

1 Minutes matter: brief hatching asynchrony adversely affects late- 2 hatched hihi nestlings, but not life beyond the nest

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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

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

82 **Methods**

83 **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 ($597.7 \pm$
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

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 ($\Delta \text{QAICc} < 2$ 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

332 **Discussion**

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

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

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438 **Data accessibility**

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

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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 (*Passer 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 cincta*). *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 Importance 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. Tullett (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.

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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

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

647 Where appropriate the difference between untreated and supplemented groups are tested statistically;
 648 tests carried out are indicated with test statistic.
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668 Table 3. Results of GLMM investigating hatch order and carotenoid treatment effects on male and female
669 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	<i>Intercept</i>	36.55	0.59	62.26	
	Hatch order * brood size	-1.92	0.49	-3.88	<0.001
	Sex				<0.000
	Female	0.00			1
Male	6.36	0.61	10.38		
2. asymptotic tarsus length	<i>Intercept</i>	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)	<i>Intercept</i>	0.39	0.03	14.45	
	Hatch order * brood size	-0.03	0.01	-3.70	<0.001
4. tarsus growth rate (N = 96)	<i>Intercept</i>	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	<i>Intercept</i>	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) $\chi^2_4 = 6.73$, $P = 0.15$; sex*treatment
671 (control/laying supp/full supp) $\chi^2_2 = 0.14$, $P = 0.93$; hatch order*sex $\chi^2_4 = 0.67$, $P = 0.71$; hatch order* treatment (control/laying
672 supp/full supp) $\chi^2_4 = 1.21$, $P = 0.88$; maternal age $\chi^2_1 = 0.06$, $P = 0.81$; hatch order*brood size $\chi^2_2 = 5.12$, $P = 0.08$; brood size $\chi^2_1 =$
673 1.62 , $P = 0.20$; date $\chi^2_1 = 2.85$, $P = 0.09$; treatment (control/laying supp/full supp) $\chi^2_2 = 3.64$, $P = 0.16$.

674 ² Removed from models: hatch order*sex*treatment (control/laying supp) $\chi^2_2 = 1.61$, $P = 0.45$; sex*treatment $\chi^2_2 = 0.61$, $P = 0.43$;
675 hatch order*treatment (control/laying supp) $\chi^2_2 = 0.94$, $P = 0.63$; hatch order* sex $\chi^2_2 = 4.13$, $P = 0.13$; maternal age $\chi^2_1 = 0.01$, $P =$
676 0.93 ; treatment (control/laying supp) $\chi^2_1 = 0.17$, $P = 0.68$; sex $\chi^2_1 = 0.34$, $P = 0.24$; date $\chi^2_1 = 3.38$, $P = 0.07$.

677 ³ Removed from models: hatch order*sex*treatment (control/laying supp) $\chi^2_2 = 2.03$, $P = 0.36$; hatch order*sex $\chi^2_2 = 0.14$, $P = 0.93$;
678 hatch order*treatment (control/laying supp) $\chi^2_2 = 0.3$, $P = 0.86$; treatment (control/laying supp)* sex $\chi^2_2 = 0.24$, $P = 0.63$; hatch
679 order*brood size $\chi^2_2 = 4.83$, $P = 0.09$; brood size $\chi^2_1 = 0.26$, $P = 0.61$; date $\chi^2_1 = 0.21$, $P = 0.65$; treatment (control/laying supp) $\chi^2_1 =$
680 1.69 , $P = 0.19$; maternal age $\chi^2_1 = 2.28$, $P = 0.13$.

681 ⁴ Removed from models: hatch order*sex*treatment (control/laying supp) $\chi^2_2 = 0.5$, $P = 0.78$; hatch order*treatment (control/laying
682 supp) $\chi^2_2 = 0.4$, $P = 0.82$; sex*treatment (control/laying supp) $\chi^2_2 = 0.38$, $P = 0.54$; hatch order* sex $\chi^2_2 = 0.88$, $P = 0.65$; maternal age
683 $\chi^2_1 = 0.09$, $P = 0.77$; date $\chi^2_1 = 0.82$, $P = 0.18$.

684 ⁵ Removed from models: hatch order*treatment (control/carotenoid supp.) $\chi^2_2 = 0.75$, $P = 0.69$; date $\chi^2_1 = 0.05$, $P = 0.83$; treatment
685 (control/ carotenoid supp) $\chi^2_1 = 0.92$, $P = 0.34$; maternal age $\chi^2_1 = 2.04$, $P = 0.15$.

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

Model	Num. Par	QAICc	Δ QAICc	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 $\phi(.)$ p(time)	17	925.29	36.21	0.00	533.62

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

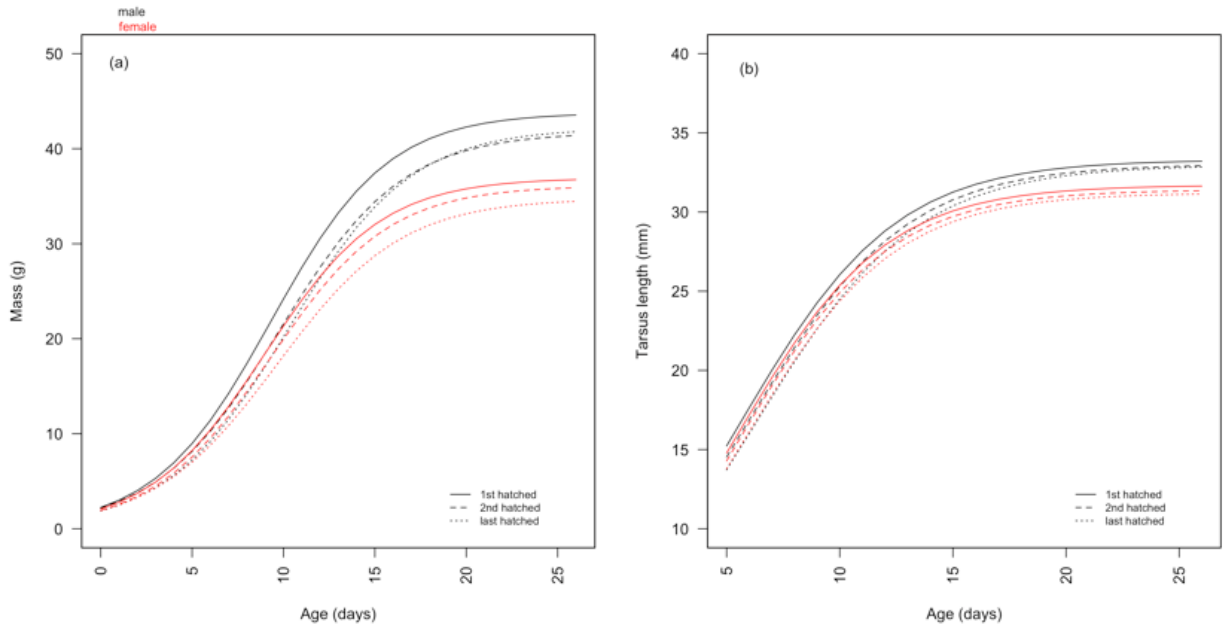
		Asymptotic mass	Growth rate (mass)	Asymptotic tarsus length	Growth rate (tarsus)	Survival to fledging	Longevity	Total offspring
a)	Hatch order	-	-	-	-	-	=	=
male	Carotenoids	=	=	=	+	=	=	=
b)	Hatch order	-	-	-	=	=	=	=
female	Carotenoids	=	=	=	=	=	=	=

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Negative effects are denoted by “-”, positive effects by “+”, and no effect by “=”.

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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.



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