# Exposure to low concentrations of pesticide stimulates ecological functioning in the dung beetle *Onthophagus nuchicornis*

Paul Manning [Coresp., 1] , G. Christopher Cutler [1]

[1] Department of Plant, Food, and Environmental Sciences, Dalhousie University, Truro, Nova Scotia, Canada

Corresponding Author: Paul Manning
Email address: paul.manning@dal.ca

Body-size is an important trait for predicting how species contribute to ecosystem functions and respond to environmental stress.Using the dung beetle *Onthophagus nuchicornis* (Coleoptera: Scarabaeidae), we explored how variation in body-size affected ecosystem functioning (dung burial) and sensitivity to an environmental stressor (exposure to the veterinary anthelmintic ivermectin). We found that large beetles buried nearly 1.5-fold more dung than small beetles, but that mortality from exposure to a range of concentrations of ivermectin did not differ between large and small beetles. Unexpectedly, we found that exposure to low concentrations of ivermectin (0.01–1 mg ivermectin per kg dung) stimulated dung burial in both small and large beetles. Our results provide evidence of ecological functioning hormesis stemming from exposure to low amounts of a chemical stressor that causes mortality at high doses.

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4  Paul Manning[1], G. Chris Cutler[1]
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6  1. Department of Plant, Food, Environmental Science, Dalhousie University
7
8  Corresponding Author:
9  Paul Manning[1]
10  Email address: paul.manning@dal.ca

12 **Abstract**

13 Body-size is an important trait for predicting how species contribute to ecosystem

14 functions and respond to environmental stress. Using the dung beetle *Onthophagus*

15 *nuchicornis* (Coleoptera: Scarabaeidae), we explored how variation in body-size

16 affected ecosystem functioning (dung burial) and sensitivity to an environmental

17 stressor (exposure to the veterinary anthelmintic ivermectin). We found that large

18 beetles buried nearly 1.5-fold more dung than small beetles, but that mortality from

19 exposure to a range of concentrations of ivermectin did not differ between large and

20 small beetles. Unexpectedly, we found that exposure to low concentrations of

21 ivermectin (0.01☐–☐1 mg ivermectin per kg dung) stimulated dung burial in both small

22 and large beetles. Our results provide evidence of ecological functioning hormesis

23 stemming from exposure to low amounts of a chemical stressor that causes mortality at

24 high doses.

26 **Introduction**

27 Insect species vary in their sensitivity and responses to environmental disturbances.

28 The framework of 'response traits' is useful for understanding mechanisms behind this

29 varied sensitivity (Hevia et al., 2017). Response traits are phenotypic or life history traits

30 that can determine species sensitivity to disturbance. For example, bee species that

31 nest below-ground are more susceptible to soil tillage than species that nest above

32 ground (Williams et al., 2010), and carnivorous carabid beetles are more susceptible to

33 landscape simplification than phytophagous or omnivorous species (Purtauf, Dauber &

34 Wolters, 2005)

35

36    Loss of insect diversity and abundance caused by environmental disturbances affects

37    the functioning of natural and managed ecosystems (Larsen, Williams & Kremen, 2005;

38    Winfree et al., 2015). A useful framework to predict and understand the implications of

39    declining abundance and diversity on ecosystem functioning is that of 'effect traits';

40    traits that influence a species contribution to an ecosystem function (Lavorel & Garnier,

41    2002). For example, the ability of a bee to successfully pollinate crops with accessible

42    flowers increases as tongue length decreases (Williams et al., 2010), and the ability of

43    carabid beetles to efficiently kill and consume prey increases with body size (Ball et al.,

44    2015).

45

46    Response traits and effect traits are sometimes interlinked where the traits that make a

47    species susceptible to an environmental disturbance also make that species more or

48    less important in providing an ecosystem function (Nervo et al., 2017). As the field of

49    trait-based research continues to grow, this relationship has become more intensively

50    studied (Hevia et al., 2017). Though most research on the interplay of effect and

51    response traits explores change at the ecosystem or community level, considerable trait

52    variability also occurs within species. Intraspecific trait variation is generated through a

53    combination of genetic and environmental factors and includes body-size, stress

54    tolerance, and personality. Variation in these traits has long been understood to have

55    implications for an individual's fitness, though variation also has significant bearing for

56    ecosystem functioning (Bolnick et al., 2011).

57

58   Here, we considered the importance of intraspecific trait variation by exploring the effect

59   of body-size as both an effect trait and response trait in the dung beetle *Onthophagus*

60   *nuchicornis (*Coleoptera; Scarabaeoidea). *Onthophagus nuchicornis* is a generalist

61   coprophage that is native to the Palearctic but has become widely naturalised within

62   North America (Floate & Kadiri, 2013). In northern North America, it is often highly

63   abundant, representing 80% or more of the dung beetle community at latitudes above

64   44N in Western USA (Jones et al., 2019), and c. 40% of the dung beetles in native

65   grasslands in Alberta  (Floate & Kadiri, 2013). As a tunnelling species, O. *nuchicornis*

66   buries dung in soil and fashions it into oblong parcels known as 'brood balls'. Females

67   lay a single egg within each brood ball where after hatching, a larva will feed for the

68   entirety of its development (Skidmore, 1991). Burial and subsequent decomposition of

69   the dung by *Onthophagus* spp. supports ecosystem functions including reductions in

70   abundance of blood-feeding flies (Macqueen & Beirne, 1975b), improved soil fertility

71   (Macqueen & Beirne, 1975a), stimulation of plant litter decomposition (Manning et al.,

72   2016), and secondary seed dispersal (Koike et al., 2012).

