Mechanisms of host exploitation by a microsporidian parasite

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Abstract

Parasites exploit their hosts to enhance their growth and reproduction, yet the mechanisms underlying host manipulation remain understudied for many taxa. The microsporidian *Vavraia culicis*, a potential biological control agent for mosquitoes, serves as an excellent model to explore such mechanisms. In this study, we investigate how infection by *V. culicis* lines that vary in virulence alters resource dynamics within the mosquito host *Anopheles gambiae*. Using metallomics and quantification of protein, carbohydrate, and lipid content, we show that infection alters host resource concentrations in ways that depend on parasite virulence. More virulent parasites led to increased protein levels and greater energy demands, evidenced by higher carbohydrate reserves. Additionally, infection with *V. culicis* impacted host metal content, particularly zinc and manganese, used by *V. culicis* independently of its evolutionary background. Iron availability, a key nutrient for parasite growth, enhanced spore production, with selected parasite lines better able to exploit host iron than unselected. These findings provide insight into the mechanisms by which *V. culicis* manipulates host resources, shedding light on the role of host exploitation in parasite virulence and the potential use of microsporidia as biological control agents in vector biology.

Keywords: microsporidia, virulence, iron, *Anopheles gambiae*, resource allocation, metallomics

1. Introduction

The infection of hosts and their consequent exploitation by parasites is a ubiquitous feature of life [1]. The negative impact a parasite has on its host, particularly on host survival, is commonly referred to as virulence [2]. Virulence arises from the interplay between host and parasite traits [3]. Hosts can influence the cost of infection by limiting parasite load or mitigating infection-induced damage - mechanisms known as resistance and tolerance, respectively [4–6]. Parasites, in turn, may exploit host resources to fuel their own growth (host exploitation or proliferation), or invest in toxins and other virulence factors that cause additional harm independent of parasite load (per-parasite pathogenicity or benevolence)[3,7,8].

Since parasites typically sequester resources from their hosts, many hosts have evolved strategies to restrict resource acquisition by parasites through both metabolic and behavioural changes [9]. The mechanisms limiting exploitation have been heavily studied in recent years and have given rise to nutritional immunity [10–13], where the main aim is to enhance host survival by manipulating the parasite's ability to exploit the host. The specific resources targeted by parasites vary depending on the stage of infection (time) and the host tissues involved (space). However, most parasites universally require a ubiquitous set of resources. These range from dietary carbohydrates to essential minerals, which are often hijacked by dedicated parasite transporters. Among these, iron is perhaps the most intensively studied [14–17]. Its critical role in microbial metabolism and structural function is underscored by the widespread evolution of siderophores - iron-chelating molecules used by microbes to extract iron from hosts [18–20]. Iron also acts as a developmental cue in some parasites, such as *Plasmodium* [21–24]. For instance, *Plasmodium falciparum* requires high iron concentrations for gametocyte fusion and successful development within the mosquito vector.

Despite increasing insight into parasite resource demands and sequestration strategies, many gaps remain, especially for obligate intracellular parasites such as microsporidia. Microsporidia are single-celled fungi that infect a wide range of hosts, from humans to dipteran insects [25–30]. Despite their compact genomes [31–33], they exhibit broad ecological diversity, colonising both aquatic and terrestrial environments [34]. Although studied for over 150 years [27], microsporidia have recently gained renewed attention due to their ability to suppress *Plasmodium* infection in *Anopheles* mosquitoes during co-infection [35–39]. Two species, *Vavraia culicis* and *Microsporidia MB*, have emerged as potential malaria control agents [35,40,41], supported by experimental [39,42] and field studies [37], respectively. However, the mechanisms by which these parasites exploit their hosts remain poorly understood. Considering *V. culicis* seems to elicit a limited host response, regardless of the vector host [43,44], here we turned focus to resource competition as a potential method of *Plasmodium*-inhibition. To date, only one study has partially characterised *V. culicis* exploitation of *Anopheles* larvae [45], and another has described limited effects on adult mosquito protein, carbohydrate and lipid content [46].

