Hidden impacts of conservation management on fertility of the critically endangered kakapo

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Background. Animal conservation often requires intensive management actions to improve reproductive output, yet any adverse effects of these actions may not be immediately apparent, particularly in threatened species with small populations and long lifespans. Hand-rearing is an example of a conservation management strategy which, while boosting populations, can cause long-term demographic and behavioural problems. It is employed in the recovery of the critically endangered kākāpō (*Strigops habroptilus*), to improve the slow population growth that is a result of infrequent breeding, low fertility and low hatching success.

Methods. We applied Bayesian mixed models to examine whether hand-rearing and other factors were associated with clutch fertility in kākāpō. We used projection predictive variable selection to compare the relative contributions to fertility of the parents' rearing environment, their age and previous mating experience, the parental kinship, and the number of mates and mating attempts. We also explored how multiple mating behaviour varied with kākāpō density.

Results. Female multiple mating behaviour and father hand-rearing status were the dominant factors in predicting fertility. Clutches produced by females mating with different males were more likely to be fertile than those from repeated matings with one male, which were more likely to be fertile than those from a single mating. The likelihood of multiple matings increased with female:male adult sex ratio, perhaps as a result of female mate guarding. Clutches were less likely to be fertile if the father was hand-reared compared to wild-reared, but there was no similar effect for mother rearing origin. Parental kinship, previous mating experience and parent age all had negligible associations with clutch fertility.

Conclusions. These results provide a rare assessment of factors affecting fertility in a wild threatened bird species, and have implications for conservation management. The increased fertility due to multiple mates, together with sperm morphology and evidence for mate guarding, suggests that an evolutionary mechanism exists to optimise fertility through sperm competition in kākāpō. The high number of clutches from a single mating in the contemporary population may therefore represent an unnatural state, perhaps due to too few females. This suggests that the opportunity for sperm competition should be maximised by increasing population densities, optimising sex ratios, and the use of artificial insemination. The lower fertility of hand-reared males may result from behavioural defects due to lack of exposure to conspecifics at critical development stages, as seen in other taxa. This potential negative impact of hand-rearing must be balanced against the short-term benefits it provides. In addition, these results of multiple matings and sex ratio affecting fertility in a lek-breeding species provide a useful test of hypotheses for avian mate-guarding and polyandry.

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ABSTRACT

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Results. Female multiple mating behaviour and father hand-rearing status were the dominant factors in predicting fertility. Clutches produced by females mating with different males were more likely to be fertile than those from repeated matings with one male, which were more likely to be fertile than those from a single mating. The likelihood of multiple matings increased with female:male adult sex ratio, perhaps as a result of female mate guarding. Clutches were less likely to be fertile if the father was hand-reared compared to wild-reared, but there was no similar effect for mother rearing origin. Parental kinship, previous mating experience and parent age all had negligible associations with clutch fertility.

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INTRODUCTION

Factors affecting fertility in conservation-managed populations

Conservation strategies for wild-living threatened species rely on improving survival and productivity to increase population growth. Methods such as habitat restoration and predator control are used to enhance survival, but often it is problems with reproductive output which most limit growth (Bunin et al., 1997; Gage et al., 2006; Comizzoli and Holt, 2019) and can have wide-ranging implications (Findlay et al., 2019). Management techniques used to address these problems include translocations, supplementary feeding and artificial insemination (Lloyd and Powlesland, 1994; Castro et al., 2003; Houston et al., 2007; Armstrong and Seddon, 2008; Blanco et al., 2009; Heber et al., 2012; Dogliero et al., 2017; Schneider et al., 2019). However, there has been little study of whether the conservation actions used to promote population growth of threatened species can in fact themselves impact productivity. This is at least partially due to any unintended consequences not being immediately apparent, especially in threatened species for which the ability to recognise significant trends is hampered by small data sets resulting from long life spans and small populations (Garamszegi, 2016). Here we consider factors which can affect fertility in conservation-dependent species, including the conservation management actions intended to improve population growth.

Rearing environment

Captive breeding and hand-rearing are often used in threatened species conservation programmes (Klusener et al., 2018). We distinguish between hand-rearing, in which animals are raised in captivity by humans, and captive breeding, in which animals mate and raise young in captivity. Since in the literature there is often little distinction between captive breeding and hand-rearing, here we consider effects of both of these interventions combined.

In bird species, eggs are sourced from either wild or captive clutches, and are artificially incubated

(Assersohn et al., 2021b). The young are hand-reared in artificial environments before release to the wild, primarily to increase productivity through improved survival during the precocial stage (Alagona, 2004; Heezik et al., 2005). As well as improving reproductive success, captive breeding and hand-rearing can lead to other benefits such as increased advocacy opportunities, and population supplementation and re-establishment to maintain genetic and demographic stability (Collazo et al., 2003; Joustra, 2018; Gilbert and Soorae, 2017).

Captive breeding and hand-rearing have well-documented diverse negative impacts (Snyder et al., 1996). Captive-bred or hand-reared juveniles can suffer from physiological, morphological and mobility differences compared to their wild counterparts (Heezik and Seddon, 1998; Liukkonen-Anttila et al., 2000; Prier et al., 2013). Captive-bred or hand-reared individuals may also have lower long-term survival rates (Aourir et al., 2013; Hampson and Schwitzer, 2016), including through loss of anti-predatory behaviour (Carrete and Tella, 2015). Survival impacts in animals raised and kept in captive environments can be multi-generational and highly variable across species (Heezik et al., 2005; Fustukjian et al., 2018; Farquharson et al., 2021). Captive individuals may also have altered microbiomes, with potential long-term health implications (West et al., 2019; Juan et al., 2021). Behavioural issues are common in captive-raised juveniles, which tend to exhibit altered social structure (Pacheco and Madden, 2021), fewer social behaviours and more aggression towards conspecifics (Meretsky et al., 2000; Utt et al., 2008; Jones, 2008), and lack of avoidance of humans (Berry, 1998; Snyder et al., 1996). These can have strong implications for threatened species management, as hand-reared individuals may be unsuited to life in the wild (Meretsky et al., 2000).

Captive breeding and hand-rearing can also affect productivity in a range of taxa. Lack of access to conspecifics in the first year is related to lower reproductive success in primates and felids, probably as a result of behavioural differences (King and Mellen, 1994; Beck and Power, 1988; Hampson and Schwitzer, 2016). Captive rhinoceroses have lower reproductive output than wild ones (Edwards et al., 2015). Captive-born animals have an overall 42% lower chance of reproductive success in captivity compared to wild-born counterparts, although impacts on fertility, hatchability and reproductive yield were not strongly significant (Farquharson et al., 2018).

Few studies have examined the effect of the captive development environment on reproductive fitness, especially in birds — perhaps due to the difficulty of monitoring reproductive success in the wild across successive generations. Most studies of fertility in birds have focussed solely on captive environments, and the majority of these have been on the single order of Galliformes. Very few studies (only 5%) have compared fertility in wild and captive reared populations (Assersohn et al., 2021a); but those have reported likely fertility impacts of captive rearing, albeit from small samples (Hemmings et al., 2012b). Since early life events can negatively impact a range of behaviours in several taxa (Anisman et al., 1998; Murgatroyd et al., 2018), it is likely that behavioural differences arising from rearing method influences reproductive behaviour in birds as it does in other taxa.

Polyandry

Differences in mating behaviour, in terms of the number of mates and mating attempts, can directly affect fertility in birds. An example of this is polyandry, which is likely to be driven by not only direct benefits such as increased access to resources (Parker and Birkhead, 2012), but also by indirect benefits, such as providing fertility advantages from post-copulation mate selection and increased likelihood of egg fertilisation — the “fertility assurance hypothesis” (Birkhead et al., 1987; Rivers and DuVal, 2019; Santema et al., 2020). There is evidence that mating with different mates improves reproductive success across many bird species (Reding, 2014), although this is not universal (Morrow et al., 2002; Rivers and DuVal, 2019). This advantage can also extend to females mating repeatedly with a single male, which can increase the likelihood of fertility (Zhang et al., 2019) and reproductive output (Heeb, 2001).

Polyandry is highly variable among species (Lank et al., 2002; Lebigre et al., 2007; Taylor et al., 2014) and among individuals of a species (Hess et al., 2012). The incidence of multiple matings (either with the same or different individuals) is likely to be affected by the number of available mates. Polyandry is likely to be more frequent with increased male density (Taylor et al., 2014), although this may be offset by other factors such as female range size (Kvarnemo, 2018). Adult sex ratio can also influence the likelihood of multiple mating, perhaps as a result of consequent changes in mate guarding (Grant and Grant, 2019; Birkhead and Montgomerie, 2020). Since fertility can be influenced by the frequency of multiple matings, which is in turn influenced by adult density and sex ratios, understanding the implications of changes in demography is particularly important in conservation-dependent bird species which have managed meta-populations.

Age

Birds commonly experience reproductive senescence, with evidence for breeding performance and output decreasing or levelling off with age in passerines, raptors, waterfowl and cranes (McCleery et al., 2008; Bouwhuis et al., 2010; Hammers et al., 2012; Murgatroyd et al., 2018; Brown et al., 2019). The likelihood of breeding also declines in some species (Berman et al., 2009), but in others there is no apparent reproductive senescence (Zhang et al., 2014; Fay et al., 2020). In many wild species the effect of ageing on reproductive output has not been measured.

Mating experience

Although increasing age can reduce reproductive output, this can be offset by experience (DuVal, 2012). In some species more experienced males have greater reproductive success (Kokko, 1997; DuVal, 2012), and as a result females select for these individuals (Kokko et al., 1999; Jouventin et al., 1999). In some species, females mating with inexperienced males may mate with more individuals to offset the lower chance of fertility, and are more likely to produce mixed broods (Rivers and DuVal, 2019). In other species, female experience is unrelated to multiple paternity (Hess et al., 2012). Collectively, there is evidence to suggest that more experienced individuals may have a higher likelihood of producing a fertile clutch (Assersohn et al., 2021a).

Inbreeding

Matings between closely related individuals (inbreeding), which is expected to occur in species that have undergone severe and prolonged population bottlenecks (Bergner et al., 2014), can lead to increased homozygosity and subsequent negative fitness consequences (inbreeding depression; Charlesworth and Willis 2009). Reduced survival and reproduction, attributed to unavoidable inbreeding in small populations, can increase extinction risk by further driving population decline (O'Grady et al., 2006; Feng et al., 2019; Hammerly et al., 2016; Harrisson et al., 2019; Robinson et al., 2019). In birds, reduced fertility due to inbreeding can be attributed to fertilization failure or very early embryo death, yet most studies to date categorise all undeveloped eggs (identified by candling) as infertile (Hemmings et al., 2012a; Assersohn et al., 2021b). Furthermore, the mechanisms causing infertility and early embryo death are poorly understood.

