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## Review article

# Expanding the actions of cortisol and corticosterone in wild vertebrates: A necessary step to overcome the emerging challenges



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## ABSTRACT

We conducted a review of scientific articles published between 2000 and 2014 and evaluated how frequently various aspects of cortisol and corticosterone (CORT) actions have been considered in studies on wild vertebrates. Results show that (1) the notion that CORT are stress-responsive hormones is central in our theoretical frameworks and it is reflected by the fact that several articles refer to CORT as “stress hormones”. (2) The large majority of studies do not contemplate the possibility of decrease and no change in CORT levels in response to chronic stressors. (3) Our ideas about CORT actions on energy balance are slanted towards the mobilization of energy, though there are several studies considering -and empirically addressing- CORT’s orexigenic actions, particularly in birds. (4) The roles of CORT in mineral-water balance, though widely documented in the biomedical area, are virtually ignored in the literature about wild vertebrates, with the exception of studies in fish. (5) Adrenocorticotrophic hormone (ACTH) independent regulation of CORT secretion is also very scarcely considered. (6) The preparative, permissive, suppressive and stimulatory actions of CORT, as described by Sapolsky et al. (2000), are not currently considered by the large majority of authors. We include an extension of the Preparative Hypothesis, proposing that the priming effects of baseline and stress-induced CORT levels increase the threshold of severity necessary for subsequent stimuli to become stressors. Studies on animal ecology and conservation require integration with novel aspects of CORT actions and perspectives developed in other research areas.

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## 1. General introduction

The field of cortisol and corticosterone (CORT) actions in free-living vertebrates has been changing and expanding constantly during the last two decades. One of the most obvious reasons underlying the development of this research area is that the hypothalamic-pituitary-adrenal axis (HPA, for mammals and birds) or interrenal axis (HPI, for reptiles, amphibians and fish) is activated in response to adverse stimuli (stressors), resulting in increased secretion of CORT from the adrenal glands. Thus, the secretion of CORT is part of the neuroendocrine response to these noxious stimuli, called the “stress response” (Sapolsky et al., 2000; Boonstra, 2005). Indeed, and due to the well-documented roles of CORT in the response to stressors, these hormones have frequently been referred to in literature as “stress hormones”.

Acute elevations of CORT levels (as a part of the “fight or flight” response) help redirect resources from temporally non-essential activities (e.g., reproduction) to immediate survival, and thus are viewed as an adaptive response (Sapolsky et al., 2000; Breuner et al., 2008). On the other hand, persistent elevations of CORT levels, associated with chronic stressors, are usually considered to produce a variety of deleterious effects upon health (Sapolsky et al., 2000; for an alternative view of chronic stress in nature see Boonstra, 2013). Recently, the “Reactive Scope Model” (Romero et al., 2009) has been proposed as an alternative to the traditional model of chronic stress and serves as an extension of the allostasis model proposed by McEwen and Wingfield (2003).

The implications of the preceding paragraphs are apparent: CORT measurements might be used as a tool to evaluate physiological stress and/or assess the effects of different types of disturbances in populations inhabiting natural environments (Mormède et al., 2007). This has produced an increasing interest in studying the HPA or HPI axis from an ecophysiological point of view in the fields of conservation biology and animal welfare (Romero and Wikelski, 2001; Mormède et al., 2007; Dantzer et al., 2014). For basic research purposes, CORT dynamics are stud-

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ied to contribute to the understanding of the interactions between ecology and physiology (Romero, 2002). As an example, CORT levels have been measured to study the interactions with other hormones in natural environments (e.g., Soto-Gamboa et al., 2005; Schradin, 2008) and CORT actions have been studied during life history processes like reproduction (e.g., Kenagy and Place, 2000; Nunes et al., 2006; Vera et al., 2013), migration (e.g., Landys et al., 2004; Eikenaar et al., 2014), hibernation (Armitage, 1991) and parental care (Rensel et al., 2010), among many other aspects.

In the last decade, several reviews were published addressing novel roles of CORT and enriching the traditional approach. Among other aspects, these studies highlighted that: (1) the roles of CORT might be better understood considering their permissive, preparative, stimulatory and suppressive actions on the stress response (Sapolsky et al., 2000). (2) Free-living vertebrates usually exhibit seasonal variations in baseline CORT levels in association with changes in activity patterns and/or environmental conditions (Romero, 2002). The term “baseline” refers originally to CORT measured in blood samples obtained within a few minutes of capture, thus avoiding the researcher’s influence on hormone profiles (Romero, 2002). By using the word “baseline”, we will refer to variations in CORT levels that occur in the absence of stressors. Noteworthy, “baseline levels” are not the same as “basal levels”, which are the concentrations needed for the basic homeostatic functions in the absence of significant predictable and non-predictable challenges (Romero, 2002; Landys et al., 2006). (3) Baseline CORT levels regulate predictable life-history processes, indicating that increases in CORT do not necessarily indicate the presence of a stress response, but might reflect allostatic adjustments to challenges within the reaction norm of the individuals at a given life-history stage (Landys et al., 2006). (4) The relationship between baseline CORT levels and fitness is highly-variable, including positive, negative and no significant relationships (Bonier et al., 2009). (5) CORT responses to chronic stressors are so variable that the literature provides essentially no predictive value on whether a study should expect to see an increase, a decrease, or no change in stress-induced CORT in response to this type of stressors (Dickens and Romero, 2013). The conclusion of this last review is very significant, as it shows that CORT alone cannot be used to determine whether an animal is chronically-stressed or not, at least, without a comprehensive understanding of how the axis functions in each study model. In addition, Boonstra (2013) has proposed that chronic stress is an adaptive trait in nature, therefore not producing a pathological condition, as it is generally considered. Moreover, during the same year, two papers were published addressing a debate on whether total or free CORT (unbound to corticosteroid-binding globulin, CBG) are the most informative measures (Breuner et al., 2013; Schoech et al., 2013). After reading these and other reviews, it becomes apparent that the field of CORT ecophysiology is an area of dynamic discussion; it is also clear that some of the most fundamental assumptions are being questioned. In other words, after becoming aware of the magnitude of the ongoing debates, it becomes clear that this research field is currently in a moment characterized by a rapid demand for change that should be understood as an opportunity for developing a renewed, expanded, and more reliable theoretical framework.

Paradoxically, it was also apparent to us that a large amount of studies are still using the most traditional approach for studying CORT or, at least, missing some of the important issues raised in the previously-mentioned reviews. For example, though fifteen years have passed since the publication of the review by Sapolsky et al. (2000), very few studies have considered the permissive, preparative, stimulatory and suppressive actions of CORT (see Section 7). As an additional example, several years have

passed since the publications by Romero (2002) and Landys et al. (2006) showing the importance of considering the variations in baseline CORT levels. In spite of this, studies using CORT as a tool to diagnose the presence of stress that do not consider this important aspect are still common in the literature, even in high-ranking journals.

The goals of the present review are: (a) to describe some general trends in this research area; (b) to evaluate to what extend some of the most common ideas of the theoretical backgrounds are empirically supported by data; (c) to identify and briefly describe some aspects of CORT actions that are not currently considered by the majority of researchers; (d) to integrate this information and make some suggestions. We undertake the review recognizing that this research field is currently needing the exploration of other, less-considered actions of CORT, which may, in the future, allow an enriching integration of new perspectives with the classic paradigm.

## 2. Sampling of articles and general analysis

### 2.1. Criteria of selection

In order to perform the present analysis, we decided to conduct our review round two high-ranking journals which frequently publish papers on CORT variations in wild vertebrates: *Hormones and Behavior* (HB) and *General and Comparative Endocrinology* (GCE). As the present analysis implies the evaluation of several issues in each article, we considered the review of hundreds of papers as impractical. Thus, we decided to review forty articles from each journal (a total amount of eighty papers). We restricted our analysis to the period comprised between 2000 and 2014, in order to perform an evaluation of the research conducted during the last years. We only considered studies about wild vertebrate species, either performed in their natural environments and/or in captivity, excluding studies conducted in laboratory with animals that do not have wild counterparts. As we were interested in original research articles, we decided to exclude reviews. Finally, we did not consider purely methodological studies. The number of studies that met the criteria was 135 out of 203 for GCE and 102 out of 174 for HB (after filtering the search for the period 2000–2014). Noteworthy, the above-mentioned criteria of selection only applies to the “reviewed papers” (i.e., studies detailed in [Supplementary Material](#)). We also included citations of several other articles and reviews published in these and other journals, which included in their research laboratory and domestic animals as well, and without a restriction of publication year, as an additional important source of information for our present discussions.

### 2.2. Sampling procedure

We used Science Direct websites for HB and GCE and performed an advanced search using the words “cortisol”, “corticosterone” and “glucocorticoid”. Subsequently, we assigned a number to every article that satisfied the previously-mentioned conditions and performed a random sampling of 40 articles per journal to determine the list of articles to be reviewed. The list of the reviewed articles is shown in the [Supplementary Material](#).

### 2.3. General analysis of articles

For each reviewed article we recorded the following issues: (1) General features of the study, such as publication year, vertebrate class studied, study goals, and hormone measured, among others (Section 3). (2) Whether CORT roles in the response to stressors are considered in the introduction and discussion of the data,

and if there are original data on CORT responses to stressors and other environmental disturbances (Section 4). (3) Whether authors acknowledge the increase in CORT as the only possible outcome in response to chronic stressors, or they also recognize other possible responses (decrease and no change, Section 5). (4) Whether authors recognize CORT roles in both the mobilization of energy reserves and acquisition of energy through the stimulation of food consumption (orexigenic roles), and if they report original data on these two issues (Section 6). (5) Whether authors cite the review by Sapolsky et al. (2000) and if they consider the permissive, preparative, stimulatory and suppressive actions of CORT on the stress response, as proposed by these authors (Section 7). (6) Whether authors consider the mineralotropic actions of CORT (balance of Na<sup>+</sup>, K<sup>+</sup> and water) for introducing or discussing their data and if they report original data on this subject (Section 9). (7) Whether authors acknowledge and/or report data on alternative endocrine pathways for CORT regulation, defined as those different to the classical regulation by adrenocorticotrophic hormone (ACTH, Section 10). These issues allowed us to achieve a descriptive panorama of the theoretical frameworks and identify the roles of CORT that are empirically evaluated or not. (8) We also developed an extension of the Preparative Hypothesis (Romero, 2002). We propose that the permissive and preparative actions of CORT might serve to decrease the likelihood of experiencing stressors in the future (Section 8).

Each section includes a first subsection explaining the rationale for the inclusion of this item in the present review, a second subsection explaining with more detail the analysis we performed, and a third part including the results and discussion.

### 3. General features of the reviewed articles

#### 3.1. Rationale for the inclusion of this item in the analysis

The goal of this section was to describe some general features of the reviewed publications in order to have an initial descriptive panorama.

