

1 **Plasticity in female timing may explain current shifts in breeding phenology of a North**  
2 **American songbird**

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18

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20 shifts; bird

21 **Abstract**

- 22 1. Climate change has driven changes in breeding phenology. Identifying the magnitude of  
23 phenological shifts and whether selection in response to climate change drives these shifts  
24 is key for determining species' reproductive success and persistence in a changing world.
- 25 2. We investigated reproductive timing in a primarily sedentary population of the dark-eyed  
26 junco (*Junco hyemalis*) over 32 years. We predicted that juncos would breed earlier in  
27 warmer springs in response to selection favouring earlier breeding.
- 28 3. To test this prediction, we compared the annual median date for reproductive onset (i.e.,  
29 egg one date) to monthly spring temperatures and examined evidence for selection  
30 favouring earlier breeding and for plasticity in timing.
- 31 4. Egg one dates occurred earlier over time, with the timing of breeding advancing up to 24  
32 days over the 32-year period. Breeding timing also strongly covaried with maximum April  
33 temperature. We found significant overall selection favouring earlier breeding (i.e., higher  
34 relative fitness with earlier egg one dates) that became stronger over time, but strength of  
35 selection was not predicted by temperature. Lastly, individual females exhibited plastic  
36 responses to temperature across years.
- 37 5. Our findings provide further evidence that phenotypic plasticity plays a crucial role in  
38 driving phenological shifts in response to climate change. For multi-brooded bird  
39 populations, a warming climate might extend the breeding season and provide more  
40 opportunities to re-nest rather than drive earlier breeding in response to potential  
41 phenological mismatches. However, as plasticity will likely be insufficient for long-term  
42 survival in the face of climate change, further research in understanding the mechanisms

43 of female reproductive timing will be essential for forecasting the effects of climate change  
44 on population persistence.

## 45 1. Introduction

46 Climate change is greatly affecting plant and animal life (Root et al. 2003; Scheffers et al.  
47 2016; Staudinger et al. 2013). Phenological shifts are common (Piao et al. 2019; Scheffers et al.  
48 2016), suggesting that many species can adjust to climate change (Charmantier and Gienapp  
49 2014; Saalfeld and Lanctot 2017). Identifying the magnitude of phenological shifts and their  
50 selective drivers in response to climate change are key for conservation efforts (Charmantier &  
51 Gienapp 2014). Numerous studies have investigated phenological shifts in passerine birds in the  
52 last two decades; however, the number of long-term datasets for unique species that can account  
53 for breeding timing as well as reproductive success is limited (i.e., <10 passerine species).  
54 Additionally, these species greatly vary in life history (e.g., migratory strategy [migrant vs.  
55 resident], breeding duration [single- vs. multi-brooded], nesting strategy [cavity vs. open cup  
56 nesting], diet, and habitat) and geography, all of which could directly affect selection pressures  
57 on breeding phenology (Dunn & Møller 2014). Investigating the potential drivers of phenological  
58 shifts in additional species with distinctive life histories will allow for more accurate predictions  
59 of which populations will be able to adapt to the changing climate. To date, many studies  
60 investigating phenological shifts in birds have been focused on European species, with some work  
61 in North America species that are predominantly migratory, but see (Wilson *et al.* 2007; Watts *et*  
62 *al.* 2019). In this study, we contribute to this growing body of literature by analysing a long-term  
63 data set of the breeding efforts of a North American, resident songbird population.

64 The relative role of microevolutionary change versus behavioural plasticity in driving  
65 phenological shifts remains under debate (Charmantier & Gienapp 2014), as it likely varies across  
66 species in relation to their life history and their ability to adapt to climate change. Climate change  
67 could drive directional selection favouring earlier breeding in birds by influencing phenology in

68 related trophic levels (e.g., prey, competitors), thus affecting offspring survival (Charmantier &  
69 Gienapp 2014). Evolutionary adaptation can then occur if directional selection favouring earlier  
70 breeding acts on heritable traits with genetic variation (Hoffmann & Sgrò 2011). Phenological  
71 changes may also reflect behavioural plasticity, or the ability of an individual to modify its  
72 behaviour based on the environment (Sih *et al.* 2010; Van Buskirk, Mulvihill & Leberman 2012;  
73 Beever *et al.* 2017). Behavioural plasticity and its underlying mechanisms can allow individuals  
74 to respond more quickly to a changing climate, as compared to microevolutionary change in  
75 response to selection (Sih *et al.* 2010; Charmantier & Gienapp 2014; Beever *et al.* 2017).  
76 However, plasticity is not always adaptive (Duputié *et al.* 2015) and is unlikely to be sufficient  
77 to allow populations to respond long-term to climate change (Ghalambor *et al.* 2007; Gienapp *et*  
78 *al.* 2013).

