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A state of non-specific tension in living matter? Stress in Australian animals

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ABSTRACT

Evidence of stress responses in Australian animals is reviewed through a series of case studies involving desert frogs and lizards, small carnivorous marsupials, desert wallabies, a dwarf kangaroo species, the quokka wallaby and a small nectarivorous bird. An operational definition of stress as "*the physiological resultant of demands that exceed an animal's homeostatic capacities*" is used to identify instances of stress responses in the field, and to gauge their intensity. Clear evidence of stress responses is found in small dasyurid marsupial carnivores, and desert agamid lizards, both of which are semelparous. Other instances of seasonal stress responses include the Rottnest Island quokka, the Barrow Island euro kangaroo and a small nectarivorous bird, the Silvereye. The review also highlights the high level of physiological adaptation of some desert wallabies, such as the Spectacled hare wallaby, which is able to maintain physiological homeostasis in the field when challenged by conditions of extreme drought. The importance of thermal and hygric refugia for the long-term survival or rock wallabies, which apparently lack any hormonal control of renal function, is also highlighted.

1. Introduction

One of the main difficulties is defining 'stress', after it was first described somewhat poetically, by Hans Selye as "*a state of non-specific tension in living matter*" (Selye, 1946, 1952). An essential step is to distinguish 'stressors', that emanate from the environment, from the condition of 'stress', which is the impact of single or multiple stressors on the organism. This avoids meaningless contradictions such as "the stress of reproduction". Examples of later definitions that confuse stressors with stress are: "*Any factor that inhibits growth and reproduction in a population*" (Brett, 1958), and "*An environmental condition that, when first applied, impairs Darwinian fitness*" (Sibley and Calow, 1989).

Australia's abysmal record in protecting its unique wildlife is well known (Burbidge, 2009; Burbidge and McKenzie, 1989; Woinarski et al., 2015) and threatened and endangered species would appear to be prime candidates for stress studies. Stress can result from predation, habitat destruction, climate change and pollution. Stressors commonly reduce fitness of animals and lower their reproductive potential and case studies have the potential to alert us to the deleterious effects of stressors impacting endangered species (Bradshaw, 1999). Key to this is an understanding of how stressors initiate adaptive activation of the hypothalamic–pituitary–adrenal axis (HPA), in an effort to maintain homeostasis; and how chronic activation of this axis leads itself to stress.

The medical concept of 'allostasis' or '*maintaining stability through change*' (Sterling and Eyer, 1988) was co-opted by (McEwen, 1998) who proposed that animals experience an 'allostatic load' divided into three levels: A = baseline corticosteroid levels, B = daily and seasonal changes in levels, and C = 'allostatic overload' leading to chronic stress if not alleviated (McEwen and Wingfield, 2003). The use of the balance between energy expenditure and intake as a measure of allostatic load, however, has been criticised by (Walsberg, 2003) and Michael Romero has countered with an innovative 'reactive scope model' of stress (Romero, 2012; Romero et al., 2009). This is based on the concept that reacting to a stress (reactive homeostasis or level B in the allostatic system) involves 'wear-and-tear' and erodes the safety gap between B and C. Chronic exposure then leads to allostatic overload (Fig. 1). An insightful application of this model was able to predict which particular individuals of a population of Galapagos iguanas would die from starvation following an El Nino event affecting their food supply of kelp (Romero, 2012).

A recent review of factors influencing the stress response in Australian marsupials documents studies that have measured either plasma or faecal levels of glucocorticoids (Hing et al., 2014). Elevated steroid levels alone, however, are not necessarily

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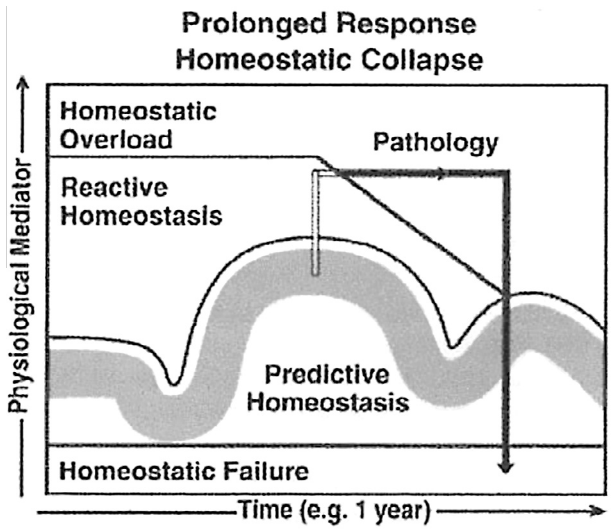

Fig. 1. The reactive scope model. A diagrammatic representation of the impact of stressors on 'wear-and-tear' as depicted by a progressive decrease in the threshold between reactive homeostasis and homeostatic overload. Prolonged exposure leads to pathology, then homeostatic failure and death (from Romero et al., 2009, with permission).

an index of stress (Johnstone et al., 2012) as they form part of the animal's normal adaptive response to the stressor (Angelier and Wingfield, 2013; Romero, 2002). Opinion is also still divided on whether total levels, or non-protein-bound 'free' levels of corticosteroids, are what should be measured in studies of stress (Breuner et al., 2013; Desantis et al., 2013; Schoech et al., 2013).

