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Between-individual variation in nematode burden among juveniles in a wild host

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1 **Between-individual variation in nematode burden among juveniles in a wild host**

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16 **Running title:** Variation in nematode burdens of juvenile birds

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18

19   ABSTRACT

20   Parasite infection in young animals can affect host traits related to demographic processes such as

21   survival and reproduction, and is therefore crucial to population viability. However, variation in

22   infection among juvenile hosts is poorly understood. Experimental studies have indicated that

23   effects of parasitism can vary with host sex, hatching order and hatch date, yet it remains unclear

24   whether this is linked to differences in parasite burdens. We quantified gastrointestinal nematode

25   burdens of wild juvenile European shags (*Phalacrocorax aristotelis*) using two *in situ* measures

26   (endoscopy of live birds and necropsy of birds that died naturally) and one non-invasive proxy

27   measure (faecal egg counts). *In situ* methods revealed that almost all chicks were infected (98%),

28   that infections established at an early age, and that older chicks hosted more worms, but faecal egg

29   counts underestimated prevalence. We found no strong evidence that burdens differed with host sex,

30   rank or hatch date. Heavier chicks had higher burdens, demonstrating that the relationship between

31   burdens and their costs is not straightforward. *In situ* measures of infection are therefore a valuable

32   tool in building our understanding of the role that parasites play in the dynamics of structured

33   natural populations.

34

KEY FINDINGS

- We quantified nematode burdens of seabird chicks using necropsy, endoscopy and FECs
- *In situ* techniques showed 98% prevalence, early establishment and higher burdens with age
- Faecal egg counts, a proxy measure, underestimated prevalence
- Chicks with higher burdens weighed more, contrary to expectations if infection is costly
- Endoscopy of juveniles enables monitoring of wild hosts' infections across their lifetime

44   INTRODUCTION

45

46   The costs that parasite infection can impose on their hosts can influence key demographic traits,

47   such as reproductive success and survival, which are crucial to the growth rate and hence viability

48   of populations (Albon et al., 2002; Newey et al., 2005; Redpath et al., 2006, Tompkins 2011).

49   However, parasitism is unlikely to affect all individuals in a population in the same way. Firstly,

50   individuals may host different burdens as a result of differences in exposure to parasites,

51   susceptibility to infection and resistance to its impacts. This contributes to parasite abundance

52   typically showing a skewed distribution among hosts, which is particularly well documented in

53   macroparasites (Randolph et al., 1999; Shaw and Dobson, 1995). Secondly, once parasitized, the

54   relationship between parasite load and host fitness may vary between individuals due to differences

55   in tolerance for a given parasite load. Siblings, for example, may vary in the level of maternal

56   antibodies they receive (Pihlaja et al., 2006), males may be affected more than females due to

57   immunosuppressive effects of testosterone (Mougeot et al., 2009), and the relative benefits of

58   allocating resources between fighting infection and reproduction may vary with age (Adamo et al.,

59   2001). These factors may lead to different types of host responding differently to infection, with

60   consequences for key host traits related to fitness such as weight gain during critical periods of

61   growth. Understanding how parasite burdens and their impacts on hosts vary between different

62   classes of individual may therefore be crucially important for understanding the impacts of parasites

63   on heterogeneous host populations.

64   A key process for population viability is the level of offspring recruitment to the breeding

65   population. Understanding how parasitism impacts on the juvenile subset of the population is

66   therefore important for modelling population growth. Infection in early life can alter juveniles'

67   developmental trajectories (Fitze et al., 2004; Romano et al., 2011), with potentially life-long fitness

68   consequences that may further influence demographic processes such as reproduction and survival

69   long after recruitment (Lindström, 1999; Metcalfe and Monaghan, 2001; Monaghan, 2008).

70   However, despite the importance of early-life infection, between-individual patterns of parasitism

71   and the development of infections in juvenile hosts have not been widely investigated in the wild.

72   Although young hosts have been shown to exhibit systematic between-individual differences in

73   their response to experimental infection or anti-parasite treatments according to characteristics such

74   as hatching order (Granroth-Wilding et al., 2014), sex (Romano et al., 2011) or timing of breeding

75   (Reed et al., 2008), it remains unclear whether variation in response is associated with differences in

76 burden or differences in tolerance. Thus, quantifying individual parasite burdens across the juvenile

77 component of the population, where individuals' responses to infection are also known by

78 measuring key fitness-related traits, is central to accurately predicting parasite impacts at the

79 population level.

80   Quantifying parasite burdens is logistically challenging in the wild, particularly for

81 endoparasites that often make up the majority of a host's parasite biomass (Hoberg, 2005). Necropsy

82 allows direct counts of parasites in the host and is widely considered to give the most reliable

83 measure. However, this destructive method prevents longitudinal studies, which are crucial for

84 detecting associated fitness consequences such as recruitment probability and future reproductive

85 success (Fitze et al., 2004). In juvenile hosts, such sublethal impacts typical of macroparasites have

86 the potential to affect key demographic parameters over a range of timescales; avoiding destructive

87 sampling is therefore particularly important to understand the full fitness consequences of infection

88 in young hosts. Moreover, necropsy may not be viable for hosts of conservation importance. Faecal

89 egg counts (FECs) are a common non-destructive and non-invasive proxy measure of endoparasite

90 burden (e.g. Bowman and Georgi, 2009; Craig *et al.* 2006; Seivwright *et al.* 2004), but may not

91 always reflect true parasite burden due to variable rates of helminth egg production (Shaw and

92 Moss, 1989; Tompkins and Hudson, 1999), poor sensitivity at low burdens (Levecke *et al.* 2009),

93 and not representing larval helminths that do not produce eggs but can nonetheless be costly to the

94 hosts (Fagerholm and Overstreet, 2008). Recent work in wild adult seabirds has pioneered

95 endoscopy as an additional, direct and reliable method to obtain an index of gastrointestinal

96 nematode burdens in live individuals (Burthe et al., 2013). This approach has great potential for

97 quantifying the development of an individual's infection from an early age, but has not previously

98 been applied to juveniles in the wild.

99   Here, we use two *in situ* measures of parasite burden – necropsy and endoscopy – and the

100 proxy measure of FECs to quantify patterns of between-individual variation in the trophically-

101 transmitted gastrointestinal nematode burden of juvenile European shags (*Phalacrocorax*

102 *aristotelis*, henceforth "shag"), a piscivorous seabird. Experimental manipulations of parasite load

103 in adults and chicks has shown that responses to treatment vary with host phenotype: treatment of

104 parents increases male chicks' survival, particularly late in the season, but not female chicks' (Reed

105 et al., 2008); treatment of chicks generally affects the growth rate of last-hatched siblings but not

106 the older brood members (Granroth-Wilding et al., 2014); and the impact of simultaneous treatment

107 of parents and their offspring differs between early- and late-nesting families (Granroth-Wilding et

108 al. 2015). Endoscopy of adults has found males to host more worms than females and late breeders

109 more than early breeders (Burthe et al., 2013), but among juveniles, patterns of variation in parasite

110 abundance and their link with variation in host fitness are not well quantified. It is hence unclear

111 whether these differences in treatment responses between types of juveniles arise from differences

112 in nematode burden or differences in the impact of a similar burden. Moreover, the link between

113 parasite burden and demographically important host traits is unexplored. Our objectives were

114 therefore to: 1. quantify individual parasite burdens of juveniles using two *in situ* methods,

115 endoscopy and necropsy, and compare these to a proxy measure of prevalence based on FECs; 2.

116 identify whether burdens vary with host age, sex, hatching order and hatch date; 3. examine whether

117 natural variation in parasite abundance is associated with a fitness-related trait, host mass.

118

119 METHODS

120

121 *Host-parasite system*

122 This study was carried out in 2012 in the breeding population of shags on the Isle of May National

123 Nature Reserve in south-east Scotland (56º11 N, 2º33 W) that has been the subject of an individual-

124 based long-term demographic study for several decades. Shags are sexually dimorphic, with males

125 growing faster to reach an adult size c. 20% bigger than females (Daunt *et al.* 2001). The modal

126 clutch size in this population is three eggs and these hatch asynchronously, with the second and

127 third siblings (B and C chicks) hatching on average 1 and 2-3 days after the first (A chick). This

128 asynchrony results in a hierarchy of size within the brood, in which youngest siblings generally

129 grow more slowly and have higher mortality but are more plastic in response to changing

130 environmental conditions than their older counterparts (Granroth-Wilding *et al.* 2014; Stokland and

131 Amundsen, 1988). Breeding success declines through the season, with later breeders fledging fewer

132 chicks and producing fewer recruits (Daunt *et al.* 1999; Harris *et al.* 1994).

133   Shags on the Isle of May are infected with the gastrointestinal nematode *Contracaecum*

134 *rudolphii* (Anisakidae: Ascaridoidea; Hartwich 1964), which occur in the GI tract of nestling and

135 adult shags in this population (Reed 2007; Burthe *et al.* 2013; E. Harris, pers. comm.; S. Burthe, J.

