1 **Minutes matter: brief hatching asynchrony adversely affects late-**

2 **hatched hihi nestlings, but not life beyond the nest**

3

4 Kirsty J. MacLeod*[1], Patricia Brekke[2], John G. Ewen[2], Rose Thorogood[1]

5
6 [1] Department of Zoology, University of Cambridge, Cambridge, United Kingdom, CB2 3EJ
7 [2] Institute of Zoology, Zoological Society of London

8 * Correspondence to: kirstyjmacleod@gmail.com

9

10 **Abstract**

11 Size hierarchies are often seen when nestlings hatch asynchronously over a period of

12 days. Shorter hatch periods are common across passerines however, and while these

13 may also give rise to asymmetries, their effects are rarely considered. Regardless of

14 hatch period, the long-term consequences for later-hatched nestlings that survive to

15 fledge is unknown for wild birds. Here we explored the timing of hatch order in a free-

16 living population of hihi nestlings (*Notiomystis cincta*) and followed any effects in and

17 out of the nest. We found that while hatching time from first to last-hatched nestlings

18 was often less than 24 hours, last-hatched individuals grew more slowly and were

19 lighter and smaller at fledging than older siblings. Last-hatched nestlings were also less

20 likely to fledge. These effects were greater in larger broods. Adult body size is

21 correlated with fledging size in hihi; however, we found no evidence that hatch order

22 affected longevity post-fledging, or lifetime reproductive success. We then explored if

23 carotenoid availability might buffer these stressful rearing conditions (through food

24 supplementation of parents) but found no evidence that increased access to carotenoids

25 for mothers and/or growing nestlings influenced incubation schedules, or the effects of

26 hatching late. Together these results suggest that while even a very short hatch period

27 can influence adult phenotype, hatching asynchrony is not maladaptive for hihi: when

28 last-hatched nestlings survive to fledge they can contribute as much to their mother's

29 fitness as first-hatched siblings.

30 **Key words:** brood hierarchies, carotenoids, hatching asynchrony, maternal effects,

31 *Notiomystis cincta*

32 **Introduction**

33 Sequential hatching of avian young driven by early onset of incubation (hatching

34 asynchrony) often establishes size hierarchies within broods (Stokland & Amundsen,

35 1988; Wiklund, 1985). This can leave younger, smaller chicks at a disadvantage from

36 sibling competition (Mock & Parker, 1997) if parents feed larger offspring preferentially

37 (Rodriguez-Girones et al. 2002), but hatching asynchrony can also be an adaptive

38 strategy if it allows a mother to maximize the overall success of her brood (for example,

39 through brood reduction to match unpredictable environments (Magrath, 1990;

40 Stenning, 1996). While the effects of hatching asynchrony on life within the nest are

41 well-known, the long-term consequences of hatch order on lifespan or reproductive

42 success are much less understood (Mainwaring, Blount, & Hartley, 2012), particularly

43 for wild bird populations.

44 It is possible that the availability of specific nutrients may influence the potential

45 for later-hatched nestlings to catch up with their elder siblings. Carotenoids, a class of

46 antioxidants synthesised by plants and acquired by birds through their diet, may act as

47 a buffer to natural stressors due to their ability to boost the immune system (Berthouly,

48 Cassier, & Richner, 2008). In birds, carotenoids mitigate the effects of stress

49 experimentally induced by increased sibling competition (Berthouly et al., 2008), and

2

50   infestation with ectoparasites (Ewen et al., 2009). In both cases, when carotenoid

51   concentration in eggs was increased by supplementing mothers, nestlings placed at a

52   disadvantage from an increased brood size (Berthouly et al., 2008), or parasite

53   infestation (Ewen et al., 2009), fared as well as nestlings from unmanipulated broods

54   (unsupplemented nestlings fared the worst). As hatching later than siblings can

55   increase the physiological stress levels of nestlings (Costantini et al., 2006; de Boer,

56   Eens, Fransen, & Müller, 2015; Eraud, Trouvé, Dano, Chastel, & Faivre, 2008),

57   carotenoids available in the nestling's diet could therefore counteract the negative

58   effects of hatching late in a brood hierarchy.

59   Here we investigate whether size hierarchies observed in the hihi (*Notiomystis*

60   *cincta*) are caused by sequential hatching driven by maternal incubation behavior, and

61   whether hatching later than siblings has long-term fitness consequences. We expect that

62   early onset of incubation should result in more asynchronously hatching clutches, and

63   that last-hatched nestlings should be smaller and grow more slowly than early-hatched

64   siblings. Body mass at fledging improves a hihi's chances of surviving its first year (M.

65   Low & Part, 2009). Therefore, if the brood hierarchy order persists throughout the

66   nestling period, it is likely to have long-term consequences for lifespan and

67   reproductive success and not just survival to fledging as is most commonly investigated

68   due to the logistical challenges of tracking individuals throughout their lives.

69   In addition, we supplemented adult hihi with carotenoids during throughout

70   breeding (including incubation) to test if increased availability of carotenoids to

71   nestlings (either in eggs or also during nestling provisioning) compensates for any

72   negative effects of late hatching. Previous research shows that carotenoids are an

73   important dietary component for hihi nestlings, but that their effects appear to be

74 compensatory: only when challenged by poor rearing conditions (ectoparasites) do

75 nestlings hatched from carotenoid-rich eggs grow better (Ewen et al., 2009). This may

76 be particularly important if brood hierarchies arise through factors other than

77 differences in maternally-driven incubation behaviour, for example, through limitation

78 of other key egg components (Nager, Monaghan, & Houston, 2000). We therefore expect

79 increased carotenoid availability to be most effective for nestlings in stressful

80 conditions: those late in the hatching order.

81

Methods

82

Supplementation experiment and data collection

84 We studied a breeding population of hihi, a bird endemic to New Zealand and listed by

85 the IUCN as Vulnerable (BirdLife International, 2013), on Tiritiri Matangi Island, a 220

86 hectare island ~25 km north of Auckland. This population is part of a conservation

87 project, with supplementary food (sugar water) and nesting boxes provided across the

88 island. Re-sighting surveys were conducted each year in February (post-breeding) and

89 in September (pre-breeding) between February 2005 and February 2015, providing 21

90 capture occasions (for further details see Thorogood *et al.* 2013). Hihi breed between

91 October and March, producing clutches of, on average, 4 eggs (this dataset, 4.21± 0.69

92 eggs), which hatch into broods of, on average, 3 nestlings (this dataset, 3.23 ± 1.05

93 nestlings). Previous work has shown that carotenoid supplementation does not

94 significantly influence these parameters (Ewen, Thorogood, Karadas, & Cassey, 2008).

95 All breeding attempts were closely monitored so the parentage of all offspring that

96 reach fledging age (30 days, blood-sampled at 21 days) could be determined via

97   genotyping and assignment using Colony 2.0 software (Brekke, Ewen, Clucas, & Santure,

98   2015).

