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 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. Hand-rearing 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 from the parents' rearing environment, their age and previous mating experience, the parental kinship, and the number of mates and copulations for each clutch. We also explored how the incidence of repeated copulations and multiple mates varied with kākāpō density.

Results. The hand-rearing status of the clutch father and the number of mates and copulations of the clutch mother were the dominant factors in predicting fertility. Clutches were less likely to be fertile if the clutch father was hand-reared compared to wild-reared, but there was no similar effect for mothers. Clutches produced by females copulating with different males were more likely to be fertile than those from repeated copulations with one male, which in turn had a higher probability of fertility than those produced by a single copulation. The likelihood of multiple copulations and mates increased with female:male adult sex ratio, perhaps as a result of mate guarding by females. 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, with implications for conservation management. The increased fertility due to multiple mates and copulations, combined with the evidence for mate guarding and previous results of kākāpō sperm morphology, suggests that an evolutionary mechanism exists to optimise fertility through sperm competition in kākāpō. The high frequency of clutches produced from single copulations in the contemporary population may therefore represent an unnatural state, perhaps due to too few females. This suggests that opportunity for sperm competition should be maximised by increasing population densities, optimising sex ratios, and using 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.

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ABSTRACT

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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 from the parents' rearing environment, their age and previous mating experience, the parental kinship, and the number of mates and copulations for each clutch. We also explored how the incidence of repeated copulations and multiple mates varied with kākāpō density.

Results. The hand-rearing status of the clutch father and the number of mates and copulations of the clutch mother were the dominant factors in predicting fertility. Clutches were less likely to be fertile if the clutch father was hand-reared compared to wild-reared, but there was no similar effect for mothers. Clutches produced by females copulating with different males were more likely to be fertile than those from repeated copulations with one male, which in turn had a higher probability of fertility than those produced by a single copulation. The likelihood of multiple copulations and mates increased with female:male adult sex ratio, perhaps as a result of mate guarding by females. 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 it is often problems with reproductive output which most limit recovery (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.

Hand-rearing, in which animals are raised in captivity by humans, is often used in threatened species conservation programmes (Klusener et al., 2018), primarily to increase productivity through improved survival during the precocial stage (Alagona, 2004; Heezik et al., 2005). However, this intervention can have negative impacts, mainly by reducing long-term survival (Aourir et al., 2013; Hampson and Schwitzer, 2016; Farquharson et al., 2021) and introducing behavioural issues (Utt et al., 2008; Jones, 2008; Pacheco and Madden, 2021) which may cause hand-raised individuals to be unsuited to life in the wild (Meretsky et al., 2000). These behavioural differences appear to affect productivity in some taxa

(King and Mellen, 1994; Beck and Power, 1988; Hampson and Schwitzer, 2016), although the impacts are poorly understood in wild bird species (Assersohn et al., 2021a).

Mating behaviour, in terms of the number of mates and copulations, can directly affect fertility in birds. Females can increase the likelihood of egg fertilisation through polyandry — the 'fertility assurance hypothesis' (Birkhead et al., 1987; Reding, 2014; Rivers and DuVal, 2019; Santema et al., 2020) — and by copulating repeatedly with a single male (Zhang et al., 2019). These behaviours are influenced by adult sex ratio (Grant and Grant, 2019; Birkhead and Montgomerie, 2020): when competition is high, females in some species use repeated copulations to 'guard' preferred males and copulate with alternative males when their preferred choice is not available (Petrie et al., 1992).

Age affects reproductive output in some bird species (Murgatroyd et al., 2018; Brown et al., 2019), but not others (Zhang et al., 2014; Fay et al., 2020) and in general is poorly studied in wild birds. Mating experience can also affect productivity: evidence suggests that more experienced individuals may have higher reproductive success (Kokko, 1997; DuVal, 2012; Assersohn et al., 2021a), and so are preferred as mates (Kokko et al., 1999; Jouventin et al., 1999). Diet is also an important factor in avian reproductive output (Selman and Houston, 1996; Klasing, 1998), but this has also not been studied in most wild bird species (Klasing, 1998; Assersohn et al., 2021a). Fertilisation failure and very early embryo death can also result from increased homozygosity due to matings between closely-related individuals (Hemmings et al., 2012; Assersohn et al., 2021b).

Kākāpō

Low productivity limits 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 2022. Remnant populations of kākāpō were translocated to predator-free island sanctuaries in the 1980s (Powlesland et al., 1995) and breeding has since occurred on five 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).

Kākāpō breeding occurs irregularly, synchronised with the mass-fruiting (masting) of certain tree species, particularly the rimu tree (*Dacrydium cupressinum*). Rimu masts every 2–4 years (Harper et al., 2006) and is the predominant food fed to chicks when available (Cottam, 2010). The kākāpō is the only parrot species with a lek mating system (Merton et al., 1984): females visit leks to choose and copulate with displaying males (Eason and Moorhouse, 2006), and both sexes often copulate with multiple partners. Females typically lay 2–3 eggs per clutch (range = 1–5). Males do not contribute to incubation or care of offspring.

Low fertility in kākāpō

Approximately 40% of kākāpō eggs are considered infertile from visual inspection ('candling'), although a recent fluorescence microscope study showed that 72% of these 'apparently infertile' kākāpō eggs were actually fertile, and instead failed due to very early embryo death (Savage et al., 2021).

There are a number of factors which may contribute to this low fertility in kākāpō. With a small founding breeding population of 35 individuals and low levels of genetic diversity, inbreeding may be an important contributor (Bergner et al., 2016; Dussex et al., 2018, 2021). Decreased female heterozygosity is correlated with lower hatching success and smaller clutch size in kākāpō (White et al., 2014), but male heterozygosity has no apparent effect on fertility, perhaps because males with the lowest heterozygosity may not mate at all (White, 2012).

