

1 **Variation in feather corticosterone levels in Alpine swift**
2 **nestlings provides support for the hypo-responsive**
3 **hypothesis**

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5 Susanne Jenni-Eiermann¹, Juanita Olano Marin¹, Pierre Bize²

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7 ¹Swiss Ornithological Institute, Seerose 1, 6204 Sempach, Switzerland

8 ²School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue,
9 AB24 2TZ, UK

10 Corresponding author: Susanne Jenni-Eiermann: susi.jenni@vogelwarte.ch

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16 Authors' contribution:

17 PB and SJE designed the study. PB did the entire field work. SJE and JOM performed the
18 lab assays and analysed the data. SJE led the writing of the manuscript. All authors
19 contributed substantially to the drafts and gave final approval for publication.

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24 **Abstract**

25 In birds, feather corticosterone values (CORTf) are increasingly used as a retrospective and
26 integrative proxy of an individual's physiological state during the period of feather growth.
27 Relatively high CORTf values are usually interpreted as an indicator of exposure to energy-
28 demanding or stressful conditions during feather growth. However, in nestlings this
29 interpretation might not always hold true. The reasons are that, firstly nestlings (especially
30 altricial ones) still develop their hypothalamo-pituitary-adrenal (HPA) reactivity during the
31 growth of their feathers. Hence, at a young age, nestlings might be unable to mount a
32 substantial adrenocortical stress response. Secondly, some species are able to down-
33 regulate their metabolism during food scarcity and therewith probably also their CORT
34 release. Consequently, CORTf values may not unambiguously reflect whether nestlings have
35 suffered from energy-demanding or stress situations. Relatively high CORTf values might
36 indicate either energy-demanding or stressful conditions ('stress responsive hypothesis'), or
37 – conversely – favourable conditions during the period of feather growth ('hypo-responsive
38 hypothesis'). In the altricial Alpine swift (*Tachymarptis melba*), we tested which factors help
39 to distinguish between the two hypotheses by considering factors which affect CORT release
40 (brood size, weather) and factors which are affected by high CORT levels (nestling size and
41 condition). We measured CORTf in 205 nestlings over 7 years and collected data on brood
42 size, body size, body condition and prevailing weather. Nestling CORTf values were
43 positively correlated with body condition and negatively with adverse weather, supporting the
44 hypo-responsive hypothesis. Results from the Alpine swift study, supplemented with a survey
45 of the literature, show that relatively easily collected parameters on brood size, nestling size
46 and condition, and environmental factors can help to distinguish between the two
47 hypotheses. A meaningful interpretation of nestling CORTf should only be made in the
48 context of species-specific traits.

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51 **1. Introduction**

52 Corticosterone (CORT) is the main glucocorticoid in birds. It is secreted by the
53 adrenal glands and its plasma levels increase to moderate levels to up-regulate the
54 metabolism (e.g. as a response to cold ambient temperature; Jimeno et al., 2017), and to
55 high levels in response to acute stressors (e.g. Müller et al., 2006) to induce a variety of
56 physiological and behavioural changes for overcoming a threatening situation (Romero and
57 Wingfield, 2016). Therefore, CORT is frequently used as an indicator to evaluate whether an
58 individual was exposed to energy demanding or stressful situations.

59 Most of our knowledge about the release of CORT in response to stressors comes
60 from research on plasma CORT which measures the momentary level of CORT circulating in

61 the blood stream (Romero and Reed, 2005). Because CORT is quickly released into the
62 blood in response to stressors, plasma levels of CORT are suited to indicate exposure to
63 immediate or ongoing stressors requiring a mobilisation of resources.

64 In birds, CORT is also deposited in feathers during their growth (CORT_f) and thereby
65 provides an integrative and historical record of an individual's CORT release during the
66 period of feather growth (Bortolotti et al., 2009; Ganz et al., 2018; Jenni-Eiermann et al.,
67 2015; Romero and Fairhurst, 2016). Hence, CORT_f is increasingly used to indicate
68 energetically demanding or stressful situations retrospectively and over a prolonged period of
69 time (e.g. Fairhurst et al., 2012; Grava et al., 2013; López-Jiménez et al., 2016), although
70 effects of relatively rare, short-lived stressors on CORT_f are considered negligible (Romero
71 and Fairhurst, 2016) and might be masked. Therefore, CORT_f has been considered more
72 useful for studying the cumulative effects of metabolic challenges and responses to
73 environmental stressors that operate over longer time periods, or persistent individual
74 differences in plasma CORT levels (Lind et al., 2020).

75 However, because of its integrative nature, CORT_f values are challenging to interpret.
76 Whether a given CORT_f value reflects increased CORT release or baseline levels, needs to
77 be evaluated indirectly by comparison with a control group (Lind et al., 2020) or by
78 considering additional factors, which are known to be related with the adrenal activity (e.g.
79 climatic factors and food availability; e.g. Bize et al., 2010; Jenni-Eiermann et al., 2008). In
80 addition, young nestlings may exhibit a limited CORT response to stressors, although their
81 hypothalamic-pituitary-adrenal (HPA) axis is fully developed (Romero and Wingfield, 2016).
82 This might be due to a) ongoing maturation of the brain's ability to regulate the HPA axis
83 (Sapolsky and Meany, 1986) and/or the sensory and brain functions, necessary to perceive a
84 threatening stimulus as such (Sims and Holberton, 2000), or to b) a down-regulation of the
85 metabolism to save energy during stressful periods (McKechnie and Lovegrove, 2002;
86 Vuarin and Henry, 2014). As a result, CORT_f of nestlings facing chronic stressors might be
87 higher, similar, or lower compared with a control group not facing those challenges, as
88 discussed below.

