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

O'Hea, N.M.[1, 2], Kirwan, L.[1, 3], Giller, P.S.[2] and Finn, J.A.[1*]

[1]*Teagasc, Environment Research Centre, Johnstown Castle, Wexford, Ireland*
[2]*Department of Zoology, Ecology and Plant Science, University College Cork, Ireland*
[3]*Centre for Scientific Computing, Waterford Institute of Technology, Co. Waterford, Ireland*

*Corresponding author:

Dr. John Finn,
Teagasc,
Environment Research Centre,
Johnstown Castle,
Wexford,
Ireland.

Tel: +353 53 9171273.
Fax: +353 53 9142213.
Email: john.finn@teagasc.ie

Running title: Ivermectin effects on *Aphodius* dung beetles

Word count up to refs = ~4500
Word count for refs only= ~1400

Word count including references: ~5900
Number of tables: 3
Number of figures: 6

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

Introduction

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

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

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

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

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

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

Materials and Methods

Treatment of animals and collection of dung

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

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

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

Bioassay test species

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

Bioassays

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

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

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

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

Data analysis

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

Results

Ivermectin concentrations in dung

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

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

Adult survival and egg-laying

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

Larval development and survival

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

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

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

Discussion

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

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

Adult survival and egg production

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

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

Larval development and survival

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

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

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

Towards evidence-based conservation

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

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

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

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

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

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

Acknowledgements
We thank Martin Danaher and Paddy Byrne for conducting HPLC analysis. We also thank Debby Britschgi, Johanna Köster, Stephen Nolan, Erin O'Rourke and Mairéad Shore for technical assistance as part of student placements. Financial support to N. O'Hea from the Teagasc Walsh Fellowship scheme is gratefully acknowledged.

Bibliography

Bernal, J. L., Del Nozal, M. J., Salas, M., Galante, E. and Lumaret, J. P. (1994). HPLC determination of residual ivermectin in cattle dung following subcutaneous injection. *Journal of Liquid Chromatography* **17**: 2429-2444.

Biström, O., Silfverberg, H. and Rutanen, I. (1991). Abundance and distribution of coprophilous Histerini (Histeridae) and *Onthophagus* and *Aphodius* (Scarabaeidae) in Finland (Coleoptera). *Entomologica Fennica* **27**: 54-66.

Carpaneto, G. M., Mazziotta, A. and Valerio, L. (2007). Inferring species decline from collection records: roller dung beetles in Italy (Coleoptera, Scarabaeidae). *Diversity and Distributions* **13**: 903-919.

Errouissi, F., Alvinerie, M., Galtier, P., Kerbœuf, D. and Lumaret, J.-P. (2001). The negative effects of the residues of ivermectin in cattle dung using a sustained-release bolus on *Aphodius constans* (Duft.) (Coleoptera: Aphodiidae). *Veterinary Research* **32**: 421-427.

Finn, J.A. and Giller, P.S. (2000). Effect of patch size on colonisation and utilisation of ephemeral resources: an experimental analysis using north temperate coprophagous dung beetles. Ecography **23**: 315-327.

Fincher, G. T. (1992). Injectable ivermectin for cattle: effects on some dung-inhabiting insects. *Environmental Entomology* **21**: 871-876.

Floate, K. D., Wardhaugh, K. G., Boxall, A. B. A. and Sherratt, T. N. (2005). Fecal residues of veterinary parasiticides: non-target effects in the pasture environment. *Annual Review of Entomology* **50**: 153-179.

Gittings, T. (1994). *The community ecology of* Aphodius *dung beetles*. Unpublished PhD thesis. University College Cork, Ireland.

Gittings, T., Giller, P. S. and Stakelum, G. (1994). Dung decomposition in contrasting temperate pastures in relation to dung beetle and earthworm activity. *Pedobiologia* **38**: 455-474.

Gustavsson, G. (1998). Dyngbagger (Coleoptera: Scarabaeidae) på kustnära betesmarker i mellersta Halland. Entomologisk Tidskrift **119**: 151-162.

Hempel, H., Scheffczyk, A., Schallnaß, H.-J., Lumaret, J. P., Alvinerie, M. and Römbke, J. (2006). Toxicity of four veterinary parasticides on larvae of the dung beetle *Aphodius constans* in the laboratory. *Environmental Toxicology and Chemistry* **25**: 3155-3163.

