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

H. M. V. GRANROTH-WILDING[1]*†, F. DAUNT[2], E. J. A. CUNNINGHAM[1]‡ *and* S. J. BURTHE[2]‡

[1] *Ashworth Laboratories, Wellcome Centre for Infection, Immunity and Evolution/Institute of Evolutionary Biology, University of Edinburgh, Charlotte Auerbach Road, Edinburgh EH9 3FL, UK*
[2] *NERC Centre for Ecology & Hydrology, Bush Estate, Penicuik EH26 0QB, UK*

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SUMMARY

Parasite infection in young animals can affect host traits related to demographic processes such as survival and reproduction, and is therefore crucial to population viability. However, variation in infection among juvenile hosts is poorly understood. Experimental studies have indicated that effects of parasitism can vary with host sex, hatching order and hatch date, yet it remains unclear whether this is linked to differences in parasite burdens. We quantified gastrointestinal nematode burdens of wild juvenile European shags (*Phalacrocorax aristotelis*) using two *in situ* measures (endoscopy of live birds and necropsy of birds that died naturally) and one non-invasive proxy measure (fecal egg counts (FECs)). *In situ* methods revealed that almost all chicks were infected (98%), that infections established at an early age and that older chicks hosted more worms, but FECs underestimated prevalence. We found no strong evidence that burdens differed with host sex, rank or hatch date. Heavier chicks had higher burdens, demonstrating that the relationship between burdens and their costs is not straightforward. *In situ* measures of infection are therefore a valuable tool in building our understanding of the role that parasites play in the dynamics of structured natural populations.

Key words: Parasite burden, endoscope, dissection, *Contracaecum*, anisakid, seabird, macroparasite, prevalence, FEC, demographic trait, growth, host–parasite interaction.

INTRODUCTION

The costs that parasite infection can impose on their hosts can influence key demographic traits, such as reproductive success and survival, which are crucial to the growth rate and hence viability of populations (Albon *et al*. 2002; Newey *et al*. 2005; Redpath *et al*. 2006; Tompkins *et al*. 2011). However, parasitism is unlikely to affect all individuals in a population in the same way. Firstly, individuals may host different burdens as a result of differences in exposure to parasites, susceptibility to infection and resistance to its impacts. This contributes to parasite abundance typically showing a skewed distribution among hosts, which is particularly well documented in macroparasites (Shaw and Dobson, 1995; Randolph *et al*. 1999). Secondly, once parasitized, the relationship between parasite load and host fitness may vary between individuals due to differences in tolerance for a given parasite load. Siblings, for example, may vary in the level of maternal antibodies they receive (Pihlaja *et al*. 2006), males may be affected more than females due to immunosuppressive effects of testosterone (Mougeot *et al*. 2009) and the relative benefits of allocating resources between fighting infection and reproduction may vary with age (Adamo *et al*. 2001). These factors may lead to different types of host responding differently to infection, with consequences for key host traits related to fitness such as weight gain during critical periods of growth. Understanding how parasite burdens and their impacts on hosts vary between different classes of individual may therefore be crucially important for understanding the impacts of parasites on heterogeneous host populations.

A key process for population viability is the level of offspring recruitment to the breeding population. Understanding how parasitism impacts on the juvenile subset of the population is therefore important for modelling population growth. Infection in early life can alter juveniles' developmental trajectories (Fitze *et al*. 2004; Romano *et al*. 2011), with potentially life-long fitness consequences that may further influence demographic processes such as reproduction and survival long after recruitment (Lindström, 1999; Metcalfe and Monaghan, 2001; Monaghan, 2008). However, despite the importance of early-life infection, between-individual patterns of parasitism and the development of infections in juvenile hosts have not been widely investigated in the wild. Although young hosts have been shown to exhibit systematic between-individual differences

* Corresponding author. P.O. Box 65, 00014 Helsinki. E-mail: hanna@granroth-wilding.co.uk
† Current address: Department of Biosciences, University of Helsinki, Viikinkaari 1, P.O. Box 65, 00014 Helsinki, Finland.
‡ Equal author contributions.

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in their response to experimental infection or anti-parasite treatments according to characteristics such as hatching order (Granroth-Wilding *et al*. 2014), sex (Romano *et al*. 2011) or timing of breeding (Reed *et al*. 2008), it remains unclear whether variation in response is associated with differences in burden or differences in tolerance. Thus, quantifying individual parasite burdens across the juvenile component of the population, where individuals' responses to infection are also known by measuring key fitness-related traits, is central to accurately predicting parasite impacts at the population level.