89 Following the interpretations of plasma CORT, most studies measuring CORT_f in
90 nestlings assume that relatively high CORT_f levels indicate energetically demanding or
91 stressful conditions during feather growth, which stimulate the adrenal activity and lead to an
92 increased CORT release (later on 'stress responsive hypothesis' e.g. Boves et al., 2016;
93 Johns et al., 2018; Sapolsky et al., 2000; Sorensen et al., 2016). However, this assumption
94 might not always be correct. For several bird species, especially altricial ones, it was shown
95 that the HPA axis reactivity is lowest in the youngest nestlings (Bebus et al., 2020; Schwabl,
96 1999; Sims and Holberton, 2000; Walker et al., 2005) and reaches a stress reactivity similar
97 to that of adults only shortly before fledging (e.g. Baos et al., 2006; Blas et al., 2006; Rensel

98 et al., 2010; Wada et al., 2007). Since the HPA axis is necessary for hatching and therefore
99 fully developed at this stage, a hypo-responsive period after hatch might be an adaption to
100 prevent adding damage of high CORT levels on the developing neurons (for review see
101 Romero and Wingfield, 2016). Such periods of hypo-responsiveness do not only occur
102 dependent on the intrinsic developmental stage (reviewed in Wada, 2008). They were also
103 observed under external stressful conditions in situations where altricial nestlings would not
104 be able to increase parental feeding (Kitaysky et al., 2001b) thereby reducing maintenance
105 energy costs and possibly growth (e.g. Müller et al., 2009). A reduced stress reactivity might
106 also be expected in altricial nestlings which down-regulate their body temperature,
107 metabolism and behavioural reactions during hunger stress and enter hypothermia (e.g.
108 swifts, swallows; reviewed in McKechnie and Lovegrove, 2002; Vuarin and Henry, 2014).
109 Since CORT is strongly positively associated with metabolic rate (Jimeno et al., 2017), one
110 might expect that CORT release is dampened during hypothermia. Hence, at a young age
111 and/or in stressful conditions low CORT_f values might - in contrast to the stress responsive
112 hypothesis - indicate stressful conditions. The scenario of a reduced stress reactivity (either
113 intrinsically depending on developmental stage or through external stressful conditions) will
114 be referred to as 'hypo-responsive hypothesis'. Therefore, for the interpretation of CORT_f in
115 nestlings, additional information is required to indicate whether nestlings up-regulate or
116 down-regulate their metabolism as a response to energy-demanding or stressful conditions.

117 The objective of our study was to test, which of the two hypotheses – the stress- or
118 the hypo-responsive hypothesis – best explains the variation in CORT_f in nestling Alpine
119 swifts (*Tachymarptis melba*). This altricial bird species exclusively feeds on the wing and has
120 the capability to enter hypothermia (Ruf and Geiser, 2015). In this study we considered
121 factors that are relatively easily collected in field studies and are known to either affect CORT
122 release (brood size, weather) or are affected by CORT (nestling size and condition) (see
123 below for the rationale and references). This allowed us to then carry out a survey of the
124 literature on CORT_f to discuss past results in the light of this new conceptual framework of
125 variation in CORT_f according to the two hypotheses (Table 1).

126 During adverse weather (low ambient temperature, rain, strong wind gusts) the
127 availability of aerial insects is strongly reduced (Grüebler et al., 2008; Jenni-Eiermann et al.,
128 2008) and Alpine swifts reduce or entirely stop feeding nestlings, which then experience
129 hunger stress. Short periods of inclement weather and food restriction lead to Cort increase.
130 However, during adverse weather Alpine swift nestlings may also down-regulate body
131 temperature to a minimum of 18°C (Bize et al., 2007) thereby reducing basal metabolic rate.
132 Large broods increase sibling competition (e.g. for Alpine swifts Bize and Roulin, 2006) and
133 begging behaviour, a behaviour facilitated by CORT (Kitaysky et al., 2001b). Therefore,
134 CORT release in large broods may be increased (Saino et al., 2003; but see Bize et al.,

135 2010; Crino et al., 2020; Gil et al., 2008). On contrary, a large brood may also hamper
136 maturation and therewith HPA responsiveness.

137 Development and growth of nestlings are retarded in early life when CORT is
138 repeatedly increased to stress levels (Almasi et al., 2012; Butler et al., 2010; Crino et al.,
139 2014; Müller et al., 2009; Sapolsky et al., 2000) and feather growth and quality were impaired
140 in experiments which applied exogenous CORT (e.g. Jenni-Eiermann et al., 2015; Romero et
141 al., 2005). However, it was also shown that feather growth is maintained even during periods
142 of reduced food intake or fasting (Jenni and Winkler, 2020) and hence no correlation
143 between wing-length (which is dominated by feather-length) and CORTf might be found.
144 Body condition is directly reduced by nutritional stress which has been shown to increase
145 CORT secretion (e.g. Jenni-Eiermann et al., 2008; Kitaysky et al., 2001a; Pravosudov and
146 Kitaysky, 2006).

