

Evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs

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Abstract

Amphibians around the world are declining from threats that cannot currently be mitigated, making it impossible to safeguard some species in their natural habitats. Amphibians in the mountainous neotropics are one example where severe disease-related declines prompted calls for the establishment of captive assurance colonies to avoid extinctions. We surveyed experts in Panamanian amphibians to determine the probability of avoiding chytridiomycosis-related extinctions using captive breeding programs. We ranked Panamanian amphibian species by perceived susceptibility to chytridiomycosis, then calculated the likelihood of avoiding extinction as the product of three probabilities, which include (1) finding sufficient founder animals, (2) successfully breeding these species in captivity and (3) becoming extinct in the wild. The likelihood of finding enough animals to create a captive founding population was low for many rare species, especially for salamanders and caecilians. It was also low for frogs which were once regularly encountered, but have already disappeared including *Atelopus chiriquiensis*, *Craugastor emcelae*, *C. obesus*, *C. punctariolus*, *C. rhyacobatrachus*, *Ecnomihyla rabborum*, *Isthmohyla calypsa* and *Oophaga speciosa*. Our results indicate that captive breeding could improve the odds of avoiding extinction for species that have severely declined or are likely to decline due to chytridiomycosis including *Atelopus certus*, *A. glyphus*, *A. limosus*, *A. varius*, *A. zeteki*, *Anotheca spinosa*, *Gastrotheca cornuta*, *Agalychnis lemur*

and *Hemiphractus fasciatus*. Priority species that experts predicted were highly susceptible to chytridiomycosis that might also benefit from *ex situ* management include *Craugastor tabasarae*, *C. azueroensis*, *C. evanescens*, *Strabomantis bufoniformis* and *Colostethus panamansis*. In spite of high levels of uncertainty, this expert assessment approach allowed us to refine our priorities for captive amphibian programs in Panama and identify priority conservation actions with a clearer understanding of the probability of success.

Introduction

Compared to other terrestrial vertebrates, amphibians are disproportionately threatened with extinction (Stuart *et al.*, 2004; Wake & Vredenburg, 2008) and receive much less conservation funding (Gratwicke, Lovejoy & Wildt, 2012b). Efficiently deploying limited resources and increasing existing capacity to conserve amphibians is imperative. Species-level conservation prioritization schemes typically evaluate level of IUCN-related endangerment (Master, 1991; Mace *et al.*, 2008), although additional criteria have been used including phylogenetic distinctiveness (Isaac *et al.*, 2007), types of conservation action (Carter *et al.*, 2000), costs and benefits (Weitzman, 1998) and probability of success (Joseph, Maloney & Possingham, 2009). However, a one-size fits all approach to setting priorities is unlikely to be useful because priorities of any program depend on specific goals and context.

Panama has 214 described amphibian species (AmphibiaWeb, 2015) that are ecologically and phylogenetically diverse, and about one third are listed by the IUCN Red List of Threatened Species as at risk of extinction. Since 1996, Panamanian amphibians have declined due to chytridiomycosis, a disease caused by an invasive fungus *Batrachochytrium dendrobatidis* (*Bd*) that has devastated naive amphibian communities (Lips, 1999; Crawford, Lips & Bermingham, 2010; Cheng *et al.*, 2011). Species declines and extirpations prompted an emergency response to create captive breeding programs (Lock, Mendelson & Gagliardo, 2006; Gagliardo *et al.*, 2008; Poole, 2008; PARC, 2010; ANAM, 2011; Zippel *et al.*, 2011). In 2000 Project Golden Frog was started to build captive assurance populations of *Atelopus zeteki* and *Atelopus varius* in the USA and after starting with a small founding population, the project now manages 1600 captive golden frogs in 50 zoos and aquaria (Poole, 2008). The last golden frog was seen in the wild in 2009, so this captive breeding project may have already avoided a *Bd*-related extinction, and an ambitious golden frog conservation plan was developed by multiple stakeholders to build the capacity, management, outreach, research and habitat goals that will allow us to pilot experimental reintroductions of this iconic species (Estrada *et al.*, 2014). A second effort to establish captive assurance colonies of more amphibian species began in 2006 involving two independent frog rescue response teams. One worked from El Copé to rescue frogs in the midst of the *Bd* epidemic and a second collected animals in the El Valle area 40 km ahead of the disease wave (Norris, 2007).

Because the die-off was so severe, the strategy involved capturing individuals of all identified frog species, even though little was known about the captive husbandry requirements of most species. Over time, much was learned about husbandry (Gagliardo *et al.*, 2008), treatment of chytridiomycosis (Baïtchman & Pessier, 2013) and specific causes of mortality including nutritional issues, polycystic kidney lesions, parasites, metabolic bone disease (Pessier *et al.*, 2014).

In 2009, the Panama Amphibian Rescue and Conservation (PARC) project began as an in-country effort to build additional capacity and manage captive assurance populations. Amphibians are held at two *ex situ* holding facilities, the El Valle Amphibian Conservation Center (EVACC) and the Gamboa Amphibian Research and Conservation Center (Gamboa ARCC). Besides improving husbandry, the goal of PARC was to establish self-sustaining, reproducing colonies, and to research methods to reduce the impact of *Bd* so that one day amphibians produced in captivity could be reintroduced to the wild (Gratwicke *et al.*, 2012a). The PARC project used an early tool developed by the IUCN's Amphibian Ark to prioritize species for captive breeding (AArk, 2009). This additive index considers extinction risk, phylogenetic significance, reversibility of threats, biological distinctiveness as well as cultural and scientific importance to produce a single numerical score ranking for priority. More recently, the process was refined to include animal availability and management capacity (AArk, 2009), but it does not explicitly evaluate the likelihood of avoiding extinction through captive breeding efforts.

