Evaluating the role of zoos and *ex situ* conservation in global amphibian recovery

by

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Abstract

Amphibians are declining worldwide, and *ex situ* approaches (e.g. captive breeding and reintroduction) are increasingly incorporated into recovery strategies. Nonetheless, it is unclear whether these approaches are helping mitigate losses. To investigate this, I examine the conservation value of captive collections. I find that collections do not reflect the species of likeliest greatest concern in the future but that non-traditional zoos and conservation-focused breeding programs are bolstering the representation of threatened amphibians held *ex situ*. Next, I examine the reproductive success of captive breeding programs in relation to species' biological traits and extrinsic traits of the program. Based on 285 programs, I find that not all species are breeding in captivity, yet success is not correlated to the suite of tested predictors. Overall, *ex situ* approaches are playing a potentially important role in amphibian conservation, but we must work to improve the representation of threatened amphibians in zoos and husbandry expertise.

Keywords: Captive breeding; Frogs; Salamanders; Threat; Zoos

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Introduction

Amphibians (frogs, salamanders, and caecillians) are an integral part of our ecosystems and their loss might not only have cascading effects through the food web but could also translate into negative impacts for human beings (Cohen 2001). Amphibians have direct impacts on human health: vast medical knowledge has been derived from frog studies, they represent an important source of protein in some countries (Ribas and Poonlaphdecha 2017), and they eat large quantities of insects including disease vectors that can transmit fatal diseases to humans (e.g. mosquitoes and malaria) (Hagman and Shine 2007). Nonetheless, current extinction rates are four orders of magnitude higher than background, and average extinction rates observed during 1971-2000 suggest that about 7% of anuran (frog) species may be lost within the next century (Alroy 2015). The causes of these declines are complex, and involve a combination of habitat loss, pollution, disease, over-harvesting, invasive species, and possibly climate change (McGregor Reid and Zippel 2008).

The Convention of Biological Diversity (CBD) and the International Union for the Conservation of Nature (IUCN) recognize that it will take more than field conservation efforts to conserve species in dire situations and that management of natural habitats will need to be combined with *ex situ* approaches (Conway 1995, McGowan et al. 2017). *Ex situ* approaches consist of management strategies under which individuals are maintained in artificial conditions under different selection regimes than those in natural conditions and include activities such as captive breeding, translocation and reintroduction programs, or head starting efforts (a technique that involves raising early-stage animals in captivity before releasing them to the wild) (McGowan et al. 2017). These activities can take place within or outside of the species' geographical range, but in a controlled or modified environment (IUCN/SSC 2014). *Ex situ* activities are absent or play a minor role in most classic conservation organizations, therefore a lot of these activities have been spearheaded by zoos (Conde et al. 2011).

At the same time, an increasing number of modern zoos have shifted institutional focus from simply keeping animals in captivity to a real commitment to conservation programs (Mallinson 2003). Partly in response to public criticism, captive breeding has become a central justification for exhibiting animals to the public, with the rationale being

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that animals kept in zoos and aquariums are ambassadors for their species and through reproduction, can serve as an insurance measure against extinction in the wild (McGregor Reid and Zippel 2008). The World Association of Zoos and Aquaria (WAZA) has publicly committed to align its activities with the goal of "improving the status of biodiversity by safeguarding ecosystems, species, and genetic diversity" (Barongi et al. 2015). Captive breeding is also being used as a recovery strategy for species in Canada: in total there are 33 federally listed species at risk whose recovery strategy references the involvement of zoos, and of these, six of them include a current captive breeding component (Olive and Jansen 2017).

One way for zoos to efficiently safeguard biodiversity is by expending resources on the conservation of small-bodied vertebrates such as amphibians (Balmford et al. 1995, McGregor Reid and Zippel 2008). Because of minimal space requirements and low costs, keeping more amphibians in zoos could increase the number of threatened species managed in population management plans overall (Amphibian TAG Regional Collection Plan; Barber and Poole 2014; Conde et al. 2015). Additionally, amphibians have been successfully reintroduced before: 25% of wild Mallorcan midwife toads (*Alytes muletensis*) are the product of a successful zoo breeding and reintroduction program, while the Kihansi Spray Toad (*Nectophrynoides asperginis*) would still be extinct in the wild if it weren't for reintroduction efforts by zoos (Krajick 2006, McGregor Reid and Zippel 2008). While reservations have been expressed on the utility of breeding amphibians in captivity (see e.g. Pounds et al. 2007), a lack of reserve area and the fact that even pristine areas may contain rapidly declining populations of chytrid-infected frogs may make field conservation efforts ineffective when used in isolation (McGregor Reid and Zippel 2008). The only realistic hope for some populations and species is veterinary treatment, population maintenance, and conservation breeding *ex situ* (McGregor Reid and Zippel 2008).

Zoos and other *ex situ* institutions have the space and expertise to contribute meaningfully to conservation activities (Olive and Jansen 2017), but are *ex situ* activities currently effective approaches for amphibian conservation? To help answer this, this thesis will first consider the conservation value of current amphibian *ex situ* collections by assessing the representation of threatened species in zoos and related institutions. Using databases of global amphibian captive holdings, I compare species held in zoos to their closest relatives not held in zoos in terms of ecological and biogeographical

indicators of threat. This matched-pair design allows to evaluate biases in captive collections and to examine to what extent zoos are housing species of current and future concern. As might be expected, zoos encompass more than just species bred for conservation purposes and species involved in captive breeding programs are not held exclusively within zoos but also in specialist captive breeding facilities run by government or non-government agencies (Harding et al. 2016). Thus, I perform a second complementary analysis using identical methodology but a completely new dataset of species being bred specifically for conservation breeding programs. Together these two analyses provide knowledge of the representation of threatened amphibians being managed *ex situ*.

Next I evaluate the outcome of captive breeding programs measured in terms of their success in producing viable offspring. Not all amphibian species thrive or reproduce in captive environments (Tapley et al. 2015), and using data from 285 captive breeding programs, I evaluate the intrinsic and extrinsic factors that may explain underlying patterns in captive breeding success. While getting a species to breed in captivity is only one measure of success and does not directly relate to conservation outcomes in the wild, it is an essential to achieving insurance populations that could be used for reintroduction in the future.

Collectively, these analyses expose biases in captive collections and identify correlates of successful captive breeding programs. This information not only provides insight into the current state of *ex situ* management of amphibians, but it can also be used to help prioritize for future breeding programs and maximize the conservation outcome of *ex situ* efforts for this imperilled group.

Chapter 1.

Representation of threatened amphibians *ex situ*[1]

Abstract

Ambitious global conservation targets have been set to manage increasing threats to amphibians. *Ex situ* institutions (broadly, "zoos") are playing an expanding role in meeting these targets. Here, we examine the extent to which zoos house species representing the greatest overall conservation priority by testing how eight variables relating to extinction risk - IUCN status, habitat specialization, obligate stream-breeding, geographic range size, body size, and island, high-altitude and tropical endemism - vary between amphibian species held in zoos and their close relatives not held in zoos. Based on 253 species found in zoos that could be confidently paired with close relatives not in zoos, and in contrast to reported patterns for birds and mammals, we find that amphibians currently held in zoos are equally as threatened as their close relatives not found in zoos. This result is entirely driven by the inclusion of data on species holdings from Amphibian Ark (AArk), an organization that helps to coordinate conservation activities in many 'non-traditional' institutions, as well as in 'traditional' commercial zoos. Such networks of small non-traditional institutions thus make meaningful contributions to *ex situ* conservation, and the establishment of other taxa-specific organisations modelled on AArk might be considered. That said, our results indicate that the *ex situ* network is still not prioritizing range-restricted habitat specialists, species that possess greater overall extinction risk in the near future. We strongly encourage zoos to continue increasing their holdings of amphibian species, but to pay greater attention to these species of particular conservation concern.

Key Words: Amphibians, Biogeography, Ex situ conservation, Extinction, Species pairs, Zoos

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[1] A version of this chapter was published as the feature article of the journal Animal Conservation in April 2017 (see Biega et al. 2017) with the following authors: A. Biega, D. A. Greenberg, A. O. Mooers, O. R. Jones & T. E. Martin. I was responsible for project management; A. Mooers and T. Martin were responsible for project conception and design; data collection was done by myself, D. Greenberg, T. Martin, and O. Jones; analyses were performed by myself, D. Greenberg, and T. Martin and writing was done in collaboration by myself, D. Greenberg, A. Mooers, and T. Martin.

Introduction

Amphibians are the most imperilled Class of vertebrates, with at least one third of extant species classified as threatened with extinction (Hoffmann et al. 2010) and 42% of species having experienced recent population declines (Stuart et al. 2004, Wake and Vredenburg 2008, Whittaker et al. 2013). These contemporary extinction rates are four orders of magnitude higher than natural background rates for anurans (Alroy 2015). It is unlikely that this situation will improve without immediate and effective conservation initiatives.

One such initiative was the 2007 Amphibian Conservation Action Plan (ACAP), a guide for implementing global amphibian conservation and research (Gascon et al. 2007). Because of the difficulty in rapidly mitigating particular extinction drivers, namely habitat loss and degradation (even within protected areas; Curran 2004), accelerating effects of climate change (Foden et al. 2013), and the spread of emerging infectious diseases (Olson et al. 2013), two of the 11 chapters within the ACAP focus on the importance of *ex situ* conservation (i.e. captive breeding and reintroduction). The Amphibian Ark (AArk) (Amphibian Ark 2017) was subsequently initiated to address the captive components of the ACAP and, in particular, to focus on species thought most difficult to safeguard *in-situ* (Zippel et al. 2011). More specifically, AArk helps advise and coordinate regional and global amphibian *ex situ* efforts while facilitating the prioritization of amphibians through their Conservation Needs Assessments (Amphibian Ark 2017). AArk maintains its own records of the institutions managing threatened species. These include smaller specialist institutions, often located within developing, high-biodiversity countries within the tropics.

Although exceptions exist (cf. Tapley et al. 2015), amphibians are generally highly suitable for *ex situ* conservation measures. They are small, relatively inexpensive to keep, and usually cope with captivity, both physiologically and behaviourally, better than do some other taxa (Bloxam and Tonge 1995, Balmford et al. 1996, Conde et al. 2015). *Ex situ* amphibian programs are also expanding: while Conde et al. (2011) estimated that only 4% of amphibian species were held in captivity worldwide at the turn of the decade (versus 25% of bird species and 20% of mammal species), Harding et al. (2016) reported a 57% increase in the number of amphibian species involved in captive breeding and reintroduction programs since the launch of the ACAP in 2007, and

Dawson et al. (2016) reported a near-doubling of *ex situ* holdings of amphibians from 1994-2014 to a total of 10.9%; this latter figure is more than double the total number of species reported by Conde et al. (2011), just four years prior. It is clear that *ex situ* institutions are playing an increasingly important role in the global conservation strategy for amphibians.

But is the growing number of amphibian species held *ex situ* representing the species of greatest conservation priority? While raw counts and proportions of International Union for the Conservation of Nature (IUCN)-listed species held in zoos have been reported (see Conde et al., 2011, Dawson et al. 2016), no existing research examines representation with respect to ecological and biogeographical indicators of threat, nor whether the emerging role of non-traditional institutions in *ex situ* conservation has affected representation of threatened amphibians across the global *ex situ* network.

Using a phylogenetically-controlled matched-pair design similar to a previous study of birds and mammals (Martin et al. 2014a), we investigate how variables correlated with extinction risk are related to the likelihood of amphibian species being held in zoos. We contrast 'in-zoo' species identified using the Zoological Information Management System (ZIMS; Species 360, 2015) and AArk (2015) databases with 'not-in-zoo' close relatives across a set of candidate predictors analyzed both individually and in multivariate logistic regressions. While the ZIMS dataset is the largest database regarding *ex situ* species holdings for regionally or nationally accredited zoos (including those accredited by the World Association of Zoos and Aquariums, the Association of Zoos and Aquariums, and the European Association of Zoos and Aquaria - WAZA, AZA and EAZA respectively), the AArk database includes species holdings from a number of institutions that are not part of a zoo association. In order to both compare the patterns in amphibians with those previously reported for birds and mammals, and to evaluate the specific effects of these non-traditional institutions identified by AArk, we compare two datasets: an 'All Institutions' dataset, comprised of species in either or both the ZIMS and AArk databases, and a ZIMS dataset. These comparisons allow us to evaluate the extent to which (a) current *ex situ* representation of amphibians aligns with species representing the most urgent global conservation priorities and (b) whether the efforts and coordination of AArk have influenced this representation.

Materials and methods

Our basic statistical approach is outlined in Figure 1: the method was first suggested by Felsenstein (1985) and is also the one used in an earlier paper that considered birds and mammals (Martin et al. 2014a). We first listed all amphibian species indicated as being held in captivity in the ZIMS and AArk databases, which summarised holdings from 516 institutions globally (of which 33 were found exclusively in Amphibian Ark institutions). From these institutions we tallied a total of 532 'in-zoo' species. To test how extinction risk varies between amphibian species involved held in zoos and their close relatives not in held in zoos, we then identify, on a phylogenetic tree, independent pairs of species that differ in a character of interest (here, contrasting in-zoo vs. not-in-zoo), and then to ask how members of each pair differ in other characters (e.g. IUCN status or range size). Because each pair (or "contrast") is phylogenetically independent of others, we can perform statistical tests (e.g. sign tests) and, using the phylogeny, construct phylogenetically corrected linear models in a multi-model inference framework (Ives and Garland 2010). This allows us to investigate which variables are most important in explaining the likelihood of a species to be held in a zoo. In total we were able to pair 253 in-zoo species with their closest relatives not in a zoo to produce 219 independent contrasts. Our complete dataset is provided as Supplementary Material (Appendix A).

Species pair construction

Species in zoos were matched to their closest relatives (i.e. those with the smallest patristic distance) not involved in a zoo using the phylogenetic hypothesis from Pyron and Wiens (2011), with an updated taxonomy (Frost 2014). A total of 459 species could be directly placed while a further 18 species were added to the phylogeny by placing the species as congeners with fewer than five species present on the tree, our cut-off for composite comparisons (see below). A total of 55 species could not be confidently placed on the tree and were dropped from further analysis, leaving 477 candidate in-zoo species.

These species were then matched with their closest 'not-in-zoo' relatives on the phylogeny (i.e. those with the smallest patristic distance) to create an in-zoo to not-in-zoo contrast (contrast A in Figure 1.1). Given that this phylogenetic tree is incomplete,

we further examined all contrasts where the two species involved belonged to two separate genera. For each of these contrasts, we checked the taxonomy of Frost (2014) and, if we found another species within the genus of the in-zoo species with data on at least four of our eight variables (see below), we added it to the phylogenetic tree to replace the original contrast. In many cases, a clade of several in-zoo species shared the same not-in-zoo closest relative, or was matched with a clade of between two and five not-in-zoo species. In these cases, in-zoo and/or not-in-zoo species were grouped to produce "composite" species for the contrast (contrast B in Figure 1.1). For these species composites, we used mean values for continuous variables and modal values for categorical variables. Where no modal value could be determined, we discarded that variable from further analysis.

In a final step, we retained all contrasts that (1) were true sister clades (i.e. we dropped paraphyletic contrasts and so several in-zoo candidates, depicted as an in-zoo species with no contrast in Figure 1.1); (2) included species that had data for at least four of our eight scoring variables and (3) had five or fewer species in either of the two sister clades involved in the contrast (species in monotypic genera could still be involved in a contrast if they could be paired with a sister clade involving five or fewer species).

Selection and scoring of variables

We scored each species for eight variables known to relate to extinction risk. Our scoring variables were as follows:

IUCN threat score. We scored a species as 'threatened' if it was classified as Data Deficient, Vulnerable, Endangered, Critically Endangered or Extinct in the Wild in the IUCN (2015) species accounts. Data Deficient species were classified as threatened because they face, on average, greater conservation risks than fully assessed amphibians (Howard and Bickford 2014). If zoos are selecting species based on conservation need, then species held in zoos will be more threatened than close relatives not held in zoos, given threatened species implicitly represent a greater conservation priority.

Habitat breadth. We quantified habitat breadth by counting the total number of suitable habitats listed for each species based on the IUCN (2015) habitat classification

scheme. Habitats listed with 'marginal' and 'unknown' suitability were excluded from these counts. If zoos are selecting species based on conservation need, then species held in zoos will have a narrower habitat breadth (i.e. they are more specialized) than their closest relatives not held in zoos, based on the observation that a high degree of habitat specialization, and the associated low ecological tolerances and adaptability, directly correlate with extinction risk in amphibians (Williams & Hero, 1998).

Stream obligate status. We scored a species as 'stream obligate' if it was listed under the 'stream, river, or creek' habitat classification (coded as 5.1 for permanent habitats and 5.2 for temporary habitats) as its sole aquatic habitat by the IUCN (2016). If zoos are selecting species based on conservation need, then species held in zoos will be more reliant on stream habitats than their close relatives not held in zoos, given that dependence on riparian habitats has been identified as one of the key correlates of amphibian threat status (Lips et al. 2003, Stuart et al. 2004), species in these habitats being particularly prone to infection by emerging diseases (Kriger and Hero 2007).

