

# Do stress hormones suppress helper reproduction in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*)?

A. L. Malueg · J. R. Walters · I. T. Moore

Received: 28 May 2008 / Revised: 10 December 2008 / Accepted: 11 December 2008 / Published online: 14 January 2009  
© Springer-Verlag 2008

**Abstract** In the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*), male helpers are subordinate to male breeders and do not mate with females, even when unrelated to the breeding female within their group or through extra-group matings, yet exhibit reproductive hormone profiles similar to those of breeders. We investigated whether reproduction might be suppressed in helper males via high levels of the stress hormone corticosterone. We also examined effects of group size and season on corticosterone levels by comparing baseline and maximal plasma levels of corticosterone between helper males and breeding males, and among helper males and breeders of both sexes living in groups of different sizes throughout the reproductive cycle. We also measured plasma levels of estrogen, progesterone, and testosterone to examine other potential hormonal differences between helpers and breeders. Male status did not explain variation in any hormones; therefore, our data do not support the hypothesis that helper males are reproductively suppressed via corticosterone or the other hormones investigated. However, the presence of two or more helper males in a group tended to reduce baseline corticosterone in breeding and helper males, but not breeding females, suggesting that helper males reduce parental effort of other male group members. Seasonally, maximal corticosterone peaked during the nestling provisioning phase for breeding and helper males, but not breeder females, suggesting that males show an increased response to stressors posing a potential threat to survival of offspring.

**Keywords** Stress · Corticosterone · Cooperative breeding · Red-cockaded woodpecker · *Picoides borealis*

## Introduction

Cooperative breeding has been described in over 100 mammal and nearly 300 bird species (Brown 1987). These systems vary from those in which group members share reproduction (e.g., Whittingham et al. 1997; Li and Brown 2000) to others in which some adults, termed helpers, remain reproductively inactive and help raise the young of breeding pairs to whom they are often, but not always, related (e.g., Dawson and Mannan 1991; Walters 1990). Ultimate causes for delayed reproduction in helpers have been investigated (Emlen 1982; Brown 1987; Walters et al. 1988; Stacey and Ligon 1991; Koenig et al. 1992; Arnold and Owens 1998; Conner et al. 2001), but studies describing proximate mechanisms controlling these differences in reproductive behavior between breeders and helpers are limited. Several hypotheses have been advanced to explain the mechanisms of suppression of reproductive behavior in male helpers. Behavioral interference may prevent even reproductively capable helper males from mating; helpers may be physically blocked from mating by breeding males, via direct aggression (Reyer et al. 1986) or mate-guarding (Mumme et al. 1983; Vleck and Brown 1999). Such mechanisms are seldom completely effective, however, and are found in systems in which subordinate males father some offspring rather than those in which their reproductive activity is completely suppressed.

Hormonal mechanisms are more likely means of total reproductive suppression of helpers. In many species, helper males exhibit lower plasma levels of testosterone than breeder males (Reyer et al. 1986; Mays et al. 1991; Schoech et al.

---

Communicated by J. Graves

A. L. Malueg · J. R. Walters · I. T. Moore (✉)  
Department of Biological Sciences, Virginia Tech,  
Blacksburg, VA 24061-0406, USA  
e-mail: itmoore@vt.edu

1991, 1996; Wingfield et al. 1991). Helpers may also exhibit high levels of progesterone (Schoech et al. 1991) or prolactin, two hormones associated with parental care (Hadley 2000) that may reduce testosterone levels or inhibit testosterone production (Nelson 2000). Low levels of testosterone may be accompanied by low body mass (Wingfield et al. 1991), small testes (Reyer et al. 1986; Wingfield et al. 1991; Schoech et al. 1996), or inability to produce sperm (Reyer et al. 1986), rendering helpers physiologically incapable of reproduction, independent of age.

In many species, helpers rarely or never assist in nest-building, incubation of eggs, or brooding of nestlings (Kinnaird and Grant 1982; Rabenold 1985; Dawson and Mannan 1991). For example, access of helper stripe-backed wrens (*Campylorhynchus nuchalis*) to the nest at this time is blocked by the breeding male, and helper Harris' hawks (*Parabuteo unicinctus*) rarely come into direct contact with young even after the nestling phase (Dawson and Mannan 1991). These patterns are consistent with hormonal differences between helpers and breeders.

In contrast in the red-cockaded woodpecker (*Picoides borealis*), helpers, which are mostly male, assist the breeding pair in territory defense, cavity construction, incubation of eggs, and feeding and brooding of young (Walters 1990); helpers even develop a brood patch (Conner et al. 2001). Furthermore, male breeders and helpers maintain similar body condition and similar levels of testosterone and prolactin throughout the breeding cycle (Khan et al. 2001). Males are capable of successful reproduction even in their first year (Lape 1990; Walters 1990) and sometimes are breeders at this age, but some males remain helpers until up to age 12 (Walters, unpublished data). Yet red-cockaded woodpeckers are nearly strictly monogamous: helpers have never been observed copulating (breeders commonly are, even outside the breeding season) or even attempting copulation (Lennartz et al. 1987; Lape 1990; Walters 1990; Conner et al. 2001), and genetic data indicate that extra-pair fertilizations are rare or non-existent (Haig et al. 1993, 1994), even though individuals commonly foray into the territories of other groups (Conner et al. 2001). In the single exception observed by Haig et al. (1994), an offspring was fathered by an extra-group male rather than by a helper from within the group, even though the helper within the group was unrelated to the breeding female.

Although most helpers originate as offspring retained on the natal territory, helpers are often unrelated to the breeding female (Fig. 1) due to two phenomena (Lennartz et al. 1987; Walters et al. 1988; Walters 1990). First, a small fraction of the males that disperse in their first year, rather than remain on the natal territory as helpers, join unassisted pairs and become unrelated helpers. Second and much more commonly, when a breeding male dies the oldest helper inherits the territory. If this male is the son of the breeding female, she



**Fig. 1** The number of helper males captured and their relationship to the breeding pair of their group, listed as relationship to breeding female, relationship to breeding male

departs and is replaced by a new female and the remaining helpers are henceforth unrelated to the new breeding female. Interestingly, if the oldest helper is unrelated to the breeding female, when the breeding male dies the female invariably stays and pairs with the helper (Daniels and Walters 2002). These patterns have two important implications. First, they suggest that kin recognition exists and results in incest avoidance. Second, they suggest that birds that recognize one another as appropriate sexual partners refrain from mating when the male holds helper status.

The means by which reproduction is completely suppressed in red-cockaded woodpecker helpers are unknown. Sexual inactivity is not due to age or sexual immaturity (Khan et al. 2001), breeding males do not appear to engage in mate-guarding to physically block helpers from mating (Lape 1990), and levels of reproductive hormones are sufficient to support breeding activity (Khan et al. 2001). Inbreeding avoidance does not fully explain helper reproductive suppression since many helpers are unrelated to the breeding female of their group. The objective of this study is to examine another possible hormonal mechanism that has not previously been investigated, suppression via glucocorticoid stress hormones.

