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Mothers front-load their investment to the egg stage when helped in a wild cooperative bird

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Keywords: plasticity, cooperation, maternal effects, load-lightening, differential allocation, maternal front-loading

Author Contributions: P.C-L. and A.J.Y. conceived and designed the study. P.C-L. contributed to long-term data collection, performed all analyses and wrote the first draft of the manuscript. A.J.Y. initiated and ran the long-term field study, provided advice on fieldwork and statistical analysis, and edited the manuscript with P.C-L. A.J.W. provided statistical advice and edited the manuscript. All authors commented on the manuscript.

Data accessibility
The main datasets generated and analysed during the current study are available from the Dryad Digital Repository:
https://datadryad.org/stash/share/zpeQlUMYXxEO4MamQW8orMkhFQyB0NPxuYYY6mLoFiE

Competing Interest Statement: No competing interests.

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Abstract

In many cooperative societies, including our own, helpers assist with the post-natal care of breeders' young, and may thereby benefit the *post-natal* development of offspring. Here we present evidence of a novel mechanism by which such post-natal helping could also have hitherto unexplored beneficial effects on *pre-natal* development: by lightening post-natal maternal workloads, helpers may allow mothers to increase their pre-natal investment per offspring. We present the findings of a decade-long study of cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali,* societies. Within each social group, reproduction is monopolized by a dominant breeding pair, and non-breeding helpers assist with nestling feeding. Using a within-mother reaction norm approach to formally identify maternal plasticity, we demonstrate that when mothers have more female helpers they decrease their own *post-natal* investment per offspring (feed their nestlings at lower rates) but increase their *pre-natal* investment per offspring (lay larger eggs, which yield heavier hatchlings). That these plastic maternal responses are predicted by female helper number, and not male helper number, implicates the availability of post-natal helping *per se* as the likely driver (rather than correlated effects of group size), because female helpers feed nestlings at substantially higher rates than males. We term this novel maternal strategy "maternal front-loading" and hypothesize that the expected availability of *post-natal* help allows helped mothers to focus maternal investment on the *pre-natal* phase, to which helpers cannot contribute directly. Such cryptic maternally mediated helper effects on pre-natal development may markedly complicate attempts to identify and quantify the fitness consequences of helping.

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Introduction

Maternal effects arising from variation in pre-natal maternal investment in the egg or fetus can have profound fitness consequences for mothers and offspring (Mousseau & Fox 1998; Krist 2011; Pick *et al.* 2016). In social organisms, mothers are predicted to evolve investment strategies that maximize their fitness returns on investment according to their social environment (Hatchwell 1999; Cunningham & Russell 2000; Russell *et al.* 2007; Bolund *et al.* 2009; Russell & Lummaa 2009). Cooperatively breeding species are of particular interest in this regard, as helpers typically contribute to the post-natal feeding of the offspring of breeding females (hereafter 'mothers') and thus have the potential to impact the optimal level of maternal pre-natal investment per offspring (Russell *et al.* 2007; Russell & Lummaa 2009; Savage *et al.* 2015; Langmore *et al.* 2016). Where mothers are assisted by variable numbers of helpers throughout their lives, selection may be expected to favor plastic strategies in which mothers adjust their pre-natal investment per offspring according to the likely availability of help during the post-natal period (Russell & Lummaa 2009).

Different maternal strategies for adjusting pre-natal investment per offspring to the presence of helpers are hypothesized to evolve depending on how helpers impact the maternal payoff from pre-natal investment. Helpers have the potential to *decrease* the mother's optimal level of pre-natal investment per offspring, leading to strategies in which mothers *reduce* pre-natal investment per offspring when assisted by more helpers (Russell *et al.* 2007; Russell & Lummaa 2009; Canestrari *et al.* 2011; Paquet *et al.* 2013). The 'Load-Lightening Hypothesis' (Hatchwell 1999; Russell *et al.* 2007) envisages that selection could favor such a maternal strategy if helpers (i) increase the overall provision of *post-natal* care to offspring (i.e., provide 'additive post-natal care'; (Hatchwell 1999)) and thereby (ii) compensate, in part or whole, for any maternal reduction in *pre-natal* investment per offspring when helped (formally modelled as the 'head start' scenario in (Savage *et al.* 2015)). Notably, this hypothesis requires that helper-derived post-natal care can compensate for reductions in maternal pre-natal investment (i.e., that investment can be 'substituted across stages'; (Savage *et al.* 2015)), which may not always be the case (Williams 1994; Royle *et al.* 2005; Savage *et al.* 2015). Indeed, there is ample evidence that pre-natal conditions, and pre-natal maternal investment in particular, can have formative effects on

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offspring phenotype and performance (Williams 1994; Henry & Ulijaszek 1996; Hales & Barker 2001; Royle *et al.* 2005; Krist 2011; Pick *et al.* 2016, 2019).

Helpers also have the potential to *increase* the mother's optimal level of pre-natal investment per offspring, leading to strategies in which mothers instead *increase* pre-natal investment per offspring when assisted by more helpers (Russell & Lummaa 2009; Savage *et al.* 2015; Langmore *et al.* 2016; Valencia *et al.* 2017). The 'Differential allocation hypothesis', for example, proposes that mothers should increase maternal investment under circumstances that increase their expected return on investment in their current breeding attempt, such as the presence of a high quality mate or more helpers (Burley 1986; Sheldon 2000; Valencia *et al.* 2006, 2017; Russell & Lummaa 2009; Horváthová *et al.* 2012; Langmore *et al.* 2016). This hypothesis was originally proposed in the context of non-cooperative species (Burley 1986; Sheldon 2000; Horváthová *et al.* 2012), before being verbally extrapolated to cooperative breeders, with the suggestion that, as helpers commonly increase the reproductive value of offspring by providing additive post-natal care, mothers should increase investment per offspring when helped (Valencia *et al.* 2006, 2017; Carranza *et al.* 2008; Russell & Lummaa 2009; Langmore *et al.* 2016; Dixit *et al.* 2017). More specifically, the provision of additive *post-natal* care by helpers may increase the mother's return on *pre-natal* investment per offspring wherever pre- and post-natal investment have positive interactive effects on offspring fitness (such that *post-natal* helping increases the effect of maternal *pre-natal* investment on offspring fitness; (Savage *et al.* 2015)). Indeed, mathematical models incorporating such interactive effects of pre- and post-natal investment per offspring (the 'silver spoon' scenario in (Savage *et al.* 2015)) predict that, where helpers contribute to post-natal care, mothers should *increase* both pre- and post-natal investment per offspring when helped.

