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Dehydration enhances cellular and humoral immunity in a mesic snake community

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Abstract

The immunocompetence of a community of free-living animals can be affected by seasonality, sex, and parasite burden. However, each of these factors is often examined independently. Recent studies have also found that dehydration can enhance aspects of immunocompetence in drought-adapted species. To explore how all of these factors interact, and their effect on the immune system in mesic-adapted species, we collected blood samples from a community of free-ranging snakes in coastal South Carolina, United States, across 2 years. We specifically examined (a) how sex and seasonality influence humoral and cellular immunocompetence and parasite burden, (b) the dynamics among hydration state, parasite burden, and immunocompetence, and (c) whether mesic-adapted species also show enhanced innate immunity with dehydration. Consistent with previous work on drought-adapted species, we found that dehydration enhances multiple aspects of humoral immunity in mesic species, and we are the first to report that dehydration also enhances aspects of cellular immunocompetence. Contrary to previous results in other squamates, sex and season did not impact immunocompetence or parasite prevalence. Our results also reveal complex interactions among parasite prevalence, immunocompetence, and hydration state demonstrating that hydration state and parasitism are two ubiquitous factors that should continue to be considered in future studies examining ecoimmunological variation.

KEYWORDS
ecoimmunology, hydration, immunocompetence, osmotic stress, parasitemia

1 | INTRODUCTION

The immune system is a remarkably dynamic and complex network involved in host defense, repair, and maintenance (Murphy, 2012). The ability of an organism to mount a normal response to an antigen (i.e., immunocompetence) has been shown to fluctuate with environmental conditions and throughout ontogeny (Bakar et al., 2016; Love, Salvante, Dale, & Williams, 2008; Nelson & Demas, 1996; Sandland & Minchella, 2003). For example, empirical evidence suggests that season, sex, and hydration can all lead to inter- and intra-individual variation in immunocompetence (Brusch, Christian, Brown, Shine, & DeNardo, 2019; Kelly, Stoehr, Nunn, Smyth, & Prokop, 2018; Walton, Weil, & Nelson, 2011; Zhang, Jin, Qu, & Caviedes-Vidal, 2017). Despite previous research detailing the internal and external conditions which influence the immune system, there remain substantial gaps in our understanding of the biotic and abiotic factors that influence different components of the immune system in ecologically relevant contexts, including in free-ranging animals (Viney, Riley, & Buchanan, 2005). Furthermore, there is a lack of data on the combined effects of these factors on immunocompetence.

Immune function competes with other physiological processes for energetic resources (Canale & Henry, 2011; Demas & Nelson, 2011; Lochmiller & Deerenberg, 2000), and, because of the high energetic requirements of reproduction (Angilletta & Sears, 2000;

Nilsson & Råberg, 2001), immunocompetence is highly influenced by a tradeoff with reproduction (Adamo, Jensen, & Younger, 2001; Cox et al., 2010; Durso & French, 2018; French, DeNardo, & Moore, 2007; Knowles, Nakagawa, & Sheldon, 2009; Stahlschmidt et al., 2013). The energy demands of reproduction can lead to a seasonal suppression of the immune system in seasonally breeding animals (Martin, Weil, & Nelson, 2008; Walton et al., 2011). Shifts in immunocompetence during the breeding season have been documented in a range of vertebrates including fishes, reptiles, birds, and mammals (Brown et al., 2016; De Coster, De Neve, Martín-Gálvez, Therry, & Lens, 2010; Hegemann, Matson, Both, & Tieleman, 2012; Martin et al., 2008; Sin et al., 2016; Zimmerman, Paitz, Vogel, & Bowden, 2010). Therefore, it is important to consider the effects of breeding season when investigating factors that may modulate the immune system.

In addition to reproductive seasonality, the sex of an individual influences variation in key features of life history and physiology across animal taxa (Zuk, 2009). Recent work highlights the role of sex in immunological variation (reviewed in Klein and Flanagan, 2016; Foo, Nakagawa, Rhodes, & Simmons, 2017; Kelly et al., 2018). A meta-analysis of 124 studies across vertebrates and invertebrates indicates that females tend to have higher measures of immunocompetence compared to males (Kelly et al., 2018). However, the significance of this sex effect is highly sensitive to phylogeny, and this study exposes the taxonomical gaps in our understanding of sex-specific variation in immune function. Although others have recently found support for greater female immunocompetence in some snakes (corn snakes: Luoma, Butler, & Stahlschmidt, 2016; garter snakes: Neuman-Lee, Van Wettere, & French, 2019), further research is needed to understand the effects of sex on immunocompetence across taxa including reptiles (Zimmerman, Vogel, & Bowden, 2010).

The vast majority of previous work has focused on energy as the primary resource affecting variation in immunocompetence (Cheynel et al., 2019; Cotter, Simpson, Raubenheimer, & Wilson, 2011; Derting & Compton, 2003; Fair & Ricklefs, 2002; Husak, Roy, & Lovern, 2017; Martin et al., 2008). While energy is clearly a vital currency, nonenergetic resources can also be limited and impact the immune system (e.g., vitamins and carotenoids: Hartley & Kennedy, 2004; Maggini, Pierre, & Calder, 2018; McGraw & Ardia, 2003). Water is an essential resource for life as it is the main constituent of cells, tissues, and organs, but water has only recently received consideration for its role as a currency that influences immunocompetence. Like food, the availability of water in the environment varies significantly across time and space, and this can lead to periods of water limitation for many animals. For example, over two-thirds of the land surface on earth undergoes 65 days or more without any measurable rainfall (Hao, Singh, & Xia, 2018). Recent work has demonstrated a link between dehydration and immune function in selected squamate reptiles. Notably, aspects of plasma-based, innate immunity (i.e., humoral immunity) are enhanced in response to dehydration, at least for the squamates studied to date that are adapted to seasonal droughts (Gila monsters: Moeller, Butler, & DeNardo, 2013; rattlesnakes: Brusch & DeNardo, 2017; Children's pythons: Brusch, Billy, Blattman, & DeNardo, 2017; water pythons: Brusch et al., 2019). However, it is unknown (a) how leukocytes (i.e., cellular immunity) or parasite burden respond to an animal's hydric state, and (b) whether dehydration-based immunoenhancement can be captured across a community, rather than solely on a species-specific level as has been previously documented.

We examined hydration state, parasitemia (as a metric of immune challenge), and several metrics of immunocompetence in a community of colubrid snakes to address three aims. First, we determined the independent and interactive effects of season and sex on aspects of humoral immunity, cellular immunity, and parasite burden. We predicted greater immunocompetence and reduced parasite burden in females relative to males and during the nonbreeding season relative to the breeding season (*sensu* Kelly et al., 2018; Luoma et al., 2016; Martin et al., 2008; Neuman-Lee et al., 2019). Second, we determined the relationship between hydration state and immune function in this community. Here, we predicted that dehydration (i.e., elevated plasma osmolality) would be positively correlated with humoral and cellular immunocompetence (*sensu* Brusch & DeNardo, 2017; Brusch et al., 2017, 2019; Moeller et al., 2013). Third, we explored the relationship between parasite burden and immune function in this community given the strong interdependence of parasites and the immune systems of their hosts (reviewed in Graham, 2013). Here, we used the correlation between these metrics to provide an initial distinction between two alternative possibilities based on work in other squamates. As many parasites illicit an immune response (i.e., inflammation, Murphy, 2012), parasite burden could be positively correlated with humoral and cellular immunocompetence (i.e., a responsive immune effect, Motz, Lewis, & Vardo-Zalik, 2014; Spence, Durso, Smith, Skinner, & French, 2017). Alternatively, animals with comparatively lower immunocompetence may be more susceptible to parasitism, and thus might have a higher parasite burden (i.e., a causative immune effect).

