



# Malnutrition and parasitism shape ecosystem services provided by dung beetles

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## ARTICLE INFO

### Keywords:

Diet quality  
Dung removal  
Immune response  
Physiological condition  
Nutrition  
Terminal investment

## ABSTRACT

Ecosystem services relies on several insects that provide fundamental functions. Despite the quality of these ecosystem services depends on insect diversity, abundance and biomass, little is known about the effects that individual body condition has over such services. One prediction is that starving or sick animals may provide a reduced service. Dung beetles bury dung in forests and cattle farms, contributing to soil fertilization and reduction of greenhouse gas emissions from livestock. However, some species are highly sensitive to habitat disturbance and this leads to important losses of ecosystem services in disturbed areas. Here we experimentally tested the effect of diet quality and parasite pressure impact on dung removal rate using three species of dung beetles from contrasting habitats. We exposed wild beetles to an immune challenge combined with experimental diets that varied in protein content. We predicted that dung removal would be better carried out by healthy and well-fed individuals. However, if a species incurs in compensatory feeding or terminal investment in reproduction, ill individuals will still exhibit intense dung removal and reproductive activity but with a physiological cost. For *Euoniticellus intermedius* beetles, the immune challenge reduced dung removal rates, and this was because the challenge reduced the weight, although not the number of built brood masses. This suggests that implanted individuals made an intense reproductive effort. Therefore, a strategy of terminal investment in reproduction might be occurring. In the same species, couples fed low-protein diets increased dung removal rates compared to control-fed animals, probably as a compensatory feeding strategy that increased energetic condition. Conversely, *Onthophagus incensus* and *O. rhinolophus* beetles did not change dung removal rates despite suffering changes in energetic condition resulting from treatment. This is the first evidence that ecosystem services provided by dung beetles depend on individual health and nutritional status that drive reproductive and feeding behavior. Understanding the environmental factors that affect individual physiology and behavior is fundamental to guarantee conditions not only for the survival of key species but also for the maintenance of ecosystem services.

## 1. Introduction

Ecosystem services depend on key organisms that, as a result of certain activities such as feeding and reproduction, provide important functions like pollination, natural pest control and soil fertilization, all of which are essential for human wellbeing (Daily, 1997; Reid et al.,

2005; Losey and Vaughan, 2006). Although the value of ecosystem services relies on the abundance and diversity of service providers (Mace et al., 2012), each individual supplies an ecosystem service that is determined by its own behavior, which is in turn dependent on self-condition and physiological status. In fact, two main drivers of the so-called pollinator crisis are starvation due to habitat loss (Naug, 2009)

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<https://doi.org/10.1016/j.ecolind.2020.107205>

Received 22 May 2020; Received in revised form 13 October 2020; Accepted 19 November 2020

Available online 10 December 2020

1470-160X/© 2020 The Author(s).

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and emerging infectious diseases (Fürst et al., 2014). The effects of both, habitat loss and diseases, can be visualized from the individual level. However, the question of how body condition shapes ecosystem services has lagged in our understanding of the factors driving the services provided by natural and productive ecosystems.

Food availability and parasite pressure are two primary environmental drivers of individual condition and behavior, defining individual development, survival and reproduction (Kitaysky et al., 1999; Bearhop et al., 2004; Lawniczak et al., 2007) and, potentially, ecosystem services. In terms of food, well-nourished individuals are expected to provide better ecosystem services. For example, bees whose nutritional budget is not satisfied, may end up reducing their pollination rate and consequently fruit production (Koch et al., 2017). Alternatively, honeybees that face nutrient deprivation may increase their foraging activity, aiming to increase pollen collection (Brodschneider and Crailsheim, 2010), thus improving the ecosystem services they provide. In response to starvation, insects may modify foraging rates according to their needs or feed on particular nutrients, with potential impacts on trophic webs and ecosystem services (Simpson et al., 2018).

Parasites also have a major effect on feeding habits in several insects, inducing sickness behavior such as anorexia, changes in diet as a form of self-medication, or increasing feeding rates to compensate energetic costs (Adamo et al., 2007, 2010; Raubenheimer and Simpson, 2009; González-Tokman et al., 2011; Simpson et al., 2018), potentially affecting ecosystem service provision in varying ways. For example, *Passalus* beetles deliver a better ecosystem service when infected with nematodes, increasing wood degradation activities (Davis and Prouty, 2019). Alternatively, worker honeybees increase their carbohydrate intake when infected with the microsporidian fungi *Nosema ceranae* (Mayack and Naug, 2009), with negative impacts on colony and brood size, honey production and survival rate (Botías et al., 2013). As the activation of immune responses are energetically costly (Siva-Jothy et al., 1998) and compromise insect survival and reproduction (Schmid-Hempel, 2005), parasitized individuals can restrict their investment in current reproduction awaiting for more favorable conditions; however, in other situations they can incur in terminal investment, such that reproductive activity is prioritized over survival (Velando et al., 2010; González-Tokman et al., 2013; Kivleniece et al., 2010; Reyes-Ramírez et al., 2019). The extent to which different species respond to environmental pressures depends on the plasticity in their behavioral and physiological processes (Hofmann and Todgham, 2010). For example, habitat or food specialization may drive differences in species responses to environmental stress, so that specialists may be more sensitive (Devictor et al., 2008; Salomão et al., 2019). However, it is unclear how parasite pressure and nutritional status affect ecosystem services when animal condition and survival expectancies vary.

