SPECIAL ISSUE | FOREWORD
https://doi.org/10.1071/RDv34n5_FO

Reproduction, Fertility and Development

Common goals, different stages: the state of the ARTs for reptile and amphibian conservation

Simon Clulow[A], John Clulow[B], Ruth Marcec-Greaves[C], Gina Della Togna[D,E] and Natalie E. Calatayud[F,G,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Natalie E. Calatayud
San Diego Zoo Wildlife Alliance, Beckman Center for Conservation Research,
15600 San Pasqual valley Road, Escondido, CA 92025, USA
Email: drncalatayud@gmail.com

Handling Editor:
Ye Yuan

Published: 11 March 2022

Cite this:
Clulow S *et al.* (2022)
Reproduction, Fertility and Development, **34**(4–5), i–ix.
doi:10.1071/RDv34n5_FO

ABSTRACT

Amphibians and reptiles are highly threatened vertebrate taxa with large numbers of species threatened with extinction. With so many species at risk, conservation requires the efficient and cost-effective application of all the tools available so that as many species as possible are assisted. Biobanking of genetic material in genetic resource banks (GRBs) in combination with assisted reproductive technologies (ARTs) to retrieve live animals from stored materials are two powerful, complementary tools in the conservation toolbox for arresting and reversing biodiversity decline for both amphibians and reptiles. However, the degree of development of the ARTs and cryopreservation technologies differ markedly between these two groups. These differences are explained in part by different perceptions of the taxa, but also to differing reproductive anatomy and biology between the amphibians and reptiles. Artificial fertilisation with cryopreserved sperm is becoming a more widely developed and utilised technology for amphibians. However, in contrast, artificial insemination with production of live progeny has been reported in few reptiles, and while sperm have been successfully cryopreserved, there are still no reports of the production of live offspring generated from cryopreserved sperm. In both amphibians and reptiles, a focus on sperm cryopreservation and artificial fertilisation or artificial insemination has been at the expense of the development and application of more advanced technologies such as cryopreservation of the female germline and embryonic genome, or the use of sophisticated stem cell/primordial germ cell cryopreservation and transplantation approaches. This review accompanies the publication of ten papers on amphibians and twelve papers on reptiles reporting advances in ARTs and biobanking for the herpetological taxa.

Keywords: assisted reproduction, biobanking, biodiversity, cryopreservation, gametes, genome resource banks, herpetofauna, IVF.

Introduction

This foreword accompanies the publication in *Reproduction, Fertility and Development* (RFD) of a two-part special issue on amphibian and reptile reproductive biology and assisted reproductive technologies (ARTs). This special issue is intended to focus attention on and promote the expanding contribution of the reproductive sciences to conservation of those taxa. The need is pressing. The biodiversity extinction crisis that marked the 20th century continues to accelerate in the 21st. Amphibians and reptiles, traditionally grouped as the herpetological taxa, are amongst the most threatened of vertebrate groups. Grouping amphibians and reptiles under the banner of the herpetological sciences is more a matter of convenience and shared discipline histories rather than reflecting real phylogenetic, ecological and reproductive homologies. However, considering the number of reproductive biologists who work on both taxa, it is useful to compare and contrast the progress of ARTs and biobanking in these groups, despite the differences in reproductive anatomy and physiology. What meaningful contribution can a better understanding of basic reproductive biology, and the development of reproductive technologies make to the conservation of these taxa? The use of assisted reproductive technologies (ARTs) in concert with genome resource banks (GRBs) are now recognised and increasingly promoted as linked tools in the fight against genetic and biodiversity loss of vertebrates (Holt *et al.* 2003; Pukazhenthi and Wildt 2004; Pukazhenthi *et al.* 2006; Ryder and Onuma 2018; Holt and Comizzoli 2021), and amphibians and reptiles are no exception. GRBs and ARTs are not a new concept in conservation *per se* (Wildt *et al.* 1986; Holt *et al.* 2003), but the investment of research resources and development of technologies has heavily favoured avian and mammalian models. Even within those taxa, the focus has been on sperm cryopreservation paired with artificial insemination (Rodger and Clulow 2021) rather than more advanced stem and cell biology approaches, with some notable exceptions involving advanced technologies such as stem cell generation and nuclear transfer (Folch *et al.* 2009; Hildebrandt *et al.* 2021; Holt and Comizzoli 2021; Sandler *et al.* 2021). For reptiles and amphibians, the development of technologies has lagged the other vertebrate taxa both in resources and time by a couple of decades (Browne *et al.* 2011; Clulow *et al.* 2012; Clulow and Clulow 2016; Della Togna *et al.* 2020). This two-part special issue of RFD (which should be read in conjunction with an upcoming book on amphibian reproductive technologies; Silla *et al.* 2022) indicates how that situation is rapidly changing, but also highlights how much more needs to be done.

ARTs and biobanking (typically cryobanks) are complementary and interdependent processes (Holt *et al.* 2003; Clulow and Clulow 2016; Della Togna *et al.* 2020; Holt and Comizzoli 2021; Silla and Kouba 2022). First, retrievable genetic material such as gametes, embryos and somatic cells are stored in long-term repositories (i.e. GRBs, typically cryobanks (Clulow and Clulow 2016) to insure against the complete loss of genes and species, or to provide the genomic material for managing the gene pools of captive and wild populations. Second, ARTs allow the stored material to be revived as viable, reproductively competent individual organisms, potentially restoring genetic diversity stored prior to declines and even extinctions many decades previously (Howard *et al.* 2016; Holt and Comizzoli 2021). The potential conservation implications of these tools are enormous. ARTs and GRBs provide conservation practitioners the tools to reverse inbreeding and restore lost genetic diversity by introducing or re-introducing critical genes to wild populations (genetic rescue; Howard *et al.* 2016; Madsen *et al.* 2020; Holt and Comizzoli 2021), manage or reverse inbreeding depression and limit selection for domesticity in captive breeding programs (Howard *et al.* 2016; Howell *et al.* 2021*a*), and even revive lost species (Jørgensen 2013), while optimising the allocation and use of conservation resources (Howell *et al.* 2021*a*).

This two-part special issue of *Reproduction, Fertility and Development* comprises 10 papers on amphibian and 12 papers on reptile ARTs. The authors form a broad international cohort, which is a hopeful sign for the future use and uptake of these conservation tools. However, while the goals and ultimate applications of ARTs in conservation for amphibians and reptiles are the same, the challenges and progress are not. The reproductive systems differ greatly between the groups, as does progress in developing ARTs. A push for the development of ARTs for amphibians gained momentum in the decade prior to the turn of the century in response to the emerging amphibian extinction crisis that became increasingly obvious from as early as the 1980s (Browne *et al.* 1998; Clulow *et al.* 1999). In large part, the acuteness of the crisis was due to the rapid spread of the emerging amphibian chytrid fungal pandemic (Berger *et al.* 1998; Bower *et al.* 2017; Scheele *et al.* 2019). The decline or extinction of more than 500 species of amphibians around the world in just a few decades has been attributed to this disease alone, with few options available to prevent or reverse ongoing severe declines in the wild (Clulow *et al.* 2018*b*; Scheele *et al.* 2019). Combined with other threats such as climate change and habitat loss, there are now estimates that more than half the world's amphibians are at risk of extinction (Stuart *et al.* 2004; González-del-Pliego *et al.* 2019).