2 | MATERIALS AND METHODS

All procedures were approved by the University of Pacific's Institutional Animal Care and Use Committee (protocol #16R02).

2.1 | Study site

As described previously (Stahlschmidt, Walman, & Mills, 2018), Spring Island is a private low-density residential community in the coastal plain of the southeastern Unites States (Beaufort County, SC). This site is characterized by a humid subtropical climate and a southern mixed deciduous-evergreen broadleaf forest. Throughout the site, sheets of steel (approx. 1 m× 2 m) are used as artificial cover objects by snakes and other terrestrial animals (Stahlschmidt et al., 2018).

2.2 | Experimental design

In 2017 and 2018, we sampled colubrid snakes ($N = 61$), which are generally long-lived mesopredators that feed on a variety of terrestrial and aquatic vertebrate prey (Gibbons & Dorcas, 2005). During March (breeding season) and September (nonbreeding season; Gibbons & Dorcas, 2005), we captured snakes by hand from beneath artificial cover objects. We then used heparinized 1 ml syringes with 25-G × 1.6 cm needles to collect a ≤0.5 ml blood sample from each snake via cardiocentesis. The total time for capture, restraint, and blood collection was ≤5 min. We placed each blood sample in a 1.5 ml microcentrifuge tube on ice and placed each snake in a cloth bag. Within 1 hr, we made 1–2 blood smears for each blood sample (see Section 2.3 below) and centrifuged the remaining blood at 7,000 rpm for 5 min to separate plasma from blood cells. We aliquoted plasma (50 μl) into separate microcentrifuge tubes that were then frozen at −80°C until we used them to measure plasma osmolality and humoral immune function (see Section 2.4 below). We then determined the body mass by placing each snake into a plastic box on a tared balance (±1 g), body size (snout-vent length [SVL]; ±0.1 cm) by gently stretching each snake along a measuring tape taped to a table, and the sex of each snake by probing the cloaca caudally. Concurrently, we also checked for ectoparasites (ticks or mites), which were rarely observed (<7% of snakes). Thus, hemoparasites were used as a proxy for parasite burden because hemoparasites are common in snakes (Davis et al., 2012; Telford, 2009), can influence fitness-related traits in snakes (Madsen, Ujvari, & Olsson, 2005; but see Brown, Shilton, & Shine, 2006), and can be determined in a minimally invasive fashion (i.e., via blood smears: see Section 2.3 below). We subcutaneously implanted each snake with a passive integrated transponder (12 mm) for future identification and returned each snake to its site of capture within 18 hr.

2.3 | Hematological methods

We made thin blood smears on glass microscope slides, which were then air-dried at ambient temperature and stored until fixation. We fixed blood smears for 10 min in absolute methanol and then Giemsa-stained the smears (*sensu* French, Fokidis, & Moore, 2008; Stahlschmidt, Shine, & DeNardo, 2012). We dehydrated stained smears for >24 hr before clearing them using xylene. We then coverslipped and sealed each slide using Cytoseal 60 (VWR, San Francisco, CA) for long-term storage.

Using a light microscope (DM750, Leica Camera AG), we randomly selected and digitized nonoverlapping microscope fields containing nonoverlapping single cell layers at ×400 magnification. We used ImageJ analysis software (v.1.52; National Institutes of Health) to identify erythrocytes (>5,000 per slide) based on morphological characteristics of their nuclei (size, circularity, and color). We included erythrocytes in the counts if their entire nucleus was visible on the digitized image. We manually counted heterophils, lymphocytes, and other leukocytes, as well as parasitized erythrocytes seen on these digitized images (Campbell, 2005; Telford, 2009).

We estimated investment into cellular immune function as the sum of leukocytes (white blood cells, WBCs) per 10^3 erythrocytes (herein, WBC count). We also determined the ratio between two leukocytes (heterophils to lymphocytes; herein, H/L ratio), which is indicative of inflammation and therefore a measure of immunocompetence (Krams et al., 2012; Norris & Evans, 2000). All hemoparasites were hemogregarines (*Hepatozoon*), and we analyzed their prevalence (i.e., binary presence/absence data) because residuals from the data for parasite load (i.e., total number of parasitized erythrocytes per 10^3 erythrocytes) were irrevocably nonnormally distributed.

2.4 | Osmolality and immune function assays

We determined plasma osmolality in a subset of thawed samples ($n = 42$) using a vapor pressure osmometer (±3 mOsm/kg; model 5100C; Wescor Inc., Logan, UT). Before use, we calibrated the osmometer using sealed osmolality standards (100, 290, and 1,000 mOsm/kg) in accordance with factory recommendations. We ran samples in triplicate and used 290 mOsm/kg standards to check the osmometer for variation after every sample. If the standard varied more than the limits of the osmometer (±3 mOsm/kg), the osmometer was recalibrated. If it continued to vary more than the limits, the osmometer head was cleaned and the machine was recalibrated before continuing triplicate analysis beginning with the last sample before calibration/cleaning to verify correct measurement of the sample. A few of the plasma samples ($n = 6$) did not contain sufficient volume for triplicate readings and were diluted (1:1) in reptile Ringer's solution (300 mOsm/kg) following methods from Secor et al. (1994) before the determination of plasma osmolality.

We used several plasma-based assays to assess humoral innate immune function ($n = 42$) and examine whether hydration state was associated with immunocompetence. To evaluate the involvement of natural antibodies and complement (C') in reacting to a novel, eukaryotic antigen, we used sheep red blood cells (sRBC; SBH050; Hemostat Laboratories, Dixon, CA) to quantify agglutination and lysis, which are measures of soluble constitutive immunity (Matson, Ricklefs, & Klasing, 2005). Briefly, we serially diluted 20 μl of each plasma sample from 1:2 to 1:2,048 with phosphate-buffered saline (PBS) along a row of a 96-well plate. We then added 20 μl 1% sRBC to each well. We did not add plasma to the final column; the top four wells contained only 20 μl PBS and 20 μl 1% sRBC (negative control, 0% lysis) and the bottom four wells contained 20 μl ammonium-chloride-potassium (ACK) lysing buffer (Lonza, Basel, Switzerland) and 20 μl 1% sRBC (positive control, 100% lysis). We incubated the plates at 37°C for 90 min and placed them at room temperature for 20 min, after which we scanned the plates at 600 dots per inch using a flatbed scanner (ScanJet 3670; Hewlett-Packard Co.) for

agglutination images. Plates remained at room temperature for an additional 70 min, and then we centrifuged them for 5 min (500 rpm; Sorvall; Newtown, CT) after which we aspirated the supernatant into a clean 96-well plate. We measured absorbance values using a microplate spectrophotometer (405 nm; Bio-Rad, Hercules, CA) to calculate lysis scores. Hemolytic-complement activity was expressed in CH_{50} units (ml plasma)$^{-1}$, where 1 CH_{50} unit equals the reciprocal of the dilution of plasma required to lyse 50% of the sRBC.