Dung beetles (Coleoptera: Scarabaeinae) use vertebrate dung and carrion to feed and provision their offspring (Halffter and Edmonds, 1982). Through these behaviors, dung beetles provide fundamental ecosystem services by burying dung and decomposing organic matter, promoting soil fertilization and bioturbation, reducing noxious fauna associated to dung, dispersing seeds, and reducing the emission of greenhouse gases (Nichols et al., 2008; Slade et al., 2016). Despite these benefits, recent declines in dung beetle diversity caused by human activities has been observed in different ecosystems, including forests and cattle pastures (Martínez and Cruz, 2009; Nichols et al., 2009; Basto-Estrella et al., 2014). These declines imply a risk for a number of dung beetle species and the ecosystem services they provide (Nichols et al., 2008). For example, strictly coprophagous species seem to be more sensitive to stressful environments than copro-necrophagous species (Salomão et al., 2019). Nevertheless, most studies aimed to analyze ecosystem services provided by dung beetles only included the whole assemblage or populations (e. g. abundance, diversity) (Andresen, 2002; Gray et al., 2014; Manning et al., 2016), ignoring individual condition. However, integrating animal condition may allow a deeper understanding of the physiological basis of ecosystem services provided by

such insects as well as an individual-based perspective of such service contribution (Salomão et al., 2018; Villada-Bedoya et al., 2019).

Environmental conditions impact the physiology and behavior of dung beetles, suggesting a link between individual condition and their associated ecosystem services. For the role of food, Shafiei et al. (2001) found that specimens of *Onthophagus taurus* pupate prematurely when subjected to food deprivation, due to larval developmental plasticity. The rapid hatching of smaller adults compared to the larvae fed with enough dung was also observed and has potential impacts on the ecosystem services that they provide. For the role of pathogens, female *Euoniticellus intermedius* injected with lipopolysaccharides (molecules from the cell wall of gram-positive bacteria that trigger an immune response) built fewer brood masses than not injected females (Reaney and Knell, 2010), highlighting the reproductive costs of being immune-challenged and potential effects on dung removal rates. Thus, the high sensitivity of dung beetles makes them important bioindicators of environmental disturbance, as some species suffer strong impacts on self-condition that are accompanied by reductions in population sizes (Salomão et al., 2020). Moreover, dung beetle individual condition has even been suggested as an important bioindicator of habitat quality that may precede population decline and reduction in ecosystem services (Cooke et al., 2013; Salomão et al., 2018, 2020).

In this paper we experimentally evaluated how diet quality and parasite pressure affect dung removal rates and energetic condition of three dung beetle species that inhabit contrasting environments: the first studied species (*Euoniticellus intermedius*) is exclusive from cattle pastures; the second (*Onthophagus rhinolophus*) is exclusive from highly conserved, shaded forest; finally, the third species (*Onthophagus incensus*) is able of inhabiting conserved and disturbed sites. We measured dung removal in beetles exposed to a combination of diets of varying protein-content and an immune challenge. As *E. intermedius* can be bread in the laboratory, we further used this species to investigate whether potential changes in dung removal depend on reproductive or feeding behavior. We predicted that dung removal is a costly activity that is better carried out by well-fed and healthy individuals. However, a terminal investment reproductive strategy could lead to an intense dung removal activity resulting in energetic depletion.

## 2. Materials and methods

### 2.1. Study subject

The first study species, *E. intermedius*, is a South African species widely distributed in wet and semi-arid regions (Barkhouse and Ridsdill-Smith, 1986; Edwards, 2007), with preference for open pastures. This species was introduced in Texas and California between 1972 and 1974, yet nowadays has become one of the most abundant dung beetle species in tropical pasturelands of the Gulf of Mexico (Montes de Oca and Halffter, 1998). In the American continent, this diurnal tunneller species inhabits pasturelands using dung from mammals, mostly cattle (Blume, 1984; Pomfret and Knell, 2006a; Cruz Rosales et al., 2012), being sometimes the most abundant dung beetle (Almendarez-Rocha, 2019). The second species, *O. incensus*, is one of the most widespread dung beetle species in American cloud forests and pasturelands. Its distribution range goes from the United States to Ecuador (Martínez et al., 1998; Pulido-Herrera et al., 2007; Barragán et al., 2014). This diurnal beetle uses cattle dung for feeding and nesting (Halffter and Edmonds, 1982) and buries manure from large herbivores directly under the dung pat through previously built galleries. Finally, *O. rhinolophus* is abundant in tropical forests and shaded plantations (Estrada et al., 1998; Favila, 2005; Halffter and Morrone, 2017). This species is a tunneller beetle that uses omnivore dung, decomposing fruit and carrion to feed and build its nests (Estrada et al., 1993; Sarges et al., 2012; Bourg et al., 2016).

