



Brood parasitism causes female-biased host nestling mortality regardless of parasite species

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Inequality in male and female numbers may affect population dynamics and extinction probabilities and so has significant conservation implications. We previously demonstrated that Brown-headed Cowbird *Molothrus ater* brood parasitism of Song Sparrows *Melospiza melodia* results in a 50% reduction in the proportion of female host offspring by day 6 post-hatch and at fledging, which modelling demonstrated is as significant as nest predation in affecting demography. Many avian brood parasites possess special adaptations to parasitize specific hosts so this sex-ratio effect could be specific to the interaction between these two species. Alternatively, perturbations associated with brood parasitism *per se* (e.g. the addition of an extra, larger, unrelated nestling), rather than a Cowbird nestling specifically, may be responsible. We experimentally eliminated the effects of Cowbird-specific traits by parasitizing nests with a conspecific nestling rather than a Cowbird, while otherwise emulating the circumstances of Cowbird parasitism by adding an extra, larger (2-day-old), unrelated Song Sparrow nestling to Song Sparrow nests. Our parasitism treatment led to few host offspring deaths and no evidence of male-biased sex ratios by day 6 post-hatch. However, after day 6, female nestling mortality rates increased significantly in experimentally parasitized nests, resulting in a 60% reduction in the proportion of females fledging. Cowbird-specific traits are thus not necessary to cause female-biased host nestling mortality and far more general features associated with Cowbird parasitism instead appear responsible. Our results suggest, however, that Cowbird-specific traits may help accelerate the pace of female host deaths. The conservation implications of our results could be wide reaching. Cowbirds are unrelated to all their hosts, are larger than the great majority, and a Cowbird nestling's presence can mean there is an extra mouth to feed. Thus, sex-biased mortality in parasitized nests could be occurring across a range of host species.

Keywords: conservation, experimental parasitism, host–parasite interactions, sex ratio.

Inequality in male and female numbers can have profound effects on population dynamics and extinction probabilities and is consequently one of four principal stochastic factors affecting species conservation (Kelly *et al.* 2001, Engen *et al.* 2003, Melbourne & Hastings 2008, Cotton & Wedekind 2009). Parasites, more than food and predators, are known to cause sex-biased mortality and although this is generally male-biased,

parasite-induced female-biased mortality is not uncommon (Moore & Wilson 2002, Zuk 2009). For example, parasites of all sorts can increase the competitive environment among avian nest-mates vying for parental care (Christe *et al.* 1996, Kilner *et al.* 2004, Reed *et al.* 2008), potentially leading to higher mortality rates of the less competitive sex (e.g. Bize *et al.* 2005). We previously demonstrated that Brown-headed Cowbird *Molothrus ater* brood parasitism of Song Sparrows *Melospiza melodia* leads to an increase in female host offspring mortality resulting in a male-biased sex ratio by

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day 6 after hatching in both naturally parasitized (Zanette *et al.* 2005) and experimentally parasitized (Pagnucco *et al.* 2008, Zanette *et al.* 2012) nests. Consistent with females being at a competitive disadvantage, sex asymmetries in begging behaviour and mass became much more pronounced in experimentally parasitized than non-parasitized nests.

Many avian brood parasites are known to possess highly specialized adaptations with which to parasitize specific hosts (Davies 2000). In species in which the brood parasitic nestling is reared together with host nestlings, the parasite commonly possesses traits such as intricate gape patterns or elaborate vocalizations which mimic and exaggerate aspects of the host's display and thus permit it to out-compete the host nestlings for parental care (Davies 2000, Hauber & Kilner 2007, Pagnucco *et al.* 2008). Like other brood parasitic nestlings, Cowbird nestlings possess traits that can increase competition within the nest. For example, Cowbird nestlings emit vocalizations that are exaggerated in amplitude, call rate and frequency relative to hosts (Briskie *et al.* 1994, Dearborn 1999, Pagnucco *et al.* 2008). Of all hosts examined to date, the contrast is greatest between the Cowbird nestlings' vocalizations and those of Song Sparrow nestlings (Briskie *et al.* 1994, Pagnucco *et al.* 2008). Thus, Song Sparrow nestlings could be particularly vulnerable to this or some other specialized trait of the Cowbird nestling, leading to male-biased sex ratios in this host. On the other hand, brood parasitism by Brown-headed Cowbirds *per se* could itself increase competition among nest-mates, thereby altering host offspring sex ratios. Cowbird nestlings are unrelated to all of their hosts, which could escalate competition for parental food provisioning (e.g. Boncoraglio *et al.* 2009). Cowbirds are also larger than the great majority of their hosts, and studies across many bird species have shown that larger nestlings receive proportionally more parental provisioning, to the detriment of their smaller nest-mates (e.g. Price & Ydenberg 1995, Oddie 2000, Forbes *et al.* 2002, Zanette *et al.* 2009), and relatively small hosts incur survival costs (Lorenzana & Selye 1999). Finally, the presence of a Cowbird nestling can mean that there is an extra mouth to feed (Hauber 2003, Kilner *et al.* 2004, Zanette & Clinchy 2010), and larger broods are expected to exacerbate competition within the nest.