We also assessed the ability of plasma to kill a Gram-negative bacterium, *Escherichia coli*, by comparing bacterial growth after exposure to plasma (French & Neuman-Lee, 2012). Briefly, we performed this bacterial killing assay (BKA) by combining 1:4 plasma dilution with CO_2-independent medium plus 4 nM L-glutamine, 10^6 colony-producing units of *E. coli* (Lot#483-478-1; ATCC 8739; MicroBioLogics, St. Cloud, MN), and agar broth on a 96-well microplate. We calculated absorbance using a microplate reader (300 nm; Bio-Rad) at 0 hr and after 12 hr of incubation at 37°C. We calculated percent bacteria killed as one minus the mean absorbance for each sample, which we ran in triplicate, divided by the mean absorbance for the positive control (triplicate wells containing only medium and bacteria), multiplied by 100.

A multivariate statistical method was used to generate an index of humoral immune function, which was subsequently analyzed (see Section 2.5 below). Specifically, a principal component analysis was performed on initial dependent variables of humoral immune function (agglutination, lysis, and bacterial killing). The initial dependent variables were significantly correlated with one another (Pearson correlation: $R > .46$, $p < .002$). The Bartlett's measure, which determines whether there is a significant pattern of correlations in a given data set, was highly significant ($p < .001$). Yet, our data set did not exhibit extreme multicollinearity (overly correlated variables) because it had a large determinant of the correlation matrix value (0.35). The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy ranges from 0 (diffuse pattern of correlations) to 1 (compact pattern of correlations), and the KMO value for our data set was 0.66, which is acceptable (Kaiser, 1974). Therefore, our humoral immune function data satisfied the assumptions of having significant and compact patterns of correlations. The first principal component extracted from our data (PC1) accounted for 71% of the total variance of the data. PC1 loaded positively onto agglutination (0.89), lysis (0.88), and bacterial killing (0.75). Thus, an individual with a high PC1 (herein, humoral immunity index) had relatively high values for agglutination, lysis, and bacteria killing.

2.5 | Statistical analyses

Several linear mixed models were performed in SPSS (v.25; IBM Corp., Armonk, NY), data were log-transformed when necessary, and two-tailed significance was determined at $\alpha = .05$. To examine the independent effects of sex and season across the community, a linear mixed model analysis was performed on each of the following traits: WBC count, H/L, plasma osmolality, and humoral immunity index. For each mixed model, sex, season, and a sex × season interaction were included as main effects, with year and species included as random effects. A binary logistic generalized linear model was used to determine the main and interactive effects of sex and season on hemoparasite prevalence (0: parasites absent; 1: parasites present) where year and species were similarly included as random effects. To determine the relationship between plasma osmolality and immunocompetence, two additional linear mixed model analyses were performed—one on WBC count and one on humoral immunity index. In these models, plasma osmolality was the fixed effect (covariate) while species, season, and year were random effects. To determine the relationship between hemoparasite prevalence and both immunocompetence and plasma osmolality, three final linear mixed model analyses were performed—one on WBC count, one on humoral immunity index, and one on plasma osmolality. In these models, hemoparasite prevalence was the fixed effect while species, season, and year were random effects.

3 | RESULTS

We captured and collected data on six snake species that varied on average body mass and SVL: northern black racer (*Coluber constrictor*), eastern hog-nosed snake (*Heterodon platirhinos*), banded watersnake (*Nerodia fasciata*), cornsnake (*Pantherophis guttatus*),

TABLE 1 Characteristics of snakes sampled on Spring Island, South Carolina, USA ($n = 61$). Body mass and snout-vent length values are displayed as mean ± s.e.m for species where more than one snake was captured

Species	*n*	Body mass (g)	Snout-vent length (cm)	Males/ females	Spring/ Fall
Coluber constrictor	36	278.1 ± 20.5	93.0 ± 2.6	12:24	13:23
Heterodon platirhinos	1	159	61	0:1	1:0
Nerodia fasciata	1	230	67.7	0:1	1:0
Pantherophis guttatus	14	315.0 ± 58.2	91.7 ± 5.1	5:9	10:4
Pantherophis obsoletus	1	168	89.5	0:1	0:1
Thamnophis sirtalis	8	101.4 ± 16.1	61.4 ± 3.8	2:6	7:1

eastern ratsnake (*Pantherophis alleghaniensis*), and common gartersnake (*Thamnophis sirtalis*) (Table 1). Because we could not gather a balanced data set (i.e., an equal number of samples from each species; see Table 1), it was important to account for species as a random effect in our linear models (results reported below).

Plasma osmolality was greater in the nonbreeding season (fall; mean ± s.e.m.: 331.7 ± 5.0 mOsm/kg) relative to in the breeding season (spring; 314.3 ± 5.4 mOsm/kg) (Table S1, available online). However, neither WBC count, H:L ratio, nor humoral immunity index varied due to sex or season (Tables S2–S4, available online).

Hemoparasite prevalence was not affected by season (Wald $\chi^2 = 1.6$, $df = 1$, $p = .21$), sex (Wald $\chi^2 = 0.83$, $df = 1$, $p = .36$) or an interaction between season and sex (Wald $\chi^2 = 1.2$, $df = 1$, $p = .27$). Relative to noninfected snakes ($n = 26$), those that were infected by hemoparasites ($n = 35$) had lower humoral immunity indices ($F_{1, 40} = 6.4$, $p = .015$; Figure 1) and higher plasma osmolality ($F_{1, 40} = 4.2$, $p = .047$; Figure 1). However, parasite prevalence was not associated with WBC count ($F_{1, 59} = 0.10$, $p = .75$).

When including species, season, and sex as random effects, plasma osmolality positively covaried with humoral immunity index ($F_{1, 38} = 9.3$, $p = .0041$; community-wide correlation: $R = .48$) and with WBC count ($F_{1, 35} = 10$, $p = .0031$; community-wide correlation: $R = .37$) (Figure 2). Covariation benefited from an individual with high osmolality (448 mOsm/kg) that was included in our analyses because our sample size was limited and the data set including this individual retained normally distributed residuals. However, removal of this individual did not change the significance of relationships between osmolality and humoral immunity index ($F_{1, 38} = 9.3$, $p = .0041$; community-wide correlation: $R = .43$) or between osmolality and WBC count ($F_{1, 35} = 5.0$, $p = .031$; community-wide correlation: $R = .24$).

4 | DISCUSSION

To date, the link between dehydration and enhanced humoral immunocompetence has been documented in studies focused on individual species of reptiles that live in areas that experience an extended seasonal drought (Brusch & DeNardo, 2017; Brusch et al., 2017, 2019; Moeller et al., 2013). Ours is the first study to explore how a community of mesic-adapted snakes responds to dehydration, and our results are consistent with previous work: dehydration enhances multiple aspects of humoral immunity (Figure 2a). Ours is also the first study to demonstrate that aspects of cellular immunocompetence is similarly enhanced by dehydration (Figure 2b). While previous studies have shown that immunocompetence can vary dramatically between sexes (Markle & Fish, 2014) and across seasons (Buehler, Piersma, Matson, & Tieleman, 2008), we found that sex and season did not impact immunocompetence or parasite load across a community of mesic-adapted snakes (Tables S2–S4). Therefore, we provide insight into the complex interactions among parasite burden, immunocompetence, and hydration state—specifically, animals with more parasites had lower humoral immunocompetence and were more dehydrated (Figure 1).

