimated probability of project success, through an identification of suitable *ex situ* samples for future de-extinction efforts and key habitat and threat information.

5.3.2 De-Extinction Efficiency Framework

The conservation project efficiency framework of Joseph *et al.* (2009) is outlined in Equation 5.1:

$$E_i = \frac{W_i \, B_i \, S_i}{C_i}, \qquad \text{Equation 5.1}$$

where *E* is the conservation (or de-extinction) project efficiency score for the species *i*, *W* is the species weight (which can represent intrinsic and extrinsic species values), *B* is the conservation benefit of conservation efforts, *S* is the probability of project success and *C* is the cost of conservation efforts. This framework and its components are based on known probabilities and associated costs, however due to a lack of quantitative data for extinct species I am unable to calculate project efficiency scores for de-extinction candidate species (Joseph *et al.* 2009). Instead, based on data availability, I assign multiple potential indicators for each component of this project efficiency framework (Table 5.1), which act to increase or decrease the value of each component and overall de-extinction project efficiency score.

5.3.3 De-Extinction Efficiency Indicators

For each of the component indicators I normalised the data available for each species to lie between zero and one, thus ranking the species and allowing me to utilise multiple indicator values per framework component. This normalisation was carried out for each indicator as shown in Equation 5.2:

$$V_{ij} = \frac{O_{ij} - Min_j}{Max_j - Min_j}, \qquad \text{Equation 5.2}$$

Where *V* is species *i*'s normalised indicator value for indicator *j* and O is species *i*'s observed indicator value for indicator *j*. *Min* represents the minimum observed value for the indicator *j*, and *Max* represents the maximum possible value for indicator *j*, across all extinct species assessed. I then sum each individual species' normalised indicator values for each framework component, generating a mean species-specific value, and therefore ranking, for each individual component (e.g. summing all species *i*'s normalised indicator values for component *W* and subsequently generating a mean species ranked component value, *RW*). Based on these multiple indicators I generate mean normalised indicator species rankings for each representative component of Equation 5.1. These ranked components are referred to as ranked species weight (*RW*), ranked conservation benefit (*RB*), ranked estimated probability of project success (*RS*) and ranked estimated cost of project (*RC*), to make clear the fact that they represent mean species ranking values and not known probabilities and associated costs. An example of this summation and mean generation for all species *i*'s normalised indicator values for the ranked component *RW* is shown in Equation 5.3:

$$RW_i = \frac{M_i + E_i + S_i + T_i + G_i}{5}, \qquad \text{Equation 5.3}$$

where *RW* is species *i*'s mean indicator value for the ranked component *RW* (indicating species weight in Equation 5.1), and where *M*, *E*, *S*, *T* and *G* represent species *i*'s normalised indicator values (calculated as per Equation 5.2, using the indicators outlined in Table 5.1) for the five assessed indicators for the ranked component, *RW*. This mean value per ranked component was necessary due to the differing number of indicators per framework component (Table 5.1). This indicator summation and mean generation procedure was followed for each representative ranked component (*RW, RB, RS* and *RC*), using the indicators outlined in Table 5.1 and discussed below.

5.3.4 Species Rankings

Although the calculation of quantitative project efficiency scores is not possible, the calculated species-specific mean indicator values per ranked component permit the generation of an overall estimated de-extinction efficiency ranking score for each extinct plant species, allowing me to rank each species for future de-extinction research initiatives as outlined in Equation 5.4:

$$RE_i = \frac{RW_i + RB_i + RS_i}{RC_i}, \qquad \text{Equation 5.4}$$

where *RE* is the estimated de-extinction project efficiency ranking score for the species *i*, *RW* is the ranked species weight (representing medicinal, economic, ecological and taxonomic values), *RB* is the ranked conservation benefit of de-extinction efforts, *RS* is the ranked estimated probability of project success and *RC* is the ranked estimate of de-extinction project costs.

5.3.5 Ranking Procedures

As four of the indicators assessed (availability of extant relatives, availability of seed samples, availability of published DNA sequences and availability of herbarium specimens) are included as indicators of both the probability of project success (*S*) and the cost of a project (*C*), I undertook two ranking procedures (Ranking Procedure 1 and 2 respectively). Ranking Procedure 1 included all available indicators as outlined in Table 5.1, including the duplicate usage of the indicators mentioned for both *RS* and *RC* calculations. Ranking Procedure 2 used the indicators above to calculate the ranked estimated cost of a project (*RC*) but did not use them to calculate the ranked estimated probability of project success (*RS*), as these indicators are the only indicators available for the cost of a project (*C*). Therefore, in Ranking Procedure 2, the indicators available for the ranked probability of project success (*RS*) were reduced to just three (time since extinction, habitat and ecological knowledge and threat knowledge and resolution) rather than the seven available for Ranking Procedure 1. Results from both ranking procedures are reported and discussed.

All analyses were carried out using the R programme (version 3.4.3; R Core Team 2017). Venn diagrams were generated using the Bioinformatics and Evolutionary Genomics web tool (2020). Plant silhouette images used throughout are provided by Phylopic under Public Domain license (http://phylopic.org/).

Table 5.1 | The indicators used in this study to measure each ranked component of Equation 5.4 and the relevant data sources for each indicator.

Indicators used only in Ranking Procedure 1 are denoted by (*), with all other indicators used in both Ranking Procedures 1 and 2.

Framework Component	Indicators	Data Sources
Ranked Species Weight (*RW*)	Medicinal Value	Royal Botanic Gardens (RBG) Kew Medicinal Plant Names Service database (RBG Kew 2020c)
	Economic Value	IUCN Red List assessment (IUCN 2018) RBG Kew Economic Botany Collection database (RBG Kew 2020b) RBG Kew SEPASAL database (RBG Kew 1999) Crop Wild Relatives assessment by Liu *et al.* (2019)
	Scientific Value	IUCN Red List assessment (IUCN 2018)
	Ecological Value	NA
	Taxonomic Value	Catalogue of Life (Roskov *et al.* 2019)
	General Interest	Web of Science (https://clarivate.com/products/web-of-science/)
Ranked Conservation Benefit (*RB*)	NA	NA

Framework Component	Indicators	Data Sources
Ranked Probability of Project Success (*RS*)	Time Since Extinction	IUCN Red List assessment (IUCN 2018)
	Habitat and Ecological knowledge	IUCN Red List assessment (IUCN 2018)
	Threat Knowledge and Resolution	IUCN Red List assessment (IUCN 2018)
	Availability of Herbarium Specimens *	RBG Kew Herbarium (RBG Kew 2020e) New York Botanical Garden (NYBG) William and Lynda Steere Herbarium (NYBG 2020a) Smithsonian Botany Collection (SNMNH 2020) Global Biodiversity Information Facility (GBIF) occurrences (GBIF 2020)
	Availability of Seed Samples *	RBG Kew Millennium Seed Bank (RBG Kew 2020d) Svalbard Global Seed Vault (SGSV 2020) European Native Seed Conservation Network Database (ENSCOBASE) (ENSCONET 2020)
	Availability of Published DNA Sequences *	RBG Kew DNA Bank (RBG Kew 2020a) NYBG William and Lynda Steere Herbarium DNA Bank (NYBG 2020b) GenBank (Benson *et al.* 2017)
	Availability of Extant Relatives *	Catalogue of Life (Roskov *et al.* 2019)
Ranked Cost of Project (*RC*)	Availability of Extant Relatives	See above
	Availability of Seed Samples	See above
	Availability of Published DNA Sequences	See above
	Availability of Herbarium Specimens	See above

5.3.6 Species Weight (*W*) Indicators

Each of the indicators listed below represent intrinsic and extrinsic species-specific values which each increase the overall species weight (*W*) value in Equation 5.1. All individual indicator values are normalised as described below, with each species being ranked between 0 (lowest possible species weight ranking, *RW*) and 1 (highest possible species weight ranking, *RW*).

Medicinal Value

Potential species-specific medicinal value was measured as the number of times the species is included in the Royal Botanic Gardens (RBG) Kew Medicinal Plant Names Service database (RBG Kew 2020c). This database provides a global reference resource for the 27,734 plants (and plant products) which have been assessed as relevant to pharmacological research, health regulation and traditional medicine (RBG Kew 2020c). Based on the number of times each extinct plant appears on the service, I assigned a species-specific normalised ranking value between 0 (species does not appear on the service) and 1 (species appears 5 times on the service).

Economic Value

Economic value is measured as the reporting of the species as economically important under the species' IUCN Red List assessment, the inclusion of the species in the RBG Kew Economic Botany Collection and SEPASAL databases and whether the species is listed as a wild relative of domestic crops by Liu *et al.* (2019). The IUCN Red List assessments of numerous species incorporate the "*Use and Trade*" of individual species, which can range from trade in ornamental plants to the use of plants and plant products in construction materials and fuels (IUCN 2018). The RBG Kew Economic Botany Collection includes more than 100,000 plant-derived objects, including textiles, wood, foods etc., representing plant species of economic importance globally (RBG Kew 2020b). Similarly, the RBG Kew Survey of Economic Plants for Arid and Semi-Arid Lands (SEPASAL) database highlights dryland plants of economic importance (RBG Kew 1999). I also assess whether the extinct species assessed are considered wild relatives of domestic crops, and therefore of potential economic value, through a comparison of the species assessed with the 4,450 crop wild relative taxa compiled by Liu *et al.* (2019) from the Harlan and de Wet Crop Wild Relative Inventory and Germplasm Resources Information Network. Based on the number of economic databases each extinct plant species is represented within, I assigned a species-specific normalised ranking value of between 0 (species does not appear in any economic data source specified) and 1 (species appears in all four economic data sources specified).

Scientific Value

Although scientific value can take many forms, here I measure scientific value as the requirement for future species-specific research under the species' IUCN Red List Assessment (IUCN 2018), with all species ranked as either 0 (no reported research required) or 1 (specified research required). Examples of reported research required include "*Taxonomy*" and "*Population size, distribution & trends*" (IUCN 2018).

Ecological Value

Although knowledge surrounding the ecological role and both the inter and intraspecific interactions of a species are likely critical to the success of any potential de-extinction efforts (Seddon *et al.* 2014), a lack of empirical and observational studies means that such information is often not available for extinct species (either quantitatively or qualitatively), with almost no real prospects of advancement (Wood *et al.* 2017). Therefore, I am unable to include any appropriate indicator of species-specific ecological value in this analysis but highlight the importance of such indicators in de-extinction research as a whole.

Taxonomic Value

Taxonomically unique species are often considered of conservation and prioritisation importance (Jones 2014). Here I measure taxonomic uniqueness as the total number of extant species per genus according to the Catalogue of Life (Roskov *et al.* 2019), with each species assigned a normalised ranked value of between 0 (2,996 extant relatives, least taxonomically unique) and 1 (zero extant relatives, most taxonomically unique).

General Interest

Although there are numerous other criteria with the potential to be included, I use the number of times the species appears when searched on Web of Science as an indicator of general interest in the species. Web of Science (https://clarivate.com/products/web-of-science/) was chosen as an appropriate indicator due to the multiple databases this search engine accesses and the breadth of academic and professional disciplines included. All species were ranked based on search results, with normalised values ranging from 0 (species does not appear when searched) to 1 (species appears 9 times when searched).

5.3.7 Conservation Benefit (*B*) Indicators

The conservation benefit, *B*, of conservation efforts is typically measured as the difference in probability of persistence of a species with and without conservation actions ($X_0 - X_i$), where X_i is the probability of extinction with conservation action and X_0 is the probability of extinction without conservation action. As all species being considered here currently have no extant populations, and therefore no probability of extinction, the conservation benefit of de-extinction efforts is the same for each species. Therefore, no conservation benefit indicators are possible or necessary for this analysis, with each species being assigned a normalised ranked conservation benefit, *RB*, value of 1 (i.e. $RB_i = 1$ for all species).

5.3.8 Probability of Project Success (*S*) Indicators

Each of the indicators listed below represent values which each increase the overall probability of de-extinction project success (*S*) in Equation 5.1. All individual indicator values are normalised as described below, with each species being ranked between 0 (lowest possible estimated probability of success ranking, *RS*) and 1 (highest possible estimated probability of success ranking, *RS*).

Time Since Extinction

It is almost universally agreed that as time since extinction increases, the probability of successful de-extinction efforts decreases, predominantly due to a lack of species-specific knowledge and habitat alterations (Abeli *et al.* 2020; Iacona *et al.* 2017; Jones 2014; McCauley *et al.* 2017; Seddon *et al.* 2014; Shapiro 2017; Wood *et al.* 2017). Here I measured time since extinction as the time since the species was last recorded (relative to 2020), according to their IUCN Red List assessment, or if no date was recorded, then the year the species was assessed as "*Extinct*" under the IUCN Red List. All species were assigned a normalised ranked value between 0 (274 years since extinction, lowest probability of project success) and 1 (four years since extinction, highest probability of project success).

Habitat and Ecological Knowledge

Species-specific habitat and ecological knowledge are necessary for de-extinction, therefore as the availability of such information increases, so does the potential success of the de-extinction project (Seddon *et al.* 2014). Habitat and ecological knowledge were measured as the availability of previous habitat and ecological information under the species' IUCN Red List assessment (IUCN 2018). All species were ranked as either 0 (no ecological information provided), 0.33 (basic habitat information provided e.g. "*Forest - Subtropical/Tropical Moist Lowland*"), 0.66

(additional abiotic habitat information provided e.g. climate, altitude, detailed abiotic aspects etc.) or 1 (habitat information and inter/intraspecific biotic information provided).

Threat Knowledge and Resolution

If the underlying threats causing the original extinction of a species are not appropriately identified and addressed, then any de-extinction effort aiming to restore ecologically meaningful populations is unlikely to be successful (Jones 2014; Seddon *et al.* 2014; Wood *et al.* 2017). Here I measured threat knowledge and any threat resolutions as the provision of species-specific threat information under the species' IUCN Red List assessment, including the current status of the threats (e.g. "*Ongoing*" or "*Past, unlikely to return*"). All species were ranked as either 0 (no threat information provided), 0.33 (threat information provided, but no threats have been resolved), 0.66 (threat information provided and some threats have been resolved) or 1 (threat information provided and all threats have been resolved). It is important to note that many threats which were labelled as "*Past, unlikely to return*", were still in fact ongoing, however due to the extinction of the species they were labelled as "*Past, unlikely to return*". This was particularly evident for invasive alien species threats. As a result, all threats were assumed to be "*Ongoing*" unless manual searches showed them to be resolved.

Availability of Herbarium Specimens

The combined number of herbarium specimens available per species from the RBG Kew Herbarium, the New York Botanical Garden (NYBG) William and Lynda Steere Virtual Herbarium, Smithsonian Botany Collection and species-specific Global Biodiversity Information Facility (GBIF) occurrences were used to measure the potential availability of herbarium samples for future de-extinction efforts. The RBG Kew Herbarium currently contains approximately 7 million plant specimens, with more than 600,000 specimens already digitised (RBG Kew 2020e). The four million digitised specimens of the NYBG William and Lynda Steere Herbarium were assessed through the C. V. Starr Virtual Herbarium (NYBG 2020a). The Smithsonian Botany Collection currently contains more than 5 million historical plant records, with 1.7 million plant specimens records currently available online (SNMNH 2020). Similarly, GBIF provides standardised access to hundreds of millions of species occurrence records, spanning numerous databases, including preserved herbarium specimens globally (GBIF 2020). Species were assigned a normalised ranked value between 0 (no known herbarium specimens, lowest probability of project success) and 1 (104 herbarium specimens recorded, highest probability of project success).

Availability of Seed Samples

The combined number of viable seed samples available per species from the RBG Kew Millennium Seed Bank, Svalbard Global Seed Vault and European Native Seed Conservation Network database (ENSCOBASE) were used to assess the availability of seed samples. The RBG Kew Millennium Seed Bank is currently the largest *ex situ* plant conservation programme in the world and contains more than 2.3 billion individual seeds, representing over 40,000 different plant species (RBG Kew 2020d). Similarly, the Svalbard Global Seed Vault currently contains more than 980,000 samples, with a focus on plants of agricultural and economic importance (SGSV 2020). ENSCOBASE provides access to a variety of seed collections across Europe, while also providing information regarding seed germination, moisture content etc. (ENSCONET 2020). Species were assigned a normalised ranked value between 0 (no known seed samples available) and 1 (known seed samples available).

Availability of Published DNA Sequences

The combined number of published DNA sequences per species from the RBG Kew DNA Bank, the NYBG William and Lynda Steere Herbarium DNA Bank and GenBank were used to measure the availability of published DNA sequences per species. The RBG Kew DNA and Tissue Bank collection currently contains more than 48,000 samples of plant genomic DNA and over 11,000 dried tissue samples which are available to researchers for a nominal fee (RBG Kew 2020a). Similarly, the DNA Bank at the NYBG William and Lynda Steere Herbarium contains more than 20,000 frozen plant, algal, and fungal tissues and extracted DNA samples (NYBG 2020b). GenBank is an open access repository for publicly available nucleotide sequences and currently contains more than 100 million individual DNA sequences (Benson *et al.* 2017). All species were assigned a normalised ranked value between 0 (no known published DNA sequences) and 1 (26 published DNA sequences).

Availability of Extant Relatives

The availability of extant relatives can be used in de-extinction research for genetic engineering and genome sequencing purposes (Steeves *et al.* 2017; Wood *et al.* 2017). This indicator is represented here by the total number of extant species per genera according to the Catalogue of Life (Roskov *et al.* 2019), with each species assigned a normalised ranked value of between 0 (zero extant relatives, lowest probability of project success) and 1 (2,996 extant relatives, highest probability of project success).

5.3.9 Cost of Project (*C*) Indicators

The availability of suitable samples and extant relatives are critical to the cost-effectiveness and feasibility of any de-extinction attempt (Iacona *et al.* 2017; IUCN SSC 2016; Shapiro 2017; Steeves *et al.* 2017; Wood *et al.* 2017). Each of the indicators listed below represent values which potentially decrease the overall cost of a de-extinction project (*C*) in Equation 5.1 through the availability of such material. All individual indicator values are normalised as described below, with each species being ranked between 0 (lowest potential project cost ranking, *RC,* higher overall estimated efficiency score ranking, *RE*) and 1 (highest possible project cost ranking, *RC*, lower overall estimated efficiency score ranking*, RE*).

Availability of Extant Relatives

The availability of extant relatives can be used in de-extinction research for genetic engineering and genome sequencing purposes (Steeves *et al.* 2017; Wood *et al.* 2017). This indicator is represented here by the total number of extant species per genera according to the Catalogue of Life (Roskov *et al.* 2019), with each species assigned a normalised ranked value of between 0 (2,996 extant relatives, lowest potential project cost) and 1 (no known extant relatives, highest potential project cost).

Availability of Seed Samples

The combined number of viable seed samples available per species from the RBG Kew Millennium Seed Bank, Svalbard Global Seed Vault and European Native Seed Conservation Network database (ENSCOBASE) were used to assess the availability of seed samples. Species were assigned a normalised ranked value between 0 (known seed samples available) and 1 (no known seed samples available).

Availability of Published DNA Sequences

The combined number of published DNA sequences per species from the RBG Kew DNA Bank, the NYBG William and Lynda Steere Herbarium DNA Bank and GenBank were used to measure the availability of published DNA sequences per species. All species were assigned a normalised ranked value between 0 (26 published DNA sequences, lowest potential project cost) and 1 (no known published DNA sequences, highest potential project cost).

Availability of Herbarium Specimens

The combined number of herbarium specimens available per species from the RBG Kew Herbarium, the NYBG William and Lynda Steere Virtual Herbarium, Smithsonian Botany

Collection and species-specific GBIF occurrences were used to measure the potential availability of herbarium samples for de-extinction efforts. Species were assigned a normalised ranked value between 0 (104 herbarium specimens recorded, lowest potential project cost) and 1 (no known herbarium specimens, highest potential project cost).

5.4 Results

5.4.1 Ranking Procedures

Despite the removal of four probability of project success (*S*) indicators in Ranking Procedure 2, both Ranking Procedure 1 and 2 produced similar results, with both procedures producing the same top five candidates and sharing a further 12 species among their respective top 20 candidates. Similarly, of the ten lowest ranking species, nine were common to both Ranking Procedure 1 and 2. As a result only the results of Ranking Procedure 1 are discussed. Comparisons of the 20 highest and lowest ranking species between Ranking Procedure 1 and 2 are shown in Appendix Tables D.1 and D.2. A comparison of the estimated de-extinction project efficiency ranking scores (*RE*) for the 50 highest ranking species between Ranking Procedure 1 and 2 are shown in Appendix Figure D.1. A comparison between the mean ranked estimated probability of project success (*RS*) and mean ranked estimated cost of project (*RC*) are shown in Appendix Figure D.2 for Ranking Procedures 1 and 2, highlighting the difference in the number of indicators used to calculate *RS* between both ranking procedures.

5.4.2 Species Rediscoveries

Of the 122 plant species recorded as "*Extinct*" under the IUCN Red List (IUCN 2018), 19 (15.6%) have since been rediscovered (Table 5.2). For example, although considered extinct under the IUCN Red List, the labelling of living botanical garden specimens as *Dracaena umbraculifera* prompted Edwards *et al.* (2018) to undertake field surveys in Madagascar, where five extant wild populations were subsequently identified. As a result, the IUCN Red List status of this species should be changed from "*Extinct*" to "*Critically Endangered*" to reflect the persistence of <50 individuals in the wild and to plan conservation actions accordingly (Edwards *et al.* 2018). Of these 19 rediscovered species, the IUCN Red List status has only been updated for six species to reflect this new information (IUCN 2020). As the majority of these species are still considered "*Extinct*" under the IUCN Red List I retain them in the analyses presented here, noting their occurrence as necessary.

Table 5.2 | The species listed as "*Extinct*" under the IUCN Red List as of October 2018 which have subsequently been rediscovered. References provided refer to the rediscovery of the species. Note some species have since had their global IUCN Red List status updated to reflect this rediscovery (*).

Rediscovered Species	**Reference**
Adiantum lianxianense (Pteridaceae)	Ebihara *et al.* 2012
Astragalus nitidiflorus (Fabaceae)	Martínez-Sánchez *et al.* 2011
Crudia zeylanica (Fabaceae)	Gunawardana 2019
Cynometra beddomei * (Fabaceae)	Sudhi 2012
Dracaena umbraculifera * (Asparagaceae)	Edwards *et al.* 2018
Madhuca insignis * (Sapotaceae)	Joshi *et al.* 2019
Melicope cruciata (Rutaceae)	Wood 2007
Melicope paniculata * (Rutaceae)	Nagendra *et al*. 2020
Ochrosia brownii (Apocynaceae)	Lorence and Butaud 2011
Ochrosia fatuhivensis (Apocynaceae)	Lorence and Butaud 2011
Ochrosia tahitensis (Apocynaceae)	Meyer and Butaud 2009
Otophora unilocularis (Sapindaceae)	Wang *et al.* 2018
Pradosia glaziovii (Sapotaceae)	Terra-Araujo *et al.* 2016
Pradosia mutisii (Sapotaceae)	Terra-Araujo *et al.* 2016
Radula visianica * (Radulaceae)	Köckinger 2016
Rauvolfia nukuhivensis (Apocynaceae)	Lorence and Butaud 2011
Shorea cuspidata * (Dipterocarpaceae)	Julia *et al.* 2014
Wendlandia angustifolia (Rubiaceae)	Viswanathan *et al.* 2000
Wikstroemia skottsbergiana * (Thymelaeaceae)	PEPP 2017

5.4.3 Species Weight (*W*) Indicators

Of the 122 de-extinction candidate species assessed, none were present in all of the five weight (*W*) indicators (Figure 5.1). One species (*Angraecopsis dolabriformis*; Orchidaceae) was represented in four *W* indicators (economic value, medicinal value, taxonomic value and general interest) and a further 16 species were represented in three *W* indicators. Of the 122 species assessed, 18 were not represented under any specified *W* indicator. 117 out of the 122 species assessed have at least one extant genus-specific relative (ranging from 1 to 2,996 extant relatives, mean = 203.42 ± 373.23 S.D.). Five species have no extant genus-specific relatives and represent the most taxonomically unique species. These *are Flabellidium spinosum* (Brachytheciaceae)*,* Macoun's shining moss (*Neomacounia nitida*; Neckeraceae)*,* the Saint Helena olive (*Nesiota elliptica*; Rhamnaceae)*, Streblorrhiza speciose* (Fabaceae) and Adams mistletoe (*Trilepidea adamsii*; Loranthaceae). Overall, the mean normalised taxonomic indicator value for all species was 0.93 (± 0.12 S.D.). Only nine species had a recorded economic value in any of the economic databases assessed, resulting in a mean normalised economic indicator value for all species of 0.05 (± 0.17 S.D.). Further scientific research was requested as part of the IUCN Red List assessment for 26 of the 122 species, giving a mean normalised scientific indicator value of 0.21 (± 0.41 S.D.) for all species. Nearly all species (80/122) appeared on Web of Science, with results of 0 - 9 appearances per species. For all species, this gave a mean normalised general interest indicator value of 0.14 (± 0.17 S.D.). Only two of the 122 candidate species, the Galapagos amaranth (*Blutaparon rigidum*; Amaranthaceae) and *Cnidoscolus fragrans* (Euphorbiaceae), had a recorded medicinal value under the RBG Kew Medicinal Plant Names Service database, giving a mean normalised medicinal indicator value for all species of 0.01 (± 0.09 S.D.). The highest-ranking species based on mean ranked species weight (*RW*) indicators alone are presented in Table 5.3.

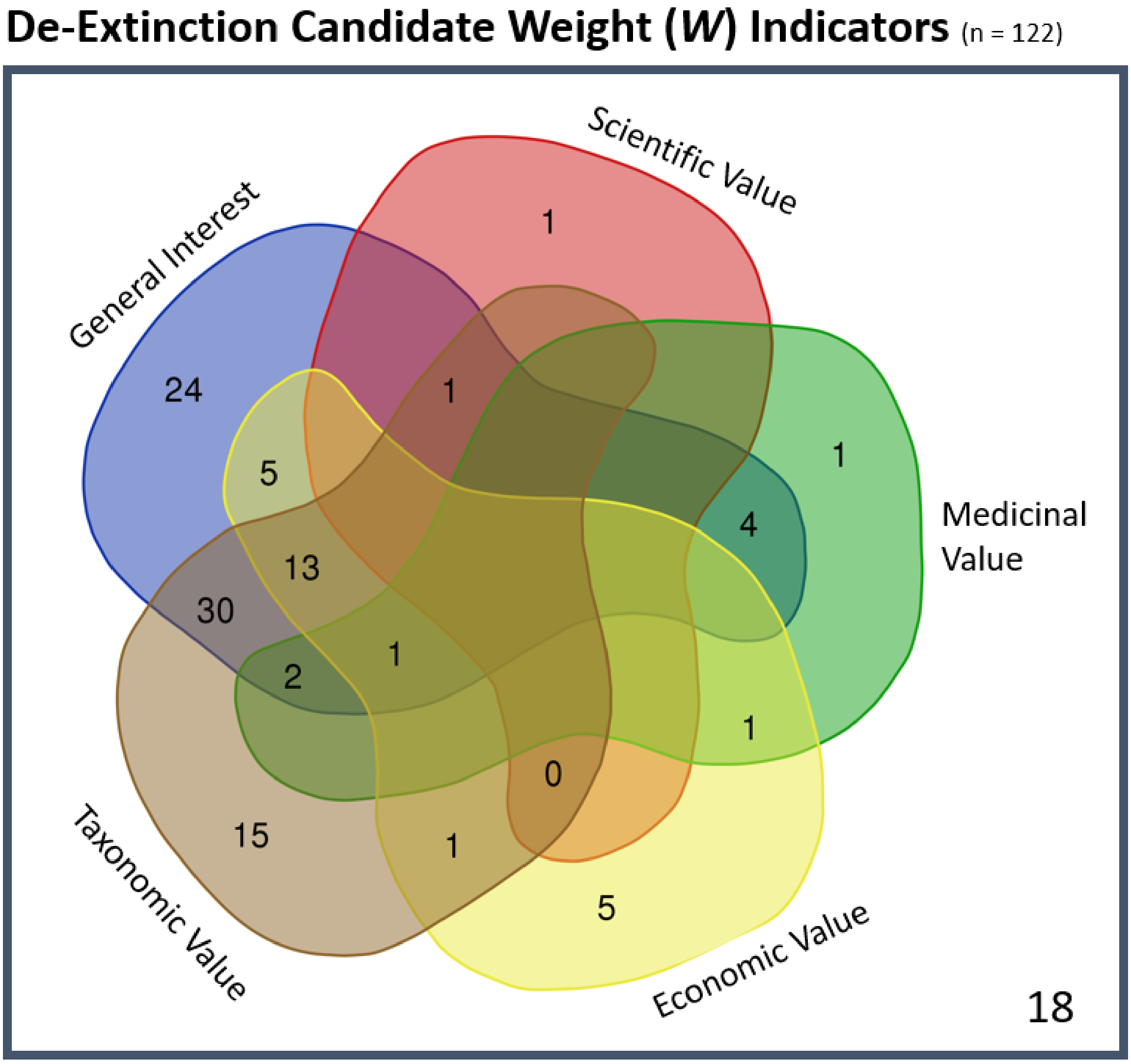


Figure 5.1 | The number of extinct plant species represented under each species weight (*W*) indicator. Values for scientific, medicinal, economic and general interest represent all species with a normalised indicator value greater than zero, due to the limited number of species represented under each indicator. Taxonomic value represents all species with a taxonomic indicator value equal to or greater than the median normalised value of 0.978.

5.4.4 Probability of Project Success (*S*) Indicators

Time Since Extinction

The recorded time since extinction ranges from four years for *Cyanea minutiflora* (Campanulaceae), which was assessed as extinct by the IUCN Red List in 2016, to 274 years for *Pausinystalia brachythyrsum* (Rubiaceae), which is only known from a type specimen collected in 1746 (IUCN 2018). The mean time since extinction is 95.98 (± 63.63 S.D.) years, resulting in a mean normalised time since extinction indicator value for all species of 0.66 (± 0.24 S.D.).

Habitat and Ecological Knowledge

Of the 122 species considered here, 33 have no available habitat information as part of their IUCN Red List assessment, while a further 47 only record basic habitat information e.g. "*Savanna - Dry*". Of the remaining 42 species, 31 provide additional detailed abiotic habitat information, e.g. the habitat of the woolly-stalked begonia (*Begonia eiromischa*; Begoniaceae) is described as "*granite rocks at 170 m altitude surrounded by dipterocarp forest*" on Penang Island, Malaysia (IUCN 2018). Only 11 species provide any biotic information as part of their IUCN Red List assessment, e.g. *Hibiscadelphus woodii* is described as being associated with native species such as *Chamaesyce celastroides* (Euphorbiaceae), *Nototrichium divaricatum* (Amaranthaceae), *Melicope pallida* and *Carex meyenii* (Cyperaceae) on the Hawaiian island of Kauai (IUCN 2018). As a result, the mean normalised habitat and ecological knowledge indicator value for all species was 0.39 (± 0.31 S.D.).

Threat Knowledge and Resolution

No threat information is recorded as part of the IUCN Red List assessment for 44 species, providing no indication for the cause of species declines or extinctions. Although species-specific threat information is provided for a further 71 species, I found no evidence that the threats had been resolved. For example, although feral pigs (*Sus domesticus*) are listed under IUCN as a "*Past, Unlikely to Return*" threat to the extinct *Acaena exigua*, a member of the rose family (Rosaceae) native to the Hawaiian islands of Kaua'i and west Maui, feral pigs are still present on both Kau'i and Maui (Else 2018). For three species I was able to confirm that some of the threats reported as part of the IUCN Red List assessment had been resolved and for four species I was able to confirm that all reported threats had been resolved. For example, according to the IUCN Red List the only threats to *Streblorrhiza speciose* (Fabaceae), a perennial shrub endemic to Phillip Island, were posed by invasive goats (*Capra hircus*), pigs (*Sus domesticus*) and rabbits (*Oryctolagus cuniculus*), all of which have subsequently been eradicated from Phillip Island

(Coyne 2010). This gave a mean normalised threat knowledge and resolution value for all species of 0.24 (± 0.22 S.D.).

Availability of Samples and Extant Relatives

None of the 122 extinct species assessed here are represented within the RBG Kew Millennium Seed Bank, Svalbard Global Seed Vault or the European Native Seed Conservation Network database (ENSCOBASE). Therefore, although herbarium specimens may also contain seeds, the mean normalised availability of seed samples indicator value is zero for all species. The number of herbarium specimens available per species from the RBG Kew Herbarium, NYBG William and Lynda Steere Virtual Herbarium, Smithsonian Botany Collection and species-specific GBIF occurrences, ranges from zero to 104 (*Delissea subcordata*; Campanulaceae), with a mean number of herbarium samples of 15.61 (± 20.12 S.D.; Figure 5.2). Eight species do not have any preserved herbarium specimens in the collections assessed here, greatly reducing the probability of project success (*Acalypha rubrinervis, Cnidoscolus fragrans, Cyanea dolichopoda, Euphrasia mendoncae, Galipea ossana, Guettarda retusa, Sicyos villosus* and *Sterculia khasiana*). Accordingly, the mean normalised availability of herbarium specimens indicator value for all species was 0.15 (± 0.19 S.D.).

The combined number of published DNA sequences per extinct species available from the RBG Kew DNA Bank, the NYBG William and Lynda Steere Herbarium DNA Bank and GenBank ranged from zero (e.g. *Kokia lanceolata*) to 26 sequences (*Melicope paniculata*), with a mean of 1.11 (± 3.93 S.D.). Importantly 102 species have no published DNA sequences, resulting in a mean normalised availability of published DNA sequences indicator value for all species of 0.04 (± 0.15 S.D.). As already stated, 117 of the 122 species assessed have at least one extant genus-specific relative, with values ranging from 1 to 2,996 extant relatives (mean of 203.42 ± 373.23 S.D.), providing a mean normalised availability of extant relatives indicator value for all species of 0.07 (± 0.12 S.D.). The combined availability of seed samples, herbarium specimens, published DNA sequences and extant genus-specific relatives for all species is shown in Figure 5.3, where we can see that 16 species have herbarium samples, published DNA sequences and extant relatives available, whereas a further 96 species have two of these three indicators available. The highest-ranking species based on mean ranked estimated probability of project success (*RS*) indicators alone are presented in Table 5.3.

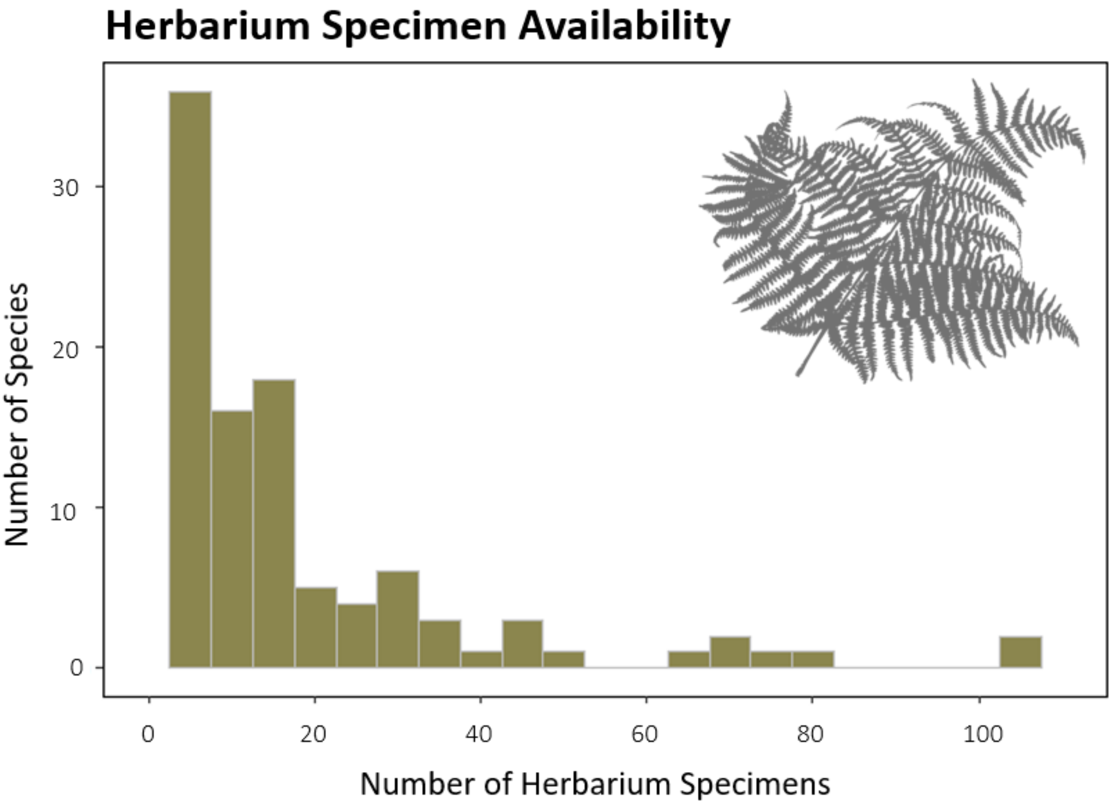


Figure 5.2 | The total number of herbarium specimens available per extinct plant species. Data represent the herbarium collection of the RBG Kew Herbarium, New York Botanical Garden (NYBG) William and Lynda Steere Virtual Herbarium, Smithsonian Botany Collection and species-specific Global Biodiversity Information Facility (GBIF) occurrences. Values range from 0 to 104 (mean = 15.61 ± 20.12 S.D.). Silhouette image provided by Phylopic under Public Domain license (http://phylopic.org/).

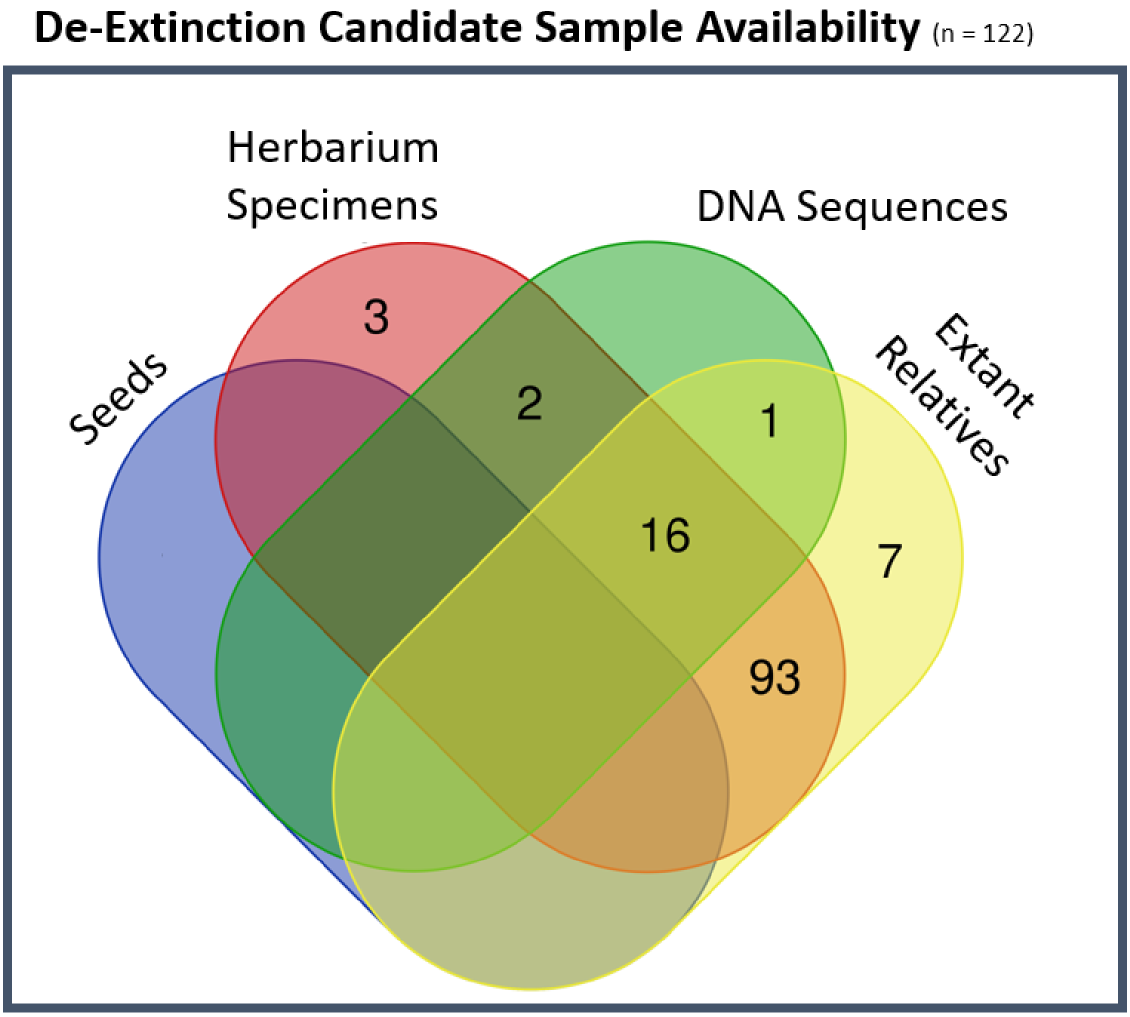


Figure 5.3 | The number of extinct plant species for which herbarium samples, DNA sequences, extant relatives and seed samples are available. All indicator values represent species with a normalised indicator value greater than zero. Herbarium samples represents the herbarium collection of the RBG Kew Herbarium, New York Botanical Garden (NYBG) William and Lynda Steere Virtual Herbarium, Smithsonian Botany Collection and species-specific Global Biodiversity Information Facility (GBIF) occurrences. The availability of extant relatives is represented by the total number of extant species per genus according to the Catalogue of Life (Roskov *et al.* 2019). DNA sequences represents the combined number of published DNA sequences per species from the RBG Kew DNA Bank, the NYBG DNA Bank and GenBank. Seed sample availability is represented the combined number of viable seed samples available per species from the RBG Kew Millennium Seed Bank, Svalbard Global Seed Vault and European Native Seed Conservation Network database (ENSCOBASE).

5.4.5 Cost of Project (*C*) Indicators

Availability of Samples and Extant Relatives

As stated above, none of the 122 extinct species assessed here are represented within any of the seed banks assessed here (RBG Kew Millennium Seed Bank, Svalbard Global Seed Vault and ENSCOBASE), resulting in a mean normalised availability of seed samples indicator value of one for all species (i.e. highest possible project cost). The number of herbarium specimens available per species from the RBG Kew Herbarium, NYBG William and Lynda Steere Virtual Herbarium, Smithsonian Botany Collection and species-specific GBIF occurrences, ranged from zero to 104, with a mean of 15.61 (± 20.12 S.D.) samples per species (Figure 5.2). Eight species do not have any preserved herbarium specimens in the collections assessed here, greatly increasing the potential project costs and decreasing project feasibility (*Acalypha rubrinervis, Cnidoscolus fragrans, Cyanea dolichopoda, Euphrasia mendoncae, Galipea ossana, Guettarda retusa, Sicyos villosus* and *Sterculia khasiana*). Accordingly, the mean normalised availability of herbarium specimens indicator value for all species was 0.85 (± 0.19 S.D.). The combined number of published DNA sequences per extinct species available from the RBG Kew DNA Bank, the NYBG DNA Bank and GenBank ranged from zero to 26 sequences, with a mean of 1.11 (± 3.93 S.D.) sequences per species. No published DNA sequences were available for 102 species, resulting in a mean normalised availability of published DNA sequences indicator value for all species of 0.96 (± 0.15 S.D.). Once again, 117 of the 122 species assessed have at least one extant genus-specific relative according to the Catalogue of Life (Roskov *et al.* 2019), with values ranging from 1 to 2,996 extant relatives (mean of 203.42 ± 373.23 S.D.). As a result, the mean normalised availability of extant relatives indicator value for all species was 0.93 (± 0.12 S.D.). The combined availability of seed samples, herbarium specimens, published DNA sequences and extant genus-specific relatives for all species is shown in Figure 5.3. The highest-ranking species with the lowest mean ranked estimate of project costs (*RC*) indicators alone are presented in Table 5.3.

5.4.6 Species Rankings

Species rankings based on both the estimated de-extinction project efficiency ranking scores (*RE*, Equation 5.4) and its individual components are presented in Table 5.3. Similarly, the 20 highest and 20 lowest ranking plant species prioritised for de-extinction research efforts are presented in Table 5.4. Based on this analysis, the top five species to be considered for de-extinction research are the Lihue melicope (*Melicope paniculata*), the pilo 'ula (*Melicope cruciata), Delissea subcordata, Astragalus nitidiflorus* and *Madhuca insignis*. It should be noted that although *Melicope paniculata*, *Melicope cruciata, Astragalus nitidiflorus* and *Madhuca insignis* are ranked within the top five species for de-extinction research, each of these species have already been rediscovered (Joshi *et al.* 2019; Martínez-Sánchez *et al.* 2011; Wood 2007). When excluding the species which have been rediscovered, the top five species to be considered for de-extinction research are *Delissea subcordata, Chrysophyllum januariense,* the Cry violet (*Viola cryana*), the Haleakala melicope (*Melicope haleakalae*) and the Saint Helena olive (*Nesiota elliptica*). Conversely, *Galipea ossana, Delilia inelegans, Pausinystalia brachythyrsum, Xanthostemon sebertii* and *Cupaniopsis crassivalvis* represent the five lowest ranking species. A complete comparison of the mean scores for each of the de-extinction efficiency ranking components (*RW, RS* and *RC*) for the 25 highest ranking species is shown in Figure 5.4 for Ranking Procedure 1 (and Appendix Figure D.3 for Ranking Procedure 2). Here we can see that although the highest-ranking species have relatively low mean ranked species weight (*RW*) values, they possess much lower mean ranked estimated cost of project (*RC*) values and higher mean ranked estimated probability of project success (*RS*) values relative to lower ranking species. This can also be seen in Appendix Figure D.2, where we see an overall negative correlation between the mean ranked estimated probability of project success (*RS*) and the mean ranked estimated cost of project (*RC*), suggesting that species which are most likely to be successful de-extinction candidates also have the lowest estimated de-extinction costs.

Delissea subcordata represents the highest-ranking plant species for de-extinction research which is still thought to be extinct. This species was endemic to the lowland forests of the Hawaiian island of Oʻahu but has not been recorded since 1934. Although not taxonomically unique, with 12 extant relatives, *Delissea subcordata* has a recorded general interest on Web of Science and a scientific value in the form of further necessary research according to the IUCN Red List assessment, however no medicinal or economic values were found. This species' IUCN Red List assessment provides detailed abiotic habitat information, however no biotic information is provided. Similarly, both habitat modification and invasive species (competition from invasive weeds, and predation by feral ungulates, rats and slugs) are listed as species-specific threats under the IUCN, however no evidence was found that any of these threats had since been resolved. In terms of sample availability, this species has the highest number of known herbarium specimens (104), 6 published DNA sequences and 12 extant genus-specific relatives, both increasing the estimated probability of project success rankings (*RS*) and decreasing estimated project cost rankings (*RC*). Conversely, the lowest-ranking species, the Cuban endemic *Galipea ossana*, has not been recorded for approximately 200 years, has no habitat or threat information available and no known herbarium or DNA samples. As a result, the estimated probability of any de-extinction project involving *Galipea ossana* being successful is negligible.

