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Body reserves influence allocation to immune responses in capital breeding female northern elephant seals

Hannah E. Peck[1], Daniel P. Costa[2] and Daniel E. Crocker*[,1]

[1]*Department of Biology, Sonoma State University, Rohnert Park, California 94928, USA; and* [2]*University of California, Santa Cruz, California 95064, USA*

Summary

1. Mounting an immune response requires substantial energy. Ecological immunology theory predicts allocation trade-offs between reproductive effort and immune responses under conditions of energy limitation. Little is known about the impact of capital breeding strategies on energy allocation to immune function in mammals.

2. Northern elephant seals (NES) forage in the marine environment, breed in dense terrestrial colonies and exhibit high rates of energy expenditure for lactation while fasting. Body reserves strongly influence reproductive effort and lactation requires elevation of plasma cortisol for energy mobilization.

3. We characterized immune response by measuring a suite of immune markers including cytokines, an acute phase protein, and immunoglobulins early and late in breeding and moult haul-outs in 197 samples from 129 female NES. We explored potential impacts of breeding, body condition and plasma cortisol on immune function.

4. Immune responses were greater and more varied during breeding. Adiposity had positive associations with innate immune responses across all life-history stages. Body mass had positive associations with both adaptive and innate immune responses early in fasts. Females with lower fat reserves showed reduced innate immune responses at the end of lactation. Immunoglobulin E, a marker of immune response to parasitic infection, exhibited a significant negative association with cortisol across all life-history stages.

5. These data suggest that breeding carries an immune cost and provide evidence for allocation trade-offs between immune function and breeding effort. These trade-offs may reflect a compromise between immune costs inherent in colonial breeding and energetic limitations that arise in use of capital breeding strategies. Variation in evidence for immunosuppressive effects of cortisol suggests that decoupling of these effects may be limited to specific aspects of the immune response during terrestrial fasting. Immune responses that are required for survival may be modulated relative to the energetic demands required for successful reproduction.

Key-words: ecological immunology, fasting, pinnipeds, reproduction, trade-offs

Introduction

Animals have limited resources available to them for survival and reproduction. Resource limitation may require animals to make trade-offs between competing physiological processes. Allocation trade-offs underlie traits and regulation of activities that can differ among life-history stages (Stearns 1989). One such trait is immune response, which is not only energetically expensive, but can also cause oxidative damage and autoimmunity (Svensson *et al.* 1998; Lochmiller & Deerenberg 2000; Costantini & Møller 2009). During resource limitation, low-intensity infections may be allowed to persist if the energetic costs outweigh the benefits of clearing the infection (Sheldon & Verhulst 1996; Martin, Hawley & Ardia 2011). Free ranging animals experience immune challenges across all life-history stages. It is critical to both life-history theory and conservation physiology to understand how animals regulate immune activity while maintaining functions that are necessary for survival and reproductive success.

*Correspondence author. E-mail: crocker@sonoma.edu

Direct trade-offs between reproductive effort and immune function have been observed in birds via brood size manipulation. Antibody response has been reported to cost 8–13% of BMR in birds (Svensson *et al.* 1998). Research in this area has mainly focused on immune response to parasites (Sheldon & Verhulst 1996). Birds have also been used to examine the impact of cold stress and energy allocation to immune response (Svensson *et al.* 1998). Evidence for trade-offs is less common in free ranging mammals, but may be especially important in species that lack energy inputs during reproduction. Several species of pinnipeds fast during energy demanding activities such as breeding and moulting. In addition to energy constraints, fasting may elevate stress hormones because of their role in regulating energy mobilization (Champagne, Houser & Crocker 2006). Glucocorticoid hormones may underlie trade-offs between energy expenditure and immune function and may prevent overshoot of the response to injury or infection during resource limitation (Sternberg *et al.* 1992; DeRijk *et al.* 1997).

Wild animals experience natural variations in stress hormones due to breeding or interactions with other animals. Additional anthropogenic stressors such as noise, habitat loss and physical disturbance increase these values (Acevedo-Whitehouse & Duffus 2009; Martin *et al.* 2010). There has been recent demand for studies that examine the effect of stress hormones on immune parameters in wildlife (Ricklefs & Wikelski 2002; Acevedo-Whitehouse & Duffus 2009). Cortisol is known to be immunosuppressive in wildlife systems (Sheldon & Verhulst 1996; Råberg *et al.* 1998) through processes such as altering antibody responses (Fowles *et al.* 1993) and inhibiting lymphocyte proliferation (Rollins-Smith & Blair 1993). Ecological immunology aims at determining how factors such as stress and resource limitation play a role in modulating immunocompetency.