99   Our data were collected during the 2004/05 and 2005/06 breeding seasons

100   (hereafter Seasons 1 and 2) as part of carotenoid supplementation experiments

101   designed to investigate effects on maternal egg investment (Ewen, Surai, et al., 2006),

102   parental provisioning of nestlings (Ewen et al., 2008), and effects of ectoparasites on

103   nestling health (Ewen et al., 2009). Our supplementation regime differed between years,

104   with females in 'Season 1' receiving supplementation from nest building to offspring

105   fledging, whereas in 'Season 2', supplementation was stopped after completion of egg

106   laying (Table 1). This allowed us to test any effect of carotenoid supplementation at

107   different times during development. Breeding pairs were allocated to treatment or

108   control groups once they settled on a nest site. Control pairs were provided with a

109   supplementary food source (sugar water), while treatment pairs were provided with

110   sugar water supplemented with carotenoids (lutein and zeaxanthin) at a concentration

111   of 100 µg/ml (Ewen et al., 2008), in both cases within 10 m of the nest box. Hihi defend

112   food resources in their territories, and food was provided *ad libitum*, so feeders were

113   rarely used by either conspecifics or other nectarivorous species, nor did use of feeders

114   differ among treatment groups (Ewen et al. 2008) Furthermore, supplementation of

115   female hihi with carotenoids during laying has shown to positively influence yolk

116   carotenoid concentration (Ewen, Thorogood, Karadas, Pappas, & Surai, 2006), and

117   supplementation of parents during nestling rearing increases circulating plasma

118   carotenoid levels of both nestlings and parents (Thorogood et al. 2008, Thorogood et al

119   2011).

120   Hihi females lay one egg per day, and incubate for 14 days after clutch

121   completion, but whether they begin incubation during laying (and therefore, whether

122   they may adaptively adjust hatching synchrony) is unknown. Incubation behavior was

123   recorded in Season 2 using temperature loggers (Thermochron iButtons® DS1921G,

124   Maxim), which were inserted into the nest cup and secured around nest material either

125   the day before or the day the first egg was laid. A second logger was attached to the

126   inside of the nest box (20 cm from the nest cup) to record ambient temperature. Both

127   loggers recorded temperature (˚C) in 2-minute intervals for up to 7 days. We counted

128   the onset of incubation from the first night when the nest cup - but not box -

129   temperature rose above 25˚C for more than one hour (Cooper & Mills, 2005; Wang &

130   Beissinger, 2009). This is 'physiological zero temperature', when embryonic growth

131   begins (Wilson, 1990). Our measurement therefore recorded incubation effort in nights

132   until clutch completion.

133   After 13 days of incubation (day final egg laid = day 1) nests were monitored to

134   determine hatching order of each egg. Nests were visited in the late afternoon (after

135   4pm) on day 13, and then at no more than two-hourly intervals from dawn until dusk

136   on day 14. If any eggs remained intact by dusk on day 15 we checked only once more on

137   day 16 before removing eggs for other analyses (Season 1, N = 39; Season 2, N = 78;

138   Thorogood & Ewen 2006): a small proportion of each clutch often fails to hatch due to

139   unviability (Brekke, Bennett, Wang, Pettorelli, & Ewen, 2010; Thorogood & Ewen,

140   2006). From these checks the time between hatching events was recorded accurate to

141   within 120 min.

142   Once nestlings hatched (day 0) they were marked on the tarsus using a

143   permanent non-toxic marker pen. This identifier was refreshed every two days until

144 nestlings were 21 days old, when birds were given numbered metal rings and plastic

145 colour ring combinations as part of standard management practice to enable

146 identification. Nestlings were weighed and the length of their tarsi measured (with

147 Vernier calipers) throughout the nestling period (Table 1). Measurements were taken

148 every 3 days until day 24, after which measurements ceased to avoid causing the

149 premature fledging of offspring. Tarsus length does not change after 21 days so

150 measurements at day 24 indicate adult tarsus length (Low, 2006).

151 For our analyses of the effects of hatch order (and mitigating effects of

152 carotenoids), we restricted our dataset to first clutches ($N = 82$ clutches); hihi

153 sometimes produce second clutches in a season, but these often fail completely

154 (Thorogood, Ewen, & Kilner, 2011). We further restricted our dataset to clutches that

155 hatched at least two nestlings as by definition, broods of 1 cannot hatch

156 asynchronously. Of these clutches, full data on mass and size were available for 167

157 nestlings from 64 nests, and full data on growth rate were available for 96 nestlings

158 from 38 nests. Reported brood sizes represent brood size at hatching.

159

160 **Statistical analyses**

161 ***Onset of incubation and hatching spread***

162 To investigate if hatching asynchrony is influenced by females' incubation behaviour,

163 and to rule out the possibility that incubation behaviour is a consequence of clutch size

164 (for example, if females always begin incubating after laying a certain number of eggs),

165 we tested for relationships between onset of incubation and hatching spread, and the

166 onset of incubation and clutch size, using Pearson's correlation tests. The sample sizes

167 for these analyses were limited by how many nests we were able to collect data on

168 incubation behaviour, and hatching spread.

169   To include broods of a range of sizes, we standardized hatch order as first,

170 second, and last-hatched. Thus, in broods of four and five, the "last-hatched" nestling

171 was either the fourth or fifth respectively. This selection allows us to compare the

172 effects of hatching late across brood sizes, as in Badyaev, Hill, & Beck (2003). In broods

173 of two nestlings, nestlings were coded as first and second-hatched in order to be most

174 comparable with first and second-hatched offspring from other brood sizes (i.e. there is

175 at least one nestling between every first and last-hatched nestling). If two nestlings

176 hatched simultaneously (within same 120 min period between nest checks) they were

177 given the same (earlier) order. Our dataset included 68 first-hatched nestlings, 49

178 second-hatched nestlings, and 50 last-hatched nestlings. Hatch order was specified as

179 an ordinal categorical variable in all models – this allowed us to retain information

180 about order, without assuming linearity in the time lag between orders. All models

181 automatically tested for linear and quadratic relationships – all relationships reported

182 are linear unless stated otherwise, as no significant quadratic relationships were found.

183

184 *Effects of hatch order and carotenoid supplementation on growth*

185 We fitted a standard logistic growth model (Ricklefs, 1968) using the SSlogis function of

186 the "stats" package in R (R Core Team, 2013) to estimate each individual's asymptotic

187 mass (g) and tarsus length (mm), and their growth rates (k). Logistic growth models fit

188 postnatal growth data well in passerines (Starck & Ricklefs, 1998), and have been used

189 to describe growth before in hihi (Ewen et al. 2009). Nestlings that died before fledging

190   were excluded. Models could not accurately predict growth rates for data from Season 1

191   due to a lack of measurements before day 10; therefore growth rate analyses included

192   only nestlings from Season 2 (asymptote analyses included nestlings from both seasons,

193   as a lack of measurements before day 10 did not affect model asymptote).

