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Does corticosterone regulate the onset of breeding in free-living birds?: The CORT-Flexibility Hypothesis and six potential mechanisms for priming corticosteroid function

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ABSTRACT

For many avian species, the decision to initiate breeding is based on information from a variety of environmental cues, including photoperiod, temperature, food availability, and social interactions. There is evidence that the hormone corticosterone may be involved in delaying the onset of breeding in cases where supplemental cues, such as low food availability and inclement weather, indicate that the environment is not suitable. However, not all studies have found the expected relationships between breeding delays and corticosterone titers. In this review, we present the hypothesis that corticosterone physiology mediates flexibility in breeding initiation (the "CORT-Flexibility Hypothesis"), and propose six possible corticosterone-driven mechanisms in pre-breeding birds that may delay breeding initiation: altering hormone titers, negative feedback regulation, plasma binding globulin concentrations, intracellular receptor concentrations, enzyme activity and interacting hormone systems. Based on the length of the breeding season and species-specific natural history, we also predict variation in corticosterone-regulated pre-breeding flexibility. Although few studies thus far have examined mechanisms beyond plasma hormone titers, the CORT-Flexibility Hypothesis is grounded on a solid foundation of research showing seasonal variation in the physiological stress response and knowledge of physiological mechanisms modulating corticosteroid effects. We propose six possible mechanisms as testable and falsifiable predictions to help clarify the extent of HPA axis regulation of the initiation of breeding.

Contents

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Introduction: fine-tuning the timing of breeding

When it comes to breeding, proper timing is key. For many species, earlier-breeding individuals have higher reproductive success than those breeding later, even after controlling for factors such as individual condition and territory quality (Dickerson et al., 2005; Perrins, 1970; Verhulst and Nilsson, 2008). However, initiating breeding too early can be associated with reproductive failure or reduced adult survival due to factors such as mismatches between available resources and the needs of growing young (Harris, 1980; Nilsson, 1994; Thomas et al., 2001). There are therefore substantial fitness consequences to breeding too late or too early, and selection should favor animals with the ability to integrate information from a variety of cues to determine when to initiate their first breeding attempt each year (Hahn and MacDougall-Shackleton, 2008).

In temperate regions, seasonally breeding animals use two kinds of cues to time reproduction (Ball, 1993; Wingfield, 1984). Photoperiod (also called initial predictive information) drives gonadal regrowth and brings animals into a pre-reproductive state (Dawson et al., 2001). Non-photoperiodic supplementary information, which includes temperature, food availability, rainfall, and the presence of a mate, then helps animals fine-tune the exact timing of breeding initiation (Bronson, 1985; Caro et al., 2013; Davies and Deviche, 2014; Schaper et al., 2012; Semlitsch et al., 1993; Wingfield and Kenagy, 1991). Even when initial predictive cues are positive, if supplementary cues indicate that conditions are not favorable for breeding – for example, a lack of suitable nesting sites – breeding initiation may be delayed. The physiological mechanisms causing this delay are not completely understood, but activation of the hypothalamic–pituitary–adrenal (HPA) axis, which results in the production of corticosteroid hormones, has been proposed as one potential mediator (Cain and Lien, 1985; Cornelius et al., 2013; Schoech, 2009; Wingfield et al., 1992; Wingfield and Kenagy, 1991; Wingfield et al., 1983). The primary corticosteroid hormone in birds, rodents, reptiles and amphibians is corticosterone, whereas in many mammals and fish, it is cortisol (Chester Jones et al., 1962).

Corticosteroids help animals regulate energy both in response to normal life processes and unpredictable environmental perturbations; they also have a variety of other effects related to immune function, behavior and reproduction (Sapolsky et al., 2000). Because of their role in energy management, even baseline concentrations of corticosteroids are quite high in most vertebrate species (typically in the nanomolar range); in response to a stressor, corticosteroid titers may increase by as much as three orders of magnitude (Romero, 2004). Unfavorable supplemental cues like low spring temperatures or late winter storms could cause increased corticosteroid release either because animals directly perceive the unfavorable supplemental cues as stressors (e.g., de Bruijn and Romero, 2013), or because these conditions cause increased energy metabolism (e.g., Voigt et al., 2011), which indirectly causes increased corticosteroid titers (McEwen and Wingfield, 2003; Romero et al., 2009). In fact, these are not mutually exclusive possibilities.

There is strong evidence, primarily in mammals, that corticosteroids can act at multiple levels to suppress activity of the hypothalamic–pituitary–gonadal axis. For example, chronic administration of corticosteroids can inhibit the pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus (Oakley et al., 2009), and reduce the responsiveness of pituitary cells to GnRH (Li and Wagner, 1983; Suter et al., 1988), thereby reducing the amplitude of the luteinizing hormone (LH) response. There is also evidence that corticosteroids can act directly on the gonads to suppress reproduction, for example by decreasing the responsiveness of ovarian granulosa cells to gonadotropic hormones (Hsueh and Erickson, 1978), or reducing androgen synthesis by the testes (Welsh et al., 1982). Furthermore, corticosteroid administration can inhibit breeding in laboratory and captive animals (Breen and Karsch, 2006; Dunlap and Schall, 1995; Rivier and Rivest, 1991; Salvante and Williams, 2003; Wingfield and Sapolsky, 2003), and cause nest abandonment and breeding failure in wild birds (Silverin, 1986; Spée et al., 2011).

Thus, it is clear that corticosteroids have potent suppressive effects on behavior and physiology associated with breeding. Earlier work tested whether increases in corticosteroid titers could transduce supplemental cues to delay breeding, but the evidence is mixed (see the section on Prediction 1, below). However, corticosteroid titers are only one aspect of an integrated physiological system that regulates the impact of corticosteroids. There are important components both upstream and downstream of hormone titers that could potentially modulate corticosteroid impacts on the body even in the absence of long-term changes in hormone secretion.

Here we present the hypothesis that "pre-breeding" is a special period characterized by the priming of corticosteroid function, which allows animals to respond flexibly to non-optimal environments (the "CORT-Flexibility Hypothesis"). In other words, the pre-breeding animal has enhanced sensitivity in its corticosteroid system, so that if it encounters a negative supplemental factor, it is primed to respond strongly in order to pause reproductive behavior and physiology. This priming of corticosteroid function may occur both upstream and downstream of hormone release. Thus, a pre-breeding animal could augment the effects of corticosteroids on the body by increasing circulating baseline or stress-induced hormone titers (Fig. 1, Prediction 1), but it could also increase the effects of corticosteroids by altering negative feedback regulation, plasma binding globulin concentrations, intracellular receptor concentrations, enzyme activity and interacting hormone systems (Fig. 1, Predictions 2–6) without necessarily increasing plasma titers over the long term. The majority of work examining the timing of breeding and corticosteroid physiology comes from avian species, and so we limit the scope of our discussion to birds; however, we believe that these ideas may help guide research across vertebrate classes. In the last part of this review, we predict which birds may (or may not) display increased pre-breeding corticosterone function, based on the length of the breeding season and species-specific natural history.

Although exposure to severe or prolonged stressors can cause reproductive failure, increased corticosterone function during the pre-breeding period may allow birds to better time their reproductive attempts in response to environmental conditions that may vary widely from year to year, and even from one day to the next. Thus far, evidence supporting the CORT-Flexibility Hypothesis is limited, but it is grounded on a solid foundation of research showing seasonal variation in the physiological stress response and knowledge of physiological mechanisms modulating corticosteroid effects. Furthermore, because our six possible mechanisms lead to testable and falsifiable predictions, we believe this framework may help physiological ecologists determine whether the HPA axis is in fact an important mechanism in delaying the onset of breeding.

The pre-breeding period: definition and clarifications

We use the term "pre-breeding" to specifically indicate the period after birds have begun expressing breeding-related physiology, morphology and behavior but before egg lay (Fig. 2). In the life history framework proposed by Wingfield (2008a, 2008b), this is the period when the termination phase of the life history stage immediately prior to breeding (often winter or spring migration) overlaps with the development phase of the breeding life history stage. Defining the pre-breeding period in wild birds is not a trivial problem, and may involve either careful field observations of behavior, multiple captures of the same individuals for specific evidence of breeding physiology and morphology, or a combination of the two.

