

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

3
4 **Abstract**

5 Size hierarchies are often seen when nestlings hatch asynchronously over a period of
6 days. Shorter hatch periods are common across passerines however, and while these
7 may also give rise to asymmetries, their effects are rarely considered. Regardless of
8 hatch period, the long-term consequences for later-hatched nestlings that survive to
9 fledge is unknown for wild birds. Here we explored the timing of hatch order in a free-
10 living population of hihi nestlings (*Notiomystis cincta*) and followed any effects in and
11 out of the nest. We found that while hatching time from first to last-hatched nestlings
12 was often less than 24 hours, last-hatched individuals grew more slowly and were
13 lighter and smaller at fledging than older siblings. Last-hatched nestlings were also less
14 likely to fledge. These effects were greater in larger broods. Adult body size is
15 correlated with fledging size in hihi; however, we found no evidence that hatch order
16 affected longevity post-fledging, or lifetime reproductive success. We then explored if
17 carotenoid availability might buffer these stressful rearing conditions (through food
18 supplementation of parents) but found no evidence that increased access to carotenoids
19 for mothers and/or growing nestlings influenced incubation schedules, or the effects of
20 hatching late. Together these results suggest that while even a very short hatch period
21 can influence adult phenotype, hatching asynchrony is not maladaptive for hihi: when
22 last-hatched nestlings survive to fledge they can contribute as much to their mother's
23 fitness as first-hatched siblings.

24 **Key words:** brood hierarchies, carotenoids, hatching asynchrony, maternal effects,
25 *Notiomystis cincta*

26 **Introduction**

27 Sequential hatching of avian young driven by early onset of incubation (hatching
28 asynchrony) often establishes size hierarchies within broods (Stokland & Amundsen,
29 1988; Wiklund, 1985). This can leave younger, smaller chicks at a disadvantage from
30 sibling competition (Mock & Parker, 1997) if parents feed larger offspring preferentially
31 (Rodriguez-Girones et al. 2002), but hatching asynchrony can also be an adaptive
32 strategy if it allows a mother to maximize the overall success of her brood (for example,
33 through brood reduction to match unpredictable environments (Magrath, 1990;
34 Stenning, 1996). While the effects of hatching asynchrony on life within the nest are
35 well-known, the long-term consequences of hatch order on lifespan or reproductive
36 success are much less understood (Mainwaring, Blount, & Hartley, 2012), particularly
37 for wild bird populations.

38 It is possible that the availability of specific nutrients may influence the potential
39 for later-hatched nestlings to catch up with their elder siblings. Carotenoids, a class of
40 antioxidants synthesised by plants and acquired by birds through their diet, may act as
41 a buffer to natural stressors due to their ability to boost the immune system (Berthouly,
42 Cassier, & Richner, 2008). In birds, carotenoids mitigate the effects of stress
43 experimentally induced by increased sibling competition (Berthouly et al., 2008), and
44 infestation with ectoparasites (Ewen et al., 2009). In both cases, when carotenoid
45 concentration in eggs was increased by supplementing mothers, nestlings placed at a
46 disadvantage from an increased brood size (Berthouly et al., 2008), or parasite

47 infestation (Ewen et al., 2009), fared as well as nestlings from unmanipulated broods
48 (unsupplemented nestlings fared the worst). As hatching later than siblings can
49 increase the physiological stress levels of nestlings (Costantini et al., 2006; de Boer,
50 Eens, Fransen, & Müller, 2015; Eraud, Trouvé, Dano, Chastel, & Faivre, 2008),
51 carotenoids available in the nestling's diet could therefore counteract the negative
52 effects of hatching late in a brood hierarchy.

53 Here we investigate whether size hierarchies observed in the hihi (*Notiomystis*
54 *cincta*) are caused by sequential hatching driven by maternal incubation behavior, and
55 whether hatching later than siblings has long-term fitness consequences. We expect that
56 early onset of incubation should result in more asynchronously hatching clutches, and
57 that last-hatched nestlings should be smaller and grow more slowly than early-hatched
58 siblings. Body mass at fledging improves a hihi's chances of surviving its first year (M.
59 Low & Part, 2009). Therefore, if the brood hierarchy order persists throughout the
60 nestling period, it is likely to have long-term consequences for lifespan and
61 reproductive success and not just survival to fledging as is most commonly investigated
62 due to the logistical challenges of tracking individuals throughout their lives.