147 Based on these findings, our predictions for the stress response hypothesis are a
148 positive correlation between CORTf and adverse weather and brood size, and a negative
149 correlation with body size measures and body condition, respectively (Table 1). In contrast,
150 for the hypo-responsive hypothesis, we predict a negative correlation between CORTf and
151 adverse weather and brood size, and a positive correlation with body size measures and
152 body condition at a given age, respectively (Table 1).

153 In a first step we verify whether the factors supposedly affecting CORT (weather and
154 brood size) are indeed correlated with the parameters supposedly affected by CORT (body
155 size and body condition). In a second step we evaluate how both, the factors affecting CORT
156 and those being affected by CORT, can be used to distinguish between the two hypotheses
157 according to the predictions given in Table 1. Thirdly, the results, complemented with a
158 survey of the literature, were used to evaluate parameters which help to distinguish between
159 the two hypotheses.

160

161 **2. Methods**

162 2.1. Study species

163 Alpine swifts are colonial and can live up to 26 years in the wild. They breed for the
164 first time at the age of 2 or 3 years and produce one clutch of 1 – 4 eggs/year until death
165 (Tettamanti et al., 2012). Alpine swifts are socially monogamous with males and females
166 sharing parental duties equally from incubation to fledging, which takes place 50 to 70 days
167 after hatching (Bize et al., 2004). Nestlings hatch naked and grow their body feathers
168 simultaneously between 10 - 50 days of age (Arn-Willi, 1960).

169

170 2.2. Study site and general field procedures

171 Data come from an Alpine swift colony of ca. 50 breeding pairs located in Solothurn,
172 Switzerland, where there is an individual based monitoring of all the nestlings and adults
173 since 1999. For the present study, feathers were collected during the years 2008 to 2014.
174 Each year, nests were visited regularly to determine clutch size and hatching date of the first
175 egg (denoted day 0), brood size at hatching and brood size at fledging (later on brood size).
176 At day 50 (mean \pm SE: 50.4 ± 0.11 ; range 47 - 64) after hatching, body mass of each nestling
177 was measured to the nearest 0.1 g, sternum-length to the nearest 0.1 mm and wing-length to
178 the nearest mm. Between 4 and 10 feathers were plucked from the white patch of the breast.
179 Nestlings were individually recognized by ringing them with a numbered ring 10 days after
180 hatching. Weather data were received from the Swiss meteorological station Bern-Zollikofen,
181 located at about 40 km from Solothurn. Note that none of the seven study years was an
182 extremely poor year in terms of environmental conditions.

183 For this study, we randomly selected one nestling per brood for keeping costs within
184 limits, which resulted in feather samples and biometric measurements at day 50 collected
185 from 205 nestlings between 2008 and 2014 (see Table 2 for a description of the sample
186 sizes per year).

187 Bird capture, ringing and measurements were performed under the legal
188 authorisation of the Federal Office for the Environment, Forests and Landscapes. The ringing
189 licence number of PB is 2235.

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191 2.3. Analysis of CORTf

192 CORTf was measured in two body feathers per nestling. The length of these two
193 feathers was measured to the nearest mm and they were weighed to the nearest μ g before
194 being minced together with scissors in small pieces (<5mm) into a soda glass centrifuge
195 tube. Feather mass was kept within narrow limits (mean \pm SE: 4.57 ± 0.038 mg). 6 ml
196 methanol (analytical or HPLC grade) were added, and the samples were placed in a
197 sonicating water bath for 30 min at room temperature, followed by incubation at 50°C in a
198 water bath for 10 h (overnight). Methanol was separated from the feather material by filtration
199 through a Whatman filter paper no. 4 into a new centrifuge tube. The feather remnants and
200 original sample tube were washed with 2 ml methanol and filtered into the tube with the
201 previous filtrate. Methanol extracts were then placed in a SBHCONC/1 Sample Concentrator
202 and evaporated under a constant stream of nitrogen gas at 50°C. Dried extracts were stored
203 in the freezer and re-suspended in 250 μ l assay buffer for CORT measurement.

204 CORTf was measured in duplicate in a total of six plates with a commercial enzyme
205 immunoassay (EIA, Enzo Life Sciences, Inc.) and calculated via a standard curve run in
206 duplicate on each plate. Crossreactivities with related steroids are: Corticosterone (100%),
207 Deoxycorticosterone (21.3%), Desoxycorticosterone (21.0%), Progesterone (0.46%),

208 Testosterone (0.31%), Tetrahydrocorticosterone (0.28%), Aldosterone (0.18%), Cortisol
209 (0.046%), and <0.03% for Pregnenolone, Estradiol, Cortisone, 11-dehydrocorticosterone
210 acetate. Plates were read with a Bio-Rad Benchmark spectrophotometer at 405 nm. A
211 chicken plasma sample (mean \pm SE: 46.86 \pm 1.65 ng/ml) was measured in duplicate as
212 internal control on each plate to calculate intra- (7.99%) and inter-assay (8.98%) variation.
213 Dilution curves of a pool sample of Alpine swift feathers extracted as described were parallel
214 to the standard curve. To test extraction efficiency, 21 Alpine swift feather samples of known
215 CORT concentration were spiked. The mean recovery was 93.37% \pm 2.33 (mean \pm SE,
216 range 74.06 - 111.88).

