Postprint

Burthe, Sarah; Newell, Mark A.; Goodman, Gidona; Butler, Adam; Bregnballe, Thomas; Harris, Eileen; Wanless, Sarah; Cunningham, Emma J.A.; Daunt, Francis. 2013 Endoscopy as a novel method for assessing endoparasite burdens in free-ranging European shags (Phalacrocorax aristotelis). *Methods in Ecology and Evolution*, 4 (3). 207-216. 10.1111/2041-210x.12015

Endoscopy as a novel method for assessing endoparasite burdens in free-ranging European shags (*Phalacrocorax aristotelis*)

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Abstract

1. Parasites are a key driver of evolutionary processes in wild animal populations. However, assessing host endoparasite burdens non-destructively is problematic. Collection of faecal samples can be difficult, and faecal egg counts may not always be a reliable indicator of infection intensity.
2. Here we report on endoscopy as a method for assessing natural burdens of nematode parasites *Contracaecum rudolphii* in a wild seabird, the European shag (*Phalacrocorax aristotelis,* L.). We aimed to measure natural individual parasite burdens and repeatability of burdens over time, and verify that treatment with ivermectin removed parasites.
3. Endoscopy was rapid, averaging 6 minutes (n=159), with no obvious adverse effects on behaviour or breeding success compared to non-endoscoped birds.
4. Nematode burdens in the oesophagus and proventriculus of conscious shags were counted and classified as absent, low, medium or high using a flexible gastroscope with a camera attachment that recorded video footage.
5. Re-assessment of worm burdens was highly accurate, with 94% of randomly selected videos (n=50) giving identical categorical scores, and 70% of worm counts (n=40) giving the same total or differing by only one worm.
6. All birds were parasitized by *C. rudolphii.* Natural burdens were significantly higher in males and in late breeders.
7. Individuals had highly repeatable categorical parasite scores over time with 65% of control birds sampled more than once (n=17; mean interval between assessments= 10.8 days) showing no change in scores. However, although the rank ordering of bird's based on categorical scores remained constant, more finely resolved quantification indicated a slight seasonal decline in worm counts within individuals.
8. Treatment with ivermectin (4mg/kg of bird weight) resulted in complete removal of parasites. There was some evidence of temporal declines in worm counts with lower doses of

ivermectin, including a dose (0.7mg/kg) previously shown to impact chick survival and growth.

9. Endoscopy has considerable potential for investigating individual variation and temporal changes in endoparasite burdens and drug efficacy. Applicability and limitations of this method for other host-parasite systems are discussed.

Keywords

Nematode; gastroscope; faecal egg count; worm; gastrointestinal; macroparasite

Introduction

Parasites are ubiquitous in wild animal populations and considered to be a key driver of evolutionary processes (Sheldon & Verhulst 1996). There is much theoretical evidence that parasites can regulate and destabilise host population dynamics (Anderson & May 1978; Dobson & Hudson 1992) and a wealth of empirical evidence indicating that they impact the mass, body condition and fecundity of domestic animals (e.g. Dimander *et al.* 2000; Nieuwhof & Bishop 2005). However, parasites comprise an overlooked but important component of ecological communities with relatively few studies having considered the impact of parasites on wild animals (see Tompkins *et al.* (2011) for a recent review) . This is despite the fact that substantial individual heterogeneity in parasite burdens can occur (Shaw & Dobson 1995), which can lead to adaptive phenotypic evolution if related to both phenotype and fitness (Wilson & Nussey 2010).

Host-parasite studies are severely impeded by difficulties associated with detecting and measuring endoparasite burdens in wild hosts. Faecal egg counts are often the only available method for quantifying individual parasite burdens without destructive sampling, and have been used in a variety of different species (e.g. Gulland & Fox 1992; Irvine *et al.* 2001). However, such counts may be an unreliable index of worm burdens, due to density dependent worm fecundity (Anderson & Schad 1985; Tompkins & Hudson 1999), temporal variation in egg shedding rates (Shaw & Moss 1989), lack of egg shedding by larval parasite stages, or poor sensitivity at low worm burdens

(Levecke *et al.* 2009). Moreover, faeces may be difficult to sample in the field. Destructive sampling of the host to quantify parasite burdens cannot be undertaken if the host is of conservation importance or longitudinal data are of interest. Manipulation of parasite burdens experimentally using anti-parasite drugs has limitations since the impacts of treatment may vary depending on initial worm burdens and evaluation of drug efficacy may not be possible without destructive sampling of hosts. Development of a non-destructive method for assessing parasite burdens is therefore crucially important in advancing our understanding of host parasite systems.

The use of endoscopy to diagnose endoparasite infections is routinely used in human and veterinary medicine (e.g. Croese & Speare 2006; Sum & Ward 2009) but to our knowledge, only one published study reports utilisation of this technique in the wild. Jackson and Cooper (1988) used a gastroscope to measure prey digestion rates in sooty albatrosses (*Phoebetria fusca*), and suggested that this also had potential for assessing parasite burdens. Here we report on the first use of this technique to measure natural endoparasite burdens and drug efficacy in a wild animal host, the European shag. Shags are a useful model species for testing endoscopy because parasite prevalence is high (Abollo, Gestal & Pascual 2001; Reed *et al.* 2008) due to their fish prey being heavily parasitized (Groenewold, Berghahn & Zander 1996). In addition, adults at our study colony can be repeatedly caught during the breeding season and diet studies on conscious birds involving stomach flushing with c15mm diameter tubes have already been undertaken without any discernible adverse effects (Wanless, Harris & Russell 1993; Daunt *et al.* 2007). Finally, there is experimental evidence that parasites have a detrimental effect in this population, with late breeding adults treated with ivermectin having higher survival of male offspring (Reed *et al.* 2008) and treated last-hatched chicks having faster growth rates than controls (Reed *et al.* 2012). However, measurement of parasite burdens and treatment efficacy was not possible and hence the mechanisms behind the observed sex and phenology differences could not be elucidated.

This study had three main aims: to (i) use endoscopy to quantify individual endoparasite burdens and assess variation in burdens between hosts; (ii) measure repeatability of individual host parasite burdens over time and (iii) demonstrate that treatment with a suitable dose of ivermectin removed parasite burdens.

Materials and Methods

Study area and species

Fieldwork was undertaken in 2010 and 2011 on the Isle of May, Scotland (56°11′N, 2°33′W). As part of a long-term population study, individual shags were uniquely marked with one metal (British Trust for Ornithology) and one plastic darvic ring. Adults are sexually dimorphic, with males being 22% larger than females (Wanless & Harris 1997), and are sexed by vocalizations (Snow 1960). The breeding season is protracted, with an average of 4.2 weeks per year between first and median laying date (*unpublished* data; estimated from weekly checks of ca. 100 pairs at monitoring plots throughout the Isle of May between 1984 and 2008). Laying dates for birds used in the study were estimated from daily checks.

Shags are infected with the anisakid nematode *Contracaecum rudolphii* Hartwich, 1964, which attaches to the lining of the proventriculus and lower oesophagus (Abollo, Gestal & Pascual 2001; Reed *et al.* 2008). Post-mortem examination of archived carcasses of 7 adults and 2 chicks from the Isle of May confirmed infection with *C. rudolphii* in the proventriculus and lower oesophagus (J. Chantrey & D. Kowalek *unpublished data*). Shags become infected with third stage larvae via their fish diet, which is predominantly lesser sandeels (*Ammodytes marinus*) and butterfish (*Pholis gunnellus*) during the breeding season (Wanless *et al.* 1993; Daunt *et al.* 2007). Larval worms moult to become sexually mature adults in the final seabird host (Anderson 1992; Moravec 2009).

