

Estimating demographic contributions to effective population size in an age-structured wild population experiencing environmental and demographic stochasticity

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1 **Estimating demographic contributions to effective population**
2 **size in an age-structured wild population experiencing**
3 **environmental and demographic stochasticity**

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18 **Running headline:** Effective size of age-structured populations

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24 **Summary**

- 25 1. A population's effective size (N_e) is a key parameter that shapes rates of inbreeding
26 and loss of genetic diversity, thereby influencing evolutionary processes and
27 population viability. However estimating N_e , and identifying key demographic
28 mechanisms that underlie the N_e to census population size (N) ratio, remains
29 challenging, especially for small populations with overlapping generations and
30 substantial environmental and demographic stochasticity and hence dynamic age-
31 structure.
- 32 2. A sophisticated demographic method of estimating N_e/N , which uses Fisher's
33 reproductive value to account for dynamic age-structure, has been formulated.
34 However this method requires detailed individual- and population-level data on sex-
35 and age-specific reproduction and survival, and has rarely been implemented.
- 36 3. Here we use the reproductive value method and detailed demographic data to
37 estimate N_e/N for a small and apparently isolated red-billed cough (*Pyrrhocorax*
38 *pyrrhocorax*) population of high conservation concern. We additionally calculated
39 two single-sample molecular genetic estimates of N_e to corroborate the demographic
40 estimate and examine evidence for unobserved immigration and gene flow.
- 41 4. The demographic estimate of N_e/N was 0.21, reflecting a high total demographic
42 variance (σ^2_{dg}) of 0.71. Females and males made similar overall contributions to σ^2_{dg} .
43 However, contributions varied among sex-age classes, with greater contributions
44 from 3 year-old females than males, but greater contributions from ≥ 5 year-old
45 males than females.
- 46 5. The demographic estimate of N_e was ~ 30 , suggesting that rates of increase of
47 inbreeding and loss of genetic variation per generation will be relatively high.

48 Molecular genetic estimates of N_e computed from linkage disequilibrium and
49 approximate Bayesian computation were approximately 50 and 30 respectively,
50 providing no evidence of substantial unobserved immigration which could bias
51 demographic estimates of N_e .

52 6. Our analyses identify key sex-age classes contributing to demographic variance and
53 thus decreasing N_e/N in a small age-structured population inhabiting a variable
54 environment. They thereby demonstrate how assessments of N_e can incorporate
55 stochastic sex- and age-specific demography and elucidate key demographic
56 processes affecting a population's evolutionary trajectory and viability. Furthermore,
57 our analyses show that N_e for the focal chough population is critically small,
58 implying that management to re-establish genetic connectivity may be required to
59 ensure population viability.

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61 **Key-words:** conservation genetics, evolutionary potential, iteroparity, life-history
62 variation, population connectivity, population management, reproductive skew.

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74 **Introduction**

75 A population's effective size, N_e , is a key parameter that shapes population-wide rates
76 of inbreeding and loss of genetic diversity and, in combination with the strength of
77 selection, determines mutation fixation probabilities (Nunney & Elam 1994; Frankham
78 1995; Charlesworth 2009). Estimation of N_e , and elucidation of key underlying
79 processes that cause the observed N_e , is therefore central to predicting evolutionary
80 trajectories of finite populations (Charlesworth 2009) and to evaluating population
81 viability (Mace & Lande 1991; Frankham, Bradshaw & Brook 2014), such that
82 appropriate population management strategies can be devised (Hare *et al.* 2011; Laikre
83 *et al.* 2016).

84 N_e is defined as the size of an idealized Wright-Fisher population that would
85 experience the same rate of genetic drift as an observed population (Wright 1931,
86 1969). Such a Wright-Fisher population is defined as a hypothetical population of
87 constant finite size with no migration or selection and random mating between
88 monoecious individuals in discrete generations, giving a Poisson distribution of
89 reproductive success (Wright 1931; Crow & Kimura 1970). However, most natural
90 populations violate key Wright-Fisher assumptions. Thus, N_e can exceed the census
91 population size N (i.e. $N_e/N > 1$), up to a theoretical maximum of $2N$ given uniform
92 reproductive success (Lande & Barrowclough 1987, but see Waples *et al.* 2013).
93 However, N_e is frequently smaller than N (i.e. $N_e/N < 1$), meaning that a population will
94 experience greater genetic drift than expected given its N , potentially reducing its
95 viability (Nunney & Elam 1994; Frankham 1995; Waples *et al.* 2013).

96 Many factors can reduce N_e below N , including varying N , skewed sex-ratio and
97 high among-individual variance in reproductive success (i.e. high reproductive skew,

98 Wright 1931; Caballero 1994; Frankham 1995; Nomura 2002; Ruzzante *et al.* 2016).
99 Indeed, high variance in reproductive success is a dominant factor reducing N_e in
100 diverse natural populations, spanning fish (e.g. red drum, *Sciaenops ocellatus*, Turner,
101 Wares & Gold 2002; steelhead trout, *Oncorhynchus mykiss*, Araki *et al.* 2007),
102 mammals (e.g. woodrats, *Neotoma macrotis*, Matocq 2004) and amphibians (e.g. Italian
103 agile frog, *Rana latastei*, Ficetola *et al.* 2010). Small populations (i.e. small N) can also
104 experience substantial demographic stochasticity, which can exacerbate variance in
105 reproductive success and further decrease N_e (Melbourne & Hastings 2008; Palstra &
106 Ruzzante 2008). Consequently, at small N , N_e might commonly be very small, further
107 increasing genetic drift and threatening population viability.

108 Conversely, some studies have found an inverse relationship between N_e/N and
109 N , implying that small populations have higher N_e than might be expected given their N
110 (Pray *et al.* 1996; Ardren & Kapuscinski 2003; Hedrick 2005; Watts *et al.* 2007; Palstra
111 & Ruzzante 2008). Such patterns can arise if the among-individual variance in
112 reproductive success decreases at small N , for example because environmental
113 stochasticity reduces the reproductive success of all population members, or because
114 male-male competition for mates or breeding sites is reduced (so-called 'genetic
115 compensation' mechanisms, Ardren & Kapuscinski 2003; Palstra & Ruzzante 2008;
116 Beebee 2009). Given the range of possible values and causes of N_e/N , pervasive aims
117 across evolutionary, population and conservation ecology are to estimate N_e/N in
118 diverse natural populations and identify the key contributing demographic processes,
119 and thereby elucidate general relationships between N_e , N , and underlying demography
120 and population dynamics (Frankham 1995; Palstra & Fraser 2012; Waples *et al.* 2013;
121 Ruzzante *et al.* 2016).

122 For most natural populations, N_e cannot be measured directly and must be
123 estimated using demographic or molecular genetic approaches. Demographic
124 approaches estimate N_e as mathematical functions of causal demographic parameters
125 and processes that generate contemporary N_e , such as the variance in lifetime
126 reproductive success, breeder sex-ratio or fluctuations in N (Lande & Barrowclough
127 1987; Caballero 1994). These demographic approaches estimate the variance effective
128 size N_{ev} (i.e. the sampling variance in allele frequencies per generation) and thereby
129 quantify N_e for the offspring generation (Kimura & Crow 1963; Caballero 1994). Such
130 methods are valuable because, beyond providing a point estimate of N_e , they directly
131 identify key demographic processes that shape N_e . They can therefore inform
132 population management strategies aiming to increase N_e and reduce future loss of
133 genetic diversity (Nunney & Elam 1994; Ruzzante *et al.* 2016). However, many
134 demographic methods rely on strong simplifying assumptions, such as discrete
135 generations with no age-structure, no density-dependence and constant N , which are
136 typically violated in natural populations (Hill 1972; Nunney 1991; Caballero 1994;
137 Nomura 2002). Even recent methods for estimating N_e in age-structured populations
138 with overlapping generations still require strong assumptions, such as constant N and
139 birth rate and hence stable age-structure (e.g. the ‘AgeNe’ method, Waples, Do &
140 Chopelet 2011) and consequently do not incorporate effects of environmental or
141 demographic stochasticity or additional demographic heterogeneity that generate
142 dynamic age-structure. However, since theory predicts that environmental and
143 demographic stochasticity and heterogeneity could substantially affect N_e , especially at
144 small N , such effects should be incorporated to avoid biased estimates of N_e (Engen,
145 Lande & Saether 2005; Engen *et al.* 2007a).

146 Accordingly, Engen, Lande and Saether (2005) and Engen *et al.* (2010) derived
147 a novel demographic method that utilises the concept of ‘reproductive value’ to relax
148 the assumption of stable age-structure and thereby capture effects of environmental and
149 demographic stochasticity and additional demographic heterogeneity on N_e .
150 Conceptually, this method considers the mean and variance in the change in frequency
151 of a rare selectively neutral allele at each time-step in a hypothetical heterozygote
152 subpopulation, given observed patterns of age-specific demographic variance (Emigh &
153 Pollak 1979; Engen *et al.* 2005). Overlapping generations and age-structure in a
154 variable environment are incorporated using Fisher’s reproductive value (i.e. the
155 contribution of an individual of a given age to population growth rate), which can be
156 summed across individuals in all age classes to give the population’s total reproductive
157 value given its age-structure (Fisher 1958; Crow & Kimura 1970; Engen *et al.* 2009).
158 Changes in total reproductive value can then be used to obtain an estimate of total
159 population growth rate, and hence the rate of increase of a neutral allele, thereby
160 generating an estimate of N_e that is not biased by dynamic age-structure (Crow &
161 Kimura 1970; Engen *et al.* 2007a, 2010).

162 The total variance in individual contributions to population growth rate in a
163 dynamically age-structured population stems from demographic and environmental
164 variances in age- and sex-specific fecundity and survival which, in practice, can be
165 defined and estimated as the variances within years and in means among years,
166 respectively (Engen, Bakke & Islam 1998). The required variance components can be
167 estimated using the distribution of individual reproductive values among individuals
168 within and among years, which can be used to estimate the total population
169 demographic variance, σ_{dg}^2 . The N_e/N ratio can then be estimated as:

170
$$\frac{N_e}{N} = \frac{1}{\sigma_{dg}^2 T}, \quad (\text{eqn 1})$$

171

172 where T is the generation time (Appendix S1; Engen, Lande & Saether 2005).

173 This calculation is potentially very insightful but imposes challenging data
174 demands, requiring individual-level information on realised sex- and age-specific
175 reproductive success and survival alongside population-level mean rates. To date, it has
176 only been implemented in a Siberian jay (*Perisoreus infaustus*) population (as a
177 methodological example considering three age classes, Engen *et al.* 2010) and a house
178 sparrow (*Passer domesticus*) metapopulation (considering two age classes, Stubberud
179 *et al.* 2017) where genetic variation and inbreeding rates are influenced by immigration
180 rather than solely local demography (Baalsrud *et al.* 2014). Indeed, immigration can
181 cause local N_e to approach that for the whole metapopulation, meaning that N_e/N is
182 largely independent of local demography (Wang & Whitlock 2003; Gilbert & Whitlock
183 2015). Consequently, studies that apply the ‘reproductive value’ estimator of N_e/N to
184 isolated populations are required to identify key demographic processes that influence
185 N_e/N given environmental and demographic stochasticity and heterogeneity, and
186 resulting dynamic age-structure.

187 Since sufficient data to implement any demographic estimator of N_e/N are often
188 unavailable, N_e is commonly instead estimated from molecular genetic data. Single-
189 sample approaches, which require DNA sampling at one time-point, are most practical
190 for species with long T and where historical samples are not available (Palstra &
191 Ruzzante 2008). Such estimators generally measure inbreeding effective size N_{ei} (i.e.
192 the rate of change in heterozygosity) and therefore reflect N_e of the parental generation
193 (Crow & Denniston 1988; Caballero 1994). Given varying N , N_{ei} is expected to lag
194 behind changes in N by at least one generation (Kimura & Crow 1963; Hill 1972;
195 Waples 2005), and might therefore give somewhat misleading estimates of current N_e .

196 Additionally, molecular genetic estimates of N_e are calculated from resultant effects of
197 N_e on genetic variation, and hence do not typically elucidate the ecological and
198 demographic processes that cause the estimated N_e (but see Wang *et al.* 2010).
199 However, such estimators can capture the genetic effects of immigration, which may
200 remain undetected based solely on observations of N and local demography (Hare *et al.*
201 2011; Baalsrud *et al.* 2014; Gilbert & Whitlock 2015). Consequently, to generate
202 overall mechanistic understanding of N_e/N and N_e and hence elucidate stochastic
203 evolutionary processes and inform population management strategies, molecular
204 genetic estimators of N_e need to be calculated alongside appropriate demographic
205 estimators.

206 Accordingly, we used detailed individual-level and population-level
207 demographic data, and molecular genetic data, to estimate N_e/N and N_e in a small and
208 apparently isolated red-billed chough (*Pyrrhocorax pyrrhocorax* Linnaeus, hereafter
209 ‘chough’) population of major conservation concern. We utilised the ‘reproductive
210 value’ demographic estimator (Engen *et al.* 2005) to account for environmental and
211 demographic stochasticity and heterogeneity and resulting dynamic age-structure, and
212 thereby identified key sex- and age-specific components of demographic variance that
213 contribute to N_e/N . We additionally computed two single-sample molecular genetic
214 estimates of N_e to infer effects of any unobserved immigration. We thereby demonstrate
215 how N_e can be estimated in dynamically age-structured populations, and identify key
216 demographic processes underlying N_e , thus aiding our general understanding of
217 evolutionary processes in finite populations and informing conservation strategy.