Diet

Diet is an important factor in avian reproductive output (Selman and Houston, 1996; Klasing, 1998). Macro- and micro-nutrients including proteins, minerals and vitamins can affect both fertility and egg production, which is also influenced by under- or over-feeding (Assersohn et al., 2021a). However, examples of nutritional impacts on reproductive success in birds are dominated by those in captivity, particularly the poultry industry, and wild populations are much less well studied (Klasing, 1998; Assersohn et al., 2021a).

Factors potentially affecting fertility of kākāpō

Low productivity is one of the major issues affecting population recovery of the kākāpō (*Strigops habroptilus*), a critically endangered, nocturnal and flightless parrot which is endemic to Aotearoa/New Zealand. Infrequent breeding, high infertility and low hatching success have hampered conservation efforts (Clout, 2006), although intensive management increased the population from 51 in 1995 to approximately 200 individuals in 2021. Conservation management of kākāpō is led by the Kākāpō Recovery Programme (KRP) of the New Zealand Department of Conservation (NZDOC), in partnership with Ngāi Tahu, a Māori iwi (tribe).

High infertility is the major limitation to kākāpō population growth, with approximately half of eggs considered infertile from visual inspection (candling). However, a recent fluorescence microscope study from one breeding season showed that 72% of 'apparently infertile' kākāpō eggs were actually fertile, and instead failed due to very early embryo death (Savage et al., 2021). This supports findings from other studies which suggest that infertility may be commonly over-estimated and that very early embryo death may be more common than suspected (Hemmings et al., 2012b; Assersohn et al., 2021b).

In this study we conduct the first assessment of the relative impacts of multiple factors on kākāpō fertility, including behavioural considerations. In order to target this analysis, we first use the information from other species listed above to consider the facets most likely to influence fertility in kākāpō.

Inbreeding

The contemporary kākāpō population was founded by 35 individuals, from a total of 62 transferred to offshore predator-free islands in the 1980s (Powlesland et al., 1995). Genetic studies revealed first order relationships (e.g., full siblings or parent-offspring) amongst founders assumed to be unrelated (Bergner et al., 2014), and found low level of genetic diversity overall (Bergner et al., 2016; Dussex et al., 2018). White et al. (2014) found that decreased female heterozygosity was correlated with lower hatching success and smaller clutch size. However, the same study found that male heterozygosity had no impact on fertility, perhaps because most males with the lowest heterozygosity may not mate at all (White, 2012).

Rearing environment

Another potential factor influencing fertility is hand-rearing, which is widely practised in kākāpō conservation, with 52% of chicks hatched since 1981 having been hand-reared for at least 10 days. All breeding occurs in the wild, since kākāpō cannot be bred in captivity, but eggs are often incubated artificially to maximise hatching success, and chicks are removed from nests for hand-rearing when they would likely die if left in the nest.

There are examples of behavioural issues arising from hand-rearing in kākāpō, with two male chicks reared individually in 1997 and 1998 exhibiting imprinting behaviours. This issue appears to affect males more adversely than females, since some females have been reared alone without imprinting, but nevertheless all kākāpō requiring prolonged hand-rearing are no longer reared alone. Alterations in behaviour might have a particularly pronounced effect in the kākāpō breeding programme since the entire population lives in the wild, whereas other psittacine breeding programmes usually take place *ex situ* where behavioural problems can be more easily mitigated.

Multiple matings

Mate choice may also affect fertility in kākāpō. The species is polygynandrous, with multiple mates and repeated matings quite common. Mating is driven by female choice at leks and so polyandry is therefore apparently unnecessary, but this occurs in kākāpō as it does in other lekking species (Lebigre et al., 2007; Rivers and DuVal, 2019). Repeated mating is poorly understood in lekking species since it requires intensive monitoring (Kempenaers, 2020), but it provides a strong test of theories of polyandry (Rivers and DuVal, 2019; Parker and Birkhead, 2012) and may have implications for fertility (Zhang et al., 2019).

Age

As in other bird species, age may also influence fertility in kākāpō. Young age is certainly a barrier: the minimum age at which both sexes have been recorded mating is just under five years old, but although females at this age have produced fertile clutches, no males younger than seven have. Impacts towards the end of life are much less certain due to difficulty in ageing kākāpō. Kākāpō are long-lived, but there is currently no method of determining their age if hatch date is unknown (Horn et al., 2011). At the time of the current analysis (2021), 23 of the extant population were of unknown age, having been found as adults from 24 to 41 years earlier. All other individuals were less than 40 years old — much less than the expected lifespan. As calculated from the number of deaths over the total period of monitoring, the mean life expectancy in the managed population is estimated to be about 60 years (KRP, 2021, unpublished data) — but this is likely to be an underestimate. In an analysis of kākāpō which included the founders and assumed they were five years old at first capture, White (2012) found no impact of male age on egg fertility. However, age-specific impacts can be difficult to measure (Clutton-Brock and Sheldon, 2010), with additional difficulties in polygynous birds (Clutton-Brock and Isvaran, 2007).

Mating experience

As a lek-breeding species, there is a high skew in kākāpō reproductive success (Eason et al., 2006). This extends to mating attempts, particularly for males, with a small number of individuals dominating the total copulations. This effect is mitigated by some over-represented and some unproductive males being removed from the breeding population (Eason et al., 2006). Yet there is still a large variation in mating experience in the breeding population, and it is possible that this may affect fertility, as in other bird species (Kokko, 1997; DuVal, 2012). There is no clear evidence that females select for more experienced males, although this has not been quantified.

Diet

As part of conservation management, kākāpō are supplementary fed during breeding years to optimise productivity (Elliott et al., 2001; Clout et al., 2002). Although feeding does not influence breeding frequency, it does increase the proportion of females nesting and clutch size and leads to a higher likelihood of mothers successfully rearing chicks (Elliott et al., 2001; Houston et al., 2007). However, there is no evidence that it affects fertility, and it is not considered here for several reasons. As a supplemental food, it may not constitute a significant proportion of an individual's diet: nutrition impacts on productivity are considered less important in wild populations — even those in modified habitats (Jamieson, 2003). A study of kākāpō showed that energy requirements obtained from feeding stations ranged from 6% to complete substitution, but that supplements constituted on average about a third of daily metabolised energy of kākāpō on the main breeding island of Whenua Hou (Bryant and Bryant, 2006). There are also incomplete feeding records for some years, and even in years where consumption was recorded, sharing of feeding stations meant it was not always possible to determine how much each individual had eaten (Bryant and Bryant, 2006). In addition, in islands on which food was provided, there were very few individuals which were not fed, and so the data were highly imbalanced.

Other factors

Other factors which can affect productivity in birds include injury, disease, stress, hormonal disruption, pollution and climate change (Assersohn et al., 2021a,b), but these were not considered in the current study. Pollution can strongly influence production in bird species (Fritsch et al., 2019; Belskii and Belskaya, 2021) but is not likely to be significant in kākāpō, which all live on offshore island sanctuaries which have had little post-industrial human habitation. Although global heating may affect kākāpō through factors such as changes to habitat availability and tree masting cycles (Hacket-Pain and Bogdziewicz, 2021), these are not thought to be significant on the timescale of this study. Other influences such as injury, stress and hormonal disruption have not been able to be measured in the wild-ranging kākāpō population. Kākāpō are affected by a range of diseases, such as exudative cloacitis (Jakob-Hoff et al., 2009; Jakob-Hoff and Gartrell, 2010) and erysipelas (Gartrell et al., 2005), but there is no evidence that they impact reproduction. Aspergillosis severely impacted kākāpō nesting on one island in 2019, but this occurred after chicks had hatched and so did not affect fertility.

MATERIALS AND METHODS

Kākāpō management

Population transfers and breeding management

Remnant populations of kākāpō were discovered in Fiordland and on Rakiura/Stewart Island in the 1970s and translocated to predator-free island sanctuaries in the 1980s (Powlesland et al., 1995). Intensive breeding monitoring and management began in 1995, and breeding has since occurred on five of these refuge sites: Whenua Hou/Codfish Island, Hauturu-o-Toi/Little Barrier Island, Te Pākeka/Maud Island, Pearl Island and Anchor Island/Pukenui (Elliott et al., 2006). Translocations are used among these and other, non-breeding islands for demographic and genetic management purposes.

Kākāpō breed irregularly in response to mass-fruiting (masting) of certain tree species. In southern New Zealand, breeding is synchronised with the masting of the rimu tree (*Dacrydium cupressinum*), which occurs every 2–4 years (Harper et al., 2006). The number of females which nest and the number of eggs laid is proportional to the abundance of rimu fruit (Elliott et al., 2001; Eason et al., 2006; Harper et al., 2006). In some years the rimu masts but the fruit does not ripen, and in these years supplementary food is important in enabling mothers to sufficiently provision their chicks (Cottam, 2010).

Supplementary feeding is controlled through use of 'smart' feeding stations, which allow individual rationing and weight management. This was implemented to correct a male-dominant sex bias in the population, after it was discovered that heavier females produce more male offspring (Clout et al., 2002). As a result of this management, the female:male sex ratio of the entire population changed from 0.68 in 2000 to 0.95 in 2013 (KRP, 2021, unpublished data).

Kākāpō are the only parrot species with a lek mating system, in which males maintain a network of 'track and bowl' systems, usually at areas of higher elevation (Merton et al., 1984). They display from these sites with low-frequency, tonal 'boom' and frequency-modulated 'ching' calls (Kelman, 2017), and during breeding seasons females visit the leks to mate (Eason and Moorhouse, 2006). The mating system is polygynandrous, with both sexes sometimes mating with multiple partners. After mating, the female returns to her home range to nest, and the male plays no part in raising the brood.

