

Brood-parasite-induced female-biased mortality affects songbird demography: negative implications for conservation

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Parasites, of all sorts, can profoundly affect host population dynamics. Parasites commonly cause sex-biased mortality and this can add to their impact. Female-biased mortality in particular can destabilize dynamics and promote population collapse. We previously reported in a correlative study that brown-headed cowbird *Molothrus ater* brood parasitism of song sparrows *Melospiza melodia* appears to cause female-biased host nestling mortality. Here, we report results from ‘infestation’ and ‘de-infestation’ experiments designed to test whether brood parasitism causes female-biased mortality, and we document the resulting demographic impact using a simulation model. Experimental cowbird infestation of song sparrow nests halved the proportion of female host nestlings (0.31 ± 0.07 vs 0.59 ± 0.06 ; infested vs unparasitized nests at day 6) replicating the halving reported in naturally cowbird-parasitized nests (0.28 ± 0.01 vs 0.57 ± 0.05 ; parasitized vs unparasitized). De-infestation of naturally cowbird-parasitized nests in turn wholly eliminated any effect on the proportion of female host nestlings (0.53 ± 0.13 vs 0.54 ± 0.06 ; de-infested vs unparasitized) confirming that brood parasitism is the cause. This halving of the proportion of females fledging is likely to be as significant as nest predation in affecting population dynamics, based on the elasticities derived from our demographic model (-0.50 vs -0.59). Experimental infestation reduced the testosterone levels, begging behaviour, and body mass of six day old female host nestlings, whereas males were largely unaffected, suggesting that it is the exacerbation of intra-brood competition that may be primarily responsible for the resulting female-biased mortality. The brown-headed cowbird is invasive in most of North America and has been implicated in regional population declines of many native species. We suggest that female-biased host offspring mortality is likely to be commonplace among the 144 host species the cowbird successfully parasitizes, and we discuss the negative implications for songbird conservation, given the projected demographic impact.

Parasites, of every kind, can have profound effects on the population dynamics of their hosts by increasing mortality and reducing reproduction (reviewed by Fenton and Rands 2006, Lefèvre et al. 2009; see also Hudson et al. 1998). In addition to increasing mortality per se, parasites also are known to commonly cause sex-biased mortality, and this may be expected to have further adverse effects on host population dynamics and extinction probabilities (Kelly et al. 2001, Engen et al. 2003, Melbourne and Hastings 2008). Although more frequently male-biased, parasite-induced female-biased mortality is not uncommon (Moore and Wilson 2002, Zuk 2009). Male-biased mortality and female-biased mortality are not equivalent in their demographic consequences. The loss of the more limiting sex, typically females, is more likely to destabilize dynamics and promote population collapse (Boukal et al. 2008). In a previous correlative study, we reported that brown-headed cowbird *Molothrus ater* brood parasitism of song sparrows

Melospiza melodia appears to cause female-biased host nestling mortality, resulting in a male-biased sex ratio at fledge (Zanette et al. 2005). Experimentally testing whether this relationship between cowbird brood parasitism and female-biased mortality is in fact causal, and quantifying the resulting impact on host demography, have important implications for songbird conservation, because the cowbird parasitizes at least 144 host species, is invasive in most of North America, and has been implicated in regional population declines of many native species (Lowther 1993, Stratford and Robinson 2005, Tewksbury et al. 2006).

Experimentally ‘infesting’ or alternatively ‘de-infesting’ hosts with a parasite (i.e. adding or removing the parasite) provides the most definitive means of testing the effects of any parasite on any aspect of host demography (Hudson et al. 1998, Bize et al. 2005). Experimental removal of brown-headed cowbirds at a regional scale has demonstrated that their presence can affect host offspring mortality per se