Rearing environment may also influence fertility in kākāpō. All breeding occurs in the wild, but eggs are often incubated artificially to maximise hatching success, and chicks are removed for hand-rearing if their life is at risk. This hand-rearing has caused behavioural issues, with two male chicks reared individually in 1997 and 1998 displaying imprinting on humans.

Repeated copulations and multiple mates could affect fertility in kākāpō, as it does in other species. Repeated copulation in lekking species can also provide a strong test of theories for polyandry (Parker and Birkhead, 2012; Rivers and DuVal, 2019).

As a long-lived species with a life expectancy of several decades, kākāpō might experience age-related changes in reproductive output. Young age is a barrier to fertility: both sexes can mate from five years old, but no males younger than eight have produced fertile clutches. Impacts towards the end of life are less clear, since the age of kākāpō discovered as adults cannot be determined (Horn et al., 2011), but White (2012) found no impact of male age on egg fertility.

As a lek-breeding species, there is a high skew in kākāpō reproductive success, with a small number of males dominating copulations (Eason et al., 2006). The subsequent large variation in mating experience may also affect fertility.

Kākāpō are supplementary fed during breeding years to optimise productivity (Elliott et al., 2001; Clout et al., 2002) and improve chick survival. Feeding increases clutch size and the proportion of females nesting and leads to a higher likelihood of mothers successfully rearing chicks, but there there is no evidence that it affects fertility (Elliott et al., 2001; Houston et al., 2007). Diet is not considered in this study since supplementary food contributes a low proportion of daily metabolised energy (Bryant and Bryant, 2006), and incomplete feeding records and sharing of food stations makes it difficult to determine individual consumption over many years.

Other factors which can affect productivity in birds include injury, disease, stress, hormonal disruption, pollution and climate change (Assersohn et al., 2021a,b). These were not included in the current study since they were not considered important in wild kākāpō living on remote islands, and because the diseases which affect kākāpō do not appear to impact reproduction (Gartrell et al., 2005; Jakob-Hoff et al., 2009; Jakob-Hoff and Gartrell, 2010).

Low fertility is one of the primary reasons for slow growth in the kākāpō population (Elliott et al., 2006), yet few studies have investigated its causes, and none have been multi-factorial. This study presents the first assessment of the relative impacts of multiple factors on kākāpō fertility, including life history, genetic and behavioural components.

MATERIALS AND METHODS

Kākāpō management

Mating and nesting detection

Kākāpō are intensively monitored in order to maximise survival and productivity, with nearly every kākāpō fitted with a VHF radio transmitter since 1995. Initially these transmitters only allowed determination of location, so breeding behaviour was assessed manually. Mating was detected by checking for mating sign at lek sites (feathers shed by the female during copulation), and nesting inferred by daily triangulation (if adult females were repeatedly in the same location they were assumed to be incubating). Remote sensing methods improved the efficiency of collecting mating data and their quality. Proximity sensors were installed at lek sites from 1997 to record male presence, and from 2012 the transmitters were fitted with activity sensors to provide mating and nesting behaviour. The activity data were initially transmitted via coded VHF pulses to telemetry receivers used by field observers or mounted in an aircraft. Then from 2016 the activity data on the main breeding islands of Whenua Hou and Anchor Island were transmitted 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 in 2014 ensured that nearly all subsequent matings were recorded. A small number of matings were not detected by the transmitters due to hardware failure or unusual mating activity, but subsequent nesting was detected. In addition, paternity of all offspring since 1997 was determined first from microsatellite genetic testing (Robertson et al., 2000) and later from genotyping-by-sequencing of blood samples taken from fertile eggs or chicks.

Artificial insemination has been attempted in kākāpō during every breeding season since 2008, 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 free-living wild bird species. Subsequent attempts failed, until three successful inseminations produced three chicks in 2019, of which one fledged (KRT, 2021, personal observations).

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’ rather than true fertility in our analyses, approximately a quarter of the eggs in which embryos died at very young age (before four days) will have instead been classed as infertile (Savage et al., 2021).

Nest management

From 1997–2019, most eggs (73%) were removed for artificial incubation, to increase hatching success. A day or two before or after hatching, the eggs or chicks were returned to nests where possible, and closely monitored. 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 multiple foster mothers and often 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 there was scarce natural food due to the rimu fruit not ripening, each nesting female could usually support only one chick, and surplus chicks were hand-reared. Between 1981 and 2019, 52% of chicks hatched were hand-reared for at least 10 days. To avoid imprinting on humans, chicks were not reared individually where feasible, and were usually kept in groups of 2–6. Where possible, chicks were reared on islands and then returned to nests, but some chicks required longer periods of hand-rearing. This long-term hand-rearing took place at a mainland facility, before the chicks were returned to islands at an age of approximately 80 days. Here they were weaned in large outdoor pens before being released into the wild at an approximate age of 120 days. Following fledging from the nest or from hand-rearing, most chicks were supported by supplementary feeding.

Data collation

Clutch data

Clutch data were collated from the Kākāpō Recovery Programme database for the breeding years between 1981 and 2019 (Table 1 and Data S1). The database contains all observed events for each individual, including transmitter activity data, captures, health checks, feeding records and copulations. These were combined with a dataset for each clutch since management began in 1981, containing clutch size, number of fertile eggs (apparent fertility), number of eggs hatched, and the number of chicks fledged, as well as paternity assumed from transmitter data and confirmed by genetic testing. 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 237 clutches.

This data set contained first ($n = 197$), second ($n = 39$) 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.

