PROCEEDINGS B

Egg size investment in superb fairy-wrens: Helper effects are modulated by climate

Journal:	*Proceedings B*
Manuscript ID	RSPB-2016-1875.R1
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Langmore, Naomi; Australian National University, Evolution, Ecology and Genetics, Research School of Biology Bailey, Liam D.; Australian National University, Research School of Biology Heinsohn, Robert; Australian National Uinversity, Fenner School of Society and Environment Russell, Andrew; University of Exeter Cornwall Campus, Centre for Ecology & Conservation, School of Biosciences Kilner, Rebecca; University of Cambridge, Department of Zoology
Subject:	Evolution < BIOLOGY, Ecology < BIOLOGY, Behaviour < BIOLOGY
Keywords:	load lightening, cooperative breeding, maternal effects, climate sensitivity, additive care
Proceedings B category:	Behavioural Ecology

SCHOLARONE™
Manuscripts

1

2

3

4   **Egg size investment in superb fairy-wrens:**

5   **Helper effects are modulated by climate**

6

7   N. E. Langmore[1], L. D. Bailey[1], R. G. Heinsohn[2], A. F. Russell[3], R. M. Kilner[4]

8

9

10   1. Division of Evolution and Ecology, Research School of Biology, Australian National

11   University, A.C.T., 2600, Australia

12   2. Fenner School of Environment and Society, Australian National University, A.C.T.,

13   2600, Australia

14   3. Centre for Ecology and Conservation, University of Exeter, Penryn, Cornwall, United

15   Kingdom

16   4. Department of Zoology, University of Cambridge, Downing St, Cambridge, United

17   Kingdom

18

19   Corresponding author: naomi.langmore@anu.edu.au

20

21   Running head: Helper effects on egg size are modulated by climate

22   Word count: 7,146

23

24   **Abstract**

25   Natural populations might exhibit resilience to changing climatic conditions if they already

26   show adaptive flexibility in their reproductive strategies. In cooperative breeders, theory

27   predicts that mothers with helpers should provide less care when environmental conditions

28   are favourable, but maintain high investment when conditions are challenging. Here we test

29   for evidence of climate-mediated flexibility in maternal investment in the cooperatively

30   breeding superb fairy-wren *Malurus cyaneus*. We focus on egg size because in this species

31   egg size influences offspring size, and females reduce egg investment when there are helpers

32   at the nest. We report that females lay larger eggs during dry, hot conditions. However, the

33   effect of temperature is modulated by the presence of helpers: the average egg size of females

34   with helpers is reduced during cooler conditions but increased during hot conditions relative

35   to females without helpers. This appears to reflect plasticity in egg investment rather than

36   among female differences. Analysis of maternal survival suggests that helped females are

37   better able to withstand the costs of breeding in hot conditions than females without helpers.

38   Our study suggests that females can use multiple, independent cues to modulate egg

39   investment flexibly in a variable environment.

40

41   Keywords: additive care, climate sensitivity, cooperative breeding, load lightening, maternal

42   effects

43   **Background**

44   Maternal investment in offspring size can be a significant determinant of variation in

45   individual fitness [1-4]. In birds, a meta-analysis demonstrated that egg size is positively

46   correlated with a range of measures of offspring quality, including hatching success, nestling

47   survival, size, growth rate, immunity, and post-fledging survival [4]. Yet resources are

48   generally finite, meaning that mothers must balance investment in current offspring against

49   conserving resources for future offspring and self maintenance [2]. Maternal investment in

50   egg size is thus likely to depend on the relative costs and benefits to females of investing in

51   current versus future offspring [2]. However, predicting the optimal solution to this life-

52   history trade-off is not straightforward in species such as birds, because the costs and benefits

53   are likely to vary in relation to both the environmental conditions at the time of egg

54   production [2] and the amount of investment provided to the offspring by other carers [5].

55

56   On the one hand, the silver spoon hypothesis [6, 7] suggests that those breeding in favourable

57   conditions should favour increased investment in offspring due to the disproportionately

58   greater fitness on offer [8, 9]. On the other hand, the 'bigger-is-better hypothesis' predicts that

59   the relationship between investment and offspring quality will be relatively stronger in

60   harsher or more competitive environments [10-13]. Specifically, mothers can benefit by

61   producing larger offspring when harsher conditions impose a greater threat to their offspring's

62   survival [4, 13-15]. This prediction has received support in studies of invertebrates, fish and

63   birds [16-21]. For example, in *Daphnia*, mothers reared on low food levels produced larger

64   eggs than genetically identical mothers grown at high food levels, and the larger offspring

65   were better able to survive long periods of starvation [22].

66

67   However, predicting optimal egg size becomes more complex in cooperatively breeding

68   species, where non-breeding helpers assist breeders with the care of young [5, 7]. Here the

69   helpers may allow breeders to reduce their investment in offspring, enhancing their own

70   survival with no (or little) net loss to current fitness (load lightening) [5, 23, 24]. This effect is

71   likely to be particularly pronounced in long-lived cooperative breeders, which are predicted to

72   favour investment in their own survival over current reproduction [5, 25] and where chick

73   provisioning by helpers can fully compensate for the reduced investment in eggs [7, 24, 26-

74   28]. Several studies have shown that females with helpers lay smaller eggs [24, 26, 27], and

75   enjoy increased survival, presumably at least partly as a consequence [24]. Thus, in contrast

76   to predictions of either silver spoon or bigger is better hypotheses, the 'load lightening'

77   hypothesis suggests that female cooperative breeders should produce smaller eggs, if

78   investment from their helpers will compensate for the shortfall in maternal pre-natal

79   investment [24].

80

81   How can we reconcile the predictions of the 'load lightening' hypothesis and the 'bigger is

82   better' hypothesis in cooperative breeders? In other words, how do the effects of helpers and

83   environmental conditions interact to influence optimal egg investment strategies [7]?

84   Hatchwell [5] proposed that parents should use the presence of helpers to reduce their own

85   effort (load lightening) when the marginal effect of helpers on reproductive success is

86   relatively low [29]. By contrast, when small increments in care have a relatively large

87   influence on offspring fitness, then breeders should maintain or even increase their level of

88   investment (see Fig. 3 in [5]). The effect of helper care on reproductive success is likely to

89   vary with environmental conditions, becoming more important as conditions worsen. In the

90   context of egg investment, Hatchwell's [5] hypothesis therefore predicts that females should

91   use provisioning by helpers to reduce egg investment under favourable environmental

92   conditions (ie. 'load-lighten'), but should maintain (or even increase) [7], investment in eggs

93   when environmental conditions deteriorate so that helper care can be used to promote

94   reproductive success in a harsh environment (ie. supporting the 'bigger is better hypothesis').