Ethical Considerations

Endoscopy is a licensed procedure and was undertaken under Home Office Project Licence PPL60/4001 and conducted by trained personnel (S. Burthe) holding a personal Licence (PIL40/6722). The work had full ethical approval from the University of Edinburgh

and CEH's Ethics Committees and the Home Office. Furthermore, as this was a novel technique that is usually undertaken in a clinical setting, the work was initially carried out under full independent veterinary supervision, first on anaesthetised and then on conscious birds. Anaesthesia was induced via a face mask with isofluorane and then delivered via a 3.5mm endotracheal tube. Our decision to endoscope conscious birds thereafter was made in conjunction with the veterinarian to improve bird welfare, by minimising the overall length of the procedure and significantly reducing (from several hours to <5 minutes) the time taken for the animal to return to normal behaviour, which was considered of primary importance since the birds were breeding. The veterinarian's overall assessment of the procedure confirmed it as being 'mild', the lowest severity banding for animal research undertaken in the UK. Discomfort to the birds was deemed to be at an acceptable level given the short duration of the procedure and the ease of entry of the endoscope to the proventriculus due to the anatomy of the species, and the method of feeding offspring, whereby food is regurgitated whilst the chick's head and neck are full inserted inside the parent's throat.

Endoscopy

Endoscopy of adult shags was undertaken from late incubation to mid chick-rearing (when the chicks were approximately 25 days old) using a 103cm long, 9mm diameter Olympus GIF-PQ20 gastroscope with a 2.8mm channel for air and water, a 100º field of view and a 150 Watt halogen light source (VES) with inbuilt air and water pump, powered by a portable EU10i generator (Honda). The gastroscope tip was flexible through 210/90º vertically and 100/100º horizontally. A compact camera (Xion) connected to the endoscope was used to view and record video images on a laptop using XION DiVASMini image software (Xion Medical, Berlin).

In 2010, endoscopy was carried out on four anaesthetised adult shags and this confirmed that parasites could be quantified in the proventriculus. Due to the ease of

147 endoscope insertion in this species and in order to minimise the time birds were absent from
148 their nests, endoscopy was then undertaken on 37 conscious birds in 2010. We established: i)
149 the best time of day for the procedure;) ii) that this methodology caused no adverse effects on
150 breeding behaviour and success; and iii) the degree of observable variation in endoparasite
151 burdens so that categorical scores could be established. As 2010 was a pilot study we only
152 analysed data from 68 conscious shags endoscoped during the 2011 breeding season.

153 To ensure that birds had empty stomachs, endoscopy was undertaken between 03:30-
154 07:30, before shags left for their first foraging trip of the day. An assistant placed a cloth over
155 the bird's eyes to reduce stress and held the neck stretched out on a cushion to prevent
156 movement, with the beak open approximately 2cm. The endoscope was lubricated with KY
157 jelly (Johnson & Johnson) and gently inserted down the oesophagus into the stomach up to a
158 length of 50cm (the base of the proventriculus) from the tip of the beak (measured using
159 gradations on the endoscope). Slight inflation with air helped introduce the endoscope into
160 the stomach and facilitated effective examination. Once a clear view was obtained on the
161 laptop, video recording was started and the scope was pulled out slowly and steadily,
162 enabling worms to be counted, and a categorical burden to be scored as: (i) absent- no live
163 worms seen; (ii) low- 1-10 worms; (iii) medium- 11-25 worms; or (iv) high- >25 worms
164 (Figure 1). Exact counts of worms were only possible for burdens of <40worms, due to
165 worms preventing good views. Counts of worms greater than this were recorded as >40. To
166 ensure that scores were not affected by viewing conditions we noted the presence of any food
167 and whether the view was satisfactory (visibility scored as 1-5, with 1 being a very poor view
168 with little confidence in the assessment, and 5 a clear view of the stomach with high
169 confidence). The endoscope was cleaned and disinfected between birds using a high-level
170 disinfectant TriGene wipe. The channels were cleaned with a soft brush and flushed with
171 diluted sterilising fluid (Milton) followed by deionised water. At the end of each session

(average 6.5 birds per session) the endoscope was soaked and cleaned with MedEzyme cleaner and MedDis disinfectant (Medichem) followed by deionised water. Ideally in a clinical setting, the endoscope would be soaked for 20-45 minutes in enzymatic cleaner and disinfectant between patients. However, this approach was not practical in the field and hence we adopted a procedure based on veterinary advice that reduced the risk of disease transmission to an acceptable level given the non-sterile, challenging field conditions.

Experimental Treatment and ivermectin efficacy

Adult shags were weighed and injected intramuscularly into the pectoral muscle with either saline solution (controls) or an anti-parasite drug treatment (ivermectin 1% w/v, Panomec, Merial Animal Health ltd., Harlow, UK) following endoscopy at first capture. Ivermectin is considered to be a safe drug for use in birds (Oksanen & Nikander 1989). Effective safe dose levels vary between bird species and parasites, for example doses of 4mg/kg in falcons and 50mg/kg in pheasants and chickens have been used without side effects (see review in Lierz (2001)). Shags were endoscoped repeatedly following treatment to investigate drug efficacy, with the aim being to find the minimum dose which removed worms from the proventriculus. Doses equivalent to 0.7 mg/kg of the birds weight had been used in previous experimental manipulations of adult birds (Reed *et al.* 2008) and found to have detectable effects on chick growth rates and survival; hence this dose was used initially. Subsequently a range of ivermectin doses were trialled (0.7; 1.0; 2.0; 3.0 and 4.0 mg/kg) with doses increased sequentially (one treatment dose per bird) to establish the dose where worm removal was complete.

Validation of observer repeatability

196 Fifty video recordings were randomly selected and rescored six months post sampling
197 by the same observer (S. Burthe) to evaluate whether categorical scores of parasite burdens
198 were repeatable. Forty randomly selected videos were also rescored for worm counts. Owing
199 to difficulties in counting high burdens, these videos were from a subset excluding those
200 originally scored with a high burden.

201

202 ***Statistical Analysis***

203 To evaluate whether endoscopy or treatment affected breeding success, a generalised
204 linear model with Poisson error structure of the number of chicks fledged per nest was fitted
205 for a subset of nests (n=147) that included the area where the endoscopy experiment was
206 undertaken plus surrounding areas which formed part of another dosing experiment but
207 without endoscopy. We fitted the experimental status of each individual bird in terms of
208 whether or not endoscopy had been undertaken. Treatment status was also fitted: whether the
209 individual had been dosed with ivermectin (low/medium (0.7-3.0mg/kg) or high (4.0mg/kg)
210 dose), was a saline treated control or a completely unmanipulated control.

211 Initial categorical worm scores per individual prior to treatment were analysed using
212 an ordinal logistic model. Previous experimental work on adult birds found variation in the
213 effects of drug treatment between the sexes and with phenology (early or late breeders; Reed
214 *et al.* (2008)), so we considered sex and phenology as potential explanatory variables.
215 Phenology was quantified using a binary variable ("earlylate") indicating whether the
216 individual laid earlier or later than the median laying date in 2011. As parasite burdens might
217 be expected to increase with exposure (age), and to investigate whether breeding success was
218 correlated with parasitism, we also considered minimum age (known age if first ringed as
219 chicks; assumed to be age 3 at ringing if first ringed as adults) and the number of chicks
220 fledged as potential explanatory variables. Finally, we also considered Julian date of

endoscopy, in order to check that patterns were not associated with seasonal changes in parasite burdens. Phenology and breeding success are highly correlated and hence not fitted together in the same model. We considered two-way interactions between sex and either age, breeding success or phenology.

