

living populations. It is important in passing to mention that although these techniques are invasive, they do not appear to debilitate free-living birds. In our study of Hawaiian honeycreepers, the percentages of unsampled birds that were recaptured are 8.8% for Common Amakihi, 3.7% for Apapane, and 8.0% for Iiwi. The percentages of sampled birds that we recaptured during the capture stress study are 18.9% for common Amakihi, 2.7% for Apapane, and 13.2% for Iiwi. These numbers are all either above or similar to recapture percentages for non-sampled birds. This study indicates clearly that collection of these kinds of data is entirely possible in endemic Hawaiian honeycreepers. It is critical that these techniques now be applied to threatened and endangered populations promptly. The information to be gleaned will shed light on many potential problems that may not be apparent from simple behavioral observations.

Baboons in an East African savannah

Olive baboons (*Papio anubis*) live in large groups (20–200 individuals) that have complex social organizations. Highest ranking males dominate these social groups. Obtaining high rank is essential if a male is to gain maximum reproductive success. Reaching that high rank, however, and then maintaining it can be extremely demanding – indeed stressful (Sapolsky 1987). There are frequent aggressive interactions to determine and maintain rank. The degree to which agonistic interactions occur varies with season, such as in a stable season when aggression is at a minimum and in an unstable season when many interactions occur, including fights and even injuries (Sapolsky 1987).

Male baboons with varying rank are captured from free-living troops by darting with an anesthetic (Sapolsky 1983). Once anesthetized, a series of blood samples is collected from the animals to assess responsiveness to capture and restraint (i.e., a form of the capture stress protocol described above). During the stable season, high ranking males have lower baseline levels of circulating cortisol (the major glucocorticosteroid in many primates) than low ranking males. In response to anesthesia and restraint, high ranking males show a greater rise in cortisol than low ranking males. During the unstable season, however, these differences disappear (Sapolsky 1983). Baseline plasma levels of testosterone also increase during the unstable season, suggesting that increased aggression increases both sex steroid levels and glucocorticosteroids. Curiously, high ranking males also have an impaired adrenocortical responsiveness

to stress during the unstable season. Chronically high baseline levels of cortisol circulating in the blood may decrease sensitivity of the testis to gonadotropins, which regulate testicular function and thus fertility, as well as increase susceptibility to disease by suppressing the immune system (Sapolsky 1987). Either of these effects could result in marked impairment of reproductive function and fertility. These findings were followed by a number of elegant field experiments to determine mechanisms (Sapolsky 1987) that do not concern us here, but this study indicates the utility of monitoring glucocorticosteroids with a capture stress protocol to assess the effects of extreme social pressure, or lack of, on reproductive function and pathology. Loss of habitat, shrinking reserves, or any environmental change that results in concentration of individuals in less and less space, therefore, could have profound detrimental effects on survival and reproduction. Baseline circulating levels of glucocorticosteroids and responsiveness to stress are highly informative ways of monitoring wild populations for the development of potential problems.

Fence lizards in western North America

The Western fence lizard (*Sceloporus occidentalis*) is an abundant lizard in western North America with a vast range extending from the Mojave Desert in Southern California to the beaches of Puget Sound in Washington State. Baseline levels of circulating corticosterone, the major glucocorticosteroid in reptiles, and responsiveness to the capture stress protocol were measured in six populations from around this species' range and during two different seasons. Baseline levels of corticosterone do not vary with population or season. As in several avian species, however, the amplitude of the increase in corticosterone levels during the capture stress protocol is correlated with physiological condition, with length adjusted for body mass. These data indicate that the capture stress protocol is useful for monitoring the health of reptiles in the wild. When physiological condition is factored out of the analysis, strong population and season effects remain. Adrenal responsiveness is higher in populations at the periphery of the species' range and is higher during the late summer than in spring (Dunlap & Wingfield 1995; Fig. 5.6). Adrenocortical responsiveness, thus, is modulated not only by changes in the external environment that affect physiology, but also by intrinsic changes associated with the annual cycle (e.g., reproductive state,