95

96   Here, we test these predictions in a long-lived, insectivorous, facultative cooperative breeder,

97   the superb fairy-wren *Malurus cyaneus*. This species is well-suited to investigating climatic

98   and helper effects on egg size, because fairy-wrens may breed as unassisted pairs or with the

99   assistance of 1-5 helpers, and do so in highly variable environments stemming from the

100   temperature differences across the protracted breeding season and significant inter-annual

101   variation in rainfall (Figures S1, S2). Climatic variables have a strong influence on breeding

102   success in fairy-wrens. Local rainfall has been shown to be a key predictor of annual

103   reproductive success [30, 31], probably because high rainfall leads to increased insect

104   abundance [32, 33]. Breeding is also affected by temperature; during heatwaves no new

105   breeding attempts are initiated, active nests may be abandoned (NEL pers. obs.) and nestlings

106   suffer a high growth cost [34]. Furthermore, females are highly variable in their investment

107   into egg size and larger eggs yield larger nestlings [24], which in turn have a greater chance of

108   being recruited to the breeding population [34].

109

110   Our previous work showed that female superb fairy-wrens laying their first clutch in spring

111   exhibit load-lightening in the presence of helpers, by laying smaller eggs with lower

112   nutritional content than pair breeding females [24]. However provisioning by helpers

113   compensated fully for this reduction in investment; chicks reared by groups grew faster than

114   chicks reared by pairs, allowing them to 'catch up' in body mass during the nestling period

115   [24]. Females with helpers were more likely to survive to breed again than females without

116   helpers [24, 30]. Thus female superb fairy-wrens benefit from concealed helper effects during

117   springtime. Whether this result holds when conditions become hotter and drier in summer is

118   unknown. Here we extend this study over ten years, encompassing several periods of both

119   drought [35] and high rainfall (Fig. S1), allowing us to test whether females modulate their

120   egg investment during harsher environmental conditions and how this affects their survival to

121   the following year.

122

123   One of the challenges in testing this prediction is that it is not always immediately apparent

124   which environmental variables are most influential in creating a 'harsh' breeding

125   environment, nor is the time window over which these variables should be measured

126   immediately obvious. We utilize a new statistical package (climwin R) [36] which detects

127   periods of climate sensitivity for a given biological response (in this case egg size), by testing

128   the effectiveness of a wide range of possible sampling periods over which climate is recorded

129   and identifying the most appropriate climate window for further use. We then test whether

130   rainfall or temperature during these key time windows interact with the presence of helpers to

131   determine egg volume.

132

133   **Methods**

134   *Study species and population*

135   Superb fairy-wrens are insectivorous, multi-brooded, cooperatively breeding passerines

136   endemic to south-eastern Australia [37]. Egg laying typically commences in September or

137   October (austral spring) and ends in January (austral summer), clutch size usually ranges from

138   three to four eggs, and up to two breeding attempts can be successful in a season. Rates of

139   nest predation are high, so females may initiate as many as eight nests in a season, leading to

140   breeding over a substantial range of environmental conditions [30]. The female builds the nest

141   and incubates the eggs, but all group members contribute to chick provisioning and nest

142   defence. In this study, helpers were present at 43% of nests, and 63% of cooperative groups

143   had just a single helper. Helpers are male offspring from previous breeding attempts, who

144   remain on their natal territory until a breeding vacancy arises on a neighbouring territory [30].

145   Incubation commences after clutch completion and chicks hatch synchronously.

146

147   The study was conducted over ten breeding seasons in Campbell Park, a 128 ha eucalypt

148   woodland in the Australian Capital Territory (149° 10' E, 35° 16' S) in 1999, 2003-2006, and

149   2011-2015. Annual population size ranged from 39 to 79 groups and 72% of females were

150   colour-banded for individual identification. The 'identity' of unbanded females with respect

151   to lay dates and helper numbers could be inferred from the banding of her group members

152   and/or because she was surrounded by banded females. Territories and group composition

153   were mapped at the start of the season and monitored with visits to the territory 1-5

154   times/week. The survival of banded females to the following breeding season was recorded

155   for all clutches except those measured in the 2015/2016 breeding season, as female survival

156   for those clutches will not be known until the 2016/2017 breeding season. We recorded the

157   number of helpers present in the group during the egg laying period for each nest. The nests

158   of all groups were monitored throughout the season, and after clutch completion the length

159   and breadth of all eggs in the clutch were measured using vernier callipers (± 0.1 mm). We

160   measured 1432 eggs from 431 clutches laid by 223 females. Measurements were made by

161   NEL in 1999, 2006 and 2011-15, by RMK in 1999 and 2003-05, and by AFR in 2003. There

162   was no significant difference in mean egg volume between measurements made by NEL

163   versus RMK/AFR (*t*-test, $t = 0.71$, $DF = 413$, $P = 0.48$). Egg volumes were calculated using

164   Hoyt's formula (0.51 x length x breadth^2) [38], and volumes were averaged within a clutch.

165   This research was conducted under approval of the Australian National University Animal

166   Ethics Committee Protocol Numbers F.BTZ.61.03 and A2012/47.

167

168 *Identifying the salient climatic window*

169 We obtained daily rainfall and temperature records from the Australian Bureau of

170 Meteorology (http://www.bom.gov.au/climate/). Weather measurements were taken from a

171 nearby weather station at Canberra Airport (4km SE of the study site).

172

173 We used the climwin package in R [36, 39] to detect and visualize periods of climate

174 sensitivity ('climate windows') for egg volumes. Climwin uses an exploratory sliding window

175 approach (eg.[34, 40]) to investigate all possible climate windows and compares their relative

176 importance using AICc [36, 41]. We investigated the influence of mean rainfall and mean

177 temperature on egg volume using a relative climate window method (climate windows

178 measured in days before the biological record) [36, 41]. Seasonal trends in rainfall and

179 temperature were included in respective analyses to ensure that standard seasonal patterns in

180 egg volume do not confound the climate window results. For example, if both egg volume

181 and seasonal rainfall increase as the season progresses, this might be detected as a strong

182 climate window, when in fact it is a repeatable seasonal trend.

183

184 Based on evidence that nestlings suffer a high growth cost when reared during heatwaves

185 [34], we also tested whether temperature thresholds might affect egg volume. We adjusted our

186 climate data to determine the number of days exceeding four temperature thresholds (20, 25,

187 30, 35 °C). We then carried out a climwin analysis to investigate the effect of the total number

188 of days over each temperature threshold on egg volume. We identified strong climate

189 windows in rainfall and temperature above 25°C (see Results). However, it is plausible that

190 these two climatic variables may influence egg volume in combination rather than

191 individually. To test this possibility, we created a principal component (PC) that integrated

192   both rainfall and temperature above 25°C. This PC was positively correlated with rainfall and

193   negatively correlated with temperature above 25°C (Table S3, Pearson's correlation

194   coefficient: 0.73 and -0.73 respectively), so that a large value of PC represented cold and wet

195   conditions while a small value of PC signified warm and dry conditions. We then carried out

196   a further climwin analysis using this PC variable. All analyses were repeated using linear,

197   quadratic and cubic terms in the models. All climwin analyses tested for a relationship

198   between climate and the biological response between 150 and 0 days before clutch initiation.