To investigate dose efficacy, worm counts were analyzed using a generalised linear mixed effects model with Poisson error structure. As it was not possible to count worm burdens in the high category that were >40 worms, these were assigned a value of 40 worms (n=14). This could potentially introduce a degree of bias, so we checked for this by fitting the same candidate model set to categorical repeated measures data (see supplementary information). Bird ID was fitted as a random effect in both cases. We included variables that were found to be important from the analysis of the natural parasite burdens, along with Julian date, treatment group and time since treatment, as potential fixed effects. The interaction between treatment group and time since treatment was also considered, and is the key variable of interest. Treatment group was either specified as a categorical variable with four groups (control, low (0.7-1mg/kg), medium (2.0-3.0mg/kg) or high (4.0mg/kg) doses), or as aggregations of adjacent dose groups (e.g. by combining the "low dose" and "medium dose" groups). Comparing the performance of different aggregations of dose groups allowed us to identify, approximately, the threshold beyond which 'dose' begins to have a substantial impact upon the parasite burden.

Analyses were undertaken in program R ("MASS" package (Venables & Ripley 2002); lme4 package (Bates *et al.* 2011); ordinal package (Christensen 2012); R Development Core Team, 2009). Models were compared using Akaike's Information Criterion (AIC; Burnham and Anderson (2002)), calculating the difference (ΔAIC) between the AIC of alternative models relative to the "best" model with the lowest AIC. Poisson GLMMs were fitted using the Laplace approximation when performing model selection (the

246 glmer function in the lme4 package), but, to account for overdispersion, the final model was
247 re-fitted using PQL (the glmmPQL function in the MASS package) to obtain parameter
248 estimates and standard errors.
249
250 **Results**
251 ***Endoscopy***

252 Parasite burdens were assessed in 68 shags (25 control and 43 ivermectin treated), 43
253 of which were repeat sampled on 2-6 occasions. Shags responded calmly to insertion of the
254 endoscope, remained calm throughout the procedure with no evidence of rapid breathing, and
255 returned to the nest within five minutes after release, unless their mate had assumed nest
256 duties. The time taken to endoscope each bird averaged 6 minutes (n=159). Occasionally the
257 endoscope became blocked during the procedure and had to be removed and cleaned before
258 re-insertion, resulting in processing time increasing up to a maximum of 12 minutes (n=8
259 assessments >8 minutes). Views of the stomach were generally excellent (83% of
260 assessments scored 4 or 5 for visibility, only 4% scored <3). There was no evidence of an
261 adverse effect on breeding success of endoscopy (mean number of chicks fledged: no
262 endoscopy 1.58 (n=80); endoscopy 1.95 (n=40)) or dosing (Table 1).

263

264 ***Validation of observer repeatability***

265 The same categorical score was assigned to 47 (94%) of the videos that were re-
266 assessed. Of the three that were scored differently (originally scored as medium, medium and
267 high and rescored as high, low and medium respectively), the category only differed by one.
268 Twenty-eight (70%) videos that were rescored for worm counts were within 1 of the original
269 count (mean difference = 1.43; max difference= 8 worms).

Patterns of worm prevalence

All birds were infected with worms (100% natural prevalence). Twenty-seven individuals (40%) had low scores, 18 medium (26%) and 23 high (34%), with counts ranging from 2 to >40 worms. The best model of natural parasite burdens included sex and phenology (early or late), such that late males had the highest parasite burdens and early females the lowest (Tables 2, 3, Figure 2). Models which included the parameters Julian date, age or breeding success were less well supported (see supplementary information).

Of the 25 control birds, 17 were endoscoped more than once (total of 65 assessments). The mean interval between repeat assessments was 10.8 days (range 3-33 days, n=40). Categorical scores for individual birds were highly repeatable over time (Figure 3). However, although the overall rank order of birds remained the same over the breeding season based on categorical data, for the more finely resolved count data there was some evidence of a slight decline in worm counts within individuals over time (see Figure 5).

Assessment of ivermectin efficacy

Forty-three shags were dosed with ivermectin, with 27 birds endoscoped more than once (total of 94 endoscopy assessments). Repeat scoping occurred 1-18 days post dosing (mean 6.3 days, n=50). Complete absence of worms was only observed after dosing with the high (4mg/kg) dose (Figure 4) but there was evidence of a consistent decline in worm counts for lower doses. Birds treated with ivermectin remained worm free for at least 18 days. However, assessment of efficacy beyond this was not possible because shags at this colony become uncatchable late in the season.

293 The best supported models of the repeated count data included a significant
294 interaction between treatment group and time from dose, with additive sex and phenology
295 ("earlylate"; Tables 4 & 5; Figure 5; see supplementary information for categorical model
296 results). Models that allowed the interaction with time from dose for the high dose group to
297 differ from that of the other treatment groups had strong support ($\Delta AIC < 8$; see Table S3c
298 supplementary information), whilst models where the high dose group was amalgamated with
299 other treatment groups had very poor support ($\Delta AIC > 50$). Therefore there is strong evidence
300 that the high dose treatment group is significantly different from the other treatment groups.

301 There was modest support for the suggestion that low and medium dose treatments
302 were significantly different from controls (difference in AIC of 5.8 between a model with low
303 and medium identical to controls, relative to a model with low and medium separate from
304 controls).

305 **Discussion**

306 Endoscopy proved to be a rapid and reliable method for quantifying endoparasite
307 burdens in shags, and to our knowledge this is the first time the technique has been used for
308 this purpose in the wild. This represents a significant methodological advance in systems
309 where destructive sampling of the host is not possible, faecal samples are difficult to collect
310 or faecal egg counts are unreliable indicators of parasite burdens.

311 Endoscopy enabled repeated quantification of natural individual parasite burdens. In
312 contrast, destructive sampling can only provide single estimates, while in the faecal egg count
313 method there may be intermittent or seasonal changes in egg shedding through the sexual
314 life-cycle of the parasites. However, adopting endoscopy requires full consideration of ethical
315 implications. Endoscopy on conscious birds significantly reduced the time taken for birds to
316 return to normal breeding behaviour in this study. Other studies have also endoscoped

conscious animals, for example the upper respiratory tract and oesophagus in small ruminants (Stierschneider, Franz & Baumgartner 2007), suggesting that adaptation of this technique to wild mammals may be possible. As in our study, Jackson and Cooper (1988) successfully endoscoped sooty albatross and rock-hopper penguins (*Eudyptes chrysocome*), in the field without anaesthetic.

However, hosts may require anaesthesia if endoscopy is overtly stressful, causes significant discomfort, or if effective restraint is not possible. Crucially, a full cost-benefit analysis should be undertaken before endoscoping conscious birds, based on the species' biology and likely responses to endoscopy with and without anaesthesia. We recommend that anaesthesia should be undertaken initially to ensure that the endoscope is appropriate for the species in question, and that parasites can be viewed in the proventriculus. Endoscopy may be of more limited use if the parasites of interest are too small in size to be effectively viewed or are located lower down in the gastrointestinal tract. Although not possible in our study, post-mortem analysis of host burdens would provide unequivocal validation of the endoscope method for quantifying parasites and would be possible in some systems.