(Smith et al. 2002) though whether they additionally cause sex-biased mortality has not been experimentally demonstrated. Whereas some brood parasites, such as cuckoos (*Cuculus* spp.; Davies 2000), are famous for directly killing the host's offspring, cowbirds are reared together with the host's nestlings and appear to benefit from the presence of some host nestmates (Kilner et al. 2004), though they nonetheless do cause host nestling mortalities (Hauber 2003, Zanette and Clinchy 2010) evidently by exacerbating intra-brood competition (Hauber 2003, Kilner et al. 2004, Pagnucco et al. 2008). In the correlative study noted above (Zanette et al. 2005), we reported that the proportion of female host nestlings in naturally cowbird-parasitized nests was half that in unparasitized nests, at six days post-hatch (0.28 ± 0.01 vs 0.57 ± 0.05) and at fledging (0.27 ± 0.06 vs 0.52 ± 0.11 ; mean \pm SE). Using data from a regional cowbird removal experiment (Smith et al. 2002) we previously developed a simulation model to evaluate the impact of each known effect of the cowbird on the annual reproductive success (fledglings per female per year) of song sparrows (Zanette et al. 2007). Sex-biased mortality was not included in this model because brood-parasite-induced sex-biased mortality had yet to be experimentally demonstrated.

In this paper, we report the results from infestation and de-infestation experiments designed to test whether brood parasitism by brown-headed cowbirds causes female-biased host offspring mortality, and we document the resulting projected impact on host demography as determined using the simulation model described above. In addition to the effect on sex-specific mortality, we also describe the effects of our infestation experiment on aspects of the physiology, behaviour, and morphology of male and female host nestlings. We report that experimentally infesting nests halved the proportion of female host nestlings, just as observed in naturally cowbird-parasitized nests, whereas the proportion in de-infested nests was virtually identical to that in unparasitized nests. Our demographic modelling revealed that this halving of the proportion of females fledging is likely to be as significant as nest predation in affecting population growth rates. Consequently, we conclude that this previously untested effect of brood parasitism indeed has important conservation implications, particularly as the changes we observed in host nestling physiology, behaviour and morphology suggest that it is the exacerbation of intra-brood competition that may be primarily responsible for the resulting female-biased mortality.

Material and methods

Study sites, species and general procedures

We studied the effects of cowbird infestation and de-infestation on song sparrows, in and near Victoria, British Columbia, Canada, over a single breeding season, at the same sites described by Zanette et al. (2005) and Zanette and Clinchy (2010), as well as nearby sites (25 km distant) where the experiments detailed in Pagnucco et al. (2008) and DeCaire et al. (unpubl.) were conducted. Sparrows lay clutches of up to 5 eggs, though 3–4 egg clutches predominate. Breeding occurs from March to August. Eggs are

incubated for 13 days and nestlings usually have fledged by 12 days post-hatch. The song sparrow is the second most commonly parasitized cowbird host, never rejects cowbird eggs, and regularly suffers nestling mortality as a result (Lowther 1993, Zanette and Clinchy 2010).

We experimentally infested sparrow nests (details below) at sites 25 km distant from Victoria and compared them with unparasitized nests found in the same area. We chose this study area to conduct the cowbird infestation experiment for two reasons. Firstly, because there are virtually no cowbirds at these sites (Zanette et al. 2006a), conducting the cowbird infestation experiment here ensured that the outcome could in no way be influenced by any variable associated with the presence of cowbirds other than the one we were manipulating (Zanette et al. 2005). Notwithstanding, the proportion of female host nestlings (0.59 ± 0.06 ; mean \pm SE) on the sixth day post-hatch in unparasitized nests at these sites was nearly identical to that Zanette et al. (2005) reported in unparasitized nests (0.57 ± 0.05) at sites in Victoria, where adult cowbirds are abundant. Secondly, because infestation was logistically challenging we wanted to maximize the number of nests in the experiment and nest predation is lower at these sites outside Victoria (Zanette et al. 2006a). De-infestation involved removing cowbirds from naturally-parasitized nests so this necessarily had to be done where cowbirds were naturally present, at sites in Victoria. De-infested nests were then compared with unparasitized nests from the same area.