Geographic range size. Geographic range sizes in km^2 were calculated for each species in our sample in R *v 3.3.3* (R Core Team 2015) using georeferenced spatial polygons depicting the current known distribution of the species within its native range. These polygon shapefiles for each species are freely available for download from the IUCN (2015). If zoos are selecting species based on conservation need, then species held in zoos will possess smaller geographic ranges than close relatives not in zoos, given that range-restricted amphibians are at greater risk of global extinction (Sodhi et al. 2008), and are inherently more at risk from localised habitat destruction and fragmentation (Pimm et al. 1995, Purvis et al. 2000).

High-altitude endemism. We scored a species as a high-altitude endemic if the IUCN (2015) species accounts listed it as living exclusively above 1000 m altitude. This 1000 m criterion based on delimitations of high altitude life-zones defined in Spehn & Körner (2005). If zoos are selecting species based on conservation need, then montane species will be better represented in zoos than non-montane close relatives given that high-altitude amphibian species face increased risks from infectious diseases (Lips et al. 2003) and climate change (Pounds et al. 1999).

Island endemism. We scored a species as being an island endemic if it occurred exclusively on island ecosystems based on IUCN (2015) range maps. If zoos are selecting species based on conservation need, then island endemic amphibians will be better represented in zoos than non-island close relatives, given that island endemics inherently possess restricted spatial ranges (see above), and the biogeographically isolated nature of these endemics often enhances extinction risk (Fordham and Brook 2010).

Tropical endemism. A species was scored as a 'tropical endemic' if it occurred exclusively within one or more of the three major tropical zoogeographic regions (Neotropical, Afrotropical, and Oriental zones; Cox 2001), based on IUCN (2015) range maps. If zoos are selecting species based on conservation need, then species restricted entirely to tropical zoogeographical zones will be better represented in zoos than non-tropical close relatives, given that tropical species face greater environmental pressures and higher extinction risks, on average, than temperate species (Vamosi and Vamosi 2008).

Body size. We obtained body size measurements from a comprehensive amphibian life history dataset (Oliveira et al. 2017) and augmented this with data from the literature and from an authoritative online database (Amphibiaweb 2015). Snout-vent lengths were used for Anurans while total body length was used for Caudates and Caecillians. If zoos are selecting species based on conservation need, then species held in zoos may be larger than close relatives not held in zoos given (i) the weak positive correlation between body size and extinction risk in amphibians (Lips et al. 2003, Sodhi et al. 2008) and (ii) its known influence on species selection for zoos in other groups (Balmford et al. 1995, Martin et al. 2014a).

Statistical analysis

To ensure the sample of in-zoo species used in our paired analysis was representative of all species held in zoos, we first completed a series of Z-tests (Zar 1999) comparing the mean scores of all variables for the 253 species in our sample with the 532 species on our original in-zoo list. Species in these tests were grouped by taxonomic Order. Next we determined differences between our in-zoo and not-in-zoo species pairs for our two datasets (All Institutions and ZIMS). Differences for binary

variables (threat status, stream obligate status, and the three measures of endemism) were assessed using simple sign tests (Zar 1999), while differences for continuous variables (habitat breadth, spatial range, and body size) were assessed using randomization tests. These randomization tests evaluated the average difference in our matched-pair comparisons against the null distribution produced by randomizing observed differences with an equal probability of being positive or negative 10,000 times (Felsenstein 1985). This created an expected distribution of differences under the assumption of no predictive power of in-zoo status for the contrast. The average observed difference for each variable could then be compared to its null distribution to determine its significance.

Finally, we investigated which variables were most important in explaining the likelihood of being held in *ex situ* institutions using a multi-model inference approach comparing models that included different combinations of all eight variables. As with the univariate analyses, we examined this across (i) the All Institutions dataset, and (ii) across species held in ZIMS institutions. We modelled the probability of a species being in a zoo (1 or 0) using phylogenetic logistic regression to account for phylogenetic autocorrelation in traits (Ives and Garland 2010). We compared all species used in the contrasts, but allowed each species to be assessed independently rather than using modes or averages of traits for contrasts composed of several species. This resulted in an All Institutions dataset of 556 species (253 in zoos, and 303 out of zoos). To facilitate the valid comparison of all factors, we removed species missing any of the eight scoring variables, resulting in a final dataset of 536 species (246 in zoos, and 290 out of zoos). The ZIMS dataset contained 468 species (216 in zoos, 252 out of zoos). All fitted values of Pagel's λ were statistically indistinguishable from 0 (all values of $p > 0.05$) for every phylogenetic logistic regression model, as expected given our selection of paired sister species on the phylogeny. We therefore analyzed the same fully-factorial models as standard generalized linear models with a Bernoulli error distribution to obtain Akaike Information Criterion (AIC) values for models, which allowed us to perform model selection and quantify the importance of each explanatory variable based on cumulative AIC weights. We compared all possible model combinations and used model selection based on AIC to assess which combination of factors best explained the probability of being held in zoos. Given that some of the explanatory variables are used as criteria for IUCN Red List status classification (e.g. range size; see Categories and Criteria *v3.1*

IUCN 2015), we checked for correlation between all 8 explanatory variables and ran models a second time without IUCN Red List status to address the problem of collinearity. Phylogenetic logistic regression models were fitted using the 'binaryPGLMM' function in the package 'ape' (Paradis et al. 2004) in R *v. 3.2.2*. Model selection results, including the five most parsimonious models and model averaged variable coefficients for each data set are available in Tables 1.2 & 1.3.

Results

Z-tests demonstrated that our sample of in-zoo species were representative of all species in their respective Orders for all variables in all analyses with one exception - body size for Caudata in the All Institutions analysis and the ZIMS analysis ($p < 0.05$ for both datasets). This was due to the presence of the two in-zoo giant salamanders (genus *Andrias*), which were dropped from the main analyses because they could not be paired with not-in-zoo close relatives. Given only two atypical outliers, we included body size for the other Caudata in further analyses.

All our contrast results are presented in Table 1.1. When all institutions are considered, we found no significant differences in threat status, high-altitude endemism, island endemism, or tropical endemism between species held in zoos and their close relatives not held in zoos (all $p > 0.05$), while stream obligates tended not to be found in zoos ($p < 0.07$). In contrast, when we considered the ZIMS subset, species held in ZIMS institutions are *less* likely to be considered threatened than their close relatives not held in zoos ($p=0.05$). All other categorical variables showed no difference for species held in ZIMS associated institutions.

For both the All Institutions and the ZIMS tests, in-zoo species were significantly larger ($p < 0.001$ for both datasets), had significantly larger geographic range sizes ($p < 0.001$ for both datasets) and broader habitat breadths ($p < 0.001$ for both datasets) than their close not-in-zoo relatives. Considering all institutions, in-zoo species are on average 13.5% larger, occupy a geographic range three and a half times the size, and occur in 27% more habitats than their not not-in-zoo close relatives. For ZIMS species, the average differences were even greater: 13.9% larger body size, over four times larger geographic range size, and 35% broader habitat breadths.

Some correlation was found between IUCN Red List status and geographic range size (r=0.68), although when models were run without IUCN Red List status, our results are consistent and our interpretations remain the same. We found that correlations between all other predictor variables were weak or moderate (all r < 0.7), indicating that our interpretation of results should be straightforward. Multi-model inference across the All Institutions dataset and for species in the ZIMS database indicated similar sets of the most parsimonious models. For both, top models suggested that a larger habitat breadth (p = 0.062, All; p = 0.017, ZIMS), larger geographic range (p < 0.001 for both) and higher threat (p = 0.001; p = 0.025) all increased the probability of being held *ex situ* (Table 1.2 & 1.3). However, differences emerged in the ranked importance of variables (averaged across models) predicting the probability of being held *ex situ* across these two datasets. While geographic range size and IUCN threat status were the two most highly weighted predictors for the All Institutions dataset, habitat breadth was more important than threat status for the ZIMS dataset (Table 1.1).

Discussion

Consistent with patterns for birds and mammals (Martin *et al.,* 2014a), amphibian species held in zoos are significantly larger-bodied, possess larger geographic ranges, and are more generalist in their habitats than their not-in zoo counterparts. Importantly, however, and in contrast to patterns for birds and mammals (Martin et al. 2014a), amphibians currently held in zoos are equally as threatened as their close out-of-zoo relatives. This result is driven by the relatively small number of amphibian captive breeding programs in 'non-traditional' zoos, which are not recorded in the ZIMS database; when species found only in these institutions are removed, amphibians in zoos are *less* threatened than their out-of-zoo close relatives (Table 1.1).

This contrast has two main implications. First, as with larger-bodied taxa, the 'traditional' zoo network is keeping amphibian species for reasons additional to threat status (Bowkett 2014). These additional reasons may relate to the other variables examined in this study: Table 1.1 indicates that biases towards keeping larger bodied, more widely distributed, and less habitat-specific species in zoos all become more pronounced when only ZIMS institutions are considered. This may relate to zoos finding generalist species easier and cheaper to hold in captivity than closely-related specialists; if such pairs of species are otherwise equally appealing to zoo visitors, it would be

logical for zoos to select the species with fewer husbandry requirements (Martin et al. 2014a). Indeed, zoos may also actively choose to keep species of low conservation concern in order to learn husbandry techniques that can be applied to holding threatened relatives in the future (K. Johnson, pers. comm). A future study tailored specifically to contrast species in conservation breeding programs with their close relatives might reveal the traits associated with amenability to captive breeding. This and other potential drivers of *ex situ* selection for amphibians (e.g. coloration and activity cycles) might be interesting avenues for further comparative research.

Although high-altitude, island, and tropical endemism are all considered to be important factors for predicting future threat status, species held in zoos are not more likely to have these traits. In contrast, species that rely on streams for breeding habitat are marginally less likely to be in *ex situ* programs. This may be noteworthy, given that many stream-associated amphibians are purported to be at a higher risk of extinction (Lips et al. 2003, Stuart et al. 2004). However, given that closely-related species tend to share many of these traits, leading to few contrasts and so low power using our approach, other analytical methods may be needed to explore these issues further.

The second implication of these patterns is that it is a relatively small number of institutions peripheral to the main zoos network, but highlighted by the AArk database, that are bolstering *ex situ* threatened amphibian representation. While ZIMS institutions are mostly (albeit not 100% restricted to) 'traditional' zoos and aquaria, these 'non-traditional' institutions include specialist breeding centres, university departments and botanical gardens (and even a nunnery). Many of these institutions are also located within high-biodiversity countries in the tropics, which allows better integration of *ex situ* and *in situ* conservation strategies, reduces the risk of the transfer of novel pathogens from other species from outside the range distribution of the species, reduces acclimatisation issues for captive species, and increases the ability to obtain species for *ex situ* breeding without having to navigate difficult international administrative and veterinary barriers (Conde et al. 2011, Martin et al. 2014b). The important positive effect of AArk includes the support and coordination of these specialized institutions, allowing them to be integrated into and make meaningful contributions to the global *ex situ* community. AArk serves to highlight and recommend priority species for *ex situ* rescue or research, but of course, such recommendations are only one step: zoos are fettered by multiple goals and so multiple selection criteria (Fa et al. 2014). Despite obvious

barriers (e.g. the costs associated with breeding large mammals ethically and sustainably *ex situ*) the establishment of other taxa-specific organisations modelled on AArk to help coordinate *ex situ* management of threatened species in less well known and less centralised institutions should be considered.

We conclude by highlighting that even with the inclusion of institutions outside the ZIMS database, *ex situ* programs as a whole are still not targeting those amphibian species predicted as being most at risk both imminently and in the near future, namely range-restricted habitat specialists. We therefore encourage all zoos to continue to increase their conservation-focussed amphibian species holdings to help meet the ambitious ACAP targets, and to do so using strategic planning efforts that include multiple facets of conservation need. Given the previously discussed benefits of establishing *ex situ* programs within the home range of target species, we also encourage North American and European zoos (where the majority of breeding programs still occur) to establish more collaborative projects with institutions within the tropics, as has recently been achieved for several threatened species in Honduras (HARCC 2016). We acknowledge that zoos play other important roles in species conservation besides keeping threatened species (Bowkett 2014, Moss et al. 2015), and that simply holding endangered species in captivity is not in itself a mark of conservation success (Harding et al. 2016) nor a guarantee of a successful breeding programme for all species held (Tapley et al. 2015). However, it is often a vital first step.

Acknowledgements

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[2] A version of this chapter was published in the April 2017 issue of *Animal Conservation* (see Biega et al. 2017), along with three scholarly commentaries (Canessa 2017, Griffiths 2017, Tapley et al. 2017) that highlight alternate means of interpreting *ex situ* conservation success and raise questions for further consideration. You can read these commentaries along with our response (Martin et al. 2017) in *Animal Conservation*.

Chapter 2.

Representation of threatened amphibians in conservation breeding programs[3]

Abstract

Conservation breeding and reintroduction programs are increasingly necessary management tools in light of rapid global amphibian declines. Here we examine whether these conservation initiatives are targeting species at the greatest risk of extinction. We compare conservation need of species involved in conservation breeding programs (CBPs) to their closest relatives not involved in such programs using eight variables related to immediate and future extinction risk. We find that species in CBPs are more likely to be threatened and equally range-restricted and specialized as their closest relatives not bred for conservation purposes. This is good news for amphibians; suggesting that in contrast to patterns reported for zoo holdings more generally, these conservation initiatives target species representing short and medium-term conservation priorities.

Keywords: Anura, Captive breeding, *Ex situ* conservation, Frog, Reintroduction, Salamander, Threat

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[3] This chapter was published in the journal Oryx (see Biega and Martin 2017) with the following authors: A. Biega and T. E. Martin. I conceived the project and collected the data. T. Martin and I analyzed the data and wrote the paper.

Introduction

In the face of the predicted global amphibian extinction crisis (Whittaker et al. 2013), the Amphibian Conservation Action Plan acknowledges that the best hope for some high-risk species is the establishment and management of captive populations (Gascon et al. 2007, Wren et al. 2015). Captive breeding, head-starting (a technique that involves raising early stage amphibians in captivity before releasing them to the wild), and reintroduction programs (collectively *ex situ* conservation) are increasingly important management tools, both as insurance policies for species at risk in the wild and in reintroducing individuals to ecosystems where they have declined or been extirpated (Gascon et al. 2007). Indeed, the number of *ex situ* programs has expanded rapidly in recent years: Harding et al. (2016) reported a 57% increase in amphibian species involved in conservation breeding and reintroduction programs since 2007, and Biega et al. (2017) listed 532 amphibian species (7% of all species) held *ex situ*, compared to 4% five years earlier (Conde et al. 2011).

However, amphibians held *ex situ* are not always those with the greatest conservation need (Dawson et al. 2016). Biega et al. (2017) reported that, although amphibians held in zoos are as threatened as their close relatives not found in zoos, the former occupy a broader range of habitats and possess larger spatial ranges than their wild counterparts. Given that range-restricted specialist amphibians may face the greatest short-term extinction risk (e.g. Sodhi et al. 2008), this bias may be problematic.

Of course, there may be meaningful differences between species simply held in zoos and those involved in Conservation Breeding Programs (CBPs). The *ex situ* conservation organization Amphibian Ark (2017) helps ensure the suitability of species and institutions selected for CBPs through its Conservation Needs Assessment and Program Implementation tool, and zoos often select species for breeding programs on the basis of recommendations from regional Amphibian Taxon Advisory Groups (Barber and Poole 2014). Characteristics of CBPs include research on species biology to inform conservation efforts, captive assurance colonies, educational exhibits, and species destined for reintroduction or wild-to-wild translocations (including head-starting programs) (Harding et al. 2016). While zoos house species for reasons other than threat (Bowkett 2014), and must consider cost, husbandry requirements, and visitor-appeal (Tapley et al. 2015), species targeted for CBPs often (albeit not always) face imminent

threats in the wild (Conde et al. 2011). Therefore, it would be useful to differentiate between species held in zoos and those actively involved in CBPs.

We investigate this issue here. We follow an identical methodology to Biega et al. (2017), but use a new dataset comprising solely of species currently bred for conservation purposes (i.e., not for medical reasons or general display in zoos) or involved in head-starting programs. We test how the same eight variables relating to extinction risk - IUCN status, habitat specialization, obligate stream-breeding, geographic range size, body size, and island, high-altitude and tropical endemism - vary between amphibian species involved in CBPs and their close relatives not in CBPs. This analysis allows us to evaluate how species involved in CBPs compare to *ex situ* holdings more generally, and how well CBPs are targeting species of both immediate and future conservation concern.

Methods

Our methods follow those previously described in Biega et al. (2017), but are applied here to focus specifically on species involved in CBPs. We explain these methods again below for the ease of the reader.