Natural parasite categorical scores were highly consistent within individuals, suggesting that a single observation using the endoscope, at least in this species, is sufficient to classify individual parasite burdens into broad categories. More detailed assessment of worm counts indicated temporal declines in burdens over the chick rearing period. This general consistency in scores between endoscope sessions, coupled with the re-analysis of video records, provides confidence in the ability of the endoscope to accurately determine parasite burdens in the host. The ability to record and archive videos is clearly of great benefit. Although not undertaken here, potentially the endoscope can be used to collect parasite samples from the gut using retrieval baskets or biopsy forceps (see Jones (1990) for

details). This would facilitate parasite identification in host systems where parasite communities are not well characterised.

Endoscopy was also used for evaluating drug efficacy, with the complete removal of worms in shags post-treatment with a 4.0mg/kg ivermectin dose. There was also some suggestion that birds treated with lower doses showed steeper declines in seasonal worm burden counts compared to controls. Evaluating drug efficacy is crucial if impacts of parasites on the host are to be fully understood. Previous experimental work on shags at this colony found that adults treated with equivalent low doses of ivermectin were significantly more successful at rearing sons than controls, but this difference was only found in late breeders (Reed *et al.* 2008). The impact of parasites in shags may therefore be even greater than suggested by this experiment, as lower doses would not have completely removed worms. Moreover, endoscopy in 2011 indicated that late breeding birds had significantly higher parasite burdens than early breeders, thus elucidating potential mechanisms for these observed differences in treatment effects.

Given our experiences we propose that endoscopy would be applicable in the field to many host endoparasite systems, including reptile, mammal and bird hosts. Endoscopy is a standard tool in veterinary medicine, routinely used on animals including: tortoise (Pizzi *et al.* 2005); cats (Kubiak *et al.* 2002); dogs (Le Sueur, Bour & Schaper 2010); rabbits (Johnson, Drazenovich & Hawkins 2007); rats (Silverman *et al.* 1980); sheep and goats (Stierschneider, Franz & Baumgartner 2007); cattle (Franz 2011); horses (Raphel 1982); and seals (reported in Jackson and Cooper (1988)). Endoscopy has been particularly well utilised as a technique in avian medicine (Hernandez-Divers (2005); Gancz (2006)), including species such as cockatoo (Oglesbee & Steinohrt 2001); falcons (Jekl *et al.* 2006); and seabirds (Jackson & Cooper 1988; Quesada *et al.* 2011). Dietary studies involving stomach flushing of conscious birds using tubes inserted via the oesophagus into the stomach have been

successfully undertaken in birds ranging in size from small shore-birds (<50g in mass; Tsipoura and Burger (1999) through to albatross (Cooper, Henley & Klages 1992). Endoscope tubes that are capable of being flushed with air/water are available as small as 4mm in diameter (Taylor & Murray 1999), opening up opportunities for a number of species.

Endoscopy potentially opens up many avenues of research into the impact of parasites on host life-history strategies and fitness. There is a wealth of long-term demographic studies across a wide range of species many of which focus on the extrinsic and intrinsic drivers of variation in individual survival and breeding success. However, the role of parasites has been largely ignored. Being able to monitor temporal changes and quantify individual heterogeneity in parasite burdens represents a major step forward for ecological research in this field. In conclusion, once a full assessment of the ethical considerations has been undertaken, endoscopy can potentially provide a rapid, reliable and repeatable method for assessing natural individual variation in hosts.

Acknowledgements

This work was funded by the Centre for Ecology & Hydrology project QUIP (QUantifying the Impact of Parasites on seabirds) and undertaken under Home Office licence PPL60/4001 and PIL40/6722. Thanks to Alan Bull, Afra Skene, Jon Green, John Harrison, Giles Constant, David Bardell, Polly Phillpot, Paul Macfarlane, Paula Redman and many others who assisted in the field. Thanks to Mike Harris, Tom Reed, Jens Peter and Hanna Granroth-Wilding for useful discussions and Deena Mobbs for video processing. We thank the Natural Environmental Research Council and the Joint Nature Conservation Committee for supporting the long-term seabird studies, and Scottish Natural Heritage for allowing us to work on the Isle of May.

Supplementary Information

S1: Video explaining the rationale for developing endoscopy as a method for assessing parasite burdens of wild shags.

S2: Four video clips showing the variation in parasite burdens encountered in the wild shags, ranging from an individual that has no worms present ("absent") following treatment with a dose of 4mg/kg of ivermectin; through low, medium and high burdens.

S3: Tables of full model results with AIC values and parameter estimates for the categorical repeated measures modelling.

References

Abollo, E., Gestal, C. & Pascual, S. (2001) Anisakid infection in the European shag Phalacrocorax aristotelis aristotelis. *Journal of Helminthology,* **75,** 209-214.

Anderson, R.C. (1992) *Nematode parasites of vertebrates : their development and transmission*. C.A.B. International, Wallingford, Oxon.

Anderson, R.M. & May, R.M. (1978) Regulation and Stability of Host-Parasite Population Interactions .1. Regulatory Processes. *Journal of Animal Ecology,* **47,** 219-247.

Anderson, R.M. & Schad, G.A. (1985) Hookworm Burdens and Fecal Egg Counts - an Analysis of the Biological Basis of Variation. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **79,** 812-825.

Bates, D., Maechler, M., Bolker, B. & (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. *http://CRAN.R-project.org/package=lme4*.

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multi-model inference : a practical information-theoretic approach,* 2nd ed. edn. Springer, New York ; London.

Christensen, R.H.B. (2012) ordinal---Regression Models for Ordinal Data R package version 2012.01-19 http://www.cran.r-project.org/package=ordinal/.

Cooper, J., Henley, S.R. & Klages, N.T.W. (1992) THE DIET OF THE WANDERING ALBATROSS DIOMEDEA-EXULANS AT SUB-ANTARCTIC MARION ISLAND. *Polar Biology,* **12,** 477-484.

Croese, J. & Speare, R. (2006) Intestinal allergy expels hookworms: seeing is believing. *Trends in Parasitology,* **22,** 547-550.

Daunt, F., Wanless, S., Harris, M.P., Money, L. & Monaghan, P. (2007) Older and wiser: improvements in breeding success are linked to better foraging performance in European shags. *Functional Ecology,* **21,** 561-567.

Dimander, S.O., Hoglund, J., Sporndly, E. & Waller, P.J. (2000) The impact of internal parasites on the productivity of young cattle organically reared on semi-natural pastures in Sweden. *Veterinary Parasitology,* **90,** 271-284.

Dobson, A.P. & Hudson, P.J. (1992) Regulation and Stability of a Free-Living Host-Parasite System - Trichostrongylus-Tenuis in Red Grouse .2. Population-Models. *Journal of Animal Ecology,* **61,** 487-498.

Franz, S. (2011) Endoscopy in cattle. *Tieraerztliche Praxis Ausgabe Grosstiere Nutztiere,* **39,** 281-288.

Gancz, A.Y. (2006) APPLICATIONS OF ENDOSCOPY FOR AVIAN MEDICINE. *Israel Journal of Veterinary Medicine,* **61**.

Groenewold, S., Berghahn, R. & Zander, C.D. (1996) Parasite communities of four fish species in the Wadden Sea and the role of fish discarded by the shrimp fisheries in parasite transmission. *Helgolander Meeresuntersuchungen,* **50,** 69-85.

Gulland, F.M.D. & Fox, M. (1992) Epidemiology of Nematode Infections of Soay Sheep (Ovis-Aries L) on St-Kilda. *Parasitology,* **105,** 481-492.

Hernandez-Divers, S.J. (2005) Minimally invasive endoscopic surgery of birds. *Journal of Avian Medicine and Surgery,* **19,** 107-120.