Quantifying parasite burdens is logistically challenging in the wild, particularly for endoparasites that often make up the majority of a host's parasite biomass (Hoberg, 2005). Necropsy allows direct counts of parasites in the host and is widely considered to give the most reliable measure. However, this destructive method prevents longitudinal studies, which are crucial for detecting associated fitness consequences such as recruitment probability and future reproductive success (Fitze *et al*. 2004). In juvenile hosts, such sublethal impacts, which are typical of macroparasites, have the potential to affect key demographic parameters over a range of timescales. Avoiding destructive sampling is therefore particularly important to understand the full fitness consequences of infection in young hosts. Moreover, necropsy may not be viable for hosts of conservation importance. Fecal egg counts (FECs) are a common non-destructive and non-invasive proxy measure of endoparasite burden (e.g. Seivwright *et al*. 2004; Craig *et al*. 2006; Bowman and Georgi, 2009), but may not always reflect true parasite burden due to variable rates of helminth egg production (Shaw and Moss, 1989; Tompkins and Hudson, 1999), poor sensitivity at low burdens (Levecke *et al*. 2009) and not representing larval helminths that do not produce eggs but can nonetheless be costly to the hosts (Fagerholm and Overstreet, 2008). Recent work in wild adult seabirds has pioneered endoscopy as an additional, direct and reliable method to obtain an index of gastrointestinal nematode burdens in live individuals (Burthe *et al*. 2013). This approach has great potential for quantifying the development of an individual's infection from an early age, but has not previously been applied to juveniles in the wild.

Here, we use two *in situ* measures of parasite burden – necropsy and endoscopy – and the proxy measure of FECs to quantify patterns of between-individual variation in the trophically-transmitted gastrointestinal nematode burden of juvenile European shags (*Phalacrocorax aristotelis*, henceforth 'shag'), a piscivorous seabird. Experimental manipulations of parasite load in adults and chicks has shown that responses to treatment vary with host phenotype: treatment of parents increases male chicks' survival, particularly late in the season, but not female chicks' (Reed *et al*. 2008); treatment of chicks generally affects the growth rate of last-hatched siblings but not the older brood members (Granroth-Wilding *et al*. 2014); and the impact of simultaneous treatment of parents and their offspring differs between early- and late-nesting families (Granroth-Wilding *et al*. 2015). Endoscopy of adults has found males to host more worms than females and late breeders more than early breeders (Burthe *et al*. 2013), but among juveniles, patterns of variation in parasite abundance and their link with variation in host fitness are not well quantified. Hence, it is unclear whether these differences in treatment responses between types of juveniles arise from differences in nematode burden or differences in the impact of a similar burden. Moreover, the link between parasite burden and demographically important host traits is unexplored. Our objectives were therefore to: (1) quantify individual parasite burdens of juveniles using two *in situ* methods, endoscopy and necropsy, and compare these to a proxy measure of prevalence based on FECs; (2) identify whether burdens vary with host age, sex, hatching order and hatch date; (3) examine whether natural variation in parasite abundance is associated with a fitness-related trait, host mass.

METHODS

Host-parasite system

This study was carried out in 2012 in the breeding population of shags on the Isle of May National Nature Reserve in south-east Scotland (56°11N, 2°33W) that has been the subject of an individual-based long-term demographic study for several decades. Shags are sexually dimorphic, with males growing faster to reach an adult size c. 20% bigger than females (Daunt *et al*. 2001). The modal clutch size in this population is three eggs and these hatch asynchronously, with the second and third siblings (B and C chicks) hatching on average 1 and 2–3 days after the first (A chick). This asynchrony results in a hierarchy of size within the brood, in which youngest siblings generally grow more slowly and have higher mortality but are more plastic in response to changing environmental conditions than their older counterparts (Stokland and Amundsen, 1988; Granroth-Wilding *et al*. 2014). Breeding success declines through the season, with later breeders fledging fewer chicks and producing fewer recruits (Harris *et al*. 1994; Daunt *et al*. 1999).

Shags on the Isle of May are infected with the gastrointestinal nematode *Contracaecum rudolphii* (Anisakidae: Ascaridoidea; Hartwich 1964), which occur in the GI tract of nestling and adult shags in this population (Burthe *et al*. 2013; E. Harris, personal communication; S. Burthe, J. Chantrey d

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D. Kowalek, unpublished data). All but one of 146 naturally infected adults endoscoped to date have hosted worms, with wide variation in burdens from 2 to >80 worms (Burthe *et al*. 2013; S. Burthe and E. Butterfield, unpublished data). *Contracaecum rudolphii* is a widely distributed seabird specialist, now recognized to comprise a complex of morphologically similar species (Anderson, 2000; Hoberg, 2005; Fagerholm and Overstreet, 2008; Moravec, 2009). Nestling shags obtain regurgitated fish directly from their parents' throats and are infected with larval worms in the fish tissue. Direct infection of chicks with adult worms dislodged from the parent's proventriculus could also occur during feeding, but the importance of this transmission route is not well understood (Dubinin, 1949; Huizinga, 1971; Hoberg, 2005; Fagerholm and Overstreet, 2008). Anisakid infection can cause costly pathology at attachment sites such as inflammation, necrosis, haemorrhaging and perforation of the stomach wall (Kuiken, 1999; Hoberg, 2005; McClelland, 2005), which may be compounded by secondary bacterial infections (Fagerholm and Overstreet, 2008), and is expected to activate a costly immune response (Colditz, 2008; Hasselquist and Nilsson, 2012). Moreover, *Contracaecum* is thought to feed on fish ingested by the bird and thus directly competes with the host for resources (Dubinin, 1949; Huizinga, 1971; Anderson, 2000; Abollo *et al*. 2001).

