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Stress in an Island kangaroo? The Barrow Island euro, *Macropus robustus isabellinus*

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ABSTRACT

Selected physiological parameters were monitored over a 4-year period in the Barrow Island euro, *Macropus robustus isabellinus*, in Western Australia in a study of this species' homeostatic capabilities in an extremely arid habitat where individuals are exposed to high environmental temperatures and a lack of free water for much of the year. Evidence was found of a significant change in the animal's *milieu intérieur* on only one occasion on Barrow Island: in November 1994, following a protracted 8-month drought. Euros had significantly elevated levels of plasma osmolality, cortisol, anti-diuretic hormone (lysine vasopressin – LVP), and a reduced eosinophil count. This suggests that these animals may have been dehydrated, despite the operation of appropriate physiological responses to water deprivation. Lower eosinophil counts also suggest that immune function may have been suppressed as a result of the elevated corticosteroid levels. Comparisons with the mainland sub-species of the euro revealed the presence of a non-generative normocytic hypochromic anaemia in Barrow Island euros that potentially compromises their aerobic capacity. Barrow Island is Australia's most important A Class Reserve, harbouring 8 species of marsupials, 4 of which are now extinct, or virtually so, on the adjacent mainland. This study reveals the remarkable effectiveness of the euro's homeostatic capacities, however, its future conservation depends on ensuring that potential stress due to declining water availability and environmental change is avoided.

1. Introduction

In Australia, many mammal species have declined in abundance and distribution, and extinctions on this continent constitute almost half of the recent mammalian extinctions worldwide (Burbidge and McKenzie, 1989; Lomolino and Channell, 1995). In an attempt to identify those factors implicated in the decline of Australia's mammal fauna, McKenzie et al. (2007) derived an index of faunal attrition (FAI) and then looked for significant correlations with a number of environmental and fauna variables. Mean annual rainfall explained more of the variation in the (FAI) than any other factor, including 'environmental change', a measure of post-European disturbance. Water and its availability is one of the critical limiting factors in the widespread arid and semi-arid environments of Australia and one that plays an important rôle in determining species' distributions, both past and present (Burbidge et al., 2008). Water availability may also be of particular significance in modified habitats where animals may ultimately succumb to dehydration as a result of environmental degradation (Jones et al., 1990; Bradshaw, 1997a).

Parameters that identify and measure stress in individuals have an important rôle to play in conservation biology because the ability of individuals to cope with environmental stressors, such as drought, will be important in determining species' long-term survival (Parsons, 1995). Monitoring the physiological status of individuals in the field may give an indication of subtle processes occurring in the population that are indicative of detrimental changes in well-being. Thus a potential threat to the population may be identified before numbers actually start to decline. In this study, evidence of stress due to water deprivation was investigated in a free-ranging population of a macropodid marsupial (*Macropus robustus isabellinus*) inhabiting the arid environment of Barrow Island, off the north-west Australian coast. Barrow Island with an area of 22,300 ha is Australia's most important A Class Nature Reserve and is home to 14 species of mammals, 8 of which are marsupials, with 4 of these being either extinct or virtually so on the adjacent mainland. The island receives cyclonic rain in the summer months (December–March) averaging 306 mm, but this can vary from as little as 54 mm to over 700 mm in any one year.

Stress has proven notoriously difficult to identify and to measure, following its early somewhat poetic description as "*a state of non-specific tension in living matter*" by Selye (1952). Other definitions such as "*any factor that inhibits growth and reproduction in a population*" (Brett, 1958) or "*an environmental condition that, when first applied, impairs Darwinian fitness*" (Sibley and Calow, 1986) are equally unhelpful. Numerous physiological variables have been employed previously as indicators of stress e.g., haematological

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changes including changes in differential leucocyte count; plasma glucose concentration; changes in regulatory hormone levels; and adenylate energy charge (Ivanovici and Wiebe, 1981). The release of adrenal corticosteroids formed the basis of the non-specificity of the vertebrate stress-response in Selye's General Adaptation Syndrome (Selye, 1946, 1952), and is commonly used as an index of stress in vertebrates. However, the corticosteroids, cortisol and corticosterone, released into the blood stream form part of the regulatory, homeostatic, response to the stressor and can only properly be used as an indicator of the presence of a stressor, not as a measure of its impact (Wingfield et al., 1997; Moberg, 2000).

Recently, the debate on what constitutes stress in man and animals has been joined by McEwens's development of the concept of 'allostasis' (McEwen, 1998a,b). This was originally conceived as "the maintenance of stability through change" by Sterling and Eyer (1988) and meant to address situations more complex than simple local feedback, involving both body and brain and the resetting of set-points. This concept of allostasis and 'allostatic load' has been developed and applied by McEwen and Wingfield (2003a), particularly in relation to the survival of Arctic birds. It involves recognising three levels of adreno-cortical activity: (a) baseline corticosteroid levels that are essential for the maintenance of carbohydrate and electrolyte homeostasis; (b) daily and seasonal changes in corticoid levels in relation to allostasis, or 'allostatic load', and (c) high transitory peaks that activate 'emergency life history stages' (ELHS) and will provoke stress if the response is not adequate, or the animal is unable to escape from the stressor ('allostatic overload').