Mating and nesting detection

Kākāpō are subject to intensive conservation management in order to maximise survival and productivity. Since 1995 nearly every kākāpō has worn a VHF transmitter to enable recapture and determination of location. Mating was originally detected by checking for mating sign at lek sites and nesting inferred by daily triangulation of adult females using VHF telemetry. Increasing use of remote sensing methods improved the quality of mating data and the efficiency with which these were collected. Proximity sensors were installed at lek sites from 1997 to record male presence, and from 2012 the transmitters were fitted with activity sensors to detect and record mating and nesting behaviour. These data were initially transmitted via coded pulses received by observers using telemetry units in the field. Then from 2016 the mating and nesting data activity on Whenua Hou and Anchor Island were monitored remotely via a radio frequency data network connected to the island base and internet.

The use of VHF transmitters ensured that all nesting attempts since 1994 were detected, except for a very small number of cases when a female's transmitter failed. The addition of activity sensors ensured that nearly all matings since 2014 were recorded. A small number of matings were not detected by the transmitters due to hardware failure or an unusual mating activity, but subsequent nesting was detected. In addition, paternity of all offspring since 1997 was determined from microsatellite genetic testing (Robertson et al., 2000) and in 2019 by genotyping by sequencing of blood samples taken from fertile eggs or chicks.

Artificial insemination has been attempted in kākāpō during every breeding season since 2005, primarily to override genetically-unsuitable matings. This is subject to significant logistical challenges, but in 2009 three chicks were produced by artificial insemination in two clutches — a first for a wild bird species. Subsequent attempts failed, until 2019 when three successful inseminations produced three chicks, of which one survived past fledging age (KRP, 2021, unpublished data).

Fertility assessment

Fertility of eggs was assessed by trained observers using 'candling': a hand-held torch was used to illuminate the egg and inspect for signs of development (e.g. embryo or blood vessels visible). This was conducted either in the nest or in an incubation facility, and was sufficient for detecting development from approximately four days after laying. Microscopic methods can detect earlier development (Savage et al., 2021), but these have only been conducted for a single breeding season for kākāpō, and so could not be used in the current study which spans multiple years. As a result of using apparent fertility in our analyses, approximately a quarter of the eggs (Savage et al., 2021) in which embryos died at very young age (before four days) will have been classed as infertile.

Nest management

Kākāpō lay 1–5 eggs per clutch, and typically two or three. Since 1997, most eggs (73%) were removed for artificial incubation, to maximise hatching success. A day or two before or after hatching, the eggs or chicks were returned to nests where possible, and closely monitored. The weight and health of each chick was recorded each night for the first week, with frequency of checks decreasing with age. Chicks were frequently cross-fostered among nests to maximise the number and growth of chicks in nests. As as result, each chick may have had several foster mothers and usually was not raised by its biological mother. Chicks fledged from nests at a mean of 73 days, but were still checked regularly until they were independent at around 219 days (Farrimond et al., 2006).

Hand-rearing

Artificial hand-rearing of kākāpō chicks was required due to health issues or if there were insufficient numbers of nests available (Eason and Moorhouse, 2006). In years when the rimu fruit didn't ripen and there was therefore less natural food, each nesting female could usually support only one chick, and surplus chicks were hand-reared. Where possible, chicks were reared on islands and then returned to nests, but some chicks required hand-rearing until fledging age. This long-term hand-rearing took place at a mainland facility.

Hand-reared chicks were placed first in brooders, then as they grew were kept in plastic tubs before being moved to open-topped pens with an area of approximately 3–6 m^2. To avoid imprinting, chicks were not penned individually where possible, particularly males, and were usually kept in groups of 2–6. Chicks requiring all of their developmental period in captivity were normally returned to the kākāpō islands at an age of 80 days, where they were weaned in large outdoor pens before being released into the wild at an approximate age of 119 days.

Data collation

All data were collected as part of routine kākāpō conservation management conducted by NZDOC, and so this study was exempt from animal ethics approval required under NZDOC's obligations to the New Zealand Animal Welfare Act (1999).

Clutch data

Clutch data were collated from the KRP database for the breeding years between 1981 and 2019 (Table 1 and Data S1). The database contained all observed events for each individual, including transmitter activity data, captures, health checks, feeding records as well as mating attempts. These were combined with fertility data collated in a separate spreadsheet, which contained information for each clutch since management began in 1981. This recorded clutch size, number of fertile eggs (apparent fertility), number of eggs hatched, and the number of chicks fledged, as well as the potential and confirmed paternity. Data prior to 1990 were excluded from the analysis since there was insufficient information for each nesting attempt. This yielded an initial data set of 242 clutches.

This data set contained first ($n = 174$), second ($n = 31$) and third ($n = 1$) clutches. Kākāpō will naturally re-nest if a nest fails early enough, and double clutching is used as a management method to improve productivity.

Year	Island	Clutches	Matings	Fertile eggs	Infertile eggs	Hatched	Fledged
1981	Rakiura	2	0	4	0	4	3
1985	Rakiura	3	0	3	6	2	0
1990	Hauturu	2	2	2	1	2	0
1991	Hauturu	4	3	6	2	4	2
1992	Whenua Hou	4	1	9	2	6	1
1993	Hauturu	2	3	1	3	1	0
1995	Hauturu	2	2	0	5	0	0
1997	Whenua Hou	6	6	7	5	4	3
1998	Maud	1	1	3	0	3	3
1999	Pearl	8	8	11	5	8	6
2002	Whenua Hou	24	34	42	25	26	24
2005	Whenua Hou	10	16	11	15	6	4
2008	Whenua Hou	5	12	10	0	8	6
2009	Whenua Hou	28	52	54	18	36	33
2011	Anchor	1	0	2	0	0	0
2011	Whenua Hou	8	13	14	4	11	11
2014	Hauturu	1	3	3	0	2	2
2014	Whenua Hou	7	14	6	9	5	4
2016	Anchor	22	32	32	38	21	15
2016	Hauturu	2	4	1	2	0	0
2016	Whenua Hou	20	31	30	19	26	20
2019	Anchor	37	60	56	67	42	37
2019	Whenua Hou	43	64	63	66	44	36
Total		242	361	370	292	261	210

Table 1. Breeding attempts since modern records began in 1981. Only data after 1990 were used in this study, since in previous years breeding information was incomplete. Note that fertility reported here is apparent fertility; not true fertility. This is the full dataset; some of these clutches were excluded from the fertility model. See text for further details.

Paternity assignment

Confirmation of paternity from DNA was available for 122 out of all 242 clutches since 1990. Of the 120 which did not have DNA paternity, 28 clutches were excluded for which multiple males were confirmed or assumed to have mated with the female, or the number of males was unknown — because in these cases the father couldn't be identified. From the 214 clutches remaining, seven with mixed paternity and/or produced by artificial insemination were excluded, since the clutch wasn't the product of a single mother and father. A further clutch without any eggs was also removed, leaving 206 clutches from a total of 59 females and 51 males.

Rearing status

For each clutch, the hand-rearing history of the mother and father was established from database records. Kākāpō were assigned as hand-reared if they had spent more than 10 days being hand-reared, at any period of their development; otherwise they were classed as wild-reared. This threshold was chosen to distinguish individuals which were only hand-reared for a short period of time: many chicks are hand-reared for just a few days to enable them to recover from ill health or weight loss. This is particularly common between the ages of two to three weeks, when chicks fed by mothers receiving supplementary food often require removal to hand-rearing for a change of diet for up to five days. There were 110 kākāpō mothers and fathers in the filtered data set of 206 clutches, of which 57 were hatched after intensive management and hand-rearing began. Of these, 18 (32%; 11 females and 7 males) were hand-reared for less than one day; 3 (5%; 1 female and 2 males) were hand-reared for between 1–10 days, and 36 (63%; 25 females and 11 males) were hand-reared for more than 10 days.

Age assignment

The ages of the male and the female producing the clutch were calculated from hatch dates if these were known. The kākāpō of unknown age comprised 17 of the 59 females and 23 of the 51 males which contributed to the 206 clutches. The ages of these individuals were estimated by assuming their hatch date was ten years prior to the discovery date, which is a typical age of first breeding for males and females. Although the inclusion of the kākāpō of unknown age introduced errors due to inaccuracies in the estimated ages, these would have been relatively small compared to the absolute ages, and the alternative of omitting these individuals would have rendered the data set too small for robust analysis. This age assumption results in the oldest kākāpō breeding at 48.5 years of age (Fig. S1), which is younger than the current best estimate of mean life expectancy in the contemporary managed population of 60 years. However, the remnant populations from which the unknown age kākāpō were sourced were under extreme predation pressure (Karl and Best, 1982; Atkinson and Merton, 2006), so would be expected to have had shorter life expectancies than the current protected population.

Previous matings

A mating history for each kākāpō was obtained from records of mating sign at display sites (‘track and bowls’), genetic paternity analysis, and electronic mating detections. This provided an estimation of the number of matings for the clutch mother and father, prior to the mating which yielded the clutch. This number was a lower limit, since not all matings were detected — even with the electronic mating detection system — and since it was assumed that all founder individuals had not previously mated at the time of their discovery. This was clearly an underestimate, but unavoidable given the lack of observation data prior to their discovery.

Parental kinship

Pairwise kinship for all male-female combinations of living and recently-deceased kākāpō were obtained from a pedigree generated from the kākāpō studbook in `PMx` (Lacy et al., 2012). To address the assumption of founders being equally unrelated to one another (Ballou, 1983), founder relatedness was incorporated into the kākāpō studbook using genomic-based estimates of relatedness. In this process whole genome resequencing data from 169 birds was used to discover SNPs using the reference-guided Deep-Variant pipeline (Poplin et al., 2018). A stringent filtering protocol using BCFTools (Li et al., 2009) and VCFTools (Danecek et al., 2011) was applied to include biallelic SNPs with a minimum coverage of three, a maximum coverage of 100, a minimum Phred quality score of 10, a genotyping rate $> 90\%$, a minor allele frequency of 0.05, and pruning for linkage disequilibrium with an r^2 of 0.8 and a sliding window of 1000 sites. This filtering resulted in 8,407 high confidence markers with high depth (average $= 19.88 \pm 8.08$SD) and low missing data (average $= 0.0002 \pm 0.0001$ SD) across individuals. Initial testing was performed to evaluate estimators for accuracy and precision with mother-offspring relatedness, including: KING (Waples et al., 2019), estimated through the package NGSrelateV2, Hanghøj et al. 2019), KGD (Dodds et al., 2015), KGD with a correction for self-relatedness (as per Galla et al. 2020, Rxy (Hedrick and Lacy 2015, estimated through NGSrelateV2), and TrioML (WANG 2007, estimated through the R program `related`, Pew et al. 2015). `Rxy` was chosen as the best relatedness estimator, given its high accuracy for mother-offspring relatedness and the benefit of bounding between 0 – 1 for ease of entry into `PMx`

(Lacy et al., 2012). Final relatedness estimators were calculated between the 35 founders identified in the kākāpō studbook and were incorporated into `PMx` as kinship (half of the relatedness value). Parental kinship for the clutches in this study were produced in `PMx` using the founder-corrected studbook. These values were in the range 0 – 0.265, with a distribution that was positively skewed (Fig. S1).

