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The influence of physiological status on the reproductive behaviour of humpback whales (*Megaptera novaeangliae*)

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ABSTRACT

For most cetacean species, there is little known about how an individual's physiology influences its behaviour. Humpback whales (*Megaptera novaeangliae*) are a good candidate to examine such links as they have a well-described distribution and behaviour, can be consistently sampled using remote biopsy systems, and have been the subject of several previous endocrine studies. The objective here was to examine whether a female humpback whale's social state (i.e. escorted by a male or not) is related to her endocrine condition, and whether male dominance ranking is related to testosterone levels. Skin and blubber biopsies were collected from the east and west Australian humpback whale populations in 2010–2016 ($n = 252$) at multiple times throughout the winter-spring breeding season. Steroid hormones were extracted from blubber and concentrations of progesterone (a marker for pregnancy), testosterone (a marker of male testicular activity) and oestradiol (a potential marker of ovarian activity) measured using enzyme-immunoassays. Principal escorts—the dominant males in mixed sex groups—had significantly higher blubber testosterone levels (mean ± SE; 1.43 ± 0.20 ng/g wet weight) than subordinate, secondary escorts (0.69 ± 0.06 ng/g wet weight). Females that were escorted by males typically possessed elevated blubber oestradiol levels (1.96 ± 0.25 ng/g wet weight; $p = 0.014$); few were considered to be pregnant ($p = 0.083$). 'Unescorted' females displayed characteristically lower blubber oestradiol levels (0.56 ± 0.06 ng/g wet weight). Together, these results are consistent with 'challenge hypothesis' theory and suggest the existence of associated reproductive patterns in humpback whales.

1. Introduction

The behaviour of animals can often be linked directly to their physiology, especially in species with associated reproductive patterns. Steroid hormones, in particular, are known to influence the activity of the nervous system and, in turn, elicit direct effects on socio-sexual behaviour (Simoncini and Genazzani, 2003; Fuxjager et al., 2017; Balthazart et al., 2018). The secretion of oestrogens, for instance, can lead females of some vertebrate species to display receptive-type behaviour (Davidson et al., 1968; Karsch et al., 1980; Findlay et al., 2002). Conversely, for many male vertebrates, increases in testicular-derived testosterone often leads to increased aggression (Wingfield et al., 1990; Klukowski and Nelson, 1998; Gould and Ziegler, 2007). Collecting endocrine information can therefore aid behavioural studies by helping to reveal the motivation behind or the function of a particular behavioural display.

For many vertebrate species, patterns of testosterone secretion can vary widely among males from the same population and for the same individual from one breeding season to the next (Wingfield et al., 1990). It is typically the dominant males of these species that display the highest levels of testosterone and, subsequently, aggression (Machida et al., 1981; Muller and Wrangham, 2004). Such observations can be largely explained by the 'challenge hypothesis' (Wingfield et al., 1990), which suggests that variation in testosterone levels relates to the degree of male-male competition experienced by the animal. The challenge hypothesis was first derived through studies on songbirds and, over the last 30 years, has been tested and supported through observations across numerous vertebrate taxa (Wingfield, 2017). Thus far, interactions between testosterone and male sexual behaviour have been examined in relatively few marine vertebrates, particular among the cetaceans.

Relatively little is known about the influence of physiology on

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cetacean behaviour, primarily due to the historical bias towards post-mortem sampling for physiological studies on these species (Chittleborough, 1954, 1955, 1958; Fukui et al., 1995). To date, dedicated behavioural endocrine studies have also been limited to those species that are maintained in captivity (Muraco and Kuczaj, 2015). Over the last 30 years, the emergence of several non-lethal sampling techniques, such as remote biopsying and faecal sampling, has made it is possible to examine the physiological status of many wild cetaceans (Amaral, 2010; Hunt et al., 2013). Blubber, which can be collected via remote biopsying, is particularly appealing for endocrine studies as it is a sink for lipophilic molecules, such as steroid hormones and their metabolites. However, as blubber accumulates these hormones from peripheral circulation over a period of hours to days (Champagne et al., 2017; Kellar et al., 2017), there is a limited capacity to detect recent or short-term fluctuations in circulating concentrations. Further, the exact composition of hormones and metabolites in blubber, and their rate of integration and clearance, is poorly known for most species. Blubber hormone (progesterone) monitoring has, nevertheless, proved reliable for detecting pregnancy in a number of cetaceans (Mansour et al., 2002; Kellar et al., 2013; Trego et al., 2013), including some baleen whales (e.g. humpback whales [*Megaptera novaeangliae*]; Clark et al., 2016; Pallin et al., 2018). Blubber testosterone has also shown promising signs as a marker of male sexual activity and maturity status (Kellar et al., 2009; Vu et al., 2015; Cates et al., 2019). The next step for many cetaceans is to establish whether changes in the concentrations of these hormones are derived from, or influence, patterns of behaviour as seen in other vertebrate species. Of particular interest is whether the concentrations of sex steroid hormones correlate with social factors, such as dominance rankings and male aggression.

