Behavioral experiments in a wild passerine cause short-term reductions in parental provisioning and nestling mass

Experimentos conductuales en un ave paserina silvestre causan reducciones de corto plazo en el cuidado parental y peso de pichones

ABSTRACT

Research on wild animals often involves the capture and temporary removal of individuals from their territory. Although the acute effects of such procedures are well understood in terms of stress hormone profiles, their effect on the behaviour of captured individuals after release is poorly known. Additionally, for socially-monogamous individuals captured whilst breeding, little is known regarding whether the remaining individual compensates for the temporary loss of its partner and whether offspring condition or survival ultimately decreases due to any reductions in parental provisioning. We investigated the influence of field-based experiments requiring temporary adult removal on adult provisioning behaviour and nestling mass in the thorn-tailed rayadito (*Aphrastura spinicauda*), a Chilean furnariid species with equal parental effort between sexes. During the absence of the removed individual, remaining individuals did not increase nestling provisioning rates while, upon their release, removed individuals did not return to nestling feeding for at least 38 minutes and typically much longer. This drastic reduction in combined parental provisioning negatively affected nestling mass, while average nestling mass increased during a control period, nestlings failed to gain weight during and subsequent to parental removal. Finally, our experiments did not affect fledging probability. Overall, our results indicate that the temporary removal of rayaditos from their territories can cause short-term reductions in parental provisioning and nestling weight, but is unlikely to lead to any long-term effects on nestling survival.

Key words: *Aphrastura spinicauda*, nestling condition, parental provisioning, temporary adult removal, thorn-tailed rayadito.

RESUMEN

Las investigaciones en animales silvestres frecuentemente requieren la captura y remoción de individuos de sus territorios. Si bien se conocen los efectos a nivel hormonal de tales métodos, sus efectos sobre la conducta de los individuos después de la liberación son escasamente conocidos. Además, para individuos de especies monógamas que son capturados durante la época reproductiva, se desconoce si el individuo restante compensa la pérdida temporal de su compañero ni si la condición o sobrevivencia de los pichones disminuye como consecuencia del cuidado parental reducido. Investigamos la influencia de experimentos de campo que remueven temporalmente un adulto sobre las visitas de aprovisionamiento y el peso de pichones en el rayadito (*Aphrastura spinicauda*), un furnárido con similar cuidado parental entre los sexos. Durante la ausencia de un adulto, los individuos restantes de cada pareja no aumentaron la frecuencia de aprovisionamiento. Después de su liberación, los individuos previamente ausentes no volvieron a alimentar a sus pichones por al menos 38 minutos. Esta acentuada reducción en cuidado parental por ambos adultos de la pareja afectó negativamente el peso de los pichones. Aunque el peso medio de los pichones aumentó durante el período control, los pichones no aumentaron su peso durante y después de la remoción parental. Nuestros experimentos no afectaron el éxito de los volantones. Globalmente, los experimentos causaron reducciones de corto plazo en cuidado parental y peso de pichones, pero es improbable que causen efectos de largo plazo en la sobrevivencia de los pichones.

Palabras clave: *Aphrastura spinicauda*, condición de pichones, cuidado parental, remoción parental temporal, rayadito.
INTRODUCTION

In order to obtain important biological information during research targeting populations of wild animals, it is often necessary to temporarily or permanently remove individuals from their territory or habitats. For example, a common practice in conservation-based research is to temporarily capture individuals to obtain morphometrical or physiological data or to attach tracking devices (Dickson & Beier 2007, Iglay et al. 2007, Martinez et al. 2007). Similarly, research in behavioral ecology often requires the long-term (e.g. aviary-based or territorial behavior studies; Ekman & Griesser 2002, Liu 2004, Zann & Cash 2008) or short-term removal of individuals from their territories (e.g. the use of captured individuals as caged decoys during simulated territorial intrusion experiments; Meddle et al. 2002, Sperry et al. 2005, van Dongen & Mulder 2007).

