

# ADOPTING A HOLISTIC APPROACH TO AMPHIBIAN CONSERVATION



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# **ADOPTING A HOLISTIC APPROACH TO AMPHIBIAN CONSERVATION**

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## **AUTHOR'S DECLARATION**

I, Benjamin Tapley declare that this thesis has been composed solely by myself with the incorporation of suggestions, feedback and editorial amendments made by Richard A. Griffiths, David L. Roberts and Jodi J.L. Rowley, and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own. The text does not exceed 100,000 words and meets the formatting guidelines of the University of Kent.

Additional comments were provided by the thesis examiners.

I hereby declare that there were no competing interests on behalf of all co-authors.

Associated datasets have been made available through online repositories at the time of publication.

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## ABSTRACT

Amphibians are significant components of healthy ecosystems and provide important ecosystem services. Amphibians are disproportionately threatened by a variety of anthropogenic threats and their current rates of extinction may be hundreds of times greater than background extinction rates. Whilst amphibians are overall highly threatened, there is an ongoing need to identify the most at-risk species and prioritise species for subsequent conservation. However, many amphibian species are poorly known, and new species are discovered on a weekly basis. Our lack of knowledge of amphibians may undermine our ability to use limited conservation resources to conserve the most imperilled species or assemblages. Conservation practitioners must decide when they know enough about a species or a threat to have some degree of certainty that conservation interventions will be effective against the backdrop of ongoing species decline and a need for imminent action. We show that we currently lack a robust understanding of the extinction risk in assessed amphibians, largely due to high rates of species discovery and financial constraints of undertaking extinction risk assessments. We demonstrate how integrative taxonomy, and the use of both traditional and non-traditional monitoring techniques may identify and robustly delimit cryptic species and aid timely extinction risk assessments by providing important data on their range, extent of available habitat and threats posed to amphibians. These data are often sufficient to inform conservation prioritisation schemes and identify candidate species for resource intensive conservation action such as *ex situ* conservation breeding programmes. However, some *ex situ* programmes have been established with insufficient data on species biology and natural history. Conversely, research on captive amphibian populations may elucidate aspects of species biology that were previously unknown and potentially difficult, time-consuming and costly to acquire. The knowledge gained through *ex situ* research may inform conservation management decisions in nature and represents an important contribution in efforts to combat global amphibian declines. Amphibians are an extremely diverse group of animals and even congeneric species may have dramatically different natural histories, differing susceptibilities to threats and differences with regard to the effectiveness of different conservation actions or interventions. Generalised Class-focused approaches to conserve amphibians that do not consider species-specific factors risk missing the subtle, yet potentially critical nuances that may be pivotal in the success of conservation programmes. Whilst there are knowledge gaps that currently impede conservation these could be overcome with the adoption of new methods, refined processes and by everyone working on amphibians taking a collective responsibility to conserve them.

Key words: conservation breeding, conservation prioritisation, monitoring, species description

**CONTRIBUTIONS**

Chapter 1									
Publication	Funding proposals	Designed study	Ethical approval	Coordinated fieldwork	Collected data	Statistical analyses	Manuscript lead	Commented on manuscript	
Tapley, B., Michaels, C.J., Gumbs, R., Böhm, M., Luedtke, J., Pearce-Kelly, P., and Rowley, J.J.L. (2018). The disparity between species description and conservation assessment: A case study in taxa with high rates of species discovery. <i>Biological Conservation</i> , 220:209–214.	NA	B. Tapley, C. Michaels, J. Rowley	NA	NA	B. Tapley & C. Michaels	B. Tapley & C. Michaels	B. Tapley	B. Tapley, C. Michaels, J. Rowley, M. Böhm, J. Luedtke, P. Pearce-Kelly, R. Gumbs.	

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Tapley, B., Cutajar, T., Mahony, S., Nguyen, C.T., Dau, V.Q., Luong, A.M., Le, D.T., Nguyen, T.T., Nguyen, T.Q., Portway, C., Van Luong, H., and Rowley, J.J.L. (2018). Two new and potentially highly threatened <i>Megophrys</i> Horned frogs (Amphibia: Megophryidae) from Indochina’s highest mountains. <i>Zootaxa</i> , 4508:301–333.	B. Tapley & J. Rowley	B. Tapley & J. Rowley	B. Tapley	B. Tapley, H.V. Luong, C.T. Nguyen	B. Tapley, J. Rowley, S. Mahony, C.T. Nguyen, V.Q. Dau, T. Cutajar, D.T. Le, T.Q. Nguyen, T.T. Nguyen, A.M. Luong	B. Tapley, J. Rowley, T. Cutajar	B. Tapley	B. Tapley, J. Rowley, S. Mahony, T. Cutajar, C. Portway, V.Q. Dau,	

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Tapley, B., Nguyen, L.T., Cutajar, T., Nguyen, C.T., Portway, C., Luong, H.V., and Rowley, J.J.L. (2020). The tadpoles of five <i>Megophrys</i> Horned frogs (Amphibia: Megophryidae) from the Hoang Lien Range, Vietnam. <i>Zootaxa</i> , 4845:035–052.	B. Tapley & J. Rowley	B. Tapley, J. Rowley, L.T. Nguyen	B. Tapley	H.V. Luong, L.T. Nguyen, B. Tapley,	B. Tapley, L.T. Nguyen, Christopher Portway, C.T. Nguyen	B. Tapley	B. Tapley	L.T. Nguyen, C. Portway, J. Rowley, Timothy Cutajar
Tapley, B., Cutajar, T., Nguyen, L.T., Nguyen, C.T., Harding, L., Portway, C., Luong, H.V., and Rowley, J.J.L. (2018). A new locality and elevation extension for <i>Megophrys rubrimeris</i> in Bat Xat Nature Reserve, Lao Cai Province, northern Vietnam. <i>Herpetology Notes</i> , 11:865–868.	B. Tapley & J. Rowley	B. Tapley, J. Rowley, L. T. Nguyen	B. Tapley	H.V. Luong, L. T. Nguyen, B. Tapley,	B. Tapley, L.T. Nguyen, C. Portway, T. Cutajar, C.T. Nguyen, J. Rowley, L. Harding	B. Tapley & T. Cutajar	B. Tapley	L. T. Nguyen, C. Portway, J. Rowley, T. Cutajar

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Tapley, B., Jervis, P., Nguyen, L.T., Portway, C., Nguyen, C.T., Luong, H.V., Kane, D., Brookes, L., Perkins, M.W., Ghosh, P., Wierzbicki, C., Shelton, J., Fisher, M.C., and Rowley, J.J.L. (2020). Low prevalence of <i>Batrachochytrium dendrobatidis</i> detected in amphibians from Vietnam's highest mountains. <i>Herpetological Review</i> . 51:726–732.	B. Tapley & J. Rowley	B. Tapley & J. Rowley	B. Tapley	B. Tapley, H.V. Luong, C.T. Nguyen	B. Tapley, L.T. Nguyen, C. Portway, C.T. Nguyen, D. Kane, L. Harding, T. Cutajar	B. Tapley, P. Jervis, L. Brookes, M. Perkins, P. Ghosh, C. Wierzbicki, J. Shelton	B. Tapley & P. Jervis	J. Rowley, L. Brookes, D. Kane, L.T. Nguyen, M. Fisher, P. Ghosh

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Chapter 4

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Michaels, C.J., Tapley, B., Harding, L., Bryant, Z., Grant, S., Sunter, G., Gill, I., Nyingchia, O., and Doherty-Bone, T. (2015). Breeding and rearing the Critically Endangered Lake Oku Clawed Frog ( <i>Xenopus longipes</i> Loumont and Kabel 1991). <i>Amphibian and Reptile Conservation</i> , 9:100–110.	NA	B. Tapley & C. Michaels	NA	B. Tapley & C. Michaels	B. Tapley, C. Michaels, L. Harding, Z. Bryant, S. Grant, G. Sunter, I. Gill	C. Michaels & B. Tapley	C. Michaels & B. Tapley	L. Harding, I. Gill, T. Doherty-Bone
Tapley, B., Michaels, C.J., and Doherty-Bone, T.M. (2015). The tadpole of the Lake Oku clawed frog <i>Xenopus longipes</i> (Anura; Pipidae). <i>Zootaxa</i> , 3981:597–600.	NA	B. Tapley & C. Michaels	B. Tapley	B. Tapley & C. Michaels	B. Tapley & C. Michaels	B. Tapley & C. Michaels	B. Tapley & C. Michaels	T. Doherty-Bone
Tapley, B., Rendle, M., Baines, F.M., Goetz, M., Bradfield, K.S., Rood, D., Lopez, J., Garcia, G., and Routh, A. (2015). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs ( <i>Leptodactylus fallax</i> ) and general recommendations for pre-release health screening. <i>Zoo Biology</i> , 34:46–52.	NA	B. Tapley	NA	B. Tapley	B. Tapley & M. Rendle	B. Tapley	B. Tapley	M. Rendle, F. Baines, M. Goetz, K. Bradfield, D. Rood, J. Lopez, G. Garcia, A. Routh

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3.6 A new locality and elevation extension for *Megophrys rubrimera* in Bat Xat Nature Reserve, Lao Cai Province, northern Vietnam.

3.7 Supplementary materials.

- IUCN Red List assessment: *Megophrys fansipanensis*.
- IUCN Red List assessment: *Megophrys hoanglienensis*.
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### CHAPTER 3: MONITORING AMPHIBIANS AND THEIR THREATS.

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- Range-wide decline of Chinese giant salamanders *Andrias* spp. from suitable habitat. Supplementary materials.

4.2 Low prevalence of *Batrachochytrium dendrobatidis* detected in amphibians from Vietnam's highest mountains.

- Low prevalence of *Batrachochytrium dendrobatidis* detected in amphibians from Vietnam's highest mountains. Supplementary materials

4.3 Supplementary materials.

- Failure to detect the Chinese giant salamander (*Andrias davidianus*) in Fanjingshan National Nature Reserve, Guizhou Province, China.
- Chinese giant salamander field survey manual.
- IUCN Red List assessment: *Andrias davidianus*.
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**STATEMENTS FROM CO-AUTHORS**

## 1.1 INTRODUCTION

The Class Amphibia is comprised of three extant orders: frogs and toads (order Anura; 7361 species), newts and salamanders (order Caudata; 766 species) and caecilians (order Gymnophiona; 213 species) (Frost, 2021). Amphibians are poorly known; there are high rates of species discovery (Catenazzi, 2015), and even basic information on species ecology and distribution is often lacking (Gower & Wilkinson, 2005; Silvano & Segalla, 2005; Rowley et al., 2010). Amphibians are present on every continent except for Antarctica, and they have colonised nearly every terrestrial and freshwater habitat (Wells, 2010). Amphibian species diversity is not evenly distributed (Wiens, 2007). Amphibians are most diverse in the tropics because of higher speciation rates, historically low extinction rates coupled with lower dispersal rates out of the tropics compared with rates of colonisation from temperate regions (Pyron & Wiens, 2013).

## 1.2 Why are amphibians important?

Amphibians are important for several reasons. Amphibians, like all taxa, provide ecosystem services and the loss of one or more species can reduce the quality of the services within an ecosystem (Hocking & Babbitt, 2014). Amphibians can be incredibly abundant and may comprise a large proportion of vertebrate biomass in some systems (Burton & Likens 1975; Petranka & Murray 2001; Gibbons et al., 2006). They are also important in ecosystem function (see review in Hocking & Babbitt, 2014) and the loss of even rare amphibian species may have a disproportionate negative impact on the stability of ecosystems as, in some systems, threatened amphibians may be more functionally distinct, thereby fulfilling a distinct functional niche (Menéndez-Guerrero et al., 2020). Recent research has indicated that amphibians can influence certain ecosystem functions through trophic cascades (Laking et al., 2021). They often facilitate nutrient exchange between aquatic and terrestrial systems (Colón-Gaud et al., 2009) and they are both important predators and prey items (Zipkin et al., 2020). The removal or reduction in numbers of amphibians in a system may also have negative consequences for human populations; although data is scarce, over-collection of frogs for the international meat trade may have resulted in an increase in agricultural pests in India (Altherr et al., 2011) and disease-mediated amphibian declines have been associated with increased incidences of malaria in human populations in Central America (Springborn et al., 2020).

Amphibians have an economic value; they are used for food, medicine, research and are increasingly popular companion animals (Collins & Crump 2009; Pasmans et al., 2017). Indeed, for some species, these are the same reasons they are threatened. Amphibians are important food items for many people (Gonwou & Rödel, 2008), and as well as subsistence hunting and local trade (e.g., Kusriani & Alford, 2006), there is a substantial international trade in frog meat. From 1996 and 2006 it was estimated that between 8,000,000 to 12,000,000

kg of frogs' legs were imported globally (Warkentin et al., 2009; Gratwicke et al., 2010). Farming amphibians for their meat may also be important to rural livelihoods (e.g., Cunningham et al., 2016). Some species are of scientific or medical importance; secretions from amphibians are known to contain antimicrobial peptides as well as painkillers (Badio & Daly 1994; Fleming et al., 2009; Azevedo Calderon et al., 2011). Furthermore, amphibians are important animal models in scientific research and frogs of the genus *Xenopus* have been used for decades in studies on human development and disease (Burggren & Warburton, 2007).

