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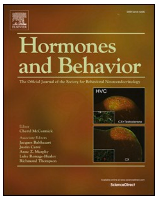

Physiological links with behavior and fitness: The acute adrenocortical response predicts trappability but not survival in male and female deermice

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ABSTRACT

The “Cort-Fitness” hypothesis predicts a negative relationship between baseline glucocorticoids (GCs) and fitness, although evidence for this hypothesis remains mixed. Such ambiguity could partially exist because blood GCs, typically used in field studies, can fluctuate too rapidly to measure accurately, while the relationship between GCs and trappability is often neglected. Here, by addressing these factors, we examined relationships between GC measures and survival of North American deermice (*Peromyscus maniculatus*; hereafter deermice) as a model system. To do this, we used more stable GC measures, including the integrated measures of baseline and stress response fecal corticosterone metabolites (FCMs), and downstream measures of neutrophil/lymphocyte ratio (N/L ratio), and body condition score (BCS), to characterize their relationships with survival and trappability. Over two years, deermice were live-trapped monthly, evaluated for BCS, and sampled for feces and blood. Stress response FCMs were evaluated only at first capture. Mark-recapture models, with GC measures as predictors of either survival or trappability, were compared to identify top models. We found that stress response FCMs negatively predicted trappability, and weaker evidence that BCS positively predicted survival. Although the latter provides some support for the “Cort-Fitness” hypothesis, there was no support when using integrated measures. Instead, our findings suggest that deermice with a lower adrenocortical response (i.e. stress response FCMs) were more likely to be captured. Therefore, GC-trappability relationships must be investigated in field studies to avoid linking the wrong GC profile to fitness, and physiological measures other than blood GCs may be useful for detecting GC-fitness patterns.

1. Introduction

Throughout their lifetime, wild vertebrates experience numerous stressors that have the potential to reduce fitness (i.e., survival and reproductive success). When vertebrates are faced with a stressor, they respond with an acute stress response, which is largely orchestrated by the catecholamines of the sympathetic nervous system and glucocorticoids (GCs) of the hypothalamus-pituitary-adrenal (or inter-renal) axis. Because GCs (i.e., cortisol/corticosterone or cort) can have long-lasting consequences on physiology, behavior, and fitness, they have been largely employed as hormone markers of health in field studies of wildlife (Busch and Hayward, 2009; Dantzer et al., 2014). GCs exert their effects at two different physiological levels, baseline and stress-induced. At baseline levels, GCs serve as critical metabolic hormones required to sustain vertebrate life through mobilizing energy, whereas at stress-induced levels, GCs mediate the acute stress response to redirect energy towards immediate challenges (Busch and Hayward, 2009; MacDougall-Shackleton et al., 2019). However, persistent elevations in GC levels can result in chronic stress, typically manifested as elevated baseline GCs and a dampened acute adrenocortical (or GC) response (Busch and Hayward, 2009).

The consequences of long-term allostatic overload (or chronic stress) on the fitness of wild vertebrates are described by the “Cort-Fitness” hypothesis, which predicts a negative relationship between baseline GCs and fitness. This stems from the idea that as environmental challenges increase, baseline GCs also increase to reallocate energy towards meeting these challenges at the expense of fitness (Bonier et al., 2009a). If consistently true, the “Cort-Fitness” hypothesis has important applied implications because GCs could then be used to preemptively identify wildlife populations at risk of decline earlier than traditional demographic measures (Dantzer et al., 2014).

Presently, a generalized relationship in vertebrates between fitness and GCs (baseline or stress response) remains elusive (Breuner et al., 2008; Bonier et al., 2009a; Crespi et al., 2013; Sorenson et al., 2017).

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This is most likely because GC-fitness relationships can be highly context-specific (Dantzer et al., 2016) and dependent on the proxy for fitness (Ebensperger et al., 2013; Milenkaya et al., 2015). However, two other factors that could potentially introduce ambiguity and generate conflicting evidence include using highly labile markers of GCs in the field (i.e., blood GCs) and neglecting to account for potential links between GCs and probability of capture/detection (or trappability).