Amongst birds, it is well established that such capture and handling of individuals typically results in an acute rise in glucocorticoid stress hormones (Wingfield et al. 1995, Romero & Reed 2005). In contrast, comparatively little is known about how bird capture can affect the behavior of individuals after their release. In addition, in monogamous species, the removal of individuals from their territory may impose detrimental effects on the individual’s partner and offspring. For example, many species undergo joint territorial defense and the removal of one individual can compromise the remaining individual’s ability to defend the territory alone (Langmore 1998). Likewise, if the removed individual was breeding, then even a short-term removal of that individual from its breeding territory could have lasting effects on offspring health due to reductions in parental feeding of offspring. Indeed, many studies have shown that the permanent removal of an adult during the breeding period (usually the male) can have drastic negative effects on nesting success. Although the remaining individuals of some species are able to compensate for the loss of their partner by increasing their own nestling provisioning rates (e.g. dark-eyed Juncos, *Junco hyemalis* [Linnaeus, 1758]; Wolf et al. 1990), permanent parental removal more typically results in a reduction in nestling mass upon fledging, a reduced immune response of nestlings or nest failure altogether (Wolf et al. 1990, Markman et al. 1996, Lynn & Wingfield 2003, Snoeijis et al. 2005, van de Pol et al. 2006).

However, much research on wild animals typically involves the temporary, and not permanent, removal of the individual from its territory. Therefore, the use of permanent-removal studies to make inferences concerning the detrimental effects on partners and offspring is less appropriate. Despite the abundance of studies requiring the temporary removal of individuals from their habitat, surprisingly little is known about the effects of such removal on the behavior of the removed and remaining individual, nor on offspring condition and survival. In addition, the potentially stress-inducing procedures typically used in field-based studies (e.g. the use of field-based aviaries or cages; Meddle et al. 2002, Sperry et al. 2005, van Dongen & Mulder 2007) could further reduce the probability the individual will return to normal behavioral activities immediately after release.

The thorn-tailed rayadito (*Aphrastura spinicauda* [Gmelin, 1789]) is a small, insectivorous, cavity-nester of the family Furnariidae (Bruce 2003). Within Chile, this species inhabits forests throughout a distribution spanning 25° of latitude from the semi-arid climate of central Chile to the sub-Antarctic conditions experienced in southern Patagonia (Jaramillo et al. 2003). We investigated the effects of a behavioral field-experiment, requiring temporary adult removal, on parental effort and nestling mass in this species. Specifically we were interested in 1) whether the remaining individual compensated for the temporary loss of its mate and how much time typically elapsed until the removed individual recommenced nestling provisioning upon release and 2) how any reduction in parental provisioning may influence short-term nestling weight loss.

METHODS

Study site

This study was conducted at isla Navarino (southern Patagonia, Chile; 54°56’ S, 67°38’
W), between 21 and 25 November 2007. Experiments occurred in a disturbed forest site predominantly consisting of Nothofagus species (N. pumilio [(Poepp. & Endl.) Krasser], N. antarctica [(Forster) Oerst] and N. betuloides [(Mirb.) Blume]). This area experiences a sub-Antarctic climate with cold temperatures (6° C mean annual temperature) and relatively high precipitation including both rain (annual rainfall: 450 mm) and snow (Di Castri & Hajek 1976). The site contains 160 artificial nesting boxes (see Moreno et al. 2005 and Moreno et al. 2007) for details concerning the nesting boxes. Active nest boxes were monitored every 1-2 days throughout the breeding season (which typically begins in October and ends in December) to determine the breeding status of each thorn-tailed rayadito pair.

Bird capture and removal

Our removal study required the capture of an individual adult from nesting boxes containing nestlings (mean nestling age on day of adult capture = 11.5 ± 0.5 days, range = 10-14 days, n = 7 nesting boxes). Although the site contains 160 nesting boxes, many natural nest cavities were also present at the site, which are also readily used by the rayaditos. Our sample size therefore only comprises of seven nests since, at the time of the study, only these seven nests were located in the nesting boxes. Our experiments could not be conducted on individuals nesting in natural cavities due to the difficulties of removing the nestlings to allow weighing.

