

Too important to tamper with: predation risk affects body mass and escape behaviour but not escape ability

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Summary

1. Escaping from a predator is a matter of life or death, and prey are expected to adaptively alter their physiology under chronic predation risk in ways that may affect escape. Theoretical models assume that escape performance is mass dependent, whereby scared prey strategically maintain an optimal body mass to enhance escape. Experiments testing the mass-dependent predation risk hypothesis have demonstrated that prior experience of predation risk can affect body mass, and the behavioural decisions about evasive actions to take. Other studies on natural changes in body mass indicate that mass can affect escape. No single experiment has tested if all of these components are indeed linked, which is a critical necessary condition underpinning the mass-dependent predation risk hypothesis.

2. We tested all components of the mass-dependent predation risk hypothesis in a repeated measures experiment by presenting predator and non-predator cues to brown-headed cowbirds housed in semi-natural conditions. Exposure to predator cues affected body mass, fat, pectoral muscle thickness and evasive actions (take-off angle and speed), but not the physiological capacity to escape, as measured by flying ability. Examining individual variation revealed that flying ability was unrelated to mass loss in either sex, unrelated to mass gain in males, and only females that gained a very large amount of mass flew poorly.

3. We next conducted a body mass manipulation in the laboratory to rigorously test whether small to large perturbations in mass can ever affect flying ability. We induced either no change in mass (control), a moderate reduction of <10% or a more extreme reduction of >10% which the literature suggests should enhance flight. Flying ability was maintained regardless of treatment. Examining individual variation revealed the same precise patterns as in the first experiment.

4. We conclude that prey may alter their mass and evasive actions in response to predation risk, but their escape ability remains robust and inelastic, presumably because disabling oneself is likely to lead to disastrous consequences. We suggest that animals may only face a mass-dependent predation risk trade-off in a narrow set of circumstances linked to life-history stages that require large amounts of mass *gain*, for example, parturition and migration.

Key-words: ecology of fear, escape performance, fit-for-escape, interrupted foraging, mass-dependent predation risk, perceived predation risk, predator–prey interactions, starvation–predation risk trade-off

Introduction

Escaping from a predator is a matter of ongoing life or immediate death, and such a powerful evolutionary force has left its imprint on animals across all taxa (reviewed in Domenici, Blagburn & Bacon 2011; Cooper & Blumstein

2015). Chronic exposure to the risk of being killed by a predator leaves long-lasting effects on prey that can enhance their ability to escape when actually attacked by a predator (Hawlina *et al.* 2011). Inducible morphological defences are probably some of the best-known alterations affecting escape performance. Here, developing prey form and maintain conspicuous outward morphological traits that enhance locomotion (e.g. tail size and shape) or

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render the prey more difficult to eat (e.g. fortified shells, spines, larger body sizes: Tollrian 1995; Relyea 2001; Benard 2006; Brookes & Rochette 2007; Urban 2007). Although well known, induced outward morphological defences can only arise from predator exposure in the developmental stage and have been thoroughly documented in only a few aquatic taxa (Tollrian & Harvell 1999; Relyea 2005). All other species and all adult animals, nonetheless, are likely not hapless victims to predator attacks but instead are expected to adaptively alter their physiology when exposed to chronic predation risk in ways that may affect escape (van der Veen & Sivars 2000), even if those physiological changes are not overtly obvious (Hawlena *et al.* 2011).

Predation risk is a key factor determining the body mass of animals, and many theoretical models have suggested that changes in body mass are necessarily linked to escape performance (e.g. Lima 1986; McNamara & Houston 1990; Houston, McNamara & Hutchinson 1993; Rogers & Smith 1993; Witter & Cuthill 1993; Bednekoff & Houston 1994; McNamara, Houston & Lima 1994; Cuthill & Houston 1997; Houston, Welton & McNamara 1997; Higginson, McNamara & Houston 2012, 2014). Foraging with predators around is a dangerous endeavour (Matassa & Trussell 2014), and prey may be expected to strategically alter body mass under predation risk to keep them 'fit-for-escape' (Metcalf & Ure 1995; Carrascal & Polo 1999; van der Veen 1999; van der Veen & Sivars 2000). The theoretical models assume that escape probability is mass dependent, whereby mass losses improve escape performance, while mass gains impair it. The assumed escape costs of gaining mass falls under the 'mass-dependent predation risk hypothesis', which was initially developed with small birds in mind (Lima 1986; Witter & Cuthill 1993; Bednekoff & Houston 1994), and for which this assumption appears to be readily plausible. Many avian species use flight to escape from a predator attack and flight requires the generation of lift force to support body mass. Consequently, Newtonian physics dictates that lifting a larger mass will require more work such that lighter birds will have a greater physiological ability to escape (Witter & Cuthill 1993). Moreover, body mass may affect escape at take-off because angles and/or speeds might decrease with increasing body mass (Witter & Cuthill 1993).

Determining whether predator-induced alterations in prey physiological condition do indeed enhance escape requires deconstructing the mass-dependent predation risk hypothesis into three constituent interactions: (i) changes in perceived predation risk must lead to changes in body mass; (ii) individuals must enjoy an escape advantage when they reduce their mass but become disadvantaged when they gain it; and (iii) animals under high perceived predation risk must show better escape performance than those under low risk. If perceived predation risk affects body mass, but predator-induced decreases and increases in body mass fail to affect escape performance, then one could conclude that alterations in an animal's

physiological condition is not a strategy to enhance escape. If perceived predation risk affects escape performance, but escape is not mediated by body mass, then the long-lasting effects of risk on individuals must be operating through a different mechanism(s). For example, prior experience of predation risk may affect the behavioural decisions that prey make about evasive actions (Stankowich & Blumstein 2005; Hawlena *et al.* 2011), which may include anticipating which direction to flee (Bateman & Fleming 2014).

Theory on the mass-dependent predation risk hypothesis has generated an enormous amount of interest and empirical work over the decades, but has provided little clarity because, surprisingly, the majority of research has focussed only on the connection between predation risk and body mass, and has produced a mix of results. Many do find that animals, across many vertebrate taxa (including birds, mammals, reptiles: e.g. Gosler, Greenwood & Perrins 1995; Pérez-Tris, Díaz & Tellería 2004; Tidhar, Bonier & Speakman 2007; MacLeod *et al.* 2007a; Zimmer *et al.* 2011) are lighter when predation risk is high, but a significant number of studies actually find mass gains (Lillien-dahl 1998; Pravosudov & Grubb 1998; MacLeod *et al.* 2007b). As for the other two connections, comparatively little is known. No study has examined whether predator-induced alterations of body mass affects escape performance in birds. Instead, natural changes in body mass over the day have been the focus, and studies typically fail to find a connection with escape performance (Kullberg, Fransson & Jakobsson 1996; Kullberg 1998; van der Veen & Sivars 2000; MacLeod 2006). These daily changes in mass are most often moderate (i.e. typically 5–8% on average, but <10% in general), compared to animals that gain a great deal of mass due to, for example, migration (at least 27%; Kullberg, Fransson & Jakobsson 1996; Lind *et al.* 1999; Kullberg, Jakobsson & Fransson 2000; Burns & Ydenberg 2002), gravid animals (at least 10%; Lee *et al.* 1996; Kullberg, Houston & Metcalfe 2002a; Kullberg, Metcalfe & Houston 2002b) and animals fitted with weights (10%; Witter, Cuthill & Bonser 1994; reviewed in MacLeod 2006). These very heavy animals do show relatively poor escape performance, leading to the suggestion that the effect of relatively small changes in mass on escape performance might exist, but are simply too modest to statistically detect (MacLeod 2006). Alternatively, it is possible that animals differ in how they accomplish daily vs. predator-induced changes in mass which could lead to different outcomes regarding escape performance. For example, daily changes in mass are due to changes in fat storage, and predator-induced changes in mass are assumed to be due to changes in fat as well (e.g. Gentle & Gosler 2001). But animals might possibly alter proteinaceous lean tissue under predation risk to give them more lift (e.g. van den Hout *et al.* 2006; Higginson, McNamara & Houston 2012). Whether the fat to lean ratio involved in predator-induced changes in mass resembles that of natural daily changes is a complete unknown. Finally, for the third and last link in the mass-dependent predation risk

chain, only three studies have examined whether predation risk affects escape ability and/or how escape behaviour is organized. Scaring grasshoppers (*Melanoplus femurrubrum*) altered their behavioural performance at take-off by angling their jump so that they could go faster and further than control grasshoppers (Hawlena *et al.* 2011). Scaring crickets did not affect their running speed, but scared crickets were more likely to engage in sustained flight, which might be expected to enhance escape. Scaring lizards (*Psammmodromus algirus*) did not affect either their ability to flee or how they fled (Pérez-Tris, Díaz & Tellería 2004).

Here, we experimentally elevated perceived predation risk for brown-headed cowbirds (*Malothrus mater*) living in semi-natural conditions and measured: (i) the effects on body mass and body composition (fat and lean mass, pectoral muscle thickness); (ii) the effects that predator-induced changes in body mass had on an animal's escape performance; and (iii) the effects perceived risk had on escape performance, as measured by the behavioural decisions made when threatened (i.e. take-off angle and speed), and the physiological capacity to escape from a predator as gauged by flying ability. We then conducted a body mass manipulation to fully test if mass loss can indeed affect flying ability. Our results indicate that perceived predation risk does have long-lasting effects on prey that may not be overtly obvious but are powerful nonetheless; affecting body mass and composition, and behavioural decisions concerning evasive actions, but the physiological ability to escape a predator was tenaciously preserved. Examining individual variation corroborated that mass loss did not affect flying ability, while also revealing that very large increases in female mass did. We suggest that flying ability may be inelastic to ensure escape when needed, because this ability is too important to survival to vary with perceived predation risk and so is maintained. We discuss the possibility that mass-dependent predation risk trade-offs may be restricted to a narrow set of circumstances linked to life-history stages requiring large amounts of mass *gain*, for example, parturition and migration.