Meanwhile, a lower profile but similarly devastating crisis of decline has unfolded among the reptiles, driven by sometimes similar and sometimes different threatening processes. Threats from exploitation due to over-harvesting, habitat loss, invasive species and emerging disease have led to recent estimates of more than 20% of reptiles now threatened with extinction (Whitfield Gibbons *et al.* 2000; Böhm *et al.* 2013; Doody *et al.* 2017). These estimates jump to as high as 50% in some groups such as the chelonians (turtles and tortoises) and crocodilians (Grigg and Kirshner 2015; Rhodin *et al.* 2018). Like amphibians, recognition of threats to the reptiles has led to more recent, but equally persuasive, calls for the development of reptile ARTs (Clulow and Clulow 2016); nevertheless, the lower profile of reptile threats and fewer resources committed in reproductive and biobanking research compared to amphibians has inevitably resulted in this group being left behind.

Here, we briefly review and summarise the recent developments in the state of reproductive technologies for amphibians and reptiles with particular emphasis on the progress captured through this two-part special issue of *Reproduction, Fertility and Development*. Comparisons to a comprehensive review on the state of the ARTs for reptiles and amphibians in 2016 (Clulow and Clulow 2016) and more recent reviews on individual technology areas and taxa (Clulow *et al.* 2018*a*, 2019; Browne *et al.* 2019; Silla and Byrne 2019; Della Togna *et al.* 2020) are illuminating. It is clear that ARTs for amphibians have come a long way over the past two decades, particularly in technology and protocol development for the storage of sperm cells and the recovery of male germlines from biobanked material. Further breakthroughs may be on the horizon for storing and retrieving maternal lines and embryos (Clulow *et al.* 2019, 2022; Holt and Comizzoli 2021). Research and application to on-ground programs translating technology to significant, applied conservation outcomes is the next stage required for amphibians (Clulow *et al.* 2019; Burger *et al.* 2021; Johnson and Mendelson 2022; Kouba 2022; Silla and Kouba 2022).

Reptiles, on the other hand, are missing many of the advances and milestones at the most basic levels of technology development (e.g. production of live young from artificial insemination in many groups, or from cryopreserved sperm in any group). Arguably, the field for reptiles is at a stage similar to the development of ARTs in amphibians in the decades before the turn of the century. The most pressing need for reptiles in the immediate future is technology development supported by studies of basic reproductive physiology and endocrinology to fill critical knowledge gaps that take into account the extreme variation in reproductive anatomy and physiology of the reptilian orders. The most appropriate targets, with the highest probability for success in the short-term, lies in the storage and retrieval of the male germline through sperm cryopreservation and artificial insemination.

Reptiles

Reptiles have been neglected for a long time compared to other vertebrates when it comes to the development of ARTs (Clulow and Clulow 2016). This may be due to indifference or negative public perceptions towards reptiles compared to other vertebrates, and to the widespread lack of awareness of the conservation issues and level of threat to reptiles. This is unfortunate, as reptiles are not only complex and social animals (Doody *et al.* 2013, 2021*a*; Gardner *et al.* 2016), a role previously considered relatively exclusive to mammals and birds, but also play critical ecosystem roles ranging from apex-predators to ecosystem engineers (Doody *et al.* 2015, 2021*b*).

The development of ARTs in reptiles is further hampered by their diverse and complex reproductive systems. These differences are a product of ancient evolutionary divergence within the Reptilia. Extant orders include Testudines (turtles and tortoises), Rhynchocephalia (tuatara), Squamata (snakes, lizards and amphisbaenians) and Crocodylia (crocodiles, alligators, caimans, gharials), which separated from one another between 150 and 250 Mya (Chiari *et al.* 2012). Highlighting just how divergent these groups are, modern crocodiles are more closely related to birds than to the other orders of Reptilia (Walker 1972; Whetstone and Martin 1979; Chiari *et al.* 2012). Thus, non-avian reptiles have evolved perhaps the most challenging variety of reproductive systems and functions of all vertebrates when it comes to the development of ARTs. These include: multiple paternity, sperm competition and post-copulatory sexual selection; significant genital variation; large, yolky amniotic eggs; development ranging from oviparity to viviparity; complex sex-determination systems including genetic and temperature-dependent sex determination; sex reversal; long-term sperm storage in the female tract; environmentally determined control over timing of birth; and parthenogenesis (reviewed in detail in Van Dyke *et al.* 2021). Despite the challenges of working with such complexity and variability, and historical neglect, the number of articles submitted on reptiles for the special issue of RFD marginally surpassed those submitted on amphibians. This is promising for the field, and reflects a recognition by reproductive scientists, especially those involved in conservation, of the urgent and growing need for the development of ARTs for this taxon.

Nevertheless, the number of published studies in the field remains low despite the valuable contributions from the impressive number of studies contributed to this two-part special issue, which represents a substantial increase in the published pool of research to date. Cryopreservation studies of reproductive cells and tissues in the reptile literature exclusively deal with the cryopreservation of sperm (Clulow and Clulow 2016), but the total number of cryopreservation studies remains low. For example, two new protocols for sperm cryopreservation in lizards (Campbell *et al.* 2021*b*; Hobbs *et al.* 2022) almost doubles the number of studies of sperm cryopreservation published for lizards worldwide, but only raises the number from three to five (Young *et al.* 2017; Campbell *et al.* 2020, 2021*a*). This compares to at least 26 reported studies on sperm cryopreservation for more than 35 species of amphibians prior to the RFD special issue (Clulow and Clulow 2016; Browne *et al.* 2019; Clulow *et al.* 2019). The small number of published lizard protocols stands in stark contrast to the more than 6000 species of lizard worldwide that constitute half of all extant reptile species (Böhm *et al.* 2013). The study by Hobbs *et al.* (2022) extends the range of cryoprotectant and extender formulations available for lizards while recognising interactions between cooling rates and cryoprotectant type. The study by Campbell *et al.* (2021*b*) demonstrates it is possible to cryopreserve sperm of the yellow-spotted monitor lizard *Varanus panoptes* after 4 days of cold-storage and still recover acceptable levels of motility (~30%) and plasma membrane integrity (~50%). This represents the first report of short-term cold-storage of lizard sperm prior to cryopreservation and retrieval and is another small step forward in reptile ARTs since the handful of reports of successful cold storage in reptile sperm without cryopreservation reviewed in Clulow and Clulow (2016).