We first compiled a list of species in CBPs using the same list and criteria as presented by Harding et al. (2016). This comprised of 213 species involved in CBPs up to the end of 2013, 77 of which were initiated after 2007 (Harding et al. 2016). To test how extinction risk varies between amphibian species involved in CBPs and their close relatives not in CBPs, we then identify, on a phylogenetic tree, independent pairs of species that differ in the character of interest (here, contrasting in-CBP vs. not-in-CBP), and then examine how members of each pair differ with regard to extinction risk (Figure 2.1). Because each pair (or contrast) is phylogenetically independent of every other pair, we can perform statistical tests (e.g. sign tests) and, using the phylogeny, construct phylogenetically corrected linear models in a multi-model inference framework (Ives and Garland 2010). This allows us to investigate which variables are most important in explaining the likelihood of a species to be involved in a CBP. In total we were able to pair 130 species in CBPs with their closest non-CBP relatives to produce 111 independent contrasts. Our complete dataset is provided as Supplemental Material (Appendix A).

Species pair construction

Species in CBPs were matched to their closest relatives (i.e. those with the smallest patristic distance) not involved in CBPs using the phylogenetic hypothesis from Pyron & Wiens (2011), with an updated taxonomy (Frost 2014). Congeners not in CBPs may or may not be held in a zoo. In the case where species were not found on the tree, they were added to the phylogeny if they had five or fewer congeners present on the tree, our cut-off for composite comparisons (see below). In many cases, a clade of several species in CBPs shared the same closest relative not in a CBP, or was matched with a clade of between two and five species not in CBPs. In these cases, in-CBP and/or not-in-CBP species were grouped to produce "composite" species for the contrast. For these species composites, we used mean values for continuous variables and modal values for categorical variables. Where no modal value could be determined, we discarded that variable from further analysis.

In a final step, we retained all contrasts that (1) were true sister clades (i.e. we dropped paraphyletic contrasts; (2) included species for which there were data for at least four of our eight scoring variables and (3) had five or fewer species in either of the two sister clades involved in the contrast (species in monotypic genera could still be involved in a contrast if they could be paired with a sister clade involving five or fewer species).

Selection and scoring of variables

We scored each species for eight variables known to relate to current and future extinction risk:

IUCN threat score. We scored a species as 'threatened' if it was classified as Data Deficient, Vulnerable, Endangered, Critically Endangered or Extinct in the Wild in the IUCN (2016) species accounts. Data Deficient species were classified as threatened because they face, on average, greater extinction risk than fully assessed amphibians (Howard and Bickford 2014). If CBPs are selecting species based on conservation need, then species involved in CBPs will be more threatened than close relatives not involved in CBPs given threatened species implicitly represent a greater conservation priority.

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Habitat breadth. We quantified habitat breadth by counting the total number of suitable habitats listed for each species based on the IUCN (2016) habitat classification scheme. Habitats listed with 'marginal' and 'unknown' suitability were excluded from these counts. If CBPs are selecting species based on conservation need, then species involved in CBPs will have a narrower habitat breadth (i.e. they are more specialized) than their closest relatives not involved in CBPs, based on the observation that a high degree of habitat specialization, and the associated low ecological tolerances and adaptability, directly correlate with extinction risk in amphibians (Williams and Hero 1998).

Stream obligate status. We scored a species as 'stream obligate' if it was listed under the 'stream, river, or creek' habitat classification (coded as 5.1 for permanent habitats and 5.2 for temporary habitats) as its sole aquatic habitat by the IUCN (2016). If CBPs are selecting species based on conservation need, then species involved in CBPs will be more reliant on stream habitats than their close relatives not involved in CBPs, given that dependence on riparian habitats has been identified as one of the key correlates of amphibian threat status (Lips et al. 2003, Stuart et al. 2004), species in these habitats being particularly prone to infection by the fungal disease, chytridiomycosis (Kriger and Hero 2007).

Geographic range size. Geographic range sizes in km^2 were calculated for each species in our sample in R *v 3.3.3* (R Core Team 2015) using georeferenced spatial polygons depicting the current known distribution of the species within its native range. These polygon shapefiles for each species are freely available for download from the IUCN (2016). If CBPs are selecting species based on conservation need, then species involved in CBPs will possess smaller geographic ranges than close relatives not involved in CBPs, given that range-restricted amphibians are at greater risk of global extinction (Sodhi et al. 2008), and are inherently more at risk from localized habitat destruction and fragmentation (Pimm et al. 1995, Purvis et al. 2000).

High-altitude endemism. We scored a species as a high-altitude endemic if it was recorded by the IUCN (2016) species accounts listed it as living exclusively above 1000 m altitude. This 1000 m criterion based on delimitations of high altitude life-zones defined in Spehn & Körner (2005). If CBPs are selecting species based on conservation need, then montane species will be better represented in CBPs than non-montane close

relatives given that high-altitude amphibian species face increased risks from infectious diseases (Lips et al. 2003) and climate change (Pounds et al. 1999).

Island endemism. We scored a species as being an island endemic if it occurred exclusively in island ecosystems based on IUCN (2016) range maps. If CBPs are selecting species based on conservation need, then island endemic amphibians will be better represented in CBPs than non-island close relatives, given that island endemics inherently possess restricted spatial ranges (see above), and the biogeographically isolated nature of these endemics often enhances extinction risk (Fordham and Brook 2010).

Tropical endemism. A species was scored as a 'tropical endemic' if it occurred exclusively within one or more of the three major tropical zoogeographic regions (Neotropical, Afrotropical, and Oriental zones; Cox 2001), based on IUCN (2016) range maps. If CBPs are selecting species based on conservation need, then species restricted entirely to tropical zoogeographical zones will be better represented in CBPs than non-tropical close relatives, given that tropical species face greater environmental pressures and higher extinction risks, on average, than temperate species (Vamosi and Vamosi 2008).

Body size. We obtained body size measurements from Biega et al. (2017), which in turn largely sourced data from a comprehensive amphibian life-history dataset (Oliveira et al. 2017), further augmented by data from the wider literature (see Supplemental Material for all literature sources used). Snout-vent lengths were used for Anurans and total body length was used for Caudates and Caecillians. We hypothesize that species held in CBPs will be larger than close relatives not held involved in CBPs given (i) the weak positive correlation between body size and extinction risk in amphibians (Lips et al. 2003, Sodhi et al. 2008) and (ii) biases towards larger bodied species found in *ex situ* holdings for other taxa (Martin et al. 2014a).

Statistical analysis

To ensure the sample of CBP species used in our paired analysis was representative of all species involved in CBPs, we first conducted a series of Z-tests (Zar, 1999) comparing the mean scores of all variables for the 130 species in our sample

with the 209 unique species listed by Harding et al. (2016). Species in these tests were grouped by taxonomic Order. We then determined differences between pairs of species included and not included in CBPs. Differences in binary variables (threat status, stream obligate status, and the three measures of endemism) were assessed using simple sign tests (Zar 1999), while differences in continuous variables (habitat breadth, spatial range, and body size) were assessed using randomization tests. The randomization tests evaluated the average difference in our matched-pair comparisons against the null distribution produced by randomizing observed differences with an equal probability of being positive or negative 10,000 times (Felsenstein 1985). This created an expected distribution of differences under the assumption of no predictive power of in-CBP status for the contrast. The mean observed difference for each variable could then be compared to its null distribution to determine its significance.

Finally, we investigated which variables were most important in explaining the likelihood of being involved in a CBP using a multi-model inference approach comparing models that included various combinations of all eight variables. We modelled the probability of a species being in a CBP (1 or 0) using logistic regression. We compared all species used in the contrasts, but assessed each species independently rather than using modes or means of traits for contrasts of several species. This resulted in a dataset of 362 species (209 in CBPs, and 153 out of CBPs). To facilitate the valid comparison of all factors, we removed species missing any of the eight scoring variables. We analyzed all possible model combinations, as generalized linear models with a Bernoulli error distribution, to assess what combination of factors best explained the probability of a species being held in CBPs. Model selection was based on Akaike information criterion (AIC) scores, and the importance of each predictor variable was based on cumulative AIC weights across all models. All analyses were completed in R, using code available upon request from Biega et al. (2017).

Results

Z-tests indicated that our sample of CBP species was representative of all Anurans and Caudates in CBPs. However, as only one Caecillian was included in our sample, Gymnophiona was found to be unrepresentative for four of the variables

(p<0.05). Following the rules for contrasts, the Caecillian contrast was removed from the analysis.

In contrast to zoo holdings more generally, we found that species involved in CBPs are *more* threatened globally than their close relatives not involved in CBPs (p=0.05). Furthermore, for these CBP contrasts, we found no significant difference between sister species for any of the other seven threat correlates (all p>0.05, Table 2.1). These patterns are supported by multi-model inference methods where threat status was the most highly weighted predictor of being part of a CBP across models, followed by range size, stream obligate status, and island endemism (Table 2.1). In order to evaluate the effect of collinearity in our anaylsis, we ran univariate models for all predictor variables. We found no significant effect of any variable, with the exception of IUCN threat status, on the probability of being involved in a captive breeding programme. This corroborates the results found in our multivariate analysis, and confirms that collinearity did not bias our results. Model selection results, including the five most parsimonious models and model averaged variable coefficients, are available in Tables 2.2 & 2.3.

Discussion

It is unsurprising that conservation-focused breeding programs are targeting threatened species, given their general purpose is to target species facing imminent extinction in the wild. Indeed, the initiation of breeding or reintroduction programs is frequently tied to regional, national or subnational environmental and legislative objectives for native species. Although some species in these programs are not listed as globally threatened, they might still be in CBPs due to more local threats. For example, the Northern Leopard Frog *Lithobates pipiens* (a CBP species bred by the Vancouver Aquarium) is listed as 'Endangered' in British Columbia while it is listed as 'Least Concern' globally (IUCN 2016). Other important reasons zoos choose to hold non-threatened species include financial and logistical constraints (Bowkett 2014). Additionally, 19 non-threatened species on our list are bred for conservation research, possibly to gain husbandry knowledge that could be applied to holding imperilled relatives in the future.

While birds, mammals, and amphibians kept in zoos possess larger body sizes than their closest not-in-zoo relatives (Martin et al. 2014a, Biega et al. 2017), amphibians involved in CBPs were no different in size to their close relatives. While smaller-bodied amphibians may be more attractive to zoos with limited space, larger-bodied, attractive and charismatic species may be more desirable for zoo visitors (Frynta et al. 2013). However, zoo-reared amphibians destined for re-release are often kept in specialist biosecure facilities, isolated from other holdings and not seen by visitors at all. Visitor expectations may therefore play a lesser role when choosing species for CBPs. Indeed, only 46% of the species in CBPs on the list used in our analysis are exclusively zoo-held: another 46% are raised in specialist facilities run by government or non-government agencies, while the remaining 8% are held within both (Harding et al. 2016).

Given the similar range sizes and habitat breadths found between amphibians in CBPs and their close relatives, it seems that there are meaningful differences between amphibian species selected for zoo holdings generally and those selected for CBPs (see Table 2.1 for a direct comparison). Biases found in global *ex situ* species holdings towards wide-ranging habitat generalists are *not* reflected in the species selected for CBPs. This is good news: CBPs are targeting species facing both immediate and medium-term extinction risks.

Although captive breeding can be a key component of imperilled species recovery, it should be acknowledged that some amphibian species fail to thrive in captivity (Tapley et al. 2015), and that a species' suitability for a breeding program must be considered before its establishment. Our modeling framework did not identify trends in specific traits associated with CPBs; however, a study examining life history traits amongst successful, and unsuccessful, captive-bred species could reveal traits associated with amenability to captivity.

In summary, species involved in CBPs are more threatened than their closest relatives not bred purely for conservation purposes. While this analysis has no bearing on the success of these programs (this was evaluated in Harding et al. 2016), it does highlight important differences between amphibians held in zoos as a whole and those actively managed for *ex situ* conservation. We encourage continued prioritisation of species facing increased extinction risk for CBPs, but emphasize that species' suitability for such programs must be assessed on a case-by-case basis.

Acknowledgements

Chapter 3.

Identifying correlates of successful amphibian captive breeding programs

Abstract

The number of amphibian species in need of *ex situ* intervention continues to increase and yet the success rate of breeding programs remains relatively low; as of October 2016, only half of the programs in the Amphibian Ark database had produced viable offspring in captivity. Given that not all species are equally well suited to captivity, I hypothesized that differences in breeding success might be predicted by life history differences of the species involved. To test this, I collected data on 285 captive breeding programs and ran phylogenetic generalized linear mixed models (MCMC-GLMMs) to investigate the relationship between the biological traits of species and captive breeding success. Extrinsic characteristics of the programs were also tested in order to assess the effects of the location of the program relative to native range, the GDP of the country hosting the institution, and measures of husbandry knowledge on program outcome. Out of 14 variables examined, none had an appreciable effect on the probability of achieving successful breeding in captivity based on model coefficients. In some cases however, differences in breeding success were observed between species in relation to certain biological characteristics: species living in aquatic habitats during development or as adults achieved breeding less frequently on average than terrestrial species, and also took longer to achieve success even when they did. This may be due to husbandry difficulties in maintaining water quality and water chemistry parameters for aquatic species. Additionally, species exhibiting parental care had greater odds of achieving breeding success in captivity than species lacking this trait. Despite between group differences in breeding success, the lack of strong predictors suggests that it is not possible to make predictions on amenability to captivity based on a species' broad biological characteristics and that more species-specific knowledge may be crucial for breeding success. These data are often not known or inaccessible to members of the zoological community, and hence the gathering and publication of fine-grained life

history data (accompanied with key environmental parameters from the wild) would likely be helpful for increasing the success rates of captive breeding programs.

Introduction

Conservation breeding is recognized as an important and occasionally necessary method of mitigating current, and sometimes irreversible, threats to amphibians worldwide (Wren et al. 2015). The Amphibian Ark, a global conservation organization that helps coordinate and support global *ex situ* conservation initiatives, has already identified over 801 species requiring rescue or *ex situ* research (Dawson et al. 2016). Given that there are more species in need of captive breeding efforts than there are programs established, it is essential that limited funds and resources are directed towards species with the greatest need and programs with the greatest chance of conservation success (Tapley et al. 2015).

Current tools (e.g. Conservation Needs Assessment and Program Implementation tool; Amphibian Ark 2016) exist that aid in the identification of priority species for *ex situ* conservation by assessing whether field approaches are sufficient for the conservation of the species and by assessing the feasibility of initiating and maintaining an *ex situ* program. Despite these measures, approximately half of the captive breeding programs on the Amphibian Ark database have failed to produce offspring in captivity, and only 19% have reproduced to the F2 generation (Amphibian Ark 2016). Some of these unsuccessful programs have been running for over ten years, suggesting that lack of effort is not a major causative factor (Michaels et al. 2014).

While amphibians are theoretically ideal candidates for *ex situ* management due to their small size and relatively low cost for husbandry, many threatened amphibians fail to meet specific criteria for suitability, and current amphibian prioritization schemes fail to acknowledge that some species may be intrinsically more difficult to maintain in captivity (Tapley et al. 2015). In fact, a recent survey conducted of zoos and aquariums identified 'difficulty meeting husbandry requirements' (regardless of existing knowledge and expertise) as the third most significant barrier to holding more threatened species in

captivity (Brady et al. 2017). Some species possess biological traits or have certain habitat requirements that may hinder their ability to thrive and reproduce in a captive environment (Michaels et al. 2014; Tapley et al. 2015). While experienced zookeepers can make hypotheses about which biological traits these may be, there are no published tests of such predictions.

Here I present the first study to investigate the correlation between life history traits and underlying differences in captive breeding success in amphibians. By comparing the biological traits of species that have successfully produced offspring in captivity to those that, despite efforts, have failed to reproduce, I aim to identify traits associated with amenability to reproduction in captivity in amphibians. I also test the effect of external factors commonly considered to be associated with program success, such as institution location in relation to a species' native range. Knowledge of the intrinsic traits of species and extrinsic characteristics of programs that influence captive breeding success could be incorporated into prioritization schemes to assess a species and institution's suitability for a future *ex situ* program.

Methods

Captive breeding data

Data on amphibian captive breeding programs were collected from three sources: the Amphibian Ark progress of programs database (Amphibian Ark 2016), the Zoological Information Management System (ZIMS) (Species360 2017), and a review of the effectiveness of amphibian conservation interventions (Smith and Sutherland 2014). For the purpose of this analysis I did not limit these programs to species being bred for conservation purposes. In cases where a species was listed as being bred by a regional zoological association on the Amphibian Ark database (such as the Association of Zoos and Aquariums (AZA), the European Association of Zoos and Aquaria (EAZA), or the Zoo Aquarium Association (ZAA)), I generated a list of institutions that had bred that species in the previous 12 months using the species holding tool in ZIMS (Species360 2017). Additionally, species in zoo population management plans (such as the AZA's Species Survival Plan program) were included in my list of captive bred species, and institutions breeding those species were found using the same species holding tool in ZIMS (Species360 2017). Since this tool was limited to breeding data from the last 12

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months, and not all institutions record the presence of non-metamorphs in the ZIMS database, this may have lead to underreporting of successful breeding programs. In total, I collected data on 285 captive breeding programs in 35 countries for 210 species (Figure 3.1).