73

74   Within agroecosystems, dung beetles and the ecosystem functions they support are

75   vulnerable to veterinary parasiticides. When parasiticides, including macrocyclic

76   lactones and synthetic pyrethroids, are applied to livestock they are excreted in dung

77   largely unmetabolized (Lumaret et al., 2012). The compound which has perhaps

78   received the most attention is ivermectin. Ivermectin exposure can increase nerve and

79   muscle cell permeability to chloride ions, leading to hyperpolarization that limits action

80   potentials (Shoop & Soll, 2002), and subsequently loss of olfaction, reduced fecundity,

81 impaired movement, and death of dung beetles (Verdú et al., 2015; Martínez et al.,

82 2017).

83

84 While studies have explored the potential of body-size on sensitivity to environmental

85 disturbances across entire dung beetle communities (e.g. Tonelli, Verdú & Zunino,

86 2018), to the best of our knowledge none have considered how intraspecific trait

87 variation influences the sensitivity of dung beetles to environmental disturbances. In

88 laboratory experiments using the dung beetle *O. nuchicornis*, we examined the

89 importance of intraspecific body size as a response trait (sensitivity to the veterinary

90 anthelmintic ivermectin) and an effect trait (burial of sheep dung).

91

92 We predicted that:

93    a) Intraspecific variation in body size is a relevant effect trait, such that larger

94       beetles would bury more dung than smaller beetles.

95    b) Intraspecific variation in body size is not a relevant response trait. Because

96       beetles are exposed to ivermectin through contact and oral exposure as they

97       feed and bury within dung, exposure occurs independently of body size. Larger

98       beetles will be similarly susceptible to ivermectin exposure as smaller beetles.

99    c) Because low level exposure to ivermectin is known to strongly and negatively

100      influence beetle condition, the importance of body size as an effect trait will

101      diminish when beetles are exposed to ivermectin.

102
103 **Materials & Methods**

104 **Collection and maintenance of beetles**

105 With permission from landowners, we collected *O. nuchicornis* from two equine facilities

106 in the vicinity of Truro, Nova Scotia (Camden Stables, Opportunity Farm) between 24□-

107 27 June 2019. The horses had not been treated with any sort of parasiticides within the

108 previous four months at either of the two locations. *Onthophagus nuchicornis* is

109 univoltine and undergoes an obligate reproductive diapause while overwintering as an

110 adult (Floate et al., 2015). Because May and June were especially cold, all beetles we

111 collected were assumed to be overwintered sexually mature adults.

112 Upon return to the laboratory, beetles were housed intermittently in vented 11.4 L

113 polypropylene containers. To each container, we added two dozen crumpled sheets of

114 unbleached paper towel that were wetted with distilled water and 250 g of horse dung

115 from a facility (Camden Stables) where we collected beetles. An additional 100 g horse

116 dung was added every three days until beginning the experiment.

117

118 **Dung collection and preparation**

119 Fresh sheep dung used in the experiment was collected from a herd of ewes housed

120 indoors at the Dalhousie University Agricultural Campus. Sheep were fed a diet of hay

121 supplemented with a 80:20 barley:high protein pellet. None of the sheep had been

122 treated with any parasiticides within eight months. Fresh sheep dung was collected

123 14□-24 June 2019. Each day following collection the dung was homogenized for 10

124 minutes using a paint mixer (Mastercraft, Toronto, Canada) attached to a cordless drill

125 and divided into two equal portions. Dung was frozen at -21°C prior to use in the

126 experiment. Twenty-four hours prior to beginning the experiment, half the dung

127  collected was removed from the freezer and thawed at room temperature. After the

128  dung was thoroughly homogenized it was weighed and hand-rolled into 100 g (+/- 2 g)

129  balls.

130

131  **Enclosure design**

132  Experimental enclosures (herein containers) were assembled using 1360 mL grease

133  resistant paper cups (Dart Container Corporation, Mason, Michigan, United States).

134  Eight 2 mm drainage holes were made in the bottom of each container. Each container

135  was filled with coarse all-purpose builder's sand (Shaw Resources, Shubenacadie,

136  Canada) to a depth of 12 cm leaving 5.8 cm between the sand surface and the mouth of

137  the container. Distilled water was slowly added to each container until water began to

138  drip from the drainage holes. Dung was placed on top of the sand and pressed gently

139  against the sand surface to ensure contact.

140

141  **Sex and size classification of beetles**

142  We collected 358 beetles (170 males, 188 females) from the field. Males were

143  differentiated from females by the presence of a single spine-like horn on the head;

144  females had a transverse ridge at the base of the head and no horn (Jessop, 1986).

145  Cohorts of females and males were placed in separate holding containers partially filled

146  with moist paper towel.

147

148  We labeled 90 mL plastic specimen cups filled half-way with moist all-purpose builder's

149  sand (N=60). Cups were labelled from S1–S30 and L1–L30, coding for small and large

150  beetles, respectively. We then created our sub-population of male beetles by selecting

151  the largest individuals based on sight and placing them individually in cups L1–L30. The

152  order of the cups was randomized, and a second large male beetle was added to each

153  cup. We repeated the same process for large female beetles, such that each cup

154  contained four beetles (two males and two females) sampled from the larger end of the

155  size distribution. Immediately after the last beetle was added, the container was closed

156  with 2-mm black fibreglass mesh secured by an elastic band. Immediately after, the

157  same process was followed for small beetles placing them in cups labeled from S1–

158  S30. All remaining beetles were released.

159

160  **Estimating the importance of body size in functioning**

161  We added beetles from cups into containers on 28 June. Beetles were left to bury and

162  feed on dung for seven days. On 5 July we gently sifted the contents of each container

163  through a 0.4 cm aperture soil sieve. After sifting, all beetles were gently removed and

164  placed in a 90 mL specimen cup, and all brood balls were placed in a Petri dish. We

165  recorded the number of brood balls formed in each container and measured the

166  cumulative mass of buried dung to the nearest tenth of a gram. We measured the mass

167  of each beetle to the nearest milligram. Three beetles died in the first part of the

168  experiment and were replaced with beetles of the same size and sex housed under

169  identical conditions.