194   To determine the interactive effects of carotenoids, sex, and hatch order on

195   nestling growth (in terms of asymptotic mass and tarsus length, and rate of growth in

196   both), we set these parameters as dependent variables in linear mixed effects models

197   constructed using the lme4 package in R (Bates et al. 2008). We included a three-way

198   interaction term: treatment * hatch order * sex. Brood size has been shown to be an

199   important determinant in the success of late-hatched red-winged blackbird nestlings

200   (Forbes, Thornton, Glassey, Forbes, & Buckley, 1997), therefore we included a separate

201   interaction term, hatch order * brood size. We also included maternal age (years) and

202   relative time in the season (days since hatching of the first clutch produced in that

203   season, a proxy for how early or late each nest is relative to other nests in the

204   population) as covariates, as these have been shown to influence other factors in hihi,

205   such as hatching failure and nestling survival (M. Low & Part, 2009). Brood identity was

206   included in all models as a random term to control for multiple individuals from the

207   same nest. Where data from both Season 1 and Season 2 were used in analyses,

208   maternal identity and season were also included as random terms to control for

209   repeated measures, and potential differences between seasons not accounted for by

210   differences in treatment regime respectively. Any interactions or terms that did not

211   contribute significantly to model fit were removed using stepwise deletion, by removing

212   the term of interest from the model, and comparing its fit to the data using chi-squared

213   tests.

214   To determine the effects of hatch order on nestling survival to fledging, we fit

215   fledging success of nestlings (0/1) in binomial linear mixed effects models, again using

216   the lme4 function in R (Bates et al. 2008), and fitting the same interactions, random

217   terms, and covariates as described above. Sex was not significant in an initial model set

218   ($\chi^2_1 = 2.73$, $P = 0.1$), so we repeated the analysis with an expanded dataset in which we

219   were able to include nestlings that died before being sexed. We report the results of the

220   second, expanded, analysis. Data were available for 242 nestlings from 76 nests.

221   For all analyses of hatch order and carotenoid treatment effects, we first looked

222   for differences among our treatment nests between seasons to determine if the timing

223   of carotenoid supplementation (Table 1) influenced brood size hierarchies and their

224   effects. If timing of carotenoid availability had no influence, we *a priori* decided to

225   combine the different supplementation regimes as an overall carotenoid treatment

226   factor (treatment variable: control/supplementation). If timing of carotenoid

227   availability (full supplementation in Season 1/laying-only supplementation only in

228   Season 2) did have an effect in a model, however, we then included this as a 3-level

229   factor (control/full supplementation/laying-only supplementation). During Season 2,

230   some nests included in this dataset ($N = 46$) were also used in a mite-removal

231   experiment (for methodology see Ewen et al., 2009). We therefore also tested for any

232   interactive effects of hatch order, mite treatment, and carotenoid treatment to

233   determine whether mite treatment had any effect on the relationships we were

234   interested in here. An effect of mite treatment was only found in the case of survival to

235   fledging, so it is only reported for that analysis.

236

237 ***Long term fitness effects of brood hierarchy position***

238 We estimated the effects of hatch order on post-fledging survival using a Cormack-Jolly-

239 Seber survival analysis in Program MARK (White & Burnham, 1999). Candidate models

240 could be time-dependent ('time'), sex-dependent ('sex'), and hatch order-dependent

241 ('hatch order) with three levels differentiating first hatched, second hatched, and last

242 hatched individuals. Most models were constructed with two age classes with

243 individuals entering the population as juveniles in each February and transitioning into

244 an adult age class the subsequent September ('age'). A global model estimated survival

245 (φ) according to age, sex, hatch order, and time, while also including time dependence

246 on detection probability (p). Alternative models were then derived by progressive

247 removal of factors thought least likely to be important based on *a priori* predictions. All

248 potential alternative models were tested and compared using QAICc (Cooch & White,

249 2008). Data were restricted to individuals for whom complete information was

250 available on sex and hatch order (N = 140). Global model fit to the data was assessed

251 using the median c-hat procedure showing a small adjustment was required (c-hat =

252 1.1).

253   To test whether position in the hatching order influenced individual lifetime

254 reproductive success, we restricted our dataset to individuals who survived to breeding

255 age (both sexes can breed in their first year, Ewen et al. 2011), and for which we know

256 their total reproductive output (i.e. excluding individuals that are still alive and

257 reproductively active) (30 females, 28 males). For each individual, we calculated the

258 total number of fledged offspring per year. Social partner identity strongly predicts

259 reproductive success in female, but not male, hihi (Brekke, Cassey, Ariani, & Ewen,

260 2013; Brekke et al., 2015). We therefore modeled hatch order effects on lifetime

261 reproductive success in males and females separately and accounted for social partner

262 identity in our analysis of females. Total number of fledged offspring produced per year

263 was set as the dependent variable in a mixed model, with hatch order as an independent

264 variable. We included age (years) as a polynomial covariate, as both male and female

265 reproductive output is best represented by a bell-shaped curve in this species (M. Low,

266 Pärt, & Forslund, 2007). Individual identity, nest of origin, mother identity, and season

267 were set as random terms.

268 **Ethical note**

269 Ethical approval for supplementing carotenoids was granted by the Zoological Society

270 of London Ethics Committee (UK). Permissions to conduct research on Department of

271 Conservation Estate and to collect samples as detailed above were also granted from the

272 Auckland Conservancy of the Department of Conservation. These protocols were

273 derived from standard monitoring protocols used for management of hihi by the

274 Department of Conservation.

275

276 **Results**

277 Our dataset included five nestlings from broods of two, 59 nestlings from broods of

278 three, 67 nestlings from broods of four, and 36 nestlings from broods of five, but not all

279 data could be collected from every nestling. Means are reported with standard

280 deviations, and sample size for each analysis is given.

281

282   *Onset of incubation and hatching spread*

283   Across all nests for which we obtained hatch order data (N = 82), there was large

284   variation in the time it took broods to hatch (hatching spread: time in minutes from first

285   egg hatching to last egg hatching). This ranged from no delay (all eggs hatched within 2

286   hours between visits) to 28 hours and 10 minutes, with a mean of ~ 10 hours (597.7 ±

287   473.9 minutes). Larger broods took longer to hatch (Pearson's r = 0.36, $P < 0.01$, N =

288   81).

289   In Season 2, we recorded incubation effort from 24 nests (14 were from the

290   carotenoid treated group). Variation among females was great, with some females

291   commencing incubation only once their clutch was complete (N = 4), but others

292   beginning 1 (N = 10), 2 (N =9), or 3 nights (N =1) before their last egg was laid.