The other two new reports of sperm cryopreservation in reptiles in this issue of RFD advanced our understanding of snake sperm cryopreservation (Young *et al.* 2021, 2022), adding to one prior published study reviewed in Mengden *et al.* (1980) and another also recently published outside of this special issue (Sandfoss *et al.* 2021). Taken together, this literature, with all the other reptile cryopreservation literature, confirms the focus of cryopreservation technology development in reptiles has been restricted to sperm, fulfilling the prediction by Clulow and Clulow (2016) that 'sperm cryopreservation will be the ART that yields the most progress in the near future' for reptiles. However, the prediction that this would be soon followed by the production of live young from cryopreserved sperm when combined with improvement and optimisation of AI protocols (Clulow and Clulow 2016) has still yet to come to fruition. The production of live young from cryopreserved sperm remains an urgent area of research and the next logical step in the continued development of reptile ARTs.

It is also worth noting that all the new cryopreservation studies in the RFD special issue, along with others recently published elsewhere (Campbell *et al.* 2021*a*; Sandfoss *et al.* 2021) were in squamates. This might not be surprising considering that squamates make up >95% of reptile species globally. However, considering that there is a large overrepresentation of testudines (turtles and tortoises) and crocodilians among threatened reptiles (Böhm *et al.* 2013; Rhodin *et al.* 2018), these other taxa should not be neglected. To date, the only published attempts for sperm cryopreservation in non-squamate reptiles include a few testudines (Platz *et al.* 1980; Perry and Mitchell 2022) and two crocodilians (Larsen *et al.* 1984; Johnston *et al.* 2014; Johnston *et al.* 2017).

Interestingly, the reptile species most represented in the special issue of RFD was the saltwater crocodile (*Crocodylus porosus*), one of the few non-squamate reptiles in which sperm cryopreservation has been previously attempted (Johnston *et al.* 2014, 2017). Four papers were submitted on the saltwater crocodile, making it the most studied reptile for ARTs in the world in recent times, and cementing it as a model species. A comprehensive review of the progress of ARTs in saltwater crocodiles reported that non-lethal semen collection through manual stimulation has been highly successful, although sperm cryopreservation attempts to date have resulted in low rates of recovery (~10–15% motility at best) (Johnston *et al.* 2021). The authors conclude that future studies are needed on fundamental anatomy, physiology and behaviour of females in particular, a point applicable to all the reptile groups. Nixon *et al.* (2021*a*, 2021*b*) and Miller *et al.* (2021) contributed three studies on fundamental reproductive physiology for the saltwater crocodile, ranging from post-testicular sperm maturation and gross and micro-anatomy of the male reproductive duct (Nixon *et al.* 2021*a*, 2021*b*) to characterising the chemical lipid composition of the sperm plasma and acrosomal membranes (Miller *et al.* 2021). Sandmaier *et al.* (2022) also contributed a study characterising sperm production in a male Philippine crocodile, further filling knowledge gaps in crocodilians.

It is worth noting that a total of six studies contributed to the special issue of RFD characterised basic reproductive physiology in reptiles, a critical step to filling the substantial knowledge gaps on fundamental reproductive physiology to enable development of ARTs in this group. These included one on the role of extracellular vesicles in the male reproductive tract of the Chinese softshell turtle *Pelodiscus sinensis* (Chen and Holt 2021) and another on predicting optimal sperm collection times in iguanas (Perry *et al.* 2022), in addition to the four on crocodiles.

Despite the significant proportional increase in studies on reptile ARTs in the RFD special issue, reptile ARTs are still in their infancy and behind our understanding and technology development for other vertebrate classes. As predicted more than 5 years ago in an earlier review (Clulow and Clulow 2016) most of the development of ARTs in reptiles has focussed on the male germ line through collection, storage and retrieval of sperm. There have been few other reports (e.g. Oliveri *et al.* 2018) of AI in reptiles since the 2016 review and successful AI with cryopreserved sperm remains elusive.

Encouragingly, while the majority of ARTs studies to date have been focussed on squamates and the saltwater crocodile, a recent study reported the first collection, characterisation, and storage of tuatara (*Sphenodon punctatus*) sperm (Lamar *et al.* 2021). Although preliminary, the authors reported low levels of live sperm recovery post-thaw using the commercial cryoprotectant Synth-a-Freeze™ (containing 10% DMSO buffered with HEPES), making it the first ART study for the ancient reptile order Rhynchocephalia (Lamar *et al.* 2021). That means there has now been at least one published study on ARTs in all four of the extant reptile orders, a small but important step forward in reptile conservation. Despite this milestone, there have still been no reports of ARTs leading to successful storage or retrieval of maternal germ lines or embryos. This is perhaps not surprising considering the substantial challenges preventing storage and retrieval of eggs or embryos in reptiles and highlights the huge task still confronting ARTs development in this historically neglected taxon.

Looking to the future, we suggest three key areas of research moving forward in the short to medium terms for reptile ARTs. These include: (1) continued development and refinement of sperm cryopreservation protocols, testing their ubiquity across a broader phylogenetic range of species; (2) development of non-lethal methods of sperm collection that lead to good quality and quantities of sperm for successful cryopreservation; and (3) development of AI with cryopreserved sperm. We note many sperm cryopreservation protocols developed to date have used sperm collected post-mortem, due to the large quantities and quality of sperm obtained and the ease of protocol development this confers (Young *et al.* 2017, 2021, 2022; Campbell *et al.* 2021*a*, 2021*b*; Sandfoss *et al.* 2021). However, encouragingly, two recent studies have looked at sperm collected non-lethally, including the first study on tuatara (Lamar *et al.* 2021; Hobbs *et al.* 2022). We also believe that further studies on fundamental reptile reproductive ecology and physiology, along with testing fundamental hypotheses around cryopreserving reptile reproductive cells and tissues

(Campbell *et al.* 2021*a*) will aid with these conservation and research goals.

Amphibians

ARTs for amphibians are much closer to a suite of utilisable technologies for conservation programmes than are reptiles, a conclusion supported by a comparison of the amphibian and reptile papers in this special issue. For example, in amphibians there are now many examples of stored sperm (cryopreserved, but also sperm held for periods without cryopreservation) resulting in the production of live offspring. In this special issue there are five papers reporting the production of live progeny in anurans (Arregui *et al.* 2022*a*; Kaurova *et al.* 2021; Silla and Byrne 2021; Upton *et al.* 2021) and caudates (McGinnity *et al.* 2022). The advent of an increasing number of caudate species from which progeny have been produced with ARTS (see also Kouba 2022) is of particular note, including with frozen sperm as reported by McGinnity *et al.* (2022) in this issue. There are also another four research papers that refine our understanding of the underpinning reproductive processes supporting these reproductive technologies including endocrinology and ovarian senescence (Jacobs *et al.* 2021), DNA integrity of induced urinic sperm (Arregui *et al.* 2022*b*), effect of over-wintering history on spermiation (Kouba *et al.* 2022), the challenges of inducing sperm release in the unique leiopelmatids of New Zealand (Germano *et al.* 2022) and finally one that modelled the econonomic and genetic benefits of utilising biobanking in amphibian captive breeding and conservation programmes (Howell *et al.* 2021*b*).