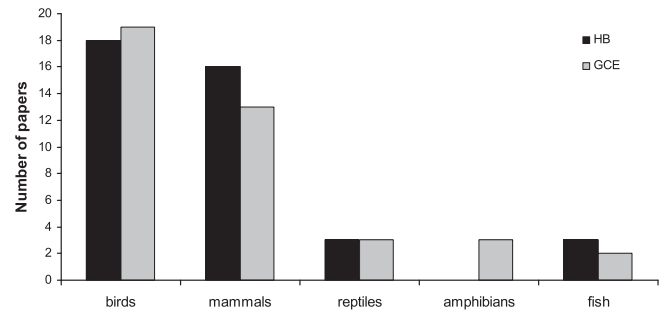
#### 3.2. Analysis

For each article we recorded: (1) year of publication, (2) vertebrate class studied (mammal, bird, reptile, amphibian, or fish), (3) hormone measured (cortisol and/or corticosterone and/or their metabolites), (4) matrix of measurement (e.g., plasma, feces, urine, hair, whole body, etc.) and (5) type of temporal CORT variation measured (e.g., acute response, chronic response, seasonal, inter-annual, during ontogeny, and others). Note that a given article usually provides data on more than one category, for instance, the measurement of cortisol and corticosterone in plasma and responses to acute and chronic stressors.

#### 3.3. Results and discussion

We reviewed 80 papers published between 2000 and 2014. When we divided this period in 5 intervals of three years, we found that 2000–2002 was the section with the smallest amount of reviewed studies (only 2 articles), periods 2006–2008 and 2012–2014 were the ones with the largest amount of reviewed articles (23 and 24, respectively) and periods 2003–2005 and 2009–2011 have intermediate numbers of reviewed studies (14 and 17, respectively).

Birds and mammals were the most represented vertebrates in the reviewed articles (Fig. 1). Indeed, pooling both journals, studies about birds and mammals represent more than 82% of the total (66 out of 80 articles). Therefore, the present review allows for a much



**Fig. 1.** Number of reviewed articles per vertebrate class. There are various studies that include more than one species of a given class. HB: articles published in *Hormones and Behavior*, GCE: articles published in *General and Comparative Endocrinology*.

more detailed analysis of CORT variations in these vertebrate classes. However, pooling the articles on reptiles, amphibians and fish (14 studies) our data suggest that trends are similar to the ones observed in birds and mammals, except for the consideration of the roles of CORT in mineral-water balance in studies about fish, and the low citation of the review by Sapolsky et al. (2000) (F. Vera, personal observation, see [Supplementary material](#)).

Most studies ( $n = 44$ ) report data exclusively on corticosterone, while a considerably smaller amount ( $n = 14$ ) report data solely on cortisol variations. Only 8 studies report concomitant data on cortisol and corticosterone levels. Finally, 7 studies present data on corticosterone metabolites, while 9 on cortisol metabolites (feces, [Supplementary Material](#)). The prevalence of corticosterone measurements is due to the fact that birds, which are usually considered to produce corticosterone as the main glucocorticoid, are highly-represented in the analysis (37 studies). Among mammals, rodents are usually (but not always) considered to produce corticosterone as the major glucocorticoid. In addition, studies about reptiles and amphibians regularly focus on corticosterone levels. Therefore, most cortisol data come from studies in mammals (a minor amount from studies in fish).

The most common matrix for CORT measurement was “plasma/serum” (data on 60 out of 80 studies), followed by “feces” (data on 18 out of 80 studies). Other matrices used for CORT measurements were urine, hair, whole body, head, kidney, and CORT water content (see [Supplementary Material](#)). Most data in birds are represented by corticosterone measured in plasma. For the case of mammals, the available CORT data are represented by measurements of both cortisol and corticosterone, mainly in plasma and feces ([Supplementary Material](#)). Recently, [Schmidt and colleagues \(2008, 2010\)](#) have shown that the identity of the dominant glucocorticoid and the receptor binding affinities for cortisol and corticosterone differ depending on the tissue considered in birds (blood, brain, bursa of Fabricius, thymus, spleen). These are, in our opinion, highly-significant results, considering that (a) evaluations of cortisol and corticosterone concentrations in matrices other than blood and feces are relatively rare in the literature and (2) this notion is virtually absent in the theoretical backgrounds of the research papers (F. Vera, personal observation). Results obtained by [Schmidt and colleagues \(2008, 2010\)](#) show that it shouldn't be assumed that the systemic concentrations of cortisol and corticosterone in a given species mirror their concentrations in other tissues, and that the predominant glucocorticoid in plasma is the only one with a significant biological role. In spite of the above-mentioned arguments, we acknowledge that it is not practical, and often undesirable, to obtain tissue samples from wild animals. Accordingly, we suggest field endocrinologists to have in mind the results by [Schmidt and Soma \(2008\)](#) and [Schmidt et al. \(2010\)](#), but to undertake the collection of this type of samples only

if deemed as indispensable, and protocols are adjusted to minimize the impact at both individual and population levels. We also suggest not assuming that cortisol and corticosterone are necessarily redundant hormones that accomplish the same physiological roles (Vera et al., 2011). The reasons explaining the presence of both cortisol and corticosterone in a given species are still unclear. It might be possible that their physiological roles do not completely overlap and, therefore, choosing to measure cortisol or corticosterone in a given matrix would create biases in our conclusions.

With regard to the temporal variation in CORT levels, we found many studies reporting data on acute responses to stressors ( $n = 48$ ), while there were also several studies showing data on seasonal ( $n = 28$ ) and inter-annual variations ( $n = 26$ ). Indeed, many studies included concomitant data on CORT levels and reproductive activity. Chronic/long term responses were reported in 18 studies. We distinguish between “chronic” and “long term” to include not only data on CORT responses to stimuli recognized as chronic stressors, but also to take into account responses to other types of prolonged stimuli (e.g., time elapsed since the transition from one social strategy to another in the striped mouse: Schoepf and Schradin, 2013). In addition, we found studies showing data on CORT variations during development/ontogeny ( $n = 10$ ), and daily-circadian variation ( $n = 3$ ). There were also a few studies with single CORT measurements (no variation in time,  $n = 5$ ). Studies from both journals were pooled because patterns are remarkably similar in this regard. Results sum much more than 80 studies because there were many articles addressing more than one type of temporal variation (e.g., data on seasonal, acute and chronic responses in the same article, Supplementary Material).

#### 4. Roles of CORT in the response to stressors

##### 4.1. Rationale for the inclusion of this item in the analysis

Clearly, it is widely acknowledged that CORT are involved in the response to stressors. In fact, we recognized as *a priori* obvious that the large majority of studies acknowledge that CORT participate in the response to stressors as mediated by the activation of the HPA or HPI axis, and that there exist plenty of data on CORT responses to stressors and other environmental disturbances. The goal of this section was to address this issue as a reference point to compare how other roles of CORT—which are later discussed—are recognized and empirically evaluated in the literature. Concordantly, we hypothesized that most authors explicitly recognize that CORT participate in the response to stressors.

##### 4.2. Analysis

For each article we recorded whether the notion that CORT are stress-responsive was explicitly used for the introduction and/or discussion of the data. An illustrative example of this is the assertion by Spencoski et al. (2012) that “the resulting defense reaction (to stressors) involves the stimulation of the hypothalamic–pituitary–adrenocortical (HPA) axis that, in turn, elicits glucocorticoid secretion from the adrenal cortex.” In addition, we recorded whether the article reports original data on CORT responses to stressors. To accomplish this goal, we divided the data in two major types of stressors considering: (a) stressors applied *ad hoc* by the researchers (e.g., capture and restraint) and (b) stressors and other disturbances occurring in the natural environment, but not applied by the researchers (e.g., agonistic social interactions and habitat degradation). We use the word “disturbance” because in some cases, variations in CORT levels were addressed in association with stimuli that, though considered as challenging, were not always assumed or verified to be stressful (e.g., bad weather, Huber

et al., 2003). For the case of stressors applied by the researchers, we also included a category for ACTH and CORT administrations because these procedures were relative frequent in the reviewed articles. We acknowledge that it is not the same to apply CORT or ACTH than to evaluate the response to non-pharmacological stressors. In part, this is because non-pharmacological stressors are identified and evaluated by the central nervous system, which then directs a physiological and behavioral response to them. Several studies included concomitant data on more than one type of stressor (Supplementary Material).

Importantly, seasonality *per se*, and particularly gestation and lactation, were not considered as “disturbances” or “stressors”, unless explicitly stated by the authors. High CORT levels associated with predictable life-history activity patterns do not necessarily indicate that the animals are stressed, only probably undergoing allostatic adjustments to changing energetic requirements (Sapolsky et al., 2000; Landys et al., 2006; Romero et al., 2009).

Finally, we decided to record whether the authors use the expression “stress hormone/s” to refer to cortisol and/or corticosterone. This fact was used as an additional indicator of how we tend to think about the physiological roles of CORT. The argument is that when we refer to a hormone in terms of one of its roles, we are implicitly considering this particular role as more relevant than other functions. Therefore, we considered that a common use of the expression “stress hormones”, together with the additional data presented here, would indicate that the roles of CORT in the response to stressors are viewed as more important than other functions.

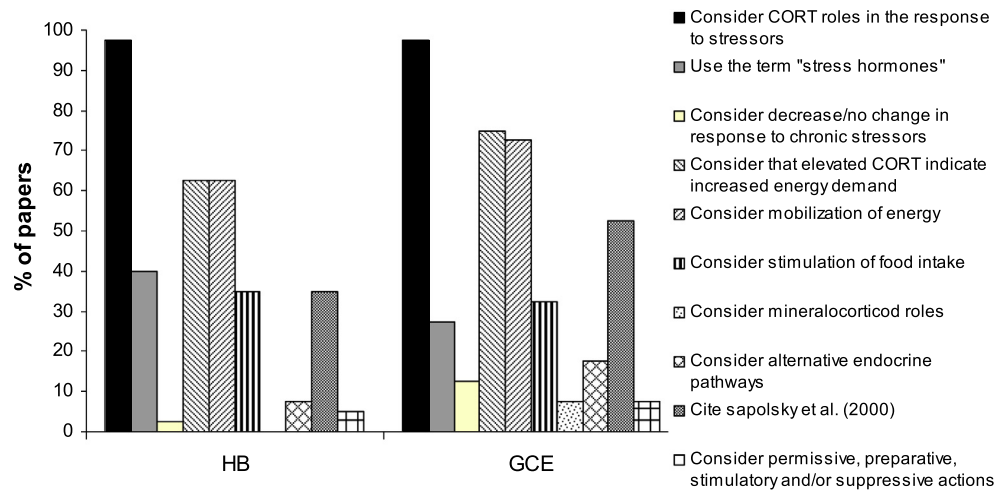
This analysis, including both the theoretical frameworks and the collected data, allowed us to evaluate how often we think about CORT roles in the response to stressors for presenting and interpreting data, and how frequently we report data on CORT responses to stressors and environmental challenges in general. Together, these two aspects allowed us to picture the dominance of roles in the response to stressors in relation to other actions exerted by these hormones.

##### 4.3. Results and discussion

As expected, the roles of CORT in the response to stressors are acknowledged in nearly all reviewed papers (78 out of 80 papers, Fig. 2). We found only two articles (one from each journal: Angelier et al., 2008; Eikenaar et al., 2014) in which the roles of CORT in the response to stressors are not explicitly considered. These results corroborate that the roles of CORT in the response to stressors are central in the theoretical backgrounds of the large majority of field endocrinologists studying CORT variations.