63 In addition, we supplemented adult hihi with carotenoids during throughout
64 breeding (including incubation) to test if increased availability of carotenoids to
65 nestlings (either in eggs or also during nestling provisioning) compensates for any
66 negative effects of late hatching. Previous research shows that carotenoids are an
67 important dietary component for hihi nestlings, but that their effects appear to be
68 compensatory: only when challenged by poor rearing conditions (ectoparasites) do
69 nestlings hatched from carotenoid-rich eggs grow better (Ewen et al., 2009). This may
70 be particularly important if brood hierarchies arise through factors other than

71 differences in maternally-driven incubation behaviour, for example, through limitation
72 of other key egg components (Nager, Monaghan, & Houston, 2000). We therefore expect
73 increased carotenoid availability to be most effective for nestlings in stressful
74 conditions: those late in the hatching order.

75

76 **Methods**

77 **Supplementation experiment and data collection**

78 We studied a breeding population of hihi, a bird endemic to New Zealand and listed by
79 the IUCN as Vulnerable (BirdLife International, 2013), on Tiritiri Matangi Island, a 220
80 hectare island ~25 km north of Auckland. This population is part of a conservation
81 project, with supplementary food (sugar water) and nesting boxes provided across the
82 island. Re-sighting surveys were conducted each year in February (post-breeding) and
83 in September (pre-breeding) between February 2005 and February 2015, providing 21
84 capture occasions (for further details see Thorogood *et al.* 2013). Hihi breed between
85 October and March, producing clutches of, on average, 4 eggs (this dataset, 4.21 ± 0.69
86 eggs), which hatch into broods of, on average, 3 nestlings (this dataset, 3.23 ± 1.05
87 nestlings). Previous work has shown that carotenoid supplementation does not
88 significantly influence these parameters (Ewen, Thorogood, Karadas, & Cassey, 2008).
89 All breeding attempts were closely monitored so the parentage of all offspring that
90 reach fledging age (30 days, blood-sampled at 21 days) could be determined via
91 genotyping and assignment using Colony 2.0 software (Brekke, Ewen, Clucas, & Santure,
92 2015).

93 Our data were collected during the 2004/05 and 2005/06 breeding seasons
94 (hereafter Seasons 1 and 2) as part of carotenoid supplementation experiments
95 designed to investigate effects on maternal egg investment (Ewen, Surai, et al., 2006),
96 parental provisioning of nestlings (Ewen et al., 2008), and effects of ectoparasites on
97 nestling health (Ewen et al., 2009). Our supplementation regime differed between years,
98 with females in 'Season 1' receiving supplementation from nest building to offspring
99 fledging, whereas in 'Season 2', supplementation was stopped after completion of egg
100 laying (Table 1). This allowed us to test any effect of carotenoid supplementation at
101 different times during development. Breeding pairs were allocated to treatment or
102 control groups once they settled on a nest site. Control pairs were provided with a
103 supplementary food source (sugar water), while treatment pairs were provided with
104 sugar water supplemented with carotenoids (lutein and zeaxanthin) at a concentration
105 of 100 µg/ml (Ewen et al., 2008), in both cases within 10 m of the nest box. Hihi defend
106 food resources in their territories, and food was provided *ad libitum*, so feeders were
107 rarely used by either conspecifics or other nectarivorous species, nor did use of feeders
108 differ among treatment groups (Ewen et al. 2008) Furthermore, supplementation of
109 female hihi with carotenoids during laying has shown to positively influence yolk
110 carotenoid concentration (Ewen, Thorogood, Karadas, Pappas, & Surai, 2006), and
111 supplementation of parents during nestling rearing increases circulating plasma
112 carotenoid levels of both nestlings and parents (Thorogood et al. 2008, Thorogood et al
113 2011).

114 Hihi females lay one egg per day, and incubate for 14 days after clutch
115 completion, but whether they begin incubation during laying (and therefore, whether
116 they may adaptively adjust hatching synchrony) is unknown. Incubation behavior was

117 recorded in Season 2 using temperature loggers (Thermochron iButtons® DS1921G,
118 Maxim), which were inserted into the nest cup and secured around nest material either
119 the day before or the day the first egg was laid. A second logger was attached to the
120 inside of the nest box (20 cm from the nest cup) to record ambient temperature. Both
121 loggers recorded temperature (°C) in 2-minute intervals for up to 7 days. We counted
122 the onset of incubation from the first night when the nest cup - but not box -
123 temperature rose above 25°C for more than one hour (Cooper & Mills, 2005; Wang &
124 Beissinger, 2009). This is 'physiological zero temperature', when embryonic growth
125 begins (Wilson, 1990). Our measurement therefore recorded incubation effort in nights
126 until clutch completion.