Understanding how microsporidia exploit their hosts is not only of interest for host-parasite interactions but also for vector biology, which entails the development of vector control strategies. In light of the long developmental time *Plasmodium* requires within its vector host, any host capable of transmitting *Plasmodium* will inherently favour parasites that also persist longer. Long-lasting parasites, such as some *V. culicis*, may thus evolve enhanced resource sequestration abilities, potentially outcompeting or excluding co-infecting parasites such as *Plasmodium spp*. Indeed, a recent study by Silva and Koella (2024) subjected *V. culicis* to artificial selection for either: i) early transmission and less time within the host, hereby referred to as *Early* treatment (i.e., parasites that killed the host under 7 days); or ii) late transmission and more time within the host, hereafter *Late* treatment (i.e., parasites that killed the host after 20 days) **(Fig. 1)** [44,47,48]. The authors found that lines selected for late transmission, and consequently persistence within the host, were more virulent, developed faster, and produced higher spore loads throughout their infective cycle (and the mosquito's life) than those in *Early* or the unselected stock population (referred to here as *Stock* treatment). However, this came at a cost: *Late* parasites were less viable outside the host, highlighting a trade-off between adaptation within and among hosts, in the outside environment [48].

In this study, we took advantage of these selected lines of *V. culicis* to: a) characterise *A. gambiae* exploitation by *V. culicis*; b) understand which mechanisms can be enhanced upon selection for early or late transmission (or less and more time within the host, respectively); and c) how changes in host exploitation relate to the different parasite lines' virulence. To address these questions, we: i) quantified protein, carbohydrate, and lipid content; ii) performed metallomics; and iii) manipulated iron availability - known to influence *V. culicis* growth - by supplementing or depleting circulating iron in the adult mosquito. All measurements were taken on days 5, 10, and 15 of adulthood, following larval-stage infection with the selected (i.e., *Early* and *Late*) and unselected (i.e., *Stock*) *V. culicis* treatments **(Table 1)**. These time points correspond to three different phases of *V. culicis* growth: beginning, highest growth, and slowdown of the growth. We believe our findings will offer important insights into microsporidian biology, parasite evolution, and disease control, as the molecular machinery studied here is likely conserved or used by other parasites.

