

# Conservation Endocrinology

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*Endocrinologists can make significant contributions to conservation biology by helping to understand the mechanisms by which organisms cope with changing environments. Field endocrine techniques have advanced rapidly in recent years and can provide substantial information on the growth, stress, and reproductive status of individual animals, thereby providing insight into current and future responses of populations to changes in the environment. Environmental stressors and reproductive status can be detected nonlethally by measuring a number of endocrine-related endpoints, including steroids in plasma, living and nonliving tissue, urine, and feces. Information on the environmental or endocrine requirements of individual species for normal growth, development, and reproduction will provide critical information for species and ecosystem conservation. For many taxa, basic information on endocrinology is lacking, and advances in conservation endocrinology will require approaches that are both “basic” and “applied” and include integration of laboratory and field approaches.*

*Keywords: hormones, endangered, stress, physiology, ecosystem*

**T**he conservation and restoration of species and ecosystems are topics of great interest to the public and form some of the most important scientific challenges of our time. Although scientists (and others) have addressed conservation and restoration from a number of disciplines for many years, in 1985, the Society for Conservation Biology was founded to provide a focal point for conservation work in the life sciences. The field of conservation biology is one of the fastest growing in biology. It is goal oriented, focused on the preservation of species and their habitats and the determination of natural and human impacts on population numbers. A major problem, however, is the *post hoc* nature of connecting disturbances with population numbers. As a consequence, substantial work in conservation biology has focused on determining methods and techniques that can discover at-risk populations and take corrective actions.

Conservation biologists have traditionally relied on established principles of ecology, and especially the development of mathematical population models, to predict the impacts of various human disturbances. Recently, some attention has shifted to the organismal biology of the species themselves. The approach is to use physiological responses as a predictor of population declines. A number of physiological systems have been studied, including reproduction, migration, immune responses, energy regulation, thermal tolerances, and body condition, and this growing body of work has been termed *conservation physiology* (Wikelski and Cooke 2006). One of the newest emerging subdisciplines in conservation biology is conservation endocrinology (Cockrem 2005, Walker et al. 2005). Although *environmental endocrinology* (assessing how the environment affects the endocrine system in both field and laboratory conditions) has an older

history, conservation endocrinology is a burgeoning field attracting numerous young investigators and is currently being applied to many traditional conservation concerns, including monitoring of wild populations, captive breeding, and reintroductions.

The endocrine system functions to communicate and coordinate internal development, homeostasis, and response to environmental change. Hormones, by definition, are secreted in one part of the body and travel through the blood to target tissues where they have physiologically relevant actions by acting through specific receptors (figure 1). Many hormones have specific releasing or inhibiting factors that promote or inhibit their secretion, binding proteins in blood and tissues that alter hormone longevity and availability to receptors, membrane transporters, and feedback systems that provide mechanisms for regulation within and among endocrine axes. The effects of hormones include relatively rapid actions such as the activation of existing proteins (seconds to minutes), the stimulation of transcription of specific genes and subsequent increase in protein abundance (minutes to hours), or proliferation and differentiation programs of cells and tissues (hours to days; McCormick and Bradshaw 2006).

The development of specific antibodies to hormones and radiolabeling techniques in the 1950s allowed for the precise measurement of the very low levels of hormones that exist in the blood (de Pablo et al. 1993). Today, there are a large number of approaches that measure critical endocrine endpoints, including radioreceptor assays for characterizing receptor binding, western blots or enzyme immunoassays for measuring protein abundance, and immunohistochemistry and *in situ* hybridization for localization and quantitation

of hormone-producing cells or their targets (de Pablo et al. 1993). Molecular approaches have taken on increasing importance in endocrinology, although it should be noted these are indirect measures of the hormones and receptors that actually carry out endocrine signaling.

The endocrine system is an attractive target for conservation research because hormones often control the reactions of physiological and behavioral systems to environmental change. Consequently, understanding how the endocrine system functions in the adaptation to anthropogenic changes will be a major component in conservation decisions. Similar to conservation biology as a whole, conservation endocrinology differs from the broader field of endocrinology by having a direct application to the conservation of species. In the following sections, we will detail and provide examples of how endocrinology can be used to understand and promote the conservation of individuals, species, and even ecosystems. However, basic research is often necessary as a foundation for application of endocrinology to conservation goals. This basic effort is especially robust among comparative endocrinologists, many of whom have been making contributions to conservation biology for many years by determining how hormones mediate physiological responses that affect the ability of animals to survive, grow, and reproduce in a changing environment. The purpose of this article is to provide an overview of endocrine systems, to offer examples of current work in conservation endocrinology, and to suggest future directions. Because of the focus of conservation efforts and our own research interests on large animals, there is a bias in the present article on vertebrates, although many of the principles and approaches will be similar for invertebrates.

### Endocrine systems

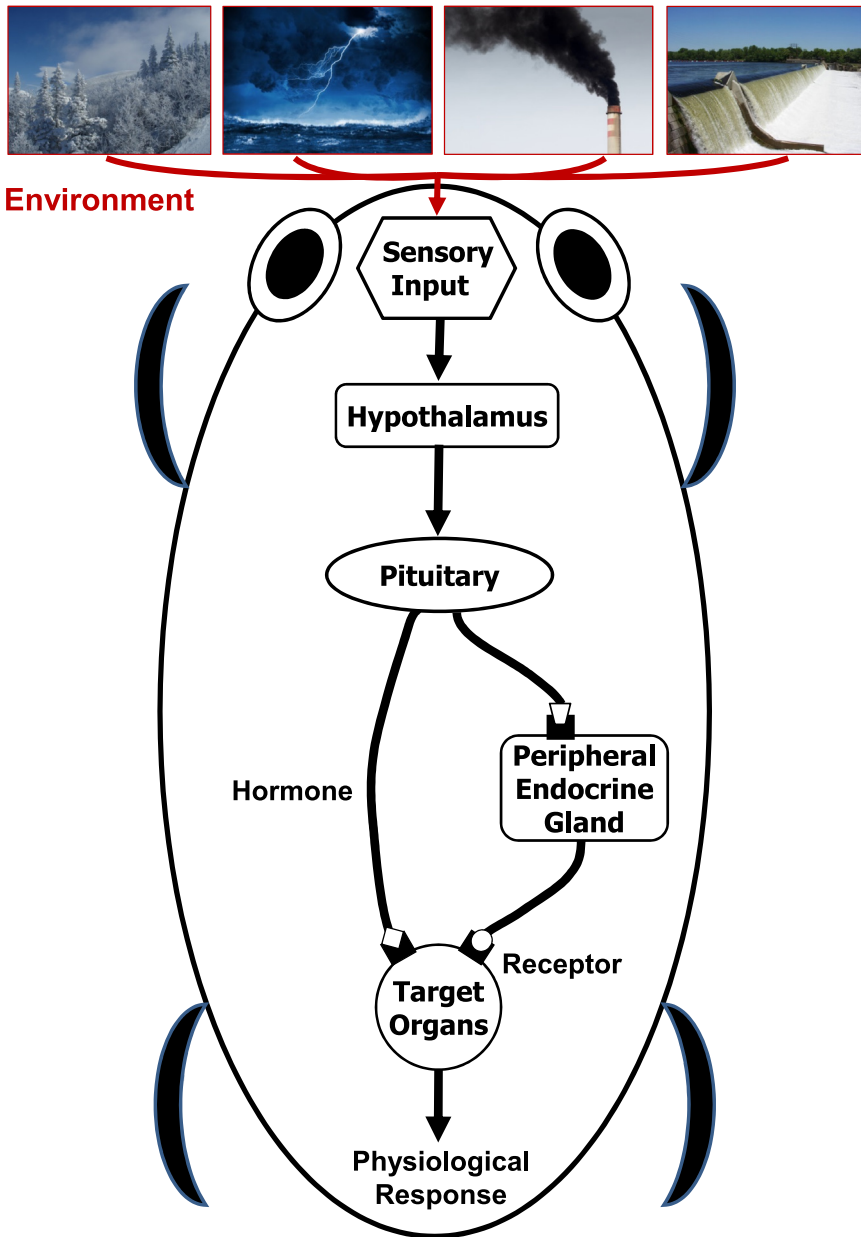
In the following sections we review how hormones control a number of physiological processes that are linked to survival, growth, and reproduction.

