The relationship between egg size and helper number in cooperative breeders: a meta-analysis across species

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Background. Life history theory predicts that mothers should adjust reproductive investment depending on benefits of current reproduction and costs of reduced future reproductive success. These costs and benefits may in turn depend on the breeding female's social environment. Cooperative breeders provide an ideal system to test whether changes in maternal investment are associated with the social conditions mothers experience. As alloparental helpers assist in offspring care, larger groups might reduce reproductive costs for mothers or alternatively indicate attractive conditions for reproduction. Thus, mothers may show reduced (load-lightening) or increased (differential allocation) reproductive investment in relation to group size. Given the growing number of studies which have investigated how cooperatively breeding mothers adjust pre-natal investment depending on group size, our aim was to assess whether in groups with more helpers mothers consistently show lower or higher pre-natal investment across species and to assess whether these changes relate to variation in post-natal investment.

Methods. We extracted data on the relationship between helper number and maternal pre-natal investment (egg size) from 12 studies on 10 species of cooperatively breeding vertebrates. We performed meta-analyses to calculate the overall estimated relationship between egg size and helper number, and to quantify variation among species. We also tested whether these relationships are stronger in species in which the addition of helpers is associated with significant changes in maternal and helper post-natal investment.

Results. Across studies, there is a significant negative relationship between helper number and egg size, suggesting that in most instances mothers show reduced reproductive investment in larger groups, in particular in species in which mothers also show a significant reduction in post-natal investment. However, even in this limited sample, substantial variation exists in the relationship between helper number and egg size, and the overall effect appears to be driven by a few well-studied species.

Discussion. Our results, albeit based on a small sample of studies and species, indicate that cooperatively breeding females tend to produce smaller eggs in larger groups. These findings on prenatal investment accord with previous studies showing similar load-lightening reductions in postnatal parental effort (leading to concealed helper effects), but do not provide empirical support for differential allocation. However, the considerable variation in effect size across studies suggests that maternal investment is mitigated by additional factors. Our findings indicate that variation in the social environment may influence life-history strategies and suggest that future studies investigating within-individual changes in maternal investment in cooperative breeders offer a fruitful avenue to study the role of adaptive plasticity.

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Summary

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These costs and benefits may in turn depend on the breeding female's social environment.

Cooperative breeders provide an ideal system to test whether changes in maternal investment are

associated with the social conditions mothers experience. As alloparental helpers assist in

offspring care, larger groups might reduce reproductive costs for mothers or alternatively

indicate attractive conditions for reproduction. Thus, mothers may show reduced (load-

lightening) or increased (differential allocation) reproductive investment in relation to group size.

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47   natal investment. However, even in this limited sample, substantial variation exists in the

48   relationship between helper number and egg size, and the overall effect appears to be driven by a

49   few well-studied species.

50   **Discussion.** Our results, albeit based on a small sample of studies and species, indicate that

51   cooperatively breeding females tend to produce smaller eggs in larger groups. These findings on

52   prenatal investment accord with previous studies showing similar load-lightening reductions in

53   postnatal parental effort (leading to concealed helper effects), but do not provide empirical

54   support for differential allocation. However, the considerable variation in effect size across

55   studies suggests that maternal investment is mitigated by additional factors. Our findings indicate

56   that variation in the social environment may influence life-history strategies and suggest that

57   future studies investigating within-individual changes in maternal investment in cooperative

58   breeders offer a fruitful avenue to study the role of adaptive plasticity.

59

60   **Introduction**

61   Life history theory suggests that, to maximize their lifetime reproductive success, breeders

62   should adjust their reproductive investment according to both current and future conditions

63   (Stearns, 1992). There is considerable evidence that increased maternal investment into offspring

64   can strongly and positively affect offspring development, survival and future chances of

65   reproduction (Bolton, 1991; Lindström, 1999; Krist, 2011), but negatively influences maternal

66   survival and investment into future reproductive attempts (Lack, 1947; Maynard Smith, 1977;

67   Berube, Festa-Bianchet & Jorgenson, 1996; Metcalfe & Monaghan, 2001; Lummaa & Clutton-

68   Brock, 2002; Hanssen et al., 2005). A mounting body of studies has shown that mothers

69   adaptively alter investment into reproduction via offspring traits, such as egg size or hormone

70   provision into eggs, depending on breeding conditions, such as food availability or mate quality

71   and contribution to care (e.g. Cunningham & Russell, 2000; Eising et al., 2001; Gil et al., 2004;

72   Bolund, Schielzeth & Forstmeier, 2009). In addition, in group-living species, mothers might

73   adjust investment according to other salient features of their social environment besides their

74   partner, in particular if there is variation in the presence or expected helping contribution of

75   alloparental carers.