Irvine, R.J., Stien, A., Dallas, J.F., Halvorsen, O., Langvatn, R. & Albon, S.D. (2001) Contrasting regulation of fecundity in two abomasal nematodes of Svalbard reindeer (Rangifer tarandus platyrhynchus). *Parasitology,* **122,** 673-681.

Jackson, S. & Cooper, J. (1988) Use of fibre-optic endoscopes in studies of gastric digestion in carnivorous vertebrates. *Comp Biochem Physiol A Comp Physiol,* **91,** 305-308.

Jekl, V., Tukac, V., Hauptman, K., Knotkova, Z. & Knotek, Z. (2006) Endoscopic removal of a bullet from the cranial thoracic air sac of a peregrine falcon (Falco peregrinus). *Journal of Avian Medicine and Surgery,* **20,** 242-246.

Johnson, L.R., Drazenovich, T.L. & Hawkins, M.G. (2007) Endoscopic evaluation of bronchial morphology in rabbits. *American Journal of Veterinary Research,* **68,** 1022-1027.

Jones, B.D. (1990) *Veterinary endoscopy*. Saunders, Philadelphia, Pa.

Kubiak, K., Nicpon, J., Jankowski, M. & Sapikowski, G. (2002) Endoscopic evaluation of the distal portion of feline digestive tract. *Medycyna Weterynaryjna,* **58,** 777-779.

Le Sueur, C., Bour, S. & Schaper, R. (2010) Efficacy of a combination of imidacloprid 10%/moxidectin 2.5% spot-on (Advocate(R) for dogs) in the prevention of canine spirocercosis (Spirocerca lupi). *Parasitology Research,* **107,** 1463-1469.

Levecke, B., De Wilde, N., Vandenhoute, E. & Vercruysse, J. (2009) Field Validity and Feasibility of Four Techniques for the Detection of Trichuris in Simians: A Model for Monitoring Drug Efficacy in Public Health? *Plos Neglected Tropical Diseases,* **3**.

Lierz, M. (2001) Evaluation of the dosage of ivermectin in falcons. *Veterinary Record,* **148,** 596-600.

Moravec, F. (2009) Experimental studies on the development of Contracaecum rudolphii (Nematoda: Anisakidae) in copepod and fish paratenic hosts. *Folia Parasitologica,* **56,** 185-193.

Nieuwhof, G.J. & Bishop, S.C. (2005) Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Animal Science,* **81,** 23-29.

Oglesbee, B. & Steinohrt, L. (2001) Gastrointestinal string foreign bodies in a juvenile umbrella cockatoo. *Compendium on Continuing Education for the Practicing Veterinarian,* **23,** 946-+.

Oksanen, A. & Nikander, S. (1989) Ivermectin as a Bird Anthelmintic - Trials with Naturally Infected Domestic-Fowl. *Journal of Veterinary Medicine Series B-Zentralblatt Fur Veterinarmedizin Reihe B-Infectious Diseases and Veterinary Public Health,* **36,** 495-499.

Pizzi, R., Goodman, G., Gunn-Moore, D., Meredith, A. & Keeble, E. (2005) Pieris japonica intoxication in an African spurred tortoise (Geochelone sulcata). *Veterinary Record,* **156,** 487-488.

Quesada, R.J., Heard, D.J., Aitken-Palmer, C., Hall, N., Conley, K.J., Childress, A.L. & Wellehan, J.F.X. (2011) Detection and Phylogenetic Characterization of a Novel Herpesvirus from the Trachea of Two Stranded Common Loons (Gavia immer). *Journal of Wildlife Diseases,* **47,** 233-239.

Raphel, C.F. (1982) ENDOSCOPIC FINDINGS IN THE UPPER RESPIRATORY-TRACT OF 479 HORSES. *Journal of the American Veterinary Medical Association,* **181,** 470-473.

Reed, T.E., Daunt, F., Hall, M.E., Phillips, R.A., Wanless, S. & Cunningham, E.J.A. (2008) Parasite Treatment Affects Maternal Investment in Sons. *Science,* **321,** 1681-1682.

Reed, T.E., Daunt, F., Kiploks, A.J., Burthe, S.J., Granroth-Wilding, H.M., Takahashi, E.A., Newell, M., Wanless, S. & Cunningham, E.J. (2012) Impacts of parasites in early life: contrasting effects on juvenile growth for different family members. *Plos One,* **7,** e32236.

Shaw, D.J. & Dobson, A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: A quantitative review. *Parasitology,* **111,** S111-S133.

Shaw, J.L. & Moss, R. (1989) The Role of Parasite Fecundity and Longevity in the Success of Trichostrongylus-Tenuis in Low-Density Red Grouse Populations. *Parasitology,* **99,** 253-258.

Sheldon, B.C. & Verhulst, S. (1996) Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution,* **11,** 317-321.

Silverman, J., Lauber, J., Zelenakas, K. & Stellman, S. (1980) ENDOSCOPIC VISUALIZATION OF INDUCED COLONIC TUMORS IN RATS. *Laboratory Animal Science,* **30,** 544-545.

Snow, B. (1960) THE BREEDING BIOLOGY OF THE SHAG PHALACROCORAX ARISTOTELIS ON THE ISLAND OF LUNDY, BRISTOL CHANNEL. *Ibis,* **102,** 554-575.

Stierschneider, M., Franz, S. & Baumgartner, W. (2007) Endoscopic examination of the upper respiratory tract and oesophagus in small ruminants: Technique and normal appearance. *Veterinary Journal,* **173,** 101-108.

Sum, S. & Ward, C.R. (2009) Flexible Endoscopy in Small Animals. *Veterinary Clinics of North America: Small Animal Practice,* **39,** 881-902.

Tompkins, D.M., Dunn, A.M., Smith, M.J. & Telfer, S. (2011) Wildlife diseases: from individuals to ecosystems. *Journal of Animal Ecology,* **80,** 19-38.

Tompkins, D.M. & Hudson, P.J. (1999) Regulation of nematode fecundity in the ring-necked pheasant (Phasianus colchicus): not just density dependence. *Parasitology,* **118,** 417-423.

Tsipoura, N. & Burger, J. (1999) Shorebird diet during spring migration stopover on Delaware Bay. *Condor,* **101,** 635-644.

Venables, W.N. & Ripley, B.D. (2002) *Modern applied statistics with S,* 4th ed. edn. Springer, New York.

Wanless, S., Corfield, T., Harris, M.P., Buckland, S.T. & Morris, J.A. (1993) Diving Behavior of the Shag Phalacrocorax-Aristotelis (Aves, Pelecaniformes) in Relation to Water Depth and Prey Size. *Journal of Zoology,* **231,** 11-25.

Wanless, S. & Harris, M.P. (1997) *Phalacrocorax aristotelis* Shag. .*BWP Update,* **1,** 3-13.

Wanless, S., Harris, M.P. & Russell, A.F. (1993) Factors Influencing Food-Load Sizes Brought in by Shags Phalacrocorax-Aristotelis during Chick Rearing. *Ibis,* **135,** 19-24.

Wilson, A.J. & Nussey, D.H. (2010) What is individual quality? An evolutionary perspective. *Trends in Ecology & Evolution,* **25,** 207-214.

Table 1: Parameter estimates for the analysis of number of chicks fledged per nest fitting treatment group (unmanipulated, control, low or high ivermectin dose) and whether endoscopy was undertaken.

