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Sublethal effects of the parasiticide ivermectin on male and female reproductive and behavioural traits in the yellow dung fly

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HIGHLIGHTS

- Ivermectin is used widely against parasites of livestock.
- We exposed juvenile and/or adult yellow dung flies of both sexes to ivermectin in a crossed laboratory study.
- Larval feeding on ivermectin-contaminated dung resulted in smaller body size, smaller male testes, and fewer offspring produced.
- Exposure of adult flies to ivermectin lowered offspring production and survival for both sexes.
- Ivermectin exposure affects dung fly performance not only at the juvenile but also the adult stage.

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ABSTRACT

The veterinary pharmaceutical ivermectin is commonly used against parasites of livestock. Excreted in dung it can have lethal and sublethal effects on non-target organisms developing in and living around cattle dung. Research in this realm typically investigates the impact of pharmaceuticals on dung-feeding insects by looking at juvenile development and survival, while fitness effects of adult exposure are largely neglected. We conducted laboratory experiments to assess combined effects of ivermectin on life history and reproductive traits of juvenile and adult yellow dung flies (*Scathophaga stercoraria*). Two treatments (12 and 24 μg ivermectin/kg wet dung) were used for the larvae reared in dung, and one much higher concentration (3000 μg ivermectin/kg sugar) for the adult flies (in addition to uncontaminated controls). Juvenile ivermectin exposure lead to smaller body size of male and female flies. Adult feeding on ivermectin-contaminated dung additionally resulted in adult male flies with smaller testes (and likely fewer sperm) that experienced reduced mating durations, resulting in lower probability of producing offspring. Exposure of adult flies to ivermectin lowered offspring production and survival for both sexes. Thus, treatment of livestock with pharmaceuticals such as ivermectin appears to have even more far-reaching sublethal ecological consequences than previously assumed by affecting not only flies at their larval stage but also adult mating behaviour and reproduction.

1. Introduction

Pharmaceutical drugs that are commonly used in veterinary medicine can have detrimental effects, such as loss of biodiversity and ecosystem functioning (e.g. degradation of dung pats), when excreted by livestock animals (Alvarado et al., 2017; Floate et al., 2016; Lumaret et al., 2012). A well-studied pharmaceutical drug is ivermectin, which is commonly applied to livestock against parasitic nematodes or arthropods such as ticks and lice (Campbell et al., 1983; Õmura, 2008). Due to incomplete metabolization of the pharmaceutical, it can enter ecosystems through livestock's feces at high concentrations (Herd et al., 1996). Experimental studies have shown that ivermectin residues in dung affect many non-target, often beneficial organisms (Campbell et al., 1983; Floate, 1998; González-Tokman et al., 2017), leading to incomplete decomposition of dung pats. Ivermectin targets the nervous and muscular systems of moulting organisms (ecdysozoa: arthropods and nematodes) by binding to γ-aminobutyric acid and glutamic acid

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receptors, ultimately disrupting their growth (Schaeffer and Turner, 1989; Puniamoorthy et al., 2014). Research in this realm typically investigates the impact of pharmaceuticals on dung-feeding insects by looking at juvenile development and survival, and the concomitant damaging effects of ivermectin in the larval stages of various invertebrates are well established (Blanckenhorn et al., 2013; Lumaret et al., 2012; Römbke et al., 2009). While comparatively rare, some recent studies have shown that this drug also negatively affects adult life stages (Conforti et al., 2018; Lumaret et al., 2012; Verdú et al., 2015).

Although toxic effects of many drugs are regularly lethal, sublethal concentrations can also be detrimental by e.g. causing prolonged development, decreased body size or reduced fertility and fecundity (Krüger & Scholtz, 1998a, 1998b; Römbke et al., 2009). Sublethal effects will thus impose additional disadvantages impairing the performance of exposed animals (terHorst et al., 2015). For example, a recent study investigating the fitness consequences of ivermectin residues in cow dung for adult sepsid flies has documented decreased offspring production when one or both of the parents were exposed to ivermectin (Conforti et al., 2018), implying a reduction in fertility or fecundity. Another study demonstrated ivermectin effects expressed in the offspring generation of dung beetles (Baena-Díaz et al., 2018). Such sub-lethal and indirect effects on adult insects are currently under-researched.

To further investigate the consequences of sublethal exposure to ivermectin in insects, we studied the common predatory yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae). This species is one of the most characteristic insects on dung of livestock in cold-temperate regions of the northern hemisphere (Blanckenhorn et al., 2010). Yellow dung fly larvae are among the predominant decomposers of cattle dung. This fly has been subject to multiple studies on reproductive physiology (Hosken and Ward, 1999; Reim et al., 2006), mating behaviour, sexual selection and conflict (Parker, 1970a,b; Blanckenhorn, 2009), but also studies of non-target effects of chemical residues in dung of livestock treated with veterinary pharmaceuticals (Floate, 1998; Sommer et al., 1992; Strong and James, 1993; Webb et al., 2007). *S. stercoraria* were approved as test species for the evaluation of the toxicity of drug residues in dung by the international regulating bodies (OECD, 2008; Blanckenhorn et al., 2010). Previous studies have already shown that *S. stercoraria* larvae feeding on dung containing ivermectin experience higher mortality, prolonged development and lower adult body mass (Römbke et al., 2009). Here, we quantify the impact of ivermectin on adult reproductive traits such as mating behaviour and egg production of this fly, traits that are highly relevant proxies for fitness (Roff, 1992). We specifically tested for interactive effects at different life stages by exposing flies to non-lethal doses of ivermectin as larvae and/or adults.

2. Material and methods

2.1. Experimental flies

Stock flies used in our experiments were originally wild-caught in Appenzell, Switzerland. About 40 copulating pairs were caught and put into separate tubes containing fresh dung for oviposition and subsequently kept in the lab for multiple generations until the start of the experiment (see Blanckenhorn et al. (2010) for details on laboratory methods and husbandry). For our experiment, eggs were collected from females mated with one random male and split into plastic pots filled with dung of three larval treatments (see ivermectin treatment below). Larvae were then reared in this dung in a climate chamber at 18 °C, 60% relative humidity and 13 h light until eclosion of adults. After ca. 20 days, when the first flies started to emerge, they were transferred to 100 ml glass bottles sealed with paper stoppers. Since *Scathophaga* flies develop their gametes only after feeding on prey, adults can become cannibalistic if no prey is present. We therefore kept flies singly in the bottles and fed them with common fruit flies *Drosophila melanogaster* (Diptera: Drosophilidae) *ad libitum*. We fed ca. 20–30 *Drosophila* flies every 2–3 days, or in shorter intervals if no prey was left in the bottle, additionally adding two ($20 \times 20 \times 15 mm^3$) plastic dishes filled with sugar and water, respectively. Under laboratory conditions, female flies require about 7–14 days for egg development, whereas males are sexually mature within 3–6 days after first feeding on prey (Blanckenhorn and Henseler, 2005).