We assessed whether Cowbird-specific traits are necessary, or more general perturbations associated with Cowbird parasitism are sufficient, to cause female-biased host nestling mortality and male-biased sex ratios in Song Sparrows. We completely eliminated the effects of Cowbird-specific traits while otherwise emulating the circumstances of Cowbird parasitism as closely as possible. Specifically, we followed the experimental protocols of Pagnucco *et al.* (2008) and Zanette *et al.* (2012), but instead of experimentally parasitizing host nests with a Cowbird chick, we used a conspecific that was as large as a Cowbird chick. The result was that experimentally parasitized nests contained a larger, extra, unrelated nestling as typically occurs in naturally Cowbird-parasitized Song Sparrow nests (Zanette & Clinchy 2010, Zanette *et al.* 2012). In addition to the effects of experimental brood parasitism on host offspring sex ratios, we also had the advantage of being able to track the fates of every individual in our experiment using video surveillance, allowing us to document the mortality rates of host offspring. Finally, we quantified the effects of experimental brood parasitism on the mass, size, parental provisioning and begging behaviour of male and female nestlings.

METHODS

Study area and species

We worked on resident pairs of Song Sparrows in the Southern Gulf Islands, British Columbia, Canada (48°43'30"N, 123°22'00"W). This is the same site where the experiments described in Pagnucco *et al.* (2008) and Zanette *et al.* (2012) were conducted. The Song Sparrow is a socially monogamous, sexually dimorphic species, in which males are slightly (5%) larger in body mass (Dunning 2008), even during the nestling phase (Zanette *et al.* 2005, 2012). Sparrows lay clutches of up to five eggs, although three- to four-egg clutches predominate. Breeding typically occurs from April to July. Eggs are incubated for 13 days and nestlings usually have fledged by 12 days after hatching. The Song Sparrow is the second most commonly parasitized Brown-headed Cowbird host, never rejects Cowbird eggs and regularly suffers nestling mortality as a result (Lowther 1993, Zanette & Clinchy 2010).

The presence of the Brown-headed Cowbird nestling results in there being an extra, larger,

unrelated nestling in the nest in naturally occurring, parasitized Song Sparrow nests (Zanette *et al.* 2005, 2012). 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 non-parasitized nests (Zanette *et al.* 2005, 2012). Adult Cowbirds are larger than adult Song Sparrows (*c.* 30 vs. 24 g), and Cowbird nestlings are larger (*c.* 19 g at day 6 of brood-rearing) than even those Sparrow nestlings in non-parasitized nests (*c.* 16.5 g at day 6 of brood-rearing).

Experimental design

Our objective was to emulate the circumstances associated with naturally occurring Cowbird parasitism as closely as possible while experimentally eliminating the effects of Cowbird-specific traits, to test whether such traits are necessary, or more general perturbations associated with Cowbird parasitism are sufficient to cause female-biased host nestling mortality. Our experimental protocol followed Pagnucco *et al.* (2008) and Zanette *et al.* (2012), who emulated Cowbird parasitism by 'infesting' Song Sparrow nests with a single Cowbird and compared results with non-parasitized nests. Zanette *et al.* (2012) found that reductions in the proportion of offspring that were female in experimentally parasitized nests were nearly identical to those found in naturally parasitized nests by day 6 after hatching (Zanette *et al.* 2005, 2012). Pagnucco *et al.* (2008) used this same experiment to examine the effect of Cowbird-specific traits on host nestling behaviour. The primary modification in our present protocol is that we experimentally parasitized nests with a conspecific nestling rather than a Cowbird, such that we could test for comparable effects on mortality and host nestling behaviour in the absence of Cowbird-specific traits. Specifically, we experimentally parasitized 11 Song Sparrow nests with a conspecific nestling while, at the same time, emulating the circumstances associated with naturally occurring Cowbird parasitism as closely as possible (as in Pagnucco *et al.* 2008, Zanette *et al.* 2012). To do so, we 'parasitized' nests with a larger, extra, unrelated conspecific nestling. To mimic the difference in size between Cowbird and Song Sparrow nestlings we added a 2-day-old (± 0.08 day) conspecific chick to host

