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Research paper

Comparative ecology of *Escherichia coli* in endangered Australian sea lion (*Neophoca cinerea*) pups

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ABSTRACT

The dissemination of human-associated bacteria into the marine environment has the potential to expose wildlife populations to atypical microbes that can alter the composition of the gut microbiome or act as pathogens. The objective of the study was to determine whether endangered Australian sea lion (*Neophoca cinerea*) pups from two South Australian colonies, Seal Bay, Kangaroo Island and Dangerous Reef, Spencer Gulf, have been colonised by human-associated *Escherichia coli*. Faecal samples ($n = 111$) were collected to isolate *E. coli*, and molecular screening was applied to assign *E. coli* isolates ($n = 94$) to phylotypes and detect class 1 integrons; mobile genetic elements that confer resistance to antimicrobial agents. *E. coli* phylotype distribution and frequency differed significantly between colonies with phylotypes B2 and D being the most abundant at Seal Bay, Kangaroo Island (55% and 7%) and Dangerous Reef, Spencer Gulf (36% and 49%), respectively. This study reports the first case of antimicrobial resistant *E. coli* in free-ranging Australian sea lions through the identification of class 1 integrons from an individual pup at Seal Bay. A significant relationship between phylotype and total white cell count (WCC) was identified, with significantly higher WCC seen in pups with human-associated phylotypes at Dangerous Reef. The difference in phylotype distribution and presence of human-associated *E. coli* suggests that proximity to human populations can influence sea lion gut microbiota. The identification of antimicrobial resistance in a free-ranging pinniped population provides crucial information concerning anthropogenic influences in the marine environment.

1. Introduction

The increasing detection of human-associated microorganisms in marine environments can potentially impact marine mammal health through the emergence of novel disease agents or altered gut microbiome composition (Pellegrini et al., 2009). Understanding the potential impact of human-associated microorganisms on marine mammals is significant for the conservation of threatened marine mammal species.

The Australian sea lion is Australia's only endemic pinniped species and is listed as endangered on the IUCN Red List (Goldsworthy et al., 2015a) with < 15,000 free-ranging individuals (Shaughnessy et al., 2011). The Australian sea lion population is highly fragmented across 80 different colonies that extend along the coast of Australia from the Houtman Albrolhos in Western Australia to the Pages Islands in South Australia (Goldsworthy et al., 2009). Australian sea lions were subject to sealing practices in the late 18th and early 19th century (Ling, 1999), resulting in a population decline for which limited recovery has occurred (Shaughnessy et al., 2011). Population decline at two of the major breeding sites for the species, Seal Bay and Dangerous Reef has been observed over numerous breeding seasons (Goldsworthy et al., 2015b) with variable, but generally high pup mortality rates that exceed 30% (Seal Bay) and 40% (Dangerous Reef) (Goldsworthy et al., 2009). Neonatal pups at these sites are endemically infected with the haematophagous nematode *Uncinaria sanguinis* (Marcus et al., 2014), which causes clinical disease and mortality due to anaemia, hypoproteinaemia and protein-losing enteropathy (Marcus et al., 2015). While other factors contributing to the establishment of disease and it's severity are currently unknown, parasite and bacterial co-infection can alter the host immune response and result in elevated levels of one pathogen due to secondary infection, leading to higher pathogenicity and mortality (Lass et al., 2013).The intestinal pathology caused by hookworm infection could facilitate secondary bacterial infections in the intestinal tract, as seen in California sea lions (*Zalophus californianus*) and South American fur seals (*Arctocephalus australis*), where a hookworm enteritis/bacteraemia complex resulted in high pup mortality (Seguel et al., 2017; Spraker et al., 2007). In this case, hookworms perforated the intestinal wall, facilitating the dissemination of enteric bacteria extra-intestinally resulting in pneumonia, meningitis and

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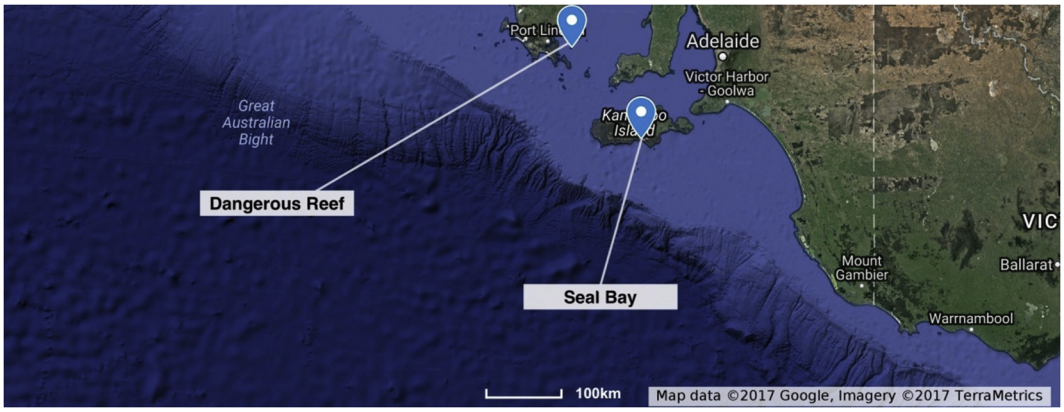

Fig. 1. Location of study sites, Dangerous Reef in Spencer Gulf and Seal Bay on Kangaroo Island.

suppurative arthritis (Spraker et al., 2007; Vedros et al., 1982).