Birds were captured in nesting box traps between 09:30 and 13:30 CSLT and were fitted with a metal ring and a unique combination of three colored leg rings. No obvious intersexual dimorphism exists in this species (Moreno et al. 2007) meaning that we could not reliably determine the sex of each adult upon capture. All individuals were instead sexed via molecular techniques (see Moreno et al. 2007 for details of the technique applied to rayaditos) revealing an approximately equal proportion of males and females that were captured for our experiments (four males and three females). Moreno et al. (2007) report that nest visitation rates are equal between sexes in this species, suggesting that sex differences will not bias our estimates of the effect of removal on provisioning effort. However, we cannot eliminate the possibility that sexes differ in their response to stress, which may also influence our estimates of mean provisioning rates after release. However, these short-comings are unlikely to affect our overall conclusions about the short-term effects of removal on parental provisioning and nestling weight.

We temporarily removed the captured individual from its territory in order to conduct a field-based ‘novel environment’ experiments for a separate study (van Dongen et al., unpublished data). After the termination of the experiment, the focal individual was recaptured and returned to the site of capture (i.e. the nesting box). The mean duration of this temporary removal (i.e. from initial capture to eventual release) was 36 ± 1 SE min (range = 32-43 min, n = 7).

Variation in parental provisioning

We investigated how our experimental removal affected the frequency of nesting box visits by the removed individual upon its release into its territory and of the remaining individual during and after its partner’s removal. Prior to bird capture and removal we quantified nest attendance rates by both individuals for a 45-minute period. During this period, one observer positioned themselves close to the nest, but hidden from view (between 10 and 15 m away), and recorded all visits by both individuals. After the capture of an individual, one observer remained at the nest to quantify visitation frequency by the remaining individual, while the other conducted the ‘novel environment’ experiment, which was carried out at least 100 m away from the territory (most activity of each rayadito pair at the site is conducted within a 30 m radius; van Dongen, personal observation). We also quantified the time taken for the remaining individual to recommence feeding after the disturbance during this period. The duration of this ‘during removal’ observation period depended on the time taken to conduct the ‘novel environment’ experiment, but variation between trials was not large (see above). After the release of the removed individual at the nest, one observer again quantified visitation
rates by both individuals during a 45-min period, including the time taken for the released individual to recommence nestling provisioning with this period. All visitation data were converted to visitation rates (visits h⁻¹). One observer (WvD) almost always conducted the pre-removal observations (6 of 7 observations), while the second observer (IL) conducted all the during-removal observations and six of seven post-removal observations. Although observer bias could contribute to differences in estimates of parental provisioning between trial stages, this is unlikely due to the high level of objectivity involved in recording the identity of individuals visiting the nest.

**Variation in nestling weight**

We also investigated how our experimental adult removal, and any resultant reduction in parental provisioning, affected nestling weights. Immediately prior to the removal of an adult (i.e. after the initial 45-min observation period), we weighed all nestlings in the nest to the nearest 0.01 g using an electronic balance (i.e. at t=0removal; mean number of nestlings per brood = 4.4 ± 0.3 SE nestlings; range = 3–6, n = 7 nests). All nestlings were fitted with a metallic leg band (National Band and Tag, Co. Newport, KY, USA; model 1242-3) to aid in identification. Nestlings were again weighed at the end of the final 45-min observation period when both individuals had been free to feed the nestlings (i.e. at approximately t=80removal min, depending on the length of the ‘novel environment’ experiment. For consistency, and to avoid confusion, this time period will always be referred to as ‘t=80removal’, regardless of the actual time of measurement). Finally, we quantified nestling weight 195removal min after the initial weighing time.

We returned to the same nest on the following day and again measured nestling weight at times t=0control (i.e. at approximately the same time as measured on the previous day) and 195control min, without any adult removal. This allowed us to quantify nestling weight variation without parent removal (i.e. the control treatment) and to monitor nestling weight over a period of approximately 24 hours. All nests were monitored until fledging to track fledging success.