There are ethical arguments supporting the conservation of amphibians; most people would concur that each species has the right to exist and has its own intrinsic value regardless of its value to humans (Collins & Crump 2009). This ethical argument is powerful as it is central to most belief systems and religions (Groom et al., 2006). There are aesthetic and cultural reasons for conserving amphibians. In many cultures amphibians are significant as they symbolise fertility, new beginnings, resurrection, wealth, happiness, healing, love and evil (Collins & Crump 2009; Crump & Fenolio, 2015), and amphibians are widely represented in popular contemporary culture (Crump & Fenolio, 2015). The cultural significance of a species can also help garner public support for conservation by facilitating conservation efforts (Negi 2010; Ceriaco 2012; Gupta et al., 2015; Schneider 2018; Nicholson et al., 2020).

### **1.3 Threats to amphibians and global amphibian declines**

Today, amphibians are disproportionately threatened by a diverse array of threats (Stuart et al., 2004). Current amphibian extinction rates may be 211 times the background extinction rate (McCallum, 2007) and at present, 41% of assessed amphibian species are considered to be threatened with extinction (IUCN, 2020) and this number is likely to be higher; 16% of amphibians are assessed as Data Deficient (IUCN, 2020) and it has been estimated that 1000 of these Data Deficient species are threatened with extinction (González-del-Piiego et al., 2019). Furthermore, many newly described amphibians are more likely to have smaller ranges and hence more likely to qualify for being assessed as threatened (Pimm et al., 2014).

Amphibians possess several traits that may make some species inherently vulnerable to rapid environmental change and pollution. Amphibians are ectotherms and even small changes in temperature may result shifts in behaviour and metabolic demands (Rohr & Palmer, 2013). This is of concern against the backdrop of habitat alteration and climate change (Thomas et al., 2004). There is limited evidence that climate change results in direct lethal effects in amphibians as researchers have not routinely eliminated other factors that may be contributing to population declines (Li et al., 2013). However, there is evidence that climate change may result in changes in foraging patterns, breeding phenology and shifts in range and elevation (see review in Li et al.,

2013; Kissel et al., 2019); shifts in elevation and range may be problematic if populations become fragmented and isolated and if there is no available suitable habitat for populations to colonise (Forero-Medina et al., 2011).

This vulnerability to rapid environmental change and pollution is further compounded by the fact that amphibians have permeable skin, a dependency on moisture for reproduction (Donnelly & Crump, 1998) and because they often have complex lifecycles (Wells, 2010). As a result, amphibians that have both aquatic larval stages and terrestrial adults are vulnerable to the effects of pollutants in both aquatic and terrestrial environments (Croteau et al., 2008). Many amphibians have extremely specific microhabitat requirements and certain environmental parameters may influence species distribution (Wyamn & Hawksley, 1987; Channing & Wahlberg, 2011). Furthermore, specific microhabitats may be important for oviposition (Nair et al., 2012; Faggioni et al., 2017), larval development (Brodman & Jaskula, 2002; Thomas et al., 2019; Bjordahl et al., 2020) or for juvenile life stages (Earl & Semlitch, 2015; Bjordahl et al., 2020). Because of their specific microhabitat requirements and permeable skin, amphibians are considered poor dispersers relative to other vertebrates, (Sinsch, 1990; Blaustein et al., 1994; Duellman & Trueb 1994) and as a result, some species have extremely limited distribution and are particularly sensitive to environmental change (Greenberg & Moores, 2017; Penner & Rödel, 2019).

The greatest driver of global amphibian population declines is habitat loss and modification (Cushman, 2006; Gallant et al., 2007; Gardner et al., 2007). Even small changes in habitat structure can impact the amount of solar radiation and wind and this can alter the thermal landscape which can have deleterious effects on amphibians (Watling et al., 2011; Nowakowski et al., 2018; Garcia & Clusella-Trullas, 2019) as they are sensitive to evaporative water loss and some have narrow thermal tolerances (Pintanel et al., 2018). The significant threat that habitat loss poses to amphibians is further exacerbated by the fact that many of the countries with the greatest diversity of amphibians are subject to the highest rates of deforestation (FAO, 2015). There is positive correlation between amphibian species with small geographic ranges and increased habitat specificity which makes these species more vulnerable to habitat modification and loss (Sodhi et al., 2008). Species with small ranges may not be particularly abundant (Murray et al., 1998). Furthermore, tropical frogs with small ranges typically have smaller clutch sizes (Cooper et al., 2007), which makes these species inherently vulnerable. As a result, a large proportion of tropical amphibian diversity is threatened by habitat loss.

Some amphibian species have disappeared from even well protected and supposedly pristine habitats (Blaustein & Wake, 1990; Halliday, 1998; Daszak et al., 1999; Lötters et al., 2009). The cause of these enigmatic declines was unknown until 1998 when amphibian declines were first linked to a pathogen (Berger et al., 1998).

Chytridiomycosis, caused by the amphibian chytrid fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*), is an infectious disease implicated in the declines of over 500 amphibian species worldwide and is thought to have caused the extinction of at least 90 species (Scheele et al., 2019). Amphibian chytrid fungi are unusual as they are non-host specific and mortality has been documented in all amphibian Orders (Gower et al., 2013), and this range in hosts and impact on host species is unparalleled. Chytridiomycosis has resulted in the most dramatic disease-mediated loss of vertebrates ever recorded (Skerratt et al., 2007), and amphibian population declines associated with the disease have been reported from all continents where amphibians occur, except for Asia (Mutnale et al., 2018) where the pathogen is thought to have originated (O’Hanlon et al., 2018). The global spread of amphibian chytrids has been linked to the global trade in amphibians (Garner et al., 2009; Schloegel et al., 2009; Wombwell et al., 2016; Fitzpatrick et al., 2018). Amphibian chytrid fungi do not cause diseases in all species (Gervasi et al., 2003; Martel et al., 2014); patterns of infection and manifestation of disease are host species, life stage and context specific. Our ability to manage and mitigate the impact of these fungal pathogens in the wild is currently extremely limited (Garner et al., 2016). Although amphibian chytrids have received the most attention, ranaviruses are another group of emerging amphibian pathogens and they have been documented to cause mass mortalities and the collapse of amphibian communities (Gray et al., 2009; Price et al., 2014). Like amphibian chytrids, ranaviruses have a global distribution (Duffus et al., 2015); can infect and cause disease in a wide range of hosts (Schock et al., 2008); have differing susceptibility of host species (Hoverman et al., 2011); and can persist outside the host (e.g. Nazir et al., 2012). Equally, the spread of ranaviruses has been facilitated by international trade (e.g. Kolby et al., 2014). There are also other diseases implicated as drivers of amphibian declines including mesomycetozoean parasites (Duffus & Cunningham, 2010; Rowley et al., 2013).

Amphibians are also directly threatened by chemical pollutants and noise pollution. Chemical pollutants such as fertilisers, pesticides, heavy metals, and road de-icers are known to negatively impact amphibian populations (Egea-Serrano et al., 2012). Pollutants can be introduced into the environment by direct application, run-off or via atmospheric deposition (Egea-Serrano et al., 2012). Pollutants can have lethal effects and cause direct mortality (de Wijer et al., 2003). They can also have an array of sublethal effects such as the incursion of fitness costs (Sanzo & Hecnar, 2006, Relyea & Diecks, 2008); cause malformations (Taylor et al., 2005; Egea-Serrano et al., 2012) and impact population demographics by changing the length of time larvae develop (de Wijer et al., 2003; Relyea & Diecks, 2008); and alter hormone systems (see review in Orton & Tyler 2015). Amphibians may also accumulate contaminants (e.g. Unrine et al., 2007) and transfer these from aquatic to terrestrial systems when they metamorphose (Roe et al., 2005) and onto to their consumers (Unrine et al., 2007). The impact of noise pollution may also be detrimental to amphibians and result in indirect impacts on fitness. Noise pollution

is known to affect call rates (Sun & Narins, 2005; Grace & Noss, 2018), or drive amphibians to avoid noisy areas such as roads altogether (Grace & Noss, 2018).

In many parts of the world, amphibians are threatened by overexploitation for human consumption (Warkentin et al., 2009; Gratwicke et al., 2010; Altherr et al., 2011; Turvey et al., 2018); for the national and international pet trade (Stuart et al., 2006; Phimmachak et al., 2012; Rowley et al., 2016); and for use in traditional medicine (Xie et al., 2007; Rowley et al., 2010; Phimmachak et al., 2012; Alves et al., 2014; Grismer et al., 2018; He et al., 2018). Over-harvesting amphibians for their meat is known to have led to population declines of exploited species (Chan et al., 2014; Turvey et al., 2018; Çiçek et al., 2020) and anecdotally, the disappearance of large sized individuals in some species (Rowley et al., 2010).

Invasive species are listed as one of the greatest threats to biodiversity and are known drivers of amphibian population declines (Falaschi et al., 2020). Invasive species can cause direct impacts on amphibians; they can be significant predators (Kats & Peerer, 2003; Mohanty & Measey, 2018), compete with native species (D'Amore et al., 2009; Mohanty & Measey, 2018) and hybridise with them (Beukema et al., 2015; Fukumoto et al., 2015; Yan et al., 2018). Invasive species can have indirect impacts too: they are a known mechanism by which pathogens (Bai et al., 2010; Miaud et al., 2012) and parasites (Hartigan et al., 2010) are spread, and they can act as important reservoirs of potential infection (Miaud et al., 2016). Finally, invasive species can also alter habitats making them less suitable for native amphibian species (Watling et al., 2011; Ransom et al., 2017).

The threats posed to amphibians are largely anthropogenic, and often synergistic (Alford & Richards, 1999; Stuart et al., 2004; Beebee & Griffiths, 2005; Gascon et al., 2007; Catenazzi, 2015). For example, climate change will likely alter disease dynamics due to shift in host-parasite interactions, particularly in montane regions where ambient temperatures may become more optimal for *Bd* (Pounds et al., 2006; Xie et al., 2016; Cohen et al., 2017; Sauer et al., 2020). There is also evidence that outbreaks of ranavirus will be more severe and prolonged under predicted climate change projections (Price et al., 2019). Understanding the particular driver of the decline in a species can be hugely challenging (Beebee & Griffiths, 2005) but important, as some threats may be easier to neutralise than others. Our ability to mitigate the impact of pathogens in the field is currently limited, however the conservation community might be able to combat the illegal trade or exploitation of a species through effective monitoring and law enforcement. Determining the relative contribution of each particular threat process to the decline of a population, species or species assemblage may be pivotal in species recovery programmes. Conservation resources are limited, and this necessitates the selection of the most impactful responses.

#### **1.4 The global response to amphibian declines**

An impactful conservation response to global amphibian declines requires a collaborative and holistic approach. The first large-scale global amphibian initiative was the launch of the Global Amphibian Assessment (GAA) in 2004, the first comprehensive global assessment of the extinction risk posed to all 5743 amphibian species described at the time. The GAA effectively highlighted the global plight of amphibian species (Stuart et al., 2004) and helped galvanise the conservation community into action and set an important baseline with which to measure the impact of the global response (Loh et al., 2005) and subsequently prioritise species for conservation action (e.g. Isaac et al., 2012). In 2005, the IUCN SSC Amphibian Conservation Summit culminated in the production of the Amphibian Conservation Action Plan (ACAP) to address global amphibian declines (Gascon et al., 2007). This plan came with an estimated funding need of US \$400 million to support activities from 2006–2010 (Gascon et al., 2007). Several organisations were subsequently formed to co-ordinate amphibian conservation on a global scale, including the Amphibian Ark (AArk) and the Amphibian Specialist Group (ASG). The ACAP was subsequently updated in 2015 (Wren et al., 2015). However, a lack of sustainable funding for the actions outlined in the ACAP continues to be a significant barrier to delivery.

Since the launch of the GAA we have learnt much more about the diversity of amphibians, the threats posed to them and some strategies to mitigate threats. However, even with the numerous organisations formed to address amphibian declines and a coordinated ACAP, the global response to amphibian declines has been inadequate relative to the scale of the problem and the rate at which species are being lost (e.g. Scheele et al., 2019).

#### **1.5 Extinction risk assessment**

Extinction risk assessments are a pivotal component of the response to global amphibian population declines. The IUCN Red List of Threatened species is a globally accepted measure to assess the extinction risk and to identify threats, highlight agreed conservation actions and identify research needs (Lamoreux et al., 2003; Rondinini et al., 2014). The Red List has significant influence over which research and conservation work is resourced, as grant funding often prioritises threatened or Data Deficient species. Finally, The Red List can also be used to track changes in extinction risk over time and is therefore an important metric in measuring the threats to biodiversity as well as evaluating the impact of a particular conservation intervention on a global scale (Hoffmann et al., 2010). The Red List underpins the Red List Index, an important biodiversity indicator steering conservation policy (Butchart et al., 2004; Butchart et al., 2007; Butchart et al., 2010). However, the IUCN Red List has a limited legislative impact as many administrative divisions have their own lists of threatened species

which are not always aligned with the IUCN Red List (e.g. Harris et al., 2012). Whilst not a conservation prioritisation tool itself, the Red List underpins many conservation prioritisation tools. Some of these tools such as Key Biodiversity Areas (KBA, 2020) and Alliance for Zero Extinction (AZE, 2020) take a site-based approach and use the presence of threatened species to highlight areas that should be priorities of conservation whilst others are species-focused and may prioritise species based on extinction risk and other factors such as evolutionary history (EDGE, 2020). Extinction risk may also be used as a criterion in tools that identify appropriate conservation interventions for threatened species (Johnson et al., 2020).