293   However, this was not explained by clutch size (Pearson's r: 0.26, P = 0.22) or

294   carotenoid supplementation (Table 2). For a subset of these nests (N = 10), we could

295   correlate onset of incubation with hatching order: more nights of incubation effort

296   showed a non-significant trend toward a longer spread in hatching from the first to the

297   last chick (Pearson's r: 0.62, P = 0.06). Therefore, it is likely that the variation in

298   hatching spread we detected in our dataset was a consequence of variation in the onset

299   of mothers' incubation behaviour, but not variation in carotenoids deposited in the eggs.

300   *Effects of hatch order and carotenoid supplementation on growth*
301
302   Hatching later had a large effect on the growth and size of nestlings (Table 3). Hatching

303   late in the hatching sequence resulted in nestlings that were significantly lighter

304   (asymptotic mass, Fig 1a) and smaller (asymptotic tarsus length, Fig 1b) than older

305   siblings at fledging, and grew more slowly (both in terms of mass, and tarsus length)

306   (Table 3). These effects were particularly pronounced in larger broods, except in the

13

307   case of tarsus length (hatch order * brood size $\chi^2_2 = 4.83$, $P = 0.09$). Male nestlings were

308   heavier and larger at fledging than female siblings, and grew faster in terms of tarsus

309   length (Table 3). There was no difference between the sexes in the rate at which they

310   gained mass (sex $\chi^2_1 = 0.34$, $P = 0.24$).

311   Regardless of when carotenoids were supplemented, there was no evidence that

312   any of these hatch order effects were influenced by carotenoid supplementation (Table

313   3). Nor did carotenoid supplementation independently influence the final mass, or rate

314   of mass gain, of nestlings, or affect the size of brood hierarchies (all results in Tables 2

315   and 3). Access to carotenoids did influence tarsus growth rate, however: carotenoid-

316   treated nestlings of all hatch orders grew more quickly than nestlings from control

317   nests (Table 3).

318   ***Long term fitness effects of brood hierarchy position***

319   Fifty-three out of 242 nestlings died before fledging. Last-hatched nestlings were more

320   likely to die before fledging than earlier-hatched siblings, and this effect was greater in

321   larger broods (Table 3). Although not the focus of our study, we detected that mite

322   treatment also had an additive effect on nestling survival: nestlings that did not have

323   mites removed were more likely to die in the nest.

324   Once nestlings fledged, however, hatch order no longer influenced survival; the

325   best models (Δ QAICc <2 of top model) contained only age and sex (Table 4), with older

326   birds surviving better than first-years, and females living longer than males. We also

327   found no influence of hatch order on the number of offspring produced by our two

328   cohorts throughout their lifetime, either in interaction with sex ($\chi^2_2 = 4.35$, $P = 0.11$), or

329   independently ($\chi^2_2 = 0.72$, $P = 0.7$). Hatch order (and carotenoid supplementation)

330   effects are summarized in Table 5.

331

Discussion

Our study demonstrates that hatching even a short time after siblings can have strong effects on offspring: later hatched nestlings grew more slowly, and remained smaller at fledging according to growth models. Last-hatched hihi nestlings were also less likely to survive to fledge, and these effects of hatching last were strongest in larger broods. As adult tarsus length does not change after fledging in hihi (Low 2006), the hatch order effects we detected on body size (as predicted by growth models) are likely to persist throughout adult life. However, we found no lasting consequences of hatch order on post-fledging longevity or reproductive success, suggesting that if they survive to fledge, later hatched offspring contribute as much to parents' fitness as their earlier hatched (and larger) nest-mates. As the period of time over which eggs hatched was related to maternal incubation behaviour, together these results are consistent with hatching asynchrony being adaptive for hihi mothers.

During the nestling period at least, hatching later than nest-mates appears to be more detrimental for young hihi than in other species with comparable or even longer hatching periods. Tree swallows hatch over a similar period to hihi (28 h on average, (Clotfelter, Whittingham, & Dunn, 2000), but unlike our study where effects persisted until fledging, for tree swallows the effects of hatch order on nestling traits have been shown to disappear by day 12 (Clotfelter et al., 2000) (but see Zach 1982). Even in species with much longer hatching periods, for example, jackdaws (which hatch over a number of days: Wingfield Gibbons, 1987), hatch order effects have been shown to disappear before fledging (Arnold & Griffiths, 2003).

354   Why are brood hierarchies so pronounced in hihi, despite their relatively short

355   hatching spread? Hihi nestlings may be particularly sensitive to hatch order effects

356   because of their long nestling period (30 days) relative to other passerines (Roff, Remes,

357   & Martin, 2005): if earlier hatched nestlings gain a head start, this may exacerbate hatch

358   order effects over this long time period (Price & Ydenberg, 1995), especially if early

359   asymmetries in sibling competition persist (Glassey & Forbes, 2002). In addition, food

360   shortages early in the post-hatching stage can have consequences for growth later on,

361   even if parental provisioning later increases (Killpack & Karasov, 2012; Lack, 1954).

362   However, lasting hatch order effects on tarsus size have been found in house finches

363   (Badyaev et al., 2002), which have a nestling period of around 16 days , so this is not an

364   entirely satisfactory explanation. More work is needed to investigate whether, for

365   example, nestling size or sex influences nestling begging, sibling competition, and/or

366   parental provisioning behaviour, and whether these factors may exacerbate hatch order

367   effects in this species.

368   If brood hierarchies create stressful growth environments for later-hatched

369   nestlings, why did carotenoids not mitigate these effects? We know that our

370   supplementation changed the phenotype of nestlings, as previous analyses of subsets of

371   these data indicate differences in nestlings' body condition (Ewen et al. 2009) and

372   tarsus length (Ewen et al. 2008) at fledging, and here we find that carotenoid

373   supplementation leads to faster growth (at least for tarsi). Other work with hihi has also

374   shown that increased access to carotenoids influences nestling begging (Ewen et al.

375   2008, Thorogood et al. 2008), but that this effect is only present when parents do not

376   provide the carotenoids themselves to their nestlings (Thorogood et al. 2011). Given

377   our lack of effect here, this suggests that parents do not favour feeding later-hatched

378   nestlings, although this requires further experiments to determine.

379   Alternatively, perhaps we detected no effect of carotenoids because they actually

380   exacerbated effects of hatch order, instead of mitigating them as we predicted.