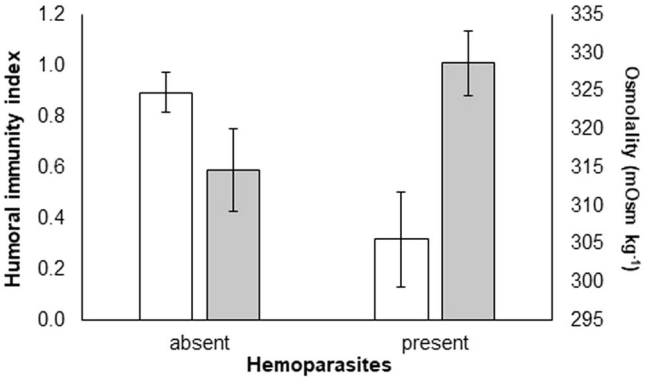

FIGURE 1 Significant relationships between hemoparasite prevalence and both log-transformed humoral immunity index (principal component explaining 71% of the variance in three metrics of immunocompetence; white columns) and osmolality (gray columns) in a community of snakes sampled in South Carolina, United States ($n = 42$). Species, sex, and season (breeding vs. nonbreeding) were included as random effects (see text for details). Values are displayed as mean ± s.e.m

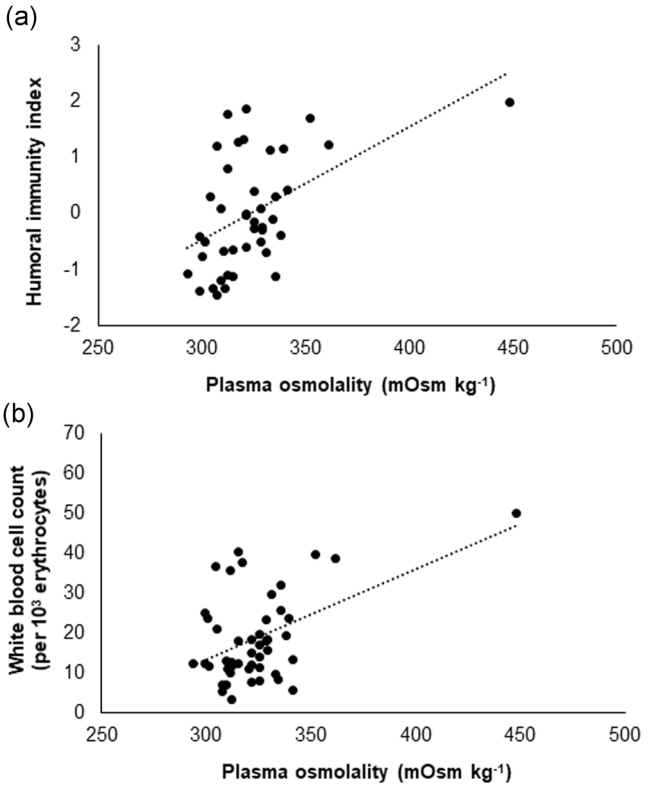

FIGURE 2 Relationships between plasma osmolality and (a) humoral immunity index ($n = 42$) and (b) white blood cell count ($n = 61$) in a community of snakes sampled in South Carolina, Species, sex, and season (breeding vs. nonbreeding) were included as random effects. Removal of the individual with high osmolality (448 mOsm/kg) did not change the significance of relationships between osmolality and humoral immunity index (see text for details). Symbols represent individual animals and a line of best fit is included for significant relationships ($p < .05$)

Periods of reproductive investment are associated with high energetic demands and reduced immunocompetence (Ardia, 2005; Deerenberg, Arpanius, Daan, & Bos, 1997; Martin et al., 2008) because energy-limited animals are unable to simultaneously invest fully in both reproduction and immune defenses (Downs, Adelman, & Demas, 2014; French et al., 2007; Sheldon & Verhulst, 1996). Conversely, animals with ample access to external (food) and internal (fat and muscle) energy resources are able to sustain investment into both reproduction and immunocompetence (French et al., 2007; Lee, 2006; Ruiz, Wang, Reinke, Demas, & Martins, 2011; but see Stahlschmidt et al., 2013). Additionally, previous studies in vertebrates have found correlations where testosterone has a suppressive effect (Beagley & Gockel, 2003; Bouman, Schipper, Heineman, & Faas, 2004; Gleicher & Barad, 2007) and estrogen an enhancing effect (Grimaldi, Jeganathan, & Diamond, 2006; Klein, 2000, 2004; Mo et al., 2005) on immunocompetence. These differences in immunocompetence are often particularly pronounced during the breeding season when levels of sex hormones are at their highest (Hasselquist, 2007). In contrast to the predictions for our first aim (effects of season and sex on immunocompetence and parasite burden), we did not detect any significant differences in humoral or cellular immunocompetence (Tables S2–S4) between breeding and nonbreeding seasons across a snake community. Similarly, we found no significant differences between males and females in humoral or cellular immunocompetence (Tables S2–S4)—in contrast to a prediction for our first aim and to previous work (reviewed in Kelly et al., 2018). Animals in our study likely had regular access to prey (Stahlschmidt et al., 2018) and sufficient body stores during the breeding season (i.e., body condition [residuals from a regression of body mass on SVL] did not change with season) to concurrently fuel reproductive investment and immunocompetence. Alternatively, some experimental evidence suggests that the energetic cost of an immune response does not form the basis for fluctuations in immunocompetence throughout an animal's lifetime (Baze, Hunter, & Hayes, 2011; Nilsson, Granbom, & Råberg, 2007; Robar, Murray, & Burness, 2011).

Similar to results for immunocompetence, hemoparasite prevalence was unaffected by sex and season in contrast to the predictions for our first aim, which were based on previous studies finding that males typically have more parasites than females (Klein, 2004; Moore & Wilson, 2002; Poulin, 1996). These sex-based differences are often greater during the breeding season (Knowles et al., 2009; Merino, Moreno, José Sanz, & Arriero, 2000; Tomás, Merino, Moreno, Morales, & Martinez-De La Puente, 2007). Sex hormones may directly impact immunocompetence (Folstad & Karter, 1992; Zuk & McKean, 1996) or cause behavioral shifts which lead to higher contact with pathogens (Eisen & DeNardo, 2000; Roved, Westerdahl, & Hasselquist, 2017; Zuk & Stoehr, 2002).

We found that humoral, but not cellular, immunocompetence was associated with hemoparasite levels where infected snakes had lower humoral immunocompetence (in support of prediction two in our third aim) and higher plasma osmolality (Figure 1). The *Hepatozoon* species that we quantified is an obligate intracellular parasite. Infections with such parasites typically result in the release of cytokines, eosinophilia, and the production of immunoglobulin E, all of which are components of the humoral response (Chakraborty et al., 2017; Frölich, Entzeroth, & Wallach, 2012). One other study exploring the relationship between parasite load and humoral immunity in a snake (water python: Ujvari & Madsen, 2006) similarly found a negative correlation between parasite load and humoral immunity. They suggest that this result is due to an energetic limitation because snakes in poor body conditions had higher parasite loads (Ujvari & Madsen, 2006). However, we found no similar relationship between body condition and parasitism. Because parasite prevalence exhibited relationships with both humoral immunocompetence and osmolality (Figure 2), future studies in free-ranging animals, especially those that use experimental manipulations, are needed to examine the interrelation between immunocompetence, parasitism, and hydration state.