nests at hatching, with the result that by the time the host nestlings were 6 days old, the added conspecific was as heavy as a 6-day-old Cowbird (18.9 ± 0.53 vs. 19.0 ± 0.82 g, conspecific vs. Cowbird; $F_{1,20} < 0.1$, $P = 0.93$; Cowbird mass from Zanette *et al.* 2012). Conspecifics were transferred to host nests from donor nests in non-adjacent territories, and consisted of an equal number of males and females (evaluated through molecular sexing, see below). We compared nests in this treatment with 30 unmanipulated nests because unmanipulated nests best emulate non-parasitized nests in brood parasite-host systems (following Pagnucco *et al.* 2008, Zanette *et al.* 2012). We refer to the conspecific used in the manipulation as the 'parasite' located in *parasitized nests* in the *parasitism treatment*, and refer to unmanipulated nests as *non-parasitized nests* in the *no parasitism treatment*.

Sexing nestlings and monitoring survival

Nestlings were individually marked at hatching (day 0) such that their fates could be followed using infrared cameras positioned 10 cm from the edge of the nest, which allowed us to continuously monitor nestlings. Nestlings either fledged or died of apparent starvation (Zanette *et al.* 2011). A blood blot was collected on day 1 for later molecular sexing (following Zanette *et al.* 2005, 2012).

Measuring nestling growth, parental provisioning and nestling begging

Treatment effects on nestling growth were evaluated on day 6 after hatching by weighing nestlings to 0.1 g and measuring their right tarsus-length to 0.1 mm (Zanette *et al.* 2005, 2012).

To measure effects on parental provisioning and nestling begging, we filmed 26 nests (13 per treatment) for 120 min each, on day 5 after hatching, between 07:00 h and 13:00 h on fine days using the camera systems described above. Prior to each trial, nestlings were individually marked with white paint on the tops of their heads, and a high-contrast ruler with 1-mm markings was held briefly at various locations around the nest to calibrate the measurement of height reached during begging bouts. We then allowed at least 20 min for the female to settle back on the nest.

We quantified parental provisioning by counting the number of feeding trips parents made to the nest, identifying which nestling was fed, and scoring

the amount on a scale of 1–4 with respect to whether the food item was: (1) not visible, (2) smaller than the parent's bill, (3) similar in size to the parent's bill or (4) larger than the parent's bill.

We measured four aspects of each nestling's begging behaviour: (1) height (mm) reached; (2) posture, quantified using a 0–5 scale wherein: 0 = not begging at all (i.e. head down, mouth closed), 1 = nestling opened its mouth but did not lift its head or extend its legs, 2 = nestling opened its mouth and raised its head, 3 = nestling opened its mouth, raised its head and lifted its breast off the bottom of the nest, 4 = nestling opened its mouth, raised its head and was standing up on its legs, and 5 = nestling opened its mouth, raised its head, was standing up on its legs and waving its wings (following Leonard *et al.* 2003); (3) start time (s) when each nestling started to beg relative to the arrival of the parent; and (4) duration (s) of begging.

Statistical analyses

We used two methods to assess the effects of our experimental treatment on host nestling survival. First, we identified whether offspring were susceptible to high mortality in a particular stage of the brood-rearing period. We categorized survival into stages corresponding to the beginning (up to day 6 after hatching) and end (after day 6 to fledging) of brood-rearing. We used these time periods because we previously found that the addition of a Cowbird nestling led to higher host mortality and male-biased sex ratios by day 6 (Zanette *et al.* 2005, 2012) and at fledging (Zanette *et al.* 2005, 2012). Survival was estimated by dividing the number of survivors at the end of each stage by the number of individuals present at the beginning of the stage, and thus indicated the proportion of host offspring that survived per nest per stage. We then carried out a mixed-model ANOVA, with treatment as a fixed effect and stage as a repeated-measures term followed by Bonferroni *post-hoc* analyses. Each of the mixed-model ANOVAs we describe included nest identity as a random effect. Next, we examined the cumulative survival probabilities of each sex in each treatment over the entire brood-rearing period. Survival probabilities were estimated using the Kaplan–Meier procedure based on the age at which each individual fledged or died (Pollock *et al.* 1989) and comparisons of survival curves were made using the most conservative version of the log-rank test (Pollock *et al.* 1989).