Although GC physiology of vertebrates is typically evaluated directly via blood samples, it can also be estimated indirectly by using integrated or downstream measures of GCs. Fecal corticosterone metabolites (FCMs) are an example of an integrated GC measure because blood GCs are gradually excreted in feces after they are metabolized, and therefore acute and rapid rises in blood GCs are smoothened in feces (Palme, 2019). Because GCs can increase neutrophils and decrease lymphocytes in the peripheral circulation, the neutrophil to lymphocyte ratio (N/L ratio) is considered a downstream GC measure and has been widely used in wildlife studies as a more stable GC measure (Breuner et al., 2013; Davis and Maney, 2018). Another downstream GC measure, also commonly used in field studies, is body condition because GCs can regulate energy metabolism and consequently, can promote catabolism of fat and muscle tissues (Sapolsky et al., 2000; Breuner et al., 2013). Because blood GCs can fluctuate greatly across time, representative estimates of GC levels can be difficult to obtain in field studies, especially from vertebrate endotherms (Romero and Reed, 2005), which could obscure existing patterns between GCs and fitness. Therefore, using integrated measures of GCs, such as FCMs, and downstream measures of GCs, such as the ones mentioned above, could improve detection of existing patterns between GC physiology and fitness because they are more stable measures.

Testing the "Cort-Fitness" hypothesis with wild vertebrates in mark-recapture studies requires the use of live-capture methods (e.g., live-traps for small mammals, Eleftheriou et al., 2020a). Consequently, this can lead to capture estimates that are biased because of individual differences in behavioral strategies, notably "proactive" and "reactive" (Koolhaas et al., 1999). The "proactive" strategy is characterized by boldness, aggression, and low HPA activity whereas the "reactive" strategy by shyness, docility, and high HPA activity (Carere et al., 2010). Therefore, how likely an individual will enter a trap (i.e. their trappability) is likely to be influenced by the individual's GC physiology where bolder individuals are expected to be more trappable. For example, eastern chipmunks (*Tamias striatus*) (Montiglio et al., 2012) with lower stress response FCMs were more likely to enter traps, and bolder Richardson's ground squirrels (*Urocitellus richardsonii*) had lower stress response FCMs (Clary et al., 2014). However, Santicchia et al. (2020) found no association between baseline FCMs and boldness in gray squirrels (*Sciurus carolinensis*). Similarly, Westrick et al. (2019) found no relationship between baseline FCMs and aggression in red squirrels (*Tamiasciurus hudsonicus*). Even if the evidence linking GC physiology to trappability remains mixed, this relationship is often ignored in field studies that test the "Cort-Fitness" hypothesis (e.g., Quirici et al., 2021), despite the development of mark-recapture statistical methods to account for heterogeneity in capture behavior (e.g., Cubaynes et al., 2010). This is problematic because mark-recapture methods employed in field studies could select for individuals with particular GC profiles that mediate trap boldness (i.e. more likely to enter traps), thereby potentially linking the wrong GC profile to fitness.

In this study, we examined relationships between four indirect measures of GCs considered to be more stable than blood GCs, and monthly survival, as a fitness proxy, using free-ranging North American deermice (*Peromyscus maniculatus*; hereafter deermice) in Montana, USA. The four physiological measures we used as GC markers were baseline FCMs, stress response FCMs (difference between stress-induced and baseline FCMs), N/L ratio, and body condition score (BCS). When long-term allostatic overload leads to lower fitness, baseline FCMs are expected to increase, stress response FCMs decrease, N/L ratio increase, and BCS decrease; all of which likely occur at different temporal scales (Sapolsky et al., 2000; Davis et al., 2008; Busch and Hayward, 2009). To ensure that we did not erroneously link a GC profile to survival, we also investigated relationships between our measures of GCs and trappability. Given that most field data came from subadults and adults, we grouped and focused on these age classes for analyses. Moreover, because demographic (e.g. sex and reproductive status) and environmental (e.g. seasonality) factors can influence survival and trappability, we included them as covariates in our models.