For our experiments, we collected eggs from multiple female clutches after copulation with one male (i.e. full-sib families). From each clutch, three times 15 eggs were transferred into dung pots containing dung of our three larval treatments (see below). Due to logistic limitations, separate experiments were conducted with treated male and female flies. For the male experiment, we used eggs from 35 clutches, yielding a total of 1575 eggs distributed across 105 dung pots of three larval treatments. For the female experiment, we obtained eggs from 25 clutches, yielding 1125 flies and 75 dung pots in total. Half of the adult flies emerging from each larval treatment were subsequently given ivermectin-treated sugar of the chosen adult concentration, and the other half control dung, which led to six different groups in total (Table 1). Since we looked at effects of ivermectin on mating behaviour and fertility/fecundity of only one sex per experiment, we used individuals of the other sex from the control/control (C0 × C0) group for the matings. All glass bottles were labelled according to family, larval and adult treatment, and the date of eclosion.

2.2. Ivermectin treatment

The dung used in the experiment was originally collected from grass-fed cattle that had not been treated with parasiticides. The collected dung was subsequently homogenized and stored at −80 °C to kill all organisms present. Yellow dung fly larvae were exposed to dung of two different ivermectin (CAS-No. 70288-86-7, dissolved in acetone) concentrations (12 and 24 μg ivermectin/kg wet dung [C12, C24]; [0.72 and 0.36 mg ivermectin/50 ml acetone]) originally based on Römbke et al. (2009), plus a control treatment (0 μg ivermectin/kg wet dung [C0] with acetone only; Table 1). The ivermectin solutions were thoroughly mixed into fresh dung and left at room temperature overnight to allow for evaporation of the acetone, which is usually used as the solvent for ivermectin (Römbke et al., 2009).

We initially conducted a range-finding pilot study to test the mortality of adult yellow dung flies exposed to five different ivermectin concentrations as adults (0, 6000, 12000, 24000, 48000 μg ivermectin/kg wet dung; Appendix Figs. A1, A2). At 6000 μg about 50% of the flies died after 6 days, so we eventually chose 3000 μg as the ideal sublethal concentration for our adult treatment in which not too many flies should die after one week of exposure. For the treatment of adult flies, we therefore prepared an additional ivermectin solution of 3000 μg ivermectin/kg sugar ([C3000]; [90 mg ivermectin/50 ml acetone]). Small plastic dishes (20 × 20 × 15mm)

Table 1
Treatment combinations used for males and females.

larval treatment	adult treatment	
	C0	C3000
C0	C0 × C0	C0 × C3000
C12	C12 × C0	C12 × C3000
C24	C24 × C0	C24 × C3000

were prepared with 1 g of coarse sugar as nutrient for the adult flies, emulating feeding of nectar. 40 µl of the adult ivermectin solution was poured over the sugar dishes, which were subsequently kept at room temperature for at least 1 h for acetone evaporation. Again, a null treatment with standard acetone was used as a control. While this may seem unnatural, it is likely that flowers around pastures, on which dung flies feed nectar, are regularly contaminated when e.g. spraying ivermectin. Adult dung flies further imbibe potentially contaminated dung when mating or ovipositing, and when eating prey.

2.3. Mating behaviour

To test the influence of ivermectin on mating behaviour we measured mating latency (l) and mating duration (d). We put a smear of dung on wet filter paper and placed the filter paper in a sealable glass bottle. One random male fly was put into the bottle and, shortly after, a random female fly from a different family was added. The starting time of the observation (t_1) was recorded. When copulation occurred, the starting (t_2) and end time (t_3) was recorded to calculate the mating latency ($l = t_2$-t_1) and mating duration ($d = t_3$-t_2). All copulations longer than 10 min were counted (cf. Blanckenhorn et al., 2003; Parker and Simmons, 2000). If no mating occurred within 45 min, the flies were separated and returned to their original containers. Mated females were left in the glass bottle to oviposit to assess their reproductive output. Males were removed after oviposition occurred, frozen, and stored in ethanol at −20 °C for later dissections. As already mentioned, we performed separate experimental blocks for the sexes, first pairing males from one of the six ivermectin treatment combinations with untreated (control) females, and later testing females subjected to one of the six ivermectin treatment combinations paired with untreated males (Table 1).

2.4. Reproductive output

To study the effects of ivermectin on female reproduction, we counted the number of eggs of the first clutch laid by females after mating (i.e. first clutch size). We considered at least 30 eggs as a full clutch (cf. Blanckenhorn, 2009). Flies that laid fewer than 30 eggs were given a fresh filter paper with dung and checked for the following 5 successive days for more eggs. We subsequently also assessed offspring egg-to-adult survival by carefully transferring 15 eggs from each clutch onto a wet filter paper, which was then transferred into a small plastic pot (100 ml) filled with fresh cow dung and left at room temperature overnight. The next day we recorded the number of larvae that had hatched using a binocular microscope (Leica MS5). The dung pots were thereafter stored at 18 °C to finally score emergence of adult offspring. From these data, we derived an estimate of larval hatching success (no. of larvae/no. of eggs) as well as an estimate of offspring egg-to-adult survival (no. adults emerged/no. eggs) per clutch.

We further measured the width (w) and length (l) of three eggs of each female clutch, and analogously the volume of the two male testes, with a binocular microscope (Leica MS5), to ultimately produce one average egg or testis volume per individual. To calculate the volume of either the testes or the eggs, we used the formula for an ellipsoid: $(4/3)\times\pi\times(l/2)\times(w/2)\times(w/2) = (1/6)\times\pi\times(l)\times(w)\times(w)$.

2.5. Statistical analyses

All outcome variables were separately analysed with linear models as a function of the larval and adult ivermectin treatments as crossed fixed factors (Table 1), specifically testing for interactive effects. We additionally entered hind tibia length, measured after death, as a continuous covariate because most life history (e.g. egg size and number), morphological (body parts) and behavioural traits vary with body size in many species, including yellow dung flies (Parker and Simmons, 2000; Blanckenhorn, 2009). All relevant two- and three-way interactions were initially included but removed if not significant (at $P > 0.15$). Mating latency, mating duration, clutch size, and testis and egg volume were analysed with normal error structure (cube-root-transformed whenever statistically necessary or biologically appropriate), whereas the probability of females laying eggs (yes/no) was analogously analysed with a binomial model. Egg-to-adult offspring survival and larval hatching success were analysed using a linear mixed model with underlying binomial error structure and clutch identity (i.e. family) as random effect, thus controlling for family variation. All analyses were conducted in R (R version 3.5.1) using the lmer-package. To correct for body size variation in all plots, we first calculated the residual trait size derived from a regression of trait size against hind tibia length and later added the grand mean to rescale the data.

3. Results

3.1. Mating behaviour

Ivermectin exposure at the larval and/or adult stage had no significant effect on mating latency (i.e. the time from pair introduction to copulation) (Fig. 1; Table 2, top). When males were exposed to ivermectin-treated sugar as adults, copula duration was significantly shorter than that for the (acetone) controls, whereas we found no effect on mating duration following larval ivermectin exposure (Fig. 1; Table 2, top). Moreover, we observed no effect whatsoever of larval or adult ivermectin exposure on female mating behaviour (Fig. 1; Table 2, bottom).