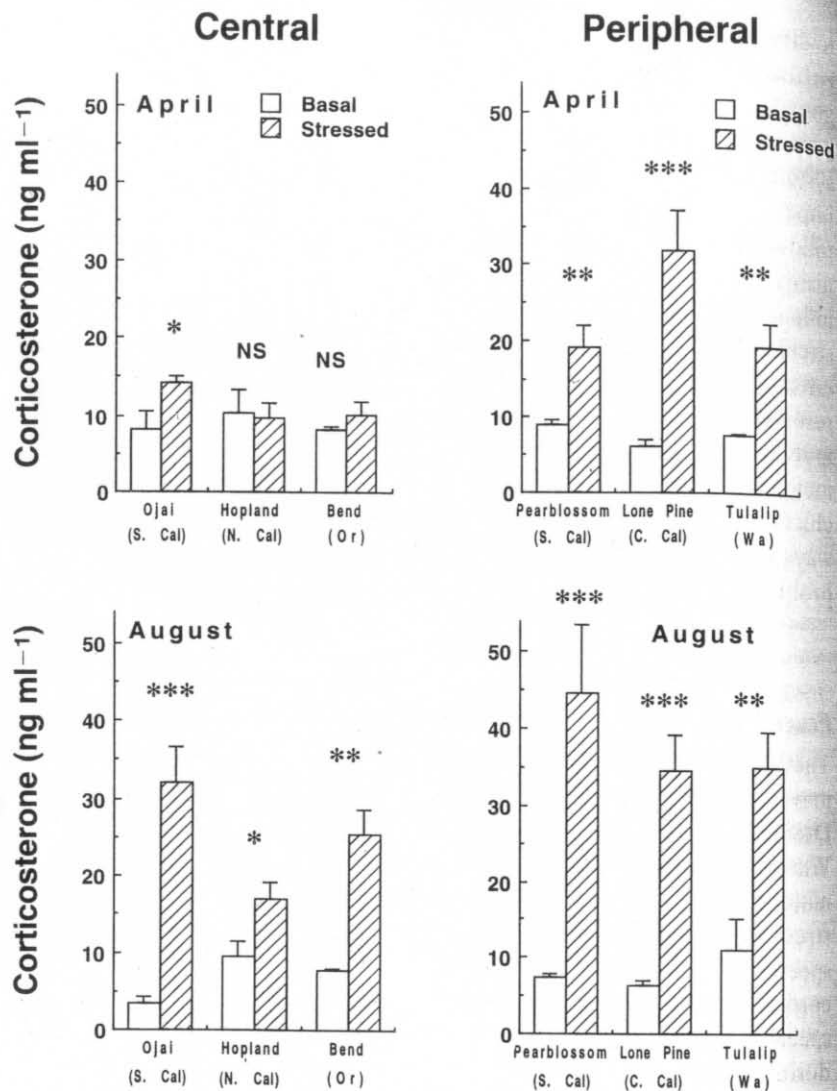


Fig. 5.6. Plasma levels of corticosterone (mean \pm SE) at capture and after 60 minutes capture stress in Western fence lizards (*Sceloporus occidentalis*) sampled in April and August in the center and at the periphery of their normal range. From Dunlap & Wingfield (1995).

emergence from hibernation, etc.), and by population differentiation (e.g., possible genetic differences).

Although Western fence lizards are not a threatened species, the population differences in adrenocortical responsiveness to stress have

considerable importance for conservation biology. If individuals of a population at the edge of the species' range, or normal habitat, are more susceptible to environmental stress, then habitat fragmentation could make that population more vulnerable to environmental stress, even if population size has not changed. Under 'average' or favorable conditions, populations in fragmented habitats may survive well, but the data above suggest that any detrimental environmental change resulting in stress (e.g., drought, increased human disturbance) would result in a much greater fraction of the population experiencing marked elevations of glucocorticosteroid levels in the blood. These increases would in turn promote reproductive failure, increase rates of disease, and trigger facultative behavioral patterns leading to animals leaving reserves, and, undoubtedly, increase mortality.

Further investigations of fence lizards reveal potential ways in which elevated adrenocortical responsiveness to stress may influence fitness. Fence lizards infected with the malarial parasite *Plasmodium mexicanum*, have similar baseline levels of corticosterone to uninfected individuals, but their responsiveness to capture and handling is higher (Fig. 5.7; Dunlap & Schall 1995). Baseline levels of testosterone and glucose are lower in infected males, but there is no difference following capture stress (Fig. 5.7). Additionally, males infected with malaria have lower testis mass, less bright nuptial coloration, give fewer courtship displays, and are less aggressive (leading to loss of territories). Females store less fat which is translated into reduced fecundity, or clutch size, the following breeding season (Dunlap & Schall 1995). Experimental implants of corticosterone, to mimic high circulating levels observed during the capture stress protocol, into healthy male fence lizards result in a decrease in testis mass, plasma levels of testosterone, and fat score, but in an increase in blood glucose concentrations. These data suggest that elevated levels of corticosterone, or increased sensitivity to stress, resulting from a natural cause such as malarial infection, directly results in several deleterious morphological, physiological, and behavioral changes, and reduced overall fitness. Again we see the potential for the capture stress protocol and measurement of baseline as well as stress levels of corticosterone to monitor wild populations.