Materials and methods

STUDY SITE AND SPECIES

We captured 80 brown-headed cowbirds in southern Ontario, Canada, as they returned from migration in 2014. Birds were given a unique combination of coloured leg bands for individual identification, and we fitted a single leg band per bird with a radiofrequency identification (RFID) tag (Phidgets Inc., Calgary, AB, Canada) using epoxy for our take-off behaviour trials (see below). We housed 10 males and 10 females in each of four, large outdoor aviaries (3.65 × 18.25 × 9.15 m) in London, ON, Canada. Cowbirds are known to behave and interact naturally in aviaries of this size (West, White & King 2002; White *et al.* 2010), with the added benefit that the birds were free from direct predation. Each aviary was configured in the same way with multiple perches, shelters, watering and feeding stations, grass and trees. Feeders were placed in open areas within the aviaries, free from

nearby protective cover. Aviaries were paired such that each pair was immediately adjacent, with an opaque barrier in between, and pairs were separated by 150 m, isolating them both visually and acoustically. Birds were provided with *ad libitum* access to a high-quality feed (see Travers *et al.* 2010) and could also forage for food that is naturally present in the aviaries. Birds spent 1 month acclimatizing to the aviaries before we manipulated perceived predation risk.

PERCEIVED PREDATION RISK MANIPULATION

We manipulated the ambient level of perceived predation risk in the environment using both acoustic and visual stimuli and tested for effects on various components of physiological condition and escape performance. We exposed birds in each aviary to either a predator or non-predator treatment for 10 days, followed by 5 days of rest which was followed by the opposite 10 days treatment. Each pair of aviaries was exposed to the same treatment at the same time providing us with a repeated measures design, along with temporal and spatial replication.

Acoustic stimuli consisted of audio playback calls of predator or non-predator species, broadcast 24 h per day from two weatherproof speakers mounted to columns inside each aviary. Each treatment had two alternating 24 h playlists, containing randomized compilations of multiple unique audio clips of eight different species of either predators or non-predators calling, at a natural pace, which are known to occur in southern Ontario during the summer. We paired calls from each predator species (e.g. *Accipiter striatus*, *Accipiter cooperii*, *Buteo lineatus*, *Buteo jamaicensis*, *Falco sparverius*, *Megascops asio*, *Aegolius acadicus* and *Strix varia*) with that of a non-predator species (e.g. *Charadrius vociferous*, *Colaptes auratus*, *Turdus migratorius*, *Setophaga coronata*, *Bombycilla cedrorum*, *Gavia immer*, *Lithobates sylvatica* and *Rana pipiens*) such that there were no significant differences in frequency characteristics (peak: $t_{1,7} = -1.22$, $P = 0.26$; maximum: $t_{1,7} = -0.26$, $P = 0.80$; minimum: $t_{1,7} = -1.56$, $P = 0.16$; range: $t_{1,7} = 0.32$, $P = 0.75$), and the volume of calls was standardized to 80 dB at 1 m. Calls were broadcast at the appropriate time of the day (e.g. daytime for diurnal species), and each call was interspersed with a period of silence at a ratio of 1 : 1.5 (call : silence) during the day and 1 : 2.3 during the night, to prevent habituation (following Zanette *et al.* 2011). Our visual stimuli included taxidermic mounts of two different predator (e.g. *A. cooperii* and *B. lineatus*) or non-predator species (e.g. *C. auratus* and *Anas acuta*), matched for size and stance. The cowbirds in each aviary were exposed to the two different mounts at a randomized time each day during the manipulation period, once between 1100 and 1400 h and the other between 1400 and 1700 h. Before each presentation, we concealed the mount under an opaque box attached to a pulley and twine leading to a blind positioned outside the aviary. A researcher located behind the blind would pull the twine to reveal the mount for a 5 min period before re-covering it with the box. The predator mounts evidently posed an immediate threat to the birds because they responded by abandoning foraging, flying up to perch and remaining vigilant (B.T. Walters, pers. obser.). We repositioned speakers and mounts to new locations (speakers every 2 days, mounts every 1 day), and presented stimuli on an 'on' vs. 'off' rotation which is effective at preventing habituation (following Zanette *et al.* 2011). Our stimuli were presented on days 1–4 and 7–8 with off periods interspersed on days 5–6 and 9–10.

Physiological responses

Our physiological assessments consisted of body mass, body composition (total fat mass, total wet lean mass and pectoral muscle thickness) and wing loading. Wing loading is a way to quantify

how changes in body mass might interfere with flight because it takes into account the mass that can be supported by the wing area of the animal. Increases in the body mass : wing area ratio, for example, may be expected to render flight more difficult (Witter & Cuthill 1993).

We caught birds at the end of each treatment between 09.30 and 16.00 h using potter and house traps, and placed them in a small holding cage within 15 min of capture, processed them for physiological data, and immediately returned them to their aviaries. Birds were weighed to 0.5 g with a Pesola spring scale. To estimate wing loading, we took a digital photograph of the right wing (positioned against a scaled board) and calculated wing area in cm² from the images using the software program IMAGEJ (Rogers 2015). Total fat and wet lean mass were quantified using Quantitative Magnetic Resonance (hereafter QMR; Echo MRI-B; Echo-Medical Systems, Houston, TX, USA; details in Guglielmo *et al.* 2011). We calculated average pectoral muscle thickness from two measurements taken at the left pectoral muscle using an Ultrasound apparatus (LOGIC Book XP Vet; GE Healthcare, Milwaukee, WI, USA) with an 8L-RS linear probe at 10 MHz. Feathers were wetted and separated at the feather tract on the breast to expose the skin over the pectoral muscle. A water-soluble, non-toxic gel was applied to the probe, placed on the skin for the measurements, and then cleaned using warm water dabbed onto paper towel (Swanson & Merkord 2013), which immediately returned skin and surrounding feathers to their original condition.

Escape performance

We assessed escape performance using two measures: take-off behaviour and physiological escape ability.

Take-off behaviour. Take-off behaviour was measured in the aviaries on days 5 and 6 of the treatment period in a specially designed apparatus that allowed us to measure the angle and speed of take-off (following MacLeod 2006). We constructed two parallel vertical 1-m² walls attached perpendicularly to a 1-m² wooden base. The walls were placed 45 cm apart to ensure the birds would engage in straight-line horizontal and vertical flight. The front wall was a transparent acrylic sheet, gridded by 2.54 cm squares to measure vertical and horizontal displacement during flight. The back wall was painted white and mounted with an automated feeder system used to entice the birds into the apparatus. When a bird landed on a single perch set next to the feeder a researcher, positioned behind a blind outside the aviary, would pull a string to raise a spring-loaded flag attached to the side of the apparatus, thereby initiating take-off. Take-off events were recorded using digital video recorders (Swann DVR4-3425, 30 frames s⁻¹, Swann Communications U.S.A. Inc., Santa Fe Springs, CA, USA) positioned perpendicular to each flight apparatus. We analysed the first 0.2 s (6 frames) of each take-off event (following Kullberg 1998; MacLeod 2006). Vertical and horizontal displacements (to the nearest 1.27 cm) and associated time (frame count) were measured relative to the 2.54 cm grid using the centre of the bird's head as the reference point (Chin *et al.* 2009). Escaping at steep angles and rapid speeds is essential to avoiding predators and maximizing the chance of survival (Kenward 1978). However, when flying at maximum capacity, animals face a trade-off between these two measures as flying at steeper angles, for example, might reduce acceleration (Witter & Cuthill 1993; Kullberg & Lafrenz 2007). We could assess whether or not animals trade-off angle and speed by calculating the mechanical energy generated to power flight. In Newtonian physics, mechanical energy output is composed of kinetic and potential energy taking into account, in one measure, the height gained during flight and the vertical and horizontal components of flight velocity (Swaddle, Williams & Rayner 1999). We calculated this measure using the equation from Williams & Swaddle (2003), $E = \frac{1}{2}$

$(V_x^2 + V_z^2) + gz$, where V is flight velocity on the vertical (V_x) and horizontal (V_z) planes, g is the acceleration due to gravity and z is height (Williams & Swaddle 2003). As it suggests, a higher output of mechanical energy would indicate stronger flying ability. Animals that do not trade-off angle and speed but instead, for example, increase angle and maintain or increase speeds would necessarily be increasing mechanical energy output. Finally, we could assess whether body mass affected take-off behaviour because when a bird landed on the perch to feed, an RFID reader (RFID Read-Write; Phidgets Inc.) recorded the individual's identity and body mass (0–780 g; Phidgets Inc.). We confirmed that the body mass levels estimated with our automated system on days 5 and 6 of each treatment period showed a strong and significant relationship with the body mass measurements taken at the end of both the non-predator (as described below; linear regression: $R^2 = 0.92$, $F_{1,48} = 530.2$, $P < 0.001$) and the predator manipulations (linear regression: $R^2 = 0.82$, $F_{1,46} = 221.7$, $P < 0.001$).

