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PERSPECTIVE

Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context and reproductive state

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Summary

1. Experimental glucocorticoid (GC) manipulations can be useful for identifying the mechanisms that drive life-history and fitness variation in free-living animals, but predicting the effects of GC treatment can be complicated. Much of the uncertainty stems from the multifaceted role of GCs in organismal metabolism, and their variable influence with respect to life-history stage, ecological context, age, sex and individual variation.

2. Glucocorticoid hormones have been implicated in the regulation of parental care in many vertebrate taxa but in two seemingly contradictory ways, which sets up a potential GC-induced 'reproductive conflict'. Circulating GCs mediate adaptive physiological and behavioural responses to stressful events, and elevated levels can lead to trade offs between reproductive effort and survival (e.g. the current reproduction vs. survival hypothesis). The majority of studies examining the fitness effects of GC manipulations extend from this hypothesis. However, when animals are not stressed (likely most of the time) baseline GCs act as key metabolic regulators of daily energy balance, homoeostasis, osmoregulation and food acquisition, with pleiotropic effects on locomotor activity or foraging behaviour. Slight increases in circulating baseline levels can then have positive effects on reproductive effort (e.g. the 'cort' fitness/adaptation hypotheses), but comparatively few GC manipulation studies have targeted these small, non-stress induced increases.

3. We review studies of GC manipulations and examine the specific hypotheses used to predict the effects of manipulations in wild, breeding vertebrates. We argue that given the dichotomous function of GCs the current 'reproduction vs. survival' paradigm is unnecessarily restrictive and predicts only deleterious GC effects on fitness. Therefore, a broader set of hypotheses should be considered when testing the fitness effects of GC manipulations.

4. When framing experimental manipulation studies, we urge researchers to consider three key points: life-history context (e.g. long vs. short lived, semelparous vs. iteroparous, etc.), ecological context and dose delivery.

Key-words: baseline levels, corticosterone, cortisol, fitness, glucocorticoids, implants

Introduction

Few ideas in life-history theory are as empirically supported as the trade-off between current reproduction and survival (Nur 1984, 1988; Stearns 1992; Love & Williams 2008). Despite a long-standing recognition that physiological processes must be at the very root of such trade-offs (Fisher 1930), we still know comparatively little about the underlying regulatory mechanisms (but see Williams 2012; Love *et al.* 2014). Ricklefs & Wikelski (2002) formalized the concept of the 'life-history/physiology nexus', which provides a framework for thinking about how physiological processes, particularly those with an endocrine basis, are linked to population-level ecological

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and evolutionary processes. However, it has only been relatively recently that studies have sought to test hypotheses about the role of hormones as key regulators of life-history variation. Experimental manipulation of hormones (and not just physiology) is particularly useful for illuminating the constraints that drive life-history trade-offs, and hence, life-history variation (Ketterson *et al*. 1996; Sinervo & Basolo 1996; Ketterson & Nolan 1999; Sinervo 1999; Zera & Harshman 2001; Harshman & Zera 2007; Williams 2012). In this respect, particular attention has been paid to the glucocorticoid hormones (e.g. corticosterone, cortisol, hereafter 'GC'), especially as these relate to the 'current reproduction vs. survival' paradigm. GCs are excellent candidate hormones for regulation of life histories because of their central role in homoeostasis, daily energy balance and the stress response, as well as their pleiotropic effects on life history and behaviour (Wingfield *et al*. 1998; Romero 2004; Love *et al*. 2005; Breuner, Patterson & Hahn 2008; Love & Williams 2008; Bonier *et al*. 2009a; Love, McGowan & Sherriff 2013; Sheriff & Love 2013).