Magellanic penguins in Patagonia, Argentina

Colonial species are particularly vulnerable to disruptive environmental events. Many individuals of a population converge on a small area, e.g., a

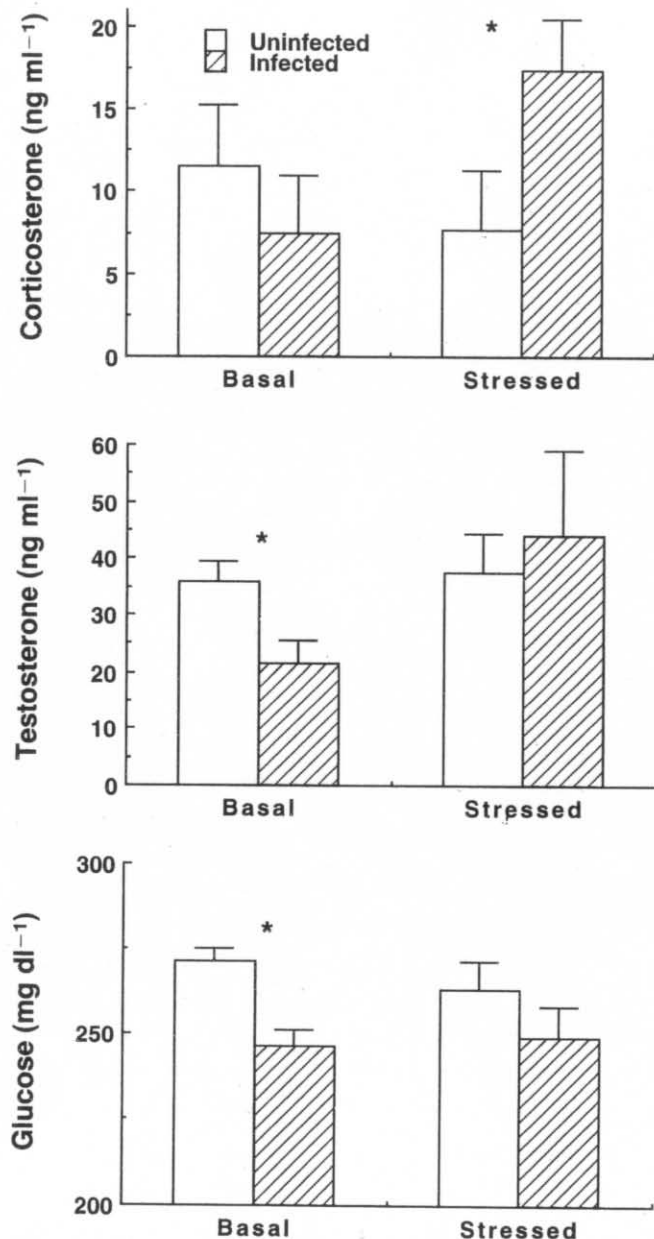


Fig. 5.7. Plasma concentrations of hormones and glucose (mean \pm SE) in uninfected and infected Western fence lizards (*Sceloporus occidentalis*). Basal measurements were taken immediately after capture, stressed measurements 1 hour after capture. Asterisks indicate statistically significant differences between infected and uninfected animals. From Dunlap & Schall (1995).

breeding colony, so there is potential for massive catastrophe. Our example here comes from a breeding colony of Magellanic penguins (*Spheniscus magellanicus*) breeding at a coastal site in the Atlantic province of Chubut in Argentina. In recent years this colony has had a chronic problem with oil spills from heavy tanker traffic offshore (e.g., Boersma 1987; Gandini *et al.* 1994). In 1991 there was a substantial oil spill near the colony at a time when investigations on the reproductive endocrinology of the colony were being conducted (Fowler 1993). This unfortunate disaster nevertheless provided a unique opportunity to assess the effects of various degrees of oiling on hormone profiles, as well as to monitor the effects of washing and rehabilitation of oiled birds.