One important note is that the role of corticosterone postulated here for the pre-breeding period is very different from the role of corticosterone postulated for the breeding season. Although corticosterone

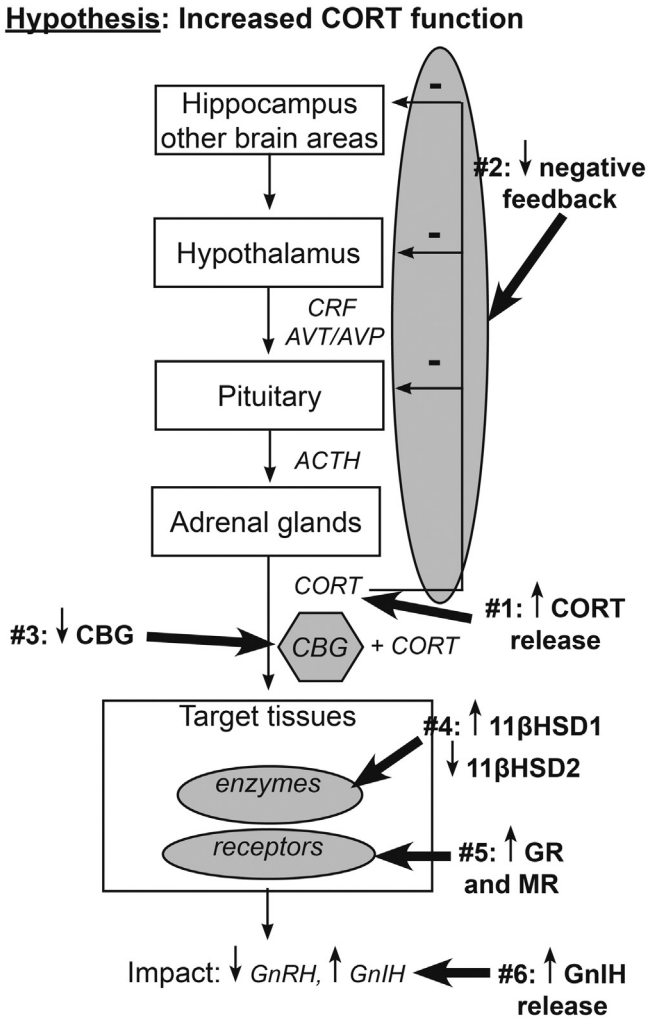

Fig. 1. The physiological impact of corticosteroids could be increased in pre-breeding animals in the following six ways: 1) by increasing corticosteroid (CORT) hormone release; 2) by decreasing corticosteroid negative feedback; 3) by decreasing plasma concentrations of corticosteroid binding globulin (CBG); 4) by altering the activity of enzymes that activate (11β hydroxysteroid dehydrogenase type 1; 11β-HSD1) or deactivate (11β hydroxysteroid dehydrogenase type 2; 11β-HSD2) corticosteroids; 5) by increasing intracellular receptor concentrations in target tissues, which in birds consist of higher-affinity mineralocorticoid receptors (MR) and lower-affinity glucocorticoid receptors (GR); and 6) by altering components of other hormone systems such as the gonadotropin-inhibitory hormone (GnIH) system. CRF: Corticotropin releasing factor. ACTH: Adrenocorticotropin hormone. AVT/AVP: arginine vasotocin/arginine vasopressin.

during the entire breeding period is generally quite high compared to times of year such as molt (Romero, 2002), substantial evidence indicates that animals decrease the physiological impact of corticosterone during later breeding stages to avoid disrupting breeding attempts (Wingfield and Sapolsky, 2003). However, if corticosterone is to serve as a transducer of supplemental cues to delay breeding until the optimal time, then it must have increased physiological impact during the pre-breeding period. Notice that these are opposite predictions — increased impact during pre-breeding shifting to decreased impact during breeding. This is the justification for proposing pre-breeding as a unique life-history stage. As stated above, however, note that increased corticosterone function in the pre-breeding period would not necessarily mean increased circulating corticosterone, which would depend on the presence of inhibitory vs. stimulatory supplemental cues in the environment. Furthermore, the opposite predictions for the two periods should be testable.

Prediction 1: pre-breeding birds increase corticosterone release to increase corticosterone's effects

The hormonal cascade resulting in the release of corticosteroids begins in the brain (Fig. 1). First, various higher brain areas stimulate the hypothalamus to secrete some combination of corticotropin releasing factor (CRF) and either arginine vasopressin (AVP, in mammals) or arginine vasotocin (AVT, in birds, reptiles and amphibians) (Antoni, 1993; Plotsky, 1991). These hormones stimulate the pituitary gland to release adrenocorticotropin hormone (ACTH), which travels through the blood to the adrenal glands, where corticosteroids are synthesized and immediately released by the adrenal cortex. Following their synthesis and release, corticosteroids circulate in blood and travel to various target tissues, where they affect cellular function through both genomic (altering transcription of hundreds of different genes) and non-genomic mechanisms (Reddy et al., 2009). Thus, the first way corticosteroids could act to suppress breeding would simply be through increased hormone release in response to supplemental cues such as a high frequency of predation attempts, cold temperatures, low food availability, or the inability to find a mate (Fig. 1, Prediction 1).

The relationship between the timing of breeding and circulating corticosterone in free-living birds is complex (for a more in-depth recent review, see Schoech et al., 2009). In fact, many studies provide contradictory data. For example, corticosterone administration caused an earlier lay date in black-legged kittiwakes (*Rissa tridactyla*) (Goutte et al., 2011), and had no effect on lay date in great tits (*Parus major*) (Ouyang et al., 2013a) or European starlings (*Sturnus vulgaris*) (Love et al., 2005). Within a species, individuals with higher endogenous corticosterone have a later date of clutch initiation in some wild bird species (Goutte et al., 2010b; Schoech, 2009), but not in others (Ouyang et al., 2013b). In free-living birds that do not breed every year, corticosterone titers are often higher in individuals that skip breeding (Goutte et al., 2010a), but this is not always the case (Tartu et al., 2014). High corticosterone titers can alter reproductive physiology and behavior in ways that should cause delays in reproduction (Breuner and Hahn, 2003; Cornelius et al., 2013; Wingfield and Silverin, 1986), but surprisingly few data exist demonstrating clear differences in the date of breeding onset between groups of wild birds with or without prolonged high corticosterone. Adding to the confusion are methodological differences among these kinds of studies, some of which have manipulated corticosterone levels within the physiological range, others which gave corticosterone in a pharmacological range, and others which were observational in nature. These methodological differences may be responsible for some of the difference in results observed.