Hypotheses and predictions concerning the role of GCs in mediating the trade-off between current reproduction and survival arose originally from the role of these hormones in the stress response, rather than from the predictable daily or life-history-related changes in baseline levels (e.g. Landys, Ramenofsky & Wingfield 2006). However, two recent reviews highlight how variation in baseline levels, and indeed changes from baseline to maximal levels (e.g. stress responsiveness), can be positively, negatively or non-significantly related to reproduction, survival or other fitness surrogates (Breuner, Patterson & Hahn 2008; Bonier *et al*. 2009a). Exogenous hormone treatments (implant studies) often aim to increase baseline GCs to stress-induced levels, and under such an experimental design, the current reproduction vs. survival paradigm is relevant. But due to the highly variable nature of the effects of implants for hormone delivery and release on circulating levels, the efficacy of GC implants in generating the desired blood level increase can be difficult to predict, as can be the duration of such increases (Quispe *et al*. 2015). This uncertainty is compounded by GC's dichotomous physiological role, where either increases in baseline or stress-induced expressions can induce very different physiological, behavioural or fitness-related responses. Layered on top of this is the variable response of GC manipulation due to life-history and ecological context, reproductive stage and individual variation (Madliger & Love 2014, 2015). Will exogenous implants set GC levels at a new baseline high, or are GCs pushed beyond a threshold level indicative of an acute stress response? Moreover, what influence do life-history and reproductive state play in shaping GC trajectories? (Fig. 1). Additionally, exogenous manipulations can be confounded by feedback and/or clearance mechanisms, which can unintentionally reduce GC levels despite the aim of increasing them (Remage-Healey & Romero 2001). To address these uncertainties, we suggest that GC implants or injection volumes should be calibrated so that a desired blood level response can be achieved in the target species (e.g. Pravosudov 2003; Criscuolo *et al*. 2005; Ouyang *et al*. 2013b), while simultaneously taking into account the specific life-history context and reproductive stage that the species is in. Without pilot studies and consideration of the specific life-history and reproductive context, it is not easy to predict the shape of the GC response curve, or how this might influence interpretations of measured experimental endpoints. Does manipulation increase baseline levels only slightly, or do levels peak at stress-induced or pharmacological levels? Does this increase influence behaviour, breeding decisions/investment or fitness? And importantly, are there any unintended effects of GC implants on other hormonal pathways or cascades (e.g. hypothalamus–pituitary–gonadal axis; Sapolsky 2002)?

These questions are important given the increasing number of experimental studies using GC manipulations in free-living animals. It is becoming apparent that the relationship between GCs and fitness varies across life-history and breeding stage (e.g. Brown *et al*. 2005) and are generally complex (Breuner, Patterson & Hahn 2008; Boonstra 2013a). Our aims in this paper were therefore twofold. First, to discuss the role of life-history and reproductive stage in shaping the response of animals to exogenous GC manipulations (e.g. silastic implants, slow-release pellets, injections, osmotic mini-pumps, etc.), especially as they relate to fitness-relevant traits in free-living animals. Secondly, to bring attention to the growing number of studies wherein the predictions of the 'current reproductive vs. survival' paradigm fail to match the observed results. We believe that for the growing number of manipulation studies being published on freely ranging animals, this paradigm may at times be too restrictive, leaving little room for alternate hypotheses, predictions or interpretations of the observed effects and variations in life-history or fitness-related outcomes. Potential reasons for the discord between predictions and results may stem from uncertainty regarding the precise life-history and reproductive context (e.g. short- vs. long-lived species, pre-breeding vs. post-breeding, etc.) and the role of GCs therein, as well as uncertainty about the precise levels (in both magnitude and duration) of GCs being delivered through exogenous manipulation.

GC dose with respect to physiological function and potential for a 'reproductive conflict'

Glucocorticoid levels have been descriptively and experimentally linked to variation in both current reproduction and survival, and in the trade-off between these key components of fitness. Extending mostly, although not exclusively, from research on birds, many studies have shown that a parent's loss of offspring, or its tendency to abandon offspring, is often linked to energetic deficiencies, poor food resource availability or foraging inefficiencies, and thus to variation in both baseline as well as stress-induced plasma GC levels (Wingfield *et al*. 1998; Dallman *et al*.