Table 5.3 | The top five plant species prioritised for de-extinction research efforts based on both the overall ranked de-extinction efficiency framework (*RE*, Equation 5.4) and its individual components (*RW, RS* and *RC*). Components reflect ranked species weight (*RW*), ranked estimated probability of project success (*RS*) and ranked estimated cost of project (*RC*).

Ranking Mechanism				
Rank	**Species Weight (*RW*)**	**Probability of Success (*RS*)**	**Cost of Project (*RC*)**	**Overall Ranking (*RE*)**
1	*Angraecopsis dolabriformis*	*Melicope cruciata*	*Melicope paniculata*	*Melicope paniculata*
2	*Angraecum astroarche*	*Melicope paniculata*	*Melicope cruciata*	*Melicope cruciata*
3	*Blutaparon rigidum*	*Wikstroemia skottsbergiana*	*Astragalus nitidiflorus*	*Delissea subcordata*
4	*Trochetiopsis melanoxylon*	*Delissea subcordata*	*Delissea subcordata*	*Astragalus nitidiflorus*
5	*Dryopteris ascensionis*	*Madhuca insignis*	*Madhuca insignis*	*Madhuca insignis*

Table 5.4 | The 20 highest and 20 lowest ranking plant species prioritised for de-extinction research efforts. Species' estimated de-extinction project efficiency ranking scores are also provided. Results reflect Ranking Procedure 1. Species which have already been rediscovered are denoted by (*).

Top 20			Bottom 20		
Species	**Score**	**Rank**	**Species**	**Score**	**Rank**
Melicope paniculata *	3.068	1	*Galipea ossana*	1.263	122
Melicope cruciata *	2.909	2	*Delilia inelegans*	1.265	121
Delissea subcordata	2.661	3	*Pausinystalia brachythyrsum*	1.290	120
Astragalus nitidiflorus *	2.550	4	*Xanthostemon sebertii*	1.291	119
Madhuca insignis *	2.245	5	*Cupaniopsis crassivalvis*	1.293	118
Chrysophyllum januariense	2.161	6	*Weinmannia spiraeoides*	1.294	117
Viola cryana	2.016	7	*Habenaria petromedusa*	1.304	116
Melicope haleakalae	1.987	8	*Pradosia argentea*	1.308	115
Nesiota elliptica	1.975	9	*Campomanesia lundiana*	1.316	114
Dracaena umbraculifera *	1.943	10	*Gomidesia cambessedeana*	1.319	113
Melicope macropus	1.923	11	*Argocoffeopsis lemblinii*	1.326	112
Achyranthes atollensis	1.921	12	*Stenocarpus dumbeensis*	1.332	111
Trilepidea adamsii	1.884	13	*Cyanea pycnocarpa*	1.334	110
Blutaparon rigidum	1.866	14	*Valerianella affinis*	1.343	109
Hibiscadelphus woodii	1.846	15	*Psiadia schweinfurthii*	1.348	108
Dryopteris ascensionis	1.831	16	*Fitchia mangarevensis*	1.352	107
Lepidium obtusatum	1.826	17	*Kokia lanceolata*	1.355	106
Melicope nealae	1.797	18	*Melicope obovata*	1.359	105
Casearia quinduensis	1.765	19	*Hernandia drakeana*	1.363	104
Angraecum astroarche	1.763	20	*Stachytarpheta fallax*	1.368	103

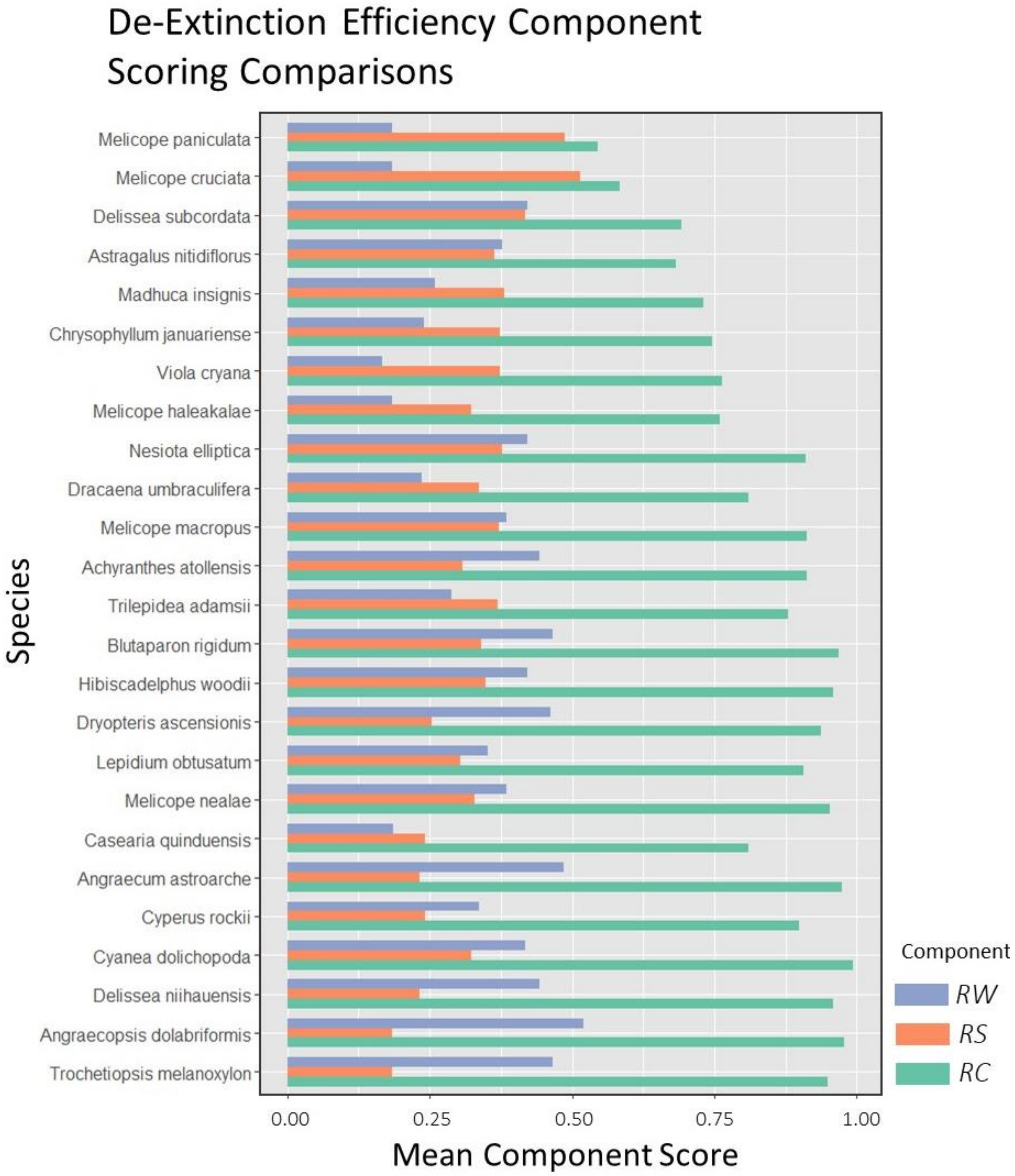


Figure 5.4 | A comparison of the mean scores for each of the de-extinction efficiency ranking components (*RW, RS* and *RC*) for the 25 highest ranking species for de-extinction research. Components reflect ranked species weight (*RW*), ranked estimated probability of project success (*RS*) and ranked estimated cost of project (*RC*). Note that high *RC* values indicate species which have higher estimated project cost rankings, and therefore lower overall estimated efficiency score rankings, *RE*. Results reflect Ranking Procedure 1, including the four shared indicators used to measure both *RS* and *RC*. The relationship between *RS* and *RC* is highlighted in Appendix Figure D.2.

5.5 Discussion

As human-induced extinctions continue to increase, the prospect of de-extinction restoring once-lost species and ecosystem functions is likely to become increasingly appealing to both conservation practitioners and the general public. However, failure to consider plants in the de-extinction conversation has resulted in a biased view of de-extinction as a whole and the technology required to achieve it. Here I show that 114 of the 122 species of extinct plants have at least one herbarium specimen, each with the potential to provide both genomic sequences and seeds capable of germination, highlighting the potential for plant de-extinction. Although I tried to incorporate as many known sample databases as possible, it is likely that additional herbarium specimens, seed samples and DNA sequences exist. Consequently, the number of specimens and samples reported here represent an overall underestimation of the total number available globally. Unsurprisingly, the extinct plants assessed have limited medicinal, economic, scientific and taxonomic values, mirroring the conventional taxonomic biases found in most disciplines (Clark and May 2002; Seddon *et al.* 2005; Troudet *et al.* 2017). Although not exhaustive, my results also highlight a clear lack of habitat, threat and ecological information for the majority of extinct plant species, greatly inhibiting the potential success of any de-extinction project and preventing informed decision-making. It is likely that herbarium specimens hold a wealth of ancillary information that has yet to be digitised, including species occurrence and ecological interaction records (Abeli *et al.* 2020). Given the physical vulnerability of herbarium specimens I encourage the prioritisation of extinct plant species over extant species for immediate digitisation efforts. Despite these limitations I show that for several species, such as *Delissea subcordata*, numerous extant close relatives, herbarium specimens and DNA sequences exist, in addition to known scientific values and habitat and threat information. Although not definitive, this implies that such species are potentially good candidates for successful de-extinction and therefore warrant further research and investigation.

The revelation that 15.6% of all plant species assessed as "*Extinct*" IUCN Red List have known extant populations highlights a disparity between conservation assessments and species occurrences. This problem is elaborated upon by Mounce *et al.* (2018) who show that 830 plant species have conflicting Regional and Global IUCN Red List assessments, with global assessments both under and over-estimating extinction risks. Similarly, of the 19 "*Extinct*" plant species rediscovered, only six have had their IUCN Red List statuses updated to reflect this information. Although not perfect, the IUCN Red List is perhaps the most conventional criteria considered when planning and prioritising conservation actions, and the difference between a species being considered “*Extinct*” and “*Threatened*” is of unarguable significance in this context (Mounce *et al.* 2018; Rodrigues *et al.* 2006). Although it takes time for the rediscovery of a species to be filtered back to the Global IUCN Red List assessment, I encourage more detailed IUCN Red List assessments, incorporating all known species distributions and occurrences wherever possible. The rediscovery of these species is however a welcome development and provides an opportunity to collect living seed and tissue samples for global seed- and bio-banking efforts, as none of the species included in this analysis had a single seed sample within any of the global seed banks assessed. Consequently, these species represent high priorities for future seed sample collection, potentially providing insurance populations should these species become extinct in the future. An unexpected, but potentially useful outcome of the ranking process employed here is the inclusion of four rediscovered “*Extinct*” plant species within the top five de-extinction candidates produced by my ranking framework, suggesting that my scoring system not only identifies potential de-extinction candidate species, but also species with the potential to be rediscovered in the wild, however this will obviously require further refinement and investigation if it is to be utilised effectively for this purpose. As rediscovery could be considered a form of de-extinction, high ranking de-extinction candidate species should also be considered a priority for further *in situ* assessments to identify potential extant populations before any further de-extinction projects are initiated.

Although herbarium specimens have been known to provide seeds, this is not a guarantee, and even if seeds are present their viability and probability of germination are extremely low (Abeli *et al.* 2020; Godefroid *et al.* 2011). As a result, even for species such as *Delissea subcordata*, with numerous extant relatives, herbarium specimens and published DNA sequences, the lack of any viable seed samples means that any potential de-extinction project is still contingent on technological and genome editing advancements. However, the availability of numerous herbarium specimens for genome sequencing does increase the genetic variation available and limit the prospect of any potential ‘resurrection genetic bottleneck’ occurring in the future (Steeves *et al.* 2017). However, a general lack of herbarium specimens means that such high levels of genetic variation are not available for the majority of extinct plant species (Sarasan *et al.* 2016). Although none of the extinct plants assessed had any seed samples stored within the global seed banks assessed, seed bank material has already been used to successfully recover two plant species (*Diplotaxis siettiana* and *Erica verticillata*) considered “*Extinct in the Wild*” under the IUCN Red List (Abeli *et al.* 2020; Hitchcock and Rebelo 2017). I therefore highlight the importance of collecting seed samples for species which are currently threatened with extinction, while extant populations and sufficient genetic variation still exist.

Unfortunately, for the 10% of angiosperms which produce recalcitrant seeds (i.e. not desiccation tolerant), standard seed banking procedures are not appropriate, leaving only orthodox species capable of benefitting from traditional seed banking efforts (Berjak and Pammenter 2008; Liu *et al.* 2018; Wyse *et al.* 2018). However, there have been promising recent advances in the successful storage of viable recalcitrant seeds using rapid cooling to ultralow temperatures (Walters *et al.* 2008; Walters *et al.* 2013). Similarly, the lack of adaptation between when seed samples were collected and stored, and the current abiotic and biotic conditions, mean that some species may no longer be able to persist in their previously suitable environments should they be reintroduced (Abeli *et al.* 2020). This was true for Yashina *et al.* (2012) who found significant morphological differences between 30,000-year-old *Silene stenophylla* compared to extant conspecifics, potentially reflecting differences in reproductive strategy (Abeli *et al.* 2020). These limitations only emphasise the fact that de-extinction cannot be seen as a substitute for the conventional *in situ* and *ex situ* conservation activities being undertaken to conserve species and their natural habitat. Even under the best-case scenario, where living adult somatic cells exist, none of the pathways to de-extinction will create an organism which is completely identical to the extinct species. For example, although SCNT results in an organism having an identical nuclear genome sequence to that of the somatic cell donor, the maternal mitochondrial DNA present in the enucleated surrogate egg cell (likely from an extant close relative) are also passed on to resulting offspring (Shapiro 2017). Therefore, in theory, true de-extinction will never be possible. However, the creation of an exact replica of an extinct species is not necessary for the goal of ecological enrichment to be realised (Shapiro 2017).

The goal of ecological enrichment and the restoration of lost ecological processes and ecosystem functions through the reintroduction of extinct species has the potential to restore ecosystem health and prevent further ecosystem degradation (Ripple and Beschta 2012). For example, it has been suggested that the reintroduction of woolly mammoths into northern Russia could fundamentally alter the landscape and even reduce the thaw of permafrost through compaction of the insulating snow layer, preventing the release of greenhouse gases and slowing global warming (Shapiro 2015a). Regardless of the predicted benefits, ecological enrichment will require a sufficiently large population of organisms to both impact ecological dynamics and maintain the genetic variation necessary for long-term population persistence and adaptation, which has been suggested to be between 500 and 5000 individuals (Steeves *et al.* 2017). However, as also noted by Steeves *et al.* (2017), each of the pathways to de-extinction will result in the production of a small, genetically depauperate population of organisms, with limited prospects of increasing the genetic variation available. Although a successful de-extinction may be seen as the generation of even a single extinct organism, if the resurrected species cannot be restored to ecologically meaningful population sizes, then the species will continue to remain functionally extinct (McCauley *et al.* 2017). Furthermore, as Wood *et al.* (2017) highlight, for many extinct species we have little to no information about their inter-specific and intra-specific interactions, let alone their ecological processes and functions. This is evident from the fact that 33 (27%) of the species assessed do not have any habitat information available as part of their IUCN Red List assessment, with only 11 species (9%) providing any inter/intraspecific information. This is concerning as the extinction of one species can also cause a cascade of subsequent secondary extinctions, each with their own functional impacts, and the reintroduction of only one species in this system may not be sufficient to result in large scale ecological changes (Dunne and Williams 2009; McCauley *et al.* 2017). Empirical studies and observations may provide this information for recently extinct species, however the extent of environmental change and the potential emergence of new threats since their extinction, mean that even if such information is available, it will only provide partial ecological knowledge (IUCN SSC 2016; Seddon *et al.* 2014). As a result, it is likely that true ecological enrichment will not be possible for the majority of current de-extinction candidate species, however for plants, with their numerous potential herbarium specimens and seeds, ecological enrichment could not only be much more feasible, but also more cost-effective.

A key consideration for any de-extinction project is the availability of suitable *in situ* habitat protected from future anthropogenic change and the resolution of the original species-specific threats leading to extinction (Abeli *et al.* 2020; Jones 2014). If these criteria are not satisfied, then the probability of a resurrected and reintroduced species becoming extinct again remains high (IUCN SSC 2016). Although this study tried to quantify the number of species-specific threats which have been resolved, 44 species (36.1%) did not provide any indication for the cause of their extinction, while for a further 71 species (58.1%) I could find no evidence that the threats had been resolved, highlighting a lack of overall ecological information and subsequently preventing informed decision-making. Even if the original extinction-causing threats have been resolved and suitable habitat exists, due to the pace and extent of environmental change, any resurrected species will also have to contend with novel ecological conditions and threats which have come into existence since it originally became extinct, such as invasive alien species (IUCN SSC 2016; Wood *et al.* 2017). Therefore, although the de-extinction and subsequent reintroduction of a species without available habitat and threat information may prove successful, it will necessitate detailed habitat and threat assessments prior to and post reintroduction in order to both identify the species' ecological niche and any potential threats, and to ensure the species is not at risk of re-extinction (IUCN SSC 2016; Seddon *et al.* 2014).

Charismatic vertebrates such as the woolly mammoth and dodo (*Raphus cucullatus*) are likely to continue dominating the de-extinction conversation, however the ability of extinct plant species to achieve the same level of ecological enrichment and avoid the significant ethical and welfare concerns of vertebrate de-extinction should also be considered a de-extinction research priority. Although de-extinction has stimulated both public and scientific interests, it remains to be seen whether or not the general public are supportive of de-extinction efforts and would welcome the return of once-extinct species, many of which became extinct due to human-induced environmental change and activities (Novak 2018). For species which are currently extinct in the wild, such as the Belgian *Bromus bromoideus*, it appears as though people are not in favour of their return (Abeli *et al.* 2020). If any de-extinction project is to be successful it is clear that such disparities between public attitudes and scientific interests must be resolved prior to any project initiation. As technology continues to advance and as more species become extinct due to anthropogenic activities, it can be viewed that we have an obligation of restorative justice to resurrect extinct species if we have the capacity to do so (Cohen 2014). However, without appropriate consideration of the motivation behind a species' de-extinction, the probability of project success and the associated costs, we risk wasting both time and valuable financial resources that could be spent on other conservation efforts, enhancing both global biodiversity loss and ecosystem degradation.

6. General Discussion

6.1 *Ex Situ* Conservation Revisited

The continuing decline in global biodiversity and associated increases in extinction rates threaten not only individual species, but also the ecosystem services vital for the persistence of human civilisation (Ceballos *et al.* 2020). Over the last four decades, the global zoo and aquarium community has committed to the conservation of biodiversity, both *in situ* and *ex situ*, collectively representing one of the largest non-governmental conservation contributors globally (Conway 2003; Gusset and Dick 2011). Despite this commitment, the limited resources of the *ex situ* community, combined with their need to fulfil multiple roles, continue to create tension between conservation objectives and economic viability (Fa *et al.* 2014; Whitworth 2012). As a result, significant knowledge gaps remain, both in terms of how to better utilise *ex situ* collections for conservation benefit, and how to integrate *in situ* and *ex situ* conservation efforts (Bowkett 2014; Pritchard *et al.* 2012; Traylor-Holzer *et al.* 2019). Contemporary research has made initial attempts to fill these knowledge gaps, highlighting the importance of visitor engagement and education, problems inhibiting the sustainability of *ex situ* populations, and the utilisation of *ex situ* collections to conserve and improve genetic variation in wild populations (Che-Castaldo *et al.* 2019; McCann and Powell 2019; Ryder and Onuma 2018; Wilson *et al.* 2019). The utilisation of globally shared *ex situ* records has the potential to radically alter *ex situ* conservation, informing collection planning and management practices. However, as of yet they remain an underappreciated and underutilised conservation tool (da Silva *et al.* 2019; Hosey *et al.* 2020; Powell *et al.* 2019). This thesis represents an important addition to the multi-faceted conservation efforts of the global *ex situ* community, highlighting the conservation value of globally shared zoological records. I provide a detailed assessment of the factors influencing zoo visitor attendance globally (Chapter 2), show the potential for globally shared zoological records to increase *ex situ* population sustainability (Chapter 3) and highlight the role of *ex situ* collections in preserving, and potentially reintroducing, genetic diversity (Chapters 4 and 5).

The role of charismatic vertebrates in zoos and aquariums has become a contentious issue owing to contemporary welfare and ethical concerns (Hosey *et al.* 2020). Their over-representation in *ex situ* collections is rooted in entrenched taxonomic biases and perceived visitor preferences, despite a lack of robust evidence to confirm the latter (Bowkett 2014; Fa *et al.* 2014; Oberwemmer *et al.* 2011). Although numerous studies have tried to measure zoo visitor preferences, this work has focussed on a limited number of species, institutions and geographic regions, and has produced conflicting results, hindering informed *ex situ* conservation and collection planning (Balmford 2000; Carr 2016; Ward *et al.* 1998; Ward 200). Chapter 2 fills important collection planning gaps and provides an integrated assessment of how zoo species composition and socio-economic factors influence both institutional visitor attendance and institutional *in situ* contributions at 458 zoos globally (Mooney *et al.* 2020). This assessment reveals a complex system of determinants, but overall indicates a net positive effect of large charismatic vertebrates on both visitor attendance, and subsequently *in situ* contributions. This seems to confirm perceived visitor preferences and suggests that large, charismatic vertebrates can act as flagship species to increase *in situ* conservation funding, protecting not only individual species, but also their habitats (Baker 2007; Leader-Williams and Dublin 2000). Although this revelation does not negate the serious welfare and ethical concerns associated with maintaining large, charismatic vertebrates in captivity, it does suggest that more serious consideration should be given to their potential role as *in situ* conservation fundraisers. This assessment comes at a time when several institutions are making the bold decision to remove large, charismatic vertebrates, such as elephants (*Elephantidae*) and cetaceans (*Cetacea*), from their collections (Simon *et al.* 2009; Thompson and Berens 2014). Once again, these decisions are based on public concern and outcry rather than robust evidence-based welfare assessments of the individuals concerned (Robeck *et al.* 2015; Waller and Iluzada 2020). Given the potential of large, charismatic vertebrates to increase both visitor numbers and *in situ* conservation contributions, whether their removal from collections will have negative economic and/or conservation consequences remains to be seen. This clearly warrants further investigation and highlights the importance of evidence-based decision-making in *ex situ* conservation and planning.

The undisputable wealth of recent research showcasing the unsustainability of nearly all managed *ex situ* populations highlights perhaps the greatest problem facing contemporary *ex situ* collections (Hibbard *et al.* 2011; Lees and Wilcken 2009; Leus *et al.* 2011; Long *et al.* 2011; Powell *et al.* 2019). Without both demographically and genetically sustainable populations, the long-term conservation value of zoo and aquarium populations is almost negligible (Powell *et al.* 2019). In Chapter 3 I utilised globally shared zoological records to address the long-term sustainability of *ex situ* flamingo (*Phoenicopteridae*) populations, investigating the influence of flock size and structure on reproductive success and critiquing current management guidelines. I provide species-specific recommendations to promote population sustainability that I hope can be incorporated into future flamingo management guidelines. These results provide strong evidence that *ex situ* flamingo flocks should be increased from the current minimum of 40 individuals to a minimum of 69-127 individuals depending on the species, confirming the importance of flock size, but conflicting previous flock size recommendations (Brown and King 2005; Pickering 1992). Although I found a limited effect of environmental factors, anecdotal evidence and *in situ* patterns suggest finer-scaled analyses are required to separate the effects of management practices from environmental suitability (Farrell *et al.* 2000; Pickering *et al.* 1992; Studer-Thiersch 2000). This chapter also suggests that the periodic exchange of individuals between flocks can increase reproductive success, mitigating the effect of small flock sizes. Logistical constraints and the current lack of breeding and transfer recommendation fulfilment suggest that such recommendations are unlikely to be successfully implemented. However, I strongly argue that such drastic changes to population management are necessary if population sustainability is to be achieved (Powell *et al.* 2019). This family-specific analysis demonstrates the potential of globally shared zoological records to inform management practices and promote *ex situ* population sustainability.

Despite the unsustainability of *ex situ* populations, zoos and aquariums currently contain 15% of all threatened terrestrial vertebrates, providing a unique opportunity to conserve a vast amount of genetic diversity in gene banks (Conde *et al.* 2011). In conjunction with assisted reproductive technologies, preserved genetic samples have the potential to conserve and reintroduce genetic variation into future populations, promoting population health and sustainability (Critser and Russell 2000; Hobbs *et al.* 2018). In Chapter 4 I investigated the current representation of species within the San Diego Zoo Frozen Zoo® living cell collection, showing that 5.1% of all IUCN Red List "*Threatened*" amphibians, birds, mammals and reptiles are currently represented within the collection and that further sampling from within existing zoo and aquarium collections can increase representation to 16.5%. This work can be incorporated into *ex situ* collection management, increasing sampling opportunities and the representation of threatened species in global gene banks, conserving global genetic diversity. Although preventing extinction is one of the primary goals of conservation, Chapter 5 highlighted the importance of *ex situ* samples (seed samples and herbarium specimens) in future plant de-extinction efforts. In spite of being an emerging field, de-extinction has already been proven in practice (Folch *et al.* 2009). However, a lack of consistent species prioritisation has created taxonomic biases in de-extinction research, which this chapter aims to rectify (Jones 2014; Martinelli *et al.* 2014; McCauley *et al.* 2017). By drawing attention to plant de-extinction I hope conservation practitioners will incorporate the collection of *ex situ* samples into their conservation management practices, particularly for species of conservation concern. Recognition of the potential for biobanking to contribute to *ex situ* and *in situ* population sustainability, species resurrections, and ecosystem stability would highlight the need for greater sample collection and biobank formation globally (Cardinale *et al.* 2012).

6.2 Future Directions

6.2.1 Integrated *Ex Situ* Collection Planning

The reliance of zoos and aquariums on visitor-generated revenue is overtly clear from the numerous fundraising campaigns, governmental supplements and even zoo closures seen during the COVID-19 pandemic (BIAZA 2020; WAZA 2020b). Consequently, as zoos and aquariums navigate a post-COVID environment, they must be strategic with their living collections in order to meet conservation objectives and remain economically viable (Kaufman 2012; WAZA 2020b). Although Chapter 2 revealed the importance of large, charismatic species in increasing visitor attendance, I also showed that such species are only one part of a complex, interlinked system of visitor attendance determinants. Additionally, Chapter 2 indicated that high species richness, number of animals and species uniqueness are as important, if not more so, than charismatic vertebrates in driving visitor attendance. This is illustrated by the addition of two giant pandas (*Ailuropoda melanoleuca*) to Adelaide Zoo in 2010. Although the giant panda is arguably the most charismatic of all species, their addition to Adelaide Zoo only resulted in increased visitor numbers lasting two years, after which attendance returned to pre-2010 levels (Driml *et al.* 2017). If zoos and aquariums are to appeal to visitor preferences, further investigation is needed into not only which species people most like to see – and the characteristics of these species that drive their appeal – but also the broader motives and expectations behind a visit to a zoological institution (Garrett 2015). For many visitors the motivation behind a zoo visit appears to be recreational and family-orientated, with a greater desire to see animals than to learn about them or their environment, but how variable these motivations are remains to be seen (Ryan and Saward 2004; Schultz and Joordens 2014). These desires and motivations are critical to the economic success of zoos and aquariums and as such should be integrated into any *ex situ* collection planning methodology.

The continued display and inclusion of large, charismatic vertebrates as part of zoological collections is unlikely to change, not only due to their public appeal, but because in general, new *ex situ* populations can no longer be sourced from the wild, greatly hindering the establishment of *ex situ* populations for species not already represented (Bowkett 2014). As a result, the ethical and welfare concerns surrounding charismatic vertebrates already in captivity must be addressed and solutions implemented, potentially including the removal of species that are unable to be maintained *ex situ* with a high standard of welfare (Young 2015). However, the decision to remove charismatic species should not be made prematurely and should be based on sound scientific evidence, including species-specific welfare assessments (Yon *et al.* 2019). For example, the decision by SeaWorld to end the captive display of killer whales (*Orcinus orca*) was predominantly based on public concern and emotion and not on evidence-based research, despite work showing similar life-history parameters between wild and captive killer whale populations (Robeck *et al.* 2015; SeaWorld 2017). Whilst this is admittedly a controversial example of this phenomenon – and ultimately the welfare of the individuals concerned warrants greater investigation – it highlights a growing trend to remove large, charismatic species from zoos and aquariums based on limited evidence, with little consideration for the potential conservation consequences of such decisions (Simon *et al.* 2009). If the welfare needs of large, charismatic species can be met under *ex situ* conditions, then their presence within a collection may represent an optimal conservation strategy, not only contributing to conservation through *ex situ* breeding and management, but also generating greater contributions for *in situ* conservation activities compared to smaller-bodied species (Mooney *et al.* 2020).

If utilised correctly, charismatic vertebrates can be combined with creative educational messaging programmes to result in behaviour change amongst visitors. This is exemplified by the "*Don't Palm Us Off*" campaign run by Melbourne Zoo which in conjunction with a living Sumatran orangutan (*Pongo abelii*) exhibit, highlighted the threat of palm oil to orangutan habitat in the wild, promoting public awareness and mandatory labelling of palm oil products (Pearson *et al.* 2014). This campaign not only raised awareness about the threats to orangutan habitat in the wild, but also resulted in a significant increase in self-reported pro-conservation behaviour amongst visitors. Similarly, the "*Seal-the-Loop*" campaign, again by Melbourne Zoo, linked interactive fur seal presentations with visitor conservation donation requests, resulting in significantly increased visitor donations from those who had seen the fur seal presentation, highlighting the role of charismatic vertebrates themselves in generating *in situ* contributions and creating behavioural change (in this case donating) amongst visitors (Mellish *et al.* 2017). These examples highlight the most efficient *ex situ* use of charismatic vertebrates in biodiversity conservation, not only attracting visitors and generating funds for *in situ* conservation, but also educating the public and creating pro-conservation behavioural change amongst visitors. However, the potential for traditionally non-charismatic species to be utilised in the same manner and achieve the same conservation outcomes has yet to be assessed, despite the fact that species uniqueness within a collection is positively correlated with visitor attendance (Mooney *et al.* 2020). For example, Smith *et al.* (2012) identified several *Cinderella species* that are both threatened with extinction, and are considered aesthetically appealing, yet are currently overlooked in terms of their potential flagship appeal. The conservation and educational potential of traditionally non-charismatic species represents a research priority, with huge potential to integrate *in situ* and *ex situ* conservation efforts in creative ways. Although the potential for zoos and aquariums to change visitor behaviour is a relatively new concept, it comes with a huge range of research possibilities, providing an exciting opportunity to create positive conservation outcomes and integrate *in situ* and *ex situ* conservation activities.

As it stands, zoos and aquariums contribute a small proportion of their operating income to *in situ* conservation activities (Bettinger and Quinn 2000; Fa *et al.* 2011; Tribe and Booth 2003). These contributions are having positive effects on *in situ* project success and viability; however, it has been strongly recommended that institutions increase this proportion or implement a conservation surcharge on entrance tickets (Conway 2003; Gusset and Dick 2010; Kelly 1997; Mace *et al.* 2007). I mirror such recommendations, but also highlight the need for greater unconventional *in situ* contributions from institutions, such as veterinary expertise, training, education, research and provision of equipment (Keulartz 2015). Similarly, I encourage individual institutions to track the effectiveness of their *in situ* contributions, ensuring their limited resources are being allocated appropriately. Although I found that the proportion of threatened species within a zoo did not influence visitor attendance, it was positively correlated with *in situ* contributions, suggesting the integration of species-specific *in situ* and *ex situ* conservation activities as encouraged under the "*One Plan Approach to Conservation*" by the IUCN Species Survival Commission Conservation Planning Specialist Group (Byers *et al.* 2013; Mooney *et al.* 2020). Although the integration of *in situ* and *ex situ* conservation activities is easier for local species and local biogeographical regions, institutions can also link charismatic and endangered species with *in situ* conservation activities globally (Dickie *et al.* 2007; Keulartz 2015). For example, the Congo Gorilla Rainforest exhibit at the Bronx Zoo has been used to generate funding for the *in situ* conservation of African forest wildlife (Dickie *et al.* 2007). Upon opening this exhibit in 1999, the Bronx Zoo not only imposed a special admission fee to support wildlife conservation in tropical African forests, but also allowed visitors to choose how their fees could be spent *in situ*. By 2009, $10.6 million had been raised and spent on African forest wildlife conservation from this source alone (Conway 2011). Such examples offer encouraging and creative solutions to immerse visitors in *in situ* conservation, while simultaneously generating important financial contributions, and I encourage other institutions to undertake similar integrated conservation programmes wherever possible.

6.2.2 Promoting *Ex Situ* Population Sustainability

Since Lees and Wilcken (2009) revealed that the majority of managed *ex situ* populations are not sustainable, and subsequently not viable long-term, the sustainability of *ex situ* populations has become a prominent concern and priority amongst stakeholders (Powell *et al.* 2019). A range of subsequent work has both confirmed the findings of Lees and Wilcken (2009) and also reiterated the need for drastic changes to collection planning and management practices, including the utilisation of large datasets and the development of new analytical tools (Hibbard *et al.* 2011; Leus *et al.* 2011; Powell *et al.* 2019). Simultaneously, recent analyses have identified the issues underpinning this *ex situ* population sustainability crisis, including limited space availability, a lack of standardised husbandry practices and poor fulfilment of breeding and transfer recommendations (Che-Castaldo *et al.* 2019; Macek 2014; Wilson *et al.* 2019). Collectively, these findings are a significant step towards the sustainability of *ex situ* populations, however limited solutions to tackle these issues have been presented. For instance, although Wilson *et al.* (2019) show that poor fulfilment of breeding and transfer recommendations affect 33% of AZA managed populations, they provide limited potential solutions to rectify this problem. The importance of population sustainability transcends the needs of individual institutions, their directors and programme leaders, and I strongly encourage global collaboration between zoos and aquariums, focusing their research on evidence-based solutions to tackle these issues and implementing necessary changes accordingly.

Chapter 3 utilised flamingos as a case study and represents one of the few attempts to use large datasets and globally shared zoological records to identify the species-specific husbandry and management practices necessary to improve *ex situ* population sustainability. The benefit of using large datasets is clearly evident from the confirmations and contradictions between this study and smaller-scale studies. Although I confirmed the importance of flock size in determining reproductive success (Pickering *et al.* 1992; Sandri *et al.* 2018; Stevens and Pickett 1994; Stevens 1991), I contradicted findings that the minimum flock size necessary is 40 individuals for all species (Brown and King 2005), instead revealing that species-specific flock sizes of 69-127 are necessary for a 50% probability of reproduction. This study benefits from the fact that flamingos are a charismatic species with a strong willingness among population managers to implement the changes necessary to increase *ex situ* population sustainability (King and Bračko 2014). Ideally, I would encourage similar analyses for other species with unsustainable *ex situ* populations. However, for non-charismatic species, and those represented within a limited number of institutions, the resources and momentum necessary are likely unavailable. Furthermore, although the suite of recent sustainability studies have shown that managed *ex* *situ* populations are unsustainable, the majority of species within zoo and aquarium collections are not considered managed populations (Traylor-Holzer 2011). For these unmanaged species there has been no assessment of population trends, genetic diversity or population sustainability, leaving immense demographic knowledge gaps in the *ex situ* conservation and population sustainability literature. Although it could be assumed that their lack of prioritisation for intensive *ex situ* management stems from their large, robust and sustainable *ex situ* populations; it could also be assumed that their populations are too small and unsustainable to warrant any further management. The difference between these assumptions is of indisputable conservation significance and subsequent population sustainability assessments should represent a top priority for *ex situ* population managers.

Unfortunately, the intensive demographic and genetic management provided by *ex situ* population management programmes, such as AZA Species Survival Plans and EAZA *ex situ* programmes, are only available for a limited number of species due to the extensive resources and investment required to manage and maintain them (Powell *et al.* 2019). However, as demonstrated in Chapter 3, the availability of globally shared zoological records in databases, such as the Zoological Information Management System (ZIMS), can be used to reveal the population structure and demographic patterns underlying *ex situ* population sustainability for many species. As ZIMS collects data from complete zoo composition and not just managed species, the data required to fill the demographic knowledge gaps of unmanaged species is theoretically already available and waiting for analysis. I recommend that regional population managers work with ZIMS to identify population sustainability issues that can benefit from evidence-based solutions derived from globally shared zoological records.

Sustainability analyses and population management programmes can help guide *ex situ* conservation and species management. However, unless more *ex situ* space is made available, the majority of species will not be able to reach the population sizes necessary to ensure long-term population sustainability (Wilson *et al.* 2019). As the creation of new zoos is relatively rare, this lack of space implies that in order for some *ex situ* populations to increase, others will have to decrease and be phased out of *ex situ* management (Kaufman 2012). Many institutions are consolidating their collections and moving in this direction, as demonstrated by St Louis Zoo, which in 2012, housed 400 more animals than it did in 2002, but had 65 fewer species overall (Kaufman 2012). This creates tough decisions, with even endangered species such as the lion-tailed macaque (*Macaca silenus*) now being phased out of North American Zoos due to a lack of available space (Kaufman 2012; Kaumanns *et al.* 2013). Fortunately for the lion-tailed macaque, European zoos are still in a position to manage the species and provide the holding capacity necessary to ensure the *ex situ* population can reach a sustainable size (Kaumanns *et al.* 2013). However, other species won't always be as fortunate. I encourage the incorporation of sustainability analyses into the collection planning and decision-making process regarding which species should, or should not, be maintained *ex situ* in the future. Continuing to manage species with small populations and limited prospects of becoming sustainable in the future will not only waste resources, but also take valuable space away from species with the potential to become sustainable if managed correctly. Matching charismatic species and sustainability analyses could provide one approach to identify the species with the greatest chance of becoming sustainable themselves, while also contributing a greater amount to *in situ* conservation projects and visitor education, representing a promising route for further investigation (Kaufman 2012).

6.2.3 Conservation Potential of *Ex Situ* Samples

The logistical limitations of conventional *ex situ* species management can be mitigated by the utilisation of *ex situ* gene banks, which when combined with genetic rescue theory and advanced reproductive technologies, have the potential to conserve genetic diversity almost indefinitely and reintroduce genetic variation into wild populations (Critser and Russell 2000; Hobbs *et al.* 2018). Chapter 4 shows that the potential of biobanking to conserve genetic diversity and biodiversity has yet to be fully appreciated, however the establishment of the EAZA biobank represents a promising step toward the routine application of *ex situ* biobanking efforts (EAZA 2020). To further integrate biobanking into conventional *ex situ* conservation practices, I recommend that biobanking and sample collection be incorporated into the accreditation process for accredited institutions, such as AZA and EAZA members, either creating new gene banks or contributing samples to existing gene banks (CPSG 2016). Similarly, I propose that *ex situ* population managers and Taxon Advisory Groups (TAGs) promote the collection of biological samples from animals under their administration wherever possible. This top-down approach would place greater pressure and requirements on individual institutions, ensuring that extant genetic diversity is not lost due to institutional complacency. Fundamentally, I would also like to see greater integration of *in situ* and *ex situ* conservation efforts, increasing not only the opportunities for sample collection, but also the opportunities to successfully restore the genetic variation of wild populations (CBSG 2015; CPSG 2016). However, I accept that the predicted conservation benefits of biobanking are currently contingent on the continued development of assisted reproductive technologies and reproductive knowledge of the species concerned, and are therefore still predominantly theoretical in nature.

Although Chapter 4 highlighted the potential of zoos and aquariums to contribute to global biobanking efforts, it also highlighted the continued taxonomic bias towards large, charismatic vertebrates (predominantly mammals). This not only reflects historical sampling biases, but also a lack of standardised methodologies for successful cell growth and cryopreservation across taxonomic groups (Ryder and Onuma 2018). I advocate for greater investment into the identification of the optimal conditions for successful cell growth in preparation for cryogenic storage across various taxonomic groups, particularly emphasising the current lack of standardised practices and knowledge for reptile and amphibian species (Benirschke 1984; Houck *et al.* 2017; Ryder and Onuma 2018). Current taxonomic limitations not only result in missed conservation opportunities for threatened species and those which have recently gone extinct, but they also greatly limit our potential to resurrect extinct species in the future.

De-extinction has already been proven in practice using *ex situ* cryopreserved living cells and germplasm (Folch *et al.* 2009), resulting in the initiation of projects to resurrect other extinct species such as the woolly mammoth (*Mammuthus primigenius*) and passenger pigeon (*Ectopistes migratorius*) (Callaway 2015; Shapiro 2015a). Almost complete failure to consider the possibility of plant de-extinction, and their potential benefits, mirrors taxonomic biases seen in traditional conservation and ecological research, and has created an entirely vertebrate-centric view of de-extinction science and the technology it requires (Jones 2014; Martinelli *et al.* 2014; McCauley *et al.* 2017). Chapter 5 not only addressed this taxonomic bias, but also highlighted the relative ease of plant de-extinction compared to vertebrates and the need for a holistic approach to de-extinction candidate selection, assessing both the feasibility of de-extinction, and the probability that the species can be returned to ecologically meaningful population levels (Iacona *et al.* 2017; Steeves *et al.* 2017). This work revealed an incredible lack of ecological knowledge for extinct species, but also highlighted the considerable *ex situ* herbarium specimens available for nearly all extinct plant species, each with the potential to provide seeds capable of germination. I advocate for further research into the potential feasibility of plant de-extinction and collection of tissue and seed samples from extant threatened plant species, providing an insurance population should the species go extinct. Seed collection should be of particular priority for the species listed as "*Extinct*" under the IUCN Red List, but which have been shown in Chapter 5 to have extant populations.

Although the taxonomic bias in de-extinction research is unlikely to change, I strongly encourage a more holistic approach to the selection of vertebrate candidate species for de-extinction research, as current species prioritisations have yet to integrate de-extinction feasibility and the subsequent probability of reintroduction success (Turner 2017). Existing de-extinction prioritisation schemes, such as that implemented by Seddon *et al.* (2014), often employ decision criteria to a pre-selected group of candidate species, potentially missing de-extinction opportunities and wasting resources on projects with a limited probability of success. For example, the availability of *ex situ* cryopreserved living cell samples from the Pyrenean ibex (*Capra pyrenaica pyrenaica*) mean that this species can be resurrected using cloning technology relatively easily (Folch *et al.* 2009). In contrast, the woolly mammoth has no cryopreserved living cell samples and will require vast alterations of the Asian elephant genome to be resurrected (Folch *et al.* 2009; Lynch *et al.* 2015; Shapiro 2017). Similarly, greater consideration should be given to the importance of ecological information in selecting de-extinction candidate species overall. Although Chapter 5 highlights a lack of such information for extinct species, ecological information could prove invaluable to the success of any de-extinction project and I encourage the continued digitisation of herbarium specimens and field notes to uncover such potential information. In agreement with Iacona *et al.* (2017), I suggest that the primary objective of de-extinction should be to restore functionally equivalent proxies of extinct species, restoring lost ecological processes and ecosystem functions (Shapiro 2017; Wood *et al.* 2017). This is a vast objective and for species with limited or no ecological information available, such endeavours may prove unsuccessful and wasteful.

The limitations of *ex situ* conservation, genetic rescue, and de-extinction technology only emphasise the importance of continued *in situ* habitat and population conservation. Similarly, the value of *ex situ* population management, genetic rescue and de-extinction are almost non-existent if no suitable *in situ* habitat or populations remain, highlighting the inextricable link between all efforts to conserve global biodiversity. Encouragingly, the amount of terrestrial and marine habitat set aside for conservation has increased drastically over the last 50 years, particularly in biodiverse regions (Naughton-Treves *et al.* 2005; Watson *et al.* 2014). When managed correctly, protected *in situ* habitat can both conserve biodiversity, and also create economic growth, empower local communities and alleviate poverty (Andam *et al.* 2010). However, these areas alone are not sufficient to conserve global biodiversity, resulting in continued *in situ* population declines and biodiversity loss (Le Saout *et al.* 2013; Stokstad 2010). Simultaneously, human pressures on the biosphere are increasing rapidly and causing extensive environmental damage and degradation (Butchart *et al.* 2010; IPBES 2019). The conservation of global biodiversity has never been more urgent, with the persistence of human civilisation directly tied to ecosystem health and functioning (Ceballos *et al.* 2020). If global biodiversity conservation is going to be effective it will require greater collaboration between all conservation stakeholders and I encourage better integration of all possible conservation approaches, both *in situ* and *ex situ*.

6.2.4 Zoological Record Sharing

Collectively, the globally shared zoological records which are included in Species360's Zoological Information Management System (ZIMS) represent the largest real-time database of comprehensive and standardised zoological information from zoos and aquariums globally (Species360 2019; ZIMS 2019). Although there are more than 1,100 Species360 member institutions, representing nearly all zoos and aquariums organised and accredited as part of internationally recognised organisations, such AZA and EAZA, they still represent only a small fraction of zoological institutions globally and are geographically biased towards North America and Europe (Chapter 3; Kelly 1997). Consequently, the *ex situ* conservation potential reported and discussed in this thesis represents an overall underestimation of the total *ex situ* conservation potential globally. This thesis echoes the recommendations of da Silva *et al.* (2019) and encourages the integration of standardised, shared zoological records across multiple data sources and stakeholders, including Species360 members and non-members. Failure to effectively manage and integrate data across stakeholders greatly inhibits the success of *ex situ* conservation breeding programmes, placing species at risk of extinction. This is epitomised by the extinction of the Catarina pupfish (*Megupsilon aporus*), a freshwater species endemic to Mexico which became extinct in the wild in the 1990's due to anthropogenic changes. Although *ex situ* populations of this species persisted, a lack of data sharing and population management resulted in *ex situ* population decline. When stakeholders realised the population required conservation intervention, only a single female remained, with the species subsequently becoming extinct in 2014 when the last individual died (Miller *et al.* 2005; Valdés González *et al.* 2020). This poignant example not only emphasises the importance of *ex situ* populations for species conservation, but also the importance of standardised, shared zoological records across institutions and stakeholders.

Species360 and ZIMS have made huge progress towards the implementation of standardised data sharing across zoological institutions, however as evident from Chapter 4, such information is not available for global gene banks and their samples (Clarke 2009). Although the San Diego Zoo Frozen Zoo® collection is the largest and most diverse wildlife gene bank in the world (SDZG 2020), it represents only a single institutional collection. Failure to consider other gene banks not only limits our ability to quantify the biodiversity contained with gene banks globally, but it can potentially result in duplicate sample collection and missed conservation opportunities, enhancing extinction risks (CBSG 2015; Ryder *et al.* 2000). As a result, numerous stakeholders and conservation practitioners have called for the establishment of a global database of gene banks and the samples within them, preventing duplicate sample collection and allowing for greater coordination of future sample selection and collection efforts (CBSG 2015; Ryder and Onuma 2018; Ryder *et al.* 2000). I echo these recommendations and suggest that biological samples and gene banks be included as part of existing databases, such as ZIMS, which already contains extensive zoological records, allowing for the integration of husbandry, veterinary, demographic and now genetic records for individual animals across the Species360 member institution network.