Previous studies have suggested that the measurement of a single immune marker can lead to improper conclusions about the state of animal health and immune function (Gruys *et al.* 2005; Yang *et al.* 2013). Immune markers do not always covary, so it is important to measure a suite of variables to characterize systemic response. However, published studies in non-model or wildlife systems frequently measure single or few immune or inflammation markers for comparison between groups or populations (Zenteno-Savin *et al.* 1997; Marquez *et al.* 1998; Krafft, Lydersen & Kovacs 2006; Thomton & Mellish 2007; Kakuschke *et al.* 2010; Kakuschke, Pröfrock & Prange 2013). Less is known about how these markers vary with each other in wildlife systems or the role that natural variation in stress plays in modulating immunocompetency.

Immune responses include rapidly acting innate responses, which include complement killing and the acute phase response, and slower acting adaptive responses, which include memory antibody formation or use. The innate response is the first line of defence against invading pathogens by secreting cytokines that cause production of acute phase proteins by the liver. The purpose of the adaptive response is to make immunological memory to elicit a faster and stronger response the next time the pathogen is seen and is characteristic of animals with slower life paces (Lee 2006). Thus, the timing of sampling relative to pathogen exposure, exposure history to various pathogens or the existence of chronic inflammation might lead to variable responses in specific immune markers. Maintenance costs of an innate immune response are high (Lochmiller & Deerenberg 2000; Freitak *et al.* 2003), while the maintenance costs of lymphocytes may be low (Svensson *et al.* 1998; Råberg *et al.* 2002). In contrast, primary and secondary adaptive immune responses can be energetically expensive and can be compromised by food limitation (Demas *et al.* 1997; Martin *et al.* 2007). These findings suggest that different components of the immune response may respond differently to energetic trade-offs.

The purpose of inflammation is to return to homeostasis after injury or trauma by eliminating infection and foreign bodies and repairing damaged tissues. The cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) are released from the site of inflammation and recruit immune cells to manage damaged and infected tissues. These cytokines are short-lived and can upregulate rapidly after an infection. Cytokines travel to the liver to induce the production of acute phase proteins such as haptoglobin. Haptoglobin scavenges free haemoglobin, inhibits microbial uptake of iron and inhibits potential oxidative damage. Haptoglobin is upregulated at times of illness because inflammation involves high erythrocyte turnover. Immunoglobulins represent the adaptive response to specific pathogens. IgG and IgM provide immunity against blood-borne diseases. All B cells express IgM before they class switch to express other antibodies. Expression of IgM can be a marker of recent and novel infections. IgG has been shown to have important implications in pinniped survival, where grey seal pups with high circulating IgG were less likely to survive (Hall, McConnell & Barker 2002). Infection by helminths and other parasites is associated with increased production of IgE and reduced systemic pro-inflammatory responses (Blackwell *et al.* 2010). Measurement of IL-1β, IL-6, haptoglobin, IgG, IgM and IgE provides a comprehensive view of innate and adaptive immune function.

Northern elephant seals (NES; *Mirounga angustirostris*; Fig. 1) haul-out (leave the marine environment and come onshore) and fast twice a year, once to breed and once to moult, and the physiology of these fasts is well studied. NES elevate cortisol to mobilize lipid resources for energy while fasting, and cortisol levels vary widely during breeding and moulting (Crocker *et al.* 2014a; Ensminger *et al.* 2014). Female NES face differing immunologic and energetic challenges during the two haul-outs. The breeding haul-out includes parturition in a highly pathogenic environment followed by producing one of the highest energy milks found in nature (Crocker *et al.* 2001). Females are susceptible to bacterial infections and viruses that can spread throughout the rookery. Males may inflict wounds

Fig. 1. Female northern elephant seal with suckling pup.

during mating attempts, and bites from conspecifics are common during breeding (Le Boeuf & Mesnick 1991). Sampling at the beginning of the breeding haul-out can provide immunological data not only about parturition, but may also reflect differences in immune status after the 8-month gestational foraging trip. Females lose 33–40% of their body mass over lactation, depleting ~25% of total body protein stores (Costa *et al.* 1986; Crocker *et al.* 2001). Immune status at the end of the breeding haul-out reflects potential trade-offs between the high energetic needs of lactation and immune function.