One of the main actions of glucocorticoids is to mobilize energy stores. Thus, in socially living species, subordinates (helpers of cooperatively breeding species) may have higher levels of the glucocorticoid hormone corticosterone (in birds) or cortisol (in most mammals) than dominant individuals if it is more energetically demanding to be subordinate than to be dominant (Goymann and Wingfield 2004). This can occur if subordinates must expend more energy than dominants to carry out daily activities such as foraging and territory defense (Goymann and Wingfield 2004). Relatively higher levels of glucocorticoids in subordinates will also occur if subordinates expend more energy enduring physical aggression from dominants compared to the energy spent by

dominant individuals in acquiring and maintaining their dominant status (Goymann and Wingfield 2004). In particular, psychological intimidation (such as eye contact in primates), as opposed to overt aggression, requires little energy from dominants, but can increase stress levels in subordinates (Sapolsky 2005). Lack of control over resources such as food and mates may also cause an increase in glucocorticoid levels of subordinates (Creel 2001; Goymann and Wingfield 2004). Stress hormones are well known for their suppressive effects on reproduction (Moore and Jessop 2003) as they can inhibit the production of reproductive hormones or directly inhibit courtship and mating behaviors without affecting other hormones (Wingfield 1988; Moore and Jessop 2003).

Several observations suggest the hypothesis that helpers experience chronic stress to be a reasonable one for red-cockaded woodpeckers. Helpers expend considerable effort on tending nests and young (Lennartz et al. 1987; Khan and Walters 2002). Helpers maintain spatially separated foraging niches from breeders, suggesting they have less control over food resources (Rudolph et al. 2007), and they have little to no control over mate choice since they usually must wait for the death of a breeder male before being able to mate (Conner et al. 2001). Furthermore, survival of breeding males, but not helper males, increases when groups are augmented by the addition of fledglings or more helpers, suggesting breeders profit more from benefits of group living than do helpers (Khan and Walters 2002). While breeder males do not actively mate-guard, they have been observed chasing or following helper males during the pre-laying and laying stages of the breeding cycle (Lape 1990). Breeder females become agitated if approached by helper males (although this is uncommon); they become noisy, fly away, or open-wing display (a defense behavior which may serve as a means of intra-pair recognition (Ligon 1970)) (Lape 1990). These acts of mild aggression and intimidation likely require little energy from breeders, but may be energetically, or at least psychologically, costly to helpers. Interestingly, Creel (2001) suggested that glucocorticoid levels may be higher mainly in subordinates of primitively social species that have not yet evolved a mechanism of direct reproductive suppression to avoid the negative consequences (such as immune suppression) of chronic stress. Cooperative breeding evolved independently in red-cockaded woodpeckers; no close relatives of this species breed cooperatively (Walters 1990). Thus red-cockaded woodpeckers may be described as a primitively social species and subsequently may be expected to use stress-mediated sexual suppression.

To test the hypothesis that stress hormones suppress reproduction by helper males, we compared plasma levels of baseline and stress-induced corticosterone between

helper males and breeding males, and among helpers and breeders living in family groups of different sizes. Since energetic demands and social interactions may vary through the breeding cycle (Silverin 1998; Romero 2002), we measured corticosterone levels during six phases of the breeding cycle. In addition, we compared testosterone levels between male helpers and male breeders to confirm, in a second population, the previous finding of Khan et al. (2001) that testosterone levels do not differ between male helpers and breeders. To investigate if there were other hormonal differences between male breeders and helpers, we also compared plasma levels of estrogen and progesterone. Finally, we sampled breeding females opportunistically, to see if their seasonal hormonal profiles differed from those of males.

## Materials and methods

### Study site

Our study was conducted on a population of red-cockaded woodpeckers on Marine Corps Base Camp Lejeune on the central coast of North Carolina, USA (34°38' N, 77°19' W). Camp Lejeune is composed of two parts, the Main Base, an area of 110,000 acres, including 26,000 acres of open water, and the Greater Sandy Run Area, encompassing 41,000 acres (Convery 2002; Walters et al. 2005, unpublished report). It is only on the Main Base that red-cockaded woodpeckers reside. The birds on the Main Base occur in two subpopulations, separated by the New River, between which there is relatively little dispersal (Walters et al. 2005, unpublished report). Camp Lejeune's red-cockaded woodpecker population has been extensively monitored since 1986 (Walters et al. 2005, unpublished report). Birds are removed from the nest as nestlings and banded with a numbered USFWS leg band and a unique color band combination. Each individual's group membership and social status is determined annually from behavioral observations, using the criteria described in Walters et al. (1988).

### Study design

We collected blood samples from male breeders, female breeders, and male helpers during the post-breeding phase in 2005 and during the pre-breeding, copulation, egg-laying/incubation, nestling provisioning, fledgling provisioning, and post-breeding phases in 2006 (see Table 1 for dates and sample sizes). Pre-breeding, copulation, egg-laying/incubation, and nestling provisioning were defined following Khan et al. (2001). Fledgling provisioning was defined as the period after the young left the nest but were

**Table 1** Sampling dates and number of days before/after clutch initiation or after hatch date for each breeding phase

Phase	Pre-breeding	Copulation	Lay/ incubation	Nestling provisioning	Fledgling provisioning	Post-breeding 2005	Post-breeding 2006
Dates	21 February–26 March	10 April–10 May	6 May–17 June	14 May–21 July	8 June–20 July	26 July–17 August	26 July–15 August
Days before (-)/after clutch initiation	-35—(-)60	-3—(-)22	3–14	–	–	–	–
Days after hatching	–	–	–	1–27	29–81	88–94	79–95
Sample size	6, 7, 2	9, 9, 2	9, 13, 8	2, 7, 4	7, 8, 5	3, 3, 3	3, 1, 1

Overlap in sampling dates across phases is due to asynchronous breeding across family groups. Sample sizes are listed in the following order: breeding females, breeding males, helper males

still fed by adults, approximately 4 to 11 weeks post-hatch (Walters 1990). Post-breeding was defined as any time after 11 weeks post-hatch. Birds were sampled from family groups ranging in size from two to four birds. Only one family group had three male helpers, and all birds from this group were sampled.

#### Blood collection

Birds were observed in the evenings to determine the tree cavities in which individuals were roosting. In the mornings, birds were captured by placing a net attached to an expandable pole over roosting cavities before birds arose at dawn. Birds were flushed into the net by kicking the trunk of the tree or banging the trunk with a wooden stake. While adults were laying and incubating eggs, birds were captured off the nest in the same manner, but were captured not only at dawn but also throughout midmorning until approximately 10:00 am. Birds were never captured the morning immediately following an evening observation on their territory.