3.2. Reproductive output

As previously known (Römbke et al., 2009), fly body size (i.e. hind tibia length) of both males and females diminished when flies were exposed to increasing ivermectin concentrations as larvae (Fig. 2; Table 2). Relative testis volume (corrected for body size) was reduced when adult males fed on ivermectin-treated sugar, and also decreased with larval ivermectin concentration (Fig. 2; Table 2). Larger females laid larger eggs, and a non-significant trend towards laying *larger* eggs with increasing ivermectin concentrations seemed evident (Fig. 2; Table 2). No significant interactions were present, which were therefore removed from the final models.

The probability of females laying eggs after copulation decreased when females or their male mating partners were exposed to ivermectin as adults, whereas no effect was detectable when they were exposed to ivermectin as larvae (Fig. 3; Table 3). Furthermore, the number of eggs laid increased with the mother's body size, and tended to decrease with her adult and larval ivermectin exposure (Fig. 3; Table 3).

Offspring egg-to-adult survival was significantly lowered when the fathers or the mothers were exposed to ivermectin as adults. This effect was entirely explained by the corresponding effects on larval hatching success (Fig. 4; Table 3). However, a significant interactive effect between the adult and larval ivermectin treatments additionally appeared in the females, which is difficult to interpret (Fig. 3; Table 3). Fly body size did not affect offspring survival, so it was removed from all final models (Table 3).

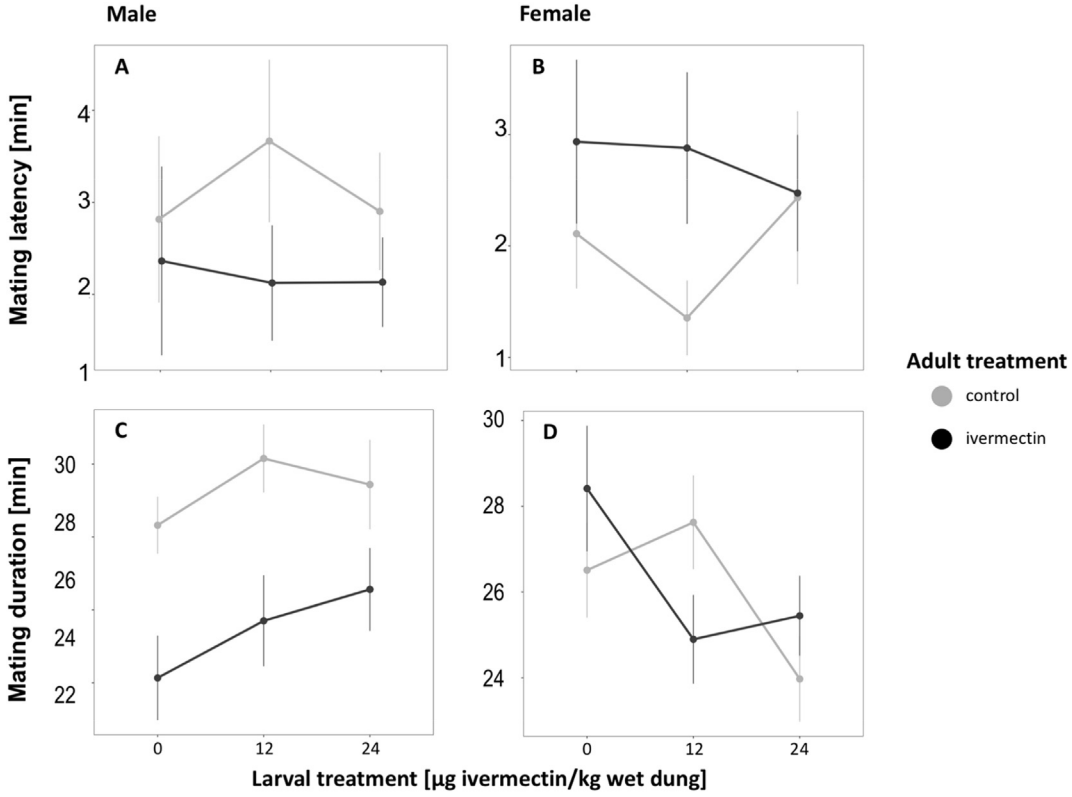

Fig. 1. Mean ± SE (**A**,**B**) mating latency and (**C**,**D**) copulation duration (all in min) of *Scathophaga stercoraria* pairs when (**A**,**C**) males or (**B**,**D**) females were exposed to ivermectin as larvae and/or as adults (n = 233 and 165 individuals in total).

Table 2
Analysis of variance for various male (top) or female (bottom) life history and behavioral traits of Scathophaga stercoraria exposed to ivermectin at their larval and/or adult stage (all non-significant interactions were removed).

males	**A) body size**				**B) testis volume**			
	MS	***df***	***F***	***P***	***MS***	***df***	***F***	***P***
Male size					0.13	1	7.26	**<0.01**
Larval exposure	1.06	2	39.80	**<0.001**	0.10	2	5.82	**<0.01**
Adult exposure	0.10	1	3.77	***0.053***	0.41	1	23.58	**<0.001**
Residuals	0.027	311			0.018	311		
	C) mating latency				**D) mating duration**			
	MS	***df***	***F***	***P***	***MS***	***df***	***F***	***P***
Male size	0.86	1	0.58	0.447	0.91	1	1.43	0.233
Larval exposure	0.79	2	0.53	0.587	1.21	2	1.90	0.152
Adult exposure	1.35	1	0.92	0.340	13.61	1	21.42	**<0.001**
Residuals	1.47	233				0.636	233	
females	**A) body size**				**B) egg volume**			
	MS	***df***	***F***	***P***	***MS***	***df***	***F***	***P***
Female size					0.01	1	6.41	**<0.05**
Larval exposure	1.11	2	58.49	**<0.001**	0.01	2	1.93	0.119
Adult exposure	0.005	1	0.24	0.622	0.00	1	0.22	0.642
Residuals	0.019	165			0.001	165		
	C) mating latency				**D) mating duration**			
	MS	***df***	***F***	***P***	***MS***	***df***	***F***	***P***
Female size	4.46	1	0.33	0.565	7.15	1	0.13	0.714
Larval exposure	2.53	2	0.19	0.828	60.00	2	1.13	0.325
Adult exposure	5.81	1	0.43	0.511	93.46	1	1.76	0.186
Residuals	13.41	165			53.09	165		

4. Discussion

The use of veterinary pharmaceuticals can have great impacts on the fitness of beneficial arthropods in grassland ecosystems. A great majority of previous studies dealing with the effects of pharmaceuticals on dung organisms focused on mortality during the juvenile (larval and pupal) stage (e.g. Blanckenhorn et al., 2013; Liebig et al., 2010; Lumaret et al., 2012; Römbke et al., 2009). In this study, we investigated sub-lethal effects of the commonly used pharmaceutical drug ivermectin at ecologically relevant concentrations. We applied approaches from evolutionary and behavioural ecology in studying mating behaviour and reproduction of the yellow dung fly *Scathophaga stercoraria*. These predatory dipterans are very common around dung pats, which they use as substrate for oviposition. Crucially here, yellow dung flies were exposed to ivermectin as larvae by spiking the dung they were reared in, and then also as adults by feeding them ivermectin-contaminated sugar. We found that sub-lethal concentrations of ivermectin can have further serious negative impacts on several standard life history, behavioural and reproductive traits of the yellow dung fly. Thus, negative consequences of ivermectin at the larval stage are reinforced by further effects accruing at their adult stage.