199   To avoid identifying a false climate window (type I error), all top windows were compared to

200   the results of climate window analyses conducted on randomised data (i.e. data with no

201   relationship between climate and egg volume; [36, 41]). To compare the strength of climate

202   windows from different climate variables, climate window results are presented using a

203   standardised predictor variable ($\mu = 0$, $\sigma = 1$).

204

205   Egg volume responses to climate may be driven by changes within females (i.e. phenotypic

206   plasticity) or changes between females (e.g. whether or not a female chooses to lay in

207   particular conditions). To test between these possibilities, we carried out within-subject

208   centring on the egg volume data, described in detail by van de Pol and Wright 2009 [42],

209   allowing us to isolate changes in egg volume within females from those between females.

210   Within-subject centring subtracts the subject mean (e.g. mean individual egg volume) from

211   each observed measurement, removing any potential between-subject variation in egg

212   volume. This within-subject centring was carried out following climate window analysis,

213   allowing us to specifically examine phenotypic plasticity as a driver of egg volume in

214   response to climate (rainfall, number of days over 25°C) in *M. cyaneus.*.

215

216   *Statistical analyses*

217   To test whether climatic variables interact with the presence of helpers to determine egg

218   volume, we constructed four restricted maximum likelihood (REML) models in GenStat v.15

219   (VSN International). GenStat calculates denominator degrees of freedom and p-values using

220   the method described in [43]. In each case, mean egg volume for each clutch was fitted as the

221   response variable. All models included two random effects, female identity and year, to

222   account for measurement of multiple clutches per female and per year. In the first model,

223   following the climate sensitivity analysis we included the 'best windows' (see results) for

224   mean rainfall and number of days over 25° C as fixed effects. We also included 'Date' (the

225   day of the year on which laying commenced for each clutch, which aims to account for any

226   natural seasonal trends in egg volume that occur independently of short term climatic

227   fluctuations), the presence/absence of helpers (yes/no), and clutch size as fixed terms, and

228   tested for interactions between helper presence and the climate variables. The final model

229   included only those terms and interactions with significant explanatory power in the model.

230   This model included the effect of helpers as a categorical 'yes/no' following our previous

231   work [24], but we also re-ran the model using number of helpers (0, 1 or 2+), as this might

232   provide greater insights into helper effects (Model 2). We also tested whether the combined

233   effects of the number of days over 25°C and rainfall had better explanatory power. The

234   analysis was the same as above, except that the best window from the PCA data was used,

235   instead of including rainfall and number of days over 25°C as separate fixed effects (Model

236   3). To test whether differences in egg volume in relation to climatic variables and/or the

237   presence of helpers in their group were the outcome of within- or between-female effects, we

238   repeated the same analysis as Model 1 above, but replaced the number of days over 25°C and

239   rainfall with the within-female effects of the number of days over 25°C and rainfall (Model

240   4).

241

242   Finally, we investigated the probability that a breeding female would survive to the start of

243   the following breeding season (n=119 females and 276 female-years) using a Generalised

244   Linear Mixed Model with binomial error and logit link function Here, the fixed effects were

245   helper presence (yes/no), mean egg volume/clutch, clutch size, number of days over 25°C and

246   rainfall from the best windows calculated above, and the two-way interactions between helper

247   presence and climatic variables, egg volume and climatic variables, and egg volume and the

248   presence of helpers. Female identity and year were included as random terms.

249

250   **Results**

251   (i) Climate windows and egg investment

252   Our analysis of climatic sensitivity revealed that egg volumes increased during dry and hot

253   conditions. Of the four fitted temperature thresholds, egg volume was most strongly affected

254   by temperatures over 25°C (Table S1), with a strong positive linear relationship between egg

255   volume and the number of days over 25°C over the two weeks prior to egg laying (Fig. 1; 12

256   – 2 days before egg laying; $\beta = 21.86$ mm^3 [95% CI: 12.05 – 31.68]). We also documented a

257   strong negative linear response in egg volume to mean rainfall prior to egg laying (Fig. 2; 20

258   – 59 days before egg laying; $\beta = -14.59$ mm^3 [95% CI: -21.84 - -7.35]; Table S2). Analysis

259   using the principal component (combining mean rainfall and number of days over 25°C)

260   showed a similar result; there was a negative relationship between the principal component

261   and egg volume, with egg volume increasing in hot and dry conditions but decreasing in cold

262   and wet conditions (Fig. 3; 44 – 27 days before egg laying; -13.90 mm^3 [95% CI: -21.33 to -

263   6.46]; Table S2). Models using linear, quadratic and cubic terms had equally high explanatory

264   power for all climate variables, so the output from the linear model is used in further analyses

265   to allow for within-female centring. Type I error was very unlikely in climate window

266   analysis of temperature over 25°C (3% chance; Table S1), but possible in analyses of climate

267   windows for mean rainfall (14%) and the principal component (22%; Table S2).

268

269   (ii) Modulating effects of helpers

270   Following identification of the best climate windows of those considered, we tested for an

271   interaction between these variables and the presence of helpers in the group on egg size

272   (Table 1). Neither clutch size nor day of the season was significantly related to egg volume in

273   any analyses. By contrast, we found that the effects of temperature on egg size were

274   modulated significantly by the presence of helpers at the nest (Table 1, Fig. 4). In cooler

275   conditions, females with helpers laid smaller eggs than females without helpers, but in hotter

276   conditions these females laid larger eggs than females without helpers (Table 1, Fig. 4). Egg

277   size generally declined with increasing mean rainfall, but this relationship was not

278   significantly altered by the presence of helpers in the group (Table 1).

279

280   Replacing helper presence/absence with helper number (0, 1, 2+) in the model (Model 2)

281   yielded qualitatively similar results; females with helpers laid larger eggs under increasingly

282   hot conditions, and the increase in egg size was more pronounced in groups with large

283   numbers of helpers (Group size x days over 25°C; $F_{1,302.4}$,= 4.36, $P = 0.038$). Just as in Model

284   1, we also found that egg size declined with increasing mean rainfall ($F_{1,300.1} = 10.66$, $P <$

285   0.001). When the analysis was repeated using the best window for the PCA on number of

286   days over 25° C and rainfall (Model 3), this term was highly significant ($F_{1,188.1} = 12.41$, $P <$

287   0.001), but did not interact significantly with the presence of helpers in the group ($F_{1,311.1}=$

288   0.10, $P = 0.748$).