Blood was collected in heparinized microhematocrit tubes after puncture of the alar wing vein with a 26-gauge needle. Approximately 50  $\mu$ L of blood was collected within 3 min of capture to assess baseline plasma corticosterone levels. Another 200  $\mu$ L of blood was collected within 10 min of capture to measure plasma levels of testosterone, estradiol, and progesterone. Birds were then held in an opaque cloth bag. Thirty minutes after the initial capture, another 50  $\mu$ L of blood was collected to assess stress-induced plasma corticosterone levels. Blood samples were transferred to microcentrifuge tubes and kept on ice in a small cooler in the field (<6 h) until centrifuged to extract plasma. Plasma was kept frozen at  $-20^{\circ}\text{C}$  until hormone analyses.

#### Hormone assays

Plasma collected at the 3- and 30-min time points was analyzed for levels of corticosterone by direct radioim-

unoassay. Plasma volumes of 9–35  $\mu$ L were equilibrated overnight with 2,000 cpm radiolabeled corticosterone for individual recovery determination. Each sample was then extracted in dichloromethane, and the organic phase was removed and dried in a warm water bath under nitrogen gas. The dried extracts were resuspended in buffer for the assay. The samples were divided into duplicate 200- $\mu$ L samples, and a 100- $\mu$ L aliquot was used to determine individual extraction efficiency. Serial dilutions for the standard curves were performed in triplicate. All samples including serial dilutions were incubated overnight with 100  $\mu$ L of antiserum and 100  $\mu$ L of radiolabeled steroid. Unbound steroid was separated using dextran-coated charcoal, and the bound steroid was decanted into scintillation vials. The samples were resuspended in 5 mL of scintillation fluid and counted on a scintillation counter. A cubic spline curve was fitted to the standard curve points, and final steroid levels were calculated from this curve and adjusted based on sample volumes and individual recoveries. Plasma corticosterone levels were measured in four assay runs with intraassay and interassay variations of 18% and 20%, respectively. Limit of detectability was  $\sim 0.76$  ng/mL.

Plasma collected at the 10-min time point was analyzed for levels of testosterone, estradiol, and progesterone. Plasma volumes of 36–164  $\mu$ L were used in these assays. The assay was similar to that used for corticosterone except that the hormones were separated from one another and from neutral lipids using column chromatography. The samples were passed through individual celite columns and increasing proportions of ethyl acetate in *iso*-octane were added to remove each steroid fraction. The purified extracts were dried and resuspended in buffer for the assay. Sample plasma testosterone, estradiol, and progesterone levels were measured in three assay runs. Intraassay variations were 8%, 12%, and 25% for testosterone, estradiol, and progesterone, respectively. Interassay variations were 14%, 22%, and 29% for testosterone, estradiol, and progesterone, respectively. Limits of detectability

were  $\sim 0.07$ ,  $\sim 0.08$ , and  $\sim 0.31$  ng/mL for testosterone, estradiol, and progesterone, respectively.

### Statistical analyses

Hormone levels were transformed as necessary to meet assumptions of normality and homogeneity of variance. Females were analyzed separately from males, as they were captured opportunistically and as our a priori interest was to compare hormone levels between male helpers and male breeders. As a preliminary analysis, we performed analyses of variance (ANOVAs) to test for a difference in baseline corticosterone, maximum corticosterone, and testosterone between helper males related and unrelated to the breeding female. No significant effect of helper kinship was found for any of these hormones ( $F_{1, 30}=0.74$ ,  $p=0.40$ ;  $F_{1, 29}=0.22$ ,  $p=0.64$ ;  $F_{1, 31}=0.85$ ,  $p=0.85$  for baseline corticosterone, maximum corticosterone, and testosterone, respectively; Fig. 2). Thus, male helpers related and unrelated to the breeding female of the group were combined for subsequent analyses. We sampled only one family group with three male helpers. Samples from this group were combined with samples from groups with two male helpers and were collectively referred to as groups with two or more helpers. All statistical analyses were performed using SAS version 9.1.

We constructed an a priori set of 17 candidate models for males and eight candidate models for females representing multiple hypotheses about factors affecting hormone levels (Table 2). The same set of models was used for each hormone. The independent variables used in the models were chosen from a pool of five factors: social status (males only), breeding phase, number of helpers in a group, year of data collection, and age of individuals. Candidate models were linear mixed models that included combinations of up to two independent variables and all possible two-way interactions. Models were restricted to two independent variables due to small sample sizes.

We used an information-theoretic approach to compare the fit of our candidate models to our dataset. We used the corrected Akaike's Information Criterion ( $AIC_c$ ), where the correction, symbolized by "c", allows for small sample sizes relative to the number of parameters (Burnham and Anderson 2002).  $AIC_c$  values were provided by PROC MIXED in SAS 9.1. The  $AIC_c$  values were used to calculate Akaike weights ( $w_i$ ), which measure a model's relative probability of being the best model for the available data compared to the other candidate models. The model with the highest Akaike weight in each set of models was chosen as the best model (Burnham and Anderson 2002). The best model for each hormone was then analyzed with ANOVA followed by post hoc Tukey pairwise comparisons when appropriate.

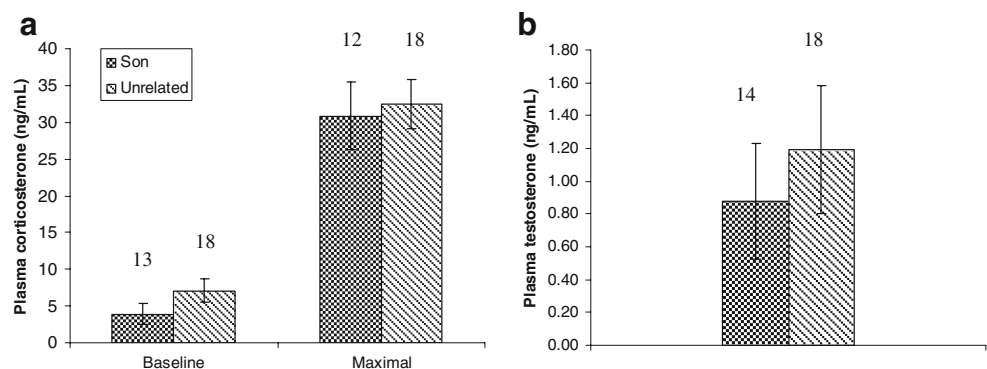
### Results

Of the candidate models, the model incorporating breeding phase, number of helpers, and their interaction (Table 3, models M5 and F1) was the model best supported by the available data for all hormones and for both males and females. This model was very strongly supported, with an Akaike weight of near or equal to 1.00 among the candidate models for every hormone. The Akaike weights and delta  $AIC_c$  values of the second-best models ranged from  $2.48 \text{ E}-4$  to  $4.00 \text{ E}-14$  and 12.2 to 61.7, respectively, indicating no support for factors not included in the best model. More importantly, there was no support for an effect of status for any hormone in the male models, that is, breeder and helper males had indistinguishable hormone profiles.