Humpback whales are a seasonal breeder with perhaps the most diverse and well-studied behavioural repertoires of all cetaceans, particularly among the baleen whales (Clapham, 1996; Stimpert et al., 2012). The behaviour of humpback whales is typical of a promiscuous or polygynous-type mating system in that there is intense male-male competition for females, and females mate with different males over successive years (Clapham, 1996; Clapham and Palsboll, 1997). A polygynous mating system is further supported by widespread observations of males guarding or 'escorting' adult females (Mobley and Herman, 1985; Glockner-Ferrari and Ferrari, 1990). Though, whether these escorts, or the females that they pursue, are indeed reproductively active is unknown. Humpback whales also possess features of a lek-based mating system, with males 'singing' low frequency, complex songs throughout the breeding season (Payne and McVay, 1971), probably as a sexual display (Winn and Winn, 1978; Tyack, 1981; Frankel et al., 1995). The frequency of both singing and escorting varies throughout the year (Winn and Winn, 1978; Tyack and Whitehead, 1983; Brown and Corkeron, 1995; Kowarski et al., 2018), which loosely correlates with seasonal changes to testosterone (Vu et al., 2015; Cates et al., 2019) and several testicular measures (Matthews, 1937; Chittleborough, 1955). Such patterns are consistent with numerous other seasonal breeding vertebrates (Yagil and Etzion, 1980; Wingfield et al., 1987; Pereira et al., 2005), and are notably comparable to those in terrestrial songbirds (reviewed in: Cholewiak et al., 2018). Despite sharing similarities to songbird mating systems, however, it is still unclear as to whether humpback whales also adhere to the predictions of the challenge hypothesis.

An essential next step in understanding the function and mechanisms behind humpback whale reproductive behaviours, such as escorting and singing, is to compare the endocrine profiles and behaviour of individual animals over time. Examining patterns of testosterone levels among 'singers' and 'non-singers', for instance, might help to clarify whether this behaviour is related to male dominance sorting (Herman, 2017; Cholewiak et al., 2018). Further, we might better understand the motivation for escorting by evaluating the endocrine condition (an indicator of reproductive status) of both escorts and the females they pursue. In turn, this should help clarify whether females in competitive groups (see: Tyack and Whitehead, 1983) are indeed sexually receptive (see: Darling et al., 1983; Mobley and Herman, 1985; Clapham et al., 1992).

There are several features of the Australian humpback whale populations that are particularly appealing for these types of behavioural endocrinology studies. For instance, both populations are at high abundance (Salgado Kent et al., 2012; Noad et al., 2019), have well-described distributions and behaviour (Dunlop, 2016; Dunlop and Noad, 2016; Kavanagh et al., 2017), and have already been sampled extensively for blubber over recent years (Mingramm et al., 2019a). The first aim of this study was to determine whether male humpback whales preferentially associate with females who possess elevated blubber oestrogen levels (a likely marker of ovarian activity) when compared to all other adult females, including those with elevated blubber progesterone levels (a marker of pregnancy). Secondly, we sought to establish whether male social rankings relate to their blubber testosterone levels, as predicted by the challenge hypothesis. This study is the first to explore potential relationships between the reproductive physiology and behaviour of humpback whales, with the purpose of improving our understanding of the motivation behind several breeding-season displays.

2. Materials and methods

2.1. Study animals

Adult and juvenile humpback whales from the east Australia (breeding stock 'E1') and Western Australia populations (breeding stock 'D') were sampled between 2013 and 2016 (Table 1). Additional samples from breeding stock 'E1' were also sourced from the Cetacean Ecology and Acoustics Laboratory (the University of Queensland), including 13 samples collected during the 'Great Barrier Reef Whale and Dolphin Research Programme' (Table 1). Only one tissue sample was collected from each study animal.

Table 1
Location, timing and number of breeding stock 'D' and 'E1' humpback whales that were biopsy sampled and behaviourally monitored.

Population	Year	Season (months)	Location	Sample size
Breeding stock D	2013	Southern migration (Sept–Oct)	Dongara, Western Australia (~29° 16′ S, 114° 51′ E)	31
Breeding stock E1	2010	Southern migration (Sept–Oct)	Sunshine coast, Queensland (~26° 30′ S, 153° 05′ E)	8[a]
	2011	Southern migration (Sept–Oct)	Sunshine coast, Queensland	2[a]
	2014	Southern migration (Sept–Oct)	Sunshine coast, Queensland	22
	2015	Northern migration (Jun–Jul)	Nth Stradbroke Island, Queensland (~27° 21′ S, 153° 32′ E)	54
	2015	Southern migration (Sept–Oct)	Sunshine coast, Queensland	30
	2016	Northern migration (Jun–Jul)	Nth Stradbroke Island, Queensland	60
	2016	Tropical breeding grounds (August)	Great Barrier Reef, Queensland (~20° 00′ S, 149° 00′ E)	13[a]
	2016	Southern migration (Sept–Oct)	Nth Stradbroke Island, Queensland	33

[a] Samples provided by the Cetacean Ecology and Acoustics Laboratory, The University of Queensland.