76   Cooperatively breeding species provide an opportunity to study the changes in parental

77   investment in response to variability in the social group environment. In these species, non-

78   breeding helpers assist breeding individuals with caring for offspring (Stacey & Koenig, 1990;

79   Clutton-Brock, 2002; Koenig & Dickinson, 2004). Differences in the number of additional group

80   members, both within and among species, generate natural variation in the social environment

81   mothers experience (Russell & Lummaa 2009). Given the trade-offs between investment into the

82   current brood and later reproductive attempts, investments by mothers into the current brood are

83   expected to vary in the presence of helpers (Crick, 1992; Hatchwell, 1999; Savage, Russell &

84   Johnstone, 2013). Detailed field observations of various cooperative breeders, particularly in

85   birds, have presented evidence that the amount of maternal investment at the nest (provisioning)

86   is associated with variation in the workforce present when breeding (Hatchwell & Russell, 1996;

87   Woxvold, Mulder & Magrath, 2006; Cockburn et al., 2008; Russell et al., 2008). Associations

88   between maternal investment and helper numbers might result from differences in territory

89   quality or maternal condition (Cockburn et al., 2008, Russell & Lummaa, 2009). In at least some

90   instances, however, reduced maternal provisioning may be interpreted as an adaptive responses

91   of females to variation in the social group environment, such that breeding females maximize

92   their lifetime fitness by reducing post-natal investment in current offspring when helpers

93   compensate for such a reduction (Wright, 1998, Russell et al., 2008, McDonald, Kazem &

94   Wright, 2009).

95   Social influences could also influence maternal pre-natal investment (Savage, Russell &

96   Johnstone, 2015): in cooperative breeders, altered maternal investment into eggs might be

97   compensated for or enhanced by the protection, provisioning or incubation of offspring provided

98   by helpers subsequent to laying (Russell et al., 2007; Taborsky, Skubic & Bruintjes, 2007;

99   Russell & Lummaa, 2009). One hypothesis that has been proposed, the primary one we test here,

100 is that cooperatively breeding mothers will show decreased investment into egg size when

101 helpers are present to compensate for such reduced investment: the 'load-lightening hypothesis'

102 (here onwards, LL hypothesis) (Russell & Lummaa, 2009; Savage, Russell & Johnstone, 2015;

103 also sometimes referred to as 'concealed helper effects', Koenig, Walters & Haydock, 2009).

104 This hypothesis argues that, since egg production is costly in terms of future maternal survival

105 and reproductive success (Visser & Lessells, 2001; Williams, 2005), lifetime reproductive

106 success will be increased if reductions in egg size have little or no effect on offspring fitness

107 owing to the compensation provided by helpers (Savage, Russell & Johnstone, 2013).

108 Observations that egg sizes decrease with an increased number of helpers at the nest would

109 provide support for the LL hypothesis (Taborsky, Skubic & Bruintjes, 2007; Russell & Lummaa,

110 2009), though such an association could also reflect factors that influence pre- and post-natal

111 conditions independently or changes in helper behaviour in response to maternal investment

112 (Savage, Russell & Johnstone, 2015).

113   An alternative hypothesis is that more helpers can cause mothers to increase investment

114 to take advantage of good conditions: the so-called 'differential allocation (DA) hypothesis'

115 (Sheldon, 2000). Usually applied to when females have a particularly attractive mate (Sheldon,

116   2000; Horvathova, Nakagawa & Uller, 2011), it has been expanded to cooperative breeding,

117   predicting that if a female is assisted by a large numbers of helpers (indicating good breeding

118   conditions), she should increase investment due to higher current potential reproductive success

119   or the increased reproductive value of the current brood (Valencia et al., 2006; Russell &

120   Lummaa, 2009, Savage, Russell & Johnstone, 2015). Thus, the DA hypothesis, unlike the LL

121   hypothesis, would predict an increase in egg investment with increased number of helpers.

122   Variation in prenatal maternal investment is likely to be associated with the care

123   offspring receive post-natally (Savage, Russell & Johnstone, 2015). Here we focus on two

124   factors that might shape the relationship between prenatal maternal investment and postnatal

125   offspring care: mothers are expected to show reduced investment in egg size if their reduced

126   investment can be directly substituted by helper care (helpers provide food, and helper efforts

127   can buffer a small size early in life) and if there are no benefits to offspring of receiving

128   investment beyond a certain threshold (for example if offspring need to reach a certain body size

129   to increase their survival, but additional increments in size do not lead to higher offspring

130   fitness). The interactions between mothers and helpers during the post-natal stage might provide

131   information on these two factors. First, if prenatal investment can be substituted by postnatal

132   care, we would predict that mothers show consistent reductions in their pre- and postnatal

133   investment whereas if prenatal investment primes offspring to benefit from increased postnatal

134   help, maternal postnatal care behaviour should be independent of the presence of helpers.