The PARC project has a diverse collection of amphibians and there is a need to focus effort on those species where captive breeding efforts will have the highest likelihood of avoiding extinction. In reality, this is easier said than done because there remains little empirical data on susceptibility to chytridiomycosis, rates of decline, wild population sizes and captive husbandry at a species level. In these situations, expert opinion surveys are often the best way to systematically capture the needed information (Fazey *et al.*, 2006; Kuhnert, Martin & Griffiths, 2010; Raymond *et al.*, 2010). The objective for this study was to conduct an expert opinion survey to evaluate the perceived susceptibility of Panama's amphibians to chytridiomycosis and filter the list of species to determine the species that had or would likely experience *Bd*-related declines. For the susceptible species, we calculate the probability of avoiding extinction through captive breeding efforts as the product of three probabilities determined from expert responses (1) finding sufficient

founder animals, (2) successfully breeding these species in captivity and (3) becoming extinct in the wild.

1 *Finding sufficient founder animals*: The probability of finding enough wild males and females to form a viable captive population varies depending on whether the species is rare, difficult to find, or has already declined. One of the greatest risks to a species in a captive breeding program is genetic deterioration resulting from inbreeding depression (Frankham, Briscoe & Ballou, 2002). To minimize the potential loss of genetic heterozygosity, a population of adequate size is required to retain 90% of existing genetic diversity for the next 100 years (Frankham *et al.*, 2002). Recommended numbers of founders vary from species to species depending on age at sexual maturity and reproductive life span (Schad, 2008). At least 10 unrelated male–female pairs is an approximate guideline for the minimum numbers needed for amphibian conservation breeding programs, and given that the probability of breeding founders is not 100%, these numbers should be increased accordingly (Schad, 2008).

2 *Successfully breeding these species in captivity*: A few colorful Latin American species such as *Dendrobates auratus*, *Oophaga pumilio* and *Agalychnis callidryas* are popular pets or widely kept in zoos and aquaria. Husbandry tactics for these species are well known to hobbyists and the zoo community. With care and attention to detail, these species can be bred in captivity, and may be useful models for understanding the husbandry requirements of related amphibians. However, the natural history of many tropical amphibian species is virtually unknown, and some species do not adapt well to captivity because we cannot sufficiently replicate natural conditions and diets (Ferrie *et al.*, 2014), do not know breeding cues, or the animals develop high levels of stress in captive conditions making captive husbandry an especially challenging research issue (Browne *et al.*, 2007).

3 *Becoming extinct in the wild*: The probability of avoiding extinction is linked to the probability that a species actually goes extinct in the wild. The IUCN Red List Authority listing process classifies species as near threatened, vulnerable, endangered or critically endangered, implying a differential degree of extinction risk between categories (Mace *et al.*, 2008). It is important to note, however, that red list categories are not tied to explicit extinction probabilities (Fieberg & Ellner, 2000; Mooers, Faith & Madison, 2008). For our purposes it is necessary to quantify extinction probability and the level of uncertainty around that estimate, because incorrectly prioritizing a species not in danger of extinction could deprive a more vulnerable species of limited *ex situ* space resources and research attention.

Materials and methods

Expert selection

We identified 35 potential amphibian experts who are members of the *Círculo Herpetológico de Panamá*, the IUCN

Amphibian Specialist Group for Panama, or who were suggested by one of the existing authors that had at least 5 years of field experience in Panama, Costa Rica or Colombia, or with equivalent captive husbandry experience with Panamanian amphibian species. We asked each expert to participate in an online questionnaire for every Panamanian amphibian species with which they had direct field or husbandry experience. We also provided information on what species were represented in captivity in Panama and at Association of Zoos and Aquariums (AZA) facilities in the United States (Table 1).

Precision and uncertainty

We devised a minimum–maximum rank weighting approach that limited responses to evenly distributed 20th percentiles as follows: very low (0–20%), low (20–40%), medium (40–60%), high (60–80%) and very high (80–100%). Where respondents had a high degree of certainty, they could also restrict the upper and lower bounds at either end of the probability range, that is 0–1% or 100%. To broaden the uncertainty bounds, respondents were allowed to select multiple categories. For each species, we asked participants to self-identify their level of experience as high, medium, low or none in both field and captive care settings so that we could weight their responses accordingly. If the participant did not know the answer to a particular question, they were asked to leave the field blank. The full survey is available in Supporting Information (Data S1) and online (available from: <http://bit.ly/1oyaKPS>).

Analysis

Due to recent amphibian name changes, all returns were checked for taxonomic consistency following the Amphibian Species of the World Database (Frost, 2014). As a first filter, species were ranked by the probability of observing declines when infected with *Bd*. These categories were as follows: 0 = not susceptible, declines in *Bd* positive situations will likely be undetectable; 1 = moderately susceptible, declines will be noticeable, but the species will likely persist in some places throughout its former range; 2 = highly susceptible, severe declines throughout its range and may only persist in a few places; 3 = severely susceptible, will likely disappear from entire range and will possibly go extinct without intervention. Individual answers were weighted for field experience, multiplying answers by 1 = no experience; 2 = low experience; 3 = medium experience and 4 = high experience. Totals for each species were divided by the total weighting score to calculate an average weighted susceptibility. Any species that received responses from fewer than three participants was omitted from the analysis as unknown. We focused subsequent analyses on the top quartile of most susceptible species.