Cooperatively breeding birds provide a fruitful testing ground for these hypotheses, given the ability to estimate maternal pre-natal investment per offspring across different helping contexts by measuring egg traits. Several studies of cooperative birds have now reported that, after controlling for variation in clutch size, mothers with (more) helpers lay smaller eggs; the pattern predicted by the load-lightening hypothesis (e.g., *Malurus cyaneus* (Russell *et al.* 2007); *Corvus corone corone* (Canestrari *et al.* 2011); *Vanellus chilensis* (Santos & Macedo 2011); *Philetairus socius* (Paquet *et al.* 2013); see also Taborsky *et al.* (2007) for an experimental demonstration in

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fish). Three studies of cooperative birds have reported no evident relationship between egg size and the availability of help (Koenig *et al.* 2009; Lejeune *et al.* 2016; Fortuna *et al.* 2021), and just one study has reported the reverse relationship. Iberian magpie (*Cyanopica cooki)* mothers with more helpers lay larger eggs *and* feed their nestlings at higher rates, consistent with the predictions of the differential allocation hypothesis (Valencia *et al.* 2006, 2017; Savage *et al.* 2015). The situation may be more complex in some cases, however, as recent work suggests that the previously reported negative relationship between egg size and the availability of help in super fairy-wrens (Russell *et al.* 2007) becomes more positive under warmer conditions (Langmore *et al.* 2016). Given the overall weight of evidence for negative relationships across species, meta-analysis of these collated findings has led to the suggestion that helpers commonly *decrease* the mother's optimal level of pre-natal investment per offspring, and that the rationale of the load-lightening hypothesis may therefore commonly apply (Dixit *et al.* 2017).

Crucially though, it has yet to be demonstrated that any of these associations between helper number and egg size in cooperative birds arise specifically from maternal *plasticity* (i.e., *within*-mother variation in egg size; see (Taborsky *et al.* 2007)). They could arise instead from among-mother variation in egg size being correlated with among-mother variation in helper number (e.g., mothers on higher quality territories might simply lay larger eggs and have more offspring that survive to become helpers). Indeed, a study that explicitly teased apart the effects of within- and among-mother variation in helper number found that the negative relationship initially detected between helper number and egg volume in red-winged fairy wrens (*Malurus elegans*) arose from among-mother variation in egg volume rather than maternal plasticity (within-mother variation), illustrating the importance of taking this approach (Lejeune *et al.* 2016). While this same approach has been used to identify plasticity in egg size according to abiotic conditions (e.g., temperature; (Langmore *et al.* 2016)), evidence of maternal plasticity in egg size according to the availability of help *per se* does not yet exist for cooperative birds. As such, it remains unclear whether avian mothers ever do adjust their pre-natal investment per offspring according to helper number, and whether any such maternal plasticity conforms to the predictions of the load-lightening or differential allocation hypotheses.

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Here, we use a long-term field study of cooperatively breeding white-browed sparrow weavers, *Plocepasser mahali*, to test the key predictions of the load-lightening and differential allocation hypotheses for the evolution of maternal plasticity in pre-natal investment. We do so by testing for maternal plasticity in both pre-natal investment per offspring (egg volume) and post-natal investment per offspring (maternal nestling provisioning rate) according to the availability of help. We test for plasticity using a maternal reaction norm approach, in which we isolate the effects of *within*-mother variation in helper number on maternal investment (i.e., maternal plasticity) from potentially confounding effects of variation among mothers (van de Pol & Wright 2009; Lejeune *et al.* 2016). White-browed sparrow-weavers live in social groups of 2-12 birds, in which a single dominant female ('the mother') and male monopolize within-group reproduction and non-breeding subordinate 'helpers' of both sexes help to feed their nestlings (Lewis 1982; Harrison *et al.* 2013). Helpers are typically past offspring of the dominant breeding pair, and hence are usually helping to rear close kin (Harrison *et al.* 2013). Female helpers feed nestlings at approximately twice the rate of male helpers, and female helper number has a causal positive effect on the total rate at which broods are fed while male helper number does not (i.e., only female helpers provide demonstrably additive post-natal care; (Capilla-Lasheras *et al.* 2021)). That only female helpers provide additive post-natal care provides an unusual opportunity to distinguish the hypothesized pre-natal maternal responses to the availability of additive help (which should manifest in this species as maternal responses to the number of *female* helpers) from maternal responses to group size more generally (which could influence maternal investment through mechanisms other than helping; (Kokko *et al.* 2001; Kingma *et al.* 2014)).

Sparrow-weaver mothers lay small clutches of 1-3 eggs (modal clutch size = 2), and do not adjust their clutch size or the number of clutches laid per year according to helper numbers (Supplementary materials A). Indeed, given their small clutch size, subtle adjustments in pre-natal maternal investment may be more readily achieved through changes in investment per egg than through changes in clutch size. The focal hypotheses assume that laying mothers are able to predict the helper numbers that they will have during the post-natal rearing period, in order to adjust their own pre-natal investment per offspring accordingly. This should be straightforward in sparrow-weaver societies, as both male and female helper numbers at laying strongly predict male and female helper numbers respectively during the post-natal rearing period (Figure S1).

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We assess pre-natal maternal investment per offspring by quantifying egg volume, which in this species is strongly correlated with egg mass at laying and strongly predicts nestling mass at hatching (see Results). Maternal variation in egg volume is therefore likely to have fitness implications for offspring (and their mothers), not least because nestling mass at hatching positively predicts nestling survival to fledging in this species (Capilla-Lasheras *et al.* 2021).

We test the following key predictions of the two focal hypotheses. The load-lightening hypothesis ('head start' scenario in (Savage *et al.* 2015)) predicts that sparrow-weaver mothers should decrease egg volume when assisted by more female, but not male, helpers. The differential allocation hypothesis ('silver spoon' scenario in (Savage *et al.* 2015)) predicts that sparrow-weaver mothers should increase both egg volume and their nestling provisioning rate when assisted by more female, but not male, helpers. To test these predictions, we first investigate whether within-mother variation in female and male helper numbers at laying predicts variation in egg volume (utilizing a large longitudinal data set; 271 clutches [490 eggs] laid by 62 mothers; 1-21 clutches [median 7] per mother). We then investigate whether within-mother variation in female and male helper numbers predicts variation in the mother's nestling feeding rate (again utilizing a large longitudinal data set; 108 broods being fed by 48 mothers; 1-10 broods [median 4] per mother). Our analyses control for the effects of variation in abiotic conditions (rainfall and temperature) as these could also influence mean levels of maternal investment (Bennion & Warren 1933; Blanckenhorn 2000) and/or any maternal response to the availability of help (Langmore *et al.* 2016).