Moulting also carries potentially unique immune challenges. Female NES fast for ~32 days while undergoing a catastrophic skin moult, which involves shedding old pelage and growing new epidermis and pelage (Worthy *et al.* 1992). Only a few species of pinnipeds (monk and elephant seals) shed the entire epidermis in this fashion (Ling 1978). While the rate of energy expenditure during moulting is low compared to breeding, females lose ~25% of their body mass during this process (Worthy *et al.* 1992). It is not known how immune response is affected by this catastrophic moult. Samples taken early in the moult, prior to the onset of pelage loss, may reflect variation in immune status after the 2-month post-breeding foraging trip. Though short in duration, this trip enables recovery of important body protein reserves lost during breeding (Crocker, Boeuf & Costa 1997). Samples taken late in the moult after pelage loss and just prior to departure may reflect combined immune impacts of moulting and fasting. Female elephant seals have an energy expenditure rate of 2·0 times standard metabolic rate (SMR) for their mass during the moulting period (Worthy *et al.* 1992) and 5·7 times SMR, when including milk energy content, during the breeding season (Crocker *et al.* 2001). Trade-offs between immune response and competing processes should be observable in animals that are operating at an elevated metabolic rate for an extended period of time.

Our objective was to determine the patterns of cytokines, an acute phase protein, and antibodies across life-history stages of female NES. Specifically, we examined influences of age, body composition, foraging success, life-history stage and plasma cortisol on immune function and looked for potential allocation trade-offs resulting from the high-energy cost of lactation.

Materials and methods

STUDY SITE AND SUBJECTS

All sampling procedures were carried out under NMFS permit #14636 and were approved by the UCSC and SSU IACUC. A total of 197 serum samples were obtained from 129 adult female NES during their biannual haul-outs at Año Nuevo State Park in San Mateo County, California, USA, between 2011 and 2014. The early breeding (EB) samples (n = 59) were taken 5 days post-partum to allow for mother–pup bonding prior to chemical immobilization. The late breeding (LB) samples (n = 53) were taken 22 days post-partum, just prior to weaning and female departure to sea. Early moult (EM) samples (n = 42) were taken as close to when the animals returned to the beach as possible (2 ± 2 days on shore), and late moult (LM) samples (n = 43) were taken after completion of moulting and just prior to departure, ~30 days later.

FIELD PROCEDURES

Animals were sedated with an intramuscular injection of telazol (~1 mg kg^{-1}), and immobilization was maintained with intravenous injections of ketamine and valium (all drugs: Fort Dodge, Fort Collins, CO, USA). Serum was obtained using an 18-G spinal needle placed in the extradural vein, collected in vacutainers and placed immediately on ice until return to the laboratory. This form of chemical immobilization does not alter baseline cortisol levels in NES (Champagne *et al.* 2012). All serum samples were centrifuged at 1400 ***g*** for 15 min at 4 °C and stored at −80 °C until analysis. Females were weighed using an aluminium tripod, weighing sling and hanging scale (±2 kg; MSI, Seattle, WA, USA; n = 185). Plastic flipper tags (Dalton, Oxfordshire, UK) applied at weaning allowed us to age 134 of the females. Body composition (% adipose tissue) was measured in 154 of the females based on morphometrics and ultrasound measurements of blubber thickness using the truncated cones method (Gales & Burton 1987; Crocker *et al.* 2001).

MEASUREMENT OF IMMUNE MARKERS

All analytes were measured in duplicate using commercially available assays (Table 1). IL-6, IL-1β and total IgE were measured using ELISA. Haptoglobin was measured using a colorimetric assay. Total IgG and total IgM were measured using antibody-sensitized microsphere microagglutination assays. ELISA kits were validated for use in NES. Serially diluted samples yielded curves that were parallel to the standard curves, and >93% of added standards were recovered from samples. The coefficient of variation was <6% for all analytes (Table 1).

DATA ANALYSIS

Data analysis was performed with JMP 11.0 (SAS Institute, Cary, NC, USA). Differences in immune markers between life-history stages were analysed using linear mixed effect (LME) models with animal ID as a random effect and stage as a fixed effect. To address whether cortisol had an effect on the immune markers, a

Table 1. Number of samples analysed, coefficients of variation and assay used for each analyte

	Analyte	*N*	CV%	Assay
Cytokine	IL-1β	194	4·70	RayBio Canine IL-1 beta ELISA
Cytokine	IL-6	196	5·51	RayBio Canine IL-6 ELISA
Acute phase protein	Hp	197	5·51	Tridelta Development Limited multispecies Hp
Immunoglobulin	IgG	197	4·79	Thermo Scientific Easy-Titer Human IgG (H+L)
Immunoglobulin	IgM	197	2·35	Thermo Scientific Easy-Titer Human IgM
Immunoglobulin	IgE	197	3·25	GenWay Biotech, Inc. Canine IgE ELISA
Hormone	Cortisol	197	1·95	Siemens–DPC RIA

IL, interleukin; Hp, haptoglobin; Ig, immunoglobulin.