2. Materials and methods

2.1. Live-trapping

We established four 1-hectare trapping grids, with 100 trap stations 10 m apart, in a 10 × 10 array, located on state-managed grasslands near Charlo, Montana, USA (see Eleftheriou et al., 2021 for site details). Deermice were trapped at four grids from November 2016 to August 2018. Grids A and B were trapped October–November 2016, February–December 2017 (excluding June and July for A, and July for B), and March–July 2018. Grids C and D were trapped over a shorter duration: October–November 2017, and March–August 2018. We typically trapped once a month over three consecutive nights, two grids at a time. Non-folding Sherman traps (H. B. Sherman, Tallahassee, Florida, USA) were baited with peanut butter and oats, and supplied with polyester bedding. Traps were opened at about dusk and checked once at about 4 h after setup when trap-induced stress is less likely to distort baseline FCMs (Harper and Austad, 2001; Eleftheriou et al., 2020b). Deermice found at the four-hour check point were processed and returned to their traps to be processed again at about dawn. This allowed us to quantify the adrenocortical response to an acute stressor (i.e. overnight trap confinement). Deermice were released on site after processing at about dawn. By systematically collecting feces at about dawn and dusk, where FCMs of deermice are typically highest at dusk and decrease gradually overnight, we avoided the effects of diurnal rhythm on FCMs. Hence, we consider overnight rises in FCMs to be attributed to the acute GC response from trap confinement (Bauer et al., 2008; Eleftheriou et al., 2020b). In between trapping sessions, Sherman traps that had captured an animal were emptied of contents (i.e. bedding, feces & bait) and washed in diluted disinfectant to be used as "clean" traps. During each trapping session (i.e. over three nights), "dirty" traps were replaced with "clean" traps.

2.2. Animal processing

At the initial check (about 4 h post setup), traps with no animals were closed, but traps with animals were taken to a central station for processing. We tagged deermice with metal ear tags (National Band and Tag Co., Newport, Kentucky, USA) and collected fecal samples. At the final trap check (at about dawn), we collected blood and fecal samples, and also weighed and sexed deermice. Only fecal pellets that were not contaminated with urine or peanut butter were collected from all soiled areas in each trap to get a representative pooled sample for each individual. Deermice were evaluated for flea absence or presence (through visual examination of the head and body and/or when a flea was observed jumping off), reproductive status (active status in females was determined via the presence of a perforate vagina, pregnancy, and/or lactation, and in males via the presence of scrotal testes), and a body condition score (BCS) via palpation of the lower back and tail base to assess fat and muscle tissue using a scale from one minus to five plus with five plus being extremely obese (Ullman-Culleré and Foltz, 1999). Similar BCS methodology has been successful in other field studies of mammals (e.g. Beldomenico et al., 2008, 2009; Pokharel et al., 2017). Although the same experienced investigator performed all field evaluations in this study, intra-observer repeatability was not formally quantified. Age was estimated from weight (juveniles <14 g, subadults 14–17 g & adults >17 g; Fairbairn, 1977). To prepare blood smears and test for the presence of Sin Nombre hantavirus (SNV), which is hosted by deermice, we collected blood samples with heparinized microcapillary tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) from the retro-orbital capillary sinus after topical anesthesia with one–two drops of proparacaine (Akorn, Inc., Lake Forest, Illinois, USA). Blood and fecal samples were frozen after collection to preserve genetic material and limit FCM artifacts, respectively. After each deermouse was processed, while in the field, we also prepared smears with remaining blood in the microcapillary tube (Eleftheriou and Luis, 2020). Safety guidelines were followed for working with deermice potentially infected with SNV, which can cause serious disease in humans that become exposed (Mills et al., 1995). All animal handling procedures were approved by our Institutional Animal Care and Use Committee (# 027-16ALDECS-051016) and land access was approved by Montana Fish, Wildlife and Parks (permit # 2017-029-W).

2.3. Sample analyses for fecal corticosterone metabolites

Fecal corticosterone metabolites (FCMs) were quantified using previously described methods (Eleftheriou et al., 2020b). Briefly, feces were initially heated in a laboratory oven at about 63 °C for 2 h, ground into powder, and 0.040 (±0.005) g of powder was weighed out for extraction. After extraction of FCMs with 80% methanol, supernatants were decanted, diluted, and assayed with a commercial corticosterone enzyme immunoassay (Arbor Assays, Ann Arbor, Michigan, USA). Intra-assay and inter-assay coefficients of variation were less than 15% and 20%, respectively.

