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Assessing stress in Western Hudson Bay polar bears using hair cortisol concentration as a biomarker

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ABSTRACT

The development of novel biomarkers to help assess whether polar bear (*Ursus maritimus*) health is impacted by long-term physiological stress associated with climate change represents an emerging area of research. Reductions in sea ice cover and food availability are potentially stressful, and chronic stress can have deleterious effects that may impair individual and population level health. Cortisol is the principal effector hormone of the stress response and has previously been linked to aspects of polar bear life history (e.g., reproduction and growth) known to be negatively influenced by environmental change. Understanding stress is important for polar bears at the southern limit of their range, such as those in Western Hudson Bay (WH), where rapidly changing sea ice phenology threatens population viability. We examined the relationships between age, reproductive status, and body condition (fatness) and hair cortisol concentration (HCC) in 729 polar bear hair samples collected in WH from 2004–2013. Overall, there was a negative relationship between fatness and HCC, suggesting that bears in poorer body condition experienced higher levels of stress. However, when reproductive status was included in our analysis, this relationship only held for male and lone female bears. Females with dependent offspring had consistently low fatness and elevated HCC, likely because of the high cost of maternal care. We also found a positive correlation between HCC and age for: (1) bears in poor body condition, possibly due to nutritional stress compounding effects of aging; and (2) male bears, potentially due to stress and injury associated with intrasexual mate competition. These findings support the use of HCC as a biomarker for polar bear health. Furthermore, we have established a HCC benchmark against which future population-level effects of climate change in WH polar bears can be compared.

1. Introduction

Arctic sea ice is the primary habitat for polar bears (*Ursus maritimus*) that use the ice as a platform to travel, hunt, and mate (DeMaster and Stirling, 1981; Stirling and Derocher, 1993). The Arctic is warming faster than more southern regions as a consequence of climate change, with the most pronounced effects being the significant reduction in sea ice extent and period of coverage due to the changing phenology of seasonal melting and freezing (Comiso et al., 2008; Stroeve et al., 2012). With positive feedback from solar absorption during an increasing and expansive open water season (Stroeve et al., 2014), as well as rising atmospheric CO_2 concentrations (IPCC, 2014; Solomon et al., 2009), further reductions in sea ice extent are predicted (Hamilton et al., 2014; Holland et al., 2006; Liu et al., 2013). Earlier ice breakup and a prolonged ice-free period has been linked to decreases in polar bear body condition (Rode et al., 2012; Stirling et al., 1999), survival (Bromaghin et al., 2015; Regehr et al., 2010, 2007) and reproductive success (Hunter et al., 2010), as well as increased human-bear interactions (Towns et al., 2009) and population declines (Bromaghin et al., 2015; Lunn et al., 2016; Regehr et al., 2007).

When exposed to environmental disturbance, animals undergo behavioural and physiological changes to increase chances of survival and maintain homoeostasis (Romero, 2004). These changes are part of the stress response that is regulated by the hypotha-

Abbreviations: HCC, hair cortisol concentration; WH, Western Hudson Bay.

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lamic pituitary adrenal axis through the release of glucocorticoid hormones (Habib et al., 2001; Sapolsky et al., 2000). Cortisol is the primary glucocorticoid (Davenport et al., 2006) and has been analyzed in various animal tissues (e.g., blood, saliva, feces, urine) as an indicator of stress levels (e.g., Bonier et al., 2004; Koren et al., 2002; Saeb et al., 2010). Acute stress is an adaptive response to threatening stimuli typified by 'fight or flight' physiological changes and behaviour (Charmandari et al., 2005; Habib et al., 2001; McEwen, 2007). However, prolonged or chronic stress can be maladaptive and has been linked to diminished growth and reproduction, reduced cognitive ability, increased catabolism of stored energy, and immunosuppression (Boonstra et al., 1998; Kitaysky et al., 2003; McEwen and Sapolsky, 1995; Sapolsky et al., 2000).

Stress hormones can be integrated over longer periods in tissues such as hair (Bechshoft et al., 2012a; Davenport et al., 2006; Macbeth et al., 2012) or feathers (Bortolotti et al., 2008; Koren et al., 2012), where free-circulating blood cortisol is incorporated via passive diffusion from the follicle throughout the growth period of the tissue; thereby representing the average stress burden over a period of weeks to months (Cook, 2012; Meyer and Novak, 2012; Sheriff et al., 2011). Unlike classically analyzed tissues (e.g., blood, saliva), hair cortisol concentration (HCC) is unlikely to be influenced by acute stress due to invasive sampling events as the time between capture and sampling is too brief for hair growth to occur (Bechshoft et al., 2012a; Webb et al., 2010). However, some evidence suggests that capture method may influence HCC even after the hair has ceased growing, although the pathway for this rapid integration of cortisol is unknown (Cattet et al., 2014). Once in the hair, HCC does not degrade over time (Bechshoft et al., 2012a; Webb et al., 2010) allowing for hair to be collected for analyses opportunistically (e.g., from hunted bears), non-invasively using hair snags (de Groot et al., 2013; Macbeth et al., 2010) and from historic samples (e.g., museums; Bechshoft et al., 2012a).

