Summary

1. Rapid, effective and enduring responses of physiology and behaviour to perturbations of the environment are key to robustness of an organism (ability to resist perturbations) and resilience (ability to resist and recover quickly from perturbations) so that the normal life cycle can be resumed quickly.

2. Perturbations of the environment can be labile (i.e. eventually subside) or permanent such as when human activity changes the environment in the long term, for example, deforestation, urbanization, etc.

3. Hormonal responses to labile perturbation factors (LPFs) allow organisms to cope during the perturbation and then return to the normal life cycle. These hormonal responses are called stress responses especially in cases when major changes in physiology and behaviour occur (emergency life-history stage).

4. Permanent perturbations require more than just temporary acclimation resulting in changes in range, adaptation or in some cases local extinction. Perturbations can be abiotic, biotic and social, but these are not mutually exclusive.

5. Here I focus on the effects of abiotic perturbation factors and their effects on the hypothalamic-pituitary-adrenal/interrenal axis in vertebrates. There is a great need for more field investigations of responses of free-living populations to perturbations of the environment, especially now that it appears the frequency and intensity of these events is increasing. However, such studies will require a high degree of opportunism on the part of the investigators to take advantage of unpredictable events when they occur.

Key-words: adrenocortical responses, CBG, emergency life-history stage, stress, stress modulation, unpredictable perturbations

Introduction

All organisms must deal with a changing environment by adjusting morphology, physiology and behaviour to cope with current conditions. However, variability of the environment presents challenges in terms of mechanisms by which change is perceived and the ways in which responses are regulated. For example, there are predictable changes in the environment such as day/night, seasons and high tide/low tide that organisms can prepare for by using environmental cues that allow them to anticipate future conditions (Wingfield 2003, 2008). On the other hand, unpredictable environmental events such as sudden storms, attacks from predators or dominant individuals are generally not predictable in the longer term, and individuals must respond during and immediately after the perturbation (Wingfield 2003, 2008). The mechanisms by which organisms such as vertebrates perceive environmental change and then transduce that information into neural and hormonal responses are fundamentally different for predictable versus unpredictable environmental events. Furthermore, types of environmental factors from the physical environment (abiotic) and biological environment (biotic, social, internal, pathological) also may determine pathways by which these factors are perceived, transduced and dealt with in the downstream response (Wingfield 2008; Wingfield, Kelley & Angelier 2011a; Wingfield et al. 2011b).

Here I will focus on the unpredictable environmental changes and the different types of perturbations and then go on to give examples of abiotic factors and the responses they elicit.
Labile versus permanent perturbation factors (also called modifying factors)

It is important first of all to distinguish between environmental factors that can be used to predict future life-cycle events, such as migration and reproduction, from sudden unpredictable environmental factors that may disrupt the life cycle temporarily or for longer periods. As an analogy, imagine that as part of your daily routine, you drive to work at 8 am and then drive back home at 5 pm. You prepare for these predictable daily journeys by filling the fuel tank in your car, regular services, etc. You can use time of day to predict when you should leave and when to return (i.e. using predictive cues). Then one day, there is an automobile accident that blocks the road and you have to decide whether to take an alternate route, which will take longer and use more fuel, or sit and wait for the road to be cleared. The former strategy will depend upon how much fuel you have in the tank and whether there is a fuel station on the new route in case you are running low. This will be important in deciding whether to sit and wait it out or leave and take an alternate route. Either way there will be an unpredicted change to your schedule that might potentially disrupt many other events during your work day (e.g. missed meetings, delayed deadlines, etc.). However, this kind of disruption is temporary, and the next day you can return to your regular routine. This is an example of an indirect labile perturbation factor because it is a single occurrence of relatively short duration and with little cost.

In another scenario, road works result in a long-term disruption of your daily commute, perhaps for days, even weeks or months. Eventually, though, you can return to your regular route, unless the alternate route has become preferable. This would be a direct labile perturbation factor because over many days, the alternate route and longer commute cost more and take time away from other pursuits. In very rare cases, your regular route may be permanently disrupted by, for example, a flood washing out the road or an equally destructive event. In this case, the only alternative is a permanent change in daily routine along with costs associated with a possibly longer trip, increased traffic congestion, etc. This requires acclimation to the new environment (adaptation) and you have to prepare accordingly. This would be a permanent perturbation factor and may ultimately require relocation or other major events to manage costs. Although these are analogies, individual organisms facing unpredictable events in their daily and seasonal routines have to make similar choices. Examples of these are described next.

