The four levels of behavioral study

reasons why parrots display hatching asynchrony, in which siblings hatch in staggered sequence and the last-hatched young usually do not survive. In some species hatching asynchrony appears to confer an advantage in the form of “insurance” offspring, whereas in other species hatching asynchrony appears to have evolved in response to physiological constraints. The authors discuss the conservation implications of the different hypotheses explaining hatching asynchrony.

In the last chapter of this section (Chapter 8, Buchholz and Clemons), we explore the current limits of the traditional systematic methods and approaches used in conservation biology for understanding the scope of biological diversity and its protection. Behavior patterns provide clues to the phylogenetic history of animals but are neglected because of largely outmoded professional biases. Integrating behavior into biodiversity analyses would result in the protection of unique and evolutionarily critical behavior patterns, facilitate the identification of cryptic species, and protect a greater variety of diversity of value in our everyday lives.


Chapter 5
Environmental stress, field endocrinology, and conservation biology

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Virtually all vertebrates use environmental signals such as day length, temperature, and availability of food to time life history events, such as reproduction and moult (e.g., Wingfield & Kenagy 1991). These cues provide predictable information about future events, such as onset of a breeding season. Reproductive maturation is thus initiated several weeks in advance so that breeding can begin as soon as environmental conditions allow. It is also critical, however, that individuals respond to unpredictable events in the environment. Severe storms can disrupt breeding, resulting in abandonment of the nest and young, or precipitate movements away from the home range in search of alternative food resources and shelter. These facultative physiological and behavioral responses to unpredictable environmental events (modifying factors, Wingfield 1983) occur in response to not only a wide spectrum of natural events described above, but also to human disturbance, pollution, and introduced parasites. Human-induced changes such as these are ‘unpredictable events’ for free-living populations of animals and elicit the same response as natural modifying factors – a facultative physiological and behavioral pattern. The relevance to conservation biology here is that unpredictable disruptions of human origin, as well as natural perturbations, will interrupt breeding. Additionally, highly mobile vertebrates such as birds and large mammals, may leave the restricted confines of reserves. These potential events are of major concern for conservation biology in general, and for refuge managers in particular.

Many modifying factors can be classified as stressors. They trigger a cascade of hormone secretions typical of stress in all vertebrates studied to date (e.g., Wingfield 1994). By measuring circulating levels of glucocorticosteroids (one of the major types of hormones involved in the orchestration of physiological and behavioral responses to stress) in free-
living individuals, we can determine whether an individual is indeed stressed, or monitor populations in relation to unpredictable and disruptive environmental change. Patterns of these hormone levels circulating in blood can provide reliable information indicative of potential and actual environmental stress, either natural or human-induced. Samples for monitoring these hormones are easy to obtain, do not harm the subject animal, and provide incisive information in a few days. Behavioral observations may require weeks or even longer to provide the same information. Another advantage of using field endocrinology techniques to monitor stress in free-living populations is that the data can provide predictive information, i.e., they allow us to anticipate likely problems before the population becomes stressed, and allow us to identify sub-populations that may be more vulnerable to disturbance or prolonged inclement weather than others. This type of information will be particularly useful to refuge managers.

Before going on to discuss how these techniques can be applied in the field, it is useful to reflect on the interface of what has been traditionally regarded as a ‘laboratory science,’ endocrinology, and conservation biology. Over the last two decades, techniques have been developed by which samples (such as blood, tissue, feces, urine, etc.) can be collected from free-living individuals without debilitating them. These samples can then be transported to the laboratory for hormone analysis. One strength of ‘field endocrinology’ techniques is that individuals continue to live normally, and so can be followed through their life cycle. Further samples for hormone analysis can then be collected when desirable (see Wingfield & Farner 1976).

Application of endocrinology to conservation biology: A general perspective

The role of endocrinology in the conservation of vertebrates, especially in captive breeding programs, is not new. Nevertheless, the application of endocrine techniques to conservation biology is still very much in its formative stages. It is pertinent here to discuss how endocrinology can contribute to conservation biology in general, and then focus on one aspect of these applications—monitoring stress. There are at least five ways in which hormone physiology can contribute to conservation issues:

1. Hormone therapy can be used in veterinary medicine to treat endocrine disorders and other diseases that may affect reproductive function (Iatropoulos 1994). This may be particularly important for endangered species, both in captivity and free-living.

2. Hormones can be used in otherwise healthy animals to enhance reproduction (e.g., Wakabayashi et al. 1992). More recently, endocrine treatment has been applied to decrease reproductive output in captive populations that have become overcrowded, or even to reduce populations of exotic introduced species and pests that may compete with endangered species (e.g., Kirkpatrick & Turner 1991; Seal 1991).