**Growth and metabolism.** Among vertebrates, the growth hormone (GH)/insulin-like growth factor I (IGF-I) axis is highly conserved and the major endocrine system regulating growth (Wood et al. 2005). A large number of hypothalamic factors influence the release of GH from the pituitary, and many are undoubtedly involved in environmental signaling. Circulating GH causes the release of insulin-like growth factor I (IGF-I) from the liver (the major source of circulating IGF-I), as well as production in target tissues. Multiple binding proteins are especially important for the regulation of IGF-I function in vertebrates and have distinct regulation and action (Kelley et al. 1996). Endocrine and paracrine (produced by the target tissue) actions of IGF-I carry out most of the growth promoting actions of GH, but GH has multiple pathways for regulating anabolic metabolism. Many endocrine axes also interact with the GH/IGF-I axis, and in particular, insulin, thyroid hormones, and corticosteroids have important roles in regulating growth and metabolism.

In primates, there is a general decline in circulating GH with age and therefore a generally positive correlation with growth rate. In teleost fish, however, growth rate is often negatively correlated with GH and positively correlated with IGF-I (Björnsson 1997). This is likely due to the downregulation of GH receptors in the liver during periods of reduced growth, which results in lower production of IGF-I that in turn leads to reduced negative feedback to the pituitary and higher GH levels. Such *GH resistance* may be a characteristic of poikilotherms in which temperature and food availability make for highly variable and/or seasonally driven growth conditions.

The importance of the GH/IGF-I axis in regulating growth and the apparent correlation between individual growth rate and circulating levels of these hormones suggest their use in monitoring growth and the environmental conditions appropriate for growth. Such an approach is already being used in the commercial aquaculture of fishes and should be applicable to the captive rearing of threatened or endangered species. It may also be possible to use circulating levels of GH/IGF-I and/or associated binding proteins as indicators of growth conditions of animals in the wild and how they may be affected by natural and anthropogenic environmental changes, but to date, there is very limited information on levels of circulating GH and IGF-I in wild animals. A strong association between plasma IGF-I levels and growth in some (but not all) fish species suggests that IGF-I may be the most likely endocrine indicator of growth (Beckman 2011). A positive relationship between circulating IGF-I levels and growth as a function of favorable habitats in snakes (Sparkman et al. 2009) and fish (Bond et al. 2014) suggests that IGF-I could be used to determine habitat suitability and critical life-history decisions (Dantzer and Swanson 2012). Plasma IGF-I levels differ in fish stocks in selection simulating fishing pressure on larger individuals (Duffy et al. 2013), suggesting that circulating levels of this hormone could also be used to monitor the long-term impacts of human harvest on fish populations. It will be important to account for species-specific impacts of seasonality, food availability, temperature, and other environmental factors that may regulate these hormones in order to interpret differences among wild animals.

**Reproduction.** The endocrine control of reproduction in vertebrates consists of gonadotropin-releasing hormone produced in the brain, which stimulates gonadotropic hormones (follicle stimulating hormone and luteinizing hormone) in the pituitary, and in turn stimulate sex steroid production by the gonads. Reproductive hormones have been targets for conservation studies for many years. Perhaps the most common purpose for reproductive hormone studies is in captive breeding. Zoos, aquaria, and wildlife sanctuaries maintain many species that are endangered, threatened, or even extirpated in the wild (e.g., Gibbons et al. 1995). Helping maintain healthy wild populations of these species is often part of the missions of these organizations. Healthy captive



**Figure 1.** A schematic representation of the integration of environmental and developmental signaling by the neuroendocrine system in the imaginary chimera, the bugfibian. External or internal changes are perceived by the brain, which results in the release of neuroendocrine factors that cause the release of hormones from the pituitary and/or peripheral endocrine glands, such as the thyroid or adrenal. These hormones travel in the blood and bind to and activate specific receptors at the cell surface or within the cell, which in turn activate (or deactivate) proteins and/or gene expression, resulting in coordinated and adaptive physiological responses. Not included in this simplified figure are other complexities of the endocrine system, such as circulating binding proteins, second messenger systems, and feedback pathways. The photos at the top represent natural and anthropogenic environmental stimuli. (Photograph credits from left to right: SD McCormick, Sergey Niven, Martin Mojziv, SD McCormick.)

populations, however, require successful breeding, either for propagation of the species in captivity or to produce a pool of individuals for release back into the wild. This creates a large need for monitoring reproductive hormone cycles and, in some cases, for providing exogenous reproductive hormones to stimulate and support successful breeding. Research in this area has become a branch of clinical veterinary medicine, in which the goal is the treatment of individual animals, but also has significant implications for the maintenance of species when only few individuals remain.

Reproductive hormones are also important to measure under field conditions. It is often unclear why a threatened population is declining, but one potential reason is reproductive failure. Monitoring reproductive hormone cycles of individuals in the population can help determine whether successful reproduction is occurring and may be especially important for animals that are difficult to breed under captive conditions. In addition, measuring reproductive hormones, such as testosterone, estradiol, and progesterone, can help assess the number of reproductive individuals in the population, the age of reproductive maturation, the timing of breeding, and ultimate breeding success. Furthermore, these data can be used to determine vital rates, such as the age of first reproduction, which are critical for robust population viability analyses (PVA), which attempt to estimate the likelihood of a population going extinct (Reed et al. 2002).

**Life-history transitions and migration.** Postnatal and posthatching development—especially rapid changes in morphology, physiology, and behavior, such as those that occur in metamorphic events—are driven by hormones. Thyroid hormones have been shown to drive metamorphic events in most vertebrates, including the tadpole-to-frog and flatfish metamorphoses (Denver 2008). Thyroid hormones also appear to be critical to the larval–juvenile transition that occurs in most teleost fishes (Brown

1997). Sea lamprey are a basal vertebrate that undergoes a dramatic metamorphosis associated with downstream migration and adoption of a parasitic lifestyle, but in these fish, thyroid hormones actually inhibit metamorphosis, which does not proceed until circulating levels decrease (Youson et al. 1997). The complex series of metamorphic stages that characterize development in many insects (*ecdysis*) are controlled by a complex interaction of ecdysone and juvenile hormones that are stimulatory and inhibitory, respectively (Zitnan et al. 2007).