217

218 2.4. Statistical analysis

219 CORT_f values were expressed per mm feather-length (pg/mm) (following Bortolotti et
220 al., 2009; Jenni-Eiermann et al., 2015) and ln-transformed before analysis in all models to
221 ensure normality of the residuals. For each individual separately, the mean of daily ambient
222 temperature (°C), daily sum of rain (mm) and daily maximum wind gusts (m/s) (later called
223 ambient temperature, rain, wind) were calculated for day 10 to 50 of each nestling (the period
224 of nestling feather development; for sternum-length, which reaches its final length at about
225 30 days of age, the first 30 days were taken). Body condition was expressed as the residuals
226 of a linear regression of body mass (g) against wing-length (mm). Annual variation in weather
227 condition, reproductive traits and nestling phenotypic traits are presented in Table 2.

228 To test the effect of environmental, individual and nest conditions on body size and
229 body condition, we fitted a General Linear Model with temperature, rain, wind, age of nestling
230 (days), season (hatching day; 1 May = 1), and brood size as explanatory covariates, year (7
231 levels: 2008-2014) as explanatory factors, and wing-length, sternum-length and body
232 condition, respectively, as dependent variables.

233 To examine intrinsic and environmental effects on CORT_f, a Linear Mixed Model was
234 fitted with lnCORT_f as dependent variable and mean ambient temperature, rain, wind, brood
235 size at fledging, wing-length, body condition as fixed explanatory covariates and year as
236 fixed explanatory factors. Plate number was included as random factor (6 levels: plates 1 - 6)
237 to account for inter-plate variation. Sternum-length was included in the initial model, but then
238 removed as sternum-length was highly correlated with wing-length and inclusion of sternum-
239 length did not show any additional effect on CORT_f.

240 All the statistical models were run in SPSS 12.0 for Windows.

241

242

243 3. Results

244 The seven study years differed markedly in CORTf, weather conditions, reproductive
245 success and nestling phenotype. Significant differences between years were found for
246 CORTf, ambient temperature, rain, wind gusts, brood size, wing- and sternum-length, but not
247 for body mass (Table 2).

248 Wing- and sternum-length of the Alpine swift nestlings were not related to the three
249 weather variables or brood size, while there was a significant year effect (Table 3). In
250 contrast, body condition was negatively related with brood size, i.e., nestlings of large broods
251 had a lower body condition, while there was no year effect.

252 CORTf showed a significant negative relationship with wind, a positive relationship
253 with body condition and differences between years, while temperature, rain, body size and
254 brood size were not significantly related with CORTf (Table 4, Fig. 1).

255

256

257 **4. Discussion**

258 Among the factors that could affect CORT, which in turn might influence traits of
259 Alpine swift nestlings, we identified that brood size had an impact on body condition, while
260 weather had no significant effect on size measures or body condition. For CORTf we found a
261 positive correlation with body condition and a negative with strong wind (Fig. 1). Hence,
262 CORTf was high during favourable environmental conditions and in nestlings in good
263 condition. These results clearly support the hypo-responsive hypothesis (Table 1).

264 Alpine swift nestlings had a lower body condition at day 50 when raised in a large
265 brood, while body size was not affected by environmental, nest- or individual factors. The
266 measures of body size used here (wing- and sternum-length), both reflecting the flight
267 machinery, seem to have a high priority; hence (moderately) adverse conditions - none of the
268 seven study years was an extremely bad year - affected body condition, rather than the size
269 of the flight apparatus, when nestlings are close to fledging. Remarkably, in nestling Alpine
270 swifts, body condition was found to be favoured at the expense of wing and sternum growth
271 earlier in the development (Bize et al., 2006). This change in allocation of resources to
272 different tissues likely mirrors a shift in priorities, with body reserves being essential to
273 ensure survival during the early growth phase and the flight apparatus being essential to
274 ensure fledging and survival in this extreme aerial bird (Liechti et al., 2013).

275 We found that CORTf was related to body condition and wind, but not significantly to
276 other weather factors, body size or brood size, although the direction of the slopes tended in
277 the same direction, i.e. better conditions were generally related to higher CORTf (Fig. 1).
278 Moreover, CORTf differed significantly between the study years. Most nestlings of a year

279 grow concurrently and hence experience similar weather conditions (averaged over the 50
280 days of growth) with little variation within a year. Therefore, we included year as a factor to
281 control for these annual variations.

282 In the following we discuss the various parameters which may be used to facilitate the
283 interpretation of CORTf in nestlings and to distinguish between the stress responsive and the
284 hypo-responsive hypotheses, in the light of other studies.

285 Adverse weather conditions reduce the availability of insects in the air (Grüebler et
286 al., 2008) which in Alpine swifts leads to reduced or no feeding by the parents and,
287 depending on the duration of adverse weather, to hunger in the nestlings, and eventually to
288 starvation (Glutz von Blotzheim and Bauer, 1980). Short bouts of adverse weather increase
289 circulating CORT in Alpine swift nestlings transiently (Bize et al., 2010), but apparently do not
290 translate into a long-lasting effect resulting in elevated CORTf (in accordance with the
291 conclusion by Romero and Fairhurst, 2016). It remains to be shown whether plasma CORT
292 in starving Alpine swift nestlings that entered a hypothermic state (Bize et al., 2007; Ruf and
293 Geiser, 2015) is indeed decreased because of a down-regulated metabolism or because of a
294 retarded development.