6.2.5 Aquariums and Aquatic Species

Chapters 2 – 4 highlight the conservation potential of zoos globally but make limited mention of the potential conservation contributions of aquariums or the sustainability of aquatic *ex situ* populations, mirroring nearly all *ex situ* conservation research to date (Conde *et al.* 2011; Hosey *et al.* 2020; Traylor-Holzer 2011). This taxonomic and terrestrial-bias is concerning given that 50–80% of the world's biodiversity is found in the oceans and that aquatic ecosystems are amongst the most threatened ecosystems globally (Millennium Ecosystem Assessment 2005; Penning *et al*. 2009). The recent work of da Silva *et al.* (2019) has made initial attempts to quantify the conservation potential of Species360 member aquariums, assessing the number of fish and coral species represented within aquariums and their population sizes. This invaluable study revealed that 14% (3,113 species) of all known fish species and 4% (257 species) of all known coral species are represented within Species360 member institutions. *Ex situ* population sustainability assessments have yet to be conducted for aquatic species and the representation of aquatic species in gene banks is almost non-existent, as shown by the inclusion of just two fish species within the San Diego Zoo Frozen Zoo® collection (Chapter 4). Given that the majority of aquarium populations still rely on the periodic importation of wild-caught individuals (Penning *et al*. 2009), it is likely that aquarium populations are as, if not more, unsustainable as zoo populations, however, this clearly warrants further research and investigation. The global aquarium community has realised that sourcing individuals from the wild is both unsustainable and not likely to continue into the future, and as a result have prioritised the sustainability of aquatic collections, as seen by the establishment in 2019 of the AZA "*Aquatic Collections Sustainability Committee*" (Richard 2020). This progressive move will hopefully bring aquarium collections closer to zoo collections in terms of sustainability initiatives, improving the sustainability of *ex situ* aquatic populations.

Given that aquariums are at the centre of the current debate surrounding the presence of large, charismatic vertebrates, such as cetaceans, in captivity, and that aquarium collections are fundamentally different to zoos in their taxonomic representation (Penning *et al*. 2009), I would encourage an integrated assessment, such as that presented in Chapter 2, to determine the collection characteristics associated with increased visitor attendance at aquariums globally. A current lack of Species360 aquarium members means that such an assessment is not yet possible, however as Species360 membership continues to grow the sample size necessary will soon be available. Compared to zoos, aquariums contain a greater number of invertebrate, fish and reptile species, with minimal mammal representation (Penning *et al*. 2009), potentially revealing novel species combinations and characteristics which can be used to guide collection planning, promoting both visitor attendance and *in situ* contributions. Conversely, such an assessment could also reveal that the limited mammal representation within aquariums is of critical importance to visitor attendance, and therefore economic viability, casting doubt on the recent decision by aquariums, such as Vancouver Aquarium and the National Aquarium in Baltimore, to phase out cetacean exhibits (Grimm 2014; Muzylowski 2019).

6.3 Conclusion

As biodiversity continues to decline globally, the role of *ex situ* collections in conserving biodiversity is almost indisputable (Conde *et al.* 2011). The variety of research presented in this thesis allowed for a holistic assessment of the multi-faceted contribution of *ex situ* collections to global biodiversity conservation, both *in situ* and *ex situ*. This work illustrates the importance of globally shared zoological records in *ex situ* conservation, allowing for the investigation and quantification of the current and future potential of *ex situ* conservation. In Chapter 2, I filled important collection planning knowledge gaps, integrating collection composition and socio-economic data to reveal the species composition necessary to increase zoo visitor attendance and *in situ* contributions, reiterating the importance of large, charismatic vertebrates in conservation fundraising (Hosey *et al.* 2020; Hutchins *et al.* 1995; Leader-Williams and Dublin 2000). Chapter 3 addressed the sustainability of *ex situ* populations and utilised globally shared zoological records to provide the management insights necessary to increase the sustainability of *ex situ* flamingo populations, both confirming the importance of flock size and contradicting current management practices (Brown and King 2005; Pickering *et al.* 1992). Chapters 4 and 5 highlighted the role of *ex situ* collections in conserving the genetic diversity of living populations in gene banks, and the potential of *ex situ* samples to contribute to future genetic rescue and de-extinction efforts, with far-reaching consequences for population sustainability and ecosystem functioning (Clarke 2009; Ryder *et al.* 2000; Shapiro 2015a; Whiteley *et al.* 2015). Although each of these chapters highlighted a unique role of *ex situ* collections in conservation, the collective effectiveness and sustainability of *ex situ* conservation efforts requires further investigation and improvement. To achieve this will involve the continued utilisation and integration of globally shared zoological and botanical records, filling knowledge gaps and providing the management guidelines necessary to increase the effectiveness of global *ex situ* conservation efforts, preventing global biodiversity loss.

Bibliography

Abeli, T., Dalrymple, S., Godefroid, S., Mondoni, A., Müller, J. V., Rossi, G., and Orsenigo, S. (2020). *Ex situ* collections and their potential for the restoration of extinct plants. *Conservation Biology* **34**, 303–313. doi:10.1111/cobi.13391

Adriaens, T., San Martin y Gomez, G., Bogaert, J., Crevecoeur, L., Beuckx, J.-P., and Maes, D. (2015). Testing the applicability of regional IUCN Red List criteria on ladybirds (Coleoptera, Coccinellidae) in Flanders (north Belgium): opportunities for conservation Eds C. Dytham and A. Watt. *Insect Conservation and Diversity* **8**, 404–417. doi:10.1111/icad.12124

Alibhai, S., and Jewell, Z. (1994). Saving the last rhino: *In-situ* conservation or captive breeding. Unpublished report for the Rhino Foundation.

Allen, R. P. . (1956). The flamingos: their life history and survival. *Audubon Society Research Report No. 5*. National Audubon Society: Manhattan, New York, United States.

Allendorf, F. W., and Leary, R. F. (1986). Heterozygosity and fitness in natural populations of animals. In ‘Conservation Biology: The Science of Scarcity and Diversity’. (Ed M. E. Soulé.) pp. 57–76. (Sinauer: Sunderland.)

Allentoft, M., and O’Brien, J. (2010). Global Amphibian Declines, Loss of Genetic Diversity and Fitness: A Review. *Diversity* **2**, 47–71. doi:10.3390/d2010047

Andam, K. S., Ferraro, P. J., Sims, K. R. E., Healy, A., and Holland, M. B. (2010). Protected areas reduced poverty in Costa Rica and Thailand. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 9996–10001. doi:10.1073/pnas.0914177107

AZA (2016a). Annual Report on Conservation and Science. Silver Spring, Maryland. Available at: https://assets.speakcdn.com/assets/2332/arcs_2015.pdf

AZA (2016b). Glossary of Common Terms. Silver Spring, Maryland. Available at: https://assets.speakcdn.com/assets/2332/glossary_of_common_terms.pdf

AZE (2018). 2018 Global AZE map. Available at: https://zeroextinction.org/site-identification/2018-global-aze-map/

Baker, A. (2007). Animal ambassadors: An analysis of the effectiveness and conservation impact of *ex situ* breeding efforts. In ‘Zoos in the 21st century: Catalysts for conservation?’. (Eds A. Zimmermann, M. Hatchwell, L. Dickie, and C. West.) pp. 139–154. (Cambridge University Press: Cambridge.)

Ballou, J. D. (1992). Potential contribution of cryopreserved germ plasm to the preservation of genetic diversity and conservation of endangered species in captivity. *Cryobiology* **29**, 19–25. doi:10.1016/0011-2240(92)90003-K

Ballou, J. D., and Traylor-Holzer, R. C. (2011). Captive populations and genetic sustainability. *WAZA Magazine 12*, 19–22.

Balmford, A. (2000). Separating Fact from Artifact in Analyses of Zoo Visitor Preferences. *Conservation Biology* **14**, 1193–1195. doi:10.1046/j.1523-1739.2000.99078.x

Balmford, A., Leader-Williams, N., and Green, M. J. B. (1995). Parks or arks: where to conserve threatened mammals? *Biodiversity & Conservation* **4**, 595–607. doi:10.1007/BF00222516

Balmford, A., Mace, G. M., and Leader-Williams, N. (1996). Designing the Ark: Setting Priorities for Captive Breeding. *Conservation Biology* **10**, 719–727. doi:10.2307/2387094

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., Marshall, C., McGuire, J. L., Lindsey, E. L., Maguire, K. C., Mersey, B., and Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature* **471**, 51–57. doi:10.1038/nature09678

Barongi, R., Fisken, F. A. ., Parker, M., and Gusset, M. (Eds.) (2015). 'Committing to Conservation: The World Zoo and Aquarium Conservation Strategy'. (World Association of Zoos and Aquariums (WAZA): Gland, Switzerland.)

Beck, B. B., Rapaport, L. G., Price, M. S., and Wilson, A. C. (1994). Reintroduction of captive-born animals. In 'Creative Conservation: Interactive management of wild and captive animals'. (Eds P. J. Olney, G. Mace, and A. Feistner.) pp. 265–286. (Springer: New York City, United States.)

Benirschke, K. (1984). The frozen zoo concept. *Zoo Biology* **3**, 325–328. doi:10.1002/zoo.1430030405

Bennett, C. (1987). Breeding and reproduction of the Chilean flamingo *Phoenicopterus chilensis* at the Santa Barbara Zoo. In 'AAZPA Regional Conference Proceedings'. pp. 313–317

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E. W. (2017). GenBank. *Nucleic Acids Research* **45**, D37–D2. doi:10.1093/nar/gkw1070

Berjak, P., and Pammenter, N. W. (2008). From Avicennia to Zizania: Seed recalcitrance in perspective. *Annals of Botany* **101**, 213–228. doi:10.1093/aob/mcm168

Berry, H. H. (1975). The great flamingo trek. *African Wildlife* **26**, 58–60.

Bettinger, T., and Quinn, H. (2000). Conservation funds: How do zoos and aquaria decide which projects to fund. In 'Proceedings of the AZA Annual Conference'. pp. 52–54

BIAZA (2020). Permanent closure during coronavirus. *British and Irish Association of Zoos and Aquariums*. Available at: https://biaza.org.uk/news/detail/permanent-closure-during-coronavirus [accessed 17 August 2020]

Bijlsma, R., Westerhof, M. D. D., Roekx, L. P., and Pen, I. (2010). Dynamics of genetic rescue in inbred Drosophila melanogaster populations. *Conservation Genetics* **11**, 449–462. doi:10.1007/s10592-010-0058-z

Bioinformatics and Evolutionary Genomics (2020). *Calculate and draw custom Venn diagrams.* http://bioinformatics.psb.ugent.be/webtools/Venn/.

Bland, L. M., Collen, B., Orme, C. D. L., and Bielby, J. (2015). Predicting the conservation status of data-deficient species. *Conservation Biology* **29**, 250–259. doi:10.1111/cobi.12372

Booth, T. H., Nix, H. A., Busby, J. R., and Hutchinson, M. F. (2014). bioclim: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies Ed J. Franklin. *Diversity and Distributions* **20**, 1–9. doi:10.1111/ddi.12144

Bowkett, A. E. (2014). *Ex situ* conservation planning is more complicated than prioritizing the keeping of threatened species in zoos. *Animal Conservation* **17**, 101–103. doi:10.1111/acv.12116

Bowkett, A. E. (2009). Recent Captive-Breeding Proposals and the Return of the Ark Concept to Global Species Conservation. *Conservation Biology* **23**, 773–776. doi:10.2307/29738795

Brichieri-Colombi, T. A., Lloyd, N. A., McPherson, J. M., and Moehrenschlager, A. (2019). Limited contributions of released animals from zoos to North American conservation translocations. *Conservation Biology* **33**, 33–39. doi:10.1111/cobi.13160

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M. and Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal* **9**, 378-400.

Brown, C., and King, C. (2005). Flamingo husbandry guidelines; a joint effort of the AZA and EAZA in cooperation with WWT.

Brown, L. H. (1958). The breeding of the Greater Flamingo *Phoenicopterus ruber* at L. Elmenteita, Kenya Colony. *Ibis* **100**, 388–420. doi:10.1111/j.1474-919X.1958.tb00405.x

Brummitt, N. A., Bachman, S. P., Griffiths-Lee, J., Lutz, M., Moat, J. F., Farjon, A., Donaldson, J. S., Hilton-Taylor, C., Meagher, T. R., Albuquerque, S., Aletrari, E., Andrews, A. K., Atchison, G., Baloch, E., Barlozzini, B., Brunazzi, A., Carretero, J., Celesti, M., Chadburn, H., Cianfoni, E., Cockel, C., Coldwell, V., Concetti, B., Contu, S., Crook, V., Dyson, P., Gardiner, L., Ghanim, N., Greene, H., Groom, A., Harker, R., Hopkins, D., Khela, S., Lakeman-Fraser, P., Lindon, H., Lockwood, H., Loftus, C., Lombrici, D., Lopez-Poveda, L., Lyon, J., Malcolm-Tompkins, P., McGregor, K., Moreno, L., Murray, L., Nazar, K., Power, E., Quiton Tuijtelaars, M., Salter, R., Segrott, R., Thacker, H., Thomas, L. J., Tingvoll, S., Watkinson, G., Wojtaszekova, K., and Nic Lughadha, E. M. (2015). Green Plants in the Red: A Baseline

Global Assessment for the IUCN Sampled Red List Index for Plants Ed C. N. Jenkins. *PLOS ONE* **10**, e0135152. doi:10.1371/journal.pone.0135152

Bucher, E. H. (1992). Population and Conservation Status of Flamingos in Mar Chiquita, Cordoba, Argentina. *Colonial Waterbirds* **15**, 179. doi:10.2307/1521451

Bucher, E. H., and Curto, E. (2012). Influence of long-term climatic changes on breeding of the Chilean flamingo in Mar Chiquita, Córdoba, Argentina. *Hydrobiologia* **697**, 127–137. doi:10.1007/s10750-012-1176-z

Buckley, Y. M. (2015). Generalized linear models. In 'Ecological Statistics: Contemporary theory and application'. (Eds G. A. Fox, S. Negrete-Yankelevich, and V. J. Sosa.) pp. 131–148. (Oxford University Press: Oxford, England.) doi:10.1093/acprof:oso/9780199672547.001.0001

Burnham, K. P., and Anderson, D. R. (2002). 'Model selection and multimodel inference : a practical information-theoretic approach'. (Springer: New York City, United States.)

Burnham, K. P., and Anderson, D. R. (2004). Multimodel Inference. *Sociological Methods & Research* **33**, 261–304. doi:10.1177/0049124104268644

Butchart, S. H. M., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J. P. W., Almond, R. E. A., Baillie, J. E. M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K. E., Carr, G. M., Chanson, J., Chenery, A. M., Csirke, J., Davidson, N. C., Dentener, F., Foster, M., Galli, A., Galloway, J. N., Genovesi, P., Gregory, R. D., Hockings, M., Kapos, V., Lamarque, J. F., Leverington, F., Loh, J., McGeoch, M. A., McRae, L., Minasyan, A., Morcillo, M. H., Oldfield, T. E. E., Pauly, D., Quader, S., Revenga, C., Sauer, J. R., Skolnik, B., Spear, D., Stanwell-Smith, D., Stuart, S. N., Symes, A., Tierney, M., Tyrrell, T. D., Vié, J. C., and Watson, R. (2010). Global biodiversity: Indicators of recent declines. *Science* **328**, 1164–1168. doi:10.1126/science.1187512

Byers, O., Lees, C., Wilcken, J., and Schwitzer, C. (2013). The One Plan approach: The philosophy and implementation of CBSG's approach to integrated species conservation planning. *WAZA Magazine* **14**, 2–5.

Callaway, E. (2015). Mammoth genomes provide recipe for creating Arctic elephants. *Nature* **521**, 18–19. doi:10.1038/nature.2015.17462

Campbell, B., and Lack, E. (1985). 'A Dictionary of Birds'. (T & A. D. Poyser: London.)

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., MacE, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., and Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature* **486**, 59–67. doi:10.1038/nature11148

Carr, N. (2016). Ideal animals and animal traits for zoos: General public perspectives. *Tourism*

Management **57**, 37–44. doi:10.1016/J.TOURMAN.2016.05.013

Cascales-Miñana, B., and Cleal, C. J. (2014). The plant fossil record reflects just two great extinction events. *Terra Nova* **26**, 195–200. doi:10.1111/ter.12086

CBD (1992). The Convention on Biological Diversity. http://www.cbd.int.

CBSG (2015). Genetic Rescue Working Group. In 'IUCN CBSG Annual Meeting'. (IUCN SSC Conservation Breeding Specialist Group: Gland, Switzerland.)

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., Garcia, A., Pringle, R. M., and Palmer, T. M. (2015). Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Science Advances* **1**, e1400253–e1400253. doi:10.1126/sciadv.1400253

Ceballos, G., Ehrlich, P. R., and Dirzo, R. (2017). Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proceedings of the National Academy of Sciences of the United States of America* **114**, E6089–E6096. doi:10.1073/pnas.1704949114

Ceballos, G., Ehrlich, P. R., and Raven, P. H. (2020). Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. *Proceedings of the National Academy of Sciences* **117**, 201922686. doi:10.1073/pnas.1922686117

Cézilly, F., Viallefont, A., Boy, V., and Johnson, A. R. (1996). Annual variation in survival and breeding probability in greater flamingos. *Ecology* **77**, 1143–1150. doi:10.2307/2265583

Chamberlain, S. A., and Szöcs, E. (2013). taxize: taxonomic search and retrieval in R. *F1000Research* **2**, 191. doi:10.12688/f1000research.2-191.v1

Che-Castaldo, J., Johnson, B., Magrisso, N., Mechak, L., Melton, K., Mucha, K., Terwilliger, L., Theis, M., Long, S., and Faust, L. (2019). Patterns in the long-term viability of North American zoo populations. *Zoo Biology* **38**, 78–94. doi:10.1002/zoo.21471

Chemnick, L. G., Kumamoto, A. T., and Ryder, O. A. (2000). Genetic analyses in support of conservation efforts for the California condor. *International Zoo Yearbook* **37**, 330–339. doi:10.1111/j.1748-1090.2000.tb00738.x

CIESIN (2017). Gridded Population of the World, Version 4 (GPWv4): Population Count, Revision 10. Available at: https://doi.org/10.7927/H4PG1PPM

CITES (2019). Checklist of CITES species. Available at: http://checklist.cites.org/

CITES (1973). Convention on international trade in endangered species of wild fauna and flora. Available at: https://www.cites.org/sites/default/files/eng/disc/CITES-Convention-EN.pdf

Clark, J. A., and May, R. M. (2002). Taxonomic bias in conservation research. *Science* **297**, 191–193. Available at: https://science.sciencemag.org/content/297/5579/191.2/tab-e-letters [accessed 12 August 2020]

Clarke, A. G. (2009). The Frozen Ark Project: the role of zoos and aquariums in preserving the genetic material of threatened animals. *International Zoo Yearbook* **43**, 222–230. doi:10.1111/j.1748-1090.2008.00074.x

Clayton, D. A. (1978). Socially Facilitated Behavior. *The Quarterly Review of Biology* **53**, 373–392. doi:10.1086/410789

Cohen, S. (2014). The Ethics of De-Extinction. *NanoEthics* **8**, 165–178. doi:10.1007/s11569-014-0201-2

Colchero, F. (2018). BaSTA.ZIMS: Age-Specific Survival Analysis from Census Data (e.g. ZIMS database). R package version 1.0.1.

Conde, D. A., Colchero, F., Gusset, M., Pearce-Kelly, P., Byers, O., Flesness, N., Browne, R. K., and Jones, O. R. (2013). Zoos through the Lens of the IUCN Red List: A Global Metapopulation Approach to Support Conservation Breeding Programs Ed M. Hayward. *PLoS ONE* **8**, e80311. doi:10.1371/journal.pone.0080311

Conde, D. A., Flesness, N., Colchero, F., Jones, O. R., and Scheuerlein, A. (2011). An Emerging Role of Zoos to Conserve Biodiversity. *Science* **331**, 1390–1391. doi:10.1126/science.1200674

Conde, D. A., Staerk, J., Colchero, F., Silva, R. da, Schöley, J., Baden, H. M., Jouvet, L., Fa, J. E., Syed, H., Jongejans, E., Meiri, S., Gaillard, J.-M., Chamberlain, S., Wilcken, J., Jones, O. R., Dahlgren, J. P., Steiner, U. K., Bland, L. M., Gomez-Mestre, I., Lebreton, J.-D., Vargas, J. G., Flesness, N., Canudas-Romo, V., Salguero-Gómez, R., Byers, O., Berg, T. B., Scheuerlein, A., Devillard, S., Schigel, D. S., Ryder, O. A., Possingham, H. P., Baudisch, A., and Vaupel, J. W. (2019). Data gaps and opportunities for comparative and conservation biology. *Proceedings of the National Academy of Sciences*, 201816367. doi:10.1073/PNAS.1816367116

Conway, W. (1987). Species carrying capacity in the zoo alone. In 'Proceedings, 1987 AAZPA Annual Conference'. pp. 20–32. (Wheeling, West Virginia, United States.)

Conway, W. (2003). The role of zoos in the 21st century. *International Zoo Yearbook* **38**, 7–13. doi:10.1111/j.1748-1090.2003.tb02059.x

Conway, W. G. (2011). Buying Time for wild animals With Zoos. *Zoo Biology* **30**, 1–8. doi:10.1002/zoo.20352

Conway, W. G. (1965). Care of James's Flamingo *Phoenicoparrus jamesi* Sclater and the Andean Flamingo *Phoenicoparrus andinus* R. A. Philippi in Captivity. *International Zoo Yearbook* **5**, 162–164. doi:10.1111/j.1748-1090.1965.tb01624.x

Conway, W. G. (1986). The practical difficulties and financial implications of endangered species breeding programmes. *International Zoo Yearbook* **24**, 210–219. doi:10.1111/j.1748-1090.1985.tb02541.x

Coonan, T. J., Varsik, A., Lynch, C., and Schwemm C. A. (2010). Cooperative conservation: zoos and *in situ* captive breeding for endangered Island fox *Urocyon littoralis ssp. International Zoo Yearbook* **44**, 165–172. doi:10.1111/j.1748-1090.2009.00092.x

Coyne, P. (2010). Ecological rebound on Phillip Island, South Pacific. *Ecological Management & Restoration* **11**, 4–15. doi:10.1111/j.1442-8903.2010.00507.x

CPSG (2016). Prioritizing the collection of samples for genetic rescue. In 'IUCN CPSG Annual Meeting'. (IUCN SSC Conservation Planning Specialist Group: Gland, Switzerland.)

Critser, J. K., and Russell, R. J. (2000). Genome Resource Banking of Laboratory Animal Models. *ILAR Journal* **41**, 183–186. doi:10.1093/ilar.41.4.183

Davey, G. (2007). An analysis of country, socio-economic and time factors on worldwide zoo attendance during a 40 year period. *International Zoo Yearbook* **41**, 217–225. doi:10.1111/j.1748-1090.2007.00007.x

DeFries, R., Hansen, A., Newton, A. C., and Hansen, M. C. (2005). Increasing isolation of protected areas in tropical forests over the past twenty years. *Ecological Applications* **15**, 19–26. doi:10.1890/03-5258

Díaz, S., Pascual, U., Stenseke, M., Martín-López, B., Watson, R. T., Molnár, Z., Hill, R., Chan, K. M. A., Baste, I. A., Brauman, K. A., Polasky, S., Church, A., Lonsdale, M., Larigauderie, A., Leadley, P. W., Van Oudenhoven, A. P. E., Van Der Plaat, F., Schröter, M., Lavorel, S., Aumeeruddy-Thomas, Y., Bukvareva, E., Davies, K., Demissew, S., Erpul, G., Failler, P., Guerra, C. A., Hewitt, C. L., Keune, H., Lindley, S., and Shirayama, Y. (2018). Assessing nature's contributions to people: Recognizing culture, and diverse sources of knowledge, can improve assessments. *Science* **359**, 270–272. doi:10.1126/science.aap8826

Dickie, L. A., Bonner, J. P., and West, C. D. (2007). *In situ* and *ex situ* conservation: blurring the boundaries between zoos and the wild. In 'Zoos in the 21st Century: Catalysts for Conservation?'. (Eds A. Zimmermann, M. Hatchwell, L. A. Dickie, and C. West.) pp. 220–235. (Cambridge University Press: Cambridge.)

Doudna, J. A., and Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science* **346**, 1258096. doi:10.1126/science.1258096

Driml, S., Ballantyne, R., and Packer, J. (2017). How Long Does an Economic Impact Last? Tracking the Impact of a New Giant Panda Attraction at an Australian Zoo. *Journal of Travel Research* **56**, 613–624. doi:10.1177/0047287516656916

Dunne, J. A., and Williams, R. J. (2009). Cascading extinctions and community collapse in model food webs. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 1711–1723. doi:10.1098/rstb.2008.0219

Duplaix-Hall, N., and Kear, J. (1975). Breeding requirements in captivity. In 'Flamingos'. (Eds N.

Duplaix-Hall and J. Kear.) pp. 131–141. (T. & A. D. Poyser: London.)

Durrell, G. (1976). ‘The stationary ark’. (Collins: London.)

EAZA (2020). EAZA Biobank. Available at: https://www.eaza.net/conservation/research/eaza-biobank/ [accessed 12 May 2020]

Ebihara, A., Fraser-Jenkins, C. R., Parris, B. S. ., Zhang, X.-C., Yang, Y.-H., Chiou, W.-L., Chang, H.-M., Lindsay, S., Middleton, D., and Kato, Masahiro; Praptosuwiryo, Titien Ngatinem; Amoroso, Victor B.; Barcelona, Ju, A. (2012). Rare and Threatened Pteridophytes of Asia 1. An Enumeration of Narrowly Distributed Taxa. *Bulletin of the National Museum of Nature and Science, Series B.* **38**, 93–119. Available at: https://rbg-web2.rbge.org.uk/thaiferns/PDF_docs/BNMNS2012_Rare-Pterid-Asia1.pdf

EDGE (2019). EDGE: Evolutionarily Distinct & Globally Endangered. Available at: http://edgeofexistence.org/

Edwards, C. E., Bassüner, B., Birkinshaw, C., Camara, C., Lehavana, A., Lowry, P. P., Miller, J. S., Wyatt, A., and Jackson, P. W. (2018). A botanical mystery solved by phylogenetic analysis of botanical garden collections: The rediscovery of the presumed-extinct *Dracaena umbraculifera*. *Oryx* **52**, 427–436. doi:10.1017/S0030605317001570

Ehrlich, P. R. (1988). The Loss of Diversity: Causes and Consequences. In ‘Biodiversity’. (Eds E. O. Wilson and F. M. Peter.) pp. 21–27. (National Academy Press: Washington D.C.) doi:10.17226/989

Ellis, E. C. (2013). Sustaining biodiversity and people in the world’s anthropogenic biomes. *Current Opinion in Environmental Sustainability* **5**, 368–372. doi:10.1016/j.cosust.2013.07.002

Else, J. (2018). Feral pig population holding steady. *The Garden Island*. Available at: https://www.thegardenisland.com/2018/01/22/hawaii-news/feral-pig-population-holding-steady/ [accessed 7 August 2020]

Emslie, R. (2020). *Ceratotherium simum*. The IUCN Red List of Threatened Species 2020: e.T4185A45813880. https://dx.doi.org/10.2305/IUCN.UK.2020-1.RLTS.T4185A45813880.en [accessed 11 May 2020]

Engler, M., and Parry-Jones, R. (2007). Opportunity or threat? The role of the European Union in global wildlife trade. Brussels, Belgium.

ENSCONET (2020). ENSCOBASE: The European Native Seed Conservation Network (ENSCONET) Virtual Seed Bank. Available at: http://enscobase.maich.gr [accessed 14 April 2020]

Fa, J. E., Funk, S. M., and OConnell, D. (2011). ‘Zoo Conservation Biology’. (Cambridge University Press: Cambridge.) doi:10.1017/CBO9780511993435

Fa, J. E., Gusset, M., Flesness, N., and Conde, D. A. (2014). Zoos have yet to unveil their full conservation potential. *Animal Conservation* **17**, 97–100. doi:10.1111/acv.12115

Fan, Y., Chen, J., Shirkey, G., John, R., Wu, S. R., Park, H., and Shao, C. (2016). Applications of structural equation modeling (SEM) in ecological studies: an updated review. *Ecological Processes* **5**, 19. doi:10.1186/s13717-016-0063-3

Farrell, M. A., Barry, E., and Marples, N. (2000). Breeding behavior in a flock of Chilean flamingos (*Phoenicopterus chilensis*) at Dublin zoo. *Zoo Biology* **19**, 227–237. doi:10.1002/1098-2361(2000)19:4<227::AID-ZOO1>3.0.CO;2-H

Foden, W. B., Butchart, S. H. M., Stuart, S. N., Vié, J.-C., Akçakaya, H. R., Angulo, A., DeVantier, L. M., Gutsche, A., Turak, E., Cao, L., Donner, S. D., Katariya, V., Bernard, R., Holland, R. A., Hughes, A. F., O'Hanlon, S. E., Garnett, S. T., Şekercioğlu, Ç. H., and Mace, G. M. (2013). Identifying the World's Most Climate Change Vulnerable Species: A Systematic Trait-Based Assessment of all Birds, Amphibians and Corals Ed S. Lavergne. *PLoS ONE* **8**, e65427. doi:10.1371/journal.pone.0065427

Foden, W., Mace, G., Vié, J.-C., Angulo, A., Butchart, S., DeVantier, L., Dublin, H., Gutsche, A., Stuart, S., and Turak, E. (2008). Species susceptibility to climate change impacts. In 'The 2008 Review of The IUCN Red List of Threatened Species'. (Eds J.-C. Vié, C. Hilton-Taylor, and S. N. Stuart.) (IUCN: Gland, Switzerland.)

Folch, J., Cocero, M. J., Chesné, P., Alabart, J. L., Domínguez, V., Cognié, Y., Roche, A., Fernández-Árias, A., Martí, J. I., Sánchez, P., Echegoyen, E., Beckers, J. F., Bonastre, A. S., and Vignon, X. (2009). First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning. *Theriogenology* **71**, 1026–1034. doi:10.1016/j.theriogenology.2008.11.005

Foose, T. J. (1983). The relevance of captive populations to the conservation of biotic diversity. In 'Genetics and Conservation'. (Eds C. M. Schonewald-Cox, S. M. Chambers, B. Macbryde, and W. L. Thomas.) (Benjamin/Cummings Publishing Company: Menlo Park, California, United States.)

Foose, T. J., de Boer, L., Seal, U. S., and Lande, R. (1995). Conservation management strategies based on viable populations. In 'Population management for survival and recovery'. (Eds J. D. Ballou, M. Gilpin, and T. J. Foose.) pp. 273–294. (Columbia University Press: New York City, United States)

Frankham, R., Ballou, J. D., and Briscoe, D. A. (2002). 'Introduction to Conservation Genetics'. (Cambridge University Press.) doi:10.1017/cbo9780511809002

Frost, W. (2010). 'Zoos and Tourism: Conservation, Education, Entertainment?' Ed W. Frost. (Channel View Publications: Bristol, United Kingdom.)

Frynta, D., Lišková, S., Bültmann, S., and Burda, H. (2010). Being Attractive Brings Advantages:

The Case of Parrot Species in Captivity Ed T. Mappes. *PLoS ONE* **5**, e12568. doi:10.1371/journal.pone.0012568

Frynta, D., Šimková, O., Lišková, S., and Landová, E. (2013). Mammalian Collection on Noah's Ark: The Effects of Beauty, Brain and Body Size Ed D. L. Roberts. *PLoS ONE* **8**, e63110. doi:10.1371/journal.pone.0063110

Gardali, T., Seavy, N. E., DiGaudio, R. T., and Comrack, L. A. (2012). A Climate Change Vulnerability Assessment of California's At-Risk Birds Ed D. Hyrenbach. *PLoS ONE* **7**, e29507. doi:10.1371/journal.pone.0029507

Garrett, E. A. (2015). 'Why Do We Go to the Zoo?: Communication, Animals, and the Cultural-Historical Experience of Zoos'. (Fairleigh Dickinson University Press: Madison, New Jersey, United States.)

GBIF (2020). Global Biodiversity Information Facility. Available at: https://www.gbif.org/ [accessed 14 April 2020]

Godefroid, S., van de Vyver, A., Stoffelen, P., Robbrecht, E., and Vanderborght, T. (2011). Testing the viability of seeds from old herbarium specimens for conservation purposes. *Taxon* **60**, 565–569. doi:10.1002/tax.602022

Golden Kroner, R. E., Qin, S., Cook, C. N., Krithivasan, R., Pack, S. M., Bonilla, O. D., Cort-Kansinally, K. A., Coutinho, B., Feng, M., Garcia, M. I. M., He, Y., Kennedy, C. J., Lebreton, C., Ledezma, J. C., Lovejoy, T. E., Luther, D. A., Parmanand, Y., Ruíz-Agudelo, C. A., Yerena, E., Zambrano, V. M., and Mascia, M. B. (2019). The uncertain future of protected lands and waters. *Science* **364**, 881–886. doi:10.1126/science.aau5525

Grace, J. B., Anderson, T. M., Olff, H., and Scheiner, S. M. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs* **80**, 67–87. doi:10.1890/09-0464.1

Grace, J. B., Anderson, T. M., Seabloom, E. W., Borer, E. T., Adler, P. B., Harpole, W. S., Hautier, Y., Hillebrand, H., Lind, E. M., Pärtel, M., Bakker, J. D., Buckley, Y. M., Crawley, M. J., Damschen, E. I., Davies, K. F., Fay, P. A., Firn, J., Gruner, D. S., Hector, A., Knops, J. M. H., MacDougall, A. S., Melbourne, B. A., Morgan, J. W., Orrock, J. L., Prober, S. M., and Smith, M. D. (2016). Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature* **529**, 390–393. doi:10.1038/nature16524

Grace, J. B., Scheiner, S. M., and Schoolmaster, D. R. J. (2015). Structural equation modeling: building and evaluating causal models. In 'Ecological Statistics: Contemporary theory and application'. (Eds G. A. Fox, S. Negrete-Yankelevich, and V. J. Sosa.) pp. 168–199. (Oxford University Press: Oxford, UK.)

Grimm, D. (2014). Free Willy? Dolphin drama riles aquaria. *Science* **344**, 1437–1438.

doi:10.1126/science.344.6191.1437

Gunawardana, J. (2019). The Sri Lankan legume: Extinct, rediscovered, and now at risk again (commentary). *Mongabay*. Available at: https://news.mongabay.com/2019/12/the-sri-lankan-legume-extinct-rediscovered-and-now-at-risk-again-commentary/ [accessed 7 August 2020]

Gunnthorsdottir, A. (2001). Physical Attractiveness of an Animal Species as a Decision Factor for its Preservation. *Anthrozoös* **14**, 204–215. doi:10.2752/089279301786999355

Gusset, M., and Dick, G. (2010). 'Building a Future for Wildlife'? Evaluating the contribution of the world zoo and aquarium community to *in situ* conservation. *International Zoo Yearbook* **44**, 183–191. doi:10.1111/j.1748-1090.2009.00101.x

Gusset, M., and Dick, G. (2011). The global reach of zoos and aquariums in visitor numbers and conservation expenditures. *Zoo Biology* **30**, 566–569. doi:10.1002/zoo.20369

Hancocks, D. (2001). 'A different nature : the paradoxical world of zoos and their uncertain future'. (University of California Press: Berkeley, California, United States.)

Hartig, F. (2019). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.2.6. https://CRAN.R-project.org/package=DHARMa

Hay, F. R., and Probert, R. J. (2013). Advances in seed conservation of wild plant species: A review of recent research. *Conservation Physiology* **1**. doi:10.1093/conphys/cot030

Heck, H. (1951). The breeding-back of the aurochs. *Oryx* **1**, 117–122. doi:10.1017/S0030605300035286

Hibbard, C., Hogg, C. J., Ford, C., and Embury, A. (2011). Maintaining the status of species management in a changing operating environment: Outcomes over outputs. *WAZA Magazine 12*, 6–10.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965–1978. doi:10.1002/joc.1276

Hitchcock, A., and Rebelo, A. G. (2017). The Restoration of *Erica verticillata*. *The International Journal of Botanic Garden Horticulture*, 39–63. doi:10.23823/SIBBALDIA/2017.222

Hobbs, R. J., O'Brien, J. K., and Spindler, R. E. (2018). Strategic Gene Banking for Conservation. In 'Scientific Foundations of Zoos and Aquariums'. (Eds A. B. Kaufman, M. J. Bashaw, and T. L. Maple.) pp. 112–146. (Cambridge University Press: Cambridge.) doi:10.1017/9781108183147.005

Hoffmann, M., Brooks, T., da Fonseca, G., Gascon, C., Hawkins, A., James, R., Langhammer, P., Mittermeier, R., Pilgrim, J., Rodrigues, A., and Silva, J. (2008). Conservation planning and the IUCN Red List. *Endangered Species Research* **6**, 113–125. doi:10.3354/esr00087

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T. M., Butchart, S. H. M., Collen, B., Cox, N. A., Darwall, W. R. T., Dulvy, N. K., Harrison, L. R., Katariya, V., Pollock, C. M., Vié, J.-C., Aguiar, J. M., Allen, D. J., Allen, G. R., Amori, G., Ananjeva, N. B., Andreone, F., Andrew, P., Luz, A., Ortiz, A., Baillie, J. E. M., Collar, N. J., Collett, L. C., Collette, B. B., Fernandez, C. F. C., Craig, M. T., Crosby, M. J., Cumberlidge, N., Cuttelod, A., Derocher, A. E., Diesmos, A. C., Garshelis, D. L., Gerlach, J., Gower, D. J., Grant, T. D., Hammerson, G. A., Harris, R. B., Heaney, L. R., Hedges, S. B., Hero, J.-M., Hughes, B., Hussain, S. A., Icochea, J., Inger, R. F., Ishii, N., Iskandar, D. T., Jenkins, R. K. B., Pourkazemi, M., Racey, P. A., Ragle, J. S., Ram, M., Rathbun, G., Reynolds, R. P., Rhodin, A. G. J., Richards, S. J., Sanciangco, J. C., Sanders, K. L., Santos-Barrera, G., Schipper, J., Shi, Y., Shoemaker, A., Sillero-Zubiri, C., Silvano, D. L., Smith, K. G., Smith, A. T., Stattersfield, A. J., Symes, A. J., Taber, A. B., Talukdar, B. K., Temple, H. J., Timmins, R., Tobias, J. A., Paul Van Dijk, P., Veiga, L. M., Williamson, E. A., Xie, F., Young, B. E., Akçakaya, H. R., Bennun, L., Blackburn, T. M., Boitani, L., Gascon, C., Lacher, T. E., Mace, G. M., Mainka, S. A., Mcneely, J. A., Mittermeier, R. A., Reid, G. M., Rodriguez, J. P., Rosenberg, A. A., and Samways, M. J. (2010). The Impact of Conservation on the Status of the World's Vertebrates. *Science* **330**, 1503–1509. doi:10.1126/science.1194442

Holt, W. V., Bennett, P. M., Volobouev, V., and Watwon, P. F. (1996). Genetic resource banks in wildlife conservation. *Journal of Zoology* **238**, 531–544. doi:10.1111/j.1469-7998.1996.tb05411.x

Hosey, G., Melfi, V., and Ward, S. J. (2020). Problematic Animals in the Zoo: The Issue of Charismatic Megafauna. In 'Problematic Wildlife II'. (Eds F. M. Angelici and L. Rossi.) pp. 485–508. (Springer International Publishing: Cham, Switzerland.) doi:10.1007/978-3-030-42335-3_15

Houck, M. (2016). Back to the (Frozen) Future. *Science Blog, San Diego Zoo Global Institute for Conservation Research*. Available at: https://institute.sandiegozoo.org/science-blog/back-frozen-future [accessed 21 April 2020]

Houck, M. L., Lear, T. L., and Charter, S. J. (2017). Animal cytogenetics. In 'The AGT Cytogenetics Laboratory Manual'. (Eds M. S. Arsham, M. J. Barch, and H. J. Lawce.) pp. 1055–1102. (Wiley-Blackwell: New York.)

Houck, M. L., Ryder, O. A., Váhala, J., Kock, R. A., and Oosterhuis, J. E. (1994). Diploid chromosome number and chromosomal variation in the white rhinoceros (*Ceratotherium simum*). *Journal of Heredity* **85**, 30–34. doi:10.1093/oxfordjournals.jhered.a111387

Howard, J. G., Lynch, C., Santymire, R. M., Marinari, P. E., and 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**, 102–111. doi:10.1111/acv.12229

Howard, S. D., and Bickford, D. P. (2014). Amphibians over the edge: silent extinction risk of Data Deficient species Ed S. Ferrier. *Diversity and Distributions* **20**, 837–846. doi:10.1111/ddi.12218

Hu, L., and Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal* **6**, 1–55. doi:10.1080/10705519909540118

Hughes, B. B., Wasson, K., Tim Tinker, M., Williams, S. L., Carswell, L. P., Boyer, K. E., Beck, M. W., Eby, R., Scoles, R., Staedler, M., Espinosa, S., Hessing-Lewis, M., Foster, E. U., Beheshti, K. M., Grimes, T. M., Becker, B. H., Needles, L., Tomoleoni, J. A., Rudebusch, J., Hines, E., and Silliman, B. R. (2019). Species recovery and recolonization of past habitats: Lessons for science and conservation from sea otters in estuaries. *PeerJ* **2019**, e8100. doi:10.7717/peerj.8100

Hutchins, M., and Wemmer, C. (1991). In defense of captive breeding. *Endangered Species Update* **9**, 5–6.

Hutchins, M., Willis, K., and Wiese, R. J. (1995). Strategic collection planning: Theory and practice. *Zoo Biology* **14**, 5–25. doi:10.1002/zoo.1430140103

Iacona, G., Maloney, R. F., Chadès, I., Bennett, J. R., Seddon, P. J., and Possingham, H. P. (2017). Prioritizing revived species: what are the conservation management implications of de-extinction? Ed K. Thompson. *Functional Ecology* **31**, 1041–1048. doi:10.1111/1365-2435.12720

IPBES (2019). Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. (Eds. S. Díaz, J. Settele, E. S. Brondízio E.S., H. T. Ngo, M. Guèze, J. Agard, A. Arneth, P. Balvanera, K. A. Brauman, S. H. M. Butchart, K. M. A. Chan, L. A. Garibaldi, K. Ichii, J. Liu, S. M. Subramanian, G. F. Midgley, P. Miloslavich, Z. Molnár, D. Obura, A. Pfaff, S. Polasky, A. Purvis, J. Razzaque, B. Reyers, R. Roy Chowdhury, Y. J. Shin, I. J. Visseren-Hamakers, K. J. Willis, and C. N. Zayas.) 56 pages (IPBES secretariat, Bonn, Germany.) Available at https://doi.org/10.5281/zenodo.3553579

Isaac, N. J. B., Redding, D. W., Meredith, H. M., and Safi, K. (2012). Phylogenetically-Informed Priorities for Amphibian Conservation Ed M. C. Fisher. *PLoS ONE* **7**, e43912. doi:10.1371/journal.pone.0043912

Isaac, N. J. B., Turvey, S. T., Collen, B., Waterman, C., and Baillie, J. E. M. (2007). Mammals on the EDGE: Conservation Priorities Based on Threat and Phylogeny Ed W. Reid. *PLoS ONE* **2**, e296. doi:10.1371/journal.pone.0000296

IUCN (2018). The IUCN Red List of Threatened Species. Version 2018-1. Available at: https://www.iucnredlist.org [accessed 7 October 2018]

IUCN (2019). The IUCN Red List of Threatened Species. Version 2019-1. Available at: https://www.iucnredlist.org [accessed 11 March 2019]

IUCN (2020). The IUCN Red List of Threatened Species. Version 2020-2. Available at: https://www.iucnredlist.org [accessed on 9 July 2020]

IUCN SSC (2014). Guidelines on the Use of *Ex situ* Management for Species Conservation. Version 2.0. (IUCN Species Survival Commission: Gland, Switzerland.) Available at: www.iucn.org/about/work/programmes/species/publications/iucn_guidelines_and__policy__statements/ [accessed 6 July 2020]

IUCN SSC (2016). IUCN SSC Guiding principles on Creating Proxies of Extinct Species for Conservation Benefit. Version 1.0. (IUCN Species Survival Commission: Gland, Switzerland.)

IUDZG/IUCN SSC (CBSG) (1993). 'Executive Summary, The World Zoo Conservation Strategy; The Role of the Zoos and Aquaria of the World in Global Conservation'. (Chicago Zoological Society: Brookfield, Illinois, U.S.A.)

IZY/ZSL (2016). Zoos and Aquariums of the World. *International Zoo Yearbook* **50**, 225–391. doi:10.1111/izy.12130

Johnson, A., and Cézilly, F. (2008). 'The Greater Flamingo'. (T & A. D. Poyser: London.)

Johnson, W. E., Onorato, D. P., Roelke, M. E., Land, E. D., Cunningham, M., Belden, R. C., McBride, R., Jansen, D., Lotz, M., Shindle, D., Howard, J. G., Wildt, D. E., Penfold, L. M., Hostetler, J. A., Oli, M. K., and O'Brien, S. J. (2010). Genetic restoration of the Florida panther. *Science* **329**, 1641–1645. doi:10.1126/science.1192891

Jones, K. E. (2014). From dinosaurs to dodos: who could and should we de-extinct? *Frontiers of Biogeography* **6**, 20–24. doi:10.21425/F5FBG19431

Joseph, L. N., Maloney, R. F., and Possingham, H. P. (2009). Optimal allocation of resources among threatened species: a project prioritization protocol. *Conservation Biology* **23**, 328–338. doi:10.1111/j.1523-1739.2008.01124.x

Joshi, G., Dhyani, A., A.N., A., and K.T., B. (2019). Rescuing *Madhuca insignis* (Sapotaceae), an extremely narrow endemic species to South Western Ghats, India. *Journal for Nature Conservation* **52**, 125760. doi:10.1016/j.jnc.2019.125760

Jule, K. R., Leaver, L. A., and Lea, S. E. G. (2008). The effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biological Conservation* **141**, 355–363. doi:10.1016/j.biocon.2007.11.007

Julia, S., Chong, L., Vilma, B., and Esther, S. (2014). Sarawak Plant Red List Dipterocarpaceae. Kuching, Malaysia.