79 Here, we used long-term data collected from Dark-eyed Juncos (*Junco hyemalis*), a north-  
80 temperate sparrow found in Canada and the United States, to investigate changes in their breeding  
81 phenology. Juncos serve as a model songbird species for studies of ecology and evolution  
82 (Ketterson & Atwell 2016). Specifically, we focused on a breeding population of Carolina Dark-  
83 eyed Juncos (*J. h. carolinensis*) that resides in the Appalachian Mountains year-round, with some  
84 individuals migrating short distances (e.g., altitudinal migrants). We first asked whether median  
85 monthly air temperature in early spring changed over the 32-year study, predicting that spring  
86 temperatures would increase over time. We next compared annual average egg one dates (i.e.,  
87 initiation of breeding, or the date of first egg laid in the year) to spring temperatures over time and  
88 predicted that egg one dates would be earlier over time in response to a warming climate. We also  
89 asked whether selection acted on earlier breeding by assessing the relationship between female  
90 annual relative fitness and egg one date. We then used a model comparison framework to identify

91 climatic drivers of the strength of selection across our study period. We predicted that selection  
92 would favour earlier breeding, especially in warmer springs. Lastly, we used a random regression  
93 model approach to evaluate the degree of female plasticity in response to spring temperatures. We  
94 predicted that individuals that bred in multiple years would vary their initiation of breeding in  
95 response to changing spring temperatures proportional to annual differences in temperature.

96

## 97 **2. Methods**

### 98 **a) Study system and breeding data**

99         Since 1983, a breeding population of Dark-eyed Juncos has been monitored at Mountain  
100 Lake Biological Station (MLBS) and the surrounding Jefferson National Forest (37°22'N,  
101 80°32'W) (Chandler *et al.* 1994). At the beginning of each breeding season (April-May), birds  
102 on the study site were caught using mist nets and Potter traps and banded with a unique USFWS  
103 metal band and distinctive combinations of colour bands. Researchers searched for nests every  
104 year, identifying parents and tracking the progress of the nest. Egg one date, expressed as Julian  
105 date, was observed directly, or for nests found after the start of egg-laying, was calculated based  
106 on the day nestlings hatched or left the nest (Nolan *et al.* 2002). Breeding data from 1983–2015  
107 were used for this study except for 2013 due to limited research effort. Records where female ID  
108 or egg one date were unknown were removed. Female subjects that were implanted with  
109 exogenous testosterone during a five-year study were (Clotfelter *et al.* 2004; Ketterson, Nolan  
110 & Sandell 2005) were also removed.

111         To calculate true egg one dates, we excluded any known re-nests. Also, knowing that the  
112 first nest found for a female might not be her true first nest, we eliminated nests whose egg one  
113 dates came later than each year's median egg one date from known re-nests. Our data filtering

114 resulted in 1,244 first nests of 936 female juncos between 1983 and 2015. Annual differences  
115 in research effort (number of nests found) did not explain variation in egg one dates (see  
116 Supplementary Materials; Fig. S1).

117 Because the distributions of egg one dates were not normal in some years, we calculated  
118 median annual egg one dates from first nests. Using both first nests and renests for each year,  
119 we calculated the annual total number of eggs and total number of fledglings produced by each  
120 female. Females were grouped into two age classes based on plumage (Pyle 1997) or records  
121 from previous breeding seasons: second years (SY; first breeding season) and after second years  
122 (ASY; second or later breeding season). Finally, since most open-cup nests fail due to predation  
123 (Ricklefs 1969), we estimated annual predation rates of nests by calculating the annual  
124 percentage of nests that failed at the egg or nestling stage before fledging.

125

## 126 **b) Temperature data**

127 Between November 16, 1971 and January 31, 1998, temperature data (daily minimum;  $T_{\min}$   
128 and maximum temperature;  $T_{\max}$ ) were collected from MLBS via a National Oceanic and  
129 Atmospheric Administration (NOAA) weather station (Network ID GHCND: USC00445828,  
130 hereafter, “Logger A”). On June 24, 1994, a second data logger (Campbell CR10) was established  
131 at MLBS that records temperature every half hour. To permit comparing data across devices, we  
132 calculated daily  $T_{\max}$  and  $T_{\min}$  from this MLBS data logger (hereafter, “Logger B”). From  $T_{\min}$   
133 and  $T_{\max}$ , we calculated daily median temperature ( $T_{\text{med}}$ ) for both loggers. Since the two weather  
134 stations overlapped from 1994-1997, we confirmed that Datasets A and B were strongly  
135 correlated and then combined the datasets (*see Supplementary Materials*).

136 Monthly average  $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{med}}$  were calculated for March–May for each year. Data  
137 were available for all years (1983–2015), except for missing March and April data for 1991 and  
138 2002 and missing March data for 2004.

139

### 140 **c) Temporal patterns**

141 All statistical analyses were conducted in R (version 4.0.0). Temperature is not expected  
142 to exclusively change linearly over time, so we first fit generalized additive models (GAMs) with  
143 a smooth term for year to flexibly determine temporal trends in average  $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{med}}$  during  
144 spring (March–May) when birds were initiating breeding.

145 We fit a GAM with a smooth term for year to first investigate change over time in median  
146 egg one date. Most female juncos lay their first egg in late April–early May, and the final stages of  
147 reproductive development can take anywhere from days to weeks (Williams 2012), such that  
148 temperatures prior to laying likely have the greatest influence on female reproductive timing.  
149 Therefore, to determine how annual spring temperatures and temporal variation related to median  
150 annual egg one date, we fit independent GAMs with smooth terms for both year and each of 9  
151 temperature variables (average  $T_{\text{med}}$ ,  $T_{\min}$ , and  $T_{\max}$  for March, April, and May). We then compared  
152 model fit using Akaike information criterion corrected for small sample size (AICc) and Akaike  
153 weight ( $w_i$ ; Burham & Anderson 2002). We considered models within two  $\Delta\text{AICc}$  of the top model  
154 to be competitive. All GAMs were fit with the *mgcv* package using a Gaussian distribution and  
155 thin plate splines (Wood 2017). We used maximum likelihood (ML) for model selection but refit  
156 all final GAMs using restricted ML (REML). We used the most competitive temperature  
157 covariates in our subsequent selection and plasticity analyses.