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221 **Materials and Methods**

222 FOCAL POPULATION

223 Choughs are of conservation concern in Europe and the United Kingdom due to
224 substantial reductions in range and population size and resulting fragmented distribution
225 (Eaton *et al.* 2015). Comprehensive censuses undertaken every 3-6 years since 1982
226 show that the island of Islay holds most (ca. 84%) of the remaining Scottish chough
227 population, yet numbers on Islay have decreased from 78 breeding pairs in 1986 to 46
228 pairs in 2014 (Finney & Jardine 2003; Hayhow *et al.* 2015; Trask *et al.* 2016, Appendix
229 S2). High neutral genetic differentiation with other British chough populations (Wenzel
230 *et al.* 2012), and a lack of observed immigration, suggest that Islay's population is
231 isolated. This isolation and small N imply that inbreeding and loss of genetic diversity
232 may compromise population viability.

233 Islay's choughs form territorial, socially monogamous breeding pairs and nest in
234 caves or farm buildings (Bignal, Bignal & McCracken 1997) with little extra-pair
235 parentage (~5% chicks, Trask *et al.* 2016). Both sexes breed once per year starting from
236 age 2-4 years and survive to breed in multiple years (maximum breeding age: 17 years),
237 generating overlapping generations (Reid *et al.* 2004). Reproductive success and
238 survival vary among ages, years, cohorts and nest sites (Reid *et al.* 2003a,b, 2004,
239 2006). Sub-adult and non-breeding individuals aged one year or older form flocks that
240 occupy known locations, meaning that all non-breeders can be readily observed and
241 censused (Bignal *et al.* 1997; Reid *et al.* 2006, 2008). First-year survival (fledging to
242 age one year) for the 2007-2009 cohorts was particularly low (Reid *et al.* 2011), causing
243 low subsequent recruitment into the breeding population. Demographic estimation of
244 N_e/N that accounts for environmental and demographic stochasticity and demographic
245 heterogeneity, and resulting dynamic age-structure, is therefore required.

246

247

248 DEMOGRAPHIC ESTIMATION OF N_e 249 *Age-specific breeding success and survival*

250 Demographic estimation of N_e using reproductive value to account for dynamic age-
251 structure (e.g. Engen *et al.* 2005, 2010) requires estimates of mean population-level sex-
252 specific demographic rates underlying the deterministic asymptotic population growth
253 rate (λ), defined for a pre- or post-breeding census. The required rates comprise the sex-
254 specific probabilities of attempting to breed at each age (c_i), age-specific breeding
255 success given a breeding attempt (m_i), and juvenile and subsequent age-specific annual
256 survival probabilities (P_i , Caswell 2001; Reid *et al.* 2004), where i denotes a sex-age
257 class. Such estimation of N_e also requires individual-level data on age-specific
258 reproductive success defined as the realised number of offspring that survived to age
259 one year (b) and realised annual survival (J) from samples of individual females and
260 males in a sample of years (Engen *et al.* 2005, 2010). All notations are summarised in
261 Appendix S1 (Table S1).

262 To estimate the required population- and individual-level demographic rates, a
263 sample of accessible though nest sites across Islay were visited each year during 1983-
264 2014. The number of nestlings that survived to ca. 3 weeks post-hatch was recorded,
265 and nestlings were marked with unique colour-ring combinations (Reid *et al.* 2003b,
266 2004). Adults breeding at nest sites across Islay were subsequently identified from their
267 colour-rings, and sexes were assigned based on reproductive behaviour and relative size
268 (Bignal *et al.* 1997). The annual breeding success of known-age individuals was thereby
269 recorded. Colour-ringed adults and sub-adults were resighted across Islay during May-
270 June each year, allowing age-specific annual resighting probabilities and apparent

271 survival probabilities to be estimated using capture-mark-recapture (CMR) models
272 (Reid *et al.* 2003a, 2004).

273 Initial year-structured CMR models showed that annual resighting probabilities
274 were typically less than one prior to 2004, but approached one subsequently due to
275 increased resighting efficiency (estimate across 2004-2014 of $0.97 \pm 0.02SE$, Appendix
276 S1). We consequently focussed on live individuals and, of those breeding, whose
277 offspring were ringed in 2004-2013, so that individual-level realised survival (J) and
278 reproductive success (b) could be directly recorded. Separate age classes for individuals
279 aged one to four, and a pooled terminal age-class for individuals aged five years or
280 older, were defined (i.e. $k = 5$ age classes). This structure captures age-specific variation
281 in key demographic rates while maintaining sufficient sample sizes within each sex-age
282 class (Appendix S1).

283 Resightings of individuals in non-breeding flocks versus at nest sites were used
284 to estimate the mean probability of breeding (c_i) for each sex-age class. Non-breeding
285 individuals typically paired and showed courtship behaviour within flocks, allowing
286 sexes to be assigned. The mean number of fledglings produced given that breeding was
287 attempted (m_i) was directly estimated from breeding records for each sex-age class.
288 Constrained CMR models were fitted to estimate separate age-specific survival
289 probabilities (P_i) for 1983-2003 and 2004-2014 while retaining full encounter histories
290 of all ringed individuals and maximising power to estimate P_i for 2004-2014. Initial
291 analyses showed that models that contained three age classes, first-year (P_1 , fledging to
292 age one year), second-year (P_2 , age one to age two) and adult (P_{ad} , all subsequent ages)
293 were strongly supported, thereby setting P_i equal across all individuals aged two years
294 or older (Appendix S1). This three age-class structure is consistent with previous
295 detailed analyses of age-specific P_i in Islay's choughs (Reid *et al.* 2004). Females and

296 males were pooled for CMR analyses because sexes of individuals that died before
297 pairing were unknown, and because previous analyses showed that P_{ad} does not differ
298 between the sexes (Reid *et al.* 2003b, 2004). Additionally, previous analyses showed no
299 evidence of strong density dependence in mean breeding success, or in P_1 , P_2 or P_{ad}
300 (Reid *et al.* 2003a, 2008).

301

302 *Population projection matrix*

303 The asymptotic population growth rate (λ), stable age distribution (u_i) and reproductive
304 values (v_i) were calculated from a $2k \times 2k$ two-sex Leslie matrix (l). The matrix
305 comprised four submatrices, formulated for a birth-pulse population with pre-breeding
306 census, which describe the contributions of females and males to female and male
307 offspring (Appendix S1). Top row fecundity f_i terms were calculated as:

$$308 \quad f_i = \frac{1}{2} q (c_i m_i P_1), \quad (\text{eqn. 2})$$

309

310 where q is the primary proportion of the focal sex and the factor of $\frac{1}{2}$ is the probability
311 that a hypothetical recessive allele is transmitted to each offspring, given a hypothetical
312 subpopulation of heterozygotes that only mate with dominant homozygotes (Engen *et*
313 *al.* 2010). Subdiagonal transition probabilities P_2 and P_{ad} were the probabilities of
314 survival from one age-class to the next (Appendix S1). Standard matrix algebra was
315 used to compute u_i and v_i from the right and left eigenvectors of l (Caswell 2001),
316 scaled so that $\sum u_i = 1$ and $\sum u_i v_i = 1$, and to compute the generation time, T , as the mean
317 projected age of parents of new offspring. Contributions to λ from the female and male
318 submatrices must be the same (Mesterton-Gibbons 1993; Caswell 2001; Engen *et al.*
319 2010). λ was therefore initially calculated as the real dominant eigenvalue of the female

320 and male submatrices separately to check that these were approximately equal, and then
321 computed for the full two-sex matrix.

322

323 *Estimating demographic variance*

324 The total population demographic variance, σ_{dg}^2 , comprises the sum of the contributions
325 to demographic variance from each sex-age class, σ_{dgi}^2 , weighted by the stable age
326 distribution, u_i :

$$327 \sigma_{dg}^2 = \sum \sigma_{dgi}^2 u_i \quad (\text{eqn. 3}).$$

328

329 To calculate each σ_{dgi}^2 , we first calculated the demographic variance component from
330 each sex-age class in each year using the mean b and J of each sex-age class i in each
331 year, the mean sum of the squared difference of each individual's b and J from its sex-
332 age class mean (S_b^2 and S_J^2), and the mean sum of squares of the cross products of b and
333 J (S_{Jb}) (Appendix S1, Engen *et al.* 2010). These calculations included all individual
334 colour-ringed choughs alive in each year during 2004-2013 whose value of b could be
335 quantified, either because they produced zero fledglings (i.e. $b=0$) or because their
336 fledglings were colour-ringed meaning that the number of one-year old offspring alive
337 in 2005-2014 was observed. Whether or not each focal colour-ringed individual was
338 alive the following year (i.e. 2005-2014), and hence individual J , was also observed
339 directly.

340 The expected contributions from females and males to demographic variance
341 from the production of sons and daughters was then calculated, conditioned on b and J
342 from the individuals and years sampled. The brood sex-ratio does not differ
343 significantly from 1:1 in Islay's choughs, meaning that $q=0.5$ (Appendix S3). The
344 contributions to demographic variance from the production of sons and daughters were

345 consequently assumed to be equal (Appendix S1). These expected contributions and the
346 reproductive values, v_i , computed from l , were used to compute σ^2_{dgi} for each sex-age-
347 year class. Each σ^2_{dgi} was then weighted by sample size (Engen *et al.* 2010). Finally,
348 N_e/N was calculated from equation 1, and N_e was then calculated by defining N as the
349 total census population size which includes both adults and sub-adults. Full details of all
350 calculations, underlying data and sample sizes are provided in Appendix S1.

351 Bootstrap confidence intervals (CIs) around estimates of demographic variance
352 for each sex-age class (σ^2_{dgi}), the total demographic variance (σ^2_{dg}) and N_e/N were
353 initially computed for the full five age-class model, using 10,000 bootstrap samples.
354 However, CIs for variances can be downwardly biased when bootstrap samples are
355 drawn from small sets of observations with skewed distributions, because rare high
356 values might not be sampled (Schenker 1985; Manly 2007; Puth, Neuhauser & Ruxton
357 2015). This is likely for b , because few individuals were observed for some sex-age-
358 year classes, and while most breeding attempts produced zero one-year olds, a minority
359 produced 2-4. Indeed, bootstrap CIs for σ^2_{dg} and N_e/N computed from the full five age-
360 class model scarcely included the point estimate. Therefore, to adequately assess
361 uncertainty around total demographic variance, σ^2_{dg} , and N_e/N estimates, these quantities
362 were re-estimated using a reduced model with three age classes (i.e. $k=3$, ages one, two
363 and three years or older), thereby increasing sample sizes for adult sex-year classes.
364 Bootstrap samples for realised survival (J) and breeding success (b) were jointly drawn
365 (with replacement) at the level of individuals within years for each sex-age-year class.
366 This sampling regime is necessary to maintain any covariance in b and J within
367 individuals, and to capture stochastic variation among individuals within years, which
368 generates the demographic stochasticity of interest (Engen *et al.* 2010, Appendix S1).

369 CMR models were fitted in program MARK (White & Burnham 1999). Other
370 analyses were run in R v2.15.2 (R Development Core Team 2012), using package
371 Popbio (Stubben & Milligan 2007) for population projections.

372

373 MOLECULAR GENETIC ESTIMATION OF N_e

374 *DNA sampling & genotyping*

375 Since adult choughs moult during breeding, DNA was non-invasively sampled by
376 collecting moulted feathers from nest sites visited during 2007-2014. This provided
377 DNA samples from a mixed-age sample of individuals nesting across Islay. DNA was
378 extracted from 3–5mm clippings of the lower feather calamus, using standard
379 ammonium acetate precipitation (Hogan *et al.* 2008; Trask *et al.* 2016). All samples
380 were genotyped at 13 microsatellite loci developed for choughs and polymorphic in the
381 Islay population (Wenzel *et al.* 2011, Appendix S4). However, one locus (Ppy-005) did
382 not conform to Hardy-Weinberg equilibrium and hence was excluded from analyses
383 (Appendix S4). Duplicate samples from the same individual were identified and
384 excluded to ensure that N_e estimates were not downwardly biased (Appendix S4).

385

386 *Genetic estimators of N_e*

387 The best-evaluated single-sample molecular estimator of N_e utilizes linkage
388 disequilibrium (LD), and measures associations between alleles at different neutral loci
389 compared to expectations given random mating and binomial sampling (Hill 1981). In
390 isolated, finite populations with random mating, LD stems from genetic drift and can be
391 used to estimate N_e (Hill 1981). We implemented a single-sample LD estimator of N_e in
392 NeEstimator v2.01 (Waples & Do 2008; Do *et al.* 2014). To relax the assumption of
393 random mating given the chough's mating system, a model that assumes random initial

394 mating followed by lifelong monogamy (Waples 2006) was used. Further, since Hill's
395 (1981) equations can give downwardly biased estimates of N_e if the sample size is less
396 than true N_e (England *et al.* 2006), a bias-corrected analysis which adjusts for sample
397 size was implemented (following Waples 2006). Finally, since low frequency alleles
398 can upwardly bias N_e estimates, alleles at frequency <0.02 were excluded (following
399 Waples & Do 2010). Sensitivity to such exclusions was examined by repeating analyses
400 with exclusion thresholds of 0.01, 0.02 and 0.05. Although the LD method assumes
401 discrete generations, it can give reasonably unbiased estimates of N_e for species with
402 overlapping generations given genotypes from a mixed-age sample of adults, and if the
403 number of cohorts represented roughly equals the generation length (Waples, Antao &
404 Luikart 2014). These conditions are fulfilled by genotype data from adult choughs
405 sampled during 2007-2014.