135   Accordingly, we predict that mothers are more likely to produce smaller eggs in groups with

136   helpers in species in which mothers also reduce their postnatal investment in the presence of

137   helpers. Second, if additional investments continue to provide benefits, helping efforts are

138   expected to increase linearly with group size, whereas if there is a limit up to which investment is

139   beneficial, helping efforts might plateau. In this case, we predict that mothers are more likely to

140   produce smaller eggs in larger groups in species where total helping efforts plateau and do not

141   increase linearly with group size.

142   In this study, we perform a meta-analysis to determine whether the currently existing

143   evidence indicates that variation in maternal prenatal investment in the form of egg size

144   systematically varies with the number of helpers across cooperative breeders, and whether the

145   direction of the relationship indicates whether load-lightening or differential allocation might be

146   more prevalent. Our study systematically combines the recently available observations to assess

147   whether consistent increases or decreases in maternal investment at the egg stage in response to

148   helper numbers have been observed and test predictions that changes in maternal pre-natal

149   investment are associated with the post-natal care offspring experience.

150

151   **Materials & Methods**

152   A literature search was conducted to find relevant papers in which egg investment traits and

153   helper number were measured. We focused on egg size as our measure of maternal investment as

154   it is clearly under maternal control while subsequent investment into offspring is frequently

155   shared among mothers, fathers, and helpers. As such, we decided not to include data on

156   mammals since offspring size at birth is not available for most cooperatively breeding mammals

157   and lactation makes it more difficult to ascertain when investment is fully under maternal

158   influence versus that of helpers. For the purpose of this analysis, cooperative breeders were

159   defined as species where additional group members act as allomaternal helpers and provide

160   resources that can replace and hence compensate for adjustments in maternal investment, such

161   that mothers could potentially alter their investment into egg size based on helper number.

162 Studies providing a quantitative measure of the link between maternal investment and group size

163 in eusocial species are rare. Moreover, we did not specifically search for these as our focus was

164 on instances where maternal investment could vary both pre- and post-natally: in eusocial

165 species, mothers tend not to provide any offspring care beyond egg production when helpers are

166 present and almost all post-natal care is performed exclusively by helpers (Keller and Chapuisat,

167 2010). We also did not include species where offspring care is shared only among reproductively

168 active females (e.g., Grinsted, Breuker & Bilder, 2014), as it is difficult to determine how

169 individual mothers differ in their investment. On the other hand, cooperatively polyandrous

170 species were included: while additional group members (in this case, males) may share paternity,

171 efforts by the males represent alloparental care and could replace female investment into the

172 provisioning and protection of offspring (Davies, 1985).

173   Six relevant papers (Legge, 2000; Taborsky, Skubic & Bruintjes, 2007; Koenig, Walters

174 & Haydock, 2009; Canestrari, Marcos & Baglione, 2011; Santos & Macedo, 2011; Lejeune et

175 al., 2016) were located using keyword searches on Google Scholar with the search terms 'egg

176 size' and 'helpers', on 30th October 2016 (search carried out by TD and confirmed by DL; search

177 criteria were specific and hence disagreements were solved easily by discussion). Forward and

178 backward citation searches were then performed to find five further relevant studies (Russell et

179 al., 2007; Paquet et al., 2013; Santos & Nakagawa, 2013; Santos, 2016; Valencia et al., 2016).

180 One study (Langmore et al., 2016) was identified as an 'online-early' abstract during the literature

181 search, and was included as full publication occurred during the course of our study. Relevant

182 studies were defined as those that investigated a relationship between egg size – measured

183 specifically as egg mass or egg volume – and number of helpers (determined by reading abstracts

184 and figures). Studies were then included if they provided the required test-statistic for use in

185   meta-analysis (see below), or authors were contacted for raw data if suitable data were not

186   provided in the paper. Where a direct measure of egg volume was not available, this was

187   calculated from length and width using Hoyt's formula (Hoyt, 1979).

188   In seven studies, the change in maternal investment per helper was not provided, rather

189   comparisons were given between investment when breeding in groups versus pairs. In these

190   cases, and in cases where data on egg size traits with helpers were referred to but not provided,

191   authors were contacted to provide the raw data for number of helpers in each group, and linear

192   models, using the command lme in the package 'nlme' (Pinheiro et al., 2015) in RStudio version

193   0.99486 (RStudio team, 2015) using R version 3.2.2 (R Core Team 2015), were used to calculate

194   test statistics. The test statistics – F, t and χ^2 – were converted to the statistic 'r' using formulae

195   provided by Rosenthal (1994), Lajeunesse (in Koricheva et al., 2013), and Rosenberg (2010).