Furthermore, although seasonal variation in circulating baseline and stress-induced corticosterone titers has been demonstrated in a wide range of different avian species (Romero, 2002), most studies have not found increased corticosterone in birds before breeding as compared to during breeding. Table 1 lists the avian studies comparing pre-breeding to breeding corticosterone. Sixteen of these studies found no difference between corticosterone in an early breeding/pre-breeding period compared to a later breeding period, compared to six which found higher corticosterone in an earlier period, and two that found lower corticosterone earlier. Two studies showed mixed effects that depended on sex: higher pre-breeding corticosterone in males, but no difference in females. However, most of these studies examined different individuals at different life history stages, and high among-individual variation may reduce the likelihood of detecting real differences in corticosterone titers between the pre-breeding and breeding life history stages (Williams, 2008). Careful examination of Table 1 also demonstrates that relatively few studies, in a limited number of species, have compared the period just prior to breeding and the period when breeding first commences. Therefore, full testing of this prediction may require collecting more baseline and stress-induced corticosterone samples within the same individuals in response to different types of

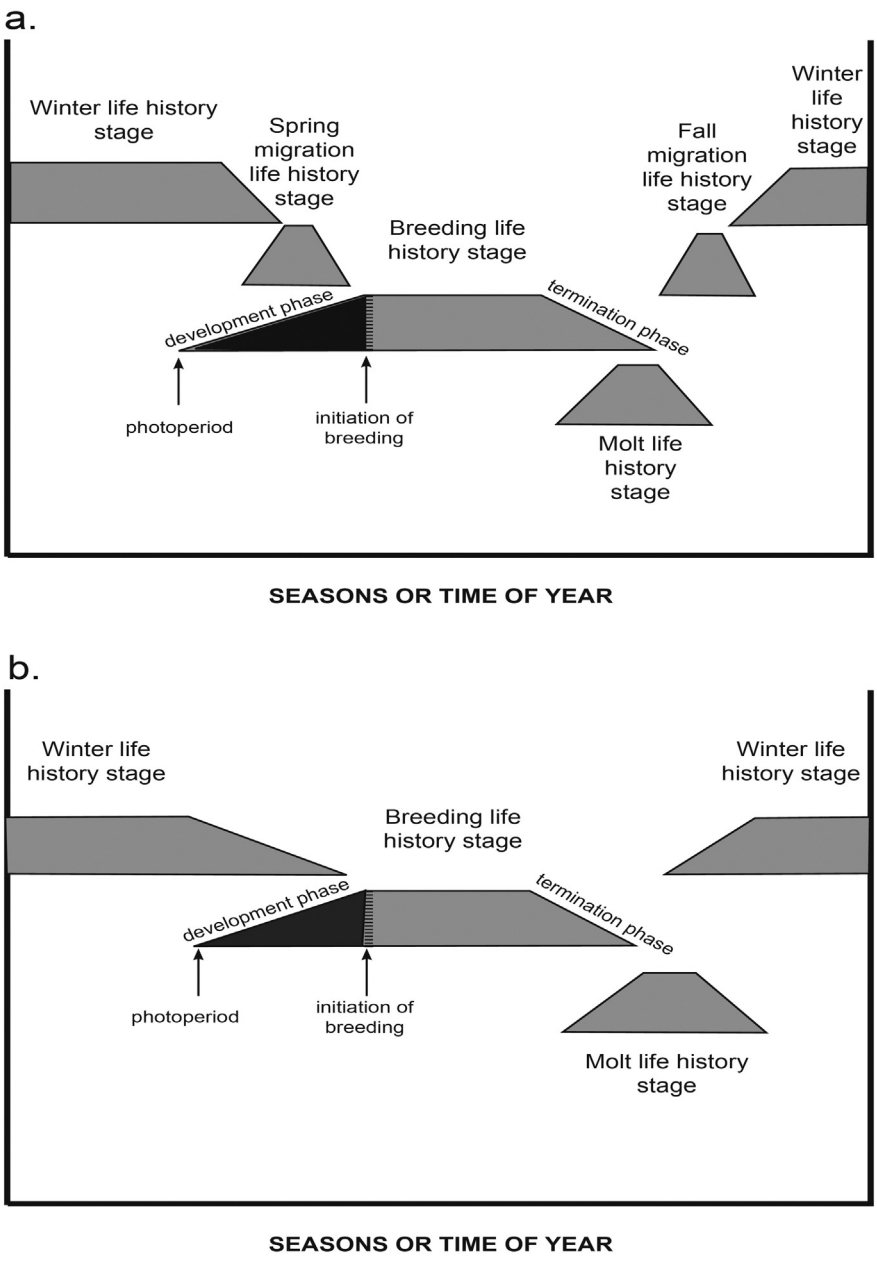

Fig. 2. Visual depiction of the pre-breeding period adapted from Wingfield (2008a, 2008b). Life history stages can be subdivided into three phases: 1) a development phase where animals begin to express stage-typical physiology, morphology and behavior, 2) a mature phase involving full expression of the life history stage, including sub-stages, and 3) a termination phase where physiology, morphology and behavior begin to shift towards the next life history phase. The termination phase of one stage overlaps with the development phase of the next. Sometimes one stage can overlap multiple stages, as can be the case with the breeding stage of migrating birds (a, top figure), which overlaps both winter and spring migration. The middle figure (b) represents the life history of a bird that is a year-round resident, because it has a molt stage and no migration stages. The development phase for the breeding life history stage is initiated by photoperiodic cues and involves physiological and behavioral changes including gonadal recrudescence, territorial behavior, courtship, and nest building. However, the actual onset of breeding (i.e., clutch initiation, represented by the hatched lines) is determined by integrating information from supplementary cues. The black-shaded area of the breeding stage thus represents the pre-breeding period, when we would expect to see increased sensitivity to corticosteroids.

natural and experimenter-induced variation in supplemental cues, combined with data on the actual date of breeding initiation in different individuals.

Prediction 2: pre-breeding birds decrease negative feedback to increase corticosterone's effects

Corticosteroids shut down their own release via negative feedback (Fig. 1). In mammals, corticosteroids are known to act at the pituitary and the paraventricular nucleus of the hypothalamus to stimulate fast negative feedback, and at the hippocampus to stimulate slow negative feedback (Herman et al., 1992; Jacobson and Sapolsky, 1991; Keller-Wood and Dallman, 1984). Because an animal's total corticosteroid exposure is not just a function of peak concentrations but also of the duration of elevated corticosteroids, knowing the strength of negative feedback can reveal patterns that might not be clear from examining hormone titers alone. For example, in Galapagos marine iguanas (*Amblyrhynchus cristatus*), reduced efficacy of negative feedback was the only HPA-related factor that predicted survival rates of iguanas through an El Niño-induced famine (Romero and Wikelski, 2010). Researchers can assess an animal's ability to shut down corticosteroid output through negative feedback by administering an injection of the synthetic corticosteroid dexamethasone. If feedback is functioning normally, dexamethasone should significantly decrease corticosteroid

Table 1
Summary of pre-breeding vs breeding plasma concentrations of corticosterone (CORT) in a range of free-living birds. Studies where pre-breeding CORT titers were found to be higher compared to breeding are in bold.

