

1 **Lethal and sub-lethal effects of ivermectin on north temperate dung beetles,**
2 ***Aphodius ater* and *Aphodius rufipes* (Coleoptera: Scarabaeidae)**

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Abstract. 1. Ivermectin is an anthelmintic veterinary medicine, and is excreted in the dung of treated livestock in a mainly unmetabolised form. Ivermectin is known to have toxic effects on dung beetles, but most studies to date have been conducted on tropical and sub-tropical species. Relatively few laboratory studies have focused on the specific effects of ivermectin on survival and development of north temperate dung beetles.

2. In this study, we experimentally investigated the effect of ivermectin concentration on various life stages of two *Aphodius* dung beetle species. Dung was collected from cattle groups that had been treated with a subcutaneous injection of ivermectin. Laboratory bioassays were conducted by feeding adults of two beetle species (*Aphodius ater* and *A. rufipes*) with dung that contained different concentrations of ivermectin. Adult survival and oviposition were measured, and the subsequent development and survival of produced larvae was monitored over time.

3. Larval development rates were significantly slowed by ivermectin. Ivermectin had significant negative effects on the survival of larvae. Overall, ivermectin concentration caused large and significant reductions in the cohort size from an individual dung pat that would potentially contribute to the next generation of beetles.

4. In general, ivermectin concentration did not have significant negative effects on adult survival. The number of eggs per female *A. rufipes* was significantly reduced by ivermectin concentration in one of two bioassays, but the magnitude of the effect was not large. The actual impacts on dung beetle population dynamics in farmland would depend on several other factors, which are discussed.

Keywords: dung beetles, ivermectin, *Aphodius ater*, *Aphodius rufipes*, bioassay, survival, larval development

64 **Introduction**

65

66 Dung decomposition is an important ecosystem service in grazed grasslands and is a
67 major contributor to efficient nutrient cycling. Dung beetles play an important role in
68 dung decomposition via their tunnelling and feeding activities, which aerate dung pats
69 and promote dung decomposition along with microbes, earthworms and other dung
70 fauna. Beetles also appear to condition dung pats for further decomposition by
71 earthworms (Holter, 1979). In addition, dung beetles constitute part of the diet of
72 several vertebrate wildlife species, including bat (e.g. horseshoe bat) and bird (e.g.
73 chough) species of particular conservation interest (McCracken, 1993).

74

75 Dung beetle diversity in north and south temperate regions is threatened and/or
76 declining due to a range of land-use changes and animal husbandry practices. Factors
77 implicated in the decline of dung beetle biodiversity include urban development and
78 associated habitat encroachment and destruction (Lobo, 2001), reduced presence of
79 livestock due to conversion of pastures to cropland (Carpaneto *et al.*, 2007), changes
80 in traditional farming methods (Biström *et al.*, 1991; Gustavsson, 1998; Roslin, 1999;
81 Hutton and Giller, 2003), forestry regrowth on traditional pasturelands (Carpaneto *et al.*,
82 2007), and use of veterinary medicines (e.g. ivermectin) (Wall and Strong, 1987;
83 Herd *et al.*, 1996).

84

85 Here, we focus on the effects on dung beetles of ivermectin (part of the avermectin
86 group of chemicals), a veterinary medicine that has been used worldwide since the
87 1980s as an anthelmintic in the effective prevention and treatment of endo- and ecto-
88 parasitic infection in livestock. It is generally administered to livestock in one of three
89 ways: injection, pour-on formulation or as an intra-ruminal sustained-release bolus
90 (Floate *et al.*, 2005). It is excreted in a mainly unmetabolised form from treated
91 animals *via* dung over a period from days to months, depending on the method of
92 administration. Susceptibility of dung beetles to the lethal and sub-lethal effects of
93 ivermectin (and other related compounds) in dung is of particular concern, because of
94 the potential for reduced dung beetle biodiversity, impaired dung decomposition and
95 reduced prey resources for wildlife. Evidence from experimental studies using tropical
96 and sub-tropical dung beetle species generally suggests that ivermectin (and other
97 avermectins) does not adversely affect adult beetles but that larval survival can be
98 severely affected by chemical residues in dung (Wardhaugh and Rodriguez-
99 Menendez, 1988; Houlding *et al.*, 1991; Fincher, 1992; Wardhaugh *et al.*, 1993;
100 Wardhaugh *et al.*, 2001a, b). However, tropical species differ markedly from north
101 temperate species in their feeding and breeding habits, and may well differ from north
102 temperate species in their response to ivermectin exposure. To date, experimental
103 laboratory-based investigation of the specific effects of ivermectin on north temperate
104 species have been very limited. More specifically, laboratory-based studies on
105 *Aphodius* species (the dominant north temperate dung beetle group) include only *A.*
106 *constans* (Duftschmid) (Kadiri *et al.*, 1999; Errouissi *et al.*, 2001; Hempel *et al.*,
107 2006; Lumaret *et al.*, 2007; Römbke *et al.*, 2007) and *A. haemorrhoidalis* (L.) (Kadiri
108 *et al.*, 1999). Several other field studies of ivermectin effects on north temperate
109 beetles have measured dung beetle colonisation and/or larval abundance in dung pats
110 with and without ivermectin (e.g. Madsen *et al.*, 1990; Sommer *et al.*, 1992; Lumaret
111 *et al.*, 1993). However, field studies generally offer little insight into how any
112 observed effects of ivermectin are manifested. Overall, strong evidence of the

113 susceptibility of north temperate dung beetles to the ecotoxicological effects of
114 ivermectin is lacking.

115

116 Reduced survival of adult and/or larval dung beetle stages could potentially have
117 indirect effects on decomposition, such as diminished dung pat suitability for
118 degradation by late-successional decomposers (i.e. earthworms) and decreased prey
119 availability for vertebrate predators (such as birds and bats) which feed on dung
120 beetles (McCracken, 1993). Ivermectin may also persist in dung over a period of
121 weeks following excretion (Sommer and Steffansen, 1993; Wratten and Forbes,
122 1996).

123

124 Current wildlife management guidelines of conservation authorities (e.g. Natural
125 England, Joint Nature Conservation Committee) recommend livestock husbandry
126 practices that at least limit the use of anthelmintics such as ivermectin in order to
127 eliminate potential ecotoxicological risks for wildlife. Nevertheless, further evidence
128 is desirable to support such recommendations in north temperate regions. This present
129 study has used an experimental approach to investigate the lethal and sub-lethal
130 effects of ivermectin on different life history stages of two widely distributed and
131 abundant north temperate beetle species. In this study, a series of bioassays were
132 conducted using two species which are abundant and have a widespread distribution
133 in north temperate areas i.e. *Aphodius ater* (de Geer) and *A. rufipes* (L.) to
134 experimentally investigate the effect of ivermectin concentration on: a) survival of
135 adult beetles, b) oviposition by adults, c) larval development rates and d) survival of
136 larvae.