196   Standard formulae were then Z-transformed, controlling for the asymptotic behaviour of the

197   statistic r. We followed the detailed advice in Jennions, Moller & Petrie (2001) for these

198   methods, and formulae therein for calculations of effect sizes, transformations, and variances.

199   We also investigated whether any relationship between maternal pre-natal investment and

200   group size varied according to how aspects of post-natal care depend on helper number

201   (specifically, whether mothers reduce post-natal care in the presence of helpers, and whether

202   helpers provide benefits to offspring additively or up to a threshold). We thus searched for

203   additional data on each species on the relationship between maternal post-natal care, helper

204   effects on offspring and group size. Specifically, for each data point, we recorded whether the

205   amount of support mothers provide to offspring (in most studies measured as the number of

206   feeds per hour) is significantly lower when at least one helper (of that sex, in instances where the

207   original study provided separate information for each sex on the relationship between egg size

208   and helper number) is present than when groups only consist of mothers and their mate. To

209   determine whether helping effort increases linearly or plateaus with group size (all studies we

210   included showed either of these two options), we checked which function authors had used to

211   model the relationship between group size and total amount of care provided at the nest

212   (measured as number of feeds per hour in bird species, and as egg caring in *Neolamprologus*

213   *pulcher*), inspected the associated graphs to see whether increases of a second, third, etc. helper

214   was similar to the increase observed in groups with just a single helper. In addition, we read

215   through the associated paper carefully to check if the description by the authors confirmed the

216   graphical pattern, for example if they stated that the amount of support increased linearly with

217   group size.

218   Functions in the R package 'metafor' (Viechtbauer, 2010) were used to compute

219   heterogeneity and average effect sizes, and to create funnel plots to visualise the data. Cochran's

220   Q-test was used to test heterogeneity between effect sizes in all analyses carried out.

221   Heterogeneity describes the variation in the data which can be attributed to actual variation

222   between species, variation in study design, or publication bias (Nakagawa & Santos, 2012). The

223   average effect size describes the overall effect of helpers on changes in maternal investment into

224   eggs, and hence indicates the strength and direction of the effect, determining whether the LL or

225   DA hypothesis receives more empirical support. Contour-enhanced funnel plots were used to

226   visualise effect sizes, and precision was defined as 1/SE where SE=√(variance). In this study,

227   with high heterogeneity predicted, a contour plot would suggest a lack of publication bias if the

228   outliers occurred at all precision values, and not solely at low precision (Macaskill, Walter &

229   Irwig, 2001). Publication bias was also quantified using Egger's regression test (Egger et al.,

230   1997) on the dataset, using the regtest function in the metafor package, and sample standard error

231   as a predictor (Viechtbauer, 2010). We followed suggested best practices to minimize bias in the

232   dataset: the literature search was comprehensive, authors were contacted if published statistics

233   were insufficient to compute effect sizes, and the analysis included a study (Russell et al., 2007)

234   even though it did not contain the information required to compute the effect size in exactly the

235   same manner as the other effect sizes (Gates, 2002). However, our sample remains small, with

236   high heterogeneity since authors might have tested hypotheses with opposite effects (negative for

237   LL and positive for DA), such that we are aware that any conclusions are likely to remain limited

238   at this stage.

239   A multilevel linear model with restricted maximum likelihood estimation (REML)

240   analysis was carried out on all effect sizes, controlling for study as a random factor, to take into

241   account the non-independence of individual effect sizes calculated from the same study. In

242   several of the studies, authors reported multiple relationships: running separate analyses on male

243   and female helpers, reporting data on both egg size and mass, using different statistical models,

244   considering variation among or within individual females, or considering different mating

245   systems. Each study focussed on a single species, and in all but two cases, studies considered

246   different species. The exceptions were Paquet et al. (2013) and Santos (2016), both of which

247   studied sociable weavers; and Russell et al. (2007) and Langmore et al. (2016), both of which

248   studied superb fairy-wrens. As all of the studies in our sample had slightly different

249   methodologies, we initially controlled for study rather than species as a random term as we

250   deemed this a more parsimonious measure for data non-independence. We also conducted a

251   separate analysis controlling for species. Given the small sample size (10 species), we decided

252   not to include phylogenetic relatedness among species as a potential covariate.