Sex ratios were compared between treatments at hatching (pre-manipulation), on day 6 and at fledging using generalized linear models with a logit link function and binomial errors, the number of female chicks being the dependent variable and total number of host offspring in the nest the binomial denominator. All models were checked for overdispersion.

We assessed whether our experimental treatment affected overall parental investment by conducting a one-way ANOVA on total day 6 brood mass. We then compared the mass and size of the 'parasite' with its host nest-mates using mixed-model ANOVAs with nest-mate identity (i.e. 'parasite' or host) as a repeated-measures term. Next, we evaluated how host offspring fared in the two treatments by calculating the coefficient of variation in mass for each brood and comparing this between treatments with a one-way ANOVA. Finally, we used mixed-model ANOVAs to examine how the mass and size of host offspring separated out by treatment (fixed effect) and sex (repeated measures).

To determine whether our experimental treatment affected overall parental provisioning we conducted one-way ANOVAs on the total number of feeding trips per chick, and total amount of food brought per chick (based on our 1–4 scoring of food items), over the 120-min observation period. We assessed how provisioning was partitioned among nest-mates by testing whether the 'parasite' was more likely to be fed than its nest-mates using a mixed-model ANOVA with nest-mate identity as a repeated-measures term. For these data, we summed the number of times the 'parasite' was fed and divided this by the number of feeding visits parents made to the nest. For hosts, we summed the number of feeds and divided this by the number of hosts, and subsequently divided by the number of feeding visits parents made to the nest. We did this to correct for the fact that there could only ever be one 'parasite' but more than one host, increasing the probability that a host would get fed. We used the same procedure to evaluate whether the 'parasite' obtained more food per bout based on our scoring of food items, which necessitated use of a Wilcoxon matched pairs test as the error residuals were not homogeneous. Finally, we tested whether the number of parental feeding trips and food brought per host nestling varied by treatment (fixed effect) and sex (repeated measures) using mixed-model ANOVAs.

The data for sex of nestlings were corrected for the number of each sex in the nest as described above.

To evaluate the effects of our experimental treatment on nestling begging we first identified which behaviours were associated with a nestling receiving food and then restricted our analysis of treatment effects to those behaviours. To begin, we categorized each nestling as successful (food received) or unsuccessful in each food provisioning bout and assessed which behaviours differed between fed and unfed nestlings. We then compared begging behaviour among host nest-mates according to treatment (fixed effect) and sex (repeated measures) using a mixed-model ANOVA, and compared the 'parasite' with its host nest-mates (repeated measures) with a mixed-model ANOVA.

All parametric models were tested for covariance with brood size, date, time of day and age of the nest. Significant covariates were retained, although we report only significant terms involving independent variables. Prior to parametric analyses the data were tested for normality and homogeneity of variances where appropriate. The descriptive statistics reported are means \pm standard errors.

RESULTS

Host offspring mortality

Experimentally parasitizing nests with an extra, larger, conspecific led to a significant increase in host offspring mortality (treatment, $F_{1,39} = 10.5$, $P = 0.002$), but only in the period between day 6 and fledging (treatment * stage, $F_{1,39} = 10.9$, $P = 0.002$). While proportionately fewer host nestlings died in the parasitism (0.03 ± 0.036) vs. no parasitism treatment (0.05 ± 0.022) from hatch to day 6, mortality increased dramatically in the parasitism treatment between day 6 and fledging (proportion of host nestlings that died, 0.30 ± 0.066) with relatively little change between stages in the no parasitism treatment (day 6 to fledging: 0.02 ± 0.040). Indeed, mortality in the parasitism treatment between day 6 and fledging was significantly different from all other Bonferroni *post-hoc* pairwise comparisons ($P < 0.008$). No other pairwise tests differed significantly ($P > 0.99$ in all cases).