Quantifying nematode burdens

We quantified the nematode burden of individual shag chicks using two *in situ* techniques, endoscopy of targeted individuals or necropsy (dissections) of a subset of the study population that died naturally during a severe storm. We also conducted FECs on fecal material collected opportunistically from both endoscoped and dissected chicks (all detailed methods below). Not all individuals produced fecal samples, precluding FECs, and no birds were both endoscoped and dissected, as endoscoped chicks were not sacrificed and endoscopy of dead animals is not reliable (S. Burthe, unpublished data).

Endoscopy. We used a refurbished 9 mm diameter medical endoscope (Olympus©, UK) to view the oesophagus and proventriculus of conscious chicks under licence (full details of endoscopy procedure in (Burthe *et al*. 2013). Endoscopy was undertaken by a trained and experienced operator (S. Burthe) while an assistant held the bird still and its bill open. A cloth was placed over the bird's eyes to reduce stress while the endoscope operator inserted the endoscope into the proventriculus. All worms that were visible were counted as the endoscope was slowly withdrawn from the bird. We noted whether the worms were large or small. Visibility was scored on a scale of 1–5 (worst to best, as in Burthe *et al*. (2013)) and included in all analyses as poorer visibility could hinder accurate quantification. Endoscopy was carried out when chicks were large enough for the endoscope to be comfortably inserted, around 25 days of age. Throughout the process, there was no evidence of discomfort (e.g. rapid breathing). All endoscoped chicks resumed normal behaviour immediately on being returned to the nest and all fledged successfully. All endoscopy was carried out early in the morning, before parents had returned with the first food load of the day, to avoid views being obstructed by recently ingested food.

At endoscopy, chicks were assigned a rank in the brood hierarchy according to size: in broods of three, the heaviest two chicks were designated AB and the lightest C, and in broods of one or two, all chicks were designated AB. Wing length was used as an additional indicator if mass difference was not >20 g. Mass at day 25–30 accurately identifies the last-hatched chick in 83% of cases but only distinguishes the first- and second-hatched (A and B) in 47% of cases, whereas A and B are accurately assigned as AB in 89% of cases (data from 27 nests, with three chicks surviving to day 10, in 2010 and 2011 with accurate hatch dates; Granroth-Wilding *et al*. 2014). We used chick mass at endoscopy as an indicator of chick performance. At endoscopy age, the majority of growth is completed (Daunt *et al*. 2001), and fledging mass has been shown to correlate with recruitment in a range of species (Magrath, 1991; Schwagmeyer and Mock, 2008). All endoscoped chicks were blood sampled for molecular sexing (Griffiths *et al*. 1996).

In total, we endoscoped 45 chicks in 20 nests, of which 18 were undisturbed before endoscopy and 27 were sham-treated controls from a parallel parasite-removal experiment (full details in online Supplementary Information; no individuals treated with anti-parasite drugs are included in the results presented here), injected with 0·05 mL saline solution at age 10–12 days and subsequently weighed at ages 10, 15 and 25 days. A subset of chicks that remained safely accessible as they got older and more mobile were endoscoped twice (2 untreated chicks and 4 sham-treated).

Necropsy. Sacrificing individuals for systematic necropsies was not possible as this would prevent longitudinal investigations of the link between parasite burden and host survival, and moreover removing individuals from this long-term study population is not desirable. However, in 2012, there was an unusually prolonged period of rain and cold weather in the middle of the peak chick-rearing period, lasting over 2 days. This caused considerable natural juvenile mortality due to waterlogging and chilling of chicks that were still downy (not

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yet waterproof) but too large to be efficiently sheltered by their parents. Mortality was thus not a direct consequence of overall poor condition nor of parasitism, though both factors may have contributed. Similar weather-related mass mortality events of chicks during the breeding season have only occurred once in the last 15 years, so this was a rare opportunity to obtain a sample of birds for dissection. When the weather improved and it was safe to approach nests, c. 12–36 h after death, we collected 28 carcasses (median 20 days old, interquartile range (IQR) 18–26 days; median hatch date 27th May, IQR 21st–29th May; cf. endoscoped chicks, median age 31 days, IQR 28–34 days, median hatch date 17th May, IQR 15th–22nd May). Nine of these were sham-treated controls from the parallel experiment. We also collected 6 further carcasses resulting from other natural mortality, found within a day of death, for necropsy (median age 25 days, IQR 25–29 days; median hatch date 2nd June, IQR 20th May–3rd June). For the 10 dissected chicks that were not of known age, we estimated age from wing length based on the growth rate of chicks from the same year with known hatch dates (Wing = 5·81 × Age – 27·75; in mixed model accounting for repeated measures within chick, $F_{1,147} = 9795$, $P < 0{\cdot}001$; without random effects, $r^2 = 0{\cdot}954$). We assigned ranks to dissected chicks in cases where the whole brood could be assessed either dead or alive, based on the structural measure of wing length: in broods of three, the two chicks with longest wings were assigned AB and the shortest C, and in broods of one or two, all chicks were assigned AB. A sample of blood or tissue was taken from every carcass for molecular sexing (Griffiths *et al*. 1996).