381   Deposition of carotenoids in egg yolk often decreases down the laying order (for

382   example, lesser black-backed gulls (Blount et al., 2002; Royle, Surai, McCartney, &

383   Speake, 1999), and barn swallows (Saino et al., 2002) so it may be that mothers skewed

384   carotenoids to earlier, "more valuable" offspring (Groothuis, Müller, Von Engelhardt,

385   Carere, & Eising, 2005; Williams, 2012). As we detected no increase in hatch order

386   effects in our carotenoid-treated group, it seems likely that if carotenoids are implicated

387   in this relationship mothers retain any extra for themselves and do not boost the yolks

388   of eggs intended to hatch last. Unfortunately the conservation status of hihi renders it

389   impossible to destructively sample eggs, or manipulate incubation schedules directly so

390   we are unable to test this possibility further.

391   Despite strong effects of hatch order on nestling size and mass at fledging, we

392   found no lasting consequences of this difference on post-fledging longevity or

393   reproductive success. This is surprising, given that previous analyses have shown that

394   body mass close to fledging correlates well with survival of females during their first

395   year (Low & Part, 2009). One possible explanation is that all nestlings in our dataset

396   were in relatively good condition when they fledged. During our experiment, both our

397   carotenoid-treated and control nests were supplied with sugar water close to the nest.

398   Furthermore, the population is provided with sugar water *ad libitum* throughout the

399   year (Thorogood, Armstrong, Low, Brekke, & Ewen, 2013). Having easy access to food

400   throughout their lives (beginning with parental provisioning) may have enabled

401 smaller, later hatched individuals to persist in the population regardless of hatch order

402 or carotenoid availability. It is also unlikely that later-hatched individuals trade

403 longevity against reproduction, as we detected no effect of hatch order on annual

404 reproductive success.

405   Finally, we expected male hihi nestlings to be more sensitive to hatching order

406 effects given that male hihi embryos are more sensitive to the negative effects of

407 inbreeding (Brekke et al., 2010). Furthermore, male hihi nestlings are larger than

408 females (Ewen et al., 2009; this study), and sex-specific differences in sensitivity to poor

409 or harsh environments are common across vertebrates where sexes are dimorphic and

410 one is more costly to produce or maintain (Anderson *et al*., 1993; Lindström, 1999).

411 Hatching order has strong sex-specific effects in certain house finch populations

412 (Badyaev *et al.*, 2002). Likewise, male house wrens were more negatively affected by

413 experimentally manipulated hatch orders than their female siblings (Bowers,

414 Thompson, & Sakaluk, 2015). However, size differences between male and female hihi

415 did not appear to result in increased male sensitivity to hatch order effects in our study.

416 Further exploration of whether parents preferentially feed one sex under certain

417 circumstances, as is seen in eastern bluebirds (Ligon & Hill, 2010) and Arabian babblers

418 (Ridley & Huyvaert, 2007) would be informative, as such a bias could mask the

419 sensitivity of males. Alternatively, any male sensitivity may be matched by

420 disadvantages of hatching late for females, given their already smaller size (Oddie,

421 2000).

422   The average period over which broods hatched in our dataset was less than 24

423 hours; so, by the accepted definition, hihi hatch synchronously (Stoleson & Beissinger,

424 1995). Nevertheless, by investigating hatch order effects in an apparently

425   synchronously hatching species, we have detected implications for hihi in early life that

426   may have lasting consequences for the adult phenotype. The increased likelihood of

427   death before fledging for last-hatched offspring is significant, however, we found that

428   later hatched nestlings that did fledge lived as long and produced as many offspring as

429   early-hatched nestlings. This suggests that hatching asynchrony is not maladaptive for

430   hihi; negative effects of hatching late are confined to the nestling period, after which

431   surviving offspring are equally likely to contribute to their mothers' fitness. Our study

432   therefore provides valuable insight into the implications and adaptive potential of

433   hatching asynchrony by broadening the scope under which it has previously been

434   studied. Closer investigation of hatch order effects in other species with apparent

435   synchronous hatching may lead to novel insights into what determines when and why

436   brood size hierarchies arise.

437

438   **Data accessibility**

439   Data will be available on Dryad upon acceptance: DOI XXXXXXX

440

441

442   **References**

443   Anderson, D. J., Reeve, J., Gomez, J. E. M., Weathers, W. W., Hutson, S., Cunningham, H. V,

444   & Bird, D. M. (1993). Sexual size dimorphism and food requirements of nestling

445   birds. *Canadian Journal of Zoology*, *71*(12), 2541–2545. doi:10.1139/z93-347

446   Arnold, K. E., & Griffiths, R. (2003). Sex-specific hatching order, growth rates and

447   fledging success in jackdaws *Corvus monedula. Journal of Avian Biology*, *34*, 275–

448   281. doi:10.1034/j.1600-048X.2003.03068.x

449   Badyaev, A. V, Hill, G. E., & Beck, M. L. (2003). Interaction between maternal effects:

450   onset of incubation and offspring sex in two populations of a passerine bird.

451   *Oecologia*, *135*(3), 386–90. doi:10.1007/s00442-003-1203-x

452   Badyaev, A. V, Hill, G. E., Beck, M. L., Dervan, A. A., Duckworth, R. A., McGraw, K. J., …

453   Whittingham, L. A. (2002). Sex-biased hatching order and adaptive population

454   divergence in a passerine bird. *Science (New York, N.Y.)*, *295*(5553), 316–8.

455   doi:10.1126/science.1066651

456   Berthouly, A., Cassier, A., & Richner, H. (2008). Carotenoid-induced maternal effects

457   interact with ectoparasite burden and brood size to shape the trade-off between

458   growth and immunity in nestling great tits. *Functional Ecology*, *22*(5), 854–863.

459   doi:10.1111/j.1365-2435.2008.01439.x

460   BirdLife International. (2013). Notiomystis cincta. Retrieved July 2, 2015, from

461   www.iucnredlist.org

462   Blount, J. D., Surai, P. F., Nager, R. G., Houston, D. C., Møller, A. P., Trewby, M. L., &

463   Kennedy, M. W. (2002). Carotenoids and egg quality in the lesser blackbacked gull

464   Larus fuscus: a supplemental feeding study of maternal effects. *Proceedings of the*

465   *Royal Society B: Biological Sciences*, *269*(1486), 29–36.

466   doi:10.1098/rspb.2001.1840

467   Bowers, E. K., Thompson, C. F., & Sakaluk, S. K. (2015). Persistent sex-by-environment

468   effects on offspring fitness and sex-ratio adjustment in a wild bird population.

469   *Journal of Animal Ecology*, *84*(2), 473–486. doi:10.1111/1365-2656.12294

470   Brekke, P., Bennett, P. M., Wang, J., Pettorelli, N., & Ewen, J. G. (2010). Sensitive males:

471   inbreeding depression in an endangered bird. *Proceedings. Biological Sciences / The*

472   *Royal Society*, *277*(1700), 3677–3684. doi:10.1098/rspb.2010.1144

473   Brekke, P., Cassey, P., Ariani, C., & Ewen, J. G. (2013). Evolution of extreme-mating

474   behaviour: Patterns of extrapair paternity in a species with forced extrapair

475   copulation. *Behavioral Ecology and Sociobiology*, *67*(6), 963–972.

