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RESEARCH ARTICLE

Fear of predators reduces body and physiological condition affecting offspring survival and the 'quality' of the survivors

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Abstract

1. Predators affect the survival of developing young by eating them. More recently, the fear of being eaten has been uncovered as a powerful driver of offspring survival reducing recruited offspring numbers by 53%, but the mechanisms driving these effects are not well understood.
2. We exposed song sparrows to predator or non-predator playbacks throughout three breeding seasons. We followed offspring as eggs through development and into maturity. We quantified the repercussions of being reared in a fearful environment on body (fat, mass, size) and physiological condition using 12 different measures categorized into six systems (oxygen carrying capacity, energy reserves, immune function, antioxidant protection, oxidative stress/damage, glucocorticoids). We further tracked offspring survival in the nest to maturity and determined which measures of condition significantly predicted survival.
3. We report that fear of predators affected offspring condition at all stages of life, with survival consequences. Predator-playback offspring hatched from heavier eggs (8%) but quickly lost this advantage and never regained it. Nestlings reared by 'frightened' parents were as heavy and much leaner than non-predator playback nestlings. This pattern extended throughout development into maturity where offspring reared by frightened parents were up to 31% leaner and 5% lighter. Predator-playback nestlings also were evidently in poorer physiological condition, having shorter telomeres, while late dependency stage fledglings carried relatively less uric acid and more haptoglobin perhaps indicative of chronic susceptibility to infection.
4. Only body condition (mass and fat) predicted survival during peak mortality periods (nestling and early fledging), with the leanest lightest offspring from the predator-playback treatment being least likely to survive. Thereafter, mass predicted survival irrespective of playback treatment. Similarly, at this later stage, offspring with less telomere loss and high uric acid levels survived best regardless of playback group.

5. Our findings reveal that fear of predators is a potent form of early life adversity which persistently alters the condition of offspring affecting early stage survival and the 'quality' of the survivors throughout their life. Development is fuelled by food and fear-induced reductions in parental provisioning at the earliest stages likely instigated these effects, with consequences on the population growth rate.

KEYWORDS
body condition and offspring survival, ecology of fear, fat mass and offspring survival, fear manipulation, offspring development, perceived predation risk, physiological condition and offspring survival, predator–prey interactions

1 | INTRODUCTION

The very earliest stages of life are most precarious for developing young. Adversity can reduce the growth trajectory, body condition, and physiology of young thereby reducing their prospects of surviving to developmental maturity (e.g. Zipple et al., 2019). The earlier development is disturbed, the stronger are the effects (Lindström, 1999). Amongst the survivors, early life adversity can permanently alter the morphological and physiological state of offspring, leaving a signature of early-life hardship with long-term consequences on their reproduction and survival as adults, regardless of the environment those adults later face (Burton & Metcalfe, 2014; Callaghan & Tottenham, 2016; Monaghan & Haussmann, 2015). If early life adversity affects entire populations then the outcome could be a reduction in the population growth rate because the number of offspring that survive sets the ceiling on the number of offspring available to recruit (reviewed in Cox et al., 2014; Dudeck et al., 2018). The long-term legacy of early life adversity imprinted for life on offspring could further interfere with the population growth rate when those survivors recruit (i.e. silver-spoon or cohort effects, e.g. Payo-Payo et al., 2016; Pigeon & Pelletier, 2018). In two classic examples, one in a bird (Reid et al., 2003, red-billed chough) one in a mammal (Albon et al., 1987, red deer), cohorts born in more favourable years (high temperatures and/or rainfall) with presumably more food available, survived longer and produced more of their own offspring, whose offspring also produced more offspring, compared to those born in years when the weather was poor and the food supply presumably low. Such effects introduce a time lag that can negatively affect the population growth rate (Sibly & Hone, 2002).

Relatively recently, the perceived presence of predators (cf. actual predation) has been uncovered as a significant cause of early life adversity for individuals that has population-level impacts on wildlife (reviewed in Zanette & Clinchy, 2020). Manipulations of perceived predation risk in both birds and mammals frequently alter the birth rate (i.e. propagules produced) in addition to the survival rates of those propagules leading to fewer offspring produced per capita (reviewed in Zanette & Clinchy, 2020), now known to be substantial enough to significantly reduce the population growth rate (Allen et al., 2022). Of offspring that survive, Allen et al. (2022) further revealed that a long-term imprint of their early rearing condition remains evident into adulthood such that cohorts reared by 'frightened' parents were expected to reduce the population growth rate even further because those adult offspring sang fewer songs (Allen et al., 2022), an indicator of reduced survival and lifetime reproductive success (Reid et al., 2005).

While predator risk can clearly affect the survival of offspring and leave a lasting legacy on the survivors, the mechanistic drivers of these 'fear' effects are less clear. Development is largely fuelled by food and a lack of food has been proposed as a key mechanism that creates early life adversity reducing both survival and the phenotype of the survivors (reviews in Burton & Metcalfe, 2014; Lindström, 1999). In both birds and mammals, reductions in the body mass of offspring, due to a lack of food, can be predictive of later survival (e.g. Magrath, 1991; reviewed in Ronget et al., 2018) presumably because body mass is often positively correlated with body fat which are the reserves necessary to tap into at times that food becomes relatively scarce (Ronget et al., 2018). The perceived presence of predators can reduce the amount of food that dependent offspring receive leading to changes in body mass and survival. For example, using audio playbacks of predators or a non-predator control, Zanette et al. (2011) reported that song sparrow nestlings reared by frightened parents were provisioned less, lighter in mass, and also less likely to survive. However, no predator risk manipulation in wildlife has examined the body condition and survival of offspring throughout each stage of their development and then determine whether body condition is directly predictive of survival at each stage.

Aside from body condition, early life adversity could also affect the physiological condition of offspring which could additionally affect survival probabilities and/or the long lasting 'quality' of the survivors (Gicquel et al., 2022; Marasco et al., 2022; Slos & Stoks, 2008), but few manipulations have examined how the physiological condition of offspring is altered by perceived predation risk. Of those that have, predation risk effects have been demonstrated on oxidative damage (e.g. Janssens & Stoks, 2013; Slos & Stoks, 2008), telomere lengths (Kärkkäinen et al., 2019; Noguera & Velando, 2019), immunity (Roncalli et al., 2020), corticosterone and androgens (Ibáñez-Álamo et al., 2011), and thermoregulatory stress of unfeathered hatchlings (Zanette et al., 2011). All manipulations

thus far have focussed on single traits or physiological systems but perceived predation risk may be expected to have pleiotropic effects on multiple traits in multiple physiological systems (e.g. Boonstra et al., 1998; Fowler & Williams, 2017; Travers et al., 2010). Moreover, components of the same physiological system do not necessarily covary suggesting that animals may make adjustments independently (e.g. Fowler & Williams, 2017) requiring measurement of multiple components for each system when possible (also see Boonekamp et al., 2018).