Programs were scored as 'successful' if they produced viable offspring, defined as animals that were bred and raised to adulthood in captivity. Of the 285 captive breeding programs in the dataset, 154 (54%) were scored as successful using this definition. The dates and outcomes of these captive breeding programs are accurate to the best of my knowledge (program managers could not verify all the information in my database) and were updated as new information became available until July 2017. The complete dataset is included as Supplementary Material (Appendix A).

Intrinsic factors

As intrinsic factors I considered life history traits and characteristics of a species' habitat. Because natural history data and measurements of wild environmental parameters are absent for many species held in captivity (Michaels et al. 2014), I chose traits whose measures are available for most species. While there are other important factors related to a species' suitability for a conservation breeding program (such as clutch size, generation length, and reversibility of threats), I focus here only on traits that relate to a species' ability to breed in captivity, and not, e.g. their potential for reintroduction. The hypotheses outlined below were developed though discussion with members of the amphibian *ex situ* community and as such are often based on managers' personal experiences. Given this, one might expect many of the tests to be confirmatory in nature.

Reproductive mode. Species with aquatic larval stages may present husbandry challenges as there is often little known on the ecology and trophic status of tadpoles and information on how to rear them is lacking in peer reviewed literature (Altig et al. 2007, Pryor 2014). Tadpole and adult life stages may differ drastically, resulting in different morphologies (Sheratt et al. 2017) and husbandry requirements (B. Tapley pers. comm). Therefore, species with aquatic larval stages may have decreased captive breeding success when compared to live bearing or direct developing species. Data on reproductive mode were collected from Sodhi et al. (2008), provided by D. Bickford and

S. Howard (pers. comm), with species being classified as either having, or not having, a tadpole stage.

Temperature and Precipitation Seasonality. Seasonal climatic events can be linked to reproduction in amphibians (Wildenhues et al. 2012), and therefore recreating seasonal events in captivity (e.g. through the use of fridges or showers) can trigger breeding in species found in highly seasonal environments in the wild. Because these are fairly straightforward triggers to replicate, such species might then be more amenable to reproducing in captivity. Data on the temperature and precipitation seasonality of a species' native range were obtained by overlaying spatial polygons of each species' extent of occurrence from the IUCN (2015) with bioclimatic layers from WorldClim v1.4 (Hijmans et al. 2005). Both temperature seasonality (BIO4) and precipitation seasonality (BIO15) layers were aggregated at a spatial resolution of $10km^2$ and values were averaged across a species' extent of occurrence.

Reproductive cycle. This hypothesis follows the one above. Species that reproduce seasonally (typically following seasonal climatic events) may be easier to breed in captivity as breeding cues can be recreated through the use of equipment (e.g. fridges and showers). However, it is worth noting that breeding seasonality may be related to other cues (e.g. the mass emergence of insects; B. Tapley pers. comm.) that are not easy to recreate in captivity. Data on reproductive cycle were collected from Sodhi et al. (2008), provided by D. Bickford and S. Howard (pers. comm), and species were classified as having either seasonal or aseasonal breeding.

Habitat. Species found in aquatic habitats may have lower breeding success in captivity due to challenges associated with recreating aquatic environments: water chemistry parameters in the wild are seldom known and often go unreported in the literature (Michaels et al. 2014). Data on spawning site, developmental site, and adult habitat were collected from Sodhi et al. (2008), provided by D. Bickford and S. Howard (pers. comm). Adult habitat was scored as aquatic, arboreal, terrestrial, or aquatic & terrestrial; spawning site was scored as aquatic & terrestrial, arboreal/phytotelms, terrestrial, parent, or aquatic; and developmental site was scored as arboreal/phytotelms, terrestrial, parent, aquatic, or larval stage absent. These factors were amalgamated into aquatic (comprised of species dwelling partially or wholly in

aquatic habitats) and terrestrial (comprised of species dwelling in terrestrial or arboreal habitats) categories in subsequent tests.

Habitat Breadth and Range Size. Species held *ex situ* have broader habitat breadths and larger range sizes than their wild relatives (Biega et al. 2017), suggesting that these broad-ranging generalists may be easier to keep in captivity. I test this here using data from the IUCN RedList (2015). Habitat breadth was scored as the total number of suitable habitats listed for each species based on the IUCN habitat classification scheme and geographic range sizes in km^2 were determined for each species using georeferenced spatial polygons of 'extent of occurrence' from the IUCN (2015).

Parental care. A lack of parental care has been cited as a reason why amphibians are generally considered suitable for captive breeding (Tapley et al. 2015) despite the fact that complex modes of parental care are indeed present in many species of amphibians (Gururaja et al. 2014). To test whether a lack of parental care is associated with captive breeding success in amphibians, data were collected from Sodhi et al. (2008), provided by D. Bickford and S. Howard (pers. comm), and species were classified as exhibiting, or not exhibiting, parental care.

Extrinsic factors

Extrinsic factors (characteristics of the program and not the species itself) may also have a bearing on the success of a captive breeding program (Conde et al. 2011). The following traits were considered for their effect on breeding success.

Location of Breeding Program. Programs established within the geographic range of the target species may have greater breeding success than programs established outside of the target species' native range due to decreased acclimatization time and decreased novel pathogen risk (Conde et al. 2011, Tapley et al. 2017). One of the reasons why the Amphibian Conservation Action Plan endorses in-range facilities is because the provision of some environmental and climatic variables that may be important for successful husbandry are simplified (Wren et al. 2015). It is worthwhile to note that some amphibians held in range may still be isolated from outside environmental conditions by being kept in controlled (e.g. air-conditioned) facilities (B.

Tapley, pers. comm). To determine whether success was related to location vis a vis native range, I simply recorded the geographical coordinates of all institutions housing programs using Google Maps (2017) and scored each as being within (1) or outside (0) of their species' native range using the spatial polygons of each species' extent of occurrence from the IUCN (2015) and the R package 'sp' (Pebesma and Bivand 2005). For those outside, I recorded the shortest distance (in kilometers) to the edge of the species' native range using the R package 'Geosphere' (Hijmans et al. 2016), and assigned a score of 0 km to species being bred inside their native range.

Funding. Captive breeding programs cost money. Because data on funding at the program level were unavailable, I used per capita gross domestic product (GDP) of the country in which the *ex situ* institution was located as a proxy for funding. Because exchange rates do not always reflect differences in price levels between countries, GDP/capita estimates were converted into international dollars using purchasing power parity (PPP) rates. This standard measure allows a comparison of real levels of expenditure among countries (The World Bank Group 2017). Data were downloaded from the world development indicators on Data Bank for the year 2015 (The World Bank Group 2017).

Species Knowledge. Species are expected to share natural history characteristics with close relatives (although exceptions exist – see Michaels et al. 2015) and therefore species with close relatives in captivity may have greater breeding success because husbandry techniques can be inferred from relatives. The presence and number of species in the same genus held *ex situ* was determined from the list of global *ex situ* holdings published by Biega et al. (2017). If close relatives share similar breeding success, then this will also be evident in the phylogenetic signal of breeding success, which can be evaluated using Pagel's λ post-analysis.

Preparing the data for analysis

When testing the effect of these variables on breeding success, it was important to incorporate an indicator of effort in the analysis; I used the number of years since a captive breeding program was initiated (i.e. program duration). The duration of the captive breeding programs is correlated strongly and positively with breeding success in my dataset, yet there are important statistical reasons for not including time as a factor

directly in the analysis. The statistical distribution of program duration differed between successful and unsuccessful programs: successful programs have continued for up to 38 years while the maximum duration for unsuccessful programs was 14 years, likely due to termination because of a continued lack of success. Such a bias would result in a false pattern linking program duration and breeding success.

I had to deal with another bias associated with recently initiated programs. Depending on the age of the founders in a captive population, it can take years for individuals to reach sexual maturity, and it takes time to develop species-specific husbandry techniques (Wren et al. 2015). Therefore, programs that were established recently but have not yet achieved breeding success should not necessarily be considered unsuccessful. To deal with both these biases, I first determined the amount of time that is generally necessary to achieve breeding success by considering data on the number of years between a program's establishment and the first offspring for the successful programs. These data were collected using either the first recorded birth in the ZIMS database (Species360 2017), the literature describing the program, or in other cases, from personally contacting a staff member at the institution managing the captive breeding program. Out of the 154 successful captive breeding programs in the database, I was able to collect data for 109. From this, I determined that the majority (86 out of 109, or 79%) of successful programs achieved breeding success within five years. Using this knowledge, I modified my dataset by (i) excluding 43 programs that were initiated in the last five years and had not yet produced viable offspring (ii) excluding 23 unsuccessful programs that were terminated before five years since the reason for termination was not known (including 1 program for which exact termination date could not be determined) and (iii) considering 21 programs that took longer than five years to achieve offspring as "unsuccessful" since perhaps these species possess characteristics that render them less amenable to breeding in captivity. Following the vetting of the dataset, I was left with 172 captive breeding programs (involving 126 species) to analyze, including 86 successful and 86 unsuccessful programs.

Phylogenetic analyses

The binary response variable of breeding success at the program level was modeled using phylogenetic generalized linear mixed models in a Bayesian framework (Hadfield 2010) with intrinsic traits of the species and extrinsic factors related to the

species or the institution as explanatory variables. Given that there are many factors at the institutional level that can affect program success for which data were not available (such as program funding, quality of equipment, and staff expertise), institution was included as a random variable in the analysis. The phylogeny was incorporated into the analysis because close relatives may share similar life histories and husbandry requirements. In cases where a single species was bred in multiple institutions, programs were considered separate observations, and this non-independence was accounted for within the phylogenetic framework (Hadfield 2010). The phylogenetic tree used was adapted from Pyron (2014); species not found on that tree were added manually half-way down the terminal branch of the sister species where they could be identified using the literature (22 species). Where no sister species could be identified (37 species), the species was added randomly to the phylogeny halfway down the branch of a species within its genus. The phylogenetic tree used is included as Supplementary Material (Appendix A).

Analyses were run using Markov chain Monte Carlo (MCMC) methods for generalized linear mixed models (GLMM) in the MCMC-GLMM package in R *v.3.3.3* (Hadfield 2010, R Core Team 2017). I ran models using a categorical distribution for the response variable and uninformative priors for 1.5 million iterations, discarding the first 10000 as burn-in and sampling every 1000 iterations thereafter, resulting in effective posterior sample sizes of 1490 for all parameters. Each explanatory variable was analyzed independently (because of the large number of factors and as to avoid the issue of collinearity) and its effect on breeding success was evaluated by examining the 95% credible interval on the logit coefficients. All continuous variables were log-transformed to improve their distributions. To investigate differences within categorical variables, posterior distributions from each level were subtracted from one another and the median difference was recorded as the effect size for the difference in odds. The consistency of this effect was measured by calculating the percentage of iterations (stored values from the MCMC chain) in which one categorical level had higher (or lower) odds of breeding than the other.

Intra-family correlation, also referred to as heritability (Villemereuil 2012), and the equivalent of Pagel's λ in a phylogenetic least squares framework, was calculated using the formula provided in Hadfield (2010), with a range from 0 (no phylogenetic signal) to 1 (high phylogenetic signal). Trace plots of posterior distributions and effective posterior

sample sizes were examined as conventional diagnostics of chain performance. All analyses were run three times, and as results did not differ among runs, only the results of the first run are reported here.

Given that funding for most captive breeding programs is short-term (Wren et al. 2015), and many species are facing immediate extinction risk in the wild (Alroy 2015), being able to breed a species quickly can be essential to its survival. Therefore, following the analysis on breeding success, I performed a second analysis to help explain the variation in time to achieve the first offspring for my successful programs. Knowledge on which traits are associated with faster successful breeding in captivity could be utilized to prioritize species for future programs. Using 109 successful programs, I ran a second MCMC-GLMM, this time using time to first successful offspring (in years) as my response variable with a Poisson distribution. All other methodology remained as reported above.

Results

Visualization of the raw data seems to indicate a moderate correlation between some of the categorical variables and mean proportion of breeding success, namely for habitat and reproductive cycle variables, although there is a lot of variance due to small sample sizes in some levels (Figure 3.2). There is no clear relationship between continuous variables and breeding success (Figure 3.3), or between each variable and time to first successful offspring (Figure 3.4 & 3.5).

I first tested the effects of life history traits and program characteristics on captive breeding success. The phylogenetic signal of the null model for breeding success was low (λ=0.21, 95% CI= 0.13, 0.26), suggesting that the residuals of the models are not correlated strongly with phylogeny.

There was no evidence of an effect of any of the variables examined on the probability of breeding success (95% credible intervals for all logit coefficients include zero; see Table 3.1, Figure 3.6 & 3.7). However, some differences were observed in the odds of breeding success between levels of a given factor. By back-transforming the distribution of logit coefficients to probability of breeding success and overlaying these distributions for factor levels, we can visualize the magnitude of these differences

(Figure 3.6). When amalgamating posterior distribution coefficients for habitat data into two categories: (1) solely terrestrial (comprised of species dwelling in terrestrial and arboreal habitats) and (2) partially aquatic (comprised of species dwelling in aquatic and aquatic & terrestrial habitats), I found that the median odds of breeding success were higher for programs involving solely terrestrial dwelling species by 2.2x in the adult life stage and 8.7x in the developmental (larval) life stage (Figure 3.6). The odds were consistently higher in terrestrial dwelling species in 79% and 92% of iterations (stored values from the MCMC chain), respectively. However, the effect of aquatic habitat on success was not observed during the spawning stage of the life cycle.

Other notable differences include: programs involving species exhibiting parental care had a median of 2.5x greater odds of breeding success (greater odds in 96% of iterations); programs involving aseasonal breeders had a median of 2.4x greater odds of breeding success (greater odds in 85% of iterations); and institutions located within a species' native range had a median of 2.3x greater odds of breeding success (greater odds in 88% of iterations). A complete summary of results for all variables, including differences between factor levels, can be found in Table 3.1.

Second, I investigated the effects of life history traits and program characteristics on time to first successful offspring in captivity, based on 109 successful captive breeding programs. The phylogenetic signal of the null model for time to first successful offspring was also low (λ=0.17, 95% CI= 0.12, 0.22), suggesting that the residuals of the models are not correlated strongly with phylogeny.

There was no relationship between any of the continuous variables and time to first successful offspring in captivity (95% credible intervals for slopes all include zero; see Table 3.2, Figure 3.8), nor were there large differences in median time to first offspring among categorical levels (Figure 3.9). Median difference in time to first successful offspring between levels of all categories varied from 1.1-1.8 years, and the direction of these effects were only consistent in 58-85% of the iterations (Figure 3.9). The most consistent difference in median time to first breeding event was observed for reproductive cycle: the median time to first offspring was 1.5 years longer for programs involving aseasonal breeders than seasonal breeders (number of years greater in 85% of iterations). This contradicts the result of higher breeding success in captivity for aseasonal breeders. The median time to first offspring was also longer for programs

involving species that dwell in aquatic habitats during development by 1.8 years or as adults by 1.6 years, although the number of years was only consistently greater in 76% or 79% of iterations respectively. Again, consistent with the breeding success patterns above, this result was not observed during the spawning stage; aquatic spawners took a median of 1.5 *fewer* years to achieve breeding in captivity (direction of effect consistent in 65% of iterations).

Discussion

Despite calls for increasing the number of threatened species in *ex situ* conservation programs (Dawson et al. 2016, Biega et al. 2017), not all amphibians will thrive and reproduce in captive environments. A large number of programs have been unable to produce offspring in captivity, sometimes even after many years of attempts. I found that none of the variables examined had significant effects on breeding success in captivity when considered in isolation (all model coefficients included zero), indicating that it may not be possible to make predictions on captive breeding success based solely on single broad biological characteristics using this sample and modelling approach. This would suggest that more fine-grained species-specific information and microhabitat data are required, consistent with the suggestion made by Michaels et al. (2014). Unfortunately this presents a challenge to practitioners as these data are often difficult to obtain, both directly and from the literature: they are simply unknown or unpublished, or if published, not easily accessible, e.g. articles cannot be identified by using search terms relevant to environmental data and natural history (Michaels et al. 2014).

However, there were differences among groups of species exhibiting particular biological characteristics. I found that programs involving species living in aquatic habitats during larval development or as adults have lower odds of breeding success than terrestrial dwelling species within the first five years. Among programs that are eventually successful, such species also took longer than species living in terrestrial habitats before success was achieved (although this effect was less consistent). This could be related to water quality: water chemistry parameters from the wild are not always known, and when known, can be difficult to recreate and maintain (Michaels et al. 2014; K. Bradfield, pers comm). However, there was no effect of occupying aquatic habitats during spawning, suggesting that the use of aquatic habitats at this stage in the

life cycle does not negatively affect captive breeding success when compared to species inhabiting terrestrial environments. Programs involving species with parental care had higher odds of producing viable offspring in captivity. Parental care has been found to decrease embryo desiccation and predation in amphibians in the field (Valencia and Delia 2016), and this may translate into increased survival in captivity. Alternatively, this could be an artefact of the previous finding: aquatic species tend to exhibit lower levels of parental care than terrestrial species (72% of programs involving species living in aquatic habitats during development lacked parental care). Conflicting results were found for reproductive cycle, with aseasonal breeders exhibiting higher odds of breeding success but taking longer on average to produce offspring. However, given that my sample was highly skewed towards seasonal breeders in both analyses and that the direction of this effect was only consistent in 85% of MCMC chain iterations, these results must be interpreted with caution.