The presence of a cowbird nestling involves having an extra, larger, unrelated nestling in the nest, in naturally-occurring, cowbird-parasitized, song sparrow nests (Zanette et al. 2005, Zanette and Clinchy 2010). The cowbird nestling's presence initially means there is an extra mouth to feed, but because one of the host nestlings typically dies due to the cowbird's presence, by day 6 of the brood-rearing period the aggregate number of nestlings (regardless of species) is the same in parasitized and unparasitized nests (Zanette et al. 2005, Zanette and Clinchy 2010). Adult cowbirds are larger than adult sparrows (ca 30 vs 24 g), and cowbird nestlings are larger (ca 19 g; day 6 of brood-rearing) than sparrow nestlings in unparasitized nests (ca 16.5 g).

Nests were located using behavioural cues from the mother (Zanette et al. 2006b) and we candled eggs to age them. The sex of sparrows and cowbirds was determined molecularly, using PCR amplification of genes located on the sex chromosomes (Griffiths et al. 1998, Zanette et al. 2005).

Experimental manipulations

Our objective in both the infestation and de-infestation experiments was to emulate the circumstances associated with naturally-occurring cowbird parasitism as closely as possible, while manipulating a single variable (the presence or absence of the parasite). Our cowbird infestation experiment followed exactly the same protocol as that described in Pagnucco et al. (2008). We emulated the cowbird mother's actions by adding a single cowbird egg to unparasitized song sparrow nests (Zanette and Clinchy 2010). Eggs added earlier in incubation are more likely to be eaten by nest predators (Zanette et al. 2007) so for this logistical reason

we added the cowbird egg just prior to hatch (3.5 ± 0.6 d prior; mean \pm SE). The pool of potential recipient nests was restricted to those that were expected to hatch on the same day as the cowbird egg, as in naturally-occurring cowbird-parasitized song sparrow nests (Pagnucco et al. 2008). The host and cowbird eggs all did hatch on the same day. The cowbird nestlings were all later humanely euthanized (following Canadian Council on Animal Care guidelines) on the sixth day post-hatch, to prevent cowbirds from establishing where there previously were none. Molecular sexing of the cowbirds revealed that there were an equivalent number of males and females.

In the de-infestation experiment we removed the cowbird egg from naturally-occurring, cowbird-parasitized nests, and replaced it with an artificial clay egg fashioned to resemble that of a cowbird, so as to prevent abandonment by the host (Smith et al. 2003), or retaliation by the cowbird mother (Hoover and Robinson 2007). Female song sparrows always accepted artificial eggs and continued to incubate them, as we have reported previously (Travers et al. 2010).

Demographic modelling

We modified the simulation model developed by Zanette et al. (2007) to include the measured effect on female-biased mortality observed in the infestation experiment described above, and evaluated the impact on a demographic parameter; the number of females fledged per breeding female per year. To assist in gauging the relative significance that this parasite-mediated effect might have on the demographic parameter of interest in addition to population growth rates, we compare the elasticity value generated from our model with the elasticity regarding nest predation since this was the model parameter with the largest elasticity in both the current simulation and Zanette et al. (2007), and nest predation is known to have a significant impact on the demography of most avian species (reviewed by Smith et al. 2010), including our populations of song sparrows (Zanette et al. 2006a). The incidence of cowbird parasitism on song sparrow nests used in the model was 62% and daily nest predation rates were 0.023 for the egg stage (i.e. 72% probability of surviving 14 days) and 0.022 for the nestling stage (i.e. 78% probability of surviving 11 days). These values were estimated from observed data (Table 1 in Zanette et al. 2007). Elasticities reflect the proportional change in a response variable (in our case, female fledglings per female per year) given a proportional change in a model parameter (i.e. sex ratio per nest and nest predation; Caswell 2001). For example, in our simulation, an elasticity value of -0.50 for a model parameter would indicate that for every 10% increase in that parameter there would be a 5.0% decrease in the number of females fledged per female per year; and a 20% increase in the model parameter, for instance, would lead to a 10% decrease in female offspring fledged per female. The elasticity values reported are the average from 10 000 iterations of the simulation. Details concerning the simulation model may be found in Zanette et al. (2007), but we provide an outline of the model below.