The launch of the GAA in 2004 was a pivotal moment for amphibian conservation. It was immediately evident that relative to other vertebrates, amphibians were disproportionately threatened and facing a conservation crisis (Stuart et al., 2004). These threats required immediate action; however our ability to conserve amphibians is dependent not only on taxonomic certainty, robust extinction risk assessment, our ability to monitor populations over time and the prioritisation of the species that are most in need of the most intensive and resource consuming conservation management. In some taxa, such as amphibians, species description rates are relatively high (Costello et al., 2012) and this presents a challenge to the sustained relevance of the Red List. IUCN Red List assessments are considered out-of-date when they are over ten years old, therefore the challenge of keeping the Red List up-to-date is further intensified by the ongoing need to regularly reassess species.

### **1.6 Unknown diversity as a challenge to the global response to amphibian declines**

Unfortunately, many taxonomic groups, including amphibians, are poorly-known and this paucity of data may undermine our ability to prioritise species in need of conservation (Iskandar & Erdelen 2006; Foufopoulos & Richards 2007; Rowley et al., 2010). Alarming, 1,136 (19.4%) of the 6,892 amphibians that have been assessed have been assigned the extinction risk of Data Deficient (IUCN, 2020). This extinction risk category is assigned to a species when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on current knowledge (IUCN, 2012). The proportion of Data Deficient species varies between amphibian orders; 55.7% of assessed gymnophionan amphibians have been assessed as Data Deficient whereas 19.6% of anuran amphibians and 6.9% of assessed caudate amphibians have been assessed as Data Deficient respectively (IUCN, 2020). Furthermore, newly described species are likely to be threatened as they often have small ranges (Pimm et al., 2014) which makes them inherently vulnerable to stochastic events and habitat loss.

The effectiveness of the IUCN Red List in the long-term, as well as the various conservation initiatives reliant on it, depend on the ability of the Red List to reflect our ever-changing understanding of species richness and species boundaries as well as trends in population size. To do this, the Red List must not only ensure that

assessments are updated regularly, but also keep pace with assessing newly described species in order for it to accurately gauge trends and prioritise taxa and regions for conservation.

### **1.7 The importance of describing and delineating species**

Over half of all amphibian species have been described since 1960 (Rodrigues et al., 2010) and this high rate of species discovery is an obstacle to amphibian conservation since species are the most used unit in amphibian conservation. Effective conservation often requires the robust and fixed delimitation of species, as species are often the unit by which conservation management decisions are made (Mace, 2004) and often the focus of conservation legislation (Aldhebiana, 2018). However, the definition of the term "species" is still controversial as there are many different concepts of what a species is (De Queiroz, 2007). Species delimitation is complicated as the process of speciation is ongoing and therefore the precise point at which it is complete is somewhat arbitrary (Zachos, 2016). Furthermore different taxonomists take different approaches to classification with some lumping or synonymising known taxa and others splitting a taxon into several new taxa (Issac et al., 2004), or elevating subspecies to species based on limited data (e.g. Hillis, 2020). To complicate things further, as species are the primary units for conservation, the description of new species may be influenced by political or economic factors (e.g. describing or splitting species with the specific purposes of serving conservation goals) rather than describing species based on established biological criteria to conserve imperilled biodiversity (Hey et al., 2003). Splitting a species may also have ramifications on how well a species is protected. Legislative changes may lag behind taxonomic changes (Garnett & Christidis, 2017) and newly described species may not benefit from national and international legislation that may have protected populations prior to any taxonomic change.

It is incredibly important to resolve taxonomic uncertainty, as uncertainty has the potential to undermine conservation action (Crawford et al., 2012; Yan et al., 2018) and there are already well-known examples of where taxonomic uncertainty has had negative consequences for some highly threatened amphibian species. A case in point is that of the Chinese giant salamander which was traditionally interpreted as a single geographically wide-ranging species *Andrias davidianus*, and thought to occur across multiple montane ecoregions and river basins in China. A nation-wide giant salamander farming industry has developed since the early 2000s, to supply animals for food markets within China which led to extensive trade and movement of animals between farms across the range of the species in China (Cunningham et al., 2016). Some of the progeny of these farmed animals are released into rivers as part of a government-promoted conservation scheme (Yan et al., 2008). Despite preliminary molecular evidence showing that there were genetic differences between giant salamanders from different regions of China (Murphy et al., 2000) there has been no pre-release genetic assessment of animals released from farms into rivers. The Chinese giant salamander has recently been shown to constitute a complex

of at least three different species, including the South China giant salamander (*A. sligoi*) and at least one other undescribed taxon (Yan et al., 2018, Liang et al., 2019, Turvey et al., 2019). Hybrid salamanders are known to occur on farms and the wide-scale and intentional releases of giant salamanders across China have resulted in genetic homogenisation of some local subpopulations (Yan et al., 2018).

### **1.8 What is currently known about amphibian species diversity and how to delineate species?**

Traditionally taxonomists have described species through the comparison of external and/or skeletal morphology of post metamorphic amphibians although the revalidation of some species has been undertaken referring to historic descriptions of morphology supported by new molecular data (e.g., Turvey et al., 2019). However, the identification of many species of amphibian cannot be reliably made based solely on morphology, even by experienced researchers (Vences, 2008). Bioacoustic comparisons are an important component of many anuran amphibian species descriptions and are increasingly used to identify and delimit species (Köhler et al., 2017). Molecular methods now play a central role in taxonomy, our understanding evolutionary relationships and delimiting species (Vogal & Monaghan, 2007). Larval characters are also an important component in species descriptions and wider research into amphibian systematics, especially for anuran amphibians (e.g., Has, 2003; Rada et al., 2019). In one notable example, a species was described based on larval morphology alone (e.g., Grosjean et al., 2015a). Differences in behaviour may also be used as additional evidence with which to delineate species (e.g., Abraham et al., 2013; Gururaja et al., 2014). Finally, folk taxonomy may also aid in the identification of undescribed species; for example, the Kalam people of New Guinea had different names for two distinct frogs that had been lumped together as *Litoria becki* by western scientists (Bulmer & Tyler, 1968). However, other studies have indicated that folk taxonomy often lumps together morphologically similar amphibians (e.g., Kanagavel et al., 2020).

### **1.9 Problems to overcome in terms of species diversity**

Taxonomy as a science is increasingly underfunded and undervalued (Giangrande, 2003; Drew, 2011). A lack of resource compromises our ability to describe, catalogue and conserve the world's biodiversity (Godfray, 2002; Mace, 2004). There is a geographic bias in amphibian species diversity and discovery. Amphibian species diversity, especially in the tropics, has been hugely underestimated (Vieites et al., 2009; Funk et al., 2012; Estupiñán et al., 2016). Approximately 85% of new amphibian species described between 2015 and 2019 came from biodiversity hotspots (Streicher et al., 2020). Unfortunately, expertise in amphibian taxonomy is often concentrated in economically rich but species-poor countries (Rodrigues et al., 2010). These points are of huge

concern against the backdrop of global amphibian declines and many species could be lost before they are formally described (Meegaskumbura et al., 2007; Rowley et al., 2015). Several amphibian species have been described after they became extinct (e.g. Coloma et al., 2007; Mendelson 2010), leading to a practice termed ‘forensic taxonomy’ (Mendelson 2010).

Many amphibian genera are known to harbour hidden species diversity within morphologically obscure species complexes (e.g., McCleod, 2010; Chen et al., 2017; Liu et al., 2018; Yan et al., 2018; Labisko et al., 2019; Jaramillo et al., 2020). Using multiple lines of evidence or an integrative approach to taxonomy is an approach increasingly adopted by taxonomists as new and increasingly affordable techniques have been developed and these are becoming progressively cheaper to utilise. Although single lines of evidence may be sufficient and, in some cases, appropriate to delimit a species, taxonomists have increasingly incorporated molecular and bioacoustics data in the delineation of amphibian species (Köhler et al., 2005; Vieites et al., 2009; Catenazzi, 2015). In a recent review of amphibian species descriptions; from 2015–2019, 89% of species descriptions could be classed as integrative; nearly all new species descriptions utilised the comparisons of external morphology to describe new amphibian species and 46% of species descriptions utilised bioacoustics and 79% and 26% of species description papers utilised mtDNA and nDNA respectively (Streicher et al., 2020). Drawing on multiple lines of evidence could facilitate greater taxonomic stability (Glaw et al., 2010; Padial et al., 2009) and therefore a stronger foundation on which to develop species-focused conservation initiatives and protective legislation. Given that many conservation actions are species-focused, species description is a prerequisite for conservation assessment. But conservation assessment requires more than just taxonomic information.

### **1.10 Monitoring amphibians and their threats**

Monitoring amphibian populations can help distinguish between natural fluctuations in population sizes and genuine population declines. Conservation action is often directed towards species that are assessed as threatened and believed to be in decline. Conservation practitioners may also target systems where a particular community of threatened species is collapsing (e.g. Gagliardo et al., 2008). Information on population size and trends as well as the likely drivers of decline is required for species conservation assessment (IUCN, 2012). A robust understanding of population trends is also important if conservation practitioners are to assess the impact of conservation interventions.

There is a lack of baseline population data on the status of most amphibian species (Gower & Wilkinson, 2005; Lips et al., 2005; Rowley et al., 2010; Kanagavel et al., 2018), which means that declines may go undetected. This is partly because amphibians are particularly challenging to monitor. Many species are extremely difficult to

detect in the field as they may be cryptic, miniature (Rakotoarison et al., 2017); seasonally active (Zacharia, et al., 2012; Vertucci et al., 2017); fossorial (Gower & Wilkinson, 2005); occur in largely inaccessible arboreal habitats (Kays & Alison 2001; Scheffers et al., 2014); or rare (Storfer et al., 2003). Some amphibian species are so poorly known that they have not been encountered since they were first described and decades may have elapsed since they were last seen (Gower & Wilkinson, 2005; Moore 2014). Whilst the presence of a species can be easily proved, the absence of a species from a particular site can only ever be inferred with varying degrees of certainty depending on search effort (Kéry, 2002). In addition, population sizes of some amphibians may exhibit a high degree of fluctuation (Green, 2003) which may make it difficult to establish if a species is declining, especially if a single population is being monitored.

Determining the presence of many amphibians at a particular site may be hampered by difficulties in identification of a particular species based on morphology alone (Vences, 2008). In some cases, particular sexes may not have been formally described, this is problematic as amphibians can be extremely sexually dimorphic (e.g. Lyu et al., 2020), and this may hinder researchers that undertake rapid biodiversity assessments. The identification of amphibian larvae in the field may help determine the presence of a particular species and provide inferences on population recruitment (e.g. Tapley et al., 2020). Larval amphibians may be easy to locate in the field as most occur within a defined area of aquatic habitat and they may be abundant, for several months (Grosjean et al., 2015b) or even years (Morrison & Hero 2003). In contrast, post-metamorphic amphibians will often disperse from breeding sites, and individuals - particularly of arboreal or fossorial species - may only be rarely encountered outside of the breeding season. However, some amphibian larvae are morphologically conserved (e.g. Grosjean, 2003) and others are known to exhibit phenotypic plasticity in response to environmental factors which may lead to morphological variation and difficulties in identifying and describing larval stages (e.g. Vences et al., 2002; Moore et al., 2004). Accurate identification of larvae is dependent on the availability of detailed larval descriptions; an estimated 40% of anuran larvae have not been described (Andrade et al., 2007).

Our lack of knowledge is compounded by the fact that long-term population monitoring can be labour intensive and because many amphibian research programmes are undertaken as part of formal academic programmes that are typically short in duration (e.g. >4 years) and therefore outside the scope of long-term study. Furthermore, there has been a lack of standardisation in the methodologies used to monitor amphibians (even the same species at different sites) which makes comparisons of data problematic (e.g. Rödel & Ernst, 2004).