Where possible, carcasses were dissected fresh within 6 h of recovery, or kept at +4 °C for up to 24 h (16 carcasses). If dissections could not be carried out within this time (17 carcasses), they were stored at −20 °C for up to 1 week and defrosted before dissection. The proventriculus was removed together with 3 cm of oesophagus and small intestine. The removed gastrointestinal portion was then opened out using one medial ventral cut and the stomach contents thoroughly examined, then rinsed with water through a fine mesh. The body cavity was examined for evidence of nematodes migrating away from the proventriculus following host death, and we additionally examined the whole intestine of four individuals; no other visible macroparasites were found (further descriptions in online Supplementary Information). All worms were counted, removed and stored in ethanol. To obtain an index of the maturity of the infection in the bird, during which stage *Contracaecum* undergoes substantial growth (Fagerholm and Overstreet, 2008), worms were categorized into size classes based on width (>0·75 mm wide, large; <0·5 mm wide, small; 159 out of 1436 worms (11%) in between the categories).

Fecal egg counts. During endoscopy, we opportunistically collected fecal samples from 19 chicks that defecated during handling. From 24 dissected chicks, we obtained a fecal sample from the cloaca after carcasses had been frozen at −20 °C for long-term storage. All fecal 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 (online Supplementary Information). FECs were carried out using a flotation technique (Bowman and Georgi, 2009; detailed methods in online Supplementary Information). Each sample was suspended in 20 mL saturated salt solution per 1 g of feces and nematode eggs were counted in 0·45 mL (0·02 g feces) of the suspension examined under a McMaster slide.

Statistical analysis

We first quantified patterns in parasite abundance obtained by each *in situ* parasite measure, endoscopy and dissection. We considered two aspects of nematode infection: total worm burden, indicating overall parasite abundance and the proportion of worms that were large, which is likely to reflect the duration of the infection. We then tested whether these indices were associated with host age, as expected if chicks are exposed to infective larvae throughout their development, and with phenotypic traits known to affect responses to infection: host sex, rank (AB *vs* C) and hatch date (Reed *et al*. 2008, 2012; Granroth-Wilding *et al*. 2014). Lastly, in endoscoped chicks, we examined the association between parasite abundance and chick performance by testing whether chick mass at endoscopy varied with worm count and the proportion of worms that were large.

In all analyses of dissected chicks, we excluded two outliers with high statistical leverage: one old chicks with a very high load (a male, 45 days old, hosting 243 worms; range of other chicks 8–148 worms) and one very young chick (ca. 2 days old), which was the only dissection that yielded a zero burden. These exclusions did not qualitatively affect any results. Although mortality is generally higher for C chicks in this population (Granroth-Wilding *et al*. 2014), all ranks were equally represented among endoscoped and dissected birds, as were males and females (for ranks across techniques, $\chi^2 = 4{\cdot}50$, D.F. = 2, $P = 0{\cdot}105$; for sexes, $\chi^2 = 1{\cdot}32$, D.F. = 1, $P = 0{\cdot}251$). Among endoscoped chicks, we confirmed that visibility score was not related to age, sex, rank or hatch date (all $P > 0{\cdot}4$ in a linear model). Among endoscoped chicks, hatch date (from which age was calculated) was only available

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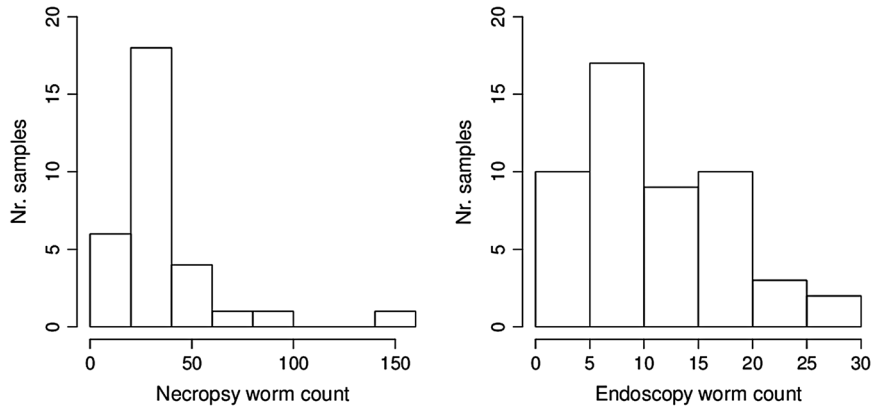

Fig. 1. Histograms showing the spread of worm counts from necropsy (left panel) and endoscopy (right panel). The dissection data does not show two high-leverage individuals excluded from the analysis, a hatchling with no worms and a near-fledgling with 243 worms.

for the first-hatched chicks in each nest, so C chicks were assigned an age 2·5 days younger than their AB siblings (median age difference across 42 nests in 2010 and 2011 with accurate hatch date data) to avoid within-brood age differences confounding rank effects. Among dissected chicks, the effects of age and hatch date could only be examined in separate models as the age-specific main mortality event meant that they were closely correlated (in linear model, $r^2 = 0{\cdot}72$, $P < 0{\cdot}001$). In these analyses, models containing hatch date instead of age gave almost identical fits (ΔAICc $\leqslant 0{\cdot}1$) and for brevity we present only the age models.