Escape ability. We assessed an animal's physiological ability to escape from a predator by measuring their mechanical energy output when required to fly directly against the force of gravity at an angle of 180°. Like others (Kullberg, Houston & Metcalfe 2002a; Kullberg, Metcalfe & Houston 2002b), we reasoned that this would represent the ultimate challenge of flight and would reveal a bird's current capacity to escape a predator because any reduction in mechanical energy output would be indicative of poorer flying ability and, consequently, an increase in predation risk. We measured escape ability immediately prior to our physiological assessments at the end of each treatment. We built a vertical flight chamber, integrating the designs of Kullberg, Houston & Metcalfe (2002a) and Chin *et al.* (2009). The chamber consisted of a metal frame (200 cm × 40 cm × 40 cm) enclosed by white wallboard and a transparent acrylic sheet – permitting observation through the front. A perch was inserted into a box topped with fine netting and placed atop the chamber where the birds were collected after each flight. A 2.54-cm grid was superimposed onto the acrylic surface to create a reference to measure vertical displacement during flights. Cowbirds were introduced into the chamber from the bottom, via a tube (30 cm length, 10 cm diameter), emerging at an upward angle of 30° (Chin *et al.* 2009). We flew each bird twice, with a 2-min rest period in between, and used the fastest measure for analyses (following Chin *et al.* 2009). All trials were recorded on a digital video recorder (Swann DVR4-3425, 30 frames s⁻¹) placed perpendicular to the flight chamber. We initiated measurement starting 21 cm upwards from where the birds emerged (i.e. 50 cm from the bottom of the chamber), which is equivalent to the length of approximately one wing beat (Chin *et al.* 2009). Using the centre of the bird's head as a reference point (Chin *et al.* 2009), we counted the number of frames it took for each bird to reach a vertical displacement of 100 cm from the starting point. We ceased measurement 50 cm from the top of the chamber to ensure that birds were not decelerating at the end of flight (Kullberg, Houston & Metcalfe 2002a). We used the same equation for mechanical energy output as we did for take-off behaviour, except here, V_z (velocity on the horizontal plane) equals zero, and z (height) is standardized. Therefore, V_x (velocity on the vertical plane) is the variable of interest as it tells us how fast an animal is capable of going, and we accordingly present escape ability in units of speed (m s⁻¹).

BODY MASS MANIPULATION, PHYSIOLOGICAL RESPONSES, AND ESCAPE ABILITY

Animals in our perceived predation risk experiments altered their mass by <10% (see Results) consistent with other predation risk studies (e.g. Carrascal & Polo 1999; van der Veen & Sivers

2000; Rands & Cuthill 2001; Pérez-Tris, Díaz & Tellería 2004). Consequently, to fully test whether mass losses can ever convey an escape advantage, we conducted a body mass manipulation. We affected changes in mass corresponding to (i) no change (control), (ii) a reduction in mass of <10% which has empirically been shown to have no effect on escape (Kullberg 1998; Kullberg, Jakobsson & Fransson 1998; van der Veen & Sivars 2000; MacLeod 2006), and (iii) a reduction in mass of >10% which is expected to significantly improve flight (MacLeod 2006). To do so, 30 male and 30 female cowbirds were brought into the laboratory and housed individually in cages (46 long × 76 wide × 46 tall cm). We first fed all cowbirds *ad libitum* until their mass had stabilized. We obtained pre-manipulation measures of body mass, total fat mass, total wet lean mass and escape ability in the vertical chamber following the same protocols as in the perceived predation risk manipulation. Then, we continued to provide *ad libitum* food to the control group, but reduced mass for the two other treatment groups by gradually reducing the quantity of food that birds received each day until they reached a target we set for them which included a 5% or 15% reduction ($N = 10$ males, 10 females per treatment). We took the same four measures post-manipulation to compare with pre-manipulation levels. We then fed birds *ad libitum* for 5 days and released them back into the wild.

STATISTICAL ANALYSES

We analysed whether perceived predation risk affected physiology (link i) and escape performance (link iii) with linear mixed models (LMM) that included treatment as a repeated measures term and sex as a fixed factor, with individual identity nested within aviary as a random effect. Because body mass can vary depending on time of day, we used two lines of evidence to confirm that changes in our manipulation were not due to when the birds were weighed. First, animals were weighed at 12.33 pm (± 12 min) on average vs. 12.17 pm on average (± 12 min) at the end of the predator vs. non-predator treatments respectively. Second, we re-ran our LMMs on body mass with time of day at which the animals were weighed as a covariate. The covariate was never significant ($P > 0.23$ in all cases), and never changed the significance level of any of our initial results.

To test link ii, we examined whether aspects of take-off behaviour were related to physiology by re-running the LMMs but this time, including measures of physiological condition that varied with perceived predation risk included as covariates (i.e. body mass, wing loading and pectoral muscle thickness). We also tested whether each covariate was quadratically related to our independent variables, but this never turned out to be the case. We tested whether predator-induced changes in body mass were associated with escape ability in the vertical chamber by following this procedure: (i) for each individual, we calculated the difference in body mass and then the difference in escape ability between the predator minus the non-predator treatments; (ii) we converted each data point to a percentage change for interpretation because only changes above 10% in mass are thought to affect escape. We divided the difference score of each individual by their body mass in the non-predator treatment and multiplied by 100, and then did the same for per cent change in escape ability; (iii) we then used an ANCOVA with percentage change in escape ability as the dependent variable, sex as a fixed factor, and per cent change in mass and per cent change in mass² as covariates. Here, we were interested in whether the covariates were significant or interacted with our independent variable.

For our body mass manipulation, we calculated the difference score for each individual (post-manipulation – pre-manipulation) for body mass, fat and lean mass in addition to escape

ability in the vertical flight chamber. We used these data to compare among the three treatment groups and between the sexes with two-way ANOVAs, followed by Tukey's honestly significant difference *post hoc* tests when appropriate. Sample sizes vary for physiological variables because four birds died during the manipulation due to causes unrelated to food manipulation (final sample sizes 17 in the control, 19 in the <10% group, 20 in the >10% group). Sample sizes vary for flying ability because five individuals did not fly straight up in the vertical flight chamber and were removed for analysis (final sample sizes, 15 in the control, 18 in the <10% group, 18 in the >10% group). We tested whether changes in escape ability varied as a function of changes in body mass between the pre- and post-manipulation periods, in the same way as in the perceived predation risk manipulation. Specifically, for each individual, per cent changes in escape ability and per cent changes in body mass were calculated ($\text{variable}_{\text{post-manipulation}} - \text{variable}_{\text{pre-manipulation}} / \text{variable}_{\text{pre-manipulation}} \times 100$), and were analysed using the same ANCOVA model.

We used parametric tests on data that had homogeneous variances and normal error distributions, and applied Box-Cox transformations when necessary. All statistical analyses were conducted using STATISTICA 6.0 (StatSoft, Tulsa, OK, USA) and SPSS Statistics for Macintosh 22.0 (IBM, Armonk, NY, USA). All figures and statistical tests report means of untransformed data ± 1 SE.

Results

PERCEIVED PREDATION RISK MANIPULATION

Physiological responses

Manipulating perceived predation risk substantially affected the physiological condition of the cowbirds (Fig. 1). Cowbirds carried a significantly greater amount of body mass when in the predator treatment compared to the non-predator control (Fig. 1a; LMM: Treatment, $F_{1,65.89} = 9.8$, $P = 0.003$), increasing mass by 2% on average. Both sexes showed the same pattern of response to perceived predation risk (Treatment × Sex, $F_{1,65.89} = 0.6$, $P = 0.44$), though as expected, males were significantly heavier (50.49 ± 0.44 g) than were females overall (40.84 ± 0.41 g; Sex; $F_{1,71.98} = 258.9$, $P = 0.001$). This mass gain in response to predation risk led to a significant 2% increase in wing loading (0.461 ± 0.006 g cm⁻²) compared to the non-predator control (0.452 ± 0.005 g cm⁻²; LMM: Treatment, $F_{1,63.16} = 11.2$, $P = 0.001$; Sex, $F_{1,69.87} = 0.3$, $P = 0.61$; Treatment × Sex, $F_{1,63.16} = 1.0$, $P = 0.30$), which would be expected to render flight more difficult for the birds during the predator treatment.