Species	Sex examined	Measure	Life history stages compared	How were life history stages assessed?	CORT titers pre-breeding relative to breeding	Source
Adélie penguins (*Pygoscelis adeliae*)	Male and female	Baseline CORT	1) Courtship, 2) incubation, 3) chick stage	Direct observation of breeding colonies	No difference	Vleck et al. (2000)
Adélie penguins (*Pygoscelis adeliae*)	Male	Baseline CORT in control males not implanted with CORT pellets	1) Post-pairing/pre-egg-laying and 2) at the end of the first incubation shift	Direct observation of breeding colonies	No difference (in successful breeders)	Spée et al. (2011)
Gray-faced petrels (*Pterodroma macroptera gouldi*)	Male and female	Baseline CORT	1) Nest burrow establishment, 2) early incubation, 3) late incubation, 4) nestling	Not specified; assumed direct observation of breeding colonies	No difference	Adams et al. (2005)
Gray-faced petrels (*Pterodroma macroptera gouldi*)	Male and female	Stress-induced CORT	See above	See above	Lower (compared to late incubation)	Adams et al. (2005)
Black-legged kittiwakes (*Rissa tridactyla*)	Male and female	Baseline CORT in animals given empty implants	1) Pre-egg-laying (avg 20–30 d before egg-laying) and 2) incubation	Direct observation of breeding colonies	No difference	Goutte et al. (2011)
Black-legged kittiwakes (*Rissa tridactyla*)	Male and female	Stress-induced CORT in animals given empty implants	See above	See above	No difference	Goutte et al. (2011)
Red knots (*Calidris canutus islandica*)	Male and female	Baseline CORT	1) After arrival on the breeding grounds, 2) pair bonding and territory defense, and 3) incubation	Brood patch size	No difference	Reneerkens et al. (2002)
Red knots (*Calidris canutus islandica*)	**Male and female**	**Stress-induced CORT**	**See above**	**See above**	**Higher**	**Reneerkens et al. (2002)**
Dusky flycatchers (*Empidonax oberholseri*)	**Male and female**	**Baseline CORT**	**1) Prenesting, 2) egg-laying, 3) incubation, 4) nestling and 5) post-fledgling**	**Direct observation of nesting sites**	**Higher**	**Pereyra and Wingfield (2003)**
Dusky flycatchers (*Empidonax oberholseri*)	**Male and female**	**Stress-induced CORT**	**See above**	**See above**	**Higher**	**Pereyra and Wingfield (2003)**
Equatorial stonechats (*Saxicola torquata rubicola*)	Male	Baseline CORT	1) Pre-breeding, 2) nest building/egg-laying, 3) incubation, 4) feeding young, 5) molt	Direct observations of nests	Lower (compared to nest building/egg-laying)	Goymann et al. (2006)
Great tits (*Parus major*)	Male and female	Baseline CORT	1) Pre-egg-laying (~2 wk before 1st egg laid in the population), 2) nestling (8 or 9 d old chicks)	Direct observation of nest boxes	No difference	J. Ouyang, personal communication (data from Ouyang et al., 2013b)
Great tits (*Parus major*)	**Male and female**	**Stress-induced CORT**	**See above**	**See above**	**Higher**	**J. Ouyang, personal communication (data from Ouyang et al., 2013b)**
House sparrows (*Passer domesticus*)	Male and female	Baseline CORT	1) Early winter, 2) late winter, 3) pre-egg-laying, 4) breeding, 5) late breeding, 6) molt	Gonadal mass, beak color (in males), brood patch development (in females)	No difference	Lattin et al. (2012a)
House sparrows (*Passer domesticus*)	Male and female	Stress-induced CORT	See above	See above	No difference	Lattin et al. (2012a)
House sparrows (*Passer domesticus*)	Male and female	Baseline CORT	1) Pre-egg-laying (24 d before first eggs laid in the population), 2) nestling (8–10 d old chicks)	Direct observation of nest boxes	No difference	J. Ouyang, personal communication (data from Ouyang et al., 2011)
House sparrows (*Passer domesticus*)	Male and female	Stress-induced CORT	See above	See above	No difference	J. Ouyang, personal communication (data from Ouyang et al., 2011)
Mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*)	Male and female	Baseline CORT	1) Pre-breeding (pre-egg lay on the breeding site), 2) breeding	Direct identification of nesting at the breeding site	No difference	C. Breuner, personal communication
Mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*)	Male and female	Stress-induced CORT	1) Pre-breeding (pre-egg lay on the breeding site), 2) breeding	Direct identification of nesting at the breeding site	No difference	C. Breuner, personal communication
Rufous-winged sparrows (*Peucaea carpalis*)	Male	Baseline CORT	1) Pre-breeding, 2) early breeding, 3) late breeding, 4) post-breeding	Climatic factors (monsoon onset), size of cloacal protuberance	No difference	Deviche et al. (2014)
Rufous-winged sparrows (*Peucaea*	Male	Stress-induced CORT	See above	See above	No difference	Deviche et al. (2014)

(continued on next page)

Table 1 (*continued*)

Species	Sex examined	Measure	Life history stages compared	How were life history stages assessed?	CORT titers pre-breeding relative to breeding	Source
carpalis)						
Snow bunting (*Plectrophenax nivalis*)	Male and female	Baseline CORT	1) Arrival on the breeding grounds before eggs in the nest, 2) incubation, 3) the nestling period, 4) molt	Presence of a brood patch, size of cloacal protuberance	No difference	Walker et al. (2015)
Snow bunting (*Plectrophenax nivalis*)	**Male** and female	**Integrated CORT (a measure of stress-induced CORT)**	**See above**	**See above**	In females: no difference; **in males: higher (compared to nestling and molt stages)**	Walker et al. (2015)
Lapland longspur (*Calcarius lapponicus*)	**Male and female**	**Baseline CORT**	**1) Arrival on the breeding grounds 2) post-arrival (establishment of a nest and initiation of breeding activities)**	**See above**	**Higher during arrival (compared to post-arrival)**	Walker et al. (2015)
Lapland longspur (*Calcarius lapponicus*)	**Male** and female	**Integrated CORT (a measure of stress-induced CORT)**	**See above**	**See above**	In females: no difference; **in males, higher during arrival compared to post-arrival**	Walker et al. (2015)
Tree swallows (*Tachycineta bicolor*)	**Female**	**Baseline CORT**	**1) Nest-building, 2) early incubation, 3) late incubation, 4) nestling**	**Direct observation of nest boxes**	**Higher (compared to nestling stage)**	**F. Bonier, personal communication (data from Bonier et al., 2009)**

secretion within a fairly short time frame (Carroll et al., 1981; Dickens et al., 2009; Sapolsky and Altmann, 1991).

Therefore, we predict that pre-breeding birds may decrease negative feedback regulation. This would increase the duration, and thereby the effects, of stress-induced corticosterone on the body potentially without increasing peak pre-breeding stress-induced corticosterone titers. Although stress physiologists generally view poor negative feedback as pathological (Dickens et al., 2009; McDonald et al., 1986; Sapolsky, 1983), it could actually be helpful in this situation: poor negative feedback would prolong corticosterone secretion, increasing hormone exposure over time (Fig. 3). Increased corticosterone exposure may increase the likelihood that animals postpone breeding to a more favorable time period.

A detailed analysis of seasonal variation in negative feedback regulation has been examined in very few studies. However, results from a recent study in wild house sparrows (*Passer domesticus*) are intriguing: sparrows had the lowest efficacy of negative feedback during the pre-egg-laying period compared to five other life history stages (Lattin et al., 2012a). Just two months later, when all animals were breeding, negative feedback regulation was at its highest level in this population. More studies examining seasonal regulation of negative feedback during pre-breeding would clarify whether this is a common pattern in other species.

Interestingly, AVP/AVT may be less sensitive to regulation by negative feedback than CRF (Bartanusz et al., 1993; Scaccianoce et al., 1991). Therefore, one potential mechanism for down-regulating negative feedback (and thereby increasing corticosteroid function) at some times of year could be seasonal changes in the use of CRF vs. AVP/AVT as a secretagogue for ACTH. The prediction would be that the stimulus to release ACTH would shift from CRF to AVP/AVT during the pre-breeding period. De-emphasizing the CRF component would make the HPA axis less sensitive to the feedback signal. Although the relative contributions of CRF and AVP/AVT to corticosteroid secretion remain to be tested specifically during the pre-breeding period, a number of studies have shown a shift in their relative contributions when comparing breeding to other life history stages (e.g., Astheimer et al., 1994; Romero, 2006; Romero et al., 1998a; Romero et al., 1998b; Romero et al., 1998c; Romero and Wingfield, 1998, 2001). Furthermore, in birds, AVT expression in several brain areas has been shown to be testosterone dependent (Panzica et al., 1999; Plumari et al., 2004), so as circulating concentrations of testosterone rise during the pre-breeding period, there may be complex, sex-specific interactions between increasing AVT expression and corticosterone function.