158



159 **d) Selection analyses**

160 Selection acting on start of breeding was defined as the slope of a regression of relative  
161 fitness (i.e., total number of fledglings per year per female divided by annual population mean  
162 total fledglings) on egg one date (Lande & Arnold 1983). We adjusted for age, annual total eggs  
163 per female, and predation rate when estimating selection acting on egg one date by including these  
164 as fixed effects (Marrot, Garant & Charmantier 2017). As relative fitness was zero inflated, we  
165 used compound Poisson generalized linear models (GLMs) or generalized linear mixed models  
166 (GLMMs) with the *cplm* package (Zhang 2013); however, we also estimated the linear selection  
167 gradient using a conventional LMM (Lande & Arnold 1983). We ran analyses on egg one dates  
168 standardized annually (zero mean and unit variance) to control for environmental covariance  
169 between fitness and this trait across years (Kingsolver *et al.* 2001; Marrot *et al.* 2018). Owing to  
170 missing values, these fitness analyses included 1,182 first nests from 898 females. We included  
171 year and female ID as random intercepts to control for multiple observations per year and females  
172 that bred more than one year.

173 We ran selection analyses and model comparisons using two approaches. First, we derived  
174 selection gradients on a per-year basis using GLMs. We then compared univariate linear  
175 regressions with each of our primary temperature variables identified from our GAM analyses  
176 using AICc and Akaike weights (Burham & Anderson 2002). To account for uncertainty in  
177 estimates of selection gradients, these models included weighting by the annual sample size, ( $\bar{x}=37$   
178 first nests  $\pm 2$  SE). Next, we tested for relationships between temperature and the strength of  
179 selection on egg one date at the individual level using GLMMs fit to our full dataset. We compared  
180 variants of our base selection GLMM with an interaction between egg one date and each  
181 temperature variable, again using AICc and Akaike weights. We derived a marginal and

182 conditional  $R^2$  as measures of fit ( $R^2_m$  and  $R^2_c$ ; (Nakagawa, Johnson & Schielzeth 2017). Owing to  
183 missing temperature data, we used a reduced dataset of 1,123 first nets from 871 females to  
184 compare GLMMs.

185

### 186 e) Behavioural plasticity

187 To assess the degree of individual female plasticity in egg one date in relation to spring  
188 temperature, we used a random regression model (RRM) approach (Nussey, Wilson & Brommer  
189 2007). RRM are a particular case of GLMMs where individuals vary in the elevation (i.e.,  
190 intercept) and slope of their reaction norms. For females that bred in at least three years of our  
191 study ( $n=62$  individuals representing 206 egg one date observations, hereafter “returning  
192 females”), we fit a RRM with a fixed effect of temperature, a random intercept of female ID, and  
193 their interaction (i.e., a random slope) using ML with the *lme4* package (Pinheiro & Bates 2006).  
194 We again included age, annual total eggs per female, and predation rate as covariates and included  
195 an additional random intercept for year. We again compared among temperature predictors using  
196 AICc and Akaike weights and refit RMMs with REML to derive  $R^2_m$  and  $R^2_c$  and estimate variance  
197 for random effects. We then used sequential likelihood ratio tests to assess if random intercepts  
198 and slopes were significantly different from zero (i.e., denoting significant inter-individual  
199 variation in reaction norms) (Nussey *et al.* 2005). To visualize individual female slopes, we held  
200 each female at the ASY age class at its mean annual clutch size and annual predation rate.

201

## 202 3. Results

### 203 a) Spring temperatures

204 March average  $T_{\text{med}}$ ,  $T_{\text{min}}$ , and  $T_{\text{max}}$  did not significantly change between 1983 and 2015 at  
205 MLBS. April average  $T_{\text{med}}$  and  $T_{\text{min}}$ , but not  $T_{\text{max}}$ , significantly increased over time. Finally,  
206 average May  $T_{\text{min}}$  significantly increased over time, but  $T_{\text{med}}$  and  $T_{\text{max}}$  did not (Table S1; Fig. 1).

207

## 208 **b) Timing of reproductive onset**

209 Median egg one date varied significantly over time ( $F_{1,1} = 4.43$ ,  $p = 0.044$ ,  $R^2 = 0.10$ ), with  
210 a 13-day difference from the first year (May 19, 1983) to the final year (May 6, 2015; Fig. 2A).  
211 Considering the largest difference in breeding phenology over the 32 years, females advanced egg  
212 one dates up to 24 days over the study (range=April 30-May 24). When testing effects of monthly  
213 temperatures after accounting for nonlinear effects of year, the best GAM included April average  
214  $T_{\text{max}}$  ( $w_i = 0.97$ ; Table S2). In this model ( $R^2 = 0.65$ ), egg one date was predicted by the average  
215 April  $T_{\text{max}}$  ( $F_{1.7, 2.1} = 14.84$ ,  $p < 0.0001$ ) and year ( $F_{2.4, 3.0} = 5.28$ ,  $p = 0.007$ ) (Fig. 2B). Since an  
216 April temperature was the best predictor of lay date, we proceeded using only the three April  
217 temperature variables for selection and plasticity analyses.