The predator-induced mass gains that the cowbirds exhibited were due to increased body fat and not changes in lean tissue. Cowbirds gained a significant amount of fat in the predator compared to the non-predator treatment (Fig. 1b; LMM: Treatment, $F_{1,64.27} = 15.1$, $P = 0.001$), regardless of sex (Treatment × Sex, $F_{1,64.27} = 0.01$, $P = 0.91$; Sex, $F_{1,61.13} = 0.1$, $P = 0.81$). By contrast, total wet lean mass was unaffected by treatment (predator, 33.95 ± 0.25 g vs. non-predator, 33.96 ± 0.22 g; LMM: Treatment, $F_{1,65.42} = 0.003$, $P = 0.96$) in either sex

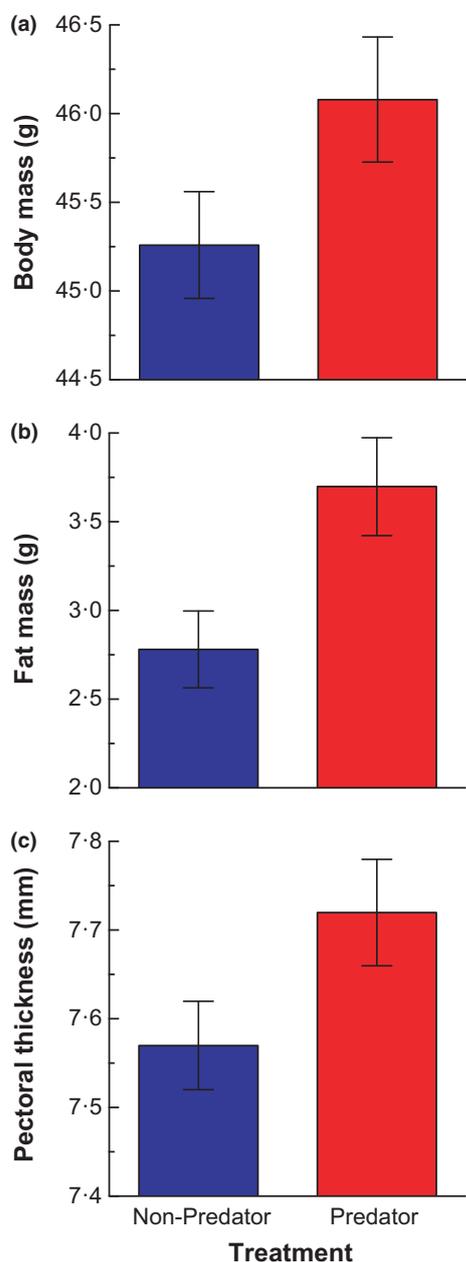


Fig. 1. Physiological responses of cowbirds including (a) body mass, (b) fat mass and (c) pectoral muscle thickness, when exposed to predator (red) vs. non-predator cues (blue). All values are mean \pm SE.

(Treatment \times Sex, $F_{1,65.42} = 1.4$, $P = 0.24$), though males carried a significantly higher total wet lean mass (37.89 ± 0.33 g) than females (30.02 ± 0.32 g; LMM: Sex, $F_{1,72.58} = 294.1$, $P = 0.001$).

Despite the lack of a treatment effect on total wet lean mass, pectoral muscle thickness significantly increased by 1.9% in the predator treatment compared to the control (Fig. 1c; LMM: Treatment, $F_{1,68.64} = 5.6$, $P = 0.02$), with both sexes displaying similar gains (Treatment \times Sex, $F_{1,68.64} = 0.3$, $P = 0.61$). Overall, males had thicker

pectoral muscles (7.88 ± 0.06 mm) than females (7.41 ± 0.06 mm; LMM: Sex, $F_{1,71.89} = 33.4$, $P = 0.001$).

Take-off behaviour

Manipulating perceived predation risk also affected behavioural decisions concerning the evasive actions taken (take-off angle and speed) when confronted with an immediate threat (i.e. a flag being raised; Fig. 2). In the predator treatment, cowbirds took-off at a significantly steeper angle than they did in the non-predator treatment (Fig. 2a; LMM: Treatment, $F_{1,50.68} = 6.5$, $P = 0.01$), regardless of sex (Sex, $F_{1,53.44} = 1.9$, $P = 0.18$; Treatment \times Sex, $F_{1,50.68} = 1.1$, $P = 0.30$). Consistent with a trade-off between take-off angle and take-off speed, both male and female cowbirds took-off at a significantly reduced speed in the predator treatment compared to the non-predator control (Fig. 2b; LMM: Treatment, $F_{1,40.90} = 4.8$, $P = 0.035$; Sex, $F_{1,49.76} = 0.05$, $P = 0.83$; Treatment \times Sex, $F_{1,40.90} = 2.4$, $P = 0.13$), and mechanical energy output was perfectly balanced, being nearly identical between the two treatments (Fig. 2c; predator vs. non-predator treatments: 3.21 ± 0.15 J kg $^{-1}$ vs. 3.35 ± 0.13 J kg $^{-1}$; LMM, Treatment, $F_{1,37.02} = 0.6$, $P = 0.44$; Sex, $F_{1,51.44} = 1.1$, $P = 0.30$; Treatment \times Sex, $F_{1,37.02} = 0.0$, $P = 0.95$). Body mass, wing loading and pectoral muscle thickness were not associated with any aspect of take-off behaviour ($P > 0.10$ in all cases).

Escape ability

While manipulating perceived predation risk did affect body mass, and thus wing loading, the escape ability of cowbirds was not affected. When required to fly straight up, cowbirds flew at nearly identical speeds in both the predator and non-predator treatments (LMM: Treatment, $F_{1,63.04} = 0.03$, $P = 0.87$; Sex, $F_{1,68.88} = 0.4$, $P = 0.53$; Treatment \times Sex, $F_{1,63.04} = 1.1$, $P = 0.30$).

We examined whether escape ability was affected by the degree to which an individual altered their body mass in response to perceived predation risk. We found a negative overall trend in this relationship, with birds flying worse the more mass they gained ($R^2 = 0.12$, $F_{1,59} = 3.2$, $P = 0.08$), but this relationship was significantly different between the sexes (% Mass Change \times Sex, $F_{1,59} = 4.3$, $P = 0.041$). In the vertical flight tests, females that maintained or lost mass in the predator treatment showed almost no change in escape ability, but the more mass females gained, the worse they flew (Fig. 3a; % Mass Change, $R^2 = 0.19$, $F_{1,34} = 7.7$, $P = 0.009$). The escape ability of males was completely unaffected by any change in their mass (Fig. 3b; % Mass Change, $R^2 = 0.002$, $F_{1,25} = 0.0$, $P = 0.84$). No relationship between changes in escape ability and pectoral muscle thickness were found (% PMT Change, $F_{1,58} = 1.4$, $P = 0.23$; Sex, $F_{1,58} = 0.02$, $P = 0.88$; % PMT Change \times Sex, $F_{1,58} = 0.6$, $P = 0.43$).

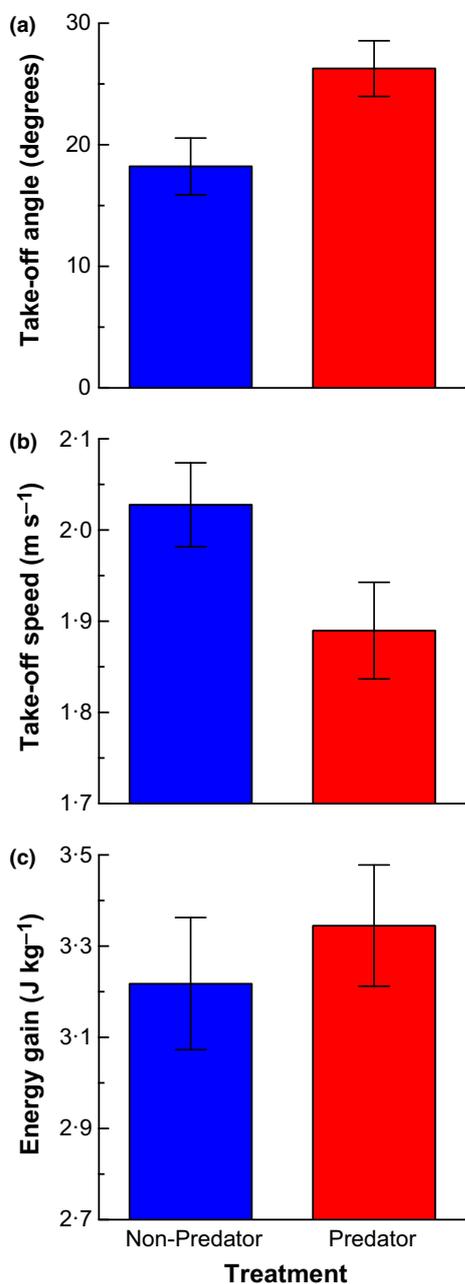


Fig. 2. Comparisons of take-off behaviour including (a) take-off angle and (b) take-off speed, which derives (c) mechanical energy output, when cowbirds were exposed to predator (red) vs. non-predator cues (blue). All values are mean \pm SE.

BODY MASS MANIPULATION

Physiological responses

We achieved our goal of creating three distinct body mass manipulation groups (two-way ANOVA: Treatment, $F_{2,50} = 96.5$, $P < 0.01$; Sex, $F_{1,50} = 0.0$, $P = 1.00$; Treatment \times Sex, $F_{2,50} = 1.4$, $P = 0.25$; all *post hoc* comparisons, $P < 0.001$). Compared to pre-manipulation levels, the control group increased their mass by $2.3 \pm 1.02\%$ (they gained 1.0 ± 0.50 g), the $<10\%$ group lost an

