Animal Conservation

Animal Conservation. Print ISSN 1367-9430

REVIEW

Why do eggs fail? Causes of hatching failure in threatened populations and consequences for conservation

K. Assersohn[1]*, A. F. Marshall[2,3]*, F. Morland[1,2]*, P. Brekke[2]† & N. Hemmings[1]†

1 Department of Animal & Plant Sciences, University of Sheffield, Sheffield, UK
2 Institute of Zoology, Zoological Society of London, London, UK
3 Department of Genetics, Evolution and Environment, University College London, London, UK

Keywords
Avian; bird breeding; captive-breeding; conservation management; development; embryo mortality; fertility; hatchability; reproductive success.

Correspondence
Dr Nicola Hemmings, Department of Animal & Plant Sciences, University of Sheffield, UK.
Email: n.hemmings@sheffield.ac.uk

Editor: Iain Gordon
Associate Editor: Jaime Ramos
*These authors contributed equally to this work.
†Joint senior authors

Received 31 July 2020; accepted 06 January 2021

doi:10.1111/acv.12674

Abstract

Reproductive failure is ubiquitous. However, research on the mechanisms underpinning reproductive failure is still lacking in most species. This gap in our understanding has particularly strong repercussions for threatened species and it hinders our ability to establish effective interventions to improve survival. In this review, we focus on why eggs fail to hatch – one of the most critical and understudied aspects of bird reproduction. We identify the main drivers of hatching failure in threatened populations of birds and the key mechanisms that cause failure at different stages of development inside the egg. We then discuss the importance of management interventions aimed at reducing hatching failure in species of conservation concern. Our review highlights the need for a better understanding of the mechanistic basis of hatching failure in non-model bird species and identifies the methodological tools necessary to achieve this.

Introduction

Around 40% of all bird species have declining populations and 13% are threatened with extinction (BirdLife International, 2020). One of the most common and important problems for threatened bird species is the failure of eggs to hatch. Many eggs are lost as a result of consumption, damage, or disturbance by humans and other animals, but even beyond these losses, some threatened bird populations experience up to 75% hatching failure as a result of indirect anthropogenic or other causes (Jamieson & Ryan, 2000; Ferreira *et al*., 2005). High rates of hatching failure not only influence individual reproductive success but can have strong repercussions for population growth and species recovery (e.g. Jamieson & Ryan, 2000; Ferreira *et al*., 2005; Brekke *et al*., 2010; White *et al*., 2015). However, the drivers of hatching failure are complex and poorly understood. In this review, we highlight the key factors associated with high levels of hatching failure beyond the impacts of predation, damage, desertion and exploitation. We then explore the underlying reproductive problems linked to hatching failure and how these are influenced by ecological and behavioural factors. We argue that a lack of understanding of the mechanistic basis of hatching failure can lead to flawed conclusions about how and why it occurs, with important implications for our understanding of avian ecology and conservation.

Major drivers of hatching failure in threatened birds

Inbreeding depression

Threatened bird populations are generally small and isolated, resulting in high levels of inbreeding and low genetic diversity (Keller & Waller, 2002). Threatened and invasive species that have undergone single or multiple bottleneck and founder events associated with low levels of genetic diversity and high inbreeding, have significantly higher levels of hatching failure (Briskie & Mackintosh, 2004; Heber &

Briskie, 2010). A large number of studies in laboratory systems and non-threatened species across multiple taxa also support that inbreeding depresses hatching success (e.g. Morrow, Arnqvist, & Pitcher, 2002; Spottiswoode & Møller, 2004; Slatyer *et al*., 2012), and a few suggest that genetic recovery (in populations of common species) can improve hatching rates (Ortego *et al*., 2010, Lindsay *et al*., 2020). Both parental and embryonic inbreeding depresses hatching success (Briskie & Mackintosh, 2004; Heber & Briskie, 2010) and egg viability (Brekke *et al*., 2010; Hemmings, Slate & Birkhead, 2012), but the effects of parental inbreeding on fertilization and embryo development are poorly understood. Although most studies show that inbreeding depresses hatching (e.g. White *et al*., 2015), some have found that parental inbreeding has no effect (e.g. Brekke *et al*., 2010) or, in a few exceptional circumstances, has a positive effect on hatching success (e.g. Weiser *et al*., 2016). Research on the effects of maternal inbreeding on fertility, egg traits and egg number in wild threatened populations is sorely lacking, despite considerable evidence of these effects in non-threatened species (e.g. Keller, 1998).

Inbreeding depression also varies with the development stage (Keller & Waller, 2002). Mutations in early acting genes that are functionally critical are generally thought to be lethal or at least highly detrimental (Keller & Waller, 2002), so the impact of inbreeding depression due to the expression of genetic load should be strongest at early stages of development (Brekke *et al*., 2010). However, our inability to correctly measure the impact of inbreeding at early stages of embryo development in birds (Hemmings, West & Birkhead, 2012) could have repercussions for the management and recovery of threatened species, as vital information on the magnitude and severity of inbreeding depression is unreliable (Grueber *et al*., 2015). This is particularly important in wild populations, where the effects of inbreeding can be exacerbated by changing environmental conditions (Keller & Waller, 2002).

Climate change

Climate change effects on hatching success in small populations are complex and confounded by other factors, such as disturbance, habitat degradation, lack of habitat connectivity, food supply and synchrony in phenology (de Villeremuil *et al*., 2019). Environmental stress as a result of climate change has, however, been shown to influence a number of different reproductive traits across a wide range of species. Changes in lay-date as a response to climate change, for example, seem to be ubiquitous (Dunn, 2019), and such shifts may have a knock-on influence on hatching success. However, the evidence for this in threatened species is unclear. In the New Zealand Hihi (Stitchbird, *Notiomystis cincta*), for example, lay-date has not shifted to match changes in climate, showing little adaptive potential (de Villeremuil *et al.*, 2019).

Experimental evidence has shown that fertility and egg viability decline with rising temperatures (Lara & Rostagno, 2013). For example, in the threatened Florida Scrub Jay (*Aphelocoma coerulescens*), females with larger clutches that experienced longer periods of pre-incubation exposure to ambient temperature had reduced hatching success (Aldredge, Leclair, & Bowman, 2012). Increased frequency of extreme weather events such as drought has also led to increased hatching failure in the Lesser Prairie Chicken (*Tympanuchus pallidicinctus*), as incubating females are unable to maintain microclimate conditions in the nest, exceeding lethal limits to embryo development (Grisham *et al*., 2016).

In species where anthropogenic incubation disturbance is frequent, the impact of environmental change may also be compounded. In ground-nesting seabirds that breed in highly vulnerable coastal regions, like in the critically endangered Tara iti (Fairy Tern, *Sternula nereis*) human disturbance and extreme weather events are the main drivers of hatching failure (Ferreira *et al*., 2005; Supplementary Material). Rising temperatures have also impacted hatching success and population sex ratios in megapodes, a family of birds in which half of species are at risk of extinction (IUCN, 2020). Megapodes rely on environmental sources of heat for incubation, and high incubation temperatures lead to male-biased mortality in the Australian Brush-turkey (*Alectura lathami*) (Eiby, Wilmer, & Booth, 2008).

Pollution

Pollution is known to interfere with reproductive function and egg viability in birds, and has been associated with widespread adult mortalities, species declines and extinctions (Giesy *et al*., 2003). Over 90,000 chemicals have been released into the environment by humans, and the vast majority of these have not been tested for their effects on humans or wildlife (Patisaul, Fenton, & Aylor, 2018). Pollutants currently known to affect bird reproduction include persistent organic pollutants (particularly chlorinated hydrocarbons such as DDT, PCBs and BFRs), non-halogenated pesticides (e.g. organophosphorus) and metal toxins (e.g. lead, mercury, cadmium, selenium) (Fry, 1995; Giesy *et al*., 2003).