253   To investigate the association between the post-natal behaviour of mothers and of helpers

254   with the relationship between pre-natal maternal investment and group size, additional analyses

255   were carried out on all effect sizes, using the type of behaviour ('mothers decrease postnatal

256   investment (in groups with on helper compared to pairs)' versus 'mothers do not decrease

257   postnatal investment (no change or increase when a helper is present); 'helper benefits increase

258   linearly (with group size)' versus 'helper benefits plateau') as a moderator (i.e. predictive

259   variable). Separate multilevel linear mixed effects models, with REML analyses, were conducted

260   for each post-natal response trait (maternal response and helping effort pattern), controlling for

261   species or for study. Following these analyses, in order to more intuitively examine the

262   difference among species, we carried out separate multilevel linear models with REML analysis,

263   controlling for species, on the subset of the data where mothers reduce postnatal care, on the

264   subset of the data where mothers do not reduce postnatal care, on the subset of the data where

265   helping efforts increase linearly with group size, and on the subset of the data where helping

266   efforts plateau with group size, in order to examine in more detail the difference in effect sizes.

267

268

269   **Results**

270   20 effect sizes from 12 studies of 10 species (9 bird species; 1 fish species) were extracted from

271   publications or calculated from raw data (see table S1). There was no evidence of publication

272   bias using Egger's regression test (test value $Z = -1.03$; $p = 0.30$). In addition, the funnel plot was

273   approximately symmetrical (figure 1), particularly for low values of 1/SE (i.e. low precision),

274   suggesting little publication bias. As visible on the funnel plot (figure 1), the overall effect size

275   (when controlling for study) was negative and significantly different from zero (effect size = -

276   0.182, p = 0.02, upper and lower confidence limits = -0.024 and -0.340; n=20 effect sizes from

277   12 studies). The Cochran's Q-test value was highly significant (Q = 128 on 19 degrees of

278   freedom, p < 0.001), suggesting that the data are highly heterogeneous. When the same dataset

279   was analysed controlling for species rather than study, the effect size was negative and

280   marginally not significant (effect size = -0.164, p = 0.08, upper and lower confidence limits =

281   0.020 and -0.348; n=20 effect sizes from 10 species).

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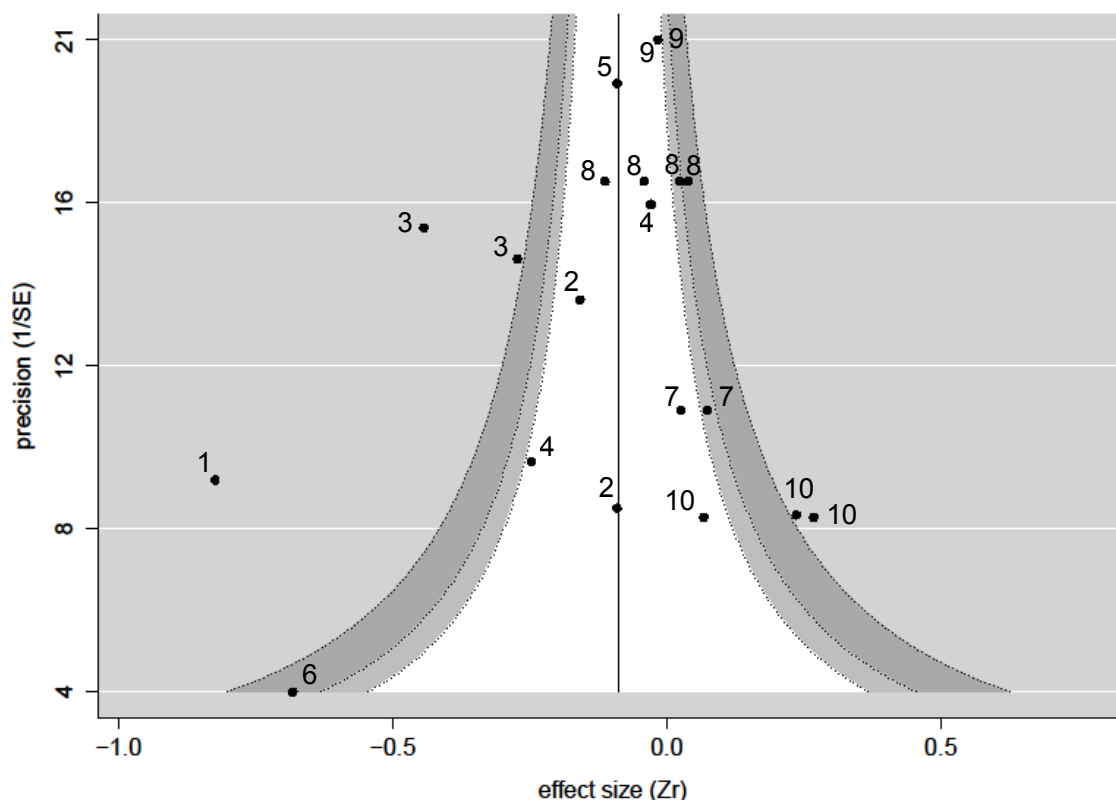
283   **Figure 1:** A funnel plot showing the 20 effect sizes extracted from 12 studies on 10 species. The