The experience-weighted upper and lower probability bounds were calculated similarly for (1) establishing a viable *ex situ* population, (2) extinction in the wild in the next 20 years and (3) breeding successfully in captivity. Any

Table 1 List of Panamanian amphibian species held for conservation purposes in Panama (April 2014), the Atlanta Botanical Gardens and accredited AZA Institutions as reported in the ZIMS (April 2014)

Species	Holding facility	Male	Female	Unsexed
<i>Agalychnis lemur</i>	EVACC	7	1	52
	19 AZA Institutions	7	2	232
	Atlanta Botanical Garden	12	20	120
<i>Anotheca spinosa</i>	EVACC	49	15	80
	5 AZA institutions		1	11
	Atlanta Botanical Garden	8	6	45
<i>Atelopus certus</i> ^a	Gamboa ARCC	93	56	89
<i>Atelopus glyphus</i> ^a	Gamboa ARCC	56	45	85
	EVACC	12	6	1
<i>Atelopus limosus</i> ^a	Gamboa ARCC	40	17	70
	EVACC	1		13
<i>Atelopus varius</i> ^a	EVACC	6	10	
	3 AZA institutions	70	44	48
<i>Atelopus zeteki</i> ^a	EVACC	12	12	
	53 AZA institutions	498	415	359
<i>Colostethus panamansis</i>	EVACC	3		3
<i>Craugastor evanesco</i>	EVACC			35
<i>Craugastor punctariolus</i>	EVACC	1	2	
<i>Craugastor tabasarae</i>	EVACC	1	4	
<i>Ecnomiohyla rabborum</i>	Atlanta Botanical Garden	1		
<i>Gastrotheca cornuta</i> ^a	EVACC	47	26	56
	Atlanta Botanical Garden	1	1	
<i>Hemiphractus fasciatus</i>	EVACC	4	13	19
<i>Hyloscirtus colymba</i>	Gamboa ARCC		2	
<i>Sachatamia albomaculata</i>	Atlanta Botanical Garden	1	1	
<i>Sachatamia ilex</i>	EVACC	4	1	3
<i>Strabomantis bufoniformis</i>	EVACC	34	13	

AZA, Association of Zoos and Aquariums; ZIMS, Zoological Information Management System; ARCC, Amphibian Research and Conservation Center; EVACC, El Valle Amphibian Conservation Center.

^aAt least 10 male and 10 female founders are known to be alive or represented in the existing captive collection. *Atelopus zeteki* and *A. varius* numbers were determined from the Golden Frog Species Survival Plan report (Estrada *et al.*, 2014).

species that received responses from fewer than three participants was omitted from the analysis as unknown.

Results

Chytridiomycosis susceptibility

Twenty-three experts returned 1387 responses for 215 Panamanian amphibian species (Supporting Information Data S1). Twelve were primarily field experts, five were primarily captive husbandry experts and six experts had experience in both field and captive settings. We received at least three independent *Bd* susceptibility responses for 134 (62%) species. We present these responses as a ranked list that also acts as our first filter (Table 2). The top quartiles (34 species) were dominated by three anuran genera: *Craugastor*, *Atelopus* and *Isthmohyla* (Table 2). Higher perceived susceptibility to *Bd* scores were associated with more endangered IUCN status (Table 2), but seven species in the top quartile were not listed by the IUCN as Endangered or Critically Endangered. *Craugastor evanesco* is a recently described species that has not yet been evaluated, but belongs to the highly chytridiomycosis-susceptible *Craugastor rugulosus* species group of Central America (Ryan *et al.*,

2010). *Silverstoneia nubicola* and *Hemiphractus fasciatus* are listed as Near Threatened, whereas *Strabomantis bufoniformis*, *Anotheca spinosa*, *Colostethus panamansis* and *Hyloscirtus palmeri* are listed as Least Concern by the IUCN Red List Authority.

Finding founders

The probability of establishing founding populations was unknown for about 15% of species identified as *Bd* susceptible, and the upper probability of finding a sufficient founding population was low for 17% of *Bd*-susceptible species (Table 2). *Atelopus zeteki*, *A. certus*, *A. glyphus* and *A. limosus* were judged to have higher probabilities of successfully establishing founding populations, in part, because they are already maintained in captive colonies established prior to declines (Tables 1, 2). The overall probability of finding founding populations for the salamanders in the genus *Bolitoglossa* was also low, largely because they are rarely encountered in the field (Table 2; Supporting Information Data S2).

Experts indicated in their narrative responses that about 4% of Panama's known amphibians are missing. *Atelopus*

Table 2 Expert survey results showing Panamanian amphibian species ranked by their perceived susceptibility to *Batrachochytrium dendrobatidis* (*Bd*) and mean upper and lower estimates of factors used to determine the percentage probability of avoiding extinction through captive breeding programs