Statistical analyses

Bayesian model structure

A Bayesian generalised linear mixed model was used to assess factors contributing to clutch fertility. The explanatory variables considered to have potential effects on clutch fertility were chosen from data exploration and knowledge of kākāpō ecology. These were: the age and hand-reared status of both mother and father; the number of matings and the number of different males the female mated with to produce the clutch; the parental kinship; and the male's previous mating experience (number of previous matings; Table 2). As previously noted, other parameters which can affect fertility in other species, such as diet, stress, injury, pollution and disease, were not included.

The mother and father hand-rearing status were set to a binary variable: one if the individual had been hand-reared for more than 10 days and zero otherwise. The number of mates/matings for the female was a categorical variable with three levels: one mating with one male, more than one mating with the same male, and multiple mates. The value 'multiple mates' included clutches in which a female mated with different males more than once ($n = 12$). Parental kinship was a continuous variable in the range 0 – 0.265. Mating experience was defined as the number of previous matings detected, prior to the matings which produced the clutch. This was calculated for both the clutch mother and father.

The numeric explanatory variables were scaled and centred to have mean of one and standard deviation of 0.5 (Gelman et al., 2008); the categorical variables were defined as factors. No interactions of the covariates were considered relevant. Collinearity of predictors was examined with correlation plots and paired posterior plots: no significant correlation among predictors were found, so none were excluded.

The response variable was the binary fertility status of each clutch (0/1), with a Bernoulli error distribution. This was used instead of the proportion of eggs fertile in a clutch, because the fertility of each egg was not independent of the fertility status of others in the clutch (Fisher exact test for association between categorical variables, $p < 0.001$).

Random effects were included for male and female identity to account for pseudo-replication, and for year, to account for unmeasured environmental variation. No effect was included for island, since this predictor was highly imbalanced, with two of the five breeding islands dominating the number clutches: Whenua Hou (126) and Anchor Island (52) produced 90% of the clutches.

Component	Variable	Type	Values (frequency)
Response	Clutch fertility	Binary	0 (66) / 1 (131)
Fixed	Mother hand-reared	Logical	true (54 clutches; 25 females) / false (143 clutches; 34 females)
	Father hand-reared	Logical	true (36 clutches; 11 males) / false (161 clutches; 38 males)
	Mother age (years)	Continuous	range = 4.8 – 48.5, mean = 21.4, median = 18.7
	Father age(years)	Continuous	range = 4.8 – 43.4, mean = 23.0, median = 22.9
	Number of matings/males	Categorical	1 mating (104) / $>$ 1 mating 1 male (50) / Different males (43)
	Female previous matings	Integer	range = 0 – 17, mean = 4.4, median = 3
	Male previous matings	Integer	range = 0 – 33, mean = 6.6, median = 5
	Mother/father kinship	Continuous	range = 0 – 0.265, mean = 0.0188, med = 0.0072
Random	Mother	Categorical	59 individuals, 1 – 9 repeats, mean = 3.3, median = 3
	Father	Categorical	49 individuals, 1 – 16 repeats, mean = 4.0, median = 3
	Year	Categorical	range = 1990 – 2019, 16 levels

Table 2. Model predictors. Parameters used in the Bayesian model relating clutch fertility to mother and father characteristics. See Fig. S1 for distributions of the numeric variables.

Observations with missing values for any of the predictors were excluded. From the initial set of 206 clutches, the final model data contained 197 clutches with complete values for all eleven input variables. This resulted in an overall number of events per variable of 17.9: greater than the minimum of 10 – 15 recommended for linear regression modelling (Heinze et al., 2018).

Bayesian model variable selection

Small datasets are common in threatened species research, leading to statistical challenges such as low precision, low accuracy and instability masking true relationships between variables (Garamszegi, 2016). To prevent the model from overfitting to the data due to the large ratio between number of parameters and number of observations, it is often necessary to limit the number of variables in the model (Heinze et al., 2018). Methods such as penalized regression and shrinkage priors are commonly used to this effect (Piironen and Vehtari, 2017b; Vehtari et al., 2017; Erp et al., 2019; Carvalho et al., 2010; Hastie et al., 2015; Narisetty and He, 2014). However, these methods do not really produce truly sparse solutions, as every variable has a non-zero probability of inclusion. Instead, we applied projection predictive variable selection (Piironen et al., 2020; Catalina et al., 2020), which effectively selects a subset of variables from a previously fitted reference model. This method ranks the variables in order of their contribution to the model predictions, replacing the posterior of the model with a constrained projection which provides predictive performance equivalent to the full model (Piironen et al., 2020; Catalina et al., 2020), as measured by the Kullback-Leibler divergence of their predictions (Goutis, 1998). Projection predictive variable selection has been shown to outperform other more established variable selection methods (Piironen and Vehtari, 2017a). Furthermore, it can be applied not only to generalised linear models, but also to generalized linear and additive multilevel models, allowing the projection of random (additive) effects.

In order to rank the variables during model search, projection predictive variable selection uses forward search for multilevel or additive models and a faster L1-like heuristic for generalised linear models. Since the model structure included random effects per individual in the sample, we restricted the search to first select the fixed effects, and only then added the random effects. This was to ensure that the predictive variance would not be completely saturated by the individual random effects and properly measure the effect of the biologically-relevant terms.

Bayesian model execution and validation

All analyses were conducted in `R` (version 4.0.3; R Core Team 2020), with the Bayesian model implemented in `R` package `brms` version 2.15.0 (Bürkner, 2017) and projection predictive variable selection applied with package `projpred` version 2.0.5.9 (Piironen et al., 2020). A regularised horseshoe prior was used (Piironen and Vehtari, 2017b), with one degree of freedom for the student-T prior for the local and global shrinkage parameters, and a scale of one for the global shrinkage and regularisation parameter (Bürkner, 2017). The model was run with four chains, with 15,000 iterations and 15,000 warm-up iterations per chain. Model code and results are available in Data S1.

Projection predictive variable selection was then used to provide a reduced model with equivalent predictive performance to the full model. The variables included in the reduced model were selected by the improvement they provided to the model. As criteria for the selection of variables we checked the ELPD improvement and each variable's marginal posterior, and selected those whose posterior mass was clearly non-zero and whose ELPD improvement was significant.

Model validity was assessed by Pareto k estimates (Vehtari et al., 2017, 2019), and by graphical residual and posterior predictive checks using the `bayestestR` package (Makowski et al., 2019b). The relative influence on clutch fertility of each predictor was assessed by the amount of intersection of the full posterior distribution of the constrained projection with the region of practical significance (ROPE; Makowski et al. 2019a,b). This region of "practically no effect" provides an equivalence test for the "importance" of a parameter, based on the proportion of the posterior which overlaps the ROPE. This is quantified by the probability of direction (p.d., the certainty of the direction of the effect) and probability of significance (p.s., the proportion of the distribution outside the ROPE). If there were values of the distribution both above and below the ROPE, the probability of significance was reported as the higher probability of a value being outside the ROPE. A range of [-0.18, 0.18] was used for the ROPE, as recommended for logistic models (Kruschke and Liddell, 2018).

Multiple matings and population density

In addition to the Bayesian fertility model, we also investigated the incidence of multiple mating with kākāpō abundance. We merged repeated matings with one male and matings with different males into a single category of 'multiple mating', in order to retain sufficient sample sizes. We correlated the proportion of clutches produced by multiple matings with the number of adult females and male kākāpō, and the adult sex ratio, for Whenua Hou for each year since 1990. This analysis was confined to a single island to avoid inter-island effects, and Whenua Hou was chosen as it produced a large proportion of all clutches from 1990 – 2019 (64%). Correlations were assessed using the `correlation` package (Makowski et al., 2020) in `R`, using the Pearson correlation coefficient and Holm adjustment method (Holm, 1979).

RESULTS

Factors affecting fertility

Projection predictive variable selection in the Bayesian mixed model showed that of the fixed terms, the multiple mating variable explained most of the variance of the model, followed by male hand-rearing status (Fig. 1). These were the only two fixed terms which had projected posterior distributions unlikely to be zero (Fig. 2). All other fixed terms had negligible impact on the model fit, and had projected posterior distributions likely to be zero (Figs. 1 and 2). Of the random terms, father and mother identity contributed most significantly to the variance, with father identity the most important of all fixed and random parameters. Random effects dominating fixed effects is common in mixed models, and can obscure the underlying fixed model structure. It suggests that there was substantial variation in the model due to individual effects which were not captured by the fixed variables. A reduced model containing number of mates/matings, father hand-rearing status and random terms for father, mother and year provided predictive performance equivalent to the full model (Fig. 3).