Host offspring mortality curves varied significantly with treatment and sex (Fig. 1; $\chi^2_3 = 23.0$, $P < 0.001$). Female chicks in the parasitism

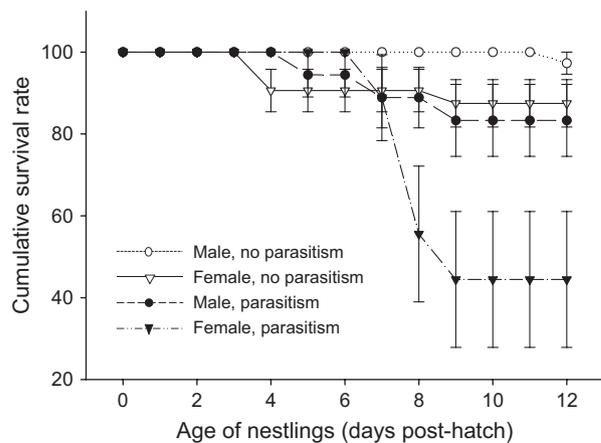


Figure 1. Survival \pm 1 se of male (circles) and female (triangles) host nestlings in the parasitism (closed symbols) and no parasitism (open symbols) treatments, compared over the entire duration of the brood-rearing period, estimated using the Kaplan–Meier procedure.

treatment suffered the highest mortality rates, with most deaths occurring after day 6 (Fig. 1). Female chicks in the parasitism treatment were more than three times as likely to die over the course of the experiment than any other category of host nestling (Fig. 1; mortality rates: females in the parasitism treatment, 0.56 ± 0.17 ; females in the no parasitism treatment, 0.12 ± 0.06 ; males in the parasitism treatment, 0.17 ± 0.09 ; males in the no parasitism treatment, 0.03 ± 0.03 ; *post-hoc* log-rank tests $P < 0.001$ – 0.049). Both sex and treatment by themselves affected mortality because females in non-parasitized nests were more likely to die than males in non-parasitized nests, and males in parasitized nests were more likely to die than males in non-parasitized nests (Fig. 1; *post-hoc* log-rank tests $P < 0.001$ – 0.043). The only two categories of host nestling that did not demonstrate a difference in mortality were females in the no parasitism treatment compared with males in the parasitism treatment (Fig. 1; log-rank test, $\chi^2_1 = 0.1$, $P = 0.72$).

Host offspring sex ratios

Experimentally parasitizing nests with an extra, larger, conspecific led to a three-fold decrease in the proportion of female host nestlings that fledged (Fig. 2). The proportion of host offspring that were female did not differ between the parasitism and no parasitism treatment either at hatch-

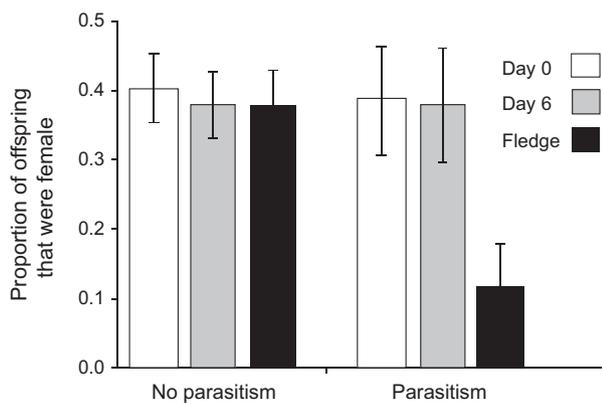


Figure 2. Sex ratios, expressed as the proportion of host nestlings that were female, compared between the parasitism and no parasitism treatments on hatch day (open bars), day 6 of brood-rearing (grey bars) and at fledging (black bars). Data from 11 nests in the parasitism treatment and 30 nests in the no parasitism treatment. Values are means \pm 1 se.

ing (Fig. 2; $\chi^2_1 = 0.1$, $P = 0.76$) or on day 6 (Fig. 2; $\chi^2_1 = 0.0$, $P = 0.99$), but then decreased significantly in the parasitism treatment only (Fig. 2; $\chi^2_1 = 4.7$, $P = 0.03$).