2.4. Sample analyses for N/L ratio

In the field, we initially air-dried blood smears prior to fixing in 100% methanol. Once in the laboratory, we stained the smears with modified Wright stain (Sigma-Aldrich, St. Louis, Missouri, USA), followed by phosphate buffer. Total WBC counts were estimated by counting cells from the feathered edge towards the center of the smear for 20 fields at 400×. The mean count of 20 fields was multiplied by 2000 to get an estimate of cells/μL. At 1000×, we counted lymphocytes, neutrophils, monocytes, eosinophils, and basophils out of 100 WBCs. The N/L ratio was calculated as the ratio of absolute number of neutrophils to lymphocytes (Eleftheriou and Luis, 2020).

2.5. Detection of SNV antibodies

Because SNV is chronic and mice do not clear this infection, SNV antibodies are a reliable marker of infection (Mills et al., 1999). We coated 96-well plates with SNV recombinant nucleocapsid antigen and performed a validated enzyme-linked immunosorbent assay (ELISA) in a biosafety cabinet (Schountz et al., 2007). SNV antibody presence was determined when the optical density (OD) value for each sample exceeded the negative control OD value by 0.2 units.

2.6. Statistical analyses

We estimated survival probability within a robust design capture-mark-recapture framework (Kendall et al., 1995; Pollock, 1982), and restricted our analyses to subadults and adults. To examine the effect of seasonality on both survival and trappability, months were grouped to create a season variable for each parameter. We expected snow cover to have the greatest impact on trappability, as deermice typically remain below the snow cover layer during winter. Thus, for trappability, we created a winter/not winter (i.e. spring/summer/fall) binary variable to examine the effect of seasonality (winter = December–February). For survival, we expected the breeding period to be influential, so we created two levels, spring/summer (March–August) and fall/winter (September–February), for the seasonality covariate. We considered monthly trapping sessions to be the primary occasions, and each trap night within a monthly session a secondary occasion. Mean survival was adjusted for primary occasions that were longer than one month (two or three months). We estimated trappability (p, probability of capture) at both the single secondary occasion (day), and the primary occasion (p^*, i.e., the probability of being captured during at least one secondary occasion within the primary occasion).

We estimated the impact of each physiological measure separately on survival and trappability, such that there were two candidate models: one with the measure as a predictor of survival and another with the measure as a predictor of trappability (Table S1). To remain consistent with previous analyses (Eleftheriou et al., 2021) and/or to promote model convergence, we used the natural log of the raw value for baseline FCMs and neutrophil/lymphocyte (N/L) ratio, and the raw value for body condition score (BCS) and stress response FCMs. Importantly, we used the rolling mean over three primary occasions for all physiological measures, except stress response FCMs for which we used the single available measurement. The rolling mean was used because we wanted to reduce any potential variation from recent, acute stressors (Bonier et al., 2009b). To further promote model convergence, we centered and scaled all values and we accounted for the effects of demographic (sex and reproductive status) and environmental (seasonality as described above) covariates on survival and trappability. Each of these covariates was implemented as a binary categorical variable on both survival and trappability (Tables S1 & S2). Although we had data on parasitized status (flea-infested $N = 85$, SNV-infected $N = 22$), we did not add parasitized status as a covariate because previous research showed no effects of parasitism on stress measures (Eleftheriou et al., 2021), and we wanted to keep the number of covariates small, thereby improving model convergence.

We fit models in a Bayesian framework using the ‘NIMBLE’ package (ver. 0.10.1; de Valpine et al., 2017, 2020) in R (ver. 3.6.1; R Core Team, 2019). We ran three chains of 45,000 iterations in total, discarded the first 5000 iterations as burn-in and thinned by ten. We visually inspected posterior distributions and trace plots using the ‘MCMCvis’ package in R (Youngflesh, 2018) and assessed model convergence using the Brooks–Gelman–Rubin convergence diagnostic (R-hat values < 1.1 indicated convergence; Gelman et al., 2013). We compared the two models for each physiological measure using widely applicable information criterion (WAIC) (Watanabe, 2010; Gelman et al., 2014) and selected the model with the lowest value as the top model (Spiegelhalter et al., 2002). If the difference in WAIC values was less than 2.0, a top model could not be selected. Importantly, we could not compare models across all four physiological measures (to decide which measure is overall the best predictor) because models had unequal sample sizes for each measure (see Results). We generated parameter and model summaries and determined significance of each covariate within the top model based on whether the 95% credible interval (CI) crossed zero.