137

138 **Materials and Methods**

139 *Treatment of animals and collection of dung*

140 The study was carried out at the Teagasc research farm, Johnstown Castle
141 Environment Research Centre, Wexford, Ireland during 2005 and 2006. Cattle were
142 divided into adults (> 1 year old, 'cattle') and juveniles (< 7 months old, 'calves').
143 The same group of animals were treated in May (period 1) and again in August
144 (period 2) of the same year to supply dung that contained ivermectin for experiments.
145 Animals were grazed on grassland swards prior to and during the treatment period.
146 Both cattle and calf cohorts were divided into four groups, a control group that was
147 untreated and three treatment groups in which animals received a subcutaneous dose
148 of ivermectin (Qualimec™) by injection (0.2 mg kg⁻¹ body weight). Following
149 subcutaneous injection, ivermectin concentrations in dung typically reach a peak at 3-
150 5 days post-treatment, and thereafter decline to low detection limits (Bernal *et al.*,
151 1994; Herd *et al.*, 1996). Thus, to vary the ivermectin concentration in dung, the
152 treatment groups were dosed at 7, 5 and 3 days prior to dung collection from all
153 groups on the same day. Dung was collected separately from all groups, homogenized
154 by stirring, and frozen at -20°C until further use. To measure ivermectin
155 concentrations, two dung subsamples from each treatment group were analysed by
156 HPLC (High Performance Liquid Chromatography). Further details are available in
157 O'Hea (2008).

158

159 In addition to determining ivermectin concentrations in fresh dung, a separate study
160 was conducted to measure changes in concentration over time. Dung collected from
161 animals in 2006 (the same dung used in bioassays 6 and 8 in Table 1) was thawed
162 from each of the treatment groups and 250 g (wet weight) placed in separate pots

163 containing soil (to simulate conditions used in bioassays). No dung beetles were
164 added. Every week for 5 consecutive weeks, two subsamples of dung per ivermectin
165 level were analysed by HPLC to determine ivermectin concentrations.

166

167 *Bioassay test species*

168 *Aphodius ater* is a small beetle (4-6 mm) which occurs in early summer (April-June)
169 in north temperate regions. Females lay eggs in cavities below the dung crust. Larval
170 development takes place in the dung pat and new adult beetles emerge at the end of
171 the larval period. Adult *Aphodius rufipes* are approximately 9-13 mm in adult form
172 and can occur in very large numbers in late summer. Eggs are laid as clutches in soil
173 beneath the dung pat. Larval development occurs within the pat and most individuals
174 overwinter as prepupae in soil, emerging as adults in the following spring. Adult
175 beetles for use in bioassays were collected from various field sites. A subsample was
176 dissected and the bioassays initiated only when the sex ratio approximated 50:50.

177

178 *Bioassays*

179 Four bioassays were carried out for each beetle species using two dung types (cattle
180 and calf), giving eight bioassays in total (Table 1). Groups of adult beetles were
181 initially added to replicate dung pats from each experimental group. Adult survival
182 and oviposition were measured, and replicates were repeatedly inspected to determine
183 larval development and survival of the eggs laid by the adults.

184

185 Experimental units consisted of plant pots (diameter 13 cm) with 10 cm depth of
186 potting soil, and either 250 g (*A. ater* bioassays) or 300 g (*A. rufipes* bioassays) of
187 dung placed on the soil surface. Adult beetles were first added to replicate pats of
188 fresh dung (containing varying levels of ivermectin, Table 2) for an initial feeding
189 period (*A. ater*: 7 days, *A. rufipes*: 5 days). Adult beetles preferentially feed on fresh
190 dung, so the adults were transferred to a new pot of soil and batch of fresh dung (from
191 the same treatment group) to feed for a further 5-7 days (*A. ater* adults fed on dung for
192 a total of 7 days only in bioassay 4.). Data gathered from both of these feeding periods
193 were pooled for each bioassay to represent a single replicate. All pots were covered
194 with muslin to prevent beetles escaping. The experiments were conducted in a potting
195 shed where ambient temperature varied from 15°C to 20°C.

196

197 At the end of the adult feeding period (*A. ater*: 14 days total, *A. rufipes*: 10 days total)
198 surviving beetles were removed, counted and preserved in 70% ethanol until
199 dissection. In bioassays with *A. rufipes*, the dung and soil were searched and the
200 number of eggs recorded. Dung pats with *A. ater* were not searched for eggs at this
201 stage because their small eggs are difficult to find and susceptible to damage. To
202 determine larval development rates, dung pats were inspected every two weeks to
203 count larvae and record their development stage in each bioassay. Five developmental
204 life stages were identified for both beetle species: *A. ater* - instar I, II, III, pupa and
205 newly emerged adults; *A. rufipes* - egg, instar I, II, III and prepupa. Eggs and larvae
206 were replaced in the dung pats after each inspection. To calculate the proportional
207 survival during the larval life stages, initial values were based on maximum number
208 of larvae found in time period 1, 2 or 3 for *A. ater* (the small size of *A. ater* larvae
209 usually resulted in the greatest abundance being recorded in the second time period),
210 and number of eggs for *A. rufipes*. Final values were based on the number of emerged
211 *A. ater* adults, and the number of prepupae of *A. rufipes*. Bioassays 2, 4 and 6 (Table
212 1) included replicates in which regular inspections were conducted (for calculation of

213 development rates), and those in which they were not conducted (see O’Hea, 2008,
214 p.82). These were treated as different bioassays, and any associated error was part of
215 the random effect of bioassay (see below). Sampling ended when all larvae had
216 metamorphosed to immature adults (*A. ater*) or reached a prepupal stage (*A. rufipes*).

217

218 *Data analysis*

219 Generalised linear mixed models (GLMMs) were used to assess the effect of
220 ivermectin concentration on beetle survival and development. In each analysis (a-e),
221 fixed effects of concentration, dung type (calf/cattle dung), beetle species (*A. ater*/*A.*
222 *rufipes*) and their interactions were fitted. A random effect was incorporated to
223 account for variation among bioassays. The number of surviving adults (a), number of
224 eggs laid by *A. rufipes* females (b), and number of individuals surviving at the end of
225 the bioassay (e) were all modelled using Poisson regression (GLMM with a Poisson
226 distribution and log link function). The effect of ivermectin concentration, dung type
227 (calf/cattle), beetle species (*A. ater*/*A. rufipes*) and their interactions on the probability
228 of reaching a particular life stage by a certain time (analysis c) were assessed using an
229 ordinal model (GLMM with a multinomial distribution and a cumulative logit link
230 function). The proportional survival of larvae (d) was modelled using logistic
231 regression with binomial distribution and logit link. All analyses were fitted using the
232 GLIMMIX procedure in SAS.

