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Health assessment of free-ranging endangered Australian sea lion (*Neophoca cinerea*) pups: Effect of haematophagous parasites on haematological parameters

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ABSTRACT

Evaluation of the health status of free-ranging populations is important for understanding the impact of disease on individuals and on population demography and viability. In this study, haematological reference intervals were developed for free-ranging endangered Australian sea lion (*Neophoca cinerea*) pups within the context of endemic hookworm (*Uncinaria sanguinis*) infection and the effects of pathogen, host, and environment factors on the variability of haematological parameters were investigated. *Uncinaria sanguinis* was identified as an important agent of disease, with infection causing regenerative anaemia, hypoproteinaemia, and a predominantly lymphocytic–eosinophilic systemic inflammatory response. Conversely, the effects of sucking lice (*Antarctophthirus microchir*) were less apparent and infestation in pups appears unlikely to cause clinical impact. Overall, the effects of *U. sanguinis*, *A. microchir*, host factors (standard length, body condition, pup sex, moult status, and presence of lesions), and environment factors (capture-type and year of sampling) accounted for 26–65% of the total variance observed in haematological parameters. Importantly, this study demonstrated that anaemia in neonatal Australian sea lion pups is not solely a benign physiological response to host–environment changes, but largely reflects a significant pathological process. This impact of hookworm infection on pup health has potential implications for the development of foraging and diving behaviour, which would subsequently influence the independent survival of juveniles following weaning. The haematological reference intervals developed in this study can facilitate long-term health surveillance, which is critical for the early recognition of changes in disease impact and to inform conservation management.

1. Introduction

Evaluation of the health status of free-ranging populations is important for understanding the impact of disease on individuals and on population demography and viability (Deem et al., 2001; Smith et al., 2009; Thompson et al., 2010). Haematological analysis is a reasonably non-invasive and efficient tool used as part of routine health assessment, permitting repeated *in situ* sampling of live individuals with minimal impact on animal welfare and survival (Clark, 2004; Wimsatt et al., 2005). Changes in haematological values provide quantifiable measures of the impact of, and host-response to, disease. However, inherent host-specific differences and dynamic temporospatial adaptations to physiological stressors also influence haematological characteristics (Gray et al., 2005; Beldomenico et al., 2008; Hufschmid et al., 2014). For this reason, the establishment of species- and context-specific reference intervals is necessary to define and assess deviations from baseline health status (Sergent et al., 2004; Ceriotti et al., 2009). This would facilitate the implementation of long-term health surveillance, essential for both the early recognition of emerging disease and to inform species conservation management (Hall et al., 2007; Thompson et al., 2010).

As high trophic-level predators exploiting a variety of ecological niches, pinnipeds act as sentinels for marine ecosystem health (Bossart, 2011). In particular, the health status of maternally-dependent pinniped pups is sensitive to changes to pathogen–host–environment relationships such as shifts in prey abundance, major climatic events, the presence of environmental toxins and contaminants, the occurrence of infectious diseases, and increasing human-impacts (Beckmen et al., 2003; Soto et al., 2004; Greig et al., 2005; Castinel et al., 2007; Melin et al., 2010; Brock et al., 2013). Haematological reference intervals have been developed for pups of several pinniped species to facilitate health assessment and several studies have investigated haematological responses to physiological changes, identifying the influential role of host factors (for example age, body condition, and sex) and environment factors (including geographic location and capture-associated stress) (Bryden and Lim, 1969; Geraci, 1971; Lane et al., 1972; Banish and Gilmartin, 1988; Castellini et al., 1993, 1996; Horning and Trillmich, 1997; Hall, 1998; Rea et al., 1998; Sepúlveda

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et al., 1999; Trumble and Castellini, 2002; Lander et al., 2003, 2014; Richmond et al., 2005; Boily et al., 2006; Clark et al., 2007; Trillmich et al., 2008; Greig et al., 2010; Brock et al., 2013). Yet, despite the widespread host distribution of haematophagous hookworm and lice species (Leonardi and Palma, 2013; Nadler et al., 2013), the effects of these parasites on the haematological values of pups and their implications for the assessment of health status remain unresolved. For example, although hookworm and lice can cause anaemia (Olsen, 1958; Dailey, 2001; Lyons et al., 2001), the population-wide occurrence of anaemia in neonates of many pinniped species has generally been attributed to a physiological host-response to the increased oxygen availability compared to the environment *in utero* and the expansion of plasma volume with growth (Richmond et al., 2005; Clark et al., 2007; Trillmich et al., 2008). A notable exception to the occurrence of neonatal anaemia is observed in land-bound northern elephant seal (*Mirounga angustirostris*) pups at Año Nuevo State Reserve (Castellini et al., 1990; Thorson and Le Boeuf, 1994) in which hookworm infection has not been detected (Lyons et al., 2012). Critically, few studies have considered parasitosis as a cause of anaemia in pinniped pups and there are no reports that characterise this anaemia by the presence or absence of reticulocytosis; classifying the erythroid response to anaemia as regenerative or non-regenerative in this way is fundamental to differentiating between pathological and physiological mechanisms (Stockham and Scott, 2008).