Examples of responses to indirect labile perturbation factors resulting in reactive responses

An individual must respond immediately to a sudden threat frequently resulting in the ‘fight-or-flight response’. The event may be over in seconds or minutes and usually does not contribute significantly to longer-term energy costs (assuming the individual survives). Examples of abiotic indirect labile perturbation factors are as follows:

- Sudden severe storm (e.g. tornado, thunderstorm, freezing rain and other ice conditions)
- Fire (localized).
- Flood from rain that fell many kilometres away and not in the local area, for example, desert stream beds (arroyos or wadis).
- Earthquakes, tsunamis, volcanic eruption.
- Accident, for example, run/crawl/fly/swim from a falling rock.

In general, the responses to indirect labile perturbation factors involve activation of the autonomic nervous system and secretions of the adrenal medulla such as epinephrine. This is classic fight-or-flight response (e.g. Axelrod & Reisine 1984; Romero, Dickens & Cyr 2009). As soon as the perturbation has passed, recovery is quick and individuals return to their predictable daily routines of the normal life cycle.

Examples of abiotic direct labile perturbation factors triggering allostatic responses

An individual is forced to abandon its normal life-history stage because of reduced resources or restricted access to resources such as food, shelter, etc., brought on by longer-term perturbations, that is, hours to days. Examples of these factors are as follows:

- Prolonged storms or temporary climate change (e.g. El Niño Southern Oscillation Event)
- Cold
- Heat and drought
- Fire (on a large scale)
- Hypoxia
- Extremes of pH
- Extremes of osmotic pressure
- Trace element deficiency
- High pressure
- Intense solar radiation

Responses to direct labile perturbation factors typically involve the hypothalamo-pituitary-adrenal cortex (HPA) axis (adrenal = interrenal in fishes) culminating in the release of glucocorticoids (e.g. Sapolsky, Romero & Munck 2000; Wingfield 2003; Romero, Dickens & Cyr 2009). Note that there are also many synergistic interactions of adrenal medulla (chromaffin) secretions and glucocorticoids (Sapolsky, Romero & Munck 2000). High levels of glucocorticoids result in the interruption of the normal life-history stage (such as reproduction, migration, etc.) and trigger an emergency life-history stage that allows the individual to temporarily move away from the source of perturbation or endure it while adopting many energy-saving behaviours that allow it to cope (Wingfield 2003).
EXAMPLES OF ABIOTIC PERMANENT PERTURBATION FACTORS LEADING TO ADAPTATION OR LOCAL EXTINCTION

Permanent changes in the environment occur following major events such changes in climate and direct disturbance of anthropogenic origin. Often, they result in death or individuals are able to show some form of acclimation/adaptation that will change many aspects of the life cycle but allow them to survive (McEwen & Wingfield 2003; Wingfield 2003). Abiotic examples are as follows:

- Global climate change
  - Human disturbance (urbanization, habitat degradation)
  - Pollution (endocrine disruption).

Because permanent perturbation factors select for those individuals that adapt to new conditions, it is unlikely that there are permanent increases in activation of the HPA axis. These individuals adapt to new conditions and then return to the normal life cycle and do not show the symptoms of chronic stress. However, as far as I know, this point has not been investigated in free-living organisms after such a permanent perturbation. It is acknowledged that habitat destruction by fire, earthquake or a volcanic eruption may also fit into this category. Habitat will eventually recover but probably will take longer than the average lifespan of the organism.

More investigations of the effects of pollutants on the adrenocortical responses to stress and life cycles are needed. For example, persistent organochlorine residues in the environment reduce reproductive function in glaucous gulls, *Larus hyperboreus*, in the Arctic (Bustnes et al. 2003). This long-term exposure was also accompanied by elevated baseline levels of corticosterone in blood of both sexes and a blunted adrenocortical response to acute stress in males (Verbven et al. 2010), suggesting possible permanent impairment of adjustments to unpredictable changes in the environment. However, although plasma levels of organochlorine compounds in the blood of another arctic-breeding gull, the black-legged kittiwake, *Rissa tridactyla*, were positively correlated with corticosterone concentrations in pre-laying females, the relationship was not found in brooding birds (Nordstad et al. 2012). More investigation will be needed to determine whether persistent pollutants in the environment can act as permanent perturbation factors. It should also be pointed out that the relationship of baseline glucocorticoid levels with fitness remains unclear (e.g. Bonier et al. 2009).