3. Field endocrinology protocols can be used to identify specific characteristics of the breeding cycle within or among populations. Temporal patterns of hormone secretion in free-living individuals, for example, can identify potential environmental requirements for successful breeding and whether a threatened population is having problems during the nesting season. Additionally, profiles of circulating reproductive hormones in free-living populations can provide a template for monitoring reproductive performance in captive breeding programs of the same or closely related species (e.g., Ishii et al. 1994).

4. Endocrine techniques can be used to determine how pollutants may affect free-living populations. Several components of petroleum and related products, for example, have been shown to have estrogenic properties. These ‘environmental estrogens’ may have potent effects on reproductive maturation and function (e.g., Hose & Guillette 1995).

5. Field endocrinology protocols can be used to identify populations vulnerable to human disturbance, development, or even natural stressors such as prolonged severe weather (drought, El Niño events, etc.). Such protocols are also able to identify populations already under stress and, thus, likely to decline rapidly, as well as monitor animals that have been reintroduced to natural conditions (e.g., Dunlap & Schall 1995; Fowler et al. 1995).

All of these ways in which endocrinology can be applied to conservation issues deserve attention, but here we will focus on only one: the application of field endocrinology protocols to assess whether free-living populations may be vulnerable to stress, or are actually exposed to debilitating stress (i.e., number 5 above).

Before presenting examples of how measuring hormonal responses to stress can be applied to conservation biology, we will next discuss the role of glucocorticosteroids in the generalized vertebrate stress response, how appropriate samples can be collected in the field, and how these hormones
can be measured. Then we will outline several examples of how these techniques can be used to identify free-living populations under stress and monitor them for vulnerability to potential future stresses.

**Glucocorticosteroids and the vertebrate response to stress**

In the vast majority of vertebrates studied to date, a broad spectrum of unpredictable and potentially stressful stimuli elicit the following responses: (1) release of catecholamines from the adrenal medulla within seconds, followed by (2) activation of the hypothalamo-adenohypophysial—adrenal cortex axis a few minutes later (culminating in the synthesis and secretion of glucocorticosteroids such as corticosterone from cortical cells of the adrenal gland), and accompanied by (3) secretion of cytokines from cells of the immune system (e.g., Axelrod & Reisine 1984; Munck et al. 1984; Greenberg & Wingfield 1987; Sapolsky 1987). Rapid secretion of catecholamines influences the vascular system, particularly cardiac output, in preparation for massive exertion in immediate social and/or physical stresses (e.g., predator avoidance) and promotes mobilization of glucose (Axelrod & Reisine 1984; Sapolsky 1987). Simultaneously, cytokines activate the immune system to reduce susceptibility to infection. Glucocorticosteroids then trigger gluconeogenesis, the formation of new glucose usually at the expense of protein and fat (Chester-Jones et al. 1972; Wingfield 1994; Table 5.1). Chronic effects of stress-induced high levels of glucocorticosteroids, usually over many days or even weeks, may reverse some earlier responses and produce several potentially detrimental effects. For example, suppression of the immune system, neuronal cell death, and disruption of second cell messenger systems occurs (Table 5.1). There are many other hormonal responses known, usually characteristic of the type of perturbation. During osmotic stress, osmoregulatory hormones are secreted, whereas in cases of metabolic stress, hormones associated with energy balance are released. Despite this complex mixture of hormone release tailored to the specific type of stress, the rapid increase of catecholamines followed by the elevation of glucocorticosteroid secretion appears common to all hormone responses to stress, regardless of what that stress may be. Catecholamine levels rise in blood extremely rapidly (a few seconds) after the onset of a sudden stress, so the adrenocortical response, as measured by release of glucocorticosteroids, is a more practical marker for stress in general and will be the focus of the discussions below.