127 After 13 days of incubation (day final egg laid = day 1) nests were monitored to
128 determine hatching order of each egg. Nests were visited in the late afternoon (after
129 4pm) on day 13, and then at no more than two-hourly intervals from dawn until dusk
130 on day 14. If any eggs remained intact by dusk on day 15 we checked only once more on
131 day 16 before removing eggs for other analyses (Season 1, $N = 39$; Season 2, $N = 78$;
132 Thorogood & Ewen 2006): a small proportion of each clutch often fails to hatch due to
133 unviability (Brekke, Bennett, Wang, Pettorelli, & Ewen, 2010; Thorogood & Ewen,
134 2006). From these checks the time between hatching events was recorded accurate to
135 within 120 min.

136 Once nestlings hatched (day 0) they were marked on the tarsus using a
137 permanent non-toxic marker pen. This identifier was refreshed every two days until
138 nestlings were 21 days old, when birds were given numbered metal rings and plastic
139 colour ring combinations as part of standard management practice to enable
140 identification. Nestlings were weighed and the length of their tarsi measured (with

141 Vernier calipers) throughout the nestling period (Table 1). Measurements were taken
142 every 3 days until day 24, after which measurements ceased to avoid causing the
143 premature fledging of offspring. Tarsus length does not change after 21 days so
144 measurements at day 24 indicate adult tarsus length (Low, 2006).

145 For our analyses of the effects of hatch order (and mitigating effects of
146 carotenoids), we restricted our dataset to first clutches ($N = 82$ clutches); hihi
147 sometimes produce second clutches in a season, but these often fail completely
148 (Thorogood, Ewen, & Kilner, 2011). We further restricted our dataset to clutches that
149 hatched at least two nestlings as by definition, broods of 1 cannot hatch
150 asynchronously. Of these clutches, full data on mass and size were available for 167
151 nestlings from 64 nests, and full data on growth rate were available for 96 nestlings
152 from 38 nests. Reported brood sizes represent brood size at hatching.

153

154 **Statistical analyses**

155 *Onset of incubation and hatching spread*

156 To investigate if hatching asynchrony is influenced by females' incubation behaviour,
157 and to rule out the possibility that incubation behaviour is a consequence of clutch size
158 (for example, if females always begin incubating after laying a certain number of eggs),
159 we tested for relationships between onset of incubation and hatching spread, and the
160 onset of incubation and clutch size, using Pearson's correlation tests. The sample sizes
161 for these analyses were limited by how many nests we were able to collect data on
162 incubation behaviour, and hatching spread.

163 To include broods of a range of sizes, we standardized hatch order as first,
164 second, and last-hatched. Thus, in broods of four and five, the “last-hatched” nestling
165 was either the fourth or fifth respectively. This selection allows us to compare the
166 effects of hatching late across brood sizes, as in Badyaev, Hill, & Beck (2003). In broods
167 of two nestlings, nestlings were coded as first and second-hatched in order to be most
168 comparable with first and second-hatched offspring from other brood sizes (i.e. there is
169 at least one nestling between every first and last-hatched nestling). If two nestlings
170 hatched simultaneously (within same 120 min period between nest checks) they were
171 given the same (earlier) order. Our dataset included 68 first-hatched nestlings, 49
172 second-hatched nestlings, and 50 last-hatched nestlings. Hatch order was specified as
173 an ordinal categorical variable in all models – this allowed us to retain information
174 about order, without assuming linearity in the time lag between orders. All models
175 automatically tested for linear and quadratic relationships – all relationships reported
176 are linear unless stated otherwise, as no significant quadratic relationships were found.

177

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

179 We fitted a standard logistic growth model (Ricklefs, 1968) using the SSlogis function of
180 the “stats” package in R (R Core Team, 2013) to estimate each individual’s asymptotic
181 mass (g) and tarsus length (mm), and their growth rates (k). Logistic growth models fit
182 postnatal growth data well in passerines (Starck & Ricklefs, 1998), and have been used
183 to describe growth before in hihi (Ewen et al. 2009). Nestlings that died before fledging
184 were excluded. Models could not accurately predict growth rates for data from Season 1
185 due to a lack of measurements before day 10; therefore growth rate analyses included

186 only nestlings from Season 2 (asymptote analyses included nestlings from both seasons,
187 as a lack of measurements before day 10 did not affect model asymptote).