136 Chantrey & D. Kowalek, unpublished data). All but one of 146 naturally infected adults endoscoped

137 to date have hosted worms, with wide variation in burdens from 2 to >80 worms (Burthe *et al.*

138 2013; S. Burthe & E. Butterfield, unpublished data). *C. rudolphii* is a widely distributed seabird

139 specialist, now recognised to comprise a complex of morphologically similar species (Anderson,

140 2000; Fagerholm and Overstreet, 2008; Hoberg, 2005; Moravec, 2009). Nestling shags obtain

141 regurgitate fish directly from their parents' throats and are infected with larval worms in the fish

142 tissue. Direct infection of chicks with adult worms dislodged from the parent's proventriculus could

143 also occur during feeding, but the importance of this transmission route is not well understood

144 (Dubinin, 1949; Fagerholm and Overstreet, 2008; Hoberg, 2005; Huizinga, 1971). Anisakid

145 infection can cause costly pathology at attachment sites such as inflammation, necrosis,

146 haemorrhaging and perforation of the stomach wall (Hoberg, 2005; Kuiken, 1999; McClelland,

147 2005), which may be compounded by secondary bacterial infections (Fagerholm and Overstreet,

148 2008), and is expected to activate a costly immune response (Colditz, 2008; Hasselquist and

149 Nilsson, 2012). Moreover, *Contracaecum* is thought to feed on fish ingested by the bird and thus

150 directly competes with the host for resources (Abollo *et al.* 2001; Anderson, 2000; Dubinin, 1949;

151 Huizinga, 1971).

152

153 ***Quantifying nematode burdens***

154 We quantified the nematode burden of individual shag chicks using two *in situ* techniques,

155 endoscopy of targeted individuals or necropsy (dissections) of a subset of the study population that

156 died naturally during a severe storm. We also conducted faecal egg counts (FECs) on faecal

157 material collected opportunistically from both endoscoped and dissected chicks (all detailed

158 methods below). Not all individuals produced faecal samples, precluding FECs, and no birds were

159 both endoscoped and dissected, as endoscoped chicks were not sacrificed and endoscopy of dead

160 animals is not reliable (S. Burthe, unpublished data).

161

162 *Endoscopy*

163 We used a refurbished 9mm diameter medical endoscope (Olympus©, UK) to view the oesophagus

164 and proventriculus of conscious chicks under licence (full details of endoscopy procedure in

165 (Burthe *et al.* 2013). Endoscopy was undertaken by a trained and experienced operator (S. Burthe)

166 while an assistant held the bird still and its bill open. A cloth was placed over the bird's eyes to

167 reduce stress while the endoscope operator inserted the endoscope into the proventriculus. All

168 worms that were visible were counted as the endoscope was slowly withdrawn from the bird. We

169 noted whether the worms were large or small. Visibility was scored on a scale of 1 to 5 (worst to

170 best, as in Burthe *et al.* (2013)) and included in all analyses as poorer visibility could hinder

171 accurate quantification. Endoscopy was carried out when chicks were large enough for the

172 endoscope to be comfortably inserted, around 25 days of age. Throughout the process, there was no

173 evidence of discomfort (e.g. rapid breathing). All endoscoped chicks resumed normal behaviour

174 immediately on being returned to the nest and all fledged successfully. All endoscopy was carried

175 out early in the morning, before parents had returned with the first food load of the day, to avoid

176 views being obstructed by recently ingested food.

177   At endoscopy, chicks were assigned a rank in the brood hierarchy according to size: in broods

178 of three, the heaviest two chicks were designated AB and the lightest C, and in broods of one or

179 two, all chicks were designated AB. Wing length was used as an additional indicator if mass

180 difference was not greater than 20g. Mass at day 25-30 accurately identifies the last-hatched chick

181 in 83% of cases but only distinguishes the first- and second-hatched (A and B) in 47% of cases,

182 whereas A and B are accurately assigned as AB in 89% of cases (data from 27 nests, with three

183 chicks surviving to day 10, in 2010 and 2011 with accurate hatch dates; Granroth-Wilding *et al*.

184 2014). We used chick mass at endoscopy as an indicator of chick performance. At endoscopy age,

185 the majority of growth is completed (Daunt *et al.* 2001), and fledging mass has been shown to

186 correlate with recruitment in a range of species (Magrath, 1991; Schwagmeyer and Mock, 2008).

187 All endoscoped chicks were blood sampled for molecular sexing (Griffiths *et al.* 1996).

188   In total, we endoscoped 45 chicks in 20 nests, of which 18 were undisturbed before

189 endoscopy and 27 were sham-treated controls from a parallel parasite-removal experiment (full

190 details in Supplementary Information; no individuals treated with anti-parasite drugs are included in

191 the results presented here), injected with 0.05ml saline solution at age 10-12 days and subsequently

192 weighed at ages 10, 15 and 25 days. A subset of chicks that remained safely accessible as they got

193 older and more mobile were endoscoped twice (2 untreated chicks and 4 sham-treated).

194

195 *Necropsy*

196 Sacrificing individuals for systematic necropsies was not possible as this would prevent longitudinal

197 investigations of the link between parasite burden and host survival, and moreover removing

198 individuals from this long-term study population is not desirable. However, in 2012, there was an

199 unusually prolonged period of rain and cold weather in the middle of the peak chick-rearing period,

200 lasting over two days. This caused considerable natural juvenile mortality due to waterlogging and

201 chilling of chicks that were still downy (not yet waterproof) but too large to be efficiently sheltered

202 by their parents. Mortality was thus not a direct consequence of overall poor condition nor of

203 parasitism, though both factors may have contributed. Similar weather-related mass mortality

204 events of chicks during the breeding season have only occurred once in the last 15 years, so this was

205 a rare opportunity to obtain a sample of birds for dissection. When the weather improved and it was

206 safe to approach nests, c. 12-36 hours after death, we collected 28 carcasses (median 20 days old,

207 interquartile range 18-26 days; median hatch date 27th May, IQR 21st-29th May; cf. endoscoped

208 chicks, median age 31 days, IQR 28-34 days, median hatch date 17th May, IQR 15th-22nd May).

209 Nine of these were sham-treated controls from the parallel experiment. We also collected 6 further

210 carcasses resulting from other natural mortality, found within a day of death, for necropsy (median

211 age 25 days, IQR 25-29 days; median hatch date 2nd June, IQR 20th May-3rd June). For the 10

212 dissected chicks that were not of known age, we estimated age from wing length based on the

213 growth rate of chicks from the same year with known hatch dates (Wing = 5.81 x Age – 27.75; in

214 mixed model accounting for repeated measures within chick, $F_{1,147} = 9795$, $p < 0.001$; without

215 random effects, $r^2 = 0.954$). We assigned ranks to dissected chicks in cases where the whole brood

216 could be assessed either dead or alive, based on the structural measure of wing length: in broods of

217 three, the two chicks with longest wings were assigned AB and the shortest C, and in broods of one

218 or two, all chicks were assigned AB. A sample of blood or tissue was taken from every carcass for

219 molecular sexing (Griffiths *et al.* 1996).

220   Where possible, carcasses were dissected fresh within 6 hours of recovery, or kept at +4°C

221 for up to 24 hours (16 carcasses). If dissections could not be carried out within this time (17

222 carcasses), they were stored at -20°C for up to one week and defrosted before dissection. The

223 proventriculus was removed together with 3cm of oesophagus and small intestine. The removed

224 gastrointestinal portion was then opened out using one medial ventral cut and the stomach contents

225 thoroughly examined, then rinsed with water through a fine mesh. The body cavity was examined

226 for evidence of nematodes migrating away from the proventriculus following host death, and we

227 additionally examined the whole intestine of four individuals; no other visible macroparasites were

228 found (further descriptions in Supplementary Information). All worms were counted, removed and

229 stored in ethanol. To obtain an index of the maturity of the infection in the bird, during which stage

230 *Contracaecum* undergoes substantial growth (Fagerholm and Overstreet, 2008), worms were

231 categorized into size classes based on width (>0.75mm wide, large; <0.5mm wide, small; 159 out of

232 1436 worms (11%) in between the categories).

233

234 *Faecal egg counts (FECs)*

235 During endoscopy, we opportunistically collected faecal samples from 19 chicks that defecated

236 during handling, and from 24 dissected chicks, we obtained a faecal sample from the cloaca after

237 carcasses had been frozen at -20°C for long-term storage. All faecal samples were therefore stored

238 at -20°C after collection; previous work in this system has given no evidence that freezing affects

239 egg counts or prevalence (Supplementary Information). FECs were carried out using a flotation

240 technique (Bowman and Georgi, 2009; detailed methods in Supplementary Information). Each

241 sample was suspended in 20 ml saturated salt solution per 1 g of faeces and nematode eggs were

242 counted in 0.45 ml (0.02 g faeces) of the suspension examined under a McMaster slide.