Nestling growth

There was no evident treatment effect on overall parental investment up to day 6, as total day 6 brood mass did not differ between the parasitism and no parasitism treatments (44.5 ± 1.36 vs. 46.3 ± 0.86 g; $F_{1,42} = 1.1$, $P = 0.30$). There were,

however, treatment effects on the differences amongst nestlings. The 'parasite' was significantly heavier than its host nest-mates (18.9 ± 0.53 vs. 15.4 ± 0.69 g; $F_{1,12} = 36.4$, $P < 0.001$), as well as being structurally larger (21.8 ± 0.23 vs. 18.6 ± 0.47 mm tarsus; $F_{1,12} = 44.3$, $P < 0.001$), and host nestlings in the parasitism treatment were significantly lighter and smaller than were nestlings in non-parasitized nests (Table 1). The presence of the 'parasite' also affected the variation in mass amongst its nest-mates, as the coefficient of variation in mass amongst host nestlings was two times greater in the parasitism than the no parasitism treatment (0.17 ± 0.03 vs. 0.08 ± 0.02 , respectively; $F_{1,37} = 7.4$, $P = 0.01$). Female host nestlings were consistently lighter and smaller than males and this did not differ significantly with treatment (Table 1).

Parental provisioning

There was no evident treatment effect on overall parental provisioning; parents in the parasitism treatment made a similar number of feeding trips as parents feeding young in non-parasitized nests (8.2 ± 0.60 per chick vs. 7.3 ± 0.60 per chick, respectively; $F_{1,24} = 1.0$, $P = 0.32$), and brought a similar amount of food (17.6 ± 1.8 per chick vs. 15.7 ± 1.8 per chick; $F_{1,24} = 0.6$, $P = 0.46$). Consistent with the results concerning mass and size, provisioning was not partitioned equally among nest-mates. The 'parasite' was more likely to get

Table 1. Means \pm 1 se for male and female host nestlings in the parasitism vs. no parasitism treatment on day 5 (provisioning and begging) and day 6 (for nestling growth) after hatch. Results from mixed-model ANOVAS using nest identity as a random effect are presented showing main effects of treatment (fixed effect) and sex (repeated measures) and their interaction.

	Parasitism treatment		No parasitism treatment		Treatment	Sex	Interaction
	Male	Female	Male	Female			
Nestling growth							
Mass (g)	16.4 ± 0.72	14.4 ± 0.88	17.9 ± 0.43	16.3 ± 0.54	$F_{1,39} = 4.5$ $P = 0.039$	$F_{1,32} = 9.4$ $P = 0.004$	$F_{1,32} = 0.1$ $P = 0.75$
Tarsus-length (mm)	19.3 ± 0.47	18.3 ± 0.55	20.7 ± 0.29	20.1 ± 0.35	$F_{1,36} = 9.4$ $P = 0.004$	$F_{1,29} = 5.4$ $P = 0.026$	$F_{1,29} = 0.5$ $P = 0.48$
Parental food provisioning							
Proportion of bouts fed	0.28 ± 0.029	0.26 ± 0.040	0.47 ± 0.031	0.35 ± 0.034	$F_{1,24} = 11.7$ $P = 0.002$	$F_{1,24} = 3.2$ $P = 0.086$	$F_{1,24} = 0.8$ $P = 0.39$
Quantity of food per bout	0.65 ± 0.097	0.53 ± 0.102	1.04 ± 0.106	0.74 ± 0.088	$F_{1,24} = 8.7$ $P = 0.007$	$F_{1,23} = 4.8$ $P = 0.039$	$F_{1,23} = 1.0$ $P = 0.33$
Nestling begging behaviour							
Begging height (mm)	31.3 ± 2.77	29.4 ± 2.82	21.4 ± 2.86	23.3 ± 2.67	$F_{1,24} = 4.8$ $P = 0.039$	$F_{1,16} = 0.0$ $P = 0.99$	$F_{1,16} = 2.0$ $P = 0.17$

fed than its nest-mates but the differences were not significant (0.37 ± 0.03 vs. 0.28 ± 0.02 ; $F_{1,12} = 4.1$, $P = 0.065$). However, the 'parasite' did receive significantly more food per food provisioning bout (0.79 ± 0.10 vs. 0.59 ± 0.07 ; Wilcoxon matched pairs test, $Z = 2.1$, $P = 0.039$), and host nestlings in the parasitism treatment were less likely to get fed and received significantly less food than nestlings in non-parasitized nests (Table 1). Female host nestlings were less likely to be fed and received less food per bout than males regardless of treatment (Table 1).