Kaufman, L. (2012). Zoos' Bitter Choice: To Save Some Species, Letting Others Die. *The New York Times*. Available at: https://www.nytimes.com/2012/05/28/science/zoos-bitter-choice-to-save-some-species-letting-others-die.html [accessed 18 August 2020]

Kaumanns, W., Singh, M., and Sliwa, A. (2013). Captive propagation of threatened primates - the example of the Lion-tailed Macaque *Macaca silenus*. *Journal of Threatened Taxa* **5**, 4825–4839. doi:10.11609/jott.o3625.4825-39

Kawata, K. (2013). Rambling Thoughts on Zoo Animal Collection and Conservation: A Historical Perspective. *Der Zoologische Garten* **82**, 26–39. doi:10.1016/j.zoolgart.2013.04.003

Keith, D. A., Mahony, M., Hines, H., Elith, J., Regan, T. J., Baumgartner, J. B., Hunter, D., Heard, G. W., Mitchell, N. J., Parris, K. M., Penman, T., Scheele, B., Simpson, C. C., Tingley, R., Tracy, C. R., West, M., and Akçakaya, H. R. (2014). Detecting extinction risk from climate change by IUCN red list criteria. *Conservation Biology* **28**, 810–819. doi:10.1111/cobi.12234

Keller, L. F., and Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**, 230–241. doi:10.1016/S0169-5347(02)02489-8

Kelly, J. D. (1997). Effective conservation in the twenty-first century: the need to be more than a zoo. One organization's approach. *International Zoo Yearbook* **35**, 1–14. doi:10.1111/j.1748-1090.1997.tb01184.x

Keulartz, J. (2015). Captivity for Conservation? Zoos at a Crossroads. *Journal of Agricultural and Environmental Ethics* **28**, 335–351. doi:10.1007/s10806-015-9537-z

King, C. E. (2008). A hypothetical husbandry point system for breeding flamingos in captivity. *Flamingo - Journal of the IUCN SSC WI Flamingo Specialist Group* **16**.

King, C. E. (2000). Captive flamingo populations and opportunities for research in zoos. *Waterbirds: The International Journal of Waterbird Biology* **23**, 142–149. doi:10.2307/1522159

King, C. E. (1994). Management and research implications of selected behaviours in a mixed colony of flamingos at Rotterdam Zoo. *International Zoo Yearbook* **33**, 103–113. doi:10.1111/j.1748-1090.1994.tb03562.x

King, C. E. (2006). Pink flamingos: atypical partnerships and sexual activity in colonial breeding birds. In 'Homosexual behaviour in animals: an evolutionary perspective'. (Eds V. Sommer and P. L. Vasey.) pp. 77–106. (Cambridge University Press: Cambridge.)

King, C. E., and Bračko, A. (2014). Nineteen years of management for Phoenicopteriformes in

European Association of Zoos and Aquaria institutions: the Fabulous Flamingo Surveys and strategies to increase reproduction in captivity. *International Zoo Yearbook* **48**, 184–198. doi:10.1111/izy.12041

Köckinger, H. (2016). Rediscovery and Redescription of the Enigmatic *Radula visianica* (Porellales, Marchantiophyta) . *Herzogia* **29**, 625–634. doi:10.13158/heia.29.2.2016.625

Kouba, A. J., Lloyd, R. E., Houck, M. L., Silla, A. J., Calatayud, N., Trudeau, V. L., Clulow, J., Molinia, F., Langhorne, C., Vance, C., Arregui, L., Germano, J., Lermen, D., and Della Togna, G. (2013). Emerging trends for biobanking amphibian genetic resources: The hope, reality and challenges for the next decade. *Biological Conservation* **164**, 10–21. doi:10.1016/j.biocon.2013.03.010

Krienitz, L., Mähnert, B., and Schagerl, M. (2016). Lesser flamingo as a central element of the East African Avifauna. In 'Soda Lakes of East Africa'. (Ed M. Schagerl.) pp. 259–284. (Springer International Publishing: Cham, Switzerland.) doi:10.1007/978-3-319-28622-8_10

Leader-Williams, N., and Dublin, H. T. (2000). Charismatic megafauna as 'flagship species'. In 'Priorities for the conservation of mammalian diversity: has the panda had its day'. (Eds A. Entwistle and N. Dunstone.) pp. 53–81. (Cambridge University Press: Cambridge.)

Lees, C. M., and Wilcken, J. (2009). Sustaining the Ark: the challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook* **43**, 6–18. doi:10.1111/j.1748-1090.2008.00066.x

Legendre, M., Bartoli, J., Shmakova, L., Jeudy, S., Labadie, K., Adrait, A., Lescot, M., Poirot, O., Bertaux, L., Bruley, C., Couté, Y., Rivkina, E., Abergel, C., and Claverie, J. M. (2014). Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 4274–4279. doi:10.1073/pnas.1320670111

Leus, K., Bingaman Lackey, L., van Lint, W., de Man, D., Riewalk, S., Veldkam, A., and Wijmans, J. (2011). Sustainability of European Association of Zoos and Aquaria bird and mammal populations. *WAZA Magazine 12*, 11–14.

Liberg, O., Andrén, H., Pedersen, H.-C., Sand, H., Sejberg, D., Wabakken, P., Åkesson, M., and Bensch, S. (2005). Severe inbreeding depression in a wild wolf *Canis lupus* population. *Biology Letters* **1**, 17–20. doi:10.1098/rsbl.2004.0266

Liu, U., Breman, E., Cossu, T. A., and Kenney, S. (2018). The conservation value of germplasm stored at the Millennium Seed Bank, Royal Botanic Gardens, Kew, UK. *Biodiversity and Conservation* **27**, 1347–1386. doi:10.1007/s10531-018-1497-y

Liu, U., Kenney, S., Breman, E., and Cossu, T. A. (2019). A multicriteria decision making approach to prioritise vascular plants for species-based conservation. *Biological Conservation* **234**, 221–240. doi:10.1016/j.biocon.2019.03.014

Loi, P., Ptak, G., Barboni, B., Fulka, J., Cappai, P., and Clinton, M. (2001). Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. *Nature Biotechnology* **19**, 962–964. doi:10.1038/nbt1001-962

Long, S., Dorsey, C., and Boyle, P. (2011). Status of Association of Zoos and Aquariums cooperatively managed populations. *WAZA Magazine 12*, 15–18.

Lorence, D., and Butaud, J.-F. (2011). A Reassessment of Marquesan *Ochrosia* and *Rauvolfia* (Apocynaceae) with two new combinations. *PhytoKeys* **4**, 95. doi:10.3897/phytokeys.4.1599

Lynch, V. J., Bedoya-Reina, O. C., Ratan, A., Sulak, M., Drautz-Moses, D. I., Perry, G. H., Miller, W., and Schuster, S. C. (2015). Elephantid Genomes Reveal the Molecular Bases of Woolly Mammoth Adaptations to the Arctic. *Cell Reports* **12**, 217–228. doi:10.1016/j.celrep.2015.06.027

Mace, G. M., Balmford, A., Leader-Williams, N., Manica, A., Walter, O., West, C. D., and Zimmermann, A. (2007). Measuring conservation success: assessing zoos' contribution. In 'Zoos in the 21st Century: Catalysts for Conservation?'. (Eds A. Zimmermann, M. Hatchwell, L. Dickie, and C. West.) pp. 322–342. (Cambridge University Press: Cambridge.)

Macek, M. (2014). Introduction to Avian Challenges. *International Zoo Yearbook* **48**, 1–6. doi:10.1111/izy.12058

Marešová, J., and Frynta, D. (2008). Noah's Ark is full of common species attractive to humans: The case of boid snakes in zoos. *Ecological Economics* **64**, 554–558. doi:10.1016/J.ECOLECON.2007.03.012

Martin, T. E., Lurbiecki, H., Joy, J. B., and Mooers, A. O. (2014). Mammal and bird species held in zoos are less endemic and less threatened than their close relatives not held in zoos. *Animal Conservation* **17**, 89–96. doi:10.1111/acv.12069

Martinelli, L., Oksanen, M., and Siipi, H. (2014). De-extinction: a novel and remarkable case of bio-objectification. *Croatian medical journal* **55**, 423–7. doi:10.3325/CMJ.2014.55.423

Martínez-Sánchez, J. J., Segura, F., Aguado, M., Franco, J. A., and Vicente, M. J. (2011). Life history and demographic features of *Astragalus nitidiflorus*, a critically endangered species. *Flora: Morphology, Distribution, Functional Ecology of Plants* **206**, 423–432. doi:10.1016/j.flora.2010.11.006

Mastromonaco, G. F., Houck, M. L., and Bergfelt, D. R. (2012). Disorders of sexual development in wild and captive exotic animals. *Sexual Development* **6**, 84–95. doi:10.1159/000332203

McCann, C., and Powell, D. M. (2019). Is there any more room on the Ark? An analysis of space allocation in four mammalian taxa. *Zoo Biology* **38**, 36–44. doi:10.1002/zoo.21469

McCauley, D. J., Hardesty-Moore, M., Halpern, B. S., and Young, H. S. (2017). A mammoth undertaking: harnessing insight from functional ecology to shape de-extinction priority setting Ed P. Seddon. *Functional Ecology* **31**, 1003–1011. doi:10.1111/1365-2435.12728

Mellish, S., Sanders, B., Litchfield, C. A., and Pearson, E. L. (2017). An investigation of the impact of Melbourne Zoo's "Seal-the-Loop" donate call-to-action on visitor satisfaction and behavior. *Zoo Biology* **36**, 237–242. doi:10.1002/zoo.21365

Meyer, J.-Y., and Butaud, J.-F. (2009). The impacts of rats on the endangered native flora of French Polynesia (Pacific Islands): drivers of plant extinction or coup de grace species? *Biological Invasions* **11**, 1569–1585. doi:10.1007/s10530-008-9407-y

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. (Island Press, Washington, DC.)

Miller, R. R., Minckley, W. L., and Norris, S. M. (2005). 'Freshwater fishes of Mexico'. (University of Chicago Press: Chicago.)

Mooney, A., Conde, D. A., Healy, K., and Buckley, Y. M. (2020). A system wide approach to managing zoo collections for visitor attendance and *in situ* conservation. *Nature Communications* **11**, 1–8. doi:10.1038/s41467-020-14303-2

Moss, A., and Esson, M. (2010). Visitor interest in zoo animals and the implications for collection planning and zoo education programmes. *Zoo Biology* **29**, 715–731. doi:10.1002/zoo.20316

Mounce, R., Rivers, M., Sharrock, S., Smith, P., and Brockington, S. (2018). Comparing and contrasting threat assessments of plant species at the global and sub-global level. *Biodiversity and Conservation* **27**, 907–930. doi:10.1007/s10531-017-1472-z

Murashige, T., and Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum* **15**, 473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

Murton, R. K., and Kear, J. (1978). Photoperiodism in waterfowl: phasing of breeding cycles and zoogeography. *Journal of Zoology* **186**, 243–283. doi:10.1111/j.1469-7998.1978.tb03368.x

Muzylowski, J. (2019). Vancouver Aquarium agrees to cetacean ban in new 35-year lease agreement. *CBC News*. Available at: https://www.cbc.ca/news/canada/british-columbia/vancouver-aquarium-drops-cetacean-ban-lawsuit-against-vancouver-park-board-1.5189507 [accessed 19 August 2020]

Nagendra, U., Nyberg, B. and Wood, K. 2020. *Melicope paniculata*. The IUCN Red List of Threatened Species 2020: e.T33675A83803153. Available at:

https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T33675A83803153.en. [accessed 7 August 2020]

Naughton-Treves, L., Holland, M. B., and Brandon, K. (2005). The Role of Protected Areas in Conserving Biodiversity and Sustaining Local Livelihoods. *Annual Review of Environment and Resources* **30**, 219–252. doi:10.1146/annurev.energy.30.050504.164507

Newhouse, A. E., Polin-McGuigan, L. D., Baier, K. A., Valletta, K. E. R., Rottmann, W. H., Tschaplinski, T. J., Maynard, C. A., and Powell, W. A. (2014). Transgenic american chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. *Plant Science* **228**, 88–97. doi:10.1016/j.plantsci.2014.04.004

Nielsen, A. M. W., Nielsen, S. S., King, C. E., and Bertelsen, M. F. (2010). Classification and Prevalence of Foot Lesions in Captive Flamingos (*Phoenicopteridae*). *Journal of Zoo and Wildlife Medicine* **41**, 44–49. doi:10.1638/2009-0095.1

Novak, B. (2018). De-Extinction. *Genes* **9**, 548. doi:10.3390/genes9110548

NYBG (2020a). C. V. Starr Virtual Herbarium - The New York Botanical Garden (NYBG) William and Lynda Steere Herbarium. Available at: http://sweetgum.nybg.org/science/vh/ [accessed 14 April 2020]

NYBG (2020b). DNA Bank - The New York Botanical Garden (NYBG) William and Lynda Steere Herbarium. Available at: http://sweetgum.nybg.org/science/dna-bank/ [accessed 14 April 2020]

O'Donnell, K., and Sharrock, S. (2017). The contribution of botanic gardens to *ex situ* conservation through seed banking. *Plant Diversity* **39**, 373–378. doi:10.1016/j.pld.2017.11.005

Oberski, D. (2014). lavaan.survey: An R Package for Complex Survey Analysis of Structural Equation Models. *Journal of Statistical Software* **57**, 1–27. Available at: http://www.jstatsoft.org/v57/i01/.

Oberwemmer, F., Bingaman Lackey, L., and Gusset, M. (2011). Which Species Have a Studbook and How Threatened Are They? *WAZA Magazine 12*, 34–36.

Ogden, L. E. (2014). Extinction is forever... Or is it? *BioScience* **64**, 469–475. doi:10.1093/biosci/biu063

Ogilvie, M. A., and Ogilvie, C. (1986). 'Flamingos'. (Alan Sutton Publishing: Cloucester.)

Okumoto, H. (2001). Establishment of Three Cell Lines Derived from Frog Melanophores. *Zoological Science* **18**, 483–496. doi:10.2108/zsj.18.483

Oo, K. T., Maung, M., Honorary, H., San, N. N., and Htwe, M. (2018). In vitro Regeneration of Sugarcane (*Saccharum officinarum*) Varieties GUI 11 and PMA 96/48. *Journal of Scientific and Innovative Research* **7**, 7–11.

Palmer, C., Kasperbauer, T. J., and Sandøe, P. (2018). Bears or Butterflies? How Should Zoos Make Value-Driven Decisions about Their Collections? In 'The Ark and Beyond: The Evolution of Zoo and Aquarium Conservation'. (Eds B. A. Minteer, J. Maienschein, and J. P. Collins.) pp. 179–191. (University of Chicago Press: Chicago.)

Palmes, P. (1981). The riddle of the fickle flamingo. *Wildfowl World*, 16–18.

Pearson, E. L., Lowry, R., Dorrian, J., and Litchfield, C. A. (2014). Evaluating the conservation impact of an innovative zoo-based educational campaign: 'Don't Palm Us Off' for orang-utan conservation. *Zoo Biology* **33**, 184–196. doi:10.1002/zoo.21120

Penning, M., Reid, G. McG., Koldewey, H., Dick, G., Andrews, B., Arai, K., Garratt, P., Gendron, S., Lange, J., Tanner, K., Tonge, S., Van den Sande, P., Warmolts, D. and Gibson, C. (Eds.) (2009). 'Turning the Tide: A Global Aquarium Strategy for Conservation and Sustainability'. (World Association of Zoos and Aquariums (WAZA): Bern, Switzerland).

PEPP (Hawai'i State Plant Extinction Prevention Program) (2017). PEP Species List, as of March 15, 2017. Hawai'i State Plant Extinction Prevention Program, Hilo.

Perry, J. (2005). Reproduction. In 'Flamingo husbandry guidelines; a joint effort of the AZA and EAZA in cooperation with WWT'. (Eds C. Brown and C. King.) pp. 68–108.

Peterson, K. (2016). Conservation hopes live at San Diego's Frozen Zoo. *The San Diego Union-Tribune*. Available at: https://www.sandiegouniontribune.com/news/zoo/sd-me-frozen-zoo-20160921-story.html [accessed 22 April 2020]

Pickering, S., Creighton, E., and Stevens-Wood, B. (1992). Flock size and breeding success in flamingos. *Zoo Biology* **11**, 229–234. doi:10.1002/zoo.1430110402

Pickering, S. P. C. (1992). The comparative breeding biology of flamingos *Phoenicopteridae* at The Wildfowl and Wetlands Trust Centre, Slimbridge. *International Zoo Yearbook* **31**, 139–146. doi:10.1111/j.1748-1090.1991.tb02377.x

Pimm, S. L., Dollar, L., and Bass, O. L. (2006). The genetic rescue of the Florida panther. *Animal Conservation* **9**, 115–122. doi:10.1111/j.1469-1795.2005.00010.x

Powell, D. M., Dorsey, C. L., and Faust, L. J. (2019). Advancing the science behind animal program sustainability: An overview of the special issue. *Zoo Biology* **38**, 5–11. doi:10.1002/zoo.21474

Pradel, R., Choquet, R., and Béchet, A. (2012). Breeding Experience Might Be a Major Determinant of Breeding Probability in Long-Lived Species: The Case of the Greater Flamingo Ed S. Descamps. *PLoS ONE* **7**, e51016. doi:10.1371/journal.pone.0051016

Praxedes, É. A., Borges, A. A., Santos, M. V. O., and Pereira, A. F. (2018). Use of somatic cell banks in the conservation of wild felids. *Zoo Biology* **37**, 258–263. doi:10.1002/zoo.21416

Pritchard, D. J., Fa, J. E., Oldfield, S., and Harrop, S. R. (2012). Bring the captive closer to the wild: redefining the role of *ex situ* conservation. *Oryx* **46**, 18–23. doi:10.1017/S0030605310001766

Puan, C. L., and Zakaria, M. (2007). Perception of visitors towards the role of zoos: a Malaysian perspective. *International Zoo Yearbook* **41**, 226–232. doi:10.1111/j.1748-1090.2007.00009.x

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RGB Kew (1999). Survey of Economic Plants for Arid and Semi-Arid Lands (SEPASAL) database. Available at: http://apps.kew.org/sepasalweb/sepaweb [accessed 14 April 2020]

RBG Kew (2020a). DNA Bank, Royal Botanic Gardens, Kew. Available at: https://www.kew.org/data/dnaBank/ [accessed 14 April 2020]

RBG Kew (2020b). Economic Botany Collection Database, Royal Botanic Gardens, Kew. Available at: https://www.kew.org/science/collections-and-resources/collections/economic-botany-collection [accessed 14 April 2020]

RBG Kew (2020c). Medicinal Plant Names Services (MPNS) Portal, Royal Botanic Gardens, Kew. Available at: https://www.kew.org/science/data-and-resources/tools-and-services/medicinal-plant-names-services [accessed 14 April 2020]

RBG Kew (2020d). Millennium Seed Bank, Royal Botanic Gardens, Kew. Available at: https://www.kew.org/wakehurst/whats-at-wakehurst/millennium-seed-bank [accessed 9 August 2020]

RBG Kew (2020e). The Herbarium Catalogue, Royal Botanic Gardens, Kew. Available at: http://www.kew.org/herbcat [accessed 14 April 2020]

Reed, D. H., and Frankham, R. (2003). Correlation between Fitness and Genetic Diversity. *Conservation Biology* **17**, 230–237. doi:10.1046/j.1523-1739.2003.01236.x

Reilly, S. B. (1992). Population Biology and Status Of Eastern Pacific Gray Whales: Recent Developments. In ‘Wildlife 2001: Populations’. pp. 1062–1074. (Springer Netherlands: Dordrecht, Netherlands.) doi:10.1007/978-94-011-2868-1_81

Richard, H. (2020). Solving Aquatic Sustainability - An Update on the Aquatic Collections Sustainability Committee. *Connect - Association of Zoos & Aquariums*. Available at: https://www.aza.org/connect-stories/stories/solving-aquatic-sustainability [accessed 19 August 2020]

Ricketts, T. H., Dinerstein, E., Boucher, T., Brooks, T. M., Butchart, S. H. M., Hoffmann, M., Lamoreux, J. F., Morrison, J., Parr, M., Pilgrim, J. D., Rodrigues, A. S. L., Sechrest, W., Wallace, G. E., Berlin, K., Bielby, J., Burgess, N. D., Church, D. R., Cox, N., Knox, D., Loucks, C., Luck, G. W., Master, L. L., Moore, R., Naidoo, R., Ridgely, R., Schatz, G. E., Shire, G., Strand, H., Wettengel, W., and Wikramanayake, E. (2005). Pinpointing and preventing imminent extinctions. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 18497–18501. doi:10.1073/pnas.0509060102

Ripple, W. J., and Beschta, R. L. (2012). Trophic cascades in Yellowstone: The first 15years after wolf reintroduction. *Biological Conservation* **145**, 205–213. doi:10.1016/j.biocon.2011.11.005

Robbins, M. M., Gray, M., Fawcett, K. A., Nutter, F. B., Uwingeli, P., Mburanumwe, I., Kagoda, E., Basabose, A., Stoinski, T. S., Cranfield, M. R., Byamukama, J., Spelman, L. H., and Robbins, A. M. (2011). Extreme Conservation Leads to Recovery of the Virunga Mountain Gorillas Ed W. M. Getz. *PLoS ONE* **6**, e19788. doi:10.1371/journal.pone.0019788

Robeck, T. R., Willis, K., Scarpuzzi, M. R., and O'Brien, J. K. (2015). Comparisons of Life-History Parameters between Free-Ranging and Captive Killer Whale (*Orcinus orca*) Populations for Application Toward Species Management. *Journal of Mammalogy* **96**, 1055–1070. doi:10.1093/jmammal/gyv113

Rodrigues, A. S. L., Pilgrim, J. D., Lamoreux, J. F., Hoffmann, M., and Brooks, T. M. (2006). The value of the IUCN Red List for conservation. *Trends in Ecology and Evolution* **21**, 71–76. doi:10.1016/j.tree.2005.10.010

Roe, D. (2008). Trading Nature: A Report, with Case Studies, on the Contribution of Wildlife Trade Management to Sustainable Livelihoods and the Millennium Development Goals. Cambridge, UK; Gland, Switzerland.

Roe, K., McConney, A., and Mansfield, C. F. (2014). The Role of Zoos in Modern Society—A Comparison of Zoos' Reported Priorities and What Visitors Believe They Should Be. *Anthrozoös* **27**, 529–541. doi:10.2752/089279314X14072268687808

Rooth, J. (1965). The flamingos on Bonaire, Netherlands Antilles: habitat, diet and reproduction of *Phoenicopterus Ruber ruber* | IUCN Library System. *Uitgaven Natuurwetenschappelijke Studiekring voor Suriname en de Nederlandse Antillen* **41**, 1–151. Available at: https://portals.iucn.org/library/node/24748 [accessed 19 May 2020]

Rose, P., Brereton, J. E., and Gardner, L. (2016). Developing flamingo husbandry practices through workshop communication. *Journal of Zoo and Aquarium Research* **4**, 115–121. doi:10.19227/jzar.v4i2.109

Rose, P. E., and Croft, D. P. (2020). Evaluating the social networks of four flocks of captive flamingos over a five-year period: Temporal, environmental, group and health influences on assortment. *Behavioural Processes* **175**, 104–118. doi:10.1016/j.beproc.2020.104118

Rose, P. E., Croft, D. P., and Lee, R. (2014). A review of captive flamingo (*Phoenicopteridae*) welfare: a synthesis of current knowledge and future directions. *International Zoo*

Yearbook **48**, 139–155. doi:10.1111/izy.12051

Rosen, G. E., and Smith, K. F. (2010). Summarizing the evidence on the international trade in illegal wildlife. *EcoHealth* **7**, 24–32. doi:10.1007/s10393-010-0317-y

Roskov, Y., Abucay, L., Orrell, T., Nicolson, D., Bailly, N., Kirk, P. M., Bourgoin, T., DeWalt, R. E., Decock, W., De Wever, A., Nieukerken, E., van Zarucchi, J., and Penev, L. (2019). Species 2000 & ITIS Catalogue of Life, 2015 Annual Checklist. Available at: www.catalogueoflife.org/annual-checklist/2018 (Naturalis, Leiden, the Netherlands)

Rosseel, Y. (2012). lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software* **48**, 1–36. Available at: http://www.jstatsoft.org/v48/i02/.

Ryan, C., and Saward, J. (2004). The zoo as ecotourism attraction - visitor reactions, perceptions and management implications: The case of hamilton zoo, New Zealand. *Journal of Sustainable Tourism* **12**, 245–266. doi:10.1080/09669580408667236

Ryder, O. A., McLaren, A., Brenner, S., Zhang, Y. P., and Benirschke, K. (2000). DNA banks for endangered animal species. *Science* **288**, 275–277. doi:10.1126/science.288.5464.275

Ryder, O. A., and Onuma, M. (2018). Viable Cell Culture Banking for Biodiversity Characterization and Conservation. *Annual Review of Animal Biosciences* **6**, 83–98. doi:10.1146/annurev-animal-030117-014556

Sandri, C., Sammarini, C., Regaiolli, B., Spiezio, C., and Piccirillo, A. (2018). Reproduction and monogamy in captive flock of greater flamingos (*Phoenicopterus Roseus*). *Journal of Applied Animal Welfare Science* **21**, 256–266. doi:10.1080/10888705.2017.1404466

Le Saout, S., Hoffmann, M., Shi, Y., Hughes, A., Bernard, C., Brooks, T. M., Bertzky, B., Butchart, S. H. M., Stuart, S. N., Badman, T., and Rodrigues, A. S. L. (2013). Protected areas and effective biodiversity conservation. *Science* **342**, 803–805. doi:10.1126/science.1239268

Sarasan, V., Buord, S., Pellicer, J., Sanchez, M., Cowan, R. S., and Lesouëf, J. Y. (2016). Approaches to develop a road map for the long-term conservation of an island endemic genus *Cylindrocline*. *Acta Physiologiae Plantarum* **38**, 1–10. doi:10.1007/s11738-015-2030-5

SCBD (2010). COP-10 Decision X/2. Secretariat of the convention on biological diversity. https://www.cbd.int/decision/cop/?id=12268

Schultz, J. G. W., and Joordens, S. (2014). The effect of visitor motivation on the success of environmental education at the Toronto Zoo. *Environmental Education Research* **20**, 753–775. doi:10.1080/13504622.2013.843646

SDZG (2020). Frozen Zoo® | San Diego Zoo Institute for Conservation Research. Available at: https://institute.sandiegozoo.org/resources/frozen-zoo® [accessed 13 August 2020]

SeaWorld (2017). SeaWorld Ends Orca Breeding Program. *SeaWorld Parks & Entertainment*.

Available at: https://seaworldentertainment.com/blog/future/ [accessed 17 August 2020]

Secher, J. O., Liu, Y., Petkov, S., Luo, Y., Li, D., Hall, V. J., Schmidt, M., Callesen, H., Bentzon, J. F., Sørensen, C. B., Freude, K. K., and Hyttel, P. (2017). Evaluation of porcine stem cell competence for somatic cell nuclear transfer and production of cloned animals. *Animal Reproduction Science* **178**, 40–49. doi:10.1016/j.anireprosci.2017.01.007

Seddon, P. J., Moehrenschlager, A., and Ewen, J. (2014). Reintroducing resurrected species: selecting DeExtinction candidates. *Trends in Ecology & Evolution* **29**, 140–147. doi:10.1016/j.tree.2014.01.007

Seddon, P. J., Soorae, P. S., and Launay, F. (2005). Taxonomic bias in reintroduction projects. *Animal Conservation* **8**, 51–58. doi:10.1017/S1367943004001799

Seymoura, D. K., Filiault, D. L., Henry, I. M., Monson-Miller, J., Ravi, M., Pang, A., Comai, L., Chan, S. W. L., and Maloof, J. N. (2012). Rapid creation of Arabidopsis doubled haploid lines for quantitative trait locus mapping. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 4227–4232. doi:10.1073/pnas.1117277109

SGSV (2020). Svalbard Global Seed Vault (SGSV) Data Portal. Available at: http://www.nordgen.org/sgsv [accessed 20 April 2020]

Shannon, P. (1985). Flamingo management at Audubon Park Zoo and the benefits of long-term research. In ‘AAZPA Regional Conference Proceedings’. pp. 226–236

Shannon, P. W. (2000). Social and reproductive relationships of captive Caribbean Flamingos. *Waterbirds: The International Journal of Waterbird Biology* **23**, 173–178. doi:10.2307/1522162

Shapiro, B. (2015a). ‘How to clone a mammoth : the science of de-extinction’. (Princeton University Press: Princeton, New Jersey, United States.)

Shapiro, B. (2015b). Mammoth 2.0: will genome engineering resurrect extinct species? *Genome Biology* **16**, 228. doi:10.1186/s13059-015-0800-4

Shapiro, B. (2017). Pathways to de-extinction: how close can we get to resurrection of an extinct species? Ed P. Seddon. *Functional Ecology* **31**, 996–1002. doi:10.1111/1365-2435.12705

Sherkow, J. S., and Greely, H. T. (2013). What If Extinction Is Not Forever? *Science* **340**, 32–33. doi:10.1126/science.1236965

Shukla, M. R., Jones, A. M. P., Sullivan, J. A., Liu, C., Gosling, S., and Saxena, P. K. (2012). *In vitro* conservation of American elm (*Ulmus americana*): Potential role of auxin metabolism in sustained plant proliferation. *Canadian Journal of Forest Research* **42**, 686–697. doi:10.1139/X2012-022 da Silva, R., Pearce-Kelly, P., Zimmerman, B., Knott, M., Foden, W., and Conde, D. A. (2019). Assessing the conservation potential of fish and corals in aquariums globally. *Journal for Nature Conservation* **48**, 1–11. doi:10.1016/j.jnc.2018.12.001

Simon, M., Hanson, M. B., Murrey, L., Tougaard, J., and Ugarte, F. (2009). From captivity to the wild and back: An attempt to release Keiko the killer whale. *Marine Mammal Science* **25**, 693–705. doi:10.1111/j.1748-7692.2009.00287.x

Smith, K. N., Shaw, J. H., Bettinger, T., Caniglia, B., and Carter, T. (2007). Conservation partnerships between Zoos and Aquariums, federal and state agencies, and nongovernmental organizations. *Zoo Biology* **26**, 471–486. doi:10.1002/zoo.20142

Smith, R. J., Veríssimo, D., Isaac, N. J. B., and Jones, K. E. (2012). Identifying Cinderella species: uncovering mammals with conservation flagship appeal. *Conservation Letters* **5**, 205–212. doi:10.1111/j.1755-263X.2012.00229.x

SNMNH (2020). Smithsonian National Museum of Natural History (SNMNH) Department of Botany Collections. Available at: https://collections.nmnh.si.edu/search/botany/ [accessed 14 April 2020]

Snyder, N. F. R., Derrickson, S. R., Beissenger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., and Miller, B. (1996). Limitations of captive breeding in endangered species recovery. *Conservation Biology* **10**, 338–348.

Soulé, M., Gilpin, M., Conway, W., and Foose, T. (1986). The millenium ark: How long a voyage, how many staterooms, how many passengers? *Zoo Biology* **5**, 101–113. doi:10.1002/zoo.1430050205

Species360 (2019). Species360. Available at: https://www.species360.org/

Spielman, D., Brook, B. W., Briscoe, D. A., and Frankham, R. (2004). Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics* **5**, 439–448. doi:10.1023/B:COGE.0000041030.76598.cd

Sprunt, A. (1975). The caribbean. In 'Flamingos'. (Eds J. Kear and N. Duplaix-Hall.) pp. 65–74. (T & A. D. Poyser: London.)

Staerk, J., Conde, D. A., Siriaroonrat, B., Ryder, O., and Hvilsom, C. (2018). Saving samples, saving species. *Zooquaria*, 8–9.

Stanley Price, M. R. (1989). 'Animal Re-introductions: the Arabian Oryx in Oman'. (Cambridge University Press: Cambridge.) doi:10.2307/3809259

Steeves, T. E., Johnson, J. A., and Hale, M. L. (2017). Maximising evolutionary potential in functional proxies for extinct species: a conservation genetic perspective on de-extinction Ed P. Seddon. *Functional Ecology* **31**, 1032–1040. doi:10.1111/1365-2435.12843

Stevens, E. F. (1991). Flamingo breeding: The role of group displays. *Zoo Biology* **10**, 53–63.

doi:10.1002/zoo.1430100107

Stevens, E. F., Beaumont, J. H., Cusson, E. W., and Fowler, J. (1992). Nesting behavior in a flock of Chilean flamingos. *Zoo Biology* **11**, 209–214. doi:10.1002/zoo.1430110309

Stevens, E. F., and Pickett, C. (1994). Managing the social environments of flamingos for reproductive success. *Zoo Biology* **13**, 501–507. doi:10.1002/zoo.1430130512

Stokstad, E. (2010). Despite progress, biodiversity declines. *Science* **329**, 1272–1273. doi:10.1126/science.329.5997.1272

Stork, N. E. (2010). Re-assessing current extinction rates. *Biodiversity and Conservation* **19**, 357–371. doi:10.1007/s10531-009-9761-9

Stralberg, D., Arseneault, D., Baltzer, J. L., Barber, Q. E., Bayne, E. M., Boulanger, Y., Brown, C. D., Cooke, H. A., Devito, K., Edwards, J., Estevo, C. A., Flynn, N., Frelich, L. E., Hogg, E. H., Johnston, M., Logan, T., Matsuoka, S. M., Moore, P., Morelli, T. L., Morissette, J. L., Nelson, E. A., Nenzén, H., Nielsen, S. E., Parisien, M., Pedlar, J. H., Price, D. T., Schmiegelow, F. K., Slattery, S. M., Sonnentag, O., Thompson, D. K., and Whitman, E. (2020). Climate-change refugia in boreal North America: what, where, and for how long? *Frontiers in Ecology and the Environment* **18**, 261–270. doi:10.1002/fee.2188

Studer-Thiersch, A. (1998). Basle Zoo's veteran flamingos. *Flamingo Specialist Group Newsletter* **8**.

Studer-Thiersch, A. (1975). Basle Zoo. In 'Flamingos'. (Eds J. Kear and N. Duplaix-Hall.) (T & A. D. Poyser: London.)

Studer-Thiersch, A. (2000). What 19 years of observation on captive greater flamingos suggests about adaptations to breeding under irregular conditions. *Waterbirds: The International Journal of Waterbird Biology* **23**, 150–159. doi:10.2307/1522160

Sudhi, K. S. (2012). Rediscovered tree still 'extinct' on IUCN Red List. *The Hindu*. Available at: https://www.thehindu.com/todays-paper/tp-national/tp-newdelhi/rediscovered-tree-still-extinct-on-iucn-red-list/article3567192.ece [accessed 7 August 2020]

Sunquist, M. E., and Sunquist, F. (2001). Changing landscapes: consequences for carnivores. In 'Carnivore Conservation'. (Eds J. L. Gittleman, S. M. Funk, D. Macdonald, and R. K. Wayne.) pp. 399–418. (Cambridge University Press: Cambridge, UK.)

Teare, A. (2014). ISIS and ZIMS: What can we learn from globally shared data? In 'Proceedings of the International Conference on Diseases of Zoo and Wild Animals'. pp. 72–74

Terra-Araujo, M. H., Faria, A. D. D., and Swenson, U. (2016). A Taxonomic Update of Neotropical Pradosia (Sapotaceae, Chrysophylloideae). *Systematic Botany* **41**, 634–650. doi:10.1600/036364416X692389

Thompson, L., and Berens, M. J. (2014). Woodland Park Zoo closing elephant exhibit. *The*

Seattle Times. Available at: https://www.seattletimes.com/seattle-news/woodland-park-zoo-closing-elephant-exhibit/ [accessed 17 August 2020]

Trask, A., Canessa, S., Moehrenschlager, A., Newland, S., Medina, S., and Ewen, J. (2020). Extinct-in-the-wild species' last stand Ed J. Sills. *Science* **369**, 516–516. doi:10.1126/science.abd4560

Traylor-Holzer, K. (2011). Identifying gaps and opportunities for inter-regional *ex situ* species management. *WAZA Magazine 12*, 30–33.

Traylor-Holzer, K., Leus, K., and Bauman, K. (2019). Integrated Collection Assessment and Planning (ICAP) workshop: Helping zoos move toward the One Plan Approach. *Zoo Biology* **38**, 95–105. doi:10.1002/zoo.21478

Tribe, A., and Booth, R. (2003). Assessing the role of zoos in wildlife conservation. *Human Dimensions of Wildlife* **8**, 65–74. doi:10.1080/10871200390180163

Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., and Legendre, F. (2017). Taxonomic bias in biodiversity data and societal preferences. *Scientific Reports* **7**, 1–14. doi:10.1038/s41598-017-09084-6

Tunstall, T., Kock, R., Vahala, J., Diekhans, M., Fiddes, I., Armstrong, J., Paten, B., Ryder, O. A., and Steiner, C. C. (2018). Evaluating recovery potential of the northern white rhinoceros from cryopreserved somatic cells. *Genome Research* **28**, 780–788. doi:10.1101/gr.227603.117

Turley, S. K. (1999). Exploring the future of the traditional UK zoo. *Journal of Vacation Marketing* **5**, 340–355. doi:10.1177/135676679900500404

Turner, D. D. (2017). Biases in the Selection of Candidate Species for De-Extinction. *Ethics, Policy and Environment* **20**, 21–24. doi:10.1080/21550085.2017.1291835

Turner, W. R., Brandon, K., Brooks, T. M., Gascon, C., Gibbs, H. K., Lawrence, K. S., Mittermeier, R. A., and Selig, E. R. (2012). Global Biodiversity Conservation and the Alleviation of Poverty. *BioScience* **62**, 85–92. doi:10.1525/bio.2012.62.1.13

Valdés González, A., Martínez Estévez, L., Ángeles Villeda, M. E., and Ceballos, G. (2020). The extinction of the Catarina pupfish *Megupsilon aporus* and the implications for the conservation of freshwater fish in Mexico. *Oryx* **54**, 154–160. doi:10.1017/S003060531800056X

Vargas, F. H., Barlow, S., Hart, T., Jimenez-Uzcátegui, G., Chavez, J., Naranjo, S., and Macdonald, D. W. (2008). Effects of climate variation on the abundance and distribution of flamingos in the Galápagos Islands. *Journal of Zoology* **276**, 252–265. doi:10.1111/j.1469-7998.2008.00485.x

Viswanathan, M. B., Premkumar, E. H., and Ramesh, N. (2000). Rediscovery of *Wendlandia*

Angustifolia Wight Ex Hook.f. (Rubiaceae), from Tamil Nadu, a Species Presumed Extinct. *The journal of the Bombay Natural History Society* **97**, 311–313.

Wake, D. B., and Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 11466–11473. doi:10.1073/pnas.0801921105

Waller, R. L., and Iluzada, C. L. (2020). Blackfish and SeaWorld: A Case Study in the Framing of a Crisis. *International Journal of Business Communication* **57**, 227–243. doi:10.1177/2329488419884139

Walters, C., Berjak, P., Pammenter, N., Kennedy, K., and Raven, P. (2013). Preservation of recalcitrant seeds. *Science* **339**, 915–916. doi:10.1126/science.1230935

Walters, C., Wesley-Smith, J., Crane, J., Hill, L. M., Chmielarz, P., Pammenter, N. W., and Berjak, P. (2008). Cryopreservation of recalcitrant (i.e. Desiccation-sensitive) seeds. In ‘Plant Cryopreservation: A Practical Guide’. (Ed B. M. Reed.) pp. 465–484. (Springer: New York.) doi:10.1007/978-0-387-72276-4_18

Wang, Q.-L., Tang, H., Wang, M.-Y., Wang, Z.-N., and Yang, Q. (2018). The rediscovery of *Lepisanthes unilocularis* (subgen. *Otophora* ; Sapindaceae), a species endemic to Hainan, China. *Nordic Journal of Botany* **36**, njb-01675. doi:10.1111/njb.01675

Ward, P. I. (2000). Zoo Visitor Preferences: Reply to Balmford. *Conservation Biology* **14**, 1196–1196. doi:10.1046/j.1523-1739.2000.00026.x

Ward, P. I., Mosberger, N., Kistler, C., and Fischer, O. (1998). The Relationship Between Popularity and Body Size in Zoo Animals. *Conservation Biology* **12**, 1408–1411. doi:10.2307/2989861

Watanabe, M. E. (2015). The Nagoya Protocol on Access and Benefit Sharing: International treaty poses challenges for biological collections. *BioScience* **65**, 543–550.

Watson, J. E. M., Dudley, N., Segan, D. B., and Hockings, M. (2014). The performance and potential of protected areas. *Nature* **515**, 67–73. doi:10.1038/nature13947

WAZA (2020a). Global Species Management Plans. Available at: https://www.waza.org/priorities/conservation/conservation-breeding-programmes/global-species-management-plans/ [accessed 3 June 2020]

WAZA (2020b). Zoo and Aquarium Emergency Operating Funds. *World Association of Zoos and Aquariums*. Available at: https://www.waza.org/news/zoo-and-aquarium-emergency-operating-funds/ [accessed 17 August 2020]

Weldon, C., Du Preez, L. H., Hyatt, A. D., Muller, R., and Speare, R. (2004). Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* **10**, 2100–2105. doi:10.3201/eid1012.030804

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., and Tallmon, D. A. (2015). Genetic rescue to the rescue. *Trends in Ecology & Evolution* **30**, 42–49. doi:10.1016/J.TREE.2014.10.009

Whitfield, J. (2002). Mirrors to help birds mate. *Nature*. doi:10.1038/news020318-2

Whitworth, A. W. (2012). An Investigation into the Determining Factors of Zoo Visitor Attendances in UK Zoos Ed J. Clifton. *PLoS ONE* **7**, e29839. doi:10.1371/journal.pone.0029839

Wickham, H. (2016). ggplot2: elegant graphics for data analysis. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* **174**, 245–246. doi:10.1007/978-3-319-24277-4

Wildt, D. E. (1997). Genome resources banking: Impact on biotic conservation and society. In 'Reproductive Tissue Banking: Scientific Principles'. (Eds A. M. Karow and J. K. Critser.) pp. 399–439. (Academic Press: San Diego.)

Wildt, D. E., Bush, M., Goodrowe, K. L., Packer, C., Pusey, A. E., Brown, J. L., Joslin, P., and O'Brien, S. J. (1987). Reproductive and genetic consequences of founding isolated lion populations. *Nature* **329**, 328–331. doi:10.1038/329328a0

Wilkinson, R. (1989). Breeding and management of flamingos at Chester Zoo. *Avicultural Magazine*, 51–61.

Williams, S. E., Bolitho, E. E., and Fox, S. (2003). Climate change in Australian tropical rainforests: an impending environmental catastrophe. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 1887–1892. doi:10.1098/rspb.2003.2464

Wilmut, I., Beaujean, N., De Sousa, P. A., Dinnyes, A., King, T. J., Paterson, L. A., Wells, D. N., and Young, L. E. (2002). Somatic cell nuclear transfer. *Nature* **419**, 583–587. doi:10.1038/nature01079

Wilson, L., Dorsey, C., and Moore, D. (2019). Challenges and solutions: An analysis of community-reported needs of AZA collaboratively managed animal populations. *Zoo Biology* **38**, 45–54. doi:10.1002/zoo.21467

Wood, J. R., Perry, G. L. W., and Wilmshurst, J. M. (2017). Using palaeoecology to determine baseline ecological requirements and interaction networks for de-extinction candidate species Ed P. Seddon. *Functional Ecology* **31**, 1012–1020. doi:10.1111/1365-2435.12773

Wood, K. R. (2007). Notes on *Melicope cruciata* (A. Heller) T. Hartley & B. Stone (Rutaceae). Kalaheo, Hawaii.

World Bank (2017). World Development Indicators: Population, total. Available at: https://data.worldbank.org/indicator/SP.POP.TOTL

Wright, A. J., Veríssimo, D., Pilfold, K., Ventre, K., Cousins, J., Jefferson, R., Koldewey, H., Llewellyn, F., and McKinley, E. (2015). Competitive outreach in the 21st century: Why we need conservation marketing. *Ocean & Coastal Management* **115**, 41–48.

doi:10.1016/J.OCECOAMAN.2015.06.029

WWF (2018). Living Planet Report - 2018: Aiming Higher. (Eds Grooten, M. and Almond, R.E.A.) (World Wide Fund for Nature (WWF): Gland, Switzerland.)

Wyler, L., and Sheikh, P. (2008). International illegal trade in wildlife: threats and U.S. policy. CRS Report for Congress. 26 pages (Congressional Research Service: Washington, D.C., United States.)

Wyse, S. V., Dickie, J. B., and Willis, K. J. (2018). Seed banking not an option for many threatened plants. *Nature Plants* **4**, 848–850. doi:10.1038/s41477-018-0298-3

Yashina, S., Gubin, S., Maksimovich, S., Yashina, A., Gakhova, E., and Gilichinsky, D. (2012). Regeneration of whole fertile plants from 30,000-y-old fruit tissue buried in Siberian permafrost. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 4008–4013. doi:10.1073/pnas.1118386109

Yon, L., Williams, E., Harvey, N. D., and Asher, L. (2019). Development of a behavioural welfare assessment tool for routine use with captive elephants Ed E. Z. Cameron. *PLOS ONE* **14**, e0210783. doi:10.1371/journal.pone.0210783

Young, R. J. (2015). Are some species just too wild for a happy life in captivity? *The Conversation*. Available at: https://theconversation.com/are-some-species-just-too-wild-for-a-happy-life-in-captivity-50482

Zimkus, B., Hassapakis, C., and Houck, M. (2018). Integrating current methods for the preservation of amphibian genetic resources and viable tissues to achieve best practices for species conservation. *Amphibian & Reptile Conservation* **12**, 1–27. Available at: http://hdl.handle.net/20.500.12634/150 [accessed 21 August 2020]

ZIMS (2019). Species360 zoological information management system. Available at: zims.Species360.org

Appendix A

Supplementary Information to Chapter 2:

A system wide approach to managing zoo collections for visitor attendance and *in situ* conservation

Contents:

Note A.1: Development of *a priori* meta-model

The *a priori* meta-model shown in Appendix Figure A.1 represents the combined prior theoretical knowledge and proposed causal relationships influencing visitor attendance and *in situ* contributions at zoological institutions globally. The development of a theoretical model based on prior knowledge is a key step in Structural Equation Modelling (SEM), as it guides model specification and modification (Grace *et al.* 2010). This meta-model represents general relationships between multiple variables, while omitting statistical details (Grace *et al*. 2010). We consulted the literature pertaining to visitor attendance and *in situ* contributions of zoological institutions globally in order to develop the main theoretical constructs involved and their interconnections. These evidence-based relationships are highlighted in Appendix Figure A.1 with appropriate citations and are further explained below.

Evidence-Based Relationships

Leader-Williams *et al.* (2007) suggest that most zoos are located in and receive more visitors in richer countries, demonstrated by a positive correlation between number of zoos and number of visitors per million people and GDP. As a result, we link GDP and ‘Country’ to ‘Institution Attendance’ in our meta-model. Davey (2007) investigated trends in visitor attendance for zoos in Australia, Germany, Japan, North America, New Zealand and the UK, and how they relate to socio-economic data. It is shown that a significant positive correlation exists between institutional attendance and national population size and between institutional attendance and gross national income (Davey 2007). This study failed to find any correlation between institutional attendance and tertiary education. As a result, we link GDP and ‘National Population Size’ to ‘Institution Attendance’ in our meta-model.