### Table 5.1 Effects of corticosterone during a “stress” response (from references cited in text)

<table>
<thead>
<tr>
<th>Rapid (i.e., short term)</th>
<th>Chronic (i.e., long term)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppress reproductive behavior</td>
<td>Inhibit reproductive system</td>
</tr>
<tr>
<td>Suppress territorial behavior</td>
<td>Suppress immune system</td>
</tr>
<tr>
<td>Increase gluconeogenesis</td>
<td>Promote severe protein loss</td>
</tr>
<tr>
<td>Increase foraging behavior</td>
<td>Disrupt second messenger systems</td>
</tr>
<tr>
<td>Promote daytime escape (irruptive) behavior</td>
<td>Neuronal cell death</td>
</tr>
<tr>
<td>Promote night restfulness by lowering standard metabolic rate</td>
<td>Suppress growth and metamorphosis</td>
</tr>
</tbody>
</table>

Originally, it was proposed that stress hormones adapt the organism to the stress and restore homeostasis. The ‘exhaustion’ phase and its attendant deleterious effects (Table 5.1) set in if homeostasis is not restored and the stress becomes chronic (Selye 1971). More recently Axelrod & Reisine (1984) stated that “stress hormones serve to adapt the body to stressors ranging from mildly psychological to intensely physical.” Sapolsky (1987) pointed out that stress hormones orchestrate an emergency reaction that suppresses unnecessary processes and promotes survival until the stress passes. Reproduction and territorial behavior, for example, are temporarily suspended following the onset of stress (Wingfield 1984, 1988; Table 5.1), but energy mobilization is elevated. When the stress has subsided, glucocorticosteroid levels decline, then reproduction and territorial behavior can resume (Wingfield 1984, 1988).

The adrenocortical response to stress is important in the short term (hours to a few days) but can become highly deleterious if it is repeatedly activated (see Table 5.1).

A rather different view has been suggested by Munck et al. (1984). They state that the function of stress hormones may be to protect against the normal defense reactions activated by stress (i.e., the immune system). These defenses may be inappropriate reactions to stressors and could be a source of stress itself. Glucocorticosteroids thus subdue immune responses activated by the onset of stress that may themselves threaten homeostasis. This view is not necessarily mutually exclusive from the others cited above, but we feel that the hypotheses of Sapolsky (1987) are most relevant to an organism in its natural environment.

In an ecological context it is difficult to see any adaptive value of the long-term (chronic) effects of elevated glucocorticosteroids listed in
Table 5.1. In its natural habitat an organism would be at a considerable disadvantage in any one of these chronic stressed states (e.g., massive protein loss from skeletal muscle, or prolonged suppression of the immune system, Table 5.1). The short-term actions of glucocorticosteroids appear more relevant to an organism in the field. The stress response is an emergency reaction that promotes survival while temporarily suspending other activities (Sapolsky 1987; Wingfield 1994). In other words, the stress-induced increase in glucocorticosteroids triggers a facultative behavioral pattern with attendant physiological changes that allow an individual to cope with the unpredictable and potentially stressful event. In this way the individual can avoid the effects of chronic high levels of glucocorticosteroids outlined in Table 5.1. On occasion chronic stressors do occur under natural conditions; indeed the literature is full of accounts of natural stressors such as extreme weather resulting in starvation and mortality (e.g., for birds, Gessaman & Worthen 1982). Facultative behavioral and physiological patterns, however, do appear effective in re-directing behavior and physiology toward survival. One obvious benefit is increased life-time fitness.

We prefer to use the term 'modifying factors' to refer to unpredictable events in the environment rather than 'stressors' because only under severe or chronic conditions are they truly stressful. In many cases the facultative behavioral and physiological patterns triggered by initial rises in glucocorticosteroids result in a physical state that is not stressed at all. Only if the modifying factor becomes chronic, thereby constantly triggering increases in glucocorticosteroid secretion, does the individual finally progress to a stressed and debilitated state.

**Glucocorticosteroids and reproductive/territorial behavior**

Elevated circulating levels of corticosterone suppress reproductive and associated behavior in birds. Subcutaneous implants of corticosterone into breeding male and female Pied flycatchers (*Ficedula hypoleuca*), in the field decrease the frequency with which they feed young resulting in lower reproductive success (Silverin 1986). Additional implants that increase circulating corticosterone even further result in all birds abandoning their nests and territories. Similarly in the Song sparrow (*Melospiza melodia*) experimental treatment with corticosterone reduces the responsiveness of free-living males to simulated territorial intrusion within 12–18 hours indicating that territorial behavior has been suppressed (Wingfield & Silverin 1986). Note that although plasma levels of testosterone are decreased in males given corticosterone implants compared with controls, they are still within the normal limits that would support territorial aggression. Plasma levels of luteinizing hormone (LH) are unaffected. Additional evidence comes from field endocrine studies of territorial Side-blotched lizards (*Uta stansburiana*). Male lizards given implants of corticosterone are less territorial than controls. Furthermore, simultaneous implants of testosterone fail to restore territorial status in corticosterone treated animals (DeNardo & Sinervo 1994). Clearly, high plasma levels of corticosterone, similar to those induced by modifying factors, suppress territorial behavior and, perhaps, reproductive behavior as well, even when testosterone levels are within the range that would normally activate these behaviors. This response may be adaptive in the long term if survival is favored by temporarily re-directing behavior away from reproduction and territoriality. Meanwhile, the reproductive system is maintained in a near functional state so that breeding can resume immediately after the perturbation has passed and corticosterone levels have subsided (Wingfield 1984, 1988). If, as indicated in Table 5.1, the modifying factor became chronic, i.e., stressful, then continued high levels of corticosterone would result in suppression of the reproductive system that could not be reversed easily within a single breeding season. In this case long-term (chronic) effects would essentially result in complete reproductive failure for that season, whereas the short-term effects and activation of facultative behavioral and physiological patterns would enable another attempt at breeding (Wingfield 1988).