We worked within the same manipulation as Allen et al. (2022) in which we reported the population-level consequences of fear. Here, our interest was to examine the extent to which the fear of predators might alter the condition of offspring at all stages of development and into maturity. We focussed on aspects of body condition (e.g. fat and mass) and physiological condition which we assessed using 12 different measures categorized into six different physiological systems including oxygen carrying capacity, energy reserves, immune function, antioxidant protection, oxidative stress/damage and glucocorticoids. We next determined which condition measures (if any) were significantly predictive of survival at each stage. Finally, we examined whether measures significantly predictive of survival were more or less affected by playback treatment. We had previously found that fear of predators led to a 53% reduction in the number of recruited offspring produced largely due to significant reductions in offspring survival in the nest and in the first week out of the nest (Allen et al., 2022). Thereafter, mortality rates were similar between the playback treatments even when offspring were still fully dependent on their parents for food. Consequently, we anticipated that condition measures would negatively affect predator playback offspring survival most at these earliest stages.

2 | MATERIALS AND METHODS

2.1 | Study area and species

We conducted our manipulation on wild, free-living, song sparrows distributed across five (≤220 ha) Gulf Islands, in B.C. (details in Allen et al., 2022). The sparrows are resident and occupy exclusive territories. They build open-cup nests typically in low shrubs and build a new nest upon each attempt. Egg-laying occurs April–July, the typical clutch size is 3–4 eggs, incubation lasts 13 days, mothers alone incubate the eggs, both parents feed the nestlings, nestlings normally fledge 12 days after hatching, all pairs can successfully rear two broods. After fledging, offspring cannot engage in sustained flight for approximately 7 days (Dybala et al., 2013; L. Zanette, unpub. data), and they remain completely dependent on their parents for food provisioning for the first 3 weeks post-fledging (i.e. 0–21 days post-fledge), and most are independent thereafter (Dybala et al., 2013). Research was conducted under Parks Canada (GINP-2008-1504) and Animal Care at Western University (2010-024).

2.2 | Experimental design

Details of the manipulation and radio-tagging are in Allen et al. (2022; also see Zanette et al., 2011). Briefly, we monitored the nests of 51 and 53 adults from the predator and non-predator playback manipulations, respectively, at 11–15 study sites over the three breeding seasons (2010, 2013, 2014). We located nests using behavioural cues from parents. We manipulated perceived predation risk by broadcasting either predator or non-predator sounds over the course of nesting. We eliminated direct killing by mammalian and large bird predators by encircling all nests with portable electric fences and covering them with seine netting. The smaller predatory (Swan et al., 2015) brown-headed cowbird was removed from each site with live traps. Playbacks included 8 species of predator and 8 species of non-predator sounds, with multiple exemplars per species, broadcast at random times. Predators included the common raven (*Corvus corax*), northwestern crow (*Corvus caurinus*), cooper's hawk (*Accipiter cooperii*), brown-headed cowbird (*Molothrus ater*), raccoon (*Procyon lotor*), barred owl (*Strix varia*), western screech-owl (*Otus kennicotti*) and northern saw-whet owl (*Aegolius arcadius*). Nonpredator species, here listed in the order matching the corresponding predator, comprised the canada goose (*Branta canadensis*), mallard duck (*Anas platyrhynchos*), northern flicker (*Colaptes auratus*), rufous hummingbird (*Selasphorus rufus*), harbour seal (*Phoca vitulina*), wood frog (*Rana sylvatica*), common loon (*Gaviaimmer*) and pacific chorus frog (*Pseudacris regilla*). All of the predators are known to kill adult song sparrows or their offspring. Sounds were interspersed with periods of silence including a 4 day on 4 day off cycle at which time speakers were moved to new locations within each site. This combination of techniques has been successful in manipulating fear in free-living wildlife, while avoiding habituation, in not only this long-term experiment (Allen et al., 2022; Zanette et al., 2011) but in others done in different systems on animals as diverse as cougars (Suraci et al., 2019) raccoons (Suraci et al., 2016), and opposums, bobcats, skunks (Suraci et al., 2019). In 2010 and 2013 playbacks were begun before first nests were built, and at hatch in 2014. In all years, playbacks ended when the last nestling fledged.

The survival of fledged offspring was established by tracking 151 randomly selected radio-tagged young from fledging to 49 days post-fledging corresponding to the end of the breeding season (31 August) in 2013 and 2014. At this stage, all offspring are well past independence from parental care and are considered independent juveniles/subadults. All radio-tags weighed <3% of the bird's body mass and were fitted with a leg-loop harness and tracked with hand-held antennas and receivers.

2.3 | Fear effects on offspring condition

2.3.1 | Assessment of body condition

We measured egg mass as an estimation of condition given that heavier eggs would hatch out heavier nestlings who would thus

have an initial mass advantage. A variety of manipulations have repeatedly reported that when food intake is restricted either by the bottom-up food supply, or via predator-induced reductions in food-intake (Zanette et al., 2006, 2009), song sparrows will lay fewer eggs that are relatively heavy. Alleviating this restriction leads to more, relatively light eggs. Consequently, we expected eggs from the predator playback treatment to be relatively heavy. Eggs were weighed to 0.01 g using an electronic balance and initial egg mass estimated following the standard procedure validated for song sparrows (see Zanette et al., 2011).

Six-day-old nestlings were measured for mass, fat, and structural size (tarsus and wing chord). Fat levels consisted of an index summed over the furculum (scored 0 to 7) and abdomen (scored 0 to 3) (following Travers et al., 2010). We expected that our index of total fat was an accurate reflection of nestling condition because it was significantly and positively correlated with nestling mass (Spearman rank correlation: $r=0.35$, $p<0.001$). We estimated hunger levels by hand-feeding nestlings after processing them but before placing them back into the nest. The food consisted of moistened egg biscuit (Living World Egg Biscuit) rolled into a standard sized 1 cm diameter ball, and our index was scaled from 0 (no food accepted) to three (three standardized balls accepted). Mass was measured as per eggs. The structural size of offspring was evaluated by measuring the right tarsus (to 0.01 mm, using callipers) and right wing chord (to 0.5 mm, using a wing ruler).

Fat, mass, tarsus and wing chord lengths were again measured for fledged, radio-tagged offspring at three stages. Once when newly fledged (1 day post-fledge, median), once again in the late dependency stage (14 day post-fledge, median), and once when the offspring were independent (40 days post-fledge, median).