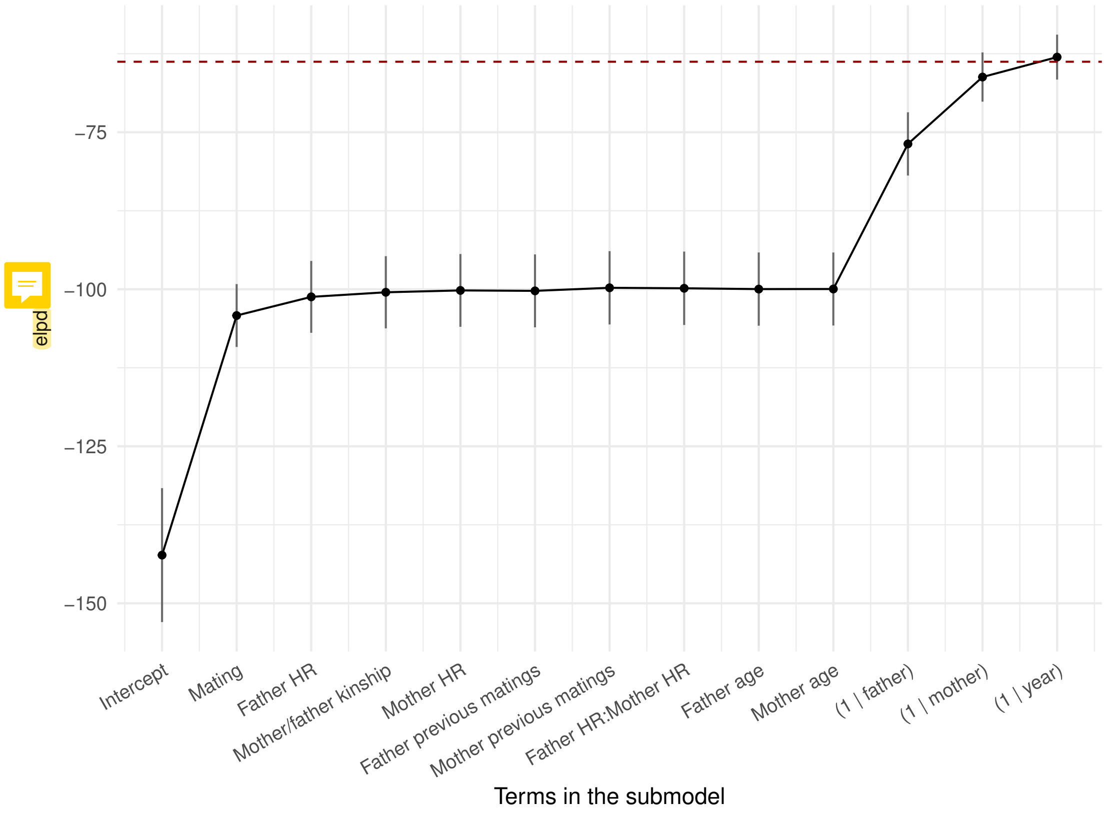

Figure 1. Projection predictive variable selection results. The contribution to the expected log predictive densities (ELPD) from each model parameter. Fixed terms are ordered in their contribution to the model variance, with random terms selected last. The dashed line shows the ELPD for the full model. The reduced model containing fixed parameters of mating and father rearing, and random terms of father, mother and year, provided equivalent predictive performance to the full model. HR = hand-rearing.

Clutches in which females mated with different males were associated with the highest increase in fertility (p.d. = 1.00; p.s. = 1.00 in the reduced model), followed by clutches in which females mated more than once with the same male (p.d. = 0.86, p.s. = 0.70; Fig. 3). Hand-reared fathers were associated with lower clutch fertility (p.d. = 0.93, p.s. = 0.88). The remaining fixed terms of mother rearing status, mother and father age, genetic relatedness of the parents, and female and male mating experience were not included in the reduced model as they all had a very low impact on clutch fertility compared to male hand-rearing status and female number of matings and mates.

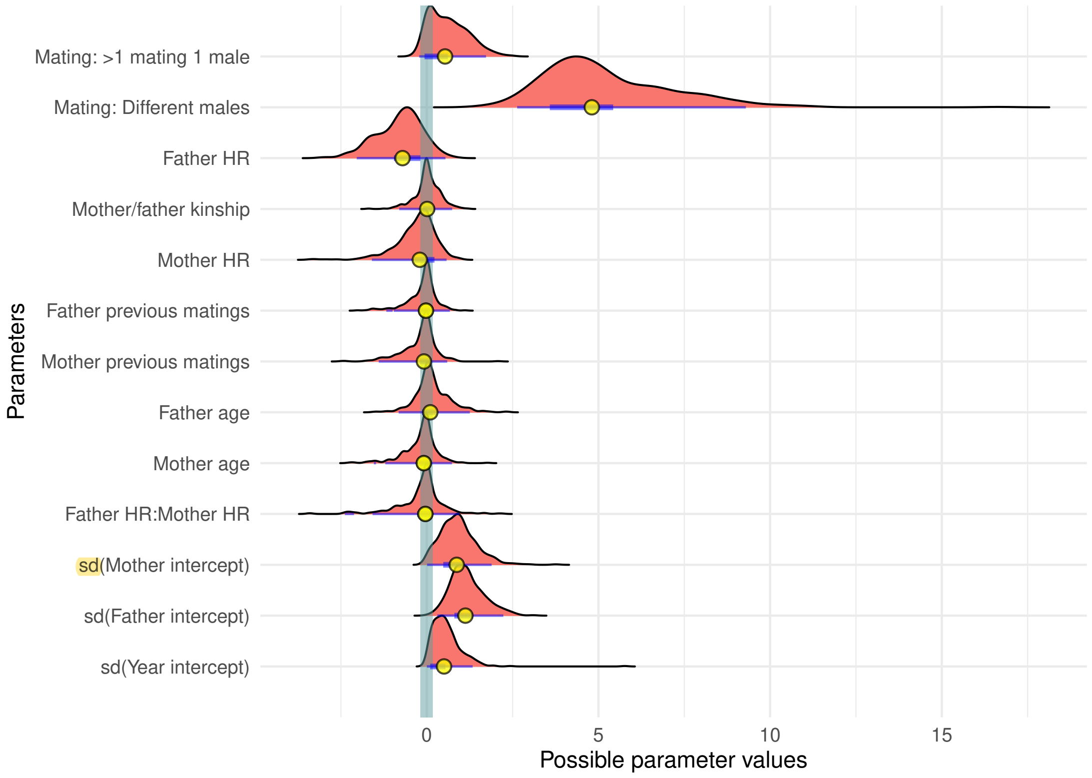

Figure 2. **Posterior coefficient distributions of the coefficients for the full projected model.** The less of the posterior distribution which intersects the ROPE (shaded vertical bar), the stronger the association of that parameter on fertility. Posterior medians are shown by filled yellow circles, with thick and thin horizontal blue bars denoting the 50th and 95th percentiles respectively. Of the fixed effects, only the female multiple mating behaviour and father hand-rearing parameters had posteriors likely to be non-zero. The multiple mating variable is split into its factor levels, with the reference level a single mating. For rearing, wild-reared (not hand-reared) is the reference level. Considering the posteriors and the projection predictive variable selection results, only these two fixed parameters were retained in the reduced model. HR = hand-rearing.

Model predictions showed that females mating with multiple males had a high probability of producing a fertile clutch, and that those mating with a single male more than once had a higher likelihood of clutch fertility than those mating just once (Fig. 4). Regardless of the number of matings and mates for a female, mating with a hand-reared male decreased the likelihood of clutch fertility.

Multiple matings and kākāpō density

The likelihood of females engaging in multiple matings (either with the same male or different males) was strongly positively correlated (Pearson correlation, $r = 0.92, p < 0.001, t = 6.46$, d.o.f. $= 8$) with the size of the adult female population on Whenua Hou from 1990 – 2019 (Fig. 5). The association between multiple matings and male abundance was much weaker (Pearson correlation, $r = 0.61, p = 0.062, t = 2.17$, d.o.f. $= 8$), but there was a strong correlation between multiple matings and the female:male sex ratio (Pearson correlation, $r = 0.89, p < 0.001, t = 5.61$, d.o.f. $= 8$).

There was substantial variation in multiple mating behaviour among females. Of the 59 females in the model data set, 28 (47%) mated with different males at least once, 27 (46%) had repeated matings with

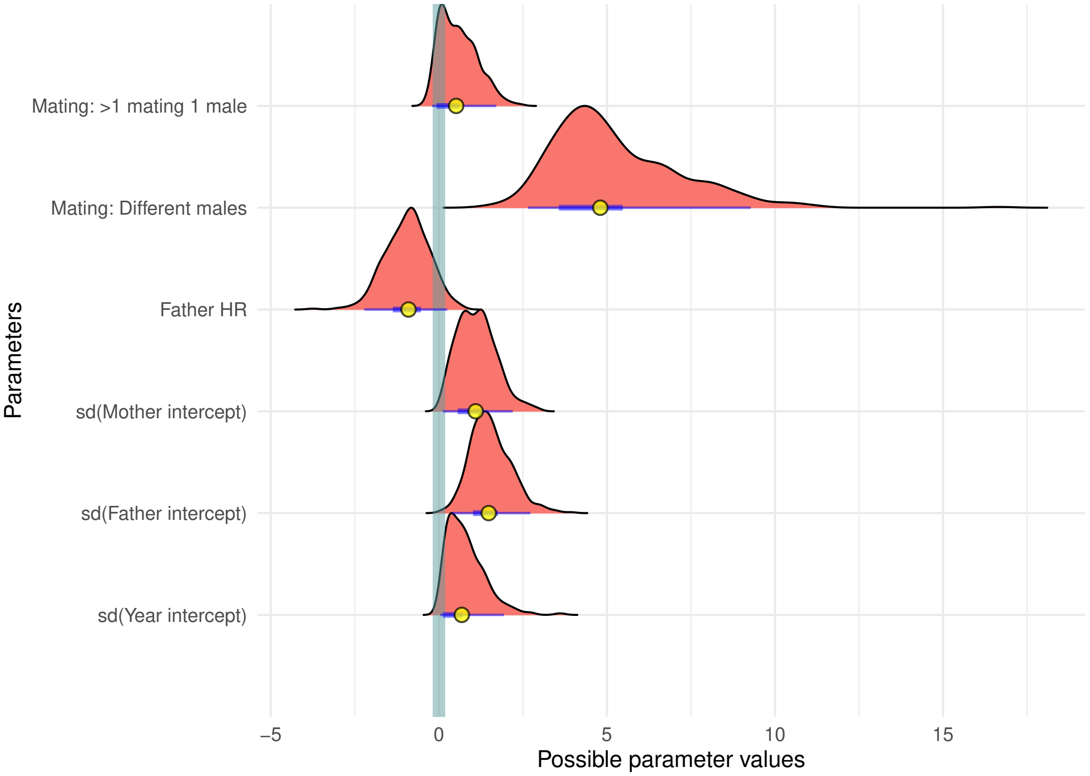

Figure 3. **Posterior distributions of the coefficients for the projected reduced model**. This model contained the two fixed effects with the highest variance contribution: female multiple mating behaviour, and father hand-rearing status. Compared to the reference level (not shown) of a single mating, the projected posterior distribution for females mating with different males was very strongly positive (p.d. = 1.00) and fully outside the ROPE (vertical shaded bar; p.s. = 1.00), indicating a very strong positive effect on clutch fertility. For clutches in which females mated with the same male more than once ('> 1 mating 1 male'), the parameter distribution was strongly positive (p.d. = 0.86, p.s. = 0.70). Compared to wild-reared fathers (the reference), hand-reared fathers were much more likely to be associated with lower clutch fertility (p.d. = 0.93, p.s. = 0.88). Symbols as in Fig. 2.

the same male at least once, and 52 (88%) produced at least one clutch from only a single mating.