Herd, R. P., Sams, R. A. and Ashcraft, S. M. (1996). Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained-release, pour-on or injectable formulations. *International Journal for Parasitology* **26**: 1087-1093.

Hirschberger, P. (1999). Larval population density affects female weight and fecundity in the dung beetle *Aphodius ater*. *Ecological Entomology* **24**: 316-322.

Holter, P. (1979). Effect of dung beetles (*Aphodius* spp.) and earthworms on the disappearance of cattle dung. *Oikos* **32**: 393-402.

Holter, P., Sommer, C., Grønvold, J. and Madsen, M. (1993). Effects of ivermectin treatment on the attraction of dung beetles (Coleoptera: Scarabaeidae and Hydrophilidae) to cow pats. *Bulletin of Entomological Research* **83**: 53-58.

Houlding, B., Ridsill-Smith, T. J. and Bailey, W. J. (1991). Injectable abamectin causes a delay in scarabaeine dung beetle egg-laying in cattle dung. *Australian Veterinary Journal* **68**: 185-186.

Hutton, S. A. and Giller, P. S. (2003). The effects of the intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology* **40**: 994-1007.

Kadiri, N., Lumaret, J.-P. and Janati-Idrissi, A. (1999). Lactones macrocycliques: leur impact sur la fauna non-cible du paturage. *Annales de la Societe de Entomologique de France* **35**: 222-229.

Krüger, K. and Scholtz, C. H. (1997). Lethal and sublethal effects of ivermectin on the dung-breeding beetles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). *Agriculture, Ecosystems and Environment* **61**: 123-131.

Krüger, K. and Scholtz, C. H. (1998). Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drought conditions. *Acta Oecologia* **19**: 425-438.

Lobo, J. M. (2001). Decline of roller dung beetle (Scarabaeinae) populations in the Iberian peninsula during the 20th century. *Biological Conservation* **97**: 43-50.

Lumaret, J.-P. and Kirk, A.A. (1987). Ecology of dung beetles in French mediterranean region (Coleoptera: Scarabaeinae). *Acta Zoologica Mexicana* (ns) **24**: 1-55.

Lumaret, J.-P., Galante, E., Lumbreras, C., Mena, J., Bertrand, M., Bernal, J. L., Cooper, J. F., Kadiri, N. and Crowe, D. (1993). Field effects of ivermectin residues on dung beetles. *Journal of Applied Ecology* **30**: 428-436.

Lumaret, J. P., Alvinerie, M., Hempel, H., Schallnaß, H.-J., Claret, D. and Römbke, J. (2007). New screening test to predict the potential impact of ivermectin-contaminated cattle dung on dung beetles. *Veterinary Research* **38**: 15-24.

Madsen, M., Overgaard Nielsen, B., Holter, P., Pedersen, O. C., Brøchner Jespersen, J., Vagn Jensen, K.-M., Nansen, P. and Grønvold, J. (1990). Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. *Journal of Applied Ecology* **27**: 1-15.

McCracken, D. I. (1993). The potential for avermectins to affect wildlife. *Veterinary Parasitology* **48**: 273-280.

O'Hea, N. M. (2008). *Impacts of ivermectin on north temperate dung beetles and the role of faunal diversity in dung decomposition*. Ph.D. thesis. National University of Ireland, Cork.

Römbke, J., Hempel, H., Scheffczyk, A., Schallna, H. J., Alvinerie, M. and Lumaret, J. P. (2007). Environmental risk assessment of veterinary pharmaceuticals: Development of a standard laboratory test with the dung beetle *Aphodius constans*. *Chemosphere* **70**: 57-64.

Roslin, T. (1999). *Spatial ecology of dung beetles.* PhD thesis. University of Helsinki, Finland.

Sommer, C. and Steffansen, B. (1993). Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. *Veterinary Parasitology* **48**: 67-74.

Sommer, C., Steffansen, B., Overgaard Nielsen, B., Grønvold, J., Vagn Jensen, K.-M., Brøchner Jespersen, J., Springborg, J. and Nansen, P. (1992). Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. *Bulletin of Entomological Research* **82**: 257-264.

Strong, L., Wall, R., Woolford, A. and Djeddour, D. (1996). The effect of faecally excreted ivermectin and fenbendazole on the insect colonisation of cattle dung

following the oral administration of sustained-release boluses. *Veterinary Parasitology* **62**: 253-266.