2.3.2 | Assessment of physiological condition

We assessed the physiological condition of 64 radio-tagged nestlings (35 non-predator, 29 predator treatment) with three variables including haematocrit (% pack cell volume), glucose (mmol L) and red blood cells' relative telomere length (T/S ratio). The physiological condition of 33 fledged offspring (17 non-predator, 16 predator treatment) was assessed using 12 physiology variables over six physiological systems including (1) oxygen carrying capacity: haematocrit (% pack cell volume); (2) energy reserves: non-esterified fatty acids (NEFAs, mmol L), glucose (mmol L); (3) immune function: haptoglobin (mg mL), lysis agglutination (natural antibody titres); (4) antioxidant protection: uric acid (mg dL), plasma antioxidant capacity (OXY; total antioxidant titres, mmol HClO L); (5) oxidative stress/damage: reactive oxygen metabolites (dROMs; mg H2O2 dL), telomere length; the rate of telomere shortening; and (6) glucocorticoids: baseline plasma corticosterone (ng mL), free plasma corticosterone (ng mL). The rate of telomere shortening was estimated as telomere length at late dependency (Day 14 post-fledge) minus the nestling stage (Day 6 post-hatch) such that high values in the negative represent the greatest rates of loss.

In all cases, blood was collected from the brachial vein within 3 min of capture and glucose was immediately measured using a glucose meter. All remaining blood was stored on ice for transport. Red blood cells (for telomeres) and plasma were extracted following centrifugation after measurement for haematocrit (packed cell volume/total volume) and were frozen at −20°C within 10 h. Nestlings were sampled in the course of banding (Day 6 post-hatch). Fledglings were sampled in the late dependency stage (Day 14 post-fledge). All procedures and assays followed those published previously (glucocorticoids: Clinchy et al., 2004, 2011; telomeres: Criscuolo et al., 2009; all other assays: Fowler et al., 2018; Fowler & Williams, 2017) with further details in the Supporting Information, Appendix 1. The physiological traits used allowed us to capture multiple physiological systems including those that reportedly vary with perceived predation risk (see Section 1). Within this context, our choice of traits was a compromise between the amount of plasma available and assay availability.

2.4 | Statistical analyses

Our previous results revealed that the fear of predators had effects on the survival of offspring during the nestling and early post-fledging stage, with no discernable fear effects on survival at later stages of life (Allen et al., 2022). We now examine whether fear also affected (i) the body and physiological condition of offspring across stages. Then, we analysed (ii) the body followed by physiology of offspring to determine which combination of variables for each were significantly predictive of survival across stages. Fear might lead to differences in offspring condition variables perhaps reflecting the consequences of being reared in a poor environment (i.e. the 'quality' of offspring) independent of condition effects on survival (i.e. the 'quantity' of offspring). Consequently, we included all indices of body and then physiological condition in models (i) and (ii). Finally, we (iii) examined whether condition traits significantly predictive of survival led to differential effects on survival according to playback treatment.

We analysed average egg mass per nest from 2010 and 2013, excluding 2014 when playbacks began at hatch. Data for the body condition of nestlings included the average fat levels and mass of each brood in 2010, 2013 and 2014. Experimental food intake of nestlings was measured in 2013 and 2014 and averaged per nest. Egg mass, nestling body condition and experimental food intake were analysed with 3-factor linear mixed-model ANOVAs (GLMMs), including playback treatment, nest number (1 or 2), and year, with parent identity as a random effect. Body condition variables of radio-tagged post-fledged young were collected in 2013 and 2014, and analysed with 3-factor GLMMs including playback treatment, year, and stage (newly fledged, late dependency, independence) entered as a repeated measures term. Individual offspring identity and nest identity were the random effects. Because neither nest number nor year interacted with playback treatment, we only present results for our main variable of interest, playback treatment.

We collected physiology data in 2013 on radio-tagged birds. We examined the physiology of nestling and late dependency stage offspring (Day 14 post-fledge) by entering all of our physiology variables into one multivariate model. Because our aim was to find the combination of variables that significantly differentiated offspring from the two playback treatments, we used discriminant function analysis (DFA, Travers et al., 2010) after testing for assumptions. We chose backward elimination as our data reduction technique, removing the least non-significant variable at each step until only significant variables remained. We cross-validated our models using a jack-knife procedure in which the data set was split into a training and testing set. The training set used all observations but one to build a model and that model is then used to predict the group membership of the one observation left out. Nestling physiology included 3 variables (telomere length, haematocrit, glucose). For late dependency stage offspring, we excluded free corticosterone because it was highly correlated with baseline corticosterone ($R^2 = 0.993$) thereby exceeding the minimum acceptable tolerance level of 0.01, leaving us with 11 physiology variables to analyse.

We next tested whether body condition variables were predictive of: (1) nestling survival to fledgling; (2) survival of newly fledged radio-tagged young to 7 days post-fledge, which is the most perilous time for fledged offspring in this species and many others; (3) survival of late dependency stage (14 days) radio-tagged offspring to the end of the study (49 days post-fledge; well past independence when offspring are moving into adulthood as independent juveniles/subadults) using Cox-proportional hazards models. We included the variables fat + mass + fat * mass, removing the interaction term if not significant and re-running the models. We tested the interaction because we reasoned that offspring of a given mass might be more prone to mortality if they also were less fat. Next, we crossed fat and mass with playback treatment to assess whether playback treatment rendered offspring more or less vulnerable to mortality, again removing higher order interactions if not significant. We also conducted principal component analysis on fat and mass and determined whether the PC1 scores for individuals were predictive of survival in Cox-proportional hazards models. At all developmental stages PC1 explained at least 66% of the variation whereby heavy offspring were also fat.

To assess how physiology might impact survival, we combined all physiology variables (3 for nestlings, 11 for late dependency stage fledglings) into two, stage specific, Cox-proportional hazards models and again conducted backward elimination (using the same criteria as above) to find the combination of variables that significantly predicted survival. Backward elimination has the benefit of considering correlations amongst variables at the outset thereby assessing their joint predictive power. For nestlings, we examined survival to the first 7 days out of the nest. Examining survival in the nest was not possible because all nestlings sampled fledged, which was intentional because one objective was to continue to monitor and further sample offspring once fledged. For late dependency stage fledglings (14 days post-fledge), we examined survival into post-independence (49 days post-fledge). All of our Cox-proportional hazards models included parent identity or nest identity as a random effect (whichever explained the most variation). We first ran our models stratifying by playback treatment to gain insight into the combination of variables that significantly predicted survival in general. Statistically significant variables remaining from backward elimination were then examined individually and crossed with playback treatment to determine whether there were any differential effects on survival as a result of fear. Integrating all 11 physiology measures into a principal component analysis provided the PC1 scores for each offspring which we also tested against survival using Cox-proportional hazards.