The underlying variation in breeding success or time to first successful offspring was not well explained by extrinsic characteristics of the program itself. While programs located at institutions within a species' native range did have higher odds of breeding success on average, my sample size was highly skewed towards programs located out-of-range (for example when Neotropical amphibians are bred in North American or European zoos). This is a problem because my sample may not be representative of all captive breeding programs located in-range. Additionally, I found no effect of distance (in km^2) between the institution and the edge of the species' native range, or when evaluating the effect of program location on time to first offspring. Overall, the data do not strongly support an effect of within-range breeding on success, but this question warrants more scrutiny. The establishment of captive facilities within a species' native range is endorsed by the Amphibian Conservation Action Plan (Wren et al. 2015): not having to transport species across borders circumvents not only logistical barriers and lowers costs (Brady et al. 2017) but it also decreases the risk of species becoming exposed to novel pathogens or of these species bringing pathogens to naïve amphibian populations destined for release (Tapley et al. 2017). However, there are also challenges to holding species within range: amphibian captive husbandry knowledge is sometimes lacking in the countries that support the greatest amphibian biodiversity, and it can be difficult to train personnel given a high degree of staff turnover (Wren et al. 2015).

Holding several members of a genus *ex situ* did not increase breeding success in captivity in congeners, nor were there strong patterns in amenability along the phylogeny. While the idea that using closely related species to gain husbandry expertise that can be applied to holding threatened relatives has been endorsed by the zoological community (Barber and Poole 2014), doubts have previously been expressed about using close relatives as analogues in this way (Michaels et al. 2014; Tapley et al. 2015). My findings are accordant with those of Michaels et al. (2015), who reported that the ease of breeding and rearing one clawed frog in captivity did not translate to another closely related species. Future research could assess the relationship between previous knowledge and success, perhaps by testing for a relationship between the number of publications on a particular species and measures of subsequent breeding success.

While the PPP GDP of the host country was not predictive of breeding success or time to first offspring, it may also not be a good proxy for the amount of funding available to a captive breeding program. For instance, the two lowest PPP GDP countries in the dataset, Tanzania and Madagascar, are both host to successful captive breeding programs: although these programs are located in low GDP countries, they are well supported by zoos in the developed world. The Kihansi spray toad (*Nectophrynoides asperginis*) captive breeding program in Tanzania was established with support from the Bronx and Toledo Zoos (among others), and the breeding program for *Boophis pyrrhus* in Madagascar is supported by a large number of North American zoos including the Woodland Park Zoo and the Toronto Zoo (Lee et al. 2006, Edmonds et al. 2012). So, while I found no effect of PPP GDP on captive breeding success, data needs to be collected on program funding directly in order to test to what extent money buys success.

In summary, many amphibians are still not breeding in captivity, and while it would be advantageous to be able to predict the chances of a species breeding if taken into captivity, this analysis has demonstrated that this is not simply a matter of assessing a species' broad biological characteristics or establishing facilities in range countries. While the odds of achieving breeding success may be greater in species with certain biological characteristics in comparison to others, achieving breeding success in general seems to rely on species-specific husbandry knowledge, which may or may not be gained by keeping a close relative in captivity. One of the main challenges to meeting captive breeding objectives remains insufficient technical expertise and therefore I

support the calls of Michaels et al. (2014) and Wren et al. (2015) for greater engagement with field biologists and the publication of environmental field data. Conservation research is cited as the main reason for establishing captive breeding programs (Harding et al. 2016) and as such we should, as a research community, also be insisting on systematic data collection on failed husbandry attempts as well as on successful ones so that we can learn from previous trials and prevent expending resources on repeating efforts. Ultimately, it is the sharing of knowledge that will increase expertise and maximize the chances of conservation successes.

Acknowledgements

Conclusion

This thesis attempted to assess the current state of *ex situ* management of amphibians by examining the current representation of threatened species held or bred *ex situ* and the success of captive breeding programs. In general, zoo collections do not reflect the species of highest conservation concern: species in zoos are no more threatened than their wild counterparts, and collections are biased towards large-ranging habitat generalists, species with lower extinction risk in the future. Fortunately, *ex situ* focused conservation initiatives (Amphibian Ark 2017) are helping to bolster the representation of high-priority amphibians in zoos and specialist breeding facilities, and I observed an increase in threat status of species in *ex situ* collections when institutions on the Amphibian Ark database were considered. Of course, there are many good reasons to hold species of lower threat status (Bowkett 2014), and not all species in zoos are involved in a captive breeding program. When I limit the analysis to species solely being bred for conservation purposes, this pattern changes: species in these programs are more likely to be threatened and are equally range-restricted and specialized as their closest relatives not being bred for conservation purposes. This shows that not surprisingly, and in contrast to zoo holdings more generally, species being bred for conservation purposes are of current, and importantly, future conservation concern.

However, simply holding threatened species in *ex situ* collections does not guarantee captive breeding success. While establishing a captive population is an important step, achieving successful breeding in captivity is essential to maintaining viable populations that could later be returned to their natural environment. Many captive breeding programs have yet to produce viable offspring in captivity, and my final analysis demonstrated that breeding success cannot be predicted using the biological traits of the species involved or extrinsic characteristics of the breeding program itself. So while species living in aquatic habitats (during development or as adults) and species lacking parental care exhibited lower breeding success on average, producing viable offspring in captivity likely comes down to having the technical expertise and species-specific natural history knowledge.

These analyses measured conservation success in two specific ways: the representation of threatened species held or managed *ex situ*, and the production of viable offspring in captivity. Naturally, there are limitations to defining success in these ways. First, the representation of threatened species in *ex situ* collections may not be the best measure of conservation value. Hutchins et al. (1995) argue that zoos should not select threatened species based solely on the possibility of a future reintroduction and that consideration should be given to the ability of a species to contribute to more immediate conservation goals, including public education and fundraising to support field conservation and scientific research (Fa et al. 2014). Whatever the reason for keeping a species in captivity may be, stakeholders must be as confident as possible that the expected positive impact on the conservation of that species will outweigh the potential risks or negative impacts, and that establishing a captive breeding program is a wise use of available resources (McGowan et al. 2017). This will require looking beyond *ex situ* representation and identifying clear objectives before establishing a captive population (Canessa 2017). Second, success depends on founder individuals being genetically and biologically representative of the species in the wild and reproducing in sufficient numbers to constitute a viable population (McGregor Reid and Zippel 2008). Third, captive breeding in itself may not be associated with conservation outcomes unless captive individuals are sustainably restored to the wild (Meredith 2015). These analyses did not examine the suitability of these breeding programs for reintroduction; this will require further investigation into the health of captive populations and, critically, threat mitigation in the wild (Wren et al. 2015).

That being said, there is still great conservation value in holding threatened species *ex situ*: zoos can perform research that may underpin wild conservation efforts, develop husbandry techniques, and uncover species biology which can be hard to observe in nature (Tapley et al. 2017). However, given the mixed breeding success of these programs, captive breeding programs should be used in conjunction with other conservation approaches. Zoos are already doing some of this: they initiate and participate in education and training programs, habitat protection projects, research, and species protection both *ex situ* and in the wild (Gusset and Dick 2011). Zoos also contribute financially to wildlife conservation, with an estimated $350 million USD of Zoo money spent on wildlife conservation in 2008 (Gusset and Dick 2011).

In the midst of a high tide of amphibian losses (Alroy 2015), we need all hands on deck. Zoos admittedly have the space and resources to dedicate to conservation initiatives (Olive and Jansen 2017), and their contributions should be heralded and encouraged. Here, I have delved into the representation of amphibian species in zoos and the success of captive breeding programs, but the role of *ex situ* management in the conservation of amphibians encompasses more than just that. Management is needed more frequently to maintain persistence of species in the wild, and the boundary between *ex situ* and *in situ* (field) management is becoming increasingly blurred (Pritchard et al. 2011), demanding new thinking about how we view *ex situ* management. Going forward, we must continue to utilize the resources and expertise provided by zoos to help achieve ambitious global conservation targets.

Tables and Figures

Table 1.1. Contrasts between species in zoos and their closest relatives not held in zoos for the All Institution dataset (both databases) and for the ZIMS institutions. Values in the 'Difference' columns show differences in positive (+) and negative (-) values between in-zoo and not-in-zoo species pairs for categorical variables, and ratio differences between these pairs for continuous variables. Values in the p (n) columns show p-values for corresponding sign tests (categorical variables) and randomization tests (continuous variables), with sample sizes for these tests provided in parenthesis. Bold entries indicate significant differences between pairs (p ≤0.05). The ΣAICw column displays relative importance of variables from multivariate analysis as indicated by cumulative Akaike weight, with asterisks denoting the top three variables by weight.

Variable	All Institutions			ZIMS Institutions		
	Difference (+ : -)	p (n)	$\underline{\Sigma AIC_w}$	Difference (+ : -)	p (n)	$\underline{\Sigma AIC_w}$
IUCN threat status	24:34	0.24 (202)	98.4 %*	**19:34**	0.05 (176)	79.8 %*
Stream obligate	12:24	0.07 (208)	27.4 %	10:18	0.20 (180)	27.9 %
Montane endemic	9:12	0.66 (212)	31.4 %	7:12	0.17 (185)	27.8 %
Island endemic	7:9	0.80 (215)	48.1 %	6:9	0.45 (187)	36.6 %
Tropical endemic	3:9	0.15 (213)	27.9 %	3:9	0.15 (185)	27.9 %
Body size	**+13.5%**	< 0.001 (210)	52.8 %	**+13.9%**	< 0.001 (185)	42.9 %
Geographic range size	**+3.5x**	< 0.001 (218)	99.7 %*	**+4.4x**	< 0.001 (190)	97.4 %*
Habitat breadth	**+27%**	< 0.001 (218)	66.6 %*	**+35%**	< 0.001 (190)	85.3 %*

Table 1.2. Top five multivariate models based on the Akaike Information Criterion (AIC) for predicting the likelihood of an amphibian species being held in a zoo, for the full dataset (a) and ZIMS only dataset (b). Delta AIC (ΔAIC) indicates the difference in the AIC value from the top model, and the Akaike weight (AICw) provides a relative weight of evidence for each model.

(a)

All Institution models	ΔAIC	AIC$_w$
Habitat breadth + Range Size + Threat status + Body size	0	6.90%
Habitat breadth + Range size + Threat status	0.15	6.40%
Habitat breadth + Range Size + Threat status + Body size + Island endemism	0.22	6.19%
Habitat breadth + Range Size + Threat status + Island endemism	0.92	4.35%
Range size + Threat status + Body size + Island endemism	1.54	3.19%

(b)

ZIMS only models	ΔAIC	AIC$_w$
Habitat breadth + Range Size + Threat status	0	9.6%
Habitat breadth + Range Size + Threat status + Body size	0.68	6.82%
Habitat Breadth + Range Size + Threat status + Island endemism	1.31	4.99%
Habitat Breadth + Range Size + Threat status + Island endemism + Body size	1.68	4.14%
Habitat Breadth + Range Size + Threat status + Stream Obligate	1.89	3.73%

Table 1.3. Results of generalized linear model analyses determining relative importance of eight traits in explaining the likelihood of a species being held in a zoo. Model-averaged logit- coefficients (Bavg), standard errors (SE), and lower and upper 95% confidence intervals for models are given for the full dataset (a) and ZIMS only dataset (b).

(a)

Parameter	B_{avg}	SE	L 95% CI	U 95% CI
IUCN threat status	0.877	0.277	0.333	1.42
Stream obligate	-0.03	0.225	-0.471	0.410
High altitude endemism	0.193	0.299	-0.393	0.780
Island endemism	0.342	0.254	-0.156	0.840
Tropical endemism	-0.047	0.195	-0.430	0.336
Body Size	0.196	0.133	-0.064	0.457
Range Size	0.175	0.048	0.080	0.269
Habitat Breadth	0.252	0.138	-0.018	0.523

(b)

Parameter	B_{avg}	SE	L 95% CI	U 95% CI
IUCN threat status	0.662	0.308	0.059	1.265
Stream obligate	0.065	0.250	-0.425	0.554
High altitude endemism	0.105	0.372	-0.624	0.835
Island endemism	0.248	0.269	-0.278	0.775
Tropical endemism	-0.056	0.205	-0.457	0.345
Body Size	0.135	0.139	-0.138	0.408
Range Size	0.151	0.054	0.044	0.256
Habitat Breadth	0.342	0.148	0.052	0.632

Table 2.1. Contrasts between species involved in conservation breeding programs and their closest relatives not involved in such programs, compared with the results of Biega et al. (2017) for global zoo holdings in general. Values in the 'Difference' columns show differences in positive (+) and negative (-) values between 'in breeding programs/zoo holdings' and 'not in breeding programs/zoo holdings' species pairs for categorical variables, and ratio differences between these pairs for continuous variables. Values in the p (n) columns show p-values for corresponding sign tests (categorical variables) and randomization tests (continuous variables), with sample sizes for these tests provided in parenthesis. Bold entries indicate significant differences between pairs (p ≤0.05). The ΣAICw column displays relative importance of variables from multivariate analysis as indicated by cumulative Akaike weight, with asterisks denoting the top three variables by weight.

Variable	Species in CBPs			Zoo Holdings (Biega et al., 2017)		
	Difference (+ : -)	p (n)	ΣAIC_w	Difference (+ : -)	p (n)	ΣAIC_w
IUCN threat status	**26:13**	0.05 (99)	90.1%*	24:34	0.24 (202)	98.4 %*
Stream obligate	9:15	0.31 (108)	56.5%*	12:24	0.07 (208)	27.4 %
High-altitude endemic	8:9	1 (107)	34.9%	9:12	0.66 (212)	31.4 %
Island endemic	5:2	0.45 (108)	55.9%	7:9	0.80 (215)	48.1 %
Tropical endemic	1:3	0.63 (108)	33.7%	3:9	0.15 (213)	27.9 %
Body size	+5.6%	0.064 (99)	36.4%	**+13.5%**	< 0.001 (210)	52.8 %
Geographic range size	+1.2x	0.37 (111)	58.4%*	**+3.5x**	< 0.001 (218)	99.7 %*
Habitat breadth	-2.7%	0.37 (110)	29.8%	**+27%**	< 0.001 (218)	66.6 %*

Table 2.2. Results of generalized linear model analyses determining relative importance of eight traits in explaining the likelihood of a species being involved in a conservation breeding program. Model-averaged logit- coefficients (B_{avg}), standard errors (SE), and lower and upper 95% confidence intervals for our conservation breeding program models are given.

Parameter	B_{avg}	SE	L 95% CI	U 95% CI
IUCN threat status	0.849	0.381	0.102	1.596
Stream obligate	-0.454	0.288	-1.019	0.112
High altitude endemism	-0.291	0.355	-0.987	0.405
Island endemism	0.586	0.385	-0.169	1.341
Tropical endemism	-0.212	0.279	-0.759	0.334
Body size	0.251	0.280	-0.298	0.801
Range size	0.650	0.441	-0.214	1.515
Habitat breadth	-0.093	0.346	-0.771	0.585

Table 2.3. Top five multivariate models based on the Akaike Information Criterion (AIC) for predicting the likelihood of an amphibian species being involved in a conservation breeding program, using general linear models. Delta AIC (ΔAIC) indicates the difference in the AIC value from the top model, and the Akaike weight (AICw) provides a relative weight of evidence for each model.

Conservation Breeding Program models	ΔAIC	AIC$_w$
Stream obligate + Range size + Island endemism + Threat status	0	3.66%
Range size + Island endemism + Threat status	0.177	3.35%
Stream obligate + Range size + Threat status	0.529	2.81%
Stream obligate + Threat status	1.02	2.20%
Range size + Island endemism + Tropical endemism + Threat status	1.19	2.02%

Table 3.1. Summary of Bayesian generalized linear models relating variables to captive breeding success, with phylogenetic and institution effects considered. Logit coefficients are given, representing the mean log-odds of breeding success for categorical variables, and slope for continuous variables. Positive coefficients signify a positive effect on breeding success and negative coefficients represent a negative effect on breeding success, with 95% credible intervals provided as a test of significance. For categorical variables, columns report the median probability of breeding success within each factor level, the effect size of the difference between factor levels, and the consistency of this effect (evaluated using the percentage of iterations in which the direction of this effect is observed). None of the variables examined had a significant effect on breeding success, although there were differences in breeding success among levels of some categorical factors.