295 Cory's Shearwater nestlings provided with less food by the parents than controls
296 showed lower CORTf levels (Fairhurst et al., 2012) which was interpreted by the authors as
297 an adaptive response to minimize the extent of physiological damage caused by chronically
298 elevated CORT. Procellaride nestlings are able to reduce their body temperature by at least
299 10°C in response to food scarcity (Boersma, 1986; Ricklefs et al., 1980) further supporting
300 the hypo-responsive hypothesis. In contrast, black kite nestlings showed elevated plasma
301 CORT during low ambient temperature (2016) which supports the stress responsive
302 hypothesis. Black kite nestlings develop in an open nest and are not known to down-regulate
303 their body temperature. It therefore seems plausible that they need to increase their
304 metabolic rate for thermoregulation.

305 In summary, adverse weather conditions reduce food intake and/or increase
306 maintenance energy requirements (thermoregulation) in nestlings. Depending on whether
307 nestlings increase metabolism (increased begging, thermoregulation) or not, weather as an
308 auxiliary parameter can help distinguishing between the stress responsive and the hypo-
309 responsive hypotheses.

310 Another factor related to nestling CORT concentration is brood size. Experimentally
311 enlarged broods lead to increased sibling competition (Bize and Roulin, 2006; Saino et al.,
312 2003) and a reduced parental provisioning rate per individual. However, whether or not
313 plasma CORT is increased in nestlings of large broods is inconsistent. Begging behavior is
314 facilitated by CORT (Kitaysky et al., 2001b), and an increase of plasma CORT in
315 experimentally enlarged broods was found in barn swallows *Hirundo rustica* (Saino et al.,

316 2003) but not in other species (Alpine swift, zebra finch *Taeniopygia guttata*, spotless starling
317 *Sturnus unicolor*, blue tit *Cyanistes caeruleus*, pied flycatcher *Ficedula hypoleuca*) (Bize et
318 al., 2010; Crino et al., 2020; Gil et al., 2008; Lobato et al., 2008).

319 In our study, brood size significantly affected body condition. Alpine swift nestlings of
320 large broods had a reduced body condition probably indicating a hampered development as
321 also reported for wild nestling zebra finches (Crino et al., 2020), but brood size did not affect
322 CORTf of the Alpine swift nestlings. In house sparrow nestlings of broods which suffered
323 nestling mortality, relatively high CORTf levels were found, suggesting sibling competition for
324 food (Salleh Hudin et al., 2017); based on the stress responsive hypothesis, the authors
325 argued that CORTf was accumulated at the beginning of the nestling phase when the broods
326 were large and sibling competition high. According to our predictions these results would also
327 support the hypo-responsive hypothesis, especially because they found a positive correlation
328 between CORTf and body condition. López-Jiménez et al. (2016) measured CORTf in black
329 kite nestlings and predicted, in line with the developmental based hypo-responsive
330 hypothesis, higher CORTf in broods with a single nestling. However, they found low CORTf
331 levels in singletons and in the first nestling of multi-nestling broods, while subordinate
332 nestlings had higher levels. They concluded an effect of the social environment on CORTf
333 levels, likely reflecting an increased allostatic load imposed by the oldest, dominant nestling
334 on its younger siblings, which is in line with the stress responsive hypothesis. In great tits
335 *Parus major*, brood size enlargement in the less favorable coniferous habitat resulted in
336 increased CORTf, while brood size reduction in lower CORTf than in controls, and there was
337 no effect of brood size manipulation on CORTf in the favorable deciduous habitat (Lodjak et
338 al., 2015). This supports the stress responsive hypothesis and indicates an increased sibling
339 competition with increasing brood size in the suboptimal habitat.

340 In summary, the effect of brood size on CORTf apparently depends on whether there
341 is pronounced sibling hierarchy and competition which may lead either to allostatic load in
342 subordinates (supporting the stress responsive hypothesis) or to a delayed development
343 (supporting the hypo-responsive hypothesis). Obviously, a mixture of both, or no effect of
344 brood size, may also occur.

345 It was repeatedly shown that an experimentally prolonged increase of plasma CORT
346 depresses growth of bone structures (e.g. tarsus, bill), feathers, and mass gain in nestlings
347 (e.g. Almasi et al., 2012; DesRochers et al., 2009; Müller et al., 2009; Spencer and Verhulst,
348 2007; Strohlic and Romero, 2008). However, whether the effect of transiently high plasma
349 CORT levels on biometrics can be detected via CORTf is controversial. In common buzzard
350 *Buteo buteo* nestlings which developed under natural conditions an inverse relationship of
351 CORTf with body mass was reported (Martínez-Padilla et al., 2013). Other studies showed
352 no relationship between CORTf and feather growth rate, mass or length (Beaugeard et al.,

353 2019; Harms et al., 2010; Will et al., 2014) or a positive relationship with feather growth rate
354 and/or feather mass in nestlings fed *ad libitum* (Patterson et al., 2015; Salleh Hudin et al.,
355 2017).