Sutherland, W. J., Armstrong-Brown, S., Armsworth, P. R., Tom, B., Brickland, J., Campbell, C. D., Chamberlain, D. E., Cooke, A. I., Dulvy, N. K., Dusic, N. R., Fitton, M., Freckleton, R. P., Godfray, H. C. J., Grout, N., Harvey, H. J., Hedley, C., Hopkins, J. J., Kift, N. B., Kirby, J., Kunin, W. E., Macdonald, D. W., Marker, B., Naura, M., Neale, A. R., Oliver, T. O. M., Osborn, D. A. N., Pullin, A. S., Shardlow, M. E. A., Showler, D. A., Smith, P. L., Smithers, R. J., Solandt, J.-L., Spencer, J., Spray, C. J., Thomas, C. D. and Thompson, J. I. M. (2006). The identification of 100 ecological questions of high policy relevance in the UK. *Journal of Applied Ecology* **43**: 617-627.

Vale, G.A. and Grant, I.F. (2002) Modelled impact of insecticide-contaminated dung on the abundance and distribution of dung fauna. *Bulletin of Entomological Research* **92**: 251-263.

Wall, R. and Strong, L. (1987). Environmental consequences of treating cattle with the antiparasitic drug ivermectin. *Nature* **327**: 418-421.

Wardhaugh, K. G. (2005). Insecticidal activity of synthetic pyrethroids, organophosphates, insect growth regulators, and other livestock parasiticides: an Australian perspective. *Environmental Toxicology and Chemistry* **24**: 789-796.

Wardhaugh, K. G. and Rodriguez-Menendez, H. (1988). The effects of the antiparasitic drug, ivermectin, on the development and survival of the dung-breeding fly, *Orthellia cornicina* (F.) and the scarabaeine dung beetles, *Copris hispanus* L., *Bubas bubalus* (Oliver) and *Onitis belial* F. *Journal of Applied Entomology* **106**: 381-389.

Wardhaugh, K. G., Mahon, R. J., Axelsen, A., Rowland, M. W. and Wanjura, W. (1993). Effects of ivermectin residues in sheep dung on the development and survival of the bushfly, *Musca vetustissima* Walker and a scarabaeine dung beetle, *Euoniticellus fulvus* Goeze. *Veterinary Parasitology* **48**: 139-157.

Wardhaugh, K. G., Holter, P. and Longstaff, B. C. (2001a). The development and survival of three species of coprophagous insect after feeding on the faeces of sheep treated with controlled-release formulations of ivermectin or albendazole. *Australian Veterinary Journal* **79**: 125-132.

Wardhaugh, K. G., Longstaff, B. C. and Morton, R. (2001b). A comparison of the development and survival of the dung beetle, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin. *Veterinary Parasitology* **99**: 155-168.

Webb, L., Beaumont, D.J., Nager R.G. and McCracken, D.I. Field-scale dispersal of *Aphodius* dung beetles (Coleoptera: Scarabaeidae) in response to avermectin treatments on pastured cattle. *Bulletin of Entomological Research* (in press).

Webb, L. (2004). *The impact of avermectin usage on the ecology of dung insect communities and the potential implications for foraging birds.* PhD Thesis, University of Glasgow.

Wratten, S. D. and Forbes, A. B. (1996). Environmental assessment of veterinary avermectins in temperate pastoral ecosystems. *Annals of Applied Biology* **128**: 329-348.