LME was fit with cortisol as a fixed effect and animal ID as a random effect. If effects of cortisol across all life-history stages were absent, stage and the interaction between stage and cortisol were included as fixed effects in the model. Degrees of freedom for F statistics were calculated using the Kenward–Rogers method. Model residuals were assessed for approximate normality. Residual plots were assessed to confirm model homoscedascity, and the response variable was log-transformed if needed. If significant differences between stages were present, *post hoc* comparisons were performed using Student's *t*-tests.

To examine the relationship between the immune markers, a mixed model was fit with one marker as the response variable and one marker as a fixed effect, along with animal ID as a random effect. To examine energy trade-offs, we ran models looking at the effects of mass, body composition and age on each immune marker. These models were fit for the early haul-out samples (EB and EM) as proxies for nutritional status after foraging and energy gain at sea. We examined these in the late haul-out samples (LB and LM) as proxies for energy depletion over the fast. Age was included in models examining the effect of mass to control for somatic growth (Hassrick *et al.* 2010) and to test for evidence of impacts of age on immune function. Mixed model R^2 was calculated for continuous fixed effects (Edwards *et al.* 2008). Slope and intercepts from the mixed model were extracted for regression plots. Results were considered significant at $P < 0{\cdot}05$.

To reduce the dimensionality of immune markers and facilitate visualization of aggregate responses, a principle components analysis was performed. Various rotations were explored to maximize unique loading of variables onto components and a Covarimax rotation was used. Variables were considered to load on an individual component when the loading was >0·6.

Results

IMMUNE MARKERS AND LIFE-HISTORY STAGE

All immune markers varied significantly with life-history stage with the general pattern being that markers were greater and more variable during the breeding haul-out than the moulting haul-out (Table 2; Fig. 2). Innate and adaptive immune response markers followed similar patterns with a few important exceptions.

Markers of the innate immune response varied significantly with life-history stage and all were greatest during the breeding haul-out. The cytokines IL-1β and IL-6 varied significantly with stage ($F_{3,119{\cdot}4} = 2{\cdot}82$, $P = 0{\cdot}04$; $F_{3,127{\cdot}3} = 32{\cdot}89$, $P < 0{\cdot}0001$, respectively). These cytokines did not change over fasting, but were higher during breeding than during moulting. Both cytokines had higher variance during breeding stages than during the moult (Levene's test $P < 0{\cdot}01$). The acute phase protein haptoglobin varied significantly with stage, but did not change across either haul-out ($F_{3,154{\cdot}7} = 15{\cdot}38$, $P < 0{\cdot}0001$). In contrast to the cytokines, haptoglobin was more variable during moulting (Levene's test $P = 0{\cdot}01$).

Markers of the adaptive immune response showed similar patterns. IgG and IgM were significantly greater during the breeding haul-out than during moulting ($F_{3,164{\cdot}8} = 11{\cdot}60$, $P < 0{\cdot}0001$, $F_{3,186{\cdot}8} = 8{\cdot}49$, $P < 0{\cdot}0001$, respectively). IgG significantly decreased across the breeding haul-out, but did not decrease across moulting. IgE was significantly greater during the early moult time point only ($F_{3,126{\cdot}7} = 4{\cdot}91$, $P = 0{\cdot}003$). Values of IgE were similar across all other time points. All adaptive immune markers had higher variance during breeding (Levene's test, $P < 0{\cdot}001$).

IMMUNE MARKERS AND BODY RESERVES

Energy allocation trade-offs were examined across all life-history stages and at the beginning and end of the

Table 2. Mean ± SD of innate and adaptive immune responses

Analyte	EB ($n = 59$)	LB ($n = 53$)	EM ($n = 42$)	LM ($n = 43$)
IL-1β (pg mL^{-1})	$38{\cdot}6 \pm 7{\cdot}3^{A}$	$43{\cdot}4 \pm 7{\cdot}7^{A}$	$21{\cdot}6 \pm 8{\cdot}5^{B}$	$18{\cdot}8 \pm 8{\cdot}3^{B}$
IL-6 (ng mL^{-1})	$0{\cdot}48 \pm 0{\cdot}03^{A}$	$0{\cdot}45 \pm 0{\cdot}03^{A}$	$0{\cdot}15 \pm 0{\cdot}04^{B}$	$0{\cdot}12 \pm 0{\cdot}04^{B}$
Hp (mg mL^{-1})	$1{\cdot}80 \pm 0{\cdot}09^{A}$	$1{\cdot}90 \pm 0{\cdot}10^{A}$	$1{\cdot}10 \pm 0{\cdot}11^{B}$	$1{\cdot}31 \pm 0{\cdot}11^{B}$
IgG (mg mL^{-1})	$10{\cdot}71 \pm 0{\cdot}50^{A}$	$9{\cdot}15 \pm 0{\cdot}52^{B}$	$7{\cdot}15 \pm 0{\cdot}58^{C}$	$6{\cdot}89 \pm 0{\cdot}58^{C}$
IgM (mg mL^{-1})	$0{\cdot}38 \pm 0{\cdot}02^{A}$	$0{\cdot}41 \pm 0{\cdot}02^{A}$	$0{\cdot}28 \pm 0{\cdot}02^{B}$	$0{\cdot}30 \pm 0{\cdot}02^{B}$
IgE (μg mL^{-1})	$2{\cdot}24 \pm 0{\cdot}10^{A}$	$2{\cdot}02 \pm 0{\cdot}11^{A}$	$2{\cdot}56 \pm 0{\cdot}12^{B}$	$2{\cdot}10 \pm 0{\cdot}12^{A}$