Corticosteroids may play a broad role in modulating the timing of many different life-history transitions in vertebrates (Crespi et al. 2015). Cortisol can play a stimulatory role in vertebrate metamorphoses by interacting at several levels of thyroid hormone regulation and action. For instance, stress events such as crowding and dehydration can hasten frog metamorphosis through the hypothalamic release of corticotropin-releasing factor, which results in increased circulating levels of thyroid hormones. The parr-smolt transformation is a series of morphological, behavioral, and physiological changes that occur during the downstream migration of juvenile salmon and are adaptive for seawater entry. Cortisol, growth hormone, and insulin-like growth factor I interact to increase salinity tolerance, which is critical for the development of salinity tolerance of smolts (McCormick 2013). Because of its strong positive association with growth rate, it has been proposed that IGF-I in vertebrates regulates (and therefore can be used to detect) a variety of life-history variations, including adult body size, growth rate, basal metabolic rate, and timing of reproduction (Dantzer and Swanson 2012).

Physiological changes associated with metamorphosis and/or habitat shifts for growth and reproduction are also critically controlled by hormones. Lipid accumulation can be important for life-history decisions (e.g., metamorphosis and reproduction) as well as survival when experiencing severe conditions such as overwintering and dramatic niche shifts during migration. There is a loss of fat content just prior to the downstream migration of salmon, perhaps reflective of the metabolic demands of a higher metabolic rate and the physiological changes that are preparatory for seawater entry (McCormick 2013). The metabolic changes that occur during the downstream migration of salmon appear to be largely driven by increased levels of thyroid hormones, growth hormone, and cortisol, and circulating levels of all of these hormones increase coincident with downstream migration (McCormick 2013). In contrast, there is an increase in fat accumulation in birds prior to migration, and thyroid hormones appear to control increased aerobic scope, whereas corticosteroids may control the increased use of metabolic fuels in migratory birds (Cornelius et al. 2013).

Although we have some understanding of the endocrine control of physiological changes that occur during migration, we have surprisingly little understanding of the neuroendocrine changes that likely underpin the behavioral changes during migration (Ramenofsky and Wingfield

2013). Prolactin is apparently causal to the water-seeking behavior of spawning salamanders known as *water drive*. The downstream migration of juvenile salmon was thought to be regulated by thyroid hormones because of the consistent elevation of circulating levels during migration, but this now appears to be a response of the thyroid axes to migration rather than causal to it (McCormick 2013). Nonetheless, thyroid hormones may have an important role in the odorant imprinting that occurs during downstream migration and return of adults to their natal stream. The downstream migration of salmon may involve the secretion of hypothalamic factors such as corticotropin-releasing factor and growth-hormone-releasing hormone, although much more research is needed to understand these control mechanisms. Seasonal or daily migrations will be driven by interaction with circannual and diel timing mechanisms (Ramenofsky and Wingfield 2013), although these also have been largely unexamined. There is evidence that the aerobic capacity of salmon during long migration at high temperatures is population specific and has been shaped by selection on the neuroendocrine system. Adults from upstream populations of sockeye salmon on the Fraser River have greater aerobic capacity at high temperature, and this trait is related to greater heart size and density of ventricular B-adrenoreceptors (Eliason et al. 2011). These evolutionary adaptations have important implications for the capacity of species and populations to respond to temperature and hydrological changes imposed by climate change and the influence of dams and fish passage on population sustainability.

Migration is especially prone to disruption by human activity because it often covers vast territories of land or water that can be difficult to safeguard or conserve (Wilcove 2012). For diadromous fishes that migrate between freshwater and seawater, large rivers and estuarine transition zones are often the most heavily affected by human activity, including dams, dredging, shipping activity, and pollution (Limburg and Waldman 2009). Measurement of circulating cortisol and estradiol levels in upstream-migrating anadromous fish can be used to demonstrate the types of fish ladders that negatively affect migration and reproductive success, whereas measurement of hormones during downstream migration can demonstrate the impact of contaminants and delays in migration on the capacity for survival in seawater. Mortality rates during migration may be inherently high and therefore critical to population persistence while at the same time presenting high susceptibility to external stressors. To date, there has been relatively little use of hormones in conservation of migration, in part because of the inherent difficulty of sampling animals that are on the move. Nonetheless, technical advances in tagging technology that include physiological and endocrine measurement and increased understanding of the neuroendocrine and physiological changes that occur normally and are associated with fitness in new habitats may help monitor anthropogenic influences that negatively affect survival during migration.

**Homeostasis.** Because of their capacity to sense and coordinate throughout the body, hormones are often involved in *homeostasis*, which is the maintenance of internal conditions such as ions, pH, metabolites, and temperature. Homeostasis is critical to normal physiological function, especially in the face of changing environmental conditions (figure 1). This is especially true for the regulation of extracellular water and electrolyte balance, which involves a number of hormone systems that respond to internal and external changes over a wide range of response periods (McCormick and Bradshaw 2006). Failure to maintain water and electrolyte balance will result in loss of cellular function, nerve signaling, muscle contraction, eventual loss of performance, and death. In most vertebrates, the kidney and gut are primarily involved in overall salt and water balance; in adult fish, the gill also has a prominent role, and in larval fish and amphibians, the skin is important.

A thorough review of the hormone systems involved in osmoregulation, even just in vertebrates, would be beyond the scope possible in this article, so only major pathways will be mentioned here. Corticosteroids in fish are involved in both ion uptake in freshwater and salt secretion in seawater, and for euryhaline (able to survive in freshwater or seawater) species, prolactin is involved in the former and growth hormone in the latter (McCormick and Bradshaw 2006). In birds and mammals, two corticosteroids have distinct but overlapping function in regulating metabolism (primarily cortisol or corticosterone, depending on the species) and promotion of sodium and water retention (aldosterone). Atrial natriuretic peptides are released by the heart and act on the kidney to promote sodium excretion.

In fish, arginine vasotocin appears to be involved in the promotion of salt secretion by the gill, whereas in mammals, arginine (or lysine) vasopressin acts at the kidney to promote water retention (McCormick and Bradshaw 2006). There is an important interaction between fluid regulation and temperature in most terrestrial vertebrates, and AVT has been shown to control behavioral changes in temperature preference during reduced water availability (Bradshaw 2007). Intracellular concentrations of calcium are very low, and extracellular levels of calcium in most vertebrates are regulated very tightly. As might be expected for such tight regulation, several endocrine systems are involved in the regulation of calcium. Calcitriol (vitamin D3) acts to increase intestinal absorption of calcium and reabsorption by the kidney, a function that appears conserved among most vertebrates. Parathyroid hormone also increases circulating calcium by increasing bone resorption, renal reabsorption and calcitriol levels. Calcitonin has effects in the intestine and kidney of fishes and birds to lower plasma calcium levels, although its importance in mammals is less certain.