188 To determine the interactive effects of carotenoids, sex, and hatch order on
189 nestling growth (in terms of asymptotic mass and tarsus length, and rate of growth in
190 both), we set these parameters as dependent variables in linear mixed effects models
191 constructed using the lme4 package in R (Bates et al. 2008). We included a three-way
192 interaction term: treatment * hatch order * sex. Brood size has been shown to be an
193 important determinant in the success of late-hatched red-winged blackbird nestlings
194 (Forbes, Thornton, Glassey, Forbes, & Buckley, 1997), therefore we included a separate
195 interaction term, hatch order * brood size. We also included maternal age (years) and
196 relative time in the season (days since hatching of the first clutch produced in that
197 season, a proxy for how early or late each nest is relative to other nests in the
198 population) as covariates, as these have been shown to influence other factors in hihi,
199 such as hatching failure and nestling survival (M. Low & Part, 2009). Brood identity was
200 included in all models as a random term to control for multiple individuals from the
201 same nest. Where data from both Season 1 and Season 2 were used in analyses,
202 maternal identity and season were also included as random terms to control for
203 repeated measures, and potential differences between seasons not accounted for by
204 differences in treatment regime respectively. Any interactions or terms that did not
205 contribute significantly to model fit were removed using stepwise deletion, by removing
206 the term of interest from the model, and comparing its fit to the data using chi-squared
207 tests.

208 To determine the effects of hatch order on nestling survival to fledging, we fit
209 fledging success of nestlings (0/1) in binomial linear mixed effects models, again using

210 the lme4 function in R (Bates et al. 2008), and fitting the same interactions, random
211 terms, and covariates as described above. Sex was not significant in an initial model set
212 ($\chi^2_1 = 2.73, P = 0.1$), so we repeated the analysis with an expanded dataset in which we
213 were able to include nestlings that died before being sexed. We report the results of the
214 second, expanded, analysis. Data were available for 242 nestlings from 76 nests.

215 For all analyses of hatch order and carotenoid treatment effects, we first looked
216 for differences among our treatment nests between seasons to determine if the timing
217 of carotenoid supplementation (Table 1) influenced brood size hierarchies and their
218 effects. If timing of carotenoid availability had no influence, we *a priori* decided to
219 combine the different supplementation regimes as an overall carotenoid treatment
220 factor (treatment variable: control/supplementation). If timing of carotenoid
221 availability (full supplementation in Season 1/laying-only supplementation only in
222 Season 2) did have an effect in a model, however, we then included this as a 3-level
223 factor (control/full supplementation/laying-only supplementation). During Season 2,
224 some nests included in this dataset ($N = 46$) were also used in a mite-removal
225 experiment (for methodology see Ewen et al., 2009). We therefore also tested for any
226 interactive effects of hatch order, mite treatment, and carotenoid treatment to
227 determine whether mite treatment had any effect on the relationships we were
228 interested in here. An effect of mite treatment was only found in the case of survival to
229 fledging, so it is only reported for that analysis.

230

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

232 We estimated the effects of hatch order on post-fledging survival using a Cormack-Jolly-
233 Seber survival analysis in Program MARK (White & Burnham, 1999). Candidate models
234 could be time-dependent ('time'), sex-dependent ('sex'), and hatch order-dependent
235 ('hatch order') with three levels differentiating first hatched, second hatched, and last
236 hatched individuals. Most models were constructed with two age classes with
237 individuals entering the population as juveniles in each February and transitioning into
238 an adult age class the subsequent September ('age'). A global model estimated survival
239 (φ) according to age, sex, hatch order, and time, while also including time dependence
240 on detection probability (p). Alternative models were then derived by progressive
241 removal of factors thought least likely to be important based on *a priori* predictions. All
242 potential alternative models were tested and compared using QAICc (Cooch & White,
243 2008). Data were restricted to individuals for whom complete information was
244 available on sex and hatch order ($N = 140$). Global model fit to the data was assessed
245 using the median c-hat procedure showing a small adjustment was required (c-hat =
246 1.1).