406 We additionally implemented an approximate Bayesian computation (ABC)
407 single-sample estimator of N_e using program ONeSAMP (Tallmon *et al.* 2008), which
408 compares eight summary statistics calculated from the focal population to the same
409 statistics for 50,000 simulated populations with N_e drawn from within specified lower
410 and upper prior boundaries. Two different priors were specified; 2-180, with the upper
411 prior reflecting the theoretical maximum N_e of $2N$, and 2-100, as N_e is generally lower
412 than $2N$ in wild populations (Nunney & Elam 1994; Frankham 1995). Since single-
413 sample genetic estimators of N_e utilise sampled breeding adults, N was taken as the total
414 number of breeding adults in the population (Palstra & Fraser 2012). As the eight
415 ONeSAMP summary statistics may be differently affected by N_e of previous
416 generations (Wang 2009) and priors were defined by current N , genotype data from
417 adults sampled during 2012-2014 were used.

418

419 **Results**

420 MEAN DEMOGRAPHIC RATES AND PROJECTION MATRIX

421 One year-old choughs never attempted to breed, and the probability of breeding (c_i)
422 increased to one in individuals aged four years or older in both sexes (Fig. 1a, Appendix
423 S1). Across sampled individuals that attempted to breed, mean breeding success (m_i)
424 increased from age two to three, and tended to be lower in four year olds and higher
425 again in individuals aged five years and older (Fig. 1b, Appendix S1). Mean annual
426 survival probabilities (P_i) increased from first-year through second-year to adult (Fig.
427 1a, Appendix S1).

428 Consequently, reproductive values, v_i , increased with age and were slightly
429 higher for males than for females in all age-classes (Fig. 1c, Appendix S1). As
430 expected, the proportional representation of age-classes measured by the stable age
431 distribution values, u_i , decreased across initial age classes but was greatest for the
432 pooled ≥ 5 age-class. Age-specific u_i values were equal for females and males because
433 values of P_i were set equal (Figs. 1a&d, Appendix S1). Population growth rate was
434 approximately equal for the male and female submatrices ($\lambda_{\text{fem}}=0.964$, $\lambda_{\text{male}}=0.967$), so
435 that for the two-sex matrix $\lambda=0.965$ and generation time $T=6.7$ years.

436

437 SEX-AGE-YEAR SPECIFIC DEMOGRAPHIC RATES

438 Reproductive success (b_i), calculated as the number of one year-olds produced per
439 individual per year, varied among sex-age-year classes (Fig. 2a, Appendix S1). Median
440 b_i was zero in all sex-age classes, but higher values occurred most frequently in females
441 and males aged five years or older (Figs. 2a&b). Realised survival (J_i) also varied
442 among sex-age-year classes; as expected given the estimated P_2 and P_{ad} , fewer one
443 year-olds survived to age two than survived through older ages (Figs. 2c&d, Appendix

444 S1). There was no consistently positive or negative covariance between J and b across
445 individuals within each year (grand mean covariance=0.04, Appendix S1).

446

447

448 DEMOGRAPHIC ESTIMATE OF N_e

449 Given the two-sex five-age class model, $N_e/N=0.21$ and $\sigma_{dg}^2=0.71$ (Fig. 3, Appendix
450 S1). Given the 2014 census of $N=141$ choughs (including adults and sub-adults) and
451 generation time $T=6.7$ years, then $N_e=30$. The reduced three-age class model returned
452 only small changes in the point estimates, with fairly tight 95% bootstrap confidence
453 intervals ($N_e/N=0.23$, 95% CI: 0.21-0.29; $\sigma_{dg}^2=0.67$, 95% CI: 0.53-0.75, Appendix S1).

454 The contribution to total σ_{dg}^2 varied among sex-age classes, such that older age
455 classes contributed more than younger age classes (Fig. 3). For one, two and four year-
456 olds the estimated components of σ_{dg}^2 were similar for females and males. However,
457 three year-old females contributed more than three year-old males to σ_{dg}^2 (Fig. 3). This
458 pattern was reversed for individuals aged five years or older, where males contributed
459 more than females to σ_{dg}^2 (Fig. 3), reflecting a strong positive covariance between J and
460 b across males in one year (Appendix S1). Despite these sex-age class differences,
461 overall male and female contributions to σ_{dg}^2 were similar (means of 0.61 and 0.57
462 respectively, Fig. 3, Appendix S1).

463

464 GENETIC ESTIMATES OF N_e

465 Across the 13 microsatellite loci, the number of alleles per locus ranged from two to six
466 and observed and expected heterozygosities ranged from 0.06 to 0.91 and 0.09 to 0.72
467 respectively (full microsatellite marker summary statistics are provided in Appendix
468 S4).

469 The single-sample LD method estimated $N_e=50$ (95% CI: 38–69, using
470 genotypes from 93 individual choughs) given a critical allele frequency of 0.02. N_e
471 estimates were larger (up to 38% larger), with wider 95% CIs, when low frequency
472 alleles were included (Appendix S4). The ABC method estimated $N_e=26$ (95% CI: 21–
473 36, using genotypes from 71 individual choughs). This estimate was robust to the
474 different upper prior boundaries (Appendix S4).

475

476 Discussion

477 Estimation of effective population size, N_e , and identification of underlying components
478 of demographic variance that reduce N_e below N , is required to understand inter-
479 relations between demography and evolutionary processes (Charlesworth 2009), and to
480 predict population viability and inform population management strategies (Mace &
481 Lande 1991; Hare *et al.* 2011; Frankham *et al.* 2014). However, estimating and
482 interpreting N_e for wild populations is extremely challenging, particularly given
483 overlapping generations, environmental and demographic stochasticity and
484 heterogeneity and resulting dynamic age-structure, and given gene-flow stemming from
485 immigration (Caballero 1994; Wang & Whitlock 2003; Waples *et al.* 2011). Such
486 effects could substantially impact N_e but are often ignored (Engen *et al.* 2005, 2007a),
487 impeding understanding of evolutionary processes and population viability analyses.
488 We used detailed individual- and population-level demographic data to estimate N_e/N ,
489 and its underlying components of sex- and age-specific demographic variance, in a
490 small and apparently isolated red-billed chough population, while accounting for
491 environmental and demographic stochasticity by considering reproductive value.
492 Additionally, we used two single-sample genetic estimators of N_e to encompass effects
493 of any unobserved immigration.

494

495 N_e AND DEMOGRAPHIC VARIANCE

496 Our demographic estimate of N_e/N for Islay's chough population was 0.21. This is
497 substantially lower than the mean value of $0.65 \pm 0.15SD$ estimated across diverse bird
498 populations, where N_e/N was calculated from mean life-table data assuming constant
499 population size and age-structure and hence no environmental or demographic
500 stochasticity or additional demographic heterogeneity (using the 'AgeNe' estimator,
501 Waples *et al.* 2013). The low N_e/N in choughs arose because the estimated total
502 demographic variance was relatively high ($\sigma_{dg}^2=0.71$) compared to other bird species
503 with similar generation times. Specifically, the female demographic variance
504 component for choughs of $\sigma_d^2=0.57$ exceeds the value of $\sigma_d^2 \approx 0.25$ for a generation time
505 of 7 years (extrapolated from Sæther *et al.* 2004b). Further, the total σ_{dg}^2 estimated for
506 choughs is notably high for a monogamous species, where reproductive skew might be
507 expected to be relatively small (Sæther *et al.* 2004a). This high σ_{dg}^2 is perhaps not
508 surprising since first-year survival is known to vary consistently among nest sites on
509 Islay, creating additional demographic heterogeneity and influencing population
510 dynamics (Reid *et al.* 2006). Similarly high demographic variance, and small N_e/N ,
511 might also arise in other populations where individual reproductive success varies with
512 territory quality (e.g. Van de Pol *et al.* 2006; Sergio *et al.* 2009; Griffen & Norelli
513 2015). However, commonly used softwares for population viability analysis often
514 assume a Poisson distribution of family sizes (e.g. RAMAS, Akçakaya 2002, although
515 see VORTEX v.10, Lacy & Pollak 2014), meaning that demographic variance will be
516 underestimated and N_e/N overestimated (Kendall & Wittmann 2010; Frankham *et al.*
517 2014). Further, our estimate of $N_e=0.21$ for choughs may itself be a slight overestimate,
518 because the Engen *et al.* (2010) demographic estimator, like other demographic

519 estimators that consider age-structure (e.g. ‘AgeNe’, Waples et al. 2011, 2013),
520 assumes zero demographic covariance within individuals across years. For relatively
521 long-lived species that show nest-site and mate fidelity, individual reproductive success
522 might be positively correlated across years (Hamel *et al.* 2009; Lee, Engen & Saether
523 2011). Such persistent individual differences can create additional demographic
524 heterogeneity, which could increase the total variance in lifetime reproductive success
525 and thereby further reduce N_e/N . Indeed, individual reproductive success (b) was
526 moderately repeatable within individuals across years in the focal chough population
527 ($R=0.22$, 95% CI: 0.00-0.40, Appendix S1). Future developments of demographic
528 estimators of N_e should aim to incorporate such persistent individual differences
529 alongside other forms of demographic stochasticity and heterogeneity.

530

531 CAUSES OF DEMOGRAPHIC VARIANCE

532 A major advantage of estimating N_e/N using demographic estimators that consider age-
533 structure is that such estimators potentially allow each sex and/or age class’s
534 contribution to total σ_{dg}^2 , and hence to reducing N_e/N , to be explicitly quantified (Engen
535 *et al.* 2010; see also Waples *et al.* 2013). Critical demographic classes that influence N_e ,
536 and thereby influence a population’s evolutionary trajectory and viability, can then be
537 identified. The detailed demographic data available for Islay’s chough population,
538 where survival and any reproduction of adults and sub-adults can be directly observed,
539 and immigration is apparently very rare or absent, provides an unusual and extremely
540 valuable opportunity to partition total demographic variance across key sex-age classes.

541 Our analyses showed that four year-olds and the defined terminal age-class
542 comprising adults aged five years or older contributed most to σ_{dg}^2 (Fig. 3). This might
543 be expected, since these classes encompass most breeding adults and hence encompass

544 substantial among-individual variation in reproductive success. Overall, male and
545 female contributions to total σ^2_{dg} were similar (Fig. 3). This concurs with the similar
546 sex-specific components of demographic variance (0.16 and 0.14 for females and males
547 respectively) estimated in Siberian jays (*Perisoreus infaustus*), a corvid with a similar
548 life-history to choughs (Engen *et al.* 2010). However, estimates of sex-specific
549 demographic variances in populations of other bird species have shown larger
550 contributions from males than females (e.g. great reed warbler *Acrocephalus*
551 *arundinaceus*, Sæther *et al.* 2004a; house sparrow, Engen *et al.* 2007b). Further,
552 although total female and male components of σ^2_{dg} were similar in choughs, the age-
553 specific contributions differed between the sexes. Specifically, ≥ 5 year-old males
554 contributed more to σ^2_{dg} than ≥ 5 year-old females, whereas two and three year-old
555 females contributed more than two and three year-old males (Fig. 3). Thus, although
556 overall sex-specific components of σ^2_{dg} were similar, our analyses illustrate that there
557 may be key sex-age-specific processes acting at small population sizes that drive
558 evolutionary processes and population viability, through their influence on N_e . These
559 sex-age specific differences may reflect persistent population or life-history
560 characteristics, or may reflect stochastic demographic processes acting at small
561 population sizes. In general, estimates of N_e from demographic data commonly only
562 consider the female component of demographic variance (Grant & Grant 1992; Nunney
563 & Elam 1994; Frankham 1995; Waples *et al.* 2013). Our results show that, even for
564 monogamous species like choughs, sex-age-class specific contributions to demographic
565 variance should be incorporated into calculations of N_e to avoid bias and identify key
566 demographic classes, and hence elucidate potential underlying ecological mechanisms.

567

568 GENETIC AND DEMOGRAPHIC APPROACHES

569 The demographic estimate of $N_e/N=0.21$ yielded an estimate of $N_e\approx 30$, given $N\approx 141$.
570 Two different single-sample genetic estimators based on linkage disequilibrium and
571 approximate Bayesian computation further supported the conclusion that $N_e\leq 50$. Direct
572 quantitative comparison between different N_e estimators is difficult (Robinson & Moyer
573 2013), not least because demographic estimators of N_e reflect processes in the offspring
574 generation and estimate N_{ev} (Kimura & Crow 1963), while single-sample genetic
575 estimators reflect processes in the parental generation and estimate N_{ei} (Crow &
576 Denniston 1988). Genetic estimates of N_{ei} may therefore lag behind changes in N by at
577 least one generation. Indeed, N_{ei} estimated from LD may be influenced by processes
578 occurring multiple generations previously, as LD can take multiple generations to break
579 down (Wang 2005; Waples 2005). Genetic estimators also incorporate effects of
580 immigration and gene flow, which demographic estimators do not (e.g. Baalsrud *et al.*
581 2014). However, effects on N_e estimates will depend on the extent of immigration.
582 Immigration of few genetically differentiated individuals could cause N_e to be
583 underestimated, because LD generated by immigration will be attributed to drift.
584 Conversely, high migration rates could cause local population N_e to approach
585 metapopulation N_e (Wang 2005; Waples & Do 2008; Gilbert & Whitlock 2015).