**Statistical analyses**

Kolmogorov-Smirnov tests revealed that the assumption of data normality was met for all our data. To compare variation in parental provisioning effort relative to the three stages of the removal experiment (i.e. prior to, during and after the removal) we initially used a repeated measure ANOVA to test whether parental provisioning varied between trial stages. Pairwise t-tests were subsequently used to determine exactly across which trial stages parental provisioning differed. Depending on the specific analysis, we either used individual parental effort (i.e. keeping the visitation rates of both members of the pair separate) or combined parental effort (i.e. summing the visitation rates of both members).

For our analyses of variation in nestling weight we used generalized linear mixed models (GLMM) due to the potentially confounding effects of pseudo-replication. This is because the sampling unit for this analysis was the nest and not each nestling within that nest since all members of the brood are subjected to the same variation in feeding effort by their parents. Therefore, GLMMs allowed for the non-independent nature of the data by incorporating nestling identity as a random factor. This controlled for differences in weight variation between nestlings of the same brood. In addition, as we compared the average nestling weight between different stages of the experiment, thus including the same nest within the two treatment groups, we also incorporated nest identity as a random factor. In all cases, the response variables followed a normal distribution and the models calculated using normal (with identity link) error variances.

GLMMs initially incorporated trial stage (t=0removal, t=80removal, t=195removal, t=0control or t=195control, depending on the specific model), brood size, brood age and all possible interactions as fixed factors. We then used Akaike’s Information Criteria (AIC; Akaike 1974) to select the most parsimonious statistical model from a range of models consisting of different combinations of the explanatory variables. AIC is calculated as the model deviance plus twice the number of estimable parameters of the model (Burnham & Anderson 1998). Trial stage was always...
included in the models (either as a main effect or within an interaction) as this was the variable we were interested in. The model resulting in the lowest AIC was considered the most parsimonious model and competing models with differences in AIC values of more than two were considered significantly different. However, when the AIC values for two competing models differed by less than two, we chose the model with the least number of parameters as the best fitting model (Quinn & Keough 2002).

Although a repeated measures approach could also be implemented to test nestling mass variation across trial stages, GLMMs are more appropriate in this case. This is because repeated measures analysis would require averaging nestling mass in each nest to avoid pseudo-replication, followed by using nest identity as the sampling unit. However, given the high number of independent variables being tested, this approach would lead to overfitting of the data (Quinn & Keough 2002). A GLMM approach coupled with AIC model selection is therefore a robust alternative. All pair-wise t-tests were conducted using SPSS 15.0 (SPSS Inc. 2006) and all GLMMs with Genstat 11.0 (VSN International Ltd 2008). Data are reported as means and standard errors and all differences were considered significant when \( P \leq 0.05 \).

**RESULTS**

**Variation in parental provisioning**

There was no effect of nestling age or brood size on combined adult provisioning rates either before the removal experiment or after the release of the focal individual into its territory (combined adult provisioning rate - before removal: nestling age - \( R^2 = 0.25, F_{1,5} = 1.7, P = 0.26 \), brood size - \( R^2 = 0.004, F_{1,5} = 0.02, P = 0.89 \); after release: nestling age - \( R^2 = 0.01, F_{1,5} = 0.06, P = 0.82 \), brood size - \( R^2 = 0.07, F_{1,5} = 0.4, P = 0.57 \)). However, the combined parental provisioning rate was reduced by 46% during the post-release trial stage (combined provisioning rate - before removal = 46.1 ± 7.0 visits h\(^{-1}\), after removal = 24.8 ± 2.3 visits h\(^{-1}\); Pairwise t-test: \( t_7 = 3.2, P = 0.02 \)), predominantly due to the reduced