After the spill, heavily oiled penguins were not seen in the breeding colony. Numerous birds with only light oil contamination did move into the colony, although most did not breed. In both males and females, plasma levels of testosterone, and its biologically active metabolite 5-alpha-dihydrotestosterone, are lower in oiled than in non-oiled birds (Fig. 5.8). Circulating levels of luteinizing hormone (LH), a pituitary protein hormone that regulates the secretion of sex steroids, are suppressed only in oiled females. Oiling in males apparently has no effect on LH (Fig. 5.9). Plasma levels of estradiol, a sex steroid that is important for the expression of female sexual behavior as well as the synthesis of yolk for eggs, is markedly reduced in oiled birds (Fig. 5.9; Fowler *et al.* 1995). The general reduction of sex steroid levels in oiled birds is consistent with observations that few pairs with oiled partners established nesting territories and laid eggs. Clearly, even very light oiling, with as little as 5% of body surface area affected, is sufficient to reduce reproductive success. In an area where oil spills are a chronic problem, there is the potential for continuous reduction of breeding success. For an endangered or threatened population, such effects could severely impair recovery, or directly cause further decreases in otherwise healthy small populations.

Baseline plasma levels of corticosterone are higher in oiled females but not in oiled males (Fig. 5.10; Fowler *et al.* 1995). Given the well-known effects of stress-induced rises of corticosterone on reproduction (see above), we expected both sexes to have high levels of glucocorticosteroids. Whether males respond to oil through a corticosterone-independent mechanism, or are more resistant to oil-produced stresses, remains to be determined.

It is now common for volunteers to attempt to rehabilitate heavily oiled birds by washing in detergent and then holding the birds captive until the

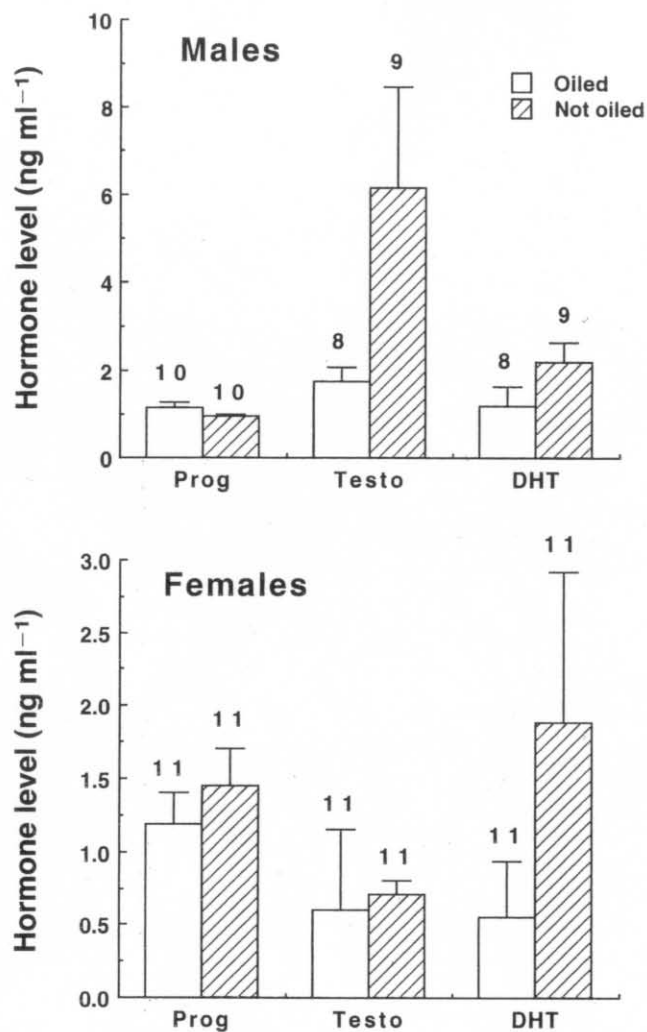


Fig. 5.8. Circulating levels of progesterone (Prog), testosterone (Testo) and 5- α -dihydrotestosterone (DHT) in oiled and non-oiled male and female Magellanic penguins (*Spheniscus magellanicus*). Bars are means (\pm SE), and numbers at tops of bars denote sample sizes. From Fowler *et al.* (1995).

plumage becomes waterproof by preening. Samples collected from heavily oiled, captive penguins as well as from individuals that had been cleaned during the week preceding blood sampling, revealed significantly higher levels of corticosterone in blood (Fig. 5.10). These data suggest