170

171  **Estimating intraspecific variation in functioning under chemical perturbation**

172  The same beetles were used for the second experiment, which began immediately after

173   the first experiment. Dung was defrosted 48 hours prior and was subsequently

174   homogenized for 10 minutes as described above. We subdivided the dung into six 1.0

175   kg portions. We added 100 mL of Ivomec, Pour-On for Cattle (5 mg·mL$^{-1}$ ivermectin)

176   diluted in acetone to each portion of dung. The final treatment concentrations of

177   ivermectin in dung were 0.01, 0.1, 1, 10, and 100 mg·kg$^{-1}$ (wet weight). A 100 mL

178   solution of pure acetone was added to the remaining 1.00 kg portion of dung to serve as

179   a control treatment. As before, we homogenized the dung using an electric paint mixer

180   and divided each parcel into n=10 dung balls each measuring 100 g. To avoid

181   contamination, when mixing ivermectin into the dung we moved sequentially from the

182   ivermectin-free controls to the highest concentration of ivermectin. The paint mixer

183   attachment was thoroughly cleaned in hot soapy water, rinsed, and dried between

184   mixing different batches of dung. We left all the dung in a well-ventilated area for 24 h to

185   allow the acetone to volatize.

186

187   We began the second experiment as the first experiment (described in section 2.5) was

188   ending (5 July). As beetles and their brood were removed from each container, we

189   repacked the sand into each enclosure and re-wetted as before. The dung balls

190   containing ivermectin were split amongst size classes (e.g. n=5 "large beetle" containers

191   with 10 mg·kg$^{-1}$ ivermectin and n=5 "small beetle" containers with 10 mg·kg$^{-1}$ ivermectin)

192   that were pre-assigned using a random number generator. Levels of ivermectin

193   exposure were then randomly assigned to containers. We added a dung ball to each

194   container, pressing the dung gently against the soil surface to ensure contact between

195   the sand and dung. Beetles were added to containers immediately after being weighed

196   and each container was closed with 2 mm black fibreglass mesh and secured with two

197   rubber bands to ensure the beetles could not escape.

198

199   Following a seven-day period (12 July) the experiment was completed. We were blinded

200   from the concentration of the ivermectin during observation. As before, we used a sieve

201   to separate beetles and brood balls from sand. We recorded the number and cumulative

202   mass of brood balls within each enclosure. We recorded the sex of each beetle, and

203   whether it was dead or alive. A beetle was considered dead if gentle prodding with a

204   pair of blunt forceps, or if breathing onto the beetle failed to elicit any significant

205   antennal or leg movements. Beetles were subsequently euthanized by freezing at -

206   21°C, and later a second estimate of body size (pronotal width) was taken using digital

207   calipers to the nearest hundredth of a millimeter. While measuring pronotal width, we

208   detected an error in differentiating male and female beetles that occurred in three

209   mesocosms. The final sample size was reduced from N=60 to N=57.

210

211   **Statistical Analysis**

212   To confirm that body size differed between the small and large groups, we used a two-

213   sample t-test that compared pronotal width and mass between the two size

214   classifications. Because *O. nuchicornis* is sexually dimorphic, we completed separate

215   tests for males and for females.

216

217   To determine whether intraspecific variation in body size is a relevant effect trait, we

218   compared small and large beetles across three measures of functioning. We used a

219   two-sample t-test to compare mean dung ball mass and cumulative mass of dung

220   buried. We used a linear model with a Poisson error structure to determine if the

221   number of brood balls varied between small and large beetles.

222

223   To determine if intraspecific variation in body size is a relevant response trait, we initially

224   intended to compare by probit analysis the sensitivity of beetles between the size

225   classes by calculating the ivermectin concentration that kills 50% of the population. This

226   was not possible because of relatively high (15 ± 5%) mortality in the control group and

227   failure to select an experimental dose high enough to generate the full sigmoidal dose-

228   response curve. We instead treated dose as a categorical factor and compared

229   mortality across the full range of doses using generalized linear models with a binomial

230   error structure. Where overdispersion occurred, models were compared using

231   generalized linear model with quasi-binomial error structure. Statistical significance was

232   calculated by through comparison to a null, intercept-only, model using an "F" test for

233   models with binomial error, and a "Chi-Square" test for models with quasibinomial error.

234

235   Lastly, to test whether ecosystem functioning provided by beetles with a larger body

236   size would be less impaired by ivermectin in comparison to beetles with a smaller body

237   size, we used generalized linear models. Ivermectin concentration (categorical: 0.01–

238   100 mg·kg$^{-1}$) and body size (categorical: small vs. large) were used as independent

239   variables along with their interaction. As above, we used three different estimates of

240   functioning as dependent variables: dung removal (Gaussian error structure), mean

241    brood ball size (Gaussian error structure), and brood ball number (Poisson error

242    structure).

243

244    All statistical models were run using R 3.6.2 (R Core Team, 2016), along with the

245    packages, dplyr (Wickham et al., 2015)) and ggplot2 (Wickham, 2016). When reporting

246    results, in all cases we have used means and associated standard errors.

247
248    **Results**
249    **Beetles varied significantly in size between population samples**

250    Our efforts to select a sample of large and small beetles from the larger population were

251    effective. The mean mass of large males (61.8 ± 1.0 mg) was 46% heavier than the

252    mass of small males (42.2 ± 0.8 mg, $t_{112}$=16.4, *P*<0.001, Fig. 1A). The pronotal width of

253    large males (4.51 mm ± 0.02 mm) was 15% greater than the pronotal width of small

254    males (3.93 ± 0.02 mm, $t_{112}$=18.8, *P*<0.001, Fig. 1B). Mean mass of large females (64.6

255    ± 1.1 mg) was 84% greater than the mass of small females (34.3 ± 0.8 mg, $t_{112}$=21.5,

256    *P*<0.001, Fig. 1C). The pronotal width of large females (4.53 ± 0.02 mm) was nearly

257    25% greater than the pronotal width of small females (3.61± 0.03 mm, $t_{112}$=22.8,

258    *P*<0.001, Fig. 1D).