#### Baseline corticosterone

*Males* Model M5 (Akaike weight=0.9997) accounted for 21% of the variation in baseline plasma corticosterone levels in breeder and helper males. ANOVA indicated that

**Fig. 2** Plasma corticosterone (a) and testosterone (b) levels (mean + SE) of helper males related and unrelated to the breeding female of their group. Sample sizes are indicated above bars. Note difference in scale between hormone levels



**Table 2** Candidate models representing multiple hypotheses of effects on hormone levels in males and females

Model number	Model structure <sup>a</sup>
M1	s * p
M2	s + p
M3	s * h
M4	s + h
M5	p * h
M6	p + h
M7	s
M8	p
M9	h
M10	s * a
M11	s + a
M12	h * a
M13	s * y
M14	s + y
M15	h * y
M16	h + y
M17	p + y
F1	p * h
F2	p + h
F3	p
F4	h
F5	h * a
F6	h * y
F7	h + y
F8	p + y

Models M1–17 represent males and models F1–8 represent females  
<sup>a</sup>Model factors included: *s* status, *p* breeding phase, *h* number of male helpers in group, *a* age of individuals, *y* year of data collection  
 Asterisk (\*) indicates a factorial model and plus sign (+) indicates an additive model

baseline corticosterone did not vary significantly due to breeding phase ( $F_{5, 73}=0.88, p=0.50$ ; Fig. 3) or the interaction of breeding phase and number of helpers ( $F_{10, 73}=0.51, p=0.87$ ).

The effect of number of helpers was also non-significant ( $F_{2, 73}=2.56, p=0.086$ ); however, baseline corticosterone levels were generally lower when two or three helpers were

present than when one helper was present (Tukey post hoc test  $p=0.071$ ; Fig. 4).

*Females* Model F1 (Akaike weight=1.0000) accounted for 48% of the variation in baseline plasma corticosterone levels in females. However, neither breeding phase ( $F_{5, 38}=1.25, p=0.32$ ), number of helpers ( $F_{2, 38}=0.23, p=0.80$ ), or the interaction of these factors ( $F_{7, 38}=1.32, p=0.28$ ) significantly influenced baseline corticosterone in the ANOVA (Fig. 3).

Maximum corticosterone

*Males* Model M5 (Akaike weight=1.0000) accounted for 34% of the variation in maximal plasma corticosterone in males. Maximal corticosterone did not vary significantly due to number of helpers ( $F_{2, 76}=1.51, p=0.23$ ) or the interaction between breeding phase and number of helpers ( $F_{9, 76}=1.05, p=0.41$ ) in the ANOVA, but varied among breeding phases ( $F_{5, 76}=3.20, p=0.013$ ; Fig. 3). Maximal corticosterone levels were significantly higher in the nestling provisioning phase than in the egg-laying/incubation, copulation, and post-breeding phases (Tukey post hoc test  $p<0.05$ ), and nearly so for the pre-breeding phase (Tukey post hoc test  $p=0.052$ ), for both breeder males and helper males.

*Females* Model F1 (Akaike weight=1.0000) accounted for 35% of the variation in maximal plasma corticosterone in females. However, neither breeding phase ( $F_{5, 38}=1.22, p=0.33$ ), number of helpers ( $F_{2, 38}=0.85, p=0.44$ ), or the interaction of these factors ( $F_{7, 38}=0.92, p=0.51$ ) significantly influenced maximal corticosterone in the ANOVA (Fig. 3).

Testosterone

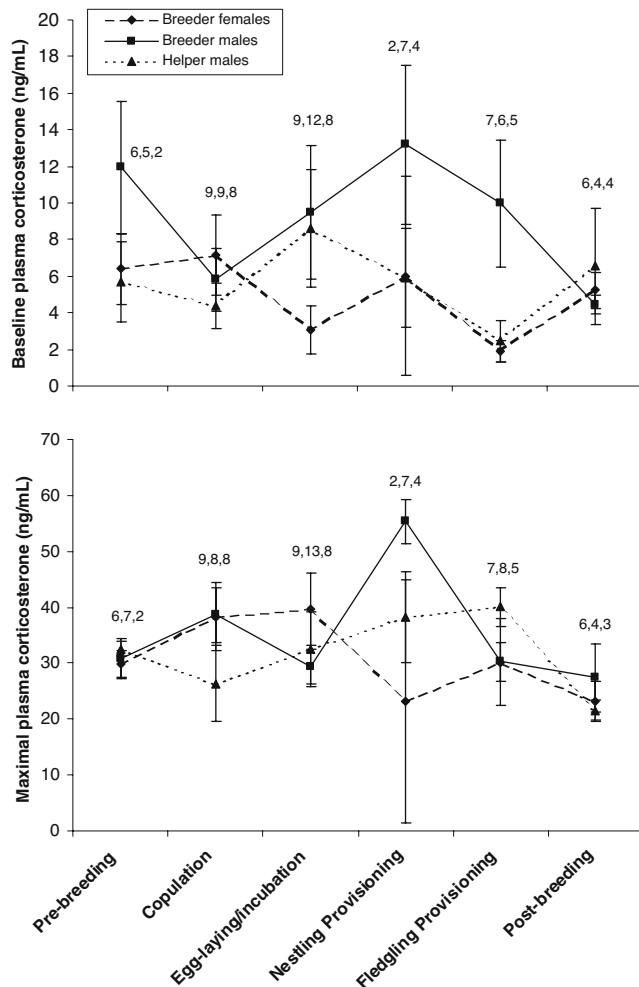
*Males* Model M5 (Akaike weight=1.0000) accounted for 57% of the variation in plasma testosterone in males. In the ANOVA, testosterone did not vary

**Table 3** AIC<sub>c</sub> values, Akaike weights (*w<sub>i</sub>*), and R<sup>2</sup> values for the best-fit model explaining variation in hormone levels in males and females