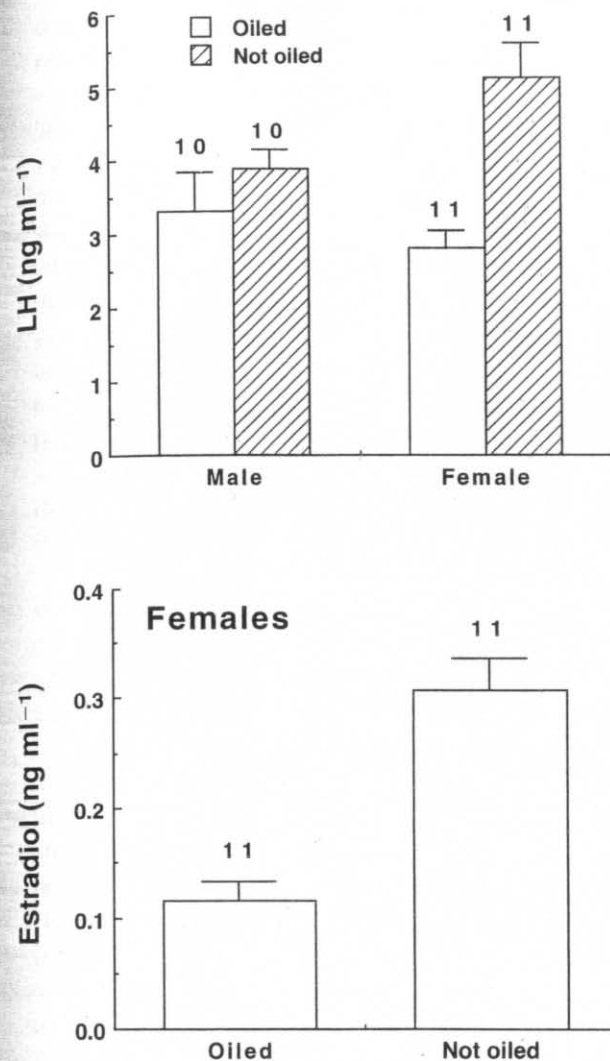


Fig. 5.9. Circulating levels of luteinizing hormone (LH) in male and female, and estradiol-17 β in female, Magellanic penguins (*Spheniscus magellanicus*) in relation to oiling. Bars are means \pm SE. Numbers at top of bars denote sample sizes. From Fowler *et al.* (1995).

that the act of cleaning is even more stressful and should be born in mind when dealing with endangered populations. Hormone profiles during recovery would be particularly useful (i.e., do they return to normal profiles of non-oiled birds?).