To gain further insight into the signal of physiology that our measures might be reflecting we conducted Spearman rank correlations of all 11 analysed variables in addition to fat and mass. We also conducted principal component analysis integrating the entirety of our condition measures (body and physiology, 13 variables) to confirm these associations. We present only eigenvectors >0.32 (explaining more than 10% of the variation), but all factor loadings can be viewed in Figure S1. We then tested whether the scores calculated from the principal component analysis were predictive of survival with Cox-proportional hazards.

For body condition, no measure of body size (tarsus length or wing chord) either differed between the playback treatments or was predictive of the survival probability of offspring at any stage (all $p > 0.18$). Consequently, we report only results for fat and mass. SAS Studio was used for our survival models and SPSS v 28 for everything else.

3 | RESULTS

3.1 | Fear effects on offspring condition

Fear effects on condition were evident at all stages in the life of offspring (Table S1). To begin, predator playback females laid fewer eggs (Allen et al., 2022), but those eggs were 8% heavier on average (predator vs. non-predator: 3.01 ± 0.043 g vs. 2.79 ± 0.047 g; GLMM: $F_{1,79} = 11.7, p = 0.001$). Although offspring from the predator playback treatment hatched from heavier eggs, nestlings ended up with 15% less fat (Figure 1; GLMM: $F_{1,76.4} = 7.8$, $p = 0.007$), and were evidently hungrier than non-predator playback nestlings given that they ate more food provided by us (predator vs. non-predator: 2.91 ± 0.075 vs 2.79 ± 0.089; GLMM: $F_{1,68.3} = 4.2$, $p = 0.043$). Nestlings from the predator playback treatment were as heavy as those from the non-predator playback group, despite their initial mass advantage at the egg stage (Figure 1; $F_{1,80.9} = 0.2$, $p = 0.62$).

Of the three physiology measures we took at the nestling stage, only telomere length significantly differentiated between the playback treatments (DFA, $F_{1,63} = 4.9, p = 0.03$; all other $p > 0.46$; Table S2), whereby nestlings had significantly shorter telomere lengths when reared in the predator versus the non-predator playback treatment (means ± SEM: 1.12 ± 0.148 vs. 1.57 ± 0.166, respectively).

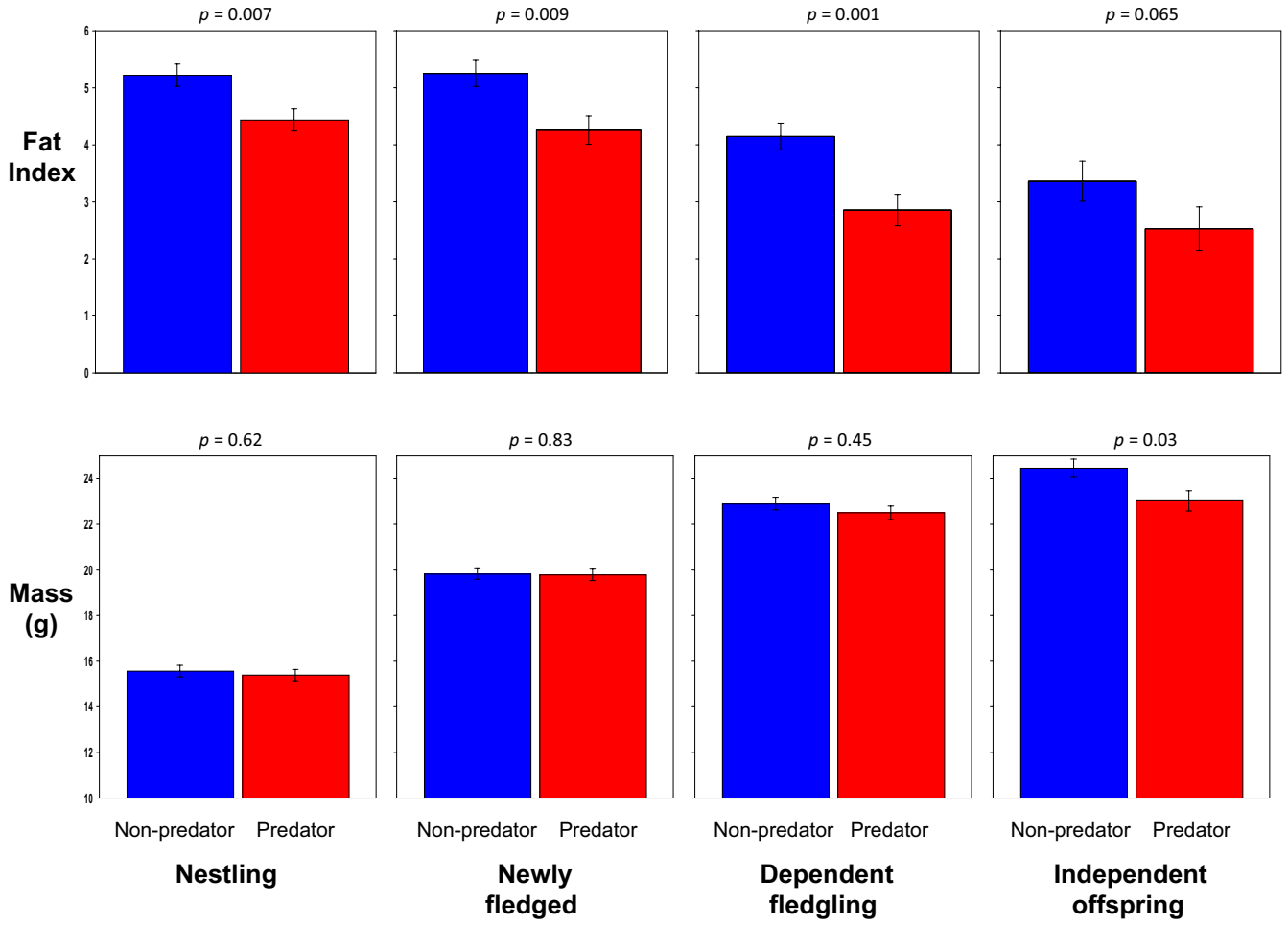

FIGURE 1 The average ± SEM of song sparrow offspring in terms of their fat score and mass from the predator (red bars) and non-predator (blue bars) playback treatment at four stages of development including the nestling stage; newly fledged (1 day post-fledge); late dependency stage (14 days); post-independent (40 days). *p*-values were calculated from our GLMM for nestlings (main effect of playback treatment) and sequential Bonferroni post-hoc tests for post-fledge offspring.