243

244 *Statistical analysis*

245 We first quantified patterns in parasite abundance obtained by each *in situ* parasite measure,

246 endoscopy and dissection. We considered two aspects of nematode infection: total worm burden,

247 indicating overall parasite abundance, and the proportion of worms that were large, which is likely

248 to reflect the duration of the infection. We then tested whether these indices were associated with

249 host age, as expected if chicks are exposed to infective larvae throughout their development, and

250 with phenotypic traits known to affect responses to infection: host sex, rank (AB vs. C) and hatch

251 date (Granroth-Wilding *et al.* 2014; Reed *et al.* 2008, 2012). Lastly, in endoscoped chicks, we

252 examined the association between parasite abundance and chick performance by testing whether

253 chick mass at endoscopy varied with worm count and the proportion of worms that were large.

254   In all analyses of dissected chicks, we excluded two outliers with high statistical leverage:

255 one old chicks with a very high load (a male, 45 days old, hosting 243 worms; range of other chicks

256 8-148 worms) and one very young chick (ca. 2 days old) which was the only dissection that yielded

257 a zero burden. Neither exclusion qualitatively affected any results. Although mortality is generally

258 higher for C chicks in this population (Granroth-Wilding *et al.* 2014), all ranks were equally

259 represented among endoscoped and dissected birds, as were males and females (for ranks across

260 techniques, $\chi^2 = 4.50$, df = 2, p = 0.105; for sexes, $\chi^2 = 1.32$, df = 1, p = 0.251). Among endoscoped

261 chicks, we confirmed that visibility score was not related to age, sex, rank or hatch date (all p > 0.4

262 in a linear model). Among endoscoped chicks, hatch date (from which age was calculated) was only

263 available for the first-hatched chicks in each nest, so C chicks were assigned an age 2.5 days

264 younger than their AB siblings (median age difference across 42 nests in 2010 and 2011 with

265 accurate hatch date data) to avoid within-brood age differences confounding rank effects. Among

266 dissected chicks, the effects of age and hatch date could only be examined in separate models as the

267 age-specific main mortality event meant that they were closely correlated (in linear model, $r^2 = 0.72$,

268 p < 0.001). In these analyses, models containing hatch date instead of age gave almost identical fits

269 (ΔAICc ≤ 0.1) and for brevity we present only the age models.

270   All analysis was carried out in R 3.0.2 (R Core Team, 2013) using the packages lme4 (Bates

271 *et al.* 2011) and nlme (Pinheiro *et al.* 2012), using (generalised) linear mixed models (LM Ms or

272 GLMMs). To account for repeated sampling of some individuals and non-independence of siblings

273 within a brood, we fitted chick within nest as nested random factors to the endoscopy data, and nest

274 as a random factor to the dissection data. Total burden was fitted as count data with poisson errors

275 and logistic link function, and proportion of large worms with binomial errors, weighted by the total

276 count, and a logit link function. Effect sizes for the proportion of large worms are presented as the

277 log odds of a worm being large. Mass was modelled in a linear mixed model including log(age) and

278 sex as fixed effects, to account for the non-linear growth curve and sexually dimorphic growth. Due

279 to the low egg prevalence in faeces preventing robust analysis of relationships between FECs and

280 host phenotypic traits or *in situ* worm burdens, we present only descriptive statistics of prevalence

281 (but see Supplementary Information for a preliminary analysis).

282   We used an information theoretic approach to model selection (Burnham and Anderson,

283 2002), identifying important explanatory variables based on the best-fitting model(s) from a

284 candidate set, which is well suited to an exploratory analysis. For each measure of parasite burden,

285 our set of candidate models contained all combinations of the explanatory variables as main effects

286 (age, hatch date, sex and rank, and additionally for endoscopy analyses, visibility) as well as an

287 intercept-only (null) model. The best-fit model was the one that had the lowest AICc (corrected

288 Akaike's Information Criterion, suitable for small sample sizes) in the set, and models with a ΔAICc

289 ≤ 2 from the best fit model were considered an equivalent fit. Model selection based on significance

290 testing gave the same conclusions. All parameters are presented ±1 standard error, not back-

291 transformed from the log (worm counts) or logit (proportion of large worms) link functions.

292 RESULTS

293 *Quantifying worm burden in situ*

294 The ages of birds available for necropsy ranged from 2 to 45 days and for endoscopy from 25 to 49

295 days. Worm burden measured using necropsy varied from 0 to 243 worms per chick; the youngest

296 and oldest chicks were excluded from further analysis due to their strong leverage, giving an age

297 range of 12–31 days and worm counts of 8 to 148 worms per chick (n = 31; mean 36.0 ± 4.9;

298 prevalence 100%) (fig. 1). Worm burden measured using endoscopy ranged from 0 to 30 worms

299 per chick (mean worm burden 11.7 ± 1.0 worms; prevalence 98%) (fig. 1). The proportion of large

300 worms ranged from 0 to 35.7% (mean 12.9 ± 1.9%) by necropsy and 0 to 100% (mean 29 ± 5%) by

301 endoscopy.

302   Using necropsy, the youngest chick to host large worms was aged 15 days and the oldest

303 chick without large worms was 18 days. Using endoscopy, large worms were found in chicks from

304 the age of 26 days, (earliest available age 25 days), although chicks with no large worms occurred

305 up to the age of 36 days.

306   Visibility during endoscopy was generally poorer for chicks than for adults endoscoped in

307 parallel studies, mainly due to the presence of semi-digested food. Visibility scores among the

308 chicks in this study ranged from 1 to 4 (mean 2.7 ± 0.1) compared to a range of 3-5 (mean 4.24;

309 n=17) for adult shags endoscoped in the same year (S. Burthe, unpublished data).

310

311 *FECs as an indicator of worm burden*

312 We obtained faecal egg counts from 19 endoscoped and 24 dissected chicks from birds aged 25–36

313 days and 12-45 days respectively. Nematode eggs were only found in one third of the 43 samples

314 available (prevalence 37%), despite a prevalence of 99% in individuals sampled using *in situ*

315 measures. Out of the 16 faecal samples that contained worm eggs, only 7 contained more than 1 egg

316 (4 samples with 2 eggs, 2 with 3 eggs and one with 42) and 5 were from chicks in which no large

317 worms were seen (1 necropsy, 4 endoscopies).

318

319

320 *Nematode burden in relation to host traits*

321   In necropsied chicks, aged 12–31 days, worm count was best explained by a model containing

322 only age, with older chicks hosting more worms. A model with age and sex had similar support

323 (table 1, fig. 2). The proportion of worms that were large was best explained by an intercept-only

324 model, with no host traits providing similar explanatory power (table 1, fig. 3).

325 Among endoscoped chicks, aged 25–49 days, total worm burden was best described by a

326 model containing age and visibility (table 1, fig. 2), with older chicks hosting more worms and

327 better visibility resulting in slightly higher worm counts (age effect size 0.10 ± 0.02

328 log(worms)/day, visibility effect size 0.10 ± 0.06 log(worms) per score increment). Age was

329 supported in all five top models. Out of three equivalent-fit models, two contained a rank term (in

330 addition to age, C chicks hosted -0.21 ± 0.16 fewer worms than AB chicks). The proportion of large

331 worms was best described by a model containing only age (effect size 0.11 ± 0.04 increase in

332 proportion of large worms/day) (fig. 3), with hatch date and rank each occurring twice in the three

333 equivalent-fit models (in addition to age, effect of hatch date: 0.05 ± 0.00 greater proportion of large

334 worms per day; effect of rank: C chicks 0.83 ± 0.50 greater proportion of large worms) (table 1).

335 A summary of the host traits identified as important to parasite abundance and size

336 distribution by the two measurement techniques is given in table 2. For both necropsy and

337 endoscopy, it is notable that individuals varied considerably in their parasite load, which contributed

338 to many analyses yielding several equivalent-fit models that made it difficult to robustly identify

339 phenotypic traits that influenced parasite load.

340

341 *Effect of infection on host performance*

342 Chick mass at endoscopy was best explained by a model containing main effects of age and worm

343 count (table 3, fig. 4): heavier chicks were older and had higher worm counts (in best-fit model,

344 effect of age 241.4 ± 43.2 g/log(day); effect of worm count, 11.8 ± 4.8 g/worm). There was one

345 model of equivalent fit, which contained an additional sex term (males 62.4 ± 46.6 g heavier than

346 females).

347

348

349 DISCUSSION

350 The juvenile period is an energetically expensive phase for an individual when the costs associated

351 with parasite infection are likely to have substantial impacts on hosts. Despite this, in comparison to

352 adults, there is very little information for wild juvenile hosts on patterns of parasite prevalence or

353 abundance, particularly internal parasites. Here we have used necropsy and endoscopy,

354 implemented for the first time in juveniles in the wild, to show that infection with gastrointestinal

355 nematodes is near-universal among nestling shags (98% prevalence) and establishes at an early age,

356 and that nematode burden increases with chick age. In contrast, the common proxy measure of

357 FECs suggested a prevalence of only 37%, demonstrating the value of endoscopy as a non-

358 destructive index of *in situ* parasite burden. Previous studies have found chick sex, hatch date and

359 rank to be important in determining responses to anti-parasite treatment (Reed *et al*. 2008, 2012;

360 Granroth-Wilding *et al*. 2014, 2015), yet we found no strong evidence that worm burden varied

361 with any of these host traits. This suggests that differences in response may arise due to variation in

362 tolerance between the subclasses of juvenile as opposed to differences in burden. Further, contrary

363 to predictions, we found that individuals with high worm burdens were heavier than similar-aged

364 individuals with lower burdens.