All analysis was carried out in R 3.0.2 (R Core Team, 2013) using the packages lme4 (Bates *et al*. 2015) and nlme (Pinheiro *et al*. 2012), using (generalized) linear mixed models (LMMs or GLMMs). To account for repeated sampling of some individuals and non-independence of siblings within a brood, we fitted chick within nest as nested random factors to the endoscopy data, and nest as a random factor to the dissection data. Total burden was fitted as count data with poisson errors and logistic link function; proportion of large worms was fitted with binomial errors and a logit link function and was weighted by the total count. Effect sizes for the proportion of large worms are presented as the log odds of a worm being large. Mass was modelled in a LMM including log(age) and sex as fixed effects, to account for the non-linear growth curve and sexually dimorphic growth. Due to the low egg prevalence in feces preventing robust analysis of relationships between FECs and host phenotypic traits or *in situ* worm burdens, we present only descriptive statistics of prevalence (but see online Supplementary Information for a preliminary analysis).

We used an information theoretic approach to model selection (Burnham and Anderson, 2002), identifying important explanatory variables based on the best-fitting model(s) from a candidate set, which is well suited to an exploratory analysis. For each measure of parasite burden, our set of candidate models contained all combinations of the explanatory variables as main effects (age, hatch date, sex and rank, and additionally for endoscopy analyses, visibility) as well as an intercept-only (null) model. The best-fit model was the one that had the lowest AICc (corrected Akaike's Information Criterion, suitable for small sample sizes) in the set, and models with a ΔAICc $\leqslant 2$ from the best fit model were considered an equivalent fit. Model selection based on significance testing gave the same conclusions. All parameters are presented ± 1 S.E., not back-transformed from the log (worm counts) or logit (proportion of large worms) link functions.

RESULTS

Quantifying worm burden in situ

The ages of birds available for necropsy ranged from 2 to 45 days and for endoscopy from 25 to 49 days. Worm burden measured using necropsy varied from 0 to 243 worms per chick; the youngest and oldest chicks were excluded from further analysis due to their strong leverage, giving an age range of 12–31 days and worm counts of 8–148 worms per chick ($n = 31$; mean 36·0 $\pm$ 4·9; prevalence 100%) (Fig. 1). Worm burden measured using endoscopy ranged from 0 to 30 worms per chick (mean worm burden 11·7 $\pm$ 1·0 worms; prevalence 98%) (Fig. 1). The proportion of large worms ranged from 0 to 35·7% (mean 12·9 $\pm$ 1·9%) by necropsy and 0–100% (mean 29 $\pm$ 5%) by endoscopy.

Using necropsy, the youngest chick to host large worms was aged 15 days and the oldest chick without large worms was 18 days. Using endoscopy, large worms were found in chicks from the age of 26 days, (earliest available age 25 days), although chicks with no large worms occurred up to the age of 36 days.

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

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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 + Sex	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

studies, mainly due to the presence of semi-digested food. Visibility scores among the chicks in this study ranged from 1 to 4 (mean 2·7 ± 0·1) compared with a range of 3–5 (mean 4·24; $n = 17$) for adult shags endoscoped in the same year (S. Burthe, unpublished data).

FECs as an indicator of worm burden

We obtained FECs from 19 endoscoped and 24 dissected chicks from birds aged 25–36 days and 12–45 days, respectively. Nematode eggs were only found in one third of the 43 samples available (prevalence 37%), despite a prevalence of 99% in individuals sampled using *in situ* measures. Out of the 16 fecal samples that contained worm eggs, only 7 contained more than 1 egg (4 samples with 2 eggs, 2 with 3 eggs and one with 42) and 5 were from chicks in which no large worms were seen (1 necropsy, 4 endoscopies).

Nematode burden in relation to host traits

In necropsied chicks, aged 12–31 days, worm count was best explained by a model containing only age, with older chicks hosting more worms. A model with age and sex had similar support (Table 1, Fig. 2). The proportion of worms that were large was best explained by an intercept-only model, with no host traits providing similar explanatory power (Table 1, Fig. 3).

Among endoscoped chicks, aged 25–49 days, total worm burden was best described by a model containing age and visibility (Table 1, Fig. 2), with older chicks hosting more worms and better visibility resulting in slightly higher worm counts (age effect size 0·10 ± 0·02 log(worms) day^{-1}, visibility effect size 0·10 ± 0·06 log(worms) per score increment). Age was supported in all five top models. Out of three equivalent-fit models, two contained a rank term (in addition to age, C chicks hosted −0·21 ± 0·16 fewer worms than AB chicks). The proportion of large worms was best described by a model containing only age (effect size 0·11 ± 0·04 increase in proportion of large worms day^{-1}) (Fig. 3), with hatch date and rank each occurring twice in the three equivalent-fit models (in addition to age, effect of hatch date: 0·05 ± 0·00 greater proportion of large worms per day; effect of rank: C chicks 0·83 ± 0·50 greater proportion of large worms) (Table 1).

A summary of the host traits identified as important to parasite abundance and size distribution by the two measurement techniques is given in Table 2. For both necropsy and endoscopy, it is notable that individuals varied considerably in their parasite load, which contributed to many analyses yielding several equivalent-fit models that made it difficult to robustly identify phenotypic traits that influenced parasite load.