DISCUSSION

Low productivity is one of the main obstacles to recovery for the critically endangered kākāpō. Using all available reproductive data for the species, this study shows that the dominant factors affecting clutch apparent fertility are female mating behaviour, in terms of the number of matings and number of males, and male hand-rearing status. Fertility was lower in clutches produced by a hand-reared father, higher if the female mated more than once with one male, and highest if the female mated with different males.

Rearing environment

This is a rare demonstration of hand-rearing affecting productivity in a bird species. In fact, evidence of similar effects across *all* taxa is extremely limited, in contrast to examples of the impact of captivity or rearing method on other traits such as survival (Farquharson et al., 2018). This is likely a result of the difficulty of measuring these effects, which usually requires longitudinal data of reproductive success across several generations (Clutton-Brock and Sheldon, 2010). This is compounded by a strong bias in fertility studies towards commercial bird species, and a tendency to focus on male reproductive issues (Assersohn et al., 2021a).

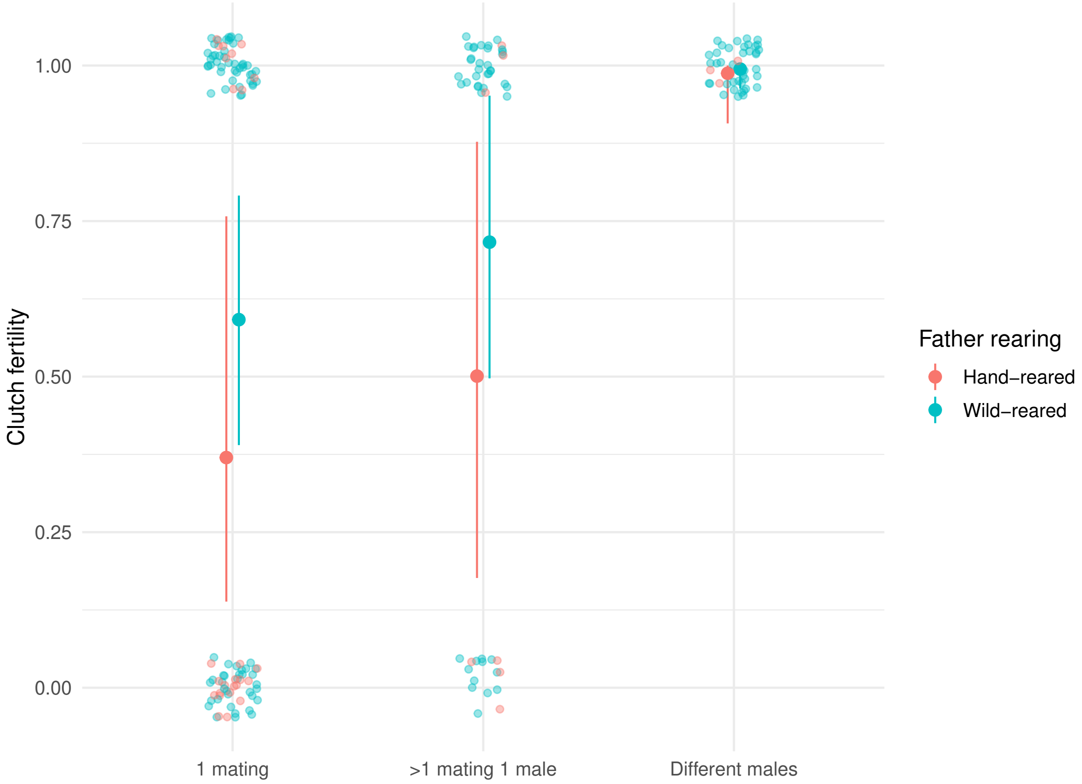

Figure 4. Fertility model predictions for the interaction of female multiple mating and male rearing status. Predictions from the reduced model of the likelihood clutch fertility with the number of mates and matings of the female, and the father rearing environment. Model predictions are shown as filled circles, with 95% highest posterior density intervals denoted by vertical bars. Filled circles denote the observational data, coloured by rearing status and jittered along both axes for clarity.

Examples from other taxa suggest that the reason for hand-rearing affecting fertility in kākāpō is likely to be behavioural. In primates, lack of access to conspecifics lowers reproductive output through suspected behavioural mechanisms (King and Mellen, 1994; Beck and Power, 1988; Hampson and Schwitzer, 2016). There is also qualitative evidence that hand-rearing affects aspects of kākāpō behaviour related to reproduction, with one individual hand-reared alone in 1997 (from three to 15 weeks of age) apparently unable to mate as a result of strong imprinting (KRP, 2021, unpublished data). Another male hatched in 1998 was also hand-reared individually for the same period and is partially imprinted, attempting to mate with humans, and although is able to mate with kākāpō, has not yet naturally produced fertile eggs. These imprinting behaviours appear to most strongly affect male chicks: females have been similarly hand-reared alone without any observed negative reproductive impacts, although these may be less immediately apparent (Harper and Joice, 2006). As a result, kākāpō chicks are not hand-reared separately from other individuals, unless it is unavoidable due to particular health issues, in which case the time that they are hand-reared without conspecifics is minimised.

The impact of the timing and length of hand-rearing on male kākāpō fertility could not be investigated in this study due to the limitations of the data set. Examples from other species demonstrate that even a short hand-rearing period may influence behaviour. In raptors, imprinting or even partial imprinting can affect pair behaviour and therefore reduces egg fertility (Jones, 2008; Lierz, 2008). Male falcons reproduce less effectively if reared by hand for more than the first week of their life (Lierz, 2019). Whereas it is clear that a fully hand-raised bird might not be able to reproduce with conspecifics, there is uncertainty over the impact of shorter hand-rearing periods. It is feasible that any time during the development period that an individual is not raised by conspecifics might later lead to behavioural alterations (Irwin and Price, 1999). Assessing whether there is a particular kākāpō life stage at which the impact of hand-rearing is

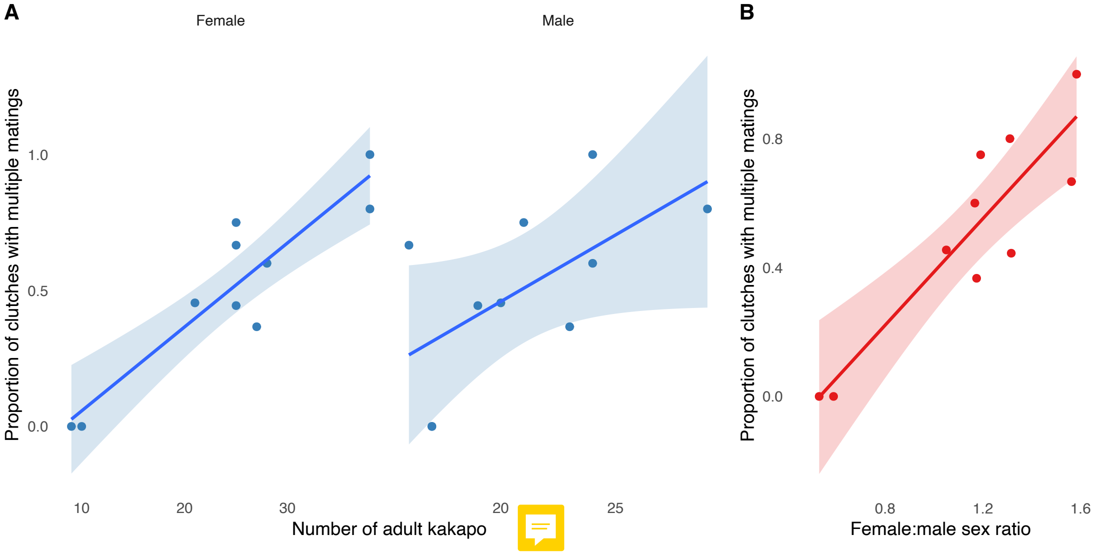

Figure 5. Multiple matings rates with the number and sex ratio of adult kākāpō. The association between the incidence of multiple matings with (A) female and male kākāpō abundance and (B) female:male sex ratio, on Whenua Hou from 1990 – 2019. The likelihood of multiple matings was strongly correlated with increasing female abundance ($r = 0.92, p < 0.001, t = 6.46, \text{d.o.f.} = 8$) and female:male sex ratio ($r = 0.89, p < 0.001, t = 5.61, \text{d.o.f.} = 8$), and moderately correlated with male abundance ($r = 0.61, p = 0.062, t = 2.17, \text{d.o.f.} = 8$).

most pronounced should be a focus for future analyses when sufficient data are available.

Hand-rearing reduces reproductive output in takahē (*Porphyrio hochstetteri*, a threatened endemic New Zealand rail), although it does not affect fertility. Hand-raised individuals fledge approximately 50% fewer offspring than their wild-reared counterparts, even though egg fertility is similar (NZDOC, 2014, unpublished data). This suggests that hand-reared takahē have reduced chick-rearing ability and that a behavioural mechanism is responsible. It supports the hypothesis that behavioural changes are responsible for the association between hand-rearing and clutch infertility in kākāpō.

The evidence for negative impacts of hand-rearing on kākāpō fertility may have profound consequences for the conservation of the species. Hand-rearing is a key part of management, used to prevent loss of chicks which would naturally have died through starvation or ill health. More than half of the 261 chicks hatched since 1981 have been hand-raised for at least 10 days, usually in the first four weeks. Hand-rearing has made the strongest contribution to population growth than perhaps any other management method. There have been no other apparent negative effects of this management to date: from 32 hand-reared females which have bred, 25 (78%) have fledged chicks, and all that have hatched chicks have fledged some.

Steps are already taken to avoid imprinting in kākāpō: chicks are reared alone, are only hand-reared if there is no alternative and are released from captivity soon after weaning. But the additional impact on fertility identified here may add greater pressure to avoid hand-rearing of males. This is at odds with the current management policy which prioritises leaving female rather than male chicks in nests, since the availability of breeding-aged females is considered one of the primary factors limiting population growth.

Multiple matings

Mating with different males

This study shows that female mate choice — in terms of the number of copulations and males mated with — has a significant effect on clutch fertility in kākāpō. To infer the conservation management implications, we first consider the consequent inferences on polyandry in this lek species.