259

260    **Intraspecific body size is a highly relevant effect trait**

261    Larger beetles buried 1.5-fold more dung (47.6 ± 2.8 g) than smaller beetles (19.0 ± 2.3

262    g, $t_{54}$=7.84, P<0.001, Fig 2A). Larger beetles buried nearly twice as many brood balls

263    (17.1 ± 0.88 g) in comparison to smaller beetles (9.62 ± 1.10, $F_{1,55}$=20.33, P<0.001, Fig.

264    2B).

265    The mean mass of brood balls formed by large beetles (2.78 ± 0.09 g) was 38%

266    heavier than the mean mass of brood balls formed by smaller beetles (2.01 ± 0.10 g,

267    $t_{51}$=5.80, P<0.001, Fig 2C).

268

269    **Beetles susceptibility to ivermectin does not vary with body size**

270    We found no significant interaction between body-size and exposure level on beetle

271    mortality ($\chi_{5,45}$=3.80, P=0.57). While beetle sensitivity to ivermectin was not significantly

272    affected by body size ($\chi_{1,54}$= 0.507, P =0.48), the level of ivermectin exposure

273    significantly affected survival ($\chi_{5,50}$= 54.5, P<0.001, Fig. 3). Beetle survival in the control

274    group was 85 ± 6%, increasing to 97 ± 3%, 93 ± 4%, and 97 ± 3% at 0.01, 0.1, and 1

275    mg ivermectin · kg$^{-1}$ dung respectively. Survival dropped to 68 ± 13% at 10 mg

276    ivermectin·kg$^{-1}$ dung and 8±4% at 100 mg ivermectin·kg$^{-1}$ dung.

277

278    **Exposure to low concentrations of ivermectin stimulates functioning**

279    Total dung buried was significantly affected by beetle size ($F_{1,54}$=4.16, $P$=0.04) and by

280    ivermectin exposure ($F_{5,49}$=3.00, $P$=0.004), but not by the interaction of these factors

281    ($F_{5,44}$=1.00, P=0.43) (Fig.4A). Low level exposure to ivermectin stimulated functional

282    efficiency. Relative to dung buried by beetles not exposed to ivermectin (1.16 ± 0.57 g

283    g), beetles exposed to 0.01mg·kg$^{-1}$ ivermectin buried 13.7-fold more dung (15.9 ± 8.4 g),

284    beetles exposed to 0.1 mg·kg$^{-1}$ ivermectin buried 10.8-fold more dung (12.6 ± 3.6 g),

285    and beetles exposed to 1 mg·kg$^{-1}$ ivermectin buried 6-fold more dung (6.9 ± 1.9 g). At

286    higher concentrations dung burial was negatively affected where exposure to 10 mg·kg$^{-1}$

287    ivermectin reduced dung burial to by 54% (0.53 ± 0.36 g) and exposure to 100 mg·kg$^{-1}$

288    ivermectin reduced dung burial by 81% (0.22 ± 0.22 g). Across all levels of ivermectin

289    exposure, larger beetles buried 8.6 ± 3.0 g of dung, which was approximately 1.75-fold

290    more dung than smaller beetles (3.1 ± 0.9 g).

291

292    We found a similar trend for brood ball production (Fig. 4B). The number of brood balls

293    buried per mesocosm was significantly affected by beetle size ($F_{1,55}$=14.36, $P$=0.01) and

294    by ivermectin exposure ($F_{5,50}$=121.9, $P$<0.001), but not by their interaction ($F_{5,45}$=4.31,

295    $P$=0.82). Low level exposure to ivermectin stimulated production of brood balls. Relative

296    to the number of brood balls produced by beetles that were not exposed to ivermectin

297    (0.60 ± 0.26 brood balls), beetles exposed to 0.01 mg·kg$^{-1}$ ivermectin buried 10-fold (5.4

298    ± 2.4 brood balls) more brood balls, and beetles exposed to 0.1 mg·kg$^{-1}$ ivermectin

299    buried more 8-fold more (4.8 ± 1.2 brood balls, Fig. 1A). Across all levels of ivermectin

300    exposure, larger beetles buried twice as many brood balls (3.0 ± 0.9) as smaller beetles

301    (1.4 ± 0.4 brood balls).

302

303    The mean size of brood balls (Fig. 4C) was not affected by beetle size ($F_{1,27}$ =2.82,

304    $P$=0.11,), ivermectin exposure ($F_{5,22}$=0.33, $P$=0.89), or the interaction of these factors

305    ($F_{3,19}$=0.50, $P$=0.69).

306

307    **Discussion**

308    Beetles with a larger body-size were more efficient at removing dung suggesting that

309    body-size is an important intraspecific effect trait for *O. nuchicornis.* This trend has been

310    observed for other species of dung beetles including *Onthophagus* (Lee & Peng, 1981).

311   The greater level of functional efficiency in large beetles can explained by two

312   complementary factors. Larger beetles produced nearly twice as many brood balls as

313   smaller beetles, and the mean size of brood balls by large beetles was 38% greater

314   than brood balls produced by smaller beetles. These observed differences can be

315   explained through biological and behavioural mechanisms.

316

317   The greater number of brood balls constructed by larger beetles may be directly related

318   to fecundity. Female body-size is positively and strongly linked to fecundity across many

319   insect groups (Honěk, 1993). However, in *O. nuchicornis,* both males and females

320   contribute to formation of brood balls (Macqueen & Beirne, 1975a), meaning the

321   number of brood balls constructed by a pair of beetles reflects more than fecundity

322   alone.