Similarly, we found abundant original data on CORT responses to stressors and other environmental disturbances. For the case of natural stressors/disturbances (i.e. not applied by the researchers), the most studied were social interactions, though all these studies were published in HB (Fig. 3A). Other studied disturbances were the effect of food/prey availability (particularly, decrease in food availability), human-induced habitat alteration and climatic variables which, together with social interactions, account for the 66% of the studied causes of environmental disturbances (Fig 3A). Among stressors applied *ad hoc* by the researchers, capture/handling/restraint and/or bleeding protocols predominated over others (especially in GCE), though there was a remarkable variability in the type of stressors that were applied (Fig. 3B). Other stressors included the maintenance of the study of individuals in captivity, social isolation and restricted access to food, among others (Fig. 3B). Protocols involving CORT or ACTH administration were relatively frequent (Fig. 3B).

In our view, the prevalence of CORT roles in the response to stressors is clearly evident in several articles in which the authors



**Fig. 2.** Consideration of different aspects of CORT actions in the theoretical frameworks of the reviewed articles. HB: Hormones and Behavior. GCE: General and Comparative Endocrinology. The permissive, preparative, stimulatory and suppressive actions represent the core idea proposed in Sapolsky et al.'s (2000) review. Therefore, the percentage of papers considering these actions should be interpreted as an indicator of the actual impact of the ideas proposed by these authors.

interpret their data based entirely (or almost entirely) on this aspect of CORT actions, overshadowing other physiological roles and variations patterns that could be relevant for the interpretation of the data. More specifically, this pattern consists in interpreting CORT data with a very marked focus on the following ideas: (1) CORT are stress-responsive; (2) the *increase* in CORT levels is the direction of change in response to both acute and chronic stressors; and therefore (3) higher CORT levels indicate physiological stress. It is still quite common to find studies with such a strong focus in these issues, that they miss other important actions of CORT mentioned in the *General Introduction*.

During the course of the present review it became apparent that sometimes the collected data are interpreted to fit or not to fit the study hypothesis in non-parsimonious ways, simply because the authors do not doubt about the fundamental premises that define their theoretical framework (e.g., that the more stressed animals are the ones with the highest CORT levels). Sometimes, data on the animals' ecology and CORT levels could be interpreted in more parsimonious ways if we allow ourselves to doubt about these common statements and formulate questions such as: "could individuals with the lowest CORT levels be actually the stressed ones? Or, "could higher CORT levels of a given category of individuals indicate allostatic adjustments within the reaction norm of this life-history stage, instead of indicating a stress response? This is critical, particularly for studies measuring CORT to assess the effect of stressors and other disturbances. It is not a goal of this review to make a list of studies that use too limited a framework about CORT actions, only to highlight some aspects that should be considered more often.

We found 27 articles that used the nomenclature "stress hormones" for CORT (sixteen in HB and eleven in GCE, Fig. 2). We acknowledge that the use of this nomenclature does not necessarily imply that the authors ignore other roles of CORT, different from the response to stressors. However and in general terms, we see the use of this expression as an additional indicator of a tendency to think about the roles of these hormones. For instance, considering that cortisol and corticosterone have also orexigenic and mineralocorticoid actions in vertebrates (Agarwal and Mirshahi, 1999; de Kloet et al., 2000; Dallman et al., 2004; Crespi and Denver, 2005; la Fleur, 2006; Dallman et al., 2007; Uchoa et al., 2014, discussed below), why is it that we do not commonly refer to them as "orexigenic hormones" or "mineralocorticoid hormones" instead of

"stress hormones"? The response is obvious: because we mainly think about them as "stress hormones". Noteworthy, to think about CORT mainly as "stress hormones" is a subjective approach, not based on data demonstrating that the roles in the response to stressors are more biologically important and/or common than their actions at baseline levels (see Sapolsky et al., 2000; Landys et al., 2006), the previously mentioned roles, or other roles not considered in the present review. To formulate it differently: what would happen to the studied animals if, for instance, CORT fail to exert their regulatory actions at baseline levels? The above-mentioned pattern exists, in part, because of historical reasons: CORT were initially described as participating in the responses to noxious stimuli (Selye, 1946). Overall, we agree with the importance of considering CORT roles in the response to stressors. We think that a problem with part of the current research is that other important physiological roles are proportionally much less considered.

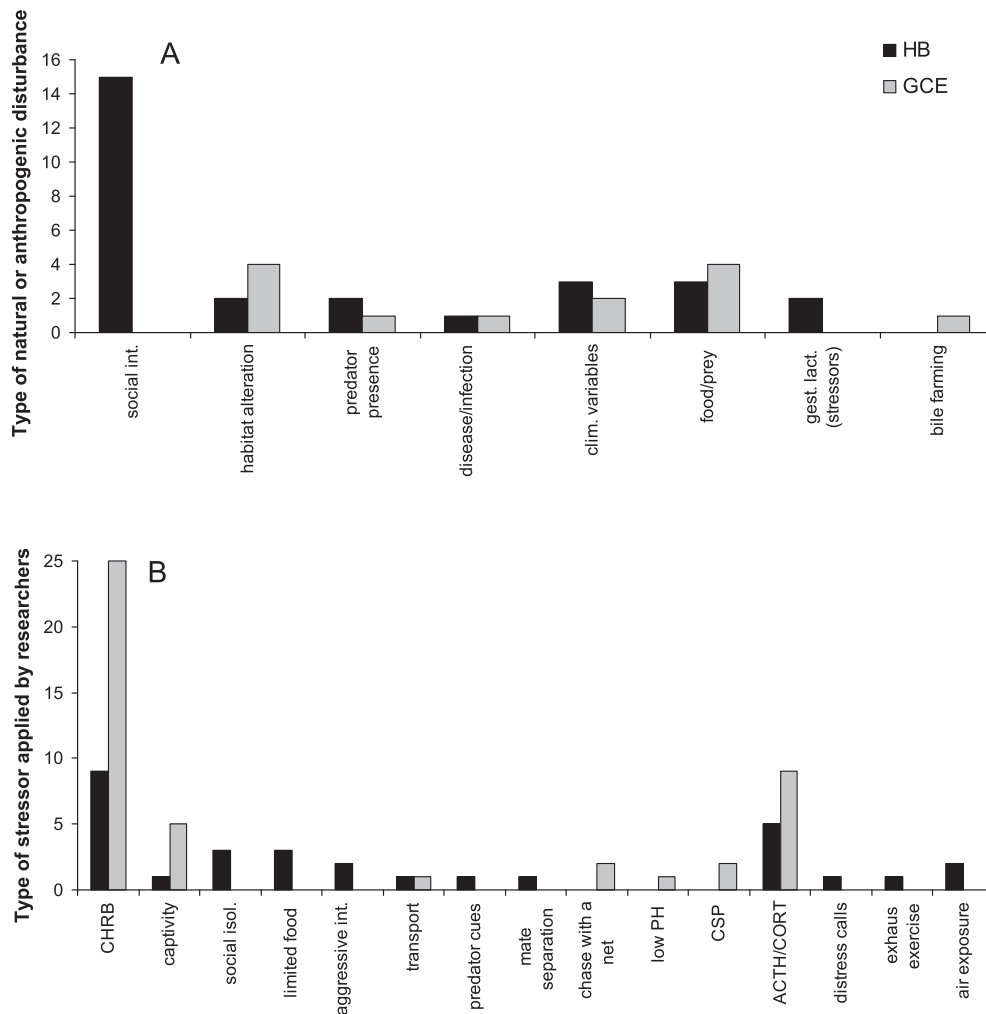
Noteworthy, the large majority of studies included the measurement of other/s indicator/s of body condition, status and/or health, such as body condition indexes, body mass, plasma steroids, reproductive status, plasma metabolites and behavior, among others (Supplementary Material). In general, these data were not used to test or infer whether the common assumptions about CORT dynamics fit. In other words, these assumptions appear to be quite rigid in several papers and are not explicitly tested, even though there are data that may allow for some testing.

## 5. Other relationships between chronic stressors and CORT levels

### 5.1. Rationale for the inclusion of this item in the analysis

As previously mentioned, the roles of CORT in the response to stressors are explicitly considered in the vast majority of papers. For many readers, it will be immediately apparent that stressors increase the activity of the HPA or HPI axis, therefore increasing CORT concentrations.

At the moment we started outlining the goals of this review, we were aware of papers stating that there exists variation in the direction of change in CORT levels in response to chronic stressors (Rich and Romero, 2005; Cyr et al., 2007; Cyr and Romero, 2007;



**Fig. 3.** Different types of stressors and disturbances assessed in relation to changes in CORT levels. Panel A shows different natural or anthropogenic disturbances assessed, but not applied by the researchers, while panel B shows the different kinds of stressors applied *ad hoc* by the researchers. HB: Hormones and Behavior, GCE: General and Comparative Endocrinology. Abbreviations and clarifications: social int = social interactions with conspecifics, frequently (but not exclusively) agonistic; habitat alteration = human-induced changes in the habitat in general; predator presence = exposure to predators in the natural environment; disease/infection = infections with gastro-intestinal parasite or *Mycobacterium bovis*; clim. variables = dry season, adverse weather or rainfall; food/prey = low or varying degrees of food resources in the environment; gest. lact. (stressors) = gestation and/or lactation viewed as stressors; bile farming = practice in which bears are kept in captivity, in extreme conditions, for the extraction of their bile. CHRB = capture-handling-restraint and/or bleeding protocols; captivity: maintenance of the study subjects in conditions of captivity; social isol = social isolation from group of conspecifics; limited food: acute or chronic exposure to limited food resources; aggressive int = aggressive interactions with conspecifics; transport = transport of the study subjects to the laboratory; predator cues = exposure of study subjects to predator cues in the water (fish); mate separation = separation from a pair bonded mate (bird); chase with a net = acute stressor in fish; low pH = 3.5 environmental PH at the laboratory (amphibian); CSP = chronic stress protocol involving various stressors; ACTH/CORT = protocols involving the administration of adrenocorticotropic hormone (ACTH) and/or natural or synthetic glucocorticoids; distress calls = exposure of nestling to nestlings distress calls; exhaust exercise = exhaustive exercise as acute stressor in fish; air exposure = exposure of study subjects to air by taking them out of the water (fish).

Linklater et al., 2010; and specially: Dickens and Romero, 2013). Together, these studies show that there are indeed three possible scenarios: increase, decrease and no change in CORT levels. The review by Dickens and Romero (2013) is critical for it indicates that there is no empirical support to assume that chronic stressors will necessarily increase CORT levels. There are identified mechanisms that can account for attenuated CORT levels in the presence of chronic stressors: habituation, exhaustion and down-regulation (discussed in Rich and Romero, 2005).