Nestling begging behaviour

Nestlings that did or did not get fed during a feeding bout differed significantly only in height obtained (27.1 ± 1.8 vs. 17.1 ± 2.0 mm; main effect $F_{1,24} = 187.4$, $P < 0.001$, treatment * feeding $F_{1,24} = 0.7$, $P = 0.40$) and posture (2.5 ± 0.10 vs. 1.8 ± 0.11 ; main effect $F_{1,24} = 6.0$, $P < 0.001$; treatment * feeding $F_{1,24} = 1.4$, $P = 0.25$). Height and posture were highly correlated (Spearman rank correlation, $r_s = 0.87$, $P < 0.001$) so we limited further analyses to height. Although the 'parasite' tended to reach higher than its nest-mates, the difference was not significant (33.7 ± 2.9 vs. 30.5 ± 2.8 mm; $F_{1,12} = 2.0$, $P = 0.17$) presumably because its presence prompted its nest-mates to reach higher, as host nestlings in the parasitism treatment obtained a significantly greater height than nestlings that were not parasitized (Table 1). Female host nestlings obtained the same height as their male nest-mates in both treatments (Table 1).

DISCUSSION

Experimentally parasitizing Song Sparrow nests with an extra, larger, conspecific nestling was sufficient to cause female-biased host nestling mortality that was extreme enough to result in a 60% reduction in the proportion of females fledged, thus demonstrating that Cowbird-specific traits are not necessary to cause these effects in Cowbird-parasitized nests. Our overall results on sex-biased mortality were similar to those of Zanette *et al.* (2005, 2012), who reported a 50% reduction in the proportion of offspring that were female when nests were naturally and experimentally parasitized with a Cowbird. Parents preferentially fed the 'parasitic' conspecific which, in turn, was heavier and structurally larger than its nest-mates. The

greater accumulated mass and greater amount of food received by the extra, larger conspecific evidently came at the cost of its nest-mates, which were correspondingly smaller and received less food than nestlings in non-parasitized nests. Female host offspring were generally the most neglected as they were fed less and were lighter and smaller than males.

Although Cowbird-specific traits are not necessary to cause female-biased host nestling mortality, our results indicate that Cowbird-specific traits may contribute to *when* females most often die in Cowbird-parasitized nests. A major difference between our current and past experiments (Zanette *et al.* 2012) is the pace at which host offspring mortality occurred. In the current experiment, mortality rates in general were relatively low up to day 6 after hatching but greatly increased thereafter in the parasitism treatment. Moreover, after day 6, the mortality rates of females were particularly elevated and it was after day 6 that we observed male-biased sex ratios in parasitized nests. In contrast, when we experimentally parasitized Song Sparrow nests with a Cowbird nestling, mortality rates were elevated and sex ratios already male-biased by day 6 (Zanette *et al.* 2012). To assess these differences between the two studies further, we compared the proportion of hatchlings that survived up to day 6, which confirmed that proportionately more hosts survived when in the nest with a conspecific (0.97 ± 0.06) vs. a Cowbird (0.72 ± 0.05 ; Mann-Whitney *U*-test, $Z = -2.1$, $P = 0.04$). When comparing between studies for non-parasitized nests, no significant differences were found (0.95 ± 0.03 vs. 0.93 ± 0.03 , conspecific-parasite vs. Cowbird; Mann-Whitney *U*-test, $Z = 0.63 \pm 0.53$, $P = 0.53$). Cowbird-specific traits clearly do have some effect on the behaviour of Song Sparrow hosts, which react to the presence of a Cowbird by exaggerating the frequency and amplitude of their vocalizations in response to the Cowbird nestling's exaggerated vocalizations (Pagnucco *et al.* 2008). That such responses to Cowbird-specific traits can translate to accelerated host mortality is supported by an experiment conducted by Hauber (2003) who found that in the early stages of brood-rearing (days 0–5), Eastern Phoebe *Sayornis phoebe* hosts experienced proportionately more brood loss in nests containing a larger Cowbird than those exposed to a larger conspecific (also see Dearborn 1998).