247 To test whether position in the hatching order influenced individual lifetime
248 reproductive success, we restricted our dataset to individuals who survived to breeding
249 age (both sexes can breed in their first year, Ewen et al. 2011), and for which we know
250 their total reproductive output (i.e. excluding individuals that are still alive and
251 reproductively active) (30 females, 28 males). For each individual, we calculated the
252 total number of fledged offspring per year. Social partner identity strongly predicts
253 reproductive success in female, but not male, hihi (Brekke, Cassey, Ariani, & Ewen,
254 2013; Brekke et al., 2015). We therefore modeled hatch order effects on lifetime

255 reproductive success in males and females separately and accounted for social partner
256 identity in our analysis of females. Total number of fledged offspring produced per year
257 was set as the dependent variable in a mixed model, with hatch order as an independent
258 variable. We included age (years) as a polynomial covariate, as both male and female
259 reproductive output is best represented by a bell-shaped curve in this species (M. Low,
260 Pärt, & Forslund, 2007). Individual identity, nest of origin, mother identity, and season
261 were set as random terms.

262 **Ethical note**

263 Ethical approval for supplementing carotenoids was granted by the Zoological Society
264 of London Ethics Committee (UK). Permissions to conduct research on Department of
265 Conservation Estate and to collect samples as detailed above were also granted from the
266 Auckland Conservancy of the Department of Conservation. These protocols were
267 derived from standard monitoring protocols used for management of hihi by the
268 Department of Conservation.

269

270 **Results**

271 Our dataset included five nestlings from broods of two, 59 nestlings from broods of
272 three, 67 nestlings from broods of four, and 36 nestlings from broods of five, but not all
273 data could be collected from every nestling. Means are reported with standard
274 deviations, and sample size for each analysis is given.

275

276 *Onset of incubation and hatching spread*

277 Across all nests for which we obtained hatch order data ($N = 82$), there was large
278 variation in the time it took broods to hatch (hatching spread: time in minutes from first
279 egg hatching to last egg hatching). This ranged from no delay (all eggs hatched within 2
280 hours between visits) to 28 hours and 10 minutes, with a mean of ~ 10 hours ($597.7 \pm$
281 473.9 minutes). Larger broods took longer to hatch (Pearson's $r = 0.36$, $P < 0.01$, $N =$
282 81).

283 In Season 2, we recorded incubation effort from 24 nests (14 were from the
284 carotenoid treated group). Variation among females was great, with some females
285 commencing incubation only once their clutch was complete ($N = 4$), but others
286 beginning 1 ($N = 10$), 2 ($N = 9$), or 3 nights ($N = 1$) before their last egg was laid.
287 However, this was not explained by clutch size (Pearson's $r: 0.26$, $P = 0.22$) or
288 carotenoid supplementation (Table 2). For a subset of these nests ($N = 10$), we could
289 correlate onset of incubation with hatching order: more nights of incubation effort
290 showed a non-significant trend toward a longer spread in hatching from the first to the
291 last chick (Pearson's $r: 0.62$, $P = 0.06$). Therefore, it is likely that the variation in
292 hatching spread we detected in our dataset was a consequence of variation in the onset
293 of mothers' incubation behaviour, but not variation in carotenoids deposited in the eggs.

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

295
296 Hatching later had a large effect on the growth and size of nestlings (Table 3). Hatching
297 late in the hatching sequence resulted in nestlings that were significantly lighter
298 (asymptotic mass, Fig 1a) and smaller (asymptotic tarsus length, Fig 1b) than older
299 siblings at fledging, and grew more slowly (both in terms of mass, and tarsus length)
300 (Table 3). These effects were particularly pronounced in larger broods, except in the

301 case of tarsus length (hatch order * brood size $\chi^2_2 = 4.83, P = 0.09$). Male nestlings were
302 heavier and larger at fledging than female siblings, and grew faster in terms of tarsus
303 length (Table 3). There was no difference between the sexes in the rate at which they
304 gained mass (sex $\chi^2_1 = 0.34, P = 0.24$).

305 Regardless of when carotenoids were supplemented, there was no evidence that
306 any of these hatch order effects were influenced by carotenoid supplementation (Table
307 3). Nor did carotenoid supplementation independently influence the final mass, or rate
308 of mass gain, of nestlings, or affect the size of brood hierarchies (all results in Tables 2
309 and 3). Access to carotenoids did influence tarsus growth rate, however: carotenoid-
310 treated nestlings of all hatch orders grew more quickly than nestlings from control
311 nests (Table 3).