233

234 **Results**

235 *Ivermectin concentrations in dung*

236 Within each dung collection event, no ivermectin was detected in dung from the
237 control group, and ivermectin concentration varied up to a maximum of 0.28 mg kg⁻¹
238 dung (wet weight) (Table 2). Maximum concentrations generally occurred in dung
239 from animals that were dosed three days prior to dung collection.

240

241 Over a 5-week period, ivermectin levels did not decrease in cattle and calf dung pats,
242 suggesting that ivermectin persists at sustained levels in dung over time (at least under
243 these conditions) (Fig. 1).

244

245 *Adult survival and egg-laying*

246 The response of adult survival to ivermectin concentration depended on the dung
247 beetle species and the dung type (Table 3a), and is therefore presented separately for
248 these factors (Fig. 2). Survival of adult *A. ater* was significantly reduced in the
249 bioassays in calf dung, but not in cattle dung. Survival of adult *A. rufipes* was not
250 significantly related to ivermectin in either cattle dung or calf dung. The positive trend
251 in the latter relationship, however, was only marginally non-significant. The number
252 of eggs per female *A. rufipes* was significantly and negatively related to ivermectin
253 concentration in the bioassays in cattle dung, although the magnitude of this decline
254 was not very large. There was no significant response in the bioassays in calf dung
255 (Fig. 3, Table 3b).

256

257 *Larval development and survival*

258 There was a highly significant and negative overall effect of ivermectin on larval
259 development (Fig. 4, Table 3c). For example, the predicted probability of a larval
260 individual developing beyond instar III was significantly affected by ivermectin for
261 both species. The largest effects occurred in the bioassays with *A. ater* in cattle dung.
262 These indicated an 80% probability of *A. ater* larvae having developed beyond larval

263 instar III after 4 weeks in the dung without ivermectin, whereas this probability
264 dropped to about 15% in dung with 0.2 mg of ivermectin per kg (wet weight of dung).
265 Negative effects on development were not as pronounced in the other bioassays, but
266 were still of considerable magnitude and significant (Fig. 4).

267
268 Ivermectin concentration had negative effects on the proportional survival of larvae of
269 both species of dung beetle (Fig. 5, Table 3d). Increased ivermectin concentration
270 consistently had a highly significant negative effect on the abundance of individuals at
271 the end of the bioassays (Fig. 6, Table 3e). Highest mean numbers of surviving newly
272 emerged adults (*A. ater*) or prepupae (*A. rufipes*) were found in the control dung pats
273 with no ivermectin. In the majority of cases, there were few, if any survivors, at the
274 end of the study in the dung pats with highest ivermectin levels (Fig. 6).

275

276 **Discussion**

277 In contrast to many similar studies, ivermectin concentrations were directly measured
278 in this study, and allowed us to directly relate ivermectin concentrations to the
279 observed effects. To our knowledge, this study is the first to simultaneously examine
280 the impacts of ivermectin on several stages of the life cycle of an *Aphodius* species
281 and the first to experimentally investigate effects of ivermectin concentration on *A.*
282 *ater* and *A. rufipes*. Overall, the results indicated that ivermectin can have differential
283 effects on different life cycle stages and on different species, and can have especially
284 strong and negative effects on the larval life stages.

285

286 Several different sources of variation may arise in field experiments and can confound
287 attempts to isolate the effects of ivermectin in general. To this end, laboratory
288 bioassays can more specifically investigate the effects of ivermectin and its effects on
289 specific groups of non-target organisms. The higher concentrations of ivermectin used
290 in this study are representative of concentrations found in dung pats in field
291 conditions. Livestock received the recommended dosage of 0.2 mg kg⁻¹ body weight;
292 thus, the concentration gradient does not exceed the expected concentrations observed
293 in fresh pats of recently treated livestock. The persistence of ivermectin in dung pats
294 is variable, and thought to be affected by temperature and sunlight, among other
295 factors. We found no decrease in ivermectin over a 5-week period (Fig. 1), but the
296 indoor conditions may have inhibited ivermectin degradation. However, Sommer *et*
297 *al.* (1992) also reported increased ivermectin concentration over a period of 45 days in
298 dung pats in field conditions, which they attributed to the metabolism of organic
299 matter and the relatively slow degradation of ivermectin.

300

301 *Adult survival and egg production*

302 In general, survival of the adult dung beetles was not negatively affected by
303 ivermectin residues in dung, and ivermectin did not inhibit egg production in *A.*
304 *rufipes* in this study. The latter result suggests that any initial cues detected by adult
305 female *A. rufipes* regarding suitability of dung for oviposition were not affected by the
306 presence of ivermectin in dung, since oviposition occurred in all dung pats. In this
307 study, the adult beetles were only exposed to ivermectin for a relatively short duration
308 of 10 to 14 days. Further work should examine the effects of a longer duration of adult
309 exposure to ivermectin, and would be needed to conclude that ivermectin has no
310 effect on adult survival and egg production in *Aphodius* beetles. Adult beetles were
311 not allowed to emigrate from the replicate dung pats in the bioassays in this study,
312 which eliminated the possibility of adult beetles responding to higher concentrations

313 of ivermectin by emigrating sooner from the dung pats. The emigration rates of dung
314 beetle can respond to dung quality and pat size (Gittings, 1994; Finn and Giller,
315 2000). The evidence across several studies provides no consistent effect of ivermectin
316 in preferentially attracting or repelling *Aphodius* species that colonised dung pats or
317 dung-baited pitfall traps (e.g. Holter *et al.*, 1993; Strong *et al.*, 1996; O’Hea, 2008;
318 Webb, in press).

319

320 *Larval development and survival*

321 Development rates of larvae were significantly and negatively affected by ivermectin.
322 Delayed development of beetle larvae in dung with ivermectin residues has been
323 previously observed (Lumaret *et al.*, 1993; Krüger and Scholtz, 1997). If ivermectin
324 results in slower larval development under field conditions, then larval survival may
325 also be adversely affected, particularly when dung is decomposing at a fast rate.
326 Under wet weather conditions in north temperate regions, the effects of rain and
327 earthworms can lead to relatively rapid dung removal which can result in mortality of
328 dung beetle larvae that have not completed their development (Gittings *et al.*, 1994).
329 Conversely, in drier conditions, dung pats may also dry out and cause mortality of
330 larvae (Lumaret and Kirk, 1987). Thus, there can be strong pressures on larvae to
331 complete their development before conditions in the dung pat become unsuitable, and
332 additional delays to larval development by ivermectin may increase larval mortality.

333

334 Due to variation in the initial numbers of eggs laid in the replicate dung pats, we
335 analysed the proportional survival of larval stages in *A. ater* and *A. rufipes*, which
336 were both significantly affected by ivermectin concentration (Fig. 5, Table 3d).