Species	IUCN ^a	<i>Bd</i> ^b	<i>P</i> finding founders		<i>P</i> extinction in wild		<i>P</i> captive breeding		<i>P</i> avoiding extinction	
			Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
<i>Atelopus chiriquiensis</i>	CR	3.0	0	1	80	100	72	92	0	1
<i>Craugastor catalinae</i>	CR	3.0					40	60		
<i>Craugastor punctariolus</i>	EN	3.0	0	1	40	60	18	42	0	1
<i>Craugastor rhyacobatrachus</i>	EN	3.0	0	1			33	60		
<i>Craugastor taurus</i>	CR	3.0	27	47			40	60		
<i>Oophaga speciosa</i>	EN	3.0					60	80		
<i>Isthmohyla calypsa</i>	CR	2.9	0	1			27	47		
<i>Atelopus zeteki</i>	CR	2.8	81	86	72	96	64	83	37	68
<i>Ecnomiophyla rabborum</i>	CR	2.8	8	17	80	100	22	34	1	5
<i>Atelopus certus</i>	EN	2.7	84	98	57	84	70	92	33	76
<i>Atelopus glyphus</i>	CR	2.7	88	95	62	87	67	91	37	75
<i>Incilius fastidiosus</i>	CR	2.6								
<i>Isthmohyla angustilineata</i>	CR	2.6					33	53		
<i>Isthmohyla tica</i>	CR	2.6	0	7			33	53		
<i>Atelopus limosus</i>	EN	2.5	60	85	67	94	58	82	23	66
<i>Craugastor azueroensis</i>	EN	2.5	38	48	38	68	40	67	6	21
<i>Craugastor obesus</i>	EN	2.5	0	7			25	45		
<i>Isthmohyla debilis</i>	CR	2.5	9	18			27	47		
<i>Craugastor evanesco</i>		2.4	33	57	77	97	27	47	7	26
<i>Atelopus varius</i>	CR	2.4	30	55	54	83	68	89	11	41
<i>Duellmanohyla uranochroa</i>	EN	2.3	37	52			27	47		
<i>Strabomantis bufoniformis</i>	LC	2.3	30	56	41	76	31	56	4	24
<i>Isthmohyla gracieae</i>	CR	2.3					27	41		
<i>Agalychnis lemur</i>	CR	2.2	35	59	37	65	61	83	7	32
<i>Anotheca spinosa</i>	LC	2.0	26	60	47	87	70	98	10	59
<i>Craugastor tabasarae</i>	CR	2.0	10	44	37	80	33	62	1	22
<i>Isthmohyla rivularis</i>	CR	2.0	20	31			40	60		
<i>Gastrotheca cornuta</i>	EN	2.0	17	45	40	68	48	74	3	23
<i>Colostethus panamansis</i>	LC	1.8	58	83	29	56	34	60	6	28
<i>Silverstoneia nubicola</i>	NT	1.8					53	73		
<i>Pipa myersi</i>	EN	1.8	0	20			40	60		
<i>Hyloscirtus colymba</i>	CR	1.7	17	42	46	77	3	28	0	9
<i>Hemiphractus fasciatus</i>	NT	1.7	24	60	45	79	53	79	6	37
<i>Hyloscirtus palmeri</i>	LC	1.7	14	39	53	83	4	29	0	9
<i>Colostethus pratti</i>	LC	1.6	47	71	29	60	65	92	9	39
<i>Oedipina grandis</i>	EN	1.5	0	15			20	34		
<i>Oophaga arborea</i>	EN	1.5					55	80		
<i>Oophaga granulifera</i>	VU	1.5	69	89			60	85		
<i>Pristimantis museosus</i>	EN	1.5	21	48	32	65	22	54	1	17
<i>Pristimantis moro</i>	LC	1.4	7	33	40	65	23	43	1	9
<i>Bolitoglossa colonnea</i>	LC	1.4	12	27	36	70	35	55	2	10
<i>Craugastor underwoodi</i>	LC	1.4	40	68	31	57	33	53	4	21
<i>Craugastor rugosus</i>	LC	1.3	13	44			33	53		
<i>Craugastor opimus</i>	LC	1.3					34	60		
<i>Hyalinobatrachium aureoguttatum</i>	NT	1.3			27	53	53	73		
<i>Cochranella euknemos</i>	LC	1.2	15	34	30	58	33	55	2	11
<i>Craugastor megacephalus</i>	LC	1.2	23	48	31	57	35	57	2	16
<i>Bolitoglossa schizodactyla</i>	LC	1.2	15	33			27	47		
<i>Phyllobates lugubris</i>	LC	1.1	52	72	24	49	50	73	6	25
<i>Hyalinobatrachium colymbiphyllum</i>	LC	1.1	58	77	5	36	44	64	1	18
<i>Silverstoneia flotator</i>	LC	1.1	73	99	4	33	53	80	2	26
<i>Craugastor monnichorum</i>	DD	1.1	22	42	38	62	32	56	3	15
<i>Sachatamia ilex</i>	LC	1.1	30	59	25	53	36	60	3	19

Table 2 Continued.