The capacity to maintain fluid and ion balance in the face of changes in water availability, humidity, and salinity is species and often population dependent. In many cases, there is an acclimation response that controls the ability of animals

to withstand future homeostatic challenges. This phenotypic plasticity may in part be controlled by hormones, and although the evolution of plasticity and endocrine systems has been recognized as important (Pigliucci 2001), it has to date not received substantial attention. Nonetheless, a thorough understanding of physiological capacities and their hormonal control will be useful tools in predicting and mitigating how human activity alters landscapes and waterscapes. Measurement of lysine vasopressin in wallabies in the wild has demonstrated important species differences in vasopressin function and its possible use to detect susceptibility to periods of limited precipitation, which are likely to increase in the face of projected climate change in Australia and other areas around the world (Bradshaw 2007).

Regulation of body temperature is critical to the survival and function of endotherms, primarily represented by birds and mammals. Mammals possess a unique thermogenic brown adipose tissue that may underlie some of their evolutionary success. The thermogenic process is stimulated by norepinephrine through  $\beta$ 3-adrenergic receptors and can be inhibited through  $\alpha$ 2-adrenergic pathways. Thyroid hormones are broadly important in controlling metabolic rate in vertebrates, and in endotherms, thyroid hormones can increase cellular metabolic rate and increase body temperature, although this latter effect appears to be largely independent of impacts on brown adipose tissue. Although the neuroendocrine control of hibernation is complex and far from being fully resolved, measurement of hormones (such as leptin) that are involved in feeding behavior and other aspects of hibernation should be useful in tracking the phenology of hibernation that may change in response to altered climate.

**Stress.** One physiological system that is particularly attractive for conservation endocrinology is the stress system (Hofer and East 1998). The endocrine stress-response systems are intimately connected with health, and high levels of stress mediators often anticipate impacts on health. Furthermore, the environment is full of *stressors* (those things that elicit a stress response), such as storms and predators, which can act additively or even synergistically with anthropogenic stressors. Although the concept of stress and its negative and positive aspects are widely recognized, defining physiological stress and recognizing its impact on individuals and populations can be problematic. In fact, there is no universally accepted definition of stress, so different researchers may come to different conclusions when studying the same phenomena. For example, many researchers will reference the “stress of reproduction,” whereas many others argue that a normal life-history event, such as reproduction, is not a stressor regardless of how energy intensive the activity is. This problem has seriously hindered the application of stress research, including in a conservation context (Romero and Wingfield 2016). Recent work in medicine, however, has begun to refine the concept of stress. A major advance has been the introduction of the concept of allostasis (Sterling

and Eyer 1988) and its extension to encompass wild animals (McEwen and Wingfield 2003, Wingfield 2005). Briefly, *allostasis* is the maintenance of homeostasis through change. Homeostasis is regulated through allostatic mediators, and the major classes of these mediators are the hormones involved in the vertebrate stress response: glucocorticoids and catecholamines. In response to a perceived stressor, corticotropin-releasing hormone is released from the brain, which stimulates adrenocorticotropic hormone release from the pituitary, which in turn stimulates glucocorticoid release from the adrenal gland (or interrenals in the case of fishes). One key concept is *allostatic load* (McEwen and Wingfield 2003), which is the integration of all the energetically costly activities the animal is currently engaged in. For example, pregnancy requires extra energy, so a pregnant female has a higher allostatic load than a nonpregnant female of reproductive age. The allostatic load on that pregnant female would be even higher if it were simultaneously using more energy to fight off an infection. Quantifying allostatic load is an area of ongoing research, but current approaches include measurement of metabolic rate (including oxygen consumption and heart rate), as well as hormone and tissue levels of glucocorticoids and catecholamines.

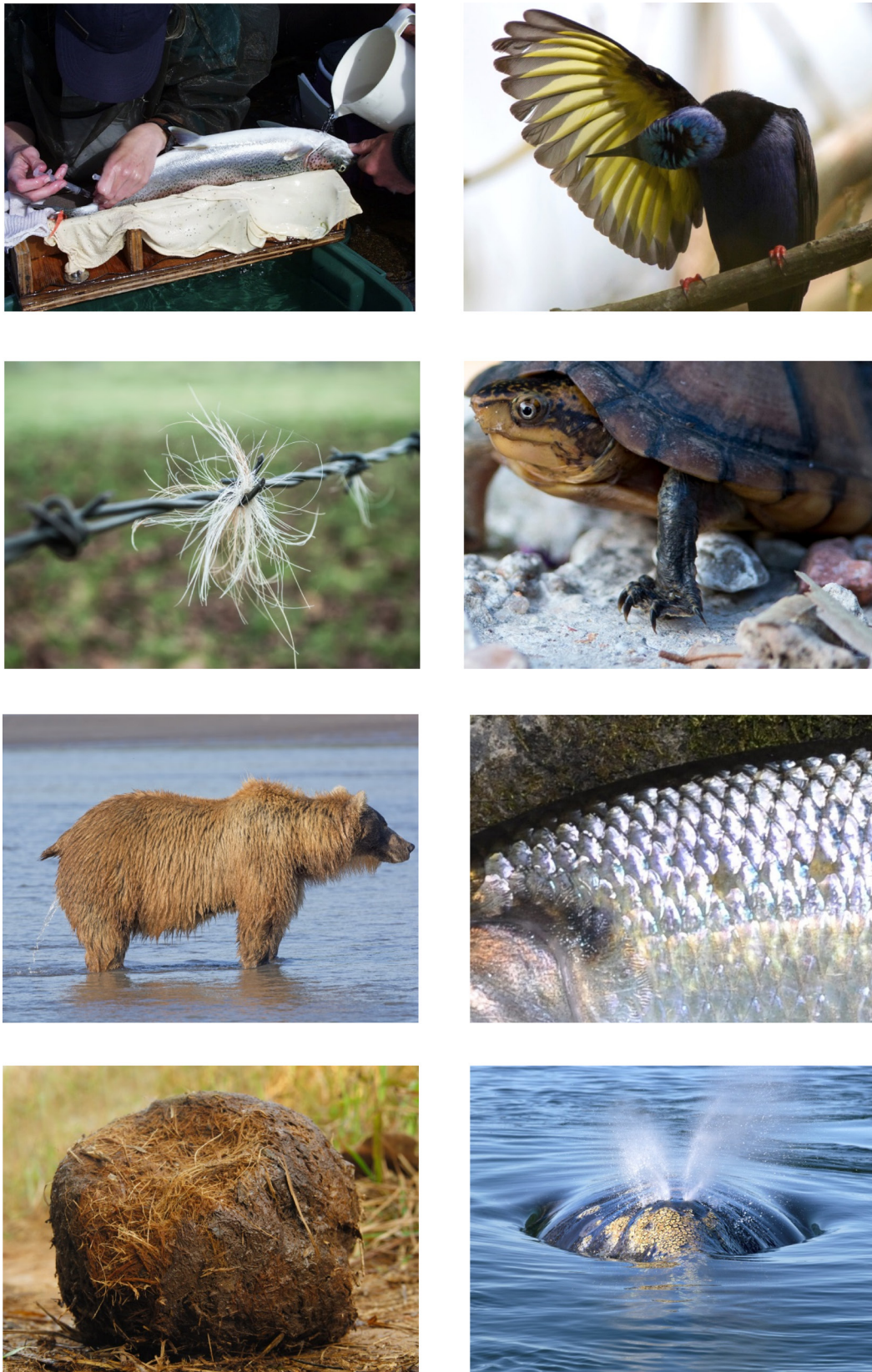
Allostatic load is not, in and of itself, problematic to the animal. If sufficient energy is available, either through internal stores or through foraging, then the animal is able to adequately cope with all these demands. However, if there is insufficient available energy, the animal enters allostatic overload (McEwen and Wingfield 2003). The reactive-scope model (Romero et al. 2009) expanded this concept to incorporate other mediators of homeostasis. Allostatic load therefore encompasses the idea that stressors can have additive effects on an animal. Although an animal might easily accommodate the increase in allostatic load from an infection, additional allostatic load from an anthropogenic stressor, such as habitat degradation, might push the animal into allostatic overload. Once allostatic overload is reached, one of two things will happen: Either the animal will decrease its allostatic load by entering an emergency life-history stage (e.g., by aborting the pregnancy; Wingfield 2005), or the allostatic mediators will start to create pathology by exceeding the animal's normal reactive scope in what has previously been referred to as *chronic stress* (Romero et al. 2009).