In addition to previously known effects of sublethal ivermectin exposure of larvae on body size in this and other dung fly species (Blanckenhorn et al., 2013; Römbke et al., 2009), we here documented relevant sub-lethal effects of ivermectin on mating behaviour and fecundity/fertility of adult yellow dung flies in terms of reduced mating duration, lower egg output, and decreased offspring production, effects that ultimately curtail these flies'

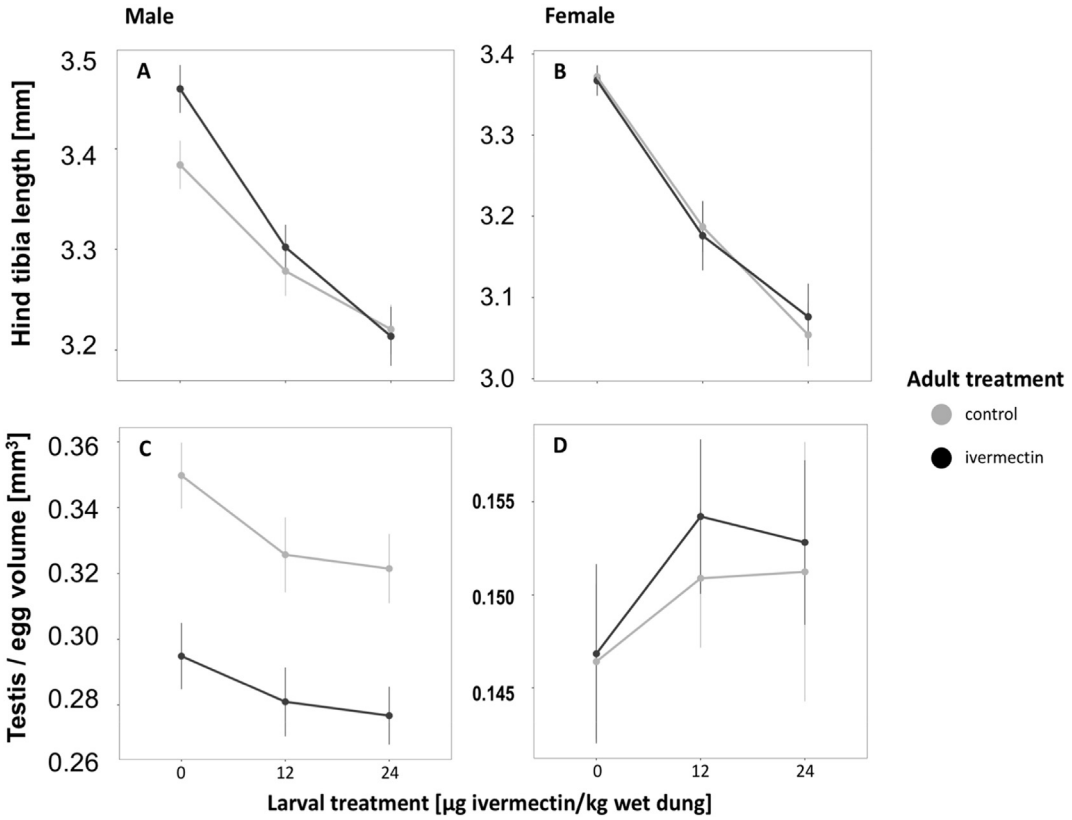

Fig. 2. Mean ± SE male (**A**) and female (**B**) hind tibia length (in mm) plus size-corrected rescaled (**C**) (male) testis volume and (**D**) (female) egg volume (both in mm^3) of *Scathophaga stercoraria* when exposed to ivermectin as larvae and/or adults (n = 311 and 165 individuals in total).

reproduction in nature. As experimental flies here were held in bottles with sugar and water, we exposed the adult flies to ivermectin by treating the sugar. In nature, dung flies are most commonly found on pastures around fresh dung pats (their mating sites) waiting for females. There these flies are in reproductive mode and only rarely feed on dung, contrary to e.g. dung beetles (Verdú et al., 2015; Martínez et al., 2017), which feed on dung as larvae and as adults. Nevertheless, besides hunting for smaller prey in the surroundings, adult dung flies regularly feed on nectar and dung as energy sources (Blanckenhorn et al., 2010; Warncke et al., 1993), and thus will likely take up ivermectin through these sources in nature when animal medications are sprayed on livestock. The ivermectin concentration we deemed effective here for the adult flies by way of range-finder pilot experiments was about a hundred-fold higher than that used to treat the larvae (Table 1), which likely relates to this somewhat unnatural way of taking up the drug. Still, such high concentrations can occur in the field (Liebig et al., 2010), though in nature the flies will be exposed to the chemicals for a much shorter time period, as they typically do not stay on any one dung pat or flower for a very long time. Ivermectin disturbs ion transport through cell walls by binding to ion channels (Lumaret et al., 2012; Ōmura, 2008), implying that flies are primarily affected during cell differentiation and growth and thus much more sensitive during their juvenile phase. So, how relevant or likely the scenario of adult exposure is in nature remains to be investigated further, and the underlying mechanisms leading to fitness reductions in adult flies also remain unclear. Negative effects on male sperm and female egg production and their fertility seem evident. Thus, dung insects encountered ivermectin residues during their juvenile and/or adult stage will experience multiple, frequently subtle and hence overlooked detrimental impacts via their regular life history functions. All these effects ultimately accumulate to disturb these insects' important ecosystem functions, including dung degradation, with likely serious ecological and economic consequences.

Numerous previous studies have shown that the use of ivermectin in livestock leads to a loss of biodiversity in non-target organisms that are often involved in dung degradation (Alvarado et al., 2017; Floate et al., 2016; Lumaret et al., 2012). According to Floate (1998), dung pats containing ivermectin show no obvious sign of degradation whereas dung not containing ivermectin degrades in about 80 days. Madsen et al. (1990) demonstrated that slowed degradation of dung pats incurs detrimental effects on the community of dung degrading insects. Ultimately, decreased numbers of dung-degrading insects and a slower degradation of dung can reduce pasture quality, because degradation of dung pats functions as fertilizer for the soil and without degradation the land underneath starts to rot. However, Tixier et al. (2016) carried out a study at 4 different locations (Canada, France, The Netherlands, Switzerland), monitoring the degradation of dung from cattle treated with ivermectin. No significant effect was found in how much time the degradation of dung required compared to untreated dung-pats, even at the highest tested concentration (7675 µg ivermectin/kg dung dry weight). Nonetheless, dung degradation depends on several other factors such as climate, spatial scale and number of treated animals (Krüger & Scholtz, 1998a, 1998b). Our findings here suggest that the effects of ivermectin treatment may be even more severe than previously thought, given that not only the offspring, but also adult flies are affected, as impaired reproductive function and offspring production of dominant decomposers like the yellow dung fly will additionally slow down dung degradation.