In addition to presence and absence studies there are several other ways in which amphibian population size and trends can be assessed. Simple counts of species are often used as an index of population size but these

data are often limited as detection probabilities are variable (Schmidt, 2003). Several methods are used to ascertain the population size of amphibians. All methods have associated assumptions and the selection of the most appropriate method requires an understanding of the natural history of the target species. Studies to estimate the population size and monitor populations of amphibians may sometimes face difficulties as amphibians are particularly challenging candidates to mark due to the permeability and sensitivity of their frequently shed skin, their relatively small size, and their often-complex life cycles (Heemeyer et al., 2007). Other monitoring methods may be further complicated by imperfect detection probabilities although these can be counteracted by modelling. Bioacoustic monitoring is a promising method for evaluating the status of anuran amphibian populations (Dorcas et al., 2009), but there are limitations of this method in estimating abundance, as it provides little information on the structure of the entire population and this method can only be used to monitor amphibians that vocalise (Dorcas et al., 2009). The measurement of environmental DNA (eDNA) is another method that is increasingly used (Rees et al., 2014), especially to detect cryptic species and those that may occur at low densities (Bohmann et al., 2014; Lopes et al., 2020). Currently this technique can be used to detect the presence of a species and the presence of threats such as pathogens (Walker et al., 2007) and invasive species (Hunter et al., 2015). However, the amount of eDNA present and therefore detectable in the environment may be affected by several biotic and abiotic factors (see review in Stewart, 2019). Recently, the concentration of eDNA has been shown to reflect the relative abundance of target species in some systems (e.g., Buxton et al., 2017).

There is a need to monitor the threats posed to amphibians and concerted effort to assess how these threats may be contributing to amphibian population declines. This may require pathogen surveillance by taking samples from the target species, or if the target species is not abundant, taking samples from syntopic species so inferences on pathogen prevalence can be made. Environmental monitoring may be undertaken at a broad or microhabitat scale to monitor pollution and climate change. Likewise, markets and resource users may be surveyed to understand the trends and scale of exploitation.

The rarity of some amphibians may make non-traditional survey methods the only viable option to make inferences on population status and drivers of population decline. Local Ecological Knowledge (LEK) can aid conservationists in understanding species occurrence, abundance, habitat use and threats (Gilchrist et al., 2005; Anadón et al., 2010; Lescureux et al., 2011). Local communities possess a diverse knowledge of the resources on which they often depend (Berkes et al., 2000) and these data may be incredibly significant with regard to threatened species or species that are rarely encountered or have extremely limited activity periods where few data would otherwise be available (Meijaard et al., 2011; Stuart, 2012; Ziembicki et al., 2013; Turvey et al., 2014,

2015, 2021; Pan et al., 2015). LEK has been shown to be effective in understanding the status of and threats posed to economically and culturally significant species (Jones et al., 2008; Pan et al., 2015) as well as species that are morphologically distinct and large-bodied (Turvey et al., 2014). However, collecting and interpreting data about focal species from untrained respondents to establish baselines for conservation is not straightforward (Gilchrist et al., 2005); the quality of the data collected may vary widely between respondent or target species and respondents may not be able to accurately identify species, may struggle to recall exact details, not report on activities that are illegal, or exaggerate (Davis & Wagner, 2003; Gilchrist et al., 2005; McKelvey et al., 2008; O'Donnell et al., 2010). Whilst LEK represents a cost-effective method to collect important data, only a handful of studies have explored the effectiveness of LEK in understanding the biology, distribution, population status, and potential threats to amphibians (e.g., Harpalini et al., 2015; Pan et al., 2015; Turvey et al., 2018, 2021; Kanagavel et al., 2020).

An understanding of how amphibian populations are faring, and the threats posed to them are important for conservation assessment and reassessment. Population monitoring and threat data, including whether threats are manageable, are also key components for identifying appropriate conservation management decisions. These can range from habitat protection to more labour and resource intensive activities such as conservation breeding and subsequent translocation.

### **1.11 *Ex situ* conservation**

*Ex situ* conservation breeding programmes have often been deemed as necessary when the threats posed to amphibians cannot be reversed or ameliorated in the short-term (Gascon et al., 2007; Griffiths & Pavajeau, 2008). Attempts to mitigate emerging infectious amphibian diseases are still in their infancy and the persistence of some species, including those assessed as Extinct in the Wild, is therefore dependent on intensive *ex situ* management (Hammerson 2004; Zippel et al., 2011; Scheele et al., 2014; IUCN SSC, 2015a). An estimated 943 species require *ex situ* conservation breeding programmes (Zippel et al., 2011). However, *ex situ* management is a costly and labour-intensive endeavour and without exit strategies for captive populations such programmes may struggle to achieve meaningful conservation objectives in the long-term (Mendelson, 2018).

Amphibians have been suggested as ideal candidates for *ex situ* conservation breeding programmes for several reasons. They are relatively small in body size and therefore have low space requirements (Balmford et al., 1996). They exhibit high fecundity (Bloxam & Tonge 1995), often have relatively short generation lengths, hardwired behaviour (Bloxam & Tonge 1995) and relatively low maintenance requirements (Browne et al., 2011a). Furthermore. This makes running such programmes relatively cost effective (Bloxam & Tonge 1995;

Balmford et al., 1996). Finally, examples of successful amphibian conservation breeding programmes (e.g. Bloxam & Tonge 1995; Griffiths & Pavajeau, 2008). As a result, some conservation breeding programmes were established in response to a perceived extinction risk and without adequate knowledge of the target species.

There is a disparity between amphibian husbandry capacity and countries where such capacity is most needed (Zippel et al., 2011). The question of who should undertake amphibian conservation breeding programmes is also controversial (e.g., Browne et al., 2018). Given that emerging infectious disease imperils many amphibian species, the consensus within the *ex situ* community is that conservation breeding programmes should be hosted in range states using facilities dedicated to sympatric species with shared management histories to minimise the risk that novel pathogens may pose to the focal species as well as syntopic taxa at release sites following translocation of captive individuals (Gascon et al., 2007, Zippel et al., 2011; Wren et al., 2015). This is because many of the pathogens that are mediating amphibian declines have been detected in cosmopolitan amphibian collections (Miller et al., 2008; Walker et al., 2008; Cunningham et al., 2015) and that some of these pathogens may be unreliably detected and difficult to eradicate (e.g., Rijks et al., 2018). Furthermore, there may be undescribed pathogens that could pose a risk to translocation programmes. Hosting facilities in range states also ensures greater integration with *in situ* conservation efforts (Gascon et al., 2007; Zippel et al., 2011). The lack of husbandry capacity in key regions is therefore of serious concern. Some amphibian programmes have been established without a detailed understanding of species natural history (Michaels et al., 2014) and some species have not been successfully maintained or bred in captivity (Norris, 2007; Gagliardo et al., 2008). Furthermore, there are high incidences of husbandry related disease in *ex situ* breeding programmes (e.g., Pessier et al., 2014; Jameson et al., 2019). Deciding whether it is possible to locate a conservation breeding facility within the range of the target species depends on an assessment of the risk, the husbandry capacity, local support, and available resources.

If conservation breeding programmes are to be successful it is essential that the appropriate species are selected (Johnson et al., 2020), and that programmes are not undermined by taxonomic uncertainty (e.g. Beauclerc et al., 2010; Yan et al., 2018). Once the appropriate species have been selected, it is critical that that programmes are hosted by appropriate institutions (e.g., Van Der Spuy et al., 2008; Edmonds et al., 2012), that field data are incorporated into captive management practices (Michaels et al., 2014) and where these data are lacking, that husbandry practitioners adopt an evidence-based approach to husbandry (Arbuckle, 2013). These approaches have not always been adopted and this has the potential to reduce the impact of conservation breeding programmes.

### 1.12 Thesis scope

In this thesis I have undertaken work that aims to address some of the issues highlighted above and present research on: (1) how we can have a more inclusive and holistic approach to conservation assessment; (2) the delimitation of cryptic species using an integrative taxonomic approach that facilitates conservation assessment; (3) how to monitor particularly cryptic species and their threats using multiple lines of evidence; (4) how some of the limitations in the way the species are prioritised for ex situ conservation can be overcome; (5) how field data can be successfully incorporated into conservation breeding programmes, and finally (6) how research on ex situ amphibian populations can address key knowledge gaps.

#### Chapter 1. Extinction risk assessment

In Chapter 1, I include a paper on the extinction risk assessment of the Class Amphibia. We used data from Amphibian Species of the World (<https://amphibiansoftheworld.amnh.org/>) to compile a list of all amphibians described between the 2004 launch of the GAA and December 2016. We used the IUCN Red List (IUCN, 2016) to record the number of species that were described in this period and subsequently assessed to gain insight into the proportion of newly described species that were assessed, their extinction risk category and the lag time to assessment. We comment on rates of reassessment since the GAA and regional trends in species discovery, subsequent assessment and reassessment to provide an overview of the challenges posed to assessing taxa with high rates of species discovery. Finally, we suggest a list of pragmatic approaches that could be adopted to increase assessment rates.

#### Chapter 2. Species description and identifying appropriate conservation units

In chapter two I include a series of published papers of a taxonomic scope to illustrate the challenges of using an integrative approach to describe new amphibian species and subsequently identify appropriate units for species conservation. I include several published papers describing amphibian larvae and range extensions to demonstrate how these combined datasets may aid subsequent extinction risk assessment and conservation.

This work focuses on the frog Family Megophryidae, an Asian radiation of frogs known to harbour cryptic species within morphologically conservative species complexes (e.g., Rowley et al., 2015; Liu et al., 2018) and high rates of species discovery. The genus *Megophrys* is a particularly difficult group of frogs to work on. The systematics of the genus *Megophrys sensu lato* have been subject to repeated change and there has been several proposals to split the genus on the basis of dubious morphological variation and limited molecular data (Delorme et al., 2006; Frost et al., 2006; Li & Wang 2008; Chen et al., 2017; Mahony et al., 2017) or for taxonomic convenience (Lyu et al., 2021). Of the seven subgenera, only two can be distinguished reliably on account of morphological

differences (Mahony et al., 2017). To compound this issue, many species descriptions are often short and vague (e.g., Rao & Yang 1997; Mathew & Sen 2007). Even the paratype series of some *Megophrys* species have been shown to include multiple species (Inger & Romer, 1961; Marx, 1976; Poyarkov et al., 2017) and many populations have been described or assigned to species without robust supporting data (Orlov et al., 2000, 2015; Saikia & Sinha, 2018) i.e., not using integrative taxonomy.

The larvae of *Megophrys* frogs also exhibit conservative morphology (Grosjean, 2003), for example, they lack labial tooth rows, one of the key larval characters used when delineating species (Dubois & Ohler 1998). Many of the *Megophrys* tadpoles that are described have extremely brief descriptions (e.g., Fei & Ye, 2016) or descriptions based solely on observations of preserved specimens (e.g. Li et al., 2011) and in many cases tadpoles have been assigned to a species as they are found in sympatry with post-metamorphic *Megophrys* at collection sites (e.g. Leong & Chou 1998; Fei et al., 2009; Wang et al., 2012). This may lead to error as several *Megophrys* species may occur in syntopy (e.g., Li et al., 2014; Wang et al., 2014; Chen et al., 2017). As a result, interspecific comparison using published tadpole descriptions is challenging.

The taxonomic uncertainty within the genus *Megophrys* has ramifications for extinction risk assessments. Extent of Occurrence and the number of locations where a species is present are some of the criteria used to assign extinction risk categories (IUCN, 2012). For example, *Megophrys parva* is currently assessed as Least Concern as it is reported from nine countries in south and southeast Asia and because it has a presumed large population size (van Dijk et al., 2004). We now know that *M. parva* is not present in southern Asia (Mahony et al., 2013, 2020) and is only known from its type locality in Myanmar (Mahony et al., 2020).

We studied megophryid frogs in Vietnam, we named and described four species using multiple lines of evidence in a way that clearly identified them as future conservation priorities. We described and redescribed the larvae of several *Megophrys* species to aid field survey work and to provide additional information on the natural history of an imperilled amphibian assemblage.

### Chapter 3. Monitoring amphibians and their threats

In Chapter 3 I include two papers on monitoring, one on using indirect evidence to make inferences on the population status of the Chinese giant salamander and the other to include a large-scale study on the prevalence of amphibian chytrids in an imperilled amphibian assemblage in northwest Vietnam.

The Chinese giant salamander (*Andrias davidianus*) is the world's largest extant amphibian and is considered a global priority for conservation on account of its evolutionary distinctiveness and global endangerment (Gumbs et al., 2018). Giant salamanders are threatened by both overexploitation and habitat degradation (Wang et al., 2004; Feng et al., 2007; Dai et al., 2009; Cunningham et al., 2016), although the contribution of each of these factors in the decline of wild giant salamanders has not been elucidated. From 2013 to 16 we undertook what was probably the largest ever wildlife survey in China to provide the evidence base needed to conserve this threatened species (Chen et al., 2018; Turvey et al., 2018, 2021; Yan et al., 2018). We developed standardised survey protocols and trained over 80 local partners to undertake three different surveys (ecological, community questionnaire and surveys on salamander breeding farms) at 97 survey sites across the range of the species. As ecological surveys only occurred once at each of the surveyed sites, the rarity of salamanders meant only broad conclusions regarding range wide decline and potential extirpation of some populations could be made. In Chapter 3 we show how multiple lines of evidence, including environmental data and information from questionnaire-based interviews can be used to make relatively robust inferences on the importance of particular threats to highly threatened, rarely seen and difficult to detect amphibian species.