356 The use of size measures may pose two difficulties. First, compensatory accelerated
357 growth may countervail a transient reduction of growth. Hence, despite stressful conditions
358 during a certain period of growth (which may or may not lead to increased CORTf), final size
359 may be similar to unstressed conspecifics (Bize et al., 2003). Second, structures that are
360 particularly important for post-fledging survival may maintain growth under stressful
361 conditions, or may be more prone to compensatory growth, than other structures (Müller et
362 al., 2009). For example, some species will not reduce their feather growth rate when
363 stressed (Grace et al., 2017). Our findings for the Alpine swift nestlings also suggest no
364 effect of adverse weather on the size of the flight apparatus (at least with the weather
365 experienced during the study years which excluded very bad periods) which is of prime
366 importance after fledging, and hence no correlation of size with CORTf. As in our study, no
367 correlation between CORTf and size was reported for wild juvenile house sparrows, although
368 urban birds had higher CORTf than rural ones (Beaugeard et al., 2019) and the authors
369 hypothesized that a potential negative relationship between CORTf and body size during
370 early development might have been blurred by compensatory growth later on.

371 Hence, even if structural development was hampered for a certain period, it might not
372 be discernible when measuring biometrics of birds shortly before or after fledging. Our
373 observation that body size was not affected by weather or brood size indicates that body size
374 does not seem a decisive parameter for distinguishing between the two hypotheses in our
375 study. A way to retrospectively identify a transient stressful phase and to achieve an
376 improved temporal resolution, is the analysis of feather growth bars and CORTf of feather
377 segments (Ganz et al., 2018; Jenni-Eiermann et al., 2015). However, this needs larger
378 feathers than the small body feathers used in our study for measuring CORTf with
379 conventional EIA.

380 For body condition, similar difficulties concerning its relationship with CORTf might
381 show up as with body size. Body condition is dependent on food intake which in turn
382 depends on weather conditions, environmental and social factors. Therefore, as for structural
383 growth, a transient low body condition may be compensated during later favourable times
384 (Müller et al., 2009). Furthermore, some species have a natural recession of body mass
385 towards the end of the nestling phase of nestlings in good condition, while those in worse
386 condition do not show such an excess of body mass. Hence, the relationship between
387 CORTf and body condition may depend on the time of measurement (i.e. during a transient
388 low or a transient peak in condition).

389 We found a significant positive correlation between body condition close to fledging
390 and $CORT_f$ in the Alpine swift nestlings, which supports the hypo-responsive hypothesis.
391 Similarly, a study in house sparrow nestlings showed that individuals of parents with access
392 to supplementary food and which were in good body condition, had significantly elevated
393 $CORT_f$ levels (Salleh Hudin et al., 2017); however the authors based their interpretation on
394 the stress responsive hypothesis and assumed a time effect with stressful events during
395 early breeding and compensatory growth and mass gain later on. In other studies negative
396 relationships between $CORT_f$ and body condition of nestlings were found which were
397 interpreted as the consequence of increased allostatic load due to a bad nutritional status
398 (Harms et al., 2010; Lamb et al., 2016; López-Jiménez et al., 2016) or due to experimentally
399 increased plasma CORT (Fairhurst et al., 2013).

400

401 **5. Conclusions**

402 The main aim of this study and brief review of the literature is to highlight that the
403 interpretation of $CORT_f$ in nestlings is not straight-forward, as it might be when investigating
404 plasma CORT levels. Contrasting interpretations of high and low $CORT_f$ values can coexist.
405 Here, we presented two alternative hypotheses associated with different underlying
406 mechanisms. Additional information is required to distinguish not only between the stress
407 responsive and hypo-responsive hypotheses, but also to differentiate whether a hypo-
408 responsive reaction to stress is due to an intrinsic, developmental process or due to
409 metabolic down-regulation triggered by external stressors. Only then $CORT_f$ can be
410 interpreted correctly. To further our understanding of $CORT_f$ in nestlings, more information is
411 needed in at least three fields.

412 First, the two proposed hypotheses need more scrupulous testing. For example, it
413 remains untested how long a period of suboptimal feeding may last until it translates in
414 elevated $CORT_f$ under the stress hypothesis; how the development of the adrenocortical
415 capacity to respond to a stressor is related to $CORT_f$ and depends on the developmental
416 mode (altricial or precocial); whether nestlings in hypothermia have low levels of plasma
417 CORT and whether or not this modifies $CORT_f$; which species may suppress CORT release
418 under stressful conditions (as shown in Cory's shearwaters; Fairhurst et al., 2012).

419 Second, as shown in the present study and literature review, many of the easily
420 obtainable parameters that may distinguish between the two hypotheses also need
421 verification. For example, weather factors and brood size are good indicators to better define
422 the ambient conditions during nestling development and to distinguish between the two
423 hypotheses. However, as discussed above, they might still leave room for contrasting
424 interpretations if standing alone. Additional factors, such as size measures and body
425 condition, might help, but are also not clear-cut. Therefore, if possible, more information

426 needs to be gathered. Of major importance is knowledge about the amount of food available
427 to the nestling because of the direct effect of hunger stress on CORT release, and
428 information about how the species in question reacts to hunger (e.g., increased begging or
429 hypothermia). Also, information about sibling competition would be helpful to assess the
430 possible level of stress the nestlings might be exposed to. In any case, findings have to be
431 interpreted in the context of species-specific traits (e.g., altricial / precocial) and ambient
432 conditions (e.g., weather). In brief, a thorough knowledge of the biology of the species is
433 needed.