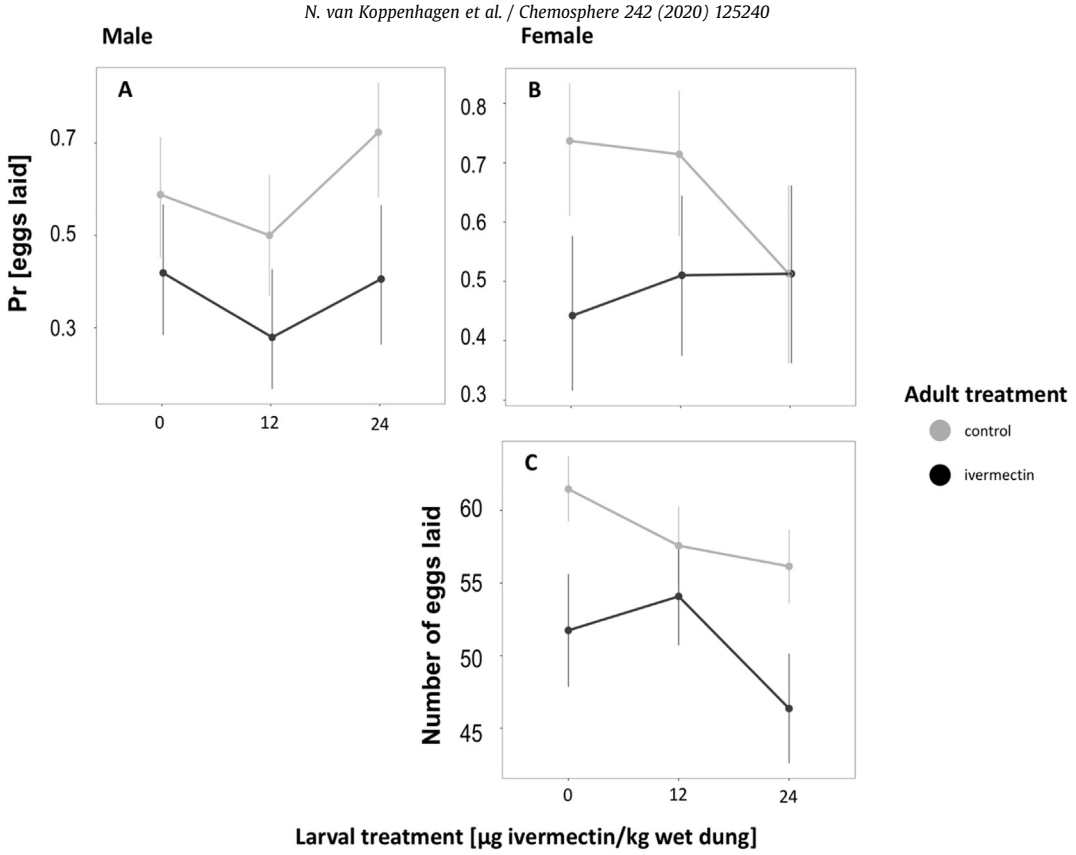

Fig. 3. Mean ± SE probability of *Scathophaga stercoraria* females laying eggs (at all) (**A**) when the male or (**B**) the female was exposed to ivermectin as larva and/or as adult; (**C**) corresponding mean ± SE number of eggs laid (clutch size) by the exposed females (n = 273 and 153 individuals, respectively).

Table 3
(**A**-**C**) Analysis of variance for offspring survival components of *Scathophaga stercoraria* when the father (males, top) or the mother (females, bottom) was exposed to ivermectin at their larval and/or adult stage; (**D**) corresponding laying probability of the untreated (top) or treated (bottom) mother, and (**E**) number of eggs laid (first clutch size) by the treated mother (bottom only). Fly body size did not affect offspring survival and was hence excluded from the final models, as were all non-significant interactions.

males	**A) larval hatching success**			**B) larva-to-adult survival**			**C) egg-to-adult survival**		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Larval exposure	0.08	2	0.963	0.112	2	0.946	0.001	2	0.999
Adult exposure	11.62	1	**<0.001**	0.061	1	0.805	6.96	1	**<0.01**
Residuals		134			134			134	

	D) p(eggs laid)		
	X^2	*df*	*P*
Larval exposure	6.74	2	0.051
Adult exposure	13.13	1	**<0.001**
Residuals		273	

females	**A) larval hatching success**			**B) larva-to-adult survival**			**C) egg-to-adult survival**		
	X^2	*df*	*P*	X^2	*df*	*P*	X^2	*df*	*P*
Larval exposure	0.58	2	0.749	2.17	2	0.337	3.51	2	0.173
Adult exposure	4.04	1	**<0.05**	1.48	1	0.224	8.84	1	**<0.01**
Interaction	15.79	2	**<0.001**				20.05	2	**<0.001**
Residuals		154			154			154	

	D) p(eggs laid)			**E) number of eggs laid**			
	X^2	*df*	*P*	*MS*	*df*	*F*	*P*
Female size				6700.6	1	51.25	**<0.001**
Larval exposure	1.63	2	0.444	384.2	2	2.95	***0.056***
Adult exposure	9.62	1	**<0.01**	505.8	1	3.87	***0.051***
Interaction	4.32	2	0.115				
Residuals		285		130.7	153		

A reduction in male fertility was observed upon feeding on ivermectin-contaminated sugar, resulting in a reduction of testis size and offspring number of the male even when mating with an untreated female. These results fall in line with the findings of