This new approach has a number of advantages, including acknowledging that elevated levels of plasma corticosteroids are not in themselves adequate indicators of stress, and focuses on their adaptive rôle in combating potentially stressful environmental changes. In an early attempt to provide a working definition that would enable one to both identify stress, and to measure its incidence, Bradshaw (1986) described stress as "*the physiological resultant of demands that exceed an organism's homeostatic capacities*". The rationale for this definition is the paradigm that under normal conditions vertebrate organisms are in a controlled state of physiological balance and that they have evolved systems specifically designed to regulate their internal environment and buffer them from changes in the external environment (Bernard, 1878; Cannon, 1929, 1939; Sapolsky, 1992). Moving away from this regulated state (a) above, to state (b) should therefore be associated with a significant and measurable perturbation of the animal's *milieu intérieur*. Applying McEwen and Wingfield's (2003a) concept of 'allostatic load', this change, if prolonged, may lead to stress with the danger of catabolic changes and a suppression of the immune system (Sapolsky, 2002).

Two obvious physiological changes should be associated with this allostatic shift: a significant deviation of the internal environment away from whatever is considered to be its normal state, and activation of the regulatory stress-response systems, whatever they may be. An example would be, evidence of significant dehydration in a mammal, despite maximal circulating levels of the anti-diuretic hormone, ADH (Bradshaw, 1992). In the current study, evidence of an increase in allostatic load, and possible stress due to seasonal lack of water, was investigated in Barrow Island euros (*Macropus robustus isabellinus*). This is an endemic sub-species of the euro or common wallaroo found in north-western and eastern Australia and is approximately half the size of the mainland form (*Macropus robustus erubescens*). Because of its much higher surface area to volume ratio, this dwarf form of euro may be more susceptible to water deprivation than the mainland form, which ranges across much of the arid interior of Australia. There is no evidence, however, that euros on Barrow Island are declining, with a population estimate of 1800 individuals (Short and Turner, 1991) that was derived originally from a continental euro population isolated from the mainland some 8–10,000 years ago by rising sea levels (Main, 1961; Dortch and Morse, 1984). Dwarfism in insular populations of mammals is common and implies adaptive changes to a restricted environment and not necessarily any loss in viability (Lomolino, 1985, 2005).

Previous studies with other macropodid marsupials on Barrow Island have shown that rates of water turnover in the driest part of the year are extremely low (King and Bradshaw, 2008), with the Spectacled hare wallaby *Lagorchestes conspicillatus* recording the lowest rate for any mammal, including those from the deserts of north Africa and north America (Bakker and Bradshaw, 1989; Bradshaw, 1990; Bradshaw et al., 2001). Despite these impressive performances, Barrow Island with its extremely arid environment, no sources of free water, and infrequent precipitation associated with high temperatures, is likely to be the most crucial factor limiting the survival and persistence of species on the island.

Seasonal variations in water balance of euros were measured using tritium to estimate both total body water content and rates of water influx and efflux. Total body condition was estimated using a condition index and hydration state was monitored through changes in plasma osmolality. Haematological parameters were also measured along with the circulating levels of cortisol, the stress-related hormone in macropodid marsupials (McDonald and Bradshaw, 1977; Lee and McDonald, 1985), and anti-diuretic hormone (ADH). Lysine vasopressin (LVP), rather than arginine vasopressin (AVP) has been identified as the major neurohypophysial peptide in macropodid marsupials (Hurpet et al., 1980; Chauvet et al., 1983a,b) and shown to function physiologically as an ADH in a number of species (Wilkes and Jannsens, 1986; Bradshaw et al., 2001; King and Bradshaw, 2008).

Our hypothesis underpinning this study was that the Barrow Island euro is well-adapted to its arid environment, but that it is nonetheless vulnerable due to its dwarfism and rare, extreme conditions of high temperature and water deprivation may induce stress responses that impact on its long-term probability of survival. The specific aims of the study were thus threefold; firstly, to monitor changes in physiological status of the Barrow Island euro, comparing body condition, plasma osmolality and water balance during wet and dry periods on the island in order to assess the efficacy of the species' homeostatic capacities; secondly, to compare circulating levels of cortisol and ADH over the same periods, to ascertain whether levels diverged from their functional range and were associated with significant changes in haematology, and, thirdly, to ascertain whether physiological changes associated with extreme drought should be classified as increases in 'allostatic load' or evidence of stress due to chronic water deprivation in this population.

2. Materials and methods

2.1. Study area

A total of 7 field trips was made to Barrow Island in December 1991, Dec '93, Nov '94, Jun '95, Sep '95, Sep '96 and Nov '96, each lasting from 2 to 4 weeks. Of these, that in June 1995 was the only 'wet trip', with 50 mm recorded that month, and following a major cyclone in February that deposited 490 mm of rain. Barrow Island is a limestone island 233 sq km in area located approximately 1400 km north of Perth, 60 km off the north–west coast of Western Australia (Lat: 20.82 S Long: 115.39 E, Fig. 1). Barrow Island has an arid climate; average annual rainfall is 306 mm, but highly variable, and average maximum and minimum shade temperatures in summer are 34.7 °C and 25.6 °C, and in winter are 24.3 °C and 16.9 °C, respectively. Most of the island's rainfall is associated with