If animals are in allostatic overload and exceed their normal reactive scope, they are likely to face lower survival and/or reproductive success. Finding an individual in allostatic overload is, therefore, a major predictor of the overall health of the animal. This is essentially what physicians do when they diagnose stress-related pathologies in humans: They are connecting the allostatic (stress) mediators with greater future risk of disease. The rationale for using stress mediators in a conservation context is similar. Demonstrating allostatic overload in individual animals exposed to human disturbances indicates decreased overall health of the animal and a resultant decrease in survival and/or reproductive

success. The aggregate of the health of the individuals in a population should provide an indication of the health of the population as a whole because overall population numbers reflect the summation of the survival and reproduction of each individual within that population. Consequently, the stress response of (many) individual animals has the potential to be used as a predictor of impact of environmental change on population numbers (Homan et al. 2003, Wikelski and Cooke 2006, Romero and Wingfield 2016). Currently, this relationship is still hypothetical, but it is critical to the emerging field of conservation endocrinology and requires further attention.

Using stress mediators in a conservation context, however, requires addressing a serious potential confounding response. Animals often habituate to repeated stressors, so a proper interpretation of changes in stress mediators such as glucocorticoids relies on assessing the degree of potential habituation to anthropogenic stressors. The term *habituation* usually implies a change in whether the animal interprets that a specific stimulus is a stressor. In other words, the individual becomes familiar with the stimulus, learns that it is not harmful, and therefore no longer interprets the stimulus to be a stressor (Cyr and Romero 2009). This results in the decrease and ultimately cessation of glucocorticoid release in response to that stimulus. Consequently, the lack of a glucocorticoid response is powerful evidence that anthropogenic disturbance is not being interpreted as a stressor and therefore not adding to allostatic load.

Unfortunately, recategorizing a stimulus from "stressor" to "innocuous" is not the only reason for a decrease in glucocorticoid release. Habituation is an important concept in assessing the impact of anthropogenic disturbances, but there is no standard definition of habituation for wild animals (Cyr and Romero 2009). As we noted above, an animal with an attenuated stress response is not necessarily habituated. In fact, because the stress response is thought to help an animal survive stressors, an inability to mount a proper stress response also likely decreases survival and fitness. However, it is difficult to observe the habituation process in the field, where a gradual decline in responses can be easily missed. A proper diagnosis of habituation would be a critical tool for the conservation endocrinologist monitoring glucocorticoid levels, and refining such a diagnosis should be a major emphasis for future work. There are a number of experimental designs that can be used to diagnose habituation in a conservation context (see table 3 in Cyr and Romero 2009). These should test whether the reduction in the stress response is generalized or specific to the stressor of interest. Only an attenuated response to a specific anthropogenic stressor would indicate habituation because learning that a stimulus is not a stressor should only apply to that stimulus, not to the generalized response. Second, habituation should not negatively affect the overall health and reproductive performance of the animal. Finally, habituation will result in reduced responses to that stimulus but should not alter baseline hormone levels; baseline



**Figure 2.** Examples of biological material that can be used to examine the endocrine status of animals in the wild, starting from upper left: blood, feathers, hair (on barbed wire), claws (and possibly carapace), urine, scales, feces, and exhalations (e.g., whale blow). (Photograph credits from upper left: SD McCormick, Sean Werle, NeydtStock, Sean Werle, Wildnerpix, SD McCormick, Hugh Lansdown, Oksana Perkins)

glucocorticoid concentrations mediate different functions from stress-induced concentrations, and baseline functions should not be altered by habituation.

### Application of conservation endocrinology

In the following sections we review case studies in which endocrine techniques have been used to aid in species conservation.

**Ecotourism.** Stress physiology has been used in a number of conservation contexts, and the number of published studies is increasing rapidly. Two examples illustrate how stress physiology, specifically glucocorticoid measurements, have been used to address longstanding questions in conservation.

The first is in assessing the impact of ecotourism. *Ecotourism* refers to touring natural habitats in a manner meant to minimize ecological impact. There has been great interest recently in determining whether ecotourism activities are major sources of stress to the observed wildlife. Tourists can clearly affect the behavior (e.g., Steven et al. 2011) and physiology (e.g., Culik and Wilson 1995) of wildlife, with activities as seemingly benign as taking photographs affecting some species (Huang et al. 2011). As a consequence, conservationists have been interested in determining whether tourist disturbance has sufficient impact to potentially outweigh the benefits of ecotourism. A major burden for addressing this question has fallen on comparative endocrinologists measuring glucocorticoids in animals affected by ecotourism.

The effect of tourism as an anthropogenic stressor on wild species has been investigated extensively (Romero and Wingfield 2016). Many species show an attenuated stress response to humans. One early study showed that baseline corticosterone (the major glucocorticoid in birds) was significantly lower in Magellanic penguins visited by tourists than in unvisited birds (Fowler 1999). Furthermore, 5 minutes of human presence at the nest failed to elicit a corticosterone response. Considering that tourists rarely physically touch the birds, this lack of response is consistent with habituation to the tourists' presence. However, further data suggest that tourist-exposed Magellanic penguins have not fully habituated to humans (i.e., they still categorize human visitation as a stressor). Tourist-visited birds were incapable of secreting equivalent amounts of corticosterone (Walker et al. 2006) and had attenuated responses to other stressors (Walker et al. 2006), which disappeared in colonies with fewer tourists (Villanueva et al. 2011). The penguins had altered their physiological responses to tourist visitation but had not habituated. Wildlife managers at penguin colonies could then use this information to appropriately regulate tourist traffic.