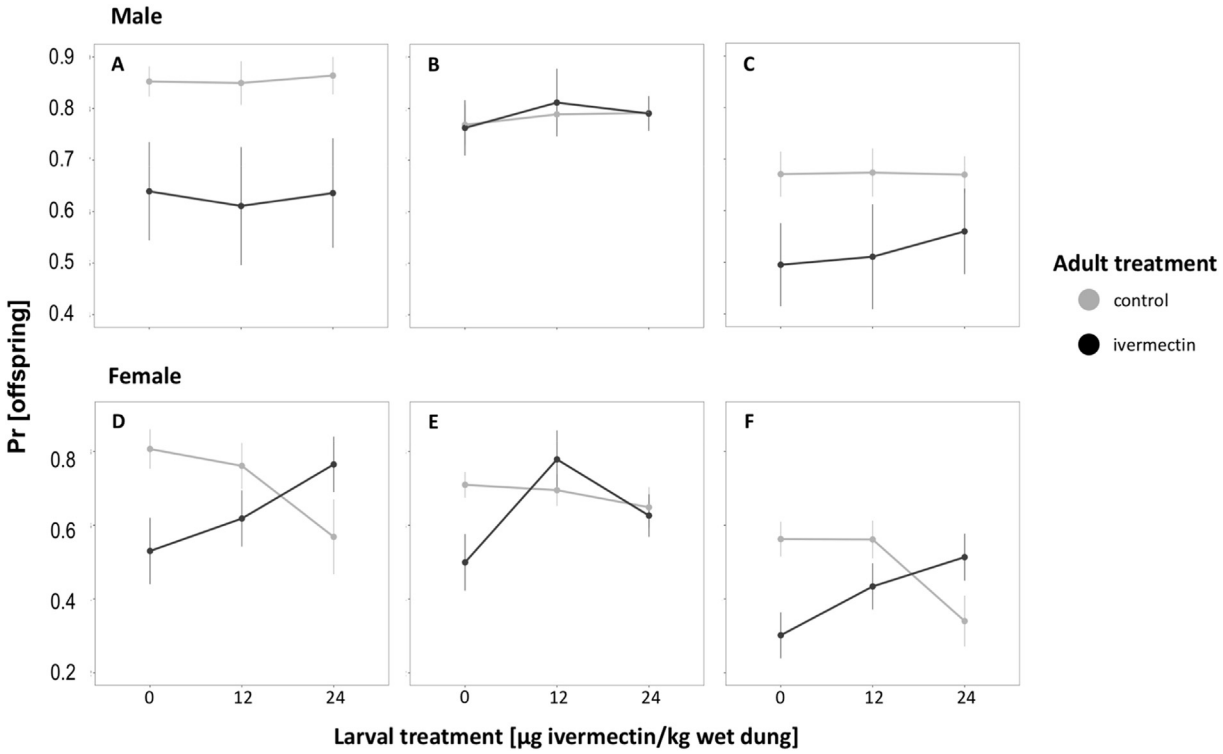

Fig. 4. Mean ± SE proportional (**A,D**) larval hatching success, (**B,E**) larva-to-adult survival, and (**C,F**) combined egg-to-adult survival (**C**) of *Scathophaga stercoraria* offspring if the father (male, top) or the mother (female, bottom) was exposed to ivermectin as larva and/or as adult (n = 134 clutches in total in both cases).

Conforti et al. (2018), who have shown similar effects in adult sepsid flies when exposed to various concentrations of ivermectin as adults. They demonstrated that feeding on contaminated dung leads to reduced reproductive success in several sepsid species by impairing male fertility via the quality or quantity of sperm. Other previous studies found a reduction in sperm production in ticks as well as reduced sperm motility in oysters (Falkenberg et al., 2017; Montasser et al., 2005). In rats, similar effects have been found in that ivermectin lowered sperm production rates and sperm motility (El-Nahas and El-Ashmawy, 2008). All this points to ivermectin impairing male reproductive function at the gamete production level and, in our case, also at the behavioural level, resulting in reduced copulation durations.

Conforti et al. (2018) further demonstrated a reduction in the number of eggs as well as reduced offspring numbers when only female flies were exposed to ivermectin. Studies by Martínez et al. (2017) and Rosales et al. (2012) observed a reduction of female fecundity in dung beetles feeding on dung contaminated with ivermectin. In our study, only marginal fecundity reductions were observed when females were exposed to ivermectin as adults (Fig. 3; Table 3). The number of offspring even seems to increase the higher the larval ivermectin concentration gets (Fig. 4). One possible explanation for this could be that the flies develop some sort of tolerance for ivermectin when reared in dung containing ivermectin. Another possibility is that they start to evolve some sort of defence mechanism against the toxic effects of ivermectin, although this will be difficult to demonstrate (see Puniamoorthy et al., 2014).

Because sublethal effects of ivermectin are not limited to the larval stage but also affect adult individuals, they will not stay local but will be spread through dispersal. Yellow dung flies are able to cover long distances by flight (up to 18 km in 18 h), although they often do not need to fly this far to find food or dung on pastures in certain landscapes such as in Switzerland (Kaufmann et al., 2013). After dispersal, ivermectin effects are also prone to accumulate in the food chain through predation. Thus, treating cattle with ivermectin likely has even more extensive impact on yellow dung flies and other organisms than previously assumed, as other coprophagues likely are similarly affected (Conforti et al., 2018; Lumaret et al., 2012).

5. Conclusion

In this study we presented evidence that ivermectin fundamentally impacts upon dung-degrading insects living on or around dung pats of livestock. The effects are not limited to larval growth and survival (Römbke et al., 2009), as non-lethal ivermectin concentrations have further detrimental effects on mating behaviour and reproduction of this species. Our results are especially important because the yellow dung fly and other insects typically cannot differentiate between treated and untreated dung even at highly lethal ivermectin concentrations (Conforti et al., 2018; Römbke et al., 2009; Webb et al., 2007). The negative non-target impacts of ivermectin and similar drugs in veterinary medicine thus have considerable ecological as well as economic consequences, such as loss of biodiversity or the retardation of dung degradation (Losey and Vaughan, 2006; Tixier et al., 2016). Further studies on the sub-lethal effects of these pharmaceuticals with other relevant arthropods need to be carried out, which as a rule should additionally concentrate on the underlying mechanisms of how ivermectin affects adult reproductive function and behaviour.

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Appendix

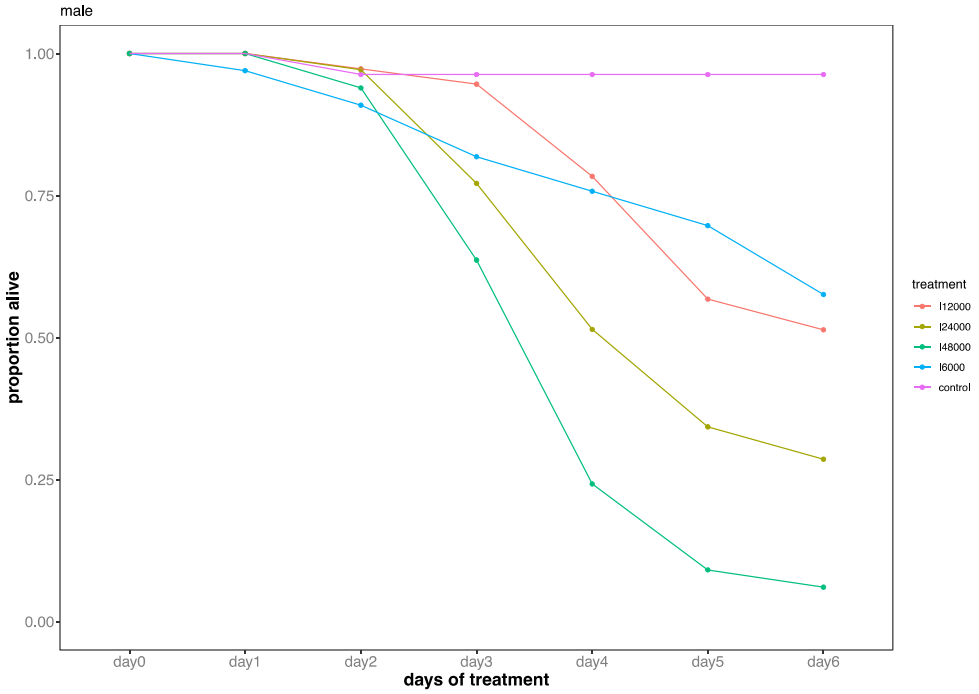

Fig. A1. Test run for the adult ivermectin treatment of **male** *Scathophaga stercoraria* flies. Concentrations ranged from 6000 to 48′000 µg ivermectin/kg wet dung.