visitation rate of the removed parent after its release into the territory (T = 7.7, df = 6, \( P < 0.001 \); Figure 1). In contrast, although the nest visitation rate of the remaining parent varied significantly among the three trial periods (repeated measures: \( F_{2,12} = 6.6, P = 0.011 \)), pair-wise t-tests revealed that their provisioning rates did not differ before and after the removal experiment (T = 0.3, df = 6, \( P = 0.77 \); Figure 1). Instead, they provisioned at lower rates during their partner’s temporary removal, compared to both pre-removal (T = 2.7, df = 6, \( P = 0.03 \)) and post-release rates (T = -4.3, df = 6, \( P = 0.005 \)). This pattern was a probable result of not immediately returning to nestling feeding (average return time of the remaining individual after partner removal: 8 min 52 s ± 1 min 2 s; range = 5 - 13 min).

Two captured individuals recommenced provisioning the nestlings relatively rapidly after their release onto their territories (within 38 and 40 min respectively), while the remaining five individuals did not return during the 45-min post-release observation period. However, all removed individuals were observed at the nest when the nestlings were re-weighed approximately 160 min after their release (i.e. 195 min after the initial weighing). All individuals were also sighted feeding the nestlings on the following day.

**Variation in nestling weight**

Nestling weights varied considerably across the different phases of our removal experiments (GLMM: Trial stage- Wald = 25.28, df = 1, \( P < 0.001 \); AIC = 8.90 vs. 12.62 for second most parsimonious model [Trial stage + Brood size]; Figure 2). Nestlings initially lost weight in the absence of the second parent (i.e. at 80\(_{\text{removal}}\) min; GLMM: Trial stage - Wald = 8.33, df = 1, \( P = 0.007 \); AIC = 27.36 vs. 29.24 for second most parsimonious model [Trial stage + Brood age]). However, they appeared to recover from this loss of weight two hours later (at 195\(_{\text{removal}}\) min), when they did not differ from their original weights (GLMM: Trial stage - Wald = 2.04, df = 1, \( P = 0.164 \); AIC = 38.12 vs. 40.31 for second most parsimonious model [Trial stage + Brood age]). In comparison, nestlings gained weight over the same period during the control period (i.e. between t = \( t_{\text{control}} \) and t = 195\(_{\text{control}}\); GLMM: Trial stage - Wald = 12.50,
df = 1, P = 0.001; AIC = 37.01 vs. 40.40 for second most parsimonious model [Trial stage + Brood age]). Finally, nestlings did not gain weight overnight, between the treatment and control days (i.e. between t=195 removal and t=0 control; GLMM: Trial stage - Wald = 0.51, df = 1, P = 0.480; AIC = 39.28 vs. 44.64 for second most parsimonious model [Trial stage + Brood age + Trial stage*Brood age]), nor was any weight gained overall, over the entire experimental and control periods (i.e. between t=0 removal and t=195 control; GLMM: Trial stage - Wald = 3.08, df = 1, P=0.090; AIC = 49.02 vs. 50.78 for second most parsimonious model [Trial stage + Brood age + Trial stage*Brood age]). Six of the seven nests where we conducted our removal experiments successfully fledged all young. The remaining nest failed due to predation.

DISCUSSION

Research in many zoological-based disciplines often requires the temporary capture and handling of wild animals (e.g. Ekman & Griesser 2002, Dickson & Beier 2007, Morgan et al. 2007). Such techniques typically provide valuable information about the study species and are often a mandatory component of research. Despite these benefits, stressed-induced negative effects on the health and behavior may also be experienced by the captured individual. Moreover, if the animal is captured during the breeding season, reductions in offspring condition and survival can also occur, if the captured individual desists in caring for offspring for a period after release. We have shown here that our temporary removal of adult rayaditos for...
environment’ experiments has short-term consequences for both adult individuals and, ultimately, the nestlings. Firstly, the remaining individuals did not increase their provisioning rates in response to the apparent loss of their partner. In addition, they did not return to feeding the nestlings for an average of approximately nine minutes after the disturbance, a period of time typically spanning 3.4 nest visits per individual. In contrast, most captured individuals did not recommence nestling provisioning during the 45-min post-release observation period, although all were observed at the nest a few hours later. This short-term reduction in combined parental effort appeared to negatively affect mean brood weight. Nestlings, on average, failed to gain any weight during the ‘experimental’ day when a parent was temporally removed. However, during the following ‘control’ day nestlings once again increased in weight, suggesting that the negative effects of adult removal are only short-term and that nestlings are able to partially or fully recover any lost weight in subsequent days.