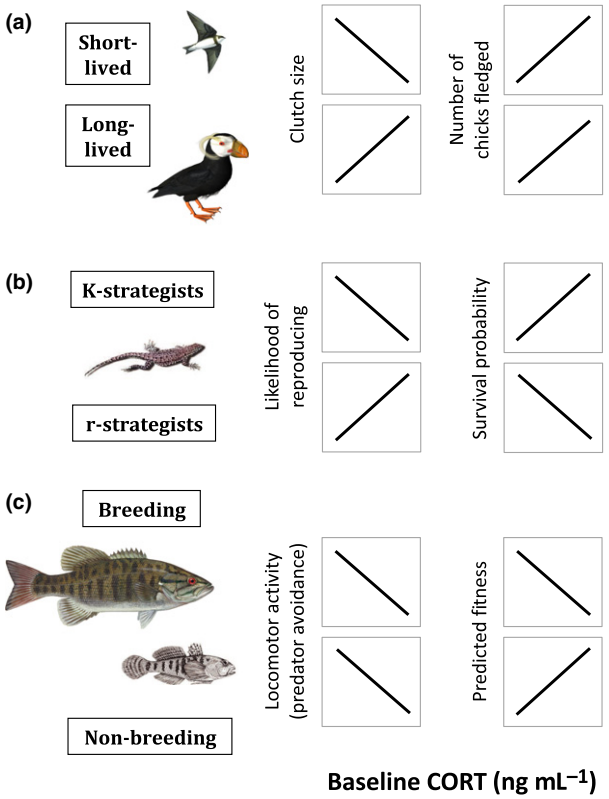

Fig. 1. Hypothetical breeding-level responses to mass-specific corticosterone (CORT) manipulation in species with different life histories (a). In tree swallows (*Tachycineta bicolor*), natural baseline variation in CORT showed a negative correlation with clutch size early in reproduction, but the relationship changed during later in the season to yield a positive correlation with fledging success (e.g. Bonier *et al*. 2009b; Bonier, Moore & Robertson 2011). We might therefore expect experimentally increased CORT to have even greater negative effects at laying and more positive effects at chick rearing. In contrast, baseline CORT in laying tufted puffins (*Fratercula cirrhata*) was positively correlated with clutch size, where as no discernable correlation with fledging success was evident later in the breeding season (Williams *et al*. 2008). At a given reproductive stage, experimental CORT manipulation in long-lived puffins might thus produce different effects than in short-lived swallows. In (b), differential responses to CORT manipulation have been observed in female side-blotched lizards (*Uta stansburiana*) possessing different life histories. CORT-implanted 'yellow' K-strategists traded reproduction for survival, whereas the opposite was observed in 'orange' r-strategists (see Lancaster *et al*. 2008). In (c), cortisol implants were found to negatively impact semelparous, female pink salmon (*Oncorhynchus gorbuscha*) by limiting spawning ground longevity and the number of eggs spawned (McConnachie *et al*. 2012), presumably via faster depletion of finite somatic energy reserves. In an iteroparous species like smallmouth bass (*Micropterus dolomieu*), it is unclear what effect that baseline cortisol manipulation might have on spawning ground longevity, but it is possible that increased cortisol could provide more energy for nest protection, and thus increasing reproductive success.

1999; Love *et al*. 2004; Groscolas, Lacroix & Robin 2008; Kitaysky *et al*. 2010). If baseline levels in a parent exceed some individual or species-specific threshold and cross into an acute or chronic stress response, the prevailing metabolic condition may result in a behavioural shift that favours self-maintenance at the expense of offspring. This is the central idea underlying the concept of the 'emergency life-history stage' found at the root of many 'current reproduction vs. survival' studies (Wingfield *et al*. 1998; Landys, Ramenofsky & Wingfield 2006). But not all studies of GCs fit this paradigm, and indeed, recent work has shown an opposite pattern where increased baseline corticosterone (CORT) levels (i.e. not stress-induced levels) relate positively with investment in offspring (e.g. during parental care; Bonier, Moore & Robertson 2011; Crossin *et al*. 2012; Love *et al*. 2014). One recent example involved the breeding tactics of wild European starlings (*Sturnus vulgaris*; Love *et al*. 2014). Here, breeding investment was manipulated via egg removals during current reproduction (first broods), resulting in an increase in baseline CORT during incubation of the second clutch which in turn facilitated greater investment in future reproduction (offspring in second broods). The result was equal overall investment between both control and treatment groups, indicating that variation in baseline CORT was proactively mediating the trade-off between current and future reproductive investment, thereby optimizing investment and maximizing the fitness of individuals.