Pathogen surveillance studies provide data on the occurrence of pathogens, whether the distribution of the pathogen is expanding and the number of species the pathogen is known to infect and may involve directly sampling the host or the presence of the pathogen in the environment (Gray et al., 2017). There are very few long-term monitoring programmes on the presence and prevalence of amphibian pathogens in southeast Asia and there has been a call for continued effort to monitor the distribution and impact of pathogens including *Bd* in southeast Asia (Rowley et al., 2010).

The Hoang Lien range in Vietnam is known to have a diverse amphibian fauna comprised of more than 80 species (Ohler et al., 2000; Nguyen et al., 2009) including many of which are highly threatened (IUCN SSC, 2015b; 2015c), including the *Megophrys* species described in Chapter 2. As a result, Mount Fansipan within the Hoang Lien Range has been identified as a priority site by the Alliance for Zero Extinction (AZE 2016). Parts of the Hoang Lien Range is within the suspected native range of *Bd* and preliminary work at lower elevations (600–900 m asl) failed to detect the presence of the pathogen (Swei et al., 2011). Most threatened amphibians in the Hoang Lien Range are stream-dwelling and occur at high elevation; high elevation, stream breeding assemblages of amphibians appear to be the most susceptible assemblages to *Bd* in the Neotropics (Berger et al., 1998; Daszak et al., 1999; Lips et al., 2006). We investigate the prevalence of *Bd* at higher elevations within the Hoang Lien

Range and patterns in *Bd* infection in space, time and host species over a five-year sampling period. We also investigated the presence of *Bsal* in the Hoang Lien Range.

#### Chapter 4. *Ex situ* conservation

In Chapter 4 I include a critical review of the commonly cited methods used to justify amphibian conservation programmes, as well as the constraints of undertaking such programmes in range states versus non-range states and in different types of institution (e.g., zoos, aquariums, academic institutions and the private sector).

I go on to illustrate key points by presenting three papers on research undertaken on two Critically Endangered anuran amphibians in the zoo setting. In one example, we show how a lack of field data can undermine a breeding programme for a highly threatened amphibian species and how the integration of environmental data collected in the field into captive management strategies can allow the goals of amphibian breeding programmes to be achieved. This study also highlights the shortcomings of the model or analogue species concept (Preece, 1998; Michaels et al., 2014) whereby common relatives of a threatened species are used as models to develop husbandry strategies before working with target, usually Critically Endangered, species. Our work illustrated how this concept may be flawed, even when working with congeneric species. In a second study we illustrate the complexities of breeding and rearing skeletally healthy anuran amphibians for a translocation programme and how a detailed understanding of nutrition, heat and light are needed if healthy offspring are to be reared; I go on to make recommendations on pre-release health screening for amphibians bred in captivity before they are released into the wild.

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## DISCUSSION

The conservation of the world's amphibians is a daunting prospect, especially against a backdrop of high rates of species description, ongoing habitat loss and the emergence of threats that we are currently unable to ameliorate. Furthermore, amphibians suffer from taxonomic bias in conservation with disproportionate focus on large-bodied mammals and birds (Clark & May, 2002; Davies et al., 2018). This body of work provides examples and strategies of how we can be more efficient and effective in conserving amphibians. We can incentivise people to increase their engagement in species conservation assessments (Chapter 1); we can provide information pertinent to species assessment in species descriptions (Chapter 2); we can adopt new and more efficient methods to monitor amphibians and their threats (Chapter 3); and we can be more selective in how we prioritise species for *ex situ* management (Chapter 4).

### Chapter 1. Extinction risk assessment

Since the launch of the Global Amphibian Assessment (GAA) in 2004 we have had an ever-decreasing understanding of the extinction risk posed to described amphibians. In 2016, 61.3% of all described amphibian species had either not been evaluated or had out-of-date extinction risk assessments (Tapley et al., 2018a). While the situation has improved since our work was published (Tapley et al., 2018a), with 7,237 of the currently described 8,326 amphibian species now assessed, 39.2% of amphibian species have either never been assessed or have out-of-date assessments (IUCN 2021; Frost, 2021). In addition, the disparity between species description and subsequent conservation assessment remains. This is largely the result of ongoing, high rates of amphibian species discovery and is compounded by the decline in species assessment rates. By comparison, other vertebrate groups such as mammals and birds with much lower species discovery rates were more up-to-date, and most described species had been assessed.

A large proportion of amphibian species described since the GAA are Data Deficient meaning that for nearly 40% of 1,730 species described since then that there are insufficient data to adequately gauge extinction risk. Data Deficient species may often be threatened (Şekercioğlu et al., 2004; Pimm et al., 2014) and we found that 53% of amphibian species that have been described over the study period and subsequently assessed for the Red List were threatened if best estimates of threats are used (IUCN, 2020). Although the information needed to resolve data deficiency may be quite basic (e.g. where a species occurs, its elevation range and the number of known localities), such data may be very expensive and / or logistically difficult to collect. The fact that these data have limited publication value (e.g. Griffiths, 2016) means that they may not be a high priority, or particularly rewarding for the research community to collect. Furthermore these areas of research may not be attractive to funders.

We proposed several recommendations to increase the rate of species assessment against the backdrop of high rates of species discovery. The foremost is for describing authors to include data including georeferenced distribution data, information on habitat and ecology, the amount of suitable habitat as well as information on ongoing, potential, and projected threats to the species being described. These data are the basic prerequisites for robust extinction risk assessment. We also encouraged authors who described species as part of a revision of species groups (e.g., taxonomic splits) to include data that may facilitate the extinction risk assessment for the species from which the newly described species has been split. Some authors describing new amphibian species provided sufficient data to assign provisional extinction risk categories to the species being described (e.g., Para-Olea et al., 2016). The four species *Megophrys* species we went on to describe in Chapter 2 were described following the recommendations we made in Tapley et al. (2018a). Three of these have now been assessed by the IUCN (IUCN SSC, In Press a, In Press b, In Press c).

There are other barriers to contributing data to the Red List. A major obstacle is the lack of sufficient acknowledgment of experts in published assessments, reducing expert engagement in the Red List assessments process. At present, Red List assessments for non-amphibian taxa (e.g., mammals, reptiles, and molluscs) recognises contributors as authors, but the Amphibian Red List does not, recognising instead the Amphibian Red List Authority. Red List assessments are recognised as scientific publications and therefore the contribution of data and intellectual input into Red List assessments should be recognised via co-authorship. Recognising the significant contribution of individuals would likely garner much more expert participation in the assessment process. This is because experts in academic sectors must balance the burden of publication quotas and paid work with the contribution of valuable scientific input and unpublished data to the Red List assessment process. Co-authorship would likely incentivise the academic community to participate in the assessment process. Furthermore, if authors describing species were to include data pertinent to Red List assessment, it would be far more likely that their work would be cited in assessment itself and included in the biography. This insufficient attribution was acknowledged as an issue and a barrier at the time this work was undertaken in 2016; unfortunately, there has been no formal change in the process and the disparity remains.

Taxonomic research at the level of species is often poorly cited (Meier, 2016), even in extinction risk assessments. Whilst the taxonomic authority may be included in the text of a publication, it is not always cited in the text (Agnarsson & Kuntner, 2007) and this reduces the impact of taxonomic work. Ultimately this in turn, could impact funding and career progression (Agnarsson & Kuntner, 2007; Meier, 2016). In IUCN Red List assessments the taxonomic authority is always listed in a species assessment, but they are not routinely referenced in the bibliography. This further alienates taxonomists, and the publication impact of their work does

not reach its full potential due to a process issue. Conversely, it is often the taxonomists who are the only people to have any knowledge of extinction risk for many newly described species. This process issue may disengage taxonomists from the Red List assessment process.

The timely assessment and reassessment of species may also be impeded by cost (Rondinini et al., 2013). We estimated that an annual investment of US \$170,478–\$319,290 was needed to have an up-to-date Red List for amphibians. The ongoing global pandemic has illustrated that assessments can be undertaken via online consultation with regional experts (Johnson et al., 2020a) and online assessments could be a potential cost-effective solution to traditional face-to-face Red List assessment workshops. However, online assessments require some level of funding and despite the need, workshops can be difficult to secure funding for (Rondinini et al., 2013).

Many conservation prioritisation schemes are reliant on the accuracy of Red List if they are to be effective. For example, the EDGE of Existence Programme prioritises four amphibians from the Western Ghats as global priorities for conservation: *Nasikabatrachus sahyadrensis* assessed as Endangered in 2004 (Biju 2004); *Melanobatrachus indicus* assessed as Endangered in 2004 (Biju et al., 2004a); *Walkerana phrynoderma* assessed as Critically Endangered in 2004 (Biju et al., 2004b) and the *Micrixalus kottigeharensis* assessed as Critically Endangered in 2004 (Biju et al., 2004c). When these species were reassessed 14 years later, they were all down-listed to Vulnerable, Vulnerable, Endangered and Vulnerable respectively (IUCN SSC Amphibian Specialist Group, unpublished data) and this will likely impact how these species are ranked as global conservation priorities and there is a risk of our limited resources being allocated to the species that are not the highest priority.

## **Chapter 2. Species description and identifying appropriate conservation units**

Frogs of the genus *Megophrys* are a salient example of the degree of undiagnosed species diversity and morphological stasis in amphibians. They also demonstrate that many undescribed species are conservation priorities. When we began describing a new species of *Megophrys* frog in 2017, there were 71 described species in the genus; and since then, there has since been a 39% increase in the number of known *Megophrys* species (Frost, 2021). Historically, bioacoustic and molecular data were not routinely included in *Megophrys* species descriptions, but it is encouraging that all species described since 2017 have been delineated from congeneric species using more than one line of evidence (i.e., morphology, bioacoustics, or molecular data) in the species description. Only one species was described in this period without the support of molecular data (Yang et al., 2018).

The genus *Megophrys* harbours cryptic diversity and highly localised species diversification as speciation has been driven by the diverse topography of the mountain ranges in which they occur, coupled with the supposed limited dispersal ability of frogs in this genus (Mahony et al., 2017; Chen et al., 2017). Despite the Hoang Lien Range being one of the better surveyed regions for amphibians in Vietnam (e.g. Ohler et al., 2000; Orlov et al., 2013), we described four new *Megophrys* species over a four-year period from the area. All the species described in this work were assigned to the subgenus *Panophrys*, a primarily Chinese radiation within the genus *Megophrys* (Liu et al., 2018). The type localities of all four species we described were all within a radius of just 30 km<sup>2</sup> and, in three cases, at above 2,000 m in elevation (Tapley et al., 2017a, 2018b, 2021a). The discovery of several new high elevation species from the Hoang Lien Range in recent years (e.g., Orlov & Ho, 2007; Nguyen et al., 2013; Rowley et al., 2013b, Tapley et al., 2018b, 2021a) reflects a lack of historic survey effort at higher elevations (e.g., Ohler et al., 2000).

An obstacle to understanding the true species diversity in the genus has been the historic misidentification of species. Molecular sequences have not always been correctly identified to species in the past. One novel aspect of our work on *Megophrys* was to provide transparent interrogation of the GenBank data used in the molecular analyses. In our species descriptions, we included the distance from the collection site of sequenced specimens and the type locality of each species. In many cases, we were able to include sequences from specimens collected at the type locality but in some instances, especially for species described historically, the only available sequences were from specimens collected more than 1000 km away. To aid species delimitation going forward, it would be beneficial if attempts could be made to collect data from the type localities where these data are currently lacking.

Even small geographical barriers may impact the dispersal of *Megophrys*, resulting in microendemism. In our paper describing *M. fansipanensis* (Tapley et al., 2018b) we predicted that this species would occur further north in the Hoang Lien Range and potentially into southern China. Subsequent survey effort failed to detect *M. fansipanensis* at sites with suitable habitat within the elevation range where this species would be expected to occur. We hypothesise that a valley, the floor of which is 250 m below the lowest reported elevations at which *M. fansipanensis* and *M. frigida* occur may act as a dispersal barrier that delimits the range of *M. fansipanensis* to the north and *M. frigida* to the south (Tapley et al., 2021a). This particular barrier may also delimit the range of other megophryid frogs; *Leptobranchella botsfordi* has not been encountered north of this barrier (Nguyen et al., 2020). This provides evidence that low elevation valley floors may act as important barriers to dispersal for megophryid frogs and that undescribed morphologically cryptic species could exist either side of such barriers.

Species may be assigned to extinction risk categories based on the best available data at the time. However, sustained field survey work has the potential to drastically change our understanding of extinction risk by revealing significant extensions in range (e.g. IUCN SSC, 2015, 2016). This is one of the reasons why it is so important to ensure that extinction risk assessments are regularly updated. Small extensions in range and elevation may result in non-genuine changes in extinction risk category, whereby the conservation status of a species has not improved, but our understanding of it has improved enough to warrant it being assigned to a less threatened category. During fieldwork in the Hoang Lien Range we collected *M. rubrimera* 21 km northwest of the type locality within the predicted range of the species (Tapley et al., 2017a) but at an elevation 430 m above previous records for the species (Tapley et al 2018c). This resulted in an increased Extent of Occurrence of 1857 km<sup>2</sup> (from 2208 km<sup>2</sup> to 4065 km<sup>2</sup>). Whilst *M. rubrimera* will still likely qualify for being assessed as Endangered, our work highlights how a single new record of elevation can drastically change our understanding of the distribution of a species and potentially have impacts in subsequent extinction risk assessment.