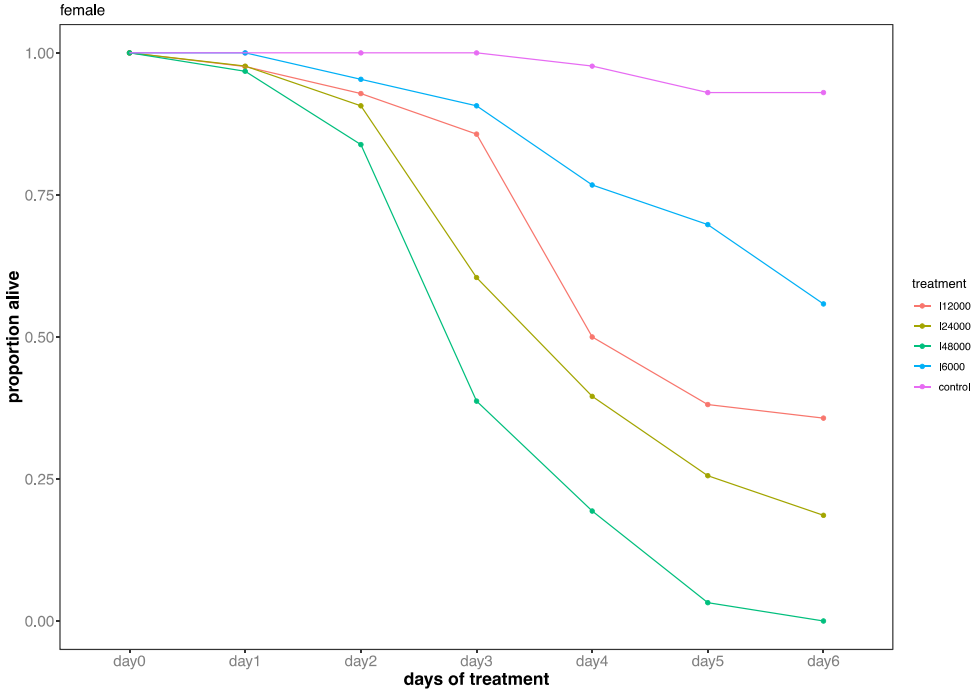

Fig. A2. Test run for the adult ivermectin treatment o **female** *Scathophaga stercoraria* flies. Concentrations ranged from 6000 to 48′000 µg ivermectin/kg wet dung.

References

Alvarado, F., Escobar, F., Williams, D.R., Arroyo-Rodríguez, V., Escobar-Hernández, F., 2017. The role of livestock intensification and landscape structure in maintaining tropical biodiversity. J. Appl. Ecol. 55, 185–194. https://doi.org/10.1111/1365-2664.12957.

Baena-Díaz, F., Martínez, M.I., Gil-Pérez, Y., González-Tokman, D., 2018. Transgenerational effects of ivermectin exposure in dung beetles. Chemosphere 202, 637–643. https://doi.org/10.1016/j.chemosphere.2018.03.109.

Blanckenhorn, W., Pemberton, A., Bussière, L., Roembke, J., Floate, K., 2010. A review of the natural history and laboratory culture methods for the yellow dung fly, *Scathophaga stercoraria*. J. Insect Sci. 10, 1–17. https://doi.org/10.1673/031.010.1101.

Blanckenhorn, W.U., 2009. Causes and consequences of phenotypic plasticity in body size: the case of the yellow dung fly Scathophaga stercoraria (Diptera: scathophagidae). In: Whitman, D., Anathakrishnan, T.N. (Eds.), Phenotypic Plasticity of Insects: Mechanisms and Consequences, pp. 369–422.

Blanckenhorn, Wolf U., Frei, J., Birrer, M., 2003. The effect of female arrivals on mate monopolization in the yellow dung fly. Behav. Ecol. Sociobiol. 54, 65–70. https://doi.org/10.1007/s00265-003-0594-3.

Blanckenhorn, Wolf U., Henseler, C., 2005. Temperature-dependent ovariole and testis maturation in the yellow dung fly. Entomol. Exp. Appl. 116, 159–165. https://doi.org/10.1111/j.1570-7458.2005.00316.x.

Blanckenhorn, Wolf U., Puniamoorthy, N., Schäfer, M.A., Scheffczyk, A., Römbke, J., 2013. Standardized laboratory tests with 21 species of temperate and tropical sepsid flies confirm their suitability as bioassays of pharmaceutical residues (ivermectin) in cattle dung. Ecotoxicol. Environ. Saf. 89, 21–28. https://doi.org/10.1016/j.ecoenv.2012.10.020.

Campbell, W.C., Fisher, M.H., Stapley, E.O., Albers-Schönberg, G., Jacob, T.A., 1983. Ivermectin : a potent new antiparasitic agent. Science 221, 823–828.

Conforti, S., Dietrich, J., Kuhn, T., van Koppenhagen, N., Baur, J., Rohner, P.T., Blanckenhorn, W.U., Schäfer, M.A., 2018. Comparative effects of the parasiticide ivermectin on survival and reproduction of adult sepsid flies. Ecotoxicol. Environ. Saf. 163, 215–222. https://doi.org/10.1016/j.ecoenv.2018.07.029.

El-Nahas, A.F., El-Ashmawy, I.M., 2008. Effect of ivermectin on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats. Environ. Toxicol. Pharmacol. 26, 206–211. https://doi.org/10.1016/j.etap.2008.03.011.

Falkenberg, L.J., Wrange, A.-L., Kinnby, A., Havenhand, J.N., Lockyer, A., Styan, C.A., 2017. Low sensitivity of reproductive life-stages in the Pacific oyster (*Crassostrea gigas*) to abamectin. Chemosphere 182, 665–671. https://doi.org/10.1016/j.chemosphere.2017.05.085.

Floate, K.D., 1998. Off-target effects of ivermectin on insects and on dung degradation in southern Alberta, Canada. Bull. Entomol. Res. 88, 25–35. https://doi.org/10.1017/S0007485300041523.

Floate, K.D., Düring, R.-A., Hanafi, J., Jud, P., Lahr, J., Lumaret, J.P., Scheffczyk, A., Tixier, T., Wohde, M., Römbke, J., Sautot, L., Blanckenhorn, W.U., 2016. Effects of ivermectin validation of a standard field test method in four countries to assess the toxicity of residues in dung of cattle treated with veterinary medical products. Environ. Toxicol. Chem. 35, 1934–1946. https://doi.org/10.1002/etc.3154.

González-Tokman, D., Martínez, M.I., Villalobos-Ávalos, Y., Munguía-Steyer, R., del Rosario Ortiz-Zayas, M., Cruz-Rosales, M., Lumaret, J.-P., 2017. Ivermectin alters reproductive success, body condition and sexual trait expression in dung beetles. Chemosphere 178, 129–135. https://doi.org/10.1016/j.chemosphere.2017.03.013.

Herd, R.P., Sams, R.A., Ashcraft, S.M., 1996. Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained-release, pour-on or injectable formulations. Int. J. Parasitol. 26, 1087–1093. https://doi.org/10.1016/S0020-7519(96)80007-5.

Hosken, D.J., Ward, P.I., 1999. Female accessory reproductive gland activity in the yellow dung fly *Scathophaga stercoraria* (L.). J. Insect Physiol. 45, 809–814. https://doi.org/10.1016/S0022-1910(98)00159-0.