337

338 Overall, ivermectin concentration caused large and significant reductions in the cohort
339 size that would potentially contribute to the next generation of beetles. The final
340 number of newly emerged adults (*A. ater*) or prepupae (*A. rufipes*) was significantly
341 and negatively related to ivermectin concentration (Fig. 6, Table 3e). Erouissi *et al.*
342 (2001) also found no emergence of *A. constans* at concentrations of 1.427 mg kg⁻¹
343 dung (wet weight), and emergence remained significantly lower than the control at
344 concentrations of 0.038 mg kg⁻¹ dung (wet weight). In the current study, note that the
345 final number of individuals in this study is a composite measure that incorporates
346 several possible effects of ivermectin on the life cycle of *A. ater* and *A. rufipes*.
347 Although we investigated several stages of the life cycle, some potentially important
348 elements were not specifically assessed. For example, we do not have data on the
349 effects of ivermectin on the hatching success of eggs of *A. ater*. In addition,
350 ivermectin may affect other characteristics such as asymmetry, body weight and
351 survival of pupae. There is definitely scope for further work to be conducted on the
352 effects of ivermectin concentration on lifetime reproductive output (see Hirschberger
353 (1999) for a study of competition on lifetime reproductive output of *A. ater*) and
354 survival of the progeny from adults that develop from larvae that have been reared on
355 dung that contains ivermectin.

356

357 *Towards evidence-based conservation*

358 In an exercise to identify ecological questions of concern to policy-makers in the
359 U.K., one of a hundred questions listed was “What are the impacts on biodiversity of
360 prophylactic treatment of farm livestock with antibiotics, anti-fungal and anti-
361 helminthic compounds?” (Sutherland *et al.*, 2006). The findings of this study, together
362 with those of other studies in north temperate environments, could be used to inform

363 policy decisions about protection of dung faunal diversity from risks associated with
364 ivermectin and other anthelmintic products. However, a range of issues need be to be
365 considered to ensure a sound evidence base that informs satisfactory trade-offs
366 between conservation targets, animal welfare and livestock production.

367
368 At the scale of individual dung pats, a number of studies on *Aphodius* beetles indicate
369 that ivermectin concentrations may affect larval survival in *A. ater* (this study), *A.*
370 *constans* (e.g. Errouissi *et al.*, 2001), *A. haemorrhoidalis* (Kadiri *et al.*, 1999) and *A.*
371 *rufipes* (this study). For experimental investigations of ivermectin effects on dung
372 beetles, these results suggest the need for detailed life history analyses and
373 consideration of toxicity effects on more than one beetle species. The Dung Organism
374 Toxicity Testing Standardisation (DOTTS) group, under the auspices of the Society of
375 Environmental Toxicology and Chemistry (SETAC), has recently proposed a protocol
376 for testing toxicity effects of veterinary medicines on dung beetles (Lumaret *et al.*,
377 2007). However, this protocol proposes to investigate the lethal effects of selected
378 chemicals on instar I larvae of *A. constans* and does not measure sub-lethal impacts.
379 This may not be the most optimal approach if residues of veterinary medicine have
380 different effects on different species that vary in their sensitivity. Use of a single
381 species to test the effects of veterinary medicines may potentially over-generalise
382 these effects and fail to accurately assess the susceptibility of other species. Despite
383 this, Lumaret *et al.* (2007) have clearly identified the need for standardised testing,
384 and the demanding nature of this will certainly limit the possible range of test species,
385 and necessarily involve some limited generality. On the basis of the results of this
386 study, use of more than one species in such tests and inclusion of a test species that is
387 known to be a more sensitive representative of a taxonomic group would be a distinct
388 advantage.

389
390 Extrapolating from controlled experiments at the scale of individual pats to field
391 conditions, however, invokes several factors that affect the levels of ivermectin in
392 dung pats, and the actual impact on dung beetle populations and other farmland
393 wildlife. A set of critical factors involves the incidence, concentration and persistence
394 of ivermectin in determining the actual exposure of dung insects to ivermectin on
395 farmland. This will be affected by the dosage and method of administration to
396 livestock e.g. ivermectin concentrations in dung following bolus administration are
397 much higher than those following injection or pour-on (e.g. Herd *et al.*, 1996;
398 Errouissi *et al.*, 2001). The timing of turnout of livestock from winter housing will be
399 important in determining the co-incidence of turnout with peak activity of dung fauna,
400 and the level of synchrony of this timing across farms would also affect the
401 landscape-scale proportion of dung pats without ivermectin. The latter proportion will
402 also be affected by farm-level decisions about whether to dose all livestock, or a
403 subset of the herd i.e. only younger animals that have not yet developed immunity to
404 grassland parasites. In individual dung pats, persistent and slowly declining
405 ivermectin levels would result in a more sustained exposure to ivermectin for both late
406 colonizing adult beetles and developing larvae in aging dung pats (Sommer and
407 Steffansen, 1993). The temporal frequency and duration of ivermectin in livestock
408 dung will also depend on the extent to which doses are repeated. In addition to the
409 above factors, the extent to which dung beetle populations in the field (and dung fauna
410 generally) are actually impacted will also depend on the extent to which beetles are
411 preferentially attracted to or repelled by dung pats that contain ivermectin. Impacts
412 may also only be evident (or else may be exacerbated) when beetle populations are

413 under stress due to other factors, e.g. unfavourable weather conditions (see Krüger
414 and Scholtz (1998) for an example from South Africa). Given the variety of factors
415 involved across several scales (and this is not an exhaustive list) it is not surprising
416 that there is considerable uncertainty about the extent to which dung beetle
417 populations are depleted by ivermectin usage, and about the knock-on effects on
418 populations of vertebrate wildlife that prey on dung beetles.

419
420 The lack of information on usage patterns of veterinary medicines remains a major
421 obstacle in establishing the extent and intensity of chemical usage (Wardhaugh
422 (2005); but see Webb (2004) and predicting short- and long-term impacts on dung
423 beetle populations and biodiversity. Longer-term field investigations of the lethal and
424 sub-lethal effects of avermectins on dung fauna populations are required in north
425 temperate regions. These will help to effectively evaluate whether anthelmintic
426 residues in livestock dung represents a single toxic event with no long-lasting effect
427 on populations of dung fauna or is an event that can have a detrimental impact on
428 successive generations of dung insects and other farmland wildlife that depends on
429 them. The data in this study can add more resolution and insight to risk assessment
430 methodologies that may better predict the impacts of avermectins on dung fauna (Vale
431 and Grant, 2002).

432

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580 avermectins in temperate pastoral ecosystems. *Annals of Applied Biology* **128**:
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585 **Figure legends**

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588 Figure 1. Ivermectin concentration in dung pats over a 5-week period. Lines represent
589 temporal sampling of the same dung pat ($n = 1$) of each of cattle (triangles) and calf
590 (squares) dung.