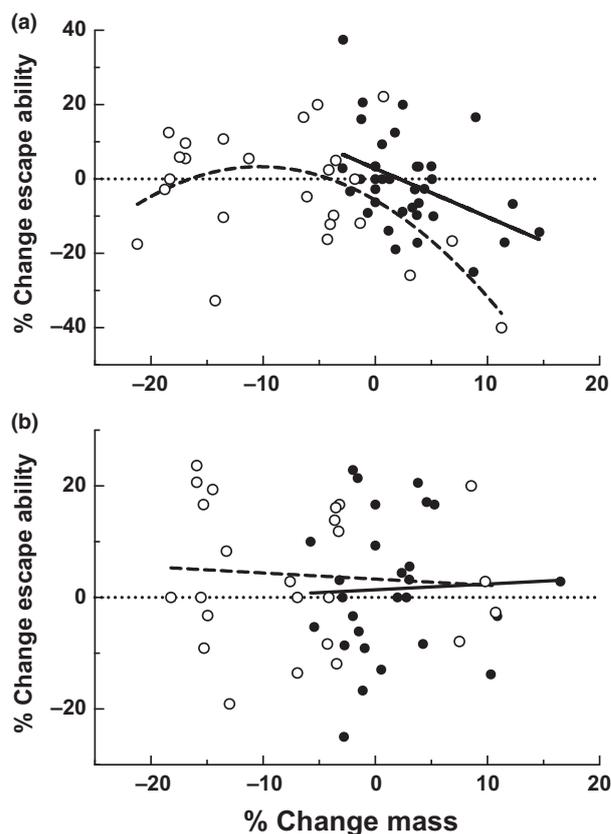


Fig. 3. Relationship between each individual's per cent change in body mass and their per cent change in escape ability for (a) female and (b) male cowbirds in our two manipulations. Filled circles and solid regression lines represent the perceived predation risk manipulation and per cent change was calculated using the equation $(\text{dependent variable}_{\text{predator treatment}} - \text{dependent variable}_{\text{non-predator treatment}}) / \text{dependent variable}_{\text{non-predator treatment}} \times 100$. Open circles and dashed regression lines represent the body mass manipulation calculated as $(\text{dependent variable}_{\text{post-manipulation}} - \text{dependent variable}_{\text{pre-manipulation}}) / \text{dependent variable}_{\text{pre-manipulation}} \times 100$. The horizontal dotted line at zero indicates no change in escape ability between two manipulation periods.

average of $6.4 \pm 0.97\%$ (they lost 2.9 ± 0.50 g) and the $>10\%$ group lost $16.9 \pm 0.94\%$ of their mass (they lost 8.0 ± 0.46 g). These changes in body mass were accounted for by changes in both fat (two-way ANOVA: Treatment, $F_{2,50} = 18.2$, $P < 0.01$; Sex, $F_{1,50} = 0.8$, $P = 0.39$; Treatment \times Sex, $F_{2,50} = 0.8$, $P = 0.42$; all *post hoc* comparisons, $P < 0.01$) and wet lean mass across our three groups (two-way ANOVA: Treatment, $F_{2,50} = 25.2$, $P < 0.01$; Sex, $F_{1,50} = 0.3$, $P = 0.88$; Treatment \times Sex, $F_{2,50} = 0.2$, $P = 0.81$; all *post hoc* comparisons, $P < 0.001$). The control group gained 1.5 ± 0.50 g of fat and lost 0.8 ± 0.27 g of lean, the $<10\%$ group lost 0.6 ± 0.47 g of fat and lost 2.3 ± 0.26 g of lean, while the $>10\%$ group lost 3.6 ± 0.46 g of fat and 3.5 ± 0.25 g of lean.

Escape ability

Escape ability was not significantly affected across our three body mass treatment groups (two-way ANOVA:

Treatment, $F_{2,45} = 0.1$, $P = 0.92$; Sex, $F_{1,45} = 2.1$, $P = 0.15$; Treatment \times Sex, $F_{2,45} = 0.6$, $P = 0.58$). Compared to pre-manipulation levels, differences in escape ability led to changes in flying speed of only 0.03 m s^{-1} in the control (± 0.072), 0.00 m s^{-1} in the $<10\%$ mass loss group (± 0.064), and 0.02 m s^{-1} in the $>10\%$ group (± 0.065).

At the individual level, we found associations between changes in mass and escape ability that were comparable to our perceived predation risk manipulation. Specifically, per cent change in body mass over the manipulation period showed a significant and negative relationship with escape ability ($R^2 = 0.24$, $F_{1,43} = 5.8$, $P = 0.02$), and once again affected females and males differently (% Mass Change \times Sex, $F_{1,43} = 6.4$, $P = 0.02$). Changes in body mass and escape ability exhibited a quadratic relationship for females (Fig. 3a; % Mass Change, $F_{1,22} = 9.1$, $P = 0.006$; % Mass Change², $R^2 = 0.30$, $F_{1,22} = 5.8$, $P = 0.025$) but no relationship at all for males (Fig. 3b; % Mass Change, $R^2 = 0.02$, $F_{1,21} = 0.009$, $P = 0.93$). Figure 3a indicates that, similar to our perceived predation risk manipulation, the benefits of losing mass were negligible for females, but females that increasingly gained mass increasingly flew worse (Fig. 3a). For males, no amount of mass loss or gain led to any appreciable change in escape ability (Fig. 3b), just as we found in our perceived predation risk manipulation.

Discussion

Our results demonstrate that perceived predation risk causes significant changes in the physiological condition of prey and behavioural decisions concerning evasive actions, without affecting an animal's physiological ability to escape. When frightened by predator cues, cowbirds increased their body mass by 2% on average (Fig. 1a), and altered their evasive actions by flying at steeper angles (nearly 10° steeper) and lower speeds (Fig. 2a,b), but though heavier on average, they maintained their escape ability. When faced with the challenge of flying straight up against the full force of gravity, cowbirds flew equally well in the predator and non-predator treatments. Our perceived predation risk experiment coupled with our body mass manipulation allowed us to completely test all three elements of the mass-dependent predation risk hypothesis, and provided complementary and consistent results definitively showing that no amount of mass loss appreciably improves the physiological escape ability for any individual prey (Fig. 3). Large gains in mass (greater than the 2% induced by perceived predation risk) were associated with decreases in escape ability in both experiments, but only for females.

Three key tenets of the mass-dependent predation risk hypothesis are that (i) animals will lose mass under heightened predation risk, (ii) as a strategy to enhance escape because light birds have more lift than heavy birds do, (iii) giving frightened prey a better physiological ability to fly. We found no evidence supporting any of these three links

in the chain. Therefore, in cases where prey lose mass in the face of heightened predation risk, it is unlikely that they strategically do so to gain an escape advantage. Frightening cowbirds did cause them to alter their behavioural decisions at take-off, flying at steeper angles and reduced speeds. This shift in escape was evidently not due to an altered ability to fly but appears to be behaviourally mediated and dependent on the individual's prior experience of predation risk. Many studies suggest that a steeper angle evades an oncoming predator's attack trajectory and allows prey to out-climb a predator which are both beneficial since it reduces the probability of capture (Howland 1974; Andersson & Norberg 1981; Lind, Kaby & Jakobsson 2002; Ilany & Eilam 2008). One possibility, therefore, is that the cowbirds in our study invested more in outmanoeuvring a predator upon attack and less in outrunning it (Lind, Kaby & Jakobsson 2002). In other taxa, snowshoe hares (*Lepus americanus*) altered their behavioural escape decisions by reducing tortuosity while increasing escape speed when confronted with a predator (Hodges, Cunningham & Mills 2014). Grasshoppers exposed to disarmed spiders had greater take-off speed and altered the angle of their body when in flight, which propelled their jump further than grasshoppers under no predation risk (Hawlena *et al.* 2011). Lizards, on the other hand, were equally likely to take a few long strides as many shorter ones regardless of perceived predation risk (Pérez-Tris, Díaz & Tellería 2004). In this case, why one combination of strides would be more advantageous in predator evasion than another is not clear. Therefore, these escape decisions might not vary for lizards if they have no net effect on the probability of being captured.

Our two manipulations revealed that prey do not strategically lose mass to enhance escape. Nonetheless, our perceived predation risk manipulation strongly suggests that mass gains might be a strategy to reduce both predator-induced starvation risk (Lilliendahl 1998; McNamara *et al.* 2005) in addition to the risk of being preyed upon. Cowbirds gained mass in the predator treatment, the average mass in the non-predator treatment being nearly identical to baseline measures taken just before the manipulations began ($45.3 \pm 0.32 \text{ g}$), indicating that mass gain was a strategy adopted in response to the predator treatment. Others also have found that animals will gain mass when perceived predation risk is elevated (Lilliendahl 1998; Pravosudov & Grubb 1998). We further confirmed that predator-induced changes in mass resulted from changes in fat loading which has always been assumed to be the case but never verified. Fat storage has many functions, but from a starvation risk point of view the primary one is to provide energy stores to buffer against possible interruptions in the food supply (Davidson & Evans 1982; Lima 1986; Higginson, McNamara & Houston 2012; MacLeod *et al.* 2014). For example, temperate species often deposit fat reserves in winter which they can draw upon when food becomes suddenly unavailable as is the case during inclement weather, such as snowfalls (McEwan & Whitehead

1984; Rogers 1987, 2015; Cresswell 1998; Rogers & Reed 2003). Laboratory experiments have shown that providing food at unpredictable time intervals leads to gains in mass (e.g. Rogers 1987; Pravosudov & Grubb 1998; Kelly & Weathers 2002) even when the average amount of food obtained remains the same (Cuthill *et al.* 2000). Increases in perceived predation risk is expected to be similar, resulting in variable and unpredictable interruptions of foraging, forcing prey to displace foraging to times or places of lower risk (Lima 1986; Houston & McNamara 1993; McNamara, Houston & Lima 1994; McNamara *et al.* 2005; MacLeod *et al.* 2007b). Our results are consistent with this predator-induced 'interrupted foraging' response (McNamara, Houston & Lima 1994; Lilliendahl 1998; McNamara *et al.* 2005; MacLeod *et al.* 2007b).