**Short-term effects of glucocorticosteroids and facultative behavioral and physiological patterns in birds**

The chronic effects of prolonged high circulating levels of glucocorticosteroids are of great importance to clinical, agricultural, and aquacultural research. The short-term effects have been less well studied, possibly because their importance as a stress-avoiding mechanism has not been fully appreciated. Here we review evidence for the effects of glucocorticosteroids in the short term. The emphasis will be on birds, although there is no reason *per se* why these effects should not be found in other vertebrates as well.
Glucocorticosteroids and mobilization of glucose

Glucocorticosteroids, especially from the breakdown of protein in muscle, is a major action of elevated circulating corticosterone levels (e.g., Holmes & Phillips 1976). For example, implants of corticosterone in the House sparrow (Passer domesticus) significantly decreases the mass of the large pectoralis muscles (a potential source of endogenous protein) indicative of possible mobilization for gluconeogenesis (Honey 1990). Further analysis reveals that the loss of protein appears to be primarily from soluble components of the pectoralis muscles as the contractile component is not affected. Whether this suggests that the massive pectoralis muscles of birds have a protein store independent of contractile components is unclear (Honey 1990). Corticosterone implants increase fat score dramatically in male Song sparrows without a change in body mass, suggesting a ‘shunt’ of energy derived from protein into a more readily accessible form – fat (Wingfield & Silverin 1986). Treatment of Dark-eyed juncos (Junco hyemalis) with corticosterone in winter also results in wasting of the pectoralis muscles and increases the deposition of fat (Gray et al. 1990). Note, however, that muscle lipoprotein lipase activity was not affected, suggesting that the ability of muscle to take up free fatty acids and glycerol is not impaired. It is possible that muscle protein is mobilized in response to corticosterone treatment but without increases in energy expenditure that would be experienced during an actual response to a modifying factor. The ‘new energy’ is then re-deposited as fat. Loss of body mass owing to protein mobilization is thus compensated for by increases of fat depot.

Glucocorticosteroids and foraging behavior

Glucocorticosteroids may be involved in the regulation of food intake (e.g., Leibowitz et al. 1984) and could be an important component of the facultative behavioral and physiological patterns triggered by modifying factors. In male White-crowned sparrows (Zonotrichia leucophrys), metyrapone, a blocker of corticosterone synthesis in adrenocortical cells, reduces foraging behavior: a combination of searching, scratching, pecking and food intake. Replacement therapy with corticosterone implants increases foraging (Wingfield et al. 1990). Later studies by Astheimer et al. (1992) show a trend for increases in foraging, but this is not significant. In the song sparrow, experimental manipulation of corticosterone levels has a greater effect on foraging but has no influence on food searching behavior when food is removed for 24 hours to simulate the effects of a passing storm. When food is returned, however, corticosterone treatment significantly increases foraging behavior. These data raise the interesting possibility that elevated circulating levels of corticosterone induced by modifying factors may not only trigger facultative behavioral and physiological patterns, but also facilitate recovery when environmental conditions ameliorate (Astheimer et al. 1992). This point deserves more investigation. Studies of other species, such as Domestic fowl (Gallus domesticus) support a role for corticosterone in the control of food intake, but effects may vary with the season (Astheimer et al. 1992). Clearly, additional experiments are needed to clarify the role of hormones on foraging behavior.

Glucocorticosteroids and activity

In birds, and perhaps other vertebrates, modifying factors such as extreme weather result in the abandonment of a breeding territory or winter home range. These movements are called 'irruptive migrations,' and they serve to remove the bird from the source of the modifying factor to refugia that provide shelter or adequate food (Elkins 1983; Wingfield 1984, 1988). This movement may be local or over hundreds of kilometers. In other cases individuals may become inactive and/or torpid, thus reducing energy expenditure, in an attempt to 'ride-out' a period of inclement weather (Elkins 1983). Combinations of these two cases are also possible in which a bird exposed to extreme weather first becomes inactive, but if inclement conditions continue, then leaves. Note that if an individual attempts to leave, it should do so while it still has sufficient reserves of fat to fuel the movement.