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593 Figure 2. Proportion of adults of *A. ater* and *A. rufipes* surviving in relation to
594 ivermectin concentration (mg per kg dung (wet weight)). Points indicate survival of
595 beetles in each replicate. Lines represent the modelled relationship (back-
596 transformed). Panels refer to bioassays with *A. ater* in cattle dung (a), *A. rufipes* in
597 cattle dung (b), *A. ater* in calf dung (c) and *A. rufipes* in calf dung (d).

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600 Figure 3. Mean number of eggs per female *A. rufipes* in ivermectin bioassays
601 conducted in a) cattle dung and b) calf dung. Lines represent the modelled
602 relationship (back-transformed).

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605 Figure 4. Effects of ivermectin concentration on larval development of *A. ater* and *A.*
606 *rufipes*. Graphs represent the predicted model estimates for ivermectin levels of 0, 0.1
607 and 0.2 mg kg⁻¹ dung (wet weight), and plot the probability of a larva developing
608 beyond larval instar III over time. Ivermectin levels of 0, 0.1 and 0.2 mg kg dung⁻¹
609 (wet weight) are shown as short-dashed, long-dashed and continuous lines,
610 respectively.

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613 Figure 5. Effects of ivermectin concentration on proportional survival of larvae of *A.*
614 *ater* and *A. rufipes*. Values were based on the final number of individuals as a
615 proportion of initial number of eggs (*A. rufipes*) or number of larval instar I (*A. ater*).
616 Fitted lines are based on model estimates (back-transformed from log scale).

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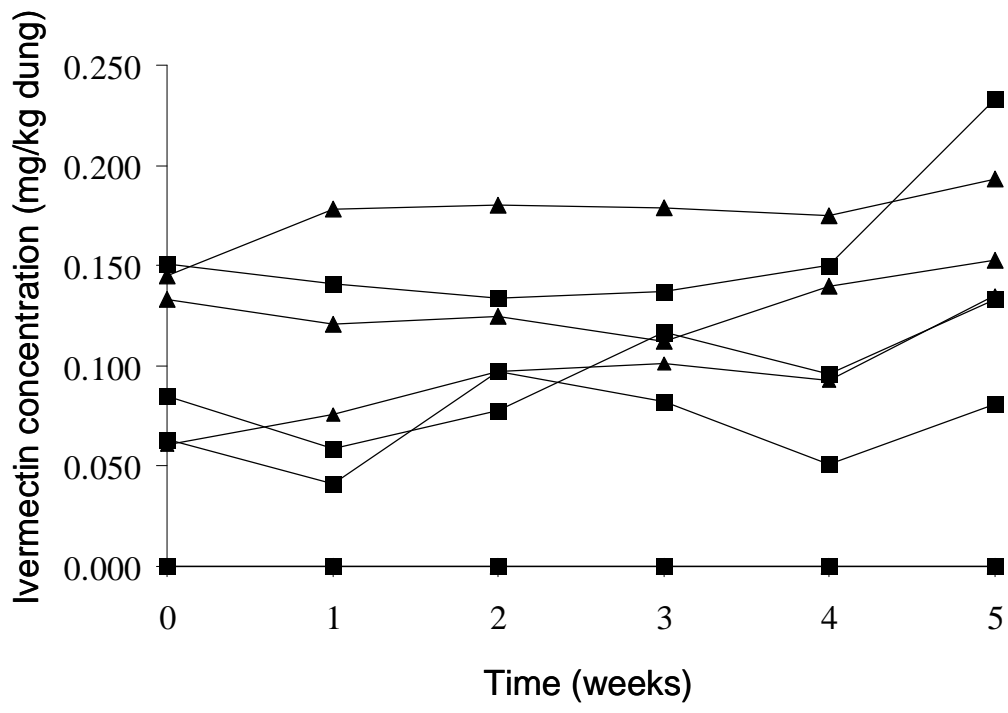
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619 Figure 6. Effects of ivermectin concentration on the final abundance of newly
620 emerged adults (*A. ater*) and prepupae (*A. rufipes*) in cattle and calf dung. Fitted lines
621 are based on model estimates (back-transformed from log scale).