## 2.2. Field work

*E. intermedius* was collected in the pastureland of Rancho San Román, Medellín, Veracruz, Mexico (18° 58' 19.37" N, 96° 04' 51.43" W) in July 2016. *O. incensus* beetles were collected in two pasturelands in Jilotepec, Veracruz, Mexico: Piedra de Agua (19° 36' 38.59" N, 96° 58' 08.10" W) and Linderos (19° 37' 44.77" N, 96° 58' 03.40" W), in June 2015. Finally, *O. rhinolophus* was collected in the rainforest of Los Tuxtlas Biological Station, Veracruz, Mexico (18° 35' 06" N, 95° 04' 30" W) in August 2016. After collection (Table 1), beetles were transported to the laboratory in groups of about 80 individuals, in 5 L ventilated plastic containers with 1.5 L of soil and 200 g of cow dung (for *O. incensus* and *E. intermedius*) or howler monkey dung (*Alouatta palliata* and *Ateles geoffroyi*) for *O. rhinolophus*.

Before the experiment, collected beetles from each species were separated in groups of 30 individuals (random mixed males and females), and placed in plastic terraria (21.5 cm diameter, 10 cm depth with 6 cm of sifted wet soil) for acclimation, thus assuring that all beetles reached sexual maturity and eliminating senescent individuals, which eventually died (Bonges, 1970). All terraria were maintained in an insectarium with the temperature regulated for each species (Table 1), 70% ± 10% RH, and a photoperiod of 12 L: 12 D. Mites that were found in beetles were removed by placing groups of ten beetles in a Petri dish with moistened paper and surrounded by water for 12–24 hrs, which led the mites to leave the beetles and drown (Favila, 1993).

## 2.3. Experimental protocol

The experiment was designed to evaluate the effect of a combination of a diet and an immune challenge (see below). This way, six treatments were formed, each comprising 20 experimental units of the combination of a diet treatment (rich, control, poor) and an immune challenge (implanted and not implanted; see below). Experimental units consisted in one or two couples placed in plastic containers (9 cm diameter, 14 cm depth, with 10 cm of sifted, sterile, wet soil) until the end of the experiment. See Table 1 for details.

Three different dung diets were used: protein rich, poor, or control diet, with sample sizes shown in Table 1. By varying protein content Cow dung used for this experiment (for *O. incensus* and *E. intermedius*) was collected from Piedra de Agua and Rancho San Román, respectively, where no herbicides are applied and where parasiticides are only used once a year (but had not been applied for more than a month before dung collection). Monkey dung (*Alouatta palliata* and *Ateles geoffroyi*, used for the experiments with *O. rhinolophus*) was obtained from Parque de la Flora y Fauna Silvestre Tropical Pipiapan (18° 26' 70" N, 95° 02' 65" W) and from Rancho los Deseos (19° 35' 02" N, 96° 55' 27" W), both located in Veracruz, Mexico.

To prepare the poor diet, homogeneous dung was filtered, considering that adult beetles only eat the smaller and more nutritious dung

particles leaving the large plant fragments present in dung (Holter et al., 2002). Filtered dung was heated in an oven during 60 min at 80 °C to reduce the availability, deaminate, and lose the essential character of some amino acids (Evans and Butts, 1949; Sang, 1956; Rafecas-Martínez et al., 2006). Then, filtered dung was mixed again with the amount of fiber needed to retrieve its original consistency and to recover its microbiota if bacteria were heat-killed (Estes et al., 2013). For the rich diet, casein (Golden-Bell) was added to the dung to increase half of its protein content, considering that approximately 15% of the cow dung dry mass is protein (Table 1; Moller et al., 2004). Casein has been widely used in experimental insect diets as a supplement because it is chemically well defined. Besides, casein has a complete composition and adequate balance of amino acids (Michelbacher, et al., 1932; Carson-Cohen, 2015). For the control diet, dung was not modified.

After an experimental feeding period ranging from 7 to 10 days in captivity (Table 1), beetles were assigned to an immune challenge treatment. The immune challenge consisted of a nylon implant (2 mm long, 0.18 mm width, previously rubbed with fine sandpaper and sterilized with ethanol 70%) fully yet gently inserted (using fine forceps) between the pronotum and elytra of beetles (see similar procedures in Rantala and Roff, 2007; González-Tokman et al., 2013). The implant acts as a standardized, non-pathogenic trigger of an energetically costly immune response of cellular encapsulation and deposition of melanin (Siva-Jothy et al., 1998). The response is similar to the one used against some metazoan parasites by a diversity of insects, such as several Coleoptera, including dung beetles (Pomfret and Knell, 2006b; Rantala and Roff, 2007; Schmid-Hempel, 2005; Steiger et al., 2011; Krams et al., 2015). Beetles from the control treatment remained unwounded. Notice that prior to the immune challenge, beetles were deprived of food for 12 h and kept at 5 °C during 5 min to anesthetize them. The same was done for the control treatment animals.