Our initial simulation model (Zanette et al. 2007) followed a female song sparrow over the course of a single

breeding season, using input data observed in the field. The song sparrow breeding season was described as beginning and ending on a randomly chosen date, based on means and variances from observed data. The season then proceeded as a series of nest cycles each of 28 days (3 days to build a nest, 1 day pre-incubation, 13 days incubation, 11 days brooding). Clutch sizes were randomly chosen from an empirically determined distribution that depended on the month. After a nest failed or fledged, there was a resting-stage after which birds could begin a new nest cycle. During each day of the egg-stage the nest could be destroyed by non-cowbird predators or parasitized by cowbirds. Parasitized nests could undergo egg-removal. A nest that was parasitized more than once or lost more than one egg to parasitism could be deserted. On day 17 of the nest cycle eggs can hatch, but some may fail to hatch. During each day of the nestling-stage the nest may be destroyed either by non-cowbird predators or by cowbirds. Nests also could experience partial brood loss. All nestlings surviving to the end of day 28 of the nest cycle were assumed to have successfully fledged, and the sex ratio per nest was assumed to be 50:50. Nest cycles were all followed to completion unless destroyed or deserted, in which case the cycle would skip directly to the rest-stage. On completion of the nest cycle another starts immediately so long as the date is prior to the end of the breeding season. If the previous nest cycle yielded no fledglings, nesting attempts for a particular female could cease. We conducted 2000 simulations of a population of breeding females consisting of 49 individuals, from which we obtained means and 95% confidence intervals for the number of female fledglings produced per female per season. Our current simulation model was identical except we altered the proportion of females:males per nest to correspond with the measured effects observed in the infestation experiment.

Host nestling physiology, behaviour and morphology

We compared aspects of the physiology, behaviour, and morphology of male and female host nestlings between experimentally-infested and unparasitized nests. All measures were taken on the sixth day post-hatch. We assayed plasma testosterone (hereafter 'T') levels because T has been shown to be positively correlated with the duration and intensity of nestling begging (Goodship and Buchanan 2007), and nestling begging is associated with whether an individual is fed (Wright and Leonard 2002). About 75 μ l of blood was collected from the brachial vein and stored on ice for transport, and all samples were centrifuged and plasma was extracted and frozen at -20°C , within eight h. T was quantified using a direct ^{125}I radioimmunoassay and an established protocol developed to detect the low concentrations present in human saliva (Moffat et al. 1997). The antiserum is highly specific for T, having negligible cross-reactivity with other steroids. The sensitivity of the assay was 0.65 pg per assay tube, and the intra-assay coefficient of variation averaged 4%.

We evaluated the begging behaviour of host nestlings by filming nests for one hour, between 07:00 and 12:00, using a miniature camera positioned 25 cm from the nest. We calculated the duration of begging bouts, which was the number of seconds, from when an individual first raised

its head to gape, to the end of a begging display. We also examined begging posture using a ranking system of 0 to 4 (following Leonard et al. 2003) where 0 = no postures; 1 = head down, gaping, sitting on tarsi; 2 = head up, gaping, sitting on tarsi; 3 = same as 2, plus neck stretched upward; 4 = same as 3, but body lifted off tarsi.

Finally, all hosts nestlings were weighed to the nearest 0.1 g using an electronic balance, following the completion of filming.

Statistical analyses

Sex ratios were compared between treatments using generalized linear models with the logit link function and binomial errors, the number of females being the dependent variable and number of offspring in the nest the binomial denominator (Zanette et al. 2005). All models were checked for overdispersion. To confirm that any resulting effect of cowbird infestation on the proportion of females present was due to differential mortality we conducted a Mann–Whitney U-test of the number of sparrow nestlings that died in experimentally-infested versus unparasitized nests.