The reduced fat levels of nestlings reared in the predator playback treatment carried through to fledge day (20% less fat), extended well into the late dependency stage (14 days post-fledge; 31% less fat), and on into independence (40 days post-fledge; 25% less fat; Figure 1; repeated measures GLMM, main effect of playback treatment: $F_{1,77.9}=15.4$, $p<0.000$; playback treatment × stage interaction: $F_{2,101.1}=0.2$, $p=0.84$). Post-fledged offspring from the predator playback treatment also tended to be lighter in mass across stages (Figure 1; Repeated Measures GLMM, main effect of playback treatment: $F_{1,120.3}=3.3$, $p=0.072$), though the differences in mass between the playback treatments were only pronounced at independence (Figure 1; playback treatment × stage interaction: $F_{2,61.4}=2.9$, $p=0.065$).

The physiology of late dependency stage fledglings also differed according to playback treatment (DFA, Wilk's $\lambda_2=0.7$, $p=0.006$). Of the 11 physiology measures included in the model, offspring from the predator playback treatment were characterized as having a combination of lower uric acid levels (factor structure coefficient, −0.44; $F_{1,30}=9.1$, $p=0.005$) and more haptoglobin (0.56; $F_{1,30}=7.0$, $p=0.013$) compared to non-predator playback birds. None of our other physiology measures remained in the model (Table S2).

3.2 | Fear effects on offspring condition and survival

Fat and mass were both important predictors of survival at all stages in the life of offspring, and also varied according to playback treatment (Table S1). At the nestling stage, the average fat levels (Spearman rank correlation, $R=-0.3$, $p=0.0006$) and mass of the brood ($R=-0.3$, $p=0.0003$) were each negatively correlated with the proportion of the brood that expired. Individual nestlings within a brood that were both lean and light were less likely to survive to fledge (Figure 2; fat * mass: *Wald* $\chi^2=4.1$, $p=0.034$), but it was the much leaner nestlings from the predator playback treatment that were most susceptible to mortality in contrast to those fatter nestlings that heard non-predator sounds (Figure 2; playback treatment * fat * mass: *Wald* $\chi^2=13.9$, $p=0.0002$). For example, as Figure 2 indicates, for a given mass on the *x*-axis, nestlings from the predator playback treatment that died were less fat

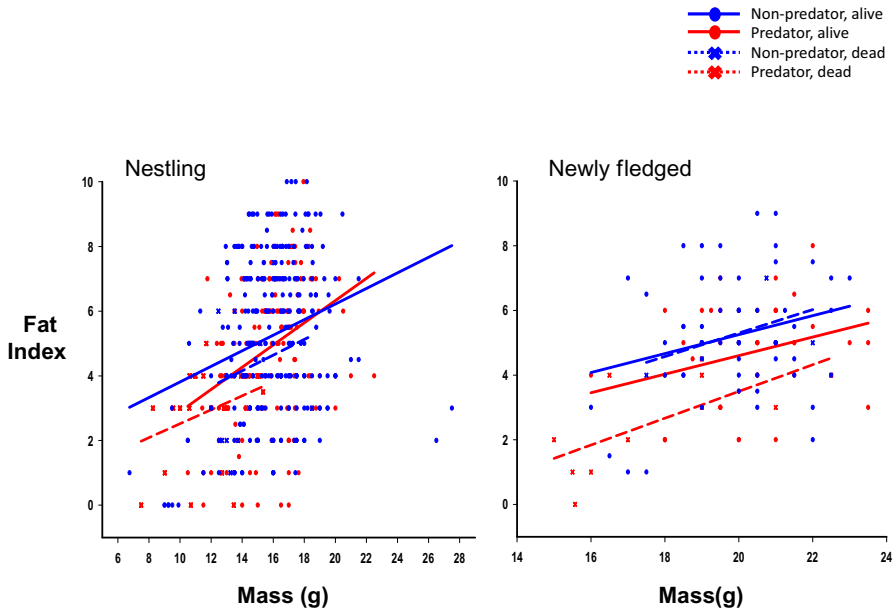

FIGURE 2 Our Cox-proportional hazards models indicated that both the fat and mass of offspring predicted the survival of nestlings and newly fledged young and did so according to playback treatment. We visualized the results by presenting the relationship between fat reserves and mass for song sparrow offspring in the predator (red) and non-predator (blue) playback treatments. Solid lines are correlations for offspring that survived from the nestling stage to fledging (solid dots are individuals); and when newly fledged to the first week out of the nest. Dashed lines are correlations for those offspring that died (represented by *x*'s).

(*y*-axis) than survivors, and also were less fat than any nestling from the non-predator playback treatment. Principal component scores integrating fat and mass also were significantly predictive of nestling survival whereby heavier and fatter nestlings survived best (*Wald* $\chi^2 = 42.8$, $p < 0.0001$). None of our three physiology measures (telomere length, haematocrit, glucose levels) were predictive of survival, nor were the principal component scores based on these three measures (all $p > 0.20$).

Newly fledged young were less likely to survive the critical first 7 days out of the nest if they were both lean (Figure 2; *Wald* $\chi^2 = 4.0$, $p = 0.037$) and light (*Wald* $\chi^2 = 5.7$, $p = 0.013$; mass * fat: Wald $\chi^2 = 3.2$, $p = 0.059$), and again, it was the leanest fledglings from the predator playback treatment that were least likely to make it through those first 7 days (Figure 2; playback treatment * mass * fat: *Wald* $\chi^2 = 4.4$, $p = 0.03$). Principal component scores on fat and mass were predictive of survival (*Wald* $\chi^2 = 13.6$, $p = 0.0002$).

Mass remained a significant predictor of survival for offspring in the late dependency stage (14 days post-fledge) whereby lighter offspring were less likely to survive than heavier ones by the end of the study when all offspring were independent juveniles/subadults (49 days post-fledge: CPH, *Wald* $\chi^2 = 8.1$, $p = 0.003$). Moreover, late dependency stage young that were relatively light were somewhat more prone to mortality if from the predator than the non-predator playback treatment, though the effects were not statistically significant (CPH, survival to independent juvenile stage: *Wald* $\chi^2 = 2.7$, $p = 0.080$). Fat no longer carried an effect on survival at this stage either as a main effect or an interaction with mass (CPH, all $p > 0.66$). Principal component scores integrating fat and mass were significantly predictive of survival at this late stage (*Wald* $\chi^2 = 8.6$, $p = 0.003$).