The contrasting, dichotomous function of GCs to breeding biology is central to the idea of a GC-induced 'reproductive conflict' (Love *et al*. 2004), the root of which stems from hormonal pleiotropy (Williams 2012). GCs are constitutively expressed to regulate daily energy balance and homoeostasis in vertebrates, and thus act as general metabolic regulators (Landys, Ramenofsky & Wingfield 2006; Crespi *et al*. 2013). GCs can thus show positive associations with current reproductive investment (Bonier *et al*. 2009a; Bonier, Moore & Robertson 2011; Ouyang *et al*. 2011, 2013a). However, GC secretion is also a component of the stress response, which helps coordinate an adaptive physiological and behavioural response to foster survival, often at the expense of current reproduction. These different functions are thought to be mediated by different cellular receptors, in which the high-affinity mineralocorticoid receptor (MR) mediates responses to changes in 'baseline' GC levels, whereas the low-affinity glucocorticoid receptor (GR) mediates the responses to 'stress-induced' GC levels (Breuner & Orchinik 2002; Romero 2004; Landys, Ramenofsky & Wingfield 2006). However, new evidence suggests that baseline GCs can mediate stress-dependent functions such as personality and immune response via GR receptor binding (Crespi *et al*. 2013; Dosmann, Brooks & Mateo 2015). Whichever the case, it is thus thought that within the context of reproduction, individuals should minimize or downregulate GC secretion, or minimize GC reactivity, when reproductive investment is high. A perhaps more suitable hypothesis to frame variations in baseline GC is the CORT-adaptation hypothesis (Bonier *et al*. 2009a), in which positive associations with reproductive (e.g. fitness) measures are predicted at certain life-history stages and relative degrees of current reproductive investment (Fig. 1).

Because of GC's dual role in the regulation of parental care and the potential for conflict, it is important that experimental studies identify, *a priori*, the appropriate theoretical framework through which increased baseline or stress-induced GC levels can then be correctly interpreted (Romero 2004). When this is unknown, that is when it is not known how exogenous implants will influence circulating GC levels, due to a lack of controlled pilot studies or previous work on the species in question, then studies should acknowledge this uncertainty and offer alternate hypotheses when predicting the effect of GC manipulations on reproductive or other fitness-related proxies.

Response to GCs varies among life-history contexts and stages of reproduction

A clear example of how baseline GC expression can change as a function of reproductive stage is found in Love *et al.* (2004). In that study, mean baseline CORT levels in breeding starlings were observed at low levels at the laying stage of the reproductive season. In this short-lived species, CORT then rose to a new baseline level during incubation, and again during nestling provisioning. From this pattern of upregulation, it was inferred that baseline CORT, as a metabolic regulator and correlate of foraging behaviour, is steadily increased during the breeding season to match parental provisioning effort to the demands of growing offspring (Crossin *et al.* 2012; Sheriff & Love 2013). Variation in breeding CORT levels can, however, vary among species with different life histories. In tufted puffins (*Fratercula cirrhata*), a long-lived seabird, the opposite pattern was observed to that of the short-lived passerine above. Mean CORT in puffins peaked just prior to laying, and then declined to lower levels at incubation and chick rearing (Williams *et al.* 2008) (Fig. 1a). High baseline CORT during pre-breeding can be very important for some species, like seabirds with single egg clutches, who have limited opportunity to exploit spatially ephemeral zooplankton prey and accrue adequate resources for egg production (e.g. the match–mismatch hypothesis; Hipfner 2008). Increased pre-breeding CORT may also benefit investment in reproduction in species like common eiders (*Somateria mollissima*) (Hennin *et al.* 2015), which must forage heavily between arrival and laying so as to accrue the resources needed for follicle development, multi-egg production and an incubation fast (e.g. Descamps *et al.* 2011).

Other short-lived species, however, such as tree swallows (*Tachycineta bicolor*), show a different pattern in which a seasonal regulation of CORT is not readily apparent, but variation among breeding individuals is apparent, revealing correlations between baseline CORT and fitness measures (Bonier *et al.* 2009a). Early in the breeding season, during incubation, a negative relationship can be observed, which then shifts to a positive correlation later during nestling provisioning (Fig. 1a). What this suggests is that, unlike long-lived puffins and eiders, increased GCs might signal a reduction in habitat quality or resource availability during the pre-breeding or incubation phase, in which case an adaptive response for swallows might be partial brood reduction or reduced investment in individual eggs. Then, during the more metabolically demanding period of chick rearing, when parents are foraging extensively, elevated baseline GCs may signal a need to increase foraging and provisioning effort to feed multiple chicks.