Our data are in accordance with previous adult removal experiments that have reported negative effects of parental removal on nestling condition and nesting success. For example, nestling Great Tits (Parus major [Linnaeus, 1758]) in nests where males were permanently removed fledged with a lighter mass than control nestlings, had a reduced immune response and were less likely to be resighted in

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**Fig. 2:** Variation in nestling weights across the different stages of the experiment trial. Bars represent means (+ SE) for all 31 nestlings present in the 7 nests sampled. Nestling weight was quantified immediately before parent removal, 45 min. after parent release into the territory and 160 min after release. Weights were also quantified on the following day as a control, at time = 0 and time = 195 min.

Variación en los pesos de los pichones a lo largo de los diferentes períodos de experimentación. Las barras indican las medias (+ SE) para todos los 31 pichones presentes in 7 nidos muestreados. El peso de los polluelos fue cuantificado inmediatamente antes de la remoción de uno de los padres, 45 y 160 min después de la liberación del padre en el territorio. Como controles, los pesos también fueron medidos al día siguiente en dos tiempos, a los 0 minutos, y a los 195 min luego de la primera medición.
the following year (Snoeij et al. 2005). Removal of male Chestnut-collared Longspurs (*Calcarius ornatus* [Townsend, 1837]), in contrast, resulted in complete nest failure (Lynn & Wingfield 2003). However, these previous studies typically focus on the benefits of bi-parental provisioning and therefore involve the permanent removal of an adult (usually the male) whilst nestling provisioning. In contrast, little information exists on the effects of temporary removal of individuals on reproductive success, a scenario which is more realistic in field-based experiments or other handling techniques commonly in use in conservation and ecological practices.

Our current knowledge on the effect of temporary adult removal on nestling mass could be augmented by several extensions of the current study. Firstly, it would be beneficial to investigate the effect of parental removal on nestlings spanning a wider range of ages - due to the logistic nature of nestling growth (Moreno et al. 2005, Brown et al. 2007) and the higher thermoregulatory energetic costs experienced by older nestlings (e.g. Weathers & Sullivan 1991, Hodum & Weathers 2003, Weathers et al. 2003) age-related differences in mass loss due to parental removal may occur. Secondly, the current study was performed on a sub-Antarctic population where weather conditions are often unfavorable (Di Castri & Hajek 1976). Since the thermoregulatory costs of nestlings are typically elevated in colder climates (Weathers et al. 2003), which in turn may affect nestling energy growth rates, the effect of parental removal on nestlings in more northerly populations (i.e. where conditions are more favorable) may be less pronounced (e.g. see Bart & Tornes (1989) and Kuitunen et al. (1996) for similar studies on other species). Finally, the current study was limited by the relatively low number of nests at which we conducted the removal experiments. Our data would therefore need to be interpreted with some caution, as these low sample sizes may influence some finer detailed conclusions of the study. In addition we were unable to include additional, yet potentially-interesting, variables in our analysis, such as previous capture experience of adults, or adult sex.

Overall it appears that behavioral experiments involving temporary removal of adults during the breeding period can have short-term negative effects on nestling weight. It remains unknown how our experiments affect nestling viability over longer periods, although all nestlings increased their weight during the following control day, indicating that nestlings are likely to be able to either fully or partially recover lost weight in subsequent days. In addition, all the focal nests successfully fledged all their nestlings (with the exception of one nest that was depredated). In light of our findings, we conclude that behavioral experiments of this nature only have short-term negative effects on both adult behavior and nestling condition, and probably cause few, if any, detrimental effects over longer periods.

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