In order to quantify dung removal (see details on Table 1), homogeneous cow dung was offered to the beetles 12 h after the immune challenge. Eighteen containers (three for each treatment group, with the same characteristics but without beetles) were used as controls for desiccation. Dung was placed over a plastic mesh with a grid of 2 × 2 cm, and after 48 h and 96 h (second and fourth day of activity) dung masses were weighed (±0.01 g) to quantify dung removal. To calculate the dung mass removed by the beetles, the difference of the initial mass and the mass at the second or fourth day was subtracted to the average mass in the control containers for desiccation. If any beetle of an experimental unit died, data from such unit was discarded. Remaining dung beetles (Table 1) were sacrificed and stored in ethanol 70% to measure body condition. For *E. intermedius*, which reproduces well in the laboratory, we also quantified reproductive output as the number and dry mass of buried brood balls (which consist of a small piece of dung covered by soil, with a larva developing inside).

**Table 1**

Details on collection methods and experimental protocol for three studied dung beetle species. Sample sizes are ordered according to Fig. 1.

Species	Collection method	Acclimation period characteristics	Rich diet preparation (50% increase of protein)	Dung mass placed for removal quantification	Experimental units for feeding period
<i>Euoniticellus intermedius</i>	Direct search in semi-dry dung pads	5 days at 27 ± 1 °C with 200 g of ape dung every 3 days	1.8 kg of cow dung with 38.25 g of casein	30 g of cow dung	One couple (male–female) with 40 g of dung for 7 days. N = 10,10,13,15,12,15
<i>Onthophagus incensus</i>	Direct search in semi-dry dung pads and 6 pitfalls per locality for 24 h (30 cm depth × 21 cm, with 7 L of soil, covered by a plastic mesh with a grid of 2x2 cm and 1.5 kg of cow dung)	17 days at 22 ± 1 °C with 200 g of cow dung every 3 days	1.8 kg of cow dung with 27 g of casein	15 g of cow dung	2 males and 2 females with 40 g of dung for 9 days. N = 12,15,10,13,7,8
<i>Onthophagus rhinolophus</i>	80 traps (0.25 L) with 50 g of dung (50% human–50% pig), 3.0 cm of soil and a triangular opening (5x5x5 cm) for 24 h during 3 days	8 days at 27 ± 1 °C with 200 g of cow dung every 3 days	1 kg of ape dung with 26 g of casein	30 g of cow dung	One couple (male–female) with 20 g of dung for 10 days. N = 12,14,11,12,10,10

## 2.4. Measured variables

As an indicator of body size, pronotum width of each beetle was measured by triplicate with a digital caliper. For dung removal analyses, beetle's mean pronotum width was calculated for all individuals in each terrarium (experimental unit). Beetles were dried (48 h in an oven at 60 °C) and weighted ( $\pm 0.1$  mg) to obtain body dry mass. To estimate energetic condition, fat content was measured by submerging dry beetles in chloroform for 48 h. The difference between the total dry mass and the dry mass after chloroform extraction was considered fat content (Plaistow and Siva-Jothy, 1996; Lee et al., 2004).

## 2.5. Statistical analyses

The effects of diet and immune challenge on dung removal and the dry mass of buried brood balls were analyzed with linear models (LM). The initial models tested included diet type (rich, control, poor), nylon implant (presence or absence), their interaction (diet\*implant) and the additive effect of beetle's mean pronotum width. The same predictors were used to analyze the number of buried brood balls with a generalized linear model with Poisson error distribution. The Akaike Information Criterion (AIC) was used to select the most parsimonious model (Johnson and Omland, 2004; Crawley, 2007).

To test the effect of diet and immune challenge over individual energetic condition after dung removal, we used mixed effects models according to Zuur et al. (2009). The response variable was fat mass and the initial predictors were diet, implant, sex and all possible two- and three-way interactions. Pronotum width was included as a covariate to control for body size. The random effect was the grouping in terraria. Models accounted for heterogeneous variances found by sex and implant in *E. intermedius* and by diet in *O. incensus* (Fligner-Killeen tests  $P < 0.05$ ). Model selection was done based on AIC and P-values of selected predictor variables were obtained by means of likelihood ratio (LR) tests. The presence of outliers was tested but none was detected (Cook's distance  $< 1$ ). All analyses were carried out in R software version 3.5.3 (R Development Core Team, 2019).

## 3. Results

### 3.1. Dung removal

The amount of dung removed by *E. intermedius* beetles was highly reduced by the immune challenge two and four days after treatment (Table 2; Fig. 1). This effect was constant across diets, as observed by the non-significant interaction diet\*implant (Table 2; Fig. 1). Regarding diet, *E. intermedius* fed with the poor diet removed more dung than the ones fed with the control diet (Table 2; Fig. 1). This effect was only significant the second day, although a similar trend was observed the fourth day after treatment (Table 2; Fig. 1). Dung removal by *E. intermedius* was highly caused by reproductive behavior: although the number of nest masses by experimental couples did not vary according to diet or implant treatments, the weight of such masses was reduced in implanted couples (Table 2; Fig. 2). Dung removal by *O. incensus* and *O. rhinolophus* at the second and fourth days of activity were not dependent on protein content in the diet or immune challenge (Table 2). Unexpectedly, body size did not explain dung removal in all species (Table 2).