After recognizing the previously-mentioned evidence showing substantial variability in CORT responses to chronic stressors and also acknowledging our almost automatic assumptions about stress and CORT (i.e., that stressors increase CORT levels), we aimed to quantify the proportion of studies that: (1) only acknowledge the possibility that CORT levels increase in response to chronic stressors and (2) contemplate other possible outcomes (i.e., decrease and/or no response). It was not a goal of this section to

address how often chronic stressors increase, decrease or do not change CORT levels, as this analysis has previously been performed by Dickens and Romero (2013). In this section, we simply address the extent to which theoretical backgrounds are impregnated with these notions. Noteworthy, for studies only focusing on acute responses (e.g., stressors, ACTH or exogenous CORT), among other aspects, it does not seem critical to have this issue into consideration. Actually, CORT increase in response to an acute stressor is considered a hallmark of the acute response. We hypothesized that the large majority of studies do not acknowledge the previously-mentioned variability in CORT responses to chronic stressors.

## 5.2. Analysis

Firstly, for all papers mentioning or testing CORT responses to stressors, we recorded whether they (1) mention and/or test only acute responses to stressors or (2) whether they mention and/or

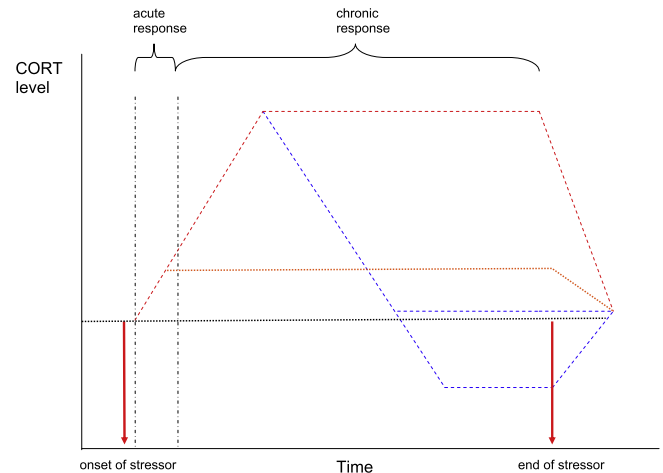
test chronic responses (note that this category frequently included the consideration of acute responses as well). We were then able to identify those studies that consider chronic stressors in their theoretical background or explicitly address their effects on the study population. For all articles that fitted this last category we recorded whether the notions that chronic stressors increase, decrease and/or do not change CORT levels are explicitly recognized. This allowed us to assess how we think about the *direction* of CORT responses to chronic stressors and contrast it with the evidence reported by [Dickens and Romero \(2013\)](#).

### 5.3. Results and discussion

For GCE, we recorded twenty studies that mention and test the effect of chronic stressors and thirteen studies that mention chronic stressors without collection of data ([Supplementary Material](#)). Only five out of these 33 studies acknowledge the variability in CORT responses to chronic stressors ([Supplementary Material](#)). For HB we recorded 25 studies that mention and test chronic responses and eight studies that mention them without presenting novel data. Only one of these studies acknowledges decrease and no response of CORT levels as a possible outcome ([Fischer et al., 2014](#)). In sum, only six out of eighty studies acknowledge decrease and no change as possibilities (five in GCE and one in HB, [Fig. 2](#)). These results confirm that the majority of authors are still unaware of the variability in CORT responses to chronic stressors evidenced in the review by [Dickens and Romero \(2013\)](#) and in other studies. In part, this is related to the historical reasons previously mentioned: CORT were originally described as participating in the response to noxious stimuli ([Selye, 1946](#)) and in this context, it is the *release* of CORT from the adrenal glands what is generally viewed as the response, or part of the response, to the stressor, and it is frequently assumed to persist for chronic stressors. We also think that this apparent paradox exists because the idea that stress levels and/or the health of natural populations can be understood and/or predicted by a single measurement (CORT levels) is a very enthusiastic one. Unfortunately, recent evidence indicates that this might not be feasible, at least without a thorough understanding of each study model.

Considering that CORT are regularly studied focusing in the response to chronic stressors, or stimuli presumed to be stressors, the previously-described bias emerges as a major concern which has previously been discussed ([Dickens and Romero, 2013](#)). It is noteworthy that we have recorded several studies with such focus that only acknowledge the notion that chronic stressors increase CORT levels (e.g., [Thompson et al., 2010](#); [Starling et al., 2010](#); [Van Meter et al., 2009](#); [Goymann et al., 2003](#); [Girard-Buttoz et al., 2014](#), among others).

Overall, the premise that higher CORT indicate the more stressed individuals should be taken as a question to be tested, and not as an assumption ([Fig. 4](#)). For instance, depending on the moment when CORT is measured after the onset of a stressor, we might encounter increased or decreased levels, if compared to unstressed individuals, or the previous baseline levels ([Fig. 4](#)). In addition, the empirical testing of this premise might allow us to evaluate whether the inclusion of additional data is necessary to interpret the observed variations in CORT levels. [Armario \(2006\)](#) suggested that in order to avoid a circular definition of stress, the situation should be considered as stressful only when two or more physiological systems involved in the response to stress are activated. It is also relevant to consider variations in the temporal response of different systems after the onset of a stressor.



**Fig. 4.** The three possible outcomes for cortisol and/or corticosterone (CORT) levels in response to chronic stressors. A few minutes after a stressor is detected (red arrow on the left), the acute response begins and CORT levels increase above baseline levels (red dashed line). If the stressor becomes chronic (e.g., lasts for more than one day), CORT levels continue to increase up to a maximum corresponding to the life history stage, sex, condition, cognitive state, past experiences, etc. of the individual and the stressor characteristics. Once this maximum is attained, three scenarios might occur: (1) CORT levels remain elevated during the whole exposure to the stressor (red dashed line), (2) CORT levels decrease to values similar to the previous baseline (the small difference in the figure is assumed as non-significant) and (3) CORT levels continue decreasing, even below baseline levels (blue dashed lines). Depending on the intensity of the stressor, among other aspects, the maximum CORT level might be attained during the acute response (orange dashed line), and this again can afterwards result in scenarios (1), (2) or (3) (after the intersection with the blue dashed line for (2) and (3)). Of course, it is feasible to accommodate variations for these possibilities; for instance, after the maximum is attained, CORT might decrease sharply, without stabilizing at a plateau, even though the stressor is still present. Once the stressor ends (red arrow on the right), CORT levels attain baseline values again. An additional possibility depicted in the figure is that CORT levels do not respond to the stressor at all (dashed black line), which affects other components of the stress response. A further possibility (not shown in the figure) is that CORT levels begin to increase only during the chronic response phase. Each of these possible outcomes should not be assumed as fixed for a given stressor or population; instead, we should consider them as probably variable among individuals and across time. Though scenarios (2) and (3) might appear as obvious at first sight, previous work ([Dickens and Romero, 2013](#)) and the present results show that they are not currently considered by the majority of field endocrinologists studying CORT variations. The model predicts that: (a) at a given moment, different individuals exposed to a particular chronic stressor will have dissimilar CORT levels, depending on the time passed since the onset of the stressor and (b) a given chronic stressor might produce increased, decreased or unchanged CORT levels depending on the moment when we measure its effects on the individuals. The model matches with the review by [Dickens and Romero \(2013\)](#), which shows that there is no empirical consensus on whether chronic stressors will increase, decrease or not change CORT levels. These ideas might be particularly useful for studies using CORT as a tool to assess the effects of environmental stimuli or physiological stress levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 6. Energy balance: Catabolic and orexigenic actions of CORT

### 6.1. Rationale for the inclusion of this item in the analysis

CORT are widely recognized as important hormones mediating energy balance ([Sapolsky et al., 2000](#); [Romero, 2002](#); [Boonstra, 2005](#); [Landys et al., 2006](#)). Indeed, the view that CORT mobilize energy reserves during energetically-demanding challenges is conspicuous in the literature. However, an integrative exploration of the roles of CORT in energy balance requires, at least, the evaluation of how CORT also influence energy acquisition through the stimulation of appetite and foraging behavior ([Crespi and Denver, 2005](#); [la Fleur, 2006](#)). The goals of this section were: (1) to evaluate

how often field endocrinologists consider the roles of CORT in the mobilization of energy reserves and the acquisition of energy for the introduction and discussion of the data and (2) to evaluate how often studies report original data on the effects of CORT on both mobilization and acquisition of energy. Based on our consideration of the literature, we hypothesized that the theoretical background of CORT actions on energy balance is biased towards the mobilization of stored energy and that there are more collected data about their effects on mobilization than about their effects on acquisition of energy.

There is plenty of evidence that CORT have powerful orexigenic actions, mainly in laboratory models (Dallman et al., 1995, 2007; Solano and Jacobson, 1999; Pecoraro et al., 2004; la Fleur, 2006; Uchoa et al., 2014), but also in humans (Dallman et al., 2004) and free-living vertebrates (Landys et al., 2004; Crespi and Denver, 2005). This dual effect of CORT occurs because their actions in the central nervous system are mainly stimulatory to energy acquisition (i.e., food intake, Dallman et al., 2007; la Fleur, 2006), though are mostly catabolic in the periphery (Dallman et al., 1995). In general, small to moderate increases in CORT levels (acting mainly through the type I receptor) have positive effect on food intake and consequently on body weight, while larger increases (stressor-induced levels acting through the type II receptor) are considered to have mainly catabolic effects (Uchoa et al., 2014). However, results from the biomedical area show that CORT levels in response to stressors can also stimulate food intake (Dallman et al., 1995, 2004). Type I and type II CORT receptor activation appear to induce different food preferences, the former stimulating intake of both carbohydrates and fat and the later only carbohydrates (Dallman et al., 2004). The mechanisms by which CORT affect energy intake include interactions with insulin, leptin and ghrelin (Dallman et al., 1999; Broberger, 2005). These interactions are mediated, in part, by neuropeptide Y (NPY, Broberger, 2005), a potent stimulator of food intake produced by neurons in various locations of the brain and the sympathetic nervous system (Dallman et al., 1995; la Fleur, 2006).

## 6.2. Analysis

First, we recorded whether the relationship between CORT secretion and high or increased energy demands is explicitly considered in the article. Afterwards, we recorded: (1) whether the roles of CORT in the mobilization of energy reserves and the acquisition of energy (i.e., stimulation of appetite and foraging behavior) are considered for the introduction and discussion of the data and (2) whether there are original data about CORT effects on mobilization and/or acquisition of energy. Mobilization of energy was recorded as the quantification of physiological variables in plasma or blood indicating catabolism, related to changes in CORT levels (triglycerides and glucose, among other metabolites). Many studies report data on body mass or body condition index, together with data on CORT levels (see [Supplementary Material](#)). However, whether an observed decrease in body mass (such as a seasonal or stressor-related decrease) is due to CORT-induced mobilization of energy reserves is difficult to ascertain from the data, specially when there are very little data on catabolic actions of CORT in the reviewed articles (see subsection 6.3). Even in Cyr et al., 2007 where the authors report concomitant data on CORT, body weight, glucose and triglycerides in response to chronic stressors in starlings, they conclude that it is difficult to establish causal relationships for the variation patterns observed for these variables. Therefore, we decided not to register data on decreases in body mass as an additional indicator of CORT-induced mobilization of energy stores, unless this was explicitly suggested by the authors. With regard to energy acquisition, we considered data on: (1) direct effects of exogenous CORT on foraging activity patterns,

body mass and/or fat scores (as related to changes in foraging behavior, i.e., experimental data); (2) relationships among CORT levels, foraging parameters (e.g., time devoted to foraging, foraging success), daily feeding schedule, food intake or availability, nestling provisioning behavior, fuel store (fat), changes in fat scores and mass gain in relation to foraging activity, among others (i.e., correlative data). We found no studies considering the possibility that CORT produce effects on body weight through their effects on water excretion.