Effect of infection on host performance

Chick mass at endoscopy was best explained by a model containing main effects of age and worm count (Table 3, Fig. 4): heavier chicks were older and had higher worm counts (in best-fit model, effect of age 241·4 ± 43·2 g log(day)$^{-1}$; effect of worm count, 11·8 ± 4·8 g worm^{-1}). There was one model of equivalent fit, which contained an additional sex term (males 62·4 ± 46·6 g heavier than females).

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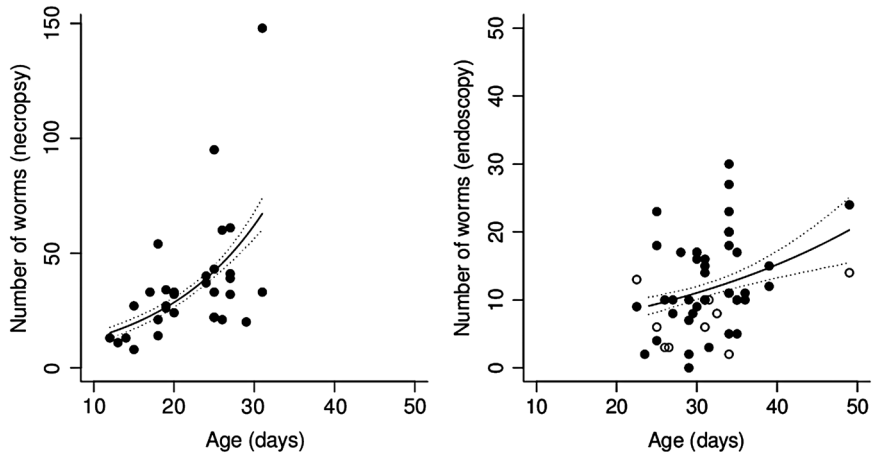

Fig. 2. Total worm burden in relation to chick age for necropsied chicks (left panel) and endosocped chicks (right panel). Among endoscoped chicks (which covered an older age range than necropsied chicks) there was some evidence that rank affected worm count, and to illustrate this, in the endoscopy panel AB chicks are shown with solid symbols and C chicks with open symbols. The regression line shown is for the best-fit model, which did not include a rank term. Excluding the oldest chicks, which did not include any C chicks were found, did not alter the ordering of best-fit models. Note the difference in scale for worm counts and age ranges between the two measures. The mean lines show a fitted model without random effects using poisson errors and a log link, with 95% confidence intervals shown by the fine-dotted lines.

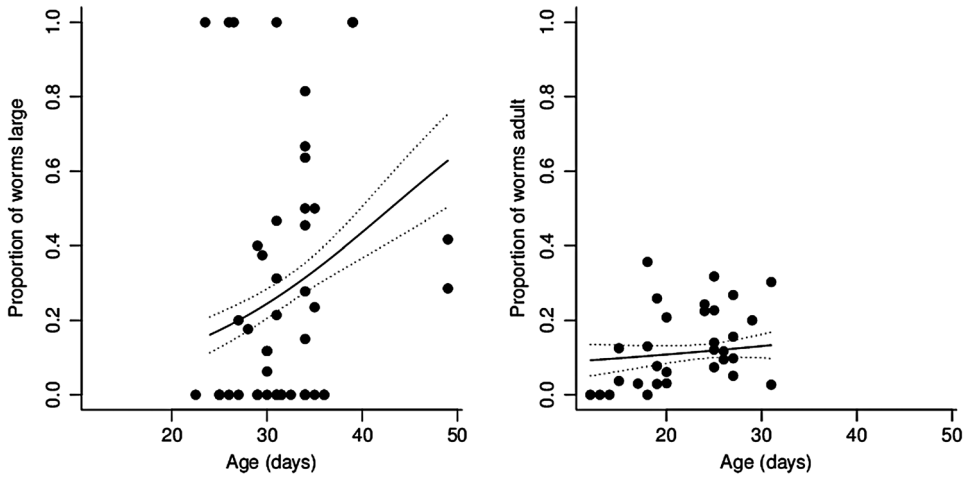

Fig. 3. The proportion of worms that were large in relation to chick age for necropsied (left panel) and endoscoped (right panel) chicks. In contrast to the worm count, excluding the oldest chicks here slightly changed the order of the best-fit models to: Age + Hatch date; Age; Age + Hatch date + Rank, Age + Rank. The mean lines show a fitted model without random effects and the fine-dotted lines show its 95% confidence intervals.

DISCUSSION

The juvenile period is an energetically expensive phase for an individual when the costs associated with parasite infection are likely to have substantial impacts on hosts. Despite this, in comparison with adults, there is very little information for wild juvenile hosts on patterns of parasite prevalence or abundance, particularly internal parasites. Here we have used necropsy and endoscopy, implemented for the first time in juveniles in the wild, to show that infection with gastrointestinal nematodes is near-universal among nestling shags (98% prevalence) and establishes at an early age, and that nematode burden increases with chick age. In contrast, the common proxy measure of FECs suggested a prevalence of only 37%, demonstrating the value of endoscopy as a non-destructive index of *in situ* parasite burden. Previous studies have found chick sex, hatch date and rank to be important in determining responses to anti-parasite treatment (Reed *et al.* 2008, 2012; Granroth-Wilding *et al.* 2014, 2015), yet we found no strong evidence that worm burden varied with any of these host traits. This suggests that differences in response may arise due to variation in tolerance between the subclasses of juvenile as opposed to differences in burden. Further, contrary to predictions, we found