### 3.2. Body condition

For *E. intermedius*, the immune challenge led to almost complete energetic depletion across diets in both males and females, that mostly had ca. zero stored fat (Table 2; Fig. 3a). Energetic depletion was not that severe (although still important) in males fed control or rich-protein diets, as indicated by the significant interaction diet\*implant (Table 2). However, in non-implanted beetles, protein-poor diets led to the highest

**Table 2**

Statistical models to evaluate the effect of diet types and immune challenge on dung removal rates and energetic condition for *Euoniticellus intermedius*, *Onthophagus incensus*, and *O. rhinolophus* beetles. Variables with  $P < 0.05$  are marked in bold. Dotted lines represent variables that were not selected by AIC and are considered non-significant. NA = Not analyzed.

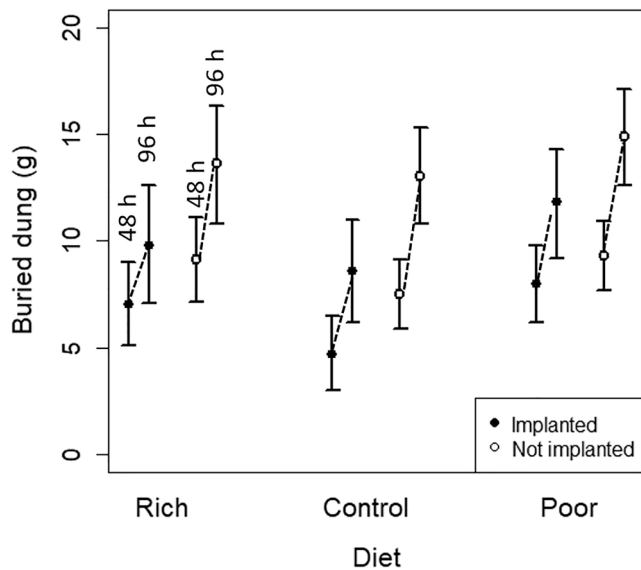
Variables and their interactions	<i>Euoniticellus intermedius</i>	<i>Onthophagus incensus</i>	<i>O. rhinolophus</i>
<b>DUNG REMOVAL AFTER 48 HOURS OF ACTIVITY</b>			
Diet	$F_{2,71} = 4.67$ , $P = 0.012$	–	–
Implant	$F_{1,71} = 8.19$ , $P = 0.005$	–	–
Diet*Implant	–	–	–
Mean pronotum width	–	–	–
<b>DUNG REMOVAL AFTER 96 HOURS OF ACTIVITY</b>			
Diet	$F_{2,71} = 2.45$ , $P = 0.093$	–	–
Implant	$F_{1,71} = 14.16$ , $P < 0.001$	–	–
Diet*Implant	–	–	–
Mean pronotum width	–	–	–
<b>NUMBER OF BROOD MASSES</b>			
Diet	Res.Dev. = 99.8, $P = 0.242$	NA	NA
Implant	Res.Dev. = 98.3, $P = 0.226$	NA	NA
Diet*Implant	Res.Dev. = 94.2, $P = 0.126$	NA	NA
Mean pronotum width	–	NA	NA
<b>DRY MASS OF BROOD MASSES</b>			
Diet	–	NA	NA
Implant	$F_{1,73} = 12.04$ , $P < 0.001$	NA	NA
Diet*Implant	–	NA	NA
Mean pronotum width	–	NA	NA
<b>FAT CONTENT</b>			
Diet	$LR = 18.86$ , $P = 0.004$	$LR = 40.52$ , $P < 0.001$	$LR = 14.88$ , $P = 0.061$
Implant	$LR = 52.89$ , $P < 0.001$	$LR = 3.52$ , $P = 0.060$	$LR = 18.29$ , $P = 0.006$
Sex	$LR = 11.78$ , $P = 0.008$	–	$LR = 11.28$ , $P = 0.080$
Pronotum width	–	$LR = 9.01$ , $P = 0.003$	$LR = 51.04$ , $P < 0.001$
Diet*Implant	$LR = 14.79$ , $P < 0.001$	–	$LR = 13.00$ , $p = 0.011$
Diet*Sex	$LR = 3.17$ , $P = 0.038$	–	$LR = 10.74$ , $P = 0.030$
Implant*Sex	–	–	$LR = 9.93$ , $P = 0.002$
Diet*Implant*Sex	–	–	$LR = 9.75$ , $P = 0.008$

fat loads, followed by control and protein-rich diets (Table 2; Fig. 3a). For *O. incensus*, on the contrary, fat content was the highest in the protein-rich, followed by control and protein-poor diets (Table 2; Fig. 3b). In this species, the immune challenge did not cause a reduction in fat load (Table 2; Fig. 3b). For *O. rhinolophus*, there was a negative effect of the immune challenge on male, but not female fat content. This effect was apparent in the rich and control diets, as revealed by the triple interaction diet\*implant\*sex (Table 2; Fig. 3c). Except for *E. intermedius*, fat content was higher in larger individuals (Table 2).