365

366 *Comparison of techniques for quantifying worm burden*

367 Both necropsy and endoscopy captured the same main pattern of infection in chicks but

368 unfortunately we did not have the opportunity to directly compare counts from the two techniques

369 in the same individuals. None of the birds that suffered natural mortality had been endoscoped,

370 endoscoped chicks could not be sacrificed for necropsy as this would prevent long-term monitoring

371 of infection and its consequences, and endoscopy of carcasses is not feasible as reliable counts are

372 difficult to obtain from the collapsed stomach of a dead bird. Comparisons of necropsied and

373 endoscoped individuals was further constrained by the limited overlap in the ages of chicks used in

374 each technique: endoscopy was carried out on generally older birds and tended to yield lower

375 overall burdens but a higher proportion of large worms than necropsies of generally younger birds.

376 Endoscopy may have yielded lower counts because chicks' stomachs frequently contained residual

377 food that partially obscured the view through the endoscope, a constraint that is more easily avoided

378 when endoscoping adults in this system (Burthe et al. 2013). Nonetheless, endoscopy counts from

379 shags have been shown to be repeatable (Burthe et al. 2013), and our successful application of this

380 technique to developing hosts thus opens opportunities for monitoring individuals' worm burdens

381 from an early stage in their long lives. Moreover, both *in situ* techniques identified similar

382 prevalences and an increase in burden with chick age, indicating that endoscopy provides a useful

383 index of between-individual variation in worm burdens. This index has already been shown to be

384 valuable for quantifying the effect of anti-parasite treatment in both adults and juveniles, even at

385 low doses (Burthe et al. 2013; Supplementary Information, this study).

386 Necropsy, on the other hand, allows complete examination of the gut of the animal at any age

387 and is likely to yield more accurate counts. However, as a destructive sampling technique, necropsy

388 is of limited application because removing individuals from the population is not desirable when

389 investigating longitudinal effects of parasite infection or working with protected natural

390 populations. In such cases, obtaining samples relies on natural mortality that may more strongly

391 affect certain parts of the population, such as those already paying the costs of a high parasite

392 burden. Moreover, necropsy of recovered carcasses may underestimate infection intensity due to

393 post-mortem migration of nematodes away from attachment sites. Given that the endoscope counts,

394 also likely underestimates, captured the same broad patterns of infection as necropsy, we suggest

395 that endoscopy provides an informative non-destructive index, albeit not true counts, of between-

396 host variation in total parasite burden. The repeated measurement of an index of infection intensity

397 across individuals' lives that this enables, while also allowing quantification of its long-term

398 consequences for host fitness, is likely also to be of practical use in other systems.

399   Measuring long-term patterns in individuals' parasite burdens could potentially be made more

400 logistically tractable if a non-invasive proxy for worm burden was available, such as FECs.

401 However, in our system, FECs failed to detect the same levels of infection revealed by *in situ*

402 measures. Although worms were found in 98% of all chicks examined, the majority of faecal

403 samples (63%) did not contain eggs, and faecal egg presence was not related to *in situ* worm burden

404 (Supplementary Information). This may be due in part to chicks hosting a high proportion of worms

405 that were small, likely immature and thus not egg-producing individuals. Variation in this

406 component of the parasite community may nonetheless be important for its impacts on host fitness,

407 as larval worms can still cause severe pathology and thus have non-negligible costs (Fagerholm and

408 Overstreet, 2008; H.-P. Fagerholm, pers. comm.). The limited presence and low counts of nematode

409 eggs in host faeces in this system appears to be a feature of this system, but we cannot rule out that

410 FECs more closely reflecting natural variation in true burdens could be obtained by examining

411 larger amount of faecal material (but see Supplementary Information), which is logistically difficult

412 in the field.

413

414 *Nematode burden in relation to host traits*

415 The positive relationship between worm burden and chick age is consistent with expectations that

416 chicks' infections should intensify throughout the nestling period. This increase suggests that chicks

417 are continuously exposed to either infective larvae in fish and/or adult worms dislodged from the

418 parent's proventriculus during feeding. Continuous exposure among chicks accords with the near-

419 universal prevalence of worms among endoscoped adult shags over 6 study years (S. Burthe,

420 unpublished data), which in turn indicates regular exposure to infected fish (Anderson, 2000;

421 Fagerholm and Overstreet, 2008; Huizinga, 1971). Two further observations can also be interpreted

422 as indicative both of larval worms establishing and growing inside the chick and of ongoing direct

423 transmission of larger worms from the parent's proventriculus: the increase in the proportion of

424 large worms with age in endoscoped chicks, and the presence of nematode eggs in the faeces of a

425 12-day-old chick (lowest estimates for maturation time of larval *C. rudolphii* in the definitive host,

426 c. 1 week; Dubinin, 1949; Huizinga, 1971). Regardless of transmission mechanism, we found

427 established nematode infections in all chicks from early on in their period of rapid growth (from 6-9

428 days; Daunt *et al.* 2001). This supports the potential of parasitism in juvenile shags to influence

429 developmental trajectories and hence long-term performance and fitness in this long-lived species

430 (Lindström 1999; Monaghan 2008).

431   Previous studies have found host sex, timing of breeding and hatching order to be important

432 in shaping individual chicks' responses to anti-parasite treatment (Granroth-Wilding *et al*. 2014,

433 2015; Reed *et al*. 2008), yet here we found little evidence that these traits were strongly associated

434 with worm burden. This contrasts with adult shags, which display variation in burdens related to sex

435 and timing of breeding, traits that also affect responses to treatment (Burthe *et al.* 2013, Reed *et al*.

436 2008). Moreover, in our opportunistic necropsies, selective mortality may have confounded the

437 effects of certain host traits: similarly-aged individuals that died in the storm event had similar

438 hatch dates, for example, yet these two traits may influence infection intensity in different ways (for

439 example, burdens increasing due to continuous exposure with age versus a seasonal increase in

440 exposure to infective larvae) whose effects we were not able to separate.

441

442 *Effect of infection on host performance*

443   Parasitism, by definition, is considered to be costly, yet we found a positive correlation

444 between parasite burden and chick mass, a fitness-related trait that is positively associated with

445 recruitment in many bird species (Schwagmeyer and Mock, 2008). This correlation may arise as

446 chicks fed at a higher rate are likely to have higher levels of exposure to parasites, yet parasite

447 infection in both parents and chicks can also affect how resources are distributed among family

448 members (Granroth-Wilding et al. 2014, 2015). Experimental approaches that tease apart the

449 relative effects of exposure, burden and host condition are therefore needed to quantify the effect of

450 parasitism on individual performance. Examining the longer-term association between juvenile

451 worm burden and success in later life should also be a priority for future endoscopy studies in this

452　system, taking advantage of the non-destructive technique to quantify the accumulation of sub-

453　lethal impacts typical of macroparasites. Such a chain of fitness effects is of particular importance

454　where parasite infection can shape hosts' developmental trajectories and life histories (Fitze *et al.*

455　2004; Granroth-Wilding *et al.* 2014; Romano *et al.* 2011).

456

457　*Conclusions*

458　　　　Measuring natural variation between hosts in parasite burdens is an essential link in

459　understanding the role of parasites in regulating natural populations. Here, we have developed

460　endoscopy as a non-destructive method to quantify relative parasite burdens in juveniles and

461　revealed prevalence to be significantly higher than expected from more traditional proxy measures.

462　Our demonstration of widespread infection that is established and increases from as early as 12 days

463　of age highlights the potential importance of nematode infection in shaping the contribution of

464　individual shags to population processes throughout their long life (over 20 years). However, we

465　found no evidence to suggest that parasite burdens differ between subgroups of hosts that have

466　previously been found to respond differently to parasite removal. Variation in tolerance among

467　different parts of the population may therefore play a role in governing variation between hosts in

468　how they are impacted by parasitism. Our findings suggest that endoscopy of live juveniles is an

469　informative index of natural variation in parasite burdens, finding the same patterns of infection

470　across the host population as the more direct but destructive index of necropsy. In addition, our

471　results showed that relationships between parasite burden and fitness-related traits in early life are

472　not straightforward. Hence, in combination with experimental approaches, endoscopy provides a

473　powerful tool to link variation in nematode burden with its impact on host success across a wild

474　animal's life and across subgroups of the population, enabling predictions of how parasitism

475　influences on demographic processes in structured natural populations.