Prediction 3: pre-breeding birds rapidly decrease plasma concentrations of corticosteroid binding globulin to increase corticosteroid effects

A large percentage (~90%) of plasma corticosteroids are bound to a carrier protein, corticosteroid binding globulin (CBG; Fig. 1) (Desantis et al., 2013; Siiteri et al., 1982). The precise role of CBG is still a matter of some debate. The "Free Hormone Hypothesis" posits that corticosteroids bound to CBG are essentially inert, and that it is only the free hormone fraction that can enter tissues and have biological effects (Mendel, 1989; Mendel, 1992). At the other extreme is the "Carrier Hypothesis" (Romero, 2002; Rosner, 1990). This hypothesis views CBG simply as a carrier protein for corticosteroids, which are thought to be able to dissociate freely; this hypothesis regards total hormone titers as biologically relevant, and is also known as the "Total Hormone Hypothesis" (Breuner et al., 2012). A third hypothesis combining aspects of the previous two, the "Reservoir Hypothesis," has also recently been proposed

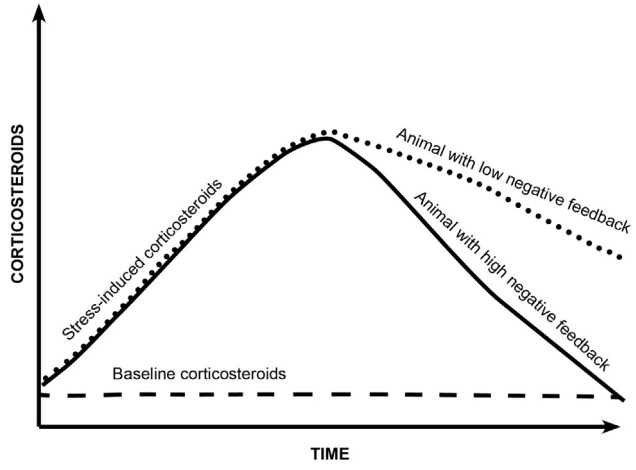

Fig. 3. Corticosteroids increase significantly above baseline (dashed line) when an animal encounters a stressor such as a predator attack or a severe winter storm. In animals with high negative feedback (solid line), corticosteroids return to baseline relatively quickly. In animals with low negative feedback (dotted line), corticosteroids remain elevated for longer, increasing the overall amount of hormone those animals are exposed to, even without changes in peak corticosteroid titers. We suggest that decreasing negative feedback could be one mechanism for birds to increase their sensitivity to corticosterone during the pre-breeding period.
Figure adapted from Romero (2004).

(Malisch and Breuner, 2010). In this hypothesis, although it is free corticosteroids that are biologically active, the remaining CBG-bound hormone serves as a reservoir that can be used as needed.

CBG likely shows all three functions, with the dominant effect simply dependent on timing (Schoech et al., 2013). Certainly, over short time scales, there is strong evidence from rodent and cell culture studies that it is the free hormone that primarily enters tissues and binds to receptors (Perogamvros et al., 2011; Qian et al., 2011). However, over longer time scales, much of the hormone secreted in response to a stressor may eventually make it into tissues (Breuner et al., 2012). These studies make it clear that CBG serves an important function - in the absence of CBG, corticosteroid clearance by the liver increases dramatically and corticosteroid half-life decreases (Schroeder and Henning, 1989).

The Free Hormone and Reservoir Hypotheses lead us to the specific prediction that birds could increase the effects of corticosterone over shorter time scales (minutes to hours) by rapid clearance or sequestration of CBG, or rapid changes to CBG that decrease its affinity for corticosterone, causing rapid increases in bioactive free hormone concentrations in blood (Fig. 4). (Note, however, that the Carrier Hypothesis does not support these predictions.) Again, this would increase corticosterone's impact on the body even in the absence of significant increases in the magnitude of stress-induced corticosterone release. Therefore, compared to other life history stages, we predict that pre-breeding birds might have more rapid clearance or degradation of CBG in response to stressors.

Is there evidence that wild birds use this mechanism to increase corticosterone's impact in the pre-breeding period? Laboratory rodents and several wild rodent and bird species are certainly capable of rapidly decreasing CBG concentrations over short time periods (Boonstra and McColl, 2000; Breuner et al., 2006; Tinnikov, 1999). However, to our knowledge no one has yet found evidence of rapid decreases in plasma CBG concentrations specifically during the pre-breeding stage in any wild animal.

What would we predict in terms of the overall size of the CBG pool in plasma before breeding as opposed to other times of year? Here, the picture is less clear because of different predictions made by the different hypotheses describing CBG's role. If the Free Hormone Hypothesis is correct, a larger CBG pool would lead to less bioactive hormone overall. The prediction would thus be less CBG before breeding, if birds were using this mechanism to augment corticosterone function. If the Carrier Hypothesis is correct, the size of the CBG pool may not matter. If the Reservoir Hypothesis is correct, a larger CBG pool would lead to less bioactive hormone in the short term, but an extended time with elevated corticosterone (i.e., a lower free hormone peak that lasts longer). The uncertainty across hypotheses makes it difficult to predict whether the overall amount of CBG in plasma should increase, decrease or show no change during the pre-breeding period.

Prediction 4: pre-breeding birds alter enzyme activity to increase corticosterone's effects

In tissues corticosteroids may be converted to inactive 11-keto metabolites by the enzyme 11β hydroxysteroid dehydrogenase (11β-HSD) type 2 (Fig. 1) (Chapman et al., 2013). Another form of this enzyme, 11β-HSD type 1, converts inactive corticosteroid metabolites back to active hormone. This means that tissues with high 11β-HSD type 1 activity (which include liver, fat, and certain regions of the brain) are exposed to higher concentrations of corticosteroids than those present in general circulation, whereas tissues with high 11β-HSD type 2 activity (such as kidney, some reproductive tissues and the colon) are exposed to lower concentrations of corticosteroids compared to circulating titers (Edwards et al., 1988; Seckl and Walker, 2001). Changes in the activity of the 11β-HSD enzymes have many important impacts on reproduction in mammalian systems, such as protecting Leydig cells from the adverse effects of corticosteroids on testosterone secretion and sperm production (Michael et al., 2003).

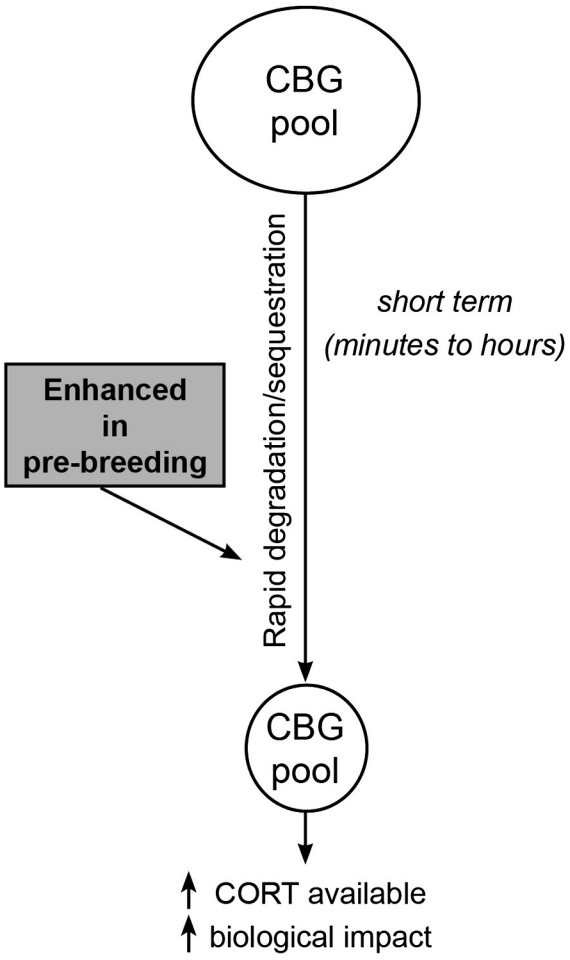

Fig. 4. In the short term (minutes to hours), decreases in the total amount of corticosteroid binding globulin (CBG) in plasma are thought to result in more bioactive corticosteroids (CORT) available to target tissues and an overall increased biological impact. Therefore, we predict that birds may enhance the rapid clearance or sequestration of CBG right before breeding compared to other times of year to enhance the biological effects of corticosterone.

Therefore, increasing 11β-HSD type 1 and decreasing type 2 activity during pre-breeding could increase the effects of corticosteroids on the body. To suppress the initiation of reproductive behavior and physiology, we specifically predict that these effects would be greatest in the gonads and/or brain. To our knowledge, no studies have examined seasonal variation in 11β-HSD in any avian species, although both isoforms of 11β-HSD are thought to be conserved across most vertebrates (Chapman et al., 2013), and studies of enzymatic activity and gene expression have reported what appears to be one or both of the 11β-HSD isoforms in birds (Katz et al., 2010; Kucka et al., 2006). Genes for these enzymes have also been cloned in chickens (Klusonova et al., 2008).