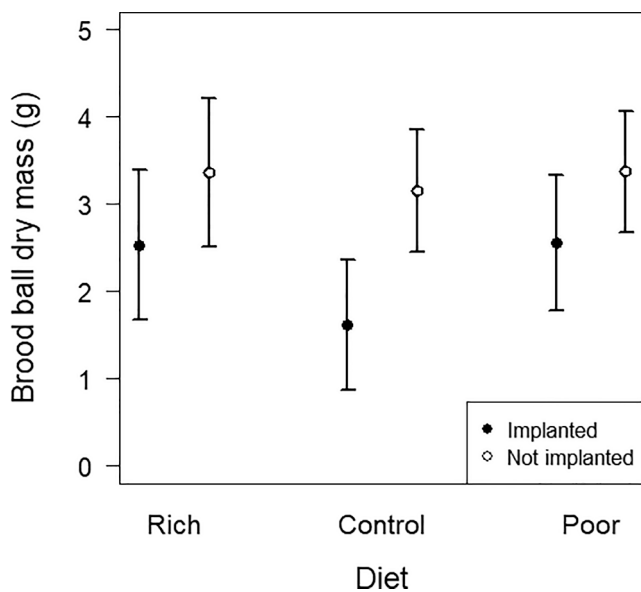
## 4. Discussion

Malnutrition and disease are main threats to organisms that provide essential ecosystem services (Nauig, 2009; Fürst et al., 2014; Vaudo et al.,





**Fig. 1.** Effect of diet (Rich, Control and Poor) and immune challenge (Implanted and Not implanted) on dung removal by couples of *Euoniticellus intermedius* dung beetles after two and four days of activity. Bars represent estimates  $\pm 95\%$  confidence intervals.

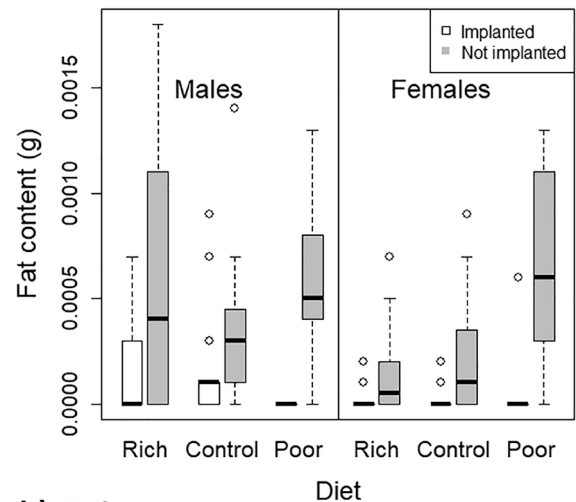


**Fig. 2.** Effect of dietary protein (Rich, Control and Poor) and immune challenge (Implanted and Non-implanted) on the mass of brood balls buried by *Euoniticellus intermedius* dung beetles. Bars represent estimates  $\pm 95\%$  confidence intervals.

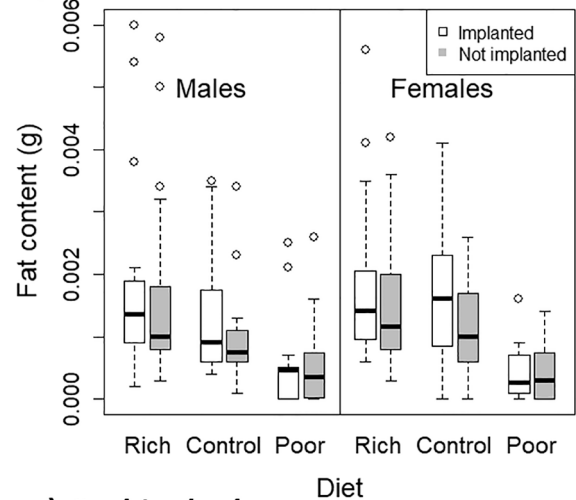
2015) and some species might be particularly sensitive to poor environmental conditions (Villada-Bedoya et al., 2019). Following this, we have shown that ecosystem services provided by dung beetles are affected by diet quality and parasite pressure. However, this result cannot be generalized for all species, as this prediction was corroborated only for *E. intermedius*, a highly invasive species which is found exclusively in livestock pastures (see below and Montes de Oca and Halfpeter, 1998).