476

477

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Table 1. The top five best-fitting models of worm burden (left columns) and the proportion of worms that were large (right columns) as measured by necropsy (top model set) or endoscopy (bottom model set) in relation to host phenotypic traits. Models in each set are shown in order of decreasing fit with their AICc and ΔAICc relative to the best-fit model. The candidate model set for each variable included all combinations of the following predictor variables: age, hatch date, rank, sex, and for endoscopy also visibility. In the necropsy models, age and hatch date and could not be included in the same models as they were closely correlated. Accordingly, models containing hatch date gave almost identical fits to those instead containing age; to illustrate a broader range of model fits, we show only the age models here.

Model (total worm count)	d.f.	AICc	ΔAICc	Model (proportion of worms large)	d.f.	AICc	ΔAICc
Necropsy							
Age	3	207.7	0.0	(intercept only)	2	117.5	0.0
Age + Sex	4	208.3	0.6	Rank	3	119.9	2.4
Age + Rank	4	210.2	2.5	Sex	3	120.0	2.5
Age + Sex + Rank	5	211.5	3.8	Age	3	120.0	2.6
Sex	3	215.6	7.9	Rank + Sex	4	122.7	5.3
Endoscopy							
Age + Visibility	5	320.2	0.0	Age	4	190.4	0.0
Age	4	320.4	0.2	Age + Rank	5	190.5	0.1
Age + Rank	5	321.1	0.9	Age + Rank + Hatch date	6	190.5	0.1
Age + Visibility + Rank	6	321.4	1.2	Age + Hatch date	5	190.9	0.4
Age + Visibility + Hatch date	6	322.6	2.5	Age + Visibility	5	192.5	2.1

503 Table 2. A summary of patterns of variation in nematode burdens between shag chicks, as quantified

504 using necropsy or endoscopy, in relation to phenotypic host traits. Patterns were investigated in both

505 the total worm burden (top set of variables) and the proportion of worms that were large, indicative

506 of how long the chick had been infected (bottom set of variables). Traits that robustly affected

507 worm measures (occurred in all equivalent-fit models) are indicated with a tick, traits that had some

508 support (occurred in more than one equivalent-fit model) are shown with a tick in brackets, and

509 traits with no robust effects are shown with a cross. Hatch date for dissected chicks is indicated with

510 a dash to show that it could not be tested simultaneously with age, as they were tightly correlated.

511

Explanatory variable	Affects necropsy counts	Affects endoscopy counts
Total burden		
Age	√	√
Hatch date	-	x
Rank	x	(√)
Sex	x	x
Proportion large worms		
Age	x	√
Hatch date	-	(√)
Rank	x	(√)
Sex	x	x

512 Table 3. The top 5 best fit models of mass of endoscoped chicks. The set of candidate models

513 included all combinations of the following variables: worm count (measured by endoscopy),

514 log(age), sex and rank.

515

Model	d.f.	AICc	ΔAICc
log(Age) + Worm count	5	553.8	0.0
log(Age) + Worm count + Sex	6	554.6	0.8
log(Age) + Worm count + Rank	6	556.1	2.2
log(Age) + Sex	5	557.1	3.3
log(Age) + Worm count + Sex + Rank	7	557.3	3.5

516 Figure 1. Histograms showing the spread of worm counts from necropsy (left panel) and endoscopy
517 (right panel). The dissection data does not show two high-leverage individuals excluded from the
518 analysis, a hatchling with no worms and a near-fledgling with 243 worms.
519
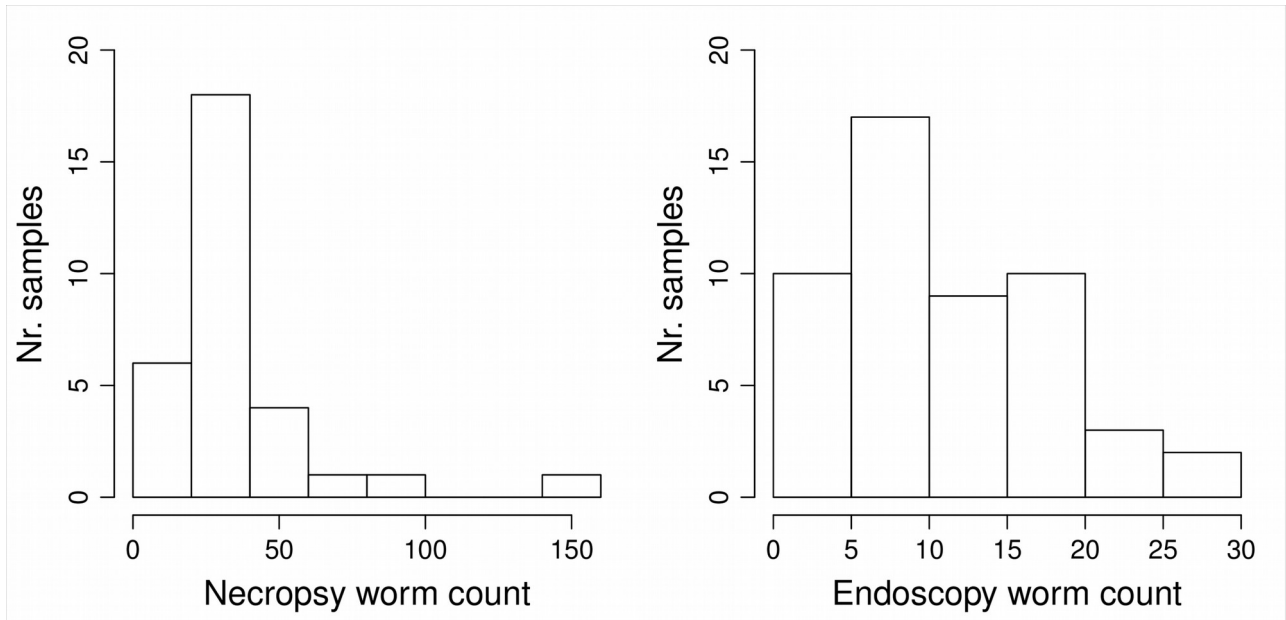
521

522

523   Figure 2. Total worm burden in relation to chick age for necropsied chicks (left panel) and

524   endosocped chicks (right panel). Among endoscoped chicks (which covered an older age range than

525   necropsied chicks) there was some evidence that rank affected worm count, and to illustrate this, in

526   the endoscopy panel AB chicks are shown with solid symbols and C chicks with open symbols. The

527   regression line shown is for the best-fit model, which did not include a rank term. Excluding the

528   oldest chicks, which did not include any C chicks were found, did not alter the ordering of best-fit

529   models. Note the difference in scale for worm counts and age ranges between the two measures.

530   The mean lines show a fitted model without random effects using poisson errors and a log link, with

531   95% confidence intervals shown by the fine-dotted lines.

532
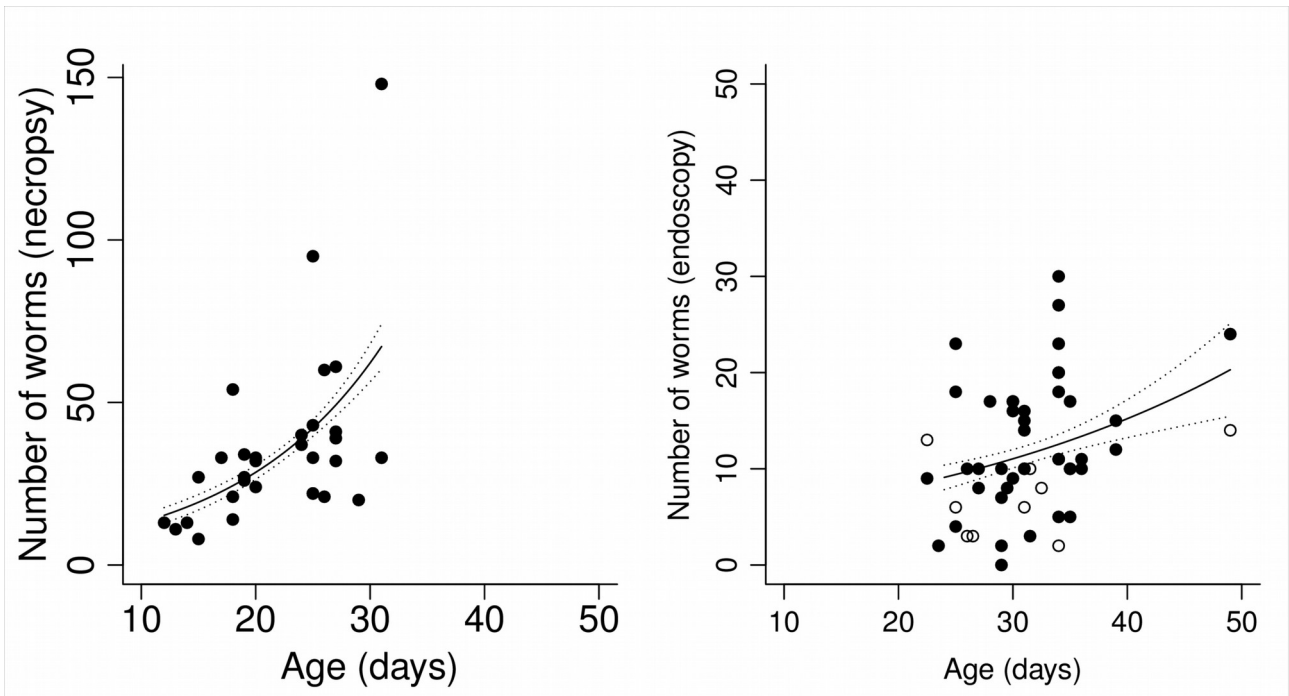
534   Figure 3. The proportion of worms that were large in relation to chick age for necropsied (left

535   panel) and endoscoped (right panel) chicks. In contrast to the worm count, excluding the oldest

536   chicks here slightly changed the order of the best-fit models to: Age + Hatch date; Age; Age +

537   Hatch date + Rank, Age + Rank. The mean lines show a fitted model without random effects and

538   the fine-dotted lines show its 95% confidence intervals.