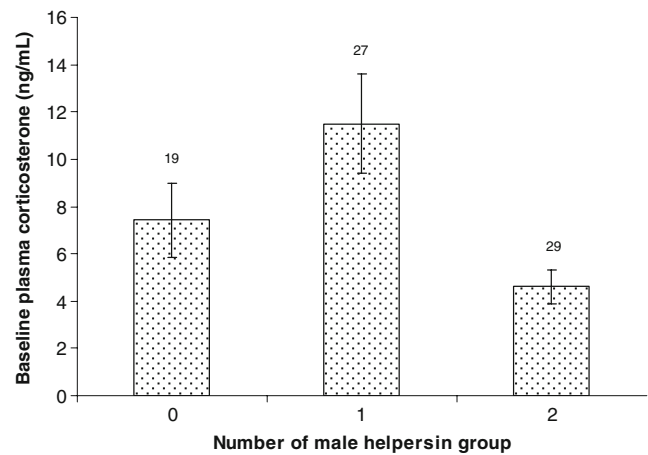
Model number	Model structure <sup>a</sup>	Baseline corticosterone			Maximal corticosterone			Testosterone			Estradiol			Progesterone		
		AIC <sub>c</sub>	<i>w<sub>i</sub></i>	R <sup>2</sup>	AIC <sub>c</sub>	<i>w<sub>i</sub></i>	R <sup>2</sup>	AIC <sub>c</sub>	<i>w<sub>i</sub></i>	R <sup>2</sup>	AIC <sub>c</sub>	<i>w<sub>i</sub></i>	R <sup>2</sup>	AIC <sub>c</sub>	<i>w<sub>i</sub></i>	R <sup>2</sup>
M5	p * h	203.8	0.9997	0.21	506.7	1.000	0.34	203.7	1.000	0.57	385.9	1.000	0.23	117.3	0.9969	0.38
F1	p * h	78.3	1.000	0.48	219.0	1.000	0.35	84.2	1.000	0.69	82.8	0.9998	0.50	64.7	0.9980	0.54

Model M5 represents males and model F1 represents females  
<sup>a</sup>Model factors: *p* breeding phase, *h* number of male helpers in group  
 Asterisk (\*) indicates a factorial model

significantly due to number of helpers ( $F_{2, 79}=0.33$ ,  $p=0.72$ ), but varied due to breeding phase ( $F_{5, 79}=6.67$ ,  $p<0.0001$ ; Fig. 5) and the interaction of phase and number of helpers ( $F_{10, 79}=2.25$ ,  $p=0.026$ ). Plasma testosterone was significantly higher during the copulation phase than during the pre-breeding, egg-laying/incubation, fledgling provisioning, and post-breeding phase (Tukey post hoc test  $p<0.05$ ) for both breeder males and helper males. Plasma testosterone was significantly lower during the post-breeding phase than during the copulation, egg-laying/incubation, and nestling provisioning phases (Tukey post hoc test  $p<0.05$ ) for both helpers and breeders. In the egg-laying/incubation phase, plasma



**Fig. 3** Baseline and maximal plasma corticosterone levels (mean  $\pm$  SE) of breeding females, breeding males, and helper males during all phases of the breeding cycle. Sample sizes are included in the plots, listed in the following order: breeding females, breeding males, helper males. Differences in sample sizes between baseline and maximal levels occur because we were unable to obtain blood for both samples from several birds and because several samples were deemed unusable due to lab error. Note difference in scale between baseline and maximal corticosterone levels



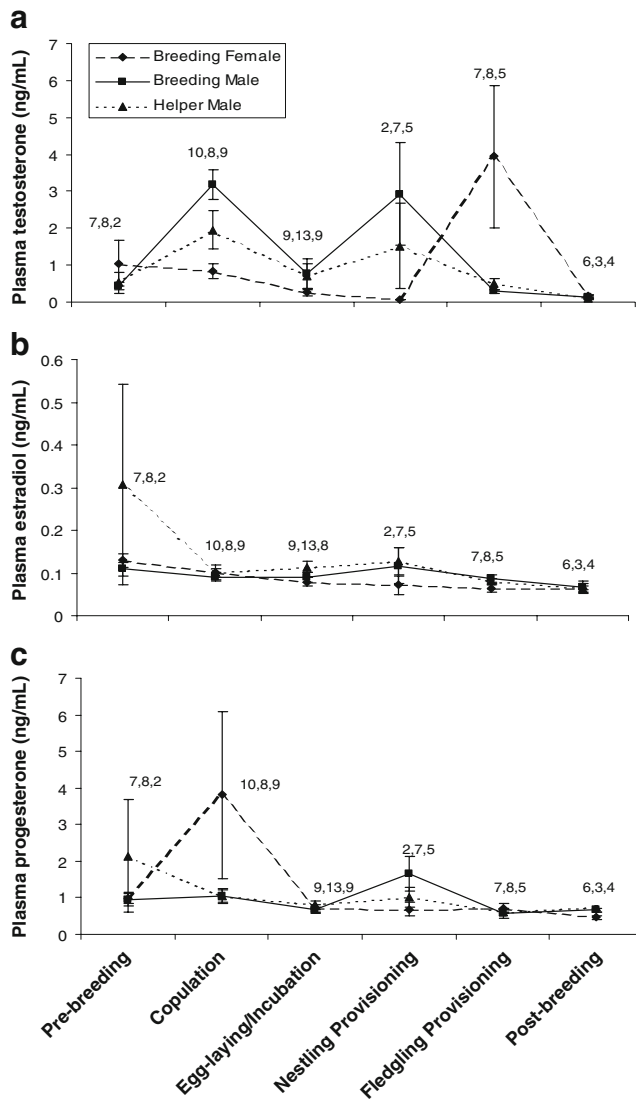
**Fig. 4** Baseline plasma corticosterone (mean  $\pm$  SE) of breeder males and helper males living in family groups with zero, one, or two or more helper males. Sample sizes are indicated above bars

testosterone was higher in groups with two or more helpers than in groups with one helper (Tukey post hoc test  $p<0.05$ ). Higher plasma testosterone in the copulation phase compared to other phases was only found in groups with one or two or more helpers during this phase (Tukey post hoc test  $p<0.05$ ). Lower plasma testosterone in the post-breeding phase compared to other phases was found only in groups with one or two or more helpers (Tukey post hoc test  $p<0.05$ ).

*Females Model F1* (Akaike weight=1.0000) accounted for 69% of the variation in plasma testosterone in females. Breeding phase ( $F_{5, 39}=3.15$ ,  $p=0.024$ ; Fig. 5), number of helpers ( $F_{2, 39}=5.54$ ,  $p=0.01$ ), and the interaction of phase and number of helpers ( $F_{10, 39}=2.69$ ,  $p=0.032$ ) all significantly influenced plasma testosterone levels in the ANOVA. Plasma testosterone was higher in the copulation and fledgling provisioning phases than in the post-breeding phase (Tukey post hoc test  $p<0.05$ ). The interaction of phase and number of helpers was completely driven by the high mean testosterone level in groups with no helpers during the fledgling provisioning phase, which was significantly higher than that of females in groups of all sizes in several phases (Tukey post hoc test  $p<0.05$ ). The high mean was likely influenced by three individuals with especially high plasma testosterone levels.