As expected, immune-challenged *E. intermedius* removed less dung than non-implanted individuals, demonstrating that the reduction in physiological condition caused by the immune challenge reduces the strength of the provided ecosystem service. This reduction in dung removal by implanted beetles was related to reproductive activities, as

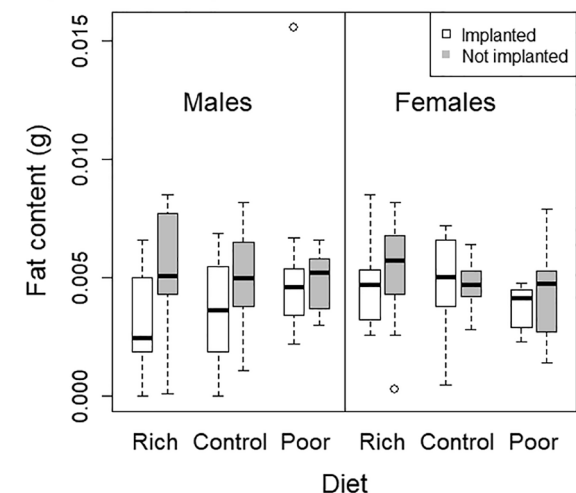
### a) *E. intermedius*



### b) *O. incensus*



### c) *O. rhinolophus*



**Fig. 3.** Effect of dietary protein (Rich, Control and Poor) and immune challenge (Implanted and Not implanted) on the energetic condition of three species of dung beetles after four days of dung removal. Bars represent estimates  $\pm 95\%$  confidence intervals.

observed by the decrease in the mass (but not the number) of buried brood balls. In this species, terminal investment in reproduction might be occurring, as immune-challenged beetles buried as many brood balls as non-implanted beetles despite suffering a severe energetic depletion (mainly females), as observed in other insects (González-Tokman et al., 2013). Despite maintaining high reproductive investment, immune-challenged beetles did not bury as much dung as healthy beetles. Our results seem thus opposite to what was previously found in the same species, that laid fewer eggs of similar size when facing a different immune challenge (lipopolysaccharides from grampositive bacteria), which would suggest a “cost of immunity” strategy (Reaney and Knell, 2010). Our contrasting findings might result from different used immune challenges, which may lead to different behavioral and physiological responses across insects (Moreno-García et al., 2013; Vale et al., 2018).

Besides reduced reproductive effort, reduced feeding could be contributing to observed low dung removal in implanted *E. intermedius*. Reduced feeding is expected for sick animals as an adaptive physiological strategy to avoid a trade-off between lipid digestion and immune response, as observed in some infected insects (e. g. crickets, lepidopterans, fruit flies) (Adamo et al., 2007, 2010; Simpson et al., 2018). This idea is supported by the observed decrease in lipid reserves that we also found in implanted *E. intermedius*, suggesting that this species might have incurred in an anorexic episode to recover. Whether terminal investment and illness-induced anorexia acted together to reduce the amount of buried dung still need to be tested.

We also predicted that diet quality would affect dung removal, but the effect of diet quality on dung removal was only evident in *E. intermedius*, as couples fed the protein-poor diet, where removed more dung than control fed couples. To overcome scenarios of low quality resources, insects may display compensatory feeding behavior, as observed in grasshoppers (*Schistocerca americana*), ground beetles (*Anchomenus dorsalis*) and mealworm beetles (*Tenebrio molitor*) (Bernays, 1998; Ponton et al., 2010; Jensen et al., 2012). By increasing dietary protein, dung beetles could increase the immune response (Verdú et al., 2013). Therefore, we suggest that dung beetles fed a protein-poor diet might have removed high amounts of dung to compensate the low protein content in food and survive for longer in the face of an immune challenge. On the other hand, dung removal by *O. incensus* and *O. rhinolophus* was not affected by the immune challenge or protein content in the diet. In these species, which hardly breed under our laboratory conditions (all authors' personal observation), dung removal revealed similar feeding rates, suggesting that protein restricted diets or immune challenge did not cause compensatory feeding or illness-induced anorexia.

An ecological comparison between the three studied species may help to explain the inter-specific differences in dung removal. On one hand, *E. intermedius* is an exotic, widespread species that rapidly invades new habitats, feeding on cattle dung, avoiding closed-canopy forests (Montes de Oca and Halffter, 1998), and tolerating some environmental pressures such as pollution (González-Tokman et al., 2017). On the other hand, both *Onthophagus* species are native at our study site and found in forested areas, where food sources are scarcer and patchier than in pasturelands (Dormont et al., 2004), but highly diverse, being composed of dung from different vertebrate species (Harvey et al., 2006; Bogoni et al., 2016). Therefore, *Onthophagus* beetles may have a wider availability of dung types and, likely, food quality than beetles that inhabit pasturelands such as *E. intermedius*, feeding exclusively on cattle dung (King et al., 2007). Moreover, dung beetles from forested sites may have physiological and behavioral adaptations (Hofmann and Todgham, 2010) that may grant them to maintain similar nutritional condition, despite there being variation in food availability. These adaptations may be a result of being selected in more complex environments compared to pasturelands (for a similar situation in other animals see Blaessing and Cruse, 2004; Coogan et al., 2018). In insects, such adaptations may result in a more efficient use of lipids, carbohydrates and proteins as

protection from future exposition to unknown agents (Lee et al., 2005) or seasons with resource scarcity (Aluja et al., 2011). The differences in species sensitivity to nutritional or parasite pressures highlight the importance of conserving diverse assemblages of species with physiological plasticity (Hofmann and Todgham, 2010) that guarantee natural functioning of forests and pastures despite environmental disturbances.