284   pooled estimate (solid line), controlling for study, is significantly smaller than zero. The funnel

285   shows the regions where 90% (white), 95% (light-grey), and 99% (dark-grey) of values are

286   expected to fall – there are several values outside of the funnel, and the heterogeneity is

287   significant. Numbers next to values refer to species identity (1: *Corvus corone*; 2: *Melanerpes*

288   *formicivorus*; 3: *Philetairus socius*; 4: *Malurus cyaneus*; 5: *Vanellus chilensis*; 6: *Neolamprogus*

289   *pulcher*; 7: *Prunella modularis*; 8: *Malurus elegans*; 9: *Cyanopica cooki*; 10: *Dacelo*

290   *novaguinea*).

291

292   When we examined how the relationship between pre-natal investment (egg size) and

293   helper number varied depending on how mothers adjust post-natal investment in larger groups,

294   we found that – in analyses controlling for study – egg sizes are significantly more likely to be

295   reduced in the presence of more helpers in species in which mothers also reduce their post-natal

296   investment in the presence of helpers (estimate ['mothers decreases postnatal investment in larger

297   groups' versus 'mothers do not decrease postnatal investment'] = -0.336±0.158, p = 0.03, upper

298   and lower confidence limits = -0.026 and -0.645; n=20 effect sizes from 12 studies). A similar,

299   but non-significant, relationship between post-natal maternal response and the relationship

300   between egg size and helper number is found when controlling for species rather than study

301   (estimate [reduction in postnatal investment present versus not present] = -0.337±0.178, p =

302   0.06, upper and lower confidence limits = -0.012 and 0.685 n=20 effect sizes from 10 species).

303   The overall effect size (i.e. of the relationship between helper number and egg size) for those

304   studies where mothers reduce their post-natal investment in the presence of helpers was

305   significantly less than zero controlling for species identity (effect size = -0.275, p = 0.02, upper

306   and lower confidence limits = -0.046 and -0.503, n=13 effect sizes from 7 species) (figure 2a),

307   with significant heterogeneity among values (Q = 98.5 on 12 degrees of freedom, p < 0.001).

308   There are only very few investigations of effects where mothers do not reduce their post-natal

309   investment in the presence of helpers, restricted to three species, but the overall effect size for

310   these is close to zero (effect size = 0.062, p = 0.33, upper and lower confidence limits = 0.187

311   and -0.064, n=7 effect sizes from 3 species) (figure 2b), with very low heterogeneity (Q = 8.85

312   on 6 degrees of freedom, p = 0.18).

313

314   The effects of helper presence on egg size do not appear to systematically differ between

315   instances in which helper efforts increase linearly with group size and those in which helping

316   efforts plateau (estimate ['helping effort increases linearly with group size' versus 'helping effort

317   plateaus'] = -0.057±0.057, p = 0.31, upper and lower confidence limits = 0.054 and -0.168; n=19

318   effect sizes from 11 studies – we excluded on study in which the relationship between helper

319   number and helper effort was unknown). The same analysis controlling for species similarly

320   shows no influence of how helping changes with group size on the relationship between egg size

321   and helper numbers (estimate [helping increase linear vs plateau] = -0.052±0.057, p = 0.37,

322   upper and lower confidence limits = 0.060 and -0.164 n=19 effect sizes from 9 species).