The change in sparrow nestling T associated with the experimental-infestation treatment was assessed using a two-factor mixed model ANOVA with treatment and sex as predictor variables and nest identity as a random effect. To evaluate the change in sparrow nestling begging behaviour we conducted a discriminant function analysis (hereafter DFA) with treatment as the grouping variable and our two measures, duration and posture, as the dependent variables (following Pagnucco et al. 2008). To avoid pseudoreplication we calculated the average for each measure for each sex in each nest, and to control for inter-brood variation we next determined the difference between the sexes (female – male) for each measure in each nest, and then used these difference scores in the DFA (Travers et al. 2010). We re-analyzed the data in a MANCOVA with brood size as a covariate and found nearly identical results. We report the DFA results only. Differences in nestling body mass were assessed, firstly by conducting a two-factor mixed model ANCOVA with treatment and sex as predictor variables, nest identity as a random effect, and brood size as the covariate, and secondly by evaluating each sex separately using one-factor ANCOVAs with brood size as the significant covariate in each case ($p < 0.05$).

Data for parametric analyses were tested for normality and homogeneity of variances where appropriate. The descriptive statistics reported are means \pm SE.

Results

Experimental cowbird infestation significantly reduced the proportion of female host nestlings ($\chi^2 = 4.8$, $DF = 1$, $p = 0.01$). The proportion of female host nestlings in experimentally-infested nests was half that in unparasitized nests (0.31 ± 0.07 vs 0.59 ± 0.06) by the sixth day post hatch. That this difference in the proportion of female host nestlings was attributable to differential mortality was corroborated by the significant difference (Mann–Whitney U, $Z = 2.7$, $p = 0.007$) in the number of sparrow nestlings

that died in experimentally-infested nests (1.3 ± 0.2 , $n = 13$) as compared to unparasitized nests (0.3 ± 0.2 , $n = 21$).

De-infestation of naturally-occurring, cowbird-parasitized nests further corroborated that cowbird brood parasitism is the cause of female-biased host nestling mortality, because there was no difference in the proportion of female host nestlings in de-infested versus unparasitized nests (0.53 ± 0.13 , $n = 11$ vs 0.54 ± 0.06 , $n = 24$, respectively; $\chi^2 = 0.0$, $DF = 1$, $p = 0.94$) on the sixth day post hatch.

Brood-parasite induced alterations in the proportions of female:male offspring per nest (elasticity value = -0.50) was similar to that of nest predation (elasticity value = -0.59) in its influence on the total number of females produced per female per year, in our demographic simulation model.

Female sparrow nestlings demonstrated lower T, a lower duration of begging, lower posture and lower body mass in experimentally cowbird-infested vs unparasitized nests (Fig. 1), whereas male sparrow nestlings demonstrated elevated T, comparable begging duration and posture, and virtually no difference in body mass (Fig. 1). The contrast between the sexes in the change in T was reflected in a significant treatment \times sex interaction ($F = 7.1$, $DF = 1$, 37 , $p = 0.01$; main effects $p > 0.47$). In cowbird-infested nests, T levels were higher in male than in female sparrow nestlings (Fig. 1a), which was the inverse of the pattern in unparasitized nests (46.3 ± 7.2 pg ml⁻¹ vs 17.9 ± 12.3 , female vs male). Cowbird-infestation significantly altered the difference in begging behaviour between male and female nestmates (DFA, $R^2 = 0.77$, $\chi^2 = 13.2$, $DF = 2$, $p = 0.001$), whereas in unparasitized nests, there was little difference between the sexes in either begging duration (7.5 ± 0.7 s vs 7.0 ± 0.7 , female vs male) or posture (3.4 ± 0.3 vs 3.4 ± 0.2). In unparasitized nests, modest sexual size dimorphism was evident as male sparrow nestlings were 5% larger in body mass (16.6 ± 0.7 g) than females (15.9 ± 0.4). This difference in body mass was significantly greater in cowbird-infested nests (treatment \times sex interaction $F = 5.1$, $DF = 1$, 18 , $p = 0.037$; sex $F = 6.8$, $p = 0.018$; treatment $p > 0.17$). Considering the sexes separately, female body mass was significantly lower in cowbird-infested nests compared to unparasitized nests ($F = 12.3$, $DF = 1$, 19 , $p = 0.003$), whereas there was almost no difference in male body mass ($F = 0.0$, $DF = 1$, 19 , $p = 0.98$) comparing between cowbird-infested and unparasitized nests.