323

324   The larger size of brood balls can be explained by both behavioural and morphological

325   differences amongst size classes. A behavioral explanation of these differences is

326   linked to reproductive investment by females. A study of a closely-related species, *O.*

327   *taurus,* found that female beetles formed larger brood balls, when mated to larger

328   bodied males (Hunt & Simmons, 2001). Because we modified male and female size

329   simultaneously, we are unable to test whether this effect occurred within our study

330   species. Onthophagine dung beetles use their legs and bodies to combine small

331   quantities of dung into tightly-packed masses known as brood balls. Because of their

332   greater size, larger beetles should build larger brood balls in comparison to smaller

333    beetles. Past research focusing on *O. gazella* found similar positive relationships

334    between body size and brood ball size (Lee & Peng, 1981).

335

336    Although mortality of *O. nuchicornis* to ivermectin was significant at concentrations of 10

337    mg·kg$^{-1}$ (~35% mortality) and 100 mg·kg$^{-1}$ (~90% mortality), larger body size continued

338    to support higher levels of functional efficiency. That is, irrespective of ivermectin

339    exposure, large beetles buried more dung than smaller beetles. Unlike in the first part of

340    the experiment, this was caused solely by brood ball abundance and not by a

341    combination of brood ball abundance and size. Comparing brood ball production from

342    the first experiment with the control group in the second experiment, shows

343    considerable loss of functional efficiency over time. This could indicate that laboratory

344    conditions were not conducive to beetle health, or that field captured beetles were

345    collected when their reproductive potential was declining.

346

347    Dung beetles exposed to low levels of ivermectin had greater functional efficiency and

348    higher survival rates than beetles in the control treatment. This response is indicative of

349    hormesis: a biphasic dose–response relationship where low doses of a stressor

350    stimulate biological effects and high doses of the same stressor inhibit biological effects

351    (Southam, 1943). While the phenomenon of hormesis has been observed across many

352    different insect taxa (Cohen, 2006; Cutler, 2013; Guedes & Cutler, 2014), to the best of

353    our knowledge this is the first time it has been recognized as such in dung beetles.

354    However, previous studies seem to have observed stimulatory effects of ivermectin on

355    other dung beetle species, but the authors have not acknowledged the hormetic effects

356    they reported. In a pair of studies by Ishikawa & Iwasa, (2020), exposure to 0.1 ppm

357    ivermectin in cow dung increased brood ball production 32% in *Onthophagus bivertex,*

358    and 52% in *Onthophagus lenzii.* A similar finding was reported by Iwasa et al., (2007)

359    who reported that exposure to 0.05 - 0.5 ppm (wet weight) ivermectin in cow dung

360    increased brood ball formation by as much as 198% in the dung beetle *Caccobius*

361    *jessoensis,* relative to an ivermectin-free control.

362

363    The range of ivermectin concentrations that stimulated a hormetic response in our

364    experiment (0.01-1 mg kg$^{-1}$), are commonly encountered in agroecosystems. A

365    pharmokinetic study of ivermectin in sheep (Vokřál et al., 2019), where animals were

366    dosed with a subcutaneous injection of 0.2 mg ivermectin kg$^{-1}$ bodyweight resulted in a

367    maximum concentration of 0.93 ± 0.50 mg kg$^{-1}$ ivermectin in dung (wet weight) 48 h

368    following injection, which quickly declined to approximately 0.05 mg kg$^{-1}$ 12 days post

369    treatment. Experiments with cattle also demonstrate the hormetic concentrations in our

370    experiments also occur in the field. For example, after treating cattle with a pour-on

371    application of 500 µg ivermectin kg bodyweight$^{-1}$, concentrations of ivermectin in dung

372    were 0.97-1.01, 0.50-1.54, and 0.07-0.14 mg kg$^{-1}$ wet weight at 3, 7 and 14 days

373    respectively (Wohde et al., 2016). Concentrations of ivermectin in dung can be above

374    these sublethal concentrations depending on the product formulation (Sommer et al.,

375    1992), species receiving the treatment (Canga et al., 2009), and occurrence of social

376    grooming behaviours (Laffont *et al.* 2001).

377

378

379    We found that ecosystem functioning by a common and highly abundant species can be

380    stimulated by exposure to low concentrations of ivermectin in dung. This has potentially

381    far-reaching beneficial effects for pasture environments. By increasing the quantity of

382    dung buried in soil, tunnelling dung beetles like *O. nuchicornis* move greater quantities

383    of nutrients into the plant root zone, which stimulates primary productivity. Increased

384    burial also results in clearing of dung from the pasture surface, which frees additional

385    pasture area for grazing livestock (Anderson, Merritt & Loomis, 1984). Dung burial by

386    tunneling beetles is well known to prevent the development of blood-feeding veterinary

387    pests such as *Haematobia irritans* (Diptera: Muscidae) (Legner & Warkentin, 1991).

388    Furthermore, enhanced dung burial and physical disruption of the dung environment

389    could work alongside the toxic effects of ivermectin on horn fly development, and might

390    complement programs developed to manage resistance to ivermectin and other

391    macrocyclic lactones (Byford et al., 1999).

392

393    While low-dose exposure to ivermectin stimulated higher levels of functioning than the

394    controls, it is important to reiterate the loss of functional efficiency between the first and

395    second experiment. Beetles that experienced no ivermectin-exposure in the second

396    experiment buried on average just $2.2 \pm 0.27$ brood balls, whereas the same cohort of

397    the beetles in the first experiment buried $10.6 \pm 2.57$ brood balls, representing a ~80%

398    reduction between the two experiments. It seems likely that the experimental conditions

399    were not conducive to prolonged insect health. The hormetic response of dung beetles

400    to ivermectin during the second part of the experiment might not have been as

401    pronounced in a less stressful environment.