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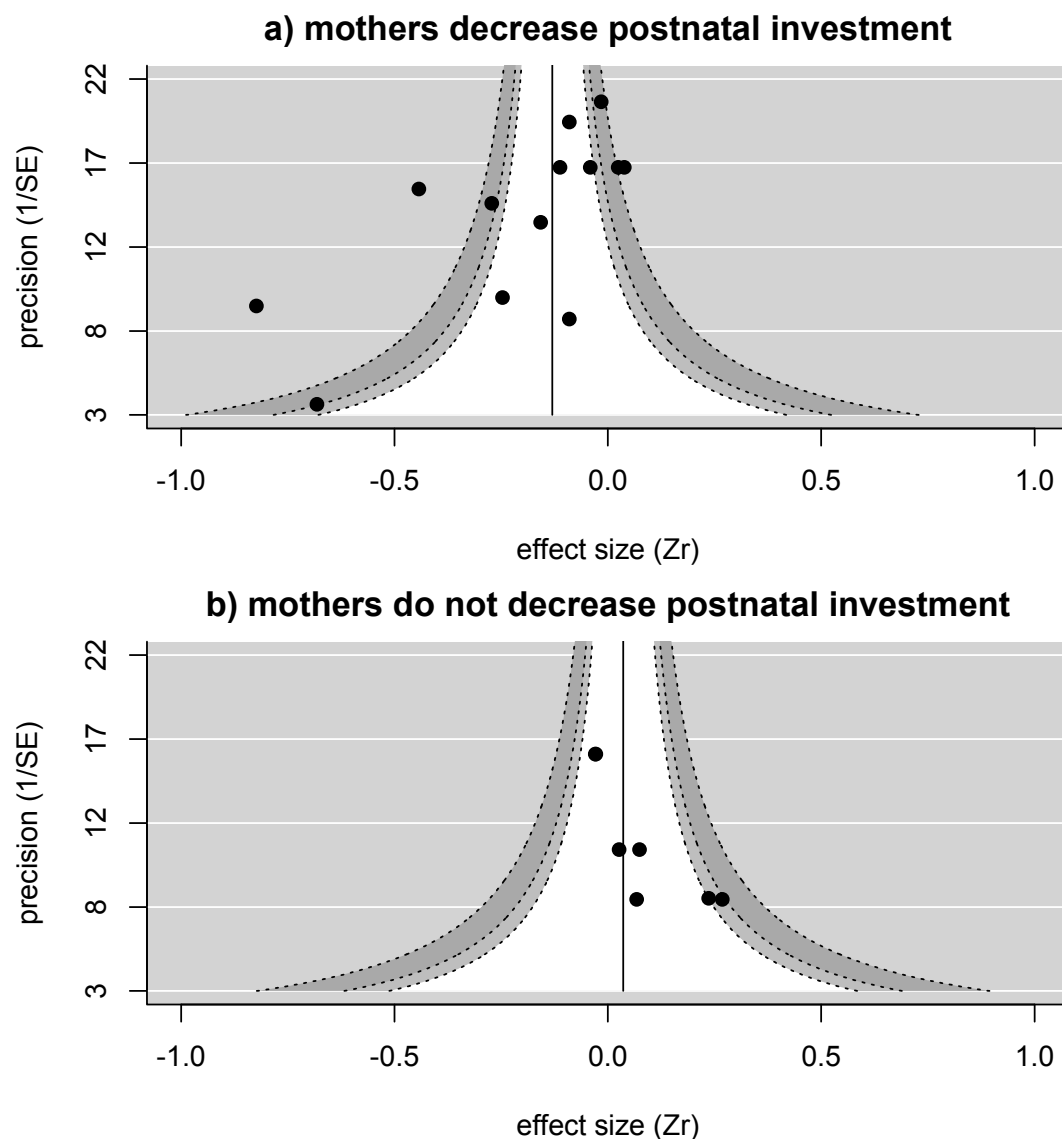
325   Figure 2: Funnel plots showing the distribution of effect sizes in species where (a) mothers

326   reduce postnatal investment in the presence of helpers (13 effect sizes from 10 studies in 7

327   species) or (b) mothers do not reduce postnatal investment in the presence of helpers (7 effect

328   sizes from 3 studies in 3 species). Solid line and shaded areas indicate pooled effect size and

329   expected values as explained in Figure 1. For instances where mothers reduce postnatal

330   investment (a), the pooled estimate (controlling for species) is significantly smaller than zero,

331   with significant remaining heterogeneity. For instances where mothers do not change or even

332 increase their postnatal investment in the presence of helpers (b), the pooled estimate (controlling

333 for species) is not different from zero, with no significant heterogeneity among effect sizes.

334

335 **Discussion**

336   Our results suggest that in most studies which have examined how investment by

337 breeding females varies with group size, results show that females produce smaller eggs if more

338 helpers are present. We also find that the association between smaller eggs and more helpers is

339 particularly pronounced when females show reduced post-natal care in the presence of helpers,

340 while egg sizes do not appear to vary with helper number when females do not adjust, or even

341 increase, their level of offspring care in the presence of helpers. These findings suggest that

342 concealed helper effects might occur in cooperative breeders, whereby helper efforts compensate

343 for reduced investment by breeding females and thus provide them opportunities for load-

344 lightening. We note that, given the small sample size and associated low statistical power, the

345 overall effect we detected is weak and that even among these few species there is large

346 unexplained variation in the association between maternal investment and the presence of

347 helpers. Moreover, only a minority of studies specifically focused on within-female adjustments

348 (3 of the 20 analyses in our dataset), and hence we cannot conclusively infer whether maternal

349 responses are an example of adaptive plasticity rather than among-female differences. Changes

350 in egg size could be due to other factors, such as greater intra-group competition, although in the

351 few detailed studies such explanations have been ruled out (e.g. Canestrari, Marcos & Baglione

352 2011). In spite of these limitations, we explain below the insights to be gained from our study for

353 our understanding of the benefits of helpers to breeding individuals.