586 In practice, the LD estimator gave the largest estimate of N_e for Islay's chough
587 population, which may reflect the somewhat larger past population size (e.g. 78
588 breeding pairs in 1986, Finney & Jardine 2003, Appendix S2). The ABC estimator gave
589 a similar estimate of N_e to the demographic estimator. Since the ONeSAMP ABC
590 approach utilises multiple different summary statistics to estimate N_e , the exact number
591 of previous generations to which the estimate applies is unclear (Wang 2009). However,
592 this estimator might be less biased by N_e of previous generations than the LD estimator
593 and may therefore better approximate current N_e in a population of varying N . The

594 alternative explanation that the LD estimator is detecting immigration seems unlikely,
595 as this is not supported by the ABC estimator. Additionally, for the LD approach to
596 give an upwardly biased estimate of N_e there would need to be considerable successful
597 immigration into the Islay population, which is unlikely to have gone unobserved
598 during population monitoring and ringing of all UK chough populations. Thus, there is
599 likely to be little or no immigration and resulting gene flow that might act to increase
600 genetic diversity and N_e in Islay's chough population beyond that calculated from
601 observed N and demography.

602

603 CONSERVATION MANAGEMENT IMPLICATIONS

604 Our insights into the magnitude and demographic causes of N_e/N given dynamic age-
605 structure are also directly relevant to conservation strategy for the focal chough
606 population. The demographic and genetic estimators all showed that $N_e \leq 50$, which is
607 below the rule-of-thumb minimum recommended to ensure short-term population
608 viability (Mace & Lande 1991; Frankham *et al.* 2014). Specifically, the baseline per-
609 generation increase in inbreeding, and the corresponding expected rate of loss of
610 genetic diversity per generation, can be calculated as:

$$611 \quad \Delta F = \frac{1}{2N_e} \quad (\text{eqn. 4})$$

612 (Falconer & Mackay 1996). From the demographic estimate of $N_e \approx 30$, ΔF will be ≈ 0.02
613 per initial generation in the continued absence of immigration. This value is relatively
614 high, implying increasing expression of inbreeding depression and reduced evolutionary
615 potential, and hence reduced population viability in both the short-term and long-term
616 (Keller & Waller 2002; Frankham *et al.* 2014). Genetic management should therefore
617 be urgently considered. Since the observed high σ_{dg}^2 stems partly from spatial variation
618 in offspring survival to age one (Reid *et al.* 2006), future strategies could aim to reduce

619 this variation through targeted management of territories with current low productivity
620 (e.g. through targeted habitat improvement or supplementary feeding of specific
621 breeding adults). Additionally, observed sex-age specific contributions to σ_{dg}^2 suggest
622 strategies to alleviate demographic variance could focus on different age classes in
623 females versus males. However, strategies to decrease σ_{dg}^2 and hence increase N_e/N may
624 be insufficient to ensure future viability of Islay's chough population, as current levels
625 of inbreeding and genetic diversity would not be decreased and increased respectively.
626 Further, because $\lambda < 1$, N will continue to decrease. Indeed, the low λ may partly reflect
627 inbreeding depression in survival and reproduction (e.g. Liberg *et al.* 2005; O'Grady *et*
628 *al.* 2006). Translocations may consequently be required to re-establish connectivity
629 between Islay and other UK chough populations and thereby increase genetic diversity
630 and ameliorate inbreeding. Re-establishment of gene-flow in small, fragmented
631 populations has been associated with increased fitness and λ ('genetic rescue') in
632 diverse species (Hostetler *et al.* 2013; Frankham 2015; Laikre *et al.* 2016). Such pro-
633 active genetic management, alongside habitat management, might be essential to ensure
634 long-term population viability.

635

636

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643

644 **Data Accessibility**

645 All data associated with this article will be archived at the Dryad Digital Repository
646 upon acceptance of the manuscript.

647

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898

899 **Figure legends**

900

901 **Fig. 1.** Age- and sex-class specific estimates of (a) annual survival probability (P_i ,
 902 diamonds) with 95% confidence intervals, and probability of attempting to breed (c_i ,
 903 grey filled symbols); (b) breeding success (number of fledglings produced) of
 904 individuals that attempted to breed (m_i), with associated standard errors; (c)
 905 reproductive values (v_i), and (d) stable age distribution (u_i). Females and males are
 906 respectively indicated by (a & b) circles and triangles, and (c & d) grey and white bars.
 907 Sample sizes are summarised in Table S2.

908 **Fig. 2.** Proportions of (a) females and (b) males in each age-class that produced 0–4
 909 one-year old offspring (b), and (c) females and (d) males in each age-class that survived
 910 to the following year (J). On panels (a) and (b), the interior x-axes show the b values
 911 and exterior x-axes show the breeder age classes, where ‘5’ includes individuals aged
 912 ≥ 5 years. N values denote sex-age specific sample sizes except for age one where sexes
 913 were unknown and hence N denotes the pooled sample size. J is consequently assumed
 914 to be equal for one year-old females and males (c & d).

915

916 **Fig. 3.** Total population demographic variance (σ_{dg}^2 , ‘Total’), sex-specific components
 917 of σ_{dg}^2 (‘Sex totals’) and contributions to σ_{dg}^2 from each sex-age class (Age 1-5+ years).
 918 Dark grey and light grey bars indicate female and male components, respectively.

919 Whiskers denote 95% bootstrapped confidence intervals. Bootstrap confidence intervals
920 did not include the point estimate for three year-old males and are not shown (see
921 Appendix S1).

922

923 **Authors' contributions**

924 AET and JMR conceived the ideas and undertook the demographic data analyses. AET
925 undertook molecular genetic analyses, and SBP assisted with labwork. EB, DMcC,
926 JMR and AET collected field data. AET wrote the manuscript, assisted by JMR.

927

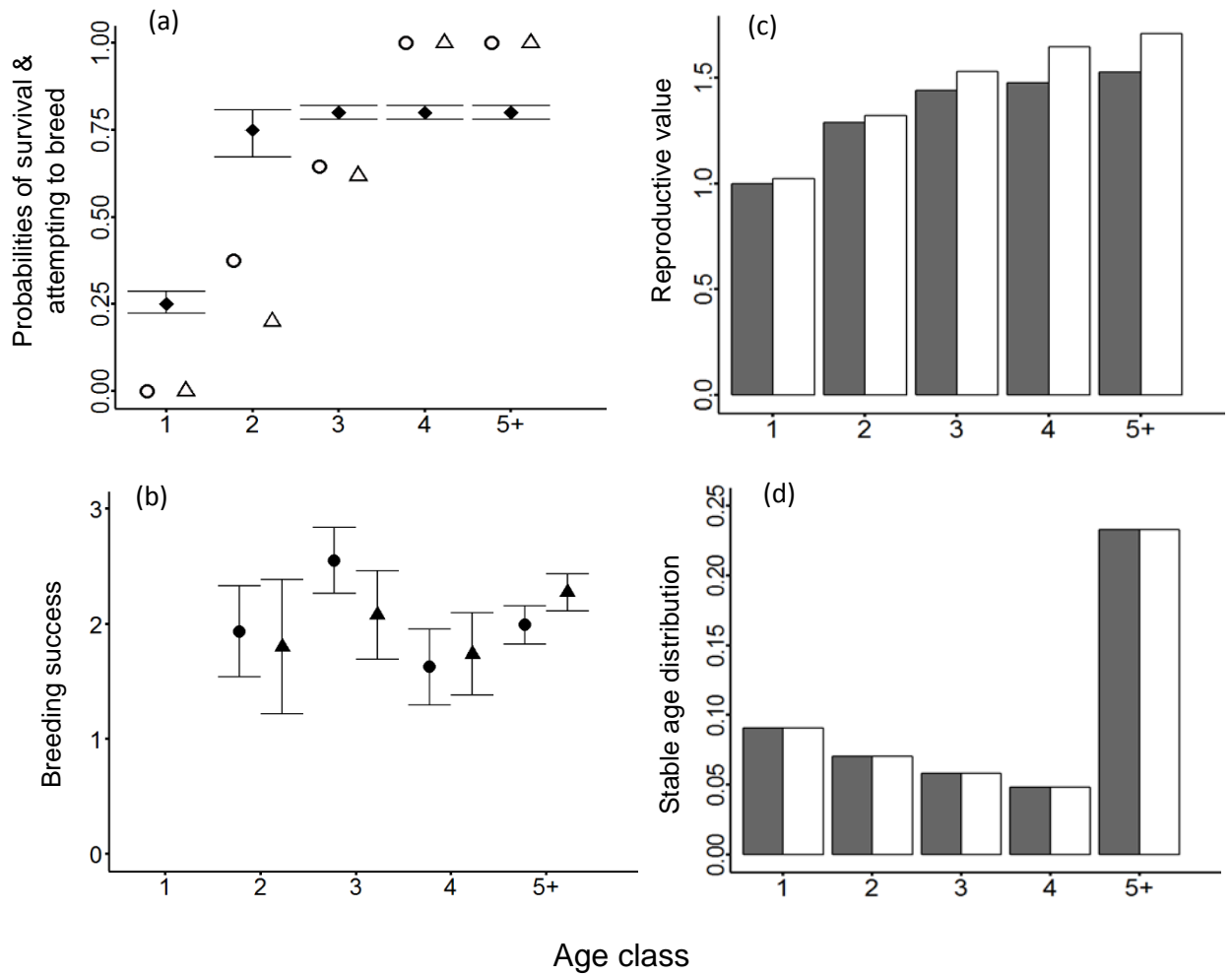
928 **Supporting information**

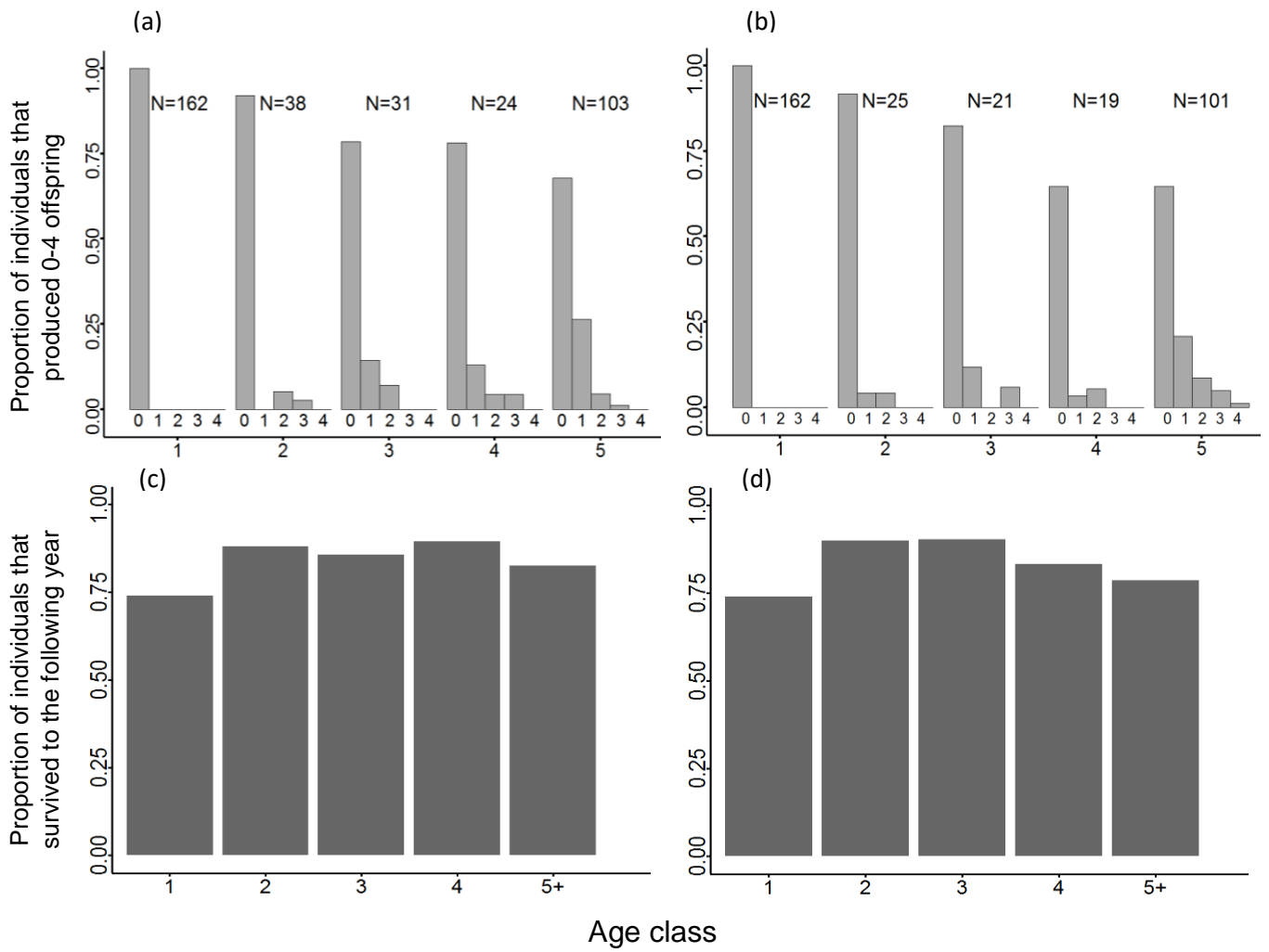
929 **Appendix S1.** Details of demographic estimation of effective population size