We found that plasma osmolality was significantly higher during the fall (nonbreeding season) compared to the spring (breeding season; Table S1) despite higher 30-day rainfall totals just before our sampling (2017: fall—23.3 cm, spring—2.2 cm; 2018: fall—11.2 cm, spring—1.6 cm; Old Tabby Links Golf Course, https://www.springisland.com/old-tabby-links). Seasonal variation in plasma osmolality may not be related to precipitation in our study, as animals on Spring Island likely have constant access to water (Z.R.S., unpublished data). Instead, these differences might be attributed to the behavioral relationship between feeding and season. For example, many snakes exhibit a lack of feeding during reproductive seasons and/or cooler body temperatures (Gregory, Crampton, & Skebo, 1999; Schneider, Wise, Benton, Brozek, & Keen-Rhinehart, 2013; Shine, 1980; reviewed in Vincent and Mori, 2008), and the 30-day average temperature just before our sampling was warmer in the fall at the field site (2017: 24.6°C, 2018: 26.2°C) than in the spring (2017: 14.9°C, 2018: 15.4°C; Old Tabby Links Golf Course, https://www.springisland.com/old-tabby-links). Increased feeding during the fall might have challenged the hydric state of snakes in our study as evidence suggests that carnivorous squamates gain no osmotic benefits from feeding (Beaupre, 1996; Wright, Jackson, & DeNardo, 2013), and meal consumption may actually increase dehydration rates (Lillywhite, 2017; Murphy & DeNardo, 2019).

Regardless of seasonal differences, we found that plasma osmolality across a community was positively correlated with multiple metrics of humoral immunocompetence (Figure 2b) as expected for our second aim (positive association between dehydration and immunity). There is now a growing body of evidence documenting a link between dehydration (i.e., hyperosmolality) and enhanced humoral immunocompetence that is (a) not the result of increased immune factor concentrations due to lower plasma volume, (b) rapidly reversible upon rehydration, and (c) found in a variety of taxa and life history stages (Brusch & DeNardo, 2017, 2019; Brusch et al., 2017, 2019; Moeller et al., 2013; this study). Additionally, our study provides the first link between dehydration and cellular immunocompetence (Figure 2b), which is an important step to further understanding the link between dehydration and the immune system.

Our study adds to a growing body of evidence showing that fluctuations in osmotic state can enhance immunocompetence. Yet, it is still uncertain if these enhancements are adaptive because the magnitude of an immune response does not always confer fitness benefits (Graham et al., 2011; Råberg, Grahn, Hasselquist, & Svensson, 1998; Roved et al., 2017; Viney et al., 2005) and an overly robust response can lead to chronic inflammation (Whitacre, 2001), autoimmunity (Ngo, Steyn, & McCombe, 2014), and self-damage (Ayres & Schneider, 2012; Sadd & Siva-Jothy, 2006). Future research is needed to uncover the proximate and ultimate mechanisms explaining the immune-modulating effects of dehydration, as well as the effect parasites have on the link between dehydration and immunocompetence. Furthermore, examining immune responses during periods of dehydration is vital for our understanding of animal survival under resource-limited conditions because rainfall events are forecasted to be less reliable in many regions, resulting in reduced water availability for many species (Marvel et al., 2019; Schlaepfer et al., 2017).

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets supporting this article can be accessed at https://doi.org/10.6084/m9.figshare.7970816

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REFERENCES

Adamo, S. A., Jensen, M., & Younger, M. (2001). Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly *G. integer*): Trade-offs between immunity and reproduction. *Animal Behavior*, *62*, 417–425. https://doi.org/10.1006/anbe.2001.1786

Angilletta, M. J., & Sears, M. W. (2000). The metabolic cost of reproduction in an oviparous lizard. *Functional Ecology*, *14*, 39–45. https://doi.org/10.1046/j.1365-2435.2000.00387.x

Ardia, D. R. (2005). Tree swallows trade off immune function and reproductive effort differentialy across their range. *Ecology*, *86*, 2040–2046. https://doi.org/10.1890/04-1619

Ayres, J. S., & Schneider, D. S. (2012). Tolerance of infections. *Annual Review of Immunology*, *30*, 271–294. https://doi.org/10.1146/annurev-immunol-020711-075030

Bakar, A. A., Bower, D. S., Stockwell, M. P., Clulow, S., Clulow, J., & Mahony, M. J. (2016). Susceptibility to disease varies with ontogeny and immunocompetence in a threatened amphibian. *Oecologia*, *181*, 997–1009. https://doi.org/10.1007/s00442-016-3607-4

Baze, M. M., Hunter, K., & Hayes, J. P. (2011). Chronic hypoxia stimulates an enhanced response to immune challenge without evidence of an energetic tradeoff. *Journal of Experimental Biology*, *214*, 3255–3268. https://doi.org/10.1242/jeb.054544

Beagley, K. W., & Gockel, C. M. (2003). Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. *FEMS Immunology and Medical Microbiology*, *38*, 13–22. https://doi.org/10.1016/S0928-8244(03)00202-5

Beaupre, S. J. (1996). Field metabolic rate, water flux, and energy budgets of mottled rock rattlesnakes, *Crotalus lepidus*, from two populations. *Copeia*, *1996*, 319–329. https://doi.org/10.2307/1446847

Bouman, A., Schipper, M., Heineman, M. J., & Faas, M. M. (2004). Gender difference in the non-specific and specific immune response in humans. *American Journal of Reproductive Immunology*, *52*, 19–26. https://doi.org/10.1111/j.1600-0897.2004.00177.x

Brown, G. P., Shilton, C. M., & Shine, R. (2006). Do parasites matter? Assessing the fitness consequences of haemogregarine infection in snakes. *Canadian Journal of Zoology*, *84*, 668–676. https://doi.org/10.1139/z06-044

Brown, M., Hablützel, P., Friberg, I. M., Thomason, A. G., Stewart, A., Pachebat, J. A., & Jackson, J. A. (2016). Seasonal immunoregulation in a naturally-occurring vertebrate. *BMC Genomics*, *17*, 369. https://doi.org/10.1186/s12864-016-2701-7

Brusch, G. A., IV, Billy, G., Blattman, J. N., & DeNardo, D. F. (2017). Reproduction alters hydration state but does not impact the positive effects of dehydration on innate immune function in children's pythons (*Antaresia childreni*). *Physiological and Biochemical Zoology*, *90*, 646–654. https://doi.org/10.1086/694834

Brusch, G. A., Christian, K., Brown, G. P., Shine, R., & DeNardo, D. F. (2019). Cane toads (*Rhinella marina*) rely on water access, not drought tolerance, to invade xeric Australian environments. *Oecologia*, *189*, 307–316. https://doi.org/10.1007/s00442-018-4321-1

Brusch, G. A., & DeNardo, D. F. (2017). When less means more: Dehydration improves innate immunity in rattlesnakes. *Journal of Experimental Biology*, *220*, 2287–2295. https://doi.org/10.1242/jeb.155028

Brusch, G. A., IV, & DeNardo, D. F. (2019). Egg desiccation leads to dehydration and enhanced innate immunity in python embryos. *Developmental and Comparative Immunology*, *90*, 147–151. https://doi.org/10.1016/j.dci.2018.09.013

Buehler, D. M., Piersma, T., Matson, K., & Tieleman, B. I. (2008). Seasonal redistribution of immune function in a migrant shorebird: Annual-cycle effects override adjustments to thermal regime. *The American Naturalist*, *172*, 783–796. https://doi.org/10.1086/592865

Campbell, T. W. (2005). *Reptile medicine and surgery*. Philedelphia, PA: WB Saunders Co.