## 6.3. Results and discussion

Results corroborate that increases in CORT levels are recognized to be related to increased energetic demands in the majority of studies (Fig. 2). The association of CORT secretion with increased energetic demands is mainly recognized in the context of CORT responses to acute and chronic stressors. The circadian variation in HPA or HPI axis activity, associated with circadian activity patterns, is also acknowledged as an additional context for CORT-related energy mobilization (e.g., Woodley et al., 2003). There are also some articles recognizing increases in seasonal baseline CORT levels as related to the higher energy demands of predictable life-history processes (Landys et al., 2004; Reeder et al., 2004). These are not mutually-exclusive categories and many papers recognize at least two of them. Noteworthy, these last contexts in which CORT are associated with increased energy demands are essentially different from the first category, as they do not necessarily imply the presence of stressors, but allostatic adjustments to predictable daily and seasonal changes in activity patterns, respectively (Landys et al., 2006; Romero et al., 2009).

Catabolic functions were predominant over the orexigenic roles in the theoretical frameworks (Fig. 2.). The percentage of papers that acknowledge that high CORT indicate increased energy demands and their roles in the mobilization of energy reserves were 62.5% for HB (both cases) and 72.5% and 75% for GCE, respectively (Fig. 2). On the other hand, stimulation of food intake was considered in 35% of studies in HB and in 32.5% in GCE. Thus, both journals were similar in this regard (Fig. 2). The mobilization of energy stores was widely considered in studies about all vertebrate classes, while the orexigenic roles were mentioned in 16 studies about birds, nine studies about mammals and two studies in reptiles (both journals considered, [Supplementary Material](#)). Results show that CORT secretion is widely recognized as related to increased energy demands with regard to the roles in energetic balance, and that our ideas are slanted towards the mobilization of stored energy.

Though CORT roles in the mobilization of energy reserves are considered in the majority of papers, we found few original data on this subject (experimental evidence: [McConnachie et al., 2012](#); [Horton and Holberton, 2009](#); correlative data: [Landys et al., 2004](#); [Cyr et al., 2007](#); [Alderman et al., 2012](#)). We also found two studies citing previous research in their own study models showing associations between CORT and mobilization of energy reserves ([Williams et al., 2008](#); [Cook et al., 2011](#)). We did find various articles in which the authors discuss CORT data with regard to energy mobilization and/or energy demands, and CORT is presumed to be involved in the mobilization of energy stores, but without direct data on this subject (e.g., [Kenagy and Place, 2000](#); [Huber et al., 2003](#); [Reeder et al., 2004](#); [Williams et al., 2008](#); [Dantzer et al., 2010](#); [Vera et al., 2011](#)). In addition, some authors emphasize the relationships between CORT and body mass/condition (e.g., [Kenagy and Place, 2000](#); [Nunes et al., 2006](#); [Ebensperger et al., 2013](#); [Bauer et al., 2014](#)).

Among wild vertebrates, there is evidence in songbirds (*Sturnus Vulgaris*) that the interplay between CORT and glucose during stress may be more complex than usually appreciated ([Remage-Healey](#)



and Romero, 2000). These birds exhibited a daily rhythm in basal glucose levels, while stress-induced levels failed to show such a pattern, even though stress-induced CORT showed a circadian rhythm (Remage-Healey and Romero, 2000). The effect of CORT on glucose appears to be context-dependent, as CORT only affects blood glucose in fasted animals (Remage-Healey and Romero, 2000).

We acknowledge that for field endocrinologists a major goal in this regard is to address whether and how CORT are related to energetically-demanding processes important for survival and reproduction. In other words, we aim to elucidate the ecological underpinnings and evolutionary implications of variations in CORT. We suggest, nonetheless, that we should be aware of the fact that a great deal of our common statements about CORT and energy mobilization come from past biomedical research and not directly from research in our own study models.

Contrary to our expectations, we found more studies reporting data on the regulatory roles of CORT on foraging behavior. However, these studies were conducted almost exclusively in birds (Breuner and Hahn, 2003; Landys et al., 2004; Martins et al., 2007; Angelier et al., 2007; Buck et al., 2007; Müller et al., 2007; Angelier et al., 2008; Brewer et al., 2008; Horton and Holberton, 2009; Eikenaar et al., 2014), though there was also one article on mammals (bats, Reeder et al., 2004), and one on reptiles (iguanas, Woodley et al., 2003, Supplementary Material). There are various articles about mammals that refer to the orexigenic roles of CORT and some of them suggest that changes observed in CORT levels might be related to the stimulation of food intake (e.g., Kenagy and Place, 2000; Soto-Gamboa et al., 2005; Nunes et al., 2006; Cabezas et al., 2007; Dantzer et al., 2010; Vera et al., 2011; Mustoe et al., 2012, Supplementary Material). Nonetheless, the research focus of these studies is not at all on the evaluation of the orexigenic actions of CORT and, in concordance, there are no direct data on this subject.

We found studies reporting concomitant data on (1) CORT and food/prey availability, (2) time spent feeding and seasonal CORT levels, and (3) corticosteroid-binding globulin (CBG) during short-term fasting, that might have benefited from considering CORT roles in the regulation of food intake (e.g., Lucas et al., 2006; Gesquiere et al., 2008; Van Meter et al., 2009; Thompson et al., 2010; Girard-Buttoz et al., 2014; Lynn et al., 2003, Supplementary Material). We suggest that these are examples showing that the regulation of foraging by CORT still needs more consideration in our theoretical backgrounds.

Overall, results show that the notion that CORT mobilize energy reserves prevails over the notion that CORT is involved in the acquisition of energy through the stimulation of foraging behavior. However, on examining the original data on both subjects published between 2000 and 2014, it appears that there are more data on the effects of CORT on energy acquisition (mainly in birds) than on energy mobilization.

In spite of the common view that increased CORT levels imply a shift to a more catabolic state, we see no reasons to think about the catabolic actions of CORT as more important than their orexigenic and anabolic effects. In general, predictable challenges and emergency responses requiring increased energy demands –such as reproduction and a predator chase, respectively– would trigger the need for mobilization of stored energy, coupled with the need of acquisition –and subsequent storage– of energy (when the stressor is no longer present). All these actions are attributable to CORT (Dallman et al., 2004). The stimulation of energy acquisition and storage might occur in advance, during or after the energetically-demanding activity. On the other hand, we can expect CORT-induced mobilization of energy to occur during and after energetically-demanding challenges. For instance, CORT may increase foraging and fat storage in anticipation for a long-distance migration, they may mobilize stored energy during the

migratory flight, and stimulate further food intake at stopover sites (Landys et al., 2004). When confronted to an acute stressor, CORT might trigger the mobilization of energy stores during and/or after the exposure to the stressor, and stimulate food consumption once the stressor ends (Sapolsky et al., 2000; see below). Therefore, the actions of CORT on energy balance, might be better understood if we think about them as a balance that depends on opposed, but complementary, actions that these hormones themselves mediate at different levels within the organism (i.e., food intake: central nervous system; mobilization of energy reserves and storage: peripheral tissues, Dallman et al., 2007). Additional relevant factors to have in consideration are: the *meaning* of the particular challenge (predictable activity pattern vs. non-predictable environmental stressor), the duration of the activity/stimulus (short term or acute vs. long-term or chronic) and the interactions of CORT with other endocrine signals regulating energy balance (e.g., insulin, leptin).

One last consideration with regard to the orexigenic roles of CORT: it is traditionally considered that, when unpredictable environmental stressors occur, the rise in CORT levels contributes to suppress food intake in the short term, as this activity is no longer essential for immediate survival (but see Crespi and Denver, 2005). This view does not completely continue to make sense after reading Sapolsky et al.'s (2000) ideas on the preparative effects of CORT (see below). If we consider that the roles of CORT in the response to acute stressors can be preparative for future stressors (Sapolsky et al., 2000), it is reasonable that increases in CORT associated with emergency states might also serve to stimulate food intake. This is because the duration of many acute stressors is not long enough for CORT actions to occur when stressors are still present, and therefore, part of CORT actions might be better understood if we think about the animals' needs after the stressor has passed (Sapolsky et al., 2000). If an energetically-demanding stressor, such as a predator chase, has decreased energy stores, it might be advantageous to replenish energy stores after the stressor has passed through the stimulation of food intake. This idea is not at all new for the biomedical area: CORT increases in response to acute and chronic stressors are known for their capacity of augmenting the motivation to eat high-caloric content food in laboratory rodents and humans (Epel et al., 2001; Dallman et al., 2004; Pecoraro et al., 2004). Noteworthy, among free-living vertebrates there is evidence that the type II CORT receptor (the one that interacts with stressor-induced CORT levels) has a permissive role in food intake in birds (Landys et al., 2004). Overall, though acute and chronic stressors can decrease food intake, it should not be *a priori* assumed that the stressor-induced CORT levels will necessarily contribute to produce this effect. In other words, the actions of CORT in the stimulation of food intake might be more salient than generally considered (Sapolsky et al., 2000; Dallman et al., 2004, 2007; Crespi and Denver, 2005). We emphasize that the ideas of Sapolsky and colleagues (2000) have the potential to significantly enrich the way we think about CORT actions in wild vertebrates, even though they have received little attention up to the present (see below).

## 7. Consideration of the permissive, preparative, stimulatory and suppressive actions of CORT (Sapolsky et al.'s, 2000 review)

### 7.1. Rationale for the inclusion of this item in the analysis

We were interested in evaluating how integrative meta-analyses about CORT actions (directed mainly to field endocrinologists) impact on original research papers. Particularly, we sought to assess to which extent integrative reviews are considered in original research articles for the demarcation of research goals and perspectives and for the introduction and discussion of the data. A considerable amount of meta-analyses have been published in the

last two decades, including high-quality reviews that explore important actions of CORT with innovative approaches (e.g., Sapolsky et al., 2000; Romero, 2002; Landys et al., 2006; Bonier et al., 2009; Romero et al., 2009; Boonstra, 2013; Dickens and Romero, 2013, among others). To evaluate the previously-mentioned issues, we chose Sapolsky et al.'s (2000) review for the following reasons: (1) it was published during 2000 and thus, it could have been read by the authors of all the papers we reviewed, (2) a substantial time has passed since its publication and thus, one might better evaluate the impact of the authors' ideas in the research field, and (3) it is a highly-integrative review that incorporates a lot of information from biomedicine and field endocrinology and describes a novel framework to study CORT actions.