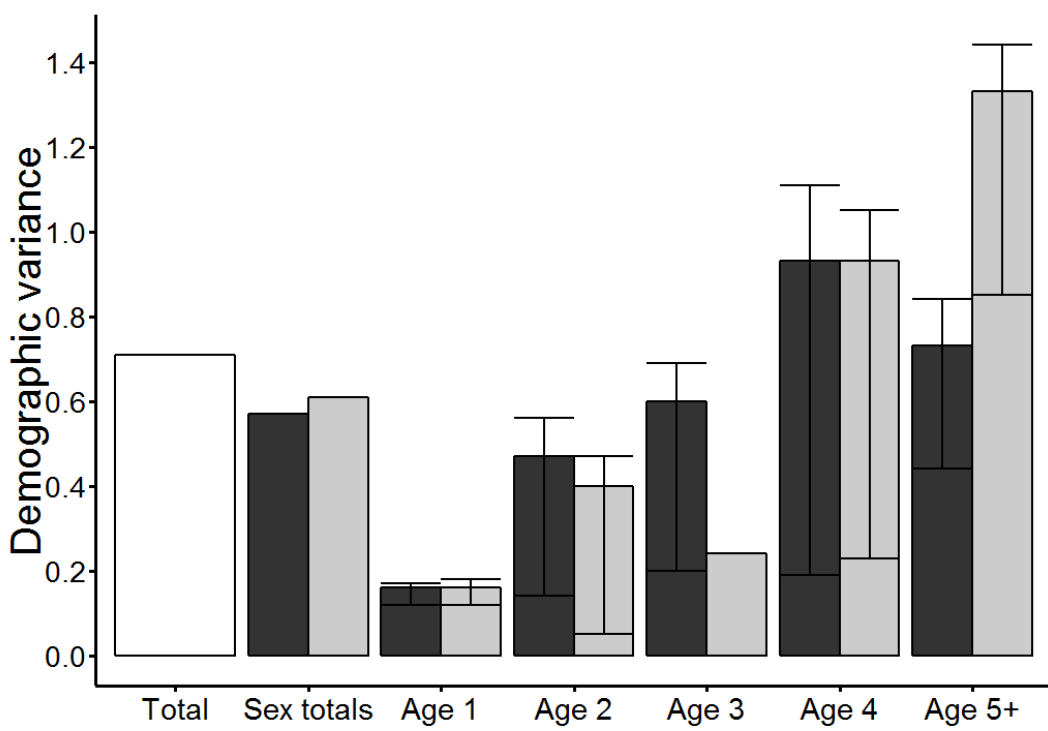
930 **Appendix S2.** Census sizes of the Islay red-billed chough population

931 **Appendix S3.** Sex ratio of nestling red-billed choughs

932 **Appendix S4.** Details of genetic estimation of effective population size







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2	
3	Estimating demographic contributions to effective population size
4	in an age-structured wild population experiencing environmental
5	and demographic stochasticity
6	
7	Amanda E. Trask, Eric M. Bignal, Davy I. McCracken, Stuart B. Piertney and Jane M.
8	Reid.
9	
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22 **Appendix S2. Census sizes of the Islay red-billed chough population**

23 **Appendix S3. Sex-ratio of nestling red-billed choughs**

24 **Appendix S4. Details of genetic estimation of effective population size**

25 S4.1 Molecular analyses and summary statistics of microsatellites

26 S4.2 Single-sample genetic estimates

27

28

29

30 **Appendix S1. Details of demographic estimation of effective population size**

31 Effective population size, N_e , was calculated for an age-structured population of red-billed
32 choughs (*Pyrrhocorax pyrrhocorax*) using data on sex- and age-specific reproductive success
33 and survival from a sample of individuals and utilising the ‘reproductive value’ method
34 derived by Engen, Lande & Saether (2005) and Engen *et al.* (2010). This approach allows
35 estimation of N_e/N in age-structured populations experiencing environmental and
36 demographic stochasticity. It can encompass sex-age-year classes that naturally include very
37 few individuals, as may be common in small populations following low breeding success or
38 high juvenile mortality in some years.

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43 **Table S1.** Summary definitions of key population-level and individual-level demographic
 44 rates, and of key estimated parameters.

Notation	Definition
N	Census population size, which may be defined as breeding adults or both adults and sub-adults.
N_{ev}	Variance effective size
N_{ei}	Inbreeding effective size.
k	Age-class.
l	Population projection matrix.
i	Index denoting a given sex-age class.
Key population-level demographic rates required for l:	
c_i	The proportion of individuals that attempt to breed in a given sex-age class i .
m_i	Mean breeding success, defined as the number of fledglings produced per breeding attempt by individuals that attempted to breed in a given sex-age class i .
q	Primary proportion of individuals that are the focal sex.
f_i	Compound fecundity of a given sex-age class i , defined as $\frac{1}{2} q (c_i m_i P_1)$ given a pre-breeding census.
P_1	Probability of first-year survival (i.e. fledging to age one year).
P_2	Probability of second-year survival (i.e. age one year to age two years).
P_{ad}	Probability of annual adult survival (i.e. age two years and older).
Parameters calculated from l:	
λ	Asymptotic population growth rate, calculated as the real dominant eigenvalue of l .
T	Generation time, calculated as the mean projected age of parents of new offspring.
u	Stable age distribution, calculated as the right eigenvector of l .
v	Age-specific reproductive values, calculated as the left eigenvector of l .

Key individual-level demographic data required:

b	Individual breeding success, defined as the number of one year-olds produced per breeding attempt (given a pre-breeding census).
J	Individual realised survival to the following year.

Final parameters estimated:

σ^2_{dgi}	Demographic variance component from each sex-age class i .
σ^2_{dg}	Total population demographic variance.
N_e/N	Ratio of effective population size to census population size.

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47 **Step 1. Mean population projection matrix**

48 Mean population-level estimates of survival and fecundity for each sex-age class were used
 49 to populate the $2k \times 2k$ population projection matrix, l , with $k=5$ age classes, and formulated
 50 for a birth-pulse population with a pre-breeding census. This population projection matrix
 51 comprised four submatrices:

$$l = \begin{bmatrix} l_{ff} & l_{mf} \\ l_{fm} & l_{mm} \end{bmatrix}$$

52 where l_{ff} is the submatrix for the female contribution to females in the next generation; l_{mm} is
 53 the submatrix for the male contribution to males in the next generation; l_{mf} is the contribution
 54 of males to females in the next generation; and l_{fm} is the contribution of females to males in
 55 the next generation. These submatrices were populated with the survival and fecundity terms:

$$56 \quad l_{ff} = \begin{bmatrix} 0 & \frac{1}{2}q(c_{f2}m_{f2}P_1) & \frac{1}{2}q(c_{f3}m_{f3}P_1) & \frac{1}{2}q(c_{f4}m_{f4}P_1) & \frac{1}{2}q(c_{f5}m_{f5}P_1) \\ P_2 & 0 & 0 & 0 & 0 \\ 0 & P_{ad} & 0 & 0 & 0 \\ 0 & 0 & P_{ad} & 0 & 0 \\ 0 & 0 & 0 & P_{ad} & P_{ad} \end{bmatrix}$$

57

58 $l_{mm} =$

$$59 \quad \begin{bmatrix} 0 & \frac{1}{2}(1-q)(c_{m2}m_{m2}P_1) & \frac{1}{2}(1-q)(c_{m3}m_{m3}P_1) & \frac{1}{2}(1-q)(c_{m4}m_{m4}P_1) & \frac{1}{2}(1-q)(c_{m5}m_{m5}P_1) \\ P_2 & 0 & 0 & 0 & 0 \\ 0 & P_{ad} & 0 & 0 & 0 \\ 0 & 0 & P_{ad} & 0 & 0 \\ 0 & 0 & 0 & P_{ad} & P_{ad} \end{bmatrix}$$

60

61 $l_{mf} =$

$$62 \quad \begin{bmatrix} 0 & \frac{1}{2}(1-q)(c_{m2}m_{m2}P_1) & \frac{1}{2}(1-q)(c_{m3}m_{m3}P_1) & \frac{1}{2}(1-q)(c_{m4}m_{m4}P_1) & \frac{1}{2}(1-q)(c_{m5}m_{m5}P_1) \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

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$$64 \quad l_{fm} = \begin{bmatrix} 0 & \frac{1}{2}q(c_{f2}m_{f2}P_1) & \frac{1}{2}q(c_{f3}m_{f3}P_1) & \frac{1}{2}q(c_{f4}m_{f4}P_1) & \frac{1}{2}q(c_{f5}m_{f5}P_1) \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

65

66 The survival probabilities, P_1 , P_2 and P_{ad} were calculated as described in Step 1.1. For the
 67 fecundity terms (probability of attempting to breed, c_i , and breeding success given that
 68 breeding was attempted, m_i , Table S1), subscripts $f2$ - $f5$ and $m2$ - $m5$ refer to females and males

69 of age class 2-5 respectively. The primary proportion of each focal sex, q , was set to be 0.5
70 (i.e. an equal brood sex-ratio, see Appendix S3).

71 The asymptotic population growth rate, λ , the stable age distribution, u_i , and reproductive
72 values, v_i , were calculated from the real dominant eigenvalue, and right and left eigenvectors
73 of l , respectively (Caswell 2001). The u_i and v_i values were then scaled so that $\sum u_i = 1$ and
74 $\sum u_i v_i = 1$. The generation time, T , was calculated directly from l . Analyses were run in R
75 v2.15.2 (R Development Core Team 2012), using the package Popbio (Stubben & Milligan
76 2007) for population projection analysis. Table S2 shows the sex-age class components of c_i
77 and m_i , sample sizes, and P_1 , P_2 and P_{ad} , and the computed u_i and v_i values. These data are
78 summarised in Fig. 1.

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92 **Table S2.** Sex-age-specific probability of breeding, c_i , and mean breeding success of
 93 individuals that attempted to breed, m_i , with associated standard error (SE) and sample sizes
 94 (N_{obs}), and probability of survival (P_i) with associated 95% confidence intervals (CI) used in
 95 the population projection matrix, l , and the computed stable age distribution, u_i , and
 96 reproductive values, v_i . Estimates of P_i were the same for both sexes. Sexes of one year-olds
 97 were unknown, thus reported sample sizes are pooled across sexes. For individuals in age
 98 class three or older, P_i is equal (i.e. P_{ad}).

<i>Age class</i>	$c_i(N_{obs})$	$m_i \pm SE(N_{obs})$	P_i (95% CI)	u_i	v_i
Females:					
1	0.00 (162)	-	0.25 (0.22-0.29)	0.09	0.69
2	0.36 (40)	1.93±0.40 (15)	0.75 (0.67-0.81)	0.07	0.89
3	0.65 (31)	2.55±0.29 (20)	0.80 (0.78 – 0.82)	0.06	1.00
4	1.00 (24)	1.63±0.31 (24)	-	0.05	1.02
5+	1.00 (103)	1.99±0.17 (103)	-	0.23	1.06
Males:					
1	0.00 (162)	-	0.25 (0.22-0.29)	0.09	0.71
2	0.20 (25)	1.80±0.58 (5)	0.75 (0.67-0.81)	0.07	0.92
3	0.62 (21)	2.08±0.38 (13)	0.80 (0.78-0.82)	0.06	1.06
4	1.00 (19)	1.74±0.36 (19)	-	0.05	1.14
5+	1.00 (103)	2.27±0.16 (103)	-	0.23	1.18

99

100 *Step 1. 1 Estimating age-specific probabilities of survival*

101 As a preliminary step to estimating N_e , capture-mark-recapture models (CMR) were used to
 102 estimate age-specific probabilities of survival from colour-ring resightings. Choughs were
 103 resighted in mid-May to mid-July each year across Islay. As dispersal from Islay is rare (Reid
 104 *et al.* 2003a, 2004, 2010; Wenzel *et al.* 2012), disappearance of colour-ringed individuals
 105 during 2004-2014 was assumed to reflect mortality. Previous analyses suggested that survival
 106 probabilities do not differ significantly between males and females (Reid *et al.* 2003b) and
 107 thus sexes were pooled. A live recaptures Cormack-Jolly-Seber model was fitted to the

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108 encounter histories of all 1695 choughs ringed during 1983-2014, with age-dependent
109 survival probabilities (P_i) and year-dependent resighting probabilities. The best supported
110 model of age-dependent survival was selected based on Akaike's Information Criterion
111 (AIC). Bootstrap goodness-of-fit tests were used to assess the fit of the model, using \hat{c} to
112 check for overdispersion.

113

114 A three age-class model, with first-year (fledging to age one year), second-year (age one year
115 to age two years) and adult (age two years and older) classes was best supported, as found
116 previously (Reid *et al.* 2004). Across the focal study years of 2004-2014, first-year survival
117 was low ($P_1=0.25$), but increased in the second year ($P_2=0.75$) and was high for adults
118 ($P_{ad}=0.80$). Resighting probability was high across all age-classes during 2004-2014
119 (mean= 0.97 ± 0.02).