Canale, C. I., & Henry, P. Y. (2011). Energetic costs of the immune response and torpor use in a primate. *Functional Ecology*, *25*, 557–565. https://doi.org/10.1111/j.1365-2435.2010.01815.x

Chakraborty, S., Roy, S., Mistry, H. U., Murthy, S., George, N., Bhandari, V., & Sharma, P. (2017). Potential sabotage of host cell physiology by apicomplexan parasites for their survival benefits. *Frontiers in Immunology*, *8*, 1261. https://doi.org/10.3389/fimmu.2017.01261

Cheynel, L., Douhard, F., Gilot-Fromont, E., Rey, B., Débias, F., Pardonnet, S., … Gaillard, J. M. (2019). Does body growth impair immune function in a large herbivore? *Oecologia*, *189*, 55–68. https://doi.org/10.1007/s00442-018-4310-4

Cotter, S. C., Simpson, S. J., Raubenheimer, D., & Wilson, K. (2011). Macronutrient balance mediates trade-offs between immune function and life history traits. *Functional Ecology*, *25*, 186–198. https://doi.org/10.1111/j.1365-2435.2010.01766.x

Cox, R. M., Parker, E. U., Cheney, D. M., Liebl, A. L., Martin, L. B., & Calsbeek, R. (2010). Experimental evidence for physiological costs

underlying the trade-off between reproduction and survival. *Functional Ecology*, *24*, 1262–1269. https://doi.org/10.1111/j.1365-2435.2010.01756.x

Davis, J. R., Boyle, S. A., Khan, A. A., Gay, A. L., Grisham, J. M., & Luque, L. E. (2012). Snake parasitism in an urban old-growth forest. *Urban Ecosystems*, *15*, 739–752. https://doi.org/10.1007/s11252-012-0234-7

De Coster, G., De Neve, L., Martín-Gálvez, D., Therry, L., & Lens, L. (2010). Variation in innate immunity in relation to ectoparasite load, age and season: A field experiment in great tits (*Parus major*). *Journal of Experimental Biology*, *213*, 3012–3018. https://doi.org/10.1242/jeb.042721

Deerenberg, C., Arpanius, V., Daan, S., & Bos, N. (1997). Reproductive effort decreases antibody responsiveness. *Proceedings of the Royal Society of London. Series B*, *264*, 1021–1029. https://doi.org/10.1098/rspb.1997.0141

Demas, G. E., & Nelson, R. J. (2011). *Ecoimmunology*. New York, NY: Oxford University Press.

Derting, T. L., & Compton, S. (2003). Immune response, not immune maintenance, is energetically costly in wild white-footed mice (*Peromyscus leucopus*). *Physiological and Biochemical Zoology*, *76*, 744–752. https://doi.org/10.1086/375662

Downs, C. J., Adelman, J. S., & Demas, G. E. (2014). Mechanisms and methods in ecoimmunology: Integrating within-organism and between-organism processes. *Integrative and comparative biology*, *3*, 340–352. https://doi.org/10.1093/icb/icu082

Durso, A. M., & French, S. S. (2018). Stable isotope tracers reveal a trade-off between reproduction and immunity in a reptile with competing needs. *Functional Ecology*, *32*, 648–656. https://doi.org/10.1111/1365-2435.13002

Eisen, R. J., & DeNardo, D. F. (2000). Life history of a malaria parasite (*Plasmodium mexicanum*) in its host, the western fence lizard (*Sceloporus occidentalis*): Host testosterone as a source of seasonal and among-host variation? *Journal of Parasitology*, *86*, 1041–1045. https://doi.org/10.1645/0022-3395(2000)086[1041:LHOAMP]2.0.CO;2

Fair, J. M., & Ricklefs, R. E. (2002). Physiological, growth, and immune responses of Japanese quail chicks to the multiple stressors of immunological challenge and lead shot. *Archives of Environmental Contamination and Toxicology*, *42*, 77–87. https://doi.org/10.1007/s002440010294

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, *139*, 603–622. https://doi.org/10.1086/285346

Foo, Y. Z., Nakagawa, S., Rhodes, G., & Simmons, L. W. (2017). The effects of sex hormones on immune function: A meta-analysis. *Biological Reviews*, *92*, 551–571. https://doi.org/10.1111/brv.12243

French, S. S., DeNardo, D. F., & Moore, M. C. (2007). Trade-offs between the reproductive and immune systems: Facultative responses to resources or obligate responses to reproduction? *The American Naturalist*, *170*, 79–89. https://doi.org/10.1086/518569

French, S. S., Fokidis, H. B., & Moore, M. C. (2008). Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban–rural gradient. *Journal of Comparative Physiology B*, *178*, 997–1005. https://doi.org/10.1007/s00360-008-0290-8

French, S. S., & Neuman-Lee, L. A. (2012). Improved ex vivo method for microbiocidal activity across vertebrate species. *Biology Open*, *1*, 482–487. https://doi.org/10.1242/bio.2012919

Frölich, S., Entzeroth, R., & Wallach, M. (2012). Comparison of protective immune responses to Apicomplexan parasites. *Journal of Parasitology Research*, *2012*, 1–11. https://doi.org/10.1155/2012/852591

Gibbons, J. W., & Dorcas, M. E. (2005). *Snakes of the southeast*. Athens, GA: University of Georgia Press.

Gleicher, N., & Barad, D. H. (2007). Gender as risk factor for autoimmune diseases. *Journal of Autoimmunity*, *28*, 1–6. https://doi.org/10.1016/j.jaut.2006.12.004

Graham, A. L. (2013). Optimal immunity meets natural variation: The evolutionary biology of host defence. *Parasite Immunology*, *35*, 315–317. https://doi.org/10.1111/pim.12073

Graham, A. L., Shuker, D. M., Pollitt, L. C., Auld, S. K., Wilson, A. J., & Little, T. J. (2011). Fitness consequences of immune responses: Strengthening the empirical framework for ecoimmunology. *Functional Ecology*, *25*, 5–17. https://doi.org/10.1111/j.1365-2435.2010.01777.x

Gregory, P. T., Crampton, L. H., & Skebo, K. M. (1999). Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: Are gravid snakes anorexic? *Journal of Zoology*, *248*, 231–241. https://doi.org/10.1111/j.1469-7998.1999.tb01199.x

Grimaldi, C. M., Jeganathan, V., & Diamond, B. (2006). Hormonal regulation of B cell development: 17β-estradiol impairs negative selection of high-affinity DNA-reactive B cells at more than one developmental checkpoint. *The Journal of Immunology*, *176*, 2703–2710. https://doi.org/10.4049/jimmunol.176.5.2703

Hao, Z., Singh, V. P., & Xia, Y. (2018). Seasonal drought prediction: Advances, challenges, and future prospects. *Reviews of Geophysics*, *56*, 108–141. https://doi.org/10.1002/2016RG000549

Hartley, R. C., & Kennedy, M. W. (2004). Are carotenoids a red herring in sexual display? *Trends in Ecolology and Evolution*, *19*, 353–354. https://doi.org/10.1016/j.tree.2004.04.002

Hasselquist, D. (2007). Comparative immunoecology in birds: Hypotheses and tests. *Journal of Ornithology*, *148*, 571–582. https://doi.org/10.1007/s10336-007-0201-x

Hegemann, A., Matson, K. D., Both, C., & Tieleman, B. I. (2012). Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. *Oecologia*, *170*, 605–618. https://doi.org/10.1007/s00442-012-2339-3

Husak, J. F., Roy, J. C., & Lovern, M. B. (2017). Exercise training reveals trade-offs between endurance performance and immune function, but does not influence growth, in juvenile lizards. *Journal of Experimental Biology*, *220*, 1497–1502. https://doi.org/10.1242/jeb.153767

Kaiser, H. F. (1974). An index of factorial simplicity. *Psychometrika*, *39*, 31–36.