Finally, perhaps the best examples how life-history context can influence the endpoints of GC manipulation comes from a study of side-blotched lizards (*Uta stansburiana*). Within populations, this species displays six distinct phenotypes, as indicated by the colour of their throats. In female lizards, two throat colours correspond to alternative reproductive strategies: females carrying orange alleles or 'orange' females, also r-strategists that lay large clutches with smaller offspring. 'Yellow' females lack the orange alleles, and are K-strategists that lay smaller clutches with larger eggs. When orange and yellow females were given similar mass-specific CORT implants, the results a classic life-history trade-off between reproduction and survival, but in opposite directions (Lancaster *et al.* 2008). Orange females invested more towards reproduction at the expense of reduced survival, whereas yellow females favoured survival over reproduction (Fig. 1b).

Despite the many examples of species differences in GC effects presented here and in Fig. 1, determining how or why differing responses emerge can be problematic. In many cases, variation in life-history strategies and tactics are useful for aiding the interpretation of taxon-specific responses. For example, it may make sense, at least theoretically (see Wingfield & Sapolsky 2003), that a short-lived or semelparous species with limited opportunities for lifetime reproduction expresses GCs differently from long-lived species (Fig. 1a), and similarly that r- and K- strategists within a taxon might also express GCs differently (Fig. 1b). However, there are situations where the underlying reasons for within-taxon differences in GC expression and responsiveness are unclear. The well-studied passerines, many of which have similar life-history strategies and tactics, can exhibit strikingly different patterns of baseline GC expression in relation to reproductive effort and success, for reasons not immediately obvious (e.g. Bonier *et al.* 2009b; Lendvai & Chastel 2010; Ouyang *et al.* 2011, 2013b; Patterson *et al.* 2014). An obvious explanation, but which is so far relatively little tested as it requires multi-year studies, is that *ecological* context can determine plasma GC patterns and functional effects even within a species, for example among years, against the broader background of life-history variation (Madliger & Love 2014, 2015). The challenge here is to delve ever deeper into the defining evolutionary and life-history characteristics of the species in question when seeking answers for any observed differences between species. Even then however, our insight may at times be limited. Collectively, what these and other studies indicate is that there is a complex interaction between GCs, life-history and breeding stages. The challenge for researchers is to be cognizant of

these factors and to carefully formulate hypotheses and predictions for a GC manipulation study, within the most appropriate theoretical framework, and to choose the most appropriate GC dose to best test those predictions.

Blocking, rather than enhancing, the effects of GCs

An alternate way to test for GC effects in vertebrates is to either block or dampen their effects, usually at the receptor level. However, most studies adopting this approach have been laboratory studies that do not assess effects on life-history variation or fitness endpoints (e.g. birds – Breuner & Orchinik 2009; fish – Dunlap, Jasharia & Pappas 2011; salamanders – Wack, Ratay & Woodley 2013). In a laboratory study of rainbow trout (*Oncorhynchus mykiss*) for example, mifepristone (RU-486, a GR antagonist) was used to suppress GR signalling, which resulted in reduced hypothalamus–pituitary–inter-renal axis (HPI) activity (Alderman *et al*. 2012). In a study of closely related pink salmon (*Oncorhynchus gorbuscha*) in an experimental spawning channel, metyrapone implants were used to suppress plasma cortisol levels in freely spawning females and fitness endpoints were considered, but ultimately treatment had no significant effect on reproductive behaviour, spawning success or physiology (McConnachie *et al*. 2012). This suggests that for semelparous Pacific salmon, certain aspects of reproduction can become refractory to stress during spawning, at a time when cortisol levels are already exceedingly high (>290 ng mL^{-1}; see Wingfield & Sapolsky 2003). Although not as commonly used as experimental increases in GCs, GC blocking studies have the potential to inform reproductive fitness studies in free-ranging animals and could be used in tandem with manipulation increases to address common hypotheses about the role of altered baseline GC levels. A recent study by Dosmann, Brooks & Mateo (2015) is one of the first to block GR in a wild vertebrate and to measure the response to exploratory behaviour and immune function. However, some caution is warranted when blocking or dampening GC receptors, as it may be difficult to compare and interpret manipulated receptor level effects vs. manipulated circulating GC levels (Sapolsky, Romero & Munck 2000), or unintended effects of handling could induce negative feedback mechanisms (Dosmann, Brooks & Mateo 2015). Endocrine blocking can also influence many types of hormones, for example RU-486 was used above to block GCs, but it is principally a progesterone antagonist, so in the context of reproduction this can be most confounding. Ultimately, this topic is beyond the scope of our discussion about baseline GC variations, and our aim was to provide some word of caution here. One final consideration regarding endocrine blocking, it is also important to adhere to relevant regulations that control the application of drugs to animals in the wild that could potentially enter the human food chain (most relevant to harvested fish or wildlife targeted by hunters) given that many pharmaceuticals can be dangerous to humans (e.g. RU-486 can abort human foetuses).