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Figure 1

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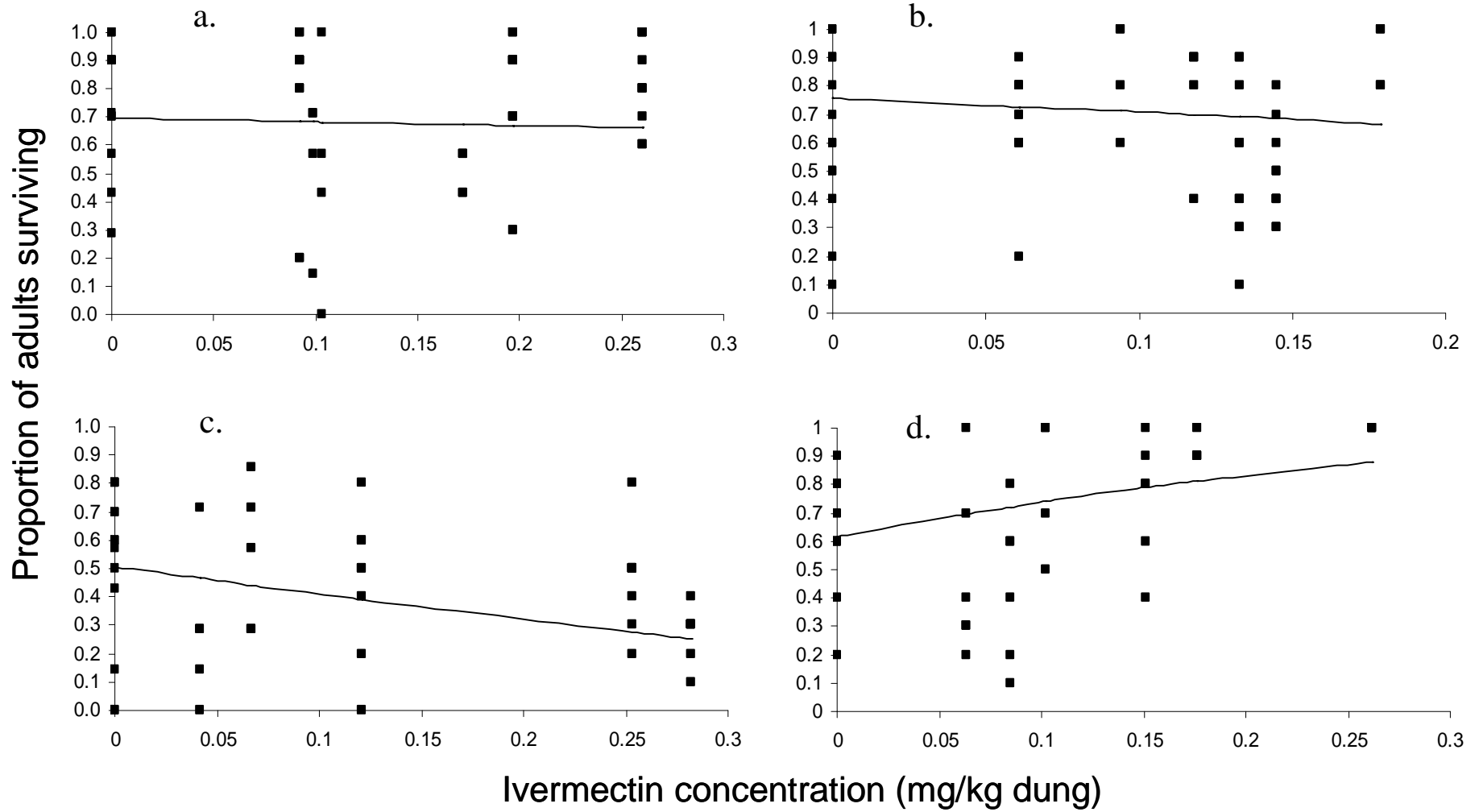
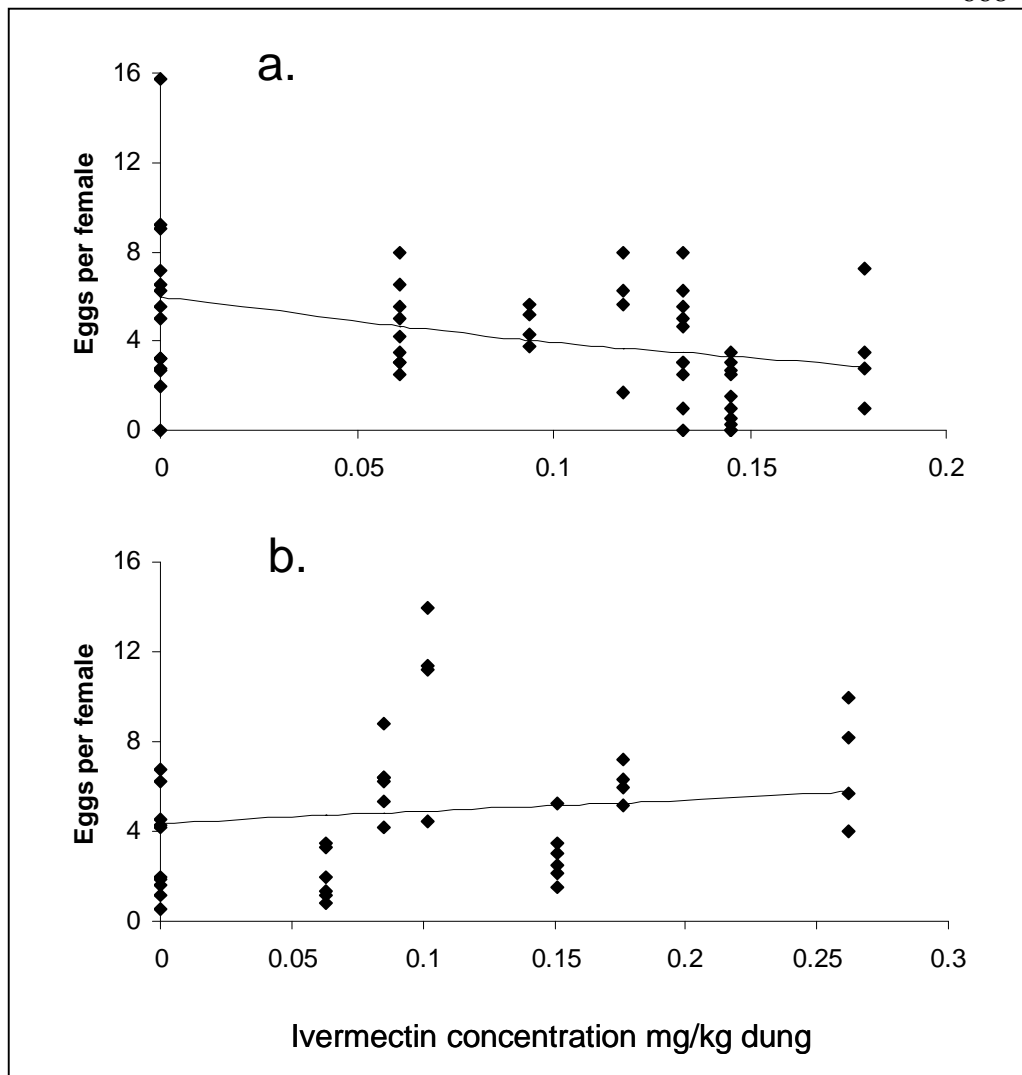