The impact of disease on the health status and population demography of the endangered Australian sea lion (*Neophoca cinerea*) is considered a key knowledge gap for understanding the impediments to population recovery in this species and for informing conservation management to mitigate the risks of population extinction (Goldsworthy et al., 2009). Whilst haematological reference intervals for free-ranging Australian sea lions older than six months of age have been reported (Needham et al., 1980; Fowler et al., 2007), data from neonatal pups is lacking. Additionally, the effects of disease on haematological values in this species have not been reported. Hookworm (*Uncinaria sanguinis*) endemically infects 100% of neonatal Australian sea lion pups at Seal Bay and Dangerous Reef in South Australia, two of the largest breeding colonies of this species, and is hypothesised to be an important agent of disease and cause of pup mortality across the species' range (Marcus et al., 2014a,b). Pups are infected *via* the transmammary route shortly after birth and demonstrate patent infection from 11–14 days of age for approximately 2–3 months (Marcus et al., 2014a; see Fig. 1). The extended breeding season of the Australian sea lion (approximately 7–9 month duration; Goldsworthy et al., 2012; McIntosh et al., 2012) facilitates the high prevalence of hookworm infection in pups by increasing the period of time in which breeding females can acquire infective free-living hookworm larvae (Marcus et al., 2014a). Additionally, the extended breeding cycle of approximately 18 months results in alternate 'summer' and 'winter' breeding seasons, occurring asynchronously between colonies (Higgins, 1993; McIntosh et al., 2012). The magnitude of colony pup mortality is associated with fluctuations in the intensity of hookworm infection, mediated by seasonal and colony-specific factors; an oscillating pattern of high-pup-mortality-with-high-infection-intensity and low-pup-mortality-with-low-infection-intensity has been observed at Seal Bay for summer and winter breeding seasons, respectively, and the opposite seasonal association has been described for Dangerous Reef, reflecting the different environmental attributes of the two colonies (Goldsworthy et al., 2012, 2013; Marcus et al., 2014a). Infestation with sucking lice (*Antarctophthirus microchir*) is also reported in Australian sea lion pups (McIntosh and Murray, 2007), although the epidemiology and clinical impact of this parasite have not been investigated in this host.

The aim of this study is to develop haematological reference intervals for free-ranging neonatal Australian sea lion pups within the context of endemic hookworm infection. In addition, this study will investigate the impact of *U. sanguinis* and *A. microchir* on pup health by estimating their effects on the variability of haematological parameters whilst considering the concurrent influence of host and environment factors. In particular, by characterising the erythroid changes in anaemia, this study will assess the hypothesis that neonatal anaemia is non-pathological, caused predominantly by physiological responses to host–environment changes.

2. Materials and methods

2.1. Sample collection

Samples were collected from Australian sea lion pups (n = 295) during consecutive winter and summer breeding seasons at two South Australian colonies, Seal Bay, Kangaroo Island (35.994°S, 137.317°E) in

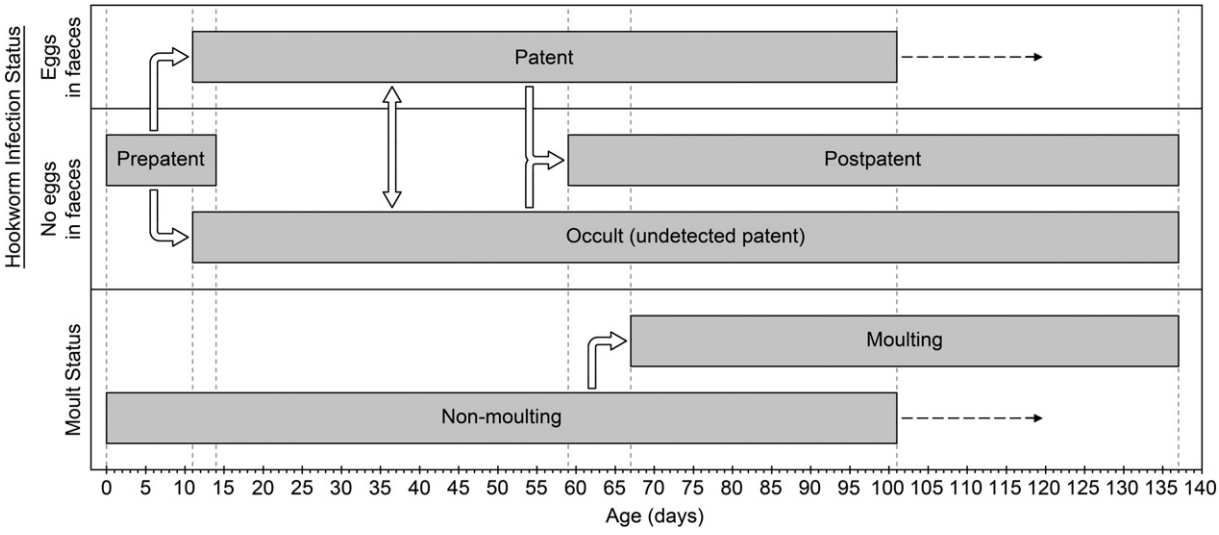

Fig. 1. Flowchart displaying the timing of hookworm (*Uncinaria sanguinis*) infection and moult status in neonatal Australian sea lion pups (*Neophoca cinerea*) from birth to 137 days of age. Grey boxes indicate the occurrence of each category with respect to pup age. White arrows indicate possible directions of change between categories. Dashed black arrows demonstrate the uncertainty in the upper limit for the occurrence of patent hookworm infection and non-moulting pups.
Adapted from Marcus et al. (2014a).