120

121 *Step 1.2. Selecting appropriate age classes*

122 Age-classes were defined to capture age-specific variation in reproductive success (c_i and m_i ,
123 Table S2) and survival probability (P_i , Step 1.1), but also maintain sufficient sample sizes so
124 that year-specific mean values of b and J for each sex-age class could be calculated. A three
125 age-class CMR model was best supported (Step 1.1) and therefore these age-classes were
126 kept separate to capture this variation. Additionally, the probability of attempting to breed (c_i)
127 and the number of fledglings produced by individuals that attempted to breed (m_i) varied
128 between ages one to four or older, thus these age-classes were also kept separate.
129 Reproductive success has previously been shown to decrease in older age-classes (Reid *et al.*
130 2003b, 2004), however sample sizes become too small such that sex-age-year class
131 demographic variances could not be calculated. Individuals aged five and older were
132 therefore pooled into a terminal age-class.

133 **Step 2. Sex-age-year class data from sampled individuals for the focal years**

134 Data from colour-ringed (and hence known-age) males and females on individual realised
135 survival to the following year (J_i) and breeding success (b_i), calculated as the number of
136 offspring surviving to one year old, for each sex-age class for the years 2004-2013 were
137 included in current analyses.

138 For each sex-age-year class, mean breeding success (b) across individuals was calculated
139 (Table S3). Mean sums of squares of breeding success (S_b^2) were then calculated as the sum
140 of the squares of each individual's difference from its sex-age class mean. The grand mean
141 was calculated by weighting each sum of squares by sample size, by dividing by the number
142 of individuals in each sex-age class in each year minus 1.

143 Mean realised survival (J) for each sex-age-year class was calculated (Table S4). Mean sums
144 of squares of adult realised survival (S_J^2) were then calculated as above; the square of each
145 individual's difference from its mean sex-age-year class realised survival was computed, the
146 sum of these squares for each sex-age class in each year was calculated, and the grand mean,
147 weighted by sample size minus 1, was computed.

148 The mean sum of squares of the cross products (S_{Jb}) between breeding success and realised
149 survival were then calculated by multiplying each individual's b and J values. The square of
150 each individual's difference from its sex-age class mean for the cross-product was calculated,
151 these squares were summed within each sex-age-year class, and the grand mean weighted by
152 the sample size minus 1 was computed.

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156 **Table S3.** Mean sex-age-year specific breeding success (b), with sample sizes (N_{obs}) in
 157 brackets. ‘-’ indicate sex-age-year classes where data were available for <2 individuals,
 158 therefore these classes were not included in analyses. One year olds did not attempt to breed
 159 and are therefore not included.

Age	2 (N_{obs})		3 (N_{obs})		4 (N_{obs})		5+ (N_{obs})	
	Female	Male	Female	Male	Female	Male	Female	Male
2004	0.00 (4)	-	-	1.00 (2)	0.00 (3)	0.00 (2)	0.22 (9)	0.38 (10)
2005	-	-	0.50 (4)	-	-	-	0.50 (8)	0.38 (12)
2006	1.25 (4)	00.0 (5)	-	-	0.00 (3)	-	0.83 (8)	0.60 (13)
2007	0.00 (4)	0.00 (4)	0.00 (4)	0.00 (6)	-	-	0.14 (9)	0.00 (10)
2008	0.00(12)	0.00 (5)	0.33 (7)	0.00 (4)	0.00 (2)	0.00 (6)	0.22 (10)	0.25 (6)
2009	-	-	0.13 (9)	0.33 (4)	0.40 (5)	0.75 (4)	0.11 (11)	0.00 (9)
2010	-	-	-	-	0.17 (6)	1.00 (3)	0.33 (14)	0.56 (11)
2011	0.50 (4)	0.33 (3)	-	-	-	-	0.36 (11)	0.91 (13)
2012	0.00 (6)	0.50 (5)	0.67 (3)	0.00 (2)	-	-	0.80 (10)	1.67 (10)
2013	0.00 (3)	0.00 (2)	0.00 (2)	3.00 (2)	1.33 (3)	-	0.50 (11)	0.44 (9)

160

161 **Table S4.** Mean realised survival to the following year (J) in each sex-age-year class, with sample sizes (N_{obs}) in brackets. Sexes of one year-
 162 olds were unknown, so sex-specific mean realised survival could not be calculated and all individuals were pooled. ‘-’ indicate sex-age-year
 163 classes where data were available for <2 individuals, therefore these classes were not included in analyses.

Age- class	1 (N_{obs})		2 (N_{obs})		3 (N_{obs})		4 (N_{obs})		5+ (N_{obs})	
	Female Male	& Female Male	Female Male	Female Male	Female Male	Female Male	Female Male	Female Male	Female Male	
2004	0.71 (21)	1.00 (4)	-	-	0.50 (2)	0.67 (3)	1.00 (2)	0.50 (9)	0.90 (10)	
2005	0.82 (17)	-	-	1.00 (4)	-	-	-	0.75 (8)	0.92 (12)	
2006	0.74 (27)	1.00 (4)	1.00 (5)	-	-	0.67 (3)	-	0.88 (8)	0.92 (13)	
2007	0.40 (5)	1.00 (4)	1.00 (4)	0.75 (4)	1.00 (6)	-	-	0.89 (9)	0.50 (10)	
2008	0.43 (7)	0.75 (12)	1.00 (5)	0.86 (7)	1.00 (4)	1.00 (2)	0.67 (6)	0.80 (10)	0.83 (6)	
2009	0.88 (8)	-	-	0.89 (9)	0.75 (4)	1.00 (5)	1.00 (4)	0.91 (11)	0.89 (9)	
2010	0.93 (14)	-	-	-	-	0.83 (6)	1.00 (3)	0.64 (14)	0.82 (11)	
2011	0.64 (14)	1.00 (4)	0.67 (3)	-	-	-	-	0.75 (11)	0.92 (13)	
2012	0.78 (27)	0.83 (6)	0.60 (5)	1.00 (3)	0.50 (2)	-	-	0.90 (10)	0.70 (10)	
2013	0.73 (22)	1.00 (3)	1.00 (2)	1.00 (2)	1.00 (2)	0.67 (3)	-	0.91 (11)	0.78 (9)	

164 **Table S5.** Summary statistics of sex-age-year class breeding success (b) and realised survival (J). Minimum (Min), maximum (Max), mean with
 165 associated standard error (SE), median and variance (Var) are shown, with sample size N_{obs} in each sex-age-year class. One year-olds did not
 166 attempt to breed and sexes were unknown, thus sex-specific realised survival could not be calculated and N_{obs} is for both sexes combined.

Age-class	1		2		3		4		5+	
	($N_{obs}=162$)	Female	Male	Female	Male	Female	Male	Female	Male	
		($N_{obs}=38$)	($N_{obs}=25$)	($N_{obs}=31$)	($N_{obs}=21$)	($N_{obs}=24$)	($N_{obs}=19$)	($N_{obs}=103$)	($N_{obs}=101$)	
Breeding success (b)										
Min	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	-	3.00	2.00	2.00	3.00	3.00	2.00	3.00	4.00	
Mean	-	0.18	0.13	0.29	0.29	0.35	0.59	0.39	0.57	
±SE		±0.11	±0.09	±0.11	±0.19	±0.16	±0.21	±0.07	±0.10	
Median	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Var	-	0.42	0.20	0.36	0.60	0.60	0.76	0.40	0.86	
Realised survival (J)										
Mean	0.74	0.90	0.88	0.90	0.86	0.83	0.89	0.79	0.83	
±SE	±0.03	±0.05	±0.07	±0.06	±0.08	±0.08	±0.07	±0.04	±0.04	
Var	0.19	0.09	0.11	0.09	0.13	0.14	0.10	0.17	0.15	

167 *Step 2.1. Phenotypic covariance between b and J*

168 Covariance between individual realised survival to the following year (*J*) and breeding
169 success (*b*) was quantified within each sex-age-year class (Table S6).

170

171 **Table S6.** Covariance between breeding success (*b*) and realised survival (*J*) within each sex-
172 age-year class. Sample sizes within each year were as for Table S3&4. ‘-’ indicate sex-age-
173 year classes where data were available for <2 individuals, therefore these classes were not
174 included.

Age- class	2		3		4		5+	
	Female	Male	Female	Male	Female	Male	Female	Male
2004	0.00	-	-	-	0.00	0.00	0.14	-0.23
2005	-	-	0.00	-	-	-	0.00	0.05
2006	0.00	0.00	-	-	0.00	-	-0.23	0.07
2007	0.00	0.00	0.00	0.00	-	-	0.00	0.00
2008	0.00	0.00	0.07	0.00	0.00	0.00	0.03	0.08
2009	-	-	0.02	0.00	0.00	0.00	0.01	0.00
2010	-	-	-	-	0.03	0.00	0.03	0.07
2011	0.00	0.17	-	-	-	-	0.11	0.09
2012	0.00	0.33	0.00	0.00	-	-	-0.02	0.63
2013	0.00	0.00	-	-	0.67	-	0.07	-0.01

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176

177 *Step 2.2. Repeatability in individual b across years*

178 Generalized linear mixed models with Poisson error distributions, using a log link function,
 179 were used to estimate repeatability in individual breeding success (b) across years, with
 180 random individual identity effects, using the R package rptR v.0.9.1 (Nakagawa & Schielzeth
 181 2010). rptR was also used to estimate 95% confidence intervals around the repeatability
 182 estimate, using 1000 bootstrap samples.

183

184 306 total observations of b from 73 individuals whose b was observed in at least two years
 185 were used. Individual reproductive success was moderately repeatable across years (back-
 186 transformed observed scale $R=0.22$, 95% CI: 0.00-0.41).

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188

189 **Step 3. Expectations for each sex-age-year class**

190 The expected contributions to demographic variance from females and males, conditioned on
 191 mean b (\bar{b}) and the sums of squares of b (S_b^2) and the sums of squares of the cross-product of
 192 breeding success and realised survival (S_{jb}) for each sex-age class in each year were
 193 computed following Engen *et al.* (2010), assuming $q=0.5$. Equations (i) and (ii), and (iv) and
 194 (v), are equal if $q=0.5$, meaning that there are equal contributions to demographic variance
 195 from the production of daughters (X) and sons (Y).

196 (i) The expected contribution from females and males to demographic variance from
 197 the production of daughters (ES_X^2):

$$ES_X^2 = \frac{1}{4}q^2(S_b^2) + \frac{1}{4}q(2-q)\bar{b}$$

- 198 (ii) The expected contribution from females and males to demographic variance from
199 the production of sons (ES_Y^2):

$$ES_Y^2 = \frac{1}{4}(1-q)^2(S_b^2) + \frac{1}{4}(1-q)(1+q)\bar{b}$$

- 200 (iii) The expected cross-product of the contribution from females and males to
201 demographic variance from the production of daughters and sons (ES_{XY}):

$$ES_{XY} = \frac{1}{4}q(1-q)(S_b^2 - \bar{b})$$

- 202 (iv) The expected contribution from females and males to demographic variance from
203 the survival of daughters (ES_{JX}):

$$ES_{JX} = \frac{1}{2}qS_{Jb}$$

- 204 (v) The expected contribution from females and males to demographic variance from
205 the survival of sons (ES_{JY}):

$$ES_{JY} = \frac{1}{2}(1-q)S_{Jb}$$

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212 **Table S7.** Expected contributions to demographic variance from the production of daughters
 213 (ES^2_X) and sons (ES^2_Y), the cross-product of the production of daughters and sons (ES_{XY}) and
 214 the survival of daughters (ES_{JX}) and sons (ES_{JY}). For age class one, where $b=0$, these
 215 expectations were 0. Sample sizes within each year were as for Table S3&4. ‘-’ indicate sex-
 216 age-year classes where data were available for <2 individuals, and were therefore not
 217 included in analyses.

Year	Age class	ES^2_X & ES^2_Y		ES_{XY}		ES_{JX} & ES_{JY}	
		Female	Male	Female	Male	Female	Male
2004	2	0.00	-	0.00	-	0.00	-
	3	-	-	-	-	-	-
	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.05	0.10	0.00	0.01	0.05	0.03
2005	2	-	-	-	-	-	-
	3	0.16	-	0.03	-	0.25	-
	4	-	-	-	-	-	-
	5	0.11	0.10	-0.01	0.01	0.07	0.14
2006	2	0.38	0.00	0.06	0.00	0.56	0.00
	3	-	-	-	-	-	-
	4	0.00	-	0.00	-	0.00	-
	5	0.22	0.17	0.01	0.02	0.18	0.23
2007	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00
	4	-	-	-	-	-	-
	5	0.04	0.00	0.00	0.00	0.04	0.00

2008	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.08	0.00	0.00	0.00	0.07	0.00
	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.05	0.06	0.00	0.00	0.05	0.06
2009	2	-	-	-	-	-	-
	3	0.03	0.08	0.00	0.00	0.03	0.08
	4	0.13	0.20	0.03	0.01	0.20	0.23
	5	0.03	0.00	0.00	0.00	0.03	0.00
2010	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	0.04	0.25	0.00	0.00	0.04	0.25
	5	0.09	0.14	0.01	0.00	0.10	0.13
2011	2	0.16	0.08	0.03	0.00	0.25	0.08
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	0.10	0.23	0.01	0.00	0.11	0.22
2012	2	0.00	0.16	0.00	0.03	0.00	0.25
	3	0.21	0.00	0.04	0.00	0.33	0.00
	4	-	-	-	-	-	-
	5	0.20	0.47	0.00	0.05	0.23	0.63
2013	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	-	-	-	-	-	-
	4	0.40	-	0.06	-	0.58	-
	5	0.11	0.10	-0.01	-0.01	0.07	0.06

219

220 **Step 4. Components of the demographic variance, σ^2_{di}**

221 Using the expected contributions calculated above and the sex-age specific reproductive
 222 values (v_i , Table S2), the demographic variance components for each sex-age-year class were
 223 estimated by parameterising the following equation. Here, v_{i+1} is the reproductive value of the
 224 subsequent sex-age class, with the final age class receiving its own reproductive value.

$$\begin{aligned}\hat{\sigma}_{dgi}^2 &= v_{i+1}^2 S_J^2 + v_1^2 ES_X^2 + v_{k+1}^2 ES_Y^2 \\ &+ 2v_{i+1}v_1 ES_{Jx} + 2v_{i+1}v_{k+1} ES_{JY} \\ &+ 2v_1v_{k+1} ES_{XY}\end{aligned}$$

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235 **Table S8.** Sex-age-year class contributions to demographic variance, $\hat{\sigma}_{dgi}^2$. Sample sizes
 236 within each year were as for Table S3&4. ‘-’ indicate sex-age-year classes where data were
 237 available for <2 individuals, therefore these classes were not included in analyses.