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Table 2. A summary of patterns of variation in nematode burdens between shag chicks, as quantified using necropsy or endoscopy, in relation to phenotypic host traits. Patterns were investigated in both the total worm burden (top set of variables) and the proportion of worms that were large, indicative of how long the chick had been infected (bottom set of variables). Traits that robustly affected worm measures (occurred in all equivalent-fit models) are indicated with a tick, traits that had some support (occurred in more than one equivalent-fit model) are shown with a tick in brackets, and traits with no robust effects are shown with a cross. Hatch date for dissected chicks is indicated with a dash to show that it could not be tested simultaneously with age, as they were tightly correlated

Explanatory variable	Affects necropsy counts	Affects endoscopy counts
Total burden		
Age	✓	✓
Hatch date	–	×
Rank	×	(✓)
Sex	×	×
Proportion large worms		
Age	×	✓
Hatch date	–	(✓)
Rank	×	(✓)
Sex	×	×

that individuals with high worm burdens were heavier than similar-aged individuals with lower burdens.

Comparison of techniques for quantifying worm burden

Both necropsy and endoscopy captured the same main pattern of infection in chicks but unfortunately we did not have the opportunity to directly compare counts from the two techniques in the same individuals. None of the birds that suffered natural mortality had been endoscoped and endoscoped chicks could not be sacrificed for necropsy as this would prevent long-term monitoring of infection and its consequences, and endoscopy of carcasses is not feasible as reliable counts are difficult to obtain from the collapsed stomach of a dead bird. Comparisons of necropsied and endoscoped individuals was further constrained by the limited overlap in the ages of chicks used in each technique: endoscopy was carried out on generally older birds and tended to yield lower overall burdens but a higher proportion of large worms than necropsies of generally younger birds. Endoscopy may have yielded lower counts because chicks' stomachs frequently contained residual food that partially obscured the view through the endoscope, a constraint that is more easily avoided when endoscoping adults in this system (Burthe *et al*. 2013). Nonetheless, endoscopy counts from shags have been shown to be repeatable (Burthe *et al*. 2013), and our successful application of this technique to developing hosts thus opens opportunities for monitoring individuals' worm burdens from an early stage in their long lives. Moreover, both *in situ* techniques identified similar prevalences and an increase in burden with chick age, indicating that endoscopy provides a useful index of between-individual variation in worm burdens. This index has already been shown to be valuable for quantifying the effect of anti-parasite treatment in both adults and juveniles, even at low doses (Burthe *et al*. 2013; online Supplementary Information, this study).

Necropsy, on the other hand, allows complete examination of the gut of the animal at any age and is likely to yield more accurate counts. However, as a destructive sampling technique, necropsy is of limited application because removing individuals from the population is not desirable when investigating longitudinal effects of parasite infection or working with protected natural populations. In such cases, obtaining samples relies on natural mortality that may more strongly affect certain parts of the population, such as those already paying the costs of a high parasite burden. Moreover, necropsy of recovered carcasses may underestimate infection intensity due to post-mortem migration of nematodes away from attachment sites. Given that the endoscope counts, also likely underestimates, captured the same broad patterns of infection as necropsy, we suggest that endoscopy provides an informative non-destructive index, albeit not true counts, of between-host variation in total parasite burden. The repeated measurement of an index of infection intensity across individuals' lives that this enables, while also allowing quantification of its long-term consequences for host fitness, is likely also to be of practical use in other systems.

Measuring long-term patterns in individuals' parasite burdens could potentially be made more logistically tractable if a non-invasive proxy for worm burden was available, such as FECs. However, in our system, FECs failed to detect the same levels of infection revealed by *in situ* measures. Although worms were found in 98% of all chicks examined, the majority of fecal samples (63%) did not contain eggs, and fecal egg presence was not related to *in situ* worm burden (online Supplementary Information). This may be due in part to chicks hosting a high proportion of worms that were small, likely immature and thus not producing eggs. Variation in this component of the parasite community may nonetheless be important for its impacts on host fitness, as larval worms can still cause severe pathology and thus have non-negligible costs (Fagerholm and Overstreet, 2008; H.-P. Fagerholm, personal communication). The limited presence and low counts of nematode eggs in host feces in this system appears to be a feature of this system, but we cannot rule out that FECs more

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Table 3. The top five best fit models of mass of endoscoped chicks. The set of candidate models included all combinations of the following variables: worm count (measured by endoscopy), log(age), sex and rank