Several hundred anthropogenic pollutants are known to be Endocrine-Disrupting Compounds (EDCs) – substances that interfere with normal hormone function (Borgeest *et al*., 2002; Patisaul, Fenton, & Aylor, 2018). Known EDCs include many organic pollutants and metal toxins, as well as phytoestrogens, PAHs, alkylphenols and phthalate esters (Borgeest *et al*., 2002; Giesy *et al*., 2003). Many EDCs are highly toxic to birds and can have severe effects on fertility, embryo viability and mating behaviour (Fry, 1995; Giesy *et al*., 2003). Embryonic exposure to pollution can occur through maternal deposition into the yolk, with significant implications for egg quality and embryo development (Ottinger *et al*., 2005). When EDCs are passed on to developing embryos, they can reduce egg quality (e.g. through eggshell thinning), disrupt development, cause abnormalities of the reproductive tract, and result in sterility or even embryo death (Leighton, 1993; Fry, 1995). The effects of the organochlorine insecticide DDT and its primary metabolite DDE is a widely known example. DDTs led to the demise of many birds of prey in the 20th century, primarily due to eggshell thinning and embryo malformations that resulted from exposure. Despite their ban in the 1980s, DDT (and similar pesticides such as MXC) still affects wild bird reproduction today (Borgeest *et al.*, 2002; Helander *et al.*, 2002, Burnett *et al.*, 2013, Hernández *et al.*, 2018, van Oosten, 2019). Understanding the consequences of EDCs on avian reproductive physiology and fertility is crucial for conservation efforts; however, the mechanisms underpinning the effects these chemicals have on birds are not fully understood (Giesy *et al.*, 2003). We also have little to no knowledge of how the majority of anthropogenic pollutants affect wildlife (Patisaul, Fenton, & Aylor, 2018), and few long-term studies have monitored the effects of EDCs on fertility in wild birds (Bernanke & Köhler, 2009).

Emerging environmental contaminants that are likely to impact avian reproduction and hatching success are those from human and veterinary health care pharmaceuticals (Espín *et al.*, 2018). The last two decades have seen a rise in the effects of veterinary pharmaceuticals on avian scavenger populations (Cuthbert *et al.*, 2014). Avian scavengers frequently eat medicated dead livestock, either opportunistically or when it is provided during supplementary feeding for conservation purposes (Cuthbert *et al.*, 2014; Blanco *et al.*, 2017). Fluoroquinolones are one of the most commonly used antimicrobial veterinary drugs for livestock (Margalida & Bogliani, 2014), and the ingestion of fluoroquinolones and other pharmaceuticals can influence embryo development and reduce hatching success (Espín *et al.*, 2016; Hruba *et al.*, 2019). With livestock carcasses still being commonly used at supplementary feeding stations (Blanco *et al.*, 2017), understanding the impact of pharmaceuticals on hatching success in wild birds remains a priority.

Mechanisms of hatching failure

Despite ample evidence that environmental change is driving increased rates of hatching failure in threatened birds, a clear understanding of the mechanistic drivers of egg failure remains elusive. The first step towards resolving this issue is to identify whether hatching failure is due to (1) fertilization failure, or (2) failure of a fertilized egg to develop into a hatched chick (i.e. embryo mortality). These two types of failure can have very different causes, so distinguishing between them is essential if we are to identify (and act upon) the ecological and/or behavioural drivers of hatching failure. Only a handful of studies distinguish between fertilization failure and embryo mortality as causes of reproductive failure in birds, and confusingly, ornithologists often universally refer to any undeveloped eggs as 'infertile' (e.g. Wetton & Parkin, 1991; Morrow, Arnqvist & Pitcher, 2002).

Fertilization failure

Fertilization is the process of sperm and egg pronuclei fusing to form a viable zygote (syngamy). Therefore, an infertile egg is one where the female pronucleus has not fused with a male pronucleus. However, infertility is often used interchangeably to describe both embryo mortality and fertilization failure, possibly due to historic difficulties in distinguishing between the two. Birkhead *et al.* (2008) described a method by which fertilization failure and early embryo mortality can be unequivocally distinguished in unhatched bird eggs, by microscopically examining the egg contents for (1) sperm on the perivitelline layer surrounding the ovum, (2) penetration points in the perivitelline layer indicating the entrance of sperm into the egg, and (3) embryonic cells/tissue in the germinal disc of the ovum, indicating the onset of development (Figure 1). This method has been used on a range of bird species and demonstrated to be relatively robust to egg degradation (Hemmings, West, & Birkhead, 2012), making it well-suited for use on eggs of endangered wild birds, which must typically be left in the nest until after other eggs hatch to eliminate the risk of removing a viable egg. We have developed step-by-step protocols and video demonstrations of this method that are openly available via https://www.zsl.org/practical-resources-for-identifying-the-causes-of-hatching-failure-in-birds. For clarity, we define infertility as fertilization failure (i.e. no syngamy) in this review, and when we talk about the causes of infertility, we refer to any processes contributing to fertilization failure.

In birds, fertilization failure is commonly assumed to be the result of a lack of sperm (Hemmings & Birkhead, 2015) or poor sperm function (Brillard, 1990; Lifjeld *et al.*, 2007), that is a problem with the male. However, there is little evidence explicitly linking sperm traits with hatching success in birds. Fertilization failure could also be female-mediated;

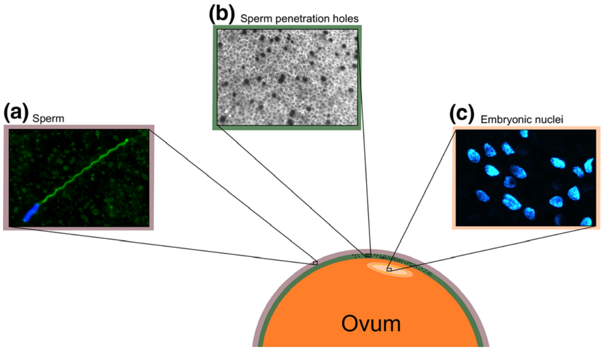


Figure 1 Microscopic examination of undeveloped eggs allows us to distinguish between fertilization failure and embryo mortality as the cause of hatching failure. (a) Zebra Finch (*Taeniopygia guttata*) sperm stained with fluorescent dyes and imaged at 200x magnification. Sperm can be found on the PVL of unhatched eggs several weeks after failure. (b) Penetration points left by sperm that have entered the ovum, imaged with darkfield microscopy at 200× magnification. (c) Embryonic cells after 24 hours incubation, stained with a fluorescent dye and imaged at 400x magnification. Cell division begins approximately 2 hours after fertilization, and by the time the egg is laid, the germinal disc typically contains thousands of embryonic cells. Polyspermy (where multiple sperm enter the ovum) is part of the normal process of fertilization in birds and is required for normal early embryo development. Diagram not to scale

recent research has revealed that females exert far more control over post-copulatory processes than was previously assumed (Pizzari & Birkhead, 2000; Hemmings & Birkhead, 2017), and that variation in female reproductive traits may have a substantial impact on fertilization success. For example, the avian vagina is thought to be highly selective, with only 1% of sperm successfully passing the vagina and entering storage. Therefore, sperm selection in the female reproductive tract (cryptic female choice) can influence which sperm are available during fertilization (Sasanami *et al.*, 2013). Ideally, this process would ensure only good quality sperm can fertilize the egg, theoretically improving fertilization success but also potentially enhancing offspring quality. It has been found in other taxa, for example, that cryptic female choice improves both egg fertilization rate and embryo survival (Rosengrave *et al.*, 2016). The exact mechanisms of sperm selection are still unclear in birds, but some females are known to preferentially eject the sperm of undesirable males (Pizzari & Birkhead, 2000), and immunological activity within the vagina can influence sperm viability and transport (Bakst, Wishart, & Brillard, 1994). If these processes are too selective, insufficient sperm may reach the site of fertilization (Hemmings & Birkhead, 2015). In domestic birds, fertilization failure has also been shown to be associated with female age (Bramwell *et al.*, 1996), female reproductive disorders (Srinivasan *et al.*, 2014), aspects of the female's environment (such as diet and stress) (Lewis, 2004; Walzem & Chen, 2014) and genetic factors that may influence the receptivity of the oviduct and/or egg to sperm (Bernier, Spencer, & Swartwood, 1951). Fertilization failure may also result from behavioural incompatibilities between males and females that impede successful courtship and copulation.