Age- class	1		2		3		4		5+	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
2004	0.17	0.18	0.00	-	-	-	0.75	0.00	0.61	0.54
2005	0.12	0.13	-	-	1.16	-	-	-	0.74	1.02
2006	0.16	0.17	2.54	0.00	-	-	0.37	-	1.20	1.55
2007	0.24	0.25	0.00	0.00	0.26	0.00	-	-	0.35	0.58
2008	0.23	0.24	0.20	0.00	0.53	0.00	0.00	0.70	0.49	0.79
2009	0.10	0.10	-	-	0.29	0.99	0.98	1.42	0.27	0.21
2010	0.06	0.06	-	-	-	-	0.41	1.60	0.84	1.14
2011	0.20	0.21	1.12	0.82	-	-	-	-	0.84	1.56
2012	0.14	0.15	0.17	1.69	1.54	0.65	-	-	1.27	4.16
2013	0.17	0.17	0.00	0.00	-	-	3.26	-	0.57	0.73

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239

240 **Step 5. Estimating total demographic variance, σ_{dgs}^2 , and N_e/N**

241 The weighted mean demographic variance for each sex-age class across years was then
 242 calculated from the components of the demographic variance, $\hat{\sigma}_{dgi}^2$ calculated above,
 243 weighted by sample size (n_{it}) minus 1, thus allowing comparison between each sex-age-year
 244 class's contributions to total demographic variance:

$$\sigma_{dgi}^2 = \frac{\sum \hat{\sigma}_{dgi}^2 (n_{ti} - 1)}{\sum n_{ti} - 1}$$

245 The total demographic variance was then calculated as the weighted mean of these sex-age
 246 class mean demographic variances (Fig. 3), weighted by the scaled stable age distribution
 247 values (u_i , Fig. 1d):

$$\sigma_{dg}^2 = \sum \sigma_{dgi}^2 u_i$$

248 where the subscript g indicates that the total demographic variance (σ_{dg}^2) has a genetic
 249 component due to Mendelian segregation. The N_e/N ratio was then calculated using σ_{dg}^2 and
 250 the generation time (T) following equation 1 in the main manuscript:

$$\frac{N_e}{N} = \frac{1}{\sigma_{dg}^2 T}$$

251

252 *Estimating the effective number of breeders (N_b)*

253 While we did not aim to estimate the effective number of breeders (N_b) in the focal chough
 254 population, N_b is a parameter of interest in other studies, in particular where it is only possible
 255 to sample or census adults in a single breeding season or from a single cohort (Palstra &
 256 Fraser 2012; Waples *et al.* 2013; Ruzzante *et al.* 2016). Indeed, N_b can be estimated from our
 257 demographic analyses, from the total demographic variance pertaining to the breeding sex-
 258 age-classes (i.e. as the weighted mean of the breeding sex-age class's demographic variances,
 259 weighted by their respective u_i values). The total demographic variance of the breeding sex-
 260 age classes can then be used to calculate N_b/N following equation 1 in the main manuscript,
 261 by replacing N_e with N_b . N_b itself can then be estimated by taking N as the total number of
 262 breeding individuals.

263 For the focal chough population, most individuals do not start to breed until age three (Fig.
264 1a). Thus, N_b/N was estimated from the weighted mean of the demographic variance
265 components pertaining to males and females aged three years or older (Fig. 3). The total
266 demographic variance of the breeding sex-age classes was 0.91, giving $N_b/N=0.16$ (from
267 equation 1). Taking $N=92$ as the total number of breeding individuals in 2014, then $N_b=15$.

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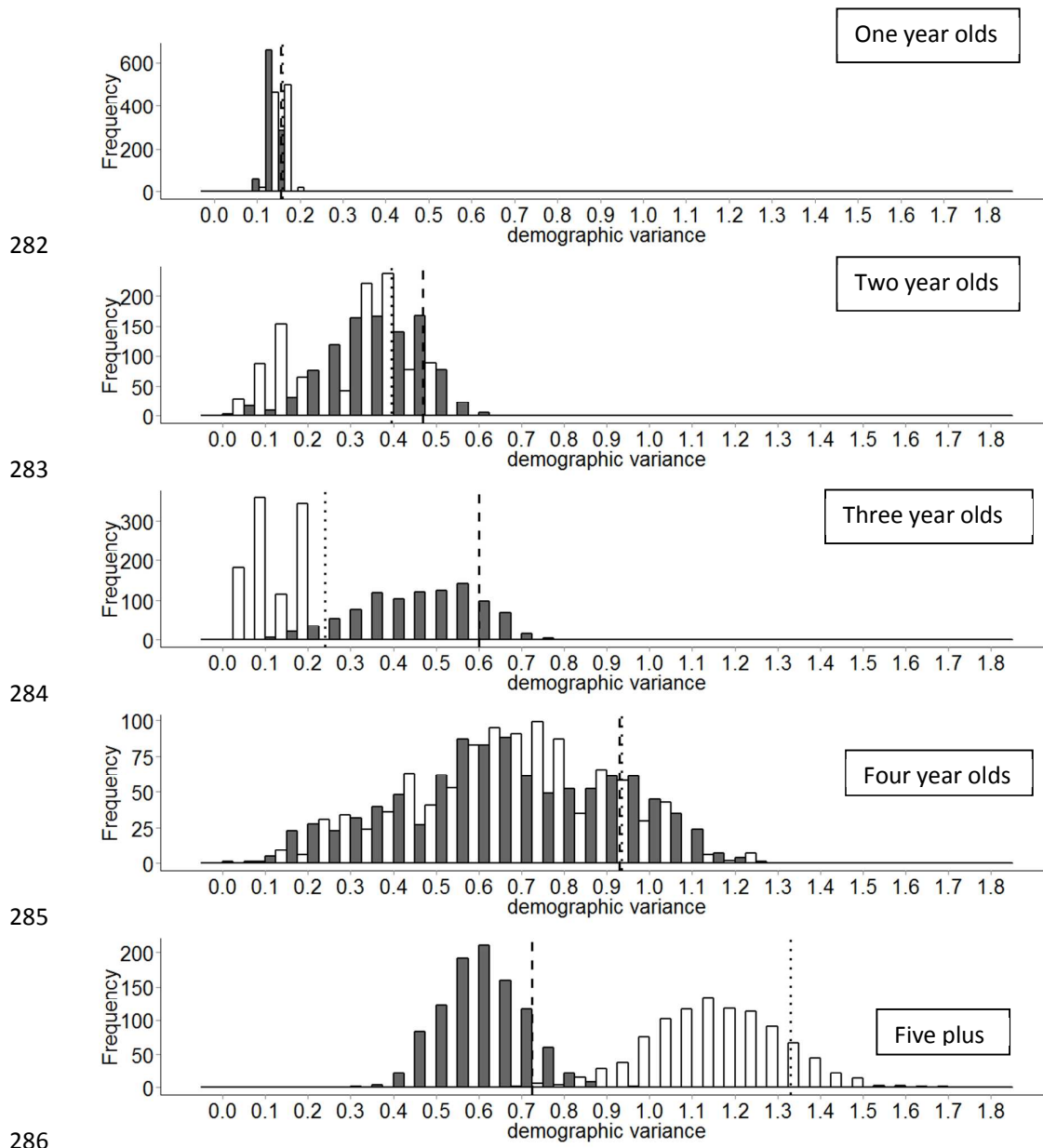
270

271 **Step 6. Estimating confidence intervals**

272 Confidence intervals around the sex-age specific components of demographic variance (σ_{dgi}^2),
273 were estimated by bootstrapping for the full five age-class model (Fig. S1). However, to
274 obtain confidence intervals around the estimates of σ_{dg}^2 and N_b/N a reduced three age-class
275 model was used, in order to increase sample sizes within sex-age-year classes (Fig. S2).

276 Realised survival (J) and breeding success (b) were jointly resampled within years for each
277 age-sex class, with replacement. Resampling within each sex-age-year class is necessary to
278 capture the stochastic variation in b and J among individuals within years and to maintain any
279 covariance, because it is this within-year variation which generates the demographic
280 variance. Confidence intervals were calculated from 10,000 bootstrap samples.

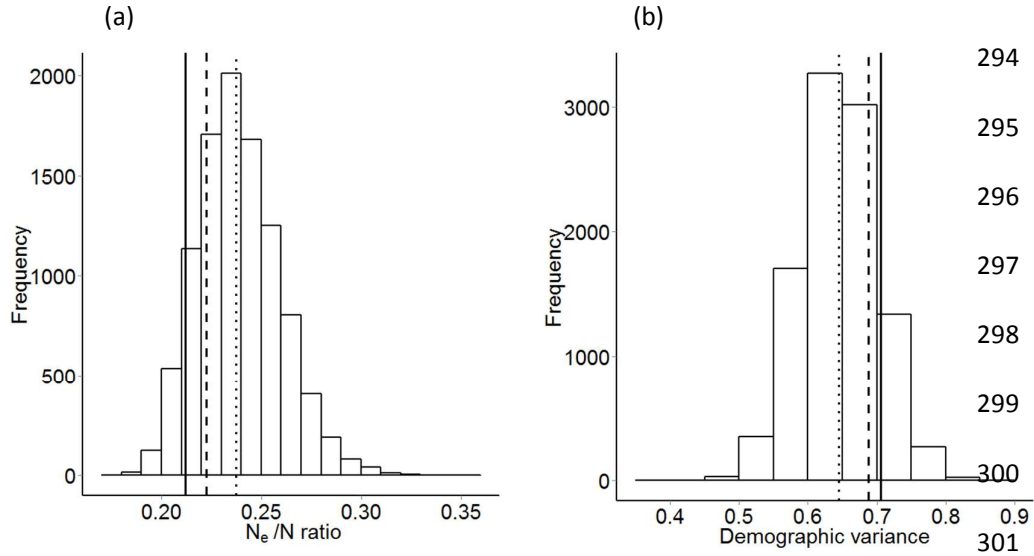
281



287 **Fig. S1.** Bootstrap distributions of each sex-age class's contribution to the total demographic
 288 variance, σ^2_{dgi} , computed from the full five age-class model (with age-class labelled). Dark
 289 grey and white bars indicate females and males respectively. Dashed and dotted lines indicate
 290 point estimates for females and males respectively. X-axis scales are standardised to facilitate
 291 comparison across age-classes.

292

293



302 **Fig. S2.** Bootstrap distribution of (a) the ratio of effective population size to census
 303 population size (N_e/N ratio) and (b) the total demographic variance (σ_{dg}^2) from a reduced two-
 304 sex model with three age classes. Dashed lines indicate the point estimates, and dotted lines
 305 are the medians of the bootstrap distributions. For comparison, solid lines indicate the point
 306 estimates from the full five age class model,

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317 **Appendix S2. Census sizes of the Islay red-billed chough population**

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318

319 **Table S9.** Census population size (number of breeding pairs) of red-billed choughs on Islay in
 320 years in which the population was fully censused between 1982 and 2014.

Year	No. of breeding pairs
1982	53
1986	78
1992	68
1998	43
2002	56
2007	53
2010	43
2013	39
2014	46

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330 **Appendix S3. Sex-ratio of nestling red-billed choughs**

331 A sample of accessible occupied nest sites across Islay were visited during the 2012-14
332 breeding seasons to colour-ring and DNA sample nestlings. Small blood samples were
333 collected through brachial venipuncture from nestlings 2-3 weeks post-hatch, and stored in
334 EDTA tubes at -20 °C or on FTA® cards (Whatman Biosciences Ltd®) stored at room
335 temperature in separate polythene sample bags. DNA was extracted from <5µl of blood using
336 DNeasy blood and tissue kits® (Qiagen Ltd®), according to the manufacturers protocol, or
337 standard ammonium acetate precipitation (Hogan *et al.* 2008). DNA from blood samples on
338 FTA cards was extracted using FTA purification reagent® (Whatman Biosciences Ltd.) and
339 5% Chelex elution (Walsh, Metzger & Higuchi 1991).