Results

Maternal plasticity in pre-natal investment per offspring: individual mothers lay larger eggs when they have more female helpers

Sparrow-weavers show appreciable variation in egg volume both within and among mothers (Figure 1a). Egg volume appears to provide a valid proxy for pre-natal maternal investment per offspring, as higher volume eggs were heavier at laying (Figure 1b; LM: $\beta_{standardized}$ ± Standard Error [S.E.] = 0.946 ± 0.016; N = 390 eggs with volume and laying mass data; ΔAIC = 878.5) and yielded heavier nestlings at hatching (Figure 1c; LM: $\beta_{standardized}$ ± S.E. = 0.468 ± 0.048; N = 342

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eggs with volume and hatchling mass data; ΔAIC = 82.56). These relationships also hold within mothers, illustrating that maternal *plasticity* in egg volume is also a key source of variation in egg mass at laying (LM: $\beta_{standardized}$ ± S.E. for the effect of *within*-mother variation in egg volume = 0.484 ± 0.016; N = 390 eggs with volume and laying mass data from 63 mothers; ΔAIC = 456.40) and nestling mass at hatching (LM: $\beta_{standardized}$ ± S.E. for the effect of *within*-mother variation in egg volume = 0.282 ± 0.048; N = 342 eggs with volume and hatchling mass data from 59 mothers; ΔAIC = 31.32). Laying larger eggs may therefore have fitness consequences for mothers and the resulting offspring, as larger eggs yield heavier hatchlings (Figure 1c) and heavier hatchlings in this species are more likely to survive to fledging (Capilla-Lasheras *et al.* 2021).

Mothers that had more female helpers at laying laid larger eggs (Figure 2a; Table 1; β ± S.E. = 20.28 ± 7.94; ΔAIC = 3.71; N = 490 eggs laid in 271 clutches by 62 mothers in 37 social groups [1-21 clutches [median = 7] measured per mother]). Partitioning the female helper number predictor within the best-supported model into its within- and among-mother components revealed evidence of maternal plasticity in egg volume (i.e., a maternal reaction norm to variation in female helper number): individual mothers laid larger eggs when they had more female helpers (Figure 2b). Specifically, the effect size for within-mother variation in female helper number (Δ female helper number; β ± S.E. = 21.26 ± 8.41; Figure 2b) matched or exceeded that for among-mother variation in mean female helper number (β ± S.E. = 12.51 ± 23.57). Accordingly, repeating model selection using the partitioned forms of both the female and male helper number predictors led to the retention of the Δ female helper number term within the best-supported model (Table S2). The observed relationships between female helper number and egg volume cannot be readily attributed to correlated variation in abiotic environmental conditions affecting both variables as our models simultaneously allowed for effects of both rainfall and temperature on egg volume (see below and Supplementary materials B).

We found weaker evidence that male helper numbers predict variation in egg volume. When modelling the effects of population-level variation in female and male helper numbers, the best supported model containing a 'male helper number' effect was 1.40 AIC points below the top model (Table 1) and would be rejected under the nesting rule (see Table 1 legend). Accordingly, in the best-supported model that contained *both* the female helper number and male helper

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number predictors, the effect size for female helper number (β ± S.E. = 17.86 ± 8.53; Table 1) markedly exceeded that for male helper number (β ± S.E. = 7.37 ± 9.51; Table 1). The same pattern was apparent when we repeated model selection using the partitioned forms of both the female and male helper number predictors: the effect size for within-mother variation in female helper number (β ± S.E. = 18.88 ± 8.99) markedly exceeded that for within-mother variation in male helper number (β ± S.E. = 7.51 ± 10.12) within the best-supported model that contained both terms (Table S2). Egg volume was also predicted by the position of the egg in the laying order (the first laid egg was consistently larger; Table 1) and by environmental temperature and rainfall (Table 1; the effects of these abiotic predictors are discussed in detail in Supplementary materials B). While our analyses allowed for an effect of clutch size on egg volume, no such association was detected (Table 1).

Maternal plasticity in post-natal investment: individual mothers provision nestlings at lower rates when they have more female helpers

Mothers that had more female helpers during the nestling period showed lower nestling provisioning rates (Figure 2c; Table 2; β ± S.E. = -0.47 ± 0.20; ΔAIC = 3.23; N = 108 broods being fed by 48 mothers in 34 social groups [1-5 broods [median = 2] per mother]). Partitioning the female helper number predictor within the best-supported model into its within- and among-mother components revealed evidence of maternal plasticity in nestling provisioning rate: individual mothers decreased their nestling provisioning rate when they had more female helpers. Specifically, the effect size for within-mother variation in female helper number (Δ female helper number; β ± S.E. = -0.47 ± 0.29; Figure 2d) matched that for among-mother variation in mean female helper number (β ± S.E. = -0.46 ± 0.30). When the within- and among-mother effect sizes match in this way, the effect size for population-level variation in female helper number (i.e., prior to partitioning; β ± S.E. = -0.47 ± 0.20) captures the within-mother effect (van de Pol & Wright 2009). Accordingly, repeating model selection using the partitioned forms of both female and male

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helper number led to the retention of Δ female helper number within the best-supported model (Table S4).

Again, we found weaker evidence that male helper numbers predict variation in maternal nestling provisioning rate. When modelling the effects of population-level variation in female and male helper numbers, the best supported model containing a 'male helper number' effect was 0.93 AIC points below the top model (Table 2) and would be rejected under the nesting rule (see Table 2 legend). Accordingly, in the best-supported model that contained both the female helper number and male helper number predictors, the effect size for female helper number (β ± S.E. = -0.43 ± 0.20; Table 2) was more strongly negative than that for male helper number (β ± S.E. = -0.26 ± 0.23; Table 2). The same pattern was apparent when we repeated model selection using the partitioned forms of both the female and male helper number predictors: the effect size for *within*-mother variation in female helper number (β ± S.E. = -0.45 ± 0.29) was more strongly negative than that for *within*-mother variation in male helper number (β ± S.E. = -0.16 ± 0.34) within the best-supported model that contained both terms (Table S4). Maternal nestling provisioning rates were also positively related to brood size (Table 2) and were predicted by environmental temperature and rainfall (Table 2; discussed in Supplementary materials B).