This two-part special issue of RFD highlights the ongoing refinements and extension of protocols for amphibian biobanking, gamete induction, and artificial fertilisation, whilst recognising some applications remain challenging and indicate the need to persist with research. The generation of live progeny for externally fertilising caudates using cryopreserved sperm (McGinnity *et al.* 2022) is one of those important achievements, an important step forward since the Clulow and Clulow (2016) review. Improving the knowledge of the reproductive biology of target species (such as the mountain yellow-legged frog, (Jacobs *et al.* 2021) with expanded use of technologies, including ultrasound for ovarian assessments and facilitated application of analysis of steroids for rapid assessment of reproductive condition, will facilitate improved protocols. As well, the use of tools to assess DNA integrity of sperm collected non-invasively (Arregui *et al.* 2022*a*) will further inform protocol development involvement utilising spermiation.

Nevertheless, despite the advances, there is still much left to do for the development of ARTs, even for the amphibians. Not all studies result in outcomes that are hoped for. Such is the case with the archaic New Zealand leiopelmatid lineage, Hamilton's frog (*Leiopelma hamiltoni*), a threatened and unique EDGE (evolutionarily distinct, globally endangered) species. Attempts to hormonally induce mating and the release of sperm met with limited success, and no matings were achieved with hormonal induction in this species (Germano *et al.* 2022). Further, despite recent successes in ARTs development in caudates, as in our 2016 review (Clulow and Clulow 2016), the third order of amphibian – the caecilians (order: Gymnophiona) – are still completely absent. This mirrors information on their ecology and status which remains elusive for the group due to their cryptic nature (Clulow and Clulow 2016).

The achievements in collection and cryopreservation of sperm, and their use in artificial fertilisation are the outstanding milestones in ARTs for amphibian species, with obvious implications for applications in conservation. In some ways, the development of amphibian ARTs for conservation based on sperm technology and artificial fertilisation parallel the development of sperm cryopreservation and AI technologies for conservation in mammal and bird species (Rodger and Clulow 2021). Nevertheless, as with mammals and birds, there are gaps in the amphibian technologies that are highlighted by the topics and papers that are not in the current issue. In particular, there are no reports of progress towards the storage and recovery of either the maternal haploid genome or the somatic diploid embryonic genome. These objectives really need to be the target of concerted research efforts by the amphibian ARTs community, but there is cause for optimism for advances in this area if the resources are available to pursue these goals (Lawson *et al.* 2013; Clulow *et al.* 2019; Clulow *et al.* 2022).

Such optimism comes from recent advances in the field of fish and aquaculture ARTs (Clulow *et al.* 2019; Holt and Comizzoli 2021; Clulow *et al.* 2022). Fish are challenging to cryopreserve because of the size and yolk content of the oocytes and embryos, and permeability barriers to cryoprotectants which together result in lethal intracellular ice formation events during thawing (Hagedorn and Kleinhans 2000; Hagedorn *et al.* 2004). For fish, there are now ways around these blocks to cryopreservation that have recently emerged such as laser warming of vitrified embyros (Khosla *et al.* 2017; Khosla *et al.* 2019; Khosla *et al.* 2020); the generation of viable gametes generated from cryopreserved primordial germ cells (Lin *et al.* 1992; Higaki *et al.* 2010; Higaki *et al.* 2013); or spermatogonial stem cell transfer into host larvae from the same or different species (Marinović *et al.* 2018; Marinović *et al.* 2019). These approaches were recently reviewed outside this special issue by Clulow *et al.* (2022). Other technologies, known to be effective in amphibians and/or fish, such as ICSI, nuclear transfer, the generation of chimeras and the xenotransplantation of ovarian tissues offer many possibilities for the future expansion of amphibian ARTs (Clulow *et al.* 2014; Clulow *et al.* 2019). Overall, there should be great optimism for technology transfer between these vertebrate classes with aquatic life history phases.

While the status quo for reptile ARTs outlined in our review half a decade ago (Clulow and Clulow 2016) has been more or less maintained, amphibian ARTs have undergone considerable refinement and generated further proofs of concept for their effectiveness in the intervening period. This can be seen both across this special issue and more broadly in the literature (Upton *et al.* 2018; Browne *et al.* 2019; Clulow *et al.* 2019; Della Togna *et al.* 2020; Upton *et al.* 2021; Silla *et al.* 2022). For amphibians, ARTs are now available and are in fact being utilised in conservation programs (for a global perspective see chapters in (Silla *et al.* 2022). In Australia, sperm of threatened amphibians are being collected from wildfire impacted ecosystems and biobanked by University of Newcastle and Taronga Conservation Society with Australian Federal Government support (Clulow *et al.* unpubl. data). Encouragingly, the broad availability of amphibian ARTs targeting storage and retrieval of sperm has resulted in a nascent focus on demonstrating not only the genetic, but also the economic value of adopting these tools and approaches in amphibian conservation programs (Howell *et al.* 2021*a*, 2021*b*). This is sure to help to increase uptake. Looking to the future, we suggest two main goals for amphibian ARTs in the short to medium terms: (1) translating the successful technology development for storing and retrieving male germ lines into tangible conservation outcomes. 'End product' proof of concept is needed to gain social licence for wildlife ARTs and encourage further funding. (2) Focus technology development on the emerging possibilities for storing and retrieving maternal haploid and embryonic diploid cell lines.

Conclusion and future directions

While the goals of developing ARTs for amphibian and reptile conservation are similar, the progress for each taxon are at different stages. There has been strong progress in understanding and developing reproductive technologies for amphibians with the goal of utilising these as conservation tools. In the case of amphibians, ARTs have developed to the point where they can be utilised now. The availability of protocols that allow the generation of live progeny from cryopreserved and biobanked sperm mean that ARTs can be used extensively in amphibian conservation programs, captive breeding and for extinction insurance, despite the lack of maternal line/embryo banking technologies. Technologies to break this block in amphibian ARTs will likely come soon for at least some species. Meanwhile, the field should be, and is in some cases, focussed on shifting technology development to application in on-ground conservation management programs for ultimate end product proof of concept and tangible conservation outcomes.

For reptiles, progress in the development of ARTs has perhaps two decades of catch up to match the range of technological applications available for amphibians, but there is the high probability that advances for reptiles will accelerate greatly if more resources are invested into the field. Technologies for sperm storage for at least some reptile species now exist and should continue to be optimised and ready for use in the near future if research resources are available. There should be no fundamental block to the application of artificial insemination with cryopreserved sperm, or even the application of advanced stem cell technologies to reptiles eventually. The storage of maternal and embryonic lines are likely still some way off, but may be accelerated by the development of these technologies in amphibians. The papers published in this two-part special issue of RFD on ARTs for reptiles and amphibians highlight the progress for both threatened taxa.