Species	IUCN ^a	Bd ^b	P finding founders		P extinction in wild		P captive breeding		P avoiding extinction	
			Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
<i>Lithobates warszewitschii</i>	LC	1.1	47	71	26	51	25	47	3	17
<i>Craugastor gollmeri</i>	LC	1.1	23	47	27	53	38	58	2	14
<i>Pristimantis caryophyllaceus</i>	NT	1.0	45	66	18	39	24	46	2	12
<i>Craugastor noblei</i>	LC	1.0	9	37	20	54	40	60	1	12
<i>Isthmohyla lancasteri</i>	LC	1.0	40	60			40	60		
<i>Andinobates claudiae</i>	DD	1.0								
<i>Pristimantis gaigei</i>	LC	1.0	14	40	29	53	49	70	2	15
<i>Craugastor talamancae</i>	LC	1.0	40	64	11	39	40	64	2	16
<i>Pristimantis pardalis</i>	NT	0.9	21	46	31	52	30	52	2	12
<i>Ctenophryne aterrima</i>	LC	0.9	3	8			30	45		
<i>Incilius aucoinae</i>	LC	0.9	87	100	10	30				
<i>Dendrobates auratus</i>	LC	0.9	73	93	10	35	70	94	5	30
<i>Hyalinobatrachium talamancae</i>	LC	0.9	46	75	33	58	36	68	6	30
<i>Pristimantis taeniatus</i>	LC	0.9	38	64	24	51	40	68	4	22
<i>Hyalinobatrachium chirripoi</i>	LC	0.9	20	45	14	42	37	57	1	11
<i>Agalychnis spurrelli</i>	LC	0.8	44	67	24	44	75	95	8	28
<i>Hyalinobatrachium valerioi</i>	LC	0.8	17	40	33	60	64	84	4	20
<i>Incilius coniferus</i>	LC	0.8	57	78	15	36	59	84	5	24
<i>Bolitoglossa medemi</i>	VU	0.8								
<i>Teratohyla pulverata</i>	LC	0.8	21	42	16	43	35	55	1	10
<i>Andinobates minutus</i>	LC	0.8	65	92	15	44	51	71	5	28
<i>Isthmohyla zeteki</i>	NT	0.8	22	40	37	57	40	60	3	14
<i>Pristimantis cruentus</i>	LC	0.8	34	67	17	44	30	57	2	17
<i>Teratohyla spinosa</i>	LC	0.8	48	73	16	39	52	72	4	20
<i>Pristimantis cerasinus</i>	LC	0.7	50	75	15	41	28	48	2	15
<i>Hypsiboas rufitelus</i>	LC	0.7	60	81	20	45	29	51	3	19
<i>Pristimantis ridens</i>	LC	0.7	52	73	13	33	30	50	2	12
<i>Craugastor melanostictus</i>	LC	0.7	40	61	20	50	40	60	3	18
<i>Cruziohyla calcarifer</i>	LC	0.7	5	20			66	86		
<i>Allobates talamancae</i>	LC	0.7	65	86	9	25	64	87	4	19
<i>Incilius signifer</i>	LC	0.7					56	80		
<i>Oophaga vicentei</i>	DD	0.7	80	94	19	46	55	75	8	32
<i>Espadarana prosoblepon</i>	LC	0.6	69	90	12	29	46	70	4	19
<i>Sachatamia albomaculata</i>	LC	0.6	59	82	11	35	50	72	3	21
<i>Craugastor podiciferus</i>	NT	0.6	60	88	33	63	33	60	7	33
<i>Bolitoglossa compacta</i>	EN	0.6	0	7			20	34		
<i>Oedipina complex</i>	LC	0.6	7	27			20	37		
<i>Ptychohyla legleri</i>	EN	0.6					27	40		
<i>Craugastor bransfordii</i>	LC	0.6	45	68	14	39	40	63	3	16
<i>Smilisca sordida</i>	LC	0.6	56	76	20	47	53	73	6	26
<i>Hyalinobatrachium fleischmanni</i>	LC	0.5	56	78	9	31	58	82	3	20
<i>Rhaebo haemitticus</i>	LC	0.5	58	80	12	35	52	77	4	21
<i>Dendropsophus ebraccatus</i>	LC	0.4	70	90	7	22	73	93	3	19
<i>Craugastor crassidigitus</i>	LC	0.4	73	93	7	31	50	73	2	21
<i>Cochranella granulosa</i>	LC	0.4	50	73	12	35	68	88	4	23
<i>Diasporus diastema</i>	LC	0.4	58	82	8	25	27	50	1	10
<i>Bolitoglossa marmorea</i>	EN	0.4					13	27		
<i>Phyllomedusa venusta</i>	LC	0.4	27	60			38	70		
<i>Bolitoglossa lignicolor</i>	VU	0.4	12	24						
<i>Bolitoglossa minutula</i>	EN	0.3								
<i>Diasporus quidditus</i>	LC	0.3	67	90	11	38	33	57	2	19
<i>Leptodactylus savagei</i>	LC	0.3	53	76	17	37	35	70	3	20
<i>Oedipina parvipis</i>	LC	0.3					33	40		
<i>Smilisca sila</i>	LC	0.3	60	83			53	80		
<i>Craugastor fitzingeri</i>	LC	0.3	67	88	6	21	57	80	2	15

Table 2 Continued.

Species	IUCN ^a	<i>Bd</i> ^b	<i>P</i> finding founders		<i>P</i> extinction in wild		<i>P</i> captive breeding		<i>P</i> avoiding extinction	
			Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
<i>Rhinella alata</i>		0.3	70	89	6	26	60	80	3	18
<i>Smilisca phaeota</i>	LC	0.3	57	84	5	29	51	76	2	18
<i>Chiasmocleis panamensis</i>	LC	0.3	40	73	20	45	33	53	3	18
<i>Rhinella centralis</i>	LC	0.2	68	96	0	5	60	80	0	4
<i>Lithobates vaillanti</i>	LC	0.2	59	79	40	60	48	68	11	32
<i>Agalychnis callidryas</i>	LC	0.2	76	98	4	22	77	100	3	21
<i>Engystomops pustulosus</i>	LC	0.2	74	100	3	21	67	93	2	19
<i>Dendropsophus microcephalus</i>	LC	0.2	75	97	3	12	67	87	1	10
<i>Crepidophryne epiotica</i>	LC	0.1	36	58	14	31				
<i>Leptodactylus melanonotus</i>	LC	0.1	60	80	11	31	40	60	3	15
<i>Craugastor stejnegerianus</i>	LC	0.1	75	95	5	28	33	60	1	16
<i>Bolitoglossa biseriata</i>	LC	0.0					20	40		
<i>Bolitoglossa magnifica</i>	EN	0.0								
<i>Dendropsophus phlebodes</i>	LC	0.0	76	96	5	12	70	90	3	10
<i>Diasporus hylaeformis</i>	LC	0.0	40	67	18	40	33	53	2	14
<i>Hypsiboas boans</i>	LC	0.0					47	73		
<i>Hypsiboas pugnax</i>	LC	0.0					47	73		
<i>Hypsiboas rosenbergi</i>	LC	0.0	55	83	12	37	33	57	2	17
<i>Isthmohyla picadoi</i>	NT	0.0	18	44			40	60		
<i>Leptodactylus bolivianus</i>	LC	0.0	45	64	8	28	40	60	1	11
<i>Leptodactylus fragilis</i>	LC	0.0	60	80	6	12	40	60	1	6
<i>Oophaga pumilio</i>	LC	0.0	76	100	5	25	65	90	3	23
<i>Pleurodema brachyops</i>	LC	0.0	76	96	0	1	55	75	0	1
<i>Rhinella marina</i>	LC	0.0	78	97	0	3	77	97	0	3
<i>Scinax alata</i>	LC	0.0					53	73		
<i>Scinax boulengeri</i>	LC	0.0	45	69	12	40	53	73	3	20
<i>Scinax elaeochrous</i>	LC	0.0	65	85			53	80		
<i>Scinax ruber</i>	LC	0.0	40	80	20	47	53	73	4	28
<i>Trachycephalus typhonius</i>	LC	0.0	40	65	20	40	53	80	4	21

Bold numbers indicate the species was ranked in the top quartile of responses. Blanks indicate that fewer than three expert responses were received. The 78 species for which fewer than three responses were received evaluating their *Bd* susceptibility are not included in this table but responses may be viewed in Supporting Information (Data S2).