Variable	Mean logit coefficient (95% CI)	Sample size (n)	Median prob (breeding success)	Median difference in odds of breeding	Consistency of difference among levels
Categorical variables					
Reproductive mode:					
Larvae present	-0.65 (-2.4, 1.0)	140	0.46	1.59x greater for species with a larval stage.	In 72% of the iterations, the odds of breeding were higher for species with a larval stage.
Larvae absent	-0.18 (-1.3, 1.1)	20	0.35		
Reproductive cycle:					
Aseasonal	0.47 (-1.4, 2.3)	17	0.62	2.4x greater for aseasonal breeders.	In 85% of the iterations, the odds of breeding were higher for aseasonal breeders.
Seasonal	-0.37 (-1.5, 0.64)	139	0.41		
Adult habitat:					
Aquatic	-0.95 (-2.8, 1.0)	14	0.27	2.2x greater for species occupying solely terrestrial habitats.	In 79% of the iterations, the odds of breeding were higher for species that occupy solely terrestrial habitats.
Arboreal	0.23 (-1.8, 2.2)	16	0.56		
Terrestrial	0.05 (-1.3, 1.3)	85	0.51		
Aquatic & Terrestrial	-0.39 (-1.7, 1.1)	45	0.41		
Spawning habitat:					
Aquatic & Terrestrial	0.13 (-2.4, 2.4)	8	0.54	1.14x greater for species occupying solely terrestrial habitats Species with parental development were excluded.	In 54% of the iterations, the odds of breeding were higher for species occupying solely terrestrial habitats. Species with parental development were excluded.
Arboreal	-0.82 (-2.9, 1.4)	12	0.31		
Terrestrial	0.06 (-1.5, 1.4)	33	0.52		
Parent	0.55 (-1.5, 2.7)	13	0.63		
Aquatic	-0.51 (-1.7, 0.6)	94	0.38		
Developmental habitat:					
Absent	-0.62 (-2.3, 1.0)	20	0.35	8.65x greater for species occupying solely terrestrial habitats. Species without a larval stage or with parental	In 92% of the iterations, the odds of breeding were higher for species that occupy solely terrestrial habitats. Species without a larval stage or with parental development
Arboreal	1.6 (-2.3, 5.1)	4	0.82		
Terrestrial	2.3 (-0.73, 5.6)	9	0.90		
Parent	-1.4 (-5.3, 2.8)	3	0.21		
Aquatic	-0.3 (-1.5, 0.8)	124	0.43		

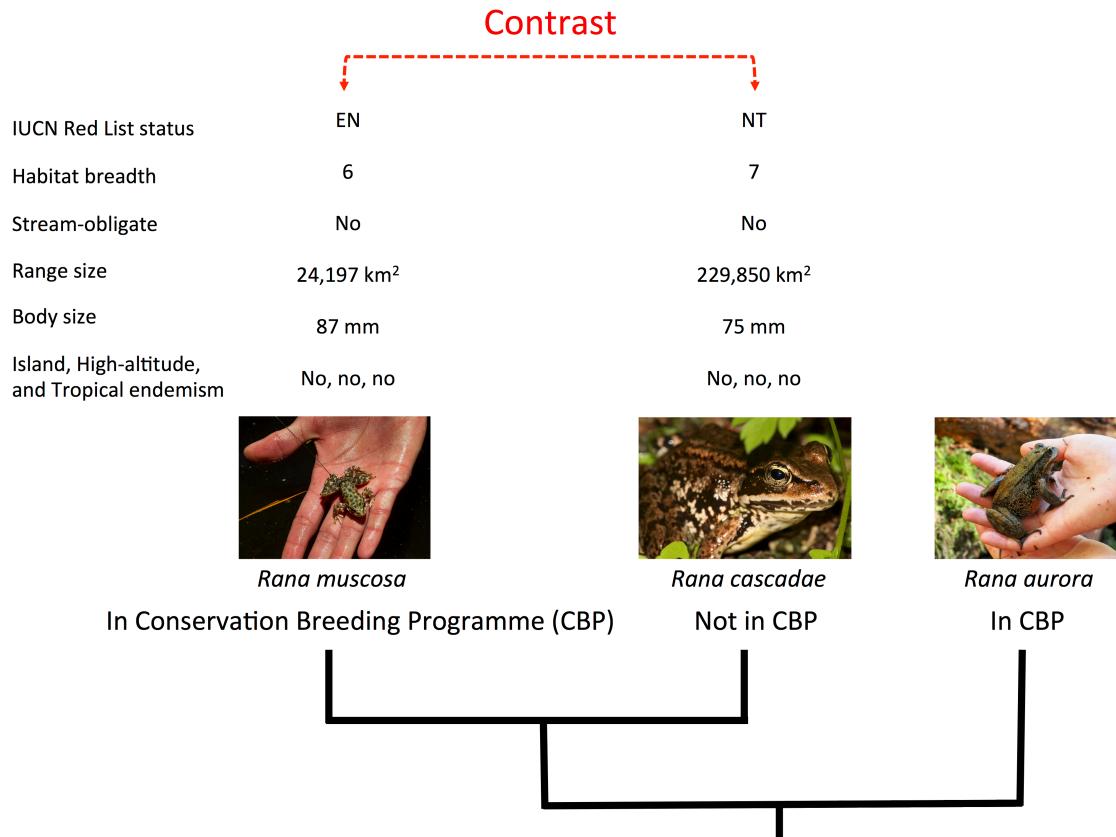|				development were excluded.	were excluded.
Parental Care					
Present	0.17 (-1.0, 1.4)	64	0.54	2.5x greater for species with parental care.	In 96% of the iterations, the odds of breeding were higher for species with parental care.
Absent	-0.75 (-1.9, 0.6)	96	0.32		
Institution location:					
In range	0.48 (-1.1, 2.3)	22	0.62	2.3x greater for institutions in range countries.	In 88% of iterations, the odds of breeding were higher for in-range institutions.
Out of range	-0.35 (-1.4, 0.96)	138	0.41		
Continuous variables:					
Temperature Seasonality	-0.07 (-0.61, 0.47)	163	NA	NA	NA
Precipitation Seasonality	0.30 (-0.67, 1.35)	163	NA	NA	NA
Habitat Breadth	0.05(-0.67, 0.72)	164	NA	NA	NA
Range Size	-0.09 (-0.22, 0.05)	162	NA	NA	NA
Distance from institution to native range	0.07 (-0.11, 0.24)	158	NA	NA	NA
GDP.PPP/capita of host country	0.24 (-0.48, 0.96)	168	NA	NA	NA
Number of congeners held *ex situ*	0.50 (-1.2, 2.3)	172	NA	NA	NA

Table 3.2. Summary of Bayesian generalized linear models relating variables to time to first successful offspring, with phylogenetic and institution effects considered. Log coefficients represent the mean log(time to first successful offspring) for categorical variables or slope for continuous variables. Positive coefficients represent an increase in time required to produce offspring and a decrease in amenability to captivity. For categorical variables, the 95% credible interval (CI) for the logit coefficient evaluates whether it takes significantly longer than the first year to produce successful offspring for each factor level, and for continuous variables the 95% CI evaluates the strength of the relationship between the variable and log(time to first successful offspring). There was no relationship between any of the continuous variables and time to first successful offspring, although there were differences in the median time to produce first offspring among categorical factor levels. The magnitudes of these differences are evaluated using the effect size of the difference in median time to first successful offspring and the consistency of this effect (using the percentage of iterations in which the direction of this effect is observed).

Variable	Log coefficient (95% CI)	Sample size (n)	Median time to first offspring (years)	Median difference in time to first offspring	Consistency of difference among levels
Categorical variables					
Reproductive mode:					
Larvae present	1.0 (0.06, 1.9)	98	2.9	1.5 years longer in species with a larval stage.	In 79% of iterations, mean time was longer for species with a larval stage.
Larvae absent	0.63 (-0.70, 1.8)	9	1.9		
Reproductive cycle:					
Aseasonal	1.3 (0.16, 2.5)	16	3.8	1.5 years longer in aseasonal breeders.	In 85% of iterations, mean time was longer for aseasonal breeders.
Seasonal	0.92 (-0.02, 1.8)	87	2.5		
Adult habitat:					
Aquatic	1.25 (-0.07, 1.4)	8	3.6	1.6 years longer in species that occupy partially or wholly aquatic habitats.	In 79% of iterations, mean time was longer for species that occupy partially or wholly aquatic habitats.
Arboreal	0.41 (-0.9, 1.8)	11	1.5		
Terrestrial	0.91 (-0.18, 1.9)	59	2.5		
Aquatic & Terrestrial	1.0 (0.04, 2.13)	29	2.8		
Spawning habitat:					
Aquatic & Terrestrial	-0.2 (-2.2, 1.6)	4	0.83	1.5 years longer in species that occupy solely terrestrial habitats. Species with parental development were excluded.	In 65% of iterations, mean time was longer for species that occupy solely terrestrial habitats. Species with parental development were excluded.
Arboreal	1.4 (0.1, 2.7)	8	3.9		
Terrestrial	0.90 (-0.22, 1.9)	23	2.4		
Parent	0.66 (-0.68, 2.0)	9	1.9		
Aquatic	1.1 (0.12, 1.9)	63	2.9		
Developmental habitat:					
Absent	0.64 (-0.53, 2.0)	9	1.9	1.8 years longer in species that occupy aquatic habitats. Species without a	In 76% of iterations, mean time was longer for species that occupy aquatic habitats. Species
Arboreal	0.98 (-0.55, 2.7)	4	2.7		
Terrestrial	0.08 (-1.4, 1.6)	8	1.1		
Parent	0.42 (-2.7, 3.2)	1	1.6		

Aquatic	1.1 (0.2, 2.0)	85	3.0	larval stage or with parental development were excluded.	without a larval stage or with parental development were excluded.
Parental Care					
Present	0.92 (-0.14, 1.8)	43	2.5	1.2 years longer in species without parental care.	In 73% of iterations, mean time was longer for species without parental care.
Absent	1.13 (0.15, 2.2)	64	3.1		
Institution location:					
In range	0.91 (-0.01, 2.1)	16	2.5	1.1 years longer in out-of-range institutions.	In 58% of iterations, mean time was longer for out-of-range institutions.
Out of range	0.95 (0.1, 1.9)	88	2.6		
Continuous variables:					
Temperature Seasonality	-0.10 (-0.47, 0.23)	108	NA	NA	NA
Precipitation Seasonality	-0.21 (-0.90, 0.50)	108	NA	NA	NA
Habitat Breadth	0.19 (-0.20, 0.59)	107	NA	NA	NA
Range Size	0.05 (-0.03, 0.12)	107	NA	NA	NA
Distance from institution to native range	-0.02 (-0.11, 0.08)	103	NA	NA	NA
GDP.PPP/capita of host country	0.02 (-0.40, 0.45)	106	NA	NA	NA
Number of congeners held *ex situ*	-0.09 (-0.50, 0.35)	109	NA	NA	NA
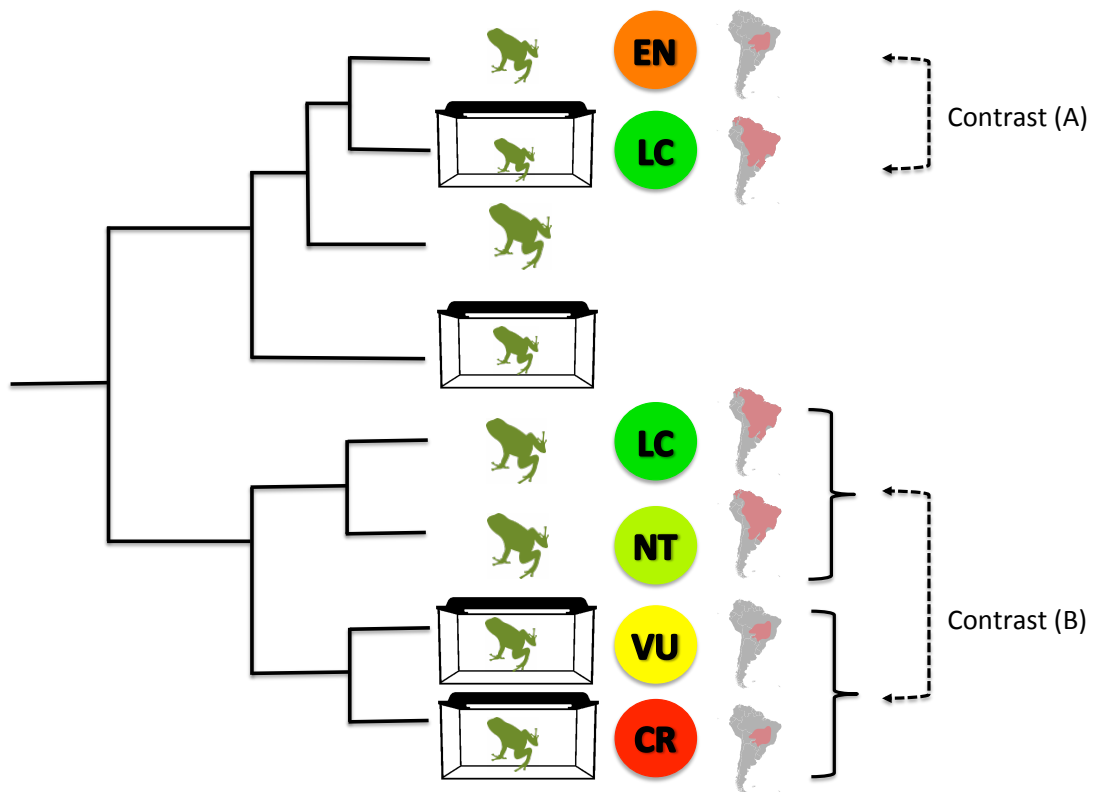
Figure 1.1 The experimental design for our comparative study. We identified independent pairs of in-zoo amphibian species (denoted here as a frog in a terrarium) and not-in-zoo species. Each pair forms a "contrast" (e.g. contrast A). We then compared species-level traits within each contrast including body size, IUCN status (coloured circles) and range size (depicted on the map). When contrasts between in-zoo and not-in-zoo species consisted of more than two species in total (as in contrast B), mean averages were used for continuous variables (e.g. for log body size and log range size) and modal averages were used for categorical variables (e.g. IUCN status). In-zoo species without an unambiguous (i.e. monophyletic) out-of-zoo sister group (depicted as the in-zoo species with no contrast) were dropped from the analyses to preserve statistical independence.

Contrast

	EN	NT
IUCN Red List status	EN	NT
Habitat breadth	6	7
Stream-obligate	No	No
Range size	24,197 km^2	229,850 km^2
Body size	87 mm	75 mm
Island, High-altitude, and Tropical endemism	No, no, no	No, no, no

Rana muscosa
In Conservation Breeding Programme (CBP)