2. An operational definition of stress

An early simple operational definition proposed for stress, which avoids some of these difficulties is "*the physiological resultant of demands that exceed an animal's homeostatic capacities*" (Bradshaw, 1986, 1987, 1997, 2003). This allows one to both discern the incidence of stress, and also measure its severity. Inherent in the definition is the understanding that the animal has maximally deployed whatever defences it has against the stressor and that, despite this, its internal state or '*milieu intérieur*' has been significantly perturbed. Thus, by simultaneously monitoring the extent of the animal's adaptive response to a particular stressor (e.g. levels of adrenocortical hormones, antidiuretic hormone, avoidance behaviour etc.) along with measures of its internal state (e.g. total body water content, plasma osmolality, body temperature etc.) one can effectively detect individuals that are experiencing stress. The following is a series of case studies illustrating the use of this approach in identifying stress responses in a range of Australian animals.

3. Dasyurid marsupials

Although a similar well-known reproductive strategy was known to occur in lower vertebrates, perhaps the most iconic example of stress in a group of Australian animals is the post-mating mortality of males that occurs in a number of species of small dasyurid marsupials (Bradley, 2003, 2007). When described, this was the first instance of semelparity[1] in a mammal, although a well-known reproductive strategy in lower vertebrates, such as salmon (Bonnet, 2007).

A PhD student, John Barnett, first reported on the bizarre male die-off following mating of *Antechinus stuartii* (now *A. agilis*) in the field (Barnett, 1973) and his discovery was quickly followed by papers describing the changes that occurred in the males, leading to their early demise during the breeding season (Lee et al., 1977, 1982). In contrast to females, males progressively lose body mass and fur (alopecia), and autopsies revealed severe gastric ulceration and haemorrhagic adrenal glands. The immunological system of the males was also compromised, exposing them to a variety of adventitious infections that were usually the ultimate cause of death (Lee and McDonald, 1985).

The underlying mechanisms responsible for this unusual life history pattern were disentangled in an important series of eco-physiological investigations where changes in blood testosterone levels (T) were measured in males prior to, and during the breeding season, and correlated with changes in cortisol (F) secretion from the adrenal gland (Bradley et al., 1980). What was innovative in these studies was that, as well as measuring total F concentrations in the plasma, equilibrium dialysis was used to measure free and protein-bound levels of the cortisol. Testosterone levels increased in the blood of males at the start of the winter breeding season and reached very high levels by early August. Males of this species are very aggressive – forming male "leks" (Cockburn and Lazenby-Cohen, 1992) and copulation with females may last for as long as 24 h. Total levels of F in the plasma changed very little in males during the breeding season suggesting, at first sight, that they were not 'stressed' as plasma corticosteroids such as cortisol and corticosterone normally rise in stressful situations. When, however, the ratio of free to bound steroid was measured by equilibrium dialysis, a very large increase in the free component was observed in the latter part of the breeding season in the males. This decrease in the amount of cortisol bound to protein was found to be due to a direct effect of T on the production and secretion of corticosteroid-binding globulin (CBG) from the liver. The homeostatic 'feedback' system, which would normally have rectified this change, failed to operate (McDonald et al., 1986). These changes are shown in Fig. 2 and it is clear from these data that the males die as a result of the effects of abnormally high free levels of F.

Field studies of other species of dasyurids have shown post-mating male die-off is not unique to the genus *Antechinus* (Bradley, 1987; Green et al., 1989; Mills and Bencini, 2000; Mills et al., 2012) but does not occur in the larger dasyurid carnivores such as *Dasyurus geoffroii*. A recent study has found that mammalian semelparity is also not unique to marsupials, with a report from an eutherian species (Smith and Charnov, 2001). There have been various attempts to interpret the evolution of mammalian semelparity (Braithwaite and Lee, 1979; Diamond, 1982; Dickman, 1993; Dickman and Braithwaite, 1992; Lee and Cockburn, 1985) including an 'adaptive-stress senescence' hypothesis (Bradley, 1997) as well as sperm competition (Dickman, 1993).

There seems little doubt that small male dasyurids are subject to stress as a result of their intense reproductive effort – regulatory hormones are maximally stimulated and there are major perturbations of body condition leading to death. The metabolic impact of elevated 'free' levels of cortisol also provides an excellent example of Romero's 'reactive scope model' of stress, with wear-and-tear eroding the gap between reactive homeostasis and allostatic overload (Romero et al., 2009).

4. The quokka

The quokka, *Setonix brachyurus* (Fig. 3), is a small macropodid wallaby, originally a mainland forest dweller, but surviving as a

[1] Named after Semele, daughter of Cadmos the King of Thebes and mother of Dionysus, who became the lover of Zeus but died when she forced him to expose himself in all his glory.

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