References

Arregui L, Kouba AJ, Germano JM, Barrios L, Moore M, Kouba CK, Clulow J (2022*a*) Fertilization potential of cold-stored Fowler's toad (*Anaxyrus fowleri*) spermatozoa: temporal changes in sperm motility based on temperature and osmolality. *Reproduction, Fertility and Development* **34**(5), 461–469. doi:10.1071/RD21037

Arregui L, Martinez-Pastor F, Arroyo F, Gosálvez J (2022*b*) Determining the effects of sperm activation in anuran cloaca on motility and DNA integrity in *Epidalea calamita* (Bufonidae). *Reproduction, Fertility and Development* **34**(5), 438–446. doi:10.1071/RD21063

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* **95**(15), 9031–9036. doi:10.1073/pnas.95.15.9031

Böhm M, Collen B, Baillie JEM, *et al.* (2013) The conservation status of the world's reptiles. *Biological Conservation* **157**, 372–385. doi:10.1016/j.biocon.2012.07.015

Bower DS, Lips KR, Schwarzkopf L, Georges A, Clulow S (2017) Amphibians on the brink. *Science* **357**(6350), 454–455. doi:10.1126/science.aao0500

Browne RK, Clulow J, Mahony M, Clark A (1998) Successful recovery of motility and fertility of cryopreserved cane toad (Bufo marinus) sperm. *Cryobiology* **37**(4), 339–345. doi:10.1006/cryo.1998.2129

Browne R, Li H, Robertson H, Uteshev V, Shishova N, McGinnity D, Nofs S, Figiel C, Mansour N, Lloyd R, Agnew D, Carleton C, Wu M, Gakhova E (2011) Reptile and amphibian conservation through gene banking and other reproduction technologies. *Russian Journal of Herpetology* **18**(3), 165–174.

Browne RK, Silla AJ, Upton R, Della-Togna G, Marcec-Greaves R, Shishova NV, Uteshev VK, Proaño B, Pérez OD, Mansour N, Kaurova SA, Gakhova EN, Cosson J, Dyzuba B, Kramarova LI, McGinnity D, Gonzalez M, Clulow J, Clulow S (2019) Sperm collection and storage for the sustainable management of amphibian biodiversity. *Theriogenology* **133**, 187–200. doi:10.1016/j.theriogenology.2019.03.035

Burger I, Julien AR, Kouba AJ, Barber D, Counsell KR, Pacheco C, Krebs J, Kouba CK (2021) Linking in-situ and ex-situ populations of threatened amphibians through genome banking. *Conservation Science and Practice* **3**(11), e525. doi:10.1111/csp2.525

Campbell L, Cafe SL, Upton R, Doody JS, Nixon B, Clulow J, Clulow S (2020) A model protocol for the cryopreservation and recovery of motile lizard sperm using the phosphodiesterase inhibitor caffeine. *Conservation Physiology* **8**(1), coaa044. doi:10.1093/conphys/coaa044

Campbell L, Clulow J, Doody JS, Clulow S (2021*a*) Optimal cooling rates for sperm cryopreservation in a threatened lizard conform to two-factor hypothesis of cryo-injury. *Cryobiology* **103**, 101–106. doi:10.1016/j.cryobiol.2021.09.001

Campbell L, Clulow J, Howe B, Upton R, Doody S, Clulow S (2021*b*) Efficacy of short-term cold storage prior to cryopreservation of spermatozoa in a threatened lizard. *Reproduction, Fertility and Development* **33**, 555–561. doi:10.1071/RD20231

Chen Q, Holt WV (2021) Extracellular vesicles in the male reproductive tract of the softshell turtle. *Reproduction, Fertility and Development* **33**, 519–529. doi:10.1071/RD20214

Chiari Y, Cahais V, Galtier N, Delsuc F (2012) Phylogenomic analyses support the position of turtles as the sister group of birds and crocodiles (Archosauria). *BMC Biology* **10**(1), 65. doi:10.1186/1741-7007-10-65

Clulow J, Clulow S (2016) Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: bringing the ARTs up to speed. *Reproduction, Fertility and Development* **28**(8), 1116–1132. doi:10.1071/RD15466

Clulow J, Mahony M, Browne R, Pomering M, Clark A (1999) Applications of assisted reproductive technologies (ART) to endangered anuran amphibians. In 'Declines and disappearances of Australian frogs'. (Ed. A Campbell) pp. 219–225. (Environment Australia: Canberra)

Clulow J, Clulow S, Guo J, French AJ, Mahony MJ, Archer M (2012) Optimisation of an oviposition protocol employing human chorionic and pregnant mare serum gonadotropins in the Barred Frog Mixophyes fasciolatus (Myobatrachidae). *Reproductive Biology and Endocrinology* **10**(1), 60. doi:10.1186/1477-7827-10-60

Clulow J, Trudeau VL, Kouba AJ (2014) Amphibian declines in the twenty-first century: why we need assisted reproductive technologies. In 'Reproductive sciences in animal conservation.' (Eds WV Holt, JL Brown, P Comizzoli) pp. 275–316. (Springer: New York)

Clulow J, Pomering M, Herbert D, Upton R, Calatayud N, Clulow S, Mahony MJ, Trudeau VL (2018*a*) Differential success in obtaining gametes between male and female Australian temperate frogs by hormonal induction: a review. *General and Comparative Endocrinology* **265**, 141–148. doi:10.1016/j.ygcen.2018.05.032

Clulow S, Gould J, James H, Stockwell M, Clulow J, Mahony M (2018*b*) Elevated salinity blocks pathogen transmission and improves host survival from the global amphibian chytrid pandemic: implications for translocations. *Journal of Applied Ecology* **55**(2), 830–840. doi:10.1111/1365-2664.13030

Clulow J, Upton R, Trudeau VL, Clulow S (2019) Amphibian assisted reproductive technologies: moving from technology to application. In 'Reproductive sciences in animal conservation'. 2nd edn. (Eds P Comizzoli, JL Brown, WV Holt) pp. 413–463. (Springer: Cham)

Clulow J, Upton R, Clulow S (2022) Cryopreservation of amphibian genomes: targeting the Holy Grail, cryopreservation of maternal-haploid and embryonic-diploid genomes. In 'Reproductive technologies and biobanking as tools for the conservation of amphibians'. (Eds AJ Silla, AJ Kouba, H Heatwole) (CSIRO Publishing: Melbourne)

Della Togna G, Howell LG, Clulow J, Langhorne CJ, Marcec-Greaves R, Calatayud NE (2020) Evaluating amphibian biobanking and reproduction for captive breeding programs according to the amphibian conservation action plan objectives. *Theriogenology* **150**, 412–431. doi:10.1016/j.theriogenology.2020.02.024

Doody JS, Burghardt GM, Dinets V (2013) Breaking the social–non-social dichotomy: a role for reptiles in vertebrate social behavior research? *Ethology* **119**(2), 95–103. doi:10.1111/eth.12047

Doody JS, Soanes R, Castellano CM, Rhind D, Green B, McHenry C, Clulow S (2015) Invasive toads shift predator–prey densities in animal communities by removing top predators. *Ecology* **96**, 2544–2554. doi:10.1890/14-1332.1