^aIUCN Red List status are indicated in the first column.

^bPerceived susceptibility to *Bd* is indicated in the second column [ranging from 3 (most susceptible) to 0 (least susceptible)].

chiriquiensis, *Craugastor emcelae*, *C. obesus*, *C. punctariolus*, *C. rhyacobatrachus*, *Ecnomihyla rabborum*, *Isthmohyla calypsa* and *Oophaga speciosa* have not been observed for 8–15 years. Therefore, the likelihood of finding sufficient numbers for captive assurance populations for these species is exceedingly low (Table 2; Supporting Information Data S1). The 78 amphibian species for which there were insufficient responses to assess *Bd* susceptibility are likely also rare; ~40% of these were salamanders or caecilians (Supporting Information Data S2). The absence of confidence by the experts to provide a formal assessment also indicates a likely inability to establish sufficient founder populations of these species.

Probability of extinction in wild

Perceived *Bd* susceptibility was strongly correlated with the probability that the species would go extinct in the wild in

the next 20 years [Pearson's $r(91) = 0.86$ $P < 0.001$]. All of the species perceived to be the most sensitive to *Bd* were within the top quartile of species at the highest risk of extinction (Table 2). Higher expert evaluations of extinction risk in the next 20 years were strongly associated with more threatened IUCN risk categories (Table 2).

Captive breeding

Of the most chytridiomycosis-susceptible species, frogs of the genera *Atelopus*, *Oophaga*, *Agalychnis* and *Anotheca* had the best chances of establishing viable, long-term captive populations, whereas frogs of the genus *Hyloscirtus* were the least likely to succeed under *ex situ* conditions (Table 2). There was no apparent relationship between the likelihood of success with captive breeding and extinction probability or the likelihood of finding founding populations (Table 2).

Probability of avoiding extinction through captive breeding

We had sufficient expert responses in all categories for 19 of the 36 species most susceptible to *Bd* (Fig. 1). Of these, the four highest ranked species were *Atelopus glyphus*, *A. certus*, *A. limosus* and *A. zeteki*, with a medium-to-high chance of avoiding extinction through captive breeding colonies. *Craugastor evanescens*, *C. tabasarae*, *C. azueroensis*, *Strabomantis bufoniformis*, *Colostethus panamansis*, *Gastrotheca cornuta*, *Agalychnis lemur*, *Hemiphractus fasciatus*, *Atelopus varius* and *Anotheca spinosa* had very low-to-medium chances of avoiding extinction through captive breeding due to a combination of difficulty finding founders and/or ability to breed them in captivity (Fig. 1; Table 2). At the lowest end of the scale were *Craugastor punctariolus*, *Atelopus chiriquiensis* and *Ecnomiohyla rabborum*. For these species, the chance of avoiding extinction through captive breeding was very low, primarily because they have already disappeared. The two *Hyloscirtus* species were ranked low primarily because of challenges associated with *ex situ* care and breeding (Table 2; Fig. 1). The prognosis of success for the other 17 species perceived to be highly susceptible to *Bd* was projected to be low, largely due to the challenges of establishing a founding population (Table 2).

Discussion

Chytridiomycosis susceptibility

There was a close association between chytridiomycosis susceptibility scores and IUCN status that was not surprising because chytridiomycosis was a major threat considered during the last systematic IUCN red list conservation assessment of Panamanian species in 2008. Of more interest were species perceived to be highly susceptible to chytridiomycosis that are not currently listed in threatened IUCN categories

such as *Craugastor evanescens*, *Strabomantis bufoniformis*, *Anotheca spinosa*, *Colostethus panamansis* and *Hyloscirtus palmeri*. This should be justification for immediate assessment or reassessment.

More than half of the species that experts considered most sensitive to chytridiomycosis belonged to the genera *Atelopus*, *Isthmohyla* and *Craugastor*. All six *Atelopus* species were ranked in the top quartile of most *Bd*-sensitive species. This genus has experienced widespread declines throughout the neotropics, and >30 of 97 species are now feared extinct, primarily because of *Bd*-related declines (La Marca *et al.*, 2005). *Atelopus* are charismatic, diurnal toads that normally exist at high densities, so their absences have been particularly noticeable compared to other more cryptic or rare species (Lindquist & Swihart, 1997; La Marca *et al.*, 2005). The hypersensitivity of *Atelopus* to chytridiomycosis has been demonstrated in both laboratory and field settings that validate this assessment (Woodhams *et al.*, 2006; Bustamante, Livo & Carey, 2010; Richards-Zawacki, 2010; Becker *et al.*, 2012; Langhammer *et al.*, 2013).

Stream-dwelling frogs of the *Craugastor punctariolus* species series (Campbell & Savage, 2000) have experienced significant *Bd*-related declines throughout much of their range (Mason, Karen & Michael, 2008; Ryan *et al.*, 2010; Zumbado-Ulate *et al.*, 2011). Panama has eight species in this species series: *C. azueroensis*, *C. catalinae*, *C. evanescens*, *C. obesus*, *C. punctariolus*, *C. ranoides*, *C. rhyacobatrachus*, *C. taurus* (Campbell & Savage, 2000; Ryan *et al.*, 2010); all were highly *Bd* sensitive with at least one species having poor innate skin defenses (Woodhams *et al.*, 2006).