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Discussion

To test the predictions of the 'load-lightening' and 'differential allocation' hypotheses for the evolution of pre-natal investment strategies in cooperative breeders, we investigated the patterns of maternal plasticity in both pre- and post-natal investment per offspring in white-browed sparrow weaver societies. Using a within-mother reaction norm approach, our analyses revealed the first formal evidence of maternal plasticity in egg investment according to the availability of help in a cooperatively breeding bird (see Introduction and Taborsky *et al.* 2007). When sparrow-weaver mothers had more female helpers they laid larger eggs (and larger eggs yield heavier hatchlings, which are more likely to survive to fledging; (Capilla-Lasheras *et al.* 2021)). This maternal plastic response runs counter to the leading 'load-lightening hypothesis' (which predicts that helped mothers should lay smaller eggs; (Russell *et al.* 2007; Savage *et al.* 2015)) and counter to general expectation given empirical work to date (Dixit *et al.* 2017). The 'differential allocation hypothesis' does predict that helped mothers should lay larger eggs (as we observe), but is thought to predict that helped mothers should also feed their nestlings at higher rates (i.e., mothers should increase both pre- *and* post-natal investment per offspring when helped; see 'silver spoon' scenario in (Savage *et al.* 2015); the pattern observed at the population level in Iberian magpies (Valencia *et al.* 2006, 2017)). By contrast, our findings reveal a novel maternal strategy in which mothers with more (female) helpers appear to *increase* pre-natal investment per offspring (lay larger eggs) but *decrease* post-natal investment per offspring (feed their nestlings at lower rates). We term this strategy 'maternal front-loading', as mothers effectively front-load their investment to the pre-natal stage when helped. We consider adaptive explanations for this strategy below, along with its implications for identifying the benefits of helping in cooperative societies. Notably, maternal front-loading provides a mechanism by which *post-natal* helping could have beneficial effects on the *pre-natal* development of young.

While relationships between helper number and egg size have previously been reported in cooperatively breeding birds ((Dixit *et al.* 2017); and see Introduction), our findings are the first to demonstrate that such a pattern arises from within-mother plasticity. This is important, as recent work has highlighted that population-level relationships between helper number and egg size (i.e., those reported to date: e.g., (Russell *et al.* 2007; Canestrari *et al.* 2011; Paquet *et al.* 2013;

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Langmore *et al.* 2016; Valencia *et al.* 2017)), can arise from among-mother variation in egg size rather than within-mother plasticity (Lejeune *et al.* 2016). Furthermore, that sparrow-weaver mothers appear to adjust egg size according to female helper number and not male helper number implicates the availability of post-natal helping *per se* as the likely driver of this plastic maternal response, rather than correlated variation in group size (as female helpers feed nestlings at twice the rate of male helpers, and only female helper number has a causal positive effect on the overall rate of nestling provisioning; (Capilla-Lasheras *et al.* 2021)). Indeed, as female and male helper numbers at laying strongly predict helper numbers during the post-natal care period (Figure S1), sparrow-weaver mothers should have sufficient information at laying to adjust their egg volume to the future availability of post-natal help, were it adaptive to do so. Such a pattern of investment per egg could conceivably emerge as a by-product of a helper effect on the mother's optimal clutch size or number of clutches per year, with which egg volume could trade off (Lejeune *et al.* 2016). This mechanism cannot readily account for our findings, however, as sparrow-weaver mothers vary neither clutch size nor clutch number according to helper numbers (Supplementary materials A). Additional analyses also suggest that maternal plasticity in egg volume cannot be readily attributed to carry-over effects on maternal condition of helper actions in previous breeding attempts (see Supplementary materials C).

The 'differential allocation hypothesis' as applied to cooperative breeders does predict the pattern of egg investment observed here: mothers should lay larger eggs when helped (Russell & Lummaa 2009; Savage *et al.* 2015; Valencia *et al.* 2017). In general terms, the hypothesis proposes that mothers should increase maternal investment under circumstances that increase their return on investment in the current breeding attempt, such as having a more attractive mate or more helpers (Sheldon 2000; Russell & Lummaa 2009). Accordingly, theoretical models that apply the differential allocation rationale specifically to pre-natal investment in cooperative breeders (by having the mother's return on pre-natal investment per offspring increase when she has help with post-natal care; (Savage *et al.* 2015)), predict that mothers should increase both their pre- *and* post-natal investment per offspring when helped. These predictions are consistent with the patterns observed in the only other species to date in which mothers are thought to consistently lay larger eggs when they have more help: Iberian magpie mothers with helpers appear to lay larger eggs *and* provision their nestlings at higher rates than those without helpers

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((Valencia *et al.* 2006, 2017); but whether either reflects maternal plasticity is unknown). It is notable then that sparrow-weaver mothers instead increase pre-natal investment per offspring while *decreasing* post-natal investment per offspring when helped (i.e., engage in 'maternal front-loading'); a pattern that is at odds with this set of predictions.

It is conceivable that the core rationale of the differential allocation hypothesis nevertheless can account for the pattern of egg investment observed here, as the biological processes and/or parameter space explored in the theoretical work to date (Savage *et al.* 2015) might not capture all relevant aspects of the biology at play. As per the differential allocation rationale, sparrow-weaver mothers could increase egg size with female helper number because the additive post-natal care that their female helpers will provide (Capilla-Lasheras *et al.* 2021) increases the mother's expected return on investment per egg. For example, producing larger hatchlings may yield a greater payoff when those hatchlings stand to be fed at higher rates (Savage *et al.* 2015; Langmore *et al.* 2016). Our findings reveal that such additive post-natal care by helpers (Capilla-Lasheras *et al.* 2021) is accompanied here by mothers decreasing their own post-natal contributions when helped; a maternal strategy of post-natal 'partial compensation' commonly observed in cooperative breeders (Hatchwell 1999). As the evolution of such maternal post-natal partial compensation is generally attributed to post-natal care yielding diminishing returns (Hatchwell 1999; Heinsohn 2004), it would be valuable to now establish whether the integration of more strongly diminishing returns of post-natal care into existing models of the differential allocation hypothesis (Savage *et al.* 2015) would leave them predicting the maternal front-loading strategy observed here. The integration of stronger maternal trade-offs between pre- and post-natal investment and/or higher costs to mothers of post-natal investment might also resolve the apparent disparity.