Whitworth (2012) investigated the factors determining visitor attendance to UK zoos. It is shown that visitor numbers are positively correlated with the popularity of institutional collections (in terms of species kept) (Whitworth 2012). It is also shown that ‘rare’ species are more popular than ‘common’ species (Whitworth 2012). We deduce from this that ‘rare’ species are more popular within a collection and that increased collection popularity results in increased visitor attendance. Therefore, we link ‘Threatened Species’ to ‘Institution Attendance’ in our meta-model. This study also highlights the importance of institutional types in determining the composition of collections, for example it is shown that safari parks, although much larger than traditional zoos, contain fewer species relative to their size. Therefore, we link ‘Institution Type’ to ‘Species Richness’ in our meta-model. Although this study found that the popularity of collections (in terms of species kept) is more important in determining visitor attendance than demographic variables, it also shows that institutions are clustered around larger cities with higher populations or areas of high tourism. Therefore, we link ‘Local Population Size’ to ‘Institution Attendance’ in our meta-model.

Dickie *et al*. (2007) provide insight into how institutions link charismatic and endangered species with *in situ* conservation activities. For instance, they highlight the example of the Fossa (*Cryptoprocta ferox*) in European zoos. The Fossa is the largest endemic carnivore on Madagascar, yet is also “*Vulnerable*” according to the IUCN Red List. The Fossa Fund, operated by Zoo Duisburg, has been instrumental in using captive Fossa populations to generate funds for *in situ* conservation and research. Any zoo wishing to acquire Fossa as part of the EAZA Fossa Endangered Species Programme (EEP) must also pay a “*conservation surcharge*” of approximately £1,000 to the Fossa Fund. This ensures *ex situ* institutions are also financially committed to the conservation of the species *in situ*. Similarly, the Congo Gorilla Rainforest exhibit at the Bronx Zoo has been used to generate funding for *in situ* African forest conservation by imposing an additional entry fee (Dickie *et al.* 2007). Between 1999 and 2009, $10.6 million had been raised and expended on African forest wildlife conservation from this source alone (Conway, 2011). As a result of these clear examples linking large, charismatic and threatened species to *in situ* conservation activities, we link ‘Threatened Species’ and ‘Institution Body Mass’ to ‘*In Situ* Project Investment’ in our meta-model.

When looking at the interconnections between the various concepts, Fa *et al.* (2011) proved invaluable. They show that larger zoos hold proportionately larger numbers of individual animals, and that a positive correlation exists between the number of individual animals and overall institutional species richness (Fa *et al.* 2011). These correlations let me link ‘Institution Area’ and ‘Institution Species Richness’ to ‘Number of Animals’ in our meta-model. However, they also note that although a positive correlation exists between the number of individual animals and overall institutional species richness, this rate is not consistent across taxonomic groups, and that the number of individual animals increases at a significantly higher rate for mammals (Fa *et al*. 2011). As a result, we link ‘Collection Taxonomy’ to ‘Number of Animals’ in our meta-model. Furthermore, it is shown that the majority of threatened species with viable population sizes across institutions are mammalian species. This mammalian bias is a constant trend throughout this work, due to the perception that mammalian species are more attractive to the public. Therefore, we link ‘Collection Taxonomy’ to ‘Threatened Species’ in our meta-model.

This mammalian bias is elaborated upon in Frynta *et al.* (2013), who explicitly investigated the influence of body size on the representation of mammals across zoological collections. They show that there is a higher probability of large and attractive mammalian families being kept in zoos (Frynta *et al*. 2013). Additionally, they show that once kept, these large and attractive mammalian species are presented in larger numbers and in more institutions (Frynta *et al*. 2013). This provides further support that taxonomic biases across institutions influence the numbers of animals kept. Based on these findings, we link 'Institution Body Mass' and 'Collection Taxonomy' to 'Number of Animals' in our meta-model.

During our review of the literature we recorded the types and number of variables measured by the various authors. As the individual studies cited in the *a priori* meta-model measured very specific relationships, we simplified their measures into broader themes. For example, Marešová and Frynta (2008) show that mammalian families, once kept in zoos, are presented in larger numbers than non-mammalian families. Therefore, in the *a priori* meta-model we interpret this and broaden it to 'Collection Taxonomy' (mammalian bias in this example) being correlated with the 'Number of Animals'. However, these are general concepts used to help define the *a priori* meta-model and they do not appear in the final model. Rather than create single indicator latent variables, we place the exact variables measured into our final models. So, instead of placing 'Collection Taxonomy' in the final model as a single indicator latent variable, we used the exact measured variable i.e. "*Mammal Species Richness [per institution]*". In this manner we use specific relationships from the literature to define general concepts in the *a priori* meta-model, these general concepts are then represented in the final models by specific measurements once again. This explains why certain variables in the *a priori* meta-model do not appear in the final models (e.g. 'Collection Taxonomy').

Proposed Causal Hypotheses

Although we gained significant insight into various aspects of the system from our literature review, we failed to find any work integrating the various institutional characteristics and socio-economic variables into a study investigating visitor attendance and *in situ* conservation contributions. Although, considerable work has been done on the links between socio-economic variables and visitor attendance, there is a noticeable lack of research on the influence of institutional characteristics (in terms of type and number of species kept) on visitor attendance. This is surprising due to the persistent belief that large, charismatic mammals are necessary to attract visitors; while this concept was found many times in our literature review, it lacks rigorous assessment.

The modelling approach used here was semi-exploratory, similar to Grace *et al.* (2016). Therefore, we combined prior theoretical knowledge with proposed causal hypotheses, capturing all evidence-based relationships and all plausible and suspected predictors of attendance and *in situ* contributions. These plausible direct pathways have no citation in the reported *a priori* meta-model due to an absence of related studies in the literature, however their potential influence was an important consideration. For example, it is repeatedly mentioned in the literature that visitors expect to see large, charismatic mammals, so in the absence of published work to demonstrate this phenomenon, we link 'Collection Taxonomy', 'Mammal Species Richness' and 'Institution Body Mass' to 'Institution Attendance' in our meta-model.

Similarly, given that there is a higher probability of large and attractive mammals being kept in zoos and that they are presented in larger numbers once kept, we also think it is plausible that 'Institution Species Richness' and 'Number of Animals' could both influence 'Institution Attendance'. It may also be expected that 'Collection Diversity' would influence 'Institution Attendance' as Whitworth (2012) has already shown that 'rare' and 'exotic' species are the most popular among visitors. Therefore, we include various metrics (diversity and dissimilarity) among the variables we include during analyses. Finally, it is logical to assume that larger institutions will attract more visitors, so we link 'Institution Area' and 'Institution Type' to 'Institution Attendance' in our meta-model. Unfortunately, the extreme lack of published literature on '*In Situ* Project Investment' meant that the only plausible and defensible link we could add was that from 'Institution Attendance' to '*In Situ* Project Investment', as we assume 'Institution Attendance' is a good proxy for the available funds for potential *in situ* investment.
All of these relationships resulted in the generation of the *a priori* meta-model shown in Appendix Figure A.1. This hypothesised causal diagram was combined with available data to test the effects of institutional compositional characteristics and socio-economic variables on visitor attendance and *in situ* contributions. This meta-model was only the first step in the modelling procedure, guiding original model specification and modifications (Grace *et al*. 2010).

Code Availability

The uncompiled R-markdown code file to reproduce the SEM analyses is available from https://github.com/yvonnebuckley/Zoo-attendance. Please see the this code for further details on how the meta-model was refined into the final models depicted in Figures 2.2, 2.3 and Appendix Figure A.4, using the approach described in Grace *et al.* (2015) and similar to that implemented in Grace *et al.* (2016).

References

CIESIN (2017). Gridded Population of the World, Version 4 (GPWv4): Population Count, Revision 10. Available at: https://doi.org/10.7927/H4PG1PPM

Conway, W. G. (2011). Buying Time for wild animals With Zoos. *Zoo Biology* **30**, 1–8. doi:10.1002/zoo.20352

Davey, G. (2007). An analysis of country, socio-economic and time factors on worldwide zoo attendance during a 40 year period. *International Zoo Yearbook* **41**, 217–225. doi:10.1111/j.1748-1090.2007.00007.x

Dickie, L. A., Bonner, J. P., and West, C. D. (2007). In situ and ex situ conservation: blurring the boundaries between zoos and the wild. In 'Zoos in the 21st Century: Catalysts for Conservation?'. (Eds A. Zimmermann, M. Hatchwell, L. A. Dickie, and C. West.) pp. 220–235. (Cambridge University Press: Cambridge.)

Fa, J. E., Funk, S. M., and OConnell, D. (2011). 'Zoo Conservation Biology'. (Cambridge University Press: Cambridge.) doi:10.1017/CBO9780511993435

Frynta, D., Šimková, O., Lišková, S., and Landová, E. (2013). Mammalian Collection on Noah's Ark: The Effects of Beauty, Brain and Body Size Ed D. L. Roberts. *PLoS ONE* **8**, e63110. doi:10.1371/journal.pone.0063110

Grace, J. B., Anderson, T. M., Olff, H., and Scheiner, S. M. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs* **80**, 67–87. doi:10.1890/09-0464.1

Grace, J. B., Anderson, T. M., Seabloom, E. W., Borer, E. T., Adler, P. B., Harpole, W. S., Hautier, Y., Hillebrand, H., Lind, E. M., Pärtel, M., Bakker, J. D., Buckley, Y. M., Crawley, M. J., Damschen, E. I., Davies, K. F., Fay, P. A., Firn, J., Gruner, D. S., Hector, A., Knops, J. M. H., MacDougall, A. S., Melbourne, B. A., Morgan, J. W., Orrock, J. L., Prober, S. M., and Smith, M. D. (2016). Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature* **529**, 390–393. doi:10.1038/nature16524

Grace, J. B., Scheiner, S. M., and Schoolmaster, D. R. J. (2015). Structural equation modeling: building and evaluating causal models. In 'Ecological Statistics: Contemporary theory and application'. (Eds G. A. Fox, S. Negrete-Yankelevich, and V. J. Sosa.) pp. 168–199. (Oxford University Press: Oxford, UK.)

Hu, L., and Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis:

Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal* **6**, 1–55. doi:10.1080/10705519909540118

Leader-Williams, N., Balmford, A., Linkie, M., Mace, G. M., Smith, R. J., Stevenson, M., Walter, O., West, C., and Zimmermann, A. (2007). Beyond the ark: conservation biologists' views of the achievements of zoos in conservation. In 'Zoos in the 21st century: Catalysts for conservation?'. (Eds A. Zimmermann, M. Hatchwell, L. A. Dickie, and C. West.) pp. 236–254. (Cambridge University Press: Cambridge.)

Marešová, J., and Frynta, D. (2008). Noah's Ark is full of common species attractive to humans: The case of boid snakes in zoos. *Ecological Economics* **64**, 554–558. doi:10.1016/J.ECOLECON.2007.03.012

Whitworth, A. W. (2012). An Investigation into the Determining Factors of Zoo Visitor Attendances in UK Zoos Ed J. Clifton. *PLoS ONE* **7**, e29839. doi:10.1371/journal.pone.0029839

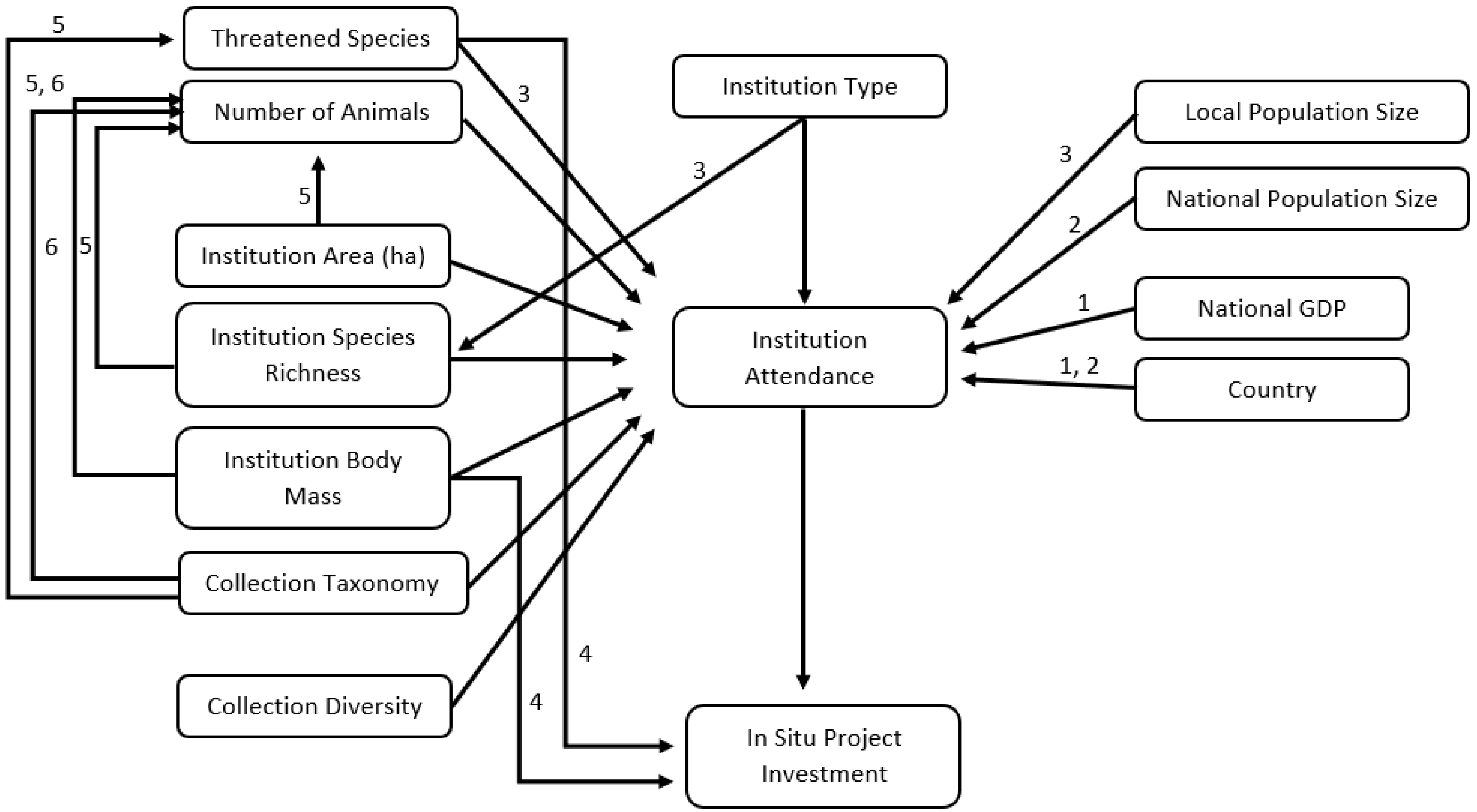


Figure A.1 | The *a priori* SEM framework meta-model, combining both theoretical knowledge and proposed causal hypotheses. Numbers indicate evidence-based relationships from the scientific literature. 1: Leader-Williams *et al*. 2007, 2: Davey 2007, 3: Whitworth 2012, 4: Dickie *et al*. 2007, 5: Fa *et al*. 2011, 6: Frynta *et al*. 2013.

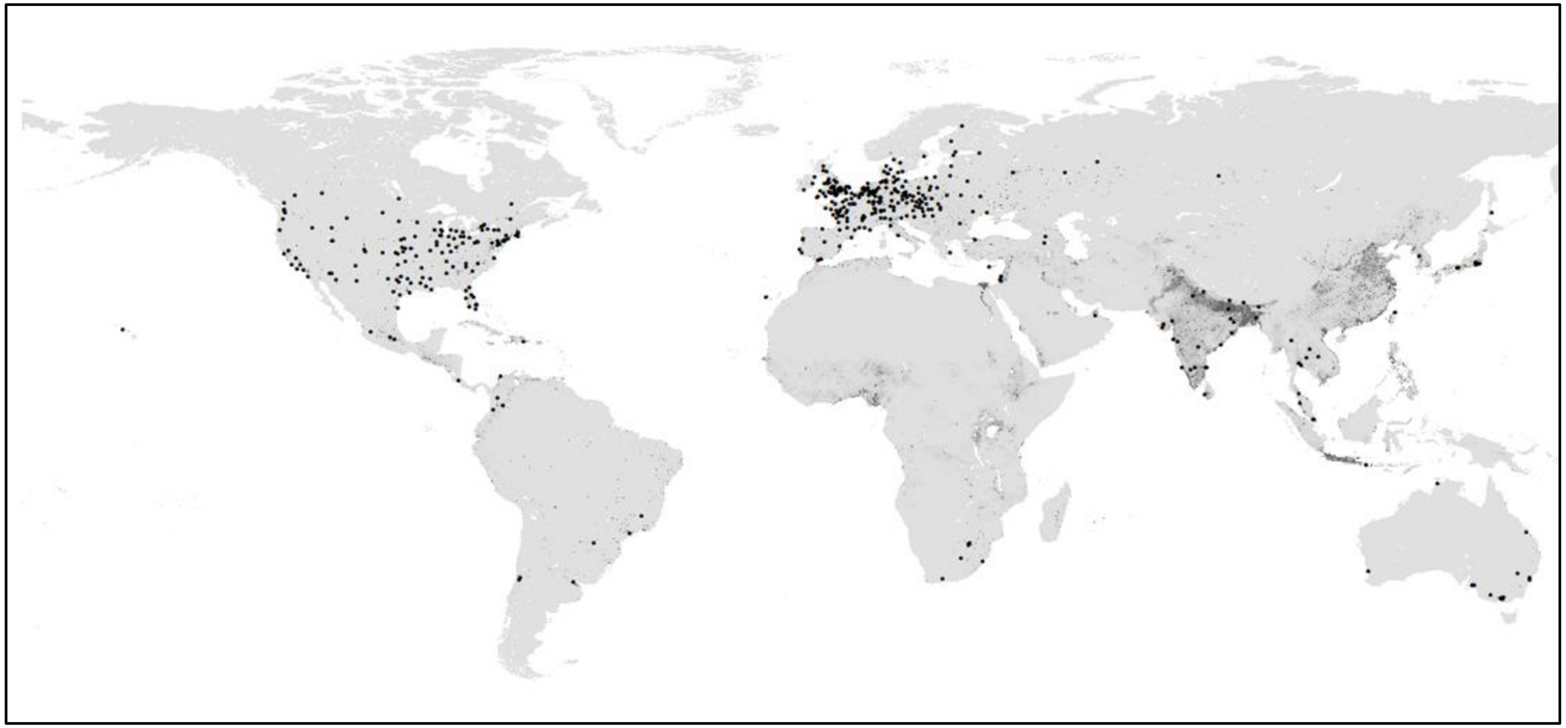


Figure A.2 | The global distribution of the Species360 and IZY member institutions used in this study (n = 458). Black dots represent individual institutions. These are presented on a global map showing population density at a resolution of 1 km (darker meaning more densely populated) (CIESIN 2017).

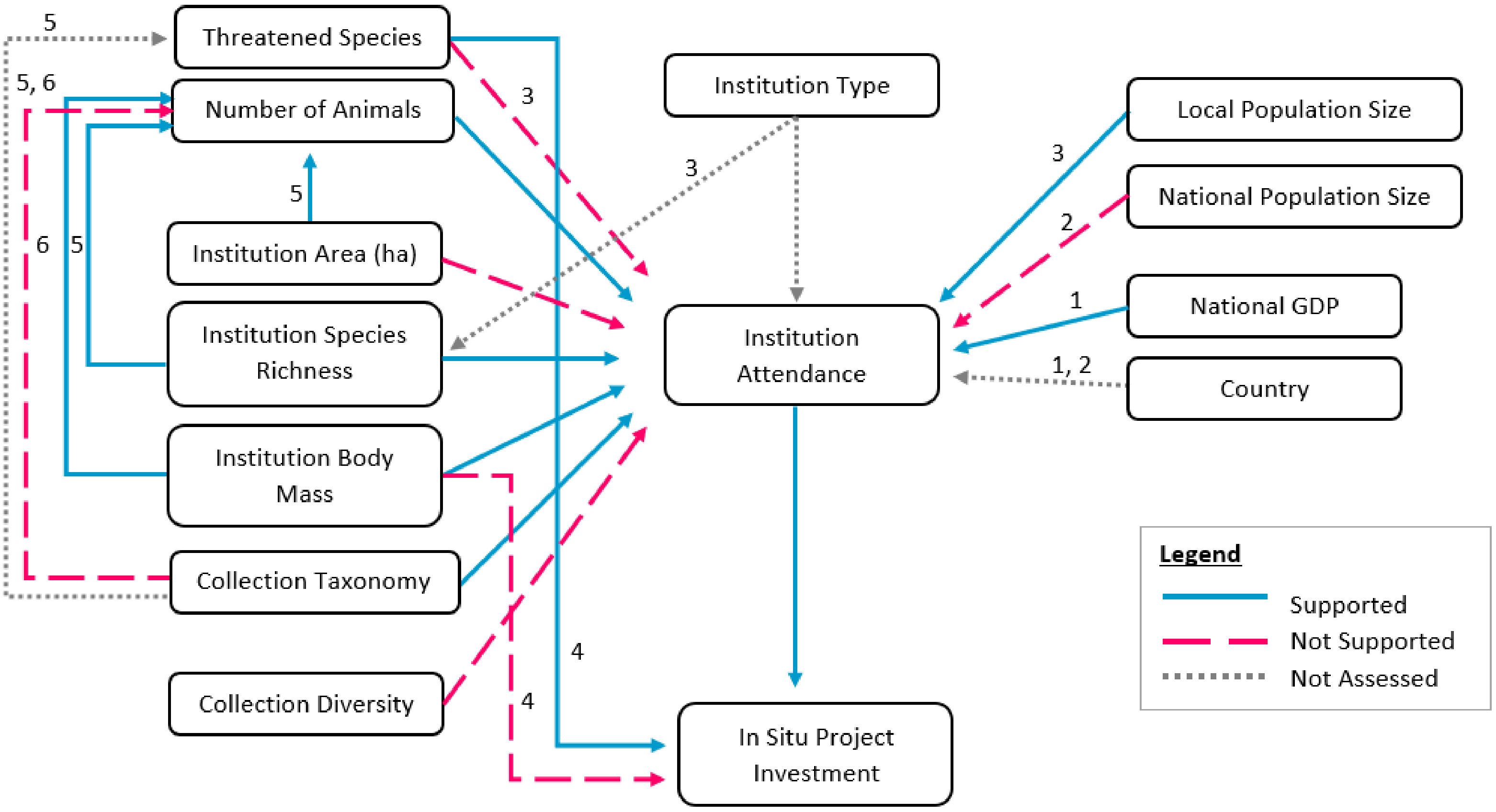


Figure A.3 | The *a priori* SEM framework meta-model updated with the results of this study. Blue lines indicate relationships supported by this study, dashed pink lines indicate relationships for which no support was found, and grey dotted lines indicate relationships that were not assessed. 1: Leader-Williams *et al*. 2007, 2: Davey 2007, 3: Whitworth 2012, 4: Dickie *et al*. 2007, 5: Fa *et al*. 2011, 6: Frynta *et al*. 2013.

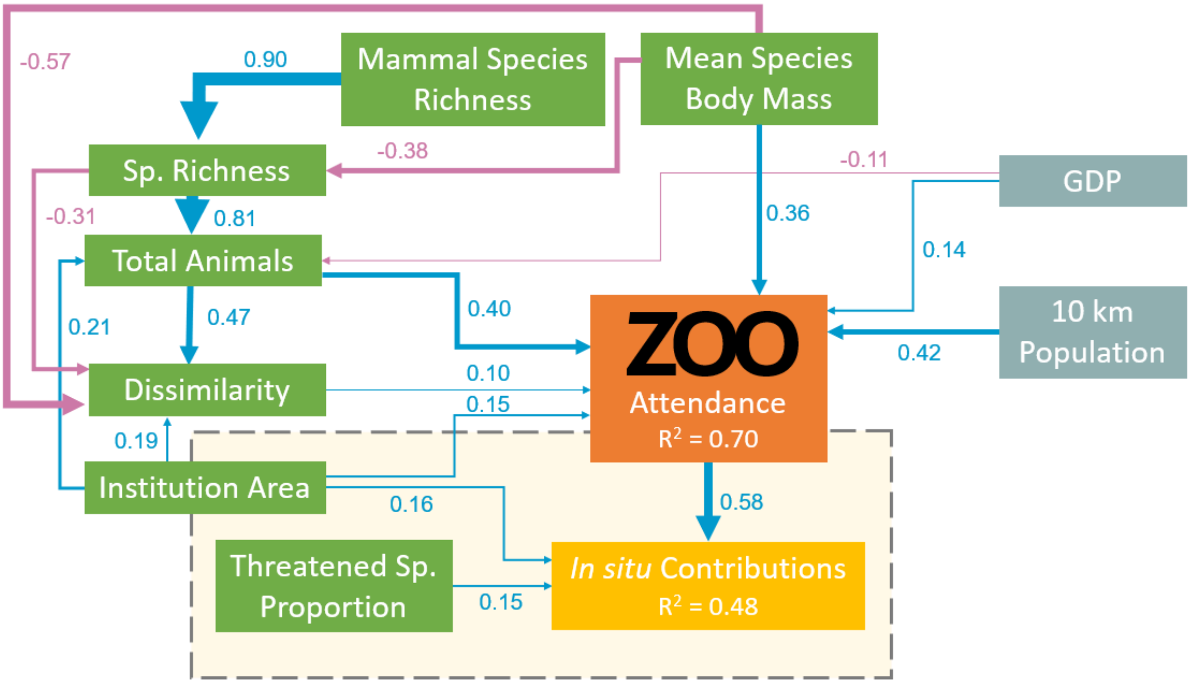


Figure A.4 | The combined Attendance (n = 458) and *In Situ* (n = 119) SEMs representing the connections between institution attendance, *in situ* contributions and various institutional and socio-economic variables. Model results presented reflect species presence-absence models. Path coefficients shown are standardised. The yellow box indicates the additional pathways included in the *In Situ* model. Effect sizes and R^2 for the attendance portion of the figure are derived from the Attendance model, with values for the pathways in the yellow box derived from the *In Situ* model. Blue arrows represent positive effects and pink arrows represent negative effects. Line width represents relative effect sizes. Grey boxes represent socio-economic variables and green boxes represent institutional variables. See Table 2.1 for variable descriptions, Appendix Table A.3 for test statistics and fit indices and Appendix Table A.4 for standardised path coefficients, total effect sizes, significance values and proposed interpretations of causal pathways.

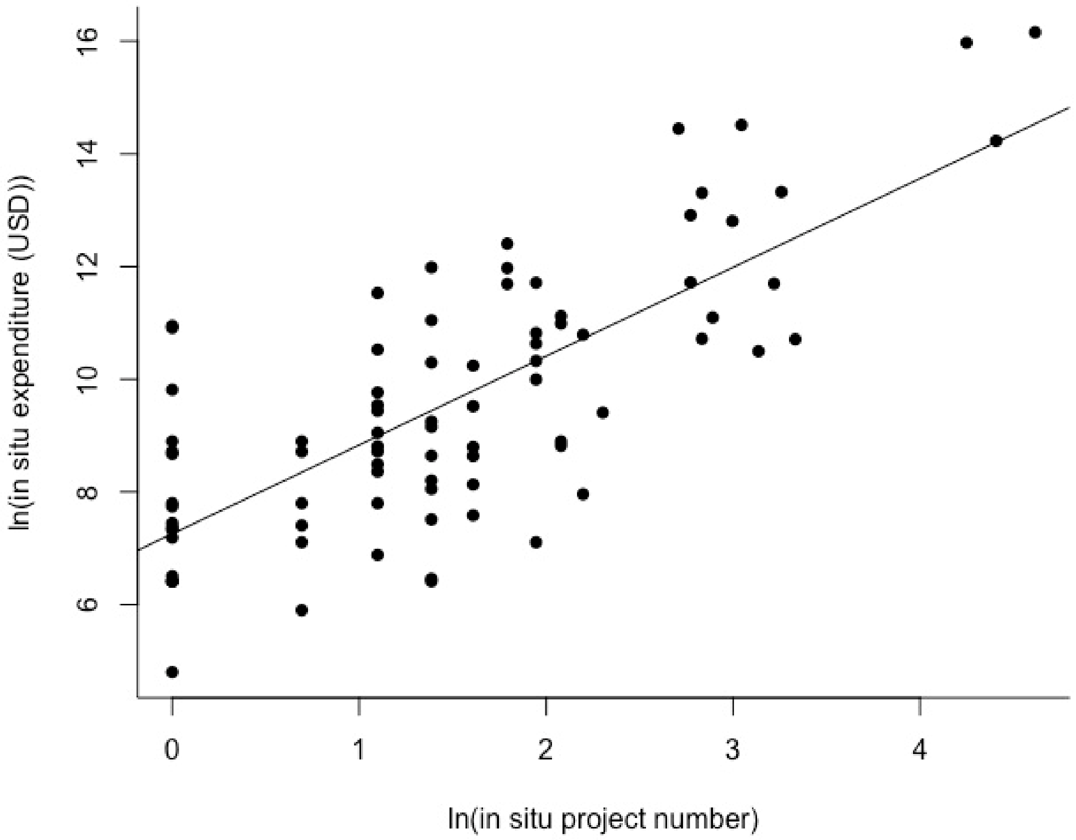


Figure A.5 | The relationship between the number of *in situ* conservation projects supported and the total financial *in situ* conservation expenditure (US Dollars) of 83 individual BIAZA institutions for the year 2018, both variables are natural log transformed. The significant effect of project number ($P < 0.0001$) is shown as the best fit line from a linear regression with an $R^2 = 0.56$.

Table A.1 | AICc values of competing Attendance and *In Situ* models, reflecting both species presence-absence and species abundance adjusted models. Shown are AICc values for the *a priori* meta-models (Model A) and the four top performing models (Models B – E), including the final models selected (Model E).

	Species Presence-Absence		Species Abundance	
	AICc	Parameters	AICc	Parameters
Attendance Model				
Model E (Final Model)	9589.54	43	9575.78	44
Model D	9715.77	42	9574.71	45
Model C	10244.94	50	9589.28	44
Model B	10252.14	49	9595.53	43
Model A (*a priori* meta-model)	14684.32	78	15315.69	88
***In Situ* Model**				
Model E (Final Model)	2672.51	34	2677.51	34
Model D	2671.34	35	2679.70	35
Model C	2683.82	34	2868.82	39
Model B	2686.40	35	2869.63	40
Model A (*a priori* meta-model)	3419.69	66	3447.56	67

Table A.2 | Chi-Squared Statistics and Absolute and Incremental Fit Indices for both the Attendance and *In Situ* models. Model results presented reflect abundance adjusted models. Good model fit ranges determined by Hu and Bentler (1999). Numbers in bold represent values that fall within the good model fit ranges. Adjusted Goodness of Fit Index (AGFI), Root Mean Square Residual (RMR), Standardised Root Mean Square Residual (SRMR), Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Non-Normed Fit Index (NNFI) and Tucker Lewis Index (TLI).

	Absolute Fit Indices					**Incremental Fit Indices**		
Goodness of Fit Measures	χ2 / (df)	AGFI	RMR	SRMR	RMSEA	CFI	NNFI	TLI
Good Model Fit Ranges	< 3.0	> 0.90	< 0.08	< 0.08	< 0.06	> 0.95	> 0.90	> 0.90
Attendance Model	**1.953 (10)**	0.802	**0.053**	**0.048**	0.122	**0.966**	**0.910**	**0.910**
In Situ Model	**2.217 (21)**	0.826	**0.056**	**0.057**	0.101	**0.959**	**0.932**	**0.932**

Table A.3 | Chi-Squared Statistics and Absolute and Incremental Fit Indices for both the Attendance and *In Situ* models. Model results presented reflect species presence-absence models. Good model fit ranges determined by Hu and Bentler (1999). Numbers in bold represent values that fall within the good model fit ranges. Adjusted Goodness of Fit Index (AGFI), Root Mean Square Residual (RMR), Standardised Root Mean Square Residual (SRMR), Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Non-Normed Fit Index (NNFI) and Tucker Lewis Index (TLI).

	Absolute Fit Indices					Incremental Fit Indices		
Goodness of Fit Measures	χ2 / (df)	AGFI	RMR	SRMR	RMSEA	CFI	NNFI	TLI
Good Model Fit Ranges	< 3.0	> 0.90	< 0.05	< 0.08	< 0.08	> 0.95	> 0.90	> 0.90
Attendance Model	**2.665 (11)**	0.787	0.063	**0.053**	0.122	**0.963**	**0.911**	**0.911**
In Situ Model	**2.020 (21)**	0.842	0.058	**0.059**	0.093	**0.965**	**0.942**	**0.942**

Table A.4 | Direct and total standardised effect sizes, R^2 values, standard errors, p-values and proposed interpretations for both the Attendance and *In Situ* models. Relationships are ranked according to direct effect size magnitude. Model results presented reflect species presence-absence models. Only the *in situ* component of the *In Situ* model is reported as all other pathways were analogous to the Attendance model.

	P-Value	**Direct Effect (SE)**	**Total Effect**	**Interpretation**
			Attendance Model	
Attendance (R^2 = 0.702)				
Attendance ~ 10km Population	< 0.001	0.416 (0.039)	0.416	Attendance is positively correlated with the local population size (10 km radius) surrounding an institution
Attendance ~ Total Animals	< 0.001	0.397 (0.035)	0.444	Attendance is positively correlated with total number of animals in an institution
Attendance ~ Body Mass	< 0.001	0.361 (0.048)	0.181	Attendance is positively correlated with the mean species body mass for an institution
Attendance ~ Institution Area	0.005	0.148 (0.053)	0.261	Attendance has a small, but positive correlation with institution area
Attendance ~ GDP	0.001	0.139 (0.042)	0.090	Attendance is positively correlated with national GDP
Attendance ~ Dissimilarity	0.006	0.101 (0.037)	0.101	Attendance is positively correlated with collection dissimilarity
Total Animals (R^2 = 0.772)				
Total Animals ~ Species Richness	< 0.001	0.812 (0.036)	0.812	The total number of animals in an institution is positively correlated with institutional species richness
Total Animals ~ Institution Area	< 0.001	0.208 (0.037)	0.208	The total number of animals in an institution is positively correlated with institutional area
Total Animals ~ GDP	0.155	-0.111 (0.078)	-0.111	The total number of animals in an institution is negatively correlated with National GDP

	P-Value	Direct Effect (SE)	Total Effect	Interpretation
Species Richness ($R^2 = 0.682$)				
Species Richness ~ Mammal Species Richness	< 0.001	0.902 (0.101)	0.902	Institutional species richness is strongly positively correlated with institutional mammal species richness
Species Richness ~ Body Mass	< 0.001	-0.376 (0.057)	-0.376	Institutional species richness is negatively correlated with the mean species body mass of an institution
Dissimilarity ($R^2 = 0.297$)				
Dissimilarity ~ Total Animals	0.001	0.470 (0.140)	0.470	Collection composition dissimilarity is positively correlated with the total number of animals in an institution
Dissimilarity ~ Institution Area	0.027	0.194 (0.087)	0.292	Collection composition dissimilarity is positively correlated with the mean species body mass of an institution
Dissimilarity ~ Species Richness	0.145	-0.313 (0.215)	0.069	Collection composition dissimilarity is negatively correlated with institutional species richness
Dissimilarity ~ Body Mass	< 0.001	-0.569 (0.076)	-0.712	Collection composition dissimilarity is negatively correlated with the mean species body mass of an institution
			***In Situ* Model**	
***In Situ* Contributions ($R^2 = 0.476$)**				
In Situ ~ Attendance	< 0.001	0.576 (0.074)	0.576	Institutional *in situ* contributions are positively correlated with institutional attendance
In Situ ~ Institution Area	0.025	0.156 (0.070)	0.295	Institutional *in situ* contributions are positively correlated with institutional area
In Situ ~ Threatened Species Proportion	0.029	0.149 (0.068)	0.149	Institutional *in situ* contributions are positively correlated with the proportion of threatened species in an institution

Table A.5 | Residual covariances for both the Attendance (n = 458) and *In Situ* (n = 119) models. Results presented reflect species abundance models.

	Estimate	Standard Error	P-Value
Attendance Model			
Mammal Species Richness ~~ Body Mass	0.227	0.078	0.003
Mammal Species Richness ~~ 10km Population	0.284	0.066	< 0.001
Mammal Species Richness ~~ GDP	-0.060	0.064	0.349
Mammal Species Richness ~~ Institution Area	0.381	0.064	< 0.001
Body Mass ~~ 10km Population	0.021	0.064	0.742
Body Mass ~~ GDP	-0.079	0.038	0.040
Body Mass ~~ Institution Area	0.522	0.101	< 0.001
10km Population ~~ GDP	-0.028	0.056	0.613
10km Population ~~ Institution Area	-0.010	0.078	0.901
GDP ~~ Institution Area	-0.027	0.041	0.501

	Estimate	**Standard Error**	**P-Value**
***In Situ* Model**			
Body Mass ~~ 10km Population	-0.209	0.093	0.025
Body Mass ~~ Institution Area	0.527	0.103	< 0.001
Body Mass ~~ Mammal Species Richness	0.219	0.093	0.018
Body Mass ~~ Threatened Species Proportion	0.092	0.091	0.312
10km Population ~~ Institution Area	-0.099	0.091	0.277
10km Population ~~ Mammal Species Richness	0.421	0.099	< 0.001
10km Population ~~ Threatened Species Proportion	0.278	0.094	0.003
Institution Area ~~ Mammal Species Richness	0.536	0.103	< 0.001
Institution Area ~~ Threatened Species Proportion	0.071	0.091	0.438
Mammal Species Richness ~~ Threatened Species Proportion	0.203	0.093	0.028

Table A.6 | Residual covariances for both the Attendance (n = 458) and *In Situ* (n = 119) models. Results presented reflect species presence-absence models.

	Estimate	Standard Error	P-Value
Attendance Model			
Institution Area ~~ Body Mass	0.570	0.136	< 0.001
Institution Area ~~ 10km Population	-0.025	0.083	0.763
Institution Area ~~ GDP	-0.020	0.074	0.788
Institution Area ~~ Mammal Species Richness	0.292	0.087	0.001
Body Mass ~~ 10km Population	0.127	0.104	0.221
Body Mass ~~ GDP	-0.145	0.063	0.021
Body Mass ~~ Mammal Species Richness	0.218	0.119	0.066
10km Population ~~ GDP	-0.119	0.071	0.094
10km Population ~~ Mammal Species Richness	0.258	0.095	0.007
GDP ~~ Mammal Species Richness	-0.057	0.093	0.540

	Estimate	**Standard Error**	**P-Value**
***In Situ* Model**			
Body Mass ~~ 10km Population	-0.023	0.091	0.804
Body Mass ~~ Institution Area	0.560	0.104	< 0.001
Body Mass ~~ Mammal Species Richness	0.373	0.097	< 0.001
Body Mass ~~ Threatened Species Proportion	0.277	0.094	0.003
10km Population ~~ Institution Area	-0.099	0.091	0.277
10km Population ~~ Mammal Species Richness	0.421	0.099	< 0.001
10km Population ~~ Threatened Species Proportion	0.287	0.095	0.002
Institution Area ~~ Mammal Species Richness	0.536	0.103	< 0.001
Institution Area ~~ Threatened Species Proportion	0.189	0.093	0.041
Mammal Species Richness ~~ Threatened Species Proportion	0.322	0.096	0.001

Appendix B

Supplementary Information to Chapter 3:

Flock size and structure influence reproductive success in four species of flamingo in 753 captive populations worldwide

Contents:

Note B.1: Detailed Species-Specific Results

Phoenicopterus chilensis

Probability of Reproduction

Phoenicopterus chilensis shows a significant decrease in the probability of reproduction between 1990 and 2018 ($p < 0.05$, SE = 0.138; Appendix Figure B.11). However, a significant positive relationship was observed between flock size and the probability of reproduction over the same period ($p < 0.05$, SE = 0.321; Figure 3.5a). Our results show that the probability of reproduction increases steadily from flocks of approximately 20 individuals and peaks at approximately 150 individuals. However, the number of institutions with flock sizes of >100 individuals is limited and as a result there is considerable variation observed within the 95% confidence intervals shown. No statistical support was found for the proportion of reproductive females or the proportion of new individuals affecting the probability of reproduction in *Phoenicopterus chilensis*. In addition to the results discussed above, both mean annual precipitation and variation in mean annual temperature had significant negative effects on the probability of *Phoenicopterus chilensis* flocks reproducing ($p < 0.05$, SE = 0.154; Appendix Figure B.12 and $p < 0.05$, SE = 0.162 respectively).

Number of Chicks

Although the probability of reproduction decreased between 1990 and 2018 for *Phoenicopterus chilensis*, the number of chicks per flock increased significantly over the same period ($p < 0.05$, SE = 0.049 Appendix Figure B.13). Similar to the probability of reproduction, flock size has a large positive effect on the predicted number of births per flock, although no peak is observed and instead the number of births continues to increase up to the maximum recorded flock sizes of approximately 160 birds ($p < 0.05$, SE = 0.059, Figure 3.7a). A small positive relationship was found between the proportion of additions in a flock and the number of chicks produced, although this was statistically non-significant ($p > 0.05$, SE = 0.026; Appendix Figure B.14), whereas a significant and much stronger positive relationship was found between the proportion of reproductive females in a flock and the number of chicks produced ($p < 0.05$, SE = 0.053; Appendix Figure B.15), suggesting an even sex ratio is beneficial for successful reproduction in *Phoenicopterus chilensis*. Significant synergistic interactions were found between flock size and both the proportion of additions and proportion of reproductive females per flock. This suggests that the addition of new individuals enhances reproductive output at smaller flock sizes, however as flock size increases the addition of new individuals does not increase the reproductive output ($p < 0.05$, SE = 0.031; Appendix Figure B.16). Conversely, the interaction between flock size and the proportion of reproductive females per flock suggests that the benefit of a balanced sex ratio enhances reproductive output at larger flocks more than smaller flocks ($p < 0.05$, SE = 0.048; Figure 3.9). Like the probability of reproduction, mean annual precipitation had a significant negative effect on the number of chicks produced per flock ($p < 0.05$, SE = 0.065, Appendix Figure B.17) and no statistical support was found for the effect of mean annual temperature or both measures of variation.

Phoeniconaias minor

Probability of Reproduction

Unlike *Phoenicopterus chilensis*, there was no statistical support to show that the probability of reproduction has changed between 1990 and 2018 in *Phoeniconaias minor* flocks. A significant positive relationship was found between flock size and the probability of *Phoeniconaias minor* flocks reproducing between 1990 and 2018 ($p < 0.05$, SE = 0.879; Figure 3.5b). We reveal that the probability of reproduction increases dramatically between flocks of approximately 100 to 150 individuals, at which point the probability of reproduction approaches one, however once again, the number of institutions with flock sizes of more than >100 individuals is limited. In contrast, we found that reproduction is unlikely to occur below flock sizes of approximately 100 individuals. A small, but significant, positive relationship was found between the proportion of new individuals in a flock and the probability of reproduction ($p > 0.05$, SE = 0.348; Appendix Figure B.18). A much stronger positive relationship was found between the proportion of reproductive females per flock and the probability of reproduction ($p < 0.05$, SE = 0.741; Appendix Figure B.19). In addition, a strong interaction between flock size and the proportion of reproductive females per flock ($p < 0.05$, SE = 0.951; Appendix Figure B.20) reveals that an approach toward an even sex ratio greatly enhances the probability of a flock reproducing, particularly at smaller flock sizes. No statistical support was found for the influence of any climatic variables on the probability of *Phoeniconaias minor* flocks reproducing between 1990 and 2018.

Number of Chicks

Similar to *Phoenicopterus chilensis*, the number of chicks per *Phoeniconaias minor* flock increased significantly over the period 1990 to 2018 ($p < 0.05$, SE = 0.204; Appendix Figure B.21). Although non-significant, flock size has an important positive effect on the predicted number of births per flock, however no drastic increase is seen in reproductive output at any flock size and the predicted number of births remains consistently low, however notably large variation can be seen in the 95% confidence intervals presented ($p > 0.05$, SE = 0.677, Figure 3.7b). The proportion of reproductive females per flock has a significant positive effect on the predicted number of births per flock ($p < 0.05$, SE = 0.672) and has a synergistic interaction with flock size where the number of chicks produced at smaller flocks can be increased through an increase in the proportion of reproductive females ($p < 0.05$, SE = 0.706; Appendix Figure B.22). Importantly, these relationships demonstrate that the number of chicks produced per flock is minimal below a flock composed of a balanced sex ratio, although a female bias does not appear to be detrimental to reproductive output. No statistical support was found for the influence of adding new individuals to *Phoeniconaias minor* flocks on the number of chicks produced. Unlike the probability of reproduction, variation in mean annual temperature had a significant positive effect on the number of chicks produced per flock ($p < 0.05$, SE = 0.812, Appendix Figure B.23), however no statistical support was found for the effect of mean annual temperature itself or mean annual precipitation and its measure of variation.

Phoenicopterus roseus

Probability of Reproduction

Like *Phoeniconaias minor*, no statistical support was found to suggest that the probability of reproduction in *Phoenicopterus roseus* flocks changed between 1990 and 2018, although a significant positive relationship was once again found between flock size and the probability of reproduction ($p < 0.05$, SE = 0.366; Figure 3.6a). The probability of reproduction increases dramatically between flocks of approximately 40 to 100 individuals. This relationship plateaus at 120 birds, followed by a sharp decrease in the probability of reproduction at flock sizes greater than 150 birds, although the number of institutions with flock sizes of >100 individuals remains extremely limited and as a result there is considerable variation observed within the 95% confidence intervals shown. A small, but significant, positive relationship was found between the proportion of reproductive females per flock and the probability of reproduction ($p < 0.05$, SE = 0.250; Appendix Figure B.24). Unusually the proportion of new individuals negatively influenced the probability of *Phoenicopterus roseus* flocks reproducing, but this relationship was non-significant ($p > 0.05$, SE = 0.115). Strong synergistic interactions were found between flock size and both the proportion of new individuals ($p < 0.05$, SE = 0.195) and the proportion of reproductive females ($p < 0.05$, SE = 0.373). Like *Phoeniconaias minor*, no significant statistical support was found for the influence of any climatic variables on the probability of *Phoenicopterus roseus* flocks reproducing between 1990 and 2018, however mean annual precipitation and measures of variation in both mean annual temperature and precipitation all show non-significant but important relationships ($p > 0.05$ for all).

Number of Chicks

Similar to both *Phoenicopterus chilensis* and *Phoeniconaias minor*, the number of chicks per *Phoenicopterus roseus* flock increased significantly over the period 1990 to 2018 ($p < 0.05$, SE = 0.075; Appendix Figure B.25). Flock size has a positive effect on the predicted number of births per flock, with the predicted number of chicks increasing steadily up to flocks of approximately 100 individuals, before plateauing and then declining from 120 birds onwards ($p < 0.05$, SE = 0.082; Figure 3.8a). Both the proportion of reproductive females and the proportion of new individuals have significant positive effects on the predicted number of chicks in *Phoenicopterus roseus* flocks ($p < 0.05$, SE = 0.054; Appendix Figure B.26 and $p < 0.05$, SE = 0.024; Appendix Figure B.27, respectively). Similar to the probability of reproduction analysis, no statistical support was found for the influence of any climatic variables on the predicted number of chicks in *Phoenicopterus roseus* flocks between 1990 and 2018.