All of the *Isthmohyla* identified by our experts as most susceptible to chytridiomycosis are listed by the IUCN as critically endangered. Disappearances of *Isthmohyla*, such as *I. rivularis*, *I. angustilineata*, *I. tica*, *I. calypsa*, have been attributed to chytridiomycosis (Lips, 1999; Cheng *et al.*, 2011; Garcia-Rodriguez *et al.*, 2012), and our experts placed these in the top quartile of the most sensitive species. Other

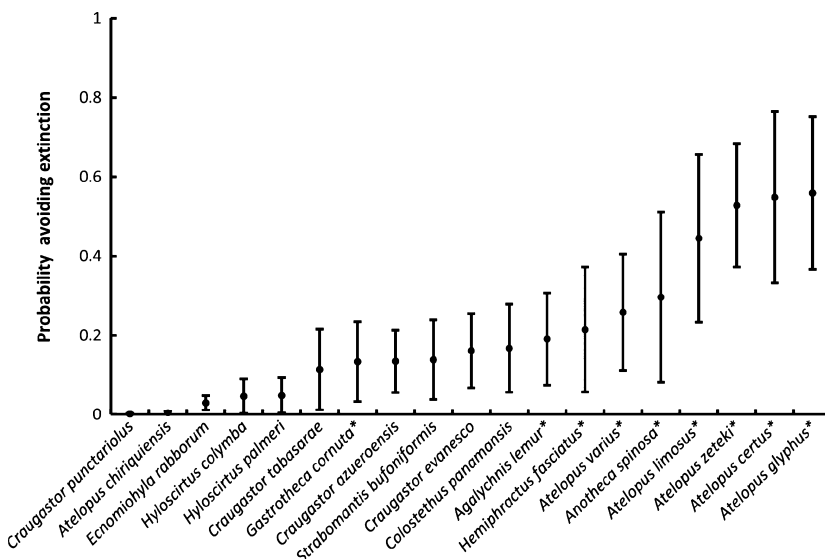


Figure 1 Ranked probability of avoiding extinction through captive breeding for Panamanian amphibian species perceived to be most susceptible to *Batrachochytrium dendrobatidis* (*Bd*). Shown are the range between mean lower and mean upper probability estimates derived through an expert assessment. Asterisk (*) indicates species has been maintained and bred reliably in captive conditions, and species for which we did not receive sufficient responses are not shown.

Isthmohyla species, such as bromeliad-dwelling *I. picadoi* and *I. zeteki* and pond-breeding *I. pseudopuma*, persist in *Bd*-positive areas (Stuckert *et al.*, 2009) and were considered less susceptible (Table 2).

Experts are powerful integrators of information and can accommodate differences between species' natural history that would be difficult to capture any other way. It is reassuring to find that existing literature corroborated this assessment, but self-reinforcement or erroneous thinking is an inherent risk of using experiential knowledge in small communities familiar with each other's work (Fazey *et al.*, 2006). Expert assessments require checks and balances in the form of empirical evidence (Fazey *et al.*, 2006). Controlled exposure experiments (Blaustein *et al.*, 2005; Martel *et al.*, 2014; Voyles *et al.*, 2014) and mucosome tests (Woodhams *et al.*, 2014) would be powerful validation tools that could help verify this study's assessment of chytridiomycosis sensitivity more extensively than covered here.

Rare and 'lost' species

Rare species of amphibians often have restricted ranges and small population sizes, which are both factors increasing vulnerability to extinction (Toledo *et al.*, 2014). While the conservation needs within this category are likely to be high, our lack of basic natural history knowledge and the inability to find many of these animals in the wild currently limits captive breeding as a potential conservation tool. Because of a dearth of information, there is a need for more natural history field studies, especially of tropical salamanders and caecilians.

For species that were once more abundant but have disappeared, rediscovery is a critical next step. Our experts identified *Atelopus chiriquiensis*, *A. zeteki*, *Craugastor emcelae*, *C. obesus*, *C. punctariolus*, *C. rhyacobatrachus*, *Ecnomiohyla rabborum*, *Isthmohyla calypsa* and *Oophaga speciosa* as 'Panama's lost frogs'. In the case of *O. speciosa*, at least 31 were exported from Panama since being listed by CITES in 1987 (Carpenter *et al.*, 2014), but it is unclear whether any descendants of that population persist in captivity. *Ecnomiohyla rabborum* is down to one last remaining captive individual held at the Atlanta Botanical Gardens (M. Mandica, pers. comm.). A few individuals presumed to be *C. punctariolus* remain at EVACC, but it is a species that is difficult to identify as it is part of cryptic species complex (Crawford *et al.*, 2013). Over the course of 7 years EVACC has produced several clutches of mostly infertile eggs and has been unable to rear young to adulthood (Supporting Information Data S2). All of these 'lost' frog species are possibly extinct in nature and only *Atelopus zeteki* is now secure in captivity in the USA and Panama.

Missing species that have been rediscovered in the wild are termed 'Lazarus' species, but what subsequent actions are needed remain open for debate (Meijaard & Nijman, 2014). Several neotropical frog species that were absent for protracted intervals, such as *Incilius holdridgei*, *Atelopus varius*, *Lithobates vibicarius*, *Craugastor taurus*, have been found again, but at low densities (Kubicki, 2004, 2006; Abarca *et al.*, 2010; Garcia-Rodriguez *et al.*, 2012; Hertz *et al.*, 2012; Delia, Whit-

ney & Burkhardt, 2013; Chaves *et al.*, 2014). In these cases there are too few animals to collect a founding population (Frankham *et al.*, 2002), and supplementing small wild populations with captive-bred animals has its own risks (Jensen, 2013). Intensive study of these populations and monitoring of threats would be recommended in order to develop a clear information-based conservation strategy.