While it may ultimately prove possible to reconcile the differential allocation hypothesis with the maternal investment strategy observed here (see above), our findings do highlight a simpler explanation for sparrow-weaver mothers laying larger eggs when helped. The differential allocation hypothesis envisages that helpers increase the maternal *benefit* of pre-natal investment per offspring (e.g., via the provision of additive post-natal care; (Savage *et al.* 2015)). However, helpers may instead reduce the maternal *cost* of pre-natal investment per offspring by reducing

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maternal post-natal workloads. Maternal front-loading may therefore reflect an anticipatory strategy in which the expected lightening of maternal *post-natal* workloads allows helped mothers to focus their investment on the *pre-natal* phase, to which helpers cannot contribute directly. Such a maternal strategy may therefore be of particular benefit when pre-natal investment has differentially large effects on offspring fitness. Under this scenario, the maternal increase in egg investment when helped is a consequence of the helper effect on the mother's post-natal workload, whereas under the differential allocation hypothesis the increase is typically considered a product of the additive effect of helpers on the overall provision of post-natal care (Russell & Lummaa 2009; Langmore *et al.* 2016; Dixit *et al.* 2017; Valencia *et al.* 2017). Species in which helpers lighten maternal post-natal workloads but do *not* have additive effects on post-natal care (because the maternal reduction in post-natal work rate completely compensates for helper contributions; (Hatchwell 1999)) would therefore provide a fruitful testing ground for testing these alternative, though not mutually exclusive, hypotheses. As maternal post-natal load-lightening is often reported in cooperative breeders (Hatchwell 1999; Heinsohn 2004; Russell *et al.* 2007; Kingma *et al.* 2010), the maternal front-loading strategy observed here could ultimately prove more commonplace once more studies formally characterize maternal plasticity in egg investment (Lejeune *et al.* 2016). Indeed, recent evidence suggesting that superb fairy-wren mothers with helpers lay larger eggs than those without when conditions are warm (Langmore *et al.* 2016) could reflect maternal front-loading in warm conditions, if the reported population-level relationship between egg size and the availability of help arose via maternal plasticity, and if post-natal load-lightening also occurred under such warm conditions (which it could well do; (Russell *et al.* 2008)).

Where mothers do front-load their investment to the pre-natal stage when helped (as observed here), post-natal helping may have hitherto unexplored beneficial effects on the pre-natal development of offspring. The potential for such cryptic 'pre-natal helper effects' has important implications for attempts to identify and quantify the benefits of helping in cooperative societies. First, while it has been suggested that studies of helper effects on offspring should control for variation in egg size in order to ensure that maternal *reductions* in egg size by helped mothers do not 'conceal' helper effects on offspring (Russell *et al.* 2007), our findings highlight a danger of this approach. If, as here, plastic mothers lay *larger* eggs when helped, controlling for variation in

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egg size could lead to the *underestimation* of helper effects on offspring, by factoring out helper effects that arise indirectly via maternal investment in the egg. Second, while helper-induced reductions in maternal post-natal workloads are typically thought to benefit mothers (e.g., by improving maternal survival; (Hatchwell 1999; Russell *et al.* 2007)), our findings highlight that anticipatory changes in egg investment may actually pass these benefits, in part or whole, to the offspring being reared. Indeed, as helpers commonly lighten maternal post-natal workloads (Hatchwell 1999; Heinsohn 2004; Russell *et al.* 2008; Kingma *et al.* 2010), it is conceivable that a maternal front-loading response of the type observed here has actually contributed to the positive relationships already described in numerous species between helper numbers and offspring survival or performance.

Conclusion

Our findings provide the first formal evidence of maternal plasticity in pre-natal investment per offspring according to the availability of help in a natural population (Taborsky *et al.* 2007). They reveal a plastic maternal pre-natal response that runs directly counter to the predictions of the leading load-lightening hypothesis and to general expectation given the limited empirical work to date (Dixit *et al.* 2017). The patterns of maternal plasticity in *post-natal* investment that we also document suggest that the overall maternal strategy does not match the existing predictions of the differential allocation hypothesis either (Savage *et al.* 2015), and instead highlight an alternative explanation for mothers increasing their egg size when helped: by lightening maternal post-natal workloads, helpers may allow mothers to focus their investment on the pre-natal stage, to which helpers cannot contribute directly. The novel ‘maternal front-loading’ strategy that sparrow-weaver mothers appear to employ has important implications for attempts to both identify and quantify the benefits of helping; the best-studied form of animal cooperation.

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Materials and Methods

General Field Methods

White-browed sparrow-weavers live in semi-arid regions of East and Southern Africa. Our study population is located in Tswalu Kalahari Reserve in the Northern Cape Province of South Africa (27°16'S, 22°25' E). Fieldwork was carried out from September to April between 2007 and 2016 inclusive. Approximately 40 social groups were monitored, each defending a small exclusive territory within an overall study site of approximately 1.5 km^2. Sparrow-weaver groups were easily monitored and distinguished in the field as all group members foraged together, engaged in communal weaving and territory defense, and roosted within in a single tree or cluster of trees close to the center of their territory. All birds in the study population were fitted with a single metal ring and three color rings for identification from the time they were first detected in the population (under SAFRING license 1444). The sex of each bird could be determined after six months of age using the sex difference in bill color (Leitner *et al.* 2009).

Each social group contains a single behaviourally dominant female. The dominant female is easily identified in the field because she displays a distinct set of behaviours: being behaviourally dominant to other females, being the only female observed to incubate the eggs or enter the nest during the incubation phase, and closely associating with and frequently duetting with the dominant male (Walker *et al.* 2016). Genetic analyses have confirmed that the dominant female is always the mother of any eggs or chicks produced on their group's territory; subordinate females never breed (Harrison *et al.* 2013). For brevity, we therefore refer throughout the paper to the dominant female as the 'mother'.

Each group's territory was regularly monitored (every one or two days while nests were present) to detect new clutches. Once a new clutch was found, egg length and maximum width were measured with a plastic calliper to the nearest 0.1 mm. Nests were then checked daily until the clutch had been completed. Clutches were then checked 8 days after the first egg was laid (to confirm the progression of incubation), before daily checks were resumed 15 days after the first egg was laid, until the fate of every egg had been determined (hatch or failure). Hatchlings were weighed on their first day of life using a portable scale to the nearest 0.01 g.

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The composition of each social group was assessed every week throughout each field season, with birds being identified on the basis of their color-ring combination. Birds were also routinely caught while roosting within their group's territory at night, and this information also contributed to group composition assessments. Group compositions were typically very stable over time, with group members residing within the same social group for many months to many years at a time (i.e., group composition not being affected by short-term fluctuations in environmental conditions). For every breeding attempt in our analyses, we used these group compositions to calculate the number of male and female helpers that the dominant female (mother) had on the day of laying (for the egg volume analyses) and on the days that provisioning behaviour was recorded (for the maternal provisioning rate analyses). All subordinate group members over the age of 6 months were considered helpers, as analyses of helper contributions suggest that subordinates < 6 months old contribute little to nestling provisioning (Lewis 1982; Capilla-Lasheras 2020).