Figure 2



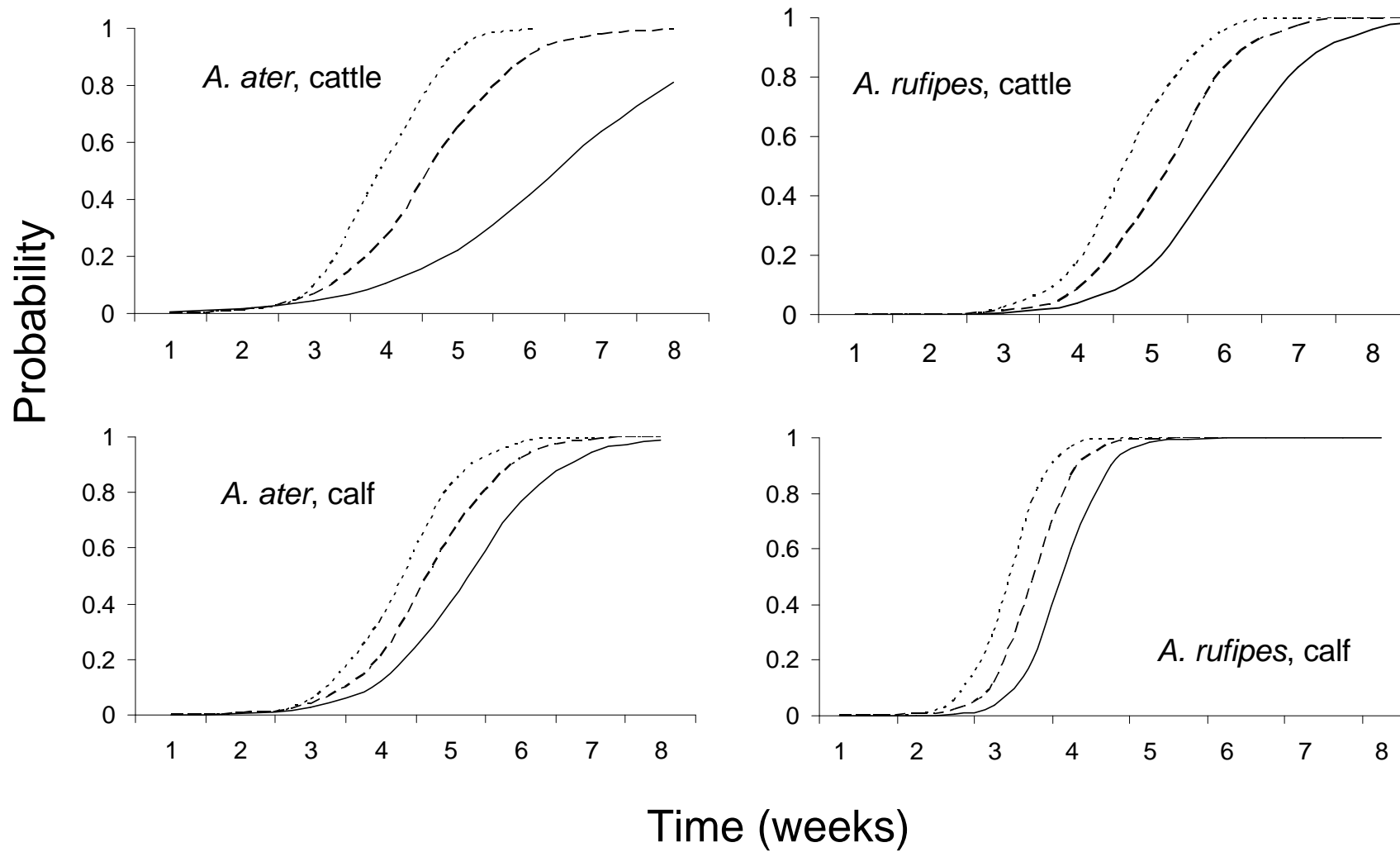
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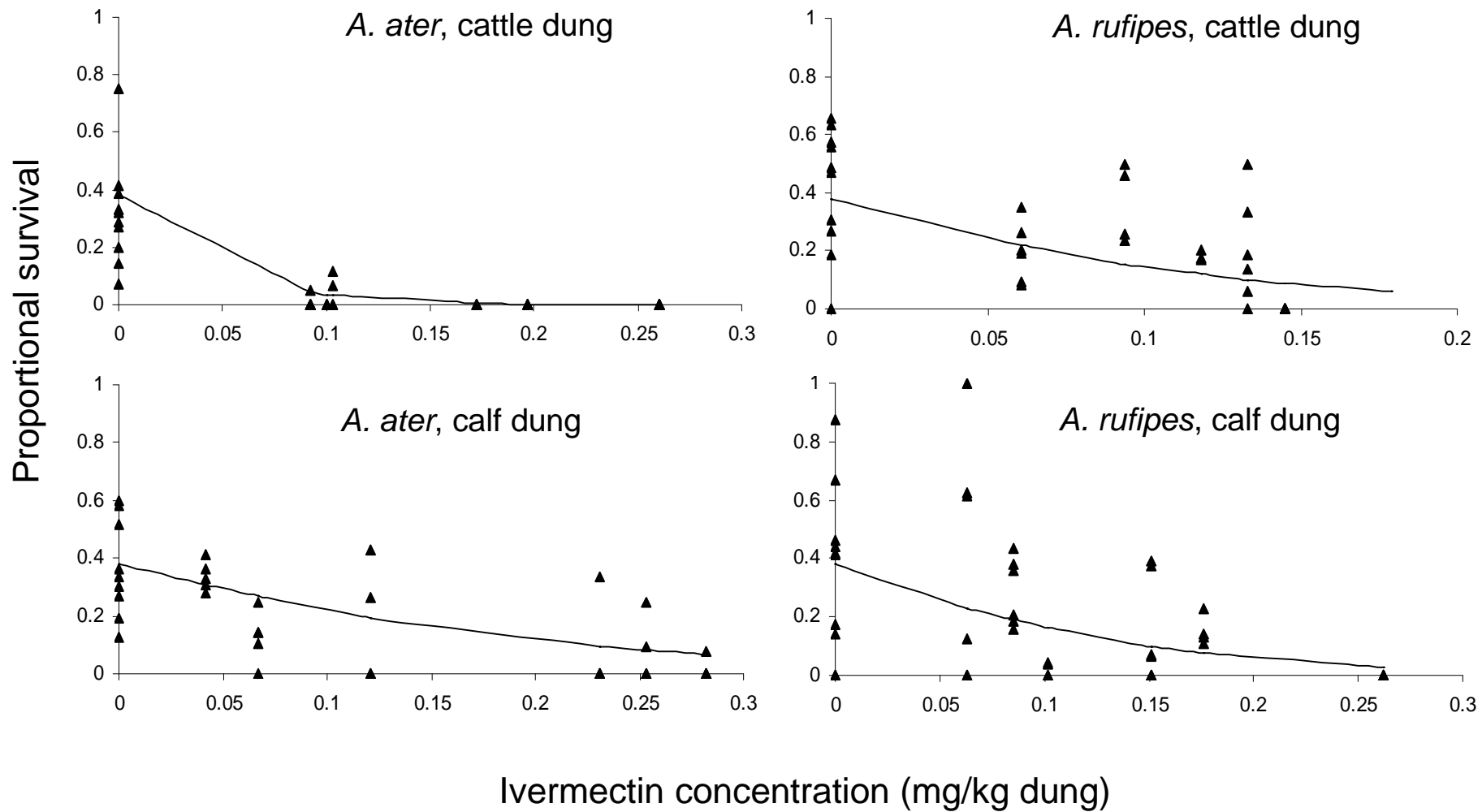
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Figure 3