Captive experience

The probability of breeding an animal in captivity for about two thirds of Panama's species fell in the medium to high range, indicating that experts were confident that husbandry challenges could be solved for a majority of the species. One of the lessons learned as a byproduct of this epidemic is that general husbandry approaches may work for the majority of species, but simply collecting a full founding population and exerting substantial effort on husbandry approaches may not work for every species. For example, founding populations were collected and maintained for *H. colymba* and *C. punctariolus*, and despite successful but limited egg laying, we were unable to rear offspring through metamorphosis (Supporting Information Data S2).

Another key factor is having enough time to resolve the limiting elements to effective husbandry, nutrition and reproduction. For example, the Panamanian Golden Frog Husbandry Manual (Poole, 2006) captured 10 years of experience and trial and error with golden frogs in captivity. These protocols have, in turn, benefited other Panamanian *Atelopus* species including *A. certus*, *A. glyphus*, *A. limosus* and *A. varius* (R. Ibáñez, pers. comm.). Such knowledge also has increased the confidence by experts in the success of future captive breeding for *Agalychnis lemur*, *Hemiphractus fasciatus*, *Gastrotheca cornuta* and *Anotheca spinosa*, all of which had high rankings in the survey for likely avoiding extinction, in part due to a successful captive breeding track record. It is clear from these examples that the information learned from captive breeding programs is an important factor that should be incorporated into the prioritization process despite the fact that the lessons learned are mostly qualitative and seldom published.

Recommendations

Less-susceptible species

About 100 species, about half of Panama's amphibian species can still be reliably found, even in *Bd*-positive areas and have not experienced severe chytridiomycosis declines (Table 2). We do not recommend establishing and maintaining *ex situ* assurance populations for these species unless non-*Bd*-related threats are a pressing concern, or new evidence is found demonstrating their susceptibility to chytridiomycosis.

Lost frogs and rare species

Our study identified 81 species, where not enough information was known to provide an evaluation, and nine species that were once fairly common but have disappeared (Sup-

porting Information Data S2). Most of the species that we have limited information for are likely to be rare species, and we know that rare species or those with very limited distributions are more likely to be in danger of extinction (Toledo *et al.*, 2014). In both cases, efforts should be made to survey known localities of these species and obtain more reliable information on their status, particularly if their congeners are *Bd* susceptible. Genomic material should be banked for these species including living cell lines, living gametes and cryopreserved tissues (Lermen *et al.*, 2009; Kouba *et al.*, 2013).

***Bd*-sensitive species that have declined severely or are likely to decline severely**

Craugastor azueroensis is not represented in current captive breeding collections, but they are extant members of the *Craugastor punctariolus* species series that can still be found reliably in the wild (Köhler *et al.*, 2012). This species is a potential candidate for captive breeding efforts even though the combined probability of avoiding extinction is low, the chances of avoiding an extinction through captive breeding are better than for several of the other species. Studying wild populations and learning more about their natural history, improving and resolving captive husbandry approaches, exploring assisted reproduction methods and collecting wild founding populations while these species are still extant will help improve the odds of success. A captive breeding population represented by at least 10 males and 10 females exists in Panama for *Atelopus glyphus*, *A. certus* and *A. limosus*, and these species are not held elsewhere in captivity (Table 1). Existing unrepresented founders should be bred as soon as possible, capturing as much of their genetic diversity as possible, with a goal of ensuring even representation of founder's genes in a total captive population of 500 individuals (Schad, 2008). A captive population represented by at least 10 males and 10 females exists for *Atelopus zeteki* and *Atelopus varius* in the USA and Panama (Table 1), and captive management steps are already being taken to coordinate the population management internationally as part of the AZA Golden Frog Species Survival Plan (Estrada *et al.*, 2014). Similarly, a captive population represented by at least 10 males and 10 females exists for *Gastrotheca cornuta* in Panama and the USA, but a formal Species Survival Plan or animal exchange plan does not exist between the two holding institutions and should be explored.

Partial founding populations of *Hemiphractus fasciatus*, *Craugastor evanescens*, *Colostethus panamansis* and *Strabomantis bufoniformis* exist only in Panama. *Hemiphractus fasciatus* has been bred in captivity, but additional expeditions to collect additional founders are needed. *Strabomantis bufoniformis* has been bred successfully, but not reliably in captivity, while *C. evanescens* and *C. panamansis* have not been bred in captivity (Supporting Information Data S2). Focused work on developing husbandry protocols and identifying breeding cues or researching assisted reproduction methods for these species is needed.

Partial founding populations exist in Panama and elsewhere for *Anotheca spinosa* and *Agalychnis lemur* (Table 1). These species have all been reliably bred in captivity, but international coordination and cooperation to manage holdings in multiple institutions is required to ensure that the genetic integrity of the captive population as a whole is maintained. Targeted collecting expeditions may be needed to collect additional founders if the combined captive population is not represented by adequate founders.

At present we do not have an ability to reverse the threat of *Bd* in the wild, so a more holistic conservation prioritization scheme that aims to get the best return on investment considering multiple threats and taxa might logically focus resources on other more tractable problems and species. Captive breeding alone does not solve the threat of chytridiomycosis, but it does buy us the time needed to continue the incremental research that may help tip the scales in favor of the frogs, and given our strong track record of solving disease problems in humans and domesticated species, we are hopeful that we will eventually be able to solve this critical wildlife disease threat. Only healthy assurance populations will help us avoid extinctions of highly susceptible species in the short term and provide the foundation for reintroductions over the longer term. This assessment has helped us to evaluate the likelihood that we will be able to avoid extinctions and to focus our resources appropriately in the longer term.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Data S1. Survey questions: evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs.

Data S2. All survey responses collected to evaluate the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs.