Short communication

Cooperative males reduce incubation in response to cues of female–female competition

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Social groups of the joint-laying Pukeko *Porphyrio porphyrio melanotus* typically contain one or two breeding females. Male Pukeko mated to two females father more offspring and therefore benefit from this mating arrangement; however, primary females should not prefer this system, because fewer eggs hatch per female in the larger joint clutches. Here, we investigated male response to simulated egg destruction, a common female competitive tactic observed in other joint-laying species. In response to egg removal, males reduced the consistency of their incubation and in some cases nests were abandoned. Such decreases in paternal effort could eliminate any putative advantage gained by a female that destroys the eggs of a co-nester. Our study demonstrates facultative adjustments in paternal care in a joint-laying species and suggests that primary females may be limited in their ability to monopolize reproduction.

The ability to fine-tune parental effort in response to changing costs and benefits of parental care has been demonstrated in a number of species. A reduction in care is thought to be adaptive when the benefits of providing care decrease, such as during a reduction in offspring number (Hegner & Wingfield 1987), quality (Clutton-Brock *et al.* 1981, Burley 1986) or relatedness (Neff 2003). Similarly, a number of studies have shown

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that changes in the costs of providing parental care can affect parental investment. For example, parental investment is known to change with parental age (Velando *et al.* 2006) and resource availability (Townshend & Wootton 1985). Theoretically, such facultative adjustment in care should increase an individual's lifetime reproductive success by optimizing how it will allocate time and energy among all of the offspring it will raise over a lifetime (Velando *et al.* 2006). If the current reproductive effort is particularly costly or the attempt is unlikely to be successful, parents may even abandon young altogether to conserve resources for future breeding events (Székely *et al.* 1996).

While a number of studies have investigated parental effort in socially monogamous species, we know much less about parental care decisions in species that live in complex social groups. We examined how cooperatively breeding male Pukeko *Porphyrio porphyrio melanotus* adjust incubation behaviour in response to experimental brood reduction. Pukeko can breed independently in mated pairs, but it is more common for groups of 3–12 individuals to defend a shared territory and cooperatively raise the group's offspring. Such groups usually contain multiple breeding males, one or two breeding females that mate promiscuously, and sometimes also non-breeding helpers (Jamieson 1997). When there are two breeding females in a group, both will lay in the same nest (termed a joint-nest). The resulting joint-clutches contain more eggs than single clutches, but are less than twice the size of single clutches (Craig 1980a, Quinn *et al.* 2012). Larger Pukeko clutches produce more chicks overall than do smaller clutches, and males therefore generally benefit whenever a joint-clutch is laid (Quinn *et al.* 2012). However, the primary female (who initiates laying and is socially dominant over other females in the group; Craig 1980b) suffers a reproductive cost as a result of joint-nesting, as a lower percentage of eggs hatch in joint-clutch nests (Quinn *et al.* 2012). As a result, joint-nesting primary females are likely to raise fewer offspring than if they had nested without a co-breeding female (Craig 1980a, Quinn *et al.* 2012).

Despite the cost of joint-nesting to primary females, it does not seem that Pukeko destroy, eject or bury the eggs of co-breeders in the nest structure (Quinn *et al.* 2012, C.J. Dey & J.S. Quinn pers. obs.). Such ovicidal behaviour is observed in several other species of joint-laying birds (Vehrencamp & Quinn 2004) and is thought to be a competitive tactic used by females to maximize their reproductive success. Male Pukeko perform the majority of incubation and all nocturnal incubation (Craig 1980b), and it has been suggested that females do not engage in ovicide because it may lead to reductions in paternal care (Quinn *et al.* 2012). We tested this idea by conducting a partial clutch removal experiment to assess whether male Pukeko adjusted

their incubation behaviour in response to simulated competition between the joint-laying females. We predicted that males would decrease incubation when clutch sizes were decreased by elimination of the secondary female's clutch and that clutch-abandonment would occur more frequently in diminished clutches.

METHODS

Nest success

We first explored the costs of joint-nesting by examining whether it resulted in egg loss during incubation or hatching failure in retained eggs. During the breeding seasons (July–December) of 2008 and 2010, we monitored Pukeko nests at the Tawharanui Open Sanctuary in New Zealand (36°22′S, 174°49′E). Nests were located by searching suitable nesting habitat. Only the first nest of the season for each group was used, and if data were collected from the same social group in both years, we only used that collected in 2008 to avoid pseudoreplication ($n = 2$ cases). Once located, nests were visited daily during laying and hatching periods, and every 3rd day during incubation. If a nest was located after laying had commenced, the clutch initiation date was estimated by floating eggs in warm water and comparing their buoyancy and angle relative to the water's surface with eggs of known age (Hays & LeCroy 1971). In nests with known initiation dates, floatation scores were highly correlated with egg age ($R^2 = 0.84$, $n = 41$ eggs). Nests were monitored for an average of 26 days (range = 10–33 days, $n = 34$), which is most of the total nesting period (c. 33 days, C.J. Dey unpubl. data). Eggs that disappeared between visits were classified as 'lost' (12% of eggs in this study). Egg losses were not likely to be due to predation, as mammalian predators are excluded from this site by a pest-proof fence. Furthermore, the only known aerial predator of Pukeko nests, Australasian Harriers *Circus approximans*, leave behind shell fragments and half-eaten eggs in the nest (Haselmayer & Jamieson 2001). We observed no such signs of predation in our study.

Clutch removal

In 2010, 21 joint-nests were alternatively assigned to either a control treatment ($n = 11$) or removal treatment ($n = 10$). Only nests in which females were still laying were used in this experiment (i.e. if a nest was located after laying had ceased, it was not used), and these nests were not included in the analysis described above. In the removal treatment, we experimentally simulated competition between females by removing one female's entire clutch. The eggs of individual female Pukeko are identifiable, as each female lays eggs with a unique colour, shape, size and spotting pattern (Craig 1980a,b, Jamieson 1997, Quinn *et al.* 2012). We removed one egg per day of the secondary female's clutch (the 'B' clutch) until the entire clutch was removed. These removals began on the first day that a 'B' egg appeared or on the day of nest discovery if the secondary female had already begun laying (mean ± se number of 'B' eggs in nest at time of discovery = 1.05 ± 0.34). In control nests, eggs were handled in a similar manner to the treatment nests (i.e. eggs were picked-up and inspected during nest visits) but no eggs were removed from the nest area. We continued this procedure at control nests until laying ceased. All nests were visited every 3rd day after clutch completion (as above), and nests found with cold eggs on three consecutive visits were considered abandoned.

In 15 of the 21 experimental nests ($n = 8$ removal and $n = 7$ control) an iButton thermologger (Embedded Data Systems, Lawrenceburg, KY, USA) was buried approximately 25 mm deep in the centre of the nest bowl. These loggers recorded the nest temperature every 20 min. Because incubation is often inconsistent during egg laying, we standardized our analysis by considering only the temperatures logged over a 10-day period beginning with the day that the last egg was laid. Furthermore, because we were primarily interested in how male Pukeko respond to cues designed to simulate female competition, we restricted our analysis of incubation temperatures to a period between sunset and sunrise (21:00–06:00 h), when only males incubate (Craig 1980b).

We analysed incubation consistency in two ways. First, the variance in nightly incubation temperature was determined, where low variance indicated high constancy of incubation (Fernandez & Reboreda 2007). Secondly, we calculated the proportion of time that eggs were left exposed. Because ambient nocturnal temperatures were always below the normal incubation temperature for Pukeko, we considered the nightly maximum nest temperature as indicative of the presence of an incubating bird. If the nest temperature was 3 °C or more below the nightly maximum nest temperature, and the nest temperature was decreasing or constant, we considered the nest to be exposed. Performing the analysis with a 2 °C threshold yielded qualitatively similar results. We are confident that any detectable differences in nest temperature reflect a true change in male incubation behaviour, as a separate experiment has shown that the clutch size *per se* does not have a significant effect on nest cooling rate (C.J. Dey unpubl. data). We were unable to use the data from two control nests because we failed to recover the thermologger from one nest, and the data logger from another was unreadable due to water damage. Thus, the final incubation analysis was performed on five control and eight removal nests.

Statistical analysis

Statistical analyses were performed using R version 2.15.1 (R Development Core Team 2012). In all statistical tests, model residuals were visually examined to determine whether they met the assumptions of parametric tests (Zuur *et al.* 2009). Welch's *t*-tests were used when comparing the means of two groups, as some data were heteroscedastic. To explore the effect of clutch size on reproductive success, we used two binomial generalized linear models (GLZ) with logit link functions. In model 1, we examined the proportion of eggs that were lost during incubation and model 2 considered the proportion of eggs that successfully hatched. In both models, we included total clutch size and clutch initiation date as fixed effects to test our main variable of interest while controlling for the timing of nesting, which can be an important correlate of avian nesting success (e.g. Hochachka 1990, Chastel *et al.* 2003, Saino *et al.* 2012). We also included the total number of days the nest was monitored as a fixed effect in model 1 to control for an increased probability of detecting egg losses when nests were monitored for longer time periods.

To understand whether our clutch removal affected male incubation behaviour, we used a linear mixed-effects model (LMM) and a binomial generalized linear mixed model (GLMM), with respectively variance in nightly nest temperature ($°C^2$) and the proportion of time the nest was exposed as the response variables, respectively. In each model, treatment (control or removal) and incubation date (e.g. day 1 of incubation period = 1) were included as fixed effects, and nest ID was included as a random intercept in each model. The residuals of the LMM were not normally distributed, and thus we transformed the response variable using the Box–Cox power transformation procedure (Box & Cox 1964) with $\lambda = 0$ (equivalent to a log transformation). Models were fitted with and without nests that were subsequently abandoned ($n = 3$ nests). As there were no qualitative differences as a result of excluding nests that became abandoned, we report only the results from the models with all nests included. Finally, we used Fisher's exact test to determine whether our egg removal caused differential nest abandonment rates. Means are presented ± 1 se.

RESULTS

Nest success

Joint-clutches were significantly larger than single female clutches (Welch's *t*-test: joint-clutch = 8.5 ± 0.49, single clutch = 4.6 ± 0.17; 95% CI for difference between groups = (2.9, 5.0), $t_{18.4} = 7.60$, $P < 0.001$). Joint-clutches had a higher proportion of eggs lost during incubation (Table 1) and also had lower hatching success in retained eggs (Table 1) than did single female clutches.

Clutch removal

The total number of eggs laid in the removal and control nests was similar (Welch's *t*-test, control = 8.7 ± 0.68, removal = 8.0 ± 0.71; 95% CI for difference between groups = (−2.5, 1.6), $t_{17.3} = −0.47$, $P = 0.65$). However, following clutch manipulations, removal nests had significantly fewer eggs than did control nests (control = 8.7 ± 0.68, removal = 4.9 ± 0.31; 95% CI for difference between groups = (−5.4, −2.2), $t_{17.3} = −5.14$, $P < 0.001$). Secondary females that had their eggs removed did not compensate for lost eggs by increasing the total number of eggs laid (mean 'B' clutch size control = 3.2 ± 0.31, removal = 3.1 ± 0.55; 95% CI for difference between groups = (−1.4, 1.2), $t_{12.0} = 0.14$, $P = 0.89$). Removal nests had higher variation in nocturnal incubation temperature (mean nightly variation in nest temperature: control = 0.83 ± 0.14 °C, removal = 3.54 ± 0.93 °C, Table 2) and were exposed for a greater proportion of time (mean nightly proportion of time exposed: control = 0.07 ± 0.02, removal = 0.17 ± 0.02, Table 2) than were control nests. Finally, removal nests tended to be abandoned more frequently than control nests but this pattern did not reach statistical significance (3/10 removal nests vs. 0/11 control nests; Fisher's exact test, $P = 0.09$).

Table 1. Nest success in Pukeko groups. The proportion of eggs not lost during incubation (egg survival) and the proportion of retained eggs that successfully hatched (hatching success) are modelled for 34 Pukeko nests. Parameter estimates are presented with 95% confidence intervals, test statistics and degrees of freedom (df). Significant *P*-values are shown in bold. See text for details of the statistical analysis.

Model	Effect	Estimate (95% CI)	Z	df	P-value
Egg survival	No. of laying females	−1.10 (−2.29, −0.12)	2.04	1,30	**0.04**
	Clutch initiation date	0.02 (−0.01, 0.04)	1.63	1,30	0.10
	Days monitored	−0.03 (−0.10, 0.04)	−0.88	1,30	0.37
Hatching success	No. of laying females	−0.94 (−1.76, 0.19)	−2.39	1,31	**0.02**
	Clutch initiation date	0.03 (0.01, 0.04)	2.91	1,31	**0.004**

Table 2. Male incubation behaviour in response to simulated egg destruction. Results of statistical models on measures of nocturnal incubation constancy are shown for 10 nights in each of 13 nests. Nest ID is included in these models as a random effect. Parameter estimates and 95% confidence intervals are presented with test statistics (*t*- or *Z*-value, respectively). Significant *P*-values are shown in bold. Treatment effects are shown for the removal group relative to the control group. See text for further details of the statistical analysis.

Response variable	Effect	Estimate (95% CI)	*t*/*Z* Value	df	*P*-value
Nest temperature variation	Treatment	0.74 (0.03, 1.44)	2.31	1,11	**0.04**
	Incubation date	−0.04 (−0.11, 0.04)	−0.96	1,116	0.34
Nest exposure	Treatment	1.07 (0.25, 1.89)	3.97	1,11	**0.01**
	Incubation date	−0.05 (−0.09, −0.02)	2.88	1,116	**0.007**

DISCUSSION

This study extends previous research showing that primary female Pukeko suffer a reproductive cost from joint-laying (Craig 1980a, Quinn *et al.* 2012). We show that joint-clutches have reduced per-egg hatching success due to both egg losses during incubation and increased hatching failure in retained eggs. Egg losses during incubation were likely due to eggs being accidentally cracked and then removed by parents. Eggs crack when nests contain large clutches in which eggs may become stacked on top of one another (Quinn *et al.* 2012; C.J. Dey pers. obs.). Furthermore, many of the 'lost' eggs in this study were observed to be cracked in the nest prior to their disappearance. Given this apparent cost of joint-laying for primary female Pukeko, the lack of overt female–female reproductive competition within Pukeko social groups is puzzling. However, our study showed that male Pukeko decreased incubation constancy in response to partial clutch removal, which may be a cost that prevents female Pukeko from destroying the eggs of co-breeders.