Parameter	Estimate	S.E.	z value	P
Intercept	0.467	0.092	5.069	<0.01
Control	-0.078	0.393	-0.197	0.844
Low dose	-0.234	0.375	-0.623	0.533
High dose	0.176	0.523	0.336	0.737
Endoscopy	0.338	0.374	0.906	0.365

Table 2: The 12 best supported models of natural categorical parasite scores based on endoscopy at initial capture (n=69; see supplementary online information for full tables). "Earlylate" refers to whether a bird laid before or after the median laying date in 2011.

Model parameters	df	ΔAIC
earlylate + sex	4	0.000
earlylate * sex	5	1.826
earlylate + sex + Julian date	5	1.869
earlylate + sex + age	5	1.993
earlylate + sex * age	6	2.279
earlylate * sex + sex * age	7	3.501
earlylate * sex + Julian date	6	3.666
sex	3	3.755
earlylate * sex + age	6	3.824
earlylate + sex + age + Julian date	6	3.865
earlylate + sex * age + Julian date	7	4.099
No. chicks fledged * sex	5	4.225

Table 3: Parameter estimates for the analysis of natural categorical parasite scores based on the best supported model (by AIC) which included sex and "earlylate" (whether a bird laid before or after the median laying date in 2011).

Model parameter	Estimate	Std. Error	t value
Sex (male)	1.726	0.521	3.312
Earlylate (late)	1.173	0.501	2.343

Table 4: The twelve best supported models for the analysis of repeated worm counts (n=159; see supplementary information for full tables). Bird ID was fitted as a random effect in all cases. Sex and "Earlylate" (whether a bird laid before or after the median laying date in 2011) were found to be important from modelling of control data and included in all models. "Time" indicates time from dose. Models were fitted with different classifications of the treatment groups: "C" is the control group; "L" is low dose (0.7-1.0mg/kg); "M" is medium dose (2.0-3.0mg/kg) and "H" is high dose (4.0mg/kg). Separation of groups is shown by a fullstop (e.g. Group (CL.M.H) would be grouped control and low, with separate medium and high dose groups). Main effects with interactions are denoted by an asterisk and interactions without main effects by a colon.

Model parameters	Main treatment effect	Interaction	df	ΔAIC
Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(C.LM.H)	low, medium and high doses	High has separate effect from low & medium	10	0.000
Sex + Earlylate + Time*Group(C.LM.H)	low, medium and high doses	High has separate effect from low & medium	9	0.731
Sex + Earlylate + Time*Group(C.L.M.H)	low, medium and high doses	All three doses have separate effects	11	1.323
Sex + Earlylate + Julian date + Time + Group(C.L.M.H) + Time:Group(C.LM.H)	low, medium and high doses	High has separate effect from low & medium	11	1.740
Sex + Earlylate + Julian date + Time*Group(C.LM.H)	low, medium and high doses	High has separate effect from low & medium	10	2.593
Sex + Earlylate + Julian date + Time*Group(C.L.M.H)	low, medium and high doses	All three doses have separate effects	12	3.028
Sex + Earlylate + Time*Group(CL.M.H)	medium and high doses	Separate effects for medium and high doses	9	3.347
Sex + Earlylate + Time + Group(CL.M.H) + Time:Group (CLM.H)	Only at high dose		8	3.835
Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(CL.M.H)	medium and high doses	Separate effects for medium and high doses	10	4.695
Sex + Earlylate + Julian date + Time*Group(CL.M.H)	medium and high doses	Separate effects for medium and high doses	10	4.925
Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(CLM.H)	Only at high dose		9	5.194
Sex + Earlylate + Time + Group(C.LM.H) + Time:Group (CLM.H)	Only at high dose		8	5.528

Table 5: Parameter estimates for analysis of worm counts based on the best supported (by AIC) model that included sex, "earlylate" (whether a bird laid before or after the median laying date in 2011), additive terms for treatment group and time from dose, and an interaction between treatment group and time from dose (high dose group separate from the other treatment groups). Model estimates were obtained using the "glmmPQL" function in R in order to account for overdispersion.

Model parameters	Estimate	S.E.	df	t-value	p-value
Intercept	2.311	0.197	86	11.735	0.000
Sex (male)	0.676	0.165	62	4.095	0.000
Earlylate (late)	0.460	0.172	62	2.681	0.009

Time from dose	-0.007	0.004	86	-1.668	0.099
Treatment group (low dose)	-0.099	0.200	62	-0.495	0.622
Treatment group (medium dose)	-0.574	0.281	62	-2.043	0.045
Treatment group (high dose)	0.009	0.265	62	0.033	0.973
time from dose:treatment group (low/medium dose)	-0.024	0.014	86	-1.647	0.103
time from dose: treatment group (high dose)	-0.316	0.058	86	-5.411	0.000

553

554 **Figure 1:** Images of the proventriculus of adult shags obtained from videos of birds exhibiting a range
555 of *C. rudolphi* burdens: a) absent; b) low c) medium and d) high.

556

557 **Figure 2**: Predicted probabilities (with 95% confidence limits) of control individuals of different sex
558 and phenology (early or late denoting whether a bird laid before or after the median laying date in
559 2011) having low, medium or high parasite burdens based on the best supported model by AIC which
560 included additive sex and phenology terms.

561

562 **Figure 3**: Categorical scores of natural parasite burdens in adult shags assessed with an endoscope.
563 Parasite scores have been offset slightly to facilitate identification of individuals.

564

565 **Figure 4**: Changes in parasite scores (categorical variable from absent to high) following initial
566 treatment with ivermectin (day 0) for individual birds. Scores have been offset slightly to assist
567 identification of individuals.

568

569 **Figure 5**: Predicted worm counts following treatment with ivermectin or controls for the best
570 supported model by AIC that included sex, "earlylate" and an interaction between treatment group
571 and time from dose. Points indicate raw worm counts. Controls are shown in pale grey; low dose (0.7-
572 3.0 mg/kg) in dark grey; high dose (4.0 mg/kg) in black. Predictions are for late breeding males.

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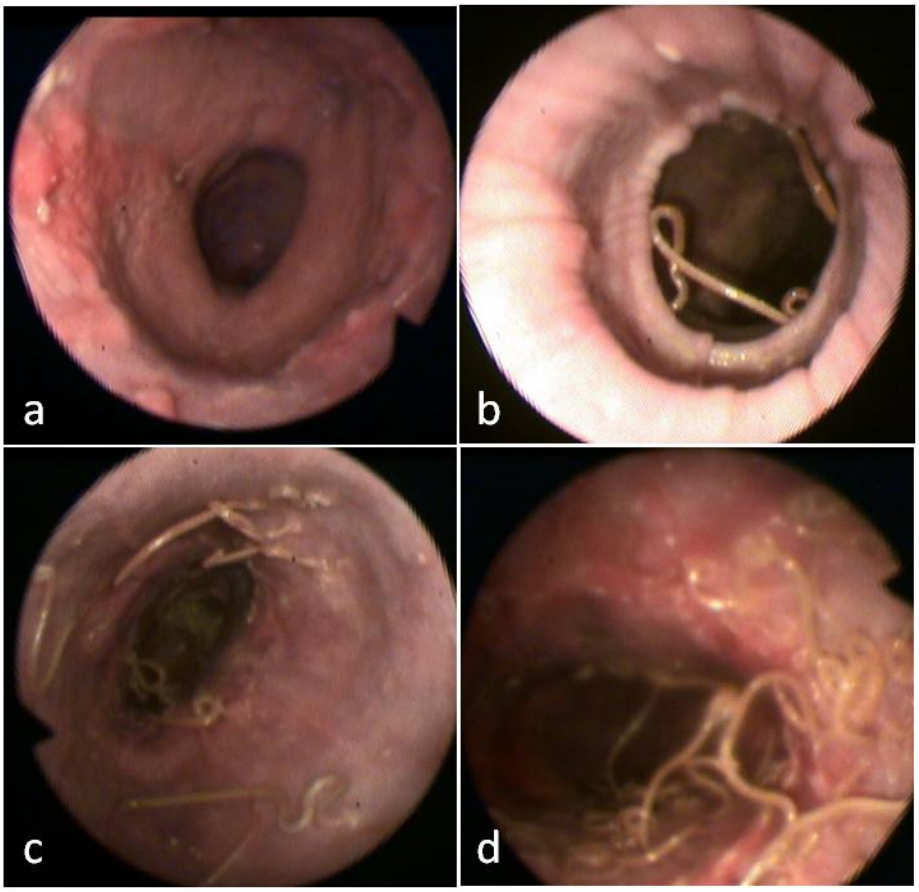
575 Figure 1:



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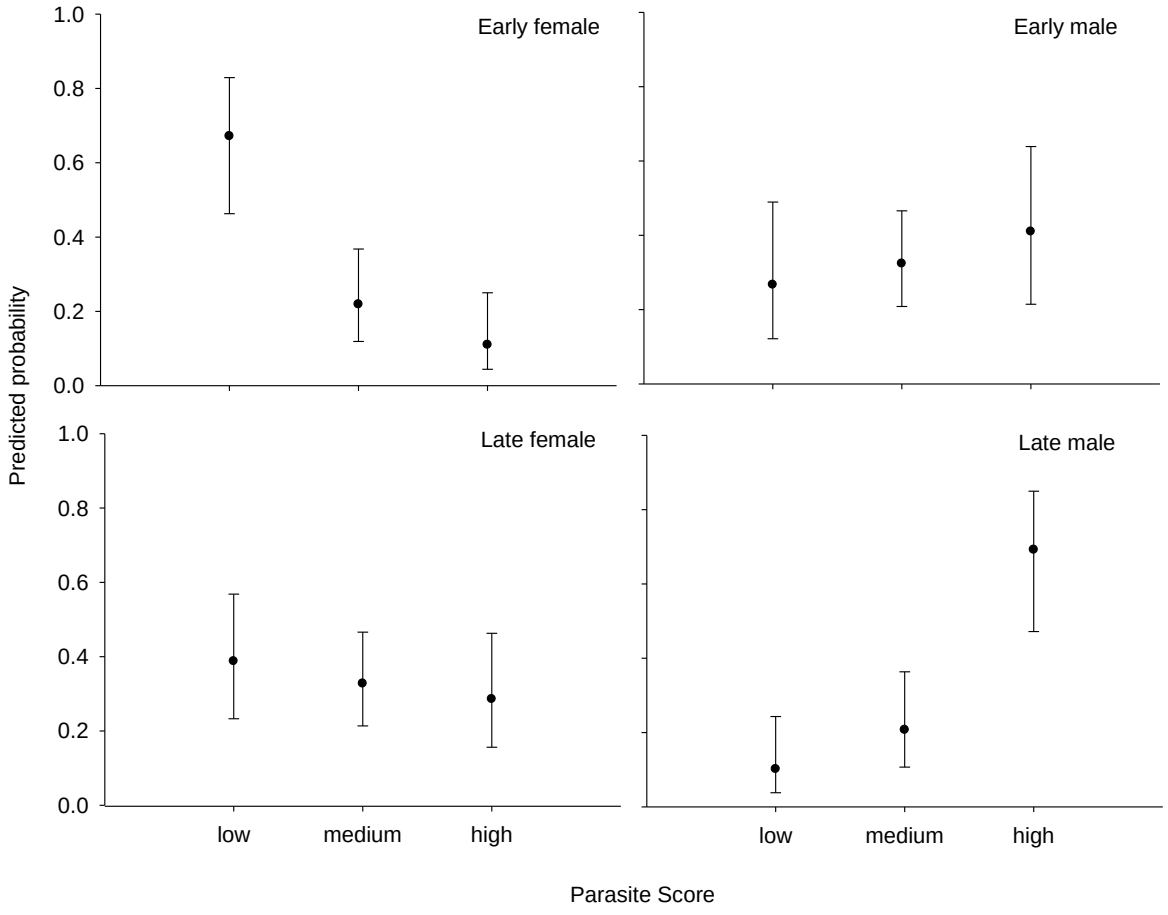
578 Figure 2



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581 Figure 3

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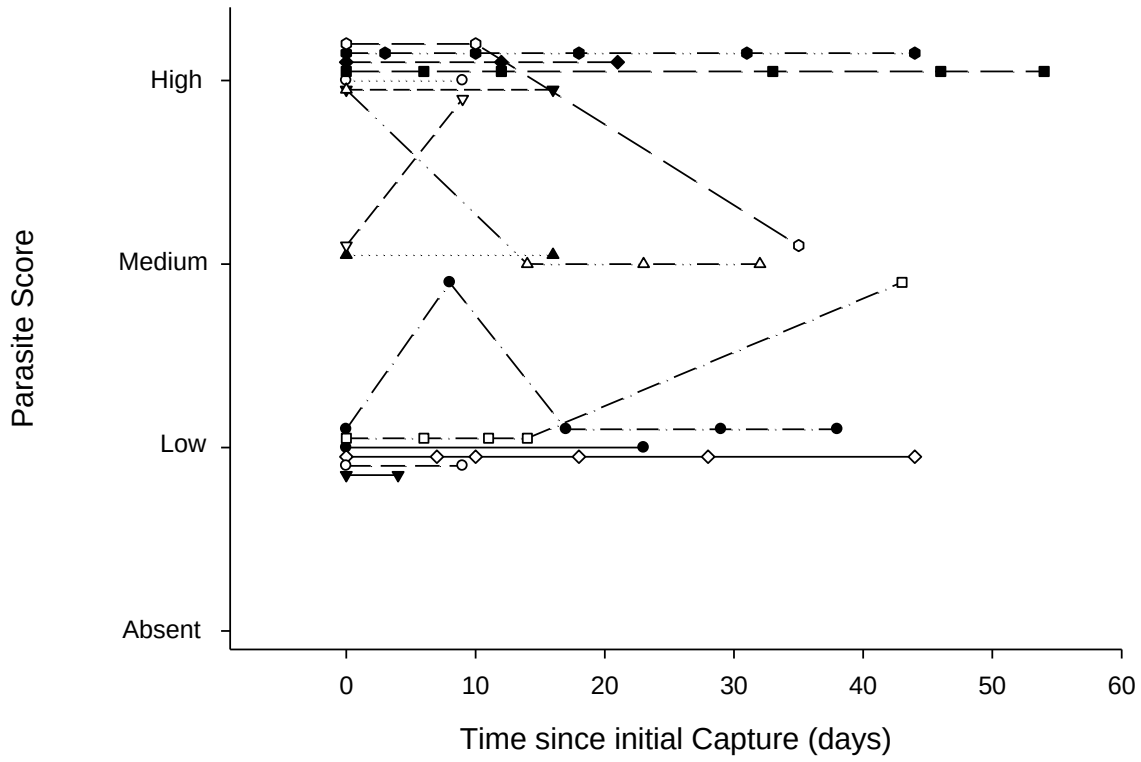