One interesting conclusion arising from studies of ecotourism is that the impact is highly dependent on the specific tourist site and does not appear to generalize. For example, two studies on marine iguanas from the Galápagos

showed divergent responses: In one study, tourist-visited animals had a lower corticosteroid response than naïve animals (Romero and Wikelski 2002), whereas the exact opposite was found on a different island (French et al. 2010). In a further complication, the elevated corticosteroid response in the second study only occurred when the animals were not breeding; there were no differences between tourist-visited and naïve animals during the breeding season. One prime suspect for the differences between the two studies is the relative intensities of tourist visitation. We currently cannot predict whether different populations of animals will respond to tourism, but when there is a response, it likely indicates that the population is affected by tourist activity.

An important finding from the majority of these studies is that ecotourism has little effect on the baseline concentrations of glucocorticoids. Most studies indicate that ecotourism's major impact is in altering the glucocorticoid responses to additional stressors (Romero and Wingfield 2016). This suggests that ecotourism is not directly acting as a stressor to these animals. If it were, we would expect to find robust and persistent changes in baseline corticosteroid concentrations across studies. Instead, tourist visitation appears to alter an individual's ability to cope with additional stressors, but if those additional stressors are mild or do not occur, the overall impact on the animal will be negligible. Furthermore, an individual animal's sensitivity can depend on its age class, life-history stage, and intensity of visitation. These data therefore provide conservation managers more information to weigh the dangers to individual animals against the benefits of the education and economic activity that help preserve these species and their habitats.

The impact(s) of ecotourism is only one example in which stress endocrinology is currently being used in a conservation context. Other examples include translocation (e.g., Dickens et al. 2010), the impacts of urbanization (e.g., Bonier 2012), invasive species (e.g., Graham et al. 2012), marine noise (e.g., Rolland et al. 2012), various field-ecology techniques (e.g., Narayan et al. 2011), hunting (e.g., Mason 1998), habitat disturbance (e.g., Homan et al. 2003, Janin et al. 2012), and many others. The goal of most of these studies is to assess the allostatic load imparted by the anthropogenic disturbance, even if the researchers themselves did not frame the work in the context of allostasis or reactive scope. As is clear from these studies, monitoring stress mediators, especially glucocorticoids, has considerable promise in helping address long-standing conservation problems. Much of the previous work, however, suffers from the considerable weakness of not assessing the role of habituation. To solve this weakness, future work should address two fundamental issues: (1) validating that glucocorticoid levels are an adequate proxy for increased allostatic load or allostatic overload and (2) designing studies to expressly account for the potential of habituation. Conservation conclusions drawn from measuring stress hormones will be on a much stronger foundation once these weaknesses are addressed.



**Endocrine disruption.** Endocrine-disrupting compounds (EDCs) are contaminants that can interact with the endocrine system and negatively affect health, reproduction, or survival. Understanding and concern about EDCs is a relatively recent phenomenon, developing in the last three decades. As research in this area has increased, there is mounting evidence that EDCs can have effects on human and nonhuman animal health (Colborn et al. 1993, Guillette 2006). There is still substantial uncertainty and controversy about the extent of EDC impacts on fish and wildlife in nature. Nonetheless, concern about EDCs is substantial enough that many developed nations are requiring testing of new and existing chemicals for their endocrine-disrupting potential.

The mostly widely recognized EDCs are estrogenic compounds that interact with the estrogen receptor resulting in altered reproductive function in both sexes. A variety of compounds including estradiol (excreted by humans and animals), ethinyl estradiol (a component of birth control pills), and nonylphenol (used in plastic manufacturing) have found their way into many watersheds (Mills and Chichester 2005). These compounds can directly bind to estrogen receptors and augment or interfere with normal estrogen-signaling pathways. Aquatic organisms or those dependent on food from water may be exposed to low levels of these compounds throughout much of their lives, resulting in interference with normal reproduction. Exposure to estrogenic compounds can result in upregulation of vitellogenin production (normally produced only by females during reproductive maturation), which has become a widely used biomarker (Jones et al. 2000). Males may also produce eggs in response to estrogen exposure, and this phenomenon of “intersex” has been found in fish species in several river systems (Blazer et al. 2007). Early developmental exposures at relatively low concentrations can have long-term effects on individuals and even transgenerational impacts, possibly through epigenetic mechanisms (Schwindt et al. 2014). These compounds are present in many freshwater (and even marine) ecosystems at concentrations that are sufficient to fish reproduction (Sumpter 1995, Kolpin et al. 2002). Experiments on whole lakes indicate that environmentally relevant levels of ethinyl estradiol can cause extirpation of fish populations and that the response differs among species (Kidd et al. 2007). The extent to which estrogenic compounds affect animal populations in nature is still an area of some uncertainty (Hamilton PB et al. 2014), as is the possible impact of EDCs in the environment on human health.

Other endocrine axes are susceptible to disruption by environmental contaminants. Synthetic androgens are widely used in feedlots for beef production, find their way into streams and ponds, and through interaction with androgen receptors can have negative effect on male and female reproduction. Other compounds such as the fungicide vinclozolin and p,p'-DDE, a persistent metabolite of DDT, can also have antiandrogenic effects. Thiocyanate, nitrate, and perchlorate interfere with iodine uptake and thyroid hormone production, although their impact at environmental levels

is unclear (Zoeller et al. 2002). Polychlorinated biphenyls (PCBs) and the flame retardant polybrominated diphenylethers (PBDEs) can interact with the thyroid hormone receptor and may affect thyroid pathways of brain development, even in the absence of detectable effects on circulating thyroid hormones levels (Zoeller et al. 2002). The pesticide atrazine, which has been shown to cause hermaphroditism in frogs (Hayes et al. 2002) and reproductive failure in fish (Tillitt et al. 2010), may exert its effects by inhibiting aromatase activity, thereby affecting estrogen production at critical developmental stages. A variety of compounds that activate the arylhydrocarbon receptor, such as PCBs, have been shown to decrease the capacity of fish to produce cortisol production following a stressor. The fact that hormone receptor binding is not a necessary step in endocrine disruption indicates that any number of endocrine pathways (e.g., hormone production, hormone-binding protein interactions, and hormone clearance) may be susceptible to interference and have impacts on reproductive success, homeostasis, and survival.

Reproduction is by far the most widely examined endpoint for the impact of endocrine disruptors. It will be important to examine other physiological and behavioral pathways for the impact of EDCs. Recent research indicates that the immune response is susceptible to interference by a number of contaminants, some of which are likely acting through endocrine pathways (Mokarizadeh et al. 2015). Interferences with normal homeostatic mechanisms, metabolism, and growth are areas that have received limited research. In salmon, the behavioral and physiological changes that are preparatory for seawater entry (known as *smolting*) can be compromised by exposure to estrogenic compounds (McCormick 2013). Exposure of recently hatched salmon to estrogen or nonylphenol for only 21 days compromises smolt development a full year later (Lerner et al. 2007), indicating a possible epigenetic effect. Developmental differences in sensitivity to EDCs are also an area requiring more research, particularly with regard to early developmental stages when sex determination is taking place (Ammara and Randhir 2016). The effect of DDT on eggshell thinning that caused local extirpations of many fish-eating birds in the 1970s may have been the result of early (*in ovo*) exposure and estrogenic effects resulting in compromised function of the eggshell gland of adult females (Colborn et al. 1993). The possible impact of pharmaceuticals and other emerging contaminants on endocrine pathways in invertebrates, fish, and wildlife is also poorly understood (Ammara and Randhir 2016).