289

290   An obvious confound of the above results would arise if females with and without helpers

291   bred at different times of the year and so under contrasting climatic conditions. First, we

292   found no evidence to suggest that the variance in lay-dates within years differed between

293   those females breeding with and without helpers (Brown-Forsythe test for equality of

294   variances, $P > 0.3$ for all years), suggesting that females with helpers did not breed over more

295   extreme climatic conditions. Second, in a REML model (with female identity and year as

296   random terms), we found no evidence of an interaction between the presence of helpers and

297   the number of days over 25 °C on lay-dates (helper presence x days over 25°C; $F_{1,368.4} = 0.46$,

298    $P = 0.5$). This suggests that females with helpers are not more likely to lay in unfavourable

299   climatic conditions than those without helpers.

300

301   That breeding phenology was uninfluenced by helper presence suggests that the reported

302   effects might stem, at least partly, from within-female adjustments in investment as a function

303   of climate and helper presence. Our subsequent analyses provide some support for this

304   hypothesis (Model 4). As in Model 1, females laid larger eggs under hotter conditions ($F_{1,206}$

305    $= 4.96$, $P = 0.027$), and there was a non-significant trend for females with helpers to lay

306   smaller eggs than females without helpers in cooler conditions and to lay larger eggs than

307   females without helpers as temperatures rose ($F_{1,208} = 3.19$, $P = 0.076$). The reduced

308   significance of this effect is likely to reflect the lower statistical power of these analyses,

309   which used a smaller number of females for whom we had measurements of multiple clutches

310   (102 vs. 223 females). Once again we found that females laid smaller eggs with increasing

311   mean rainfall ($F_{1,204.2} = 7.55$, $P = 0.007$).

312

313   (iii) Female survival

314   Finally, we examined whether egg investment was correlated with female survival. There was

315   no significant effect of egg volume or the interaction between egg volume and the presence of

316   helpers in the group on female survival (Table 2). This is not surprising given that females

317   with helpers generally lay smaller eggs early in the season and larger eggs later in the season

318   (Fig. 1), so their overall investment in egg volume over the course of the season is likely to be

319   similar to that of females without helpers. However, rainfall in the two months prior to egg

320   laying was a significant predictor of female survival to the next season. There was also a

321   significant interaction between the presence of helpers in the group and the number of days

322   over 25°C (Table 2, Fig. S6), indicating that under cool conditions females with and without

323   helpers were equally likely to survive to the following season, whereas under hotter

324   conditions females with helpers were significantly more likely to survive to the following

325   season than females without helpers (Table 2, Fig. S6).

326

327   **Discussion**

328   Our results suggest that superb fairy-wren egg volume varies in relation to two independent

329   ecological conditions: climate and helper presence. In accordance with the 'bigger is better'

330   hypothesis, females laid larger eggs when breeding in dry conditions. In accordance with our

331   previous findings [24], and the predictions of the load-lightening hypothesis [5, 7], females

332   breeding during cool conditions and in the presence of helpers reduced their investment in egg

333   volume. Additionally, and in support of the 'bigger is better' hypothesis, females with helpers

334   laid larger eggs during hot conditions. Finally, females without helpers breeding in hot

335   conditions suffered reduced survival to the following year.

336

337   Egg volume was related to mean rainfall, being smaller following wetter periods prior to egg

338   laying. The association between egg volume and rainfall is likely to reflect the predicted load

339   lightening by females during favourable conditions, because higher rainfall increases

340   vegetation growth, leading to increased insect biomass [32, 33]. We found that the time

341   window during which rainfall influenced egg size did not extend right up to the point of egg

342   laying, but instead closed 20 days beforehand. This may indicate that insect abundance or

343   female body condition exerts more influence on egg volume than rainfall per se, and there is a

344   lag time in the chain of events from rainfall to increased insect abundance to improved body

345   condition. In support, the relationship between rainfall and chick mass in superb fairy-wrens

346   also shows a similar two week lag time [34].

347

348   Of the climatic variables tested, temperature was most strongly correlated with egg volume,

349   but its effect was modulated by the presence of helpers in the group. Females with helpers

350   laid smaller eggs than females without helpers when it was cooler in the two weeks prior to

351   egg laying, but laid larger eggs than females without helpers when conditions were hotter

352   prior to egg laying (Fig 4). Existing theory of parental investment strategies in the presence of

353   helpers predicts load-lightening in species breeding in favourable conditions but not in those

354   breeding in more challenging environments [5, 7]. Our results show that such theory can be

355   extended to different investment strategies among and within females in a population: in the

356   presence of helpers, egg investment was lower when offspring growing conditions were

357   favourable but increased when growing conditions deteriorated. Production of large eggs

358   during heatwaves is likely to be particularly beneficial for nestling survival, because superb

359   fairy-wren chicks suffer weight loss during such conditions [34]. This has been attributed to a

360   combination of a direct effect of dehydration or lethargy of chicks, and an indirect result of

361   reduced foraging or provisioning effort by parents or helpers [34] (see also [44] for similar

362   evidence in common fiscal shrikes *Lanius collaris*). Larger chicks may be less vulnerable to

363   heat stress, because the rate of evaporative water loss decreases with increasing body mass

364   [45] or because they are more quickly able to reach internal homeostatis during development.

365   Thus, females that lay larger eggs and thereby produce larger hatchlings during heatwaves can

366   buffer their chicks against the risks associated with reduced provisioning by carers, weight

367   loss and dehydration, thereby increasing the probability of offspring survival in the first few

368   days after hatching [46]. The extent to which large egg size can ameliorate the effects of hot

369   weather could be tested explicitly in future work by examining the relationship between egg

370   size, climatic conditions and provisioning rates on nestling mass and survival.

371

372   An obvious question, however, is that if the production of larger eggs is so beneficial under

373   hotter conditions, then why are they not also produced by females without helpers? Our

374   results suggest that production of larger eggs by females without helpers may be constrained

375   in two ways by the extra effort they must put into their offspring. Unlike helped females,

376   these females cannot afford to under-invest in their eggs during the cool conditions early in

377   the season (Fig. 4) because there are no helpers to compensate for the shortfall in egg

378   nourishment. Producing larger eggs at the start of the season may limit the extent to which

379   these females can increase egg size further in hotter conditions later in the season. In addition,

380   females breeding without helpers work harder to provision their chicks than females with

381   helpers [29], and this too might limit the extent to which they can increase investment in their

382   eggs, particularly when breeding conditions are harsh. Further, our analysis of female survival

383   (Fig. S6) suggests that mothers without helpers may not be able to afford to produce larger

384   eggs because their survival after breeding in hot conditions was already compromised, even

385   without additional egg investment. The hypothesis emerging from these results is that the

386   presence of helpers liberates female fairy-wrens to modify their investment to different

387   extents during egg laying versus nestling provisioning depending on prevailing climatic

388   conditions.