218

## 219 **c) Selection analyses**

220 We observed strong selection on egg one date, and the gradient from our full dataset  
221 GLMM was significantly negative (i.e., selection favouring earlier breeding;  $\beta = -0.16$ ,  $t = -4.42$ ,  
222  $p < 0.001$ ). This estimate was identical to the selection gradient from an analogous LMM ( $\beta = -0.16$ ,  
223  $t = -4.91$ ,  $p < 0.001$ ). Annual total eggs per female was under positive selection, in which individuals  
224 that produced more eggs also had more successful fledglings ( $\beta = 0.05$ ,  $t = 5.28$ ,  $p < 0.001$ ). Older  
225 females had marginally higher relative fitness ( $\beta = 0.12$ ,  $t = 1.89$ ,  $p = 0.06$ ). Annual nest predation  
226 rates did not predict relative fitness ( $\beta = -0.09$ ,  $t = -0.38$ ,  $p = 0.71$ ). The overall (GLMM) selection

227 gradient was similar to the mean of the per-year estimates ( $\bar{x}=-0.18 \pm 0.03$  SE). Annual selection  
228 gradients showed strong inter-year variation ( $\sigma^2=0.04$ ) and became significantly more negative  
229 (i.e., more strongly favouring earlier breeding) with time (Fig. 3;  $\beta=-0.01$ ,  $p=0.04$ ,  $R^2=0.11$ ).

230 When comparing temperature-dependent models of selection on egg one date, all three  
231 April temperature measures received equivalent support (Table S3). When analysing selection  
232 gradients directly, models including average April  $T_{\max}$  and  $T_{\min}$  received the most support from  
233 AICc ( $w_i=0.36$  and  $0.35$ ), but warmer temperatures were nevertheless not associated with selection  
234 gradients ( $T_{\max}$ :  $\beta=0.01$ ,  $p=0.55$ ,  $R^2=0$ ; Fig. 4A;  $T_{\min}$ :  $\beta=-0.01$ ,  $p=0.59$ ,  $R^2=0$ ). Individual-level  
235 GLMMs with interactions between egg one date and temperature did not differentiate between  
236 April temperature predictors ( $w_i=0.32-0.35$ ; Table S3). We similarly found no significant  
237 interaction between egg one date and April temperature (e.g.,  $T_{\max}$ :  $\beta=-0.0004$ ,  $t=-0.02$ ,  $p=0.98$ ;  
238 Fig. 4B). Thus, in both analyses, warmer April temperatures were not associated with selection  
239 favouring earlier breeding.

240

#### 241 **d) Behavioural plasticity**

242 We used RMMs to assess phenotypic plasticity in the relationship between timing of  
243 breeding and temperature. For 62 females studied across at least three years, RMMs found most  
244 support for an association between egg one date and April average  $T_{\min}$  ( $w_i=0.60$ ) and  $T_{\text{med}}$   
245 ( $w_i=0.36$ ,  $\Delta\text{AICc}=1.04$ ), but not with  $T_{\max}$  ( $w_i=0.04$ ,  $\Delta\text{AICc}=5.33$ ; Table S4). Individuals started  
246 breeding significantly earlier with warmer April average  $T_{\min}$  ( $\beta=-1.33$ ,  $t=-2.50$ ,  $p=0.02$ ) and  $T_{\text{med}}$   
247 ( $\beta=-1.67$ ,  $t=-2.70$ ,  $p=0.01$ ). Importantly, individuals did not vary in their elevation (i.e., estimated  
248 egg one date at the average temperature) nor their slope (i.e., individual response to inter-year  
249 variation in temperature) for either competitive temperature measure (Fig. 5; Table S5). Therefore,

250 females displayed significant population-level phenotypic plasticity, but not inter-individual  
251 variation in plasticity.

252

#### 253 **4. Discussion**

254 We investigated shifts in phenology over a 32-year period and in relation to spring  
255 temperatures and found a net change of 12 days in egg one date and a maximum between-year  
256 advance of 24 days. Springs have grown warmer, and females are initiating reproduction earlier  
257 than in the past. We also found evidence of selection favouring earlier breeding that has also  
258 become stronger over time. However, unlike studies of other avian species [25, 30], spring  
259 temperatures did not predict strength of selection on egg one dates, suggesting that other factors  
260 may be driving shifts in phenology. Among the many possibilities are some combination of abiotic  
261 factors (Dunn & Winkler 2010) or even advances in male reproductive stimulating earlier egg-  
262 laying in females (Watts, Edley & Hahn 2016). It is also possible that females respond to a  
263 temperature threshold in the spring to initiate laying, explaining why our temperature measures  
264 were not related on selection on timing of breeding.

265 We found strong evidence for plasticity in driving these phenological changes. For a subset  
266 of returning females with sufficient multi-year data, females bred earlier in the warmer of the three  
267 or more springs in which they bred. However, females exhibited very little inter-individual  
268 variation in the degree of plasticity, leaving little variation on which selection might act. While  
269 selection and plasticity both likely played a role in earlier breeding associated with warmer springs,  
270 the pattern may be more a result of plasticity than selection.