The increase in clutch fertility from matings with different males supports the hypothesis of direct benefits from polyandry (Reding, 2014). With no male parental care in the kākāpō as a lek species, there are no clear benefits from increased access to resources from multiple mates. Therefore, improved fertility is instead likely to be the key driver for polyandry (Birkhead et al., 1987). This is apparent in other species:

for example, in the passerine blue tit (*Cyanistes caeruleus*), extra-pair copulations ensure a higher fertility when the partner is infertile (Santema et al., 2020). This effect is also likely to apply to lekking species, as mating with one single male, which might be infertile, has a higher risk of clutch infertility.

Competition between sperm from different males in the female reproductive tract might also be important for increasing egg fertility in kākāpō through post-copulatory sperm selection (Birkhead et al., 1987; Pizzari and Birkhead, 2000; Calhim et al., 2008; Santema et al., 2020). Evidence supporting this "sperm competition hypothesis" in kākāpō is provided by sperm morphology. Carballo et al. (2019) demonstrated that parrot species which are gregarious, sexually dichromatic and/or have a high level of extra-pair paternity all have longer sperm, and thus a higher level of sperm competition, than monogamic psittacine species. Their results therefore support the hypothesis that variation in sperm morphology is driven by sperm competition in psittacines, as it is in passerines. Interestingly, they also demonstrated that kākāpō sperm morphology is longer than many other parrots, and in the range of species with a high level of sperm competition. This suggests that the kākāpō has a naturally high level of sperm competition, which is in accordance with their polyandrous lek breeding system.

Further support for the sperm competition hypothesis driving female kākāpō to mate with multiple males is provided by the incidence of mixed paternity broods. Under the hypothesis, mating with multiple males should be common, but mixed-paternity within broods should be rare (Rivers and DuVal, 2019). This is the case for kākāpō: only 2% (one out of 48) of multiple matings resulted in broods with mixed paternity.

Mating with multiple males may also be a result of mate guarding, which is common in polygamous species (Birkhead and Montgomerie, 2020). In a mating system driven by female choice, it could be expected that since females can assess male quality before mating, there would be little driver for mating with multiple males (Balmford, 1991). However, if mate guarding takes place, then copulations with multiple males can result from females having to 'wait' to mate with their preferred male, and mating with a non-preferred male first (Petrie et al., 1992). There is also observational evidence that kākāpō mate guard: at least 13 females have been detected at the display sites of males either the night before and/or after mating (KRP, 2021, unpublished data).

The correlation of the likelihood of multiple matings with increasing female:male sex ratio supports the hypothesis that female kākāpō mate guard. As the threat of competition for mates grows with a changing sex ratio, there may be more mate guarding through monopolisation of preferred males with repeated matings, and subsequently more instances of females mating with different males when their preferred choice is not available (Petrie et al., 1992). Similar variations in mate guarding behaviour with changing levels of competition from varying sex ratio are evident in other species (Grant and Grant, 2019; Birkhead and Montgomerie, 2020).

Multiple matings with the same male

The kākāpō reproductive data provide a rare opportunity to assess fertility benefits of females mating repeatedly with the same male. Close observation of individual mating behaviour is rare in wild bird species, so there have been limited opportunities to assess the impact of repeated matings to help determine the reason for this behaviour. Some of the hypotheses for repeated matings require a pair bond or paternal investment, which are not present in kākāpō (Hunter et al., 1993). Remaining explanations include: to reduce the likelihood that the male can mate successfully with other females, to devalue the sperm from an inferior male, or by increasing fertility through a higher likelihood of the female receiving sufficient sperm (Petrie et al., 1992; Heeb, 2001; Hunter et al., 1993). The first of these hypotheses is less likely to apply to kākāpō, since males mate relatively infrequently, despite apparently having the capacity to do so more often. The second explanation is not supported by examples in which female kākāpō mate only with one male, or with one male before and after a second ($n = 6$). The final explanation, the increased fertility hypothesis (Birkhead et al., 1987), is supported in flycatchers, in which repeated inseminations from the same individual increased the number of sperm reaching the perivitaline layer (PVL; Torok et al. 2003). Savage et al. (2021) provided evidence for this in kākāpō, reporting that multiple matings may increase sperm reaching the PVL. There is not accordance across all species: Rivers and DuVal (2019) reported that the number of matings did not affect reproductive success in a lek-breeding bird, and Hunter et al. (1993) found no evidence to support the hypothesis from a review of several species. However, our observed association of higher kākāpō clutch fertility with multiple matings, together with that of Savage et al. (2021), suggests that the fertility assurance hypotheses for multiple matings applies to kākāpō.

Mate guarding can also explain the instances in which females mate with the same male multiple times. With competition for preferred males, female kākāpō may monopolise their preferred mate with repeated courtship and matings, as hypothesised in other lekking species (Petrie et al., 1992). That study reported approximately half of feral female peahens engaged in multiple matings, which is the same proportion as we found in kākāpō (47%).

Conservation implications of multiple mating effects

From their sperm morphology, mating system and our finding of lower fertility from single matings, we speculate that it is usual for female kākāpō to copulate multiple times and with multiple males. The current situation in which females usually mate with just one male (53% of clutches) may therefore represent an abnormal state.

This situation may be the result of management practices, in which the density of kākāpō on breeding islands (15 – 20 ha/bird; Whitehead et al. 2011) has been limited to reduce the likelihood of male deaths from fighting, to ensure sufficient habitat for females, and to reduce nest interference. If the subsequent density of kākāpō was lower than their natural state, particularly for females, this may have resulted in fewer multiple matings. Coupled with possible behavioural deficiencies in hand-reared males, this could have led to reduced sperm competition and lower fertility in the contemporary population.

Having sufficient males available at leks was previously assumed to be important to encourage females just to visit and mate, but now takes greater significance in ensuring sufficient sperm competition by encouraging multiple matings. Kākāpō sites should therefore be stocked with high densities of breeding males, while recognising that too many males on leks can lead to higher mortality among males due to fighting. However, the potential impact of female density on fertility, not previously considered in management, appears to be more important. Female densities should be kept as high as the habitat can support, with a high female:male adult sex ratio. There is no evidence of reduction in the number of multiple matings at high sex ratios, so it appears that adult female:male ratios could be at least as high as 1.6. However, this must be balanced against ensuring that nesting females have sufficient quality habitat to enable them to rear chicks in nests.

Artificial insemination should also be continued, as a way to introduce sperm competition when females copulate with only one male. Savage et al. (2021) demonstrated that artificial insemination had a significant impact on numbers of sperm reaching the PVL in kākāpō, and this is also true in other species (Brown et al., 2019). Increasing sperm competition may be as important as the primary reason artificial insemination was initiated in kākāpō, which was to override any natural matings with a genetically unsuitable (e.g., highly relatedness) mate.

Finally, from our finding of lower fertility of clutches produced by hand-reared males, hand-rearing should be reduced as much as possible, particularly for males.

Age effects

There was no strong impact of either mother or father age on clutch fertility. This is not particularly surprising given the relatively young age of the contemporary population (mean age = 19.1 and 23.0 respectively for females and males in the model clutch data), and considering that factors such as individual condition, food availability and population density can outweigh age effects (Hammers et al., 2012; Oro et al., 2014).

Accordingly, there were no strong differences in the contribution of mother and father age to clutch fertility. Sex differences in senescence are often more pronounced in polygamous vertebrate species, with males tending to have declining reproductive success at an earlier age than females (Clutton-Brock and Isvaran, 2007). This is thought to be a result of males being less likely to win fights as they age, and are excluded access to females (Clutton-Brock and Isvaran, 2007). This might be expected in kākāpō, with older, less fit males less able to defend their position in the lek and attract females. However, with the 'exploded' lek system in kākāpō (Merton et al., 1984), direct competition among males may be less important.

It was not possible to investigate differences in fertility with increasing age between hand-reared and wild-reared kākāpō since all hand-reared kākāpō were under 25 years old. However, this should be a focus of future analysis when the data set is sufficiently large, since the development environment, including rearing method, has been shown to affect reproductive senescence in other bird species (Balbontín and Møller, 2015; Murgatroyd et al., 2018; Cooper and Kruuk, 2018).

Mating experience

Mating experience (in terms of the number of previous matings) no impact on clutch fertility in kākāpō for either sex, unlike in other species (DuVal, 2012; Kokko, 1997). This is in accordance with observational evidence which indicates that females kākāpō do not preferentially select for the most experienced males (KRP, 2021, unpublished data). Some males have displayed for decades, but have never or rarely mated and produced offspring, despite being visited at the lek by females (Eason et al., 2006). Conversely, other, younger males have produced offspring from first-time matings.

Inbreeding

Our model showed no discernible effect of parental kinship on apparent infertility. This combines both 'true' infertility and very early embryo deaths, but the results of Savage et al. (2021) suggest that our sample was likely to dominated by the latter. Embryo death has been attributed to maternal and environmental effects as well as genetic incompatibility (Savage et al., 2021; Assersohn et al., 2021b), one measure of which can be parental kinship. However, our results suggest that parental kinship is not a strong driver of early reproductive failure in kākāpō, relative to the behavioural effects.

This appears to be in contrast with findings from another bird species with low rates of fertility, the whooping crane (*Grus americana*), in which higher parental kinship values are a dominant predictor of apparent egg infertility (Brown et al., 2019). Similarly, Jamieson and Ryan (2000) reported that takahē on islands with higher apparent infertility than their mainland counterparts was at least partially attributable to genetic factors. However, environmental factors were considered to dominate in takahē fertility, and both the whooping crane and takahē studies did not distinguish true infertility from early embryo death (Assersohn et al., 2021b).

The results of most other studies assessing effects of parental kinship on fertility cannot be compared to ours, since they use different measures of reproductive success, such as fledging rates (Morrison, 2020). In addition, in the majority of bird species, small sample sizes combined with low rates of infertility have led to reduced statistical power to detect genetic effects on fertility (Garamszegi, 2016; Assersohn et al., 2021b).

Our analyses of kākāpō fertility were not limited by sample sizes and low rates of infertility, but were unavoidably restricted by a low kinship range (0 – 0.265) with a positively skewed distribution. This was perhaps at least partially a result of genetic management methods such as translocations reducing the likelihood of closely related matings. Survivorship bias may have also contributed to the low kinship values: in cases when females mated with different males, only clutches with DNA confirmed paternity were included, so males which mated but did not produce fertile eggs were excluded. However, the whooping crane data also had low kinship values (a median of zero), yet still detected a strong association between parental kinship and apparent fertility (Brown et al., 2019). It is unclear why this was not the case with kākāpō, although their different breeding ecology could have led to a different relative contribution of genetic and behavioural effects. Regardless, future studies should more closely examine the relationships between other genetic metrics and low rates of fertility in kākāpō. For example, very early embryo death can also be attributed to gross chromosomal abnormalities (Assersohn et al., 2021a) which would not have been detected in our study.