Paternity assignment

Confirmation of paternity from genetic testing was available for 120 out of all 237 clutches laid from 1990–2019. Of the 117 clutches which did not have confirmed genetic paternity, it was necessary to identify the male which ‘fathered’ the clutch, so that its hand-rearing status, age, mating experience and the parental kinship could be compared to clutch fertility. Four clutches were excluded for which an unknown number of males copulated with the female, leaving 113 clutches without confirmed genetic paternity and 233 in total. In 92 clutches without genetic paternity confirmation, only one male mated with the female, so assigning the ‘father’ was straightforward.

For a further 21 clutches (14 infertile, 7 fertile) with unconfirmed genetic paternity, different identified males were confirmed or assumed to have copulated with the female. These clutches could not be excluded since doing so would remove the entire set (14) of infertile clutches produced by copulations with different males, biasing the clutches from multiple males to higher fertility by only leaving the fertile clutches. So to retain these 21 clutches, a ‘father’ was assigned from the 2–3 males identified to have copulated with the female, based on a likelihood of paternity from male copulation order. The likelihood of paternity was determined from clutches with confirmed genetic paternity, calculated as the proportion of clutches fathered by a male copulating first, last, middle, or first and last out of all the males which copulated with the female (Table S1). These probabilities were then used to select a ‘father’ from the candidate males using weighted sampling.

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 determined from ‘candling’; not true fertility from microscopic analysis. This is the full data set; some of these clutches were excluded from the fertility model. See text for further details.

This method of selecting a clutch ‘father’ will have introduced errors due to the incorrect male being chosen in some cases, but these instances would have been few compared to the overall number of clutches. Moreover, this method would have caused less bias to the measured impact on fertility of copulations with multiple males compared to omitting the 21 clutches without genetic paternity confirmation. Furthermore, reducing the sample of clutches from multiple males would have greatly reduced the ability to assess the effect of sperm competition on fertility, which may be greater than the influence of the characteristics of the male which fathered the clutch. We acknowledge that the term ‘father’ cannot strictly be applied to an infertile clutch, but it is used to signify the copulating male which had the highest likelihood of fertilising the eggs — and noting that in many cases, the eggs of these apparently infertile clutches were in fact fertilised.

A further seven clutches with mixed paternity and/or produced by artificial insemination were excluded, since the clutch wasn’t the product of a single male and female. The resulting 226 clutches were therefore the product of a single identified female and a male designated as the clutch ‘father’. A further nesting attempt without any eggs was also removed, leaving 225 clutches from a total of 60 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. A binary hand-rearing variable was chosen over a continuous one as it is more practicable to apply to management and because it simplified the statistical analysis. The binary variable was also more suited to the bimodal distribution of hand-rearing periods, with kākāpō chicks tending to be hand-reared for either a short period or for most of their development (Figure S1). Many chicks are hand-reared for just a few days to enable them to recover from ill health or weight loss, particularly 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. Alternatively, prolonged ill-health or lack of available nests means that they are hand-reared until they reach weaning age. Of the 111 adult kākāpō which contributed to the 225 clutches, 59 were hatched after intensive management and hand-rearing began. Of these, 21 (36%) were hand-reared for up to 10 days and 38 (63%) for more than 10 days, of which only 4 were hand-reared between 10 and 60 days (Figure S1).

Age assignment

The ages of the male and the female producing the clutch were calculated from hatch dates if these were known. Kākāpō of unknown age comprised 17 of the 60 females and 22 of the 51 males which contributed to the 225 clutches. These were assigned a minimum age of 10 years at discovery, 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 these estimated ages, these were likely to have been relatively small compared to the absolute ages at breeding, 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. S2), which is younger than the presumed mean life expectancy in the contemporary managed population. However, the remnant populations from which the kākāpō of unknown age were sourced were under extreme predation pressure (Karl and Best, 1982; Atkinson and Merton, 2006), so would have had shorter life expectancies than the current protected population.

Previous matings

A mating history for each kākāpō was obtained from recorded copulation attempts and genetic paternity analysis, providing an estimated cumulative number of copulations for the clutch mother and father before the clutch was produced. This number was a lower limit, since not all copulations were detected — even with the electronic mating detection system — and since it was assumed that all founder individuals had not previously copulated at the time of their discovery. This was clearly an underestimate, but unavoidable given the lack of observational 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 median of 0.0074 and a distribution that was positively skewed (Fig. S2).

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, hand-reared status and previous mating experience (number of previous copulations) of both clutch mother and father; the number of copulations and the number of different males the female copulated with to produce the clutch; and the parental kinship.

The clutch 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 and copulations for the female was a categorical variable with three levels: one copulation with one male, more than one copulation with the same male, and multiple mates. The value 'multiple mates' included clutches in which a female copulated with different males more than once ($n = 17$). Parental kinship was a continuous variable in the range 0–0.265. Mating experience was defined as the number of previous copulations detected prior to those which yielded the clutch. This was calculated for both the female and male which produced the clutch.

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 in a clutch that were fertile, 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 clutch mother and father 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 (145) and Anchor Island (59) produced 91% of the 225 clutches.

Component	Variable	Type	Values (frequency)
Response	Clutch fertility	Binary	0 (80) / 1 (137)
Fixed	Mother hand-reared	Logical	true (64 clutches; 26 females) / false (153 clutches; 34 females)
	Father hand-reared	Logical	true (43 clutches; 12 males) / false (174 clutches; 38 males)
	Mother age (years)	Continuous	range = 4.8 – 48.5, mean = 20.9, median = 17.8
	Father age(years)	Continuous	range = 4.8 – 43.4, mean = 22.7, median = 20.8
	Number of copulations/mates	Categorical	1 mating (104) / > 1 mating 1 male (50) / Different males (63)
	Female previous copulations	Integer	range = 0 – 17, mean = 4.4, median = 4
	Male previous copulations	Integer	range = 0 – 33, mean = 6.5, median = 4
	Mother/father kinship	Continuous	range = 0 – 0.265, mean = 0.021, med = 0.0074
Random	Mother	Categorical	60 individuals, 1 – 9 repeats, mean = 3.6, median = 3
	Father	Categorical	50 individuals, 1 – 16 repeats, mean = 4.3, median = 4
	Year	Categorical	range = 1990 – 2019, 16 levels

Table 2. Model predictors. Parameters for the 217 clutches used in the Bayesian model relating clutch fertility to clutch mother and clutch father characteristics. See Fig. S2 for distributions of the numeric variables.