3. Results

In total, we trapped 327 unique individuals over 18 primary periods from October 2016 to August 2018 across four sites, resulting in 992 individual captures. Capture occasions occurred in all months of the year except January. We restricted our analyses to subadults and adults, resulting in a dataset of 307 unique individuals and 970 captures. Trapped individuals were generally balanced between sexes (131 female, 175 male, 1 unidentified), and 198 individuals were reproductively active at some point during the study. We removed 11 individuals that were captured for the first time at the last primary occasion, given they could not provide information for estimating survival. Thus, the dataset used for analysis contained 296 unique individuals and a total of 944 captures.

Physiological measures were taken once per primary occasion that an individual was captured or recaptured, except for stress response FCMs, which were only evaluated once per individual during the course of the study. This means that the number of measurements was lower than the number of captures for all measures. We collected 448 baseline FCM measurements (mean = 12,311 ng/g, range = 808–108,838 ng/g) and were able to calculate 126 stress response FCM values (i.e., stress-induced FCMs subtracted from baseline FCMs; mean = 26,350 ng/g, range = −19,953–174,704 ng/g). Because we were interested in the magnitude of the acute GC response, we elected to subtract based on previous recommendations (Romero, 2004; Breuner et al., 2013). Neutrophil/lymphocyte (N/L) ratio measurements were assessed for 463 captures (mean = 1.73, range = 0.04–30.3). Body condition score (BCS) was evaluated for 453 captures (mean = 2.74, range = 0.67–4.33). For a graphical summary of the physiological variables, please see the Supplementary Information (Fig. S1).

All models reached convergence (assessed with traceplots and R-hat values) and therefore, generated usable parameter estimates. Across all models, mean survival over the primary occasion (one month) did not vary greatly (Fig. S2). For the models where the physiological measure was a covariate on survival, mean monthly survival ranged from 0.509 to 0.545 (Table S3). For the models where the measure was a covariate on trappability, mean monthly survival ranged from 0.536 to 0.566 (Table S3). In contrast, mean daily trappability was more variable across models (Fig. S2), the greatest being a difference of 0.161 for stress response FCMs (Table S3). Mean trappability per secondary occasion ranged from 0.362 to 0.550 when the physiological measure was a covariate on trappability whereas it ranged from 0.519 to 0.525 when the measure was a covariate on survival (Table S3). Taken together, estimates of mean survival and trappability were similar across model candidates, with the exception of stress response FCMs where the difference between the two trappability estimates was 0.161.

We used WAIC values to decide which model, survival or trappability, was considered the top candidate for each physiological measure. When stress response FCMs was the predictor variable, the trappability model was selected as the top candidate, in which the physiological measure influenced trappability only, not survival (Table 1). Conversely, when baseline FCMs or BCS was the predictor variable, the survival model where the physiological measure influenced survival only and not trappability, had the lowest WAIC value by almost three points, making it the top candidate (Table 1). For N/L ratio, WAIC values did not differ substantially ($\Delta < 2$) and thus, a top model could not be selected (Table 1). Therefore, we were able to select a top model, survival or trappability, for all physiological measures except N/L ratio.

The magnitude and direction of the effect on survival and trappability varied greatly across physiological measures (Fig. 1, Table S4). Stress response FCMs had a significant negative association with trappability (mean = −0.799, 95% CI = −0.997, −0.614). In contrast, BCS had a significant positive association with survival (mean = 0.249, 95% CI = 0.048, 0.447). Baseline FCMs, our other integrated GC measure, had no significant association with survival (mean = 0.011, 95% CI = −0.223, 0.241). N/L ratio did not associate with survival (mean = −0.156, 95% CI = −0.386, 0.074), or trappability (mean = 0.101, 95% CI = −0.153, 0.346). Therefore, the evidence for an effect based on the 95% credible intervals was strongest for only two physiological measures, stress response FCMs and BCS.

Table 1

Widely Applicable Information Criterion (WAIC) results for comparisons between the survival and trappability models, where the physiological predictor either influenced only survival or trappability, respectively, for all measures examined. A Δ in WAIC of <2 was used as the cutoff threshold for selecting the top model (shown in bold). FCMs = fecal corticosterone metabolites.