Very briefly, the focus of Sapolsky et al.'s (2000) review is to describe how CORT influence stress responses integrating different kinds of actions: (1) *permissive actions*, defined as those exerted by CORT levels present before the stressor (i.e., basal or baseline levels), which prime the stress responses, (2) *preparative actions*, defined as those that do not affect the immediate response to a stressor, but modulate the organism's response to a subsequent stressor, (3) *stimulatory actions*, those that enhance the effects of the first wave of hormonal responses, and (4) *suppressive actions*, considered as those that prevent the stress response from overshooting (Sapolsky et al., 2000).

## 7.2. Analysis

For each paper we registered whether (a) Sapolsky et al.'s (2000) review was cited, (b) permissive, suppressive, stimulating and/or preparative actions were explicitly considered for the introduction and discussion of the data and (c) the study of permissive, suppressive, stimulating and/or preparative actions was included in the research goals. We registered both the citation of the review and the penetration of the authors' ideas because we assumed that it is not the same to cite a paper than to consider what is written in the paper in instances such as the demarcation of goals and interpretation of the data.

## 7.3. Results and discussion

Sapolsky et al.'s (2000) review was highly cited, especially in GCE, with more than 50% of papers including this citation. For HB the amount of papers including this citation was 35% (Fig. 2). In spite of the elevated citation impact, the quantity of papers considering the distinction among preparative, permissive, stimulatory and/or suppressing actions was much smaller (only 5 out of 80 papers, Fig. 2). The evaluation of these types of CORT actions, as described by Sapolsky et al. (2000), was not included explicitly in the goals of any of the reviewed papers. In addition, the consideration of these types of actions was in all cases very brief and limited to one or a few sentences in the introduction or discussion sections (Reeder et al., 2006; Horton and Holberton, 2009; Spencoski et al., 2012; Bauer et al., 2014; Dantzer et al., 2010). Mostly, Sapolsky et al.'s (2000) review was cited as a reference text to introduce or discuss basic aspects of CORT actions, such as their roles in the response to stressors and effects on the immune system, among others (e.g., Vera et al., 2011; Koren et al., 2012; George et al., 2014). Noteworthy, many articles explicitly acknowledge that CORT can suppress reproduction, immune function and other processes not essential for survival during acute and/or chronic responses to stressors, which is not the same as considering CORT's suppressive actions, as defined by Sapolsky et al. (2000), implying that the increase in CORT levels functions to suppress the stress response itself, preventing it from overshooting.

These results show that the elevated citation impact of Sapolsky et al.'s (2000) review is not correlated with a high impact of the

main ideas raised by these authors, at least in this research field. This does not mean that the analysis made by Sapolsky and colleagues is not considered as important by field endocrinologists. Indeed, the review contains a great amount of information and is highly-integrative. We suggest that researchers working in field endocrinology still find difficult to integrate this information with their research perspectives (ourselves included). Methodologically, assessing Sapolsky et al.'s (2000) proposal implies the evaluation of multiple systems involved in the response to stressors and not just solely focusing on CORT levels. To prove some of these actions of CORT might require studies under very controlled conditions. We suggest that the use of antagonists of CORT receptors (e.g., Landys et al., 2004) might be a valuable tool to assess how CORT influence other components of the stress response.

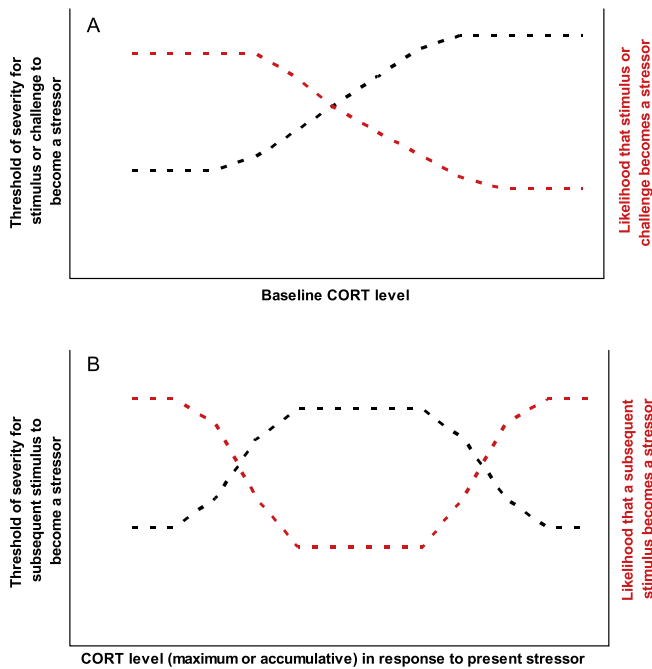
It is worth mentioning that we do not expect that similar results would occur for all the integrative reviews that have been published. In fact, if the main ideas of the review were more familiar for field endocrinologists and could be easily contrasted with experimental data, we would expect a closer association between the citation impact and the actual impact of the authors' ideas. The review by Bonier et al. (2009), which addresses the Cort-Fitness and Cort-Adaptation Hypothesis, is probably an example as it has received both numerous citations and empirical contrasting of the authors' proposals (F.V. personal observation). In our opinion, the core of the analysis made by Sapolsky et al. (2000) is worth of more attention than that received up to now.

## 8. Expanding the idea of permissive and preparative effects

The feature that permissive and preparative actions have in common is that CORT levels present *before* a stressor are meaningful and influence the physiological response to it (Sapolsky et al., 2000; Romero, 2002). The main difference between both types is that permissive actions are related to baseline (or basal) levels, while preparative actions are related to stressor-induced levels during the response to a previous stressor, which in time influences the response to a later stressor (Sapolsky et al., 2000).

An issue that emerges from these ideas is that CORT may not only prepare the organism for a "better" response to a subsequent stressor through their permissive and preparative actions, but also they may decrease the likelihood that certain stimuli will indeed become stressors (Figs. 5 and 6). This is because what CORT do is to modify the systems within the animal, previous to the exposure to the potentially stressing agent. The ways in which CORT modify the systems involved in the response to environmental challenges are addressed in detail in Sapolsky et al. (2000). Briefly, the permissive actions of CORT (1) augment and sustain catecholamine production and release and allow them to exert their full actions, (2) help mediate the immune activation during the first moments of response to a variety of stressors, (3) help mediate the metabolic stress response, synergizing with catecholamines and glucagon to stimulate lipolysis and to elevate circulating glucose concentrations and (4) stimulate food consumption and deposition of energy stores (see Sapolsky et al., 2000), among others. Preparative actions of CORT might also involve stimulation of food intake and deposition of hepatic glycogen and fat reserves (Sapolsky et al., 2000).

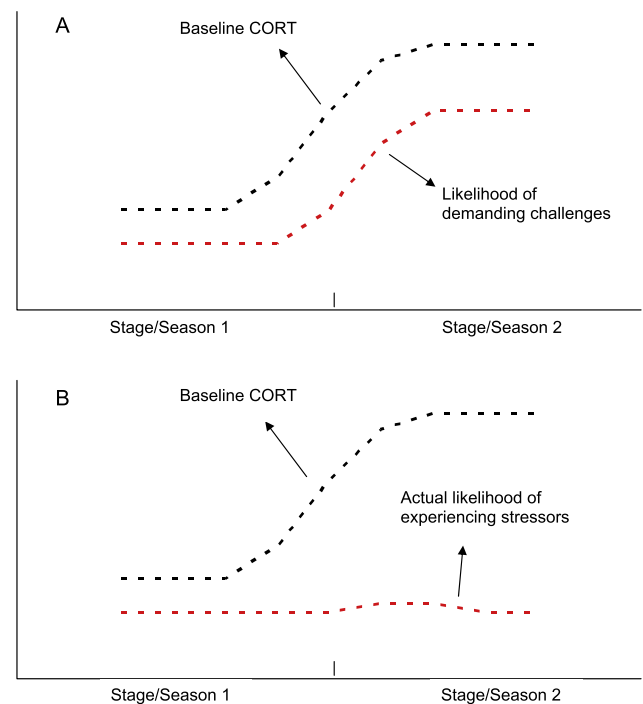
We propose that the permissive and preparative actions of CORT increase the threshold of severity necessary for the subsequent stimuli to become stressors (Armario, 2006, Figs. 5 and 6). As the threshold of severity increases due to the priming effects of CORT, there will be less environmental stimuli capable of threatening homeostasis and producing allostatic overload (Figs. 5 and 6). Perhaps, the most important concern here is that CORT are viewed as capable of modifying the meaning of the environmental



**Fig. 5.** Relationships between baseline CORT (A) and stressor-induced CORT (B), and both the likelihood that subsequent stimuli become stressors and the threshold of severity necessary for a stimulus to become a stressor. The permissive actions of baseline CORT prime the systems within the animal, making the organism better prepared to face future challenges. This actions increase the threshold of severity necessary for stimuli to become stressors (therefore lowering the probability that a given stimulus indeed becomes a stressor) (A). The preparative actions of CORT levels in response to a present stressor tend to produce the same effect: prepare the animal for a subsequent challenge. However, if the CORT response is too exacerbated, it will tend to produce the opposite effect (B). These models might be considered as an extension of the Preparative Hypothesis (Romero, 2002).

stimuli (i.e., change what the external stimuli represent for the animal).

Possibly, these actions apply mainly for long-term predictable demanding stimuli or activities. We suggest that the term “preparative” (originally used for the actions of stress-induced CORT, Sapolsky et al., 2000) could be extended to the actions at baseline levels, which change in anticipation for predictable long-term demanding activities (Landys et al., 2006). An example is the case in which increased baseline CORT levels anticipate or occur during a demanding period, stimulating food intake and deposition of fat stores. If energetic stores are increased we can reasonably expect that challenges requiring energy reallocation (migration, lactation, bad weather, decreased time to foraging, moderate intensity agonistic interactions) will have less probability of taking the physiological systems out of balance. The energetic status of the animal previous to the onset of a challenge is also expected to influence the animals’ perception of whether the stimulus is a stressor, because energy stores affect the ability of animals to cope with allostatic load (Landys et al., 2006). Thus, the increase in baseline CORT would contribute to maintain a stable likelihood of experiencing stressors, even during stages when we might expect the probability of stressors to increase (Fig. 6, see also Landys et al., 2006). The baseline level (and the change from one season to another) is expected to vary between males and females, if both sexes differ in the probability of facing potential stressors. Clearly, this does not mean that an individual could reach a state in which it will be able to deal with all environmental stimuli and there will no longer be stressors; it means that a lower proportion of them will be perceived as stressors because of its improved physical condition and/or its capacity to mount a response to them. Logically,



**Fig. 6.** The effect of baseline CORT levels on the perception of stimuli and challenges as “stressors”. In the original Preparative Hypothesis (Romero, 2002) it is proposed that baseline CORT levels increase in moments when the likelihood of experiencing stressors increase (A). However, because baseline CORT levels prime many systems within the organism (Sapolsky et al., 2000), they are able to change the meaning that these stimuli and challenges have for the organism. The effect is that these actions contribute to maintain a stable likelihood of experiencing stressors, even in moments when challenges clearly increase (e.g., reproduction, long distance migration, B). Logically, variation can be accommodated for the model. For instance, the likelihood of experiencing stressors during stage 2 could still be significantly increased in relation to that of stage 1, but lower than in the scenario where CORT did not prime the systems within the animal.

certain chronic stressors, such as human-induced habitat degradation, might largely override the permissive and preparative effects of CORT (Brearley et al., 2013; Balestri et al., 2014).