Observations with missing values for any of the predictors were excluded. From the initial set of 225 clutches, the final model data contained 217 clutches with complete values for all eleven input variables (Table 2). This resulted in a mean of 19.7 events per variable, 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 generalised 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 4.1.2; R Core Team 2020), with the Bayesian model implemented in `R` package `brms` version 2.16.3 (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 equivalence (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 copulations with one male and copulations with different males into a single category of 'multiple mating', in order to achieve sufficient sample sizes. We correlated the proportion of clutches produced by multiple matings with the number of adult female 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 clutch father hand-rearing status explained most of the variance of the model, followed by the multiple mating variable (Fig. 1, Supplementary Data S2). These terms contributed 15% and 11% respectively of the total difference in expected log predictive density (ELPD); all other fixed terms contributed just 5% combined. These proportions should only be used as a guide to the relative contribution to the model variance, since the ELPD depends on the order of the projected terms. Multiple mating and clutch father hand-rearing status were the only two fixed terms which had projected posterior distributions distinguishable from zero (Fig. 2). All other fixed terms had negligible impact on the model fit, and had projected posterior distributions indistinguishable from zero (Figs. 1 and 2). Of the random terms, clutch father and mother identity contributed most significantly to the variance (50% and 12% of the total ELPD variation, compared to 8% for the year random term), 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/copulations, clutch father hand-rearing status and random terms for clutch father, mother and year provided predictive performance equivalent to the full model (Fig. 3). This reduced projected model explained approximately 30% (estimated $R^2 = 0.030$) of the total observed variation in clutch fertility.

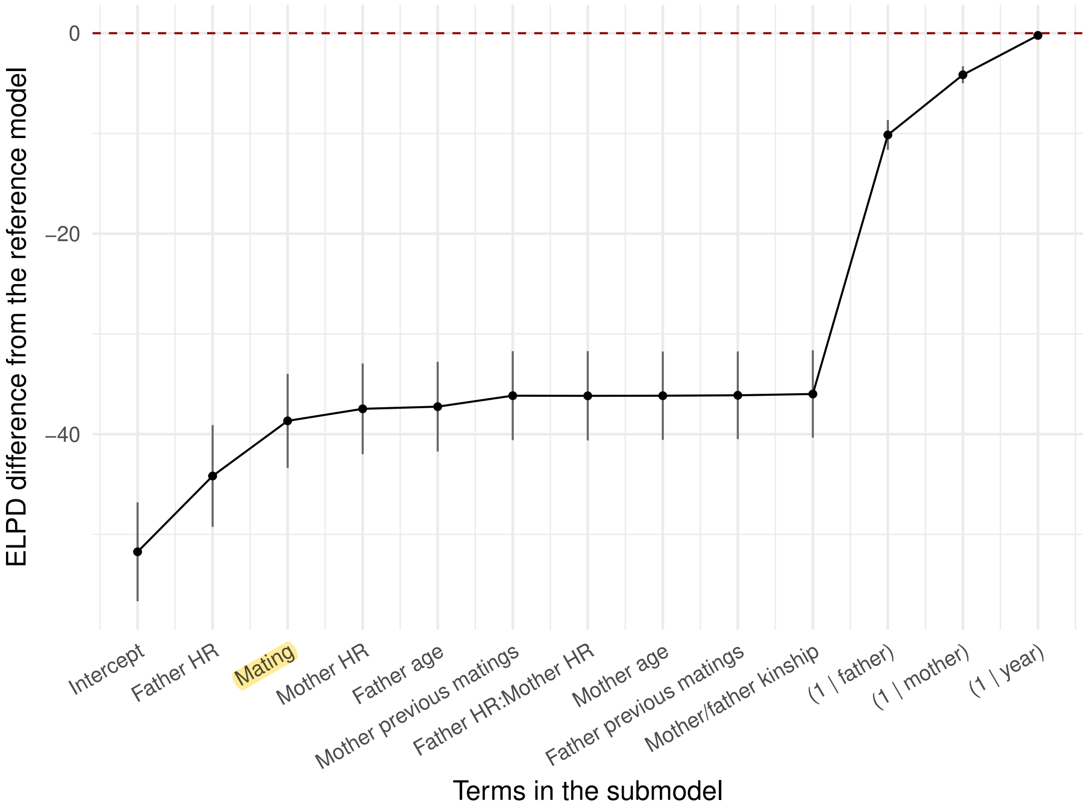

Figure 1. Projection predictive variable selection results. The contribution to the expected log predictive densities (ELPD) from each model parameter. Fixed terms are ordered in their contribution to the model variance, with random terms selected last. The dashed line shows the ELPD for the full model,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

Clutches from hand-reared fathers were associated with the highest change in clutch fertility, which was negative (probability of direction, p.d. = 0.98, probability of significance, p.s. = 0.96 in the reduced model). Clutches in which females copulated with different males were associated with the highest increase in fertility (p.d. = 0.96; p.s. = 0.90), followed by clutches in which females copulated repeatedly with the same male (p.d. = 0.84, p.s. = 0.61; Fig. 3). The remaining fixed terms of clutch mother rearing status, clutch mother and father age, genetic relatedness of the parents, and clutch mother and father mating experience were not included in the reduced model as they all had a very low impact on clutch fertility compared to hand-rearing status of the clutch father and the number of copulations and mates of the clutch mother.