434 Third, the way of CORT deposition into the feather must be considered. CORT_f is
435 incorporated in a time-dependent manner and therefore dependent on feather growth rate.
436 The slower feathers grow, i.e. the longer a feather segment is exposed to the circulation in
437 the feather pulp, the higher is its CORT_f concentration (Bortolotti et al., 2008; Jenni-
438 Eiermann et al., 2015). Hence, if feather growth rate varies between groups or with factors of
439 interest, CORT_f may reflect feather growth rate rather than an integrated measure of
440 exposure to plasmatic CORT. Therefore, feather growth rate, although not considered in this
441 study, is potentially an important measure that can be determined retrospectively by
442 measuring growth bars if repeated measurements of growing feathers are not feasible.

443

444

445 Table 1. Expected correlations of nestling CORTf with factors known to either affect CORT
 446 release (adverse weather, brood size) or to be affected by CORT (nestling size, body
 447 condition) in nestlings based on two different hypotheses. The stress responsive hypothesis
 448 predicts an increase in CORT release under stressful conditions. The hypo-responsive
 449 hypothesis predicts an increase of CORT release with nestling development or because of a
 450 down-regulation of CORT release under (transient) adverse conditions. The arrows indicate
 451 an expected positive or negative correlation, a dash no correlation. The significant
 452 correlations found in this study for Alpine swift nestlings are indicated in bold.

453

	Correlation of CORTf under the	
	stress responsive hypothesis	hypo-responsive hypothesis
Adverse weather	↗	↘
Brood size	↗	↘
Nestling size	↘ ¹ — ²	↗¹ —²
Body condition	↘	↗

454 ¹for body size measurements

455 ² for wing-length

456

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459

460 Table 2. Annual means (\pm SD) of CORTf, weather parameters recorded during nestling
 461 feather development (day 10 to 50 for each nestling), reproductive success, and nestling
 462 biometric traits at day 50 after hatching. Feathers were collected at 50 days of age from one
 463 randomly chosen nestling per brood per year (number of nestlings). F and P values are
 464 results from one-way ANOVAs testing for annual variation in traits. For each trait, years not
 465 connected by the same letter are significantly different using post-hoc Student t-test
 466 comparisons.

467

	2008	2009	2010	2011	2012	2013	2014	$F_{6,198}$	P
Number of nestlings	30	29	32	36	23	27	28		
Feather CORT at day 50 (pg/mm)	1.32 ^A ± 0.40	2.13 ^{A,B} ± 1.89	4.48 ^C ± 2.60	4.43 ^C ± 1.64	2.36 ^B ± 1.54	5.40 ^D ± 2.16	1.34 ^A ± 0.34	27.60	<0.001
Weather condition during nestling feather development (10-50 days)									
Mean ambient temperature ($^{\circ}$ C)	18.83 ^A ± 0.10	17.98 ^B ± 0.26	19.48 ^C ± 0.41	17.35 ^D ± 0.10	18.38 ^E ± 0.14	19.86 ^F ± 0.50	17.39 ^D ± 0.29	341.1	<0.001
Sum of rain (mm)	2.98 ^{A,B} ± 0.06	4.65 ^D ± 0.29	2.67 ^C ± 0.79	4.05 ^E ± 0.20	2.76 ^{B,C} ± 0.16	3.05 ^A ± 0.17	6.73 ^F ± 0.58	360.7	<0.001
Maximum wind gust (m/s)	6.06 ^A ± 0.04	6.30 ^C ± 0.20	5.82 ^B ± 0.29	5.74 ^B ± 0.14	6.20 ^{A,C} ± 0.05	6.21 ^C ± 0.18	6.27 ^C ± 0.57	22.10	<0.001
Reproductive success									
Brood size at fledging	2.63 ^A ± 0.62	2.63 ^A ± 0.49	2.13 ^B ± 0.61	2.22 ^B ± 0.76	2.35 ^{A,B} ± 0.65	2.11 ^B ± 0.75	2.36 ^{A,B} ± 0.83	2.95	0.009
Nestling phenotype at day 50									
Body mass (g)	95.17 ^A ± 8.16	98.19 ^{A,B} ± 6.27	98.02 ^{A,B} ± 9.66	100.14 ^B ± 8.25	96.62 ^{A,B} ± 9.97	97.43 ^{A,B} ± 7.92	94.77 ^A ± 9.43	1.49	0.183
Wing-length (mm)	215.87 ^{A, B} ± 7.41	215.76 ^{A, B} ± 9.76	216.61 ^{A, B} ± 7.64	216.08 ^{A,B} ± 6.81	210.15 ^C ± 9.04	218.37 ^A ± 8.15	213.93 ^{B,C} ± 9.66	2.42	0.028
Sternum-length (mm)	40.19 ^A ± 1.48	39.81 ^{A,B} ± 1.36	39.33 ^{B,C} ± 1.16	39.71 ^{A,B} ± 1.31	38.71 ^C ± 1.30	39.22 ^{B,C} ± 1.80	39.63 ^{A,B} ± 1.30	3.09	0.006

468

469 Table 3. General Linear Models with wing-length (mm), sternum-length (mm) and body
 470 condition at ca. 50 days of age as response variable and mean ambient temperature (°C),
 471 rain (mm) and wind (m/s), respectively, during days 10 - 50 of each nestling (days 10 – 30 for
 472 sternum-length), brood size at fledging, hatching day (1 = 1 May), exact age (days) as fixed
 473 explanatory variables and year (7 levels: 2008-2014) as fixed factor. Estimates ± SE (P-
 474 value) are reported for the explanatory variables only. Differences between years are
 475 presented in Table 2. Significant effects were indicated in bold.