Implications of global change

One of the more serious consequences of global change over the past 50 years is the increase in the frequency and intensity of labile and permanent perturbation factors (Wingfield, Kelley & Angelier 2011a). For example, there is accumulating evidence that climate change is resulting in more extreme weather events such as severe storms, record-breaking heat or cold, prolonged droughts, floods, increasing oceanic winds and wave height (citations reviewed in Wingfield & Ramenofsky 2011; Wingfield, Kelley & Angelier 2011a; Wingfield et al. 2011b). Therefore, in addition to changes in the predictable environment such as earlier spring and later autumn, there are changes in unpredictable events as well. The sum impact of these environmental events plus other changes of anthropogenic origin such as human disturbance, urbanization, pollution, etc., on endocrine response systems remains to be explored fully (reviewed in Walker, Boersma & Wingfield 2005; Wingfield 2008; Wingfield & Mukai 2009). Although coping mechanisms of vertebrates involve several hormone suites, activation of the hypothalamo-pituitary–adrenal cortex axis in tetrapod vertebrates and the homologous interrenal cells in fish are universal responses to most, if not all, perturbation types (e.g. Sapolsky, Romero & Munck 2000). I will focus on this axis next.

The hypothalamo-pituitary–adrenocortical axis as a transducer of environmental perturbations

The adrenocortical response to acute environmental perturbations potentially triggers an emergency life-history stage that redirects the individual away from daily routines or seasonal life-history stages into coping and survival modes. The results are savings of energy and behavioural changes that promote survival and/or extend the period an organism can endure the perturbation. Secretions and regulation of the HPA axis are summarized in Fig. 1. The left-hand part of the figure represents the hormone secretion cascade, in this case the HPA axis of birds. Perturbations of the environmental are perceived by sensory modalities and that information is transduced into neuropeptide secretions such as corticotropin-releasing hormone (CRH), arginine vasotocin (AVT) and mesotocin that regulate expression of a precursor or pro-peptide hormone, pro-opiomelanocortin (POMC), in the anterior pituitary (e.g. Nelson 2011). Pro-opiomelanocortin is cleaved to give several peptides including adrenocorticotropic (ACTH). Release of ACTH from the pituitary gland into the blood is also regulated by CRH and AVT. ACTH acts on adrenocortical cells to activate CYP enzymes including hydroxysteroid dehydrogenases that synthesize glucocorticoids such as corticosterone and cortisol. Release of glucocorticoids into the blood is a major end point of the cascade of events that are part of the adrenocortical response to stress. Glucocorticoids provide negative feedback signals for ACTH release from the pituitary as well as CRH release from the hypothalamus (Sapolsky, Romero & Munck 2000; Romero, Dickens & Cyr 2009; Nelson 2011). Once in the blood, glucocorticoids circulate bound to a carrier protein corticosteroid-binding globulin (CBG) that is the transport part of the system (red lines). More than 90% of glucocorticoids circulating in avian blood are
bound by CBG. On reaching target cells such as liver or neurons in the brain involved in the emergency life-history (coping) stage, it is thought that only glucocorticoids unbound to CBG can enter cells (Breuner et al. 2003; Malisch & Breuner 2010). Note that glucocorticoids bound to CBG may also be accessible to target cells if CBG levels are also regulated (Malisch & Breuner 2010). Once inside the cell, there are two types of genomic receptor that can bind corticosterone or cortisol and become gene transcription factors. The mineralocorticoid type receptor (MR) binds with high affinity and so can be saturated at low circulating levels of glucocorticoids. The glucocorticoid type receptor (GR) has a lower affinity for glucocorticoids and is saturated only at higher concentrations (Nelson 2011). Thus, GR has been proposed as the ‘stress’ receptor (Breuner et al. 2003). Note also that there is strong evidence for a membrane receptor (non-genomic) that mediates rapid behavioural effects within minutes (Breuner, Greenberg & Wingfield 1998). The genomic receptors have effects through gene transcription, different genes affected by each receptor type, and thus require up to several hours for biological effects to be manifest.