Hypotheses and predictions regarding experimental GC manipulations

Three published examples show how different life-history hypotheses can be used to provide a framework and foundation for predictions of effects of exogenous GC manipulations. In a classic example of a current reproduction vs. survival study, breeding black-legged kittiwakes (*Rissa tridactyla*) were given a GC implant aimed at increasing baseline levels to concentrations associated with chronic stress (Angelier *et al*. 2009), or more specifically 'to mirror prolonged energy constraints (type I allostatic overload, McEwen & Wingfield 2003) and the activation of an emergency life-history stage (Wingfield *et al*. 1998)'. This manipulation is predicted to have negative effects on reproductive investment in the form of parental care, and ultimately negative fitness effects, similar to that observed in Silverin (1998). Indeed, there was a correlated decrease in prolactin levels (a hormone involved with some aspects of incubation behaviour and parental care; Williams 2012), as well as reduced nest attendance and reduced breeding success in GC-implanted individuals, which supports the hypothesis that increasing GCs will favour survival over reproduction. In contrast, a study by Crossin *et al*. (2012) of breeding female macaroni penguins (*Eudyptes chrysolophus*) manipulated baseline levels of GCs only moderately in order to test predictions of the GC-adaptation hypothesis, which predicts positive associations between baseline GC levels and reproductive and other fitness proxies (as defined by Bonier *et al*. 2009a). In that penguin study, the implants were specifically targeting an increase within the range of normal baseline levels, not stress-induced levels and this was confirmed because higher CORT levels were positively related to foraging behaviour, foraging success and chick growth (i.e. fitness). As in the study of black-legged kittiwakes, the predictions of the hypothesis matched the results. In a third study, breeding male Adélie penguins (*Pygoscelis adéliae*) were implanted with CORT, but there was sufficient uncertainty about whether the implants would raise plasma levels within the baseline range, or beyond this to stress levels (Thierry *et al*. 2013). As such, the authors invoked both the current reproduction vs. survival paradigm as well as the GC-adaptation hypothesis in the introduction to their study. By embracing this approach, the authors were open to alternate interpretations of their data, which is commendable given that they could not predict the precise effect of the CORT implants on reproductive investment, and given that the results ultimately fit both paradigms. What the study showed was that compared with sham-implanted penguins, CORT-implanted penguins experienced both costs and benefits. CORT manipulation was associated with brood reduction, with treated birds tending to hatch a single egg from their 2-egg clutches. However, the surviving chicks of GC-treated

penguins did not differ in final mass or fledging success from control penguins, which could be interpreted as either a neutral or a positive effect. By reducing clutch size to a single egg, the parent bird is ensuring that at least one chick will fledge, thus maximizing relative fitness. In a study of captive black-legged kittiwake chicks, GC treatment had again both costs and benefits (Kitaysky *et al.* 2003). Treated chicks out-competed their nest mate for food delivery from parents (resulting in brood reduction), which was deemed a positive effect for the treated chick. But this early developmental exposure to increased GC led to cognitive deficiencies later in life, thus representing a longer-term cost of the treatment.