Figure legends

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

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

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

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

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

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

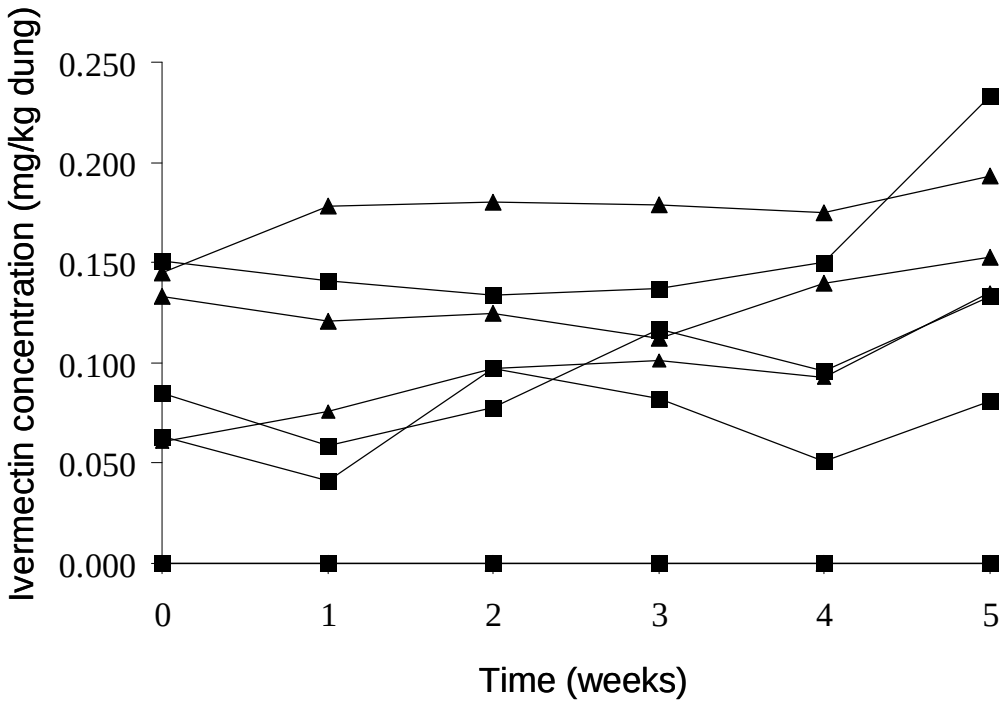

Ivermectin concentration (mg/kg dung)
0.250
0.200
0.150
0.100
0.050
0.000
0
1
2
3
4
5
Time (weeks)