EB, early breeding (5 days post-partum); LB, late breeding (22 days post-partum); EM, early moult; LM, late moult; IL, interleukin; Hp, haptoglobin; Ig, immunoglobulin. Different superscripts denote significant differences between life-history stages in least square means from a linear mixed effects model with female ID included as a random effect.

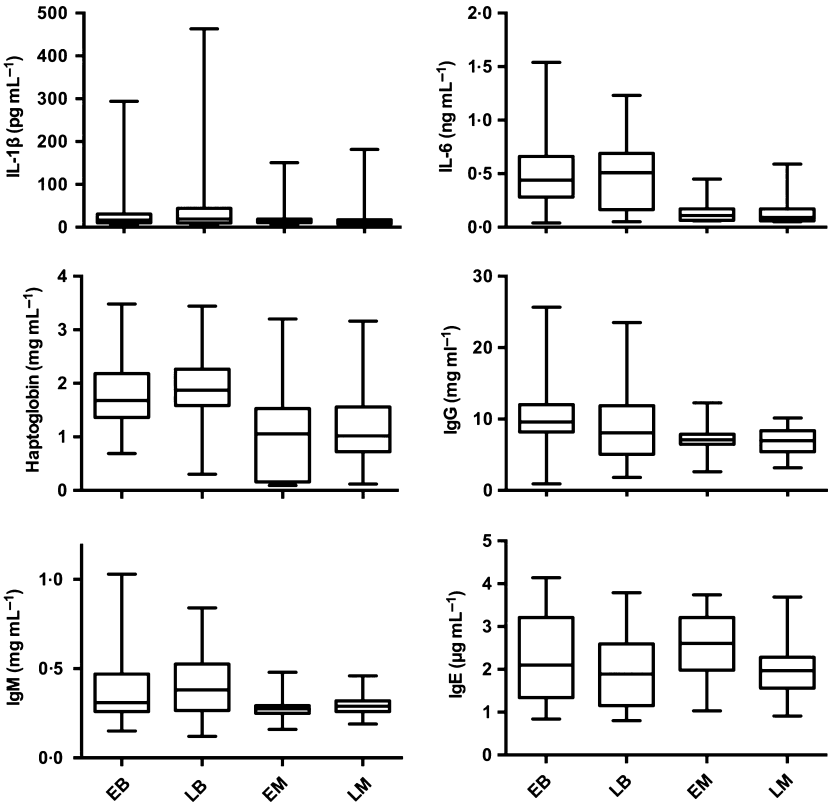

Fig. 2. Box plots of immune marker measurements from female NES across life-history stages. Box shows 1st to 3rd quartile. Central line is the median. Whiskers show minimum and maximum values. EB, early breeding; LB, late breeding; EM, early moult; LM, late moult.

haul-outs (Table 3). Mass had a significant positive effect on IgE across all life-history stages. Adiposity had a significant positive effect on both cytokines, IL-1β and IL-6, across all life-history stages. Early in the fasts, mass controlled for age, a proxy for post-foraging nutritional status, had a significant positive effect on both cytokines (IL-1β and IL-6) and two of the antibodies (IgG and IgM). Late in the fasts, body fat percentage, a proxy for energy reserve depletion over the fast, had a significant positive effect on both cytokines (IL-1β and IL-6). Body composition was not related to adaptive immune responses in any analysis. No associations of female age with innate or adaptive immune function were evident for any marker ($P > 0{\cdot}05$).

Table 3. Results from linear mixed effect models examining the impact of body reserves on innate and adaptive immune responses at various life-history stages in northern elephant seals. Models included age to control for somatic growth

Stage	Body reserves	Immune marker	R^2	F	Den df	P
All	Mass	IgE	0·19	11·7	102·1	<0·001
	Adipose %	IL-1β	0·04	5·8	143·6	0·02
	Adipose %	IL-6	0·16	27·6	149·7	<0·0001
Early fast	Mass	IL-1β	0·09	6·6	64·0	0·01
	Mass	IL-6	0·07	4·7	62·4	0·03
	Mass	IgG	0·51	17·3	16·3	<0·001
	Mass	IgM	0·15	5·4	30·0	0·03
Late fast	Adipose %	IL-1β	0·14	7·3	15·1	0·02
	Adipose %	IL-6	0·32	11·8	74·0	0·001

Ig, immunoglobulin; IL, interleukin. Female ID was included in each model as a random effect. Only significant effects are shown.