389

390   In a previous study [24], we reported that females reduced egg investment in the presence of

391   helpers with such reductions explaining why the mass of nestlings was equivalent across

392   helped and unhelped nests despite their receiving more food with helpers [24, 47]. Our results

393   here suggest that egg investment strategies are more complicated than we previously

394   supposed. Our finding that females reduced egg volume in the presence of helpers when

395   laying during cool conditions corroborates our previous analyses, which were restricted to

396   first attempts and so more cool temperatures. How then do we reconcile the lack of helper

397   effects on nestling mass in previous studies, when females apparently increase egg size when

398   laying in hot weather? There are three possible explanations. (1) The proportion of nests

399   monitored during hot weather has been too low to detect helper effects overall. This is likely

400   to be a contribution factor because: (a) prolonged hot conditions occur relatively infrequently

401   (Fig. S7) and late in the season (Fig. S2), when a reduced percentage of clutches are initiated

402   (Fig S2), and (b) heatwaves have increased in frequency in recent years [34]. (2) Increases in

403   egg investment give offspring a 'head start'[7], which allows group members to reduce

404   provisioning investment during hot conditions (see Discussion above). (3) Alternatively,

405   increases in egg volume arising during hot weather might primarily derive from increased

406   water content to reduce the probability of dehydration [48], rather than from increases in

407   nutrient content. Further work is required to test the validity of these hypotheses.

408

409   Either way, our finding that, on average, only those females with helpers increased egg

410   volume when breeding during hot weather suggests that it is adaptive to do so. Several studies

411   suggest that the benefits of helpers to breeders are greater when environmental conditions are

412   harsh [5, 49-53], and that cooperative breeding is a bet-hedging strategy in which helpers

413   reduce variance in reproductive success associated with environmental uncertainty [54, 55].

414   Our previous results suggested that load lightening resulted in greater survival of breeding

415   females [24]. Our new results suggest that load lightening during good conditions might also

416   enable females to conserve additional resources to allocate to eggs during harsh conditions,

417   when additional egg investment is likely to make the greatest difference to nestling and

418   fledgling survival. If this is the case, then reduced egg investment with helpers early in the

419   breeding season might additionally be viewed as a bet hedging strategy, enabling greater

420   investment late in the season should temperatures rise. Nevertheless, it is noteworthy that

421   prolonged hot conditions occur relatively infrequently (Fig. S7) and late in the season (Fig.

422   S2), so females initiate relatively few clutches under these circumstances (Fig. S2), which

423   might explain why females with helpers exhibit greater survival than those without, despite

424   greater investment in eggs during hot conditions.

425

426   In conclusion, our results suggest a novel mechanism by which helpers may benefit female

427   breeders during harsh conditions: by facilitating strategic increases in egg size under harsh

428   environmental conditions. Such buffers are likely to become increasingly beneficial as climate

429   change leads to an increase in the frequency and magnitude of extreme climatic events [56],

430   potentially even resulting in greater resilience and lower rates of population extinctions in

431   cooperatively breeding species than in pair breeders in response to climate change.

432

433   **Competing interests**

434   We have no competing interests

435

436   **Data accessibility**

437   Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.087b9

438

439   **Author contributions**

440   The study was conceived by NEL. Field data were collected by NEL, RMK and AFR.

441   Analyses were conducted by NEL, LDB and RGH. NEL drafted the manuscript. All authors

442   gave feedback on the ms and final approval for publication.

443

449

450   **References**

451   1.   Benton T.G., Plaistow S.J., Beckerman A.P., Lapsley C.T., Littlejohns S. 2005 Changes

452   in maternal investment in eggs can affect population dynamics. *Proc. R. Soc. B* **272**,

453   1351-1356.

454   2.   Bernardo J. 1996 The particular maternal effect of propagule size, especially egg size:

455   patterns, models, quality of evidence and interpretations. *Am. Zool.* **36**, 216-236.

456   3.   Clutton-Brock T.H. 1991 *The Evolution of Parental Care*, Princeton University Press.

457   4.   Krist M. 2011 Egg size and offspring quality: a meta-analysis in birds. *Biol. Rev.* **86**,

458   692-716.

459   5.   Hatchwell B.J. 1999 Investment strategies of breeders in avian cooperative breeding

460   systems. *Am. Nat.* **154**, 205-219.

461   6.   Grafen A. 1988 On the uses of data on lifetime reproductive success. In *Reproductive*

462   *Success* (ed. Clutton-Brock T.H.), pp. 454-471. Chicago, IL, University of Chicago

463   Press.

464   7.   Savage J.L., Russell A.F., Johnstone R.A. 2015 Maternal allocation in cooperative

465   breeders: should mothers match or compensate for expected helper contributions? *Anim.*

466   *Behav.* **102**, 189-197.

467   8.   Lummaa V., Clutton-Brock T. 2002 Early development, survival and reproduction in

468   humans. *Trends Ecol. Evol.* **17**, 141-147.

469   9.   Lindstrom J. 1999 Early development and fitness in birds and mammals. *Trends Ecol.*

470   *Evol.* **14**, 343-348.

471   10.   Brockelman W.Y. 1975 Competition, fitness of offspring, and optimal clutch size. *Am.*

472   *Nat.* **109**, 677-699.

473   11.   McGinley M.A., Temme D.H., Geber M.A. 1987 Parental investment in offspring in

474   variable environments: theoretical and empirical considerations. *Am. Nat.* **130**, 370-398.

475   12.   Parker G.A., Begon M. 1986 Optimal egg size and clutch size: effects of environment

476   and maternal phenotype. *Am. Nat.* **128**, 573-592.

477   13.   Sogard S.M. 1997 Size-selective mortality in the juvenile stage of teleost fishes: a

478   review. *Bull. Mar. Sci.* **60**, 1129-1157.

479   14.   Kuijper B., Johnstone R.A. 2013 How should parents adjust the size of their young in

480   response to local environmental cues? *J. Evol. Biol.* **26**, 1488-1498.

481   15.   Parker G.A., Begon M. 1986 Optimal egg size and clutch size: effects of environment

482   and maternal phenotype. *Am. Nat.* **128**, 573-592.

483   16.   Bashey F. 2006 Cross-generational effects and the evolution of offspring size in the

484   Trinidadian guppy *Poecilia reticulata. Evolution* **60**, 348-361.

485   17.   Fox C.W. 2000 Natural selection on seed-beetle egg size in nature and the laboratory:

486   variation among environments. *Ecology* **81**, 3029-3035.

487   18.   Fox C.W., Czesak M.E. 2000 Evolutionary ecology of progeny size in arthropods.

488   *Annu. Rev. Entomol.* **45**, 341-369.

489   19.   Garant D., Kruuk L.E.B., McCleery R.H., Sheldon B.C. 2007 The effects of

490   environmental heterogeneity on multivariate selection on reproductive traits in female

491   great tits. *Evolution* **61**, 1546-1559.