Embryo mortality

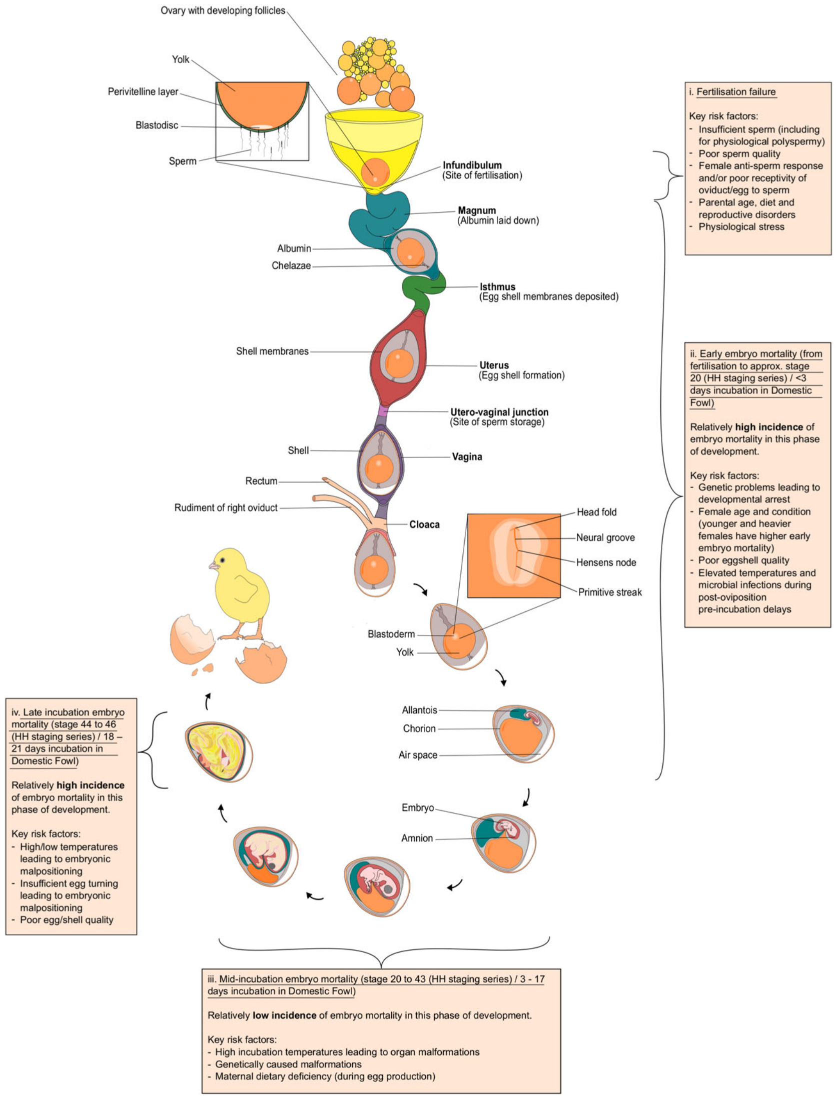
If an ovum is successfully fertilized, hatching failure may still occur as a result of embryo mortality. Embryo mortality can occur at any stage of development (including prior to oviposition) and for a variety of reasons (Figure 2), although deaths are more common during the early and late stages (Romanoff, 1949). Early embryo mortality (within 72 hours of fertilization) is commonly associated with lethal genetic factors, such as chromosomal abnormalities (Shook, Stephenson, & Biellier, 1971). Genetic perturbations are more likely in inbred individuals, and accordingly, inbreeding has been shown to significantly depress early embryo survival (Hemmings, Slate, & Birkhead, 2012). However, the mechanisms by which inbreeding depresses embryo development remain largely unknown. While most genetic problems manifest early in development, some result in death at a later stage of development, typically due to gross morphological abnormalities (Romanoff, 1949).

Although sperm quality is more typically expected to influence fertilization success, prolonged sperm storage in the male or female reproductive tract before fertilization has been shown to increase the incidence of early embryo mortality (Lodge, Fechheimer, & Jaap, 1971). This effect may be explained by age-related deterioration of sperm and/or a reduction in the number of sperm surviving to reach and penetrate the ovum (Eslick & McDaniel, 1992). Fewer viable sperm may limit the scope for physiological polyspermy, which is essential for normal early embryo development in birds (Hemmings & Birkhead, 2015).

In the early stages of development, embryos are vulnerable to fluctuations in ambient climatic conditions (particularly elevated temperatures) and trans-shell infections during the period between oviposition and incubation onset (Meijerhof, 1992). In many species that lay a clutch of eggs, incubation does not begin until the end of the egg-laying period to ensure synchronous hatching, so eggs laid earlier in the clutch have a longer pre-incubation exposure time. Early embryo mortality also appears to be more common when (1) females are younger (Fairchild *et al.*, 2002); (2) females have greater body weight (Coleman & Siegel, 1966) and (3) eggs are small and/or poor quality (including the eggshell) (Lerner *et al.*, 1993), which can be the result of poor female condition or stress/disturbance during egg production (Reynard & Savory, 1999).

Mid-development embryo mortality is relatively infrequent, although hyperthermia at this stage can result in developmental arrest or malformations (Christensen, 2001). The nature of these malformations depends on the stage at which the embryo is exposed to high temperatures. For example, around day 3 of incubation, during early brain formation, elevated temperatures can lead to abnormal brain and neural tube development (Alsop, 1919), whereas a week or so into development, high temperatures are more likely to lead to circulatory system failure, for example heart enlargement and cardiac arrest. Romanoff (1949) identified a critical period in the mid-stage development (at 12–14 days of incubation) of Domestic Fowl (*Gallus gallus domesticus*) when embryo mortality can peak if the maternal diet during egg production is deficient in animal protein, vitamins and minerals. Towards the end of development, high or low temperatures, as well as insufficient egg turning, can increase the incidence of embryonic malpositioning, limiting the ease with which the developed chick can successfully break out of the egg.

Although it is relatively easy to identify by eye whether a freshly laid egg is fertilized (Christensen, 2001), the structure of the blastoderm degenerates rapidly following early developmental arrest, particularly in the warm conditions of a nest. Therefore, if an embryo from a wild nest dies within the first 72 hours of development, and several days or weeks elapse before it is collected, the egg can be mistaken as unfertilized upon macroscopic examination (Birkhead *et al.*, 2008). Using fluorescence microscopy methods, Hemmings & Evans (2020) found that early embryo deaths were mistaken for fertilization failure in 52% of Blue Tit (*Cyanistes caeruleus*) and 33% of Great Tit (*Parus major*) eggs left in the nest for 2 weeks after hatching. The fact that early embryo mortality can be so easily mistaken for fertilization failure in wild populations is of particular concern, given that the majority of embryo mortalities may happen during these early stages of development (Christensen, 2001).



i. Fertilisation failure

Key risk factors:
- Insufficient sperm (including for physiological polyspermy)
- Poor sperm quality
- Female anti-sperm response and/or poor receptivity of oviduct/egg to sperm
- Parental age, diet and reproductive disorders
- Physiological stress

ii. Early embryo mortality (from fertilisation to approx. stage 20 (HH staging series) / <3 days incubation in Domestic Fowl)

Relatively **high incidence** of embryo mortality in this phase of development.

Key risk factors:
- Genetic problems leading to developmental arrest
- Female age and condition (younger and heavier females have higher early embryo mortality)
- Poor eggshell quality
- Elevated temperatures and microbial infections during post-oviposition pre-incubation delays

iii. Mid-incubation embryo mortality (stage 20 to 43 (HH staging series) / 3 - 17 days incubation in Domestic Fowl)

Relatively **low incidence** of embryo mortality in this phase of development.

Key risk factors:
- High incubation temperatures leading to organ malformations
- Genetically caused malformations
- Maternal dietary deficiency (during egg production)

iv. Late incubation embryo mortality (stage 44 to 46 (HH staging series) / 18 – 21 days incubation in Domestic Fowl)

Relatively **high incidence** of embryo mortality in this phase of development.