271

272 **a) Plastic versus evolutionary responses to climate change**

273           Microevolutionary responses to climate change are predicted to result from directional  
274 selection favouring earlier breeding to alleviate the negative effects of phenological mismatches  
275 (Charmantier & Gienapp 2014). Without knowledge of the heritability of timing of reproduction,  
276 however, evidence of selection favouring earlier breeding is insufficient to conclude that  
277 microevolutionary change is occurring, as genetic and environmental effects can be difficult to  
278 disentangle (Merilä 2012; Helm *et al.* 2019). There is some evidence of microevolutionary changes  
279 in phenology across taxa that are likely adaptive shifts in response to climate change (Van Asch *et*  
280 *al.* 2013; Manhard, Joyce & Gharrett 2017). In the case of the junco, while we found strong overall  
281 selection favouring earlier breeding, and this selection has become stronger over time, the strength  
282 of selection was not associated with the observed changes in spring temperature. We note that we  
283 did not measure genetic variation or heritability of reproductive timing. Therefore, we cannot  
284 conclude whether microevolutionary change might account for the observed shifts in timing.  
285 Future work should integrate genomic quantitative genetics approaches with this breeding data to  
286 determine the role of microevolution in phenological shifts, which could in turn have important  
287 implications for conservation genomics (Gienapp *et al.* 2017).

288           Additionally, behavioural plasticity, which can allow for more rapid changes in phenotype  
289 than microevolutionary changes, may account for why earlier breeding was related to increases in  
290 fitness over time (Van Buskirk, Candolin & Wong 2012; Charmantier & Gienapp 2014; Beever *et*  
291 *al.* 2017). Numerous studies support behavioural plasticity as a mechanism for coping with climate  
292 change (Charmantier *et al.* 2008; Phillimore *et al.* 2016; Verhagen *et al.* 2020), despite its  
293 limitations in promoting population persistence in the face of climate change (Ghalambor *et al.*  
294 2007; Gienapp *et al.* 2013; Duputié *et al.* 2015). We found that returning females initiated egg  
295 laying earlier in warmer springs. However, there was very little among-individual variation in the

296 degree of plasticity upon which selection might act, suggesting that microevolutionary change in  
297 plasticity itself is not a likely explanation for the observed change.

298

## 299 **b) Winners versus losers in relation to climate change**

300 Global change biologists often discuss ‘winners’ and ‘losers’ in relation to climate change,  
301 typically in the context of range shifts (Crick 2004; Bateman *et al.* 2016; Tayleur *et al.* 2016).  
302 Here, we extend these concepts of winning and losing to migratory strategy and breeding season  
303 length. Short-distance migrants and residents often experience longer breeding seasons than long-  
304 distance migratory species. This is true in part because they typically breed at lower latitudes  
305 where spring comes earlier and also because they do not lose time to the migratory journey leaving  
306 time for multiple broods (Newton 2010). Juncos in our study population have a longer breeding  
307 season than closely related long-distance migrant populations (Nolan *et al.* 2002). Females can re-  
308 nest as many as five times and can fledge up to three successful nests.

309 The advancement in breeding phenology reported here is supported by a previous finding  
310 that multi-brooded species tend to exhibit larger advances in breeding phenology than single-  
311 brooded species, likely because multi-brooded species are experiencing longer breeding seasons  
312 with warmer springs (Dunn & Møller 2014). Thus multi-brooded populations are expected to have  
313 higher reproductive output than migratory populations that are typically single- or double-brooded  
314 (Halupka & Halupka 2017), an effect echoed in our finding that juncos that bred earlier tended to  
315 fledge more offspring that year, presumably an effect of having more time for breeding attempts  
316 (Dunn & Møller 2014). Warmer springs may benefit this population by allowing females to breed  
317 earlier and extend their breeding season, despite the lack of evidence that warmer spring  
318 temperatures predicted stronger selection favouring earlier breeding.

319 Overall, females can respond flexibly to changes in temperature, but individuals do not  
320 strongly vary in their plastic response to temperatures. However, plasticity alone will likely be  
321 insufficient for populations to survive in the long-term when facing climate change (Gienapp *et*  
322 *al.* 2013). Since our study population occurs at high elevation, persistent increases in temperature  
323 could eventually result in population decline, as the population cannot shift any further up the  
324 mountains.

325

### 326 **c) Future directions**

327 Accurate predictions of future responses to climate change will require further  
328 consideration of mechanisms of female reproductive timing (Williams 2012; Chmura, Wingfield  
329 & Hahn 2020; Kimmitt 2020). Past and ongoing work in the junco is elucidating the physiological  
330 mechanisms driving reproductive timing in females based on life history, including endocrine  
331 systems and costs of early breeding (Greives *et al.* 2016; Graham *et al.* 2019; Kimmitt *et al.* 2019;  
332 Kimmitt, Sinkiewicz & Ketterson 2020). However, more research is necessary to understand how  
333 females integrate supplementary cues, such as temperature, to regulate the final stages of their  
334 reproductive development and ovulation (Wingfield *et al.* 2016; Chmura, Wingfield & Hahn  
335 2020). Via our analysis of this 32-year dataset, we found that flexibility in female timing is likely  
336 relevant for population persistence, and further work on the proximate mechanisms of female  
337 timing will improve forecasts on the effects of climate change on birds.

338

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352

### 353 **Authors’ Contributions**

354 AAK conceived and designed the study, curated data, conducted statistical analysis and was the  
355 primary author of the manuscript; DJB conducted statistical analysis, drafted sections of the  
356 manuscript, and revised the manuscript; SND participated in data curation and analysis and  
357 revised the manuscript. NMG conceived and designed the study, curated data, and revised the  
358 manuscript. KAR conceived and designed the study and revised the manuscript. EDK conceived  
359 and designed the study, secured funding for data collection, and revised the manuscript. All  
360 authors gave final approval of this publication.