Doody JS, Rhind D, Green B, Castellano C, McHenry C, Clulow S (2017) Chronic effects of an invasive species on an animal community. *Ecology* **98**(8), 2093–2101. doi:10.1002/ecy.1889

Doody JS, Dinets V, Burghardt GM (2021*a*) 'The secret social lives of reptiles.' (JHU Press: Baltimore)

Doody JS, Soennichsen KF, James H, McHenry C, Clulow S (2021*b*) Ecosystem engineering by deep-nesting monitor lizards. *Ecology* **102**(4), e03271. doi:10.1002/ecy.3271

Folch J, Cocero MJ, Chesné P, Alabart JL, Domínguez V, Cognié Y, Roche A, Fernández-Arias A, Martí JI, Sánchez P, Echegoyen E, Beckers JF, Sánchez Bonastre A, Vignon X (2009) First birth of an animal from an extinct subspecies (Capra pyrenaica pyrenaica) by cloning. *Theriogenology* **71**(6), 1026–1034. doi:10.1016/j.theriogenology.2008.11.005

Gardner MG, Pearson SK, Johnston GR, Schwarz MP (2016) Group living in squamate reptiles: a review of evidence for stable aggregations. *Biological Reviews* **91**(4), 925–936. doi:10.1111/brv.12201

Germano JM, Cree A, Molinia F, Arregui L, Bishop PJ (2022) Hormone treatment does not reliably induce spermiation or mating in Hamilton's frog from the archaic leiopelmatid lineage. *Reproduction, Fertility and Development* **34**(5), 447–452. doi:10.1071/RD21061

González-del-Pliego P, Freckleton RP, Edwards DP, Koo MS, Scheffers BR, Pyron RA, Jetz W (2019) Phylogenetic and trait-based prediction of extinction risk for data-deficient amphibians. *Current Biology* **29**(9), 1557–1563.e3. doi:10.1016/j.cub.2019.04.005

Grigg G, Kirshner D (2015) 'Biology and evolution of crocodylians'. (CSIRO Publishing: Clayton)

Hagedorn M, Kleinhans F (2000) Problems and prospects in cryopreservation of fish embryos. In 'Cryopreservation in aquatic species'. (Eds T Tiersch, C Green) pp. 161–178. (The World Aquaculture Society: Baton Rouge)

Hagedorn M, Peterson A, Mazur P, Kleinhans F (2004) High ice nucleation temperature of zebrafish embryos: slow-freezing is not an option. *Cryobiology* **49**(2), 181–189. doi:10.1016/j.cryobiol.2004.07.001

Higaki S, Eto Y, Kawakami Y, Yamaha E, Kagawa N, Kuwayama M, Nagano M, Katagiri S, Takahashi Y (2010) Production of fertile zebrafish (Danio rerio) possessing germ cells (gametes) originated from primordial germ cells recovered from vitrified embryos. *Reproduction* **139**(4), 733–740. doi:10.1530/REP-09-0549

Higaki S, Kawakami Y, Eto Y, Yamaha E, Nagano M, Katagiri S, Takada T, Takahashi Y (2013) Cryopreservation of zebrafish (Danio rerio) primordial germ cells by vitrification of yolk-intact and yolk-depleted embryos using various cryoprotectant solutions. *Cryobiology* **67**(3), 374–382. doi:10.1016/j.cryobiol.2013.10.006

Hildebrandt TB, Hermes R, Goeritz F, Appeltant R, Colleoni S, de Mori B, Diecke S, Drukker M, Galli C, Hayashi K, Lazzari G, Loi P, Payne J, Renfree M, Seet S, Stejskal J, Swegen A, Williams SA, Zainuddin ZZ, Holtze S (2021) The ART of bringing extinction to a freeze – History and future of species conservation, exemplified by rhinos. *Theriogenology* **169**, 76–88. doi:10.1016/j.theriogenology.2021.04.006

Hobbs RJ, Upton R, Keogh L, James K, Baxter-Gilbert J, Whiting MJ (2022) Sperm cryopreservation in an Australian skink (*Eulamprus quoyii*). *Reproduction, Fertility and Development* **34**(5), 428–437. doi:10.1071/RD21031

Holt WV, Comizzoli P (2021) Opportunities and limitations for reproductive science in species conservation. *Annual Review of Animal Biosciences* **10**, 5.1–5.21. doi:10.1146/annurev-animal-013120-030858

Holt W, Abaigar T, Watson P, Wildt D (2003) Genetic resource banks for species conservation. In 'Reproductive science and integrated conservation'. Vol. 8. (Eds W Holt, A Pickard, J Rodger, D Wildt) pp. 267–280. (Cambridge University Press: Cambridge)

Howard JG, Lynch C, Santymire RM, Marinari PE, Wildt DE (2016) Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret. *Animal Conservation* **19**(2), 102–111. doi:10.1111/acv.12229

Howell LG, Frankham R, Rodger JC, Witt RR, Clulow S, Upton RMO, Clulow J (2021*a*) Integrating biobanking minimises inbreeding and produces significant cost benefits for a threatened frog captive breeding programme. *Conservation Letters* **14**, e12776. doi:10.1111/conl.12776

Howell LG, Mawson PR, Frankham R, Rodger JC, Upton RMO, Witt RR, Calatayud NE, Clulow S, Clulow J (2021*b*) Integrating biobanking could produce significant cost benefits and minimise inbreeding for Australian amphibian captive breeding programs. *Reproduction, Fertility and Development* **33**, 573–587. doi:10.1071/RD21058

Jacobs LE, Hammond TT, Gaffney PM, Curtis MJ, Shier DM, Durrant BS, Righton A, Williams CL, Calatayud NE (2021) Using reproductive technologies to assess the development of secondary sexual characteristics, ovarian senescence and hermaphroditism in the endangered mountain yellow-legged frog Rana muscosa. *Reproduction, Fertility and Development* **33**(9), 610–614. doi:10.1071/RD21029

Johnson K, Mendelson JR (2022) Status of global amphibian declines and the prioritisation of species for captive breeding. In 'Reproductive technologies and biobanking as tools for the conservation of amphibians'. (Eds AJ Silla, AJ Kouba, H Heatwole). (CSIRO Publishing: Melbourne)

Johnston SD, Lever J, McLeod R, Qualischefski E, Brabazon S, Walton S, Collins SN (2014) Extension, osmotic tolerance and cryopreservation of saltwater crocodile (*Crocodylus porosus*) spermatozoa. *Aquaculture* **426–427**, 213–221. doi:10.1016/j.aquaculture.2014.02.014

Johnston SD, Qualischefski E, Cooper J, McLeod R, Lever J, Nixon B, Anderson AL, Hobbs R, Gosálvez J, López-Fernández C, Keeley T (2017) Cryopreservation of saltwater crocodile (*Crocodylus porosus*) spermatozoa. *Reproduction, Fertility and Development* **29**(11), 2235–2244. doi:10.1071/RD16511