As with many newly discovered species in the region, the *Megophrys* species we described are likely to be highly threatened. All four *Megophrys* species described by our team qualify for being assessed as Endangered (IUCN, 2012; IUCN SSC, In Press a, In Press b, In Press c) and have an Extent of Occurrence of less than 4,100 km<sup>2</sup> and are probably restricted to the Hoang Lien Range in Vietnam and, in some cases, the adjoining Ailao Mountain Range in China (Tapley et al., 2018b, 2018c; 2021a). To assist in conservation assessments, when describing the new megophryid frogs we included the georeferenced distribution data, information on habitat and ecology, the amount of suitable habitat as well as information on ongoing, potential, and projected threats to the species being described, as these data are the basic prerequisites for robust extinction risk assessment (Tapley et al., 2018a). Our work had far wider ramifications than species description and subsequent extinction risk assessment for the newly described species. Populations of *M. rubrimera* were previously assigned to *M. kuatunensis* in Vietnam; the description of *M. rubrimera* resulted in a range contraction of *M. kuatunensis* (Tapley et al., 2017a), this species is now restricted to Fujian and Jiangxi provinces in China approximately 1780 km north-east of where it was previously thought to occur in Vietnam (Tapley et al., 2017a). The effect of this range contraction has not yet been reflected by the IUCN Red List assessment for this species (Huiqing et al., 2004) but it is possible that *M. kuatunensis* would be assigned to a Threatened IUCN Extinction Risk Category on account of its much smaller distribution.

Like many frog species, the life stages of many *Megophrys* species are unknown. We described the tadpoles of three species of *Megophrys* frogs for the first time and redescribed the larvae of three more in detail (Tapley et al., 2020a, 2020b), representing a significant contribution to the field. In cases where larvae had been previously

described, specimens had not been attributed to species with supporting molecular data and there were several incongruencies with our descriptions and published descriptions (e.g., Fei et al., 2009). Furthermore, it was apparent that some tadpole descriptions had been written without the authors ever examining the specimens in life (e.g., Li et al., 2011), and as a result, an important diagnostic character had been missed. Drawing conclusions about the robustness of diagnostic morphological characters based on limited data should be avoided as some larval anurans are known to exhibit phenotypic plasticity (e.g. Vences et al., 2002; Moore et al., 2004). Whilst our tadpole descriptions suggest that certain characters may be used to differentiate tadpoles of specific *Megophrys* subgenera, the sample size was insufficient to draw any robust conclusions. Morphological differences between tadpoles from species within the subgenus *Panophrys* were insufficient to clearly delineate all species.

Larval descriptions have been demonstrably important to this body of work allowing us to survey for species in the absence of adults. The presence of two *Megophrys* species at one site was first confirmed due to the presence of the larvae (Tapley et al., 2020a), and the ongoing presence of another when post-metamorphic specimens had not been observed at the site for four years. In another example, we reported a significant range extension for a highly threatened species in the Hoang Lien Range by documenting the presence of the larvae (Tapley et al., 2020c), saving us the significant cost of mounting repeat expeditions to a remote and challenging field site. In addition, knowing more about the tadpoles provides important information on the habitat of this life stage and, the presence of larvae provides important evidence that frogs are breeding. Information inferred from tadpoles can therefore be vital in informing species conservation assessments.

The work in Chapter 2 illustrates how resource intensive it can be to formally describe species, gain new insights into the natural history of species and prioritise species for conservation. The work took place over a six-year period and involved more than 10 field expeditions at an estimated total cost of approximately £20,000 (not inclusive of key project personnel wages). It is highly likely that despite being one of the better studied regions in Vietnam, there are further amphibian species awaiting discovery in the Hoang Lien Range and it will be several more years before a species inventory can be considered complete. Alarming, microendemism coupled with habitat loss means that there is a risk that some species may be lost before they are described. Whilst new species and larval stages were described and important regional amphibian research capacity was built, it was already established that the region was of global significance due to the threatened amphibian assemblage it supports, and these new discoveries only strengthen the argument to protect the site. Parts of the Hoang Lien Range are clearly prioritised for conservation by the Alliance for Zero Extinction and Key Biodiversity Areas (AZE, 2020; KBA, 2020). Despite this prioritisation, species that are endemic to the Hoang Lien Range or even a single

protected area within it, are still threatened by ongoing habitat loss (Rowley et al., 2013; Tapley et al., 2017a, 2018b, 2018c; 2021a, Nguyen et al., 2020).

This work describing new species, their tadpoles and undertaking surveys to better understand them would not have been prioritised without a species-focused approach. Amphibian conservation is usually species-focused rather than system-focused. Some attempts have been made to adopt area-based conservation approaches that include amphibians (e.g. Myers et al., 2000), but some area-based approaches are also species-focused. For example, Alliance for Zero Extinction sites are locations where 95% or more of the known population of an Endangered or Critically Endangered species occur (Ricketts et al., 2005). Other conservation prioritisation schemes adopt a species-based approach underpinned by both taxonomic certainty and robust conservation assessment (e.g. Isaac et al., 2012; Johnson et al., 2020). Many conservation organisations have shifted from the traditional species-focused approach to a more system-focused approach (e.g. Franklin 1993) whilst using charismatic flagship, keystone, or umbrella species to garner public support for associated biodiversity (Barua, 2011). Would a site-based or system-based approach be more suited to conserve this threatened amphibian assemblage in Vietnam? Should we invest additional resources in describing new species and learning more about their natural history? And do we now know enough about this assemblage to conserve it?

Many regions that support diverse amphibian assemblages are not true wilderness areas as they are used and modified by people, and the Hoang Lien Range and the protected areas within it is no exception. There are no well-known conservation flagships or umbrella species in the Hoang Lien Range and many primates and birds have been locally extirpated due to overhunting (e.g. Rawson et al., 2011; Vietnam, 2017). In recent years, nature-based tourism is a market that has undergone significant growth (Kuenzi & McNeely 2008) and the increase in tourism and outdoor recreational activities is now considered a major threat to global biodiversity (Christ et al., 2003). All the species described in this work occur inside at least one protected area within the Hoang Lien Range. On paper these sites are protected; however, the region is under increasing pressure from tourists and new infrastructure is being developed within protected areas at an alarming rate, including a record-breaking cable car in terms of length and elevation climbed (Michaud & Turner 2017). These infrastructural developments are primarily focused on the summit of Mount Fansipan in the same elevational range of *M. fansipanensis* and two other highly threatened megophryid frogs. Even if a system-based approach were to be adopted, there would still be a need to prioritise sites for conservation within these protected areas to ensure a balance between biodiversity conservation, economic growth and poverty reduction. This prioritisation itself requires a detailed knowledge of local biodiversity, the distribution of species and information on species natural history such as larval development and larval ecology, as some of the species within the Hoang Lien Range have

a limited distribution and / or extreme microhabitat specificity (e.g. Nguyen et al., 2020; Tapley et al., 2018b, 2021a).

Rather than a species or a site-based approach, some researchers have called for more solutions-based approaches to amphibian declines (Grant et al., 2019). However, potential solutions can only be identified once basic research has been undertaken on the species assemblage. Currently, in many parts of the world, our lack of knowledge of species diversity, species ecology and distribution remain an impediment to amphibian conservation. Unfortunately, much of the data that underpin a more solutions-based approach to conservation may be incredibly difficult to collect. The target species are often cryptic and / or rare resulting in issues with sample size, replication, and data collection may be time consuming and expensive to collect (Griffiths, 2016). Furthermore, some data that underpin solutions-based approaches may have limited publication value or impact and this could act as a barrier as academics are under increasing pressure to publish in high impact journals (Griffiths, 2016). Finally, insufficient data or the lack of knowledge of a particular conservation issue may result in the selection of an inappropriate conservation intervention which could incur the loss of valuable conservation funds and the loss of valuable time, during which the target species may have undergone further declines in population size. Whilst some of these data gaps may be addressed through collaboration with zoos and academic institutions, conservationists face an ongoing dilemma, regarding the robustness and completeness of the data to inform interventions versus the time in which they have to act in order to save species (see discussion in McCoy, 1994). Ultimately there will be a point at which we can say we know enough about a species assemblage to conserve it and that we do not need to undertake more research; however, this is some time away in the Hoang Lien Range. For the time being, research must be prioritised and proceed in tandem with conservation action.

### **Chapter 3. Monitoring amphibians and their threats**

In Chapter 3, I included two papers which help increase our understanding of the threats posed to a particular amphibian species (Tapley et al., 2021b) or assemblage (Tapley et al., 2020d). Both surveys were ambitious in their scale and costly to undertake.

The first paper on the drivers of Chinese giant salamander declines is one in a series of papers that were outputs from a large-scale project to generate the evidence base needed to inform conservation strategies for Chinese giant salamanders. Ecological surveys were trialled and validated in 2013 (Tapley et al., 2015a). It was apparent during the trials that whilst many of our collaborators reported using established cryptobranchid survey techniques previously, including snorkelling surveys (e.g., Browne et al., 2011b; Tapley et al., 2017b) that there

were limitations with the historic work undertaken. We had to teach some of the participants how to swim and how to identify larval and juvenile giant salamanders so it was evident that the historic data could have had serious flaws.

We used a combination of ecological surveys and questionnaire-based interviews across the range of giant salamanders in China. At each ecological survey site, the survey teams recorded a suite of environmental parameters and recorded anything that could be construed as a threat to the species (e.g., evidence of aquatic traps or electrofishing). We took cloacal and skin swabs from giant salamanders encountered during our surveys and evidence for ranavirus and amphibian chytrid infection. The samples were processed following for amphibian chytrids and for ranaviruses. Unfortunately, very few salamanders were encountered during this work; we detected a total of just 25 giant salamanders at just four of the 97 selected survey counties (Turvey et al., 2018).

We did not detect amphibian chytrids or ranavirus infection in any of the giant salamanders we swabbed (N=20) and we recorded the presence of diverse amphibian assemblages at many survey sites. The small number of wild salamanders that were directly detected during our surveys reflects some of the issues of working with incredibly rare or cryptic species. The sample size was insufficient to provide us with a robust understanding of some of the potential threats to this species. For example, data from the analysis of 20 swabs would be insufficient to state with confidence whether amphibian chytrids infect wild giant salamanders (Skerratt et al., 2008).

The nature of the threats posed to the species precluded certain survey methods. The isolation of environmental DNA (eDNA) from rivers and streams has been effective in detecting the presence of cryptobranchid salamanders in North America (Spear et al., 2015; Pitt et al., 2017; Takahashi et al., 2018) and Japan (Fukumoto et al., 2015; Bjordahl et al., 2020). This survey technique is deemed less invasive and less resource intensive than traditional ecological survey techniques. However, eDNA analysis was precluded across our entire study area due to the ubiquity of giant salamander farms. These farms discharge effluent into nearby rivers and streams, and false positives were therefore deemed highly likely.

Whilst we did not detect many salamanders, the analyses of environmental data both direct and indirect evidence that the extraction of giant salamanders from the wild was ongoing. This supports the hypothesis that the decline of giant salamanders across China has been primarily driven by overexploitation rather than habitat loss and degradation. This is important as this new evidence-base can guide conservation planning. This body of

work highlights the urgent need for the existing protective legislation prohibiting the hunting of giant salamanders to be better implemented and more strictly enforced in tandem with the existing efforts for strict habitat protection. This is a recommended conservation action that has been adopted in the recently completed IUCN Red list assessments for *A. davidianus* and *A. sligoi* (IUCN SSC, In Press d, IUCN SSC, In Press e). This work provides important baseline data on environmental parameters that can inform husbandry protocols for *ex situ* conservation programmes. Conservation breeding programmes have been identified as a necessary component of the conservation strategy for each of the Chinese giant salamander lineages and species (Turvey et al., 2018, 2019).

The use of non-traditional techniques from which to infer the status of Chinese giant salamander populations and primary drivers of decline was a key aspect of this work. Community questionnaires provided important data that would not have been recorded had we been entirely reliant on ecological survey techniques. Ongoing hunting by respondents was directly reported across 14 of the 16 surveyed provinces or equivalent administrative areas. Local ecological knowledge (LEK) has not been widely used by amphibian conservationists, but this work demonstrates that it could be a far more efficient and cost-effective means to gather information on threatened species provided that the species is known by the local community and that folk taxonomy is broadly congruent with scientific taxonomy. There are other threatened amphibians that are both morphologically distinct and exploited by people that could be candidates for research using these methods, including iconic amphibians such as *Conraua goliath* (e.g. Hermann et al., 2005), *Phyllobates terribilis* (Myers et al., 1978) and *Laotriton laonesis* (e.g. Phimmachak et al., 2012). In addition to LEK, there are other methods that can be used to address key knowledge gaps. The Delphi method is a process by which the opinions of experts are elicited and scores these opinions in scenarios where there is much data uncertainty and or a lack of data (MacMillan & Marshall 2006). Statistical models can combine the expert opinion on the status of a species with quantitative data (MacMillan & Marshall 2006; Choy et al., 2009; Kuhnert et al., 2010; Griffiths et al., 2015) and these methods are beginning to be applied to amphibian conservation (e.g. Smith et al., 2020).