Rana cascadae
Not in CBP

Rana aurora
In CBP

Figure 2.1 Diagram showing the experimental design of our paired species analytical approach. Amphibian species in conservation breeding programs were first paired to their closest relative(s) not involved in such programs and then scored for eight variables relating to extinction risk (IUCN Red List status, habitat breadth, stream-obligate status, geographical range size, body size, and island, high-altitude, and tropical endemism). Differences between pairs were calculated and statistical tests (e.g. sign tests and randomization tests) were performed based on these differences. Species in conservation breeding programs for which no monophyletic out-of-breeding program relative could be identified (e.g. *Rana aurora*) were dropped from the analysis, to preserve statistical independence. Photograph credits (left to right): U.S. Geological Survey/Jenny Mehlow, Walter Seigmund, Dan Greenberg.
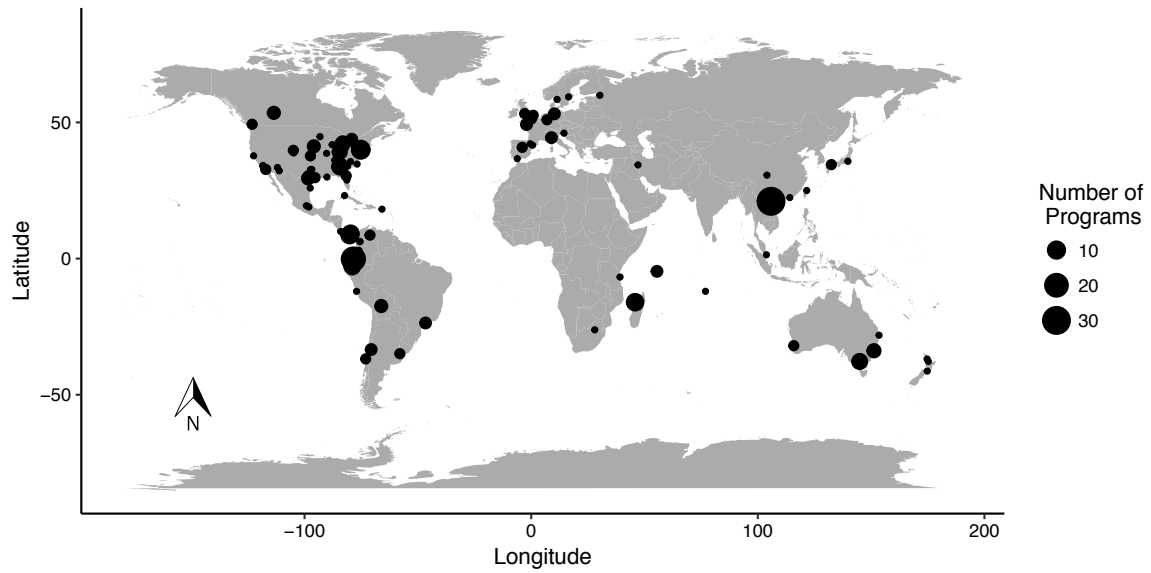
Figure 3.1 Location of all 285 captive breeding programs in 35 countries worldwide. The size of the dot represents the number of breeding programs at a particular institution.
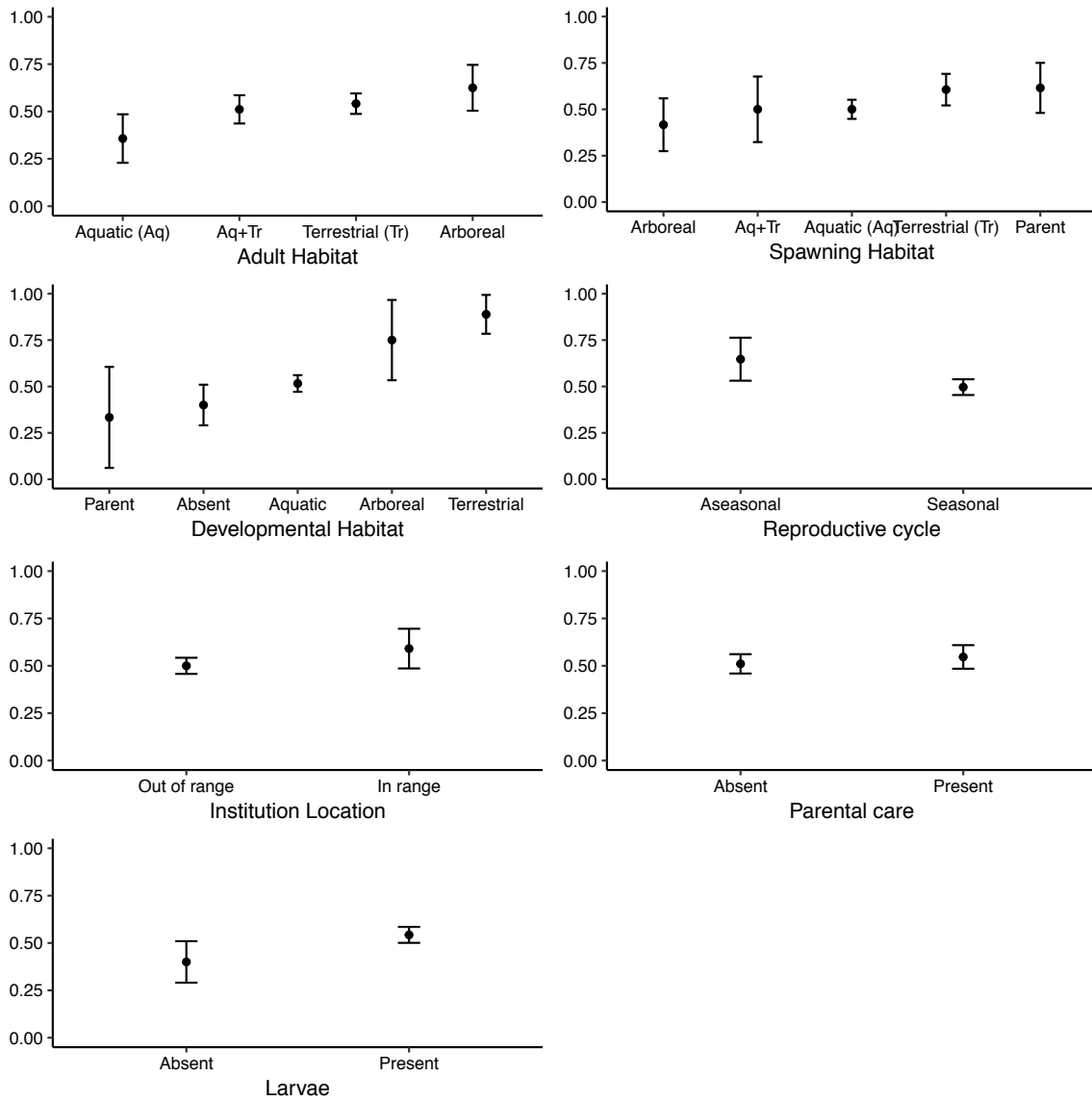
Figure 3.2. The mean (±SE) proportion of successful breeding programs for each categorical level. Standard error bars indicate the variation in the mean proportion estimate and reflect the sample size.
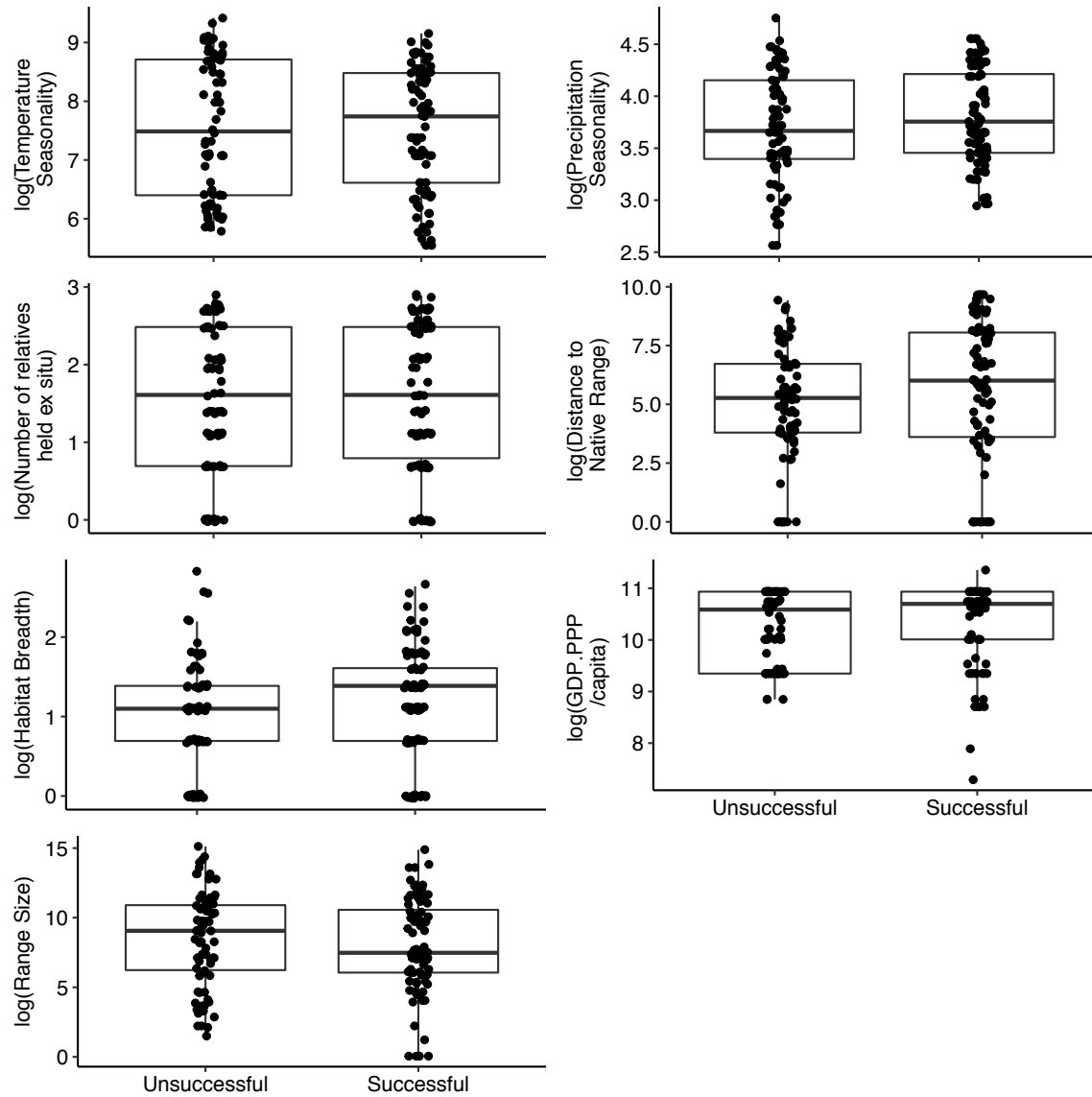
Figure 3.3. Boxplots of raw data for the continuous variables for successful and unsuccessful captive breeding programs. Variables include biological traits of the species involved in the captive breeding programs and characteristics of the institution itself, such as such as distance to the edge of a species' native range, or the GDP-PPP/capita of the country where the institution is located. Each data point represents an individual program.
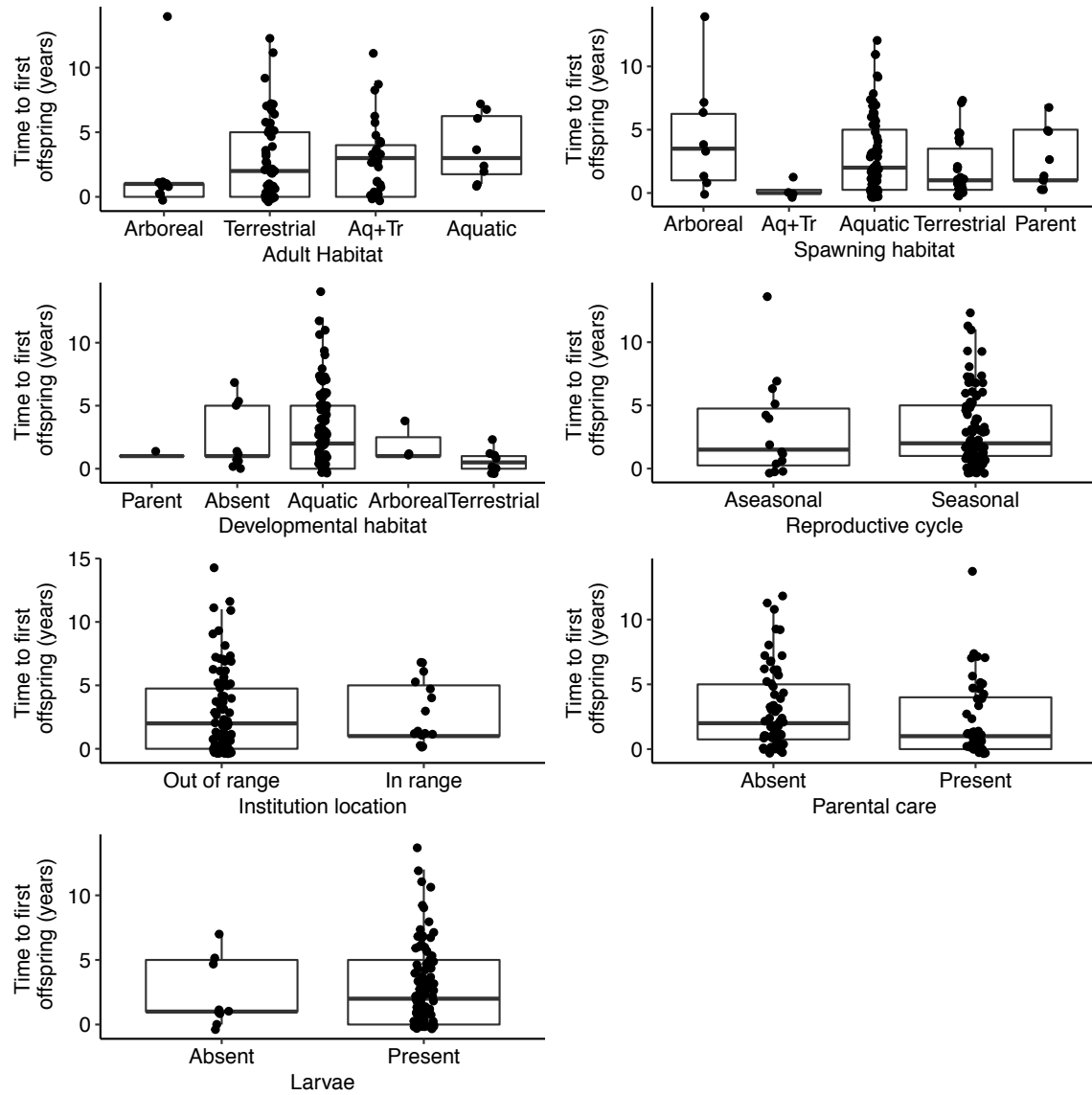
Figure 3.4. Boxplots of raw data representing the time to first successful offspring in captivity for 109 successful captive breeding programs, blocked by categorical variable level. Each data point represents an individual program.
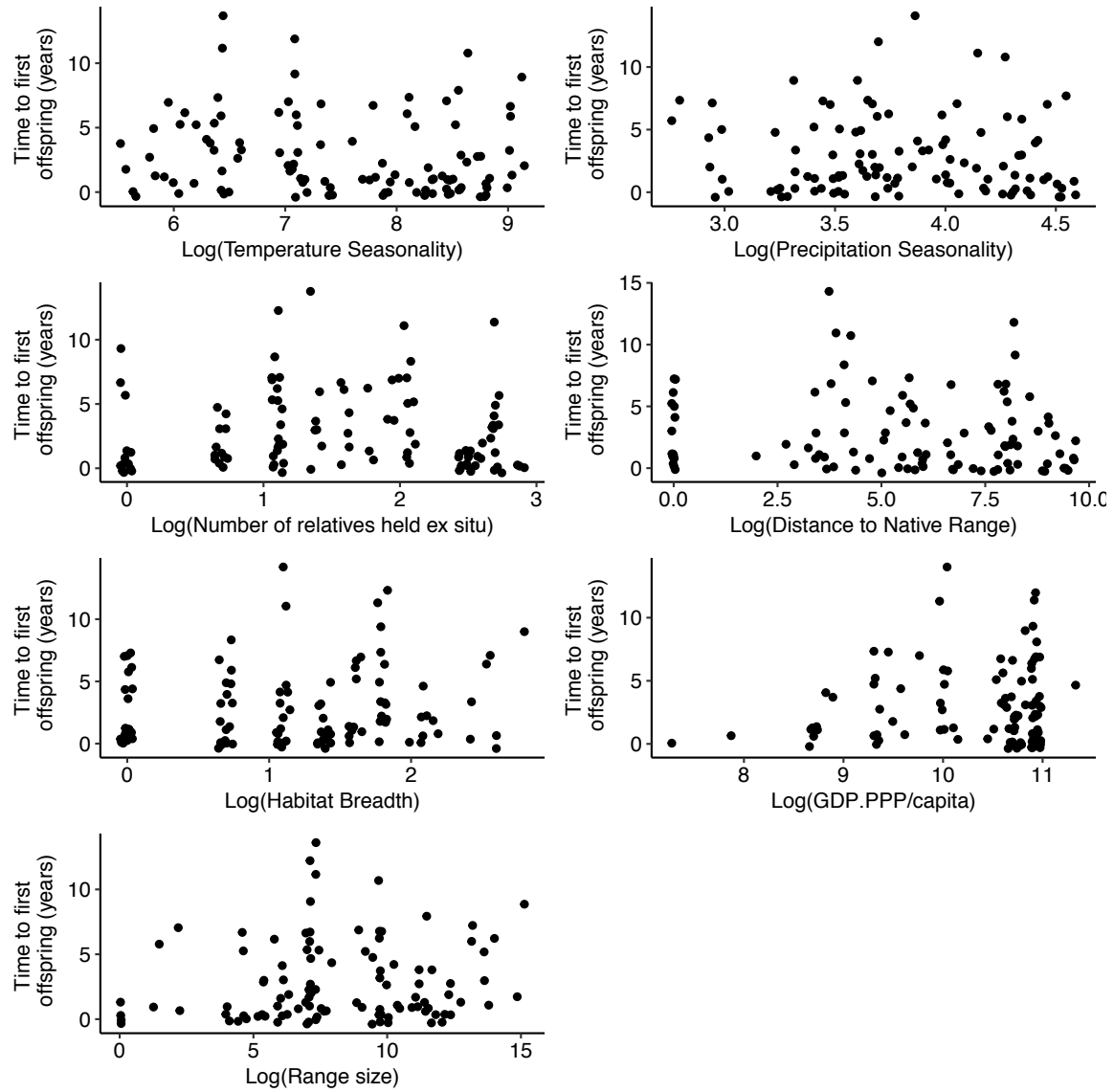
Figure 3.5. Raw data representing the relationship between continuous variables and time to first successful offspring in captivity for 109 successful captive breeding programs. Variables include biological traits of the species involved in the captive breeding programs and characteristics of the institution itself, such as distance to the edge of a species' native range, or the GDP-PPP/capita of the country where the institution is located. Each data point represents an individual program.
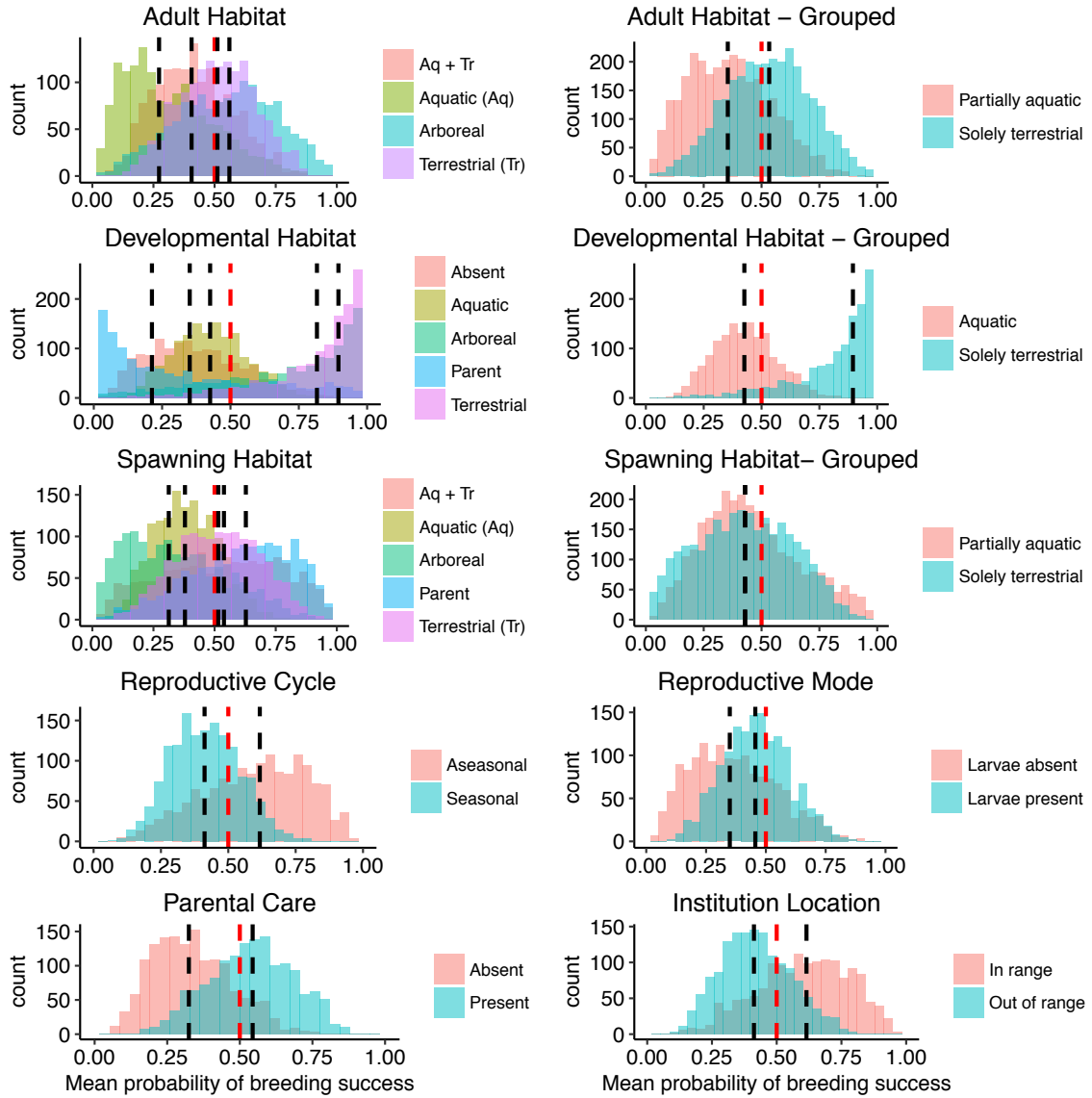