Key risk factors:
- High/low temperatures leading to embryonic malpositioning
- Insufficient egg turning leading to embryonic malpositioning
- Poor egg/shell quality

Figure 2 Key risk factors associated with egg failure at different stages of egg formation and embryo development. I. Fertilization failure refers to factors that reduce the likelihood of sperm reaching and penetrating the ovum; II. Early embryo mortality refers to embryo death occurring between fertilization and approximately developmental stage 20 (~3 days incubation in Domestic Fowl). III. Mid-incubation mortality refers to embryo death during developmental stages 20-43 (~3-17 days incubation in Domestic Fowl); IV. Late incubation mortality refers to embryo death during developmental stages 44-46 (~18-21 days incubation in Domestic Fowl). Developmental stages and incubation phases are provided as a guide but vary depending on developmental rate and mode of different species. Embryo death is most common during the early (<3 days incubation) and late (pre-hatch) stages of development. Diagram not to scale

Hatching failure in wild populations

Despite differences in the mechanisms that cause fertilization failure versus embryo mortality, the majority of studies of hatching failure in wild birds consider only whether or not eggs hatch, without investigating the underlying cause of failure and/or the stage at which the embryos died (e.g. Spottiswoode & Møller, 2004). Of those studies that have attempted to look at embryo mortality rates in wild birds, most have assumed undeveloped eggs to be unfertilized and therefore restricted their analyses to analysing mid- and late-term embryos (Jamieson & Ryan, 2000; Brekke *et al.*, 2010). Results from the limited number of studies that have distinguished between fertilization failure and early embryo death as causes of hatching failure in wild birds suggest that early embryo mortality is more common (Hemmings & Evans, 2020). Hemmings, West, & Birkhead (2012) microscopically examined eggs classed as 'infertile' from five endangered species and found that only 26% of these eggs were truly unfertilized. If extrapolated to another study such as Jamieson & Ryan (2000), which compares infertility rates in New Zealand endangered species, this suggests that infertility may be strongly overestimated, while the incidence of early embryo mortality is underestimated (Figure 3).

Recognizing the role of early embryo mortality in the hatching failure of wild populations can improve conservation research but is also important for studies in other fields. For example, a study on a wild population of Eurasian Tree Sparrows (*Passer montanus*) – one of the few studies that has accurately discriminated between unfertilized eggs and early embryo mortality – found that the female-biased secondary sex ratio in this population was due to higher mortality of male embryos, most (62%) of which occurred at the early embryo stage (Kato *et al*., 2017). Previous studies have attributed skewed sex ratios to temperature-dependent sex-biased embryo mortality (Eiby, Wilmer, & Booth, 2008) and biased parental investment (Spelt & Pichegru, 2017), but failure to consider individuals that die very early in the population creates a potential bias in these studies.

Accurate monitoring of early embryo mortality in wild populations can also provide important and formerly lacking data on extra-pair paternity. The role of extra pair paternity in connection with reproductive success is controversial; there is some evidence that engaging in extra-pair copulations is a female strategy for directly improving fitness via decreased hatching failure (Yuta *et al*., 2018). However, there is opposing evidence for whether within-pair or extra-pair offspring have higher fitness themselves (Sardell *et al*., 2012; Hsu *et al*., 2014) and meta-analyses have come to contradictory conclusions about the correlation between extra-pair paternity and hatching success rates across species (Morrow, Arnqvist, & Pitcher, 2002; Reding, 2015). The paternity assignment of early embryos, previously assumed to be unfertilized eggs, provides more accurate data on paternity and reopens lines of enquiry on this issue – not only on the occurrence of extra-pair paternity, but also the consequences for hatching and survival of extra-pair offspring, their distribution in the laying order, differential parental investment and other related questions.

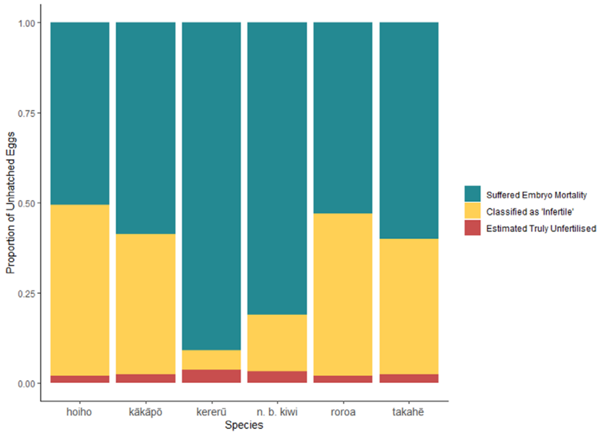


Figure 3 The proportion of failed eggs classified as infertile in six endangered bird species (Northern Brown Kiwi (*Apteryx mantelli*), Roroa (Great Spotted Kiwi, *Apteryx haastii*), Kākāpō (*Strigops habroptilus*), Kererū (*Hemiphaga novaeseelandiae*), Takahē (*Porphyrio hochstetteri*), and Hoiho (Yellow-eyed Penguin, *Megadyptes antipodes*; data from Jamieson & Ryan (2000), Table 1), and estimated proportion of truly infertile eggs based on results of Hemmings, West, & Birkhead (2012), who found on average 74% of undeveloped eggs from endangered species that were classed as unfertilized by ornithologists actually showed evidence of fertilization/development

Conservation management interventions for hatching failure: benefits and challenges

Birds that are bred in captivity for conservation management purposes often suffer notably high levels of hatching failure. However, unlike in the wild, where unhatched eggs tend to be fertilized but suffer early embryo mortality, fertilization failure may be a common cause of hatching failure in captive birds (Hemmings, West, & Birkhead, 2012). While captive birds benefit from medical care, a stable food supply and absence of predation (Mason, 2010), captivity can also be stressful due to frequent human disturbance and handling, unnatural or inadequate environment (e.g. artificial lighting), atypical group sizes, and forced mate pairing or separation (Morgan & Tromborg, 2007; Griffith *et al*., 2017; Fischer & Romero, 2019). Such captive stress could lead to fewer breeding attempts, reduced parental investment/abnormal parental behaviour, and overall, reduced production of successful eggs. For example, in Houbara Bustards (*Chlamydotis [undulata] macqueenii*) hatching failure is higher in captivity than in the wild (Saint Jalme *et al*., 1996), and captive (domesticated) Zebra Finches (*Taeniopygia guttata*) experience around twice the level of hatching failure reported for their wild counterparts (Hemmings, Slate, & Birkhead, 2012). Elevated rates of hatching failure impact the effectiveness of captive-breeding programmes, so it is important that management techniques are implemented to counteract these issues and improve egg hatchability (Supplementary material).

Egg manipulations are commonly used by conservation programmes of endangered birds to improve hatching success and population growth. A common conservation management practice for both captive and wild populations is 'egg pulling' – removal of eggs from nests for artificial incubation and/or fostering. These eggs are then either returned to the wild at a later stage of incubation or hatched in captivity, with the chicks being captive-reared and either retained for breeding programmes or released into the wild as juveniles or adults. Egg pulling may be employed if there are 'surplus' eggs, for example in the Whooping Crane (*Grus americana*) where two eggs are typically laid but only one chick usually survives (Supplementary Material; Kuyt, 1996). Eggs may also be pulled if they are at risk in the nest, for example to prevent incubating Peregrine Falcons (*Falco peregrinus*) from accidentally smashing eggs that were thin-shelled due to DDE contamination (e.g. Burnham *et al.*, 1988). Alternatively, eggs may be removed to encourage the breeding pair to lay a replacement clutch, increasing the overall number of eggs laid in the population (e.g. Wood & Collopy, 1993). However, egg fertility, hatchability and quality have all been shown to decline in replacement clutches (e.g. Jones *et al.*, 1994) and forced re-clutching may negatively impact fledgling survival (e.g. Parmley *et al.*, 2015) and/or future reproductive success of adults (e.g. Wood & Collopy, 1993). This indicates that egg pulling can have important costs as well as benefits for breeding management, and accordingly, some conservation protocols enforce limitations on the number of replacement clutches that can be laid in a season.