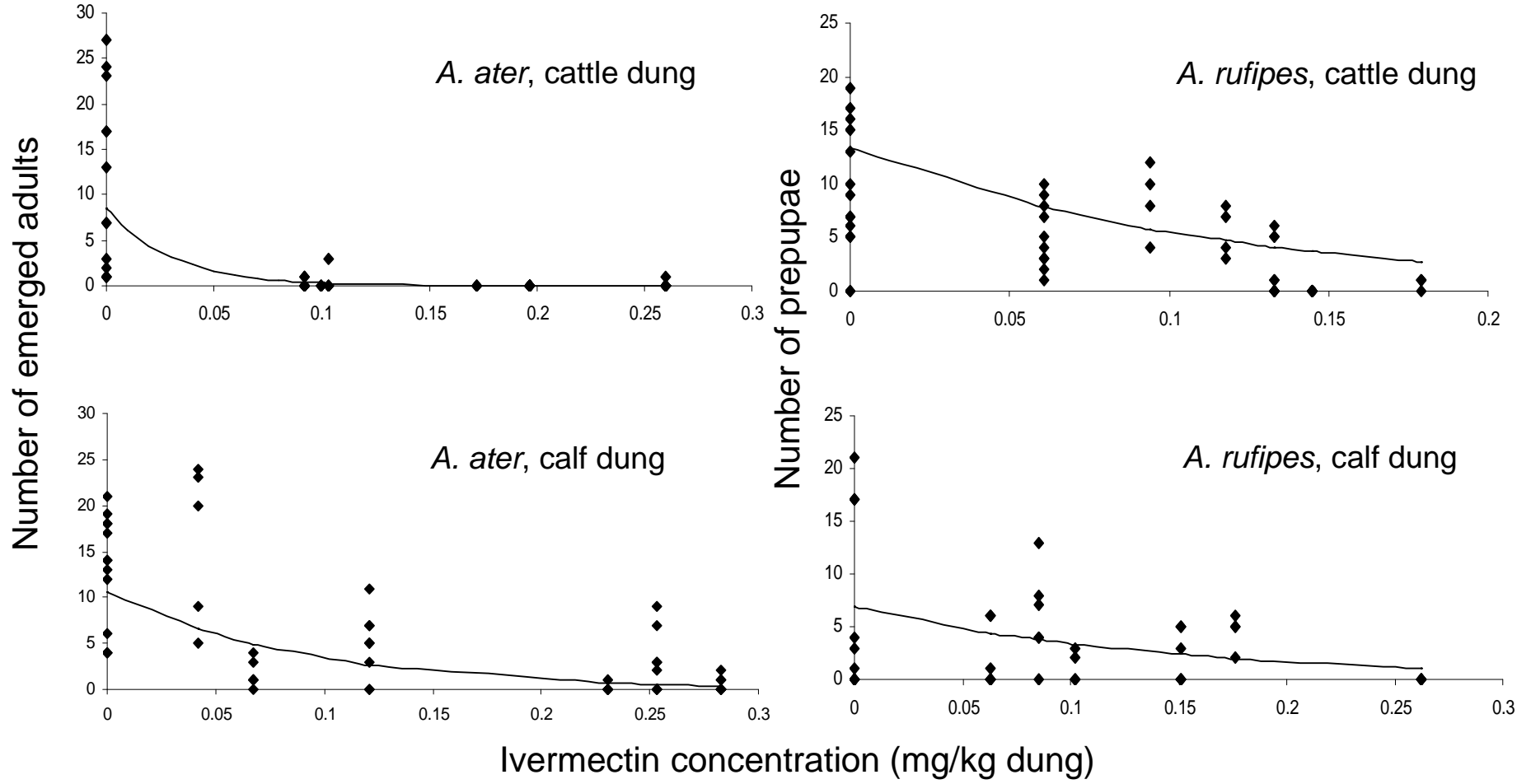


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707 Figure 4



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709 Figure 5

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Figure 6

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715 **Table 1.** Details of bioassay studies. Four bioassays were carried out for each
716 beetle species (two each in cattle dung and calf dung). The number of adult beetles
717 per replicate and the number of replicates varied across the bioassays.

Bioassay	Year	Cattle type	Beetle species	Number of beetles	<i>n</i>
1	May 2005	Cattle	<i>A. ater</i>	7	4
2	May 2006	Cattle	<i>A. ater</i>	10	10
3	May 2005	Calf	<i>A. ater</i>	7	5
4	May 2006	Calf	<i>A. ater</i>	10	8
5	August 2005	Cattle	<i>A. rufipes</i>	10	4
6	August 2006	Cattle	<i>A. rufipes</i>	10	10
7	August 2005	Calf	<i>A. rufipes</i>	10	4
8	August 2006	Calf	<i>A. rufipes</i>	10	6

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722 **Table 2.** Mean (\pm SE) ivermectin concentrations (mg kg^{-1} , wet weight of dung) ($n =$

723 2), in dung collected following treatment with a subcutaneous injection. Columns

724 indicate the control group, and groups dosed 7, 5 and 3 days prior to dung collection.

725 $^{\dagger}n = 1$

Bioassay no.	Cattle type	Control	-7 days	-5 days	-3 days
1	Cattle	0	0.099 (0.0055)	0.103 (0.0075)	0.172 (0.0110)
2	Cattle	0	0.092 (0.0045)	0.260 (0.0005)	0.197 (0.0055)
3	Calf	0	0.042 [†]	0.067 (0.0005)	0.23 (0.0165)
4	Calf	0	0.121 (0.0170)	0.253 [†]	0.282 (0.0235)
5	Cattle	0	0.094 (0)	0.179 (0.0045)	0.118 (0.0010)
6	Cattle	0	0.061 (0.0010)	0.133 (0.0240)	0.145 (0.0165)
7	Calf	0	0.176 (0.0150)	0.102 (0.0015)	0.262 (0.0050)
8	Calf	0	0.063 (0.0045)	0.085 (0.0040)	0.151 (0.0030)

726

727 **Table 3.** Summary of fixed effects in GLMM analyses of different life history
 728 measurements (a-e). Included are the F-value, and level of significance (P). Degrees
 729 of freedom for analyses a-d were: $F_{1,187}$, $F_{1,91}$, $F_{1,8957}$ and $F_{1,129}$, respectively. For
 730 analysis e, degrees of freedom were $F_{1,100}$ for *A. ater* and $F_{1,87}$ for *A. rufipes*.

Life stage analysed	F	P
a) proportion of adults surviving		
Concentration	0.07	0.7879
Dung	1.09	0.2987
Beetle	0.28	0.5953
Beetle*Dung	0.01	0.9271
Concentration*Dung	1.20	0.2751
Concentration*Beetle	3.16	0.0773
Concentration*Beetle*Dung	6.83	0.0097
b) eggs per female <i>A. rufipes</i>		
Concentration	5.93	0.0168
Dung	0.54	0.4623
Concentration*dung	12.86	0.0005
c) larval development		
Concentration	50.83	<.0001
Time	3693.57	<.0001
Concentration*Time	139.41	<.0001
Concentration*Beetle	35.05	<.0001
Time*Beetle	699.12	<.0001
Concentration*Time*Beetle	3.76	0.0524
Concentration*Dung	55.23	<.0001
Time*Dung	35.23	<.0001
Concentration*Time*Dung	47.86	<.0001
Time*Beetle*Dung	13.17	0.0003
d) proportion of larvae surviving		
Concentration	105.39	<.0001
Beetle	2.9	0.0908
Dung	0.07	0.7847
Dung*Beetle	1.29	0.2579
Concentration*Beetle	3.14	0.0788
Concentration*Dung	14.26	0.0002
Concentration*Dung*Beetle	9.62	0.0024
e) final abundance		
<i>A. ater</i>		
Concentration	123.26	<.0001
Dung	0.41	0.5249
Concentration*Dung	40.45	<.0001
<i>A. rufipes</i>		
Concentration	109.67	<.0001
Dung	3.74	0.0564
Concentration*Dung	7.41	0.0079
Number of Eggs	80.23	<.0001