361

### 362 **Competing Interests**

363 The authors declare no competing interests.

364

365 **Data Accessibility**

366 Data will be made available on Dryad pending manuscript acceptance.

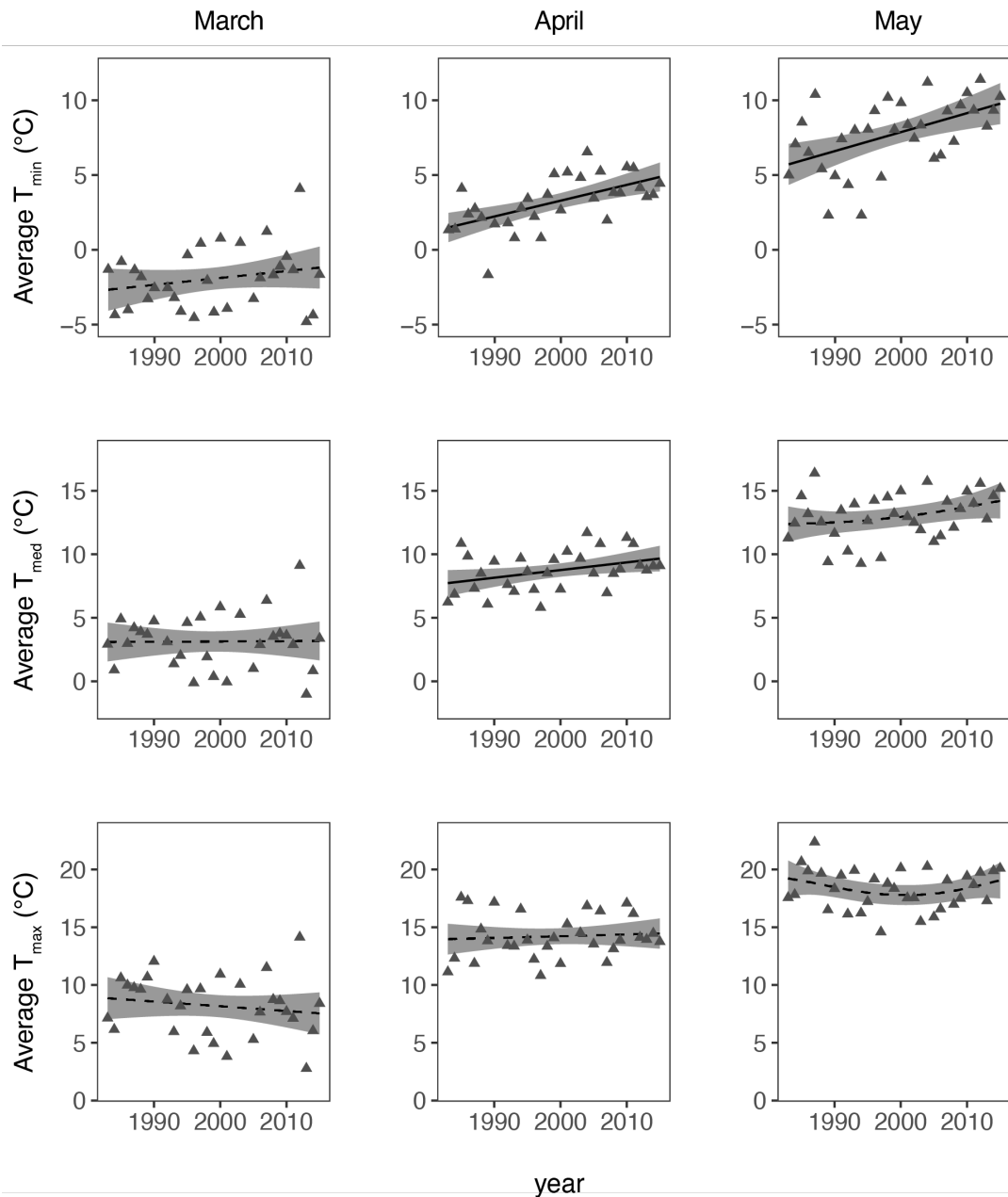
367 **Figures**

368

369 Figure 1. Independent relationships between year and median, minimum, and maximum  
370 temperatures in March-May from 1983 to 2015. All prediction lines and confidence bands from  
371 the GAMs are created as a function of year as a smooth term and overlaid with original data.

372 Average April  $T_{med}$ , April  $T_{min}$ , and May  $T_{min}$  change significantly over time (**April  $T_{med}$** :  $F_{1,1} =$   
373  $4.79$ ,  $p = 0.037$ ,  $R^2 = 0.11$ ; **April  $T_{min}$** :  $F_{1,1} = 15.86$ ,  $p < 0.001$ ,  $R^2 = 0.33$ ; **May  $T_{min}$** :  $F_{1,1} = 11.41$ ,  $p =$   
374  $0.002$ ,  $R^2 = 0.25$ )