Corticosterone implants significantly decrease perch-hopping activity in male White-crowned sparrows (a measure of possible irruptive migratory activity) consistent with a strategy of reduced activity and 'riding out' the storm. Alternatively, if food is removed from the cages to simulate the effects of severe weather on food resources (Wingfield 1988), corticosterone-treated birds show greatly enhanced perch-hopping, including escape-like behavior in which individuals are attempting to get out of the cage (Astheimer et al. 1992). These data suggest that elevated circulating levels of corticosterone, similar to those induced by direct modifying factors, activate behavioral strategies consistent with the observations of free-living birds exposed to extreme weather. Additional factors obviously must be involved in the transition from inactivity to
greatly enhanced perch-hopping and escape behavior. Interestingly, increased irruptive migration occurs during the day, whereas normal migration occurs mostly at night in White-crowned sparrows (e.g., Wingfield et al. 1990). Thus, irruptive migration may have different ecological and hormonal bases from regular seasonal migration.

**Glucocorticosteroids and metabolic rate**

Evidence to date indicates that rises in plasma levels of corticosterone may increase perch-hopping activity, at least when combined with reduced availability of food. The question then arises as to whether the metabolic rate is also affected. Implants of corticosterone in White-crowned sparrows, Pine siskins (Carduelis pinus) and other species have no effect on basal metabolic rate compared with birds sampled before implants were given. All birds were sampled at night when activity levels were very low. Control birds show periodic surges in oxygen consumption perhaps as a result of sleep–wake rhythms. In contrast, corticosterone-treated birds show virtually no surges in oxygen consumption during the night (Buttemer et al. 1991). Corticosterone treatment appears to suppress extended metabolic rate when asleep and promote 'nocturnal restfulness,' with a projected 20% savings of energy over the 16 hour night. This conserved energy can then be utilized the following day to recommence irruptive migration or continue to ride out the storm.

**Faculative behavioral and physiological patterns and the 'emergency state'**

The experimental evidence cited above is consistent with the role of elevated corticosterone secretion, induced by direct modifying factors, in the activation of facultative behavioral and physiological patterns. High plasma levels of corticosterone trigger an emergency life history state designed to maximize fitness during and after temporary disruption of activities normally expressed at that stage in an individual's life cycle. There are three major components to this emergency reaction in birds (modified from Wingfield 1994):

1. Corticosterone may suppress 'unnecessary' physiological and behavioral functions such as reproductive behavior and territoriality. This suppression may be a direct action rather than through the inhibition of reproductive hormones.

2. Corticosterone may activate temporary, facultative behavioral and physiological patterns that promote survival. For example, foraging behavior may be elevated, and activity either decreased ('ride it out' strategy), or increased (irruptive migration) depending upon severity and duration of a direct modifying factor such as extreme weather. At night corticosterone promotes restfulness with up to 20% savings in energy.

3. Corticosterone promotes gluconeogenesis, including the mobilization of protein as a substrate. It is possible that protein is stored in flight muscles, as the initial loss of pectoralis muscle mass appears to be from soluble fractions rather than contractile elements.

Facultative behavioral and physiological patterns triggered by short-term elevations of circulating corticosterone serve to promote survival in the best physical condition and avoid the deleterious long-term effects characteristic of a stressed state. Note that there is no reason why these components of short-term behavioral and physiological responses to modifying factors should not be applicable to other vertebrates.

**How to measure adrenocortical responsiveness to modifying factors**

Clearly, changes in circulating levels of glucocorticosteroids indicate responsiveness to modifying factors, some of which could potentially be stressful. Investigators interested in conservation biology, however, may not be familiar with field and laboratory techniques that have been designed specifically to monitor the adrenocortical responses of individuals under free-living conditions. In this section we outline some techniques that have proved practical, relatively inexpensive, and highly reproducible. It should be borne in mind that these techniques require a period of learning and adjustment, but we feel that the results obtained provide unique and highly useful information that could be vitally important for monitoring free-living populations of threatened and endangered species. These techniques have the potential to provide predictive information concerning (1) whether the population under investigation is stable and healthy, (2) more vulnerable to modifying factors, or (3) currently in trouble and likely to be chronically stressed. Such information could be of considerable importance for management decisions.