Physiological predictor	Model	WAIC	Δ WAIC
Baseline FCMs	**Survival**	**6730.029**	–
	Trappability	6732.603	2.574
Stress response FCMs	**Trappability**	**3860.294**	**–**
	Survival	3897.882	37.588
Neutrophil/lymphocyte ratio	Survival	6868.207	–
	Trappability	6868.550	0.3
Body condition score	**Survival**	**7299.694**	**–**
	Trappability	7302.563	2.869

The demographic and environmental covariates (included in all models) had significant impacts on survival and trappability (Fig. S3, Table S5), and the effect sizes were largely similar across models, suggesting that whether the physiological measure was allowed to affect survival versus trappability did not matter greatly in inferring their effects (Fig. S3). Overall, we found evidence that males had lower survival than females, and that deermice had lower trappability in the winter, but higher survival in the fall/winter than spring/summer. Conversely, reproductive status did not impact survival or trappability. In summary, seasonality predicted both survival and trappability whereas sex predicted survival only.

4. Discussion

Although evidence for GC-fitness relationships as predicted by the “Cort-Fitness” hypothesis is currently mixed, further testing of this hypothesis can help facilitate the use of GC measures that best identify wildlife populations at risk of decline, earlier than traditional demographic methods (Madliger and Love, 2014; Dantzer et al., 2014). In this study, we found that stress response fecal corticosterone metabolites (FCMs), an integrated GC measure, did not predict monthly survival of free-ranging deermice, an example of a short-lived rodent. Instead, this measure was a significant predictor of daily trappability, suggesting that deermice with a lower adrenocortical response are more likely to be captured. Conversely, body condition score (BCS), a downstream measure of GCs, positively predicted survival although the evidence was weak. We found no significant relationships for neutrophil to lymphocyte ratio (N/L ratio) and baseline FCMs, despite identifying a top model (survival versus trappability) for the latter. However, we did find that seasonality was a significant predictor of both survival and trappability whereas sex was a significant predictor of survival only.

Taken together, our study emphasizes three key points. Firstly, the strong link between stress response FCMs and trappability highlights that GC-fitness links could become biased if relationships between GCs and capture/detection behavior are not explicitly considered. Secondly, because we found that BCS predicted survival, instead of the integrated markers of blood GCs (i.e., FCMs), our study suggests that downstream measures of GCs, could indeed be useful predictors of fitness. Thirdly, relevant demographic, and environmental factors should be considered during field investigations of GC relationships with fitness or trappability.

4.1. GC physiology and survival

We found that BCS was a positive predictor of monthly survival in deermice, although we do acknowledge that the evidence for this inference is weak. Nevertheless, this finding supports the “Cort-Fitness” hypothesis where deermice with better body condition have higher monthly survival. Similarly, other field studies found a positive relationship between measures of body condition and survival in birds (e.g., Bowers et al., 2014), mammals (e.g., Rakotoniaina et al., 2017) and reptiles (e.g. Romero and Wikelski, 2001). However, this relationship may not be always present (e.g., Ebensperger et al., 2013; Cox and Calsbeek, 2015; Milenkaya et al., 2015).

In contrast to BCS, our other three measures, including the integrated GC markers (i.e. FCMs), were not predictive of survival, either because the trappability model received more support, or because the estimated effect was not significant (CI overlapped zero). Although links between GCs and fitness were first investigated with blood GCs, considered highly labile measures (Breuner et al., 2008; Bonier et al., 2009a), field studies over recent decades have employed integrated measures of GCs, such as FCMs. Although a negative relationship between baseline FCMs and survival has been found in some studies of mammals, such as yellow-bellied marmots (*Marmota flaviventris*) (Wey et al., 2015), and