Discussion

Our experimental results demonstrate that brood parasitism can cause female-biased mortality and our demographic modelling reveals that this previously untested effect of brood parasitism may be as significant as nest predation in its impact on host demography. Experimental cowbird infestation significantly reduced the proportion of female host nestlings present by the sixth day post-hatch and the concomitant increase in sparrow nestling deaths confirms that female-biased mortality was the cause. Experimental infestation halved the proportion of female host nestlings (0.31 ± 0.07 vs 0.59 ± 0.06 ; infested vs unparasitized) thereby almost perfectly replicating the halving of the

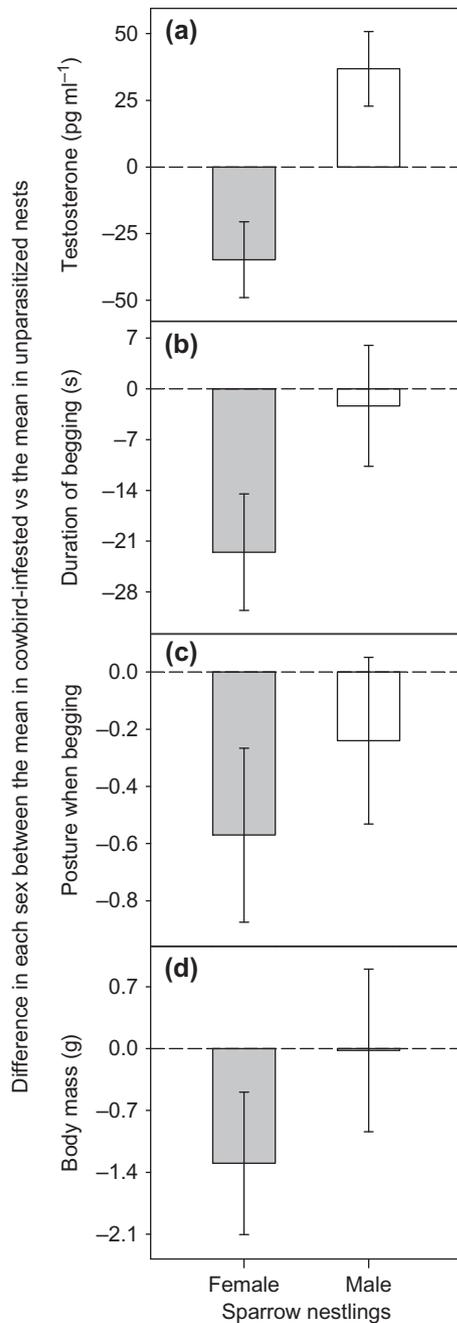


Figure 1. Changes in response to cowbird-infestation in the physiology, behaviour and morphology of female (grey bars) and male (white bars) sparrow nestlings, as measured on the sixth day post-hatch. Bars illustrate the difference in each sex between the mean in cowbird-infested nests and the mean in unparasitized nests in: (a) plasma testosterone (pg ml^{-1}), (b) duration of begging (s), (c) posture during begging (based on a rank scale described in the text), and (d) body mass (g). Bars falling below the zero line indicate that the measure had a lower value in cowbird-infested nests, whereas those falling above the zero line signify that the measure was elevated in cowbird-infested nests. SEs shown are for cowbird-infested nests. Means \pm SE in unparasitized nests are reported in the text.

proportion of female host nestlings evident in naturally cowbird-parasitized nests (0.28 ± 0.01 vs 0.57 ± 0.05 ; parasitized vs unparasitized; Zanette et al. 2005). De-infestation of naturally cowbird-parasitized nests fully verified that cowbird brood parasitism is the cause of female-biased host nestling mortality because de-infestation wholly eliminated any effect on the proportion of female host nestlings (0.53 ± 0.13 vs 0.54 ± 0.06 ; de-infested vs unparasitized). Cowbirds are an invasive species in the region in which we work and our results demonstrate that this previously untested effect of brood parasitism has important conservation implications for sparrows in this area (Jewell and Arcese 2008).