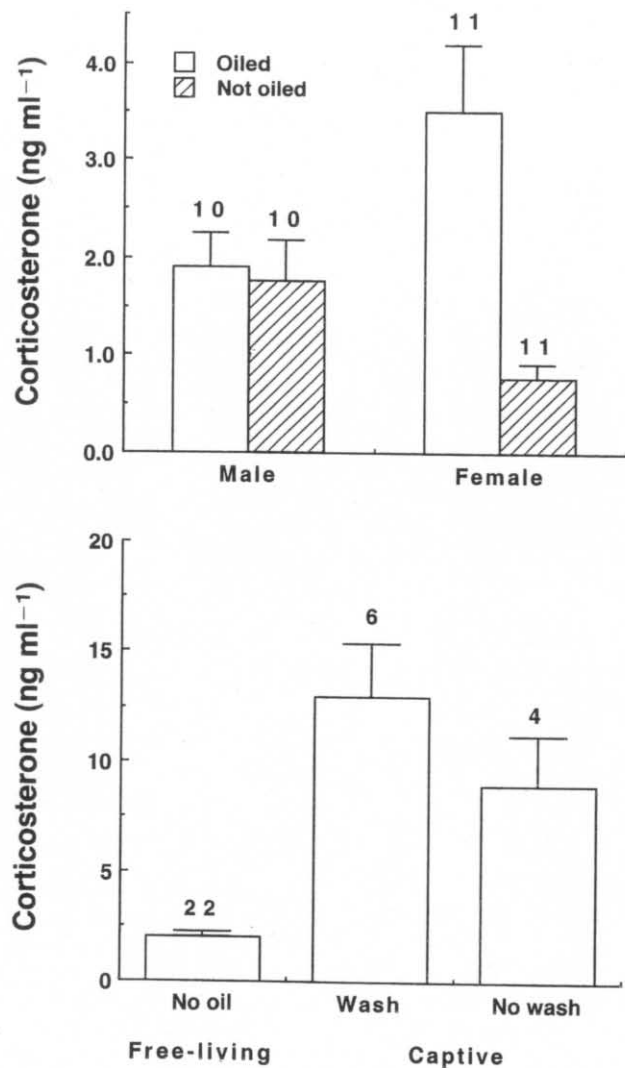


Fig. 5.10. (a) Circulating levels of corticosterone in oiled and non-oiled male and female Magellanic penguins (*Spheniscus magellanicus*). (b) Levels of corticosterone in relation to washing in captives. Bars are means (\pm SE), and numbers at tops of bars denote sample sizes. From Fowler *et al.* (1995).

Conclusions

An organized, efficient protocol is described as a way of assessing responses to modifying factors (i.e., environmental modifications that are potentially stressful). Measurement of circulating levels of

corticosterone, a glucocorticosteroid secreted by the adrenal gland in response to a variety of unpredictable and disruptive environmental events, is used as an indicator of stress. This response is also known as the 'adrenocortical response to stress' and is common virtually throughout the vertebrate classes. The protocol is an organized way of obtaining the maximum information on vulnerability of a population to environmental stressors as well as a direct measure of the degree of stress at the time when samples are collected. Classical field techniques using entirely non-invasive methods such as censusing and observation may take several years to reach the same conclusions. The protocol is designed to give unequivocal information and may be particularly useful when recovery biologists need to establish a program for endangered or threatened populations for which there is little, or no, background information. Note also that these protocols can be modified to fit all vertebrate classes, thus raising the possibility of applying the techniques to many species within a threatened ecosystem.

The elevation of plasma glucocorticosteroid levels is common to a wide spectrum of stressor stimuli, including capture and handling. In birds, capture and restraint results in a rapid increase of corticosterone, usually within 5–10 minutes, and reaches a maximum within 30–60 minutes after capture. We have developed a technique called the capture stress protocol in which birds are captured and very small (20–30 μ l) samples of blood collected as soon as possible after capture. The birds are then held in cloth bags for 60 minutes and further samples collected at 5, 10, 30 and 60 minutes after capture for measurement of changing corticosterone levels. Data in the field from species representing several vertebrate classes indicate that sensitivity to stress may change with season and reproductive state, as well as body condition of individuals. Collection of capture stress series in free-living animals and captive populations can tell us a great deal about sensitivity to stress and current stress conditions. Adrenocortical responses are sensitive to human disturbance as well as to natural environmental conditions, disease, exposure to pollution, and other potentially stressful situations.

Several field studies of marked individuals now indicate that capture and blood sampling does not harm the animal. Individuals are re-sighted in the field and appear to breed and behave normally after sampling. Future developments using fecal and urine analysis will reduce the invasive nature of this approach even further. Any investigator beginning this type of work should pay attention to these issues. Individuals must be monitored not just for survival but also for normal behavior following

sampling. When working in the field with threatened or endangered species, it is particularly important that sound judgment be used concerning the timing of the sampling and whether local conditions (e.g., severe weather) constitute an additional factor that may be detrimental to the individual after release.

Interpretation of the patterns of glucocorticosteroid levels following the capture stress protocol must also be made with caution. Stress series should be collected as a 'standard' from populations under benign conditions for comparison with further series during times when the population may be threatened. If individuals in a population, for example, show a very rapid and marked elevation of glucocorticosteroids during the capture stress protocol, they may 'over react' to an otherwise relatively minor stress in the natural environment. The result could be deleterious, because such functions as reproduction may be disrupted unnecessarily. This may be especially critical if the breeding season is short and there are few possibilities to breed, and less so if the breeding season is long with many opportunities to initiate a breeding attempt.

We feel that the techniques described will be highly useful additions to the arsenal of methods used to monitor threatened and endangered populations. Although rises in glucocorticosteroids have wide ranging effects on physiology, they also have important ramifications for behavior in several ways. They can promote survival ('facultative behavioral and physiological patterns') in the face of stress – obviously an advantage. If susceptibility to stress is increased by pollution or habitat modification, however, then a relatively minor stress in the future, which normally would *not* have triggered a facultative behavioral and physiological pattern, will become deleterious. Clearly, there is an urgent need to apply these approaches to a broad spectrum of vertebrate species in the field. In this way we can rigorously test and further characterize the adrenocortical responses to stress. It will be then possible to develop guidelines with a heightened awareness and accuracy for monitoring free-living populations.

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