Kelly, C. D., Stoehr, A. M., Nunn, C., Smyth, K. N., & Prokop, Z. M. (2018). Sexual dimorphism in immunity across animals: A meta-analysis. *Ecology Letters*, *21*, 11885–11894. https://doi.org/10.1111/ele.13164

Klein, S. L. (2000). The effects of hormones on sex differences in infection: From genes to behavior. *Neuroscience and Biobehavioral Reviews*, *24*, 627–638. https://doi.org/10.1016/S0149-7634(00)00027-0

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology*, *26*, 247–264. https://doi.org/10.1111/j.0141-9838.2004.00710.x

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*, 626–638. https://doi.org/10.1038/nri.2016.90

Knowles, S. C., Nakagawa, S., & Sheldon, B. C. (2009). Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: A meta-regression approach. *Functional Ecology*, *23*, 405–415. https://doi.org/10.1111/j.1365-2435.2008.01507.x

Krams, I., Vrublevska, J., Cirule, D., Kivleniece, I., Krama, T., Rantala, M. J., … Hõrak, P. (2012). Heterophil/lymphocyte ratios predict the magnitude of humoral immune response to a novel antigen in great tits (*Parus major*). *Comparative Biochemistry and Physiology Part A*, *161*, 422–428. https://doi.org/10.1016/j.cbpa.2011.12.018

Lee, K. A. (2006). Linking immune defenses and life history at the levels of the individual and the species. *Integrative & Comparative Biology*, *46*, 1000–1015. https://doi.org/10.1093/icb/icl049

Lillywhite, H. B. (2017). Feeding begets drinking: Insights from intermittent feeding in snakes. *Journal of Experimental Biology*, *220*, 3565–3570. https://doi.org/10.1242/jeb.163725

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, *88*, 87–98. https://doi.org/10.1034/j.1600-0706.2000.880110.x

Love, O. P., Salvante, K. G., Dale, J., & Williams, T. D. (2008). Sex-specific variability in the immune system across life-history stages. *The American Naturalist*, *172*, E99–E112. https://doi.org/10.1086/589521

Luoma, R. L., Butler, M. W., & Stahlschmidt, Z. R. (2016). Plasticity of immunity in response to eating. *Journal of Experimental Biology*, *219*, 1965–1968. https://doi.org/10.1242/jeb.138123

Madsen, T., Ujvari, B., & Olsson, M. (2005). Old pythons stay fit; effects of haematozoan infections on life history traits of a large tropical predator. *Oecologia*, *142*, 407–412. https://doi.org/10.1007/s00442-004-1742-9

Maggini, S., Pierre, A., & Calder, P. C. (2018). Immune function and micronutrient requirements change over the life course. *Nutrients*, *10*, 1531. https://doi.org/10.3390/nu10101531

Markle, J. G., & Fish, E. N. (2014). SeXX matters in immunity. *Trends in Immunology*, *35*, 97–104. https://doi.org/10.1016/j.it.2013.10.006

Martin, L. B., Weil, Z. M., & Nelson, R. J. (2008). Seasonal changes in vertebrate immune activity: Mediation by physiological trade-offs. *Philosophical Transactions of the Royal Society, B*, *363*, 321–339. https://doi.org/10.1098/rstb.2007.2142

Marvel, K., Cook, B. I., Bonfils, C. J., Durack, P. J., Smerdon, J. E., & Williams, A. P. (2019). Twentieth-century hydroclimate changes consistent with human influence. *Nature*, *569*, 59–65. https://doi.org/10.1038/s41586-019-1149-8

Matson, K. D., Ricklefs, R. E., & Klasing, K. C. (2005). A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental and Comparative Immunology*, *29*, 275–286. https://doi.org/10.1016/j.dci.2004.07.006

McGraw, K. J., & Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: An experimental test. *The American Naturalist*, *162*, 704–712. https://doi.org/10.1086/378904

Merino, S., Moreno, J., José Sanz, J., & Arriero, E. (2000). Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proceedings of the Royal Society of London. Series B*, *267*, 2507–2510. https://doi.org/10.1098/rspb.2000.1312

Mo, R., Chen, J., Grolleau-Julius, A., Murphy, H. S., Richardson, B. C., & Yung, R. L. (2005). Estrogen regulates CCR gene expression and function in T lymphocytes. *The Journal of Immunology*, *174*, 6023–6029. https://doi.org/10.4049/jimmunol.174.10.6023

Moeller, K. T., Butler, M. W., & DeNardo, D. F. (2013). The effect of hydration state and energy balance on innate immunity of a desert reptile. *Frontiers in Zoology*, *10*, 23. https://doi.org/10.1186/1742-9994-10-23

Moore, S. L., & Wilson, K. (2002). Parasites as a viability cost of sexual selection in natural populations of mammals. *Science*, *297*, 2015–2018. https://doi.org/10.1126/science.1074196

Motz, V. L., Lewis, W. D., & Vardo-Zalik, A. M. (2014). Leukocyte profiles for western fence lizards, *Sceloporus occidentalis*, naturally infected by the malaria parasite *Plasmodium mexicanum*. *The Journal of Parasitology*, *100*, 592–597. https://doi.org/10.1645/13-371.1

Murphy, K. (2012). *Immunobiology* (8th ed.). Hamden, CT: Garland Science.

Murphy, M. S., & DeNardo, D. F. (2019). Rattlesnakes must drink: Meal consumption does not improve hydration state. *Physiological and Biochemical Zoology*, *92*, 381–385. https://doi.org/10.1086/704081

Nelson, R. J., & Demas, G. E. (1996). Seasonal changes in immune function. *The Quarterly Review of Biology*, *71*, 511–548. https://doi.org/10.1086/419555

Neuman-Lee, L. A., Van Wettere, A. J., & French, S. S. (2019). Interrelations among Multiple Metrics of Immune and Physiological Function in a Squamate, the Common Gartersnake (*Thamnophis sirtalis*). *Physiological and Biochemical Zoology*, *92*, 12–23. https://doi.org/10.1086/700396

Ngo, S. T., Steyn, F. J., & McCombe, P. A. (2014). Gender differences in autoimmune disease. *Frontiers in Neuroendocrinology*, *35*, 347–369. https://doi.org/10.1016/j.yfrne.2014.04.004

Nilsson, J. Å., Granbom, M., & Råberg, L. (2007). Does the strength of an immune response reflect its energetic cost? *Journal of Avian Biology*, *38*, 488–494. https://doi.org/10.1111/j.0908-8857.2007.03919.x

Nilsson, J. Å., & Råberg, L. (2001). The resting metabolic cost of egg laying and nestling feeding in great tits. *Oecologia*, *128*, 187–192. https://doi.org/10.1007/s004420100653

Norris, K., & Evans, M. R. (2000). Ecological immunology: Life history trade-offs and immune defense in birds. *Behavioral Ecology*, *11*, 19–26. https://doi.org/10.1093/beheco/11.1.19

Poulin, R. (1996). Sexual inequalities in helminth infections: A cost of being a male? *The American Naturalist*, *147*, 287–295. https://doi.org/10.1086/285851

Robar, N., Murray, D. L., & Burness, G. (2011). Effects of parasites on host energy expenditure: The resting metabolic rate stalemate. *Canadian Journal of Zoology*, *89*, 1146–1155. https://doi.org/10.1139/z11-084

Roved, J., Westerdahl, H., & Hasselquist, D. (2017). Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. *Hormones and Behavior*, *88*, 95–105. https://doi.org/10.1016/j.yhbeh.2016.11.017

Ruiz, M., Wang, D., Reinke, B. A., Demas, G. E., & Martins, E. P. (2011). Trade-offs between reproductive coloration and innate immunity in a natural population of female sagebrush lizards, *Sceloporus graciosus*. *The Herpetological Journal*, *21*, 131–134.