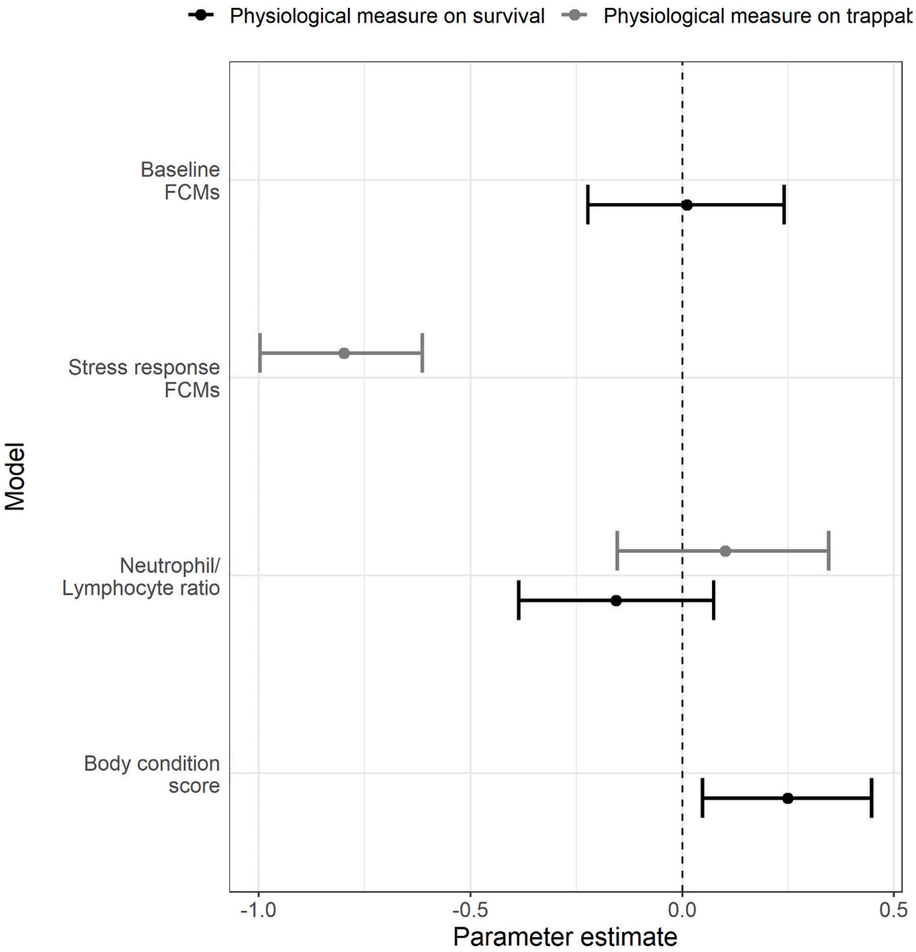

Fig. 1. Parameter estimates with 95% credible intervals for physiological measures examined with their respective survival and trappability models. FCMs = fecal corticosterone metabolites.

ring-tailed lemurs (*Lemur catta*) (Pride, 2005), it was not found in degus (*Octodon degus*) (Ebensperger et al., 2013). Indeed, a recent meta-analysis found no relationship between FCMs and survival in mammals, albeit it is unclear if these were baseline or stress-induced levels (Schoenle et al., 2021). In fact, there is scant evidence about how survival links to stress response FCMs (or even stress-induced FCMs) in mammals, warranting the need for further field research. However, another integrated GC measure that is increasingly being used to examine GC-fitness relationships in the field is hair GCs. For example, Rakotoniaina et al. (2017) found that hair cortisol negatively predicted survival in gray mouse lemurs (*Microcebus murinus*). Although hair GCs, similar to FCMs, are a non-invasive measure, they represent GC levels accumulated over weeks to months (compared with hours to days for FCMs) and are similarly subject to various confounding factors (Heimbürge et al., 2019). Nevertheless, as we learn more about how GCs are metabolized in hair, the field will certainly benefit from these integrated measures.

Taken together, our findings suggest that downstream measures of GCs could potentially serve as the more robust predictors of survival compared to integrated GC measures (i.e., FCMs). However, other integrated GC measures that are increasingly being employed in field studies of mammals (i.e. hair GCs) could serve as an alternative measure for testing the "Cort-Fitness" hypothesis, although physiological and biological validations of immunoassays, as for FCMs, are still necessary (e.g. Mastromonaco et al., 2014).