340

341 DNA samples were sex-typed using PCR amplification of the CHD1 gene with the primer
342 pairs P2 and P8 (P2: 5'-TCTGCATCGCTAAATCCTTT-3' and P8: 5'-
343 CTCCAAGGATGAGRAAYTG-3'; Griffiths *et al.* 1998). PCR reactions were carried out
344 with reaction volumes and cycling conditions as described in Wenzel *et al.* (2012). PCR
345 products were visualised via electrophoresis on 2% agarose gels, stained with Web Green
346 DNA stain. Individuals with a putative male sex-type show a single gel band and thus are
347 indistinguishable from a null allele at the female-specific second band (Robertson &
348 Gemmell 2006). All putative male samples were therefore additionally sex-typed using the
349 2550 and 2718 primer pair, which amplify a different region of the CHD1 gene (2550F: 5' –
350 GTTACTGATTCGTCTACGAGA-3' and 2718R: 5'- ATTGAAATGATCCAGTGCTTG-3';
351 Fridolfsson & Ellegren 1999). The PCR reaction profile for the 2550F and 2718R primer pair
352 consisted of an initial denaturation step of 94 °C for 2 min, followed by 10 TouchDown
353 cycles from 60 °C to 50 °C with 1 °C decrements (denaturation at 94 °C for 30 s, annealing
354 for 30 s, elongation at 72 °C for 30 s). Then 25 additional cycles were run with denaturation
355 at 94 °C for 30 s, constant annealing temperature of 50 °C for 30 s and elongation at 72 °C

356 for 30 s and a final elongation step at 72 °C for 5 min. The sex-ratio of nestlings was then
357 compared to a 1:1 expected ratio, using χ^2 goodness-of-fit tests.

358

359 In total, 157 nestlings were sex-typed. These individuals were identified as 81 (51.6%) males
360 and 76 (48.4%) females. Consequently, the offspring sex-ratio did not differ significantly
361 from 1:1 ($\chi^2_1=0.16$, $P=0.69$).

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379 **Appendix S4. Details of genetic estimation of effective population size**

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381 **S4.1 Molecular analyses and summary statistics of microsatellites**

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383 PCRs for microsatellite markers were performed individually, following Wenzel *et al.* (2012)
384 and with annealing temperatures as specified in Table S10. Genotyping procedures are
385 described in Trask *et al.* 2016 and with primers fluorescently-tagged as specified in Table S10.

386

387 Microsatellite data were checked for genotyping errors and heterozygote deficiency using
388 MICRO-CHECKER v2.2.3 (van Oosterhout *et al.* 2004). Allele frequencies and observed (H_O)
389 and expected (H_E) heterozygosity at each locus were calculated using GenAlex v6.5 (Peakall &
390 Smouse 2012). Departures from Hardy-Weinberg equilibrium for each locus were assessed
391 using Fisher's Exact tests in GENEPOP v4.2.2 (Rousset 2008), using a Markov Chain Monte
392 Carlo approach (1,000 dememorisation steps, 100 batches, 5,000 iterations). GENEPOP v4.2.2
393 was also used to test for genotypic linkage disequilibrium between all possible loci
394 combinations. For tests for both Hardy-Weinberg equilibrium and linkage disequilibrium,
395 Bonferroni corrections for multiple comparisons were applied, using p.adjust in R v2.15.2 (R
396 Development Core Team 2012).

397

398 The number of alleles per locus ranged from two to six and observed and expected
399 heterozygosities ranged from 0.06 to 0.91 and 0.09 to 0.72 respectively (Table S10). There was
400 no evidence for null alleles at any of the 13 loci. Significant linkage disequilibrium was found
401 in 9 of 58 loci combinations, and persisted in two combinations after Bonferroni corrections
402 (between Ppy-008 and Ppy-010 and Ppy014-Ppy011). However, Wenzel *et al.* (2012) has
403 previously shown no consistent linkage between loci across different chough populations, thus

404 suggesting no physical linkage on chromosomes. Observed linkage disequilibria can therefore
405 be attributed to chance associations between alleles at freely-recombining loci arising due to
406 finite population size (Hill 1981; Waples 2005). Significant deviations from Hardy-Weinberg
407 equilibrium, which persisted after Bonferroni corrections, were found at locus Ppy-005. This
408 locus was therefore excluded and twelve loci used in further analyses.

409

410 Genetic estimates of N_e will be downwardly biased if duplicate samples from the same
411 individual are included (Waits, Luikart & Taberlet 2001). Use of DNA samples from moulted
412 feathers collected across multiple years creates risk of duplicate sampling, especially since
413 individual choughs typically breed at the same nest site in multiple years (Trask *et al.* 2016).
414 Duplicate feather samples from the same individual were identified and excluded by
415 comparing the genotype obtained from each feather sample together using the program Gimlet
416 v1.3.3 (Valière 2002). Additionally, at nest sites where colour-ring observations showed that
417 the same individual was present across consecutive years, samples that differed at <2 loci or
418 had missing data were assumed to be from the same individual, and such duplicates were
419 excluded. In total, from 109 feather samples genotyped, 16 were duplicates and were excluded
420 from further analysis.

421

422

423 **Table S10.** Microsatellite locus name, GenBank accession number, PCR primer sequence with fluorophore shown in square brackets, annealing
 424 temperature (T_a), repeat motif and allele size ranges are shown. Number of alleles per locus for the Scottish red-billed chough population (N_a)
 425 and observed (H_o) and expected (H_e) heterozygosity values were calculated using GenAlex v6.5 (Peakall & Smouse 2012). Frequencies of null
 426 alleles, $F(\text{null})$, were calculated in MICRO-CHECKER v2.2.3 (van Oosterhout *et al.* 2004). These loci were developed specifically for use in
 427 red-billed choughs (Wenzel *et al.* 2011).

Locus name	GenBank accession no.	Primer sequence (5'-3')	T_a (°C)	Repeat motif	Allele size range	N_a	H_o	H_e	$F(\text{null})$	
Ppy-001	JF304556	F: TCCCAACAAAGCAACAAACA	60 →50	(TACA) ₂ TACT(TACA) ₃ T	150-179	3	0.50	0.51	0.17	
		R: [NED]TGGCAAAAACGAAAGACTAGC	^{TD}	GCA(TACA) ₃ TAGA(TAT						
				A) ₂ (CA) ₄						
Ppy-003	JF304558	F: [6 FAM]	60 →50	(ATCT) ₈	292-344	3	0.26	0.23	0.21	
		CAGCAGTCCGGATAAGAACA	^{TD}							
		R: CTTCCACCTTAGCATTTTT								
Ppy-004	JF304559	F:CCTTGCTGTCTGTTCAAATAA	60 →50	(AGAT) ₂ AGGT(AGAT) ₁₂	174-295	3	0.19	0.17	0.16	
		R: [6 FAM]	^{TD}							

TTGGCATGCATGAAATTTGT										
Ppy-005	JF304560	F: CTGTCTCCCAGCAGAGAACC	60	→50	(TATC) ₃ TCTC(TATC) ₇ GA	222-242	2	0.91	0.50	0.37
		R: [NED] TCGCTCCATGCTTTTATTCC	TD		TCTATCTGTC(TATC) ₂					
Ppy-007	JF304562	F: [NED]	60	→50	(GATA) ₁₅	161-193	5	0.71	0.72	0.67
		AGGCTCTAAACGTGAGGAATT	TD							
		R: CTTCTCCTTTAGAGATATC								
Ppy-008	JF304563	F: AGAGAGATTTTACCATGGGAGAT	55	→45	(GATA) ₉ GACA(GATA) ₅	233-340	4	0.50	0.51	0.03
		R: [HEX]	TD							
		AGACTGATTGCCGGACTTTG								
Ppy-009	JF304564	F: CACAGGTCAATATGGGCATC	60	→50	(GT) ₃ (AAGT) ₉	222-238	2	0.06	0.09	0.09
		R: [PET]	TD							
		CCGACTGAGCATTAAAGGTG								
Ppy-010	JF304565	F: AACCTGTTGCTTGGCATT	60	→50	(CA) ₂₇	108-146	3	0.28	0.31	0.27
		R: [6 FAM]	TD							
		ACAAACGTGAAGACAGAGAGAGC								
Ppy-011	JF304566	F: [HEX]	60	→50	TAGA(TA) ₂ GA(TAGA) ₁₂	160-191	3	0.76	0.63	0.55
		GAGAGATGTCGTTATCACTTCCAA	TD							

		R: CCAGCAGAATATGCCATTCC								
Ppy-012	JF304567	F: [PET] 60 →50 TAGA(TA) ₂		210-266	2	0.28	0.27	0.23		
		AGGGAAGGGCAACGTATGTA	^{TD}							
		R: TCATGACAGTTTCCCCAAAA								
Ppy-013	JF304568	F: AGCTCACTTCTTGCTCACAGTTT	60 →50	(TAGA) ₂ (GATA) ₁₃ (GACA	197-221	4	0.70	0.59	0.49	
		R: [6 FAM] ^{TD}) ₂ (GATA) ₄						
		GCTTCAGGCTGTTCTATCTATC								
Ppy-014	JF304569	F: GGCCTTGAAAGAAGTGTGCT	60 →50	(GATG) ₇ GACAGATT(AG	239-275	2	0.32	0.27	0.23	
		R: [HEX] ^{TD}		AT) ₃ (AGAC) ₂ (AGAT) ₃ (G						
		GCCTGATCCTCTTCTTGCTTT		GAT) ₄						
Ppy-016	JF304571	F: [NED]GTCTTCTCCAACCCAAACCA	60 →50	(GGAT) ₂₂	210-266	6	0.43	0.37	0.35	
		R: TCTCCTTCCTTTGCAACACA	^{TD}							

428 **S4.2 Single-sample genetic estimates**

429 N_e can be estimated from molecular genetic data using temporal or single-sample approaches.
430 The temporal approach requires genotype samples from two or more time-points separated by
431 at least one generation time T , to measure allele frequency change (Waples 1989). This
432 approach is consequently impractical for species like choughs that have long T , and where
433 historical DNA samples are not available (Palstra & Ruzzante 2008). Single-sample
434 approaches utilizing linkage disequilibrium (LD, Do *et al.* 2014) and approximate Bayesian
435 computation (ABC, Tallmon *et al.* 2008), which require one sampling time-point, were
436 instead used to estimate N_e for the focal population. Genotype data from a mixed-age sample
437 of breeding adults from across Islay was used and, due to our non-invasive sampling of
438 surviving adults, comprehensive genetic data from single cohorts were not available.

439

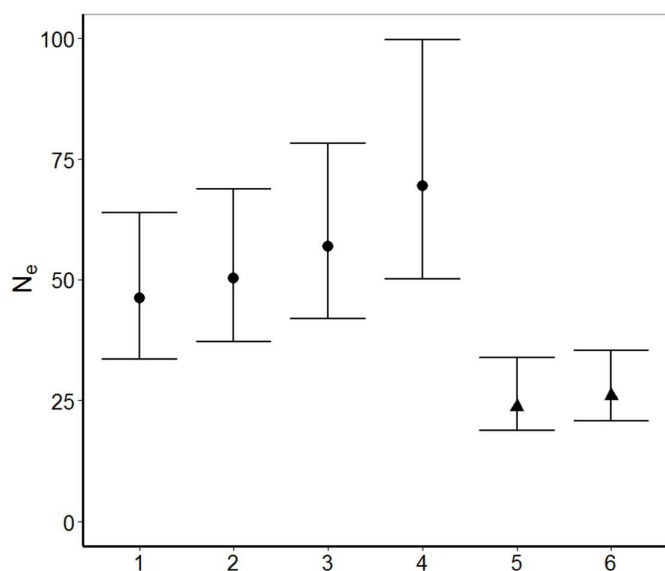
440 The LD method has been suggested to be reasonably unbiased by overlapping generations
441 when a mixed-age sample of mature adults is used, if the number of cohorts represented in
442 the sample is roughly equal to the generation length (Waples, Antao & Luikart 2014).
443 However, the LD method has also been postulated to underestimate N_e when sampling adults
444 from different cohorts as the Wahlund effect can also cause LD, which is then attributed to
445 genetic drift (Waples *et al.* 2014). In our study, a large downward bias in the LD estimate of
446 N_e seems unlikely as this approach yielded the highest N_e estimate.

447

448 Including low frequency alleles in the LD estimation of N_e can upwardly bias estimates
449 (Waples & Do 2010). We therefore re-estimated N_e excluding alleles that occurred below
450 frequencies of 0.05, 0.02 and 0.01. In concordance with previous studies (Waples 2006;

451 Waples & Do 2010), including alleles that occurred at a low frequency gave higher N_e
452 estimates (Fig. S3).

453 We also tested whether N_e estimated from summary statistics in ONeSAMP was sensitive to
454 different upper prior boundaries. However, estimates were very similar given priors of 2-100
455 and 2-180 (Fig. S3).



456

457 **Fig. S3.** Effective population size (N_e) estimates using single-sample linkage disequilibrium
458 (LD, filled circles) and ONeSAMP summary statistics (filled triangles) methods, with
459 associated 95% confidence intervals. Estimates from LD use different critical allele
460 frequency values of 0.05 (1), 0.02 (2), 0.01 (3) and all alleles included (4). Estimates from
461 ONeSAMP use two different upper priors of 180 (5) and 100 (6).

462

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466 **References**

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Colour-ringed red-billed choughs (*Pyrrhocorax pyrrhocorax*). Photograph by Gordon yates, provided by the Scottish Chough Study Group.

244x158mm (96 x 96 DPI)