In the late dependency stage, physiology was also predictive of survival to post-independence (49 days post-fledge) regardless of playback treatment (all *p*-values >0.24; Table S2). Specifically, of our 11 physiology measures included in the model, it was the combination of telomere shortening rate (Figure 3; CPH: *Wald* $\chi^2 = 7.5$, $p = 0.004$) and uric acid level (Figure 3; CPH: *Wald* $\chi^2 = 3.9$, $p = 0.034$)

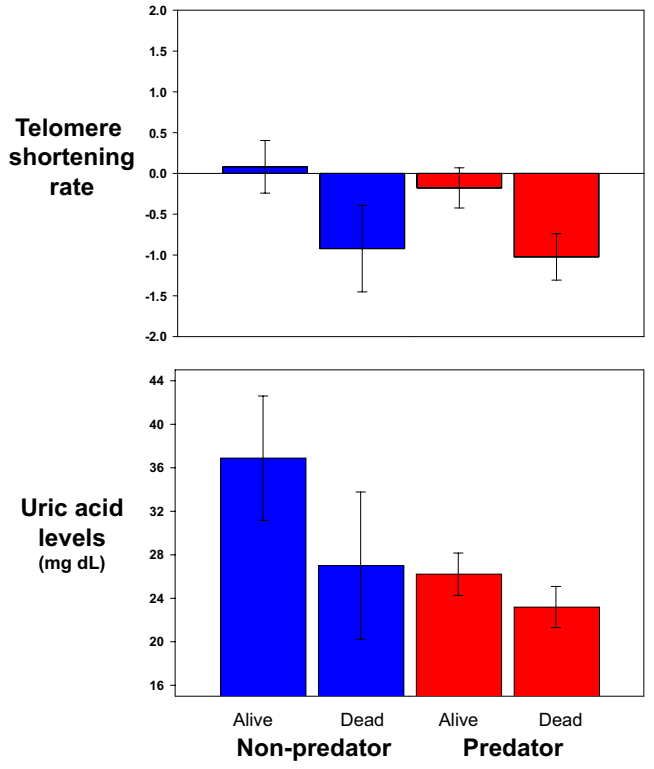

FIGURE 3 Our Cox-proportional hazards model on our 11 physiology measures analysed, identified the combination of telomere shortening rates and uric acid levels as significant predictors of the survival of offspring from the late dependency stage to post-independence. Here, we visualized the results for the predator (red bars) and non-predator (blue bars) playback treatments, by calculating the median values of telomere shortening and uric acid for offspring that survived to independence versus those that died. SEMs were estimated by bootstrapping the data.

that remained after backward elimination (see Table 1 for the order of elimination). Here, the hazard ratios indicated that there was a 5.3 fold (±1.84) and a 1.1 fold increase (±1.03) in the likelihood of

TABLE 1 Spearman rank correlations (*R*) and *p*-values of all 11 physiology variables used in the Cox-proportional hazards model conducted to assess which combination of variables best predicts survival of late dependency stage fledged offspring (14 days) to post-independence (49 days after fledging). Backward elimination sequence 1 indicates that that variable was removed first. Also included are correlations with fat and mass. Cox proportional hazards revealed that telomere shortening rates and uric acid best predicted survival. Correlations ≥0.30 are highlighted with *p*-values ≤0.10 in red and *p*-values ≤0.05 in red and bold.

Backward elimination process	Physiological system	Physiological variable	Telomere shortening rate[a]		Uric acid		Corticos-terone		Lysis
			R	*p*	*R*	*p*	*R*	*p*	*R*
Remains	Oxidative stress/damage	Telomere shortening	*x*	*x*					
Remains	Antioxidant protection	Uric acid	−0.05	0.80	*x*	*x*			
1	Glucocorticoids	Corticosterone	−0.16	0.37	**−0.39**	**0.03**	*x*	*x*	
2	Immune function	Lysis	0.07	0.70	**−0.45**	**0.01**	0.11	0.54	*x*
3	Oxidative stress/damage	dROMS	0.04	0.83	0.02	0.90	0.01	0.94	0.04
4	Immune function	Haptoglobin	−0.18	0.34	**0.41**	**0.02**	−0.20	0.26	−0.21
5	Oxidative stress/damage	Telomere length	0.12	0.51	0.05	0.78	−0.01	0.96	−0.22
6	Antioxidant protection	Antioxidant capacity	0.02	0.89	0.31	0.07	0.12	0.49	0.07
7	Energy reserves	Glucose	−0.23	0.21	0.05	0.79	0.14	0.42	**0.36**
8	Oxygen carrying capacity	Haematocrit	0.18	0.32	−0.17	0.36	0.01	0.96	−0.17
9	Energy reserves	NEFA	**0.41**	**0.02**	0.09	0.62	−0.23	0.19	−0.01
	Body condition	Fat	0.30	0.09	0.01	0.96	−0.24	0.18	0.22
	Body condition	Mass	0.33	0.06	0.10	0.58	**−0.37**	**0.04**	0.24

Note: Sample sizes for physiology = 33; Sample sizes for fat and mass = 40.

[a]Telomere shortening rate: positive correlations = high telomere loss associated with low quantities of the corresponding variable, whereas low telomere loss associated with high quantities; negative correlations = high telomere loss and high quantities of the corresponding variable, whereas low telomere loss associated with low quantities.

mortality with every one unit drop in each of these variables, respectively, for all offspring (interactions with playback treatment, all $p > 0.26$). Principal component scores integrating all 11 physiology measures were not significantly predictive of survival in the late dependency stage (*Wald* $\chi^2 = 1.2$, $p = 0.20$).

That 9 of 11 physiology measures were removed from our survival model does not suggest that those 9 variables were unimportant. Indeed, examining the physiology variables more closely revealed that 5 of 9 excluded variables were correlated with the two that remained (Table 1). That is, telomere shortening rate was most closely correlated with measures associated with energy supply (non-esterified fatty acids), and fat and mass to some extent. Uric acid was most closely correlated with our two measures of immune function (lysis and haptoglobin), in addition to corticosterone, and antioxidant protection (OXY) to some extent. Principal component analysis integrating all body and physiology variables (Figure S1) confirmed that less telomere shortening (eigenvector = 0.35) positively loaded with energy supply (NEFA, 0.47), fat (0.45), and mass (0.53; PC1: $\lambda = 2.6$, explaining 20% of the variation), while uric acid levels (eigenvector = 0.56) negatively loaded with immune function (lysis, −0.47; PC2: $\lambda = 2.1$, explaining 16% of the variation). The scores for PC1 integrating both body and physiological condition were significantly predictive of whether or not late stage dependent offspring would survive to become independent juveniles/subadults (49 days post-fledge; *Wald* $\chi^2 = 4.3$, $p = 0.029$). The scores for PC2 were not predictive of survival (*Wald* $\chi^2 = 0.2$, $p = 0.54$).