476   doi:10.1007/s00265-013-1522-9

477   Brekke, P., Ewen, J. G., Clucas, G., & Santure, A. W. (2015). Determinants of male floating

478   behaviour and floater reproduction in a threatened population of the hihi

479   (*Notiomystis cincta*). *Evolutionary Applications*, *8*(8), 796–806.

480   doi:10.1111/eva.12287

481   Clotfelter, E. D., Whittingham, L. A., & Dunn, P. O. (2000). Laying order, hatching

482   asynchrony and nestling body mass in Tree Swallows *Tachycineta bicolor*. *Journal*

483   *of Avian Biology*, *31*(3), 329–334. doi:10.1034/j.1600-048X.2000.310308.x

484   Cooch, E., & White, G. C. (2008). *Program MARK: a gentle introduction*. Retrieved from

485   http://www.phidot.org/software/mark/docs/book

486   Cooper, C. B., & Mills, H. (2005). Software to quantify incubation behavior from time

487   series recordings. *Journal of Field Ornithology*, *76*(July), 352–356.

488   Costantini, D., Casagrande, S., De Filippis, S., Brambilla, G., Fanfani, A., Tagliavini, J., &

489   Dell'Omo, G. (2006). Correlates of oxidative stress in wild kestrel nestlings (*Falco*

490   *tinnunculus*). *Journal of Comparative Physiology B*, *176*(4), 329–337.

491   doi:10.1007/s00360-005-0055-6

492   de Boer, R. A., Eens, M., Fransen, E., & Müller, W. (2015). Hatching asynchrony

493   aggravates inbreeding depression in a songbird (*Serinus canaria*): An inbreeding-

494   environment interaction. *Evolution*, *69*(4), 1063–1068. doi:10.1111/evo.12625

495 Eraud, C., Trouvé, C., Dano, S., Chastel, O., & Faivre, B. (2008). Competition for resources

496   modulates cell-mediated immunity and stress hormone level in nestling collared

497   doves (*Streptopelia decaocto*). *General and Comparative Endocrinology*, *155*(3),

498   542–551. doi:10.1016/j.ygcen.2007.09.008

499 Ewen, J. G., Surai, P., Stradi, R., Moller, A. P., Vittorio, B., Griffiths, R., & Armstrong, D. P.

500   (2006). Carotenoids, colour and conservation in an endangered passerine, the hihi

501   or stitchbird (*Notiomystis cincta*). *Animal Conservation*, *9*(2), 229–235.

502   doi:10.1111/j.1469-1795.2006.00028.x

503 Ewen, J. G., Thorogood, R., Brekke, P., Cassey, P., Karadas, F., & Armstrong, D. P. (2009).

504   Maternally invested carotenoids compensate costly ectoparasitism in the hihi.

505   *Proceedings of the National Academy of Sciences of the United States of America*,

506   *106*(31), 12798–802. doi:10.1073/pnas.0902575106

507 Ewen, J. G., Thorogood, R., Karadas, F., & Cassey, P. (2008). Condition dependence of

508   nestling mouth colour and the effect of supplementing carotenoids on parental

509   behaviour in the hihi (*Notiomystis cincta*). *Oecologia*, *157*(2), 361–8.

510   doi:10.1007/s00442-008-1073-3

511 Ewen, J. G., Thorogood, R., Karadas, F., Pappas, A. C., & Surai, P. F. (2006). Influences of

512   carotenoid supplementation on the integrated antioxidant system of a free living

513   endangered passerine, the hihi (*Notiomystis cincta*). *Comparative Biochemistry and*

514   *Physiology*, *143*(2), 149–154. doi:10.1016/j.cbpa.2005.11.006

515 Forbes, S., Thornton, S., Glassey, B., Forbes, M. J., & Buckley, N. j. (1997). Why parent

516   birds play favourites. *Nature*, *390*, 351–352. doi:10.1038/37025

517 Glassey, B., & Forbes, S. (2002). Begging and asymmetric nestling competition. In J.

518   Wright & M. Leonard (Eds.), *The Evolution of Begging: Competition, Cooperation &*

519   *Communication* (pp. 269–281). Dordrecht, The Netherlands: Kluwer Academic

520   Publishers.

521   Groothuis, T. G. G., Müller, W., Von Engelhardt, N., Carere, C., & Eising, C. (2005).

522   Maternal hormones as a tool to adjust offspring phenotype in avian species.

523   *Neuroscience and Biobehavioral Reviews*, *29*(2), 329–352.

524   doi:10.1016/j.neubiorev.2004.12.002

525   Killpack, T. L., & Karasov, W. H. (2012). Growth and development of house sparrows

526   (P*asser domesticus*) in response to chronic food restriction throughout the nestling

527   period. *The Journal of Experimental Biology* , *215* (11), 1806–1815. Retrieved from

528   http://jeb.biologists.org/content/215/11/1806.abstract

529   Lack, D. (1954). *The natural regulation of animal numbers*. Oxford, UK: Oxford

530   University Press.

531   Ligon, R. A., & Hill, G. E. (2010). Sex-biased parental investment is correlated with mate

532   ornamentation in eastern bluebirds. *Animal Behaviour*, *79*(3), 727–734.

533   doi:10.1016/j.anbehav.2009.12.028.Sex-biased

534   Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends in*

535   *Ecology & Evolution*, *14*(9), 343–348. doi:10.1016/S0169-5347(99)01639-0

536   Low, M. (2006). Sex, age and season influence morphometrics in the New Zealand

537   Stitchbird (or Hihi; *Notiomystis cinct*a). *Emu*, *106*(4), 297–304.

538   doi:10.1071/MU06003

539   Low, M., & Part, T. (2009). Patterns of mortality for each life-history stage in a

540   population of the endangered New Zealand stitchbird. *Journal of Animal Ecology*,

541   *78*, 761–771. doi:10.1111/j.1365-2656.2007.0

542   Low, M., Pärt, T., & Forslund, P. (2007). Age-specific variation in reproduction is largely

543   explained by the timing of territory establishment in the New Zealand stitchbird

544   *Notiomystis cincta*. *Journal of Animal Ecology*, *76*(3), 459–470. doi:10.1111/j.1365-

545   2656.2007.01234.x

546   Magrath, R. D. (1990). Hatching Asynchrony in Altricial Birds. *Biological Reviews*, *65*(4),

547   587–622. doi:10.1111/j.1469-185X.1990.tb01239.x

548   Mainwaring, M. C., Blount, J. D., & Hartley, I. R. (2012). Hatching asynchrony can have

549   long-term consequences for offspring fitness in zebra finches under captive

550   conditions. *Biological Journal of the Linnean Society*, *106*(2), 430–438.