As seen in Fig. 1, there are also steroidogenic enzymes expressed in target cells that can modulate how much corticosterone or cortisol encounters at least genomic receptors. 11β-hydroxysteroid dehydrogenase (11β-HSD) has two major forms – 1 and 2. 11β-HSD2 converts corticosterone to deoxycorticosterone, or cortisol to cortisol. These metabolites cannot bind to any known receptor, and this enzyme system is a deactivation shunt (Holmes et al. 2001). 11β-HSD1 tends to have the opposite effect enhancing glucocorticoids and thus likelihood of binding to MR or GR. As in Fig. 1, co-repressors and co-activators also are points of regulation for gene transcription and responses that control the emergency life-history stage and affect the immune system (Shibata et al. 1997; Edwards 2000). The adrenal medulla of mammals (medullary cells in birds are called chromaffin) is a key component of the fight-or-flight response (right-hand part of the figure) secreting epinephrine. This neuroendocrine system is also involved in the emergency life-history stage.

There is growing evidence in mammals and birds that some target cells in liver and brain express enzymes allowing them to synthesize cortisol or corticosterone de novo from cholesterol (Taves, Gomez-Sanchez & Soma 2011b; Taves et al. 2011a). Local production of glucocorticoids may allow differential regulation of responsiveness of tissues to specific stimuli without broadcasting the hormone signal throughout the organisms as would be the case if
they were secreted directly into blood (Schimdt et al. 2008; Taves, Gomez-Sanchez & Soma 2011b).

The three-part system – hormone cascade, transport in blood and response networks in the target cells – is also well conserved across vertebrates, but the diversity of ways by which specific components can be regulated to modulate responsiveness to stress is very great. How this flexibility is used to regulate responses to perturbation factors remains to be determined (Wingfield 2012).

Responses of the hypothalamo-pituitary–adrenal cortex axis to abiotic labile perturbation factors

Field studies have allowed us to evaluate the potential for abiotic perturbations of the environment to elicit an adrenocortical stress-like response under natural conditions. In some cases, these investigations have been followed up by experiments in the laboratory to further identify mechanisms by which organisms cope with a capricious environment. It is important to acknowledge that any abiotic perturbation probably acts through biotic ways such as imbalance of homeostasis, disruption of food resources, increased energetic demand, exposure to predators, etc. Nonetheless, much remains to be done to fully understand the breadth of responses to unpredictable perturbations and how free-living populations might fare in a changing world where the frequency and intensity of perturbations of all kinds appear to be increasing (Wingfield, Kelley & Angelier 2011a; Wingfield et al. 2011b). Summaries of what is known of how free-living vertebrates respond to abiotic factors follow, and it is hoped that they will stimulate more field studies of responses to perturbations and how investigators must be opportunistic to take advantage of these events and document the responses accordingly.

Weather events that disrupt the normal life cycle

There are now numerous reports of weather events such as sudden storms and more prolonged inclement weather on the life cycles of vertebrates. Most information on adrenocortical responses to these events come from birds (e.g. Romero, Reed & Wingfield 2000) and has been reviewed in detail by Wingfield & Ramenofsky (2011). Vertebrates in general can incur considerable energetic costs during the normal life cycle (e.g. to breed successfully), making them particularly vulnerable to weather events. Spring weather such as a late snowfall or heavy rains can result in total loss of young (e.g. Perrins 1979). Linking these effects of weather with biotic effects such as increased numbers of predators can affect survival of adults and young even more (Perrins 1979). Although there are now numerous accounts of HPA axis responses to weather events, much remains to be done to understand the complex ways in which organism and environment interact to customize the physiological and behavioural responses. Some examples follow (see Wingfield & Ramenofsky 2011 for more details).

Organisms at lower latitudes are frequently exposed to extreme heat and periods of drought. Exposure to heat and drought is usually associated with deserts, but such conditions can also be found when an individual finds itself exposed to direct sunlight, for example, open terrain or to extreme hot, dry weather. Desert species cope with xeric conditions by ceasing all foraging activity during the hottest part of the day and seek refugia where exposure to extreme heat is lowest (e.g. Wolf 2000; Williams & Tiellman 2001; Tiellman 2002). Use of microhabitats can greatly ameliorate potentially disruptive effects of weather but also can detract from other routine activities (summarized in Wingfield & Ramenofsky 2011).