402

403 Trade-offs may have occurred with biological endpoints not measured in our study. For

404 example, others have shown that exposure of dung beetles to ivermectin in dung

405 stimulates broodball formation, but thereafter results in reduced emergence of F1

406 offspring (Ishikawa & Iwasa, 2020). This may be because of greater ivermectin

407 sensitivity in developing larvae relative to adult beetles, which has been reported in

408 multiple studies (Beynon et al., 2012; Pérez-Cogollo et al., 2015). More work is needed

409 to clarify whether ecological benefits of enhanced dung processing stemming from

410 exposure to low doses of ivermectin results in increased functioning of the system

411 overall, or neutralized or reduced functioning as a result of trade-offs.

412

413 Our findings must be considered against landscape-scale studies that indicate negative

414 impacts of veterinary parasiticides on dung beetles. Sands and Wall (2018) sampled

415 dung beetles across 24 beef farms across Southwest England, finding farms that used

416 macrocyclic lactones had 19% fewer species of dung beetles, but no reduced

417 abundance, compared to farms that used no parasiticides. While not specifically

418 targeting the effect of veterinary parasiticides, a study of 24 cattle farms in Ireland by

419 Hutton and Giller (2003) found that organic farms supported dung beetle abundance

420 50% greater than conventional farms. Short-term benefits for ecosystem functioning

421 caused by hormesis may be short-lived if dung beetle populations experience decline in

422 abundance and diversity: both of which have been shown to negatively affect

423 ecosystem functions provided by dung beetles (e.g. Beynon *et al.* 2012; Manning and

424 Cutler 2018).

425

426    Relatively little is known about how short-term exposure to parasiticide residues

427    impacts beetles later in life. When *Aphodius fossor* fed on dung containing 0.5 mg kg$^{-1}$

428    ivermectin, dung removal was suppressed 48% relative to beetles feeding on

429    ivermectin-free dung, but such effects subsided two weeks after exposure (Manning,

430    Beynon & Lewis, 2017). If low-dose exposure to ivermectin can stimulate reproduction,

431    hormetic effects could have a net positive effect on ecosystem functioning. Past

432    research indicates this is possible: males of the dung beetle *Euoniticellus intermedius*

433    exposed to 0.01 mg kg$^{-1}$ ivermectin in cattle dung had 39% increased testis volumes,

434    which is indicative of higher fertility (Hunt & Simmons, 2001), and females exposed to

435    the same ivermectin treatment had 25% more mature oocytes (a measure of fecundity),

436    with no effect on the number of brood masses formed; a second measure of fecundity.

437

438    Our current understanding of insecticide-induced hormesis is mostly limited to

439    laboratory conditions, despite the ramifications this phenomenon could have for

440    ecosystems (Cutler, 2013). Dung insect communities perturbed by parasiticide residues

441    could be an excellent model system to better understand the strength and significance

442    of hormetic relationships in complex environments subject to ecological flux and

443    chemical disturbance. In conjunction with feeding on the dung of livestock, dung beetles

444    support a wide range of ecosystem functions within agroecosystems that vary from

445    enhancing primary productivity (Manning et al., 2017) to disrupting parasite

446    development (Sands & Wall, 2017). This offers a range of endpoints and experimental

447    designs that can be used to better understand hormetic influences of stressors on

448    ecosystem functioning.

449

**Conclusions**

450

451 We found that body size was a strong predictor of functional efficiency for the dung

452 beetle *Onthophagus nuchicornis*. This benefit of larger body size for functional efficiency

453 persisted, even when beetles were exposed to the veterinary anthelmintic ivermectin.

454 High concentrations of ivermectin in dung (10-100 mg·kg$^{-1}$) caused mortality; but low

455 concentrations (0.01-1 mg·kg$^{-1}$) stimulated the amount of dung buried via increasing the

456 number of brood ball production. This hormetic response of *Onthphagus* dung beetles

457 to ivermectin has been reported in earlier publications but was not recognized as

458 hormesis. Because dung beetles support healthy agroecosystems by providing a

459 number of ecological functions, we suggest that dung beetles could be a useful system

460 for understanding the significance of hormesis within ecosystems.