Phoenicopterus ruber

Probability of Reproduction

Like *Phoenicopterus chilensis*, the probability of reproduction in *Phoenicopterus ruber* flocks decreased significantly over the period 1990 to 2018 ($p < 0.05$, SE = 0.182; Appendix Figure B.28). As expected, flock size had a strong positive effect on the probability of reproduction ($p < 0.05$, SE = 0.218; Figure 3.6b). Similar to *Phoenicopterus chilensis* the probability of reproduction increases steadily from flocks of approximately 20 individuals and peaks at approximately 180 individuals. However, the number of institutions with flock sizes of >100 individuals is limited and as a result there is considerable variation observed within the 95% confidence intervals shown. We found no support to suggest that the addition of new individuals influences the probability of reproduction, however like *Phoenicopterus roseus*, the proportion of reproductive females had a small but significant positive effect ($p < 0.05$, SE = 0.198; Appendix Figure B.29). Similar to *Phoenicopterus chilensis* once again, mean annual precipitation had a significant negative effect on the probability of *Phoenicopterus ruber* flocks reproducing ($p < 0.05$, SE = 0.280; Appendix Figure B.30), but mean annual temperature and measures of variation were not statistically supported.

Number of Chicks

Similar to *Phoenicopterus chilensis, Phoeniconaias minor* and *Phoenicopterus roseus*, the number of chicks per *Phoenicopterus ruber* flock increased significantly between 1990 and 2018 ($p < 0.05$, SE = 0.062; Appendix Figure B.31). Flock size had a positive effect on the predicted number of births per flock, with the predicted number of chicks increasing steadily up to flocks of approximately 150 individuals, however there is considerable variation observed within the 95% confidence intervals shown at larger flock sizes ($p < 0.05$, SE = 0.066; Figure 3.8b). The proportion of new individuals per flock had a significant positive effect on the predicted number of births ($p < 0.05$, SE = 0.018; Appendix Figure B.32) and has a synergistic interaction with flock size whereby the number of chicks produced at smaller flocks can be increased through an increase in the proportion of new individuals added to the flock in the previous year ($p < 0.05$, SE = 0.017; Figure 3.10). No statistical support was found for the influence of the proportion of reproductive females on the predicted number of chicks in *Phoenicopterus ruber* flocks between 1990 and 2018. Unlike any other species of flamingo, mean annual temperature had a significant positive effect on the predicted number of chicks per *Phoenicopterus ruber* flock ($p < 0.05$, SE = 0.089; Appendix Figure B.33), however mean annual precipitation and measures of variation were not statistically supported.

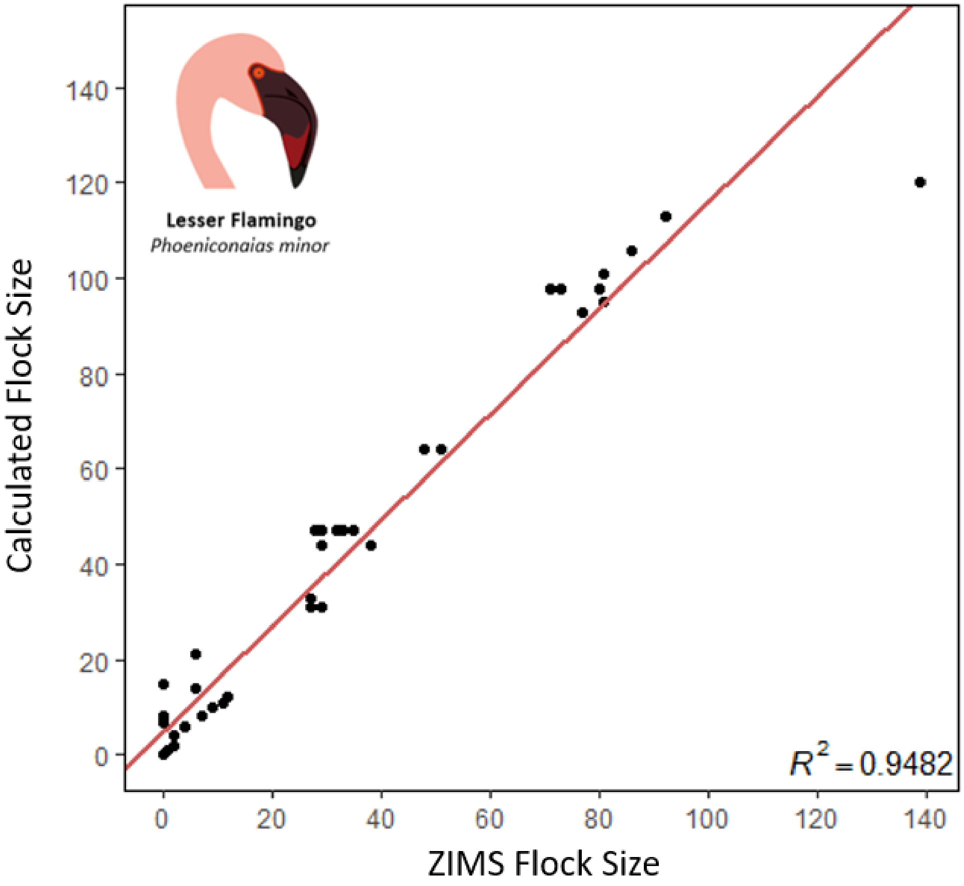


Figure B.1 | The relationship between the calculated flock sizes and those from ZIMS inventory reports for a subset of *Phoeniconaias minor* data (n = 51). The mean difference between flock sizes is 7.71 ± 9.21 birds, with upper and lower 95% confidence intervals of 10.30 and 5.12 respectively.

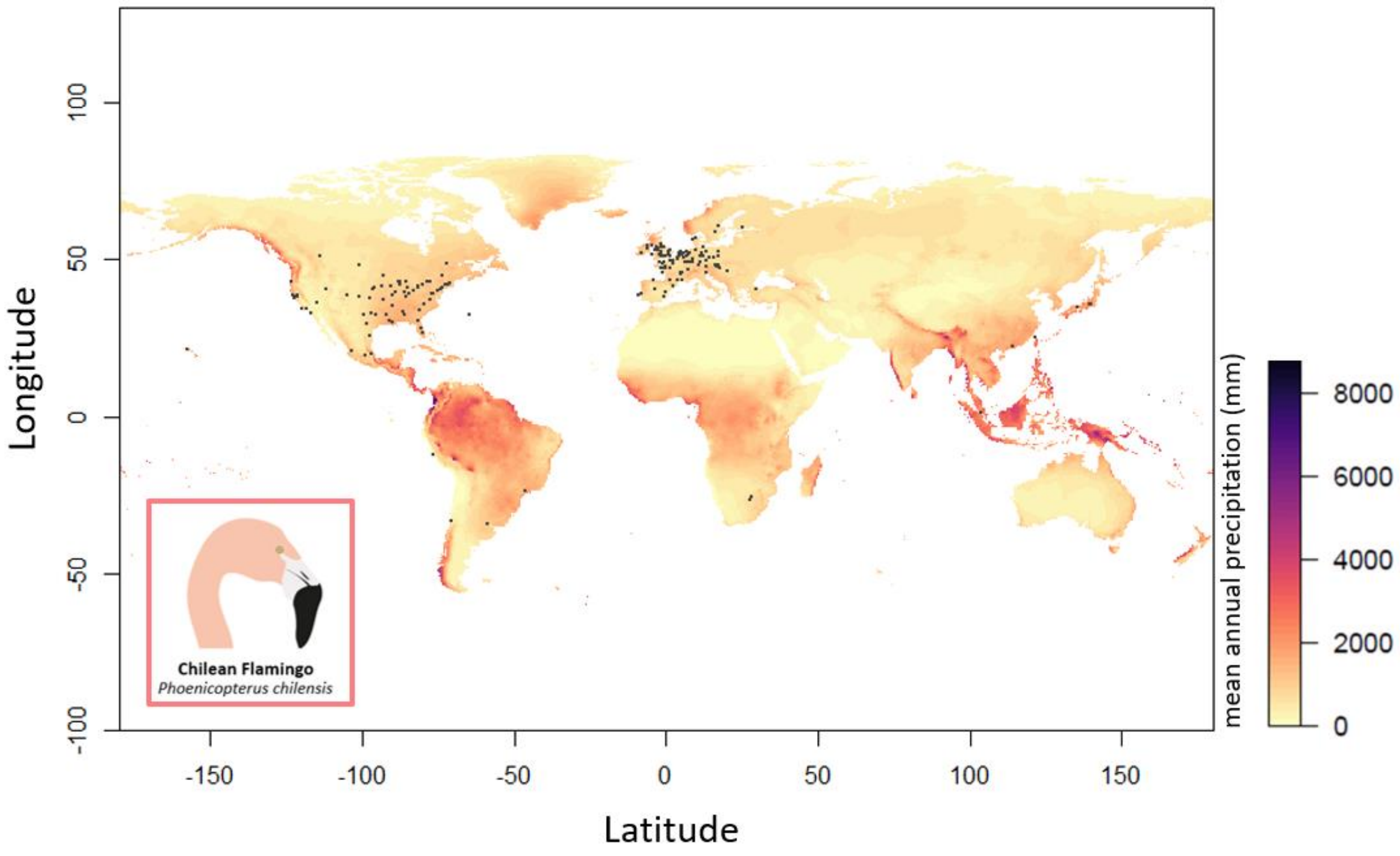


Figure B.2 | The global distribution of current Species360 member institutions which contain flocks of *Phoenicopterus chilensis*. These are presented on a global map showing mean annual precipitation (mm) at a resolution of 1 km (darker meaning higher precipitation).

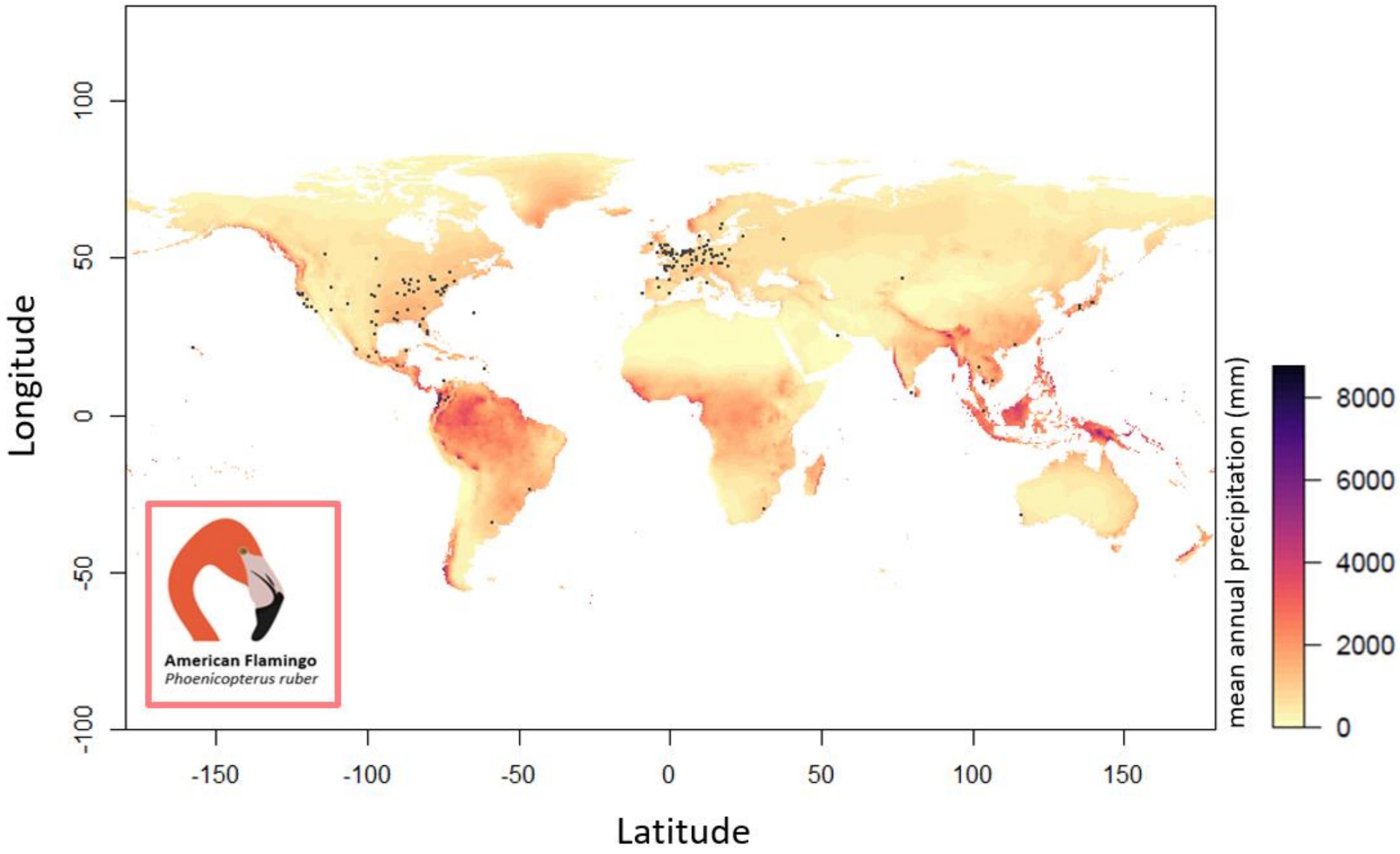


Figure B.3 | The global distribution of current Species360 member institutions which contain flocks of *Phoenicopterus ruber*. These are presented on a global map showing mean annual precipitation (mm) at a resolution of 1 km (darker meaning higher precipitation).

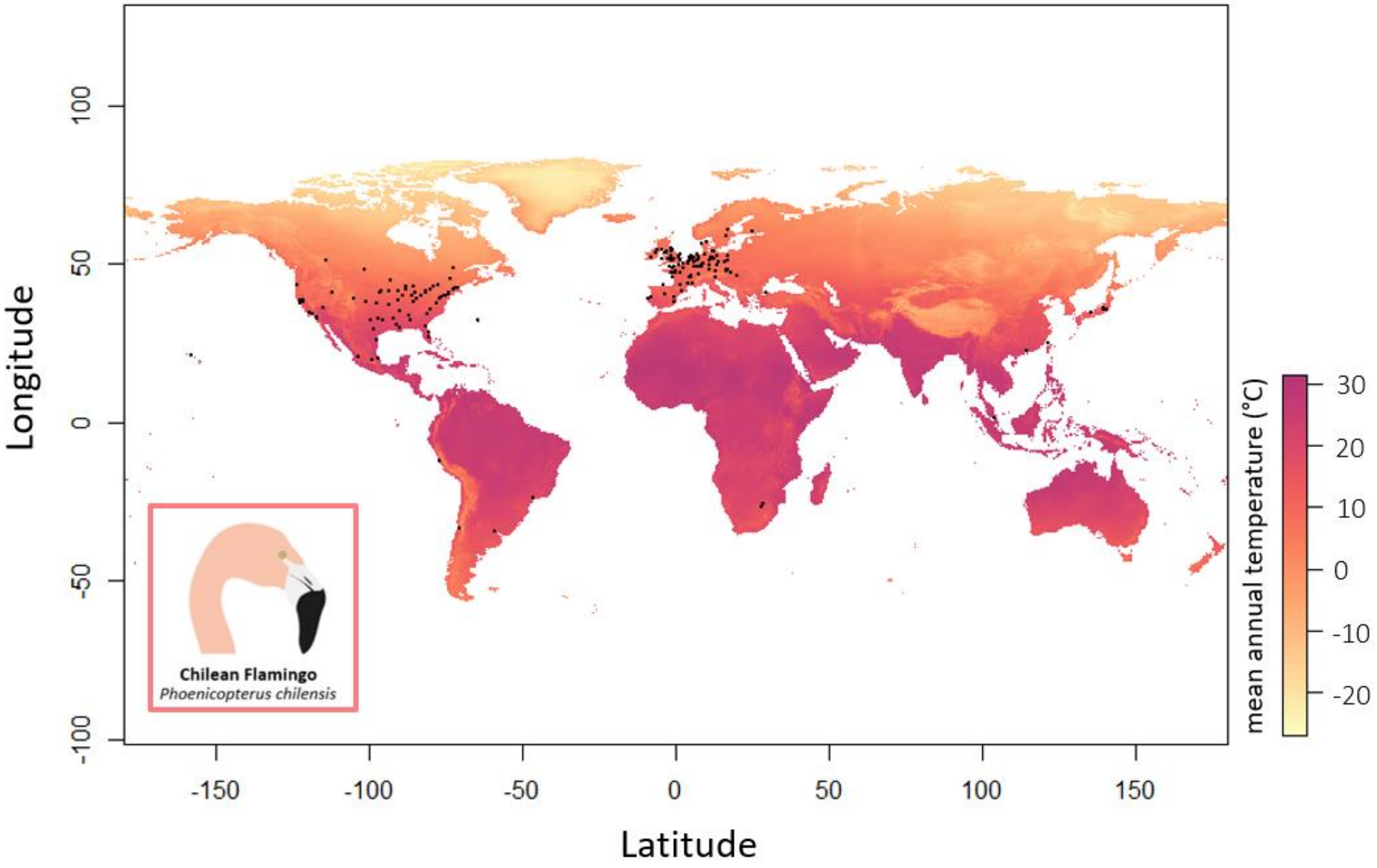


Figure B.4 | The global distribution of current Species360 member institutions which contain flocks of *Phoenicopterus chilensis*. These are presented on a global map showing mean annual temperature (°C) at a resolution of 1 km (darker meaning higher precipitation).

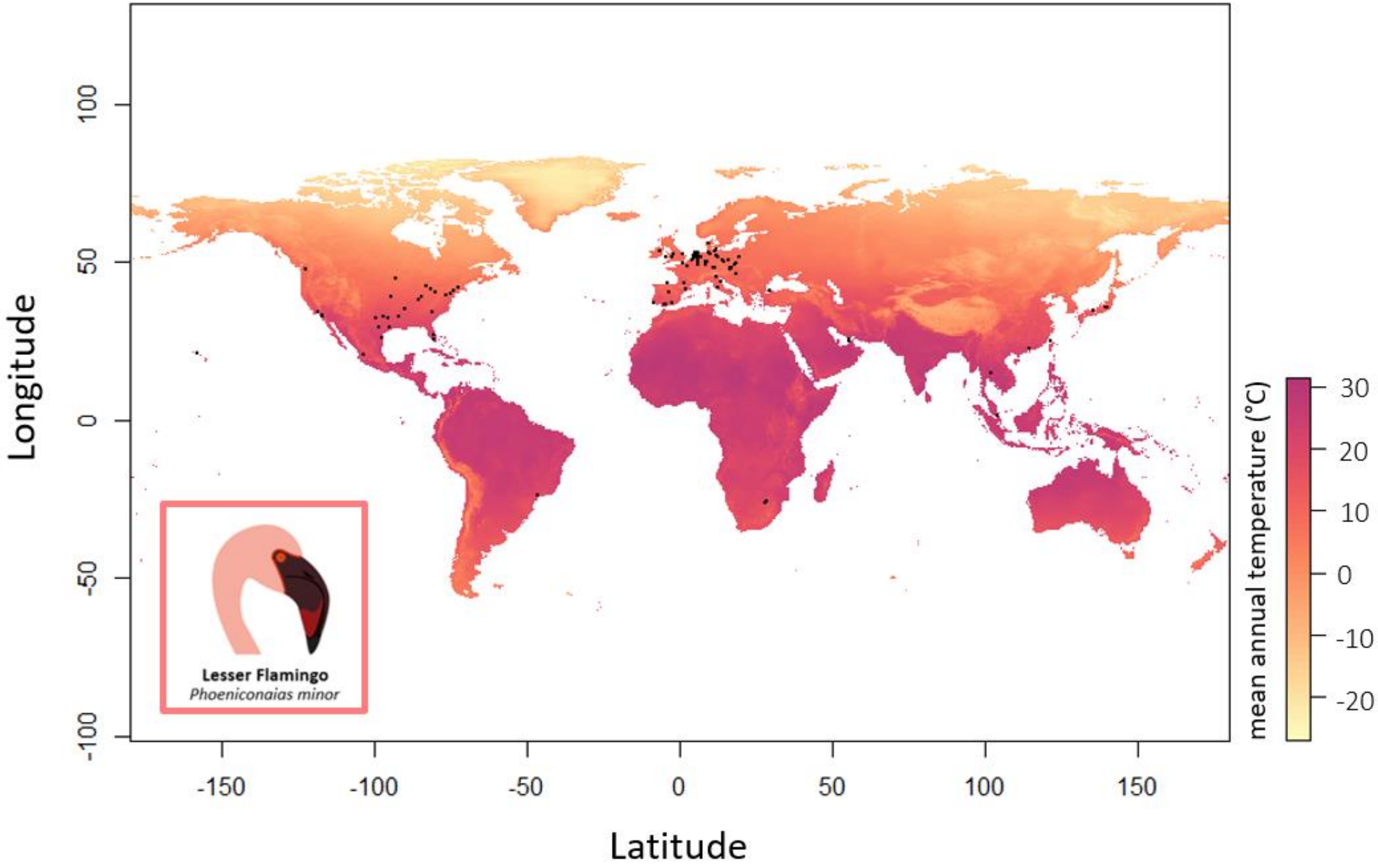


Figure B.5 | The global distribution of current Species360 member institutions which contain flocks of *Phoeniconaias minor*. These are presented on a global map showing mean annual temperature (°C) at a resolution of 1 km (darker meaning higher precipitation).

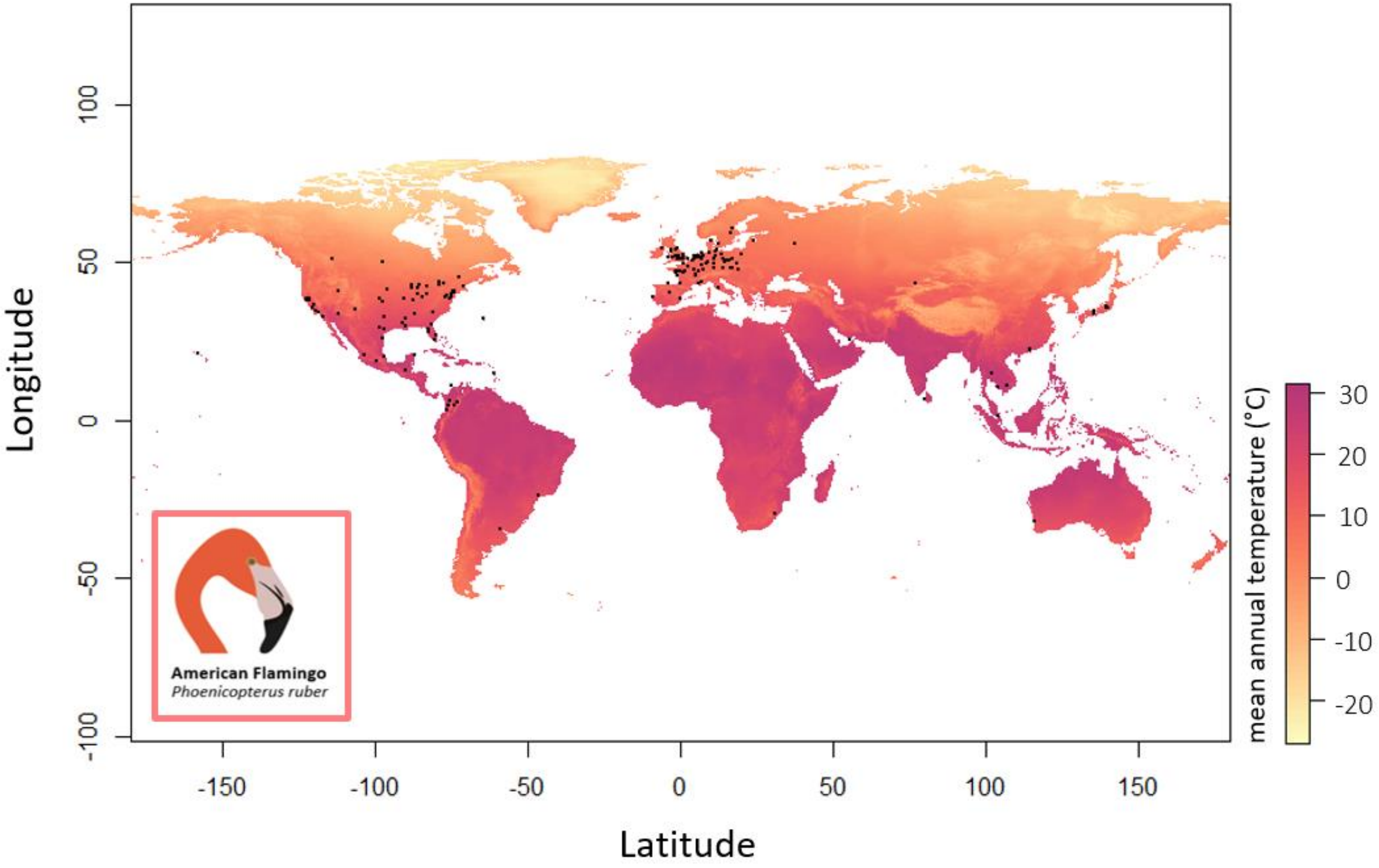


Figure B.6 | The global distribution of current Species360 member institutions which contain flocks of *Phoenicopterus ruber*. These are presented on a global map showing mean annual temperature (°C) at a resolution of 1 km (darker meaning higher precipitation).

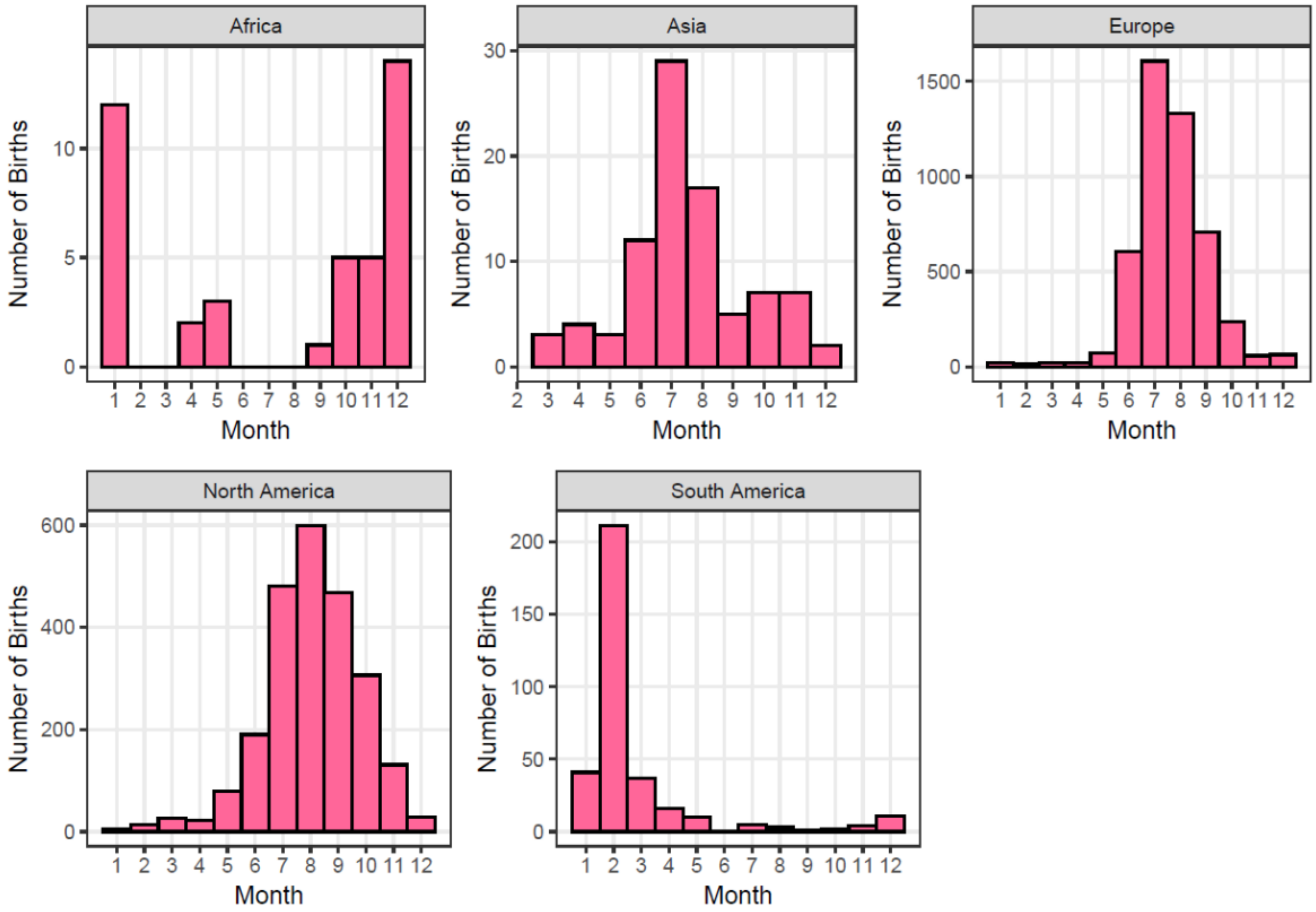


Figure B.7 | The reproductive seasonality of *Phoenicopterus chilensis*, showing the total number of births in each month across different geographic locations. Months; 1-12 = January – December.

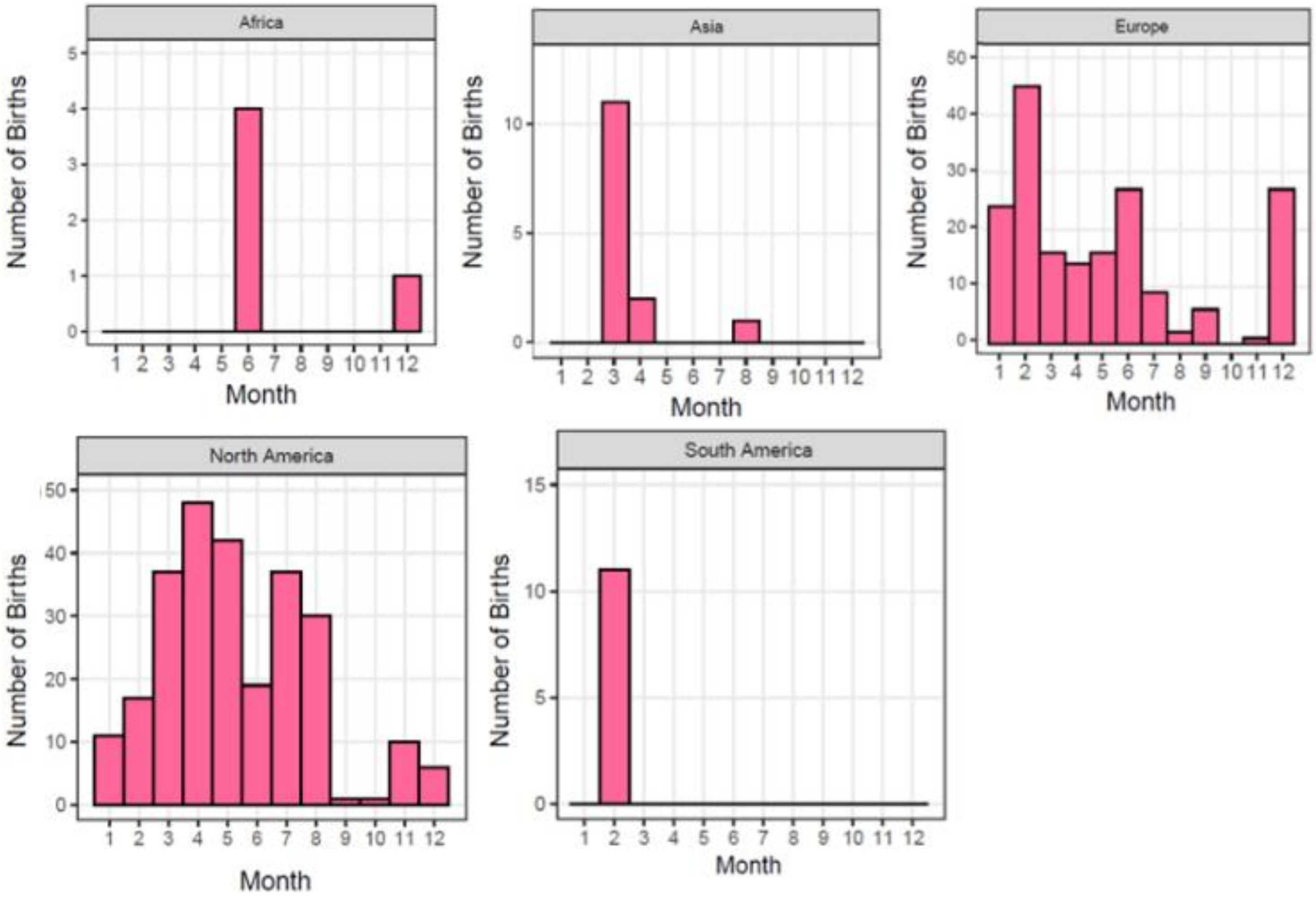


Figure B.8 | The reproductive seasonality of *Phoeniconaias minor*, showing the total number of births in each month across different geographic locations. Months; 1-12 = January – December.

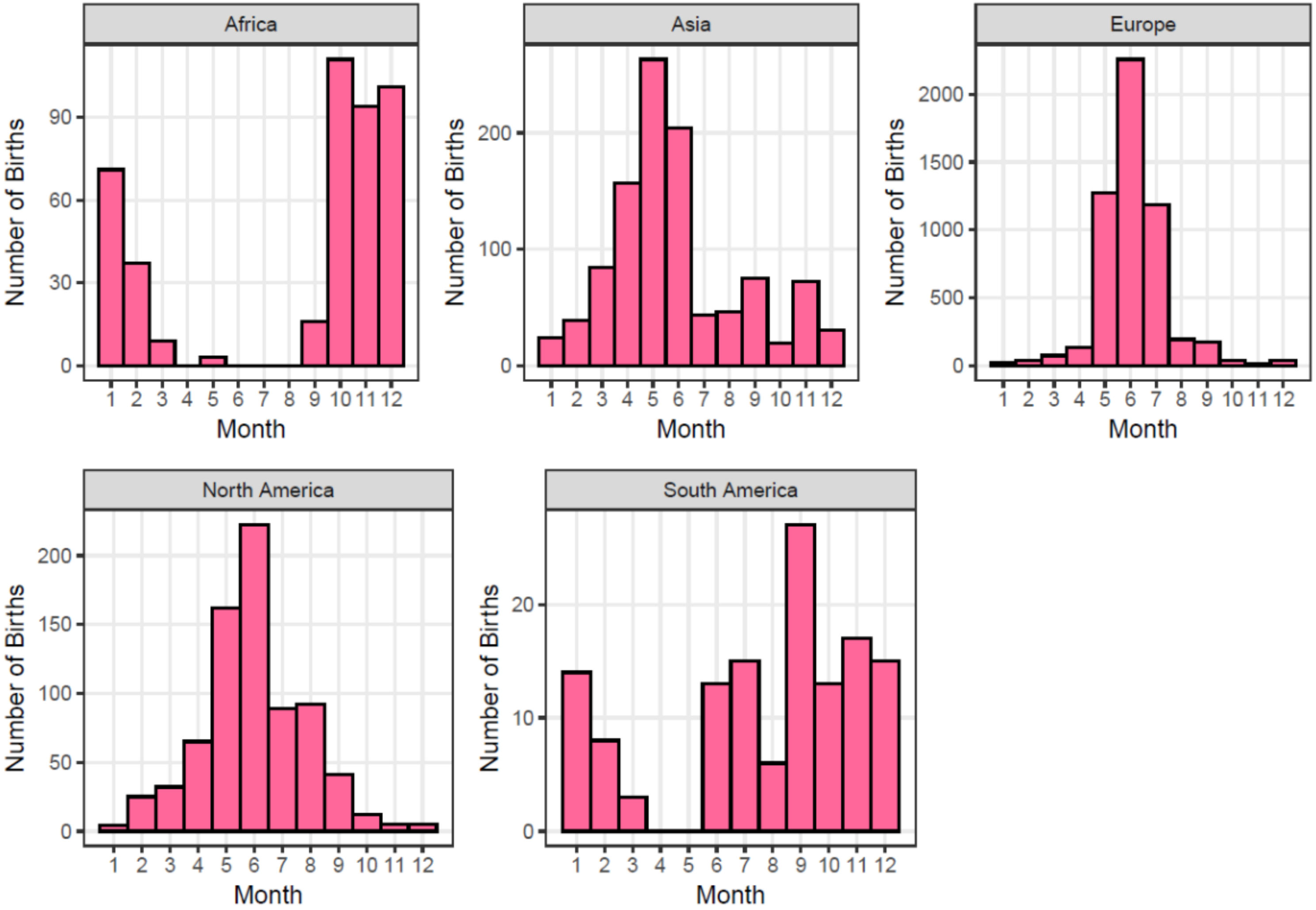


Figure B.9 | The reproductive seasonality of *Phoenicopterus roseus*, showing the total number of births in each month across different geographic locations. Months; 1-12 = January – December.

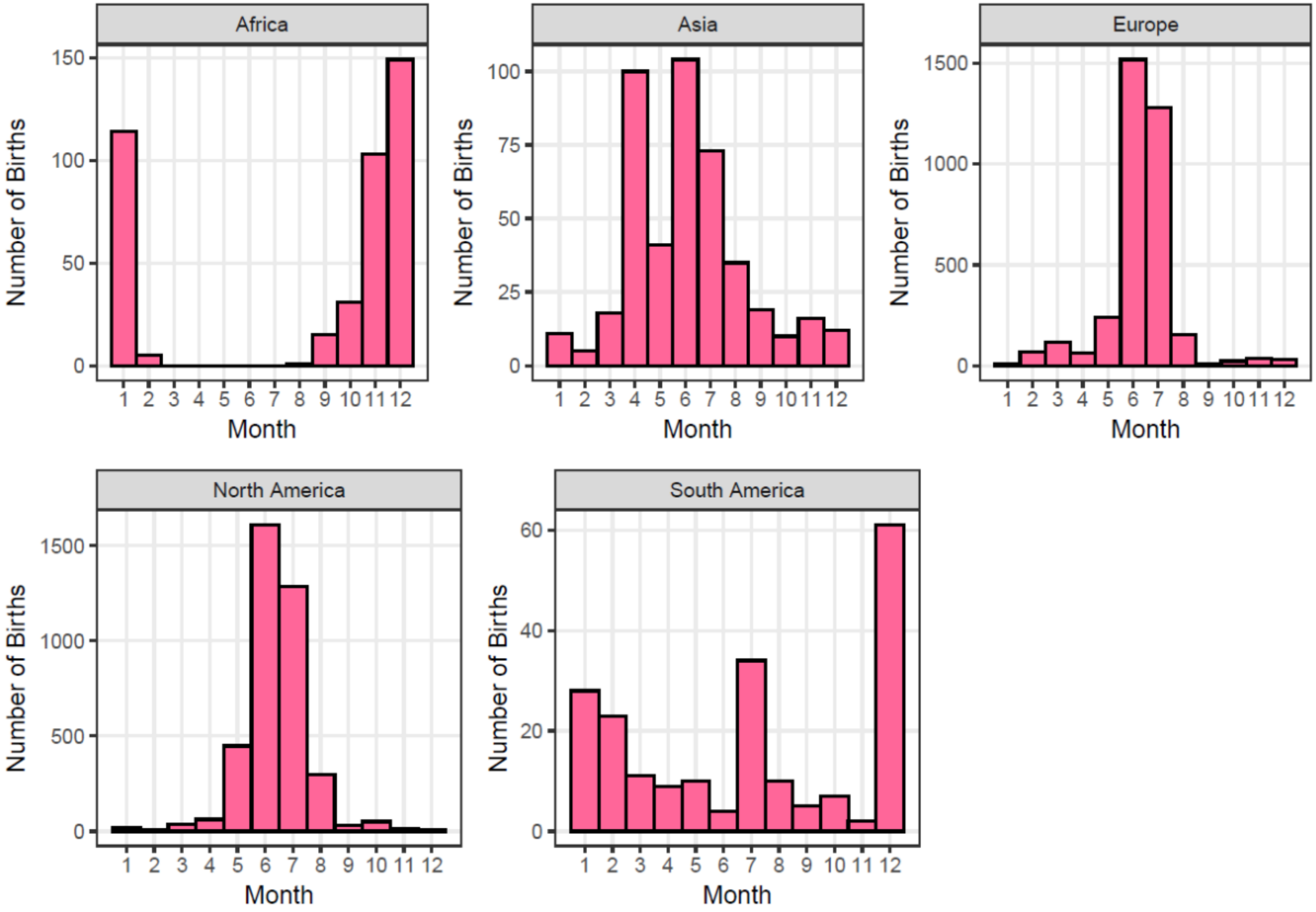


Figure B.10 | The reproductive seasonality of *Phoenicopterus ruber*, showing the total number of births in each month across different geographic locations. Months; 1-12 = January – December.

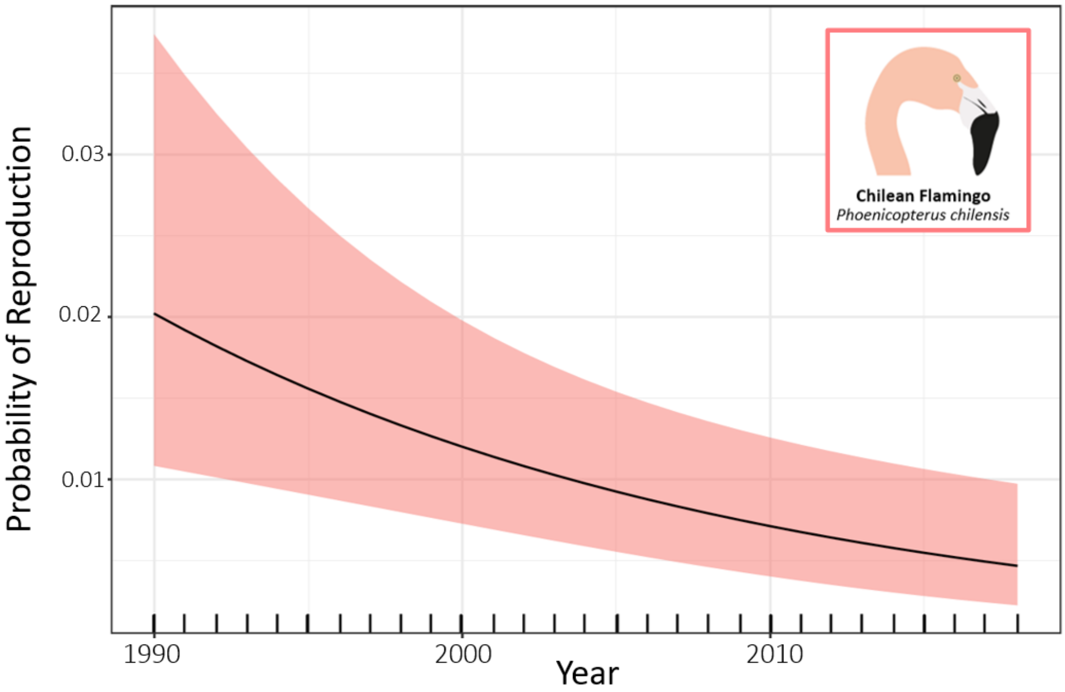


Figure B.11 | The probability of reproduction for *Phoenicopterus chilensis* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

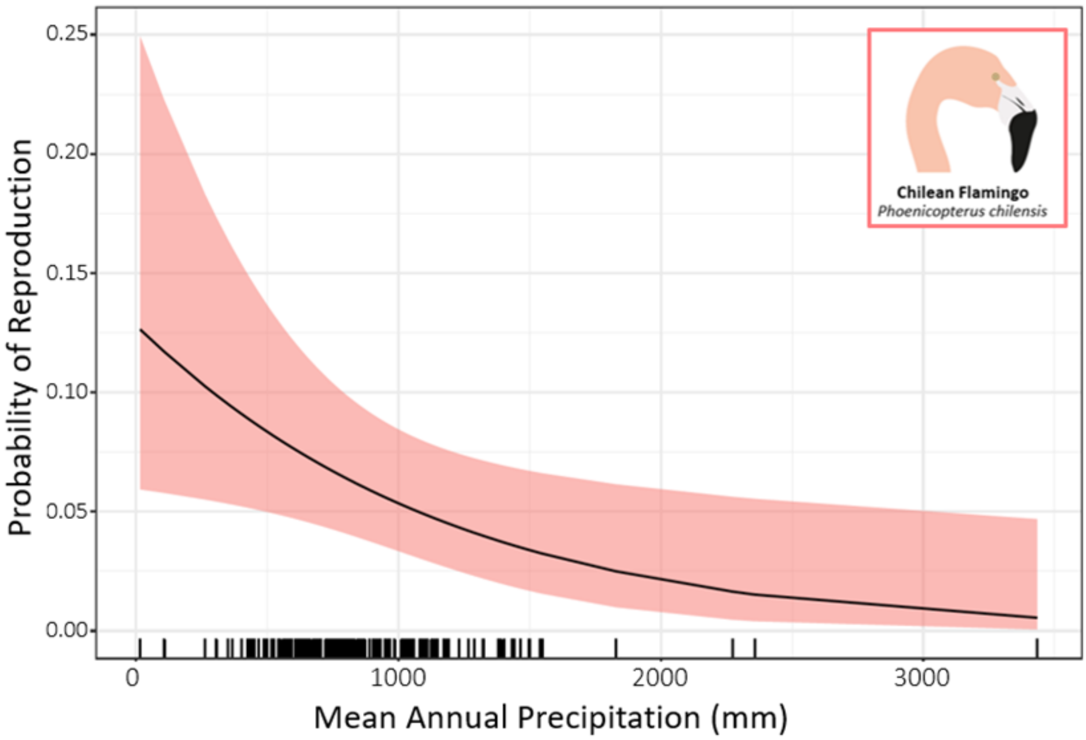


Figure B.12 | The relationship between mean annual precipitation (mm) and the probability of reproduction for *Phoenicopterus chilensis* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

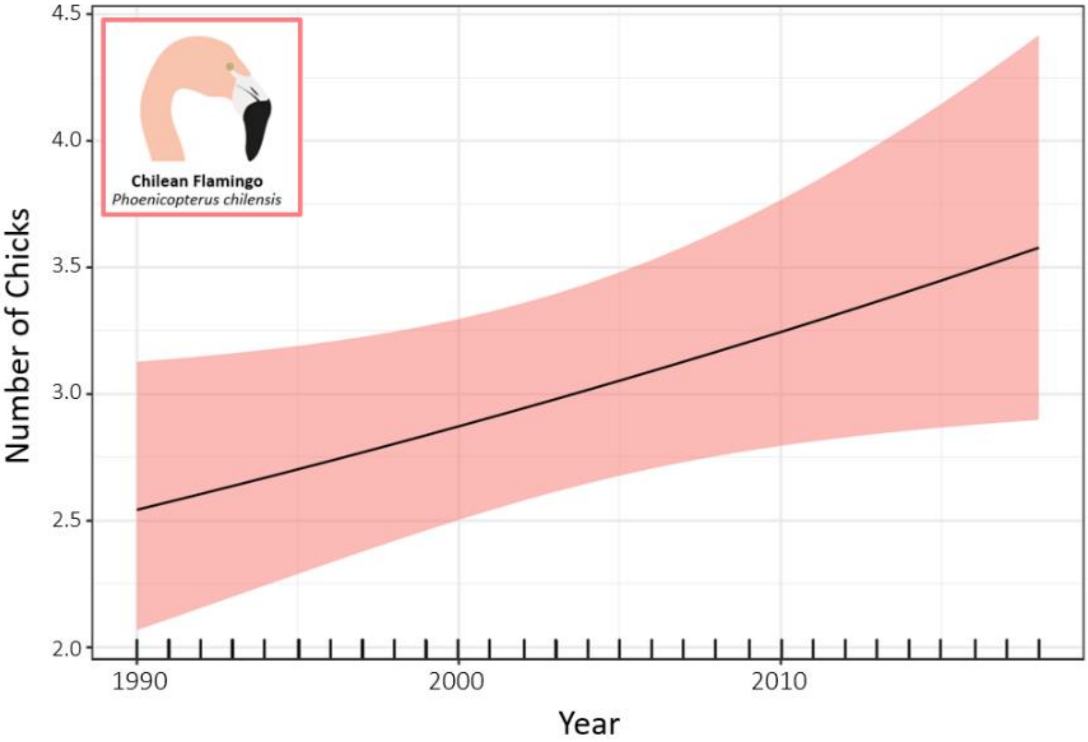


Figure B.13 | The number of chicks produced for *Phoenicopterus chilensis* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

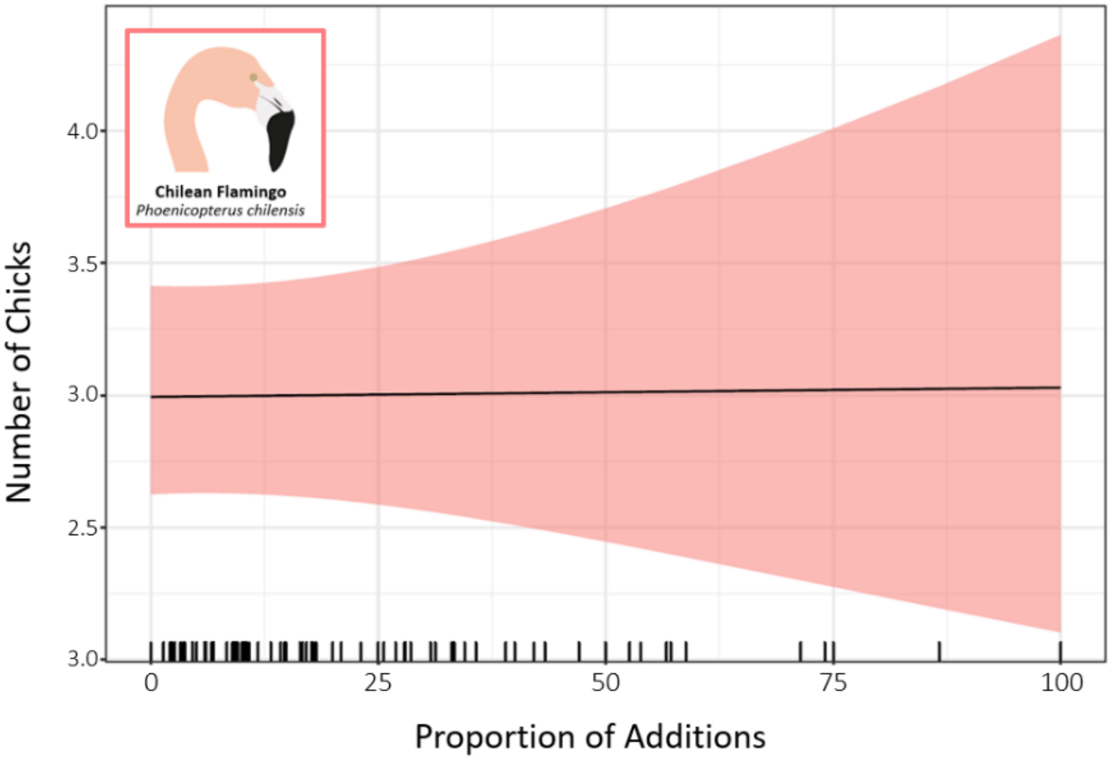


Figure B.14 | The relationship between the proportion of new individuals in a flock and the number of chicks produced for *Phoenicopterus chilensis* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

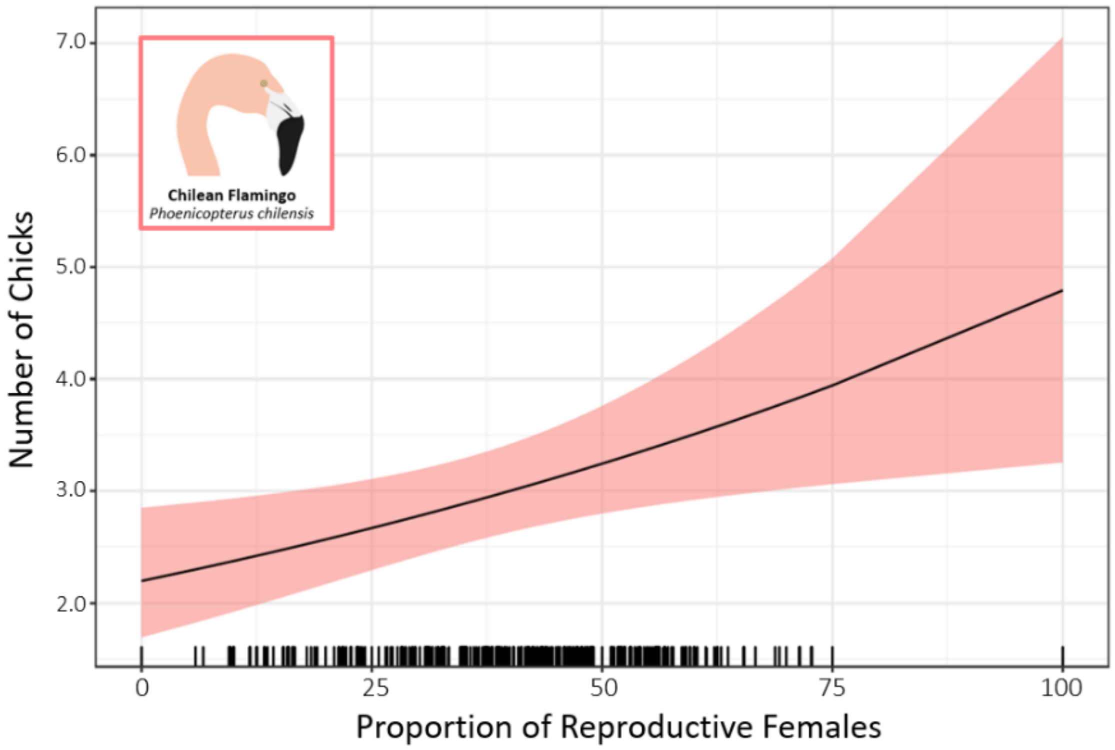


Figure B.15 | The relationship between the proportion of reproductive females per flock and the number of chicks produced for *Phoenicopterus chilensis* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

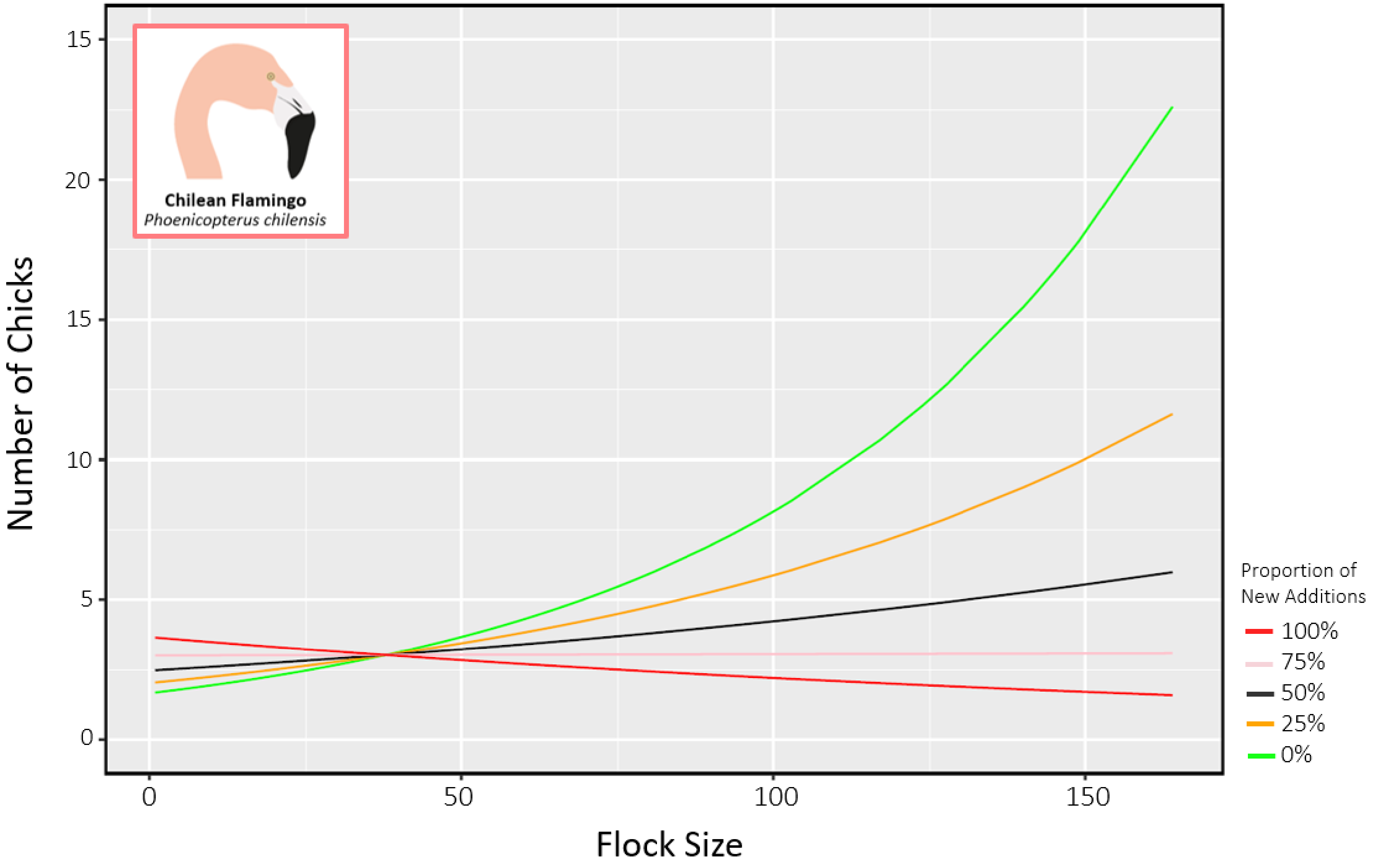


Figure B.16 | The relationship between flock size and the number of chicks produced for *Phoenicopterus chilensis* at varying proportions of new additions per flock between 1990 and 2018. Solid lines represent predicted values.

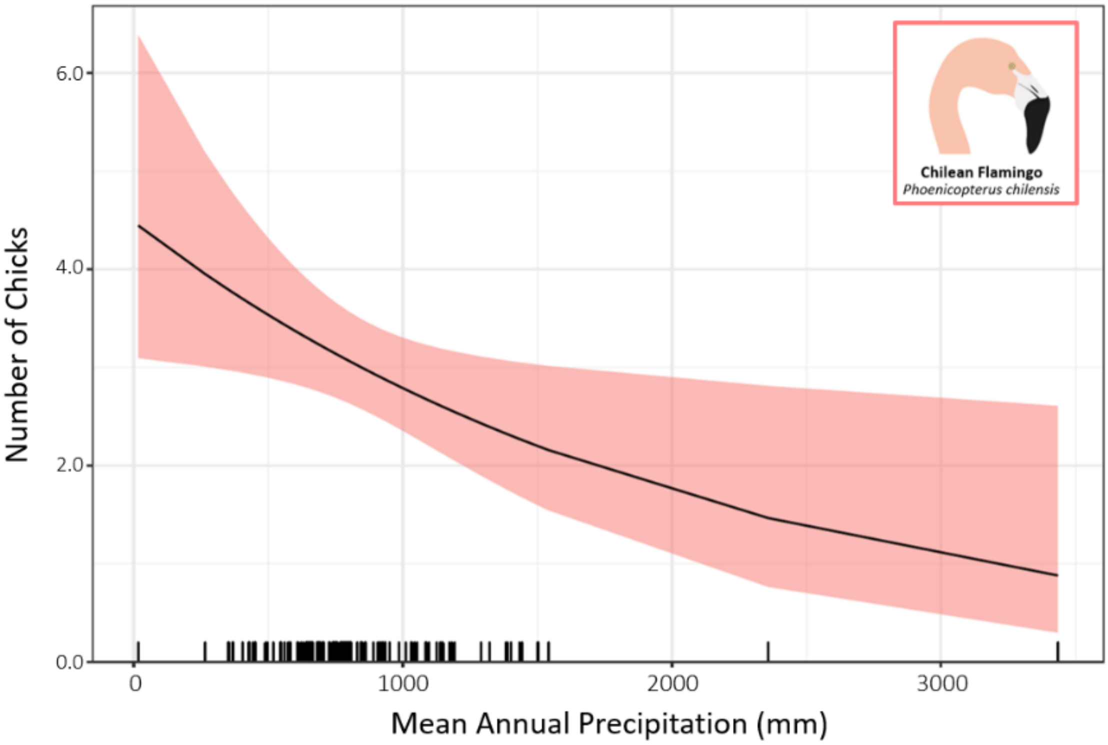


Figure B.17 | The relationship between the number of chicks produced and mean annual precipitation for *Phoenicopterus chilensis* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

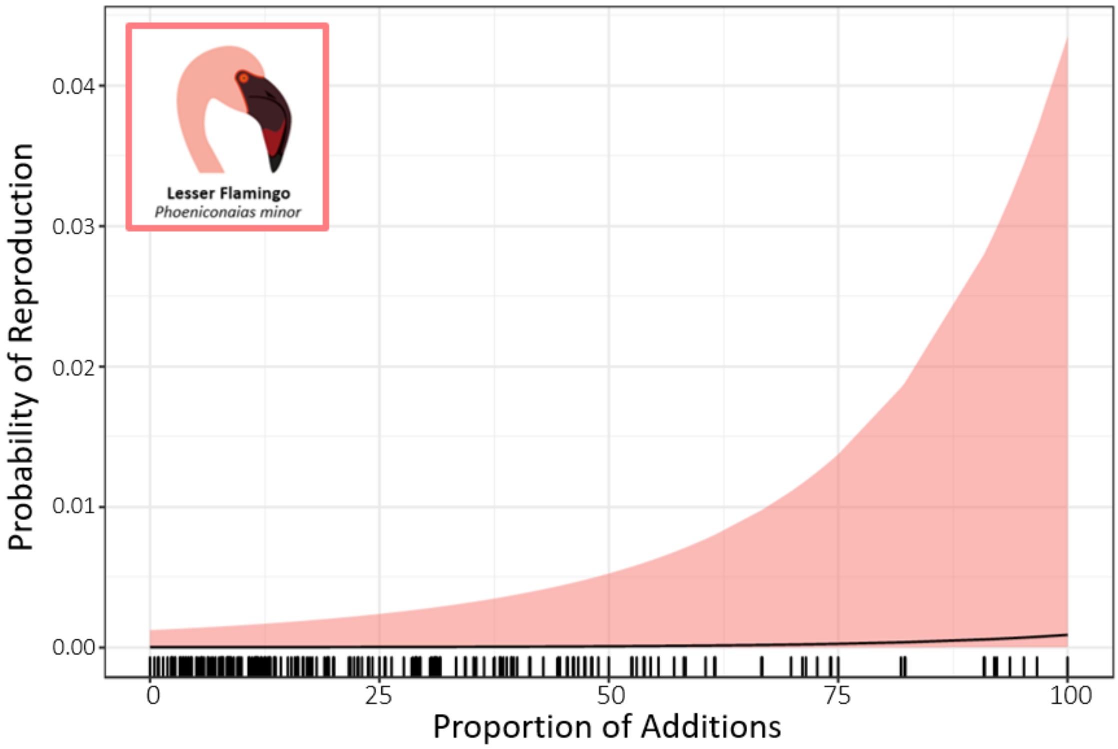


Figure B.18 | The relationship between the proportion of new individuals in a flock and the probability of reproduction for *Phoeniconaias minor* between 1990 and 2018. The black line represents predicted values and the shaded area represent 95% confidence intervals.

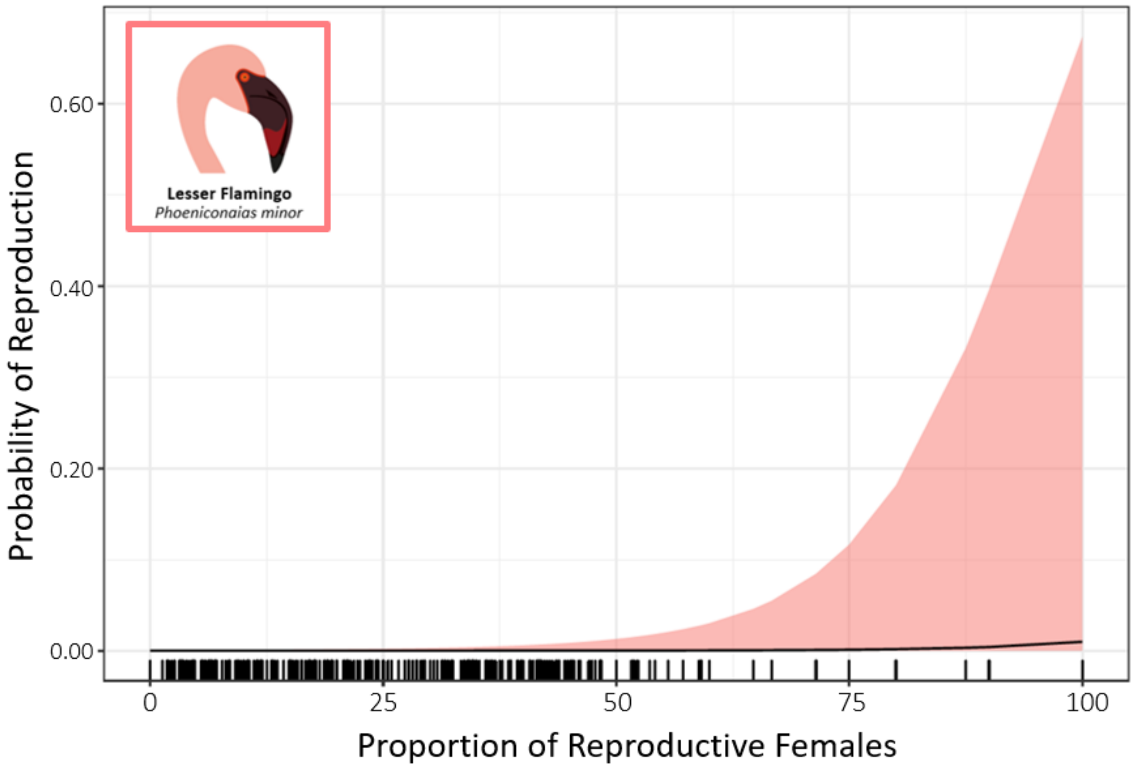


Figure B.19 | The relationship between the proportion of reproductive females in a flock and the probability of reproduction for *Phoeniconaias minor* between 1990 and 2018. The black line represents predicted values and the shaded area represent 95% confidence intervals.

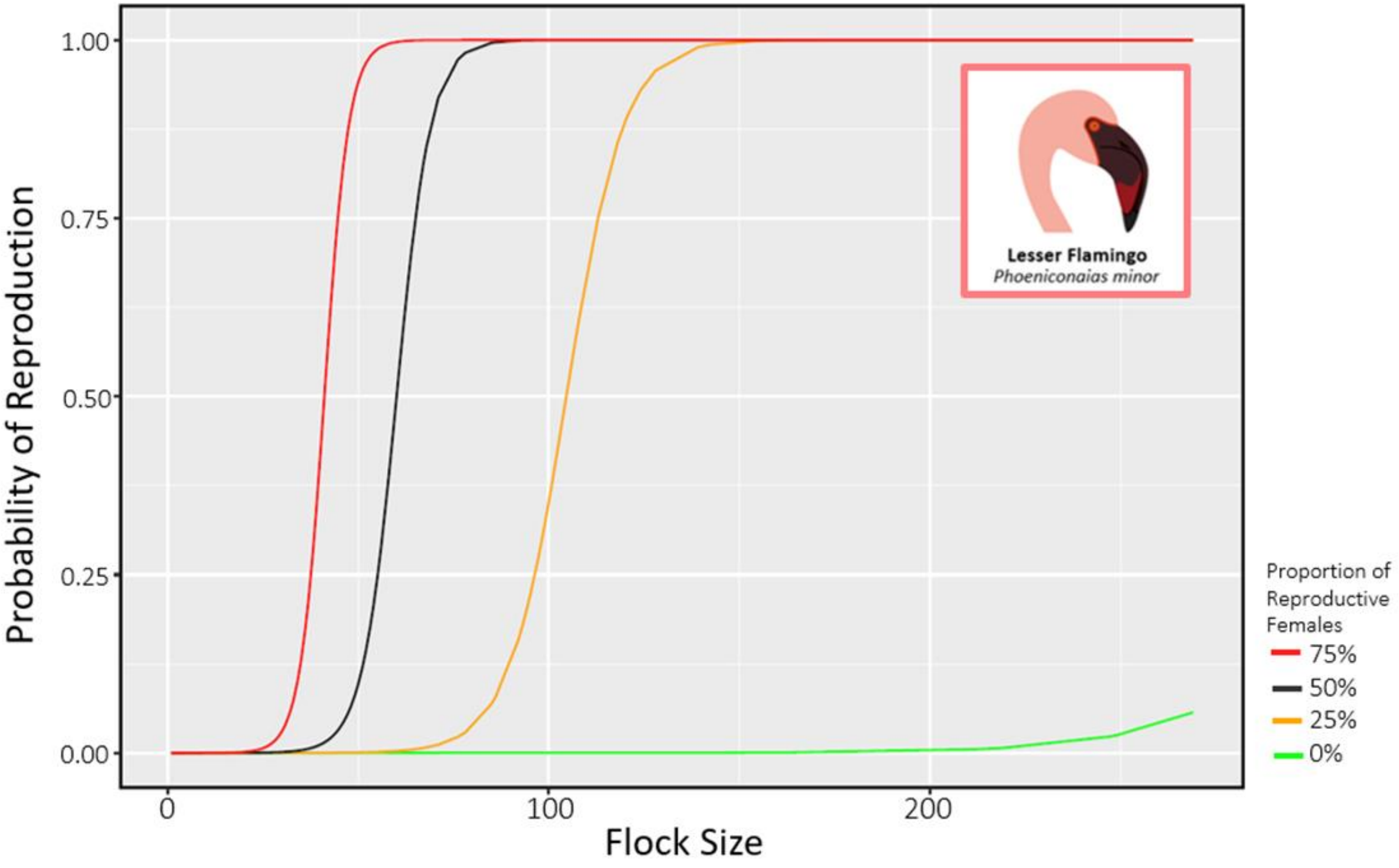


Figure B.20 | The relationship between flock size and the probability of reproduction for *Phoeniconaias minor* at varying proportions of reproductive females per flock between 1990 and 2018. Solid lines represent predicted values

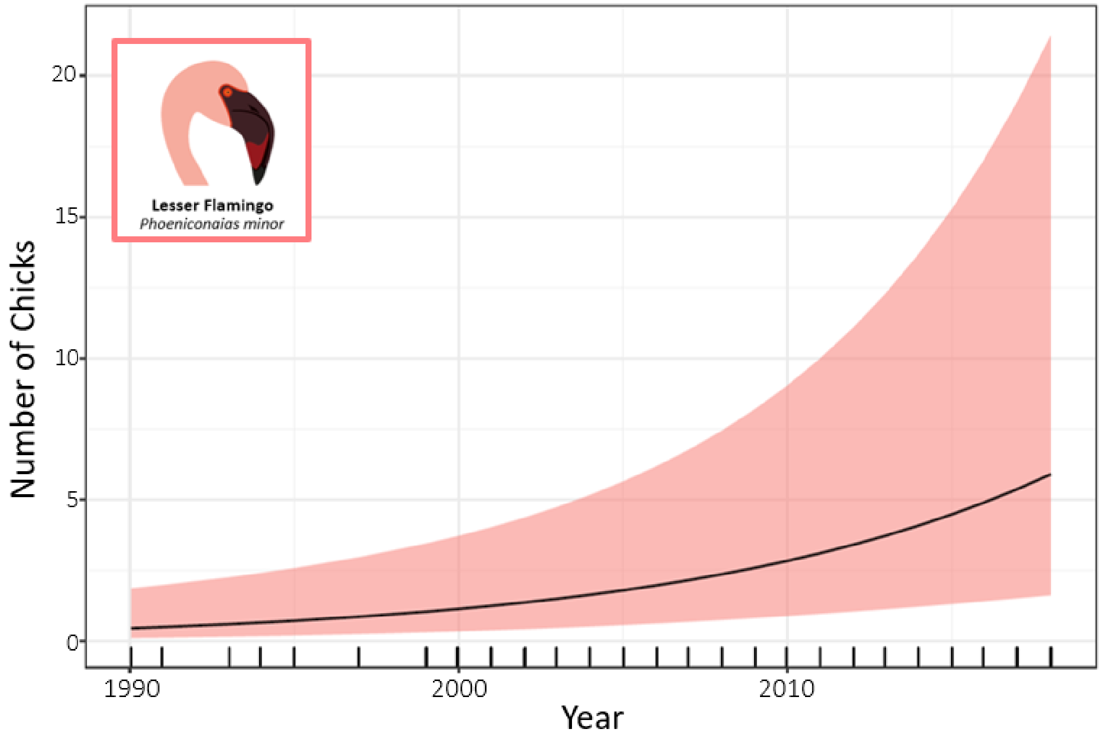


Figure B.21 | The number of chicks produced for *Phoeniconaias minor* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

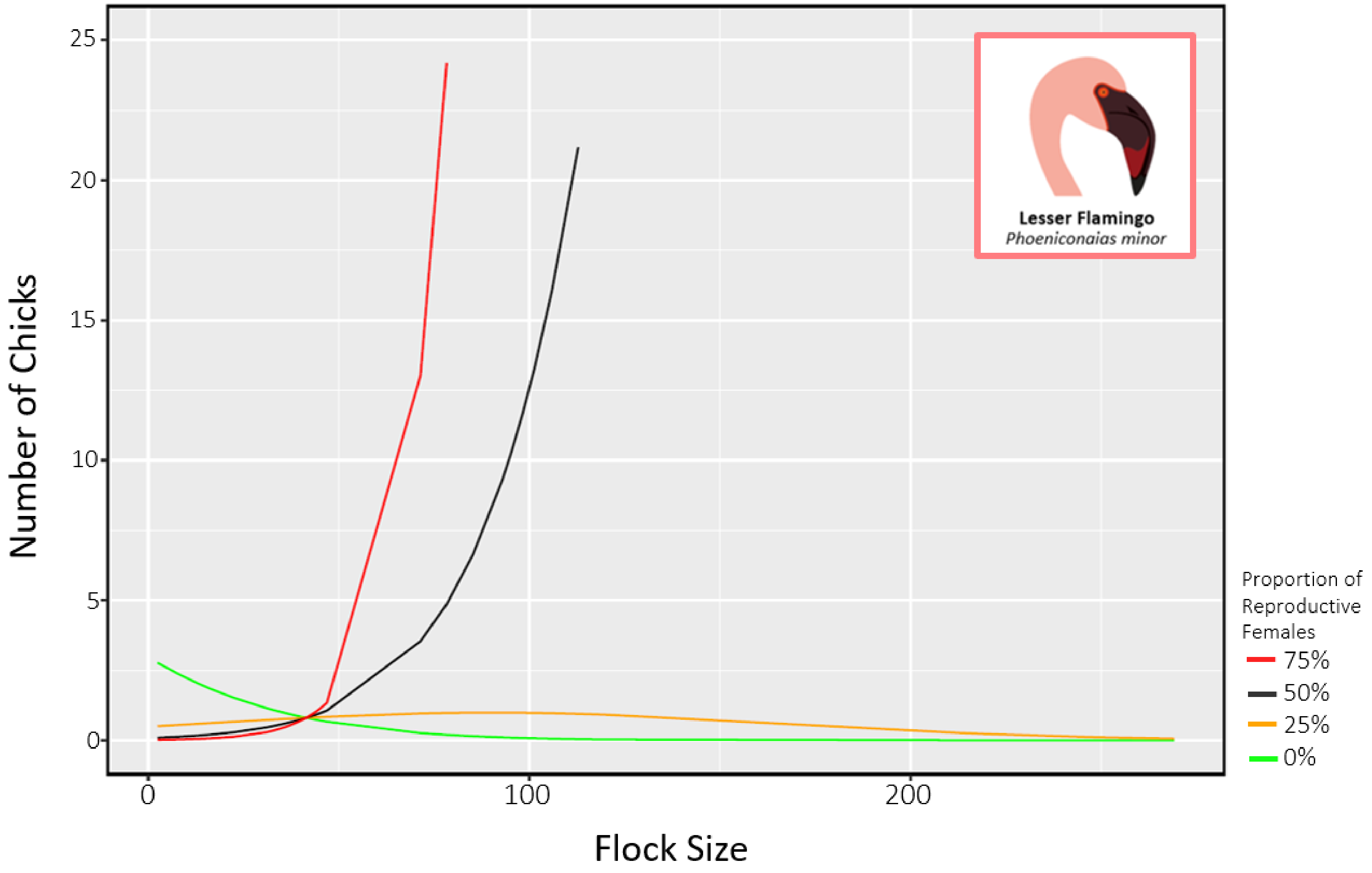


Figure B.22 | The relationship between flock size and the number of chicks produced for *Phoeniconaias minor* at varying proportions of reproductive females per flock between 1990 and 2018. Solid lines represent predicted values

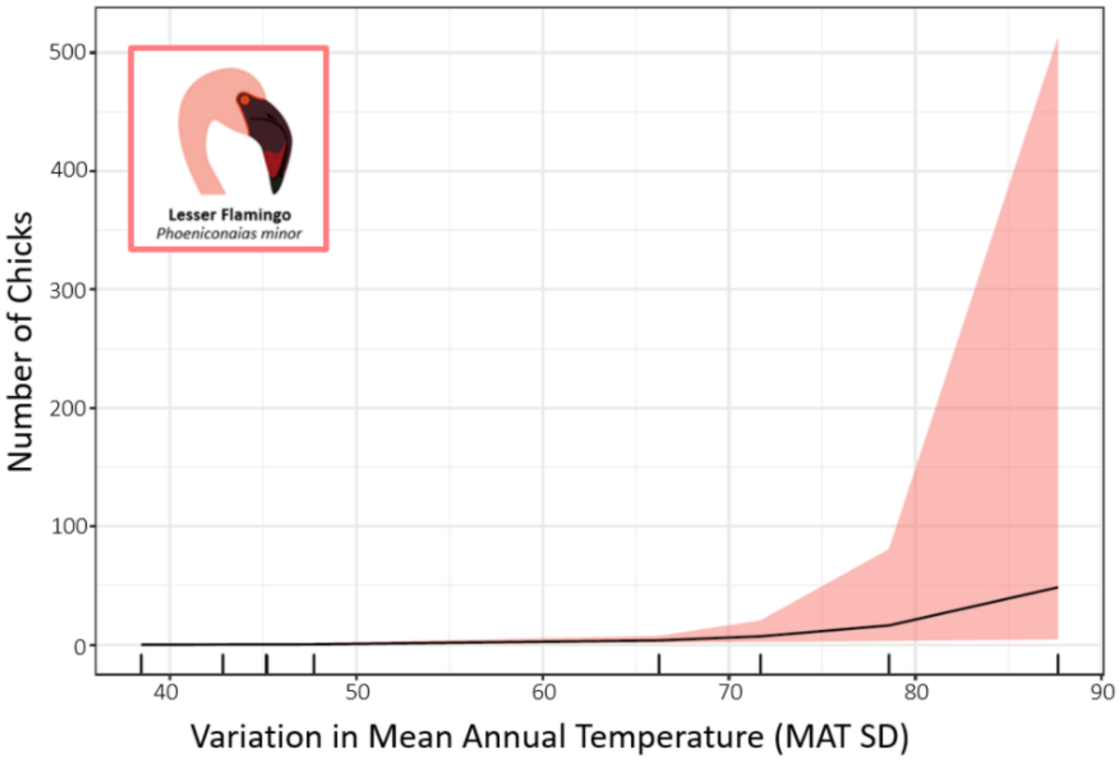


Figure B.23 | The relationship between the number of chicks produced and variation in mean annual temperature (MAT standard deviation) for *Phoeniconaias minor* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

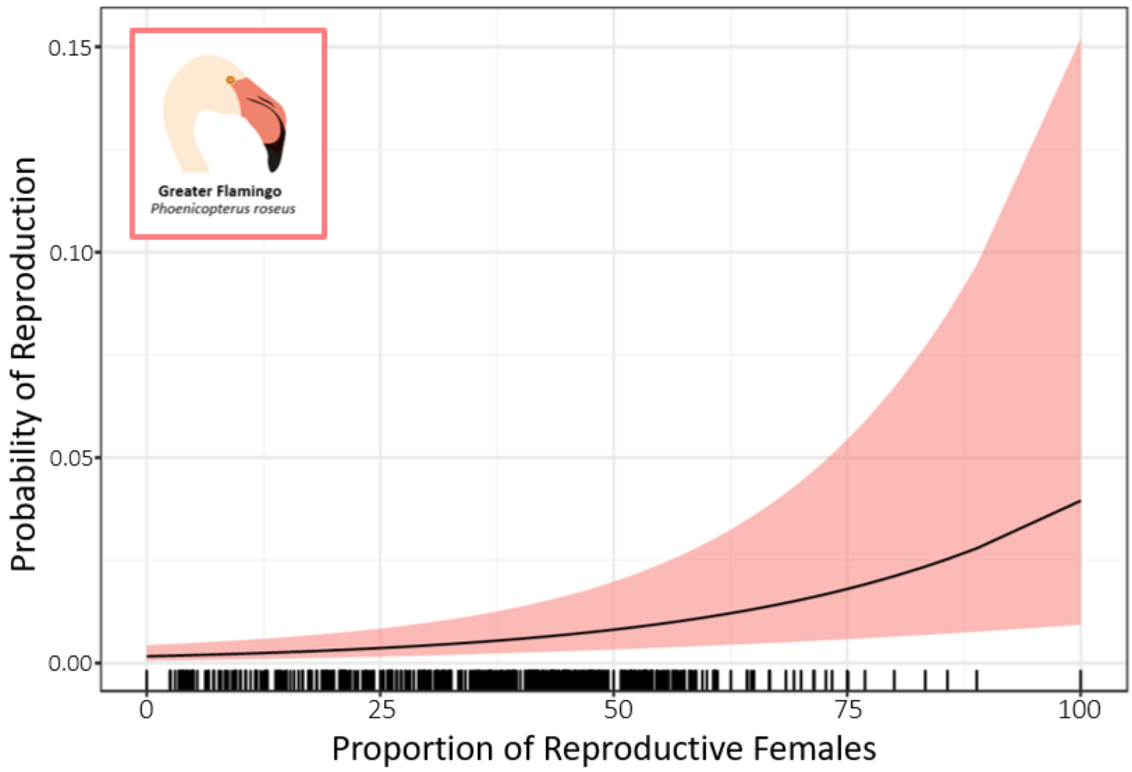


Figure B.24 | The relationship between the proportion of reproductive females in a flock and the probability of reproduction for *Phoenicopterus roseus* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals

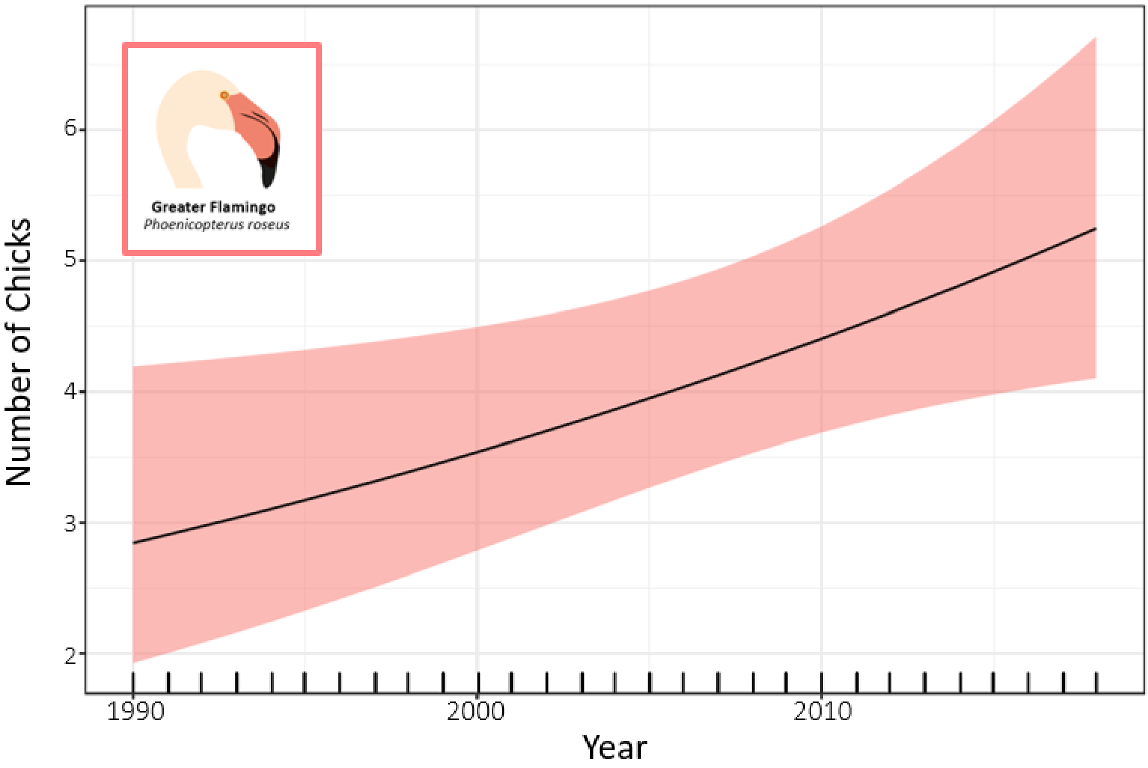


Figure B.25 | The number of chicks produced for *Phoenicopterus roseus* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

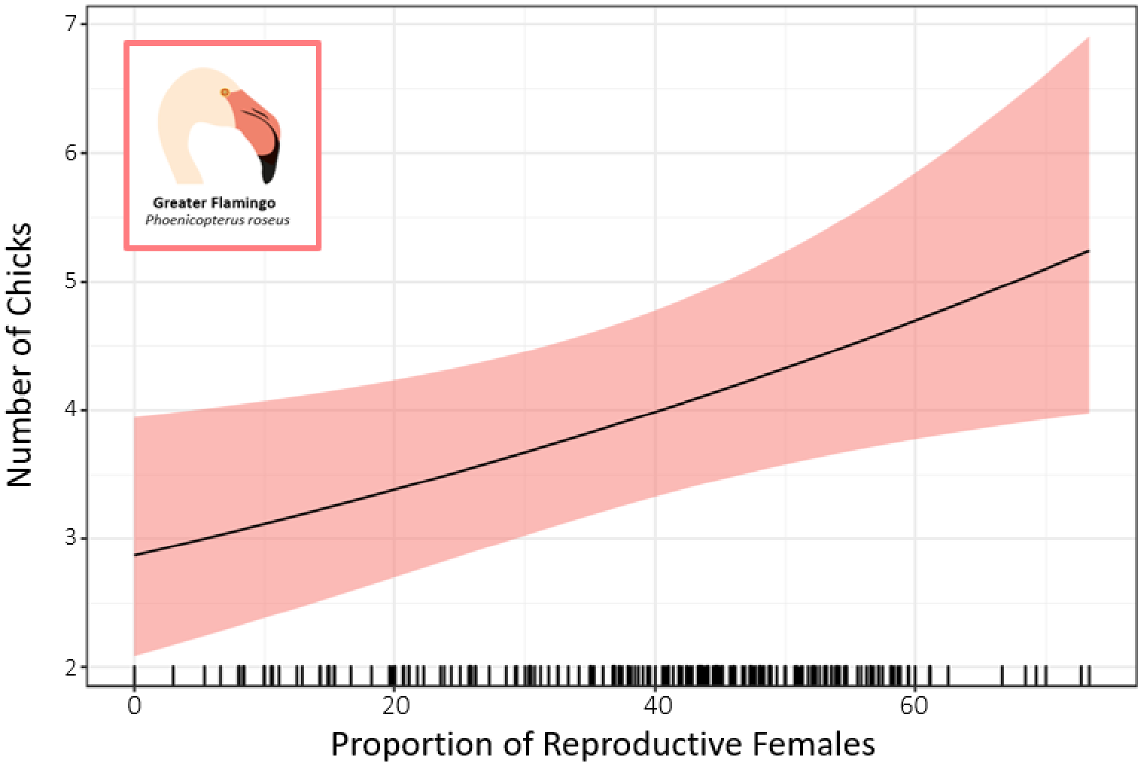


Figure B.26 | The relationship between the proportion of reproductive females in a flock and the number of chicks produced for *Phoenicopterus roseus* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

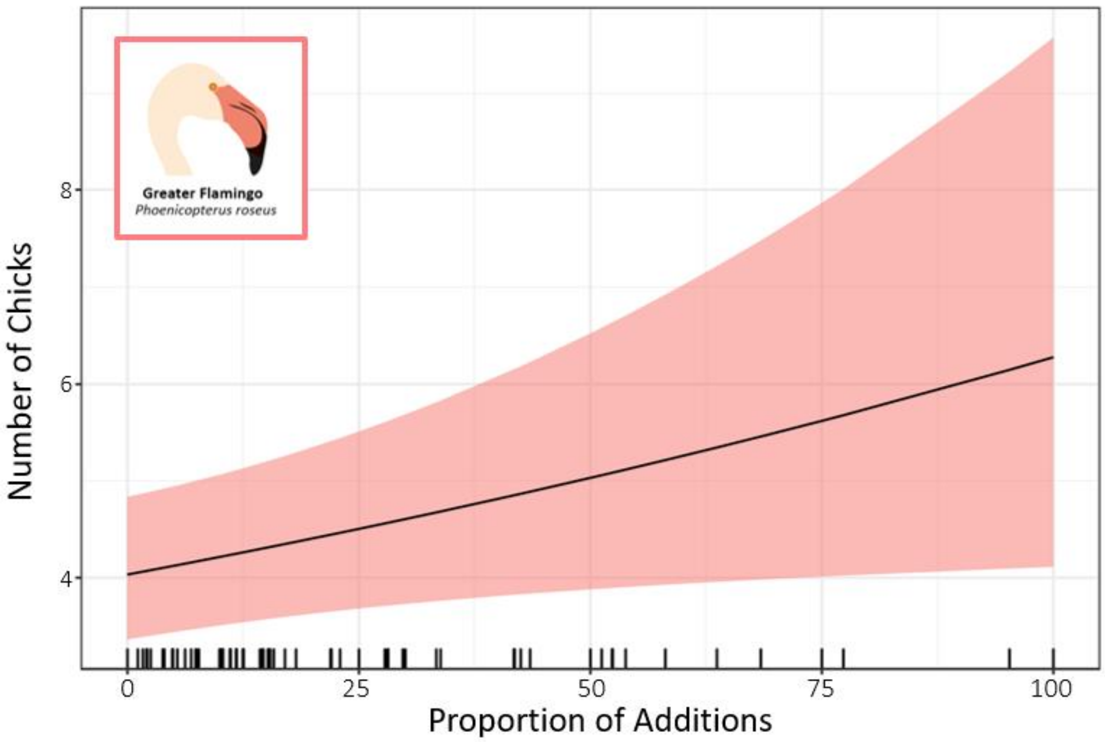


Figure B.27 | The relationship between the proportion of new individuals in a flock and the number of chicks produced for *Phoenicopterus roseus* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

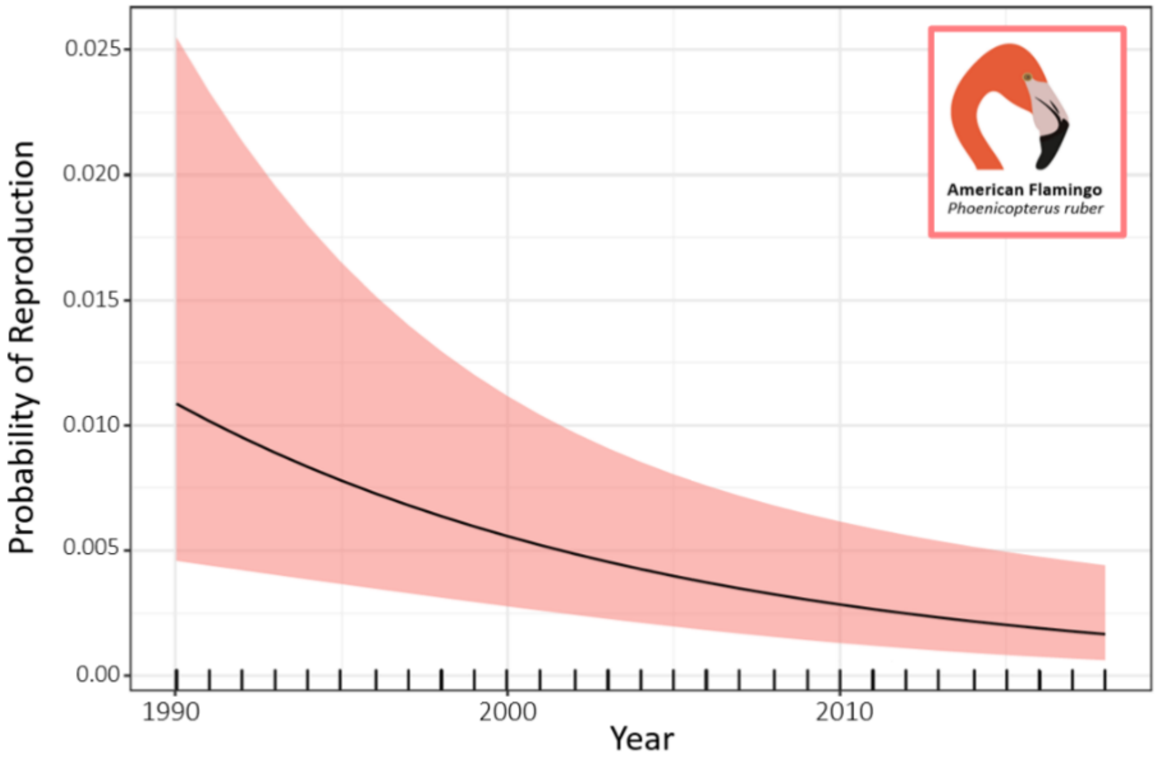


Figure B.28 | The probability of reproduction for *Phoenicopterus ruber* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