476

	Temperature	Rain	Wind	Brood size	Hatching day	Age	year
Wing (mm)	-2.571±2.43 (0.292)	0.099±1.80 (0.957)	- 0.397±2.96 (0.894)	-0.964±0.87 (0.267)	-0.052±0.12 (0.663)	0.292±0.41 (0.475)	(0.010)
Sternum (mm)	0.176±0.42 (0.679)	0.303±0.31 (0.337)	0.191±0. 52 (0.713)	-0.007±0.15 (0.961)	-0.031±0.02 (0.140)	- 0.092±0.07 (0.198)	(0.005)
Body condition	3.496±2.34 (0.137)	- 2.558±1.73 (0.142)	0.035±2.85 (0.990)	-2.00±0.83 (0.017)	-0.026±0.12 (0.820)	- 0.263±0.39 (0.504)	(0.114)

477

478

479 Table 4. Linear Mixed Model with lnCortf (pg/mm) as response variable, plate number as
 480 random factor, and mean daily ambient temperature (°C), rain (mm) and wind (m/s) during
 481 days 10 - 50 of each nestling, brood size at fledging, wing-length (mm) and body condition at
 482 ca. 50 days of age, respectively, as fixed explanatory variables and year (7 levels: 2008-
 483 2014) as fixed factor. Estimates ± SE, F-value and P-values are indicated (bold if significant
 484 at the P< 0.05 level).

485

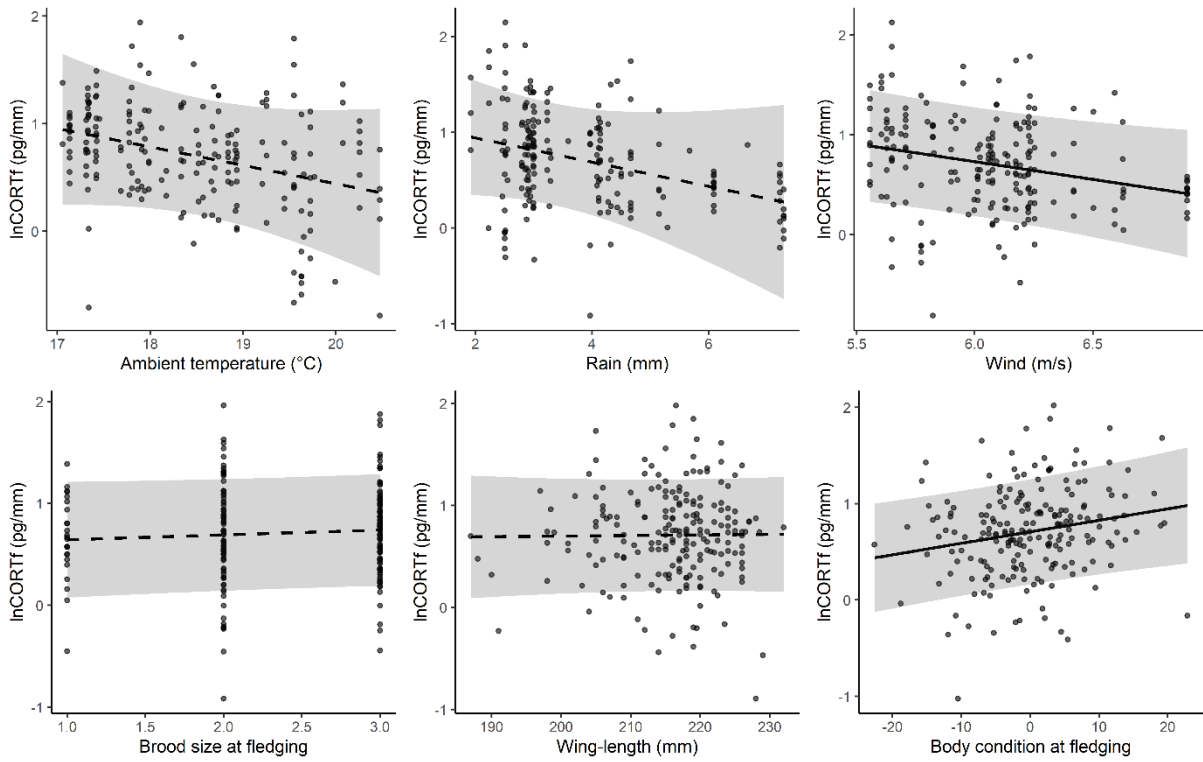
	Estimate ± SE	F-value	P
Temperature	-0.171±0.15	1.337	0.249
Rain	-0.127±0.11	1.350	0.247
Wind	-0.355±0.18	4.054	0.046
Brood size	-0.048±0.05	0.779	0.379
Wing-length	0.001±0.00	0.016	0.900
Body condition	0.012±0.01	6.811	0.010
Year		2.723	0.044

486

487

488

489 Figure 1. Relationships of lnCORTf (pg/mm) with mean daily ambient temperature (°C), rain
490 (mm), wind (m/s) during days 10 – 50 of each nestling, respectively, brood size at fledging,
491 and wing-length (mm) and body condition at day 50, respectively. Plots show regression
492 lines from the Linear Mixed Model in Table 4 (solid: significant, dashed: non-significant) with
493 95% confidence intervals. Dots are partial residuals of the model given in Table 4.
494



495

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