#### Estradiol

*Males Model M5* (Akaike weight=1.0000) accounted for 23% of the variation in plasma estradiol in males. However, neither breeding phase ( $F_{5, 78}=1.20$ ,  $p=0.32$ ), number of helpers ( $F_{2, 78}=1.01$ ,  $p=0.37$ ), or the interaction of these



**Fig. 5** Plasma testosterone (a), estradiol (b), and progesterone (c) levels (mean  $\pm$  SE) of breeding females, breeding males, and helper males during all phases of the breeding cycle. Sample sizes are included in the plots, listed in the following order: breeding females, breeding males, helper males. Note difference in scale for each hormone

factors ( $F_{10, 78}=1.19$ ,  $p=0.31$ ) significantly influenced plasma estradiol in the ANOVA (Fig. 5). Generally, estradiol levels were low except for a high value for helpers in the pre-breeding phase.

*Females* Model F1 (Akaike weight=0.9998) accounted for 50% of the variation in plasma estradiol in females. The number of helpers ( $F_{2, 39}=0.16$ ,  $p=0.86$ ) and the interaction of breeding phase and number of helpers ( $F_{10, 39}=0.77$ ,  $p=0.62$ ) did not influence plasma estradiol levels in the ANOVA. There was no significant effect of breeding phase ( $F_{5, 39}=2.49$ ,  $p=0.059$ ; Fig. 5).

## Progesterone

*Males* Model M5 (Akaike weight=0.9969) accounted for 38% of the variation in plasma progesterone in males. Progesterone levels did not vary significantly due to number of helpers in the ANOVA ( $F_{2, 79}=0.24$ ,  $p=0.79$ ), but did vary due to breeding phase ( $F_{5, 79}=3.09$ ,  $p=0.015$ ; Fig. 5). Plasma progesterone was significantly higher during the nestling provisioning phase than during the fledgling provisioning phase (Tukey post hoc test  $p<0.05$ ) for both breeder males and helper males. The interaction of breeding phase and number of helpers did not significantly influence progesterone levels ( $F_{10, 79}=1.85$ ,  $p=0.071$ ).

*Females* Model F1 (Akaike weight=0.9980) accounted for 54% of the variation in plasma progesterone in females. The number of helpers ( $F_{2, 39}=1.53$ ,  $p=0.24$ ) and the interaction of breeding phase and number of helpers ( $F_{10, 39}=0.75$ ,  $p=0.63$ ) did not significantly influence progesterone levels in the ANOVA. There was a significant effect of breeding phase ( $F_{5, 39}=3.12$ ,  $p=0.025$ ; Fig. 5), with higher progesterone in the pre-breeding and copulation phases than in the post-breeding phase (Tukey post hoc test  $p<0.05$ ).

## Discussion

### Reproductive suppression of helper males

The results of this study do not support the hypothesis that increased plasma corticosterone levels are associated with reproductive inactivity in helper male red-cockaded woodpeckers. To our knowledge, studies comparing corticosterone levels between breeders and helpers have been conducted in only three cooperatively breeding bird species: Florida scrub jays (*Aphelocoma coerulescens*; Schoech et al. 1991, 1997), white-browed sparrow weavers (*Plocepasser mahali*) (Wingfield et al. 1991), and Harris' hawks (Mays et al. 1991). No significant differences in either baseline or maximal (measured in scrub jays only) corticosterone levels were found to exist between male helpers and breeders of these species either. However, we predicted that red-cockaded woodpeckers would contradict these previous results because the reproductive suppression of these other species has been explained by other physiological and behavioral mechanisms that have not been observed in red-cockaded woodpeckers, such as low levels of testosterone (Mays et al. 1991; Schoech et al. 1991, 1996; Wingfield et al. 1991) and low body mass (Wingfield et al. 1991) in helper

males. Helper red-cockaded woodpeckers also partake in more helping activities than helpers of other species do (Kinnaird and Grant 1982; Rabenold 1985; Dawson and Mannan 1991). Instead, our findings support the emerging, consistent pattern that corticosterone levels do not differ between male breeders and helpers in cooperatively breeding birds.

Our results do support general ideas about the relationship of dominance hierarchies to stress. A growing number of studies investigating the physiology of social living have shown that the relationship between social status and stress hormone levels is highly variable. Therefore, although we had several reasons to suspect that helper males would exhibit higher levels of corticosterone than breeder males, it is not unreasonable that corticosterone levels are similar between helpers and breeders. The fact that red-cockaded woodpeckers live in stable social groups with established dominance relationships appears to have a significant impact on individual stress hormone levels in this species. Commonly, subordinates living in such permanent social groups maintain relatively low levels of stress hormones because the outcome of social interactions are predictable and severe agonistic encounters are uncommon (Creel 2001). A species' breeding system alone, however, does not necessarily predict relative stress levels of dominants and individuals (Goymann and Wingfield 2004).

The results of our study confirmed the finding of Khan et al. (2001) that red-cockaded woodpecker helpers maintain levels of testosterone similar to those of breeder males. We also measured plasma estradiol and progesterone to examine alternative hypotheses for reproductive inhibition in male helpers, but found no support for an effect of these hormones on reproductive suppression of helper males. It thus appears unlikely that the proximate basis of reproductive suppression in red-cockaded woodpeckers is hormonal.

At this point, as suggested by Khan et al. (2001), direct behavioral suppression by either breeder males or females (or both) appears to be the best hypothesis to explain why seemingly reproductively capable helper red-cockaded woodpeckers do not attempt to breed. Although red-cockaded breeder males do not appear to follow fertile females and are not overtly aggressive toward helper males, mate guarding may take form other than tending females (Lape 1990). Breeder males have occasionally been observed following or chasing helper males, and if breeder females are approached by helper males, they become noisy and fly away; in one instance, a female displayed aggressively (Lape 1990). Such social interactions may not be of sufficient frequency or intensity to influence corticosterone levels in helper males, but may be sufficient to prevent mating attempts by helpers.

#### Effect of helper presence on baseline corticosterone

Although we failed to reject the null hypothesis of no effect of the number of helpers on the corticosterone levels of breeding males, we are not confident in this inference because of a modest sample size and because breeder corticosterone decreased as the number of helpers increased. There are reasons to expect an effect of helper number. Reduced baseline corticosterone levels generally correlate with reduced energy expenditure (Goymann and Wingfield 2004). The results of this study therefore provide a physiological correlate to the finding by Khan and Walters (2002) that breeding males significantly reduced their incubation efforts and their nestling provisioning rates when assisted by helpers, and that survival of breeding males increases as group size increases. The fact that the reduction in corticosterone did not occur until two or more helpers were present could indicate that a threshold number of helpers may be necessary before a significant increase in fledging success occurs. Indeed, a threshold of at least two helper males before an increase in reproductive success is attained has been suggested in red-cockaded woodpeckers (Conner et al. 2004) as well as cooperatively breeding splendid fairy-wrens (*Malurus splendens*; Webster et al. 2004) and stripe-backed wrens (Rabenold 1984). Khan and Walters (2002) found that in the presence of helpers, breeder females also reduced their incubation efforts and tended to reduce their feeding rates, but the reduction in their workload was less than that of breeder males. This may explain why female corticosterone levels were not significantly influenced by the presence of helpers.