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

313 Fifty-three out of 242 nestlings died before fledging. Last-hatched nestlings were more
314 likely to die before fledging than earlier-hatched siblings, and this effect was greater in
315 larger broods (Table 3). Although not the focus of our study, we detected that mite
316 treatment also had an additive effect on nestling survival: nestlings that did not have
317 mites removed were more likely to die in the nest.

318 Once nestlings fledged, however, hatch order no longer influenced survival; the
319 best models ($\Delta \text{QAICc} < 2$ of top model) contained only age and sex (Table 4), with older
320 birds surviving better than first-years, and females living longer than males. We also
321 found no influence of hatch order on the number of offspring produced by our two
322 cohorts throughout their lifetime, either in interaction with sex ($\chi^2_2 = 4.35, P = 0.11$), or
323 independently ($\chi^2_2 = 0.72, P = 0.7$). Hatch order (and carotenoid supplementation)
324 effects are summarized in Table 5.

325

326 **Discussion**

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

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

348 Why are brood hierarchies so pronounced in hihi, despite their relatively short
349 hatching spread? Hihi nestlings may be particularly sensitive to hatch order effects
350 because of their long nestling period (30 days) relative to other passerines (Roff, Remes,
351 & Martin, 2005): if earlier hatched nestlings gain a head start, this may exacerbate hatch
352 order effects over this long time period (Price & Ydenberg, 1995), especially if early
353 asymmetries in sibling competition persist (Glasse & Forbes, 2002). In addition, food
354 shortages early in the post-hatching stage can have consequences for growth later on,
355 even if parental provisioning later increases (Killpack & Karasov, 2012; Lack, 1954).
356 However, lasting hatch order effects on tarsus size have been found in house finches
357 (Badyaev et al., 2002), which have a nestling period of around 16 days, so this is not an
358 entirely satisfactory explanation. More work is needed to investigate whether, for
359 example, nestling size or sex influences nestling begging, sibling competition, and/or
360 parental provisioning behaviour, and whether these factors may exacerbate hatch order
361 effects in this species.

362 If brood hierarchies create stressful growth environments for later-hatched
363 nestlings, why did carotenoids not mitigate these effects? We know that our
364 supplementation changed the phenotype of nestlings, as previous analyses of subsets of
365 these data indicate differences in nestlings' body condition (Ewen et al. 2009) and
366 tarsus length (Ewen et al. 2008) at fledging, and here we find that carotenoid
367 supplementation leads to faster growth (at least for tarsi). Other work with hihi has also
368 shown that increased access to carotenoids influences nestling begging (Ewen et al.
369 2008, Thorogood et al. 2008), but that this effect is only present when parents do not
370 provide the carotenoids themselves to their nestlings (Thorogood et al. 2011). Given

371 our lack of effect here, this suggests that parents do not favour feeding later-hatched
372 nestlings, although this requires further experiments to determine.

373 Alternatively, perhaps we detected no effect of carotenoids because they actually
374 exacerbated effects of hatch order, instead of mitigating them as we predicted.
375 Deposition of carotenoids in egg yolk often decreases down the laying order (for
376 example, lesser black-backed gulls (Blount et al., 2002; Royle, Surai, McCartney, &
377 Speake, 1999), and barn swallows (Saino et al., 2002) so it may be that mothers skewed
378 carotenoids to earlier, “more valuable” offspring (Groothuis, Müller, Von Engelhardt,
379 Carere, & Eising, 2005; Williams, 2012). As we detected no increase in hatch order
380 effects in our carotenoid-treated group, it seems likely that if carotenoids are implicated
381 in this relationship mothers retain any extra for themselves and do not boost the yolks
382 of eggs intended to hatch last. Unfortunately the conservation status of hihi renders it
383 impossible to destructively sample eggs, or manipulate incubation schedules directly so
384 we are unable to test this possibility further.

385 Despite strong effects of hatch order on nestling size and mass at fledging, we
386 found no lasting consequences of this difference on post-fledging longevity or
387 reproductive success. This is surprising, given that previous analyses have shown that
388 body mass close to fledging correlates well with survival of females during their first
389 year (Low & Part, 2009). One possible explanation is that all nestlings in our dataset
390 were in relatively good condition when they fledged. During our experiment, both our
391 carotenoid-treated and control nests were supplied with sugar water close to the nest.
392 Furthermore, the population is provided with sugar water *ad libitum* throughout the
393 year (Thorogood, Armstrong, Low, Brekke, & Ewen, 2013). Having easy access to food
394 throughout their lives (beginning with parental provisioning) may have enabled

395 smaller, later hatched individuals to persist in the population regardless of hatch order
396 or carotenoid availability. It is also unlikely that later-hatched individuals trade
397 longevity against reproduction, as we detected no effect of hatch order on annual
398 reproductive success.