Figure 1

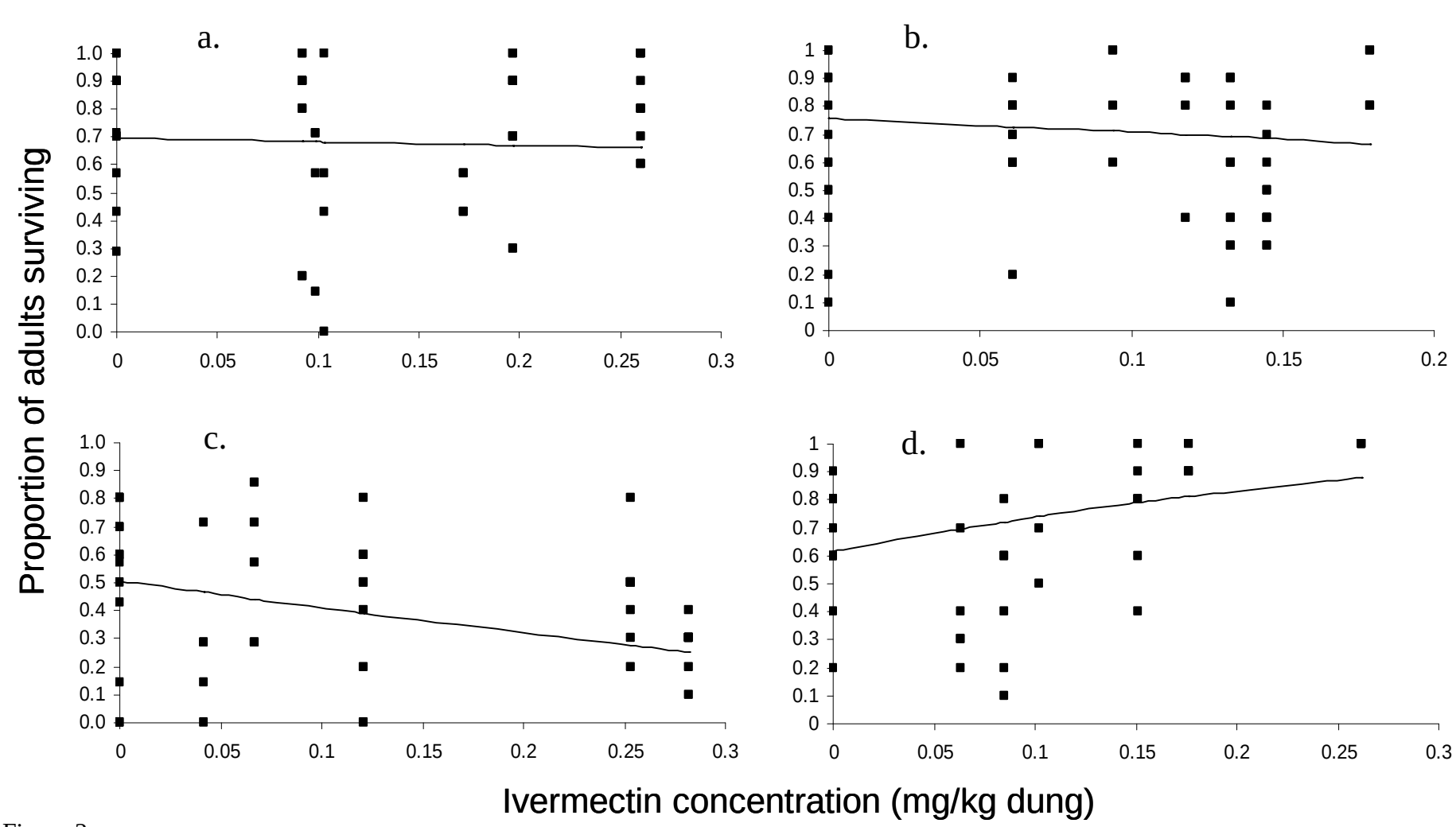
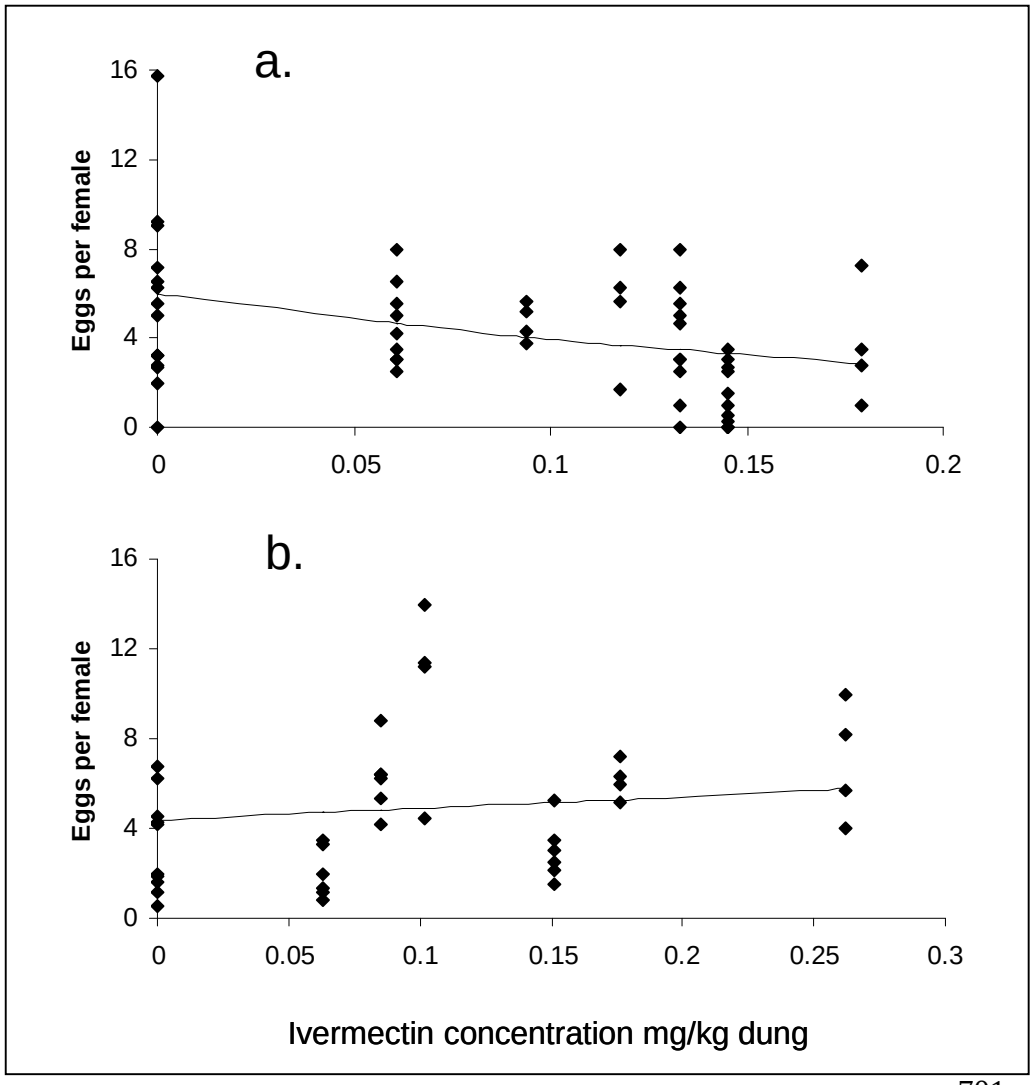

a.
b.
c.
d.
Proportion of adults surviving
Ivermectin concentration (mg/kg dung)