Johnston SD, Lever J, McLeod R, Qualischefski E, Madrigal-Valverde M, Nixon B (2021) Assisted breeding technology in the saltwater crocodile Crocodylus porosus: a review and look to the future. *Reproduction, Fertility and Development* **33**, 503–518. doi:10.1071/RD20217

Jørgensen D (2013) Reintroduction and de-extinction. *BioScience* **63**(9), 719–720. doi:10.1525/bio.2013.63.9.6

Kaurova SA, Uteshev VK, Gapeyev AB, Shishova NV, Gakhova EN, Browne RK, Kramarova LI (2021) Cryopreservation of spermatozoa obtained postmortem from the European common frog Rana temporaria. *Reproduction, Fertility and Development* **33**(9), 588–595. doi:10.1071/RD20336

Khosla K, Wang Y, Hagedorn M, Qin Z, Bischof J (2017) Gold nanorod induced warming of embryos from the cryogenic state enhances viability. *ACS Nano* **11**(8), 7869–7878. doi:10.1021/acsnano.7b02216

Khosla K, Zhan L, Bhati A, Carley-Clopton A, Hagedorn M, Bischof J (2019) Characterization of laser gold nanowarming: a platform for millimeter-scale cryopreservation. *Langmuir* **35**(23), 7364–7375. doi:10.1021/acs.langmuir.8b03011

Khosla K, Kangas J, Liu Y, Zhan L, Daly J, Hagedorn M, Bischof J (2020) Cryopreservation and laser nanowarming of zebrafish embryos followed by hatching and spawning. *Advanced Biosystems* **4**(11), 2000138. doi:10.1002/adbi.202000138

Kouba AJ (2022) Genome Resource Banks as a tool for amphibian conservation. In 'Reproductive technologies and biobanking as tools for the conservation of amphibians'. (Eds AJ Silla, AJ Kouba, H Heatwole). (CSIRO Publishing: Melbourne)

Kouba AJ, Langhorne CJ, Willard ST, Smith T, Kouba CK (2022) Spermiation response to exogenous hormone therapy in hibernated and non-hibernated boreal toads (*Anaxyrus boreas boreas*). *Reproduction, Fertility and Development* **34**(5), 453–460. doi:10.1071/RD21033

Lamar SK, Nelson NJ, Moore JA, Taylor HR, Keall SN, Ormsby DK (2021) Initial collection, characterization, and storage of tuatara (*Sphenodon punctatus*) sperm offers insight into their unique reproductive system. *PLoS ONE* **16**(7), e0253628. doi:10.1371/journal.pone.0253628

Larsen RE, Cardeilhac PT, Lane T (1984) Semen extenders for artificial insemination in the American alligator. *Aquaculture* **42**(2), 141–149. doi:10.1016/0044-8486(84)90361-2

Lawson B, Clulow S, Mahony MJ, Clulow J (2013) Towards gene banking amphibian maternal germ lines: short-term incubation, cryoprotectant tolerance and cryopreservation of embryonic cells of the frog, *Limnodynastes peronii*. *PLoS ONE* **8**(4), e60760. doi:10.1371/journal.pone.0060760

Lin S, Long W, Chen J, Hopkins N (1992) Production of germ-line chimeras in zebrafish by cell transplants from genetically pigmented to albino embryos. *Proceedings of the National Academy of Sciences of the United States of America* **89**(10), 4519–4523. doi:10.1073/pnas.89.10.4519

Madsen T, Loman J, Anderberg L, Anderberg H, Georges A, Ujvari B (2020) Genetic rescue restores long-term viability of an isolated population of adders (*Vipera berus*). *Current Biology* **30**(21), R1297–R1299. doi:10.1016/j.cub.2020.08.059

Marinović Z, Li Q, Lujić J, Iwasaki Y, Csenki Z, Urbányi B, Horváth Á, Yoshizaki G (2018) Testis cryopreservation and spermatogonia transplantation as a tool for zebrafish line reconstitution. *Cryobiology* **85**, 145–146. doi:10.1016/j.cryobiol.2018.10.104

Marinović Z, Li Q, Lujić J, Iwasaki Y, Csenki Z, Urbányi B, Yoshizaki G, Horváth Á (2019) Preservation of zebrafish genetic resources through testis cryopreservation and spermatogonia transplantation. *Scientific Reports* **9**(1), 13861. doi:10.1038/s41598-019-50169-1

McGinnity D, Reinsch SD, Schwartz H, Trudeau V, Browne RK (2022) Semen and oocyte collection, sperm cryopreservation and IVF with the threatened North American giant salamander *Cryptobranchus alleganiensis*. *Reproduction, Fertility and Development* **34**(5), 470–477. doi:10.1071/RD21035

Mengden G, Platz C, Hubbard R, Quinn H (1980) Semen collection freezing and artificial insemination in snake. In 'Contributions to herpetology. No. 1. Reproductive biology and diseases of captive reptiles'. (Eds JB Murphy, JT Collins) pp. 71–78. (Society for the Study of Reptiles: St Louis)

Miller Jr RR, Beranek F, Anderson AL, Johnston SD, Nixon B (2021) Plasma and acrosomal membrane lipid content of saltwater crocodile spermatozoa. *Reproduction, Fertility and Development* **33**(9), 596–604. doi:10.1071/RD21007

Nixon B, Anderson AL, Bromfield EG, Martin JH, Cafe SL, Skerrett-Byrne DA, Dun MD, Eamens AL, De Iuliis GN, Johnston SD (2021*a*) Post-testicular sperm maturation in the saltwater crocodile Crocodylus porosus: assessing the temporal acquisition of sperm motility. *Reproduction, Fertility and Development* **33**(9), 530–539. doi:10.1071/RD20204

Nixon B, Anderson AL, Bromfield EG, Martin JH, Lord T, Cafe SL, Roman SD, Skerrett-Byrne DA, Eamens AL, De Iuliis GN, Johnston SD (2021*b*) Gross and microanatomy of the male reproductive duct system of the saltwater crocodile *Crocodylus porosus*. *Reproduction, Fertility and Development* **33**(9), 540–554. doi:10.1071/RD20303

Oliveri M, Bartoskova A, Spadola F, Morici M, di Giuseppe M, Knotek Z (2018) Method of semen collection and artificial insemination in snakes. *Journal of Exotic Pet Medicine* **27**(2), 75–80. doi:10.1053/j.jepm.2018.02.034

Perry SM, Mitchell MA (2022) Reptile assisted reproductive technologies: can ART help conserve 300 million years of evolution by preserving extant reptile biodiversity? *Reproduction, Fertility and Development* **34**(5), 385–400. doi:10.1071/RD21034

Perry SM, Park T, Mitchell MA (2022) Sex, drugs and rock iguanas: testicular dynamics and plasma testosterone concentrations could predict optimal semen collection times in *Cyclura*. *Reproduction, Fertility and Development* **34**(5), 417–427. doi:10.1071/RD21032

Platz C, Mengden G, Quinn H, Wood F, Wood J (1980) Semen collection, evaluation and freezing in the green sea turtle, Galapagos tortoise, and red-eared pond turtle. In 'Proceedings of the American Association of Zoo Veterinarians, Arlington'. pp. 47–48. (American Association of Zoo Veterinarians)

Pukazhenthi BS, Wildt DE (2004) Which reproductive technologies are most relevant to studying, managing and conserving wildlife? *Reproduction, Fertility and Development* **16**(2), 33–46. doi:10.1071/RD03076.