Nestling provisioning behaviour

Nestling provisioning behaviour was recorded for 174 breeding attempts between September 2007 and April 2016. We collected provisioning data using video recordings of the birds visiting the nest (viewed from below the nest) between the 10th and 12th day inclusive after the first egg of a given clutch had hatched (this is the period of highest nestling post-natal demand; the nestling period lasts approximately 20-25 days). At least five days before video recording started, we (i) caught and marked the vent of each group member other than the dominant female using hair dye (Capilla-Lasheras *et al.* 2021) to aid their identification on the video and (ii) deployed a tripod on the ground beneath the nest to acclimatize the birds to its presence prior to recording. On recording days, the video camera was set up and recording started soon after sunrise, at standard times relative to sunrise in order to track seasonal changes in sunrise timings. Provisioning behaviour was recorded for approximately three hours per day per brood. Video recordings were then watched using VLC media player to determine the rate at which each group member visited the nest (here after their 'provisioning rate'), identifying each visitor via their sex (based on bill coloration (Leitner *et al.* 2009)), unique vent pattern and color-ring combination. Prior analyses using within-nest cameras have confirmed that during this peak provisioning period all nest visits by all group members entail the delivery of a single food item by the visitor that is then eaten by

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the chicks (the only exception being nest-maintenance visits that were easily excluded from the data set on the basis of the visitor conspicuously carrying grass (Walker 2015)).

We then calculated the provisioning rate of mothers (feeds / hour). In some cases, we were unable to reliably identify every visiting bird within the provisioning video, yielding some uncertainty in our estimate of maternal provisioning rate. We therefore only carried forward maternal provisioning rate estimates to our statistical analyses where the maximum possible maternal provisioning rate (i.e., if one considered the mother the feeder in *all* cases of uncertain feeder identity) did not exceed the observed maternal provisioning rate (calculated solely on the basis of the mother's identified visits) by more than 33% or 3 feeds / hour. Applying this filtering criteria, there was less than 10% uncertainty for more than 90% of maternal provisioning rate estimations. Where estimates of maternal provisioning rate were available for multiple mornings for a given breeding attempt, the measures were averaged to yield a single mean maternal provisioning rate for each breeding attempt for analysis (as maternal provisioning rate estimates for a given breeding attempt were highly correlated over successive mornings of video recording). This yielded a data set for analysis of mean maternal provisioning rate for 48 different dominant females (mothers) feeding 108 broods in 34 social groups.

Environmental data

Daily rainfall data were collected from two rainfall gauges located to the west (27° 16' 58.9'' S, 22° 23' 02.1'' E) and east (27° 17' 42.1'' S, 22° 27' 34.9'' E) of the study site, 7.60 km apart from each other. These two rainfall measurements were highly correlated during the study period (Pearson's product-moment correlation: r = 0.875, 95% CI = 0.867 – 0.882, df = 3,347). We therefore calculated average daily values across both gauges and used this as a proxy for rainfall conditions at the study site.

Temperature data for a 0.25 degree latitude x 0.25 degree longitude area that encompassed the study site was extracted from the GLDAS-2.1 Noah 0.25 degree 3-hourly data set (Rodell *et al.* 2016), accessed via the NASA's Goddard Earth Sciences Data and Information Services Center online data system (Giovanni; http://disc.sci.gsfc.nasa.gov/giovanni). From this, we calculated the daily maximum temperature and daily mean temperature (i.e., the average of all eight measures

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available per 24 hour period) for all days of our study. The daily mean temperatures from this data set were highly correlated with those obtained directly within our study site using a 2700 Watchdog weather station (Spectrum Technologies Inc) deployed for part of the study period (partial coverage of 2010-2015; Pearson's product-moment correlation: r = 0.973, 95%CI = 0.970 – 0.975, df = 1,771).

Statistical analysis

Modelling maternal pre-natal investment per offspring: egg volume.

Linear mixed models with Gaussian error structure were used to investigate the predictors of egg volume (calculated based on length and maximum breadth following the formula given in (Narushin 2005)). Four terms were included as random intercepts: breeding season (referring to each of the nine different September-April breeding seasons studied), social group ID, clutch ID and maternal ID. The following were included as fixed effect predictors: egg position within the clutch, clutch size, number of female helpers, number of male helpers and the interaction between helper number (both females and males) and (i) egg position, and (ii) clutch size. To control for the potential effects of temperature and rainfall on egg volume, we also fitted the following two indices as fixed effect predictors: a 'heat waves' index (the number of days in which the maximum daily temperature exceeded 35°C within a time window spanning the 13 days prior to egg laying) and a rainfall index (the total rainfall that fell within a time window spanning 44-49 days prior to egg laying). The specific time windows used for the calculation of these indices were determined objectively by the application of a sliding window approach prior to this modelling step (see Supplementary materials B). The 'heat waves' index as defined here (i.e., number of days above 35°C) has been shown to appropriately capture hot-weather events in the Kalahari and it impacts the reproductive biology of several Kalahari bird species (Cunningham *et al.* 2013b, a). Between 2007 and 2016 inclusive, we collected egg length and width information (and therefore volume) from 906 eggs that were detected in the field with less than four days of uncertainty around their laying date. We focused our analysis on the 490 of these for which we also knew laying order (allowing determination of the 'egg position within the clutch' variable): 490 eggs from 271 clutches laid by 62 dominant females (mothers) across 37 social groups (mean = 7.90 eggs per mothers; range 1 – 21 eggs per mother).

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Modelling maternal post-natal investment: maternal nestling provisioning rate

Linear mixed models with Gaussian error structure were used to investigate the predictors of maternal provisioning rate (calculated as a single mean value for each breeding attempt; see above). Three terms were included as random intercepts: breeding season (see above), social group ID, and maternal ID. The following were included as fixed effect predictors: brood size, number of female helpers, number of male helpers and the interactions between helper number (both females and males) and brood size. To control for the potential effects of temperature and rainfall on maternal provisioning rate, we also fitted the following two indices as fixed effect predictors: 'heat waves' index (the total number of days within a time window spanning 51-58 days prior to egg laying in which the maximum daily temperature exceeded 35°C) and a rainfall index (the total amount of rainfall that fell within a time window spanning 61-78 days prior to egg laying). The specific time windows used for the calculation of these indices were determined by the application of a sliding window approach prior to this modelling step (see Supplementary materials B for methods and interpretation). The final data set contained 108 measures of mean maternal provisioning rate for 108 broods born to 48 dominant females (mothers) across 34 social groups.