PRINCIPLE COMPONENT ANALYSIS

Three principle components described 72% of the variation in the immune markers. The antibodies IgG and IgM, representing the adaptive response, loaded onto the 1st component (PC1) with factor loadings of 0·86 and 0·77,

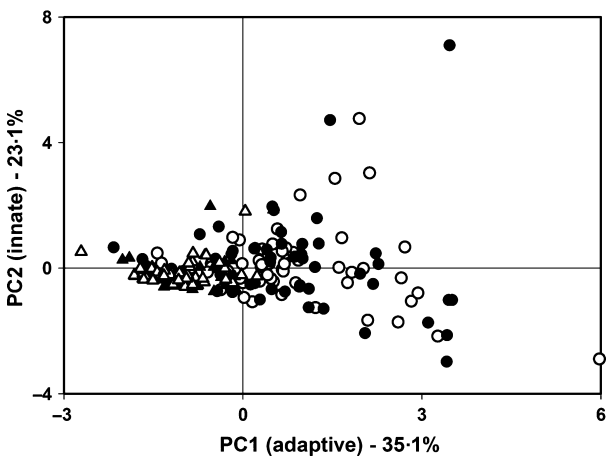

Fig. 3. Ordination of individual females on a plot of PC1 vs. PC2. Breeding females showed greater adaptive immune responses and more variable innate responses. Open circles = early breeding, closed circles = late breeding, open triangles = early moult, closed triangle = late moult. PC1 loaded the immunoglobulins IgG and IgM. PC2 loaded the cytokines IL-1β and IL-6.

respectively. The cytokines IL-1β and IL-6, representing the innate response, loaded together onto the 2nd component (PC2) with factor loadings of 0·92 and 0·66, respectively. IgE loaded uniquely onto the 3rd component (0·93). Haptoglobin did not load uniquely onto a component with any rotation. PC1 (adaptive response) varied between life-history stages ($F_{3,117·2} = 48·0$, $P < 0·0001$) and was greater during breeding ($P < 0·05$; Fig. 3). Body mass was positively associated with PC1 across all life-history stages ($F_{1,182·9} = 9·63$, $P = 0·002$). Body composition was positively associated with PC2 across all life-history stages ($F_{1,123} = 11·55$, $P < 0·001$, respectively).

RELATIONSHIPS BETWEEN IMMUNE MARKERS AND CORTISOL

We examined relationships between immune markers to observe whether they covaried and to see whether the secretagogues IL-1β and IL-6 had downstream effects on the production of haptoglobin. There was a significant positive relationship between IL-1β and IL-6 ($R^2 = 0·12$, $F_{1,161·2} = 21·28$, $P < 0·001$) and between IL-6 and haptoglobin ($R^2 = 0·12$, $F_{1,179·9} = 23·53$, $P < 0·0001$). There was a significant positive relationship between IgM and IgG ($R^2 = 0·23$, $F_{1,189·7} = 57·54$, $P < 0·0001$, Fig. 4). No other immune markers covaried ($P > 0·05$). IgE concentrations varied inversely with cortisol ($R^2 = 0·12$, $F_{1,158·4} = 21·97$, $P < 0·0001$). No other variables had significant relationships with cortisol even when controlled for variation due to life-history stage.

ANNUAL DIFFERENCE IN IMMUNE MARKERS

Most markers showed significant annual differences ($P < 0·05$), but lacked a consistent pattern to these differences with the exception of IgE. When controlling for differences due to life-history stage, IgE decreased significantly over each of the 4 years ($F_{3,179·1} = 134·06$, $P < 0·0001$). The least squares means decreased threefold from 3·14 ng mL^{-1} in 2011 to 1·05 ng mL^{-1} in 2014.