In the United States, the Environmental Protection Agency will soon require that all of the 87,000 chemicals currently in use and any new chemicals be tested for their endocrine-disrupting potential (Schapaugh et al. 2015). The proposed tests known as the Endocrine Disruptor Screening Program will focus on the ability of chemicals to interfere with the estrogen, androgen and thyroid axes. The recommended tier 1 screening will include *in vitro* assays

for alteration of estrogen and androgen receptor binding, steroidogenesis and aromatase activity, and *in vivo* assays for fish and rat reproductive development and amphibian metamorphosis. These are certainly laudable goals, although it should be remembered that such assays are less likely to detect the potential for impacts on invertebrates, effects on endocrine systems other than the sex steroid and thyroid axes, and endpoints other than vertebrate reproduction and amphibian metamorphosis. In addition to identifying new chemicals, a number of strategies have been developed to reduce existing levels of EDCs entering aquatic ecosystems (Ammara and Randhir 2016).

**Endocrine intervention.** One major problem in conservation is what to do with nuisance vertebrate species whose populations have exceeded environmentally sustainable levels, largely because of human removal of predators. The traditional solution has been to lethally remove sufficient animals to keep the population at an appropriate level. For many charismatic species, however, lethal removal is socially and politically difficult. The public is generally against the widespread killing of species such as deer and feral cats, even though these animals can be destructive to economic activities and natural habitats.

A potential solution to this problem is the use of contraceptives to disrupt breeding. Just as for human contraceptives, the goal is to disrupt the endocrine system that regulates reproduction. There have been two general approaches (Fagerstone et al. 2010). One is to use exogenous hormones, usually steroids, to stimulate negative feedback on the hypothalamic–pituitary–gonadal axis so as to disrupt normal release of gonadotropin releasing hormone (GnRH). These techniques are similar to those used in human medicine and often are the same compound. The second approach is to inject an anti-GnRH vaccine that will disrupt interaction of GnRH with its pituitary receptors (Kirkpatrick et al. 2011). A combination of the GnRH agonist deslorelin and the progestin melengestrol acetate is widely used in zoos when reproduction in captive populations is detrimental (Asa et al. 2012). Recently discovered gonadotropin inhibiting hormone (GnIH) may also be a candidate for use in wildlife contraception.

Over the years, there have been many attempts to use contraceptive technologies in wild animals, and the technique is growing in popularity. Even though contraception doesn't solve the immediate problem of too many individuals in the population, at least the problem doesn't become worse with continued breeding. Furthermore, population numbers will eventually decrease as animals die naturally.

One recent paper includes an intriguing prediction in reference to the use of contraception to control feral cat populations. Spaying and neutering (removing the testes or ovaries) of feral cats appears to result in increased lifespan of the treated adults (Hamilton JB et al. 1969) and increased survival of kittens (Gunther et al. 2011). The mechanism appears to be the loss of normal hormonally mediated behaviors, such as intraspecific aggression, that

increase mortality. In contrast to spaying and neutering, hysterectomy and vasectomy also remove the ability to breed but keep the treated animals hormonally intact. A recent population-modeling study suggests that maintaining the hormonally mediated behaviors via hysterectomy and vasectomy would result in far superior population control of feral cats than spaying and neutering (McCarthy et al. 2013). Testing predictions such as these would be an excellent opportunity for a conservation endocrinologist.

Unfortunately, it is not clear how effective contraception is for controlling nuisance wildlife populations. Most contraceptives are very good at inhibiting reproduction in individual animals and contraception has produced modest reductions in population sizes in some studies (Rutberg et al. 2004). However, contraception can also have a variety of physiological and behavioral side effects (Gray and Cameron 2010) that may be unacceptable to the public. Furthermore, there is a serious question whether contraceptive control of wildlife will ever be cost effective or even possible in large populations. Modeling studies suggest that nearly 70% of females need to be successfully inhibited from breeding in order to prevent a population from increasing if it is below carrying capacity (Porter et al. 2004). The number of breeding females that must be treated combined with the sustained effort required to maintain that coverage can make the attempt expensive and logistically daunting. Most successful attempts to use fertility control to reduce populations have occurred in small, closed populations (Ransom et al. 2014); therefore, it will be important to place fertility control projects in an ecological context. Conservation endocrinologists could make a major impact in this area with improved contraceptives that are cost effective and easy to deliver.

**Climate change.** Climate change is perhaps the most important environmental challenge that we will face in the twenty-first century. Human activity, especially the burning of fossil fuels, has increased atmospheric concentrations of greenhouse gases at an unprecedented rate and to levels that have not occurred for millions of years (IPCC 2007). The resulting rise in environmental changes will have substantial impact on the Earth's biota through a number of pathways, including increased temperature, high atmospheric and aquatic CO<sub>2</sub>, ocean acidification, and altered geographic and temporal patterns of rainfall, to name just a few. Animals will be affected directly by these abiotic factors and also indirectly through the distribution and abundance of prey items, predators, and disease agents. Climate-change impacts have already been demonstrated in some areas of the world through the alterations in animal and plant distributions, abundances, and the timing of life-history events (Saino et al. 2015).

Although it is beyond the scope of this article to present an exhaustive review of the biotic impacts of climate change, we can point out several areas in which conservation endocrinology can be important in understanding and predicting the impacts of climate change. The absolute

**Box 1. Noninvasive Endocrine Techniques.**

The use of noninvasive techniques in endocrinology has expanded rapidly and can greatly contribute to conservation endocrinology. Hormones and/or their breakdown products are present in living and nonliving tissues, such as hair, antlers, and claws, or are excreted in waste products, including feces, urine, saliva, and exhalations. To date, stress corticosteroids and reproductive steroids have been most widely examined because of their relatively high stability, but it should be possible in some cases to examine peptide and protein hormones. As with circulating hormones, it will be important to establish baseline values for a given species and/or population and determine what are normal seasonal and ontogenetic changes. It will also be important to validate the approach and methods that are being used: Is the hormone or hormone metabolite present in the biological material being accurately and specifically measured? Is the hormone or hormone metabolite reflective of the circulating hormone levels and/or the physiological state of the animal? Validation approaches will be dependent on the hormone and tissue being examined (Gaswindt et al 2012, Kersey and Dehnhard 2014) but will be critical for understanding the value and limitations of noninvasive approaches.