Although removing eggs for artificial incubation is generally considered the safest option for conservation managers, artificially incubated eggs often experience lower hatching success than eggs left in the wild, and therefore represent an important source of mortality in captive-breeding programmes (e.g. Sancha *et al.*, 2004). While many aspects of the artificial incubation environment can be tightly controlled, what is lacking is the fine-scale control and adjustments that may be provided by parent birds throughout development as they respond to the developing embryo's requirements (Tong *et al.*, 2013). Artificial incubation will also inevitably lack factors that natural nesting environments and parental incubation provide such as growth of beneficial microbes, periodic cooling, natural turning patterns and stimulation provided by parental and sibling vocalizations (Deeming, 2002). One technique that has been shown to increase the hatchability of wild eggs in artificial incubation is delaying the removal of eggs from the nest (e.g. Burnham, 1983), suggesting that allowing a small amount of early incubation by the parent may be beneficial. However, delayed egg removal can also increase predation risk and exposure to adverse climatic conditions and may reduce the likelihood that parents will lay a replacement egg or clutch, which is often the primary objective of this intervention.

Artificial incubation is also used for eggs produced by birds in captive breeding programmes. Hatching success of captive-laid eggs under artificial incubation is often lower than that of wild-laid eggs (e.g. Burnham, 1983), but this may reflect lower rates of fertilization success in captive birds. Indeed, wild-laid Whooping Crane eggs (Supplementary Material) had greater hatching success than captive-laid eggs even when they were both naturally incubated (by foster parents; Kuyt, 1996). Differences between wild and captive-laid eggs may also be a consequence of the presence or absence of pre-collection incubation, respectively (see above), and/or health problems affecting egg/embryo quality in the captive population. For example, a sudden increase in late-incubation embryo deaths in captive Kakī (Black Stilt, *Himantopus novaezelandiae*) eggs, but not wild-laid eggs subjected to the same artificial incubation environment, indicated differences in egg quality between captive and wild birds. This was subsequently shown to be the result of iodine deficiency in the captive population (Sancha *et al.*, 2004).

A major risk to eggs during the incubation period are trans-shell microbial infections, which can lead to embryo mortality. Parental incubation has been shown to limit bacterial and fungal growth on eggshells relative to unincubated eggs, reducing the risk of infection and increasing hatching success (Cook *et al.*, 2005). However, the precise mechanisms underpinning this effect remain unclear. In the absence of parental incubation, cleaning eggs with alcohol has been shown to reduce trans-shell infection and increase hatching success (Cook *et al.*, 2005), and egg-cleaning before artificial incubation is common practice within some areas of the poultry industry (Rideout, 2012). However, support for egg-cleaning is mixed, since resulting damage to the shell cuticle could potentially reduce natural barriers against microorganisms (Baggott & Graeme-Cook, 2002).

Fostering of eggs is sometimes used in breeding management practices in combination with, or as an alternative to, artificial incubation. A study comparing parentally incubated, fostered and artificially incubated wild-laid Killdeer (*Charadrius vociferous*) eggs showed that hatching success was similar after parental incubation and fostering (in this case by another species, Spotted Sandpipers *Actitus macularia*) (Powell & Cuthbert, 1993). Artificial incubation resulted in significantly higher hatching success than both parental incubation and fostering, but this was primarily because a large proportion of wild nests were predated rather than due to failure in artificial incubation. While fostering by both conspecifics and heterospecific parents has been successful (e.g. Byrd *et al.*, 1984), fostering by heterospecifics carries the risk of incorrect imprinting (e.g. Butler & Merton, 1992) and inter-species disease transfer (e.g. Snyder et al., 1985). Hence, fostering by conspecifics is generally preferred where possible.

Conditions in captivity may influence reproductive behaviour, ultimately resulting in decreased fertilization success (Saint Jalme *et al.*, 1996; Hemmings, West, & Birkhead, 2012). Captive birds are often kept in pairs or small groups, limiting the potential for mate choice and extra-pair copulations, and potentially leading to a higher incidence of incestuous and/or same-sex pairings than found in the wild (Driscoll, 2008). Commonly in captive breeding programmes, unsuccessful individuals are separated and provided with alternative mates, a technique which may also be used to manage genetic diversity. However, multiple studies in both wild and captive populations have indicated that birds that retain their mates over multiple seasons have greater reproductive success than those that 'divorce' and change mate (e.g. Yamamoto *et al.*, 1989), and several studies of captive birds (albeit with small sample sizes) have shown that reproductive success – particularly fertilization success – improves with increasing time spent as a pair (e.g. Brosset, 1981). Hence, there is a trade-off in terms of management decisions between allowing sufficient time for captive pairs or groups to establish normal socio-sexual behaviour, gain experience and improve their reproductive success, and avoiding the risk of wasted mating opportunities. This is particularly important in seasonal and/or unpredictable breeders.

To address issues with reproductive behaviour and timing, artificial insemination has been introduced in many captive populations, and in the special case of the free-living Kākāpō

(*Strigops habroptilus*) (Supplementary Material). Artificial insemination can compensate for a lack of copulation, an absence of extra-pair copulations, and/or unsuitable or unsuccessful pairings. For example, in a captive-bred population of Houbara Bustard, 'natural breeding' scenarios yielded 20-50% fertility (in this study, fertility refers to eggs that showed an obvious sign of embryonic development), while artificial insemination achieved up to 85% fertility (Saint Jalme *et al*., 1996). It has been shown that even when fertility levels are high (80-85%) they can be improved by an additional 5-10% through artificial insemination, with the best results being obtained from repeated deep inseminations of a large volume of semen as soon as possible after collection (Gee *et al*., 2004). The application of artificial insemination can be expanded through the use of frozen semen, which removes the temporal and spatial constraints imposed by the decline in sperm function over time post-ejaculation (Lodge, Fechheimer, & Jaap, 1971). However, the use of frozen semen results in lower egg fertilization rates (e.g. Parks & Hardaswick, 1987; Gee *et al*., 2004) and improvements in cryopreservation methods are essential to make this a viable management approach. Despite its benefits, artificial insemination is labour intensive and invasive, hence many programmes continue to focus on improving fertilization success in natural breeding.

Conclusions and guidelines for best practice

Hatching failure is one of the most crucial factors limiting the recovery of threatened bird populations. Here we have highlighted the key drivers of hatching failure and explored how these might differ between wild and managed/captive populations. Our overarching conclusion is that a better understanding of the mechanistic causes of hatching failure is required in order to ensure conservation management interventions are appropriately targeted. Distinguishing accurately between infertility and early embryo death and the rates at which each of these occur will enable bird conservation managers to adapt their approaches and provide more tailored solutions to egg failure. We have developed a set of openly available protocols and video demonstrations to facilitate the integration of egg examination techniques into conservation management (https://www.zsl.org/practical-resources-for-identifying-the-causes-of-hatching-failure-in-birds), and we advocate the use of these methods for the following reasons. First, these techniques allow us to establish if sufficient sperm are reaching eggs. The absence of sperm on the perivitelline layer of unhatched eggs strongly indicates a male sperm production or copulation problem, facilitating quick intervention. In captivity, for example, unsuccessful males (no sperm reaching eggs) can be removed to allow the female to form a new pair bond with a male of proven fertility within the same breeding season. Alternatively, females could be artificially inseminated with sperm from a proven male. Either approach would maximize the production of fertilized eggs within a season. Second, the identification of male fertility status from the presence/absence of sperm on eggs provides crucial information for translocation decisions – inclusion of an infertile male could potentially threaten the successful establishment of a small founder population. Third, if undeveloped eggs are fertilized but suffer early embryo death, management interventions can shift focus to incubation conditions and maternal health/nutrition to ensure optimal conditions for early embryo survival, as well as considering the genetic compatibility of the parents. Methods for examining unhatched eggs have so far been used to inform the management of a small number of captive and managed bird populations (e.g. Hemmings, West, & Birkhead, 2012; Croyle, Durrant, & Jensen, 2015). We hope that conservation practitioners will make use of the open resources now available (https://www.zsl.org/practical-resources-for-identifying-the-causes-of-hatching-failure-in-birds), and that examinations of unhatched eggs will be widely adopted in the future to maximize our understanding of avian reproductive failure.