According to starvation-predation risk trade-off theory, gaining fat is beneficial as it ensures survival for longer periods without food, mitigating starvation risk (Davidson & Evans 1982; Lima 1986; MacLeod *et al.* 2014), but that increases in fat increase predation risk under the assumption that mass gain impairs escape performance (Lima 1986; Kullberg, Fransson & Jakobsson 1996). We found no evidence that the 2% predator-induced mass gain in our experiment had any appreciable negative effect on either one of our two escape measures. Instead, our evidence would suggest that predator-induced increases in fat may provide a two-fold benefit by minimizing both predator-induced starvation risk (as discussed above) in addition to the risk of being preyed upon. The latter benefit would only be realized if cowbirds can gain mass when out of harm's way, during the least risky times; for example, when predator cues are not actually on. Such fine-scale temporal tactics to eat and avoid being eaten are theoretically outlined by the 'predation risk allocation hypothesis' (Lima & Bednekoff 1999), and empirical tests do find that when under high predation risk, prey respond to temporal variations by being more active during periods of safety (e.g. Sih & McCarthy 2002; Creel *et al.* 2008).

Across studies, perceiving high predation risk has led to significant gains (Lilliendahl 1998; Pravosudov & Grubb 1998) and losses in mass (Gosler, Greenwood & Perrins 1995; Lilliendahl 1997; van der Veen & Sivars 2000). In a study investigating predator-induced mass responses of 30 bird species in the United Kingdom, MacLeod *et al.* (2007b) suggested that mass gains would most often occur in food-rich (quantity or quality) environments, where birds can meet their daily energy requirements even when predators impose foraging constraints. Because the birds in our perceived predation risk manipulation were fed *ad libitum*, food quantity could explain our results. However, predation risk can reduce food intake even when the food supply is unlimited. Zanette *et al.* (2013) reported that when predation risk was experimentally elevated, free-living female song sparrows (*Melospiza melodia*) ate relatively less from supplemental sources and were in poorer physiological condition as a result. These results indicate that an increase in risk may have caused the sparrows to shift

from feeding on the high-quality supplemental food to foraging on natural sources of lower quality food in protective cover, which may have been perceived as a safer option (e.g. Creel *et al.* 2005; Wirsing, Heithaus & Dill 2007). In our experiment, because the feeders were the primary source of food, the cowbirds would have had to primarily forage there even if the locations were perceived as unsafe. Having only the feeders available, the cowbirds might have opted to intensify foraging during the least risky times (e.g. when predator stimuli were not immediately present; Lilliendahl 1998) consistent with the predation risk allocation hypothesis, leading to an increase in mass consistent with the interrupted foraging hypothesis. If during the predator treatment cowbirds had been given the option of foraging on high-quality food but out in the open, vs. lower quality food in protective cover, and they opted for cover, then conceivably they would have lost mass rather than gained it.

Animals also could gain fat under high perceived risk as a result of predator-induced stress. Animal models demonstrate that exposure to chronically stressful situations can deregulate the brain's hypothalamus-pituitary-adrenal axis, causing amplified glucocorticoid secretion that, in turn, promotes increased food intake, fat deposition and a predilection for energy-dense foods (Yau & Potenza 2013). Animals can show elevated levels of glucocorticoids when faced with high predation risk and unpredictable food sources (Pravosudov *et al.* 2001; Clinchy *et al.* 2004, 2011; Sheriff, Krebs & Boonstra 2009; Travers *et al.* 2010).

In addition to strategically increasing mass in response to perceived predation risk, the results from our two manipulations further indicate that the magnitude of mass gain may have been strategically orchestrated to ensure the probability of being killed by a predator was not increased. Scared females in particular may have ensured that any gains in body mass were not so extreme as to tamper with their escape ability. While losses in mass, even up to 20%, had no appreciable effect on escape performance for either sex, the heaviest females did not fly well (Fig. 3a). The fattest female in the dataset gained 14.6% mass in the predator treatment and flew 16.2% worse. This was not the norm, however, because cowbirds gained only 2% mass (2.8% for females, 1.4% for males) in the predator treatment on average. As Figure 3a illustrates, only once female cowbirds gained more than 2.8% mass (i.e. more than that induced by perceived predation risk), did their escape ability actually begin to become impaired.

The magnitude of mass change (increases and decreases) has long been implicated in affecting escape performance. No studies have examined how predator-induced mass changes affect flight in birds and instead have relied on natural, daily changes in body mass which typically fail to affect escape (Kullberg 1998; Kullberg, Jakobsson & Fransson 1998; van der Veen & Sivars 2000; MacLeod 2006; but see Krams 2002). Daily changes in mass typically run under 10% on average and it has been suggested that this change might be too small to statistically detect effects

on escape (MacLeod 2006). Experiments that manipulate predation risk, including ours (e.g. Carrascal & Polo 1999; van der Veen & Sivars 2000; Rands & Cuthill 2001; Pérez-Tris, Díaz & Tellería 2004) show average changes in mass of similar magnitude (<10%) and we too found no effect of perceived predation risk on escape ability. However, because the individual variation in mass changes exhibited by cowbirds in both manipulations varied from losses to gains, we were able to reveal that relatively large changes in mass are required to affect flying ability, but only for females, and only when they gain mass not when they lose it. Working on lizards in captivity, Pérez-Tris, Díaz & Tellería (2004) demonstrated that frightening lizards caused them to lose 1–3% mass on average, but escape ability (i.e. endurance and speed) was unaffected by perceived risk for either sex. The authors also reported that the amount of mass an individual lost did not affect their escape ability, but the authors were unable to assess whether increases in mass (a component of the mass-dependent predation risk hypothesis) affect escape because that variation did not exist and was not induced.

Gravid females typically gain >10% of their body mass and do not fly well (Lee *et al.* 1996; Kullberg, Houston & Metcalfe 2002a; Kullberg, Metcalfe & Houston 2002b). It is conceivable that in our predator treatment, females tempered their mass gain to levels that would account for the added weight that they would have gained if they had formed eggs. Cowbird eggs weigh 3.17 g on average (Ankney & Johnson 1985) translating to a 7.7% increase in female body mass. When added to the mass they gained in the predator treatment would equal 10.5%, which is over the 10% threshold proposed by MacLeod (2006). We never saw cowbirds copulate and do not know how often they would have laid eggs, but this remains a possibility.

Migrants are a group of animals that seasonally gain a great deal of mass, typically beyond what we observed in our manipulations (migrants gain mass upwards of 40%; Blem 1976), and such massive increases in mass does reduce escape performance (Lind *et al.* 1999; Burns & Ydenberg 2002). Being extremely fat, our results indicate that migrants (likely of both sexes given the magnitude of increase) might enjoy an escape benefit by losing mass, and that the trade-offs assumed by the mass-dependent predation risk hypothesis would thus be pertinent to this life-history stage. Some evidence in support of this comes from a study by Ydenberg *et al.* (2004), who found that migrating western sandpipers (*Calidris mauri*) were lighter in areas of recovering predator populations. Migratory birds, however, will always be constrained in how much mass they can lose because enormous energy stores are required to complete a migration route. In these cases, birds would have to make other behavioural adjustments to mitigate risk, such as reducing stopover times in predator heavy areas (Ydenberg *et al.* 2004) and altering their spatial and temporal patterns of migration to avoid predators (Lank *et al.* 2003).

Scared cowbirds significantly increased their pectoral muscle thickness by 1.9% on average, but this neither improved their escape performance nor did it lead to any significant differences in total lean mass between the two perceived predation risk treatments. Our findings complement those of van den Hout *et al.* (2006) showing that ruddy turnstones (*Arenaria interpres*) gained pectoral muscle thickness (by 4.1% on average), but not lean mass, when predation risk was heightened. Birds do show rapid flexibility in pectoral muscle size, even at the expense of other lean tissue (Piersma, Gudmundsson & Lilliendahl 1999) during physiologically demanding periods (i.e. moulting and migration; Piersma, Gudmundsson & Lilliendahl 1999; Lind & Jakobsson 2001) presumably to compensate for the negative effects that wing loading may have on escape capacity at these times. While gaining pectoral muscle may be beneficial in improving escape performance in such cases, the magnitude of change in our study may have simply been too small to have any biological relevance to escape.

Although the starvation–predation risk trade-off literature is vast, the majority of studies typically examine only one of three links in the mass-dependent predation risk hypothesis chain and this is almost always the link between predation risk and body mass (link i). Here, any instances in which animals are relatively light when predation risk is assessed as high has been taken as evidence confirming the theoretical models that because carrying fat carries the cost of impaired escape, animals lose mass under high risk as a strategy to gain an escape advantage (e.g. Carrascal & Polo 1999; Gentle & Gosler 2001). We examined how prey respond to predation risk in terms of physiological and behavioural changes in escape performance, in addition to assessing, in two manipulations, whether mass changes affect escape. Our work has demonstrated that animals may alter their mass according to perceived predation risk but that escape ability remains robust and inelastic in both high and low risk environments. That animals preserve their ability to escape from a predator regardless of the level of perceived predation risk makes adaptive sense because actually disabling oneself would presumably lead to a greater chance of death when a predator is encountered. As such, the theoretical underpinnings of the mass-dependent predation risk hypothesis appear to be problematic and require some reworking. Notwithstanding, our research has shown that prey certainly do put several strategies regarding optimal body mass into play, in addition to using different behavioural evasion tactics, according to the level of predation risk they perceive in the environment in which they live.

Authors' contributions

B.T.W., L.Y.Z. and M.C. conceived the study. B.T.W., L.Y.Z., M.C., T.N.N.C., J.D. and C.G.M. designed the methodological approach. B.T.W., T.N.N. and J.S. collected the data. L.Y.Z. and B.T.W. analyzed the data. L.Y.Z. wrote the manuscript with contributed feedback from

B.T.W., M.C., T.N.N., J.S. and C.G.M. All authors gave final approval for publication.