492   20.   Smith H.G., Bruun M. 1998 The effect of egg size and habitat on starling nestling

493   growth and survival. *Oecologia* **115**, 59-63.

494   21.   Styrsky J.D., Eckerle M.P., Thompson C.F. 1999 Fitness-related consequences of egg

495   mass in nestling house wrens. *Proc. R. Soc. B* **266**, 1253-1258.

496   22.   Gliwicz Z.M., Guisande C. 1992 Family planning in Daphnia: resistance to starvation in

497   offspring born to mothers grown at different food levels. *Oecologia* **91**, 463-467.

498   23.   Crick H. 1992 Load-lightening in cooperatively breeding birds and the cost of

499   reproduction. *Ibis* **134**, 56-61.

500   24.   Russell A.F., Langmore N.E., Cockburn A., Astheimer L.B., Kilner R.M. 2007 Reduced

501   egg investment can conceal helper effects in cooperatively breeding birds. *Science* **317**,

502   941-944.

503   25.   Ghalambor C.K., Martin T.E. 2001 Fecundity-survival trade-offs and parental risk-

504   taking in birds. *Science* **292**, 494-497.

505   26.   Canestrari D., Marcos J.M., Baglioni V. 2011 Helpers at the nest compensate for

506   reduced maternal investment in egg size in carrion crows. *J. Evol. Biol.* **24**, 1870-1878.

507   27.   Paquet M., Covas R., Chastel O., Parenteau C., Doutrelant C. 2013 Maternal effects in

508   relation to helper presence in the cooperatively breeding sociable weaver. *PLoS ONE* **8**,

509   e59336.

510   28.   Santos E.S.A., Macedo R.H. 2011 Load lightening in southern lapwings: group-living

511   mothers lay smaller eggs than pair-living mothers. *Ethology* **117**, 547-555.

512   29.   Russell A.F., Langmore N.E., Gardner J.L., Kilner R.M. 2008 Maternal investment

513   tactics in superb fairy-wrens. *Proc. R. Soc. B* **275**, 29-36.

514   30.   Cockburn A., Sims R.A., Osmond H.L., Green D.J., Double M.C., Mulder R.A. 2008

515   Can we measure the benefits of help in cooperatively breeding birds: the case of superb

516   fairy-wrens *Malurus cyaneus*? *J. Anim. Ecol.* **77**, 430-438.

517   31.   van de Pol M., Brouwer L., Brooker L.C., Brooker M.G., Colombelli-Negrel D., Hall

518   M.L., Langmore N.E., Peters A., Pruett-Jones S., Russell E.M., et al. 2013 Problems

519   with using large-scale oceanic indices to compare climatic sensitivities across

520   populations and species. *Ecography* **36**, 249-255.

521   32.   Recher H.F., Majer J.D., Ganesh S. 1996 Seasonality of canopy invertebrate

522   communities in eucalypt forests of eastern and western Australia. *Aust. J. Ecol.* **21**, 64-

523   80.

524   33.   Woinarski J.C.Z., Cullen J.M. 1984 Distribution of invertebrates on foliage in forests of

525   south-eastern Australia. *Aust. J. Ecol.* **9**, 207-232.

526   34.   Kruuk L.E.B., Osmond H.L., Cockburn A. 2015 Contrasting effects of climate on

527   juvenile body size in a Southern Hemisphere passerine bird. *Glob. Chang. Biol*. (doi:

528   10.1111/gcb.12926).

529   35.   Steffen W. 2015 Thirsty Country: Climate Change and Drought in Australia. Climate

530   Change Council of Australia Ltd.

531   36.   Bailey L.D., van de Pol M. 2016 climwin: An R Toolbox for Climate Window

532   Analysis. bioRxiv http://dx.doi.org/10.1101/069427

533   37.   Rowley I., Russell E. 1997 *Fairy-wrens and Grasswrens*. Oxford, Oxford University

534   Press.

535   38.   Hoyt D.F. 1979 Practical methods of estimating volume and fresh weight of bird eggs.

536   *Auk* **96**, 73-77.

537   39.   R Core Team. 2016 R: A language and environment for statistical computing. Vienna,

538   Austria, R Foundation for Statistical Computing.

539   40.   Stopher K.V., Bento A.I., Clutton-Brock T.H., Pemberton J.M., Kruuk L.E.B. 2014

540   Multiple pathways mediate the effects of climate change on maternal reproductive traits

541   in a red deer population. *Ecology* **95**, 3124-3128.

542   41.   van de Pol M., Bailey L.D., McLean N., Rijskijk L., Lawson C.R., Brouwer L. 2016

543   Identifying the best climatic predictors in ecology and evolution. *Methods Ecol. Evol.* **in**

544   **press**. (doi:10.1111/2041-210X.12590).

545   42.   van de Pol M., Wright J. 2009 A simple method for distinguishing within- versus

546   between-subject effects using mixed models. *Anim. Behav.* **77**, 753-758.

547   43.   Kenward M.G., Roger J.H. 1997 Small sample inference for fixed effects from

548   restricted maximum likelihood. *Biometrics* **53**, 983-997.

549   44.   Cunningham S.J., Martin R.O., Hojem C.L., Hockey P.A.R. 2013 Temperatures in

550   excess of critical thresholds threaten nestling growth and survival in a rapidly-warming

551   arid savanna: a study of common fiscals. *PLoS ONE* **8**, e74613.

552   45.   McKechnie A.E., Wolf B.O. 2010 Climate change increases the likelihood of

553   catastrophic avian mortality during extreme heat waves. *Biol. Lett.* **6**, 253-256.

554   46.   Williams T.D. 1994 Intraspecific variation in egg size and egg composition in birds:

555   effects on offspring fitness. *Biol. Rev.* **68**, 35-39.

556   47.   Mulder R.A., Dunn P.O., Cockburn A., Lazenby-Cohen K.A., Howell M.J. 1994

557   Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proc. R.*

558   *Soc. B* **255**, 223-229.

559   48.   Nager R.G., Monaghan P., Houston D.C. 2000 Within-clutch trade-offs between the

560   number and quality of eggs: Experimental manipulations in gulls. *Ecology* **81**, 1339-

561   1350.

562   49.   Cockburn A. 1998 Evolution of helping behaviour in cooperatively breeding birds. *Ann.*

563   *Rev. Ecol. Syst.* **29**, 141-177.

564   50.   Covas R., du Plessis M.A., Doutrelant C. 2008 Helpers in colonial cooperatively

565   breeding sociable weavers *Philetairus socius* contribute to buffer the effects of adverse

566   breeding conditions. *Behav. Ecol. Sociobiol.* **63**, 103-112.

567   51.   Jetz W., Rubenstein D.R. 2011 Environmental uncertainty and the global biogeography

568   of cooperative breeding in birds. *Curr. Biol.* **21**, 72-78.