Energetic condition was affected in the three studied species by diet quality and immune challenge. Whereas *E. intermedius* stored more fat when fed the poor-protein diet, *O. incensus* stored less fat and *O. rhinolophus* did not suffer notable changes in energetic condition in response to dietary protein. Immune challenge also affected the physiological condition of the studied beetles, leading to severe energetic depletion in *E. intermedius* and *O. rhinolophus*, indicating that the immune challenge severely deteriorated physiological condition in these species, possibly because these beetles re-allocated resources to favor immune function (Ponton et al., 2010). On the other hand, there was a general increase in the condition of *O. incensus* implanted beetles compared to non-implanted individuals. In this regard, it is important to consider that this is a eurytopic species, in terms of inhabiting closed and open vegetation sites (Escobar and Chacón de Ulloa, 2000). Eurytopic species are tolerant to a wide range of environmental conditions, facing high pathogen loads, obtaining food from more diverse sources and thriving under anthropogenic habitat disturbances caused mainly by human activity (Davis et al., 2004; Martin et al., 2012). However, this species showed reduced energetic condition with reduced dietary protein, indicating that *O. incensus* is tolerant to parasitic pressures but sensitive to diet quality.

The present study was carried-out with field-caught beetles, which were inevitably exposed to a number of environmental factors that we did not control during development, potentially including malnutrition and parasitism. Despite not controlling developmental conditions, our experiment considered an acclimation period where all individuals received the same diet and control of ectoparasites, and where senescent individuals were discarded. Moreover, by experimenting with field-caught beetles, we guaranteed the use of a realistic sample with natural variation in terms of sensitivity to disturbance, which could not be obtained with laboratory-reared insects (Sikes and Paul, 2013). Although using wild animals increase the potential sources of variation in our experiments, we detected clear effects of diet and parasite pressure on beetle physiology and function that varied across species. Further similar research, with individuals reared in the laboratory under controlled conditions, is suggested to evaluate the impacts of developmental conditions or maternal effects defining dung beetle health and function (Baena-Díaz et al., 2018).

Finally, our paper illustrates the need to understand how ecosystem services can be shaped at individual level in wild animals. Ecosystem services are usually estimated from biomass, abundance and/or diversity of key species (Larsen et al., 2005; Mace et al., 2012; Nervo et al., 2014). However, the situation may be more complex than this, as individual condition and behavior define the effectiveness of the service provided. Physiological parameters are gaining attention as indicator tools that reflect individual response towards environmental quality (e. g. Rimbach et al., 2013; Deikumah et al., 2015; Salomão et al., 2018), presenting faster and clearer responses than other approaches, as community structure (Cooke et al., 2013). Thus, our results support the idea that physiological parameters of key insect species should be considered as indicators of ecosystem functioning and service maintenance (Cooke et al., 2013; Villada-Bedoya et al., 2019). The contrasting results found across our studied species show that each dung beetle responds differently to environmental pressures, likely depending on its biology, feeding and reproductive strategies. At the individual level, it would be interesting to scale how an entire dung beetle community responds when facing a number of stressors, to assess the resulting service provided.

## 5. Conclusions

We have shown that dung beetles, which are important indicators of environmental quality, suffer important changes in dung removal rate in response to parasitism and malnutrition. However, the three studied species responded differently, revealing the importance of conserving physiologically diverse species to maintain ecosystem services in stressful environments. Thus, our approach provides a cornerstone for further studies as it reveals that ecosystem services start from an individual level.

## 6. Data availability statement

Data will be uploaded in a public repository upon article acceptance.

## CRediT authorship contribution statement

**Mariana Servín-Pastor:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Renato Portela Salomão:** Methodology, Writing - original draft, Writing - review & editing. **Francisco Caselín-Cuevas:** Methodology, Writing - review & editing. **Alex Córdoba-Aguilar:** Conceptualization, Funding acquisition, Writing - review & editing. **Mario E. Favila:** Funding acquisition, Writing - review & editing. **Alberto Jácome-Hernández:** Methodology, Writing - review & editing. **Daniel Lozano-Sánchez:** Methodology, Writing - review & editing. **Daniel González-Tokman:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Writing - original draft, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

Mariana Servín-Pastor is a masters student from Programa de Maestría en Ciencias Biológicas, Universidad Nacional Autónoma de México (UNAM) and received the fellowship 417302 from Consejo Nacional de Ciencia y Tecnología (CONACYT). The project was funded by project CONACYT (Ciencia Básica 257894, granted to DGT). The authors declare that no competing interests exist.

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