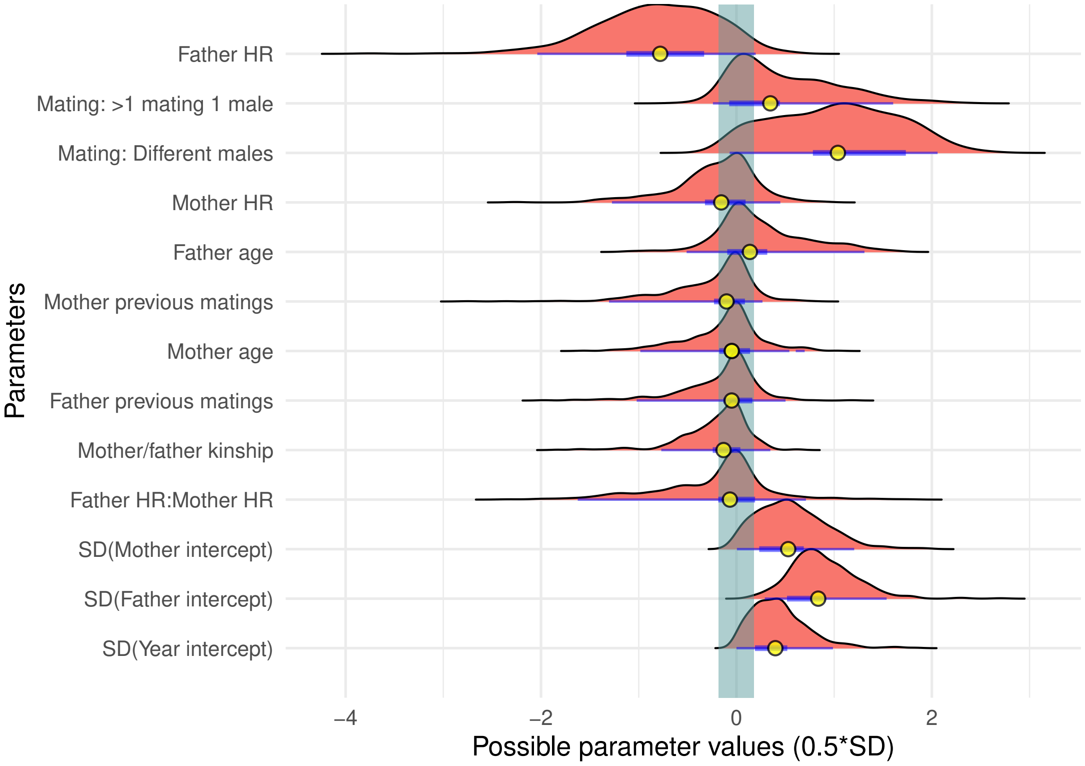

Figure 2. **Posterior coefficient distributions of the coefficients for the full projected model.** The less of the posterior distribution which intersects the ROPE (region of practical equivalence, denoted by shaded vertical bar — see Statistical Analyses for details) indicates a stronger association 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 clutch mother multiple mating behaviour and clutch 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 status, wild-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 (Fig. 4) showed that females copulating with multiple males had a high probability of producing a fertile clutch, especially if the clutch father was wild-reared (84% for a wild-reared father and 63% for a hand-reared father). Females copulating repeatedly with a single male had a higher likelihood of clutch fertility than those copulating just once (73% vs 64% for a wild-reared mate, and 48% vs 38% for a hand-reared mate). Irrespective of the number of copulations and mates, mating with a hand-reared male decreased the likelihood of clutch fertility compared to a wild-reared male.

Multiple matings and kākāpō density

The likelihood of females engaging in multiple copulations (either with the same male or different males) was strongly positively correlated (Pearson correlation, $r = 0.93$, 95% CI $= [0.74, 0.98]$, $p < 0.001, t = 7.44, \text{d.f.} = 8$) with the size of the adult female population on Whenua Hou from 1990–2019 (Fig. 5). The association between multiple copulations and male abundance was much weaker (Pearson correlation,