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

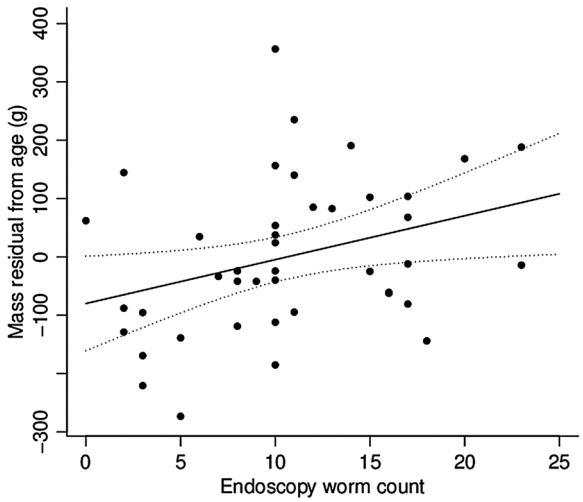

Fig. 4. The relationship among endoscoped chicks between mass at endoscopy and worm count. The solid line shows the fitted relationship and the dotted line the 95% confidence intervals. To account for other factors affecting mass it is shown as the residual from a LMM containing age as the only predictor, following the best-fit model for chick mass.

closely reflecting natural variation in true burdens could be obtained by examining larger amount of fecal material (but see online Supplementary Information), which is logistically difficult in the field.

Nematode burden in relation to host traits

The positive relationship between worm burden and chick age is consistent with expectations that chicks' infections should intensify throughout the nestling period. This increase suggests that chicks are continuously exposed to either infective larvae in fish and/or adult worms dislodged from the parent's proventriculus during feeding. Continuous exposure among chicks accords with the near-universal prevalence of worms among endoscoped adult shags over 6 study years (S. Burthe, unpublished data), which in turn indicates regular exposure to infected fish (Huizinga, 1971; Anderson, 2000; Fagerholm and Overstreet, 2008). Two further observations can also be interpreted as indicative both of larval worms establishing and growing inside the chick and of ongoing direct transmission of larger worms from the parent's proventriculus: the increase in the proportion of large worms with age in endoscoped chicks, and the presence of nematode eggs in the feces of a 12-day-old chick (lowest estimates for maturation time of larval *C. rudolphii* in the definitive host, c. 1 week; Dubinin, 1949; Huizinga, 1971). Regardless of transmission mechanism, we found established nematode infections in all chicks from early on in their period of rapid growth (from 6 to 9 days; Daunt *et al*. 2001). This supports the potential of parasitism in juvenile shags to influence developmental trajectories and hence long-term performance and fitness in this long-lived species (Lindström, 1999; Monaghan, 2008).

Previous studies have found host sex, timing of breeding and hatching order to be important in shaping individual chicks' responses to anti-parasite treatment (Reed *et al*. 2008; Granroth-Wilding *et al*. 2014, 2015), yet here we found little evidence that these traits were strongly associated with worm burden. This contrasts with adult shags, which display variation in burdens related to sex and timing of breeding, traits that also affect responses to treatment (Reed *et al*. 2008; Burthe *et al*. 2013). Moreover, in our opportunistic necropsies, selective mortality may have confounded the effects of certain host traits: similarly-aged individuals that died in the storm event had similar hatch dates, for example, yet these two traits may influence infection intensity in different ways (for example, burdens increasing due to continuous exposure with age *vs* a seasonal increase in exposure to infective larvae) whose effects we were not able to separate.

Effect of infection on host performance

Parasitism, by definition, is considered to be costly, yet we found a positive correlation between parasite burden and chick mass, a fitness-related trait that is positively associated with recruitment in many bird species (Schwagmeyer and Mock, 2008). This correlation may arise as chicks fed at a higher rate are likely to have higher levels of exposure to parasites, yet parasite infection in both parents and chicks can also affect how resources are distributed among family members (Granroth-Wilding *et al*. 2014, 2015). Experimental approaches that tease apart the relative effects of exposure, burden and host condition are therefore needed to quantify the effect of

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parasitism on individual performance. Examining the longer-term association between juvenile worm burden and success in later life should also be a priority for future endoscopy studies in this system, taking advantage of the non-destructive technique to quantify the accumulation of sub-lethal impacts typical of macroparasites. Such a chain of fitness effects is of particular importance where parasite infection can shape hosts' developmental trajectories and life histories (Fitze *et al*. 2004; Romano *et al*. 2011; Granroth-Wilding *et al*. 2014).

Concluding remarks

Measuring natural variation between hosts in parasite burdens is an essential link in understanding the role of parasites in regulating natural populations. Here, we have developed endoscopy as a non-destructive method to quantify relative parasite burdens in juveniles and revealed prevalence to be significantly higher than expected from more traditional proxy measures. Our demonstration of widespread infection that is established and increases from as early as 12 days of age highlights the potential importance of nematode infection in shaping the contribution of individual shags to population processes throughout their long life (over 20 years). However, we found no evidence to suggest that parasite burdens differ between subgroups of hosts that have previously been found to respond differently to parasite removal. Variation in tolerance among different parts of the population may therefore play a role in governing variation between hosts in how they are impacted by parasitism. Our findings suggest that endoscopy of live juveniles provides an informative index of natural variation in parasite burdens, finding the same patterns of infection across the host population as the more direct but destructive index of necropsy. In addition, our results showed that relationships between parasite burden and fitness-related traits in early life are not straightforward. Hence, in combination with experimental approaches, endoscopy provides a powerful tool to link variation in nematode burden with its impact on host success across a wild animal's life and across subgroups of the population, enabling predictions of how parasitism influences demographic processes in structured natural populations.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182016001700.

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