461

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465

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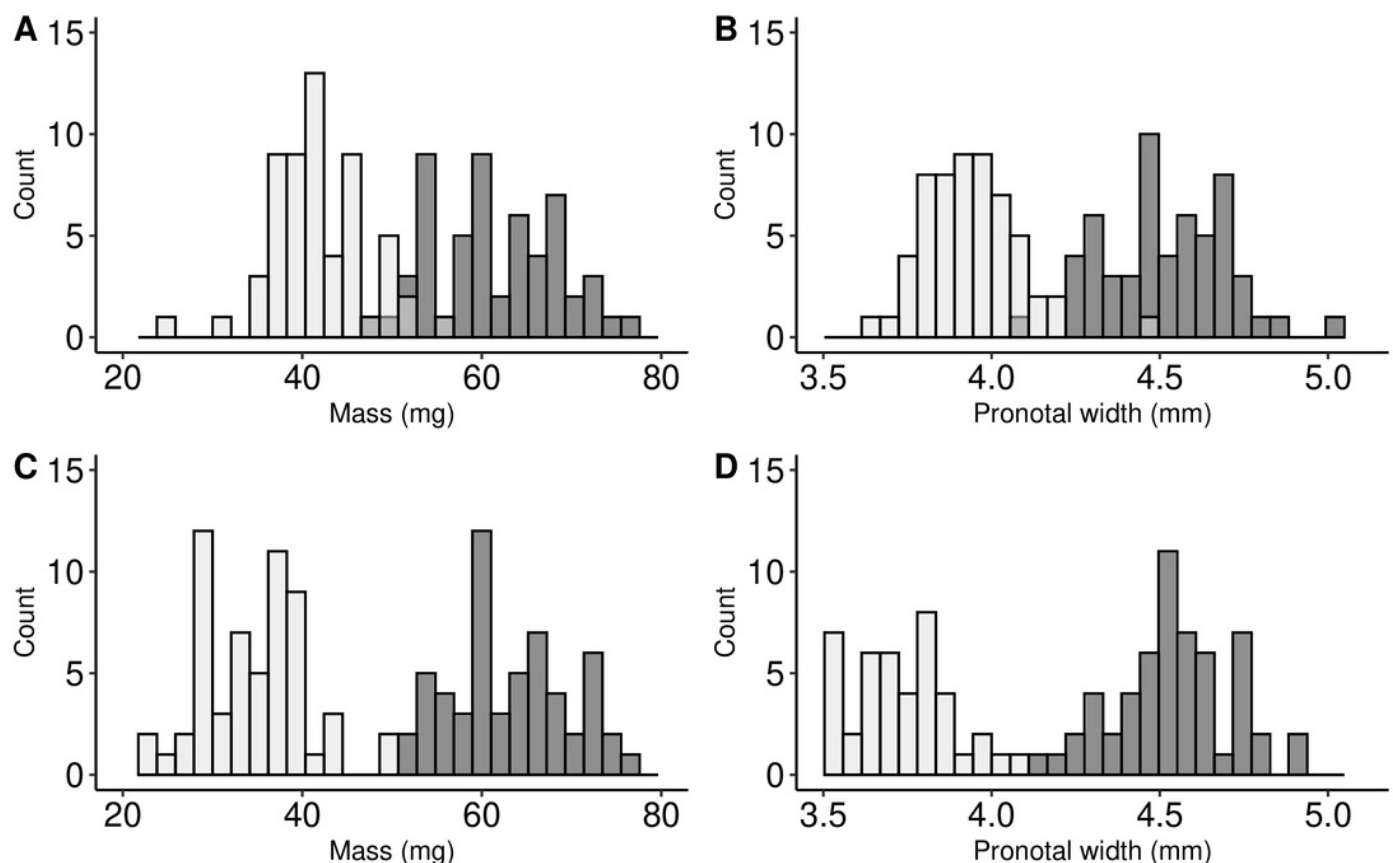
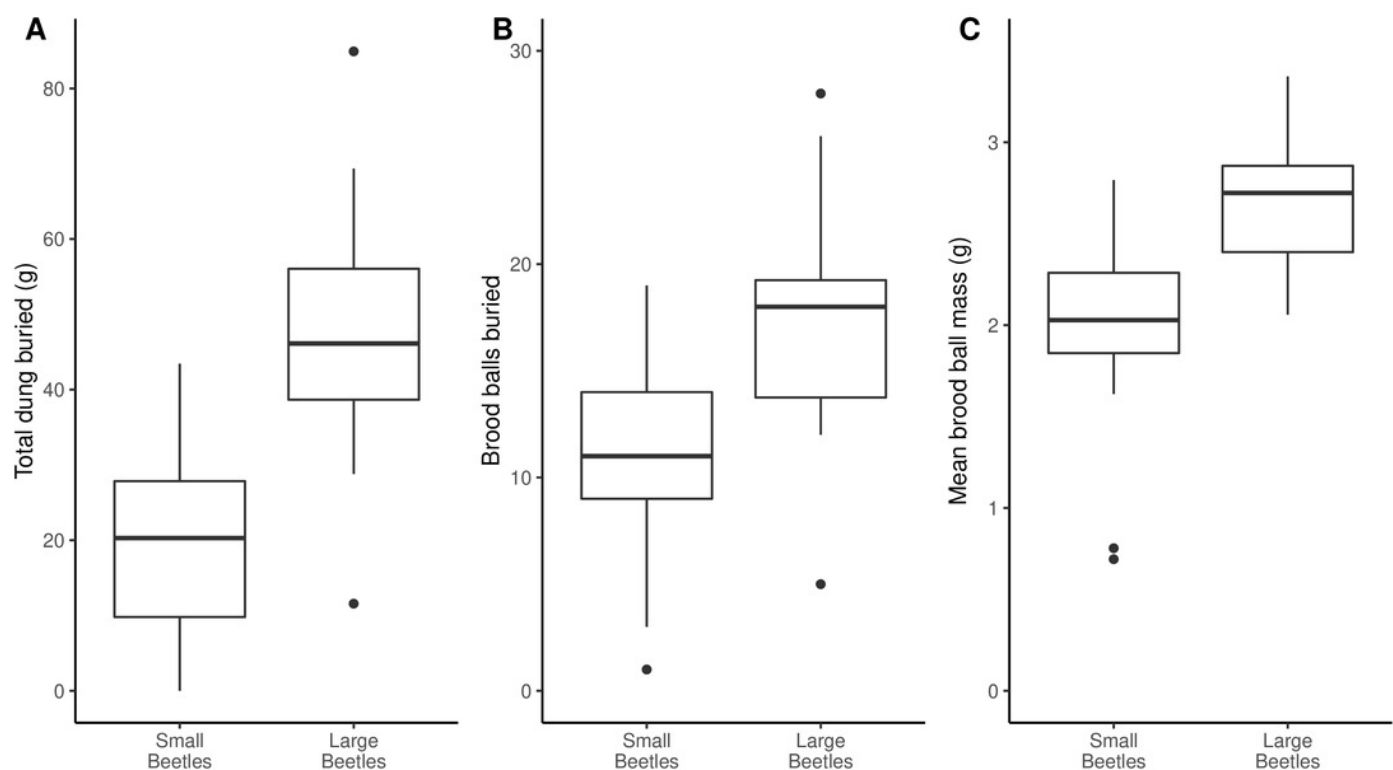
# Figure 1