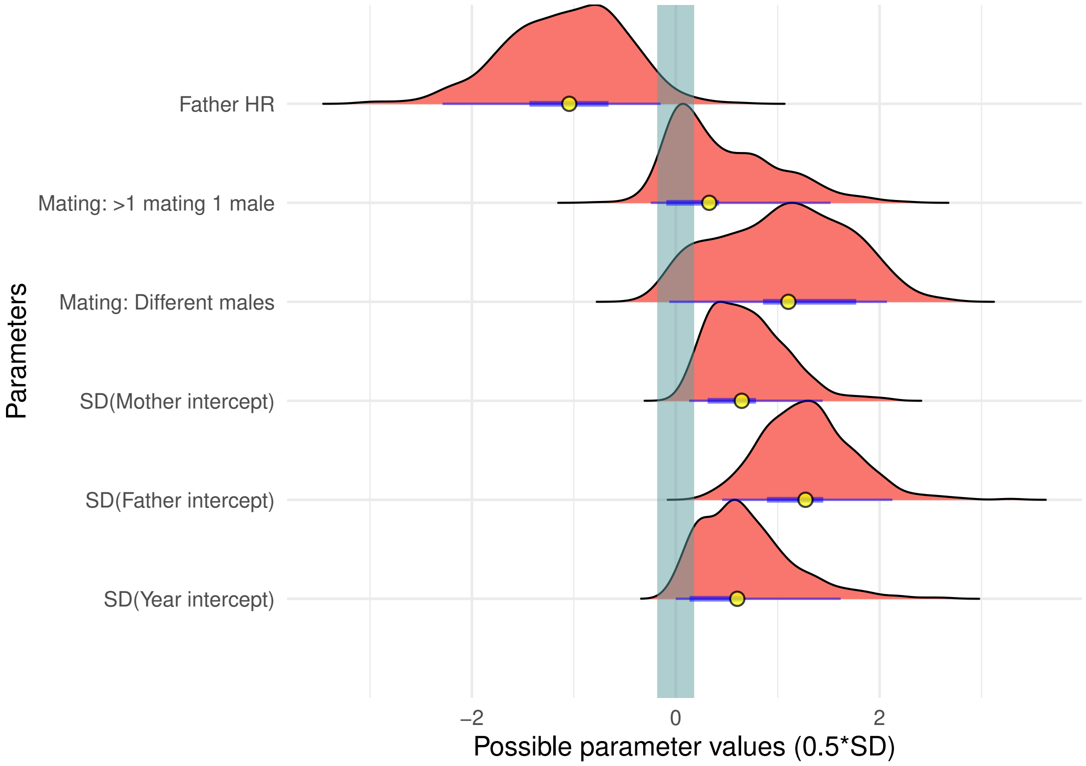

Figure 3. Posterior distributions of the coefficients for the projected reduced model. This model contained the two fixed effects with the highest variance contribution: clutch father hand-rearing status and clutch mother multiple mating behaviour. Compared to wild-reared fathers (the reference level), the projected posterior distribution for the hand-reared father parameter was strongly negative (probability of direction, p.d. = 0.98) and nearly entirely outside of the ROPE (region of practical equivalence, denoted by the vertical shaded bar) with a probability of significance, p.s. = 0.96. This indicates that hand-reared fathers were much more likely to be associated with lower clutch fertility. Compared to the reference level of a single copulation, the projected posterior distribution for females copulating with different males was very strongly positive (probability of direction, p.d. = 0.96) and mostly outside the ROPE (p.s. = 0.90), indicating a very strong positive effect on clutch fertility. For clutches in which females copulated with the same male more than once ('> 1 mating 1 male'), the parameter distribution was strongly positive (p.d. = 0.84, p.s. = 0.61). Symbols as in Fig. 2.

$r = 0.61$, 95% CI $= [-0.02, 0.90]$, $p = 0.059, t = 2.20, \text{d.f.} = 8$), but there was a strong correlation between multiple copulations and the female:male sex ratio (Pearson correlation, $r = 0.92$, 95% CI $= [0.71, 0.98]$, $p < 0.001, t = 6.88, \text{d.f.} = 8$).

There was substantial variation in multiple mating behaviour among females. Of the 60 females in the model data set, 38 (63%) copulated with different males in at least one breeding season, 27 (45%) had repeated copulations with the same male at least once, and 52 (87%) produced at least one clutch following a single copulation.

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 of those assessed, the dominant factors affecting clutch fertility are male hand-rearing status and female mating behaviour, in terms of the number of copulations and number of mates. Fertility was lower in clutches produced by a hand-reared father, higher if the mother copulated more than once with one male, and highest if the mother copulated with

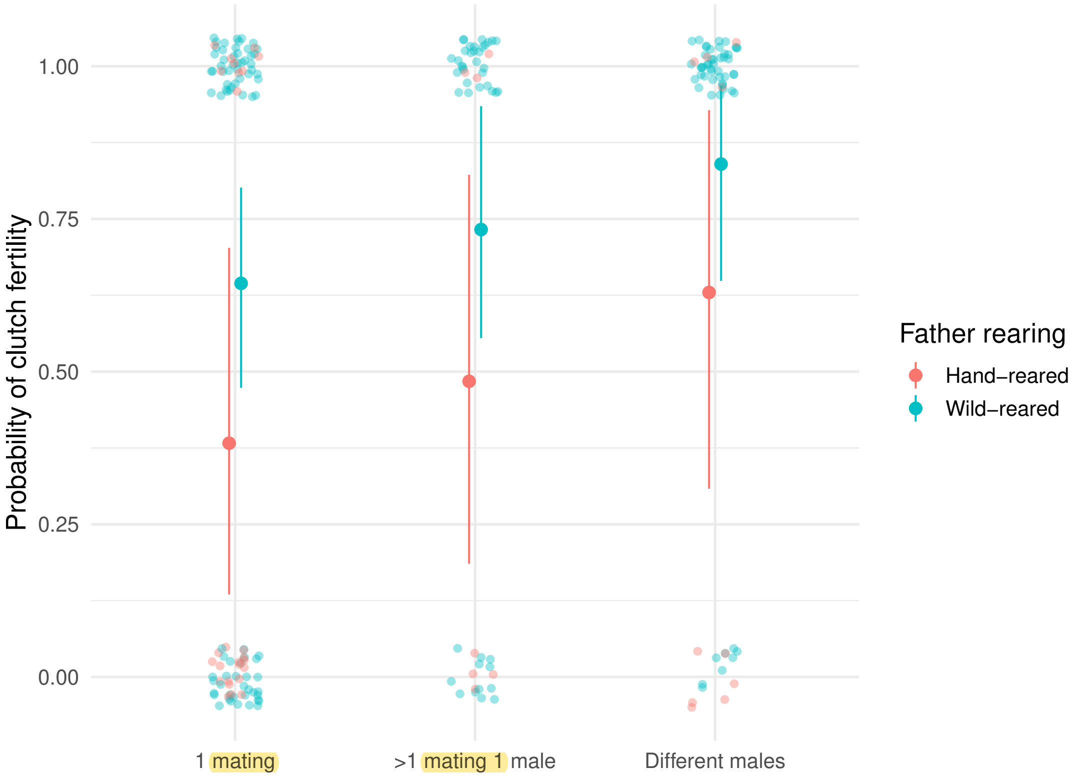

Figure 4. **Fertility model predictions for the interaction of clutch mother multiple mating and clutch father rearing status.** Predictions from the reduced model of how the likelihood of clutch fertility varied with the number of mates and copulations of the clutch mother, and with the rearing environment of the clutch father. A clutch is considered fertile if at least one egg is fertile, and infertile if all eggs are infertile. Model predictions are shown as large filled circles, with 95% highest posterior density intervals denoted by vertical bars. Small filled circles denote the observational data, coloured by father rearing status and jittered along both axes for clarity.

different males.