Acknowledgements

Thanks to Ruthven Park Bird Banding Station and the Long Point Bird Observatory in addition to P. Duenk, C. Rasenberg, J. Barr, S. Taylor, A. Aggarwal, E. Hobbs, E. Mullen, L. Lester and Zoological Collections, UWO for assistance. Funding was provided by the Natural Science and Engineering Research Council of Canada and Western University research grants to L.Y.Z., and Canada Foundation for Innovation grants to L.Y.Z. and C.G.G. This study was approved by the Institutional Animal Care Committee of Western University (protocol #2010-024) and conforms to the ethical and legal requirements of Canada.

Data accessibility

Data are deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.s5935> (Walters *et al.* 2017).

References

- Andersson, M. & Norberg, R.A. (1981) Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. *Biological Journal of the Linnean Society*, **15**, 105–130.
- Ankney, C.D. & Johnson, S.L. (1985) Variation in weight and composition of brown-headed cowbird eggs. *The Condor*, **87**, 296–299.
- Bateman, P.W. & Fleming, P.A. (2014) Switching to Plan B: changes in the escape tactics of two grasshopper species (Acrididae: Orthoptera) in response to repeated predatory approaches. *Behavioral Ecology and Sociobiology*, **68**, 457–465.
- Bednekoff, P.A. & Houston, A.I. (1994) Dynamic models of mass-dependent predation, risk-sensitive foraging, and premigratory fattening in birds. *Ecology*, **75**, 1131–1140.
- Benard, M.F. (2006) Survival trade-offs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). *Ecology*, **87**, 340–346.
- Blem, C.R. (1976) Patterns of lipid storage and utilization in birds. *American Zoologist*, **16**, 671–684.
- Brookes, J.I. & Rochette, R. (2007) Mechanism of a plastic phenotypic response: predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*. *Journal of Evolutionary Biology*, **20**, 1015–1027.
- Burns, J.G. & Ydenberg, R.C. (2002) The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). *Behavioral Ecology and Sociobiology*, **52**, 128–136.
- Carrascal, L.M. & Polo, V. (1999) Coal tits, *Parus ater*, lose weight in response to chases by predators. *Animal Behaviour*, **58**, 281–285.
- Chin, E.H., Love, O.P., Verspoor, J.J., Williams, T.D., Rowley, K. & Burness, G. (2009) Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 499–505.
- Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J.C. & Smith, J.N.M. (2004) Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B-Biological Sciences*, **271**, 2473–2479.
- Clinchy, M., Zanette, L., Charlier, T.D., Newman, A.E.M., Schmidt, K.L., Boonstra, R. & Soma, K.K. (2011) Multiple measures elucidate glucocorticoid responses to environmental variation in predation threat. *Oecologia*, **166**, 607–614.
- Cooper, W.E. & Blumstein, D.T. (2015) *Escape From Predators: An Integrated View of Escape Decisions*. Cambridge University Press, Cambridge, UK.
- Creel, S., Winnie, J., Maxwell, B., Hamlin, K. & Creel, M. (2005) Elk alter habitat selection as an antipredator response to wolves. *Ecology*, **86**, 3387–3397.
- Creel, S., Winnie, J.A., Christianson, D. & Liley, S. (2008) Time and space in general models of antipredator response: tests with wolves and elk. *Animal Behaviour*, **76**, 1139–1146.
- Cresswell, W. (1998) Diurnal and seasonal mass variation in blackbirds *Turdus merula*: consequences for mass-dependent predation risk. *Journal of Animal Ecology*, **67**, 78–90.
- Cuthill, I.C. & Houston, A.I. (1997) Managing time and energy. *Behavioural Ecology*, 4th edn (eds J.R. Krebs & N.B. Davies), pp. 97–120. Blackwell Scientific, Oxford, UK.
- Cuthill, I.C., Maddocks, S.A., Weall, C.V. & Jones, E.K.M. (2000) Body mass regulation in response to changes in feeding predictability and overnight energy expenditure. *Behavioral Ecology*, **11**, 189–195.
- Davidson, N.C. & Evans, P.R. (1982) Mortality of redshanks and oystercatchers from starvation during severe weather. *Bird Study*, **29**, 183–188.
- Domenici, P., Blagburn, J.M. & Bacon, J.P. (2011) Animal escapology II: escape trajectory case studies. *Journal of Experimental Biology*, **214**, 2474–2494.
- Gentle, L.K. & Gosler, A.G. (2001) Fat reserves and perceived predation risk in the great tit, *Parus major*. *Proceedings of the Royal Society B-Biological Sciences*, **268**, 487–491.
- Gosler, A., Greenwood, J.D. & Perrins, C. (1995) Predation risk and the cost of being fat. *Nature*, **377**, 621–623.
- Guglielmo, C.G., McGuire, L.P., Gerson, A.R. & Seewagen, C.L. (2011) Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. *Journal of Ornithology*, **152**, 75–85.
- Hawlena, D., Kress, H., Dufresne, E.R. & Schmitz, O.J. (2011) Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation. *Functional Ecology*, **25**, 279–288.
- Higginson, A.D., McNamara, J.M. & Houston, A.I. (2012) The starvation-predation trade-off predicts trends in body size, muscularity, and adiposity between and within taxa. *The American Naturalist*, **179**, 338–350.
- Higginson, A.D., McNamara, J.M. & Houston, A.I. (2014) The starvation-predation trade-off shapes the strategic use of protein for energy during fasting. *Journal of Theoretical Biology*, **359**, 208–219.
- Hodges, K.E., Cunningham, J.A.F. & Mills, L.S. (2014) Avoiding and escaping predators: movement tortuosity of snowshoe hares in risky habitats. *Ecoscience*, **21**, 97–103.
- Houston, A.I. & McNamara, J.M. (1993) A theoretical investigation of the fat reserves and mortality levels of small birds in winter. *Ornis Scandinavica*, **24**, 205–219.
- Houston, A.I., McNamara, J.M. & Hutchinson, J.M.C. (1993) General results concerning the trade-off between gaining energy and avoiding predation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **341**, 375–397.
- Houston, A.I., Welton, N.J. & McNamara, J.M. (1997) Acquisition and maintenance costs in the long-term regulation of avian fat reserves. *Oikos*, **78**, 331–340.
- van den Hout, P.J., Piersma, T., Dekinga, A., Lubbe, S.K. & Visser, G.H. (2006) Ruddy turnstones *Arenaria interpres* rapidly build pectoral muscle after raptor scares. *Journal of Avian Biology*, **37**, 425–430.
- Howland, H.C. (1974) Optimal strategies for predator avoidance – relative importance of speed and maneuverability. *Journal of Theoretical Biology*, **47**, 333–350.
- Ilan, A. & Eilam, D. (2008) Wait before running for your life: defensive tactics of spiny mice (*Acomys cahirinus*) in evading barn owl (*Tyto alba*) attack. *Behavioral Ecology and Sociobiology*, **62**, 923–933.
- Kelly, J.P. & Weathers, W.W. (2002) Effects of feeding time constraints on body mass regulation and energy expenditure in wintering dunlin (*Calidris alpina*). *Behavioral Ecology*, **13**, 766–775.
- Kenward, R.E. (1978) Hawks and doves – factors affecting success and selection in Goshawk attacks on Woodpigeons. *Journal of Animal Ecology*, **47**, 449–460.
- Krams, I. (2002) Mass-dependent take-off ability in wintering great tits (*Parus major*): comparison of top-ranked adult males and subordinate juvenile females. *Behavioral Ecology and Sociobiology*, **51**, 345–349.
- Kullberg, C. (1998) Does diurnal variation in body mass affect take-off ability in wintering willow tits? *Animal Behaviour*, **56**, 227–233.
- Kullberg, C., Fransson, T. & Jakobsson, S. (1996) Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proceedings of the Royal Society B-Biological Sciences*, **263**, 1671–1675.
- Kullberg, C., Houston, D.C. & Metcalfe, N.B. (2002a) Impaired flight ability – a cost of reproduction in female blue tits. *Behavioral Ecology*, **13**, 575–579.
- Kullberg, C., Jakobsson, S. & Fransson, T. (1998) Predator-induced take-off strategy in great tits (*Parus major*). *Proceedings of the Royal Society B-Biological Sciences*, **265**, 1659–1664.
- Kullberg, C., Jakobsson, S. & Fransson, T. (2000) High migratory fuel loads impair predator evasion in Sedge Warblers. *The Auk*, **117**, 1034–1038.