Sperm quality

Many male kākāpō in the contemporary population have poor sperm quality, with low concentration and morphological abnormalities (White et al., 2014). This is quite unusual for polyandrous parrots. Bublat et al. (2017) demonstrated that Eclectus parrots, which also have a polyandrous breeding strategy, had a high sperm density, very high total sperm count and few morphological issues compared to monogamous macaws, which had a low sperm density, low total sperm count, lower motility and many altered sperm cell. The authors speculated that sperm competition in polyandrous birds is an evolutionary force for high semen quality. Therefore the low semen quality and quantity found in the contemporary kākāpō population is not expected from their breeding biology, and may instead be due to other reasons such as inbreeding (White et al., 2014) or diet.

Recent evidence suggests that male sperm quality may not be such a limiting factor in kākāpō fertility. The microscopic egg analysis of Savage et al. (2021) showed that the true egg infertility rate in 2019 was 14%, rather than the 52% assumed. Infertility was still higher in males than females (17% and 2% respectively), but this suggests that embryo deaths, rather than insufficient sperm reaching the egg, are the biggest factor in kākāpō infertility.

Environmental impacts

The year random effect in the fertility model accounted for only a relatively small amount of the total variance compared to the random effects of father and mother identity. This suggests that variation among years was less important than among individuals, and that unmodelled individual effects dominated unmodelled inter-annual ones. Factors which varied among years would have included environmental factors such as climatic conditions, which may affect fertility, although this is poorly studied in wild species (Walsh et al., 2019). Inter-annual variation would also have occurred in food supply, particularly rimu abundance and whether ripe rimu fruit was available. Rimu abundance is correlated with clutch size in kākāpō (Harper et al., 2006), but our results indicate that it is not strongly associated with clutch fertility, along with any other environmental dietary or climatic variations.

Small samples sizes and longitudinal data

The effect of small data sets must be considered when evaluating these results. Small sample sizes are often an unavoidable consequence in threatened species analysis, which can lead to imprecise, inaccurate or unstable results, and important effects being missed due to apparently non-significant results arising from high uncertainty (Garamszegi, 2016). This is why robust statistical methods which provide reliable and useful uncertainty measures and can rank predictors by their contribution to the response are important for these data, such as the Bayesian predictive projection variable selection utilised here. Even with these methods, the impact on fertility of the effects reported here is likely to be underestimated. This must be considered when using these results to make conservation management decisions, and emphasises the importance of reanalysis when data sets become larger with further monitoring.

The sample size of 197 clutches in this study is statistically small, but represents a substantial and long-term monitoring effort utilising advanced technologies. Few wild species are monitored as intensively as the kākāpō, with individuals closely followed over decades, so that nearly all mating attempts are recorded. This longitudinal data set has enabled analysis of potential impacts on fertility, highlighting the importance of adequate monitoring to assess the effects of management methods which may not be apparent, and the importance of long-term, individual-based studies (Clutton-Brock and Sheldon, 2010).

Other species

The implications from this study can also be considered in conservation programmes on other species, particularly the impacts of hand-rearing. In a review of global psittacine re-establishment projects, Joustra (2018) reported that nearly a quarter (24%) used hand-reared individuals, with two-thirds of those relying on them entirely. Although there are widely-reported negative impacts on behaviours such as predator-avoidance, increased human interactions and aggression or avoidance to conspecifics (Carrete and Tella, 2015; Utt et al., 2008; Joustra, 2018), further attention should be paid to the more subtle but potentially more damaging impacts on fertility.

CONCLUSION

Determining the cause of their low productivity is the highest research priority for kākāpō conservation. This analysis of a long-term mating data set suggests that the biggest factors affecting apparent clutch fertility are behavioural: female mating behaviour and male developmental environment. Clutch fertility increased with the number of mates and mating attempts a female had, and was lower for hand-reared males compared to those which were wild-reared.

Together with sperm morphology and a mating system which indicates high levels of sperm competition, these results suggest that current kākāpō mating frequencies are lower than those which have previously been selected for, and that this is perhaps a result of low population size which may have been compounded by management. The sex difference in hand-rearing impacts indicates that hand-rearing affects mating behaviour in males more than females, in accordance with imprinting behaviours found in hand-reared male but not female kākāpō. Age, previous mating experience and parental kinship were found not to be important predictors of apparent clutch fertility.

These findings have immediate applications in kākāpō conservation management. Hand-rearing should be limited as much as possible for males; a reversal from previous strategies in which retaining female chicks in nests was prioritised. Population densities should be maximised so that there are sufficient males at leks to ensure adequate mate choice for females, but that the female:male sex ratio is kept as high as the habitat can support. Artificial insemination should also be continued, to ensure sufficient sperm competition as well as maximizing founder representation.

That female mate choice affects fertility in the lek-breeding kākāpō also has implications for hypotheses for polyandry and repeated matings. Our results, combined with those on kākāpō sperm morphology, indicate that these behaviours are driven by high levels of sperm competition in kākāpō to improve the likelihood of fertilisation. The increase in multiple matings with increasing female:male adult sex ratio also provides evidence that female mate guarding occurs in this species.

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This study relied upon the observational data of the Kākāpō Recovery Programme database, which has been contributed to by hundreds of NZDOC staff and volunteers over more than 40 years. Huge respect and admiration are especially due to the personnel who worked under difficult field conditions without the benefit of remote monitoring methods, often for low kākāpō productivity, from the 1970s to early 2000s. Particular thanks also go to Daryl Eason, Graeme Elliott and Ron Moorhouse for the generation, maintenance and accessibility of this data set.

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ADDITIONAL INFORMATION AND DECLARATIONS

Author Contributions

Conceptualisation: AD, DE, DV and ML.
Data curation (clutch fertility and demography): DE, AD, DV, KRP (many staff and volunteers since 1981).
Data curation (parental kinship data): SG, DE, JG, ML, LU, TJ, CL, TS, PD.
Formal analysis (clutch fertility and demography): AC, AD, DE.
Formal analysis (parental kinship): SG, JG, ML, LU, TJ, CL, TS, PD.
Writing - original draft: AD.
Writing - review and editing: AD, DE, ML, AC, DV, LU, TS, JG, PD, TJ, TD, KRP, CL.
Supervision: DV, TD, TS, PD.
Funding: DV.

Data Availability

The clutch data, demographic data and `R` code used in the fertility models and analysis of multiple mating are available as Supplementary Files.

- Supplemental Figure S1: distribution of the numeric predictors used in the Bayesian model.
- Supplemental Data S1: `R` data file containing clutch data used in the Bayesian model.
- Supplemental Data S2: `R` data file containing numbers of kākāpō per island per year used in the multiple mating – density analysis.
- Supplemental Data S3: `html` file produced by `RMarkdown`, containing code and results for the fertility models and multiple mating – density analysis.

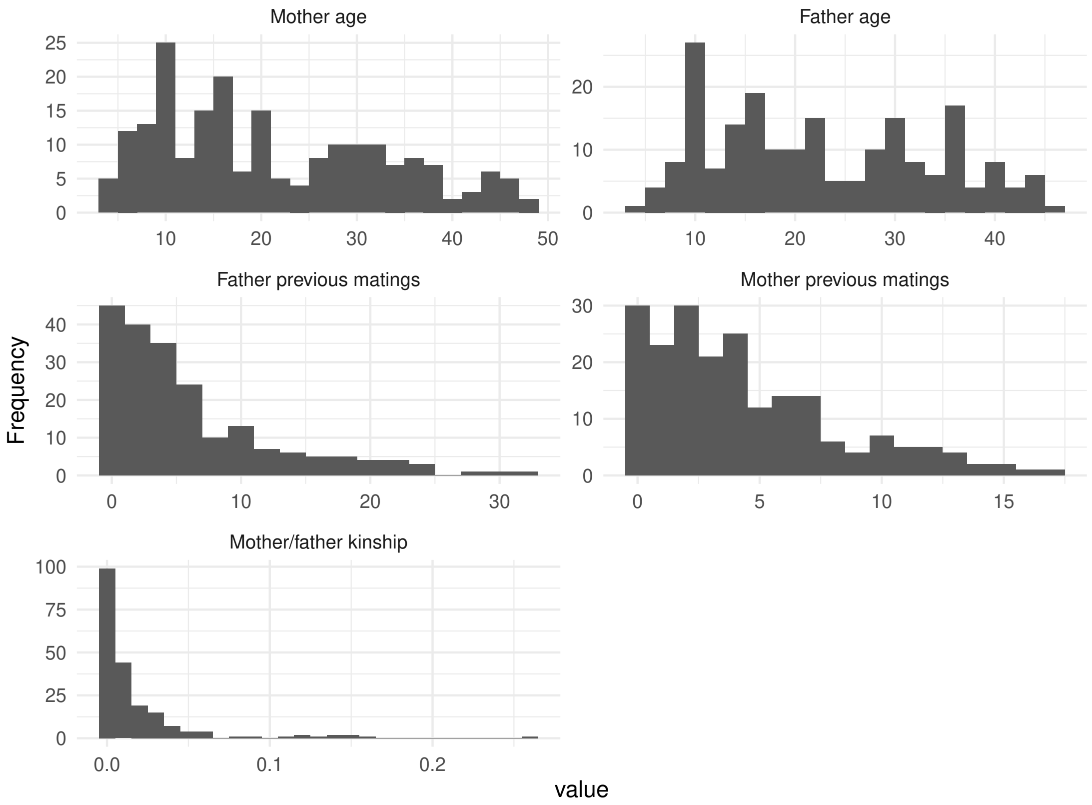

Figure S1. **Model predictor distributions.** Distributions for the numeric fixed parameters in the Bayesian model of clutch fertility.

Supplementary Information

Animal Ethics

The following information was supplied relating to ethical approvals:

The data used in this study were collected as part of routine kākāpō conservation management conducted by NZDOC as required by the New Zealand Conservation Act (1987), and so this study was exempt from the requirement of animal ethics approval under NZDOC's obligations to the New Zealand Animal Welfare Act (1999).

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