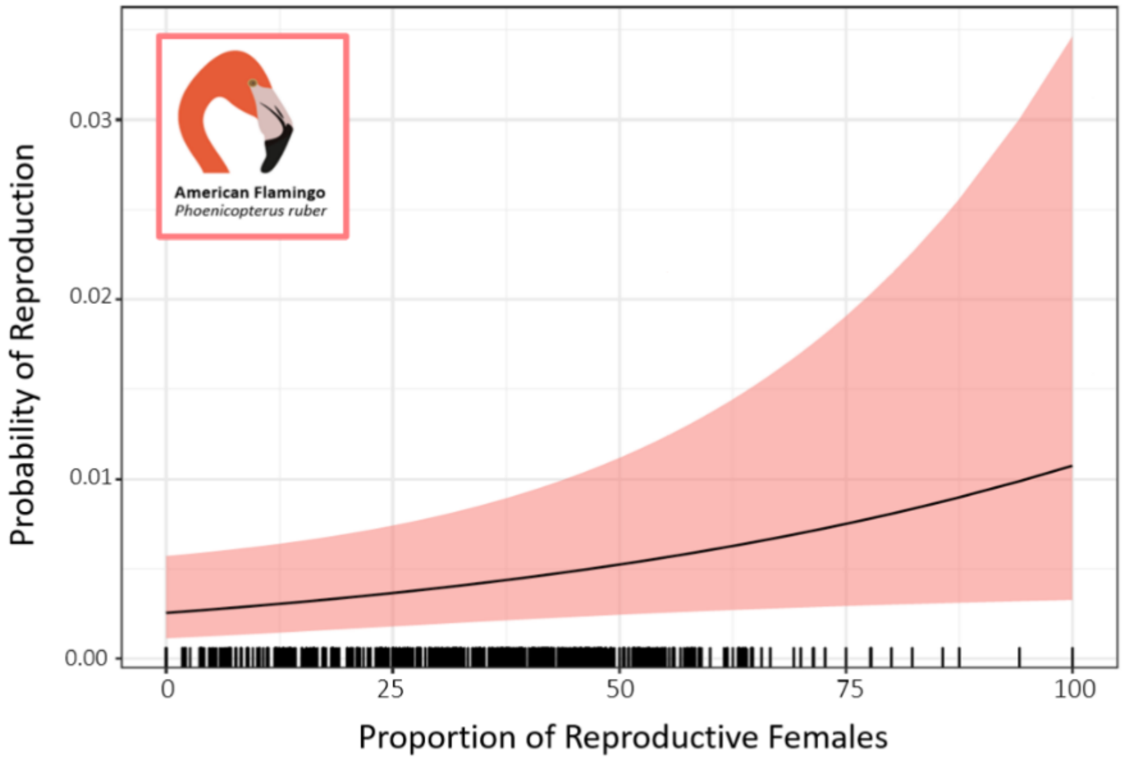


Figure B.29 | The relationship between the proportion of reproductive females in a flock and the probability of reproduction for *Phoenicopterus ruber* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

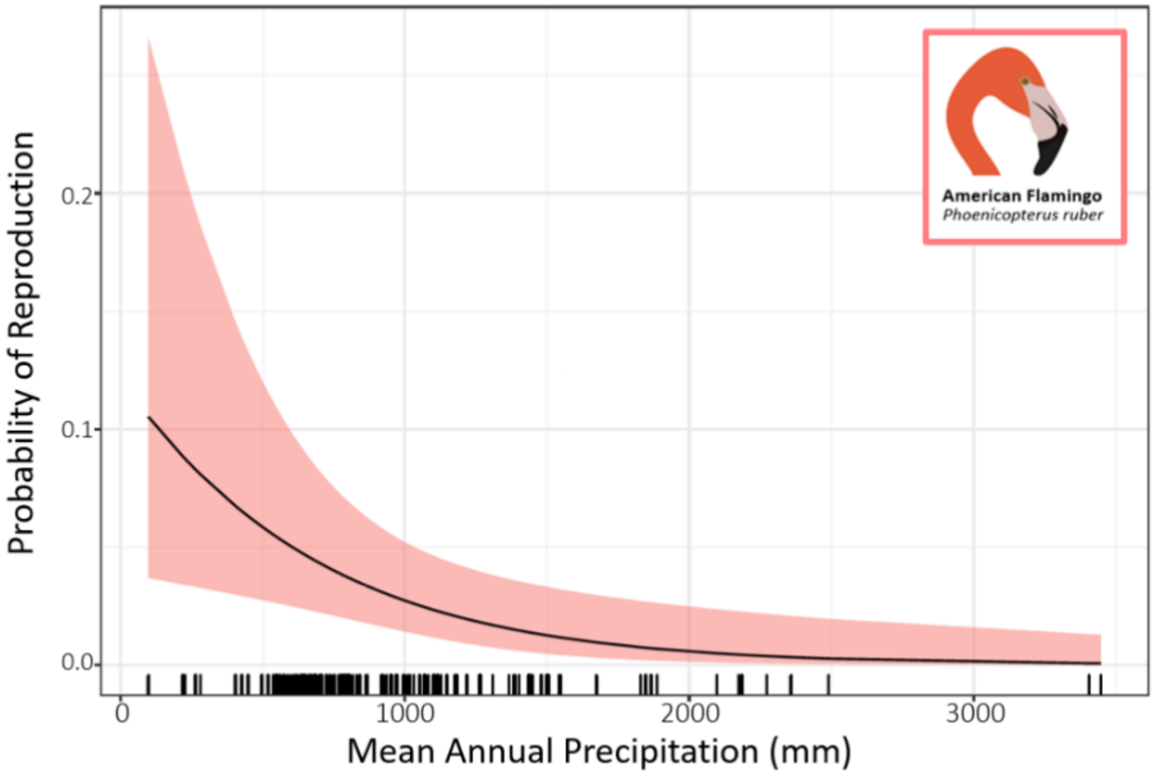


Figure B.30 | The relationship between mean annual precipitation (mm) and the probability of reproduction for *Phoenicopterus ruber* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

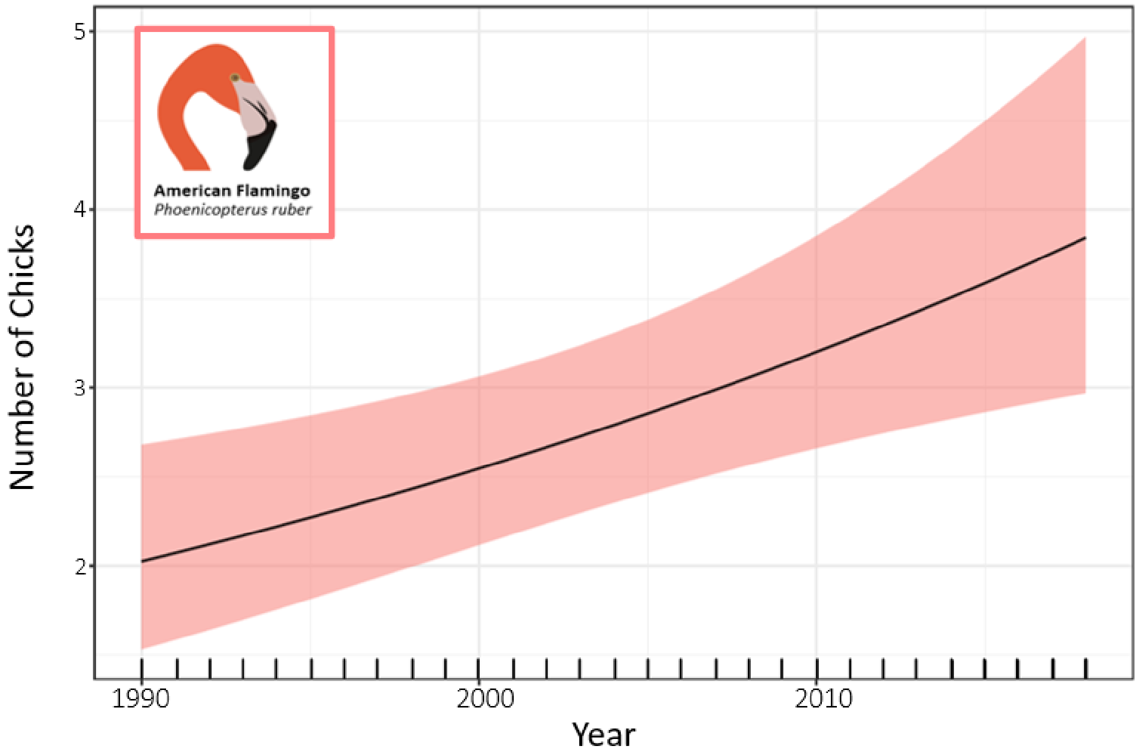


Figure B.31 | The number of chicks produced for *Phoenicopterus ruber* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

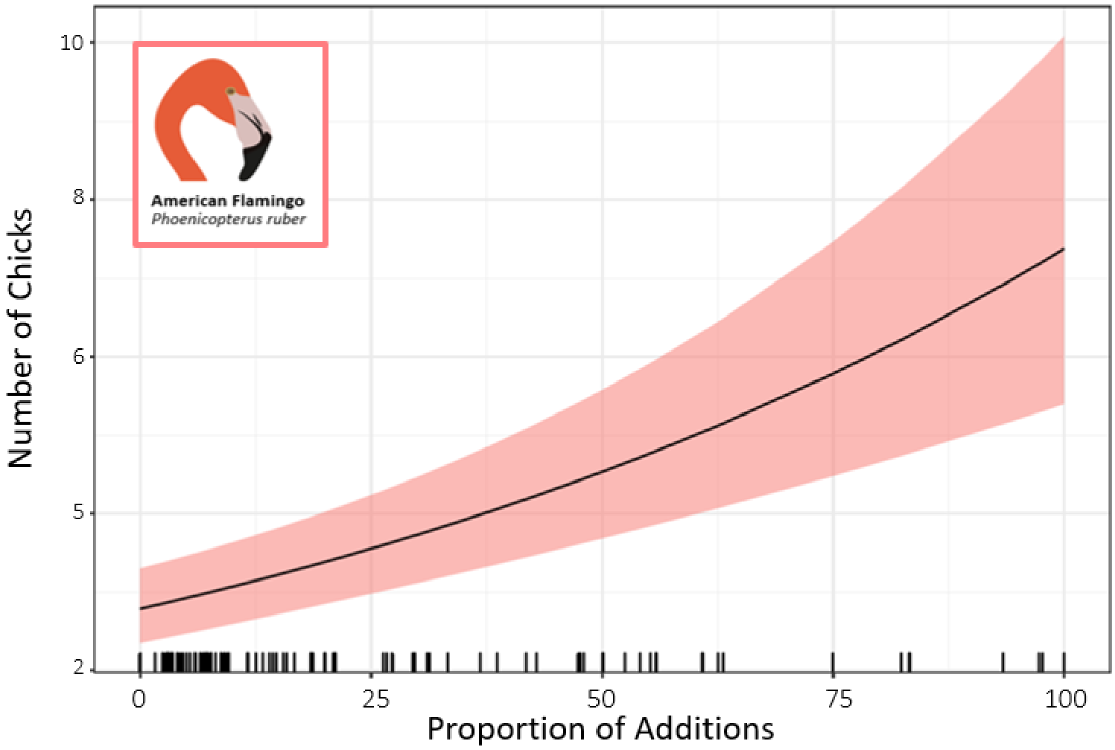


Figure B.32 | The relationship between the proportion of new individuals in a flock and the number of chicks produced for *Phoenicopterus ruber* between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.

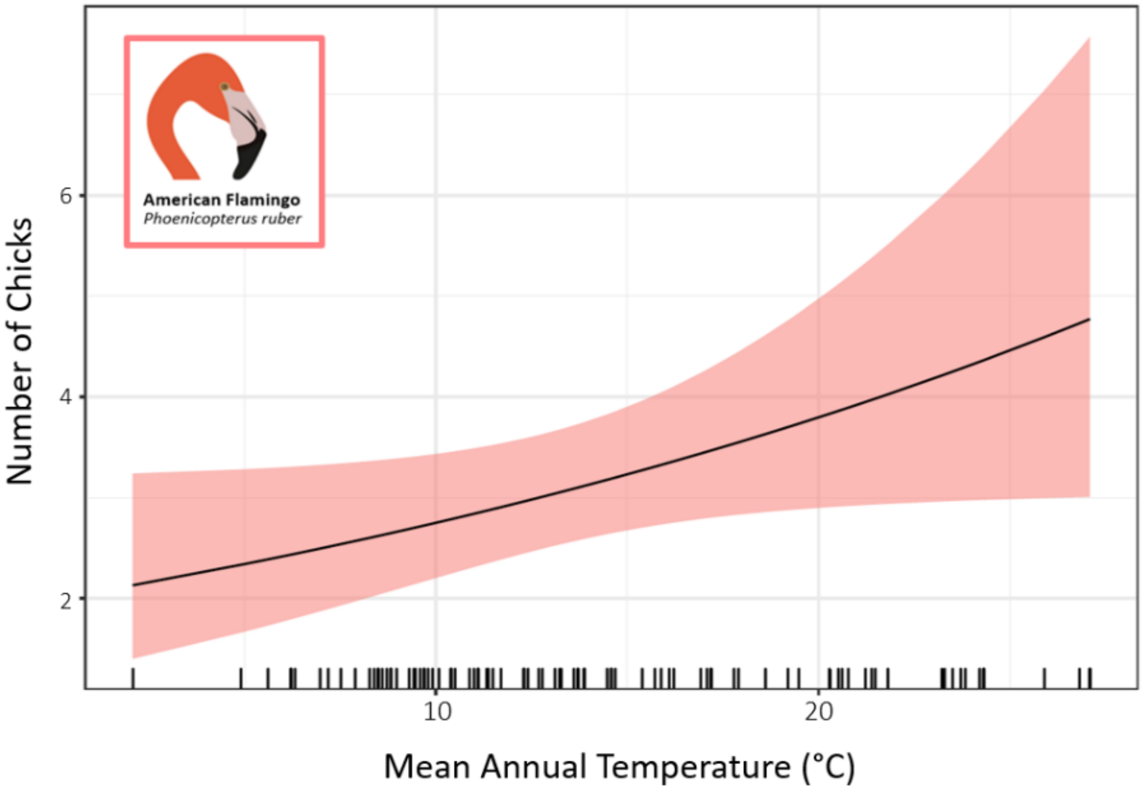
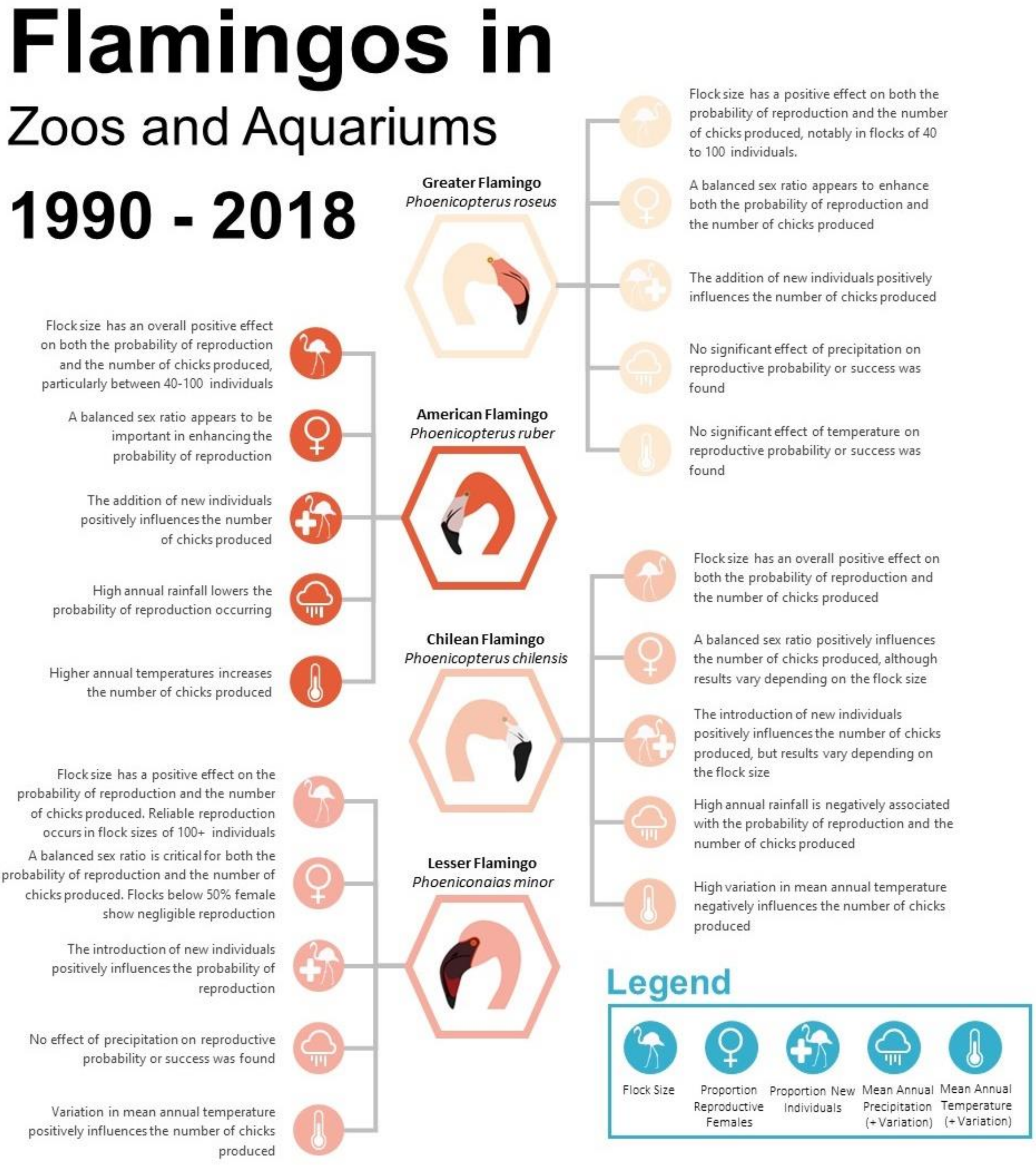


Figure B.33 | The relationship between mean annual temperature (°C) and the number of chicks produced for *Phoenicopterus ruber* flocks between 1990 and 2018. The black line represents predicted values and the shaded area represents 95% confidence intervals.



Flamingos in
Zoos and Aquariums
1990 - 2018

Greater Flamingo
Phoenicopterus roseus

Flock size has a positive effect on both the probability of reproduction and the number of chicks produced, notably in flocks of 40 to 100 individuals.

A balanced sex ratio appears to enhance both the probability of reproduction and the number of chicks produced

The addition of new individuals positively influences the number of chicks produced

No significant effect of precipitation on reproductive probability or success was found

No significant effect of temperature on reproductive probability or success was found

American Flamingo
Phoenicopterus ruber

Flock size has an overall positive effect on both the probability of reproduction and the number of chicks produced, particularly between 40-100 individuals

A balanced sex ratio appears to be important in enhancing the probability of reproduction

The addition of new individuals positively influences the number of chicks produced

High annual rainfall lowers the probability of reproduction occurring

Higher annual temperatures increases the number of chicks produced

Chilean Flamingo
Phoenicopterus chilensis

Flock size has an overall positive effect on both the probability of reproduction and the number of chicks produced

A balanced sex ratio positively influences the number of chicks produced, although results vary depending on the flock size

The introduction of new individuals positively influences the number of chicks produced, but results vary depending on the flock size

High annual rainfall is negatively associated with the probability of reproduction and the number of chicks produced

High variation in mean annual temperature negatively influences the number of chicks produced

Lesser Flamingo
Phoeniconaias minor

Flock size has a positive effect on the probability of reproduction and the number of chicks produced. Reliable reproduction occurs in flock sizes of 100+ individuals

A balanced sex ratio is critical for both the probability of reproduction and the number of chicks produced. Flocks below 50% female show negligible reproduction

The introduction of new individuals positively influences the probability of reproduction

No effect of precipitation on reproductive probability or success was found

Variation in mean annual temperature positively influences the number of chicks produced

Legend

- Flock Size
- Proportion Reproductive Females
- Proportion New Individuals
- Mean Annual Precipitation (+Variation)
- Mean Annual Temperature (+Variation)

Figure B.34 | A summary of the findings and management recommendations for each flamingo species, considering both the probability of reproduction and the number of chicks produced. Flamingo profile images adapted with permission from Krienitz *et al*. (2016). Flamingo silhouette image used throughout provided by Phylopic under Public Domain license

Table B.1 | The original maximal and final species-specific 'Probability of Reproduction' models (Total Analysis and Climate Analysis).

Maximal Model	
Total Analysis	Probability of Reproduction ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (Year|Institution Code)
Climate Analysis	Probability of Reproduction ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + Mean Annual Precipitation + Mean Annual Temperature + Variation in Mean Annual Precipitation + Variation in Mean Annual Temperature + (Year|Institution Code) + (1|Country)
Phoenicopterus ruber (Final Models)	
Total Analysis	Probability of Reproduction ~ log(Flock Size) + log(Flock Size)^2 + Year + Proportion of Reproductive Females + (Year|Institution Code)
Climate Analysis	Probability of Reproduction ~ log(Flock Size) + log(Flock Size)^2 + Proportion of Reproductive Females + Mean Annual Precipitation + (Year|Institution Code) + (1|Country)
Phoenicopterus chilensis (Final Models)	
Total Analysis	Probability of Reproduction ~ log(Flock Size) + log(Flock Size)^2 + Year + (Year|Institution Code)
Climate Analysis	Probability of Reproduction ~ Flock Size + (Flock Size)^2 + Year + Mean Annual Precipitation + Variation in Mean Annual Temperature + (Year|Institution Code) + (1|Country)
Phoenicopterus roseus (Final Models)	
Total Analysis	Probability of Reproduction ~ Flock Size + (Flock Size)^2 + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (Year|Institution Code)
Climate Analysis	Probability of Reproduction ~ Flock Size + I(Flock Size^2) + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + Mean Annual Precipitation + Variation in Mean Annual Precipitation + Variation in Mean Annual Temperature + (Year|Institution Code) + (1|Country)
Phoeniconaias minor (Final Models)	
Total Analysis	Probability of Reproduction ~ Flock Size + Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (1|Institution Code)
Climate Analysis	Probability of Reproduction ~ Flock Size + Proportion of Reproductive Females + (1|Institution Code) + (1|Country)

Table B.2 | The original maximal and final species-specific 'Number of Chicks' models (Total Analysis and Climate Analysis).

Maximal Model	
Total Analysis	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (Year|Institution Code)
Climate Analysis	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + Mean Annual Precipitation + Mean Annual Temperature + Variation in Mean Annual Precipitation + Variation in Mean Annual Temperature + (Year|Institution Code) + (1|Country)
Phoenicopterus ruber (Final Models)	
Total Analysis	Number of Births ~ log(Flock Size) + log(Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + (Year|Institution Code)
Climate Analysis	Number of Births ~ log(Flock Size) + log(Flock Size)^2 + Year + Proportion of Additions + Flock Size:Proportion of Additions + Mean Annual Temperature + (Year|Institution Code) + (1|Country)
Phoenicopterus chilensis (Final Models)	
Total Analysis	Number of Births ~ Flock Size + Year + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (Year|Institution Code)
Climate Analysis	Number of Births ~ Flock Size + Proportion of Additions + Flock Size:Proportion of Additions + Proportion of Reproductive Females + Mean Annual Precipitation + (Year|Institution Code) + (1|Country)
Phoenicopterus roseus (Final Models)	
Total Analysis	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Additions + Proportion of Reproductive Females + (Year|Institution Code)
Climate Analysis	Number of Births ~ Flock Size + I(Flock Size^2) + Year + Proportion of Reproductive Females + (Year|Institution Code) + (1|Country)
Phoeniconaias minor (Final Models)	
Total Analysis	Number of Births ~ Flock Size + (Flock Size)^2 + Year + Proportion of Reproductive Females + Flock Size:Proportion of Reproductive Females + (1|Institution Code)
Climate Analysis	Number of Births ~ Flock Size + (Flock Size)^2 + Proportion of Reproductive Females + Variation in Mean Annual Temperature + (1|Institution Code) + (1|Country)

Table B.3 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the probability of reproduction in *Phoenicopterus ruber* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Probability of Reproduction ~ log(Flock Size)	2.490	0.218	< 0.05
Probability of Reproduction ~ log(Flock Size)^2	0.295	0.178	0.098
Probability of Reproduction ~ Proportion of Reproductive Females	0.414	0.198	< 0.05
Probability of Reproduction ~ Year	-0.555	0.182	< 0.05
Climate Analysis			
Probability of Reproduction ~ Mean Annual Precipitation	-0.738	0.280	< 0.05

Table B.4 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the probability of reproduction in *Phoenicopterus chilensis* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Probability of Reproduction ~ log(Flock Size)	2.215	0.321	< 0.05
Probability of Reproduction ~ log(Flock Size)^2	0.181	0.144	0.208
Probability of Reproduction ~ Year	-0.433	0.138	< 0.05
Climate Analysis			
Probability of Reproduction ~ Mean Annual Precipitation	-0.418	0.154	< 0.05
Probability of Reproduction ~ Variation in Mean Annual Temperature	-0.410	0.162	< 0.05

Table B.5 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the probability of reproduction *in Phoenicopterus roseus* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Probability of Reproduction ~ Flock Size	4.307	0.366	< 0.05
Probability of Reproduction ~ (Flock Size)^2	-0.761	0.090	< 0.05
Probability of Reproduction ~ Proportion of New Individuals	-0.037	0.115	0.747
Probability of Reproduction ~ Proportion of Reproductive Females	0.913	0.250	< 0.05
Probability of Reproduction ~ Flock Size:Proportion of New Individuals	-0.545	0.195	< 0.05
Probability of Reproduction ~ Flock Size:Proportion of Reproductive Females	1.717	0.373	< 0.05
Climate Analysis			
Probability of reproduction ~ Variation in Mean Annual Temperature	0.185	0.364	0.610
Probability of reproduction ~ Variation in Mean Annual Precipitation	-0.026	0.329	0.937
Probability of reproduction ~ Mean Annual Precipitation	0.527	0.366	0.150

Table B.6 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the probability of reproduction in *Phoeniconaias minor* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Probability of Reproduction ~ Flock Size	3.136	0.879	< 0.05
Probability of Reproduction ~ Proportion of New Individuals	0.912	0.348	< 0.05
Probability of Reproduction ~ Proportion of Reproductive Females	2.179	0.741	< 0.05
Probability of Reproduction ~ Flock Size:Proportion of Reproductive Females	2.474	0.951	< 0.05
Climate Analysis			
NA			

Table B.7 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the number of chicks produced in *Phoenicopterus ruber* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Number of Chicks ~ log(Flock Size)	0.373	0.066	< 0.05
Number of Chicks ~ log(Flock Size)^2	0.068	0.027	< 0.05
Number of Chicks ~ Year	0.186	0.062	< 0.05
Number of Chicks ~ Proportion of New Individuals	0.122	0.018	< 0.05
Number of Chicks ~ Flock Size:Proportion of New Individuals	0.074	0.017	< 0.05
Climate Analysis			
Number of Chicks ~ Mean Annual Temperature	0.175	0.089	< 0.05

Table B.8 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the number of chicks produced in *Phoenicopterus chilensis* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Number of Chicks ~ Flock Size	0.434	0.059	< 0.05
Number of Chicks ~ Year	0.100	0.049	< 0.05
Number of Chicks ~ Proportion of New Individuals	0.002	0.026	0.949
Number of Chicks ~ Flock Size:Proportion of New Individuals	-0.084	0.031	< 0.05
Number of Chicks ~ Proportion of Reproductive Females	0.137	0.053	< 0.05
Number of Chicks ~ Flock Size:Proportion of Reproductive Females	0.111	0.048	< 0.05
Climate Analysis			
Number of Chicks ~ Mean Annual Precipitation	-0.147	0.065	< 0.05

Table B.9 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the number of chicks produced in *Phoenicopterus roseus* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Number of Chicks ~ Flock Size	0.730	0.082	< 0.05
Number of Chicks ~ (Flock Size)^2	-0.133	0.028	< 0.05
Number of Chicks ~ Year	0.173	0.075	< 0.05
Number of Chicks ~ Proportion of New Individuals	0.053	0.024	< 0.05
Number of Chicks ~ Proportion of Reproductive Females	0.136	0.054	< 0.05
Climate Analysis			
NA			

Table B.10 | Standardised effect sizes, standard errors and p-values for both the Total and Climate Analyses considering the number of chicks produced in *Phoeniconaias minor* flocks between 1990 and 2018. Only the climate components of the Climate Analysis model are reported, all other relationships are derived from the Total Analysis due to its larger sample size and higher statistical power.

	Estimate	Standard Error	P-Value
Total Analysis			
Number of Chicks ~ Flock Size	1.144	0.677	0.091
Number of Chicks ~ (Flock Size)^2	-0.513	0.246	< 0.05
Number of Chicks ~ Year	0.720	0.204	< 0.05
Number of Chicks ~ Proportion of Reproductive Females	1.412	0.672	< 0.05
Number of Chicks ~ Flock Size:Proportion of Reproductive Females	2.206	0.706	< 0.05
Climate Analysis			
Number of Chicks ~ Variation in Mean Annual Temperature	2.180	0.812	< 0.05

Appendix C

Supplementary Information to Chapter 4:

Maximising the potential for living cell banks to contribute to global conservation priorities

Note C.1

San Diego Zoo Global is not obligated to provide biomaterials to any party. Existence of samples does not guarantee availability. To request samples for non-commercial, conservation research projects, please contact BRG@sandiegozoo.org. All requests undergo a comprehensive review process to ensure alignment with San Diego Zoo Global's mission and vision. Requests with no wildlife conservation value will not be approved. Samples may be subject to regulatory restrictions. Requestors are solely responsible for obtaining any required permits, and San Diego Zoo Global cannot provide advice or counsel on regulatory requirements.

Appendix D

Supplementary Information to Chapter 5:

A conservation project efficiency approach to the prioritisation of plants for de-extinction research

Contents:

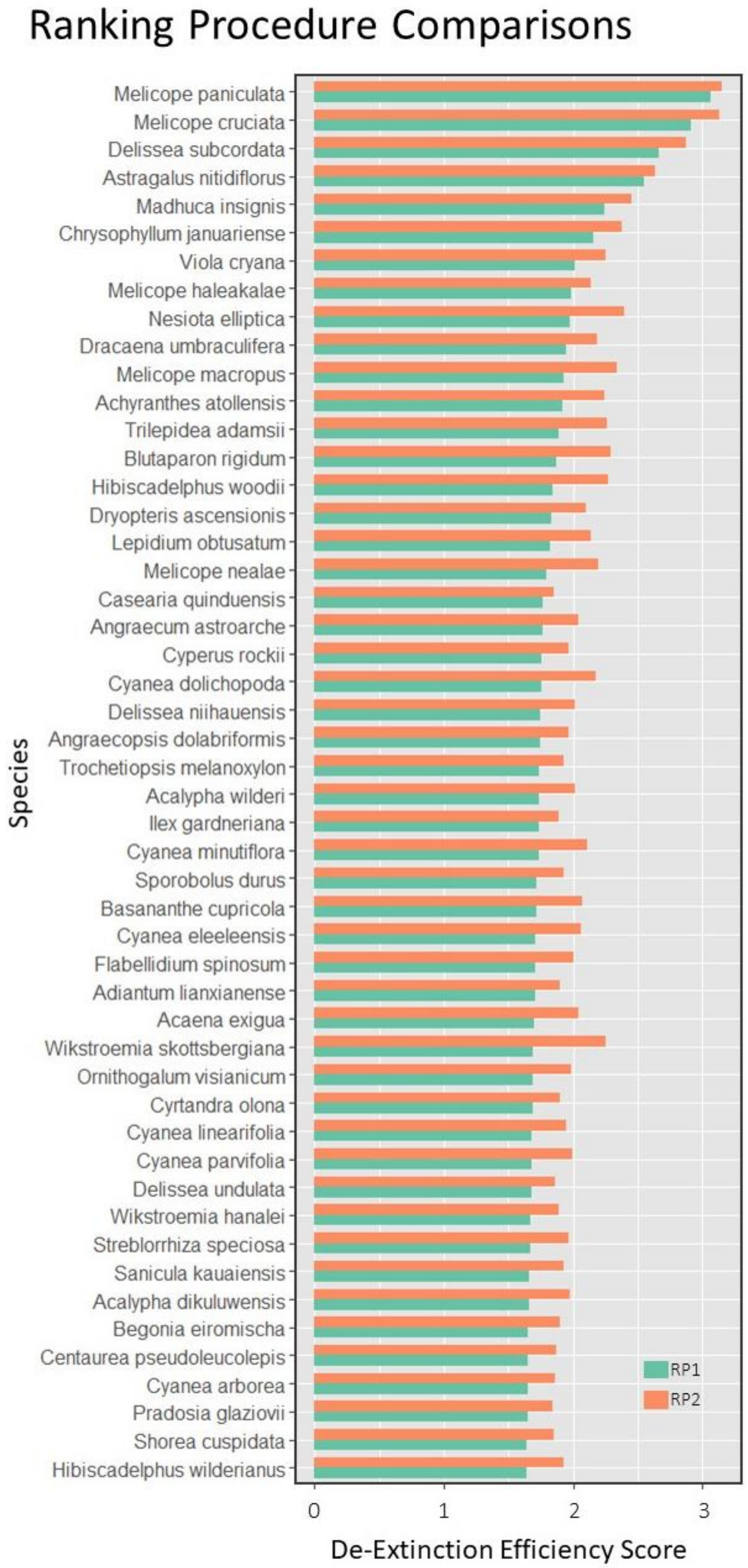


Figure D.1 | A comparison of the estimated de-extinction project efficiency ranking scores (*RE*) for the 50 highest ranking species between Ranking Procedure 1 (RP1) and Ranking Procedure 2 (RP2).

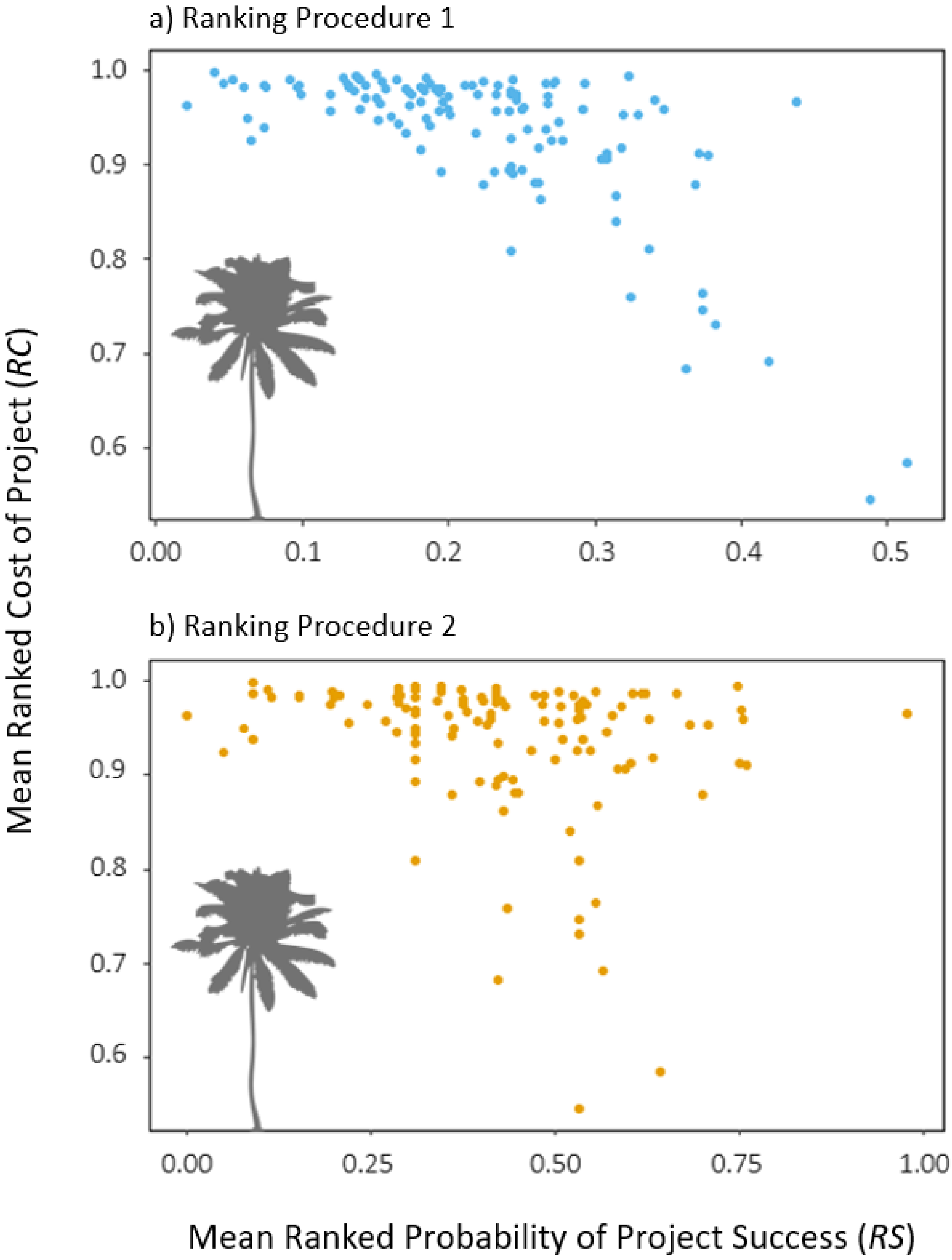


Figure D.2 | The relationship between the mean ranked estimated probability of project success (*RS*) and the mean ranked estimated cost of project (*RC*) for all species assessed (n = 122). Panel (a) represents Ranking Procedure 1 and the seven indicators used to measure *RS*, four of which were also used to measure *RC*. Panel (b) represents Ranking Procedure 2 and the three indicators used to measure *RS*, none of which were used to measure *RC*.

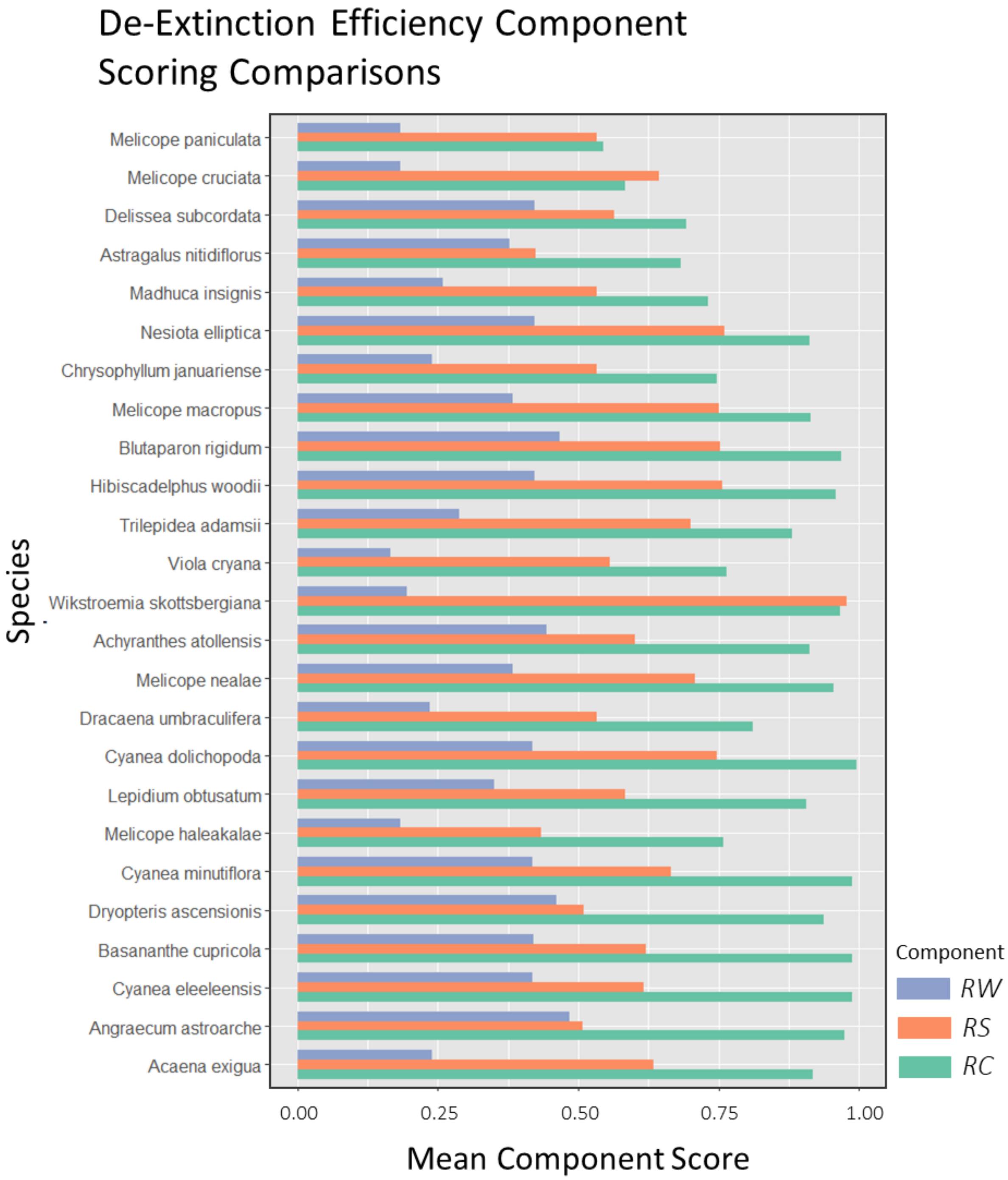


Figure D.3 | A comparison of the mean scores for each of the de-extinction efficiency ranking components (*RW, RS* and *RC*) for the 25 highest ranking species for de-extinction research. Components reflect ranked species weight (*RW*), ranked estimated probability of project success (*RS*) and ranked estimated cost of project (*RC*). Note that high *RC* values indicate species which have higher estimated project cost rankings, and therefore lower overall estimated efficiency score rankings, *RE*. Results reflect Ranking Procedure 2, which does not use any shared indicators to measure *RS* and *RC*. The relationship between *RS* and *RC* is also highlighted in Appendix Figure D.2.

Table D.1 | The top 20 plant species prioritised for de-extinction research efforts following the Ranking Procedures 1 and 2, also provided are the species' overall ranked de-extinction efficiency scores, *RE*. Species in bold appear in the top 20 ranked species using both ranking procedures. Species which have already been rediscovered are denoted by (*).

Ranking Procedure 1			Ranking Procedure 2		
Species	**Score**	**Rank**	**Species**	**Score**	**Rank**
Melicope paniculata *	3.068	1	***Melicope paniculata*** *	3.149	1
Melicope cruciata *	2.909	2	***Melicope cruciata*** *	3.132	2
Delissea subcordata	2.661	3	***Delissea subcordata***	2.872	3
Astragalus nitidiflorus *	2.550	4	***Astragalus nitidiflorus*** *	2.638	4
Madhuca insignis *	2.245	5	***Madhuca insignis*** *	2.451	5
Chrysophyllum januariense	2.161	6	***Nesiota elliptica***	2.396	6
Viola cryana	2.016	7	***Chrysophyllum januariense***	2.374	7
Melicope haleakalae	1.987	8	***Melicope macropus***	2.339	8
Nesiota elliptica	1.975	9	***Blutaparon rigidum***	2.292	9
Dracaena umbraculifera *	1.943	10	***Hibiscadelphus woodii***	2.271	10
Melicope macropus	1.923	11	***Trilepidea adamsii***	2.261	11
Achyranthes atollensis	1.921	12	***Viola cryana***	2.255	12
Trilepidea adamsii	1.884	13	*Wikstroemia skottsbergiana* *	2.248	13
Blutaparon rigidum	1.866	14	***Achyranthes atollensis***	2.243	14
Hibiscadelphus woodii	1.846	15	***Melicope nealae***	2.193	15
Dryopteris ascensionis	1.831	16	***Dracaena umbraculifera*** *	2.185	16
Lepidium obtusatum	1.826	17	*Cyanea dolichopoda*	2.176	17
Melicope nealae	1.797	18	***Lepidium obtusatum***	2.136	18
Casearia quinduensis	1.765	19	***Melicope haleakalae***	2.133	19
Angraecum astroarche	1.763	20	*Cyanea minutiflora*	2.110	20

Table D.2 | The 20 lowest ranking plant species prioritised for de-extinction research efforts following the Ranking Procedures 1 and 2, also provided are the species' overall ranked de-extinction efficiency scores, *RE*. Species in bold appear in the 20 lowest ranked species using both ranking procedures.

Ranking Procedure 1			Ranking Procedure 2		
Species	Score	Rank	Species	Score	Rank
Galipea ossana	1.263	122	***Pausinystalia brachythyrsum***	1.268	122
Delilia inelegans	1.265	121	***Habenaria petromedusa***	1.289	121
Pausinystalia brachythyrsum	1.290	120	***Galipea ossana***	1.314	120
Xanthostemon sebertii	1.291	119	***Delilia inelegans***	1.323	119
Cupaniopsis crassivalvis	1.293	118	***Gomidesia cambessedeana***	1.338	118
Weinmannia spiraeoides	1.294	117	***Weinmannia spiraeoides***	1.352	117
Habenaria petromedusa	1.304	116	***Pradosia argentea***	1.352	116
Pradosia argentea	1.308	115	***Xanthostemon sebertii***	1.370	115
Campomanesia lundiana	1.316	114	***Cupaniopsis crassivalvis***	1.371	114
Gomidesia cambessedeana	1.319	113	***Stachytarpheta fallax***	1.384	113
Argocoffeopsis lemblinii	1.326	112	***Campomanesia lundiana***	1.428	112
Stenocarpus dumbeensis	1.332	111	***Stenocarpus dumbeensis***	1.432	111
Cyanea pycnocarpa	1.334	110	***Argocoffeopsis lemblinii***	1.435	110
Valerianella affinis	1.343	109	***Melicope obovata***	1.487	109
Psiadia schweinfurthii	1.348	108	*Cyanea cylindrocalyx*	1.490	108
Fitchia mangarevensis	1.352	107	***Cyanea pycnocarpa***	1.495	107
Kokia lanceolata	1.355	106	***Valerianella affinis***	1.504	106
Melicope obovata	1.359	105	*Sicyos villosus*	1.505	105
Hernandia drakeana	1.363	104	***Psiadia schweinfurthii***	1.507	104
Stachytarpheta fallax	1.368	103	***Kokia lanceolata***	1.511	103