Pukazhenthi B, Comizzoli P, Travis AJ, Wildt DE (2006) Applications of emerging technologies to the study and conservation of threatened and endangered species. *Reproduction, Fertility and Development* **18**(2), 77–90. doi:10.1071/RD05117

Rhodin AGJ, Stanford CB, Van Dijk PP, *et al.* (2018) Global conservation status of turtles and tortoises (order Testudines). *Chelonian Conservation and Biology* **17**(2), 135–161. doi:10.2744/CCB-1348.1

Rodger JC, Clulow J (2021) Resetting the paradigm of reproductive science and conservation. *Animal Reproduction Science* doi:10.1016/j.anireprosci.2021.106911

Ryder OA, Onuma M (2018) Viable cell culture banking for biodiversity characterization and conservation. *Annual Review of Animal Biosciences* **6**, 83–98. doi:10.1146/annurev-animal-030117-014556

Sandfoss MR, Whittington OM, Reichling S, Roberts BM (2021) Toxicity of cryoprotective agents to semen from two closely related snake species: the endangered Louisiana pinesnake (*Pituophis ruthveni*) and bullsnake (*Pituophis cantenifer*). *Cryobiology* **101**, 20–27. doi:10.1016/j.cryobiol.2021.06.008

Sandler RL, Moses L, Wisely SM (2021) An ethical analysis of cloning for genetic rescue: case study of the black-footed ferret. *Biological Conservation* **257**, 109118. doi:10.1016/j.biocon.2021.109118

Sandmaier SES, Shepard T, Reeves A, Bohr K, Krebs J, Herrick JR (2022) Characterisation of sperm production and morphology in the male

Philippine crocodile *Crocodylus mindorensis* via voluntary behavioural training. *Reproduction, Fertility and Development* **34**(5), 410–416. doi:10.1071/RD21016
Scheele BC, Pasmans F, Skerratt LF, *et al.* (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* **363**(6434), 1459–1463. doi:10.1126/science.aav0379
Silla AJ, Byrne PG (2019) The role of reproductive technologies in amphibian conservation breeding programs. *Annual Review of Animal Biosciences* **7**, 499–519. doi:10.1146/annurev-animal-020518-115056
Silla AJ, Byrne PG (2021) Hormone-induced ovulation and artificial fertilisation in four terrestrial-breeding anurans. *Reproduction, Fertility and Development* **33**(9), 615–618. doi:10.1071/RD20243
Silla AJ, Kouba AJ (2022) Integrating reproductive technologies into the conservation toolbox for the recovery of amphibian species. In 'Reproductive technologies and biobanking as tools for the conservation of amphibians'. (Eds AJ Silla, AJ Kouba, H Heatwole). (CSIRO Publishing: Melbourne)
Silla AJ, Kouba AJ, Heatwole H (2022) 'Reproductive technologies and biobanking as tools for the conservation of amphibians'. (CSIRO Publishing: Melbourne)
Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* **306**(5702), 1783–1786. doi:10.1126/science.1103538
Upton R, Clulow S, Mahony MJ, Clulow J (2018) Generation of a sexually mature individual of the Eastern dwarf tree frog, *Litoria fallax*, from cryopreserved testicular macerates: proof of capacity of cryopreserved sperm derived offspring to complete development. *Conservation Physiology* **6**(1), coy043. doi:10.1093/conphys/coy043
Upton R, Clulow S, Calatayud NE, Colyvas K, Seeto RGY, Wong LAM, Mahony MJ, Clulow J (2021) Generation of reproductively mature offspring from the endangered green and golden bell frog Litoria aurea using cryopreserved spermatozoa. *Reproduction, Fertility and Development* **33**(9), 562–572. doi:10.1071/RD20296
Van Dyke JU, Thompson MB, Burridge CP, *et al.* (2021) Australian lizards are outstanding models for reproductive biology research. *Australian Journal of Zoology* **68**(4), 168–199. doi:10.1071/ZO21017
Walker AD (1972) New light on the origin of birds and crocodiles. *Nature* **237**(5353), 257–263. doi:10.1038/237257a0
Whetstone KN, Martin LD (1979) New look at the origin of birds and crocodiles. *Nature* **279**(5710), 234–236. doi:10.1038/279234a0
Whitfield Gibbons JW, Scott DE, Ryan TJ, Buhlmann TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT (2000) The global decline of reptiles, déjà vu amphibians. *BioScience* **50**, 653–666.
Wildt DE, Schiewe MC, Schmidt PM, Goodrowe KL, Howard JG, Phillips LG, O'brien SJ, Bush M (1986) Developing animal model systems for embryo technologies in rare and endangered wildlife. *Theriogenology* **25**(1), 33–51. doi:10.1016/0093-691X(86)90182-2
Young C, Ravida N, Curtis M, Mazzotti F, Durrant B (2017) Development of a sperm cryopreservation protocol for the Argentine black and white tegu (*Tupinambis merianae*). *Theriogenology* **87**, 55–63. doi:10.1016/j.theriogenology.2016.08.006
Young C, Ravida N, Durrant B (2021) Challenges in the development of sperm cryopreservation protocols for snakes. *Reproduction, Fertility and Development* **33**(9), 605–609. doi:10.1071/RD21038
Young C, Ravida N, Rochford M, Mazzotti F, Curtis M, Durrant B (2022) Sperm cryopreservation in the Burmese python *Python bivittatus* as a model for endangered snakes. *Reproduction, Fertility and Development* **34**(5), 401–409. doi:10.1071/RD21023

Conflicts of interest. The authors declare no conflict of interest.

Author affiliations
[A]Centre for Conservation Ecology & Genomics, Institute for Applied Ecology, University of Canberra, Bruce, ACT 2617, Australia.
[B]University of Newcastle, Conservation Biology Research Group, University Drive, Callaghan, NSW 2308, Australia.
[C]Honduras Amphibian Rescue and Conservation Center, Honduras.
[D]Universidad Interamericana de Panama, Direccion de Investigacion, Campus Central, Avenida Ricardo J. Alfaro, Panama City, Panama.
[E]Smithsonian Tropical Research Institute, Panama Amphibian Rescue and Conservation Project, Panama.
[F]San Diego Zoo Wildlife Alliance, Beckman Center for Conservation Research, 15600 San Pasqual valley Road, Escondido, CA 92025, USA.
[G]Conservation Science Network, 24 Thomas Street, Mayfield, NSW 2304, Australia.