539
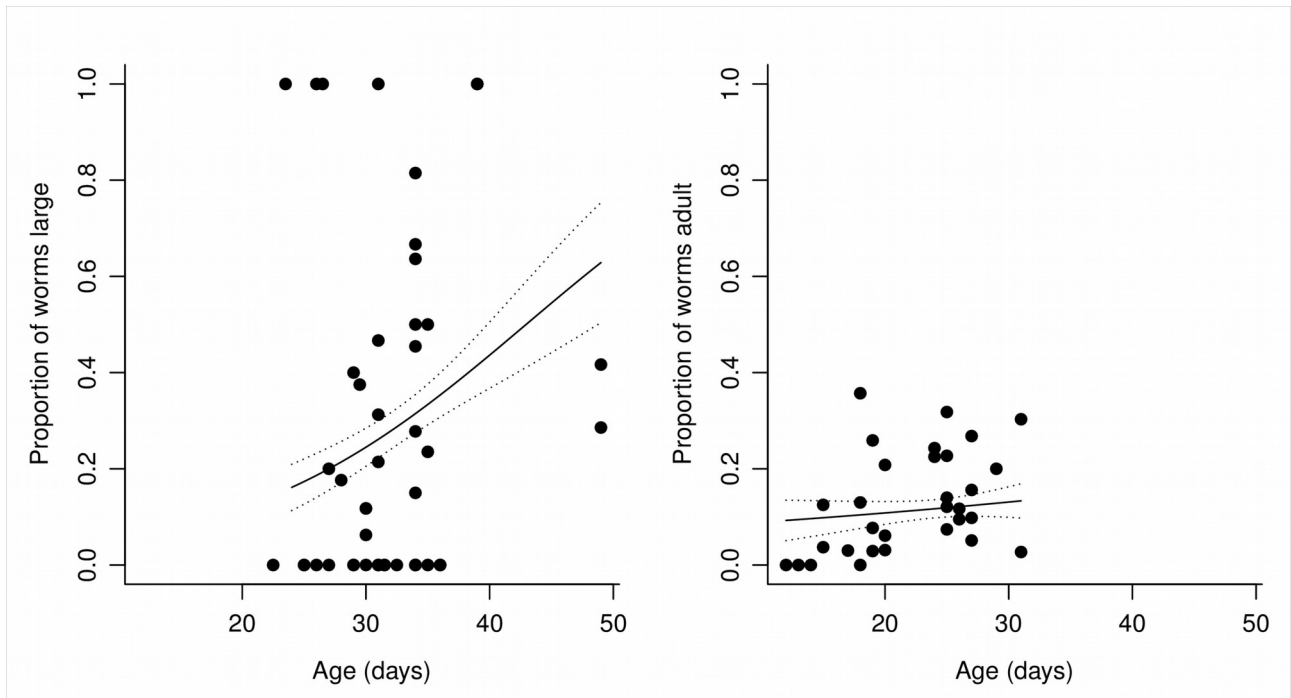
541 Figure 4. The relationship among endoscoped chicks between mass at endoscopy and worm count.

542 The solid line shows the fitted relationship and the dotted line the 95% confidence intervals. To

543 account for other factors affecting mass, mass is shown as the residual from a LMM containing age

544 as the only predictor, following the best-fit model for chick mass.

545
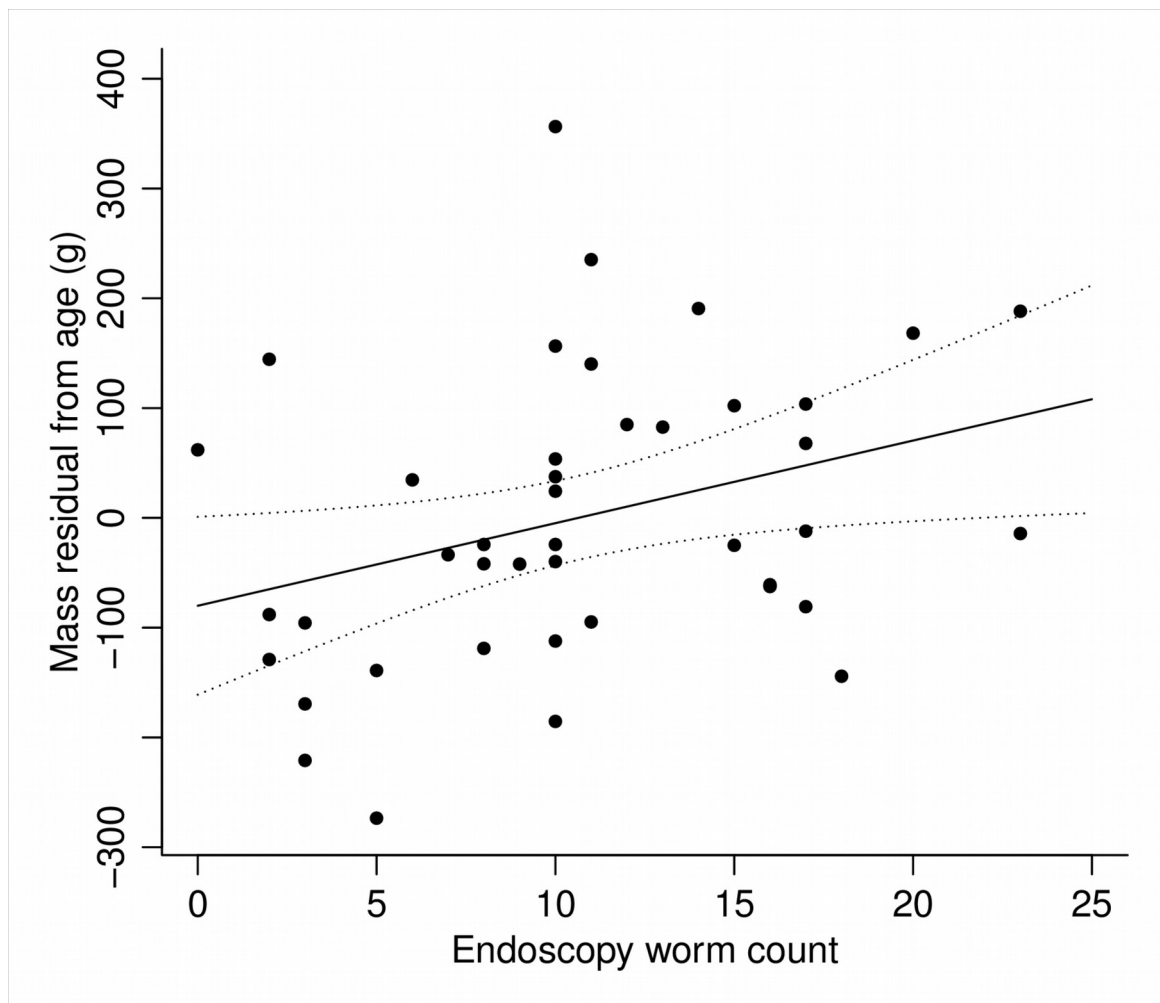
546 **Between-individual variation in nematode burden among juveniles in a wild host**

547 Supplementary Information:

548 *Using endoscopy to test the efficacy of anti-parasite treatment,*

549 *observations from dissections, and patterns in faecal egg counts*

550

551

552 **Efficacy of anti-parasite treatment**

553

554 *Introduction*

555 Our study system, the European shag (*Phalacrocorax aristotelis*, henceforth "shag") and its

556 gastrointestinal nematodes, is increasingly yielding valuable insights into the effects of parasitism

557 on individual fitness-related traits and population-level consequences in wild hosts. Although

558 parasites are known to be important influences on host demography and evolution in wild

559 vertebrates (Hudson *et al.* 2002; Tompkins *et al.* 2011), they are rarely considered as factors in

560 ecological processes in seabirds, a globally threatened group whose members are often used as

561 indicators of the state of their marine environment (Piatt, Sydeman & Wiese 2007; Croxall *et al.*

562 2012).