Known and potential threats to species can also be monitored. The second paper in Chapter 3 examines the prevalence of *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) in the Hoang Lien Range in northwest Vietnam (Tapley et al., 2020d). We sampled 601 individual amphibians representing 40 species at 10 different sites from 2015–2019. All samples were analysed for *Bd* but only 180 samples (representing 27 species) were analysed for *Bsal* due to funding constraints.

We did not detect *Bsal* infection in any of the analysed samples and report a *Bd* prevalence of 1% (0.37–2.2% (CI). This typical endemic pattern of low-prevalence and low-intensity of infection corresponds with other studies in Asia (Kusrini et al., 2008; Mendoza et al., 2011; Savage et al., 2011; Swei et al., 2011; Gilbert et al., 2012; Rowley et al., 2013b; Le et al., 2017; Mutnale et al., 2018). It is estimated that samples from 60 individuals should be tested to achieve 95% certainty of detecting a single *Bd* positive individual using qPCR if the prevalence of infection is  $\geq 5\%$  (Skerratt et al., 2008). Given the extremely low prevalence of overall *Bd* infection at our study sites and others in mainland southeast Asia it is likely that a sample size of 300 swabs would be required to make inferences about the prevalence of *Bd* infection in the endemic range of *Bd*. Assessing the prevalence of *Bd* infection at the level of an individual species may be extremely problematic as many species were not particularly abundant. We did not detect 60 amphibians, let alone 300, at any visit to our survey sites at any point during our five-year survey and we only encountered 26 *L. botsfordi* (a Critically Endangered species we were specifically targeting) over the five-year study period. Whilst we could make broad inferences about the prevalence of the surveyed pathogens to the scale of site and amphibian assemblage, it was not logistically possible to collect data that would allow for any study on the prevalence of *Bd* infection in a single species. This was also the case when we attempted to assess the prevalence of amphibian chytrids in giant salamanders in China (Tapley et al., 2021b).

Five of the six known strains of *Bd* are known from Asia (Byrne et al., 2019) and we attempted to culture and isolate *Bd* from toe clips of 168 different frogs following Fisher et al. (2018). These attempts failed either due to the absence of infection or due to competition with other microorganisms during the culturing stage. The collection, storage and transport of this number of tissue samples is logistically challenging due to the remoteness of the field sites and lack of refrigeration units. Recently, new qPCR assays have been developed to discriminate between two lineages of *Bd* (Ghosh et al., 2020), but assays do not currently exist to discriminate between the other lineages. Developments in fungal metabarcoding may also provide opportunities to determine the lineage/s of *Bd* infecting amphibian hosts (e.g., Nillson et al., 2019). Further work should aim to identify the lineage/s of *Bd* infecting amphibians in the Hoang Lien Range. Cost and site remoteness was a limiting factor in this work, and we attempted to use lateral flow tests to detect *Bd* and *Bsal* infections following Dillon et al. (2017). Eighty-three samples were processed (representing 19 species). There were three chytrid positive samples using the lateral flow kits but none of these samples were positive for *Bd* (qPCR) although they were not screened for *Bsal*.

This body of work represents one of the most intensive surveys for amphibian chytrids undertaken at a site in mainland southeast Asia to date and will be an important baseline against which to measure changes in infection

prevalence and / or intensity. However, the pathogen surveillance study in the Hoang Lien Range was costly. Analysis of pooled swab samples alone would have amounted to £3,420.00. Cost is a limiting factor to the long-term surveillance of amphibian pathogens, especially if enough swabs are collected to ensure that robust conclusions can be drawn. This cost could be beyond the means of many. Furthermore, swabs often have to be exported for analyses abroad and there is limited capacity for many countries to undertake amphibian pathogen surveillance studies without collaborating with foreign researchers.

Conservation programmes often operate with limited budgets and some components of these programmes such as pathogen surveillance and molecular genetics may be disproportionately costly. This means that conservationists could have little funding left to undertake practical conservation work and once again, they must make difficult decisions on how much research should be undertaken prior to undertaking any conservation intervention. Lateral flow assays to detect *Bd* and *Bsal* infection offer a potential solution (Dillon et al., 2017) as samples do not need to be sent to a laboratory for costly analysis. However, these lateral flow assays do not provide information on the intensity of infection, the lineage or even the species of amphibian chytrid infecting the host. Furthermore they have not been widely used and are not currently commercially available. Portable PCR devices are now being used in the field (Marx, 2015) and their use may make it easier to process samples in remote locations before they degrade via transport routes (Marx, 2015). Recent field trials indicated that there were more false negatives when compared to laboratory extractions, a reduced sensitivity and overall, it may be more expensive and time consuming to process large numbers of samples than in the laboratory setting (Kamoroff et al., 2020). One can hope that the current pandemic and the increased focus on wildlife health and zoonosis will result in cost-effective, technological and methodological advances that will benefit the amphibian conservation community.

#### **Chapter 4: *Ex situ* conservation**

*Ex situ* conservation became widely accepted as a viable tool to conserve highly threatened species in the early 1990s (IUDZG/CBSG, 1993). However, this approach was increasingly questioned as a strategy by the wider conservation community due to the limitations of captive breeding in the recovery of threatened species (Snyder et al., 1996; Bowkett 2009). In the early 2000s, the potential value of *ex situ* conservation gained traction once again as there was no other viable option to safeguard susceptible amphibian species when it was identified that many population declines and potentially recent extinctions has been mediated by a pathogen (Gascon et al., 2007; Bowkett 2009).

Amphibians have often been considered ideal candidates for *ex situ* conservation programmes on account of traits such as small size and associated low space requirements, high fecundity, innate behaviours, amenability to assisted reproductive techniques, relative cost-effectiveness of amphibian conservation breeding programmes and also because captive husbandry capacity exists (Bloxam & Tonge, 1995; Balmford et al., 1996; Griffiths & Pavajeau, 2008; Browne et al., 2011; Smith & Sutherland, 2014). However, given the sheer diversity of amphibian species, we argue that it is impossible to make generalisations about the biology or geo-political context of an entire class when undertaking costly, resource intensive conservation actions (Tapley et al., 2015b). Furthermore, amphibian husbandry capacity does not often exist where it is most needed i.e., in regions with the most diverse and most threatened amphibian assemblages (Gagliardo et al., 2008; Edmonds et al., 2012). Finally, to implement successful *ex situ* programmes for amphibians, it is essential that there is taxonomic certainty regarding the population or evolutionary significant unit being targeted (e.g. Hudson et al., 2016; Yan et al., 2018). Taxonomic uncertainty and / or an incomplete knowledge of the genetic structure of different populations may undermine *ex situ* conservation breeding programmes or lead to compromises being made in later management decisions (e.g. Beauclerc et al., 2010)

Conservation breeding programmes should not be established as a response to extinction risk alone. There are many amphibians that need conservation breeding programmes and there are only limited resources available. It is therefore crucial that species that have been assessed as high priorities for *ex situ* conservation action are subsequently individually reassessed to determine their suitability for inclusion in conservation breeding programmes to ensure that these limited resources are most wisely invested (Tapley et al., 2015b). We highlight the advantages and disadvantages of hosting conservation breeding programmes in different types of institutions namely zoos and aquariums; museums and other academic institutions as well as the private sector and go on to discuss the pros and cons of conservation breeding programmes being established in the range state of the target species versus the target species being housed in facilities in non-range states.

Some amphibian conservation breeding programmes have been established as an emergency response due to threats that could not conceivably mitigated in the short-term. The mountain chicken frog (*Leptodactylus fallax*), the largest native frog in the eastern Caribbean underwent what is probably the fastest documented decline of a vertebrate after the arrival of *Bd* (Hudson et al., 2016). The founding stock of the conservation breeding programme had to be exported to European zoos from Montserrat as capacity did not exist on the island to undertake such a venture. However, a husbandry and veterinary capacity was built in tandem on Dominica, a much larger island within the native range of *L. fallax* and this included the establishment of husbandry facilities and a molecular diagnostics laboratory (Tapley et al., 2014). *Leptodactylus fallax* had been maintained and bred

successfully in European Zoos prior to the establishment of the conservation breeding programme (Gibson & Buley, 2004).

A population of *L. fallax* established earlier in the United States failed to thrive and captive animals exhibited clinical signs of nutritional secondary hyperparathyroidism (NSHP) commonly known as nutritional metabolic bone disease (NMBD) (King et al., 2011) and incidences of NSHP were also reported in the population in Europe (pre-dating the establishment of the conservation breeding programme; Tapley et al., 2015b). The population in the United States had not been provided with UVB emitting lamps. We demonstrate that the provision of vitamin D<sub>3</sub> via the diet alone was insufficient to sustain skeletal health in *L. fallax*. However, skeletally healthy animals were reared when vitamin D<sub>3</sub> was provided in the diet in tandem with the provision of UVB emitting lighting arrays (Tapley et al., 2015b). This scenario would probably best reflect conditions in nature where *L. fallax* have been seen actively basking (Tapley et al., 2015b). Whilst the screening for some pathogens is routine in many translocation programmes, the assessment of skeletal health is often not undertaken. Given the prevalence of metabolic bone disease in captive amphibians we call for skeletal health to be included in the routine pre-release health assessment of captive amphibians (Tapley et al., 2015b).

The Lake Oku frog (*Xenopus longipes*) is a Critically Endangered frog that occurs in a single crater lake on Mount Oku in Cameroon (Loumont & Kobel, 1991; Stuart et al., 2008). In 2008, a conservation breeding programme was established in European zoos in response to enigmatic die offs of Lake Oku frogs between 2006 and 2010 (Doherty-Bone et al., 2013) and the perceived risk of predatory fish being introduced to Lake Oku as a food source for local people (Tinsley & Measey, 2004). Amphibian husbandry capacity did not exist in Cameroon at this time. It was assumed that the analogue concept could be applied i.e. that husbandry protocols that have been used to manage less threatened congeneric species (e.g., *X. laevis*) in labs for decades could be adopted. In our work we concluded that whilst adult *X. longipes* can tolerate established captive management protocols and conditions used for other *Xenopus* species (e.g., Green 2012), the tadpoles are more sensitive, especially to the mineral content of the water. It was only by replicating the precise water parameters of Lake Oku that we were able to successfully rear tadpoles to metamorphosis (Michaels et al., 2015; Tapley et al., 2015c), and this process was extremely labour intensive and took several years of trial and error (Michaels et al., 2015). There were differing life history traits between *X. longipes* and other *Xenopus* species including smaller clutch size and prolonged larval development (Michaels et al., 2015; Tapley et al., 2015c). This work highlights the risk of the analogue species concept and additional work has shown the limitations of this concept in frogs of the Family Alytidae (e.g., Michaels et al., 2016); even closely related species may have very different husbandry needs.

Both the first and revised Amphibian Conservation Action Plans (ACAP) state that amphibian conservation breeding programmes should be established in range states (Gascon et al., 2007; Wren et al., 2015) and this view is also maintained by the Amphibian Ark (Zippel et al., 2011), the organisation established to oversee the *ex situ* components of the ACAP. Hosting the facilities in range states reduces the risk of exposing target species to novel pathogens (Zippel et al., 2011) and ensures that the programme is well integrated with wider species recovery plans which may involve habitat restoration and the mitigation of other threats (Johnson et al., 2020b). Amphibian husbandry and veterinary capacity should be built in the areas where it is most needed. The aspiration that every country will be equipped with the expertise to establish and effectively run amphibian conservation breeding programmes, should they be needed, is laudable. However, some range states that support globally threatened amphibian assemblages and / or highly threatened species are relatively small and not particularly populous and it may not be realistic to expect that the capacity to initiate and run programmes that require such long-term commitment and funding will always exist. Conversely, it can be relatively easy to establish conservation initiatives when working with small governments and at a small spatial scale (pers. obs.).

There may be several barriers to overcome when establishing conservation breeding programmes in range states. On Dominica, it took approximately four years to establish live food colonies that were productive enough to maintain a captive population of *L. fallax*; commonly cultured live food species used by zoos throughout the world could not be imported due to the potential risk these non-native invertebrates posed if they were to escape (Nicholson et al., 2017). Furthermore, specialist equipment such as UVB emitting lamps are not commercially available on Dominica and shipping costs were prohibitively expensive (pers. obs). In Madagascar, half the footprint of an amphibian conservation breeding facility was dedicated to local live food production (Edmonds et al., 2012) and the facility was originally established with no running water or electricity (D. Edmonds, pers. com.). These barriers are a particular issue if programmes are to be funded by small range-state governments which may have limited resources to dedicate to conservation breeding programmes. Finally, the ultimate success of these programmes may be undermined by proximity of the initial threat; there were repeated outbreaks of chytridiomycosis in the captive facility on Dominica and cycles of disease and treatment could be one of the reasons that the frogs never successfully bred in the captive facilities there (pers. obs). Ultimately the facility was irreparably damaged by a cyclone in September 2017.