- Kullberg, C. & Lafrenz, M. (2007) Escape take-off strategies in birds: the significance of protective cover. *Behavioral Ecology and Sociobiology*, **61**, 1555–1560.
- Kullberg, C., Metcalfe, N.B. & Houston, D.C. (2002b) Impaired flight ability during incubation in the pied flycatcher. *Journal of Avian Biology*, **33**, 179–183.
- Lank, D.B., Butler, R.W., Ireland, J. & Ydenberg, R.C. (2003) Effects of predation danger on migration strategies of sandpipers. *Oikos*, **2**, 303–319.
- Lee, S.J., Witter, M.S., Cuthill, I.C. & Goldsmith, A.R. (1996) Reduction in escape performance as a cost of reproduction in gravid starlings, *Sturnus vulgaris*. *Proceedings of the Royal Society B-Biological Sciences*, **263**, 619–623.
- Lilliendahl, K. (1997) The effect of predator presence on body mass in captive greenfinches. *Animal Behaviour*, **53**, 75–81.
- Lilliendahl, K. (1998) Yellowhammers get fatter in the presence of a predator. *Animal Behaviour*, **55**, 1335–1340.
- Lima, S.L. (1986) Predation risk and unpredictable feeding conditions: determinants of body mass in birds. *Ecology*, **67**, 377–385.
- Lima, S.L. & Bednekoff, P.A. (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist*, **153**, 649–659.
- Lind, J. & Jakobsson, S. (2001) Body building and concurrent mass loss: flight adaptations in tree sparrows. *Proceedings of the Royal Society B-Biological Sciences*, **268**, 1915–1919.
- Lind, J., Kaby, U. & Jakobsson, S. (2002) Split-second escape decisions in blue tits (*Parus caeruleus*). *Naturwissenschaften*, **89**, 420–423.
- Lind, J., Fransson, T., Jakobsson, S. & Kullberg, C. (1999) Reduced take-off ability in robins (*Erithacus rubecula*) due to migratory fuel load. *Behavioral Ecology and Sociobiology*, **46**, 65–70.
- MacLeod, R. (2006) Why does diurnal mass change not appear to affect the flight performance of alarmed birds? *Animal Behaviour*, **71**, 523–530.
- MacLeod, R., MacLeod, C.D., Learmonth, J.A., Jepson, P.D., Reid, R.J., Deaville, R. & Pierce, G.J. (2007a) Mass-dependent predation risk and lethal dolphin porpoise interactions. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 2587–2593.
- MacLeod, R., Lind, J., Clark, J. & Cresswell, W. (2007b) Mass regulation in response to predation risk can indicate population declines. *Ecology Letters*, **10**, 945–955.
- MacLeod, C.D., MacLeod, R., Learmonth, J.A., Cresswell, W. & Pierce, G.J. (2014) Predicting population-level risk effects of predation from the responses of individuals. *Ecology*, **95**, 2006–2015.
- Matassa, C.M. & Trussell, G.C. (2014) Prey state shapes the effects of temporal variation in predation risk. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141952.
- McEwan, E.H. & Whitehead, P.M. (1984) Seasonal changes in body weight and composition of dunlin (*Calidris alpina*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **62**, 154–156.
- McNamara, J.M. & Houston, A.I. (1990) The value of fat reserves and the tradeoff between starvation and predation. *Acta Biotheoretica*, **38**, 37–61.
- McNamara, J., Houston, A. & Lima, S. (1994) Foraging routines of small birds in winter – A theoretical investigation. *Journal of Avian Biology*, **25**, 287–302.
- McNamara, J.M., Barta, Z., Houston, A.I. & Race, P. (2005) A theoretical investigation of the effect of predators on foraging behaviour and energy reserves. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 929–934.
- Metcalfe, N.B. & Ure, S.E. (1995) Diurnal variation in flight performance and hence potential predation risk in small birds. *Proceedings of the Royal Society B-Biological Sciences*, **261**, 395–400.
- Pérez-Tris, J., Díaz, J.A. & Tellería, J.L. (2004) Loss of body mass under predation risk: cost of antipredatory behaviour or adaptive fit-for-escape? *Animal Behaviour*, **67**, 511–521.
- Piersma, T., Gudmundsson, G.A. & Lilliendahl, K. (1999) Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiological and Biochemical Zoology*, **72**, 405–415.
- Pravosudov, V.V. & Grubb, T.C. (1998) Management of fat reserves in tufted titmice *Baeolophus bicolor* in relation to risk of predation. *Animal Behaviour*, **56**, 49–54.
- Pravosudov, V.V., Kitaysky, A.S., Wingfield, J.C. & Clayton, N.S. (2001) Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). *General and Comparative Endocrinology*, **123**, 324–331.
- Rands, S.A. & Cuthill, I.C. (2001) Separating the effects of predation risk and interrupted foraging upon mass changes in the blue tit *Parus caeruleus*. *Proceedings of the Royal Society B-Biological Sciences*, **268**, 1783–1790.
- Relyea, R.A. (2001) Morphological and behavioural plasticity of larval anurans in response to different predators. *Ecology*, **82**, 523–540.
- Relyea, R.A. (2005) Constraints on inducible defenses. *Ecology of Predator-Prey Interactions* (eds P. Barbosa & I. Castellanos), pp. 189–207. Oxford University Press, New York, NY, USA.
- Rogers, C.M. (1987) Predation risk and fasting capacity: do wintering birds maintain optimal body mass? *Ecology*, **68**, 1051–1061.
- Rogers, C.M. (2015) Testing optimal body mass theory: evidence for cost of fat in wintering birds. *Ecosphere*, **6**, 55.
- Rogers, C.M. & Reed, A.K. (2003) Does avian winter fat storage integrate temperature and resource conditions? A long-term study. *Journal of Avian Biology*, **34**, 112–118.
- Rogers, C.M. & Smith, J.N.M. (1993) Life-history theory in the nonbreeding period: trade-offs in avian fat reserves? *Ecology*, **74**, 419–426.
- Sheriff, M.J., Krebs, C.J. & Boonstra, R. (2009) The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology*, **78**, 1249–1258.
- Sih, A. & McCarthy, T.M. (2002) Prey responses to pulses of risk and safety: testing the risk allocation hypothesis. *Animal Behaviour*, **63**, 437–443.
- Stankowich, T. & Blumstein, D.T. (2005) Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 2627–2634.
- Swaddle, J.P., Williams, E.V. & Rayner, J.M.V. (1999) The effect of simulated flight feather moult on escape take-off performance in starlings. *Journal of Avian Biology*, **30**, 351–358.
- Swanson, D.L. & Merkord, C. (2013) Seasonal phenotypic flexibility of flight muscle size in small birds: a comparison of ultrasonography and tissue mass measurements. *Journal of Ornithology*, **154**, 119–127.
- Tidhar, W.L., Bonier, F. & Speakman, J.R. (2007) Sex- and concentration-dependent effects of predator feces on seasonal regulation of body mass in the bank vole *Clethrionomys glareolus*. *Hormones and Behavior*, **52**, 436–444.
- Tollrian, R. (1995) Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology*, **76**, 1691–1705.
- Tollrian, R. & Harvell, C.D. (eds) (1999) *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton, NJ, USA.
- Travers, M., Clinchy, M., Zanette, L., Boonstra, R. & Williams, T.D. (2010) Indirect predator effects on clutch size and the cost of egg production. *Ecology Letters*, **13**, 980–988.
- Urban, M.C. (2007) Risky prey behavior evolves in risky habitats. *Proceedings of the National Academy of Sciences*, **104**, 14377–14382.
- van der Veen, I.T. (1999) Effects of predation risk on diurnal mass dynamics and foraging routines of yellowhammers (*Emberiza citrinella*). *Behavioral Ecology*, **10**, 545–551.
- van der Veen, I.T. & Sivars, L.E. (2000) Causes and consequences of mass loss upon predator encounter: feeding interruption, stress or fit-for-flight? *Functional Ecology*, **14**, 638–644.
- Walters, B.T., Cheng, T.N.N., Doyle, J., Guglielmo, C.G., Clinchy, M. & Zanette, L.Y. (2017) Data from: Too important to tamper with: predation risk affects body mass and escape behaviour but not escape ability. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.s5935>.
- West, M.J., White, D.J. & King, A.P. (2002) Female brown-headed cowbirds', *Molothrus ater*, organization and behaviour reflects male social dynamics. *Animal Behaviour*, **64**, 377–385.
- White, D.J., King, A.P., West, M.J., Gros-Louis, J. & Tuttle, E.M. (2010) Effects of singing on copulation success and egg production in brown-headed cowbirds, *Molothrus ater*. *Behavioral Ecology*, **21**, 211–218.
- Williams, E.V. & Swaddle, J.P. (2003) Moulting, flight performance and wing-beat kinematics during take-off in European starlings *Sturnus vulgaris*. *Journal of Avian Biology*, **34**, 371–378.
- Wirsing, A.J., Heithaus, M.R. & Dill, L.M. (2007) Fear factor: do dugongs (*Dugong dugon*) trade food for safety from tiger sharks (*Galeocerdo cuvier*)? *Oecologia*, **153**, 1031–1040.
- Witter, M. & Cuthill, I. (1993) The ecological costs of avian fat storage. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **340**, 73–92.
- Witter, M.S., Cuthill, I.C. & Bonser, R.H.C. (1994) Experimental investigations of mass-dependent predation risk in the European starling, *Sturnus vulgaris*. *Animal Behaviour*, **48**, 201–222.

- Yau, Y. & Potenza, M. (2013) Stress and eating behaviors. *Minerva Endocrinologica*, **38**, 255–267.
- Ydenberg, R.C., Butler, R.W., Lank, D.B., Smith, B.D. & Ireland, J. (2004) Western sandpipers have altered migration tactics as peregrine falcon populations have recovered. *Proceedings of the Royal Society B-Biological Sciences*, **271**, 1263–1269.
- Zanette, L.Y., White, A.F., Allen, M.C. & Clinchy, M. (2011) Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, **334**, 1398–1401.
- Zanette, L.Y., Hobson, K.A., Clinchy, M., Travers, M. & Williams, T.D. (2013) Food use is affected by the experience of nest predation: implications for indirect predator effects on clutch size. *Oecologia*, **172**, 1031–1039.
- Zimmer, C., Boos, M., Poulin, N., Gosler, A. & Petit, O. (2011) Evidence of the trade-off between starvation and predation risks in ducks. *PLoS ONE*, **6**, e22352.

Received 8 June 2016; accepted 2 February 2017

Handling Editor: Sheila Patek