Figure 2

667

668



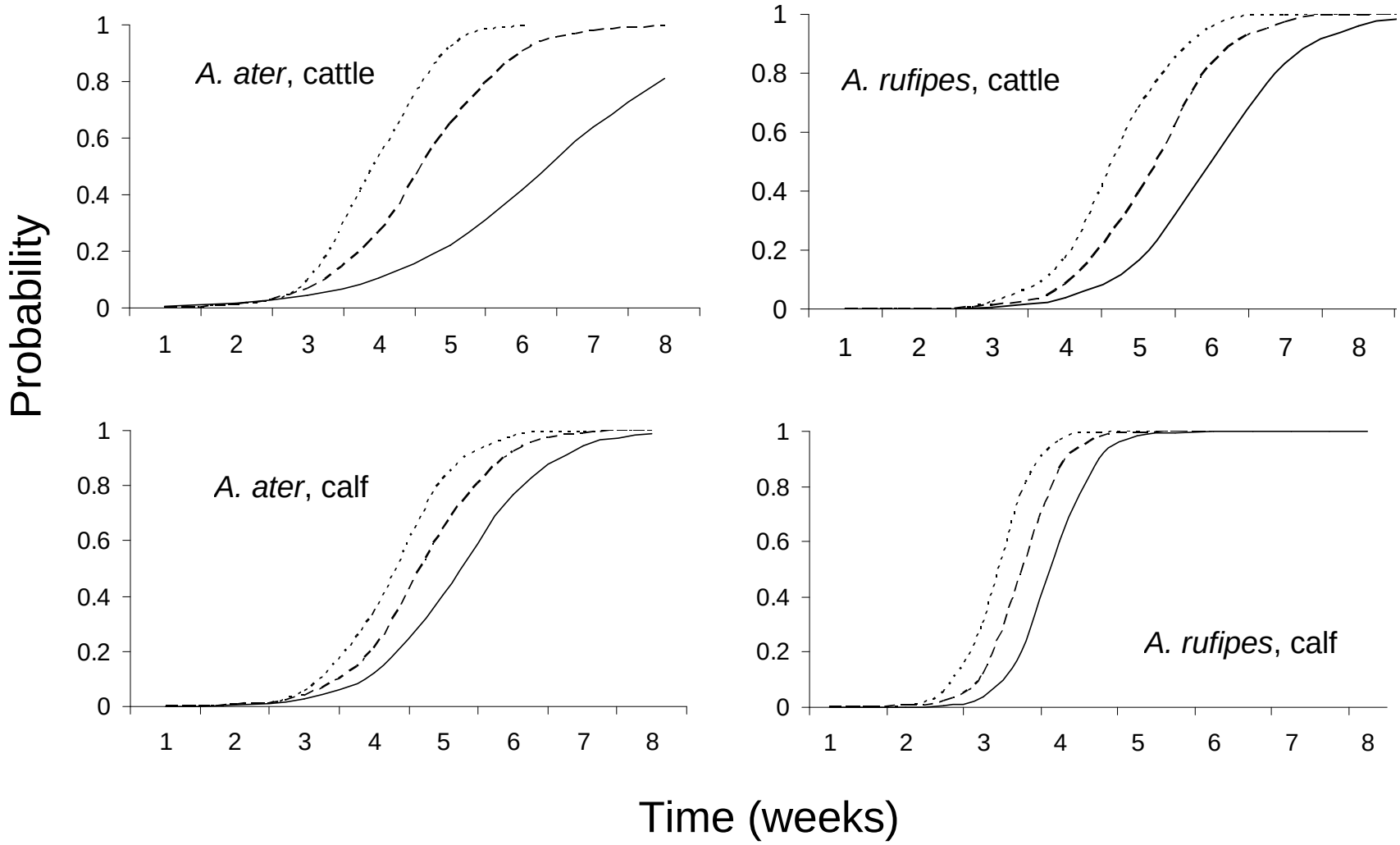
701

702

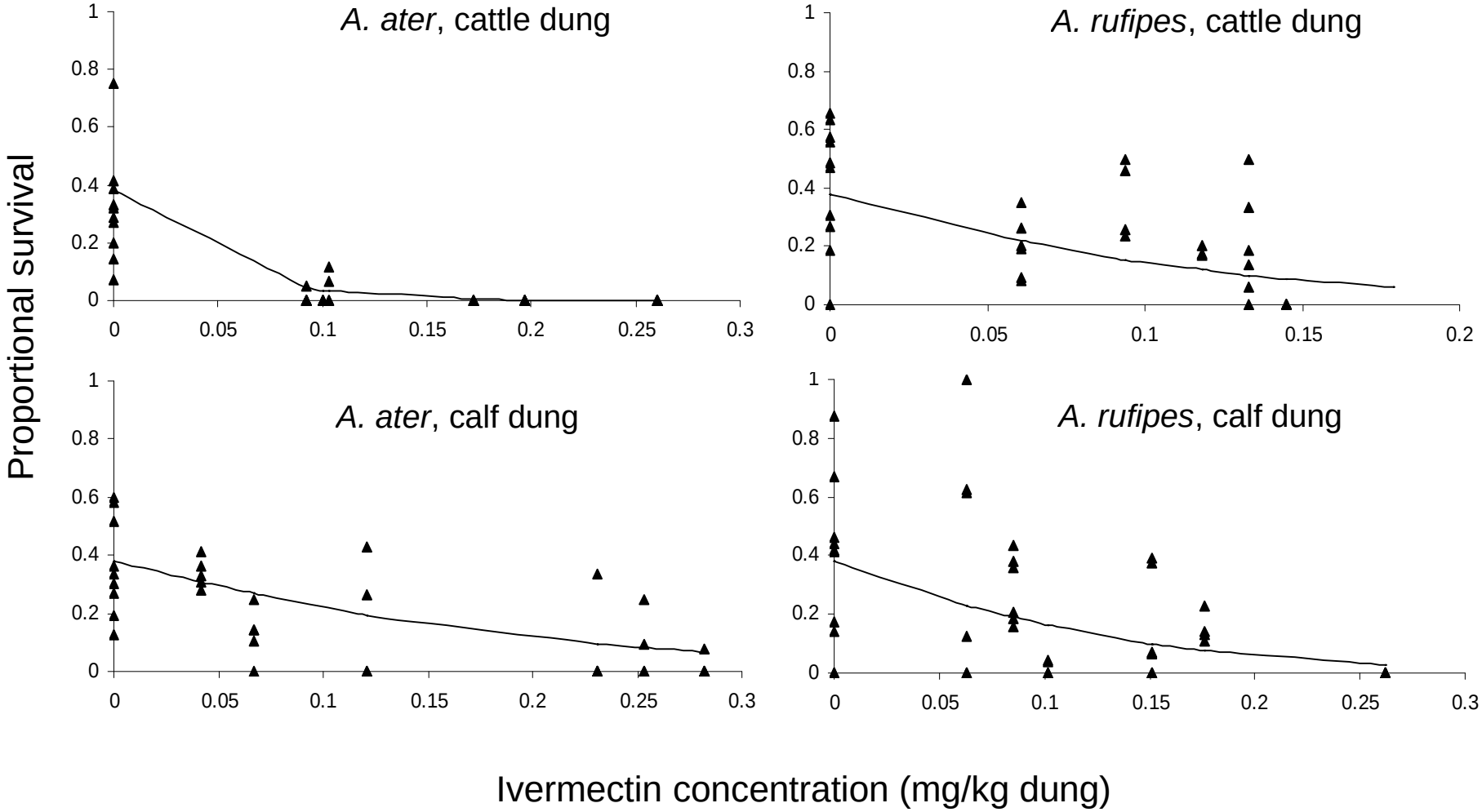
703 Figure 3

704

705

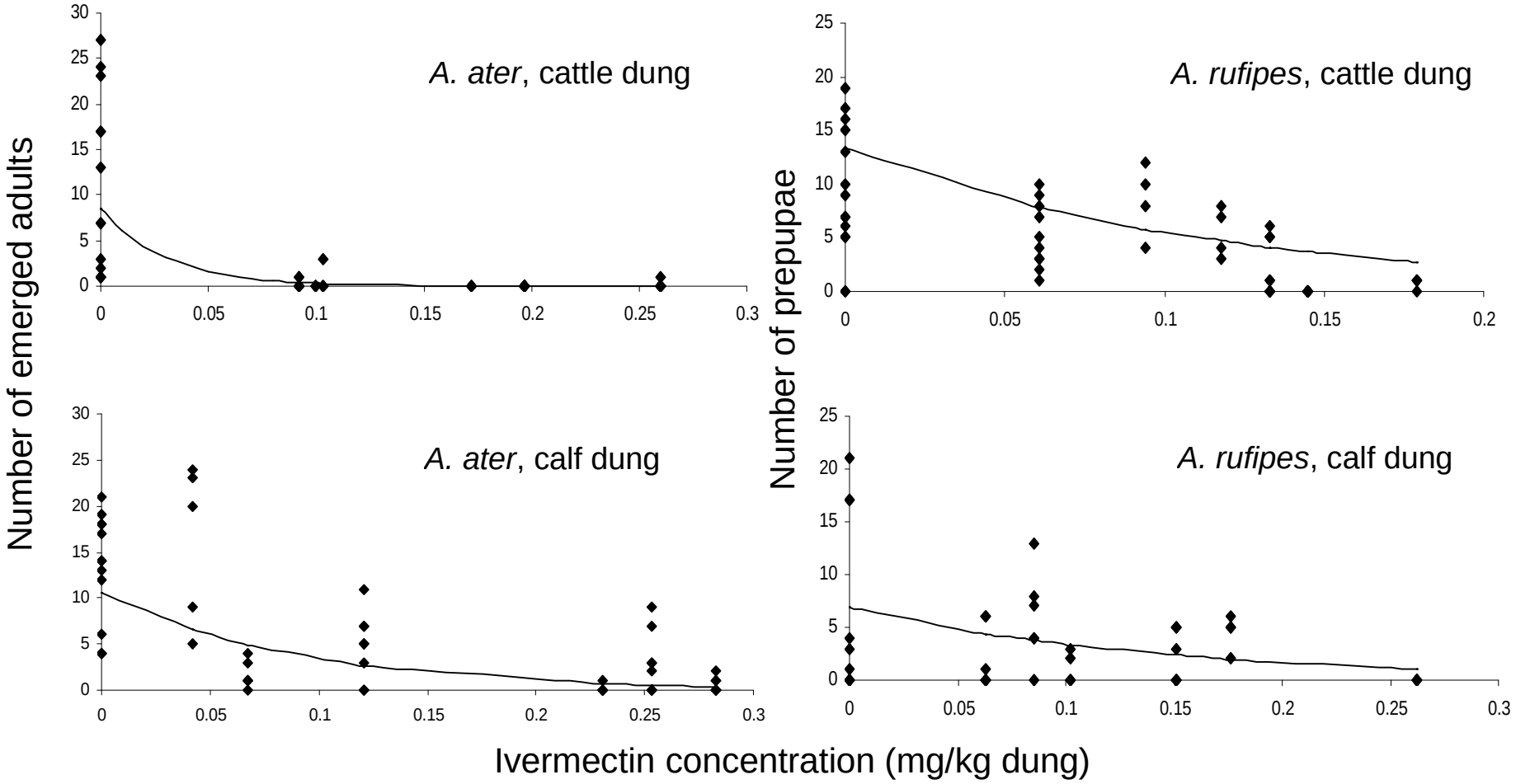


706
707 Figure 4



708
709 Figure 5

710



711
712
713 Figure 6

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

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

Table 2. Mean (± SE) ivermectin concentrations (mg kg^{-1}, wet weight of dung) (n = 2), in dung collected following treatment with a subcutaneous injection. Columns indicate the control group, and groups dosed 7, 5 and 3 days prior to dung collection. [†]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)

Table 3. Summary of fixed effects in GLMM analyses of different life history measurements (a-e). Included are the F-value, and level of significance (P). Degrees of freedom for analyses a-d were: $F_{1,187}$, $F_{1,91}$, $F_{1,8957}$ and $F_{1,129}$, respectively. For 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 *A. rufipes*		
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		
A. ater		
Concentration	123.26	<.0001
Dung	0.41	0.5249
Concentration*Dung	40.45	<.0001
A. rufipes		
Concentration	109.67	<.0001
Dung	3.74	0.0564
Concentration*Dung	7.41	0.0079
Number of Eggs	80.23	<.0001

731