However, in the opportunistically breeding toad *Bufo arenarum*, testes concentrations of an enzyme similar to mammalian 11β-HSD type 2 (the corticosteroid inactivating isoform) were lower in both the pre-reproductive and reproductive periods compared to the post-reproductive period (Denari and Ceballos, 2005). This suggests that, in this species, the testes are potentially more sensitive to the effects of high circulating corticosteroids during the entire reproductive period than just following it, which may be related to this toad's opportunistic breeding habits. Avian studies examining seasonal changes in 11β-HSD type 1 and 2 activity will help us determine to what extent wild birds may use this as a mechanism for modulating corticosterone's actions.

Prediction 5: pre-breeding birds increase receptor density to increase corticosterone's effects

Corticosteroid effects are primarily mediated through two populations of receptors - higher-affinity mineralocorticoid receptors and lower-affinity glucocorticoid receptors (Fig. 1). These intracellular receptors are found throughout the body in both mammals and birds, although the exact distribution of each receptor depends on tissue type (Ballard et al., 1974; Breuner and Orchinik, 2009; Lattin et al., 2012b). After binding to corticosteroids, these receptors form dimers, bind to specific DNA responsive elements and alter gene transcription (Stahn et al., 2007). There is also evidence that corticosteroids can cause rapid changes in behavior through a membrane-bound receptor subtype in the brain (Breuner and Wingfield, 2000; Groeneweg et al., 2011; Haller et al., 2008; Moore and Orchinik, 1994; Olijslagers et al., 2008).

Receptor number contributes strongly to tissue response (Vanderbilt et al., 1987). Therefore, we predict that increased corticosteroid receptor density in target tissues may increase corticosteroid effects in the pre-breeding period. Again, this would cause greater corticosteroid impacts on the body independent of increases in hormone titers, and, similar to 11β-HSD effects, we predict increased receptors in brain and gonads in particular. In a series of studies examining different hypotheses for seasonal modulation of corticosteroids in wild house sparrows, corticosterone receptors in seven different target tissues were found to vary seasonally (Table 2) (Lattin and Romero, 2013, 2015; Lattin et al., 2013). Receptors were examined during six different life history stages (early and late winter, pre-egg-laying, breeding, late breeding and molt), and in 5 of the 7 tissues, the highest receptor density occurred during the pre-egg-laying period. This was true of tissues involved in functions as diverse as central control (whole brain), immune function (spleen) and metabolism (two muscle types and subcutaneous fat). One of the weaknesses of these studies was the lack of anatomical detail, particularly in the brain. It is possible that increased receptor density in brain areas involved in negative feedback (such as the hypothalamus and hippocampus) could actually cause *decreased* corticosterone function. However, in house sparrows caught at the same place and time as those used in the receptor studies, negative feedback was shown to be at a seasonal nadir during pre-breeding (Lattin et al., 2012a), suggesting that the increased receptors are not in brain areas involved in negative feedback. This is further supported by data showing no increase in hippocampal glucocorticoid or mineralocorticoid receptor binding in the pre-breeding period (Lattin and Romero, 2013), although hippocampus is just one potential brain area regulating negative feedback.

Similarly, Gambel's white-crowned sparrows were also found to have higher corticosterone receptor expression in brain in the pre-breeding period compared to the breeding period (Krause et al., 2015). These two studies suggest that increasing receptor density may indeed be one way for birds to increase the impact of circulating corticosterone in the pre-breeding period. However, it remains to be seen whether this pre-breeding increase in corticosterone receptor density is widespread across other avian species, and whether membrane-bound corticosterone receptors show a similar pattern of seasonal modulation.

Prediction 6: pre-breeding birds alter the activity of other hormone systems to increase corticosterone's effects

Another likely mechanism for modulating the pre-breeding impact of corticosteroids on different target tissues is via seasonal changes in other hormones that may exert permissive effects on corticosteroid activity (Fig. 1). For example, European starling (*S. vulgaris*) gonadal tissue is more sensitive to corticosterone and markers of metabolic stress in the pre-breeding period (McGuire et al., 2013), although no differences in gonadal corticosterone receptor levels were detected in house sparrows (Lattin and Romero, 2013). The authors suggest that these seasonal changes in gonadal receptivity to corticosterone may be regulated via changes in some hormonal system that interacts with corticosterone, such as the melatonin or gonadotropin-inhibitory hormone (GnIH) systems.

Increasing evidence points to a relationship between corticosterone and GnIH. For example, captive male zebra finches (*Taeniopygia guttata*) increased plasma corticosterone in response to a 10 h fast, and the magnitude of this increase was correlated with increased GnIH expression in testes (Lynn et al., 2015). Similarly, Calisi et al. (2008) found that wild-caught house sparrows showed a greater increase in immediate-early gene expression in the hypothalamus in response to the stress of capture and handling at the beginning of the breeding season compared to late in the breeding season. More hypothalamic GnIH neurons were also activated in response to stress early in the breeding season. Although the role of corticosterone was not investigated in this study, these data suggest that if corticosterone is involved in shutting down reproduction during the pre-breeding period, it could occur at least in part via the GnIH system.

Methodological considerations

Ideally, many investigators studying wild birds want to take multiple samples from the same animals, to control for some of the among-individual differences discussed earlier and to provide opportunities for different kinds of experimental interventions and the determination of fitness effects. One of the advantages of assessing corticosterone's impact via hormone titers is ease of sampling — baseline corticosterone can be assessed through rapid blood sampling (Romero and Reed, 2005), and stress-induced corticosterone by blood sampling after some kind of standardized stress protocol, such as the widely used cloth bag stressor (Wingfield and Romero, 2001). Even in cases where blood samples cannot be collected, integrated corticosteroids can be assessed using feces, feathers, or some other body tissue or fluid (Sheriff et al., 2011).

Because CBG and negative feedback are measured from plasma samples, sampling is fairly straightforward and multiple samples can also be collected from the same individuals. Assessing negative feedback regulation is slightly more invasive, as it involves the administration of injections and holding animals for longer periods of time than typical stress

Table 2
Summary of significant seasonal trends in corticosterone receptor density in either glucocorticoid receptors (GR) or mineralocorticoid receptors (MR) in different corticosteroid target tissues of wild-caught house sparrows (*Passer domesticus*). Sparrows were caught at six different life history stages: early and late winter, pre-egg-laying, breeding, late breeding and molt (n = 12 at each stage).

Tissue type	Receptor type that varied	Highest receptor density	Source
Liver	GR	Early winter, molt	Lattin and Romero (2015)
Subcutaneous fat	GR	Pre-egg-laying (males only)	Lattin and Romero (2015)
Gastrocnemius muscle	GR	Pre-egg-laying	Lattin and Romero (2015)
Pectoralis muscle	GR	Pre-egg-laying	Lattin and Romero (2015)
Kidney	GR	Late winter	Lattin and Romero (2015)
Spleen	GR and MR	Pre-egg-laying (for both receptors)	Lattin et al. (2013)
Brain	GR	Pre-egg-laying	Lattin and Romero (2013)

hormone sampling. Enzyme sampling typically involves euthanizing animals and extracting tissues for use in enzyme activity assays, through protein quantification (e.g., using enzyme-linked immunoassays or Western blots with validated antibodies), or by examining expression for 11β-HSD genes. However, researchers may be able to take small biopsies of tissues such as muscle or liver, and in vivo assays of 11β-HSD activity based on urine samples have been developed for use in humans (Zuckerman-Levin et al., 2011).

Sampling for corticosterone receptors also typically involves euthanizing animals, although it might be possible to take non-lethal biopsies from tissues such as skin, liver, and muscle. Corticosterone receptors can be quantified using a number of different methods, including radioligand binding assays, autoradiography and Western blots (Qume, 1999; Spencer et al., 2000). Examining receptor gene expression is also an option, but investigators should be cautious in interpreting a change in gene expression to mean that there is a change in protein expression, as the two are not always correlated (Feder and Walser, 2005; Medina et al., 2013).