399 Finally, we expected male hihi nestlings to be more sensitive to hatching order
400 effects given that male hihi embryos are more sensitive to the negative effects of
401 inbreeding (Brekke et al., 2010). Furthermore, male hihi nestlings are larger than
402 females (Ewen et al., 2009; this study), and sex-specific differences in sensitivity to poor
403 or harsh environments are common across vertebrates where sexes are dimorphic and
404 one is more costly to produce or maintain (Anderson *et al.*, 1993; Lindström, 1999).
405 Hatching order has strong sex-specific effects in certain house finch populations
406 (Badyaev *et al.*, 2002). Likewise, male house wrens were more negatively affected by
407 experimentally manipulated hatch orders than their female siblings (Bowers,
408 Thompson, & Sakaluk, 2015). However, size differences between male and female hihi
409 did not appear to result in increased male sensitivity to hatch order effects in our study.
410 Further exploration of whether parents preferentially feed one sex under certain
411 circumstances, as is seen in eastern bluebirds (Ligon & Hill, 2010) and Arabian babblers
412 (Ridley & Huyvaert, 2007) would be informative, as such a bias could mask the
413 sensitivity of males. Alternatively, any male sensitivity may be matched by
414 disadvantages of hatching late for females, given their already smaller size (Oddie,
415 2000).

416 The average period over which broods hatched in our dataset was less than 24
417 hours; so, by the accepted definition, hihi hatch synchronously (Stoleson & Beissinger,
418 1995). Nevertheless, by investigating hatch order effects in an apparently

419 synchronously hatching species, we have detected implications for hihi in early life that
420 may have lasting consequences for the adult phenotype. The increased likelihood of
421 death before fledging for last-hatched offspring is significant, however, we found that
422 later hatched nestlings that did fledge lived as long and produced as many offspring as
423 early-hatched nestlings. This suggests that hatching asynchrony is not maladaptive for
424 hihi; negative effects of hatching late are confined to the nestling period, after which
425 surviving offspring are equally likely to contribute to their mothers' fitness. Our study
426 therefore provides valuable insight into the implications and adaptive potential of
427 hatching asynchrony by broadening the scope under which it has previously been
428 studied. Closer investigation of hatch order effects in other species with apparent
429 synchronous hatching may lead to novel insights into what determines when and why
430 brood size hierarchies arise.

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432 **Data accessibility**

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

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435

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625 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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640 Table 2. Descriptive statistics on all nests from both seasons ($N = 119$).

	Group		Test statistic	<i>P</i>
	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

641 Where appropriate the difference between untreated and supplemented groups are tested statistically;
 642 tests carried out are indicated with test statistic.
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662 Table 3. Results of GLMM investigating hatch order and carotenoid treatment effects on male and female
663 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		

664 1. Removed from models: hatch order*sex*treatment (control/laying supp/full supp) $\chi^2_4 = 6.73, P = 0.15$; sex*treatment
665 (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
666 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 $\chi^2_1 =$
667 1.62, $P = 0.20$; date $\chi^2_1 = 2.85, P = 0.09$; treatment (control/laying supp/full supp) $\chi^2_2 = 3.64, P = 0.16$.

668 2. Removed from models: hatch order*sex*treatment (control/laying supp) $\chi^2_2 = 1.61, P = 0.45$; sex*treatment $\chi^2_2 = 0.61, P = 0.43$;
669 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 $\chi^2_1 = 0.01, P =$
670 0.93; treatment (control/laying supp) $\chi^2_1 = 0.17, P = 0.68$; sex $\chi^2_1 = 0.34, P = 0.24$; date $\chi^2_1 = 3.38, P = 0.07$.

671 3. Removed from models: hatch order*sex*treatment (control/laying supp) $\chi^2_2 = 2.03, P = 0.36$; hatch order*sex $\chi^2_2 = 0.14, P = 0.93$;
672 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
673 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) $\chi^2_1 =$
674 1.69, $P = 0.19$; maternal age $\chi^2_1 = 2.28, P = 0.13$.