563 Several studies of parasitism in the shag have used anti-helminthic treatment as an

564 experimental approach to investigate the effects of nematode infection (Reed *et al.* 2008, 2012;

565 Burthe et al. 2013; Granroth-Wilding *et al.* 2014, 2015). The injectable, broad-spectrum

566 antihelminthic drug, Ivermectin (Panomec©, Merial UK) has thus been shown to affect chick

567 survival and growth, adult condition, and behaviour of both adults and chicks, strongly suggesting

568 that treatment affects worm burden. Ivermectin also impacts on ectoparasites, yet previous evidence

569 from this system suggests that ectoparasites contribute little to the cost of a shag's overall parasite

570 burden (Daunt *et al.* 2001). Indeed, Burthe *et al.* (2013) used endoscopy to show that a high dose of

571 ivermectin significantly reduced or removed worm burdens in the proventriculus of adult shags,

572 with no evidence that infection returned for at least 20 days after treatment. In chicks, faecal egg

573 counts (FECs) have provided an indication that treatment reduces affects worm burden, but direct

574 evidence is lacking in chicks of how treatment at the doses used in previous studies affects worm

575 burden. Demonstrating a real effect of treatment on *in situ* nematode burden is particularly

576 important given that, as we show in the main manuscript, FECs in this low-shedding system may

577 not be sensitive to small-scale variation in worm burdens.

578   Understanding the effect of treatment on parasite load is an important link in understanding
579   how between-individual variation in fitness is linked to infection status in juveniles, given that anti-
580   parasite treatment experiments have suggested that infection in chicks can affect both chick growth
581   and parental condition, with long-lasting effects that may be important in population processes
582   (Granroth-Wilding *et al.* 2014, 2015). Here, we use endoscopy of chicks to quantify the effect of
583   treatment with ivermectin on worm burden in shag chicks, at the dosage used in previous work.
584
585   *Methods*
586   We combined the main endoscopy study of natural variation in parasite burden with an experiment
587   to investigate the efficacy of anti-parasite treatment, following protocol from previous parasite
588   removal experiments in shag chicks (full details in Granroth-Wilding *et al.* 2014). We visited nests
589   of three eggs every two days around predicted hatching to obtain hatching dates. When the oldest
590   chick in a brood was 10–12 days old, if all three chicks were still alive, the whole brood was
591   injected with either 0.05ml ivermectin (Panomec© by Merial, 1% wt/vol) (drug-treated broods) or
592   veterinary saline solution (sham-treated control broods). At treatment, we blood sampled chicks for
593   molecular sexing (Griffiths, Daan & Dijkstra 1996) and assigned a rank in the brood hierarchy to
594   each chick according to size, with the heaviest two assigned AB and the lightest C. We have
595   previously shown that mass at this age correctly identifies the C chick in 90% of broods (Granroth-
596   Wilding *et al.* 2014). Previous work has shown that responses to treatment, and therefore potentially
597   the effect of treatment on worm burden, varied between chicks according to differences in rank, sex
598   and hatch date (Reed *et al.* 2008, 2012; Granroth-Wilding *et al.* 2014, 2015). At or after age 25
599   days, we endoscoped all surviving experimental chicks (66 chicks in 29 nests; detailed endoscopy
600   methods in the main text). We also endoscoped unmanipulated chicks from 6 nests known to have
601   had an initial brood size of three.
602   We examined the efficacy of treatment on worm burden by testing whether it affected the total
603   number of worms and the proportion of worms that were large)an indicator of the maturity of the
604   infection). We also examined the impact of treatment on chick performance by testing whether it
605   affected mass at endoscopy, which was positively associated with natural worm burdens in the main
606   investigation. Unmanipulated and sham-treated chicks were pooled as the control group (see main
607   text). All models included age as a predictor, given that older chicks host more worms and a greater
608   proportion of large worms (see main text) and are heavier. For all three response variables (worm
609   count, proportion large, chick mass), treatment was tested as a main effect and in interactions with

sex, rank or hatch date, factors which have previously been shown to influence the impact of treatment (Granroth-Wilding *et al.* 2014, 2015; Reed *et al.* 2008, 2012). In this directed analysis we used hypothesis-testing to assess the importance of each tested factor, in contrast to the more exploratory AIC-based model selection in the main manuscript. We were unable to robustly test the effect of ivermectin treatment on FECs as only 3 drug-treated chicks yielded faecal samples, but we provide a qualitative discussion of these data. All modelling was conducted in R 3.0.2 (R Core Team 2013) using the packages lme4 (Bates, Maechlar & Bolker 2011) and nlme (Pinheiro *et al.* 2012). Worm count was modelled with poisson errors and a log link, the proportion that were large was modelled as a binomial response (weighted by total count), and mass at endoscopy was modelled as a Gaussian response. All parameters are presented as the mean ±1 standard error.

Results & discussion

Ivermectin-treated chicks had lower worm burdens than control chicks (mean burden of ivermectin-treated chicks 8.7 ± 1.3 worms; mean burden of control chicks 11.0 ± 1.1 worms; log-transformed effect size in addition to age -0.54 ± 0.26 log(worms), $z = -2.12$, $p = 0.034$) (fig. S1). However, treatment did not affect the proportion of worms that were large (in addition to age, effect of treatment 0.34 ± 0.55, $z = 0.62$, $p = 0.537$). Sex, age and hatch date did not change the effect of treatment on either worm count or the proportion of worms that were large (all interactions $p > 0.1$). This demonstrates that ivermectin is an effective anti-helminthic in live juveniles in the wild, and indicates that it acts equally on all parts of the worm population. These results support the continued use of ivermectin in long-term experiments into the fitness impacts of parasite infection in the wild, enabling experimental work that is valuable in teasing apart correlative patterns in natural burdens and concurrent variation in host fitness.

Chick mass at endoscopy did not differ between any ivermectin-treated and control chicks (in addition to age, effect of treatment 18.3 ± 61.9, $t = 0.30$, $p = 0.771$; interactions with sex, rank and hatch date all $p > 0.3$). This is perhaps unexpected given that treatment reduced worm burden and that, among naturally infected chicks, heavier chicks had higher burdens (see main text). However, the lack of an effect of treatment on mass is consistent with between-year variation in the impacts of anti-parasite treatment on shag chicks: breeding conditions in the experimental year were such that we would expect little impact of treatment or variation between individuals (Granroth-Wilding *et al.*, 2014).

642   Although we could not explicitly test the effect of treatment on FECs as a proxy indicator of

643   worm burden, we noted that eggs were detected in the faeces of 16 out of 43 control or

644   unmanipulated chicks (37% prevalence) but in none of the four drug-treated chicks for which we

645   had faecal samples (0% prevalence). This points towards treatment reducing FECs as well as

646   reducing worm burdens measured *in situ*. Although our main study found that egg presence in

647   faeces does not, in this system, provide sufficient resolution to reflect natural variation in worm

648   burdens, it is notable that previous work has shown ivermectin treatment to prevent an increase in

649   FEC with age in shag chicks (Granroth-Wilding *et al*., 2014). Together, this suggests that FECs may

650   be a useful indicator of artifical differences in worm burden in this system, providing an accessible

651   though crude tool to validate the efficacy of experimental anti-parasite treatment.

652

Observations from dissections

As part of the main study, 33 chicks that had died naturally were dissected to obtain an alternative, direct measure of worm burden. Findings concerning between-individual variation in worm burdens are described in the main text; here, we provide a qualitative summary of observations made during dissections concerning the biology of the parasite within the host and pathology of infection.

All dissected chicks contained food, ranging from a heavily digested paste to almost-whole fish from recent feeds. Worms were found almost exclusively in the proventriculus; some worms were present in the oesophagus of two chicks, but never in the intestine. On no occasion were worms or other visible parasites observed in the body cavity outside the digestive tract. Smaller worms were found predominantly in digested food at the bottom of the stomach, whereas larger worms were found predominantly in or on recently ingested or semi-digested boluses of fish. In most dissections, worms were also found in and under the mucous lining of the stomach. Some attachment points on the stomach wall were characterised by hardened ulcerations, which were all in the upper part of the stomach, more concentrated towards the oesophagus.

Patterns in FECs

As part of the main study, we collected faecal material from 43 unmanipulated or control-treated chicks to examine how well this proxy measure reflects the more reliable indices of worm burden obtained through endoscopy and necropsy. FECs are commonly used as a non-invasive indicator of variation in worm burden, but their reliability is variable and must be verified in each new system in which they are used. In this paper, our main study revealed that eggs could only be detected at very low levels in faecal material (see main manuscript), and that FECs therefore did not capture the full extent of infection in juveniles, possibly as worms have not yet reached sexual maturity at these early stages of infection. We therefore instead investigate whether the presence/absence of eggs, indicative of an established infection,varies with *in situ* indices of worm burden and with host phenotypic traits that have previously been reported to affect how individual traits are affected by infection.