375



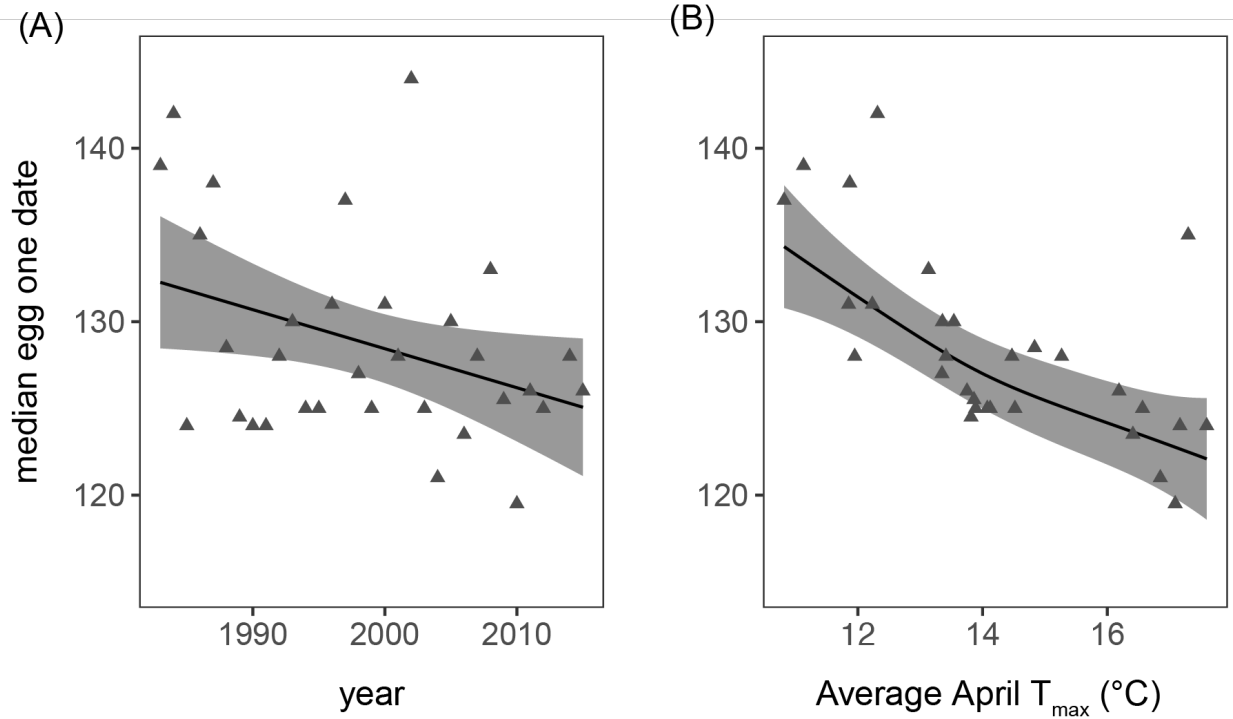
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378 Figure 2. (A) Average egg one date of females is shown as a function of year only ( $R^2=0.10$ ). (B)

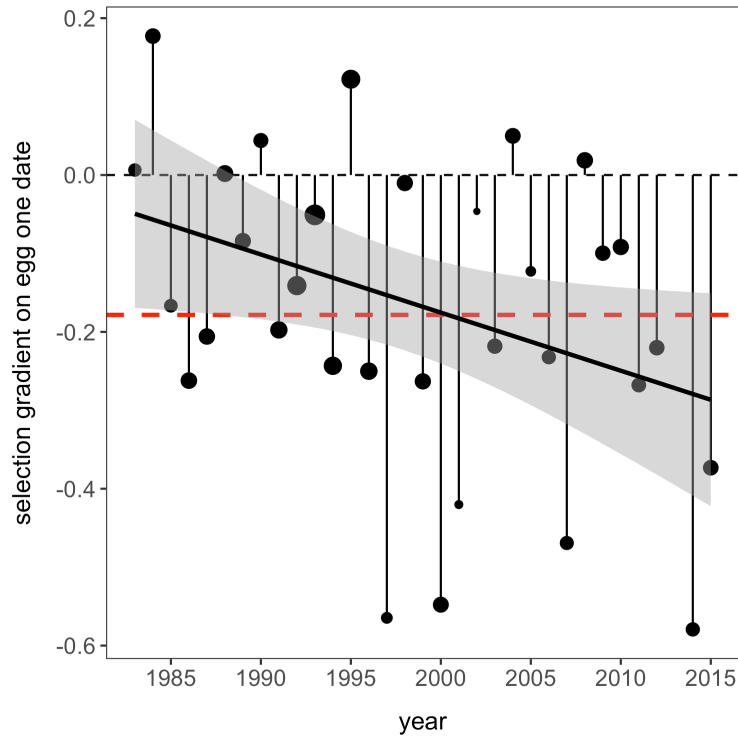
379 Average egg one date is shown as a function of April average maximum temperatures when the

380 model also accounts for nonlinear effects of year ( $R^2=0.65$ ). Fitted values and 95% confidence  
381 bands from the GAMs are overlaid with original data.  
382