Figure 3.6. Distribution of posterior coefficients for mean probability of breeding success for categorical variables. Red dashed line denotes the null hypothesis of no effect on breeding success, and black dashed lines represent the median probability of breeding success in each category.
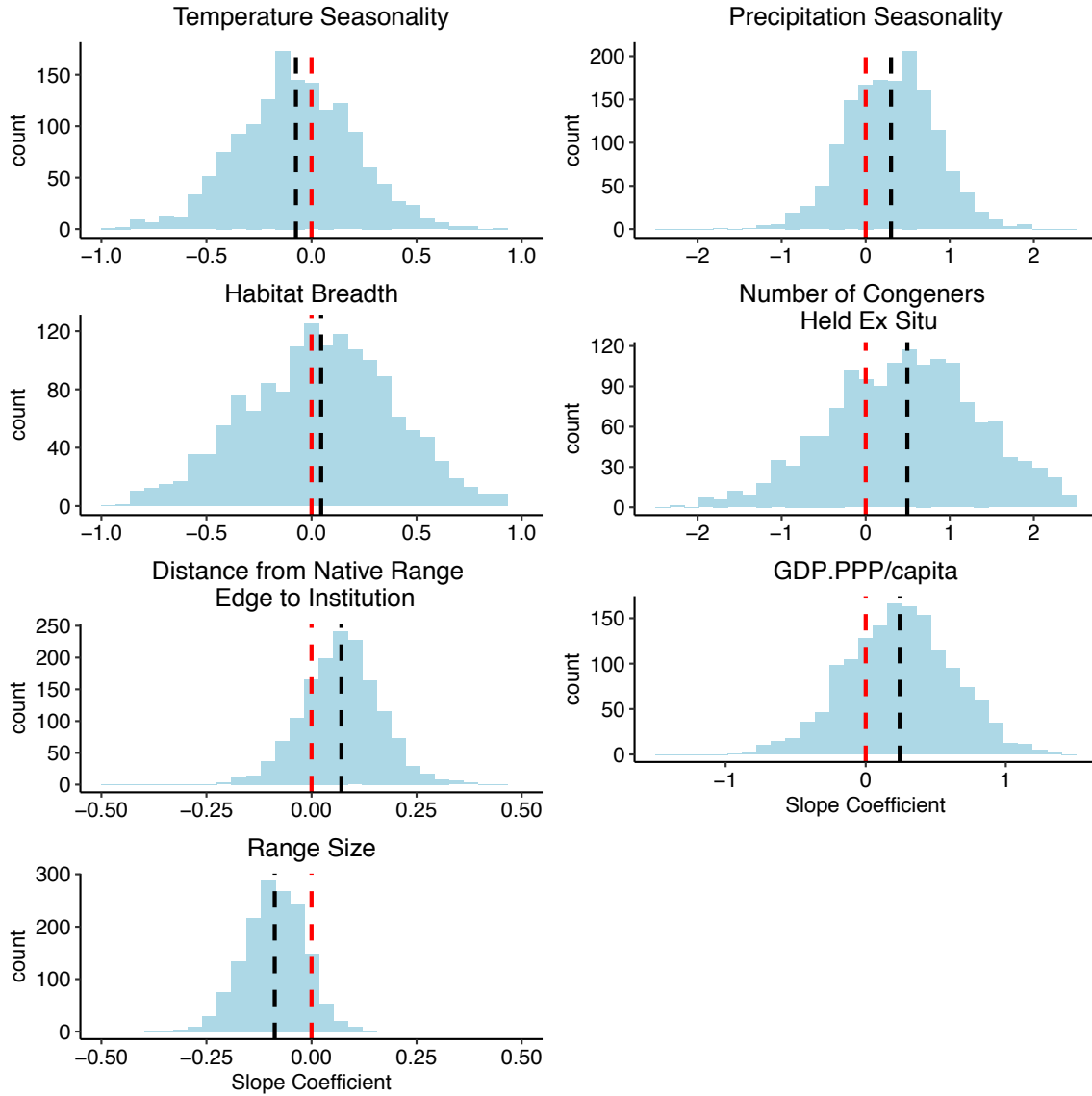
Figure 3.7. Slope coefficients relating the log of continuous traits to logit of breeding success. Red dashed line represents the null hypothesis of no relationship to breeding success, and the black dashed line represents the mean slope coefficient. Positive coefficients signify a positive effect on breeding success (as the variable increases, so does the probability of breeding success), and negative coefficients represent a negative effect on breeding success.
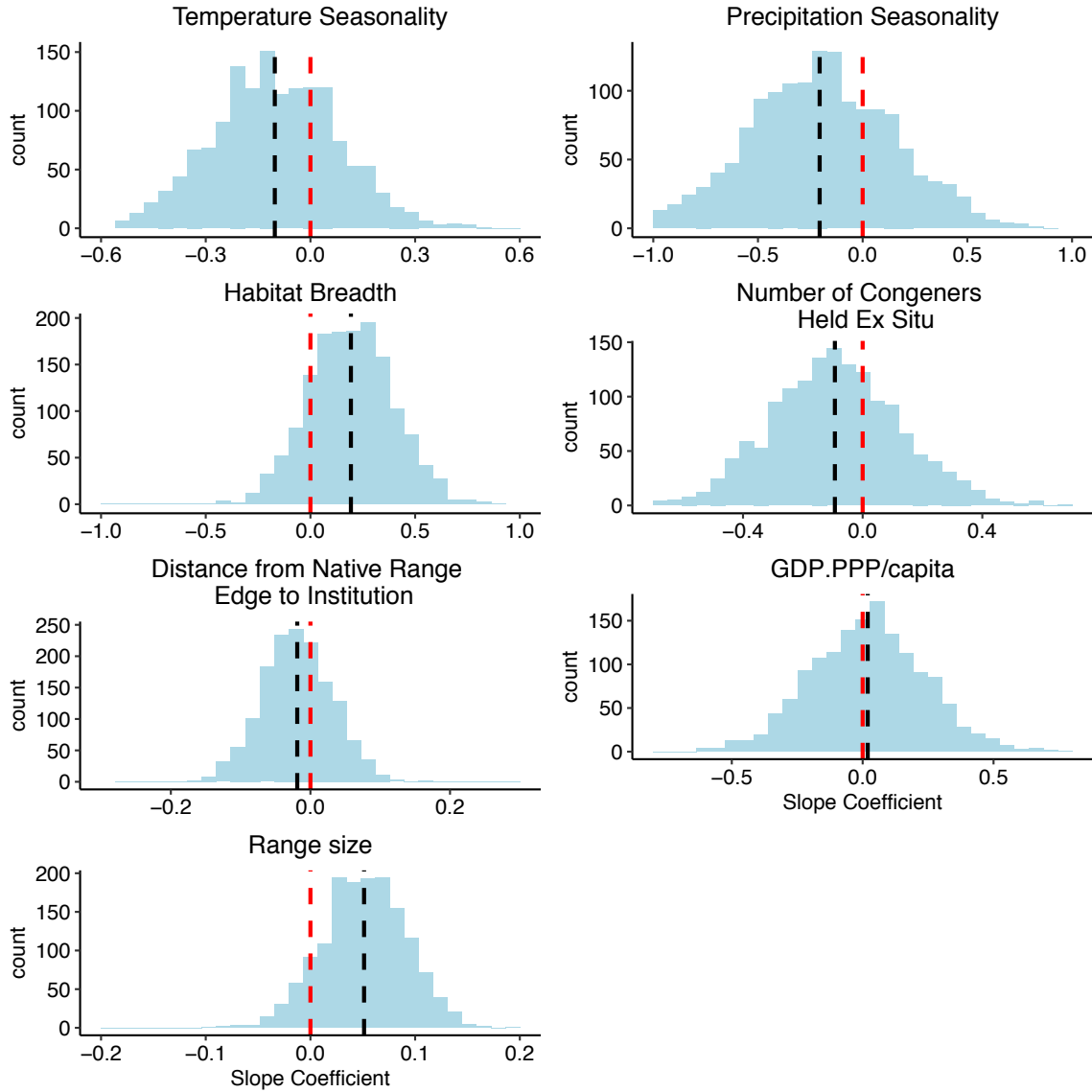
Figure 3.8. Slope coefficients relating log of continuous traits to log of the mean time (in years) to first successful offspring in captivity. Red dashed line represents the null hypothesis of no relationship to time to first offspring, and the black dashed line represents the mean slope coefficient. Positive coefficients signify a positive relationship with time to first offspring (as the variable increases, so does the time it takes to produce viable offspring), and negative coefficients represent a negative relationship with time to first offspring.
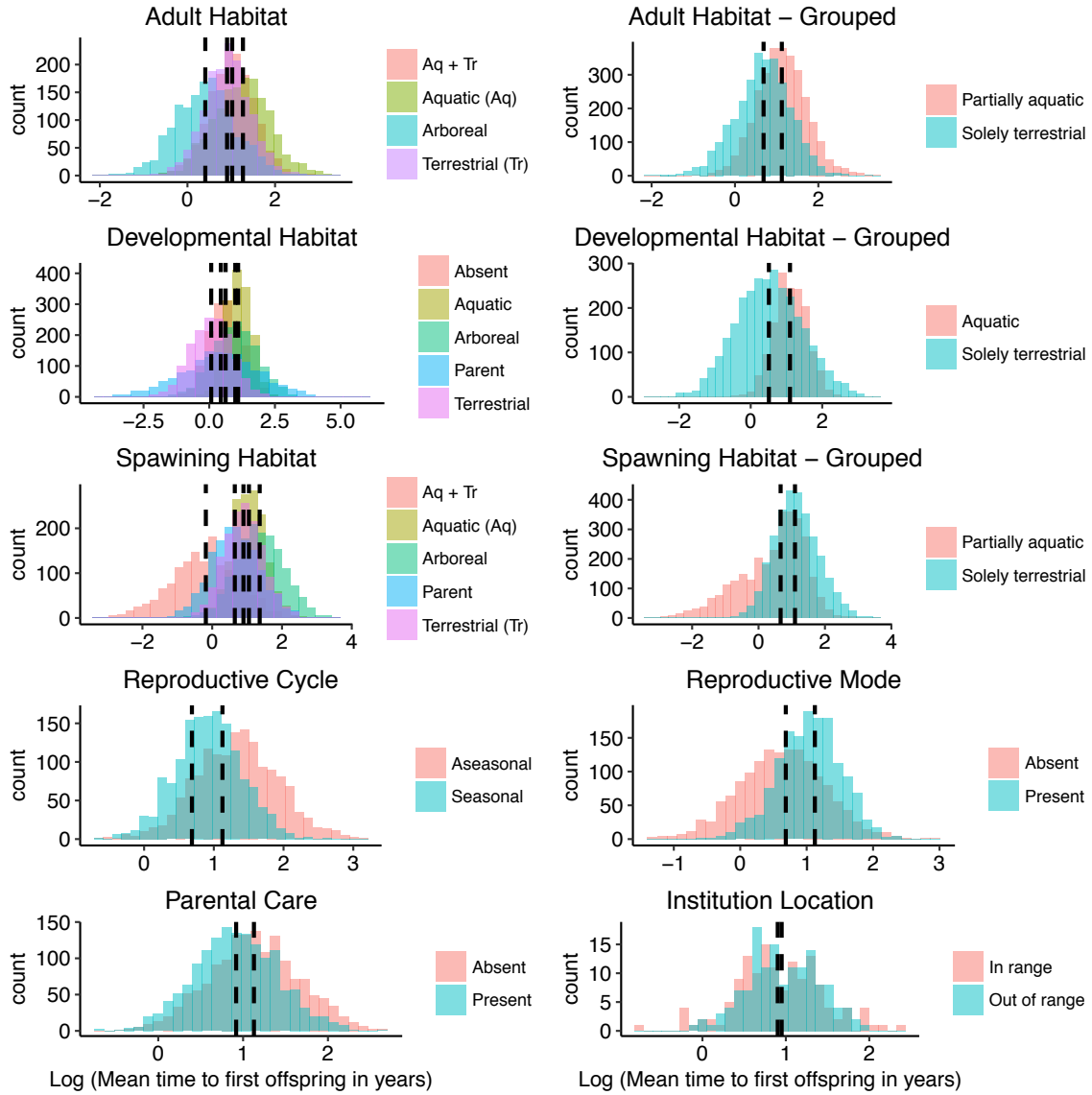
Figure 3.9. Distribution of categorical posterior coefficients on the log scale for log of the mean time (in years) to first successful offspring. Black dashed lines represent median time to first offspring on the log scale in each category.

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Appendix A. Supplementary Data Files

Chapter 1:

Description:

The accompanying spreadsheet contains the raw data associated with chapter one.

Filename:

Biega_Chapter 1 data.xlsx

Chapter 2:

Description:

The accompanying spreadsheet contains the raw data associated with chapter two.

Filename:

Biega_Chapter 2 data.xlsx

Chapter 3:

Description:

The first accompanying file includes one spreadsheet containing the raw data associated with chapter three of this thesis. Details on scoring variables can be found in the second sheet of the spreadsheet under "Notes".

The second accompanying text file contains the phylogenetic tree used in the analyses in chapter three of this thesis. The phylogenetic tree used was adapted from Pyron (2014); species not found on the tree were added manually half-way down the terminal branch of the sister species where they could be identified using the literature (22 species). Where no sister species could be identified (37 species), the species was added randomly to the phylogeny halfway down the branch of a species within its genus.

Filenames:

Biega_Chapter 3 data.xlsx

Biega_Chapter 3 tree.txt