569   52.   Magrath R.D. 2001 Group breeding dramatically increases reproductive success of

570   yearling but not older female scrubwrens: a model for cooperatively breeding birds. *J.*

571   *Anim. Ecol.* **70**, 370-385.

572   53.   Rubenstein D.R., Lovette I.J. 2007 Temporal environmental variability drives the

573   evolution of cooperative breeding in birds. *Curr. Biol.* **17**, 1414-1419.

574   54.   Cockburn A., Russell A.F. 2011 Cooperative breeding: A question of climate? *Curr.*

575   *Biol.* **21**, R195-R197.

576   55.   Rubenstein D.R. 2011 Spatiotemporal environmental variation, risk aversion and the

577   evolution of cooperative breeding as a bet-hedging strategy. *Proc. Nat. Acad. Sci.* **108**,

578   10816-10822.

579   56.   Easterling D.R., Meehl G.A., Parmesan C., Changnon S.A., Karl T.R., Mearns L.O.

580   2001 Climate extremes: observations, modeling and impacts. *Science* **289**, 2068-2074.

581

582

583

584   **Table 1**. Mixed model analysis of egg volume, including the main effects of the presence of

585   helpers, day number, clutch size and the 'best' windows for weather variables of those tested.

586   Temp25 refers to the number of days $> 25°$ C in the 2-12 days prior to egg laying. Mean

587   rainfall: window from 20 - 59 days prior to egg laying.

588

Fixed term	*F*	n.d.f	d.d.f	*P*
Temp25 x Helped	5.66	1	294.4	**0.018**
Mean rainfall x Helped	2.07	1	302.6	0.152
Temp25	13.17	1	146.7	**<0.001**
Mean rainfall	11.88	1	276.2	**<0.001**
Clutch size	0.50	1	333.7	0.479
Day number	1.36	1	244.2	0.244
Helped	0.12	1	406.4	0.725
Random effects	Variance component			
Female identity	6430			
Year	0			
Model AIC = 4134.16				

589

590

591

592   **Table 2**. Mixed model analysis of the probability of female survival to the following breeding

593   season, including the main effects of the presence of helpers at the time of egg laying, mean

594   egg volume, clutch size and the 'best' windows for weather variables of those tested. Temp25

595   refers to the number of days > 25° C in the 2-12 days prior to egg laying. Mean rainfall:

596   window from 20 - 59 days prior to egg laying.

597

Fixed term	F	n.d.f	d.d.f	P
Helped x Egg volume	0.38	1	251.9	0.536
Rainfall x Egg volume	0.23	1	212.2	0.630
Temp25 x Egg volume	0.09	1	204.7	0.762
Temp25 x Helped	4.82	1	188.1	**0.029**
Temp25	1.51	1	167.0	0.221
Mean rainfall	8.73	1	179.5	**0.004**
Egg volume	0.02	1	256.8	0.900
Clutch size	1.53	1	223.9	0.218
Helped	3.34	1	235.5	0.069
Random effects	Variance component			
Female identity	9.533			
Year	0.007			
Dispersion parameter	0.422			

598
599
600

601   Figure legends.

602   Figure 1: Raw data and model predictions showing the mean (± SE) egg volume of *Malurus*

603   *cyaneus* clutches in relation to the number of days above 25°C (2-12 days prior to egg laying;

604   Table S1, Fig. S3). Data was collected over 10 years between 1999 and 2015. The colour of

605   points signifies the year of collection, with darker points sampled in earlier years and lighter

606   points sampled in later years.

607   Figure 2: Raw data and model predictions showing the mean (± SE) egg volume of *Malurus*

608   *cyaneus* clutches in relation to mean rainfall (mm) (59 – 20 days prior to egg laying; Table

609   S2, Fig. S4). Data was collected over 10 years between 1999 and 2015. The colour of points

610   signifies the year of collection, with darker points sampled in earlier years and lighter points

611   sampled in later years.

612   Figure 3: Raw data and model predictions showing the mean (± SE) egg volume of *Malurus*

613   *cyaneus* clutches in relation to a principal component integrating mean rainfall and

614   temperature above 25°C (44 – 27 days prior to egg laying; Table S2, Fig. S5). A positive

615   value represents cold and wet conditions, while a negative value represents hot and dry

616   conditions. Data was collected over 10 years between 1999 and 2015. The colour of points

617   signifies the year of collection, with darker points sampled in earlier years and lighter points

618   sampled in later years.

619   Figure 4. Raw data and model predictions showing the mean (± SE) egg volume of *Malurus*

620   *cyaneus* clutches in relation to the number of days over 25°C (2-12 days prior to egg laying;