551   doi:10.1111/j.1095-8312.2012.01868.x

552   Mock, D. W., & Parker, G. A. (1997). *The Evolution of Sibling Rivalry*. Oxford, UK: Oxford

553   University Press.

554   Nager, R. G., Monaghan, P., & Houston, D. C. (2000). Within-clutch trade-offs between the

555   number and quality of eggs: Experimental manipulations in gulls. *Ecology*, *81*(5),

556   1339–1350. doi:10.1890/0012-9658(2000)081[1339:WCTOBT]2.0.CO;2

557   Oddie, K. R. (2000). Size matters : competition between male and female great tit o □

558   spring. *Journal of Animal Ecology*, *69*, 903–912. doi:10.1046/j.1365-

559   2656.2000.00438.x

560   Price, K., & Ydenberg, R. (1995). Begging and provisioning in broods of asynchronously-

561   hatched yellow-headed blackbird nestlings. *Behavioral Ecology and Sociobiology*,

562   *37*(3), 201–208. doi:10.1007/BF00176718

563   R: A language and environment for statistical computing. (2013). Vienna, Austria: R

564   Core Team, R Foundation for Statistical Computing. Retrieved from http://www.r-

565   project.org/

566   Ricklefs, R. E. (1968). Patterns of growth in birds. *Ibis*, *110*(4), 419–451.

567   doi:10.1111/j.1474-919X.1968.tb00058.x

568   Ridley, A. R., & Huyvaert, K. P. (2007). Sex-biased preferential care in the cooperatively

569   breeding Arabian babbler. *Journal of Evolutionary Biology*, *20*(4), 1271–1276.

570   doi:10.1111/j.1420-9101.2007.01356.x

571   Roff, D. A., Remes, V., & Martin, T. E. (2005). The evolution of fledging age in songbirds.

572   *Journal of Evolutionary Biology*, *18*(6), 1425–1433. doi:10.1111/j.1420-

573   9101.2005.00958.x

574   Royle, N. J., Surai, P. F., McCartney, R. J., & Speake, B. K. (1999). Parental investment and

575   egg yolk lipid composition. *Functional Ecology*, *13*, 298–306.

576   Saino, N., Bertacche, V., Ferrari, R. P., Martinelli, R., Møller, A. P., & Stradi, R. (2002).

577   Carotenoid concentration in barn swallow eggs is influenced by laying order,

578   maternal infection and paternal ornamentation. *Proceedings of the Royal Society B:*

579   *Biological Sciences*, *269*(1501), 1729–33. doi:10.1098/rspb.2002.2088

580   Starck, J. M., & Ricklefs, R. E. (1998). *Avian growth and development: evolution within the*

581   *altricial-precocial spectrum*. Oxford, UK: Oxford University Press.

582   Stenning, M. J. (1996). Hatching asynchrony, brood reduction and other rapidly

583   reproducing hypotheses. *Trends in Ecology & Evolution*, *11*(96), 243–246.

584   Stokland, J. N., & Amundsen, T. (1988). Initial size hierarchy in broods of the shag:

585   relative significance of egg size and hatching asynchrony. *The Auk*, *105*(April), 308–

586   315. Retrieved from http://www.jstor.org/stable/4087495

587   Stoleson, S. H., & Beissinger, S. R. (1995). Hatching Asynchrony and the Onset of

588   Incubation in Birds, Revisited. In D. Power (Ed.), *Current Ornithology* (Vol. 12, pp.

589   191–270). Springer US. doi:10.1007/978-1-4615-1835-8_6

590   Thorogood, R., Armstrong, D. P., Low, M., Brekke, P., & Ewen, J. G. (2013). The value of

591   long-term ecological research: Integrating knowledge for conservation of hihi on

592   Tiritiri Matangi Island. *New Zealand Journal of Ecology*, *37*(3), 298–306.

593   Thorogood, R., & Ewen, J. G. (2006). Rare occurrence of embryonic twins in the Hihi (

594   Stitchbird) Notiomystis cincta : an endangered passerine of New Zealand. *Ibis*, *148*,

595   828–829.

596   Thorogood, R., Ewen, J. G., & Kilner, R. M. (2011). Sense and sensitivity: responsiveness

597   to offspring signals varies with the parents' potential to breed again. *Proceedings of*

598   *the Royal Society B: Biological Sciences*, *278*(1718), 2638–45.

599   doi:10.1098/rspb.2010.2594

600   Wang, J. M., & Beissinger, S. R. (2009). Variation in the onset of incubation and its

601   influence on avian hatching success and asynchrony. *Animal Behaviour*, *78*(3), 601–

602   613. doi:10.1016/j.anbehav.2009.05.022

603   White, G. C., & Burnham, K. P. (1999). Program MARK: Survival estimation from

604   populations of marked animals. Bird Study 46 Supplement, 120-138.

605   Wiklund, C. G. (1985). Fieldfare Turdus pilaris Breeding Strategy: The Improtance of

606   Asynchronous Hatching and Resources Needed for Egg Formation. *Ornis*

607   *Scandinavica*, *16*(3), 213–221. doi:10.2307/3676633

608   Williams, T. D. (2012). *Physiological Adaptations for Breeding in Birds*. Princeton, NJ:

609   Princeton University Press.

610   Wilson, H. R. (1990). Physiological requirements of the developing embryo:

611   temperature and turning. In S. G. Tulett (Ed.), *Avian Incubation* (pp. 145–156).

612   London, UK.: Butterworths.

613   Wingfield Gibbons, D. (1987). Hatching Asynchrony Reduces Parental Investment in the

614   Jackdaw. *Journal of Animal Ecology*, *56*(2), 403–414.

615

616

617

618

619

620

621

622

623

624

625

626

627

628

629

630

631 Table 1. Details of between-year differences in our carotenoid supplementation regime..

Season	Supplementation regime				Incubation monitored	Nestlings weighed from:
	Nest-building	Egg-laying	Incubation	Nestling rearing		
2004/2005 (1)	X	X	X	X	No	Day 10
2005/2006 (2)	X	X			Yes	Day 3

632

633

634

635

636

637

638

639

640

641

642

643

644

645

646 Table 2. Descriptive statistics on all nests from both seasons (N = 119).

	Group		Test statistic	P
	Untreated	Carotenoid supplemented		
Total clutches	62	57		
Clutch size (mean ± SD)	4.26 ± 0.68	4.16 ± 0.70	T = 0.79	0.43
Average egg mass (per egg mean g ± SD)	2.95 ± 0.19 (N=104)	2.92 ± 0.26 (N=71)	T = 0.97	0.34
Nights of incubation prior to laying of final egg (mean nights ± SD)	1.2 ± 0.92 (N = 10)	1.4 ± 0.74 (N = 14)	χ^2= 0.43	0.51
Hatch failures	23% (62/264)	22% (53/237)	χ^2= 0.09	0.76
Average hatching spread (mean mins ± SD)	571.7 ± 483.0 (N=91)	593.06 ± 477.18 (N=90)	T = -0.21	0.84
Brood size (mean ± SD)	3.24 ± 1.10	3.21 ± 1.01	T = 0.16	0.87
Range of nestling masses at fledging (mean coefficient of variation)	15.61	16.58	χ^2= 0.003	0.95

647 Where appropriate the difference between untreated and supplemented groups are tested statistically;
648 tests carried out are indicated with test statistic.
649

650

651

652

653

654

655

656

657

658

659

660

661

662

663

664

665

666

667

668
669 Table 3. Results of GLMM investigating hatch order and carotenoid treatment effects on male and female chick size and growth (mass in grams, tarsus length in mm).