Acknowledgements

For fruitful discussions about eggs while writing this review, we thank: G. Ward, E. Flach, B. Zimmerman, A. Walls, J. Graham, C. McSweeney, J. G. Ewen, T. and McMurdo Hamilton (ZSL), J. Crane, A. Digby, and D. Eason (NZ DOC Kākāpō Recovery Group), L. Brown, M. Marot, and J. Cooper (NZ DOC/Kakī Recovery Centre), A. Richardson and L. Percasky (Isaac Conservation and Wildlife Trust), A. Santure (University of Auckland), T. Steeves, S. Galla, J. Wold, and I. Cubrinovska (University of Canterbury), M. McCready (NZ DOC/Hihi Recovery Group), T. Makan (NZ DOC/Tara iti Recovery Group), and D. Shanahan, R. Empson, M. Booth, and N. Anderson (Zealandia Sanctuary). K.A. was supported by the Natural Environment Research Council ACCE Doctoral Training Partnership (DTP) (grant number NE/S00713X/1). A.F.M. was supported by a ZSL Collaborative Award in Science and Engineering (CASE) Studentship/London Natural Environmental Research Council DTP (grant number NE/L002485/1). F.M. was supported by the Royal Society (grant number RGF\R1 \180101). P.B. was supported by an Institute of Zoology Fellowship. N.H. was supported by the Royal Society (Dorothy Hodgkin Fellowship DH160200).

References

Aldredge, R.A., Leclair, S.C. & Bowman, R. (2012). Declining egg viability explains higher hatching failure in a suburban population of the threatened Florida Scrub-jay *Aphelocoma coerulescens*. *J. Avian Biol.* **43**, 369–375.

Alsop, F.M. (1919). The effect of abnormal temperatures upon the developing nervous systems in the chick embryos. *Anat. R.* **15**, 307–331.

Baggott, G.K. & Graeme-Cook, K. (2002). Microbiology of natural incubation. In *Avian Incubation: Behaviour, Environment and Evolution*: 179–191, Deeming, D.C. (Ed.). New York: Oxford University Press.

Bakst, M.R., Wishart, G. & Brillard, J.P. (1994). Oviducal sperm selection, transport, and storage in poultry. *Poult. Sci. Rev.* **5**, 117–143.

Bernanke, J. & Köhler, H.-R. (2009). The impact of environmental chemicals on wildlife vertebrates. *Rev. Environ. Contam. Toxicol.* **198**, 1–47.

Bernier, P.E., Spencer, J.V. & Swartwood, J.L. (1951). The relative effects of inbreeding and outbreeding on reproduction in the domestic fowl. *Hilgardia* **20**, 529–628.

BirdLife International. (2020). *State of the world's birds: taking the pulse of the planet*. Cambridge, UK: Birdlife International.

Birkhead, T.R., Hall, J., Schut, E. & Hemmings, N. (2008). Unhatched eggs: methods for discriminating between infertility and early embryo mortality. *Ibis* **150**, 508–517.

Blanco, G., Junza, A. & Barrón, D. (2017). Occurrence of veterinary pharmaceuticals in golden eagle nestlings: unnoticed scavenging on livestock carcasses and other potential exposure routes. *Sci. Total Environ.* **586**, 355–361.

Borgeest, C., Greenfeld, C., Tomic, D. & Flaws, J.A. (2002). The effects of endocrine disrupting chemicals on the ovary. *Front. Biosci.* **7**, 1941–1948.

Bramwell, R.K., Mcdaniel, C.D., Wilson, J.L. & Howarth, B. (1996). Age effect of male and female broiler breeders on sperm penetration of the perivitelline layer overlying the germinal disc. *Poult. Sci.* **75**, 755–762.

Brekke, P., Bennett, P.M., Wang, J., Pettorelli, N. & Ewen, J.G. (2010). Sensitive males: inbreeding depression in an endangered bird. *Proc. R. Soc. Lond. B: Biol. Sci.* **277**, 3677–3684.

Brillard, J.P. (1990). *Control of fertility in birds*. Paris: INRA.

Briskie, J.V. & Mackintosh, M. (2004). Hatching failure increases with severity of population bottlenecks in birds. *Proc. Nat. Acad. Sci.* **101**, 558–561.

Brosset, A. (1981). Breeding the Black Sparrow Hawk *Accipiter melanoleucus* in captivity. *Raptor Res.* **15**, 58–64.

Burnett, L.J., Sorenson, K.J., Brandt, J., Sandhaus, E.A., Ciani, D., Clark, M., David, C., Theule, J., Kasielke, S. & Risebrough, R.W. (2013). Eggshell thinning and depressed hatching success of California Condors reintroduced to Central California. *The Condor* **115**, 477–491.

Burnham, W. (1983). Artificial incubation of falcon eggs. *J. Wildl. Manage.* **47**, 158–168.

Burnham, W.A., Heinrich, W., Sanfort, C., Levine, E., O'Brien, D. & Konkel, D. (1988). Recovery effort for the Peregrine Falcon in the Rocky Mountains. In *Peregrine Falcon Populations, Their Management and Recovery*: 565–575, Cade, T.J., Enderson, J.H., Thelander, C.G. & White, C.M. (Eds.). Boise, Idaho: The Peregrine Fund Inc.

Butler, D. & Merton, D. (1992). *The Black Robin: Saving the world's most endangered bird*. Oxford: Oxford University Press.

Byrd, G.V., Sincock, J.L., Telfer, T.C., Moriarty, D.I. & Brady, B.G. (1984). A cross-fostering experiment with Newell's race of Manx Shearwater. *J. Wildl. Manage.* **48**, 163–168.

Christensen, V.L. (2001). Factors associated with early embryonic mortality. *Worlds Poult. Sci. J.* **57**, 357–372.

Coleman, J.W. & Siegel, P.B. (1966). Selection for body weight at eight weeks of age: 5. Embryonic stage at oviposition and its relationship to hatchability. *Poult. Sci.* **45**, 1003–1011.

Cook, M.I., Beissinger, S.R., Toranzos, G.A., Rodriguez, R.A. & Arendt, W.J. (2005). Microbial infection affects egg viability and incubation behavior in a tropical passerine. *Behav. Ecol.* **16**, 30–36.

Croyle, K.E., Durrant, B.S. & Jensen, T. (2015). Detection of oocyte perivitelline membrane-bound sperm: a tool for avian collection management. *Conserv. Physiol.* **3**, cou060. https://doi.org/10.1093/conphys/cou060.

Cuthbert, R.J., Taggart, M.A., Prakesh, V., Chakraborty, S.S., Deori, P., Galligan, T., Kulkarni, M., Ranade, S., Saini, M., Sharma, A.K., Shringarpure, R. & Green, R.E. (2014). Avian scavengers and the threat from veterinary pharmaceuticals. *Philos. Trans. R. Soc. B: Biol. Sci* **369**, 20130574. https://doi.org/10.1098/rstb.2013.0574.

Deeming, D.C. (Ed.). (2002). *Avian incubation: behaviour, environment and evolution*. Oxford: Oxford University Press.

Driscoll, E.V. (2008). Bisexual species. *Sci. Am. Mind* **19**, 68–73.

Dunn, P. (2019). Changes in timing of breeding and reproductive success in birds. In *Effects of climate change on birds*: 108–119.Dunn, P. & Møller, A.P. (Eds.), Oxford: Oxford University Press.

Eiby, Y.A., Wilmer, J.W. & Booth, D.T. (2008). Temperature-dependent sex-biased embryo mortality in a bird. *Proc. R. Soc. B: Biol. Sci.* **275**, 2703–2706.

Eslick, M.L. & McDaniel, G.R. (1992). Interrelationships between fertility and hatchability of eggs from broiler breeder hens. *J. Appl. Poult. Sci.* **1**, 156–159.

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P. & Jaspers, V.L.B. (2016). Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? *Ecotoxicology* **25**, 777–801.

Fairchild, B.D., Christensen, V.L., Grimes, J.L., Wineland, M.J. & Bagley, L.G. (2002). Hen age relationship with embryonic mortality and fertility in commercial turkeys. *J. Appl. Poult. Res.* **11**, 260–265.

Ferreira, S.M., Hansen, K.M., Parrish, G.R., Pierce, R.J., Pulham, G.A. & Taylor, S. (2005). Conservation of the endangered New Zealand fairy tern. *Biol. Conserv.* **125**, 345–354.