Putting GC levels into a predictive context

As mentioned earlier, the GC dose delivered to study animals should be calibrated, *a priori*, to best suit the overarching hypotheses and predictions. Although one of our aims in this paper was to draw attention to the potentially restrictive scope of the current reproduction vs. survival paradigm in studies of GC manipulations, this can be a perfectly suitable hypothesis in studies seeking to explore the effects of acute or chronic stress on some fitness measures, and when the exogenous GC dose needed is already known. However, when the effect of GC manipulation is less certain or unknown, then we suggest that alternate hypotheses that leave room for interpretation are necessary (e.g. the CORT-adaptation hypothesis). This could be necessary in situations where the GC manipulation inadvertently pushes individuals away from their endocrine optimum, which would then predict different effects of experimental GC increases as opposed to natural, endogenous GC increases. In other words, an HPA axis that is fighting to restore a phenotype to its baseline state after an experimental GC manipulation might be very different from an HPA axis that induced a particular endocrine phenotype naturally. This introduces the idea of 'individual optimization', in which different individuals have different sensitivities to specific circulating hormone levels, the result of which is that, depending on the individual, different amounts of hormones are needed to sustain a common physiological function (Williams *et al.* 2008). As such, individuals maintain individually 'optimized' endocrine profiles in relation to their own individual constraints and investment history, and balance the relative costs and benefits accordingly. This has the potential to greatly complicate GC manipulations.

Interpreting the effects of manipulations in the absence of pilot studies or previous research is important to the increasing number of studies using GC manipulations to test mechanisms of life-history variation, especially those using serial physiological sampling techniques and electronic tracking technologies in freely ranging animals (e.g. biotelemetry and biologging; Cooke *et al.* 2004; Ropert-Coudert & Wilson 2005; Rutz & Hays 2009; Crossin *et al.* 2014). Table 1 lists several recent studies that have used GC implants to explore mechanisms underlying reproductive investment strategies, as well as the overarching hypotheses that predict the effects of increased GC levels. The first thirteen studies are framed within the current reproduction vs. survival paradigm, and for eleven of these the observed effect of GC manipulation on reproductive performance matched predictions. However, for two studies, the results did not match prediction. Criscuolo *et al.* (2005) is unique in having conducted a pilot study in which the study species, the common eider, was held captive so that the temporal change in plasma GC induced by the GC implant could be measured. This provided evidence that GC levels in the target species reached stress-induced levels, thus providing a good rationale for predicting negative effects of GC treatment on reproduction in the subsequent field study with the same species. However, the observed results did suggest a negative effect of GC increase on reproductive effort, as was predicted. The authors therefore discussed other potential factors that could have offset the negative effects of the implants, in this case re-feeding by parents. In the next study (Patterson, Winkler & Breuner 2011), results of GC manipulation in breeding tree swallows might not have matched predictions in part because the implants increased GC within what was likely a baseline range rather than stress-induced levels. Here, an alternate interpretation wherein effects of baseline GC on reproduction could be predicted to be positive (e.g. the GC-fitness hypothesis; Bonier *et al.* 2009a). Alternately, a different hypothesis could have been used to frame the study and its predictions (e.g. GC adaptation). We do not mean to suggest that a hypothesis should be chosen to match results, only that alternate hypotheses should be considered in the discussion (or preferably before) about why a given hypothesis did not match predictions. Given the varied and seemingly contrasting results of GC manipulations in the published literature, we urge researchers to identify, *a priori*, alternative hypotheses as they design future studies, and devise experiments that can then distinguish among those, rather than just testing one or the other. This would bring greater clarity to the canon of GC manipulation studies.

Unfortunately, most studies of wild, freely ranging animals do not include pilot studies on captive individuals of the species under study (as in Criscuolo *et al.* 2005; and Ouyang *et al.* 2013b). Such studies can be very useful for targeting implant doses, and for choosing appropriate hypotheses and predictions. We realize, however, that this is not always possible due to logistical or other constraints. Barring such pilot studies, or prior knowledge of what specific implant are likely to achieve in terms of circulating plasma GC levels, it is important to choose inclusive, more broadly scoped, hypotheses or multiple alternate hypotheses, so that results can be interpreted more freely without being forced into a single paradigm. From a technical perspective, we also urge that, when possible, controlled pilot studies are conducted so that GC implant doses can be calibrated to desired plasma level increases, and so that the shape of the dose–response curve (e.g. the time course of circulating hormone levels in response to exogenous

Table 1. Studies where direct metrics of current reproductive effort were measured in response to exogenous glucocorticoid (GC) treatment. Studies where exogenous GC and survival relationships were measured outside the period of current reproduction were excluded