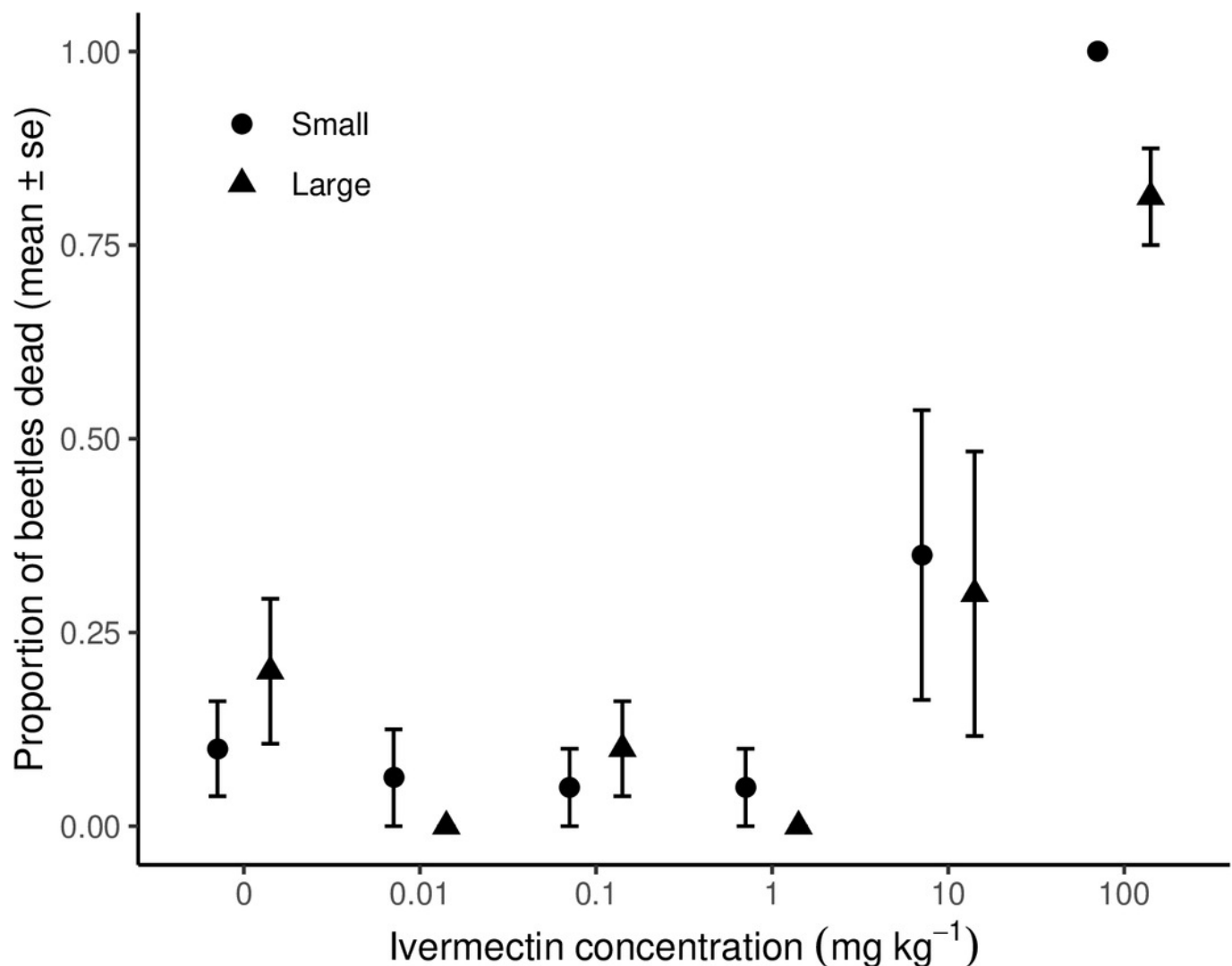
Demonstrating the large differences in body-size for Onthophagus nuchicornis

Distributions in body-size variation of *Onthophagus nuchicornis* across male body mass (A), male pronotal width (B), female body mass (C), and female pronotal width (D). Beetles from the smaller end of the body-size spectrum are filled in light grey. Beetles from the larger end of the body-size spectrum are filled in dark grey. In all cases differences in means were statistically significant with large effect sizes (minimum 15% difference).

# Figure 2

Larger dung beetles are more functionally efficient than smaller dung beetles.

Comparison of three different measures of functional efficiency in response to body size in the dung beetle *Onthophagus nuchicornis*. Body size is an important predictor of functional efficiency. Larger beetles bury more dung than small beetles (A), which is facilitated by larger beetles burying a larger number of brood balls than smaller beetles (B) in greater abundances (C). The lower and upper limits of the box represent the inter quartile range (IQR) of the data. The horizontal line within each box represents the median. Whiskers extend from the boxes to show maximum and minimum values; if this distance is >1.5× the IQR, observations appear as a filled point.

# Figure 3

High doses of ivermectin cause mortality in the dung beetle *Onthophagus nuchicornis*

Mortality of *Onthophagus nuchicornis* exposed to ivermectin in dung. Mortality varied with ivermectin concentration, but body size did not affect susceptibility to ivermectin by itself, or via any exposure-body size interaction. Points are mean values with associated standard errors.

# Figure 4

Exposure to low doses of ivermectin stimulate functioning in the dung beetle *Onthophagus nuchicornis*

Low exposure to ivermectin enhances the ecosystem function of dung burial. Larger beetles continue to be more functionally efficient than smaller beetles, regardless of ivermectin exposure. In each case, the higher level of functioning observed (A) was driven by greater brood ball production (B), rather than the production of heavier brood balls (C). Points represent means and associated standard errors.