The broader conservation implications of our results obviously depend upon their generalizability and we suggest there are good reasons to expect that brood-parasite-induced female-biased mortality may be commonplace. The changes we observed in host nestling physiology, behaviour and morphology all point to female-biased mortality being primarily due to exacerbated intra-brood competition. Cowbird-infestation caused significantly different changes in T, begging behaviour and body mass in male and female host nestlings (Fig. 1). The lower T, reduced begging and lighter mass of female host nestlings in cowbird-infested nests (Fig. 1) all suggest that females were 'losing-out' due to exacerbated intra-brood competition, whereas the elevated T and unaffected begging behaviour and body mass of males (Fig. 1) all suggest that males in contrast were able to 'hold their own'. In our system, cowbird infestation (naturally and experimentally) entails having an extra, larger, unrelated nestling in the nest (Zanette et al. 2005, Zanette and Clinchy 2010), all of which may be expected to exacerbate intra-brood competition. Conceivably, the female-biased mortality reported here may not have been due to these general perturbations associated with cowbird parasitism but some specific cowbird trait that female song sparrow nestlings are peculiarly vulnerable to. This is not the case however as we tested this hypothesis in a separate experiment (DeCaire et al. unpubl.), by adding an extra, larger, unrelated song sparrow nestling to song sparrow nests, and documented a comparable effect on female-biased mortality to that reported here.

Since some general perturbation associated with brood parasitism rather than any cowbird-specific trait is sufficient to cause female-biased mortality (DeCaire et al. unpubl.), this may be expected to be quite a general phenomenon. Considering all its hosts, the cowbird nestling is unrelated to all its host nestmates, is larger than the great majority (Dearborn 1998), and its presence commonly means there is an extra mouth to feed (Hauber 2003, Kilner et al. 2004, Zanette and Clinchy 2010). If relatedness is the determining factor (Boncoraglio et al. 2009) then female-biased host nestling mortality may be expected to be universal. If the cowbird nestling's larger size is the key then several recent reviews suggest that female-biased mortality is likely in the majority of cowbird hosts. Song sparrows are representative of 15 of the 17 most commonly parasitized cowbird hosts in being both smaller than the cowbird and demonstrating a modest degree of sexual size dimorphism (males ca 5% larger than females; Lowther 1993, Råberg et al. 2005, Dunning

2008). Many studies across avian species have shown that larger nestlings receive the lion's share of parental provisioning at the cost of their smaller nestmates (Price and Ydenberg 1995, Oddie 2000, Forbes et al. 2002, Zanette et al. 2009). Where host nestlings are smaller than the cowbird, female host nestlings may be expected to be the most disadvantaged (Fig. 1). Moreover, a number of recent reviews indicate that female nestlings are more sensitive to harsh environmental conditions particularly in species where sexual size dimorphism is modest (Råberg et al. 2005, Uller 2006, Nicolaus et al. 2009).

Finally, if female-biased host nestling mortality is contingent on brood augmentation, i.e. there being more nestlings at hatch than host eggs laid (Hauber 2003, Kilner et al. 2004, Zanette and Clinchy 2010), then the likelihood of female-biased mortality may be expected to vary inversely with the probability of cowbird egg removal. Though cowbirds sometimes remove eggs from host nests the frequency of egg removal varies among host species, and among populations of the same host species, and can range from almost never to virtually always (Peer and Bollinger 2000, Peer 2006). Egg removal causes abandonment when the host's clutch size is reduced below a desertion threshold (Smith et al. 2003). Egg removal is less common among host species that are smaller than the cowbird, and it has been proposed that this is because there are fewer benefits, that consequently do not outweigh the cost of potentially inducing nest abandonment (Peer and Bollinger 2000, Kosciuch et al. 2006, Peer 2006). We previously experimentally demonstrated that in song sparrows, egg removal is less likely the smaller the host's clutch (Zanette and Clinchy 2010), consistent with cowbirds avoiding reducing host clutches below a desertion threshold. Because egg removal is less likely among smaller hosts, and those with small clutch sizes, female-biased mortality would thus be expected to be more common among such hosts. As already noted, the great majority of cowbird hosts are smaller than the cowbird. The expectation that female-biased mortality may be more likely where clutch size is smaller is worrisome since such species and populations may be expected to be more adversely affected, given that having a smaller clutch size limits the capacity for population growth (Zanette et al. 2006a, 2007).