Rearing environment

These results provide 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).

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 on humans. 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 it is able to mate with kākāpō, it has not yet (to 2019) 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

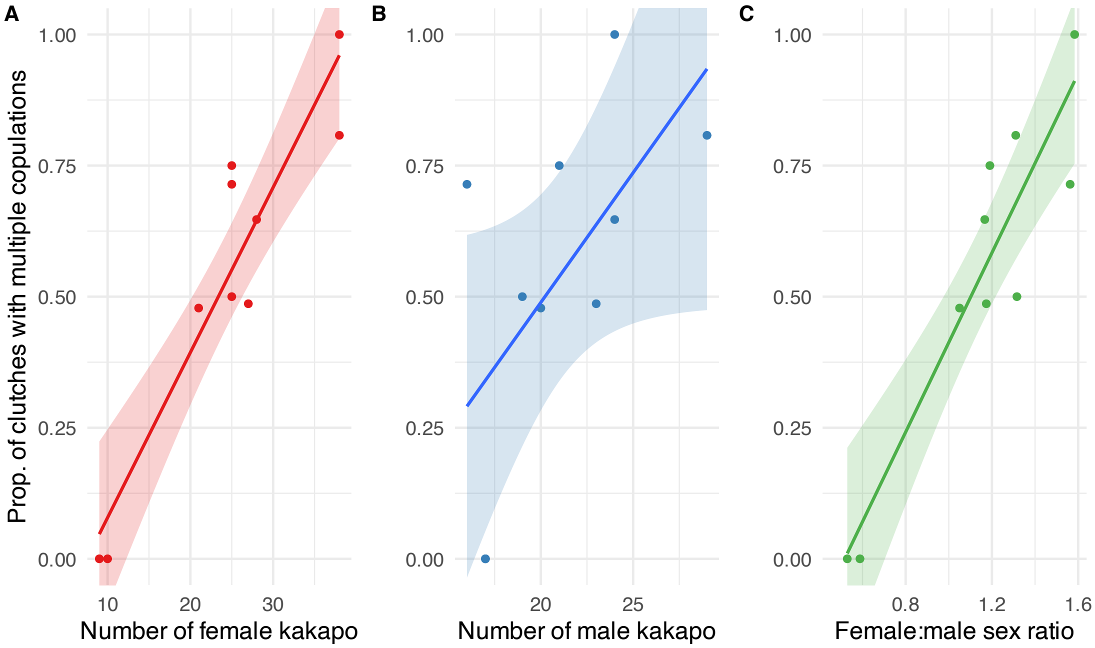

Figure 5. Proportions of clutches with multiple copulations compared to the number and sex ratio of adult kākāpō. The association between the incidence of clutches produced by multiple copulations with (A) female and (B) male kākāpō abundance and (C) female:male sex ratio, on Whenua Hou from 1990–2019. Clutches from multiple copulations are those produced by a female repeatedly copulating with a single male or by copulating with multiple males. The likelihood of clutches being produced from multiple copulations was strongly correlated with increasing female abundance and female:male sex ratio, and moderately correlated with male abundance (see text for details).

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

While there is limited data on the impact of hand-rearing on avian fertility, hand-rearing has been shown to reduce reproductive output — although not fertility — in takahē (*Porphyrio hochstetteri*), a threatened endemic New Zealand rail. Hand-raised takahē fledge approximately 50% fewer offspring than their wild-reared counterparts, even though egg fertility is similar (G. Greaves, New Zealand Department of Conservation, 2015, pers. comm.). 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 from 1981–2019 were 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 practice: from 32 hand-reared females which bred up to 2019, 25 (78%) fledged chicks, and all that hatched chicks fledged some.

Steps are already taken to avoid imprinting in kākāpō: chicks are not 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 adds 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 mates — 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 copulations with different males supports the hypothesis of direct benefits from polyandry (Reding, 2014). With no male parental care in kākāpō due to their lek breeding system, 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 a 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 than monogamic psittacine species, indicating a higher level of sperm competition. Their results therefore support the hypothesis that variation in sperm morphology is driven by sperm competition in psittacines, as it is in passerines. Interestingly, Carballo et al. (2019) also demonstrated that kākāpō sperm is longer than many other parrots and is 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. This is because the multiple matings are assumed to be driven by post-copulatory sperm assessment — for example, if the initial mate is unlikely to fertilise the eggs due to infertility or insufficient sperm (Birkhead et al., 1987; Rivers and DuVal, 2019). The frequency of mixed paternity is low in kākāpō: only 2% (one out of 66) of clutches produced by natural matings with multiple males resulted in mixed paternity.

Copulations with multiple males may also be a result of mate guarding by females, 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 by females 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 females practise mate guarding: at least 13 females have been detected at the display sites of males either the night before and/or after mating (KRT, 2021, personal observations).

The correlation of the likelihood of multiple mates and copulations with increasing female:male sex ratio supports the hypothesis that there is mate guarding by female kākāpō. As the threat of competition for mates grows with a changing sex ratio, there may be more mate guarding by females through monopolisation of preferred males with repeated copulations and subsequently more instances of females copulating 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).