Several bacterial species cause disease and mortality in pinniped species, including *Klebsiella pneumoniae* in New Zealand sea lions (*Phocarctos hookeri*) and *E. coli* in harbor seals (*Phoca vitulina*) (Gulland et al., 1996; Lockwood et al., 2006; Wilkinson et al., 2006). Higher *P. hookeri* pup mortality during the 2001/02 and 2002/03 breeding seasons was attributed to infection with *K. pneumoniae* (Wilkinson et al., 2006), a bacterium not previously known to be pathogenic in *P. hookeri*. The origin of *K. pneumoniae* isolated from the pups is unknown, and although *K. pneumoniae* can proliferate in the environment, it is considered a commensal of the human microbiome and is commonly isolated from the human gastrointestinal tract (Gorrie et al., 2017).

Water sources of importance to, and frequently used by humans, are routinely monitored for human-associated bacteria for water quality determination (Beversdorf et al., 2007; Hartz et al., 2008). Levels of faecal coliforms such as *E. coli* provide an indication of faecal contamination in drinking and recreational waters and the marine environment (Beversdorf et al., 2007). *E. coli* belongs to the diverse *Enterobacteriaceae* family and can function as a commensal or pathogen (Clermont et al., 2011; Russo and Johnson, 2000). *E. coli* has been categorised into eight phylotypes (A, B1, B2, C, D, E, F, clade I) that exist in varying ecological niches, display differing life histories, and vary in their pathogenic ability (Carlos et al., 2010; Gordon et al., 2008). Phylotypes B2 and D are more specialized and commonly isolated from humans, with clinically relevant strains such as uropathogenic (UPEC), neonatal meningitis-associated (NMEC), and sepsis-causing *E. coli* (SEPEC) belonging to these phylotypes (Bidet et al., 2007; Dale and Woodford, 2015). Strains of extraintestinal pathogenic *E. coli* (ExPEC) predominantly belong to the B2 and D phylotypes and can be present in humans without causing disease, depending on the presence of virulence factors (Johnson and Russo, 2002). Although virulence factors are needed for ExPEC to establish and cause disease, host factors associated with susceptibility and severity of infection are more important determinants of whether colonisation will result in disease (Dale and Woodford, 2015).

E. coli has previously been isolated from free-ranging adult Australian sea lions, with the B2 phylotype found to be most common, suggesting that these free-ranging populations have already been colonised by bacteria from human sources (Delport et al., 2015). The prevalence of *E. coli* was higher in captive individuals, indicating that proximity and interactions with humans can alter the presence of enteric bacteria (Delport et al., 2015). *E. coli* isolated from captive Australian sea lions also carried class 1 integrons (Delport et al., 2015), a genetic mechanism that confers antibiotic resistance in gram negative bacteria (Gillings, 2014). Class 1 integrons are mobile genetic elements that are transferred horizontally between bacteria of a single species or different species; they have been integral components to the rapid emergence and spread of antimicrobial resistance (Hall and Collis, 1995). Integrons contain a highly conserved integrase gene (*IntI1*), recombination site (*attI*) and promoter (*Pc*), that together have the ability to capture genes from the environment and transfer them between bacterial cells (Gillings, 2014). In addition to their detection in Australian sea lions, class 1 integrons have been reported in bacteria from various marine associated wildlife species such as Black-headed gulls (*Larus ridibundus*) (Dolejska et al., 2007) and the Little penguin (*Eudyptula minor*) (Lundbåck and Power, unpublished).

Australian sea lions have high female natal site fidelity (Campbell et al., 2008; Goldsworthy et al., 2013; Lowther et al., 2012). Populations in closer proximity to human populations could be at higher risk of exposure to human-associated bacteria (Delport et al., 2016, 2015; Power et al., 2013). Pathogenic strains of *E. coli* can cause intestinal or extraintestinal disease in humans (Russo and Johnson, 2000) and other hosts, including avian species (Mora et al., 2009). Given this pathogenicity, understanding the risks that human pathogens and antibiotic resistant bacteria pose to vulnerable wildlife populations is paramount.

The objectives of this study were to determine whether Australian sea lion pups are being colonised by human-associated bacteria and to compare phylotype distribution across two colony sites. A comparison of *E. coli* phylotype and parameters of health, including pup weight, standard length and haematological values, as well as mercury concentration (a metalloid known to cause immunosuppression), was also conducted.

2. Methods

2.1. Study sites and sample collection

Faecal samples from Australian sea lion pups ($n = 111$) were collected at two South Australian colonies; Seal Bay, Kangaroo Island (35.994°S, 137.317°E) during September and November 2016 and Dangerous Reef, Spencer Gulf (34.815°S, 136.212°E) during July 2017 (Fig. 1). These colonies are two of the largest Australian sea lion (*Neophoca cinerea*) colonies in terms of pup production but differ in host density (greater at Dangerous Reef) and substrate.

As part of an ongoing health investigation, pups were captured by hand when mothers were foraging at sea and were placed in a ventilated canvas bag. Faecal samples were collected from each pup by the insertion of a sterile swab (Copan, Brescia, Italy) directly into the rectum or by swabbing a faecal sample passed by the pup during capture and restraint. These swabs were subsequently sub-sampled into Sterile FecalSwab™ (Copan, Brescia, Italy). Some pup faecal samples were collected directly off substrate (designated as “unknown”) or were collected by a rectal or colonic swab from dead pups during necropsy. For samples from unknown and dead pups, haematological data and some health parameters were unavailable. All swabs were refrigerated at 4 °C until culture, usually within seven days of collection. A physical