Our results on mortality rates and sex ratios clearly indicated that female nestlings suffered disproportionately more than males in the parasitism vs. no parasitism treatment. However, these disproportionate effects on mortality rates were not reflected in our measures of parental food provisioning or nestling mass. We found that females were at a disadvantage compared with males because females were fed less and were lighter than males. However, females were neither fed disproportionately less in experimentally parasitized nests nor were they disproportionately lighter than males in this treatment (in contrast to Zquette *et al.* 2012). We suggest that because provisioning and mass were measured by day 6, before the majority of host mortality occurred, sex-biased asymmetries in provisioning and mass had not yet become extreme in the parasitism treatment. Interestingly, our current results on food provisioning and mass between treatments perhaps did provide a signal that a rise in female mortality rates was imminent when we parasitized nests with a conspecific. The presence of the extra, larger conspecific did not cause parents to provide more food to the brood, as there was no treatment effect on either brood mass or overall parental provisioning per chick. Consequently, the greater mass and greater amount of food received by the 'parasite' came at the cost of its nest-mates, who were correspondingly smaller and received less food than nestlings that were not parasitized (Table 1). Consistent with the known sexual size dimorphism in Song Sparrows, male host nestlings were larger and received more food than females (Table 1). Due to the combination of both treatment and sex effects, female host nestlings in experimentally parasitized nests were the smallest and least well fed among the four categories of host nestlings. The presence of the extra, larger conspecific significantly increased the variation in mass among its nest-mates, compared with nestlings that were not parasitized, and female host nestlings in experimentally parasitized nests were notably also the most variable in mass (see Table 1). Being the smallest, least well fed and most variable presumably made female host nestlings in experimentally parasitized nests the most vulnerable of the four categories of host nestlings, consistent with their significantly greater mortality (Fig. 1).

Our results indicate that some general perturbation associated with brood parasitism *per se* can lead to female-biased mortality rates and male-biased

sex ratios at least in Song Sparrows. Our experiment was not designed to determine which features of brood parasitism are involved but it is likely that more than one comes into play. Brood parasitism by Cowbirds leads to there being a larger, extra and unrelated individual in the nest. Accumulating evidence indicates that each of these factors on their own can enhance sex differences during development with respect to competitive ability and/or nestling size (Uller 2006). Relatively few studies have been designed to test whether these sex differences translate into differential effects in terms of mortality or brood sex ratios (Uller 2006) but of those studies that have done so, few find any sex differences in survival (e.g. Sheldon *et al.* 1998, Oddie 2000, Råberg *et al.* 2005, Rowland *et al.* 2007). One possible reason for this could be that most experiments have typically been interested in how one factor specifically affects nestlings, and therefore have manipulated that factor while keeping the others constant. Cowbird parasitism, by contrast, can alter all three at the same time, as is the case in Song Sparrows. Across host species, Cowbird parasitism can be expected to regularly alter at least two of these factors (size and relatedness) and potentially all three in many cases (Zquette *et al.* 2012), which may heighten competition to such an extent that sex-biased mortality results.

The conservation implications of our results could be wide-reaching. Zquette *et al.* (2012) found that the loss of female Song Sparrow offspring as a result of Cowbird-mediated nestling competition was an important determinant of Song Sparrow demography. Specifically, this mechanism was shown to be as important as nest predation in its influence on host reproductive success (i.e. number of females fledged per female per year). Any mortality that is sex-biased can affect populations, but in general it is the loss of females that is more likely to destabilize population dynamics and promote their collapse (Boukal *et al.* 2008). Thus, establishing how widespread this effect is among the 144 species known to be parasitized by Brown-headed Cowbirds has important implications for songbird conservation, because the Cowbird is an invasive species across most of North America and has been implicated in regional population declines of many native species (Lowther 1993, Stratford & Robinson 2005, Tewksbury *et al.* 2006). Cowbirds are unrelated to all their hosts, are larger than the great majority and a Cowbird nestling's presence can mean there is an extra mouth to feed (Hauber

2003, Kilner *et al.* 2004, Zanette & Clinchy 2010). Thus, sex-biased mortality in parasitized nests may be expected to occur across a range of host species (Zanette *et al.* 2012). Moreover, 15 of the 17 most commonly parasitized Cowbird hosts are not only smaller than the Cowbird, but the hosts also demonstrate a modest degree of sexual size dimorphism (males *c.* 5% larger than females; Lowther 1993, Råberg *et al.* 2005, Dunning 2008), which is expected to put females (more than males) at a disadvantage, particularly when conditions become harsh (Råberg *et al.* 2005, Uller 2006, Benito & González-Solís 2007, Nicolaus *et al.* 2009), as when a nest has been parasitized. Because sex ratios can affect the extinction probabilities of populations, it is critical that the factors that influence them be investigated and results incorporated into demographic models (Donald 2007). For Cowbird–host systems specifically, the need to do so is even more pressing, given that Cowbirds are a source of conservation concern.

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