4.2. GC physiology and trappability

Trappability of wild vertebrates, including rodents, can be driven by various intrinsic and extrinsic factors (e.g., Krebs and Boonstra, 1984; Adler and Lambert, 1997). These factors could confound links between GCs and fitness if they are neglected in field studies. Our results indicate that stress response FCMs predicted trappability of deermice when baited live-traps were used for capture. In particular, we found that deermice with lower adrenocortical responses (i.e. stress response FCMs) were more likely to be trapped. This finding supports previous work that considers individuals with lower stress response FCMs (i.e. lower HPA activity) to follow a behavioral strategy that is more "proactive" (in our study, more likely to enter traps) (Réale et al., 2007; Carere et al., 2010). Indeed, such association has been shown in some mammals (Montiglio et al., 2012; Clary et al., 2014), but not in all (Ferrari et al., 2013; Lapointe et al., 2015; Qu et al., 2018), the latter of which supports an alternative conceptual framework where HPA reactivity is considered to be independent from coping behaviors (Koolhaas et al., 2010).

In summary, our study cautions that links between GCs and trappability must be considered in tandem when analyzing GC relationships with fitness in studies of free-ranging wildlife. If we had not explicitly examined them, we would have erroneously concluded that a significant relationship between stress response FCMs and survival does indeed exist.

4.3. Modulators of fitness and trappability

Given that various factors can modulate fitness or trappability, it is essential they are carefully considered when investigating fitness or trappability relationships with GCs in field studies with wildlife. Because we had many of these types of data available, we were able to incorporate and account for them in our analyses. Indeed, in this study, season was a significant predictor of both survival and trappability whereas sex only predicted survival.

We found that male deermice had lower survival than females, and that during fall/winter, survival was higher than in spring/summer. The latter findings agree with Luis et al. (2010) that used a larger dataset and found that deermice had a higher survival in winter than spring at a grassland in central Montana, USA. This is most likely because the breeding period, typically over spring/summer, brings a multitude of stressors (e.g. lactation, fighting) that can negatively impact deermouse survival. Our findings also agree with Hannebaum et al. (2017), who found male white-footed mice (*Peromyscus leucopus*) had lower survival than females but disagree with Breininger et al. (2018) that found male southeastern beach mice (*Peromyscus polionotus niveiventris*) had a slightly higher survival than females.

In our study, deermouse trappability was lower in winter. This would be expected to occur given harsh environmental conditions (e.g. precipitation, temperature) that discourage deermouse exploration outside of nests and burrows. Although previous studies with free-ranging rodents have found effects of seasonality on trappability, this is not a consistent finding. For example, in agreement with our study, Madikiza et al. (2010) found that trappability for woodland dormice (*Graphiurus murinus*) in South Africa was lower in winter than summer. In contrast, Krebs and Boonstra (1984) found that trappability of four *Microtus* species in North America was lowest in the summer.

In light of our findings, we recommend that relevant demographic, and environmental modulators are carefully considered during field investigations of relationships between GCs and fitness or trappability, and whenever possible, included as covariates in statistical analyses.

4.4. Study limitations

We considered baseline FCMs as reflective of baseline GC levels because we (1) checked traps within 4 h of setup to limit effects from trap-induced stress (Harper and Austad, 2001; Eleftheriou et al., 2020b) and (2) used a rolling mean over three months to avoid effects from acute stressors prior to capture (e.g., acute predation event; Bonier et al., 2009b). Although we cannot control for any residual effects from recent stressors (e.g. predation event), we believe our findings can still be interpreted as baseline values because (1) acute rises in blood GCs from recent stressors would have been smoothened in feces and (2) any contamination from recent stressors would have been diluted from pooling of fecal pellets deposited during confinement.

4.5. Conclusions

An integrated marker of blood GCs, namely stress response FCMs, strongly predicted trappability but not survival. Although we acknowledge weak evidence, BCS was the only physiological measure associated with survival, where the relationship was positive, and thus supports the “Cort-Fitness” hypothesis. These results suggest that integrated markers of GCs (i.e. FCMs) may not be as useful predictors of fitness when compared to downstream measures of GCs (i.e. BCS). Through our analytical approach, we also demonstrate that it is critical for researchers to consider the potential links between trappability and GC physiology, which could inadvertently bias GC-fitness relationships in field studies. Lastly, we show that demographic and environmental factors able to influence fitness or trappability should be considered in field studies that examine links between GC physiology and fitness or trappability of wildlife.

Data availability

Example R code is included in supplementary information.

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CRediT authorship contribution statement

AE and ADL conceived study design. AE collected and SHW analyzed the data. AE led the writing of the manuscript. All authors contributed to manuscript drafts.

Data statement

Data are available per request from the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2022.105183.

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