

PROCEEDINGS B

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

Journal:	<i>Proceedings B</i>
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 University, 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. Langmore¹, L. D. Bailey¹, R. G. Heinsohn², A. F. Russell³, R. M. Kilner⁴

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 ($0.51 \times \text{length} \times \text{breadth}^2$) [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; $\beta = 21.86 \text{ mm}^3$ [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; $\beta = -14.59 \text{ mm}^3$ [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

444 **Acknowledgements**

445 Warmest thanks to the many field assistants that helped with nest searching, particularly

446 Wendy Dimond, and thanks to the Australian Bird and Bat Banding Scheme for supplying

447 bird bands. The work was funded by grants from the Australian Research Council and

448 National Geographic (NEL), and the Royal Society (AFR and RMK).

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., Rijksijk 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 $> 25^{\circ}$ 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	<i>F</i>	n.d.f	d.d.f	<i>P</i>
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	<i>F</i>	n.d.f	d.d.f	<i>P</i>
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 (\pm 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 (\pm 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 (\pm 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 (\pm 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

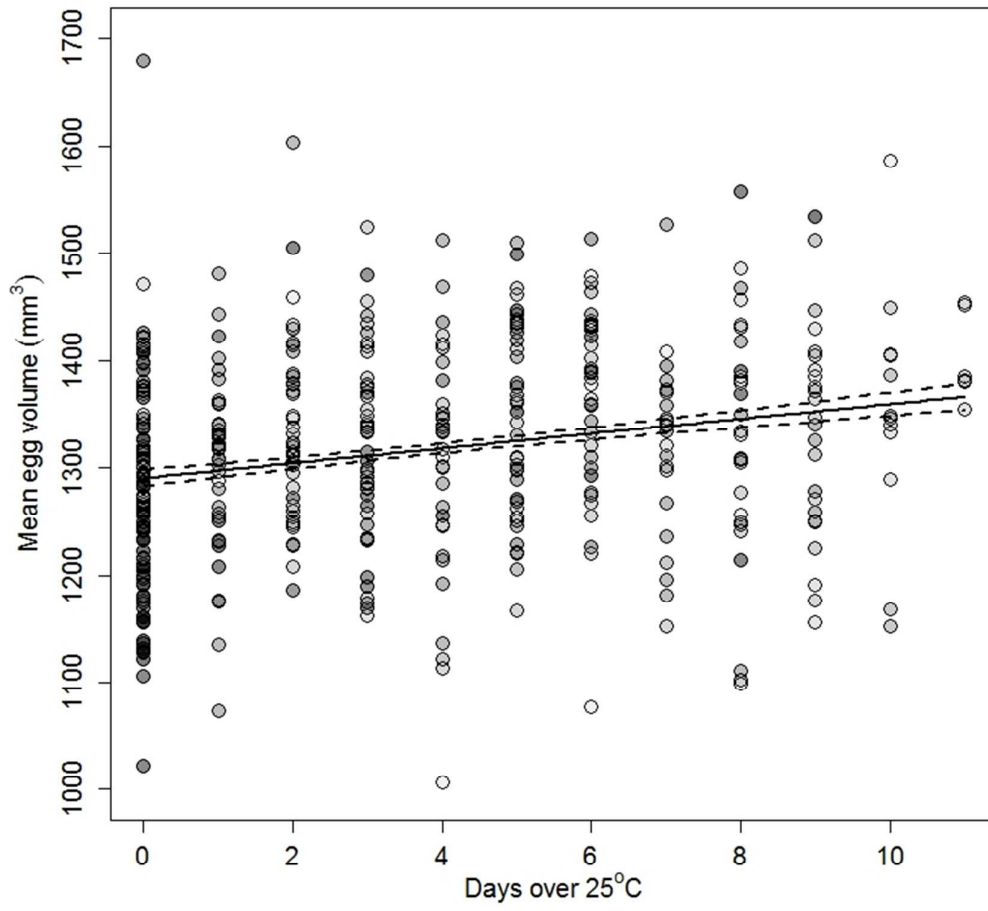


Figure 1

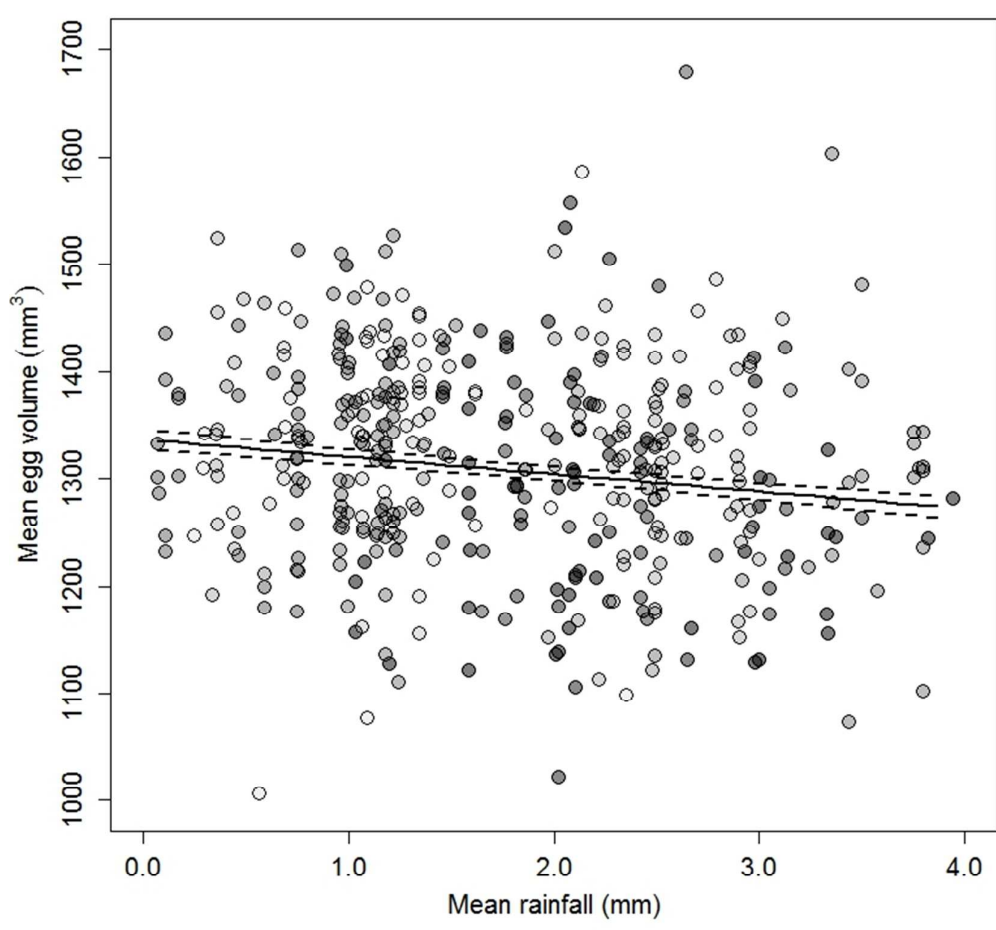


Figure 2

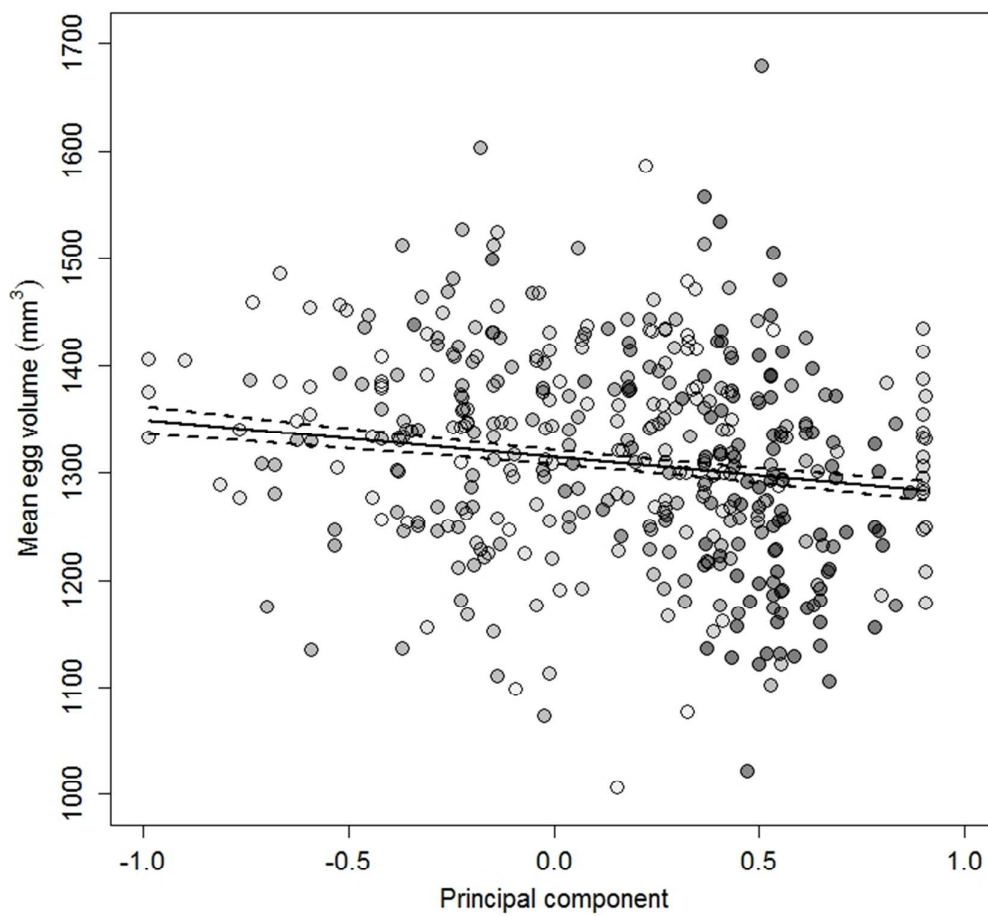


Figure 3

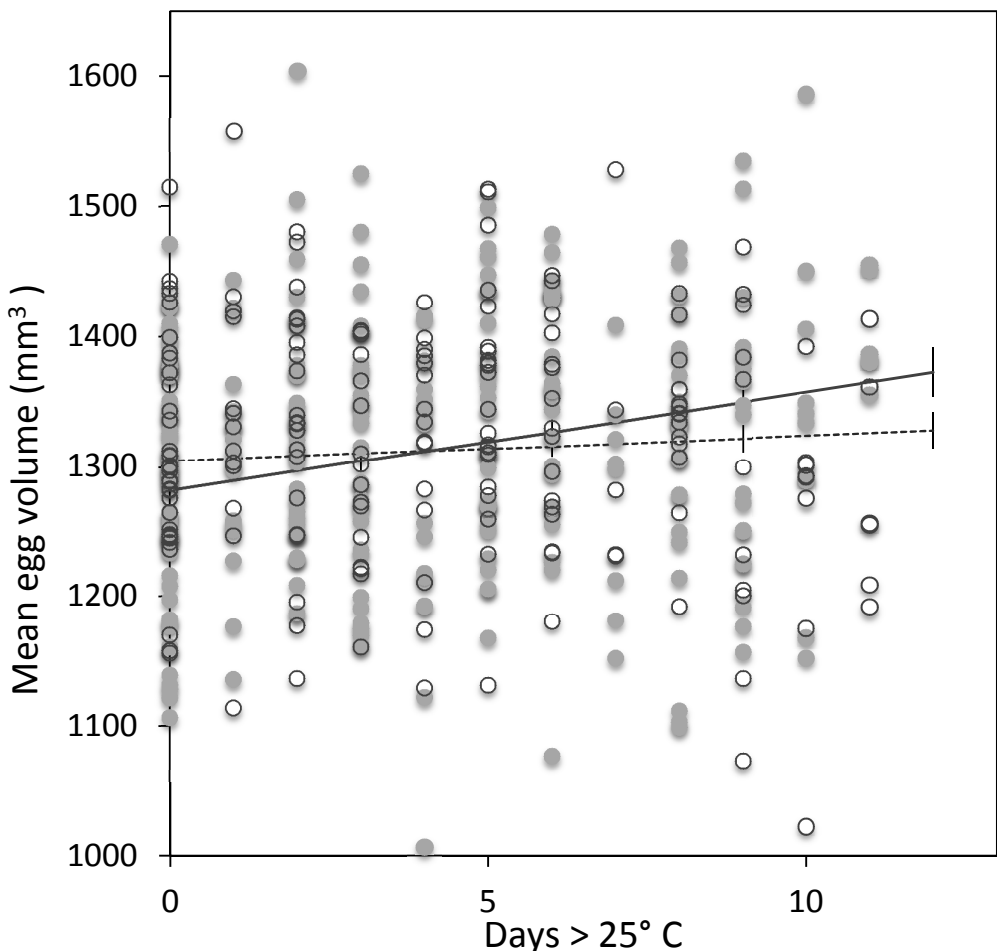


Figure 4