Cowbird infestation definitely causes female-biased mortality in our system and, as we have discussed, there are good reasons to expect this in many other systems. Similarly there are sound reasons to expect that the demographic impact will be comparable. Nest predation is generally considered to be the principal cause of egg and nestling losses in most birds (reviewed by Smith et al. 2010) and this is true of our system as well (Zanette et al. 2006a, 2007). How then can the elasticities concerning brood-parasite-induced female-biased mortality (-0.50) and nest predation (-0.59) be comparable? Assume each host mother produces two broods of four young each year, and the primary sex ratio is 50:50, so there are two females per brood. One of these two females (50%) may be expected to die as a consequence of brood-parasite-induced female-biased mortality, given our experimental results, so with two broods each mother may be expected to fledge two

daughters. Doubling the number of deaths (i.e. a 100% increase) due to this cause would reduce the number of females fledging to zero (a 100% decrease). Similarly, if nest predation is 50%, with two broods each mother may be expected to fledge two daughters, and doubling the number of deaths (a 100% increase) would reduce this to zero (a 100% decrease). The respective elasticities do not depend upon the absolute number of losses but the strength of the sex-biased mortality caused by brood parasitism, the population sex ratio, and the assumption that nest predation does not cause sex-biased mortality. Any system with a comparable population sex ratio to ours and no obvious sex bias as regards nest predation should demonstrate a comparable elasticity concerning brood-parasite-induced female-biased mortality.

Cowbird infestation led to corresponding reductions in T, begging behaviour, and body mass in female host nestlings (Fig. 1). Losing out in social contests has been shown to depress plasma T in a variety of birds and mammals (Hsu et al. 2006). Thus, the depression of T alone would suggest that female nestlings were losing out in intra-brood competition when together with both their brothers and a cowbird nestling (Fig. 1a). Winning is associated with elevated T, though this may be cause or consequence, depending upon species and circumstances (Hsu et al. 2006, Rutte et al. 2006, Gleason et al. 2009). Female nestlings demonstrated elevated T when together with just their brothers and males demonstrated elevated T when with a cowbird nestling (Fig. 1a) and we suggest the most parsimonious explanation is that in this circumstance T is a cause of competitive success, and both females and males elevate T when faced with a competitor they can win against, even though the competitor is in a heavier weight class. When up against two heavyweights, as females are when with both their brothers and a cowbird nestling, winning is likely no longer a possibility, which may then be expected to depress their plasma T (Fig. 1a; Hsu et al. 2006).

Sex-biased mortality in response to infestation by a parasite is probably the norm rather than the exception (Moore and Wilson 2002, Zuk 2009). In birds, female-biased host nestling mortality has previously been demonstrated in response to experimental infestation with an ectoparasite (Bize et al. 2005), and the authors of this study pointed to exacerbated intra-brood competition as being the most likely proximate cause. Our results demonstrate that experimental infestation with a brood parasite can similarly cause female-biased host nestling mortality. We suggest that the reason brood-parasite-induced sex-biased mortality has rarely been considered (Zanette et al. 2005), and has never before been tested, is that infestation by a brood parasite is often viewed as being different from infestation by other parasites. Viewed as just like any other parasite (Kilner 2005), the fact that parasites in general cause sex-biased mortality may be added to the various specific reasons we have proposed as to why brood-parasite-induced female-biased mortality may be expected to commonplace. Whether viewed generally or for the specific reasons we have discussed, the fact that brood-parasite-induced female-biased mortality may be expected to commonplace has serious implications for the conservation of probably most cowbird hosts.

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