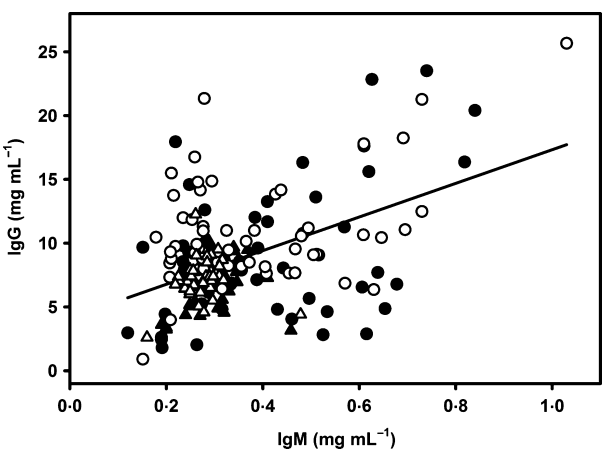

Fig. 4. Relationship between IgM and IgG in female NES during all life-history stages. Fitted line is based on the linear mixed model parameter estimates $y = 13·18x + 4·14$, $R^2 = 0·23$, $P < 0·0001$). Open circle = early breeding, closed circle = late breeding, open triangle = early moult, closed triangle = late moult.

Discussion

Female NES mounted an immune response during breeding after giving birth in a dense, pathogenic environment. The increase in adaptive immune response and more variable increase in innate immune response during breeding in comparison with moulting is seen clearly in the plot of PC1 and PC2 (Fig. 3). In addition to the process of parturition itself, females are in close physical proximity to other females and are frequently injured by conspecifics during agonistic encounters (Le Boeuf & Mesnick 1991). As females approach the end of lactation, they enter oestrus and are copulated with through mating behaviour that can include bites from males (Le Boeuf & Mesnick 1991). The immune response to breeding included elevation in both innate and adaptive immune markers. The wide individual variation in markers during this period indicates that some females experienced greater post-partum inflammation and response to pathogens than conspecifics. The lack of association between innate and adaptive responses suggests differential activation of the two types of responses.

Mass and adiposity influenced the magnitude of different aspects of immune response. Across all life-history stages, larger females showed evidence for more robust responses to parasite infection (IgE). When controlled for age, females that were the largest when returning from the two foraging trips exhibited greater IL-1β, IL-6, IgG and IgM responses. These data suggest available energy reserves impact allocation of energy towards immune response at the beginning of periods of extended fasting. Similarly, adiposity was positively associated with cytokine concentrations across all life-history stages. Females with greater body fat percentage exhibited higher concentrations of markers for innate immune response compared to females that had greater depletion of adipose tissue reserves close to weaning. In contrast, individual adaptive immune responses showed no relationship to body composition at any life-history stage. When characterized using principle components, impacts of body mass on adaptive responses and body composition on innate responses were evident across all life-history stages. While the effects of body reserves sometimes represented a relatively small proportion of the total variance in individual immune markers (4–51%), we did not expect high predictive power due to the unmeasured variation in pathogen exposure and wounds. The consistent impacts of body reserves on various immune markers across life-history stages, which comprise varying immune challenges and energy constraints, provide strong evidence for allocation trade-offs between immune function and the energy costs of breeding and moulting.

In NES, body mass and composition at parturition are important determinants of reproductive effort (Crocker *et al.* 2001). Larger females expend more energy over lactation and provide higher rates of milk energy delivery to their offspring. Depletion of fat reserves appears to be an important constraint on energy expenditure during breeding. Loss of adiposity across breeding influences the ability to spare body protein from catabolism (Crocker *et al.* 1998) and directly influences release and metabolic responses to hormones that regulate energy mobilization and use (Fowler *et al.* 2008; Crocker *et al.* 2014a,b). Use of energy for maternal metabolism, which includes allocation to immune response, influences lactation efficiency. Females make behavioural adjustments to reduce energy expenditure and increase the energy available for milk production (McDonald & Crocker 2006). During moulting, body size impacts on surface to volume ratios influence the relative nutrient resources required for pelage synthesis, but changes in adiposity are less dramatic during this similar duration fast (Worthy *et al.* 1992). Consistent impacts of mass and adiposity on immune response across both fasts, despite greater variability and depletion of reserves during breeding, suggest consistent mechanisms for mediating allocation towards immune response relative to energy reserves while fasting.

Theoretical structures make several distinct predictions about the impacts of body size, energy expenditure and life-history strategies on energy allocation to immune response (Lee 2006; Downs & Dochtermann 2014). Specifically, species with 'slow' speeds of life, such as NES, should invest more in long-term survival and maintenance of the immune system instead of current reproduction when under energy constraints. In agreement with this idea, we found that despite extreme energy constraints, female NES still exhibited immune responses. This trait may be critical to survival in a colonial breeding environment. Ecological immunology theory also suggests that in times of energy constraint, animals will shift immune investment from adaptive responses to innate responses, especially during breeding. While we did not observe a full shift from adaptive to innate immune responses, IgG was suppressed over the breeding haul-out, indicating that some energy may have been shifted away from adaptive responses. Finally, theory suggests a positive correlation between resource acquisition and the ability to allocate those resources towards an immune response. We saw that larger females had an increased immune response early in the fast resulting from a positive association between body size and allocation to immune responses.