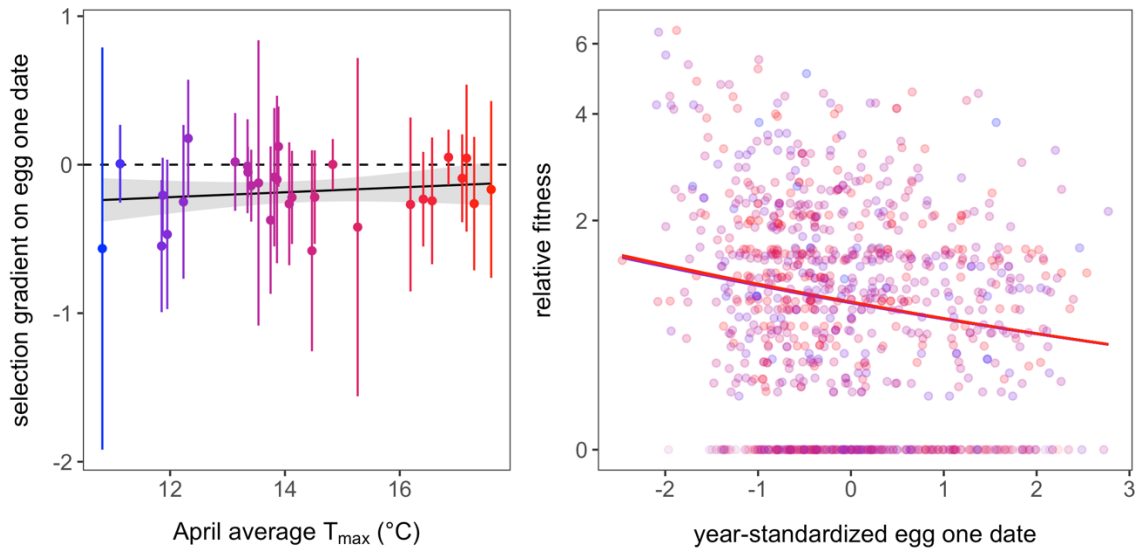
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384

385 Figure 3. Per-year selection gradients on egg one date estimated with compound Poisson GLMs  
386 after adjusting for annual total eggs per female and female age. The dashed line shows  $\beta=0$ ,  
387 whereas the red line displays the mean selection gradient across the 32 years. The solid line and  
388 grey band show fitted values and 95% confidence intervals from a linear model that included  
389 weighting by annual sample size (points are scaled by sample size).  
390



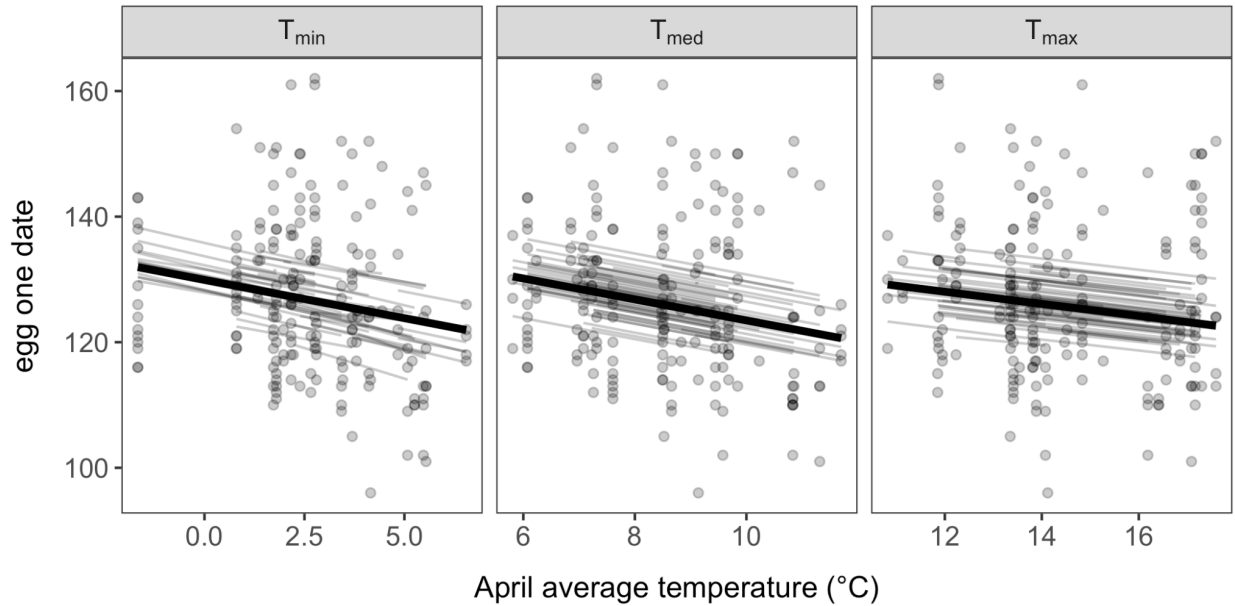
391

392 Figure 4. April temperature does not predict variation in selection on egg one date. (A) Results of  
393 a linear model using the per-year selection gradients as the response variable (after adjusting for  
394 female age and annual total eggs per female), with the solid line and grey band showing fitted  
395 values and 95% confidence intervals. The linear model included weighting by annual sample size,  
396 which is illustrated through point size. (B) Results of a GLMM predicting relative fitness as a  
397 function of the interaction between maximum April temperature and egg one date (after adjusting  
398 for female age, annual total eggs per female, and annual predation rate). Points display individual  
399 nest data and lines show fitted values, with colours indicating temperature as in A. Relative fitness  
400 is shown with a modulus transformation given the right-skew in this variable.  
401



402  
403

404 Figure 5. Fitted values of RRM testing for temperature-driven plasticity in egg one date. Thick  
405 lines show the overall reaction norm for each April temperature measure (displayed in order of  
406 Akaike weights) after controlling for female age, annual total eggs per female, and annual  
407 predation risk, whereas thin lines show reaction norms for each individual female. Random effects  
408 were visualized by holding each female (assumed to be ASY) at its mean annual total eggs per  
409 female and annual predation rate.  
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