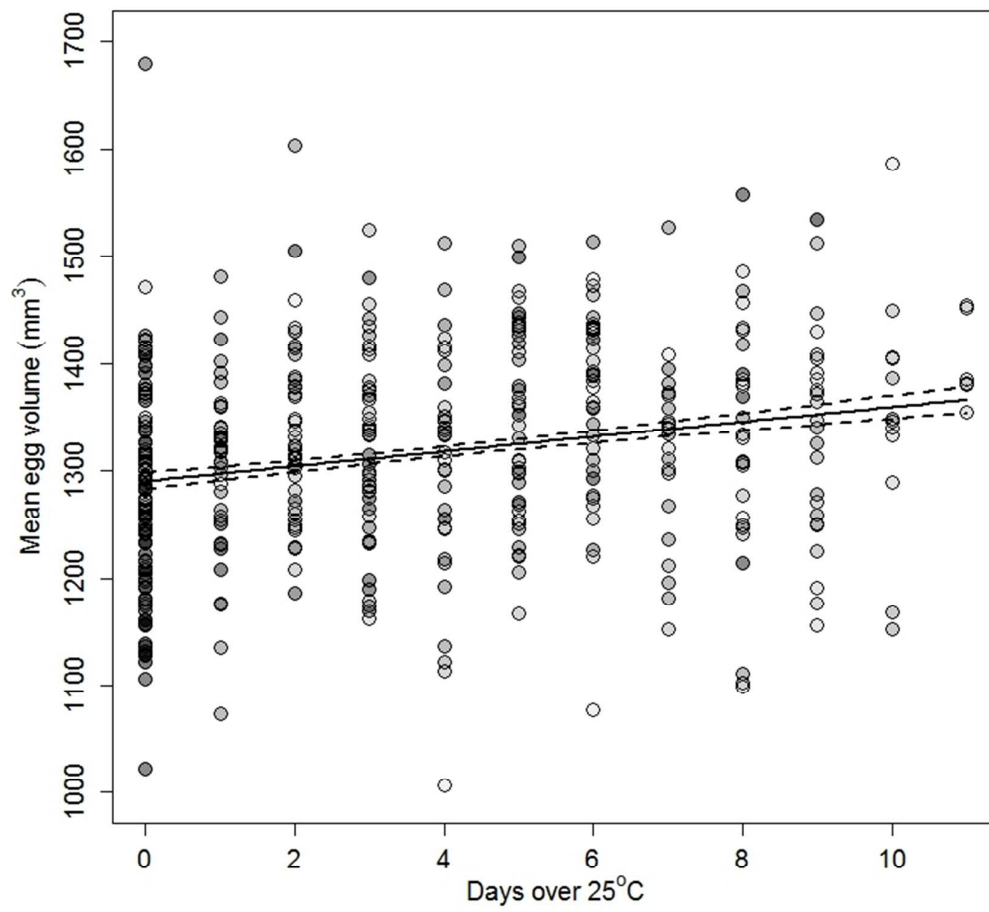621   Table S1, Fig. S3), for pairs with helpers (solid circles, solid line) and pairs without helpers
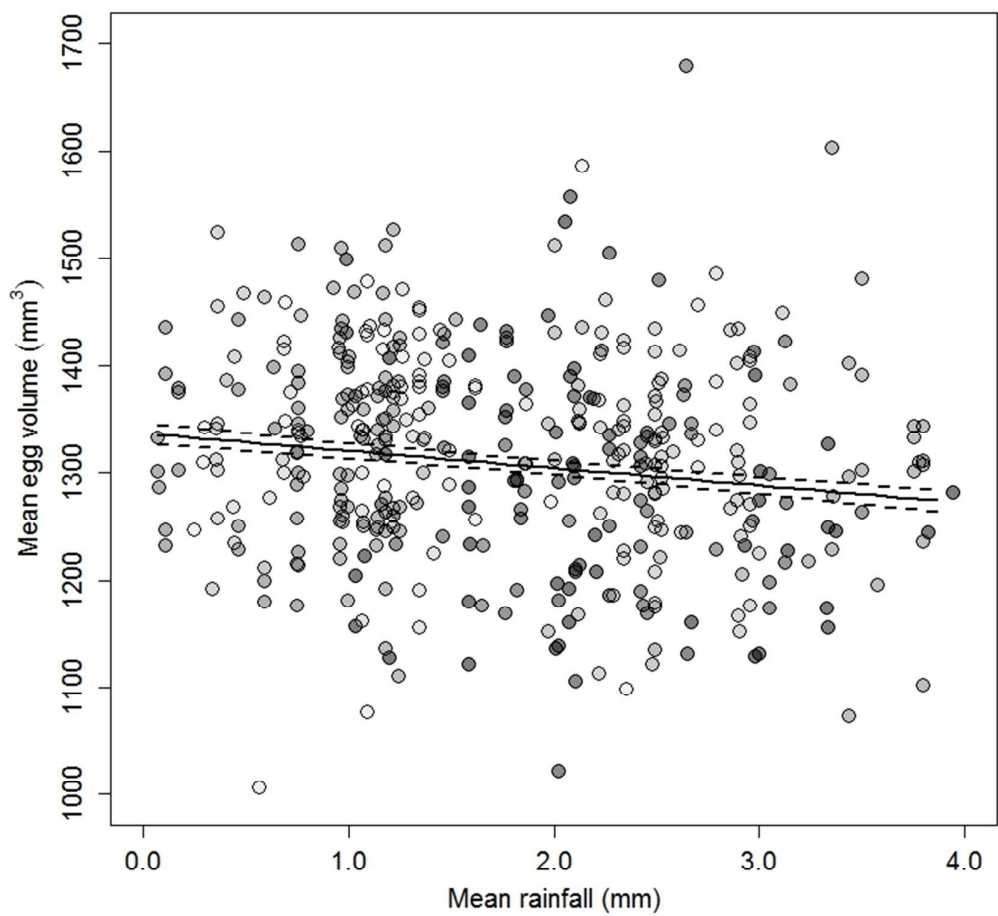
622   (open circles, dashed line).
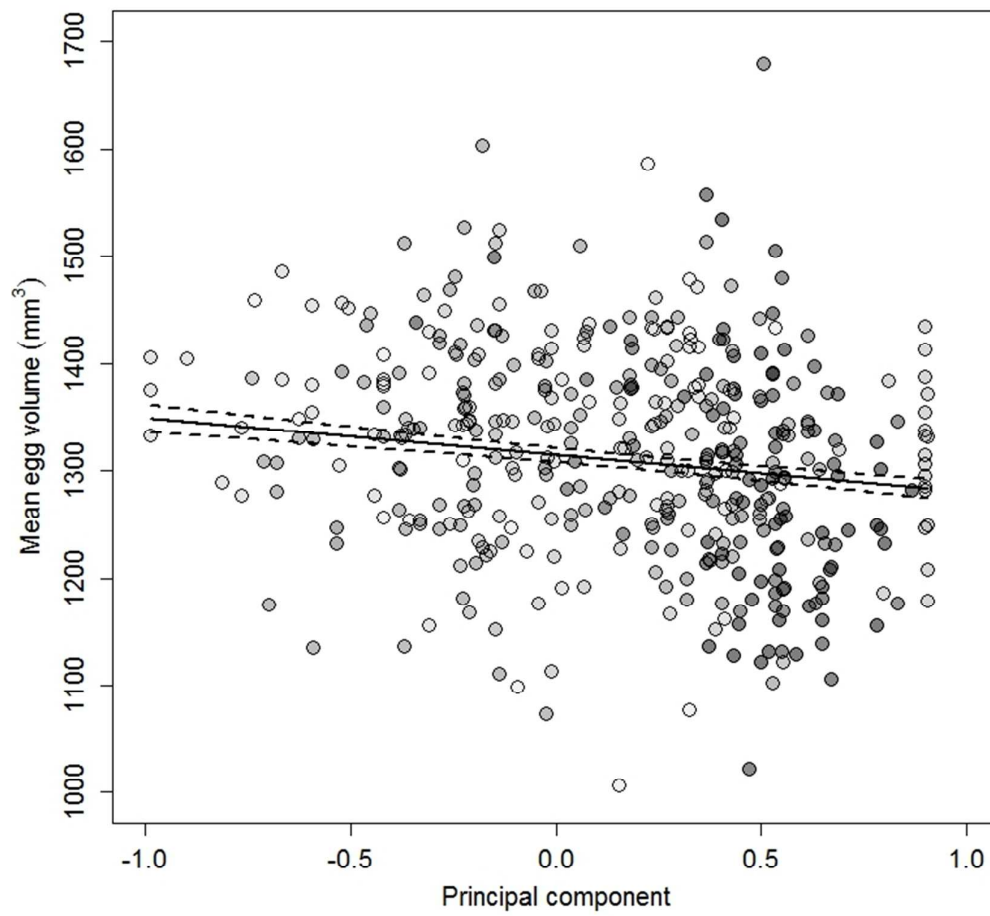
623

Figure 1

Figure 2

Figure 3

Figure 4