Kaufmann, C., Reim, C., Blanckenhorn, W.U., 2013. Size-dependent insect flight energetics at different sugar supplies. Biol. J. Linn. Soc. 108, 565–578. https://doi.org/10.1111/j.1095-8312.2012.02042.x.

Krüger, K., Scholtz, C.H., 1998a. Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drought conditions. Acta Oecol. 19, 425–438. https://doi.org/10.1016/S1146-609X(98)80048-9.

Krüger, K., Scholtz, C.H., 1998b. Changes in the structure of dung insect communities after ivermectin usage in a grasslansd ecosystem. II. Impact of ivermectin under high-rainfall conditions. Acta Oecol. 19, 439–451.

Liebig, M., Fernandez, Á.A., Blübaum-Gronau, E., Boxall, A., Brinke, M., Carbonell, G., Duisy, K., 2010. Environmental risk assessment of ivermectin: a case study. Integr. Environ. Assess. Manag. 6 (Suppl. 1), 567–587. https://doi.org/10.1002/ieam.96.

Losey, J.E., Vaughan, 2006. The economic value of ecological services provided by insects. Bioscience 56, 311. https://doi.org/10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2.

Lumaret, J.P., Errouissi, F., Floate, K., Römbke, J., Wardhaugh, K., 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Curr. Pharmaceut. Biotechnol. 13, 1004–1060.

Madsen, M., Overgaard Nielsen, B., Holter, P., Pedersen, O.C., Brøchner Jespersen, J., Vagn Jensen, K.-M., Nansen, P., Grønvold, J., 1990. Treating cattle with ivermectin: effects on the fauna and decompsition of dung pats. J. Appl. Ecol. 27, 1–15.

Martínez, M.I., Lumaret, J.-P., Ortiz Zayas, R., Kadiri, N., 2017. The effects of sublethal and lethal doses of ivermectin on the reproductive physiology and larval development of the dung beetle Euoniticellus intermedius (Coleoptera: scarabaeidae). Can. Entomol. 12, 1–12. https://doi.org/10.4039/tce.2017.11.

Montasser, A.A., Gadelhak, G.G., Tariq, S., 2005. Impact of ivermectin on the ultrastructure of the testis of argas (persicargas) persicus (ixodoidea: argasidae). Exp. Appl. Acarol. 36, 119–129. https://doi.org/10.1007/s10493-005-1270-2.

OECD, 2008. Test No. 228: Determination of developmental toxicity of a test chemical to dipteran dung flies (*Scathophaga stercoraria* L. (Scathophagidae), *Musca autumnalis* De Geer (Muscidae)). -OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris, France, p. 16.

Õmura, S., 2008. Ivermectin: 25 years and still going strong. Int. J. Antimicrob. Agents 31, 91–98. https://doi.org/10.1016/j.ijantimicag.2007.08.023.

Parker, G.A., Simmons, L.W., 2000. Optimal copula duration in yellow dung Flies : ejaculatory duct dimensions and size- dependent sperm displacement. Evolution 54, 924–935.

Parker, G.A., 1970a. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera : scatophagidae): I . Diurnal and seasonal changes in population density around the site of mating and oviposition. J. Anim. Ecol. 39, 185–204.

Parker, G.A., 1970b. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera : scatophagidae): II . The fertilization rate and the spatial and temporal relationships of each sex around the site of mating and oviposition. J. Anim. Ecol. 39, 205–228.

Puniamoorthy, N., Schäfer, M.A., Römbke, J., Meier, R., Blanckenhorn, W.U., 2014. Ivermectin sensitivity is an ancient trait affecting all ecdysozoa but shows phylogenetic clustering among sepsid flies. Evol. Appl. 7, 548–554.

Reim, C., Teuschl, Y., Blanckenhorn, W.U., 2006. Size-dependent effects of larval and adult food availability on reproductive energy allocation in the Yellow Dung Fly. Funct. Ecol. 20, 1012–1021. https://doi.org/10.1111/j.1365-2435.2006.01173.x.

Roff A., D., 1992. The Evolution of Life Histories. Chapman and Hall, New York, NY, USA.

Römbke, J., Floate, K.D., Jochmann, R., Schäfer, M.A., Puniamoorthy, N., Knäbe, S., Lehmhus, J., Rosenkranz, B., Scheffczyk, A., Schmidt, T., Sharples, A., Blanckenhorn, W.U., 2009. Lethal and sublethal toxic effects of a test chemical (ivermectin) on the yellow dung fly (*Scathophaga stercoraria*) based on a standardized international ring test. Environ. Toxicol. Chem. 28, 2117–2124. https://doi.org/10.1897/08-599.1.

Rosales, M.C., Imelda Martinez, M., López-Collado, J., Vargas-Mendoza, M., González-Hernández, H., Fajersson, P., 2012. Effect of ivermectin on the survival and fecundity of Euoniticellus intermedius (Coleoptera: scarabaeidae). Rev. Biol. Trop. 60, 333–345.

Schaeffer, M., Turner, M.J., 1989. Ivermectin and Abamectin, first ed. Springer, New York.

Sommer, C., Steffansen, B., Nielsen, B.O., Grønvold, J., Vagn Jensen, K.M., Brøchner Jespersen, J., Springborg, J., Nansen, P., 1992. Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. Bull. Entomol. Res. 82, 257–264. https://doi.org/10.1017/S0007485300051804.

Strong, L., James, S., 1993. Some effects of ivermectin on the yellow dung fly, *Scathophaga stercoraria*. Vet. Parasitol. 48, 181–191. https://doi.org/10.1016/0304-4017(93)90154-F.

terHorst, C.P., Lau, J.A., Cooper, I.A., Keller, K.R., La Rosa, R.J., Royer, A.M., Schultheis, E.H., Suwa, T., Conner, J.K., 2015. Quantifying nonadditive selection caused by indirect ecological effects. Ecology 96, 2360–2369. https://doi.org/10.1890/14-0619.1.

Tixier, T., Blanckenhorn, W.U., Lahr, J., Floate, K.D., Scheffczyk, A., Düring, R.-A., Wohde, M., Rämbke, J., Lumaret, J.-P., 2016. Effects of ivermectin the function of coprophilous communities of arthropods in breaking down livestock dung. Environ. Toxicol. Chem. 35 https://doi.org/10.1002/etc.3243, 1953–1958.

Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martinez-Pinna, J., Lumaret, J.P., Lobo, J.M., Numa, C., Sánchez-Piñero, F., 2015. Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. Sci. Rep. 5, 1–10. https://doi.org/10.1038/srep13912.

Warncke, E., Terndrup, U., Michelsen, V., Erhardt, A., 1993. Flower visitors to Saxifraga hirculus in Switzerland and Denmark, a comparative study. Bot. Helv. 103, 141–147.

Webb, L., Beaumont, D.J., Nager, R.G., McCracken, D.I., 2007. Effects of avermectin residues in cattle dung on yellow dung fly Scathophaga stercoraria (Diptera: scathophagidae) populations in grazed pastures. Bull. Entomol. Res. 97, 129–138. https://doi.org/10.1017/S0007485307004683.