354   Our findings are based on a small sample of correlative studies, most of which compared

355   conditions across, rather than within, individual females. There are multiple, potentially non-

356   exclusive explanations for why, in most species, egg sizes are smaller in nests that are tended for

357   by a large number of alloparental carers. For example, it has previously been suggested that

358   competition among females (Russell & Lummaa 2009) or the relative costs of pre- versus post-

359   natal care (Savage, Russell & Johnstone, 2015) might influence patterns of maternal investment.

360   However, our results do appear to reject the prediction of the differential allocation hypothesis,

361   as females do not appear to increase investment in the presence of helpers. Instead, female

362   investment – both pre- and postnatal – appears to be consistently reduced in groups with more

363   helpers, which supports the prediction of the load-lightening hypothesis. The prevalence of

364   effects consistent with load-lightening in our dataset could explain the common observation that

365   helper effects on offspring size or fledging success are often weak or absent, as care efforts by

366   helpers might be directly counterbalanced by a decrease in maternal investment (i.e. load-

367   lightening), hence leading to little difference in offspring traits in groups with or without helpers

368   (Russell et al., 2007; Cockburn et al., 2008; Koenig, Walters & Haydock. 2009; Paquet et al.,

369   2015). The evidence existing thus far indicates that adaptive plasticity in maternal investment in

370   response to helper number is at least possible. Further detailed studies focusing on variation

371   within individuals offer the opportunity to assess the role of pre-natal maternal investment in

372   understanding selection on cooperative systems (Taborsky, Skubic & Bruintjes, 2007; Russell &

373   Lummaa, 2009). The scope of comparative studies could be increased by not only focusing on

374   quantitative changes in egg size, but by also including other evidence of helper effects, such as

375   indications of load-lightening in eusocial insects including bees (Shpigler et al., 2013), ants

376   (Villet, 1990), and termites (Matsuura & Kobayashi, 2010).

377   We observed large variation across species in the effect of helper numbers on egg size.

378   Differences among species might reflect that in some instances changes in female investment

379   reflect non-adaptive processes, but even in cases where plasticity in egg size results from

380   adaptive processes, the direction and magnitude of changes in maternal investment likely reflect

381   trade-offs. (Savage, Russell & Johnstone 2015). One factor which may influence adjustment of

382   maternal investment with a greater number of helpers (i.e. favourable conditions) is reproductive

383   lifespan, with increased investment in favourable conditions in species with short lifespan and

384   decreased investment in favourable conditions in long-lived species (Paquet et al. 2015). The

385   occurrence of LL or DA may also depend on environmental conditions (Hatchwell, 1999,

386   Langmore et al. 2016) - in poor conditions, females should increase investment when helpers are

387   present in order to gain some reproductive success, because the marginal impact of care has a

388   relatively large influence on offspring fitness. In good conditions, the impact of small amounts of

389   additional care is low, and hence mothers would be selected to reduce care in the current

390   generation (Hatchwell, 1999). Furthermore, when helpers provide assistance which increases the

391   survival of offspring or reproductive success beyond that which would be expected from

392   provisioning alone (for example by reducing predation risk), breeders may be selected to

393   increase investment in the presence of helpers (Carranza et al., 2008), due to the high

394   reproductive value of the current brood in comparison with potential future broods (Valencia et

395   al., 2006; Carranza et al., 2008; Russell et al., 2010; Valencia et al., 2016). These predictions

396   primarily apply when variation in maternal pre-natal investment is in the size of each offspring,

397   and different relationships might occur if changes in maternal investment are associated with the

398   number of offspring – if mothers increase their investment by producing a higher number of

399   small offspring rather than the same number of larger offspring, more help is likely to be

400   beneficial (e.g. Liebl et al., 2016).

401   Our findings support the idea that adaptive plasticity in reproduction, with females

402   reducing investment in current broods to prioritise future survival and reproduction, might occur

403   in response to changes in the social environment. Further studies are needed to test whether

404   effects hold consistently on a within-female level and whether increases in fecundity and

405   survival are seen when load-lightening occurs. Identifying the conditions favouring the evolution

406   of plasticity in reproductive investment has wider implications, as in many animal species the

407   social environment is more variable than the physical environment and, indeed, social factors

408   often mediate physical environmental factors (e.g. through foraging competition).

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