Measurements of adaptive immune response (IgG and IgM) in breeding female NES have been reported previously (King *et al.* 1998) and followed a different pattern than the one detected in our much larger sample size. The previous work described a decrease in IgM across the breeding haul-out, while we found a significant decrease in IgG. The decline in IgG over lactation is consistent with an investigation in harbour seals (Ross *et al.* 1993), which showed that female harbour seals had a decrease in IgG over the lactation period. The authors attributed this to possible stressors such as malnutrition. In the current study, improved body condition allows animals to elicit a greater innate immune response, but all animals exhibited a reduction in IgG levels across lactation, and adaptive immune responses late in breeding were not associated with body reserves. Based on mean values in the current study and assuming an average plasma volume (Hassrick *et al.* 2010), post-partum females would have to mobilize 0·44 kg of protein from 1·63 kg of lean tissue to provide the protein content of serum IgG at that time point. Loss of the ability to spare protein across lactation (Crocker *et al.* 1998) may cause females to reduce allocation towards the more expensive adaptive response. The correlation between both adaptive immune markers in the current study suggests that females are exposed to a variety of novel and familiar pathogens during breeding and that some mechanisms of exposure (e.g. parturition, wounds and proximity to infected conspecifics) require simultaneous responses to a diverse group of pathogens.

While there was a weak positive relationship between the inflammatory cytokine IL-6 and its target haptoglobin, no relationship was evident with IL-1β. This could be due to a timing delay between the upregulation of cytokines at the site of infection and the liver releasing acute phase proteins and the state of this process at the time of sampling. This could also be due to haptoglobin levels being influenced by other functions unrelated to immune response. Haptoglobin also plays an important role in scavenging iron from breakdown of haem and erythrocytes (Boretti *et al.* 2009), and NES have an unusually high erythrocyte mass (Hassrick *et al.* 2010). NES retain highly consistent mass-specific blood volumes despite the loss of 33–40% of body mass during breeding (Hassrick *et al.* 2010). This requires significant breakdown of erythrocytes while fasting. Despite this process, circulating haem levels are similar across the breeding fast (Champagne *et al.* 2013). This may reflect extensive haem scavenging by haptoglobin. However, the correlation of IL-6 with haptoglobin and the elevation of haptoglobin during breeding suggest activation as an acute phase protein. These factors may complicate the use of haptoglobin as an immune marker in fasting pinnipeds and suggest the importance of using a suite of immune markers to assess innate immune status.

Females appeared to avoid the immunosuppressive effects of glucocorticoids in that dramatic elevations of cortisol during breeding were not associated with suppression of immune response. One immune marker, IgE, showed a strong negative association with cortisol across all life-history stages. IgE is a marker of parasitic infection, and parasites are common in marine mammals (Dierauf & Gulland 2001). IgE was highest after the post-breeding foraging trip and thus may reflect increased parasite exposure while foraging. IgE also declined significantly over the four study years, despite similar foraging success (data not shown), suggesting declining parasite exposure or response

across the study period. The female seals are either experiencing fewer parasites or mounting less of an immune response to the same parasitic load. A decrease in parasites could be due to a shift in diet. The diet of the female NES is comprised mainly of mesopelagic squid and fish (Antonelis *et al*. 1987), which carry diverse groups of helminth parasites which vary with food web structure (Marcogliese 2002). The strong negative association of serum cortisol with IgE at the return from foraging suggests that immune responses to parasites may be influenced by variation in plasma cortisol during foraging.

Immune responses vary between life-history stages in female NES and are greatest during the breeding haul-out. Breeding was associated with innate and adaptive immune responses in most individuals while the magnitude of response was associated with variation in body reserves. Elephant seals appear to minimize potential deleterious effects of wide variation in cortisol levels during fasting by avoiding the immunosuppressive effects of sustained cortisol levels. Measurable impacts of cortisol levels on antiparasite responses after foraging suggest this decoupling may be limited to specific aspects of the immune response during terrestrial fasting. Consistent impacts of body mass and composition on immune status suggest allocation trade-offs between body reserves and the nutrient demands of breeding and moulting. These trade-offs may reflect a compromise between immune costs inherent in colonial breeding and energetic limitations that arise in use of capital breeding strategies. These findings provide insight into the forces that may shape allocation towards immune response in iteroparous mammals. Immune responses that are required for survival may be modulated relative to the energetic demands required for successful reproduction. Allocation to immune response during a reproductive attempt may reflect state variables, the social context of breeding, pathogen exposure and the magnitude of parental investment.

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Data accessibility

Data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.g1994 (Peck, Costa & Crocker 2014).

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