Behavioural and physiological responses to environmental and ecological disturbances, such as chronically elevated stress, have implications for the stability of a population (Boonstra et al., 1998; Harvell et al., 1999). As the proportion of chronically stressed individuals in a population increases, the combined influence of reduced reproduction, lower body condition, and immunosuppression may impact population viability. Understanding stress in a population, including differing vulnerabilities, is important for health monitoring and informing conservation and management decisions. Further, a meta-survey identified sea ice habitat loss, nutritional stress, and chronic physiological stress as the top three threats to the long-term health and sustainability of polar bears (Patyk et al., 2015).

Polar bears near the southern limit of their range, such as those in the Western Hudson Bay (WH) population, are particularly vulnerable to impacts of a warming climate because of the rate and magnitude of changing sea ice phenology (Castro de la Guardia et al., 2013; Gagnon and Gough, 2005; Hochheim and Barber, 2014). For these bears, building and maintaining fat reserves is important because of reduced access to their primary prey species, the ringed seal (*Pusa hispida*), during a progressively longer ice-free period in Hudson Bay (Castro de la Guardia et al., 2013; Derocher and Stirling, 1990; Hochheim and Barber, 2014; Parkinson, 2014). During this ice-free period, polar bears fast onshore (Derocher and Stirling, 1995; Polischuk et al., 2002) and use stored fat for metabolic maintenance (Atkinson and Ramsay, 1995; Derocher and Stirling, 1990). Although polar bears are opportunistic foragers and some use terrestrial food sources (e.g., berries, seaweed, caribou, seabird eggs) when available (Derocher et al., 1993b; Gormezano and Rockwell, 2013; Iverson et al., 2014; Thiemann et al., 2008), the caloric value of these items relative to the energetic cost of acquisition is low and unlikely to compensate for reduced access to ringed seals (Ramsay and Hobson, 1991; Rode et al., 2015). Bears that are in poorer condition during the ice-free period are at risk of nutritional stress and increased loss of lean mass (Molnár et al., 2009; Polischuk et al., 2002). Further, stress can increase lipolysis and exacerbate declines in body condition (Adam and Epel, 2007). Progressively earlier ice breakup and the subsequent extension of the fasting period may compromise the overall health and long-term viability of the WH population (Molnár et al., 2010, 2014; Stirling and Derocher, 2012).

In this study, we used hair samples to test whether HCC can be used as a proxy (i.e., a biomarker) to assess aspects of health in free-ranging polar bears in WH. We also examined the combined influence of body condition and demographic variables (i.e., age, sex, and the presence of dependent offspring) on HCC.

2. Materials and methods

2.1. Study system – Western Hudson Bay

Hudson Bay is a large inland sea that is ice covered in winter and ice free in summer (Hochheim et al., 2010). Seasonal ice forms over Hudson Bay from mid-November to December and reaches maximum thickness in April. Breakup begins in May, followed by a full open water season by August (Hoover, 2010). Significantly earlier breakup and later freeze-up (each defined as 50% ice coverage) has decreased the number of days of ice cover in Western Hudson Bay with a corresponding increase in the number of days the bears spend ashore (Cherry et al., 2013; Gagnon and Gough, 2005; Stirling et al., 1999).

2.2. Hair collection

Hair samples were collected in the autumn of each year as part of ongoing research in WH in 2004–2013. Ninety-four percent of samples were collected in September with the remaining 6% of samples collected in the 4 days immediately preceding and following this month. While onshore, bears were captured and handled within a 12,000 km^2 area between the town of Churchill, Manitoba and the mouth of the Nelson River (Lunn et al., 2013; Fig. 1) following methods described by Stirling et al. (1989). All captures were opportunistic and, therefore, some bears were sampled multiple times over the study. Hair samples with a minimum weight of 50 mg, consisting of both guard hair and underfur were collected by shaving a patch on the rump as close to the skin as possible with a single-use Feather® disposable surgical scalpel. The hair in these samples would have grown between May and September (Derocher, 2012; Pedersen, 1945) following the spring moult.

The age of bears was determined by counting the cementum growth rings in a vestigial premolar tooth extracted during capture (Calvert and Ramsay, 1998). Body condition was assessed in the field using a qualitative index of fatness ranging from 1 to 5, with 1 being emaciated and 5 being obese (Stirling et al., 2008). The sex of each bear and the presence of dependent offspring were also recorded.

2.3. Cortisol analysis

Guard hairs were subsampled for cortisol analysis, which was performed at the Toxicology Centre, University of Saskatchewan (Saskatoon, SK, http://www.usask.ca/toxicology/). Hair samples were initially washed to remove external contamination. The wash and analysis procedures follow Davenport et al. (2006), which were subsequently modified and validated for use with polar bear hair (Macbeth et al., 2012). In brief, 50–80 mg of guard hairs were washed three times with 3 min agitations in 0.04 ml methanol/mg of hair, then left to dry at room temperature for 48 h. Washed guard hairs were ground to a fine powder in a mixer mill (Retsch Inc., Newtown, PA). Cortisol was extracted from a