4 | DISCUSSION

We previously reported that fear of predators led to a 53% reduction in the number of recruited offspring produced largely due to significant reductions in offspring survival (Allen et al., 2022) that occurred primarily in the nest and in the first week out of the nest with no playback treatment differences in survival thereafter. Here, we demonstrate that fear of predators affected the body and physiological condition variables of offspring at these earliest stages, such that predator playback offspring put on less fat and had shorter telomeres lengths than non-predator playback offspring. We further report that, being especially lean and light, predator playback offspring were the least likely to survive those early stages. The condition of offspring continued to diverge between playback treatments even at later stages of life, as expected if early life adversity sets an irreversible trajectory on condition. Predator playback offspring remained leaner and lighter and had higher haptoglobin and lower uric acid levels. None of these condition variables, however, differentially affected the survival of predator playback birds at later stages. Instead, lighter offspring, and those with both high telomere

	dROMS		Hapto-globin		Telomere length		Anti-oxidant capacity		Glucose		Haemato-crit		NEFA		Fat	
p	*R*	*p*	*R*	*p*			*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
x																
0.83	*x*	*x*														
0.23	0.06	0.73	*x*	*x*												
0.22	0.07	0.70	−0.15	0.41	*x*	*x*										
0.71	0.12	0.50	0.24	0.17	−0.16	0.39	*x*	*x*								
0.04	−0.06	0.72	−0.15	0.40	−0.03	0.89	0.26	0.14	*x*	*x*						
0.35	0.33	0.06	0.01	0.97	−0.03	0.85	0.13	0.49	−0.16	0.36	*x*	*x*				
0.97	−0.07	0.69	0.00	0.98	0.30	0.08	−0.12	0.50	−0.29	0.10	−0.01	0.94	*x*	*x*		
0.21	0.17	0.36	0.09	0.61	−0.03	0.85	0.00	0.99	0.07	0.70	0.14	0.44	0.22	0.21	*x*	*x*
0.18	−0.07	0.68	0.00	0.99	0.15	0.40	−0.10	0.58	−0.08	0.65	−0.33	0.06	**0.61**	**<0.001**	**0.43**	**0.01**

shortening rates and low levels of uric acid were less likely to survive irrespective of treatment.

Predator playback offspring hatched from heavier eggs but quickly lost this body condition advantage and never regained it. Instead, their early poor body condition stayed with them throughout the entire developmental period, into independence, and beyond (Table S1). The importance of body condition is that it was linked to the survival prospects of offspring. Offspring survival was, in turn, a key driver of the population growth rate in a fear manipulation conducted on these same individuals (Allen et al., 2022). Measures of early offspring body condition are often reported as strong predictors of later offspring survival in both birds (Cox et al., 2014; Dybala et al., 2013; Magrath, 1991; Naef-Daenzer & Grüebler, 2016; Ronget et al., 2018), and mammals (e.g. Plard et al., 2015; Ronget et al., 2018), as we too found. Moreover, that early offspring survival drives variation in the population growth rate for many birds and mammals also is known (Bjorkvoll et al., 2016; Cox et al., 2014; Dybala et al., 2013; Gaillard et al., 1989; Gaillard & Yoccoz, 2003; Reid et al., 2011). The insight from our manipulation is that the perceived presence of predators is a key mechanism involved in compromising body condition, thereby reducing survival, and the population growth rate of prey.

Most studies examining the relationship between early body condition and later survival have focussed on mass. Body mass is often positively correlated with fat such that lighter animals have fewer body reserves to rely on when challenged with restrictions in food intake (reviewed in Ronget et al., 2018), as we also found. The effects of reduced fat on survival would be expected to be particularly severe during early growth when energy demands are highest. The results from our manipulation would appear consistent with these ideas. That predator playback offspring were fed less (Zanette et al., 2011) and were evidently hungrier likely led to lower offspring fat levels, and both fat and mass were predictive of survival during the nestling and early fledging stages, the times of highest growth. The effects of fat and mass on survival were particularly harsh on predator playback chicks and newly fledged young, who had a much reduced chance of surviving those early stages of life compared to non-predator playback birds (Allen et al., 2022). The next time we measured fat and mass was when growth was largely complete (late dependency stage). Here, even though predator playback offspring were still leaner and somewhat lighter than were offspring from the non-predator playback treatment, mortality rates were similar between treatments. At this time, it was mass, but not fat, that was a significant predictor of survival for offspring regardless of playback treatment. Taken together, the implications here are that (i) the mechanism driving the often reported relationship between early mass and later survival may be primarily due to fat levels during early development; (ii) because the fat levels of predator playback offspring were lower at all stages measured, but dropped out as a

predictor of survival near independence, suggests that fat levels in later life may continue as a signal of early life adversity.

Physiology may contain another possible signal of early life adversity because predator playback young at the late dependency stage were characterized as having relatively low uric acid and high haptoglobin levels. Uric acid is a by-product of protein metabolism and reduced parental food provisioning (Zanette et al., 2011) would mean less protein to metabolize for predator playback young. Haptoglobin is a first-line of defence in fighting the inflammation associated with infection (Roast et al., 2020) and can be a reliable indicator of infection status in birds (Sebastiano et al., 2017). One other manipulation examining predator effects on immune responses demonstrated that predator exposure increased the susceptibility of adult house sparrows to infection and did so at least 6-weeks post exposure (Navarro, 2004), thereby persistently triggering the immune system long-term. It is possible then that predator playback offspring had been, and perhaps continued to be, more susceptible to infection and so chronically engaged in immune responses beginning at least as early as the nestling stage.

The combination of low uric acid and high haptoglobin levels also may interact in their effects on offspring condition. Immune responses can result in the production of reactive oxygen species which are beneficial in damaging pathogens, but harmful because they can also damage host cells (Armour et al., 2020). Uric acid is a by-product of protein metabolism that also has antioxidant functionality, which can neutralize oxidative damage (Cohen et al., 2007) and having less uric acid, would reduce this capacity. Similarly, if predator playback offspring were more susceptible to infection in early life, then this might have exacerbated the negative effects on their body condition evident as a result of being fed less by their parents (Roncalli et al., 2020). Poor feeding generally results in poor body condition and animals in poor body condition are least able to fend off infection which can then worsen condition thereby increasing the incidence of infection (the "vicious circle", reviewed in Strandin et al., 2018). Infection itself can degrade body condition because immune defences and challenges require the mobilization of energy and nutrients which the body has to tap into, diverting those resources from other processes including growth (Armour et al., 2020; Strandin et al., 2018; but see Hasselquist & Nilsson, 2012). Food manipulation experiments, mostly on young birds (Hasselquist & Nilsson, 2012), indicate that food does affect responses to immune challenges (meta-analysis in Strandin et al., 2018).