General statistical procedures

To identify the predictors of the focal response term in our mixed effects models we used an information-theoretic (IT) approach. Starting from a global model that contained the fixed effect variables and interactions predicted to have an effect on the focal response term (see above for details), we fitted all possible models containing simpler combinations of these fixed effect predictors and ranked them for model fit based on Akaike's Information Criterion (AIC, (Burnham & Anderson 2002)). With this approach, the best-supported model is the one with the lowest AIC value. ΔAIC values were then calculated for every model, as the difference between the AIC of the focal model and that of the best-supported model (thus, the ΔAIC for the best-supported model is zero, and models with progressively weaker fits have progressively larger positive ΔAIC values). We gave consideration to models with ΔAIC values < 6 (Richards 2008; Richards *et al.* 2011) and subsequently reduced this Δ6 top model set by applying the 'nesting rule' described in (Richards 2008). Simulations have shown that the addition of uninformative variables to a top model can weaken AIC support by less than six points, leading to the retention within the Δ6 top

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model set of more complex versions of better supported models, which contain such uninformative variables (Richards 2008; Arnold 2010). The nesting rule aims to avoid this scenario by discarding models that are more complex versions of better-supported simpler (nested) models (Richards 2008; Arnold 2010). The subset of models retained after applying the nesting rule are flagged in the right-hand column in all model output tables. When quadratic terms were included in a given model, linear coefficients were always present. Intercept-only models were always considered. For AIC comparisons, models were fitted using maximum likelihood. All predictors were standardized prior to analysis by mean centering and dividing by one standard deviation, with the exception of those relating to helper numbers (to facilitate comparisons of the effect size estimates for male and female helper numbers by keeping them on the same scale; using standardized helper number predictors instead yielded identical conclusions). Statistical analyses were performed in R version 3.6.1. (R Core Team 2019), and statistical models were fitted using the R package 'lme4' (Bates *et al.* 2015).

A common concern in studies of the effects of helper numbers on fitness-related traits in cooperative species, is that positive correlations between the two could arise not from a causal effect of helpers on the focal trait but instead from both helper numbers and the focal trait being positively impacted by territory (and/or maternal) quality (Cockburn 1998; Cockburn *et al.* 2008). We addressed this concern in two different ways. First, we excluded young individuals (< 6 six months old) from our calculations of the number of male and female helpers (see above; as they contribute little to helping), given that transient resource peaks could leave recent and current productivity positively correlated, potentially yielding a spurious correlation between helper number and current productivity if recently fledged young were considered helpers. Second, we first carried out our analyses using the number of (male and female) helpers as the focal predictor, and then partitioned this variable into its within- and among-mother components: Δ (male or female) helper number and μ (male or female) helper number respectively (van de Pol & Wright 2009). 'μ helper number' is the mean helper number that a mother had across all of her breeding attempts in the relevant data set, whereas 'Δ helper number' is the difference between her helper number in the focal clutch or brood and 'μ helper number'. This approach allows us to statistically isolate the effects of within-mother (Δ) variation in helper number (which is both within-mother

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and within-territory, as each mother in our analyses only ever held one territory), which are indicative of maternal *plasticity*, in the knowledge that its effects cannot be attributed to variation in quality *among* mothers or their territories. A recent study has shown that partitioning within- and among-individual effects following this approach provides a robust estimation of the within-individual effect size, the parameter of interest in this study (Westneat *et al.* 2020).

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Acknowledgments

We would like to thank the many team members who contributed to the collection of the long-term data over the years (in particular Tom Reed, Jenny York, Dom Cram, Lindsay Walker, Emma Wood and Xavier Harrison), Northern Cape Conservation for permission to carry out the research, Nigel Bennett for invaluable assistance with in-country permissions, and E. Oppenheimer & Son, the Tswalu Foundation, and all at Tswalu Kalahari Reserve for their support in the field and the collection and sharing of the reserve-wide rainfall data. We also thank Ben Hatchwell and Erik Postma for insightful discussions. The long-term field study was funded by a BBSRC David Phillips Research Fellowship to A.J.Y. (BB/H022716/1) and P.C.-L. was supported by a BBSRC-funded PhD studentship (BB/M009122/1).

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Figures and Tables

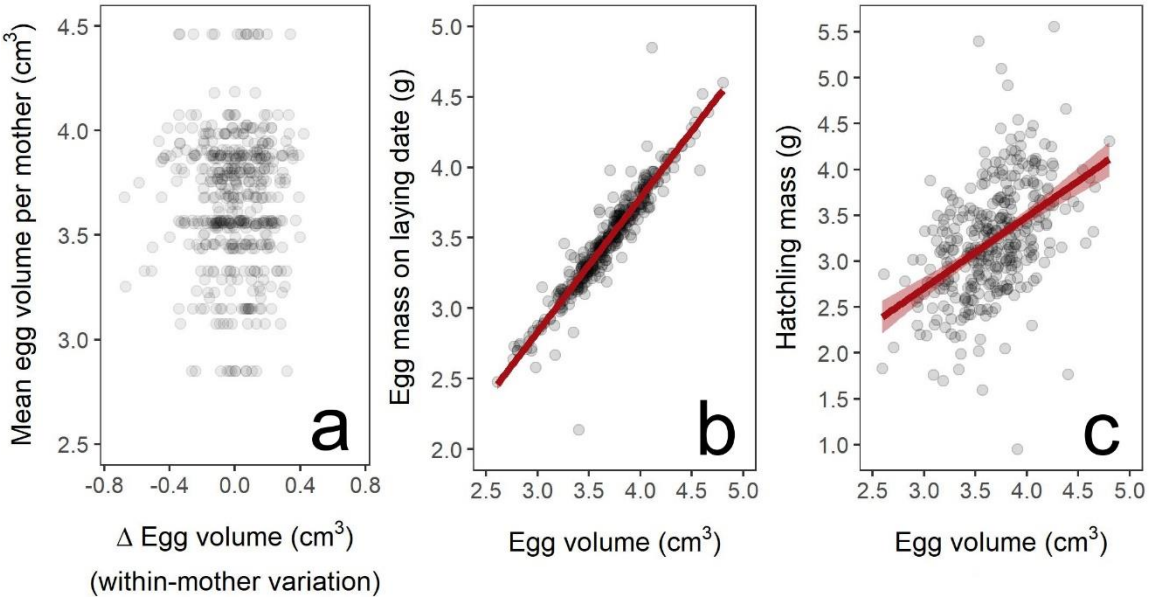

Figure 1. Patterns and implications of maternal variation in egg volume. (a) Egg volume showed high variation both within (x-axis) and among mothers (y-axis). Δ egg volume represents the difference in egg volume between the focal egg and that mother's own mean egg volume (i.e., within-mother variation; hence the negative and positive values). (b) Variation in egg volume positively predicted egg mass (g) on the day of laying and (c) nestling mass (g) on the day of hatching. Mean model predictions ± standard error (SE) are plotted in red.