The last 20 years have seen a rapid advance in the use and validation of nonlethal and noninvasive approaches for obtaining information on the endocrine status of animals, and advances in this area will continue. For instance, unmanned vehicles (drones) have been used to collect blowhole spray from whales that can be analyzed for hormone levels (akin to salivary measurements widely used in humans) and general health (Hunt et al. 2013). In addition to whale blow, earplug and baleen have also been used to determine the endocrine status of cetaceans. Cortisol can be measured in the water of fish reared in captivity and used to assess response to stressors (Scott and Ellis 2007), and it should be possible under some circumstances to detect stress of fish in the wild. Radio-transmitting heart-rate monitors already provide us with indirect measures of stress of free-living animals (Bisson et al. 2011). In the near future, it should be possible to measure hormones in wild animals using implantable devices that can transmit blood hormone levels in real time. In addition to the measurement of hormones in these materials, it is also possible to obtain genetic (transcriptional) and genomic information. Comprehensive reviews of currently used, minimally invasive approaches in threatened mammals can be found in Kersey and Dehnhard (2014), and for corticosteroids in wild animals in Sheriff and colleagues (2011).

level of temperature and daily fluctuations in temperature will increase in response to accumulating greenhouse gases in many areas of the world (IPCC 2007), and both can substantially affect survival, fitness, and animal distribution. In extreme situations, temperatures can surpass the thermal tolerance of animals and result in large but often localized mortalities (McKenchnie and Wolf 2010). In other cases, high temperatures may have sublethal impacts, interfering with normal growth, development, or reproduction. Such sublethal impacts of either acute or chronic altered temperature exposure may be perceived as stressful by animals and result in higher levels of glucocorticoids. Even elevated winter temperatures may be energetically stressful if they increase metabolic rate (as they would in ectotherms) without a concomitant increase in food availability. As we noted above, altered levels of glucocorticoids can be detected in a variety of low- or no-impact methods (figure 2, box 1) and serve as early warning systems for the impact of temperature at the individual and population level.

Patterns of rainfall, storms, and other extreme weather events are also predicted to change in certain areas of the globe. These episodic events could influence not just terrestrial systems but also streams, estuaries, fresh- and saltwater marshes, mangroves, and many near-shore marine communities. Such unpredictable events can have substantial impacts on sensitive developmental stages, such as recently hatched birds. They may also act as stressors, stimulating glucocorticoids and with subsequent impacts on movement, migration, or reproduction. High water and associated increases in turbidity have been shown to elicit higher

cortisol in some fish species. Sea-level rise will inundate many estuaries (and may act synergistically with rainfall patterns), altering the location and timing of salinity change. Cortisol has been shown to be critical to the process of acclimation to changing salinity in fish, aiding both ion uptake in freshwater and salt secretion in seawater (McCormick 2013). Hypoxic zones in the ocean have human causes that may increase with climate change; examination of gonad histology and aromatase activity (which converts androgens into estrogens) indicates that impairment of reproduction in marine fish in hypoxic zones can occur over large coastal regions (Thomas and Rahman 2012).

Early increases in temperature in spring have advanced the cycles of primary and secondary productivity, which are critical to the survival of higher trophic levels. Such changes in phenology are particularly important to newly birthed or hatched animals that rely on abundant food resources for early survival and growth (Visser et al. 2016). In some cases, animals appear to have responded to changes in the timing of spring productivity by altering the timing of migration or reproductive events, possibly by responding to temperature itself. The impact of temperature on reproduction of endotherms has been demonstrated in both the wild and under laboratory conditions, although the mechanism of this altered timing is unknown (Caro et al. 2013). Animals that use photoperiod to time critical life-history events have relied on past selection to determine the timing of events and therefore may be mismatched to current timing of events that are controlled by temperature (McCormick 2013). Migratory birds in Europe that have experienced the greatest *thermal delay* (a gap in current versus historical

temperatures) in arrival times to breeding grounds have experienced the greatest population declines (Saino et al. 2015). This gap may be due to the photoperiodic control of migration, and it is unclear whether the pace of evolution to alter timing in photoperiod-controlled animals is sufficient to keep pace with phenological changes driven by early increases in temperature. Such a mismatch makes it critical to know the underlying control mechanisms for seasonal events in species at risk. For many if not most species, this information is absent. As has been pointed out by Wingfield (2015), it is increasingly important that we know both the environmental cues and their attendant neuroendocrine-signaling pathways for reproduction and migration if we are to understand, predict, and detect the impacts of climate change on animals.

### Conclusions

In this article, we have outlined a number of areas in which endocrinology is being or can be used in the service of conserving species and their ecosystems. The in-depth examples described above are “biased” toward corticosteroids and sex steroids and are representative of work in this field, in part because of their integrative nature but also because of their relative ease of measurement and preservation in nonliving tissue. We have included syntheses of other endocrine axes in the hope of advancing approaches in these areas that could also contribute to this field. As the need for conservation grows with increasing human impacts on habitats, we see increasing need and opportunity for conservation endocrinology to aid in this endeavor. Growth in this field will be facilitated by developing new approaches and techniques; increasing our understanding of “basic” endocrinology in both model and nonmodel organisms; and training endocrinologists that have knowledge of endocrine mechanisms, of how natural systems affect the neuroendocrine system, and of the conservation issues faced by species of concern.

We have already seen a large advance in our ability to monitor the endocrine status of animals without handling them or by using minimally invasive techniques (figure 2). Technological advances in many arenas can be used or adapted to achieve previously difficult goals. In many cases, our ability to apply endocrine techniques to conservation issues is limited by our understanding of how environmental factors affect the endocrine system. Although there are many common endocrine mechanisms for the control of homeostasis, growth, development, and reproduction, as we noted above, there are many differences in the details of these pathways and how they are influenced by environmental change. Indeed, given the importance of the endocrine system as a signaling pathway for environmental cues to affect critical life-history events, we should expect that evolution will have shaped endocrine responses that differ for species with different habitat demands and that there will in many cases be strong species differences in response to environmental change. In many cases, it may be necessary to have long-term monitoring to determine what is “baseline” for a given

species or population (Crespi et al. 2015). Such efforts would allow for establishing “cause and effect” for environmental perturbations (Cooke et al. 2013), as well as aiding in the determination of appropriate interventions where possible.

Although we are fairly knowledgeable of the endocrine control of some taxa, such as mammals, birds, teleost, and insects, we are woefully ignorant of the hormonal regulation of homeostasis, growth, and reproduction in a number of other critical taxa. Freshwater mussels are one of the most endangered taxa in North America (Williams et al. 1993), but we know little of their reproductive endocrinology. Therefore, there is a great need to expand our understanding of “basic” endocrinology in a broader range of taxa, as well as to determine how environmental factors regulate the neuroendocrine system.

There is great opportunity to combine new approaches in genomics and transcriptomics with more classical endocrine and physiological approaches to expanding our knowledge in comparative and environmental endocrinology. For example, the expression of sex steroid receptors in homologous rhinoceros fibroblasts allowed for the characterization and prediction of species sensitivity to phytoestrogens and subsequent increased reproductive capacity in captivity after a change in diet (Tubbs et al. 2014). Advances in techniques and the application of conservation endocrinology can be facilitated by breaking down discipline barriers and providing training for a new generation of conservation endocrinologists. In many cases, the most productive research is likely to involve collaboration with conservation ecologists, geneticists, and endocrinologists. Students with broad training in comparative endocrinology and the principles of ecology and conservation biology will be especially well positioned to make contributions to conservation endocrinology. We encourage young investigators to gain experience in molecular, biochemical, physiological, and organismic approaches and to develop a thorough understanding of the major endocrine axes and their interactions. A combination of novel techniques, basic research, and training of students will help stimulate conservation endocrinology and its contributions to the global environment.

### Acknowledgments

We thank Erica Crespi and three anonymous reviewers for providing insightful comments on earlier versions of this manuscript.

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