The analysis of the effect of group size on helper males' corticosterone levels is actually a test of the effect of the presence of *additional* helper males in a group, and thus suggests that the presence of additional helpers decreased the baseline corticosterone levels of other helper males. Since helpers reduce the workload of breeder males, presumably the presence of an additional helper also reduces the workload of other helpers. Also, when two or more helpers are present, each helper may receive less aggression (described above) from the breeder male, and helpers may provide each other with social support (Goymann and Wingfield 2004).

The effect of helper presence may be confounded with habitat quality, since groups with more helpers inhabit higher quality territories (Lennartz et al. 1987). However, previous studies that found a reduction in breeder workload (Khan and Walters 2002) and an increase in reproductive success (Lennartz et al. 1987) in the presence of helpers showed that these effects still held when territory quality was accounted for. Thus, while higher quality habitat in the presence of two helpers may influence baseline corticoste-

rone levels to some extent, the decreased workload of breeders appears to be a direct effect of helper presence.

#### Seasonal patterns of hormone secretion

The majority of avian species studied thus far modulate their baseline corticosterone levels seasonally, exhibiting a peak during the breeding season. This peak may occur because breeding is an energetically costly time of year since birds are often caring for young, and corticosterone may function to mobilize energy stores (Romero 2002). Our results are not consistent with the typical seasonal pattern of avian baseline corticosterone levels, however. Neither male nor female red-cockaded woodpeckers in our study experienced significant seasonal variation in baseline corticosterone. Although caring for the young is energetically costly, perhaps other activities in which red-cockaded woodpeckers participate year-round, such as cavity excavation and territory defense, are equally costly, such that the net energy expenditure remains relatively steady throughout the year. Furthermore, unlike most bird species, cooperative breeders share parental responsibilities among parents and helpers. This may result in red-cockaded woodpeckers expending relatively less energy individually during the breeding season than do other avian species.

Of the bird species reviewed by Romero (2002), approximately half exhibit their highest maximal corticosterone levels during the breeding season, and approximately half exhibit their lowest levels during this time. Male red-cockaded woodpeckers exhibit an elevated stress response during the nestling provisioning phase of the breeding season. This may reflect an increased need to respond quickly to a stressor that poses a potential threat to survival of offspring. Loss of red-cockaded nestlings is often due to conflicts with intruders, either other red-cockaded woodpeckers (Johnston 2006) or other cavity-nesting species, which intensify during the breeding season (Walters 1990). Males may mount a large stress response to guard their nest against intruders. Participating in defensive behavior conflicts with time spent caring for young, but in cooperative breeders, additional adult group members are available to tend to young while others chase intruders. Furthermore, unlike species in which the stress response is muted, which often have short breeding seasons and must invest much energy into a single nesting attempt (Silverin 1998), red-cockaded woodpeckers often re-nest if eggs or nestlings are lost (Walters 1990).

Breeder females do not exhibit a seasonal change in their maximal corticosterone levels. Khan (2001) suggested that females are more sensitive to nest stimuli (such as vocalizations from nestlings) than are males. Thus, they may be less likely than males to respond to stimuli outside the nest. Also, females must not only incubate, but also produce and

lay eggs, and therefore have a physiologically higher energy investment in a clutch than do males. Females may be physically unable to mount an increased stress response during the breeding season.

The seasonal pattern of reproductive hormones in red-cockaded woodpeckers generally matched the typical endocrine profiles of most biparental, monogamously breeding avian species (Wingfield et al. 1990). The increase in male plasma testosterone and female plasma estradiol during the copulation and pre-breeding phases, respectively, corresponds with the expression of sexual behaviors during this time of year (Wingfield et al. 1990; Nelson 2000). Females' increase in plasma testosterone during the pre-breeding and copulation phases may be due to increased female–female competition for breeding positions during this time of year. Female “floaters” sometimes affiliate with groups to whom they are unrelated, and female helpers may compete with breeding females for breeding status (Walters 1990). Also, many juvenile females remain with their natal group through the winter and engage in forays to other groups just prior to, or in the early stages of, the breeding season (Walters, unpublished data). The peak in female testosterone levels during the fledgling provisioning stage is curious, but was also observed by Khan et al. (2001) and thus is not an anomaly. Males exhibited elevated plasma progesterone during the nestling provisioning stage, as expected since progesterone is involved in the promotion of parental behavior (Nelson 2000). That a concomitant peak in plasma progesterone was not seen at this time in females is surprising; however, only two females were captured during the nestling provisioning stage.

#### Conclusions

The results of this study add to the data describing proximate mechanisms of cooperative breeding and lend further support to the hypothesis that corticosterone is not a mechanism mediating reproductive suppression of helpers in cooperatively breeding species. Red-cockaded woodpeckers remain unique among cooperatively breeding bird species in that reproductive suppression of helpers is unexplained by hormonal differences that have been demonstrated in other species. Although we found no effect of social status on corticosterone levels of male red-cockaded woodpeckers, this study provided physiological evidence for benefits of social living in this species. Reduced stress in both breeder and helper males in large groups, due to decreased workloads and possibly to social support, may translate into fitness benefits for male members of larger groups. This study further supports behavioral and demographic evidence (Heppell et al. 1994; Khan and Walters 2002) that red-cockaded

woodpecker breeders benefit from the presence of helpers. To our knowledge, this is the first study to examine the physiological effects of helpers on other helpers in addition to their effects on breeders, and the first to examine changes in corticosterone levels due to group size in a cooperatively breeding bird. Further research in these areas may provide interesting insight into the complex physiological effects of social interactions in cooperative breeders.

**Acknowledgments** This project was supported financially by the Virginia Tech Graduate School (A. L. M.), Sigma Xi (A. L. M.), the Graduate Women in Science Program (A. L. M.), the U. S. Department of Defense (J. R. W.), Marine Corps Base Camp Lejeune (J. R. W.), and the National Science Foundation (IOS-0545735 to I. T. M.). This work would not have been possible without the tremendous field assistance of the Camp Lejeune field technicians Kristina Hudgins, Charlie Clarkson, and Cari Nycum. We also thank Kerry Brust, Jen Maynard, Dan Kuefler, Koan Heindel, and Erika Gonzalez for additional assistance in the field. We are grateful to John Townsend and Craig Ten Brink for their support and assistance in providing data, and the members of the Virginia Tech RIA lab for advice and assistance with lab work. We thank Carola Haas for her input and constructive criticism in all aspects of this project. We thank two anonymous reviewers for helpful comments on this manuscript.