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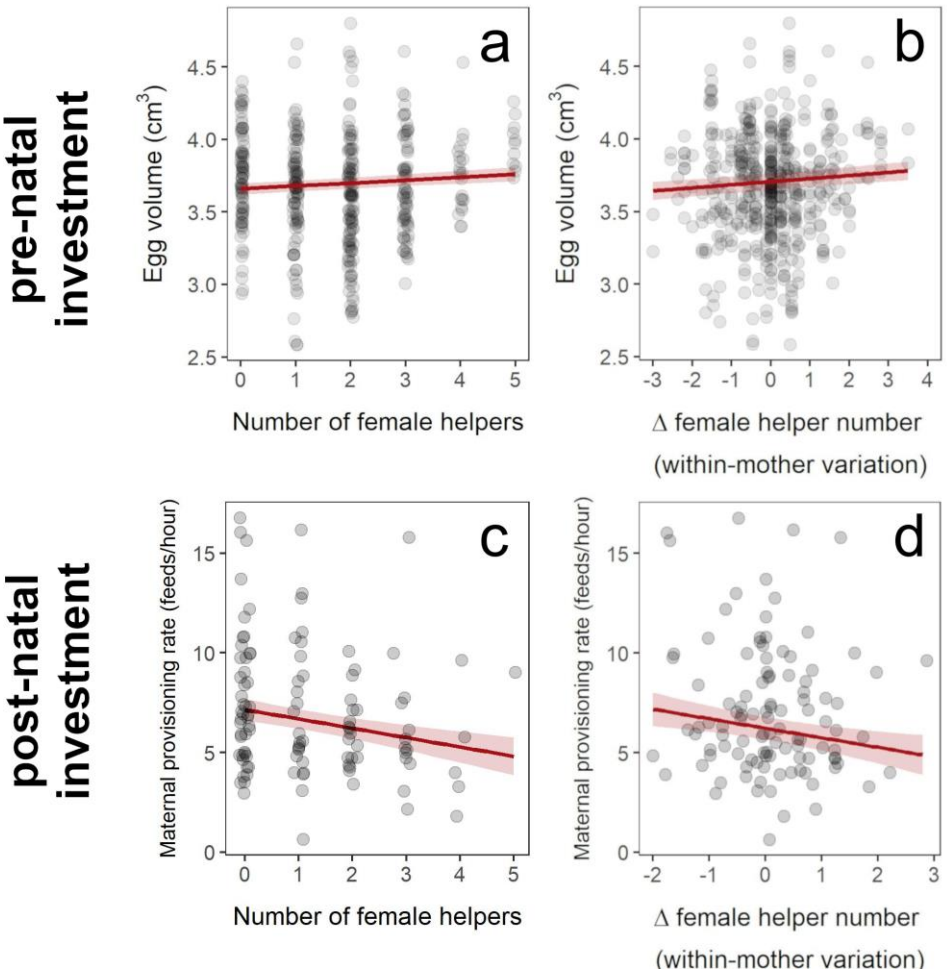

Figure 2. Maternal plasticity in pre-natal (egg volume) and post-natal (nestling provisioning rate) investment according to female helper numbers. (**a**) Female helper number positively predicts egg volume at the population level (Table 1; prior to partitioning variation in helper number). (**b**) Within-mother variation in female helper number ('Δ female helper number') also positively predicts egg volume, providing evidence of maternal plasticity (see results & Table S2). (**c**) Female helper number negatively predicts maternal nestling provisioning rate at the population level (Table 2). (**d**) Within-mother variation in female helper number ('Δ female helper number') also negatively predicts maternal nestling provisioning rate, providing evidence of maternal plasticity (see results & Table S4). Grey dots illustrate raw data points and red lines present model predictions (± SE).

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Table 1. The top-performing models (i.e., those within ΔAIC < 2 of the top model) explaining variation in egg volume. Only the top model would be retained under the nesting rule ('Retained' (Richards 2008); see methods). The longer list of models within ΔAIC < 6 of the top model is presented as Table S1. The first model not containing number of female helpers as a predictor scored a ΔAIC value of 3.71 (see Table S1). Model coefficients (effect sizes) are shown along with number of model parameters ('k'), AIC, and ΔAIC. Other tested predictors not shown in this table (as they were not present in the models within this set) were as follows: clutch size, clutch size x female helper number, egg position x female helper number and egg position x male helper number.

Intercept	Number female helpers	Number male helpers	Rainfall[1]	Rainfall[2]	Egg position	Heat waves	Clutch size x Number male helpers	k	AIC	ΔAIC	Retained
3638.00	20.28		-553.60	-859.80	-43.20	-41.71		11	6671.1	0.00	✓
3631.00	17.86	7.37	-545.70	-855.40	-42.96	-42.46		12	6672.5	1.40	
3638.00	20.51		-555.30	-865.60	-47.81	-41.56	3.77	12	6672.7	1.59	

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Table 2. The top-performing models (i.e., those within ΔAIC < 2 of the top model) explaining variation in maternal provisioning rate. Only the top model would be retained under the nesting rule ('Retained' (Richards 2008) ; see methods). The longer list of models within ΔAIC < 6 of the top model is presented as Table S3. The first model not containing number of female helpers as a predictor scored a ΔAIC value of 3.23 (see Table S3). Model coefficients (effect sizes) are shown along with number of model parameters ('k'), AIC, and ΔAIC.

Intercept	Number female helpers	Number male helpers	Brood size	Rainfall[1]	Rainfall[2]	Heat waves	Number female helpers X Brood size	Number male helpers X Brood size	k	AIC	ΔAIC	Retained
7.63	-0.47		1.51	4.98	7.18	0.69			10	526.3	0.00	✓
7.85	-0.43	-0.26	1.92	4.86	7.34	0.62		-0.34	12	527.2	0.93	
7.88	-0.45	-0.21	1.49	4.76	7.15	0.64			11	527.5	1.18	
7.63	-0.46		1.48	5.07	7.15	0.69	0.04		11	528.3	1.97	