Fischer, C.P. & Romero, L.M. (2019). Chronic captivity stress in wild animals is highly species-specific. *Conserv. Physiol.* **7**, 1–38.

Fry, D.M. (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ. Health Perspect.* **103**, 165–171.

Gee, G.F., Bertschinger, H., Donoghue, A.M., Blanco, J. & Soley, J. (2004). Reproduction in nondomestic birds:

physiology, semen collection, artificial insemination and cryopreservation. *Avian Poult. Biol. Rev.* **15**, 47–101.

Giesy, J.P., Feyk, L.A., Jones, P.D., Kannan, K. & Sanderson, T. (2003). Review of the effects of endocrine-disrupting chemicals in birds. *Pure Appl. Chem.* **75**, 2287–2303.

Griffith, S.C., Crino, O.L., Andrew, S.C., Nomano, F.Y., Adkins-Regan, E., Alonso-Alvarez, C., Bailey, I.E., Bittner, S.S., Bolton, P.E., Boner, W., Boogert, N., Boucaud, I.C.A., Briga, M., Buchanan, K.L., Caspers, B.A., Cichoń, M., Clayton, D.F., Derégnaucourt, S., Forstmeier, W., Guillette, L.M., Hartley, I.R., Healy, S.D., Hill, D.L., Holveck, M.J., Hurley, L.L., Ihle, M., Tobias Krause, E., Mainwaring, M.C., Marasco, V., Mariette, M.M., Martin-Wintle, M.S., McCowan, L.S.C., McMahon, M., Monaghan, P., Nager, R.G., Naguib, M., Nord, A., Potvin, D.A., Prior, N.H., Riebel, K., Romero-Haro, A.A., Royle, N.J., Rutkowska, J., Schuett, W., Swaddle, J.P., Tobler, M., Trompf, L., Varian-Ramos, C.W., Vignal, C., Villain, A.S. & Williams, T.D. (2017). Variation in reproductive success across captive populations: methodological differences, potential biases and opportunities. *Ethology* **123**, 1–29.

Grisham, B.A., Godar, A.J., Boal, C.W. & Haukos, D.A. (2016). Interactive effects between nest microclimate and nest vegetation structure confirm microclimate thresholds for Lesser Prairie-Chicken nest survival. *Condor* **118**, 728–746.

Grueber, C.E., Hogg, C.J., Ivy, J.A. & Belov, K. (2015). Impacts of early viability selection on management of inbreeding and genetic diversity in conservation. *Mol. Ecol.* **24**, 1645–1653.

Heber, S. & Briskie, J.V. (2010). Population bottlenecks and increased hatching failure in endangered birds. *Conserv. Biol.* **24**, 1674–1678.

Helander, B., Olsson, A., Bignert, A., Asplund, L. & Litzén, K. (2002). The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. *Ambio* **31**, 386–403.

Hemmings, N. & Birkhead, T.R. (2015). Polyspermy in birds: sperm numbers and embryo survival. *Proc. R. S. B: Biol. Sci.* **282**, 20151682. https://doi.org/10.1098/rspb.2015.1682.

Hemmings, N. & Birkhead, T.R. (2017). Differential sperm storage by female zebra finches Taeniopygia guttata. *Proc. R. S. B: Biol. Sci.* **284**, 20171032. https://doi.org/10.1098/rspb.2017.1032.

Hemmings, N. & Evans, S. (2020). Unhatched eggs represent the invisible fraction in two wild bird populations. *Biol. Lett.* **16**, 20190763. https://doi.org/10.1098/rsbl.2019.0763.

Hemmings, N.L., Slate, J. & Birkhead, T.R. (2012). Inbreeding causes early death in a passerine bird. *Nat. Commun.* **3**, 863. https://doi.org/10.1038/ncomms1870.

Hemmings, N.L., West, M. & Birkhead, T.R. (2012). Causes of hatching failure in endangered birds. *Biol. Lett.* **8**, 964–967.

Hernández, M., Colomer, M., Pizarro, M. & Margalida, A. (2018). Changes in eggshell thickness and ultrastructure in the Bearded Vulture (*Gypaetus barbatus*) Pyrenean population: A long-term analysis. *Sci. Total Environ.* **624**, 713–721.

Hruba, Hana, Abdelsalam, Ehdaa Eltayeb Eltigani, Anisimov, Nikolay, Bandouchova, Hana, Havelkova, Barbora, Heger, Tomas, Kanova, Miroslava, Kovacova, Veronika, Nemcova, Monika, Piacek, Vladimir, Sedlackova, Jana, Vitula, Frantisek & Pikula, Jiri (2019). Reproductive toxicity of fluoroquinolones in birds. *BMC Vet Res* **15**, 209.

Hsu, Y.-H., Schroeder, J., Winney, I., Burke, T. & Nakagawa, S. (2014). Costly infidelity: low lifetime fitness of extra-pair offspring in a passerine bird. *Evolution* **68**, 2873–2884.

IUCN. (2020). The IUCN. Red List of Threatened Species. Version 2020–1. https://www.IUCN.redlist.org.

Jamieson, I.G. & Ryan, C.J. (2000). Increased egg infertility associated with translocating inbred Takahe (*Porphyrio hochstetteri*) to island refuges in New Zealand. *Biol. Conserv.* **94**, 107–114.

Jones, C.G., Heck, W.R., Lewis, R.E., Mungroo, Y., Slade, G. & Cade, T.J. (1994). The restoration of the Mauritius Kestrel *Falco punctatus* population. *The Ibis* **137**, 173–180.

Kato, T., Matsui, S., Terai, Y., Tanabe, H., Hashima, S., Kasahara, S., Morimoto, G., Mikami, O.K., Ueda, K. & Kutsukake, N. (2017). Male-specific mortality biases secondary sex ratio in Eurasian Tree Sparrows *Passer montanus*. *Ecol. Evol.* **7**, 10675–10682.

Keller, L.F. (1998). Inbreeding and its fitness effects in an insular population of Song Sparrows (*Melospiza melodia*). *Evolution* **52**, 240–250.

Keller, L.F. & Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241.

Kuyt, E. (1996). Reproductive manipulation in the Whooping Crane *Grus americana*. *Bird Conserv. Int.* **6**, 3–10.

Lara, L. & Rostagno, M. (2013). Impact of heat stress on poultry production. *Animals* **3**, 356–369.

Leighton, F.A. (1993). The toxicity of petroleum oils to birds. *Environ. Rev.* **1**, 92–103.

Lerner, S.P., French, N., McIntyre, D. & Baxter, C. (1993). Age-related changes in egg production, fertility, embryo mortality, and hatchability in commercial turkey flocks. *Poult. Sci.* **72**, 1025–1039.

Lewis, P.D. (2004). Responses of domestic fowl to excess iodine: a review. *Br. J. Nut.* **91**, 29–39.

Lifjeld, J., Laskemoen, T., Fossøy, F., Johnsen, A. & Kleven, O. (2007). Functional infertility among territorial males in two passerine species, the Willow Warbler *Phylloscopus trochilus* and the Bluethroat *Luscinia svecica*. *J. Avian Biol.* **38**, 267–272.

Lindsay, W.R., Madsen, T., Wapstra, E., Lillie, M., Loeb, L., Ujvari, B. & Olsson, M. (2020). Long term effects of outbreeding: experimental founding of island population eliminates malformations and improves hatching success in sand lizards. *Biol. Conserv.* **249**, 108710. https://doi.org/10.1016/j.biocon.2020.108710.

Lodge, J.R., Fechheimer, N.S. & Jaap, R.G. (1971). The relationship of *in vivo* sperm storage interval to fertility and embryonic survival in the chicken. *Biol. Reprod.* **5**, 252–257.

Margalida, A., Bogliani, G., Bowden, C.g r, Donazar, J.a, Genero, F., Gilbert, M., Karesh, W.b, Kock, R., Lubroth, J., Manteca, X., Naidoo, V., Neimanis, A., Sanchez-Zapata, J.a, Taggart, M.a, Vaarten, J., Yon, L., Kuiken, T. & Green, R.e (2014). One Health approach to use of veterinary pharmaceuticals. *Science* **346**, 1296–1298.