We also observed nest abandonments by some of our clutch removal groups. Quinn *et al.* (2012) also observed a high rate of nest abandonment (32%) when one clutch was removed from a joint-nest. Nest abandonment in response to partial clutch loss may be a male strategy that encourages re-nesting with a larger overall clutch. Indeed, male Wilson's Phalarope *Phalaropus tricolor* are more likely to incubate large clutches and may abandon small clutches early in the breeding season (Delehanty & Oring 1993). Although male Pukeko that decrease parental care or abandon nests suffer an immediate fitness cost in terms of reduced success of their current brood, their lifetime fitness may be increased, as they will not waste time and energy raising a small brood. Such a strategy may be especially beneficial when the breeding season is long (Delehanty & Oring 1993), re-nesting can occur quickly and the parental investment required for offspring to survive to adulthood is high. Pukeko at our study site regularly breed from July to February and are capable of breeding year-round (Dey & Jamieson 2013). Furthermore, in the two groups we monitored following nest abandonment, new clutches were initiated within 10 days. Finally, although Pukeko chicks are nidifugous, they are fed, guarded and brooded by adults for up to 4 months (Dey & Jamieson 2013). Hence, Pukeko fit the profile of a species in which facultative adjustments in parental effort in response to perceived brood value would be beneficial.

To our knowledge, this study is the first to demonstrate facultative adjustments in parental care in a joint-laying species. However, our study could not distinguish the specific cues used by males when decreasing their investment in incubation. Our partial clutch removal may have decreased the total clutch size to a point where the costs of incubation for males are no longer repaid by the benefits of raising the small brood. In Pukeko groups, paternity is usually shared among multiple males and thus each male breeder can only expect to sire a small number of offspring. It is possible that the additional eggs provided by a secondary laying female are required to entice breeding males to provide parental care. Indeed, many animals reduce paternal care in response to a decrease in perceived paternity (e.g. Hegner & Wingfield 1987, Dixon *et al.* 1994, Neff 2003) and such an effect could be responsible for the observed decrease in incubation consistency in the current study. An alternative explanation is that males perceived our egg removals as a predation event and reduced parental care in response to the nest no longer being considered a safe place to raise young. In joint-laying species in which ovicide occurs, males are unlikely to know the cause of missing eggs because egg destruction usually occurs when the male is absent from the nest (Vehrencamp & Quinn 2004). However, even if males could determine eggs lost to reproductive competition from those lost to predation, their response to any decrease in clutch size should be to decrease paternal investment, as both predation and ovicide decrease the benefits of providing parental care relative to the costs. As such, reductions in male care in response to decreases in clutch size may eliminate the potential benefits to females of destroying a co-breeder's clutch.

Although the available evidence suggests that primary female Pukeko would produce more offspring by nesting without a co-breeding female, it appears they have few options to monopolize reproduction. Because male Pukeko perform most of the incubation, females are probably unable to completely exclude co-breeding females from access to the nest. Furthermore, we show that destroying the eggs of a co-breeder may not be beneficial due to corresponding decreases in paternal care. The best option for females may be to evict or aggressively prevent secondary females from joining the group in the first place. Female Pukeko defend their territory from females that are not part of their group (C.J. Dey pers.obs.). However, whether eviction occurs and who performs evictions is not known for this species. Clearly, the fact that Pukeko females tolerate co-breeders suggests there are benefits of joint-nesting that have not yet been identified or that there are constraints preventing females from excluding competitors altogether.

In cooperative breeders, intrasexual competition will not occur if breeders are constrained in their ability to control the reproduction of same-sex rivals (Hodge 2009). While these constraints have typically been considered a property of competition and transactions among same-sex group members (Nonacs & Hager 2012), researchers are becoming increasingly aware that the behaviour of opposite-sex group members can influence the distribution of reproduction (e.g. Cant & Reeve 2002, Hamilton & Heg 2007). In particular, we suggest that more attention should be given to plasticity in parental and alloparental care in plural breeding co-operative breeders, as this may provide a widespread mechanism by which individuals can influence reproductive dynamics of opposite-sex group members.

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