		Est.	SE	Z	P
ASYMPTOTIC MASS AND SIZE – 167 nestlings from 64 nests					
1. asymptotic mass	*Intercept*	*36.55*	*0.59*	*62.26*	
	Hatch order * brood size	-1.92	0.49	-3.88	<0.001
	Sex				<0.0001
	Female	0.00			
	Male	6.36	0.61	10.38	
2. asymptotic tarsus length	*Intercept*	*31.34*	*0.12*	*253.75*	
	Hatch order	-0.43	0.13	-3.38	<0.01
	Sex				<0.001
	Female	0.00			
	Male	1.77	0.15	11.62	
GROWTH RATE – 96 nestlings from 38 nests					
3. mass growth rate (*N* = 96)	*Intercept*	*0.39*	*0.03*	*14.45*	
	Hatch order * brood size	-0.03	0.01	-3.70	<0.001
4. tarsus growth rate (*N* = 96)	*Intercept*	*0.33*	*0.02*	*19.16*	
	Treatment				<0.001
	Control	0.00			
	Laying supplementation	0.01	0.006	2.16	
	Sex				<0.001
	Female				
	Male	-0.02	0.004	-4.02	
	Hatch order * brood size	-0.01	0.005	-2.74	<0.01
SURVIVAL TO FLEDGING – 242 nestlings from 76 nests					
5. survival to fledging	*Intercept*	*2.22*	*1.24*	*1.79*	
	Hatch order * Brood size	-2.00	0.55	-3.66	<0.001
	Mite treatment				<0.001
	Mites removed	0.00			
	Mites present	-2.66	0.60	-4.42	

670 [1.] Removed from models: hatch order*sex*treatment (control/laying supp/full supp) χ^2_4 = 6.73, P = 0.15; sex*treatment
671 (control/laying supp/full supp)χ^2_2 = 0.14, P = 0.93; hatch order*sex χ^2_4 = 0.67, P = 0.71; hatch order* treatment (control/laying
672 supp/full supp) χ^2_4 = 1.21, P = 0.88; maternal age χ^2_1 = 0.06, P = 0.81; hatch order*brood size χ^2_2 = 5.12, P = 0.08; brood size χ^2_1 =
673 1.62, P = 0.20; date χ^2_1 = 2.85, P = 0.09; treatment (control/laying supp/full supp) χ^2_2 = 3.64, P = 0.16.
674 [2.] Removed from models: hatch order*sex*treatment (control/laying supp) χ^2_2 = 1.61, P = 0.45; sex*treatment χ^2_2 = 0.61, P = 0.43;
675 hatch order*treatment (control/laying supp) χ^2_2 = 0.94, P = 0.63; hatch order* sex χ^2_2 = 4.13, P = 0.13; maternal age χ^2_1 = 0.01, P =
676 0.93; treatment (control/laying supp) χ^2_1 = 0.17, P = 0.68; sex χ^2_1 = 0.34, P = 0.24; date χ^2_1 = 3.38, P = 0.07..
677 [3.] Removed from models: hatch order*sex*treatment (control/laying supp) χ^2_2 = 2.03, P = 0.36; hatch order*sex χ^2_2 = 0.14, P = 0.93;
678 hatch order*treatment (control/laying supp) χ^2_2 = 0.3, P = 0.86; treatment (control/laying supp)* sex χ^2_2 = 0.24, P = 0.63; hatch
679 order*brood size χ^2_2 = 4.83, P = 0.09; brood size χ^2_1 = 0.26, P = 0.61; date χ^2_1 = 0.21, P = 0.65; treatment (control/laying supp) χ^2_1 =
680 1.69, P = 0.19; maternal age χ^2_1 = 2.28, P = 0.13.
681 [4.] Removed from models: hatch order*sex*treatment (control/laying supp) χ^2_2 = 0.5, P = 0.78; hatch order*treatment (control/laying
682 supp) χ^2_2 = 0.4, P = 0.82; sex*treatment (control/laying supp) χ^2_2 = 0.38, P = 0.54; hatch order* sex χ^2_2 = 0.88, P = 0.65; maternal age
683 χ^2_1 = 0.09, P = 0.77; date χ^2_1 = 0.82, P = 0.18.
684 [5.] Removed from models: hatch order*treatment (control/carotenoid supp.) χ^2_2 = 0.75, P = 0.69; date χ^2_1 = 0.05, P = 0.83; treatment
685 (control/ carotenoid supp) χ^2_1 = 0.92, P = 0.34; maternal age χ^2_1 = 2.04, P = 0.15.

686

687

688

690   Table 4. Results of Cormack-Jolly-Seber survival analysis in Program MARK.

Model	Num. Par	QAICc	ΔQAICc	Weight	Deviance
φ (age) p(time)	22	889.08	0.00	0.63	486.56
φ (age + sex) p(time)	23	890.44	1.36	0.32	485.72
φ (age + sex + hatch order) p(time)	25	894.17	5.09	0.05	485.03
φ (age + sex + hatch order + time) p(time)	44	904.16	15.08	0.00	451.08
φ (sex) p(time)	22	934.59	45.51	0.00	532.07
Null model φ(.) p(time)	17	925.29	36.21	0.00	533.62

691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724

725
726
727 Table 5. Summary of results showing effects of hatch order and carotenoid supplementation on a) male
728 and b) female nestlings.

		Asymptotic mass	Growth rate (mass)	Asymptotic tarsus length	Growth rate (tarsus)	Survival to fledging	Longevity	Total offspring
a) male	Hatch order	-	-	-	-	-	=	=
	Carotenoids	=	=	=	+			
b) female	Hatch order	-	-	-	=	=	=	=
	Carotenoids	=	=	=	=			

729 Negative effects are denoted by "-", positive effects by "+", and no effect by "=".
730
731

732

733

734

735

736

737

738

739

740

741

742

743

744

745

746

747

748

749

750

751   Fig. 1. Effects of hatch order on (a) mass, and (b) tarsus length. Male nestlings are depicted in black, and female
752   nestlings are depicted in red.

753

754
755