For this type of actions predictability of events across life-history stages is a key factor (Fig. 6). This includes predictability in the patterns of energy demand and/or supply, as well as predator and/or parasite presence. If these patterns can be essentially predicted, then we would expect these actions of CORT to explain, in part, the observed seasonal variations in wild vertebrates (Romero, 2002). Therefore, biological interactions, such as co-evolution among the study species with predators and parasites might also be expected to influence the shifts in CORT levels that occur during the life of many species.

Acute and less predictable stressors requiring a flight or fight response are less likely to become interpreted as less of a stressor because the components of the stress response are primed to act (permissive effect), or stores are being rebuilt (preparative). Mainly, these actions of CORT would help the organism deal with the acute stressor, reducing its negative impact, as proposed in the Original Preparative Hypothesis (Romero, 2002). On the other hand, considering that the permissive/preparative actions affect the overall body condition and the ability to mount a response to demanding stimuli, it can still be expected that the proportion of acute stimuli that become stressors would tend to decrease up to a point. Clearly, this effect applies to rather moderate stimuli that need to be quantitatively excessive to become stressors (e.g., social conflict), as opposed to those that are qualitative stressors (e.g.,

presence of predators, [Vigas, 1980](#)). It should be noted that a “good” stress response also involves suppressive actions of CORT that will prevent it from overshooting ([Sapolsky et al., 2000](#)).

For preparative actions -mediated by the levels in response to a stressor- an increase of CORT in response to a present stressor would decrease the likelihood of a subsequent challenge becoming a stressor or, if it does, they would help decrease its negative impact. However, if the response of CORT to the present stressor is too exacerbated, the condition of the animal at the moment of facing the second challenge (e.g., altered cognitive state, waste of stored energy) will not be optimal ([Fig. 5B](#)). For these actions, the balance between the cost of the response to a current stressor and the likelihood of experiencing a second demanding challenge in the short term is a key factor. In a scenario where the response to a present stressor does not have a significant biological cost and/or the likelihood of a subsequent challenge is low, these actions would be unimportant. However, if the response to a current stressor (e.g., predator chase, agonistic social interaction) entails a significant cost, this means that it renders the individual more susceptible to a second demanding event occurring in the short term (i.e., minutes to several hours, [Sapolsky et al., 2000](#)). Thus, if a second challenge has a non-depreciable likelihood, the preparative actions are meaningful in terms of survival and future reproduction. Overall, the importance of the preparative actions would increase with both the cost of the response to a present stressor and the likelihood and intensity of subsequent challenges.

These actions are expected to have different relevance depending on life-history traits, such as expected life span and reproductive strategy (i.e., semelparous or iteroparous, [Crespi et al., 2013](#)). For instance, in semelparous species, the HPA or HPI axis might become insensitive to the negative feedback and animals experience very high stress levels, leading to the death of the individuals (e.g., [Boonstra, 2005](#)). In such cases, there is not an adaptive value in avoiding high stress levels, but solely in maximizing the current reproductive output. On the other hand, for iteroparous species with an expected life span exceeding the next reproductive event or demanding activity (e.g., migrating bird) the model presented in [Fig. 6](#) might be much more pertinent. Particularly, it might explain why animals can go through these activities without experiencing significant levels of stress, while having concomitantly increased CORT levels ([Landys et al., 2006](#); [Vera et al., 2013](#)).

It is noteworthy that this view highlights that increased CORT levels may not be necessarily associated with increased stress levels (classical approach), or increased likelihood of being subjected to stressors (the Preparative Hypothesis, [Romero, 2002](#)). Our present proposal is different from the original Preparative Hypothesis, as it involves the notion of changing the probabilities of subsequently experiencing stressors as a direct consequence of the priming effects of CORT levels before challenges occur. We suggest that the three scenarios should be considered for the interpretation of data in studies about wild vertebrates.

From the previous reasoning follows that the inability to increase baseline CORT levels, or to increase CORT in response to an acute stressor to transfer the hormonal signal into the appropriate biological effects through interactions with CORT receptors, might preclude an individual to be well prepared for subsequent challenges. There is already evidence in this regard ([Sapolsky et al., 2000](#); [Landys-Ciannelli et al., 2002](#); [Landys et al., 2004](#)).

## 9. Mineralocorticoid roles of CORT

### 9.1. Rationale for the inclusion of this item in the analysis

Cortisol and corticosterone have been studied in relation to mineral-water balance in the fields of biomedical and domestic

animal research during the last 30 years. Many studies indicate that these hormones exert their mineralotropic actions by acting both systemically and centrally ([Agarwal and Mirshahi, 1999](#); [de Kloet et al., 2000](#); [Thunhorst et al., 2007](#); [Shelat et al., 1999a](#); [Liu et al., 2010](#)). In the brain, CORT increase the binding of angiotensin and aldosterone to their respective receptors and potentiate their effects on water drinking and salt appetite ([Ganesan and Sumners, 1989](#); [Ma et al., 1993](#); [Shelat et al., 1999a](#); [Thunhorst et al., 2007](#)). For example, CORT can increase the numbers of angiotensin II (Ang II) type I receptor ([Sato et al., 1994](#); [Shelat et al., 1999a,b](#)) and amplify intracellular signaling processes induced by Ang II ([Sumners et al., 1991](#); [Daniels and Fluharty, 2004](#)). Systemically, CORT increase glomerular filtration rate, augmenting urine volume and sodium excretion, promoting further ingestion of water and sodium ([Thunhorst et al., 2007](#)). In the large intestine, CORT increase sodium absorption ([Agarwal and Mirshahi, 1999](#)). In the heart (atrial myocytes) CORT augment atrial natriuretic peptide mRNA expression (ANP, an hormone that increases the excretion of sodium and increases the rate of blood filtration, [Baxter et al., 1988](#); [Hill et al., 2012](#)). Thus, it appears that, depending on the volume of the extracellular fluid, CORT might stimulate the actions of Ang II and aldosterone (e.g., during hypovolemia) or counteract them, through their effects on ANP (e.g., hypervolemia). It has been recently reported that these hormones increase the number of the primary receptor of ANP in the kidney and hypothalamus ([Liu et al., 2010](#)). On the other hand, CORT secretion from *zona fasciculata* (ZF) cells of the adrenal cortex can be modulated by the rennin-angiotensin system. Among mammals, Ang II has been shown to stimulate the secretion of corticosterone and cortisol in mice and bovines, respectively, by acting through the angiotensin type I receptor in ZF cells ([Rábano et al., 2004](#)). In non-mammalian wild vertebrates, including fish, amphibians and reptiles, there is also evidence that Ang II stimulates cortisol or corticosterone secretion ([Norris and Carr, 2013](#)), though this is not always the case ([Klingbeil, 1985](#)). Overall, there is much evidence from laboratory models, but also in wild vertebrates, indicating mineralocorticoid functions for CORT.

The goal of this section was to assess how often the mineralocorticoid actions of CORT are currently acknowledged in the literature about wild vertebrates. Based on our previous reading of the literature, we hypothesized that at present, these roles are very poorly considered. As with other issues addressed in this review, the detailed description of how CORT achieve roles in the regulation of body fluids is not a goal of field endocrinology. However, we suggest that the acknowledgment of the mineralotropic actions might be pertinent for the interpretation of data in some studies, as well as for the generation of hypothesis and delineation of new research perspectives.

### 9.2. Analysis

In each article we assessed whether (1) the mineralocorticoid actions of CORT are considered in the theoretical framework, (2) the evaluation of cortisol and/or corticosterone roles in mineral-water balance is included in the research goals and (3) there are experiments designed in order to test mineralotropic actions of cortisol and/or corticosterone and/or correlative data on this subject.

### 9.3. Results and discussion

Results show that the roles of CORT in mineral-water balance are currently very scarcely and briefly considered in the literature about wild vertebrates ([Fig. 2](#)). The mineralocorticoid actions of CORT were not included in the research goals of any of the reviewed articles ([Supplementary Material](#)). Accordingly, there

are no original data on this subject in the articles. The two GCE papers related to fish refer to the mineralocorticoid roles of cortisol because fish lack a mineralocorticoid hormone, such as aldosterone, and therefore, the roles of cortisol in body fluid balance have been the focus of much research (e.g., McCormick, 2011).

Logically, the relevance of having this in mind will depend on the goals and experimental design of each study. For example, for studies addressing CORT responses to acute stressors or ACTH, or the regulation of the HPA axis by negative feedback, it is not obviously critical to have the mineralocorticoid actions in mind. On the other hand, studies using CORT measurements to evaluate the condition of animals or assess physiological adjustments to changing environmental conditions (e.g., dry season, natural or anthropogenic disturbances, changes in habitat type) or activity patterns (e.g., reproduction, migration) might probably benefit from considering these less-prominent roles (e.g., Landys et al., 2004; Gesquiere et al., 2008; Van Meter et al., 2009; Spencoski et al., 2012). Accounting for this possibility will contribute to expand our theoretical background beyond the classic roles in the response to stressors and the regulation of energy balance, which is especially important for studies focusing conservation and animal welfare. Though virtually all biomedical research indicating the effects of CORT on mineral-water balance uses laboratory mice and rat models, the distinction between gluco and mineralocorticoids appears to be even more vague in non-mammalian species, like teleosts, amphibians, marine reptiles and certain birds (Agarwal and Mirshahi, 1999).

From a more epistemological point of view, this sub-consideration of mineralotropic actions is probably related to the definition of cortisol and corticosterone as “glucocorticoids”, as opposed to aldosterone, which is defined as a “mineralocorticoid”. Indeed, it may appear somewhat confusing to speak about the “mineralocorticoid roles of glucocorticoids”. From a historical perspective, this terminology is related to the fact that cortisol and corticosterone were originally characterized with regard to carbohydrate metabolism, while aldosterone was described in relation to its salt conserving actions. Also, the fact that cortisol and corticosterone are produced in the *zona fasciculata* of the adrenal cortex, while aldosterone is produced in the *zona glomerulosa*, probably reinforces the view that their roles do not overlap.

In addition, it is known that the access of CORT to the type I receptor is restricted in certain tissues (e.g., kidney, paraventricular nucleus of hippocampus) due to the co-expression of the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase, which converts CORT into inactive forms, conferring specificity for aldosterone binding (reviewed by de Kloet et al., 2000). This is a major reason explaining why the mineralocorticoid effects of CORT are understudied. Nonetheless, apart for the central actions of CORT regulating aldosterone and Ang II actions above-mentioned, there is evidence that binding to the type II receptor can also account for mineralocorticoid actions of CORT in kidney. For example, in adrenalectomized rats, the type II receptor agonist RU 28362 increased renal Na<sup>+</sup> absorption, which was unaffected by the antimineralocorticoid RU 28318, but could be reversed by the antigluco-corticoid RU 38486 (Agarwal and Mirshahi, 1999). Among amphibians (toad), there is evidence that the bladder, which expresses both the dehydrogenase and the type II receptor, responds to corticosterone with enhanced sodium absorption (Broillet et al., 1993; Chen et al., 1998). Thus, CORT effects on mineral-water balance involve, depending on the case, actions through both type I and type II receptors in vertebrate taxa.