Cain & Lien (1985) showed that water restriction of bobwhite quail, Colinus virginianus, elevated levels of corticosterone. Treatment of quail with corticosterone at doses that mimicked circulating levels during water restriction resulted in decreased testes, ovaries and oviduct weights accompanied by reductions in sperm and egg production. Cain and Lien (1985) proposed that inhibition of reproduction by heat and drought was a result of stress-induced corticosterone increases that suppressed reproductive function.

White-browed sparrow weavers, Plocepasser mahali, in the Luangwa Valley of Zambia, Africa, showed an almost complete failure to initiate breeding during the period August - December 1987 because of a longer than usual dry season. These data agree with many other reports showing inhibitory effects of drought on breeding in birds and other vertebrates (Wingfield & Ramenofsky 2011). However, plasma levels of corticosterone were not affected, and mechanisms underlying adjustments of breeding in relation to heat and drought are enigmatic.

Low environmental temperature can be challenging for ectothermic and endothermic vertebrates alike and particularly small birds. As for responses to extreme heat, thermoregulatory responses to cold involve the use of microhabitats such as a cavity in a tree, an insulated nest or cavity beneath snow. Huddling behaviour is common in birds and mammals in cold regions, although huddling may also occur for a variety of reasons including protection from predators and for social reasons (e.g. Vickery & Millar 1984; Wingfield & Ramenofsky 2011 for review). Rogers et al. (1993) showed that dark-eyed juncos, Junco hyemalis, wintering in Tennessee had higher plasma levels of corticosterone during a cold spell and snow storm than before or after. Corticosterone levels were highest during the storm when the juncos were actually on the move and had left their home range, that is, in an emergency life-history stage (Rogers et al. 1993).

The incidence of torpor in the rufous hummingbird, Selasphorus rufus, varies with season being higher in autumn and lower in spring and summer. Torpor can be induced at any time of the year providing flexibility in relation to weather conditions. Experiments on captive rufous hummingbirds showed that when torpor use increased,
excreted corticosterone levels in cloacal fluid prior to a torpor bout were elevated (Hiebert et al. 2000). Furthermore, experimental administration of corticosterone in nectar increased use of torpor and depressed food intake (Hiebert et al. 2004).

Wind and heavy rain can have dramatic effects on the normal life cycle. The common diving petrel, Pelecanoides urinatrix, normally thrives in high winds and large oceanic swells, but in June 1991, a severe storm with extreme winds, low temperatures and snow led to reduced feeding efficiency. These birds flew to nearby oceanic islands to shelter (Veit & Hunt 1991; ). Body weights of diving petrels captured during the storm were lower compared with those captured during calm weather, and plasma levels of corticosterone were elevated (Smith, Wingfield & Veit 1994). Such refuge-seeking behaviour during severe weather conditions is analogous to that of ground-feeding songbirds in snow described above. Furthermore, the responses of corticosterone secretion appear identical.

Hypoxic conditions, or dead zones, occur seasonally in shallow coastal seas, estuaries and brackish lagoons as a result of high temperatures and increasing salt concentrations that decrease oxygen content. These conditions result in mortality of marine life, or movement away from the area in, for example, fish (e.g. Thomas et al. 2006, 2007). In the northern Gulf of Mexico, seasonal moderate hypoxia may affect up to 30% of the regions’ shallow coastal waters in late summer (Engle, Summers & Macaulay 1999). Increased eutrophication of coastal waters due to agricultural use of fertilizers rich in nitrogen and raw sewage has resulted in expansion by 2- to 10-fold of sea areas (Thomas 2006, 2007). These dead zones likely result in major changes in coastal ecosystems and represent a major perturbation for many marine plants and animals (Joyce 2000). It is important to note that natural states of hypoxia occur seasonally, and many organisms can prepare for these extreme conditions. However, increased area of hypoxic zones and the development of new zones represent an unpredictable source of perturbation that could be particularly devastating locally.