Methods

We opportunistically collected faecal samples from 19 endoscoped chicks that defecated during handling. From 24 dissected chicks, we obtained a faecal sample from the cloaca after carcasses had been frozen at -20°C for long-term storage. All faecal samples were therefore stored at -20°C after collection. Previous work in this system has given no evidence that freezing affects egg counts or prevalence (in 138 faecal samples across 3 years of chicks with natural worm burdens, stored either frozen or at room temperature in the non-distorting preservative DESS (Yoder et al., 2006), in a binomial GLMM including year as a random effect and storage method and age as fixed effects: effect of freezing compared to room-temperature DESS on egg count 0.09 ± 0.8, $z = -0.11$, $p = 0.910$; effect on egg presence -1.0 ± 0.8, $z = -1.31$, $p = 0.191$).

FECs were carried out using a flotation technique (Bowman and Georgi, 2009). The sample was fully defrosted and mixed well with 20ml saturated salt solution per 1g of faeces (sample sizes, including a variable proportion of nitrogenous waste, ranged from 0.1 to 1.2 g; mean 0.6 g). The mixture was left for c. 10 minutes to allow organic debris to settle out and the lipid-rich eggs to float up. The upper two-thirds of the water column was then mixed gently using a pipette, and an aliquot taken while raising the pipette slowly through the liquid to ensure sampling of any eggs that had not yet reached the surface. The aliquot was placed in a McMaster slide and the portion under the grid (0.15 ml) was systematically searched for nematode eggs at 40x magnification using a light

702 microscope. Three aliquots were examined from each bird, totalling 0.0225g of faecal material. This

703 is sufficient to detect egg presence in adult birds in this low-shedding system (egg presence/absence

704 in 42 adult shags with natural burdens quantified using a variable number of aliquots: using 4-10

705 aliquots, effect of number of aliquots on egg presence 0.02 ± 0.15, z = 0.14, p = 0.882; mean

706 prevalence with 95% confidence intervals across 23 individuals with 10 aliquots $32 \pm 20\%$, across

707 19 individuals with 4-8 aliquots $35 \pm 23\%$; across 43 chicks with 3 aliquots in this study, $37 \pm$

708 15%).

709   Most of our 43 faecal samples contained no eggs and only 7 contained more than 1 egg (9

710 with 1 egg, 4 samples with 2 eggs, 2 with 3 eggs and one with 42). To overcome the statistical

711 challenges presented by such a skewed distribution, FECs were analysed as a binary

712 presence/absence response with binomial errors and a logit link. Fitting egg counts with poisson

713 errors and an observation-level random effect to allow for this overdispersion gave qualitatively

714 similar results. We tested whether the probability of egg presence in FECs varied with total worm

715 burden or the number of large worms (more likely to be mature and thus producing eggs) as

716 quantified using either endoscopy or necropsy. Model selection used AICc (details in main

717 manuscript).

718

719 *Results and discussion*

720 Among models examining the effect of worm burden as measured *in situ* (endoscopy and necropsy

721 combined) on FECs, nematode egg presence in faeces was best explained by a model containing

722 only a measurement technique term (log odds of egg presence in endoscoped chicks 1.17 ± 0.70

723 compared to dissected chicks), although this was of an equivalent fit to an intercept-only model

724 (ΔAIC = 0.3) and a model containing containing a single large worm count term (log odds of egg

725 presence -0.05 ± 0.06 per large worm). In relation to chick phenotypic traits, egg presence was best

726 explained by chick age, which appeared in all three best-fit models (table S1, fig. S2). There was no

727 strong support for any other chick traits being associated with egg presence in faeces.

728   Despite the lack of evidence for any relationship between the presence of nematode eggs in

729 faeces and the more direct *in situ* indices of infection intensity, this proxy measure did reflect the

730 increase in worm burden with chick age that we found with both necropsy and endoscopy. As worm

731 eggs were more likely to be found in older chicks, FECs may thus have some utility for capturing

732 natural variation (or experimental changes to natural burdens; see above) in established infections

733 across the population. However, the variation in the data resulting from the low prevalences mean

734   that we cannot confidently rule out some zero counts being false negatives, and the results of the
735   FEC analyses should therefore be interpreted with caution. Thus, endoscopy remains a more useful
736   technique for capturing the full extent of infection for any given bird and across the population at
737   any point in its lifetime.

738   **Bibliography**

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743 Table S1. The top five models of best fit explaining the presence of nematode eggs in shag chick
744 faeces. The top set of models investigated the relationship of FECs with worm counts as measured
745 by one of two *in situ* techniques (endoscopy or dissection) on a set of candidate models using the
746 variables technique, worm count and proportion of large worms. The bottom set investigated
747 variation in FECs in relation to host traits, and explanatory variables used in building the candidate
748 model set were age, sex, rank and hatch date. Models are shown with their ΔAICc relative to the
749 best-fit model, in order of decreasing fit. All models included a random effect of nest.
750

Model terms	d.f.	ΔAICc
Relationship with in situ *worm measures*		
Technique	3	0.0
(intercept only)	2	0.3
Large worm count	3	1.9
Large worm count + technique	4	2.3
Total worm count + technique	4	2.4
Host traits		
Age	3	0.0
Age + Hatch date	4	1.0
Age + Sex	4	1.7
(intercept only)	2	2.1
Age + Rank	4	2.1

751   Figure S1. Worm counts measured using endoscopy in chicks of varying ages that had been treated

752   with ivermectin (solid symbols and line) or sham-treated not manipulated before endscopy (hollow

753   symbols, dotted line). The line shows the fitted mixed-effects model.

754
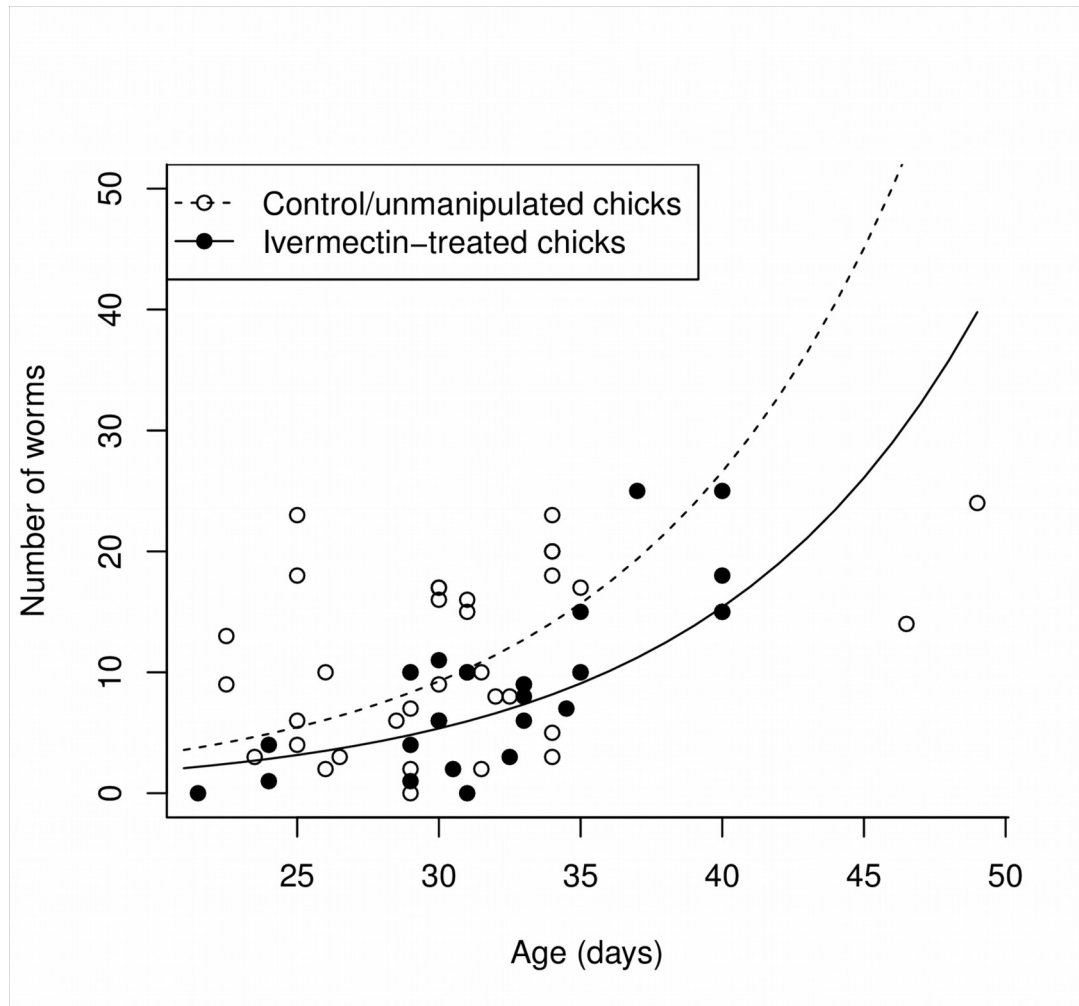
755   Figure S2. The relationship of egg prevalence, quantified using FECs, with chick age. The solid line

756   shows the fitted relationship and the dotted lines its 95% confidence intervals.

757