We suggest that the categorization of cortisol and corticosterone using the term “glucocorticoids”, though sustained by historical reasons and tradition, is currently contributing to narrowing the way we think about the actions of these hormones. In other words, the nomenclature “glucocorticoid” for cortisol and

corticosterone applies to a previous state of knowledge that is no longer highly-descriptive, but restrictive, at the present moment.

A comparable misunderstanding exists with the names of type I and type II CORT receptors, which are usually called “mineralocorticoid” and “glucocorticoid” receptors, respectively, though they both bind CORT (with different affinity, de Kloet et al., 2000; Uchoa et al., 2014). It should be acknowledged that nature does not always perfectly fit in fixed categories, incorporating redundancy into important physiological systems.

## 10. Alternative endocrine regulatory pathways

### 10.1. Rationale for the inclusion of this item in the analysis and goals

The literature on biomedical and domestic animal research contains plenty of evidence indicating that a wide variety of factors, apart from pituitary ACTH, regulate CORT secretion from the adrenal cortex. These include: interactions with the immune system through the release of cytokines, paracrine control from endothelial cells, adipocyte-derived adipokines and/or fatty acids, angiotensin II, and direct neural control (Bornstein et al., 2008; Rábano et al., 2004). Clearly, the detailed study of these endocrine pathways is not a goal of field endocrinology. However, as field endocrinologists, we usually desire to understand causes underlying temporal, geographical, inter-individual and developmental variations in CORT levels, among other sources of variation. Therefore, it is interesting to assess the degree to which we think about CORT as regulated solely by ACTH, or we attempt to integrate additional information about the regulation of the adrenal cortex. Surprisingly, even in the biomedical literature, it has been generally accepted that adrenocortical CORT secretion is exclusively linked to pituitary ACTH release (Bornstein et al., 2008). Thus, the goal of this section was to assess how often field endocrinologists recognize and discuss about endocrine pathways controlling CORT secretion, different from the classic regulation by ACTH. This is also related to the acknowledgment of other physiological roles of CORT. For instance, if we focus classically on CORT actions during responses to stressors, we will be predisposed to think mainly about their regulation by ACTH, while having in mind the roles of CORT in the regulation of food intake predisposes us to think about interactions with other hormones that regulate energy balance, such as leptin and insulin (Dallman et al., 1999; Solano and Jacobson, 1999).

### 10.2. Analysis

In each article we registered whether ACTH-independent regulatory mechanisms of CORT secretion are explicitly considered and if there are original data on this subject. By “ACTH-independent regulatory mechanism” we mean regulatory pathways other than the classical control of CORT secretion, mediated by corticotropin-releasing factor (CRF), arginine-vasopressin (AVP) (hypothalamus), and ACTH (pituitary). Therefore, the classic negative feedback of the axis (Boonstra, 2005) was excluded from this category. The regulation of CORT actions by CBG (Breuner et al., 2013; Schoech et al., 2013) and the expression of CORT receptors (e.g. Breuner and Orchinik, 2001) were not considered either. To ponder an alternative pathway as present in an article, the criteria was the consideration that an endocrine, neuroendocrine or neural signal (other than the ones previously mentioned) affects CORT secretion (cause-effect) and/or is associated with increased or decreased CORT concentrations (correlative evidence). The reverse (i.e., effects of CORT on other endocrine systems or signals) was not considered here, as we focused on the causes underlying variations in CORT levels.

### 10.3. Results and discussion

Results show that a minority of papers recognize endocrine pathways others than the classic ACTH control of CORT secretion (Fig. 2, [Supplementary Material](#)). The strongest pattern is the consideration of regulation of CORT by reproductive steroids. Other less common regulatory agents included are Ang II, opioids, neuropeptides, growth factors and prostaglandins ([Supplementary Material](#)). There were no original data on this subject, with the exception of [Cartledge and Jones \(2007\)](#), reporting the effects of estrogens on adrenal function. Bearing in mind that even in the biomedical literature, adrenocortical CORT secretion has been generally viewed as solely linked to pituitary ACTH ([Bornstein et al., 2008](#)), it is noteworthy that we have found some articles considering ACTH-independent regulation in wild vertebrates. It is not surprising that articles from GCE included more references to ACTH-independent regulation than articles from HB, as the former journal is more focused on regulatory mechanisms. We consider that the relatively low acknowledgement of ACTH-independent regulation also reflects, in part, the prevailing focus on the classical roles of CORT in the response to stressors.

## 11. Conclusions and perspectives

The present review shows some clear patterns in the research of CORT dynamics in wild vertebrates: (1) the roles of CORT in the response to stressors are central in the theoretical framework and study goals and, therefore, there are plenty of data on CORT responses to stressors. (2) Chronic stressors are very frequently assumed to increase CORT levels. (3) Our ideas about the effects of CORT on energy balance are slanted towards the mobilization of energy reserves, though the stimulation of food intake is considered in a significant amount of articles (and completely overlooked in others). However, the idea that CORT mobilize energy reserves seems to come mainly from past research and not from data in our own study models, as we found few original data on this subject. On the other hand, there are more data on CORT roles on foraging activity, particularly in birds. (4) The mineralocorticoid roles of CORT are virtually ignored, with the exception of studies in fish. Though fish are poorly represented in the present revision (only 5 studies), we have checked that there is indeed much research on this subject (e.g., [McCormick, 2011](#)). (5) Several studies recognize the importance of baseline CORT levels in the regulation of life-history processes (or refer briefly to the “baseline levels”), but others do not. This aspect is critical and since authors do not recognize it, they frequently assume that higher CORT levels indicate that animals are “stressed” and data are interpreted on the basis of this idea (see [Landys et al., 2006](#) and [Dickens and Romero, 2013](#)). It is not always easy to objectively determine whether this notion is present or absent in an article, though we found quite a few examples in which it is clearly absent. (6) CORT is mainly regarded as regulated solely by ACTH, with fewer studies recognizing other regulatory pathways, largely androgens and estrogens. (7) The proposal by [Sapolsky and colleagues \(2000\)](#) to study the permissive, preparative, stimulatory and suppressive roles of CORT has received very little attention, even though the review has received numerous citations to refer to basic aspects of CORT actions. This observation probably reflects the fact that novel approaches need plenty of time to penetrate in the theoretical framework of the field, especially when they are highly integrative, as is the case of that review. (8) We suggest that the permissive and preparative actions of CORT might serve, in part, to decrease the likelihood of experiencing stressors. This can be seen as an extension of the Preparative Hypothesis ([Romero, 2002](#)). However, the present proposal implies a significant change

in the way we think about the relationship between CORT and stressors, contrasting with the view that CORT is positively associated with the presence stressors or the likelihood of experiencing stressors (the original Preparative Hypothesis, [Romero, 2002](#)).

*General and Comparative Endocrinology* and *Hormones and Behaviors* were rather similar with regard to the general patterns above-mentioned, with relatively small differences related to their differences in scopes (Fig. 2). In these regard, HB has a clear emphasis in the relationship between CORT and social interactions, which was not observed in GCE. Also, the trends observed were similar for the different vertebrate classes. Perhaps, the most noticeable difference is that the orexigenic roles of CORT are being currently studied (i.e., collection of data) in birds much more than in the other classes. Because reptiles, amphibians and fish were less represented in the analysis, we evaluated the general trends pooling the articles about these three classes (n = 14), and we found that they are very similar to those observed for birds and mammals (F. Vera, personal observation, see [Supplementary Material](#)). The two exceptions are that mineralocorticoid roles were considered in two out of five studies in fish and the review by [Sapolsky et al. \(2000\)](#) was only cited in one article ([Fischer et al., 2014](#), fish).

The present data show that our consideration of CORT is frequently too narrow. As plenty of data have been collected during the last two decades, a significant proportion no longer fits in the traditional way of thinking about CORT. Many issues illustrate this: the relationship between CORT and fitness is too variable ([Bonier et al., 2009](#)), the responses of CORT to chronic stressors can include increases, decreases and no-response ([Dickens and Romero, 2013](#)), increases in CORT (or differences among individual categories) do not necessarily indicate the presence of stress, as baseline CORT levels also play crucial regulatory roles ([Landys et al., 2006](#); [Romero et al., 2009](#)). There are many regulatory pathways for CORT secretion other than the classical regulation by ACTH, which are rarely considered ([Bornstein and Chrousos, 1999](#); [Bornstein et al., 2008](#)). Bornstein and Chrousos (1999) have suggested that contradictory results are a consequence of treating the physiological markers as independent indicators of some aspects of functioning, rather than as regulatory components of a unified system responsible for maintaining homeostasis (Physiological Regulatory Networks). In agreement with the latter, we suggest that part of the inconsistent results about CORT dynamics in free-living vertebrates might be the consequence of traditionally focusing them with a marked bias on one -or a few- aspects of CORT actions.

Enriching our view about CORT actions beyond the classical paradigm will help to integrate information and have a wider perspective on the reasons explaining CORT dynamics. Though the classical approach has been undoubtedly very fruitful and has produced much valuable information, we seem to have arrived to a dead end. In other words, the traditional view of CORT as “stress hormones, regulated by ACTH, that indicate the presence of physiological stress when their concentrations are augmented” is currently too restrictive. Thus, we need to enrich and expand our way of thinking about CORT actions, and there are several papers already going in this direction ([Sapolsky et al., 2000](#); [Landys et al., 2006](#); [Romero et al., 2009](#); [Dickens and Romero, 2013](#), among others).

There seems to be a confusion related to the fact that the actions exerted by CORT depend on both the target tissue and the type of receptor bound (type I and type II receptors). It is critical to identify the context in which CORT variations occur (e.g., response to predictable life-history process or response to unpredictable, potentially life-threatening challenge). The effect of an increase in CORT in one or another context can be expected to vary. For instance, increases in baseline CORT might favor reproductive activity, while stressor-induced CORT inhibit reproductive activity

(Sapolsky et al., 2000; Landys et al., 2006). This is related to the fact that baseline CORT primarily acts through the type I receptor, while stressor-induced CORT are able to activate the type II receptor (Sapolsky et al., 2000; Landys et al., 2006).

At first sight, it might be assumed that the previously described issues take away some of the importance of studying CORT dynamics in wild vertebrates, because the attractive idea of reducing stress levels to CORT levels is recognized as too simplistic (note: we acknowledge that it sometimes works). However, these challenges represent a valuable opportunity to explore other less considered actions of CORT and think about these hormones with different perspectives. Not only in science, but in every other area of interest, thinking about a problem from different perspectives is generally more recommended than focusing on a single, or a few points of view. We recognize that the data presented here and in previous reviews show that it is time to incorporate other actions of CORT more frequently in our theoretical framework and study goals. Hopefully, these steps will lead to an enriching integration of information in the future.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ygcen.2017.01.010>.

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