It is highly likely that attempts to raise *Xenopus longipes* in aquaria in Cameroon would have failed as attempts in a relatively resource-rich zoo with decades of cumulative aquarist knowledge were only marginally successful. Acquiring specialist equipment such as UVB emitting lamps, veterinary drugs and even disinfectants can be problematic in some range states (pers. obs.). Often, even when local capacity is built there may be a lack of

succession planning and this can compromise the longevity of conservation breeding programmes when someone with a particularly niche skill set moves on from the programme (pers. obs).

Political instability may also undermine the viability of conservation breeding programmes in some regions or pose a threat to facilities, those that manage them or even the animals being housed within them (e.g., Kümpel et al., 2015). In Cameroon, there is currently political unrest in the anglophone regions of the country (Pommerolle & Heungoup, 2017) and this would make operating any conservation breeding programme close to the range of *X. longipes* in the anglophone region extremely challenging. Should any stochastic events occur that result in the extinction of *X. longipes* in the wild, the zoo colony would be the only viable population on the planet. However, this is a population that was not managed in isolation from other amphibian species and using this population as a source population could present a real risk to other threatened amphibians in the vicinity of Lake Oku, as the frogs may host pathogens that are not present in Lake Oku. Political support may also undermine conservation breeding efforts. The Asiatic lion is restricted to the Indian State of Gujarat and the population is vulnerable to extinction (Johnsingh et al., 2007). A Supreme Court Ruling mandated the creation of a second population in the State of Madhya Pradesh; this has not been realised as a change in national political leadership has supported the State of Gujarat's reluctance to translocate lions to another State (Dutta, 2019). Whilst there are no published accounts of political support undermining the goals of amphibian conservation programmes, it is likely that conservation practitioners may not have disseminated such information due to the need of maintaining important relationships, the potential for political support to undermine amphibian conservation initiatives should not be underestimated.

An effective *ex situ* response may require a more pragmatic approach. For example, one of the barriers to *ex situ* conservation is the risk that novel pathogens may pose to a programme, especially if amphibians are housed outside of the range state. These risks should be carefully measured against the risk of not undertaking the programme at all because of excessive biosecurity costs, and the potential loss of a species or assemblage. Whilst it is difficult to mitigate against unknown and undescribed pathogens (e.g., Walker et al., 2008), there are now well-established processes to assess the risk that pathogens may pose to translocation programmes (e.g., Sainsbury et al., 2017; Suarez et al., 2017). However, these are also time consuming and potentially costly to undertake and their robustness is dependent on our existing knowledge of pathogens which may be entirely lacking in some regions.

Conservation breeding for translocation is just one of the justifications for managing *ex situ* populations of amphibians. The *ex situ* community is in a unique position to undertake research that may underpin conservation

efforts (Browne et al., 2011; Tapley et al., 2017c). Maintaining threatened amphibians in captivity provides the opportunities to document and describe unknown aspects of species biology, many of which may be difficult to observe in nature (Michaels et al., 2015; Tapley et al., 2015c; Tapley et al., 2017c) as well as demographic information. A recent analysis of the data stored on zoo and aquarium animals in an international online database concluded that a huge amount of demographic data are available for the world's threatened tetrapods, although data on amphibians was the most limited (Conde et al., 2019). Maintaining populations of amphibians in captivity also provides the opportunity to develop, trial and validate techniques that may facilitate both the research of a particular threatened species and novel conservation interventions that may benefit threatened amphibians in nature (e.g. Rendle et al., 2015; Tapley et al., 2019). Husbandry techniques can also be developed outside of range states and the knowledge gained can be shared with those wishing to develop *ex situ* conservation breeding programmes within the range of the target threatened species (Tapley et al., 2017c). Husbandry practitioners rarely have the publication pressures of those working within academia and have the luxury of undertaking research projects that address the small, yet often critical gaps, in our knowledge that currently impede amphibian conservation.

## Conclusions

Although amphibian declines are a global problem, the capacity to deal with amphibian declines is geographically skewed. An effective global response must continue to involve long-term support, capacity building and long-term international partnerships. The effective and impactful response to amphibian declines requires a network of stakeholders and long-term, sustainable funding.

While describing and subsequently assessing new species may take considerable time and effort it is important that there is a continued work to understand the true diversity of amphibians by describing species and subsequently assessing them so that they can be prioritised for conservation attention. An integrative approach to delimiting new species helps resolve species boundaries, especially in taxa that exhibit a high degree of morphological stasis and this approach using multiple lines of evidence may, in the long-term, facilitate a more accurate taxonomy upon which long-term conservation management decisions and legislation can be based (Fig. 1).

For known species, our lack of basic knowledge of many amphibians remains a major impediment to their conservation. Even basic information on distribution and reproductive biology are entirely lacking for many species. Gathering these data may not always be easy. Furthermore, a species may undergo further decline whilst such data are being collected which may undermine the success of any subsequent conservation

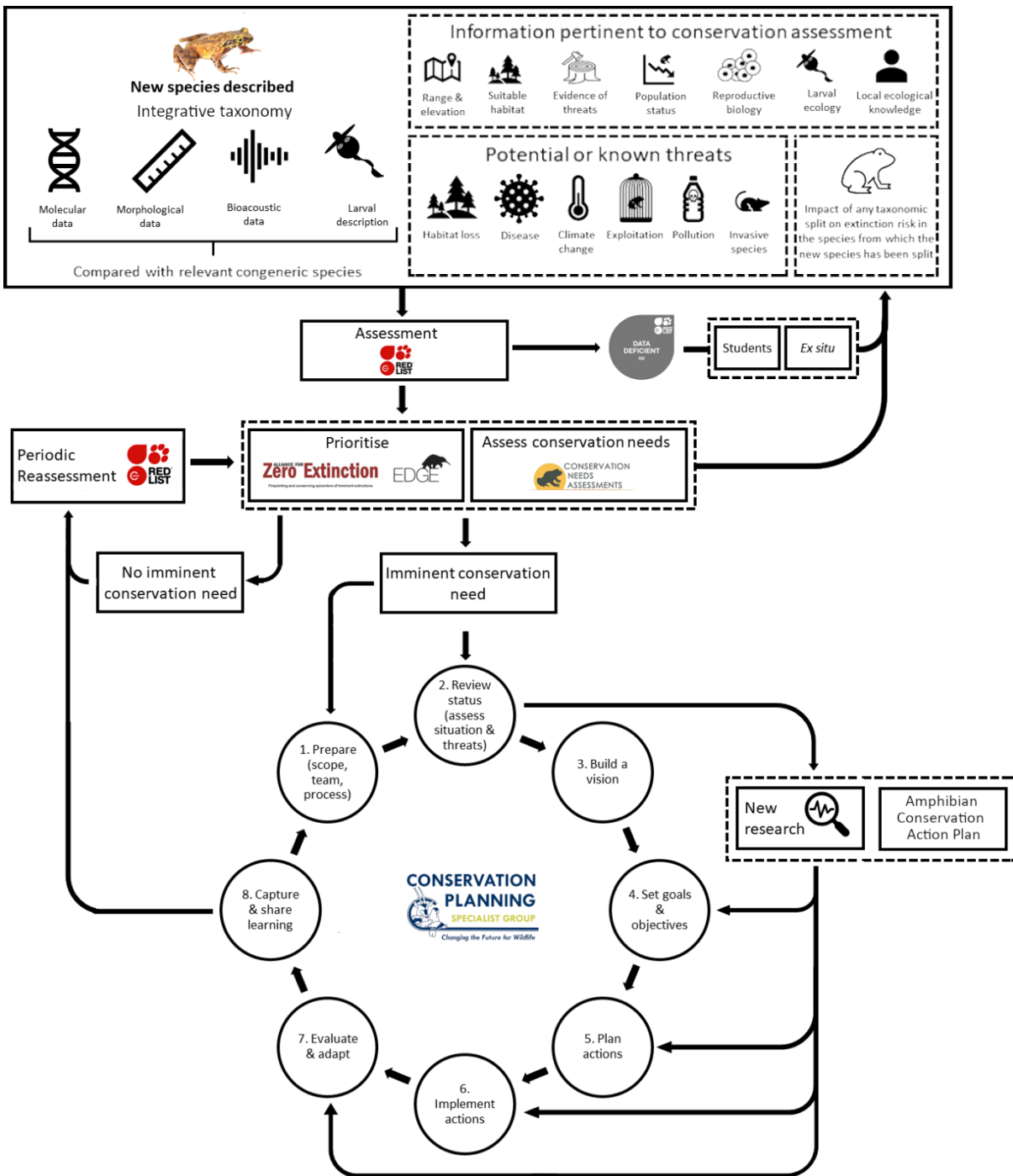


Figure 1. Flow chart to illustrate a potential, iterative pathway and feedback loops from the point at which a species is described through to assessment, prioritisation and the implementation of a conservation programme incorporating the Conservation Specialist Group’s species conservation planning steps (after CPSG, 2020).

programme. Understanding the species and the precise cause of decline will help identify the most appropriate and most impactful intervention. This is important as resources are extremely limited relative to the scale of global amphibian declines. Whilst some would advocate a proactive approach to addressing perceived threats to amphibians, it is essential that conservation interventions are underpinned by sufficient research and robust

prioritisation in tandem with conservation action. Some data gaps may be efficiently addressed if everyone working on amphibians took a collective responsibility to conserve them. For example, many authors describing new species likely have enough information on the species being described to recommend provisional IUCN extinction risk categories to the new species and to consider the conservation ramifications of taxonomic splits. Making these data accessible in the species description itself saves valuable time and resources, may aid timely conservation assessment, inform conservation prioritisation schemes, and inform conservation action (Fig. 1).

This body of work demonstrates how knowledge deficits can be addressed by using LEK. People working on amphibians should embrace new cost-effective approaches, such as the use of LEK, to gain new insights into the status of a species and the specific threats posed to it. Whilst there may be errors associated with LEK, and some reporting bias or exaggeration, these issues may be outweighed by the cost effectiveness of such data and counteracted by research design. Furthermore, in cases where there are data gaps or data uncertainty emerging statistical models that combine quantitative data with expert opinion offer further opportunities to address knowledge deficits (e.g., MacMillan & Marshall 2006; Choy et al., 2009; Kuhnert et al., 2010; Griffiths et al., 2015).

This body of work also demonstrates how new and important information on species natural history can be collected from captive animals. Furthermore, husbandry techniques can be developed and refined in countries with existing *ex situ* husbandry capacity for dissemination to regions where there is the greatest need, yet where *ex situ* husbandry capacity may be less developed. The *ex situ* community, particularly zoos and aquaria, should place greater emphasis on undertaking research (Fig. 1) and capacity building as these contributions are important responses to global amphibian declines. For some organisations with a focus on *ex situ* conservation, the exchange of knowledge, rather than animals is probably a much wiser use of resources; not only is the need for costly biosecure facilities and health screening reduced, but it also aids in the decolonisation of conservation by ensuring that all stakeholders have the opportunity and means to participate in species recovery efforts.

It is important that the current species-focused approach to amphibian conservation is maintained. Many amphibians, especially threatened amphibians, have small distributions and there is a risk that species may be missed should broader system-based approaches be adopted for amphibian conservation. For example, 24% of amphibian species are not known to be present in any protected area (Nori et al., 2015). The importance of a species-focussed approach to conservation is further supported by the fact that susceptibility to threats varies between species, even those within the same assemblage. Furthermore, the potential interventions to reduce the threat may not be suited to all species. A case in point is the fungal pathogen *Bsal*. Some species are highly susceptible to this pathogen (e.g. Martel et al., 2014), and current recommended treatment options include

prolonged exposure to high temperatures (e.g. Blooi et al., 2015) which could be lethal to salamander species with low tolerances to high temperatures. Conservation breeding might be an option for some species should *Bsal* cause catastrophic population declines. However, not all salamanders might be suited to the establishment of conservation breeding programmes should there be a requirement to do so (Gilbert et al., 2020). There may be regional differences regarding the severity of particular threats to amphibians; emerging infectious diseases do not currently appear to be a major threat to amphibians in Asia, whereas habitat loss is a more imminent concern. As a result, there may be a greater urgency to establish *ex situ* assurance colonies of threatened amphibians in regions where population declines are being mediated by disease, whereas *ex situ* conservation may not be a priority in other regions.

Amphibians are an extremely diverse, poorly known, and ancient group of animals. There is substantial evidence to suggest that generalised Class-focused approaches to conserve amphibians should be avoided. Instead, species-specific conservation strategies that are informed by robust extinction risk assessment and knowledge of species natural history and threatening processes should be developed. Generalised approaches that do not consider species-specific factors risk missing the subtle, yet potentially critical nuances that may be pivotal in the success of conservation programmes. Whilst there are knowledge gaps that currently impede conservation, the adoption of new methods, new processes, adjustments in the focal areas of work by some stakeholders and the development of a more collective responsibility by everyone working on amphibians to conserve them could put us in a better position to conserve these undeniably important denizens of our planet.

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