## References

- Arnold KE, Owens IPF (1998) Cooperative breeding in birds: a comparative test of the life history hypothesis. *Proc R Soc Lond* 265:739–745
- Brown JL (1987) Helping and communal breeding in birds. Princeton University Press, Princeton, NJ
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York
- Conner RN, Rudolph DC, Walters JR (2001) The red-cockaded woodpecker: surviving in a fire-maintained ecosystem. University of Texas Press, Austin
- Conner RN, Saenz D, Schaefer RR, McCormick JR, Rudolph DC, Burt DB (2004) Group size and nest success in red-cockaded woodpeckers in the West Gulf Coastal Plain: helpers make a difference. *J Field Ornithol* 75:74–78
- Convery KM (2002) Assessing habitat quality for the endangered red-cockaded woodpecker (*Picoides borealis*). M. S. Thesis, Virginia Polytechnic and State University, Blacksburg
- Creel S (2001) Social dominance and stress hormones. *Trends Ecol Evol* 16:491–497
- Daniels SJ, Walters JR (2002) Between-year breeding dispersal in red-cockaded woodpeckers: multiple causes and estimated cost. *Ecology* 81:2473–2484
- Dawson JW, Mannan RW (1991) Dominance hierarchies and helper contributions in Harris' hawks. *Auk* 108:649–660
- Emlen ST (1982) The evolution of helping. I. A ecological constraints model. *Amer Nat* 119:29–39
- Goymann W, Wingfield JC (2004) Allostatic load, social status and stress hormones: the costs of social status matter. *Anim Behav* 67:591–602
- Hadley ME (2000) Endocrinology, 5th edn. Prentice Hall, Upper Saddle River, NJ
- Haig SM, Belthoff JR, Allen DH (1993) Examination of population structure in red-cockaded woodpeckers using DNA profiles. *Evolution* 47:185–194
- Haig SM, Walters JR, Plissner JH (1994) Genetic evidence for monogamy in the cooperatively breeding red-cockaded woodpecker. *Behav Ecol Sociobiol* 34:295–303
- Heppell S, Walters JR, Crowder LB (1994) Evaluating management alternatives for red-cockaded woodpeckers: a modeling approach. *J Wildl Manag* 58:479–487
- Johnston PA (2006) Reproductive success and intraspecific competition of the red-cockaded woodpecker. M. S. Thesis, University of South Carolina, Columbia
- Khan MZ, Walters JR (2002) Effects of helpers on breeder survival in the red-cockaded woodpecker (*Picoides borealis*). *Behav Ecol Sociobiol* 51:336–344
- Khan MZ, McNabb FMA, Walters JR, Sharp PJ (2001) Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*). *Horm Behav* 40:1–13
- Kinnaird MF, Grant PR (1982) Cooperative breeding by the Galapagos mockingbird, *Nesomimus parvulus*. *Behav Ecol Sociobiol* 10:65–73
- Koenig WD, Pitelka FA, Carmen WJ, Mumme RL, Stanback MT (1992) The evolution of delayed dispersal in cooperative breeders. *Q Rev Biol* 67:111–150
- Lape JJ (1990) Mate guarding in the red-cockaded woodpecker. M. S. Thesis, North Carolina State University, Raleigh
- Lennartz MR, Hooper RG, Harlow RF (1987) Sociality and cooperative breeding of red-cockaded woodpeckers, *Picoides borealis*. *Behav Ecol Sociobiol* 20:77–88
- Li S, Brown JL (2000) High frequency of extrapair fertilization in a plural breeding bird, the Mexican jay, revealed by DNA microsatellites. *Anim Behav* 60:867–877
- Ligon JD (1970) Behavior and breeding biology of the red-cockaded woodpecker. *Auk* 87:255–278
- Mays NA, Vleck CM, Dawson JW (1991) Plasma luteinizing hormone, steroid hormones, behavioral role, and nest stage in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk* 108:619–637
- Moore IT, Jessop TS (2003) Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm Behav* 43:39–47
- Mumme RL, Koenig WD, Pitelka FA (1983) Mate guarding in the acorn woodpecker: within-group reproductive competition in a cooperative breeder. *Anim Behav* 31:1094–1106
- Nelson RJ (2000) An introduction to behavioral endocrinology, 2nd edn. Sinauer, Sunderland, MA
- Rabenold KN (1984) Cooperative enhancement of reproductive success in tropical wren societies. *Ecology* 65:871–885
- Rabenold KN (1985) Cooperation in breeding by nonreproductive wrens: kinship, reciprocity, and demography. *Behav Ecol Sociobiol* 17:1–17
- Reyer H, Dittami JP, Hall MR (1986) Avian helpers at the nest: are they psychologically castrated? *Ethology* 71:216–228
- Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen Comp Endocrinol* 128:1–24
- Rudolph DC, Conner RN, Schaefer RR, Koerth NE (2007) Red-cockaded woodpecker foraging behavior. *Wilson J Ornithol* 119:170–180
- Sapolsky RM (2005) The influence of social hierarchy on primate health. *Science* 308:648–652
- Schoech SJ, Mumme RL, Moore MC (1991) Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *Condor* 93:354–364

- Schoech SJ, Mumme RL, Wingfield JC (1996) Delayed breeding in the cooperatively breeding Florida scrub jay (*Aphelocoma coerulescens*): inhibition or the absence of stimulation? Behav Ecol Sociobiol 39:77–90
- Schoech SJ, Mumme RL, Wingfield JC (1997) Corticosterone, reproductive status, and body mass in a cooperative breeder, the Florida scrub-jay (*Aphelocoma coerulescens*). Physiol Zool 70:68–73
- Silverin B (1998) Stress responses in birds. Poult Avian Biol Rev 9:153–168
- Stacey PB, Ligon JD (1991) The benefits-of-philopatry hypothesis for the evolution of cooperative breeding: variation in territory quality and group size effects. Amer Nat 137:831–846
- Vleck CM, Brown JL (1999) Testosterone and social and reproductive behavior in *Aphelocoma* jays. Anim Behav 58:943–951
- Walters JR (1990) Red-cockaded woodpeckers: a “primitive” cooperative breeder. In: Stacey PB, Koenig WD (eds) Cooperative breeding in birds. Cambridge University Press, Great Britain
- Walters JR, Doerr PD, Carter JHI (1988) The cooperative breeding system of the red-cockaded woodpecker. Ethology 78:275–305
- Webster MS, Tarvin KA, Tuttle EM, Pruett-Jones S (2004) Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction. Behav Ecol 15:907–915
- Whittingham LA, Dunn PO, Magrath RD (1997) Relatedness, polyandry and extra-group paternity in the cooperatively-breeding white-browed scrubwren (*Sericornis frontalis*). Behav Ecol Sociobiol 40:261–270
- Wingfield JC (1988) Changes in reproductive function of free-living birds in direct response to environmental perturbations. In: Stetson MH (ed) Processing of environmental information in vertebrates. Springer, New York, pp 121–148
- Wingfield JC, Hegner RE, Dufty AMJ, Ball GF (1990) The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Amer Nat 136:829–846
- Wingfield JC, Hegner RE, Lewis DM (1991) Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. J Zool, London 225:43–58