Råberg, L., Grahn, M., Hasselquist, D., & Svensson, E. (1998). On the adaptive significance of stress-induced immunosuppression. *Proceedings of the Royal Society of London. Series B*, *265*, 1637–1641. https://doi.org/10.1098/rspb.1998.0482

Sadd, B. M., & Siva-Jothy, M. T. (2006). Self-harm caused by an insect's innate immunity. *Proceedings of the Royal Society B*, *273*, 2571–2574. https://doi.org/10.1098/rspb.2006.3574

Sandland, G. J., & Minchella, D. J. (2003). Costs of immune defense: An enigma wrapped in an environmental cloak? *Trends In Parasitology*, *19*, 571–574. https://doi.org/10.1016/j.pt.2003.10.006

Schlaepfer, D. R., Bradford, J. B., Lauenroth, W. K., Munson, S. M., Tietjen, B., Hall, S. A., … Lkhagva, A. (2017). Climate change reduces extent of temperate drylands and intensifies drought in deep soils. *Nature Communications*, *8*, 14196. https://doi.org/10.1038/ncomms14196

Schneider, J. E., Wise, J. D., Benton, N. A., Brozek, J. M., & Keen-Rhinehart, E. (2013). When do we eat? Ingestive behavior, survival, and reproductive success. *Hormones and Behavior*, *64*, 702–728. https://doi.org/10.1016/j.yhbeh.2013.07.005

Secor, S. M., Stein, E. D., & Diamond, J. (1994). Rapid upregulation of snake intestine in response to feeding: A new model of intestinal adaptation. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *266*, G695–G705. https://doi.org/10.1152/ajpgi.1994.266.4.G695

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution*, *11*, 317–321. https://doi.org/10.1016/0169-5347(96)10039-2

Shine, R. (1980). "Costs" of reproduction in reptiles. *Oecologia*, *46*, 92–100. https://doi.org/10.1007/BF00346972

Sin, Y. W., Newman, C., Dugdale, H. L., Buesching, C., Mannarelli, M. E., Annavi, G., … Macdonald, D. W. (2016). No compensatory relationship between the innate and adaptive immune system in wild-living european badgers. *PLOS One*, *11*, 0163773. https://doi.org/10.1371/journal.pone.0163773

Spence, A. R., Durso, A. M., Smith, G. D., Skinner, H. M., & French, S. S. (2017). Physiological correlates of multiple parasitic infections in side-

blotched lizards. *Physiological and Biochemical Zoology*, *90*, 321–327. https://doi.org/10.1086/691059

Stahlschmidt, Z. R., Lourdais, O., Lorioux, S., Butler, M. W., Davis, J. R., Salin, K., … DeNardo, D. F. (2013). Morphological and physiological changes during reproduction and their relationships to reproductive performance in a capital breeder. *Physiological and Biochemical Zoology*, *86*, 398–409. https://doi.org/10.1086/670918

Stahlschmidt, Z. R., Shine, R., & DeNardo, D. F. (2012). The consequences of alternative parental care tactics in free-ranging pythons in tropical A ustralia. *Functional Ecology*, *26*, 812–821. https://doi.org/10.1111/j.1365-2435.2012.02003.x

Stahlschmidt, Z. R., Walman, R. M., & Mills, A. M. (2018). Red imported fire ants (*Solenopsis invicta*) and seasonality influence community refuge use. *Biological Invasions*, *20*, 2849–2859.

Telford, S. R. (2009). *Hemoparasites of reptilia, color atlas and text*. Boca Raton, FL: CRC Press.

Tomás, G., Merino, S., Moreno, J., Morales, J., & Martinez-De La Puente, J. (2007). Impact of blood parasites on immunoglobulin level and parental effort: A medication field experiment on a wild passerine. *Functional Ecology*, *21*, 125–133. https://doi.org/10.1111/j.1365-2435.2006.01214.x

Ujvari, B., & Madsen, T. (2006). Age, parasites, and condition affect humoral immune response in tropical pythons. *Behavioral Ecology*, *17*, 20–24. https://doi.org/10.1093/beheco/ari091

Viney, M. E., Riley, E. M., & Buchanan, K. L. (2005). Optimal immune responses: Immunocompetence revisited. *Trends in Ecology and Evolution*, *20*, 665–669. https://doi.org/10.1016/j.tree.2005.10.003

Vincent, S. E., & Mori, A. (2008). Determinants of feeding performance in free-ranging pit-vipers (Viperidae: Ovophis okinavensis): key roles for head size and body temperature. *Biological Journal of the Linnean Society*, *93*, 53–62. https://doi.org/10.1111/j.1095-8312.2007.00928.x.

Walton, J. C., Weil, Z. M., & Nelson, R. J. (2011). Influence of photoperiod on hormones, behavior, and immune function. *Frontiers in Neuroendocrinology*, *32*, 303–319. https://doi.org/10.1016/j.yfrne.2010.12.003

Whitacre, C. C. (2001). Sex differences in autoimmune disease. *Nature Immunology*, *2*, 777–780. https://doi.org/10.1038/ni0901-777

Wright, C. D., Jackson, M. L., & DeNardo, D. F. (2013). Meal consumption is ineffective at maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*. *Journal of Experimental Biology*, *216*, 1439–1447. https://doi.org/10.1242/jeb.080895

Zhang, Z., Jin, C., Qu, K., & Caviedes-Vidal, E. (2017). Immune responsiveness to phytohemagglutinin displays species but not sex differences in three anuran species. *PeerJ*, *5*, e3181. https://doi.org/10.7717/peerj.3181

Zimmerman, L. M., Paitz, R. T., Vogel, L. A., & Bowden, R. M. (2010). Variation in the seasonal patterns of innate and adaptive immunity in the red-eared slider (*Trachemys scripta*). *Journal of Experimental Biology*, *213*, 1477–1483. https://doi.org/10.1242/jeb.037770

Zimmerman, L. M., Vogel, L. A., & Bowden, R. M. (2010). Understanding the vertebrate immune system: Insights from the reptilian perspective. *Journal of Experimental Biology*, *213*, 661–671. https://doi.org/10.1242/jeb.038315

Zuk, M. (2009). The sicker sex. *PLOS Pathogens*, *5*, 1000267. https://doi.org/10.1371/journal.ppat.1000267

Zuk, M., & McKean, K. A. (1996). Sex differences in parasite infections: Patterns and processes. *International Journal for Parasitology*, *26*, 1009–1024. https://doi.org/10.1016/S0020-7519(96)80001-4

Zuk, M., & Stoehr, A. M. (2002). Immune defense and host life history. *The American Naturalist*, *160*, S9–S22. https://doi.org/10.1086/342131

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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