To date, most research on GnIH in "non-model" organisms has used experimental administrations of GnIH via intracerebroventricular cannula (Bentley et al., 2006), measurements of gene expression of GnIH or its receptor (McGuire et al., 2011), or direct quantification of the hormone in tissue using methods such as immunohistochemistry (Calisi et al., 2011; Calisi et al., 2008). These are all fairly invasive measures and most require euthanizing animals for tissue collection.

Which animals do we expect to increase corticosteroid function before the onset of breeding?

We expect the CORT-Flexibility Hypothesis to apply to populations of wild birds that have at least some scope for changing the timing of breeding onset from one year to the next (Fig. 5). For example, house sparrows, one of the most common avian species used in studies of corticosterone physiology, show substantial variation in the timing of breeding onset in North America. Murphy (1978) found ~2 and ~4-week variation across years in median clutch initiation in house sparrows breeding in Kansas (USA) and Alberta (Canada), respectively. Indeed, many temperate wild bird populations show significant variation in the timing of breeding initiation across different years, due to ecological factors such as variation in food availability, temperature and storm frequency among different years (Cornelius et al., 2013; Goutte et al., 2014; Nussey et al., 2007; Potti, 1999). Researchers could assess a population's scope for postponing breeding onset by examining the variance in the dates of first breeding attempts over several years. This type of assessment should certainly be done on a population rather than a species level, given that different populations of the same species can show large variation in the dates of breeding onset based on factors such as latitude (Breuner et al., 2003) and habitat type (Caro et al., 2006).

At the other extreme, animals are generally expected to be resistant to corticosteroid-induced reproductive suppression when future reproductive opportunities are severely limited (reviewed in Wingfield and Sapolsky, 2003). This includes older individuals with limited future opportunities for breeding, semelparous species with only a single breeding opportunity, and animals with very short breeding seasons. In particular, a number of studies have shown that many bird species breeding in the high Arctic, where the breeding season is temporally constrained, are relatively insensitive to corticosterone-mediated reproductive suppression (Astheimer et al., 2000; Wingfield et al., 1996; Wingfield and Hunt, 2002). Species breeding in the short Arctic summer may not be able to postpone breeding by even a few days because of the minimum amounts of time required for them to successfully breed in this environment while conditions are favorable (Hahn et al., 1995). However, most of these studies were done in animals that had already begun breeding, and less is known about possible corticosterone resistance in Arctic breeders before eggs are laid.

We might therefore expect to see a continuum in sensitivity to corticosterone-induced delays in breeding. At one extreme would be avian species where the window of opportunity for breeding is too short to permit delays, and which should be relatively corticosterone insensitive in the pre-breeding period; at the other, species where the window of opportunity is long enough that breeding can be postponed by up to several weeks, and which should be relatively corticosterone sensitive. More data on the variance in timing of breeding in wild bird populations would be useful for making these kinds of predictions. Again, it is important to note that "pre-breeding" and "breeding" are quite different physiological states, and we can imagine circumstances where animals have increased corticosterone function immediately prior to breeding, but corticosterone resistance once breeding has begun. For example, because older individuals have fewer future breeding opportunities than younger individuals, it may be especially important that they time breeding initiation just right and do not waste resources on failed breeding attempts (the "Residual Brood Value Hypothesis": (Bokony et al., 2009; Wingfield et al., 2011)).

Interestingly, HPA activation alone may also not be enough to cause breeding delays when the corticosterone signal contradicts salient environmental cues such as weather and food availability. For example, female Florida scrub-jays (*Aphelocoma coerulescens*) provided with supplementary food and dosed with exogenous corticosterone did not lay eggs any later than birds who received food only; however, both groups of food-supplemented birds laid eggs earlier than non-food-supplemented females (Schoech et al., 2007). Similarly, while corticosterone implants extended the time away from the breeding site in poor weather conditions in breeding white-crowned sparrows, those same implants given in good weather did not induce territory abandonment (Breuner and Hahn, 2003). This suggests that when information from other environmental cues is good (pro-breeding), corticosterone signals may either be ignored or overruled by information from other physiological systems. Interestingly, animals breeding at higher latitudes also seem less sensitive to supplemental cues such as food supplementation and temperature (Maney et al., 1999; Schoech and Hahn, 2007). In keeping with these data, we predict that some species, such as some Arctic birds, may be insensitive to both corticosterone and environmental cues while others, such as the Florida scrub-jay, may be sensitive to corticosterone combined with cues. It remains to be seen whether there could be a third class of animal (such as tropical species, or "opportunistic" temperate species), which might be sensitive to corticosterone even in the absence of appropriate environmental cues. Future studies examining the integration of different kinds of signals in the brain (sensory, hormonal, etc.) should clarify the mechanisms of corticosterone-induced reproductive suppression in wild birds.

There may also be differences within a single species in which sex will be more sensitive to corticosterone effects during pre-breeding. Many avian species have inequitable distributions of parental effort. In a study on multiple shorebird species, for example, the sex that showed the most parental care was the least sensitive to the stress of capture and handling during the breeding season (O'Reilly and Wingfield, 2001). A similar response, although in reverse, might be predicted during pre-breeding, with the sex investing most in parental care being the most sensitive to corticosterone in order to better time the disproportionate reproductive effort. To our knowledge, this prediction has not yet been tested.

Finally, we believe many of the predictions of the CORT-Flexibility Hypothesis could be applied to other vertebrate taxa that display associated reproductive stages (i.e., where gamete maturation and maximum sex steroid secretion occurs immediately before or concurrent with courtship and copulation (Crews, 1984)). This includes many small mammals, and some reptiles, amphibians and fish. However, if avian evidence supporting this hypothesis is limited, evidence from other free-living taxa is almost nonexistent. More studies are needed across a wider range of vertebrates that specifically examine individuals just before the onset of breeding.