Animal model	Study framework	Exogenous GC effect on plasma levels	Predicted effect on reproduction	Observed effect on reproduction	Results match predictions?	References
Pied flycatchers, *Ficedula hypoleuca*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	Silverin (1986)
Common geckos, *Hoplodactylus maculatus*	Current reproduction vs. survival*	Stress-induced	Negative	Negative	Yes	Cree *et al.* (2003)
Red-sided garter snake, *Thamnophis sirtalis parietalis*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	Moore & Mason (2001)
Common lizards, *Lacerta vivipara*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	Cote *et al.* (2006)
Atlantic salmon, *Salmo salar*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	Eriksen *et al.* (2006)
Pink salmon, *Oncorhynchus gorbuscha*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	McConnachie *et al.* (2012)
Black-legged kittiwakes, *Rissa tridactyla*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	Angelier *et al.* (2009)
Black-legged kittiwakes, *R. tridactyla*	Current reproduction vs. survival	Stress-induced	Negative (parents)	Negative	Yes	Kitaysky, Wingfield & Piatt (2001)
Adélie penguins, *Pygoscelis adéliae*	Current reproduction vs. survival*	Stress-induced†	Negative	Negative	Yes	Spée *et al.* (2011)
Adélie penguins, *Pygoscelis adéliae*	Current reproduction vs. survival	Stress-induced	Negative	Negative	Yes	Thierry, Ropert-Coudert & Raclot (2013)
Smallmouth bass, *Micropterus dolomieu*	Current reproduction vs. survival*	Stress-induced	Negative	Negative	Yes	Dey *et al.* (2010)
Common eiders, *Somateria mollissima*	Current reproduction vs. survival	Stress-induced	Negative	None	No	Criscuolo *et al.* (2005)
Tree swallows, *Tachycineta bicolor*	Current reproduction vs. survival	Increased baseline	Positive	None	No	Patterson, Winkler & Breuner (2011)
Adélie penguins, *Pygoscelis adéliae*	Current reproduction vs. survival*, GC-adaptation‡	Uncertain	Negative/ positive	Negative/ positive	Yes	Thierry *et al.* (2013)
Common lizards, *Lacerta vivipara*	Current reproduction vs. survival*, GC-adaptation‡	Moderate, stress-induced	Moderate = pos stress-Induced = neg	Moderate = pos stress-Induced = neg	Yes	Gonzalez-Jimena & Fitze (2012)
Macaroni penguins, *Eudyptes chrysolophus*	GC-adaptation	Increased baseline	Positive	Positive	Yes	Crossin *et al.* (2012)
Great tits, *Parus major*	GC-adaptation‡	Increased baseline	Positive	Positive	Yes	Ouyang *et al.* (2013a)

*Although not explicitly stated, the predictions in this study match those of a current reproduction vs. survival hypothesis.
†Individuals were given either a 'high'-dose GC implant or a 'low'-dose implant. The high dose led to nest abandonment, whereas the low dose did not.
‡Although not explicitly stated, the predictions of this study match those of the GC-adaptation hypothesis as outlined by Bonier *et al.* (2009a,b).

implantation) can be determined *a priori*. Pilot studies also allow researchers to screen for any unintended effects of GC manipulation on the expression of other hormones or hormonal pathways (e.g. HPG axis). And as we discussed earlier GC dose considerations must also take into account the specific life-history context and reproductive stage of study animals, as these can have significant bearing on the response of individuals to GC manipulation. Of course, as

suggested previously, complimentary studies of MR and GR receptor activity may be very useful for interpreting the varied responses of GC manipulation, especially when the delivered dose is either unknown or uncalibrated. Some recent studies have in fact identified phenotypic differences in receptor expression that run counter to expected differences in blood hormone titres; Peterson *et al*. (2013) identified differences in receptor expressions and activity in male and female birds in response to the same hormonal manipulation. Finally, GC manipulations (or any hormonal manipulations) should be conducted in tandem with studies of natural endocrine variation (e.g. circadian, circannual, etc.), or they should at least reference relevant literature if already previously examined. This would allow for a more thorough understanding of endocrine mechanisms underlying adaptation.

By considering all of these factors, researchers will be better positioned to design experimental studies that reveal the mechanisms underlying variation in fitness-related traits. Understanding the effects of GC variation is all the more salient given Boonstra's (2013b) recent suggestion that, despite the potential for pathological effects, chronic stress is assuredly an adaptive phenomena that ultimately promotes the fitness of animals in nature, although this may depend to some extent on the life-history context of the organisms under study (e.g. semelparous vs. iteroparous, short lived vs. long lived, etc.).

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Data accessibility

This manuscript does not use data.

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