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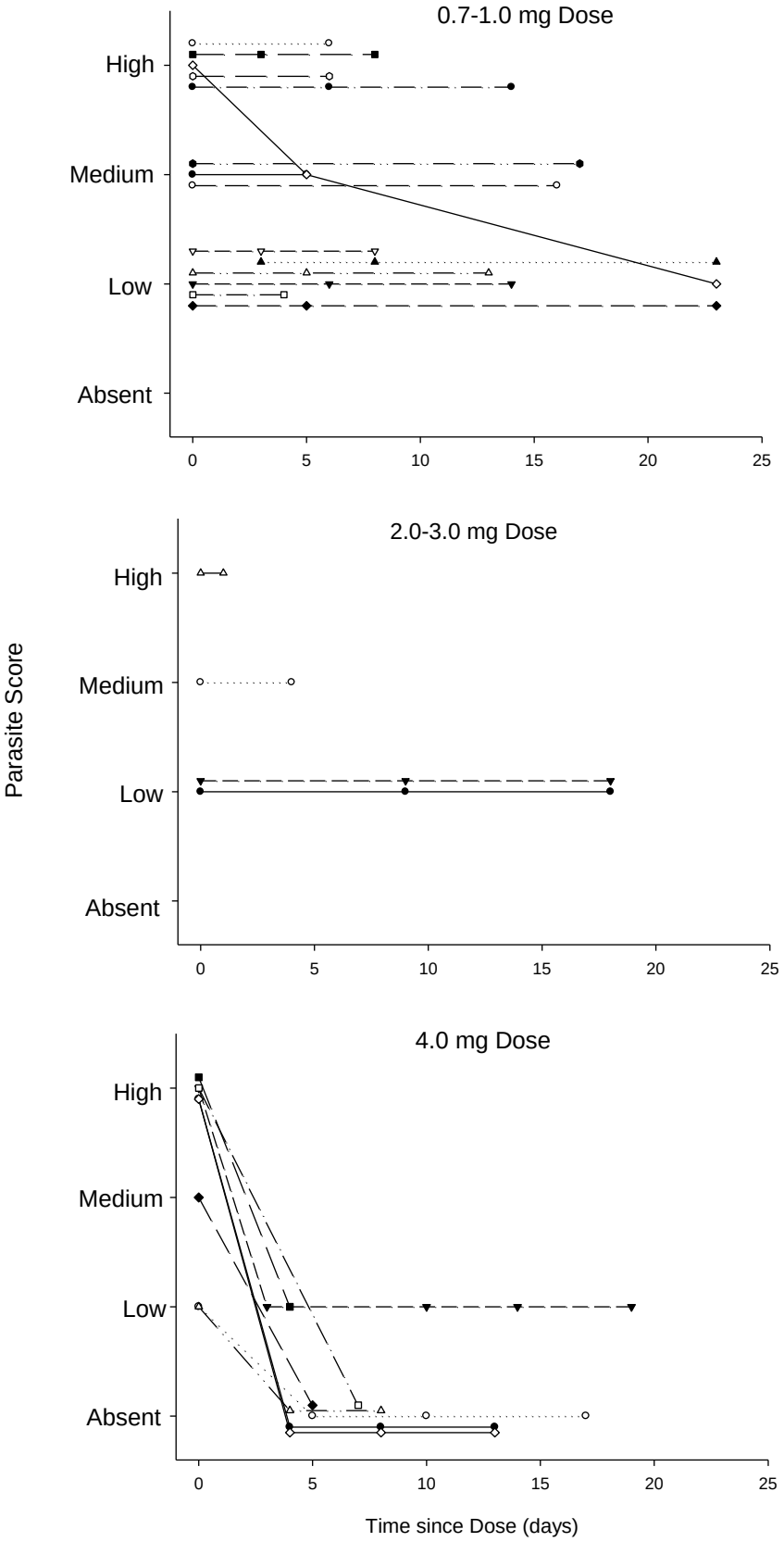
587 Figure 4



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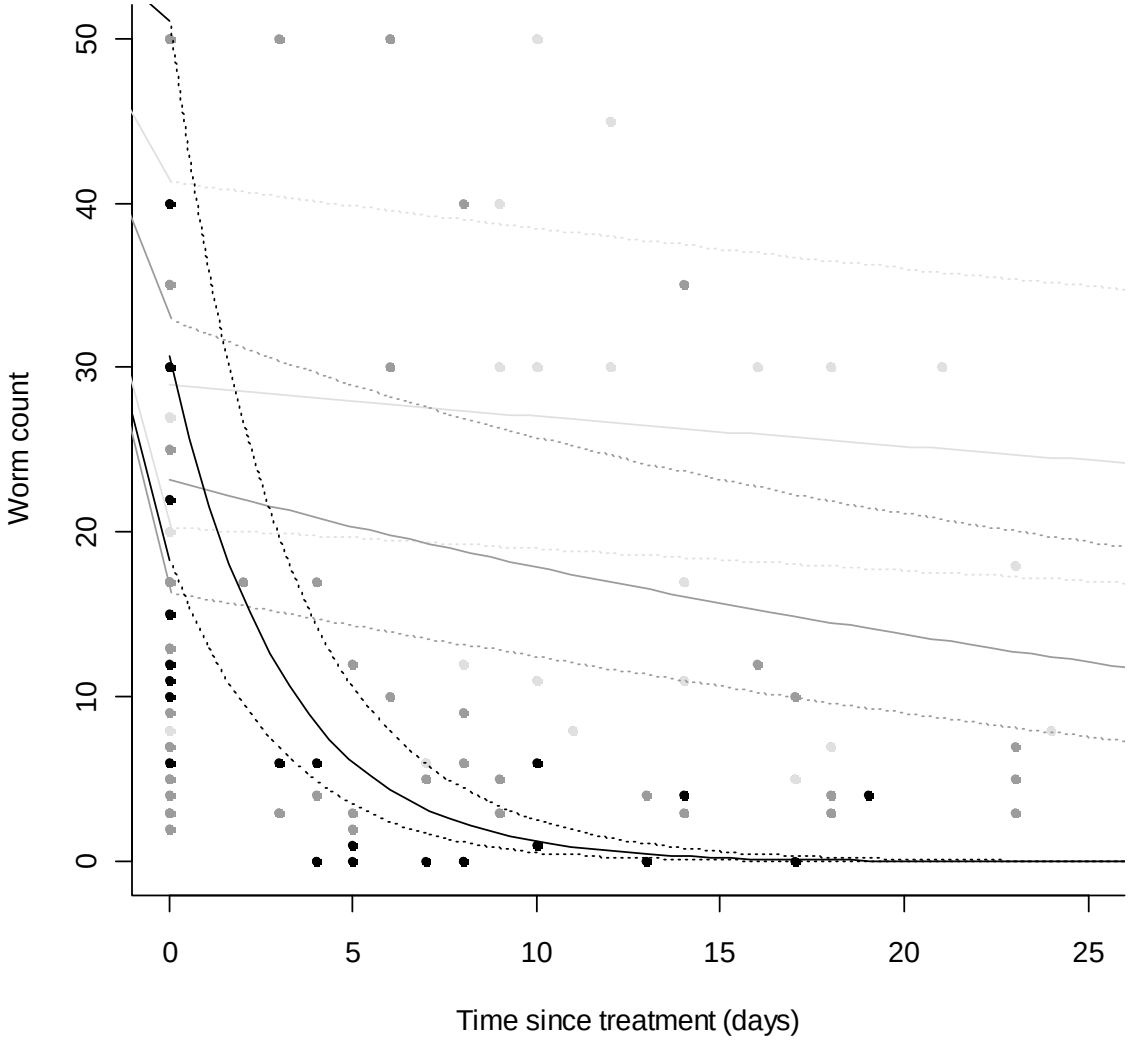
590 Figure 5



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