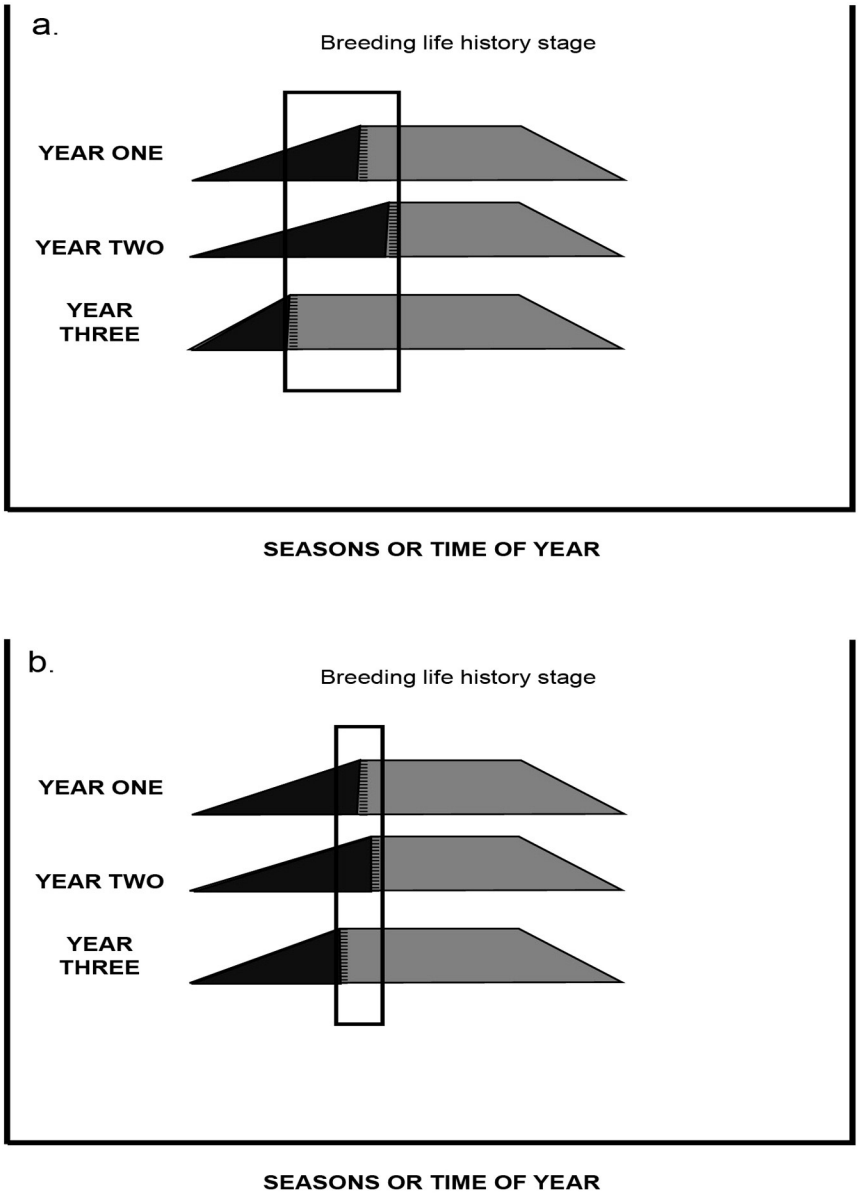

Fig. 5. A population of wild birds showing wide year-to-year variation in the timing of breeding initiation (a) would be expected to rely more upon supplemental cues, and therefore increase overall pre-breeding corticosterone function more than a population of birds showing little year-to-year variation in the timing of breeding initiation (b). Life history stages other than breeding are not shown here for the sake of simplicity.

Synthesis and future directions

Current data provide evidence for seasonal modulation of corticosteroid physiology, from the hormone titers themselves, to negative feedback, concentrations of binding globulins, enzyme activity and receptor density in different target tissues. However, it is less clear exactly why all this seasonal modulation exists. Three different hypotheses have been proposed to explain the seasonal patterns that occur in corticosteroid hormones (Romero, 2002). The Energy Mobilization Hypothesis suggests that corticosteroids should be highest during the most energetically costly times of year, given the important role of these hormones in energy acquisition and mobilization (Dallman et al., 1993). The Preparative Hypothesis posits that animals should have the highest corticosteroid titers at times of year when adverse conditions are most likely to occur, given the importance of corticosteroids in priming the body to respond to stressors (Sapolsky et al., 2000). The Behavior Hypothesis suggests that annual modulation of corticosteroids results from animals having different requirements for expressing corticosteroid mediated behaviors (like fleeing and relocating to a new area) at different times of year (Wingfield et al., 1998). There is some support for each of these three hypotheses, and they are not mutually exclusive — they may all be supported in certain circumstances and for certain species.

The "pre-breeding period" is potentially one where all three of these hypotheses intersect. Relevant to the Energy Mobilization Hypothesis, this period can be energetically costly in many species due to the challenges of preparing for breeding (finding mates, defending territories) when food may not be abundant (Bronson, 1985; Thomas et al., 2001). Similarly, pre-breeding birds may be exposed to more stressors relative to other times of year, so the Preparative Hypothesis may also apply. For example, male birds that spend much of the day singing in conspicuous breeding plumage may be more obvious to predators (Gotmark and Post, 1996), and may encounter more challenges from conspecifics resulting in injury (Townsend et al., 2011), compared to non-breeding males. However, the most relevant of these three seasonal hypotheses to pre-breeding corticosteroid physiology is probably the Behavior Hypothesis. Although this hypothesis is often invoked to explain the apparent down-regulation of corticosteroid physiology during

breeding to avoid stress-induced reproductive failure (Bokony et al., 2009; Lendvai et al., 2007; O'Reilly and Wingfield, 2001), an up-regulation of corticosteroid physiology immediately prior to breeding to ensure animals start breeding at an appropriate time also fits into the framework of this hypothesis. Furthermore, in this review we would like to suggest that these kinds of seasonal hypotheses should be applied to corticosteroid physiology beyond hormone titers. Corticosteroids primarily affect the body by changing gene expression, and there are multiple ways to increase the effect of corticosteroids on target tissues beyond just increasing corticosteroid release.

It is important to note that there are other ways to increase the impact of corticosteroids beyond the six mechanisms described here, including changing expression of the nuclear receptor coactivators that facilitate transcription initiation (York and O'Malley, 2010), or the heat shock proteins that help stabilize corticosteroid receptors in their high-affinity steroid binding conformation (Pratt and Toft, 1997). However, whereas evidence supporting the six mechanisms presented here is still limited and preliminary, evidence for seasonal modulation of these other mechanisms in free-living birds is nonexistent. Also, although we recognize that it is not possible to examine every component of a physiological system, it may be helpful to look at other types of evidence (i.e., receptor concentrations, CBG, etc.) when one type of evidence (i.e., hormone data) is contradictory from species to species as seems to be the case for the CORT-Flexibility Hypothesis.

Thus far, there is intriguing evidence suggesting that some avian species may increase the impact of corticosterone on the body during the pre-breeding period through mechanisms other than altering hormone titers, such as decreased negative feedback regulation and increased receptor density in target tissues. However, because there are so few studies of these kinds of measures, support for these predictions of the CORT-Flexibility Hypothesis must be considered preliminary, and there are still many unanswered questions. There is certainly the need for more studies that specifically examine birds just before the onset of breeding, to determine if this is indeed a unique period characterized by increased corticosterone function. More studies of corticosterone physiology beyond circulating hormone titers are needed, even though there can be considerable technical challenges involved in quantifying receptors, enzyme activity and other components of the HPA axis, and not all investigators are able or willing to euthanize animals for these kinds of studies. In addition, cross-sectional studies may produce misleading patterns not found in longitudinal studies (Beecher et al., 2000; Nussey et al., 2009), so investigators must interpret results from cross-sectional studies with a certain degree of caution.

We also know very little about how these different mechanisms interact. Should we expect to see a suite of co-occurring changes in a particular species, or do different species regulate corticosterone function differently? So far, the only evidence we have suggests that these changes tend to co-occur — we know that wild house sparrows increase receptor density in several different target tissues, decrease negative feedback regulation and increase activity of gonadotropin-inhibitory hormone neurons in the brain in response to stress right before the onset of breeding (Calisi et al., 2008; Lattin et al., 2012a; Lattin and Romero, 2013, 2015; Lattin et al., 2013). However, this may not be typical. With more data, we can determine whether any general patterns exist across different species. We also know little about the mechanisms that cause animals to switch from relatively corticosterone-sensitive to relatively corticosterone-insensitive, although there is evidence from house sparrows that the switch from pre-breeding to breeding life history stages is accompanied by a decrease in corticosterone receptors in at least some tissues, and an increase in negative feedback regulation (Lattin et al., 2012a; Lattin and Romero, 2013).

Finally, although it is clear that there are costs both to initiating breeding too early and too late, these costs may not be symmetrical, and they may depend on a species' life history. For example, in some species it may be worse to start breeding too late than to start breeding too early, and this could affect how corticosterone physiology is regulated. These costs could also differ for males and for females, based upon factors such as the relatively greater energetic cost of egg laying in female birds (Ball and Ketterson, 2008; Williams, 2012). Indeed, there is already evidence in some avian species that pre-breeding corticosterone function may differ between the sexes, including many of the studies discussed above (Lattin and Romero, 2015; McGuire et al., 2013; Silverin, 1986). Furthermore, in different individuals within a species, there could also be differences in how the pre-breeding impacts of corticosterone are mediated based on different physiological or behavioral syndromes (Carere et al., 2010).

Although the CORT-Flexibility Hypothesis remains to be fully tested, we think the data presented here suggest that this is a fruitful area for future research. Due to climate change, photoperiod and other formerly predictive cues may no longer accurately reflect the suitability of the environment for breeding (Visser, 2008). The key role of the HPA axis in helping organisms cope with environmental perturbations makes it an essential component in responding to a changing world (Angelier and Wingfield, 2013), and understanding how corticosterone physiology may be modulated to help wild birds successfully time the onset of breeding will help us predict how they might respond to future changes.

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