675 4. Removed from models: hatch order*sex*treatment (control/laying supp) $\chi^2_2 = 0.5, P = 0.78$; hatch order*treatment (control/laying
676 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
677 $\chi^2_1 = 0.09, P = 0.77$; date $\chi^2_1 = 0.82, P = 0.18$.

678 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
679 (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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684 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 $\varphi(.)$ p(time)	17	925.29	36.21	0.00	533.62

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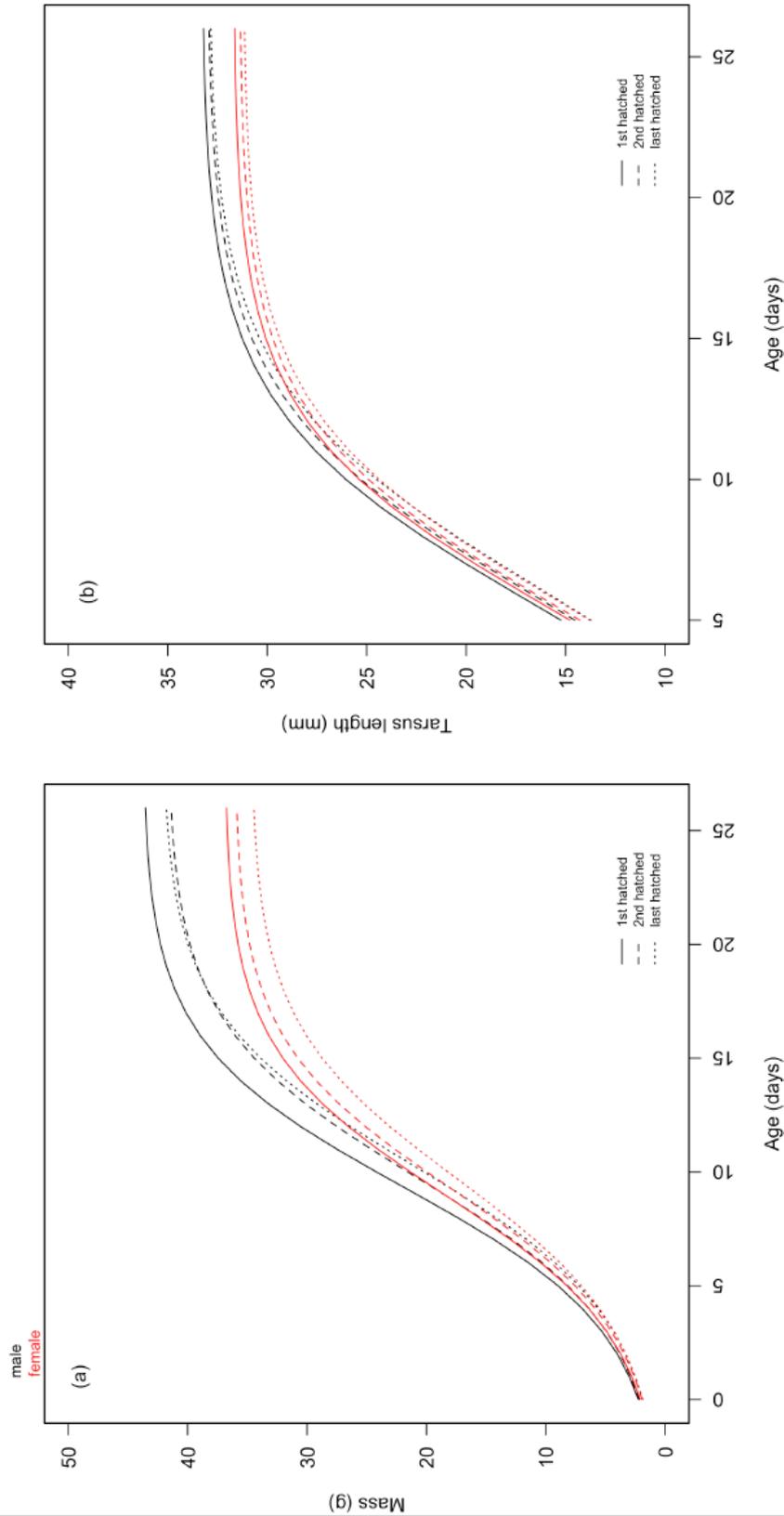
Table 5. Summary of results showing effects of hatch order and carotenoid supplementation on a) male and b) female nestlings.

		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	=	=	=	=			

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

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

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