It should be emphasised that the estimated effect on fertility from copulation with multiple males will have been subject to errors from assigning a putative clutch 'father' to the 21 such clutches which had no genetic paternity confirmation. However, we consider this bias to be much lower than if these clutches were omitted entirely, which would overestimate the fertility from mating with different males. Furthermore, this method made use of the available information of the identity of the 2–3 candidate fathers known to have copulated with the female, which the alternative method of imputing missing values (van Buuren and Groothuis-Oudshoorn, 2011) would not. That this process affected less than 10% of all clutches also suggests that the impact of any incorrectly assigned paternity was relatively small. This was confirmed by a comparison of model results with and without these clutches included, which showed that the overall conclusions were preserved.

Multiple copulations with the same male

The kākāpō reproductive data provide a rare opportunity to assess fertility benefits of females copulating 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 copulations to help determine the reason for this behaviour. Some of the hypotheses for repeated copulations 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 to increase 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 copulations may increase sperm reaching the PVL. However, 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. Nevertheless, our observed association of higher kākāpō clutch fertility with repeated copulations, together with the results of Savage et al. (2021), suggests that the fertility assurance hypotheses for multiple copulations applies to kākāpō.

It is also possible that the incidence of repeated copulations is a function of female condition. However, we do not consider this to be a major contributor in kākāpō, since all breeding females were provided with supplementary food and were maintained at an optimal weight range (Clout et al., 2002).

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

Conservation implications of multiple mating effects

From their sperm morphology, mating system and our finding of lower fertility from single copulations, we speculate that it is usual for female kākāpō to copulate multiple times and with multiple males. The current situation in which females often copulate once with one male (48% of 217 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 to visit and mate, but now takes greater significance in ensuring sufficient sperm competition by encouraging repeated copulations and multiple mates. 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 than that of males. 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 copulations 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. 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 (i.e. closely related) mate.

Additionally, our finding that hand-reared males are more likely to produce infertile clutches suggests that hand-rearing should be reduced as much as possible, particularly for males.

Age effects

There was no strong impact of either clutch mother or clutch father age on clutch fertility. This is not particularly surprising given the relatively young age of the contemporary population (mean age = 20.9 and 22.7 respectively for females and males in the model dataset), 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 therefore having reduced 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 developmental 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) had no impact on clutch fertility in kākāpō for either sex, unlike in other species (DuVal, 2012; Kokko, 1997). This is in accordance with female kākāpō not preferentially mating with the most experienced males (Supplemental Data S1). 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, some young 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, although the results of Savage et al. (2021) suggest that our sample was likely to be 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 higher apparent infertility of takahē on islands compared to 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 low kinship values and range (0.0–0.265; median = 0.0074; Figure S2). This was perhaps at least partially a result of genetic management methods such as translocations reducing the likelihood of closely related matings. However, the whooping crane data also had low kinship values and a lower spread (range = 0–0.125; median = 0.0), yet Brown et al. (2019) still detected a strong association between parental kinship and apparent fertility. 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 cells. The authors speculated that sperm competition in polyandrous birds is an evolutionary force for high semen quality. Calhim et al. (2007) also suggested that sperm competition can lead to convergence to an optimum sperm morphology within a species. 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. It is however still possible that sperm abnormalities could be a result of genetic defects which in turn cause embryo deaths.

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 clutch father and mother identity (7% of total ELPD variation for year; 50% and 11% for father and mother respectively). This suggests that variation among years was less important than among individuals (particularly the clutch father), 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. A further benefit of the Bayesian methods employed here is that they make such reanalysis straightforward.

The sample size of 217 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 and nearly all mating attempts recorded. This longitudinal data set has enabled analysis of potential impacts on fertility, highlighting the importance of adequate monitoring to assess effects of management methods which may not be immediately 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 of 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 reduced predator avoidance, increased human interactions and aggression toward or avoidance of 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: male developmental environment and female mating behaviour. Clutch fertility was lower for hand-reared fathers compared to those which were wild-reared, and increased with the number of mates and copulation attempts the clutch mother had.

Together with sperm morphology and a mating system which indicates high levels of sperm competition, these results suggest that current kākāpō copulation 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 such 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 mating behaviour 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 collected and managed over more than 30 years by the Kākāpō Recovery Team. Members of this team who collected field data from 1990 – 2019 are listed in Supplementary Data S4. Thanks are also due to the many volunteers, veterinarians and others who contributed to kākāpō conservation over this period. Huge respect and admiration are especially due to the personnel who worked under difficult field conditions without the benefit of remote monitoring methods 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. Lydia Uddstrom provided helpful comments on the manuscript.

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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 length of hand-rearing for parents of the clutches in the Bayesian model.
- Supplemental Figure S2: distribution of the numeric predictors used in the Bayesian model.
- Supplemental Table S1: likelihood of paternity based on male mating order, used to sample from candidate males to select a clutch father for clutches without genetic confirmation of paternity.
- Supplemental Data S1: Comma-separated file containing clutch data used in the Bayesian model.
- Supplemental Data S2: Comma-separated 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.
- Supplemental Data S4: text file listing employees of the Kākāpō Recovery Team who collected field data from Jan 1990 – May 2019.

Supplementary Information

Figure S1. Distribution of length of hand-rearing. Distribution of the number of days for which kākāpō were hand-reared, from the 59 kākāpō (38 females and 21 males) hatched after intensive management and hand-rearing began which contributed to the data set of 225 clutches.

Figure S2. Model predictor distributions. Distributions for the numeric fixed parameters in the Bayesian model of clutch fertility.

Table S1. Probability of paternity from mating order. The likelihood of paternity, given the order in which males copulated with the female. These probabilities are calculated from clutches produced by the female copulating with multiple males which had genetically confirmed paternity.

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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