Mason, G.J. (2010). Species differences in responses to captivity: stress, welfare and the comparative method. *Trends Ecol. Evol.* **25**, 713–721.

Meijerhof, R. (1992). Pre-incubation holding of hatching eggs. *Worlds Poult. Sci. J.* **48**, 57–68.

Morgan, K.N. & Tromborg, C.T. (2007). Sources of stress in captivity. *Appl. Anim. Behav. Sci.* **102**, 262–302.

Morrow, E.H., Arnqvist, G. & Pitcher, T.E. (2002). The evolution of infertility: does hatching rate in birds coevolve with female polyandry? *J. Evol. Biol.* **15**, 702–709.

van Oosten, H.H., van den Burg, A.B., Arlt, D., Both, C., van den Brink, N.W., Chiu, S., Crump, D., Jeppsson, T., de Kroon, H., Traag, W. & Siepel, H. (2019). Hatching failure and accumulation of organic pollutants through the terrestrial food web of a declining songbird in Western Europe. *Sci. Total Environ.* **650**, 1547–1553.

Ortego, J., Cordero, P.J., Aparicio, J.M. & Calabuig, G. (2010). Parental genetic characteristics and hatching success in a recovering population of Lesser Kestrels. *J. Ornithol.* **151**, 155–162.

Ottinger, M.A., Quinn, M.J., Lavoie, E., Abdelnabi, M.A., Thompson, N., Hazelton, J.L., Wu, J.M., Beavers, J. & Jaber, M. (2005). Consequences of endocrine disrupting chemicals on reproductive endocrine function in birds: establishing reliable end points of exposure. *Domest. Anim. Endocrinol.* **29**, 411–419.

Parks, J.E. & Hardaswick, V. (1987). Fertility and hatchability of falcon eggs after insemination with frozen Peregrine Falcon semen. *J. Raptor Res.* **21**, 70–72.

Parmley, E.J., Pearl, D.L., Vogt, N.A., Yates, S., Campbell, G.D., Steiner, J., Imlay, T.L., Hollamby, S., Tuininga, K. & Barker, I.K. (2015). Factors influencing mortality in a captive breeding population of Loggerhead Shrike, Eastern subspecies (*Lanius ludovicianus* ssp.) in Canada. *BMC Vet. Res.* **11**, 129.

Patisaul, H.B., Fenton, S.E. & Aylor, D. (2018). Animal models of endocrine disruption. *Best Pract. Res. Clin. Endocrinol. Metab.* **32**, 283–297.

Pizzari, T. & Birkhead, T.R. (2000). Female feral fowl eject sperm of subdominant males. *Nature* **405**, 787–789.

Powell, A.N. & Cuthbert, F.J. (1993). Augmenting small populations of plovers: an assessment of cross-fostering and captive-rearing. *Conserv. Biol.* **7**, 160–168.

Reding, L. (2015). Increased hatching success as a direct benefit of polyandry in birds. *Evolution* **69**, 264–270.

Reynard, M. & Savory, J. (1999). Stress-induced oviposition delays in laying hens: duration and consequences for eggshell quality. *Br. Poult. Sci.* **40**, 585–591.

Rideout, B.A. (2012). Investigating embryo deaths and hatching failure. *Vet. Clin. North Am. Exot. Anim. Pract.* **15**, 155–162.

Romanoff, A.L. (1949). Critical periods and causes of death in avian embryonic development. *Auk* **66**, 264–270.

Rosengrave, P., Montgomerie, R. & Gemmell, N. (2016). Cryptic female choice enhances fertilization success and embryo survival in chinook salmon. *Proc. R. Soc. B.: Biol. Sci.* **283**, 20160001. https://doi.org/10.1098/rspb.2016.0001.

Saint Jalme, M., Combreau, O., Seddon, P.J., Paillat, P., Gaucher, P. & van Heezik, Y. (1996). Restoration of *Chlamydotis undulata macqueenii* (Houbara Bustard) populations in Saudi Arabia: a progress report. *Restor. Ecol.* **4**, 81–87.

Sancha, E., van Heezik, Y., Maloney, R., Alley, M. & Seddon, P. (2004). Iodine deficiency affects hatchability of endangered captive Kakī (Black Stilt, *Himantopus novaezelandiae*). *Zoo Biol.* **23**, 1–13.

Sardell, R.J., Arcese, P., Keller, L.F. & Reid, J.M. (2012). Are there indirect fitness benefits of female extra-pair reproduction? Lifetime reproductive success of within-pair and extra-pair offspring. *Am. Nat.* **179**, 779–793.

Sasanami, T., Matsuzaki, M., Mizushima, S. & Hiyama, G. (2013). Sperm storage in the female reproductive tract in birds. *J. Reprod. Dev.* **59**, 334–338.

Shook, J.G., Stephenson, A.B. & Biellier, H.V. (1971). Heritability estimates of differences in arbitrary and embryonic mortality traits in turkeys. *Poult. Sci.* **50**, 1255–1260.

Slatyer, R.A., Mautz, B.S., Backwell, P.R. & Jennions, M.D. (2012). Estimating genetic benefits of polyandry from experimental studies: a meta-analysis. *Biol. Rev.* **87**, 1–33.

Snyder, B., Thilstead, J., Burgess, B. & Richard, M. (1985). Pigeon herpesvirus mortalities in foster reared Mauritius Pink Pigeons. *Annu.l Proc. Am. Assoc. Zoo Vet.* 69–70.

Spelt, A. & Pichegru, L. (2017). Sex allocation and sex-specific parental investment in an endangered seabird. *Ibis* **159**, 272–284.

Spottiswoode, C. & Møller, A.P. (2004). Genetic similarity and hatching success in birds. *Proc. R. Soc. Lond. B: Biol. Sci.* **271**, 267–272.

Srinivasan, P., Balasubramaniam, G.A., Gopala Krishna Murthy, T.R. & Balachandran, P. (2014). Prevalence and pathology of oviduct impaction in commercial white leghorn layer chicken in Namakkal region of India. *Vet. World* **7**, 553–558.

Tong, Q., Romanini, C.E., Exadaktylos, V., Bahr, C., Berckmans, D., Bergoug, H., Eterradossi, N., Roulston, N., Verhelst, R., McGonnell, I.M. & Demmers, T. (2013). Embryonic development and the physiological factors that coordinate hatching in domestic chickens. *Poult. Sci.* **92**, 620–628.

de Villemereuil, P., Rutschmann, A., Lee, K.D., Ewen, J.G., Brekke, P. & Santure, A.W. (2019). Little adaptive potential in a threatened passerine bird. *Curr. Biol.* **29**, 889–894.e3. https://doi.org/10.1016/j.cub.2019.01.072.

Walzem, R.L. & Chen, S. (2014). Obesity-induced dysfunctions in female reproduction: lessons from birds and mammals. *Adv. Nutr.* **5**, 199–206.

Weiser, E.L., Grueber, C.E., Kennedy, E.S. & Jamieson, I.G. (2016). Unexpected positive and negative effects of continuing inbreeding in one of the world's most inbred wild animals. *Evolution* **70**, 154–166.

Wetton, J. & Parkin, D. (1991). An association between fertility and cuckoldry in the House Sparrow, *Passer domesticus*. *Proc. R. Soc. B. Biol. Sci.* **245**, 227–233.

White, K.L., Eason, D.K., Jamieson, I.G. & Robertson, B.C. (2015). Evidence of inbreeding depression in the critically endangered parrot, the Kakapo. *Animal Conserv.* **18**, 341–347.

Wood, P.B. & Collopy, M.W. (1993). Effects of egg removal on Bald Eagle productivity in northern Florida. *J. Wildl. Manage.* **57**, 1–9.

Yamamoto, J.T., Shields, K.M., Millam, J.R., Roudybush, T.E. & Grau, C.R. (1989). Reproductive activity of force-paired Cockatiels (*Nymphicus hollandicus*). *Auk* **106**, 86–93.

Yuta, T., Nomi, D., Ihle, M. & Koizumi, I. (2018). Simulated hatching failure predicts female plasticity in extra-pair behavior over successive broods. *Behav. Ecol.* **29**, 1264–1270.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1