Merrill et al. (2017) suggested that haptoglobin levels measured during development is an indicator of early life adversity and an excellent predictor of the quality of individuals once they become breeders themselves as measured by their sexual signals. Merrill et al. (2017) demonstrated that when given an immune challenge, developing male zebra finches in the lab had higher haptoglobin levels which were negatively correlated with song complexity later in life as adults (uric acid levels were not measured). Like most cells in the body, brain nuclei associated with song learning grow rapidly during development in early life and are sensitive to perturbations in development the most common being reductions in parental food provisioning (MacDonald et al., 2006; Nowicki et al., 1998, 2000), which can be predator-induced (Allen et al., 2022; Zanette et al., 2011). Consequently, the songs adults sing frequently reflects their past levels of development stress (Buchanan et al., 2003; MacDougall-Shackleton & Spencer, 2012). Because song is a sexually selected trait, song complexity can be predictive of an individual's longevity and lifetime reproductive success, as is the case in song sparrows (Reid et al., 2005). Indeed, song sparrows in Allen et al. (2022) did sing fewer songs as adults when raised by fearful parents. It is possible that the haptoglobin levels (and perhaps uric acid levels) we found were forecasting the effect that predator-induced early life stress would have on this important sexual signal.

In addition to reflecting the effect of early life developmental stresses, and possibly signifying future quality as an adult, uric acid was also an important predictor of the probability that late dependency stage offspring would survive to the end of the breeding season, but in this case it affected all offspring regardless of playback treatment. Another significant and even stronger predictor here was the rate of telomere shortening (also see Criscuolo et al., 2019; Sheldon et al., 2022). Indeed, the overall physiological profile of late stage offspring (integrating all 11 physiology measures) did not predict survival while telomere shortening rates and uric acid levels together did.

Telomere shortening rates (reviewed in Monaghan & Ozanne, 2018) are often associated with oxidative stress which can occur when reactive oxygen species are produced in excess of the circulating antioxidants available to counteract their effects, potentially leading to DNA damage (Gormally et al., 2019) including the DNA caps formed by telomeres. We did not find, however, that telomere shortening rates were correlated to our measures of oxidative stress/damage. Instead, high telomere shortening rates were associated with low levels of fat and mass (also see Boonekamp et al., 2014) and energetics (non-esterified fatty acid levels, NEFA). NEFA is produced mainly by hydrolysis of triglycerides during body fat reserve mobilization indicating that individuals that did not mobilize much fat lost the most telomere sequences. Interestingly, that our principal component analyses were significantly predictive of survival at all stages but only when body condition was part of the model may indicate that body condition best signals the survival prospects of offspring which might be expected if body condition drives physiology for example. Unravelling the complexity of both the direct and indirect effects of all condition indices on survival warrant further study.

Although telomere shortening rates predicted offspring survival, it did not vary according to the playback treatment, perhaps indicating that telomere shortening is not a good indicator of past rearing stresses and/or predictive of the future quality of the survivors. On the other hand, measuring telomere shortening between two points of time requires that the individual be alive at each of those two points. As mentioned, differential mortality between the playback treatments occurred only in the early

dependency stages, from nestlings to the first few days out of the nest and was similar thereafter. Repeated measures of individuals during the peak mortality phase might have revealed differences in telomere shortening according to playback treatment. We know of only one other experiment on this topic. Kärkkäinen et al. (2019) placed nest boxes for pied flycatchers near nesting eurasian pygmy owls or at control sites. Telomere shortening occurred amongst the flycatchers between 5 and 12 days post-hatch but shortening rate was the same between predator treatments. Thus, telomere shortening rate may not be a function of perceived predation risk, and is perhaps more of a genetic trait than one that reflects early life hardships (e.g. Boonekamp et al., 2014). Alternatively, the manipulation also did not differentially affect parental care as measured by chick growth, such that there would be little a priori reason to expect telomere shortening rates to differ between treatments (effects on survival were not measured).

Kärkkäinen et al. (2019) did find that nestlings reared near owls had longer telomere lengths than did control chicks. We found the opposite; offspring reared with predator playbacks had shorter telomere lengths as nestlings. Few other studies have examined telomere length in developing young but Noguera and Velando (2019) reported shorter telomeres due to predator exposure as did Tablado et al. (2022) for great tits, while Monteforte et al. (2020) found no predator-induced effects in guppies. A relatively short telomere length is expected to reflect, amongst other things, poor biological state and increased disease susceptibility, and potentially decreased survival (McLennan et al., 2016; Watson et al., 2015). Telomere length was not predictive of survival at any stage in our study. Nonetheless, nestlings reared in the predator playback treatment were evidently in poorer body condition and perhaps more susceptible to infection. As mentioned, we do not have the telomere lengths of nestlings that died to compare with the survivors. Of those nestlings destined to fledge, however (i.e. the survivors), predator-induced reductions in telomere lengths may be a reflection of their relatively poor upbringing.

5 | CONCLUSIONS

Manipulating the fear of predators in free-living wildlife is logistically challenging but results to date reveal that fear has effects on the number of offspring produced in addition to the survival of those offspring which have a significant impact on the population growth rate. Reductions in parental care and feeding behaviour appear to be the most likely driver of these responses. Consistent with this, we demonstrate that offspring reared by frightened parents suffer a reduction in body and physiological condition in the very earliest stages which offspring carry with them throughout much, if not all, of their life. Also, predator-induced reductions in body condition, particularly fat levels again in the earliest stages, was the best explanation for the mechanism leading to the differential mortality that occurs at this time between the playback treatment groups. Once the period of predator-induced differential mortality is over, aspects of body and physiological condition continue to predict survival but for all offspring in both treatments. Our findings point to fear of predators as a potent form of early life adversity which has a two-fold effect on offspring in altering both survival and the 'quality' of the survivors. That fear can generate this dual effect might explain why fear of predators can have such large impact on populations.

AUTHOR CONTRIBUTIONS

LYZ and MC conceived the study. All authors contributed to the design of at least one aspect of the methodology. LYZ, MC and MCA collected the data. TDW, MAF, FC and SZ conducted the bulk of the physiological assays. LYZ and MCA analysed the data. LYZ wrote the manuscript with feedback from authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest. Tony Williams is an Associate Editor of Functional Ecology but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

Data are made available on Borealis: https://doi.org/10.5683/SP3/R9CZMW.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Overview of condition results.

Table S2. DFA and CPH backward elimination procedure.

Figure S1. Principal component factor loadings integrating all condition variables.

Appendix S1. Assays for physiology variables.

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