Until recently, very little was known about how fish cope with hypoxic conditions. Initially, hypoxia-tolerant fish increase their capacity to deliver oxygen to tissue and reduce aerobic metabolism and oxygen demand (Hochachka & Somero 2002), and hypoxia-induced gene expression inhibits reproductive function in Atlantic croaker, Micropogonias undulatus. This species also appears to shift distribution and possibly decrease metabolism at times of year when hypoxic zones occur naturally (Eby & Crowder 2002; Bell & EGGLESTON 2005). This suggests that mild-to-moderate hypoxia may signal changes in metabolic, behavioural and reproductive functions to cope with seasonal hypoxia. Now that these dead zones are expanding and becoming severely hypoxic, then the potential for greater mortality and loss of biodiversity in these regions is critical.

In croakers sampled from an estuary in Florida, exposure to moderate hypoxia resulted in significant reduction in ovarian and testicular development. Hypoxia-induced gene expression triggered a decrease in serotonin content in the hypothalamus through inhibition of tryptophan hydroxylase, an enzyme critical for the synthesis of serotonin. Experimental replacement of serotonin restored reproductive function suggesting that this mechanism may be adaptive in regulating reproduction in response to unfavourable conditions (Thomas et al. 2006, 2007). These authors point out that because the intensity and extent of hypoxic zones is increasing, then the metabolic, behavioural and reproductive changes triggered by hypoxia may severely disrupt the life cycle of this fish and other organisms.

In parrotfishes from Caribbean Islands, experimental exposure to hypoxia increased glucocorticoid metabolite levels in faeces of these aquarium-adapted fish (Turner, Nemeth & Rogers 2003). Similarly, in the channel catfish, Ictalurus punctatus, another estuarine fish, low oxygen content of the water resulted in a significant increase in plasma cortisol levels that then declined within 30 min when fish were returned to normal oxygen concentrations in sea water (Tomasso, Davis & Parker 1981). Indeed, there is growing evidence that glucocorticoids may play a major role in responses to, and acclimation to, hypoxic conditions in vertebrates in general (Kodama et al. 1997). In mammals, hypoxia-inducible transcription factor (HIF-1) is upregulated by glucocorticoids acting through GR (Kodama et al. 1997).

Rainbow trout subjected to severe hypoxia showed marked increases in blood cortisol and catecholamines (Swift 1981; van Raaij et al. 1996). About 60% of the fish did not survive recovery probably because their behavioural response to hypoxia included bursts of activity and strenuous avoidance behaviour. Those fish that survived showed much less activity and conservation of energy with reduced oxygen demand. Non-surviving fish had higher levels of catecholamines, but lower cortisol (van Raaij et al. 1996). The physiological and behavioural responses to unpredictable hypoxic conditions deserve more study to clarify coping strategies.

**Conclusions**

Coping with environmental perturbations involves a finely tuned spectrum of responses that orchestrate physiological and behavioural responses to immediate threats as well as long-term adjustments to more severe conditions. The responses to abiotic factors resemble those to biotic and social factors further suggesting a ‘common’ set of reactions to perturbations that are highly conserved across vertebrates. Fig. 1 summarizes the hormone cascades and responses of the hypothalamo-pituitary-adrenal axis in, for example, a bird as well as indicating that other components of the emergency life-history stage such as the fight-or-flight responses, activation of the immune system and
cellular responses to stress including heat-shock proteins, DNA repair and mitigation of oxidative stress are also important. Hormonal mechanisms that integrate all these responses, especially under natural conditions, await further investigation. Focusing on the hormonal cascades, it is critical to understand that there are three major levels of regulation by which the adrenocortical responses to perturbation factors can be modulated individually or seasonally at the population level. Fig. 1 summarizes the neuroendocrine/endocrine cascade that can be regulated independently of the transport system and ultimately responsiveness of the target cells. Although these three levels are highly conserved, there are many ways in which the overall responses to perturbations of the environment can be modulated. These could vary across individuals within a population or within an individual over seasons.

There is also an important need to explore the repercussions of responding to environmental perturbations at the population level. Although individual responses are now the subject of broader interest, population effects resulting from increasing frequency and intensity of perturbations remain to be seen. For example, do those populations that endure perturbations of the environment have a more flexible emergency life-history stage, and regulatory mechanisms underlying it, than those that cannot and undergo rapid decline? Such future investigations will be important but challenging.

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