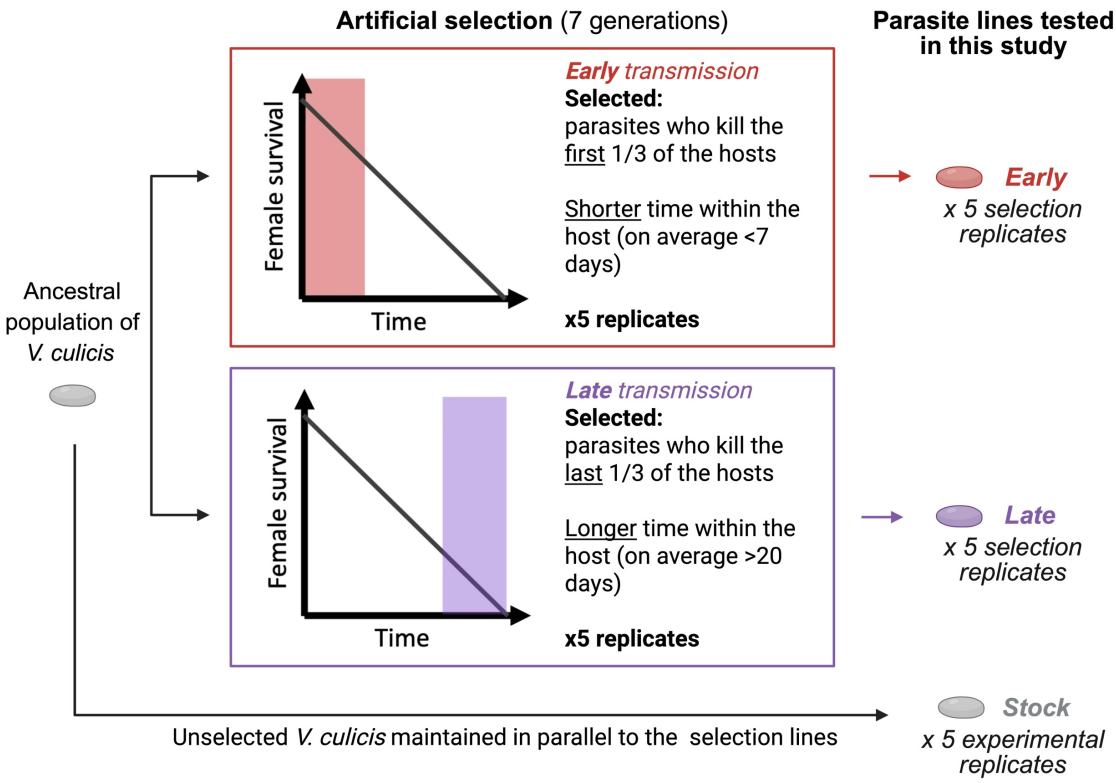


Figure 1. Illustration of the artificial selection experimental setup. An ancestral population of *V. culicis* was used to infect a Kisumu colony of the mosquito host *A. gambiae*. Two selection treatments were generated: i) *Early*, encompassing the parasites that were retrieved from the first third of the mosquitoes that died in the population; and ii) Late, including the parasites that were collected from the last third of the mosquitoes to die. The parasite went through seven serial passages before conducting the experiments in this study. Each selection treatment was replicated five times. As a baseline reference, the unselected parasite kept in a similar rearing parallel to the selection lines was also included in this study (i.e., *Stock*). To facilitate statistical comparisons, the *Stock* was also replicated five times. Further details can be found in Section 2.1 of the Materials and Methods and in the original study describing the selection lines, Silva and Koella (2024), bioRxiv.

Table 1. Experiments conducted and respective replication. Experiments performed in this study (Figures 2 to 4), their treatments, and the number of replicates. The factor(s) of interest are the level at which conclusions are drawn. The scale at which the factor is applied represents the level at which treatments or factors are implemented, whereas the number of replicates at the appropriate scale shows the count of independent replicates for each treatment or factor level. Parasite treatment includes selected treatments, i.e., *Early* and *Late*, and the unselected *Stock*. Time consists of three time-points during mosquito adulthood, 5-, 10-, and 15-day post-adult emergence. Diet encompasses a standard sugar diet, supplemented with iron or chelated with BPS. The replication statement was adapted from

135 the guidelines provided by the journal Functional Ecology (Wiley-Blackwell and British Ecological
136 Society).

Dependent variable(s)	Factor(s) of interest	Scale at which the factor is applied	Number of replicates at the appropriate scale
Figure 2. Protein, carbohydrate and lipid content throughout infection.			
Protein, carbohydrate and lipid content	Parasite treatment and time	Individual mosquito	10 mosquitoes per replicate (5) per time-point (3)
Figure 3. Metallomic profiles throughout infection.			
Fe, Zn, Cu, Ca and Mn content	Parasite treatment and time	Pool of 10 mosquitoes	1 pool per replicate (5) per time-point (3)
Figure 4. Effect of iron-supplementation and -chelation on parasite growth.			
Spore load	Parasite treatment and diet	Individual mosquito	10 mosquitoes per replicate (5) per time-point (3) per diet (3)

137

138 ## 2. Materials and Methods

139 *(a) Experimental model and mosquito rearing*

140 We used the Kisumu strain of the mosquito host *Anopheles gambiae* (s.s.) and differently selected and
141 unselected lines of the microsporidian parasite *Vavraia culicis floridensis* **(Fig. 1)**. To generate the
142 selected lines, we used an ancestral population of *V. culicis* to infect naive *A. gambiae* mosquitoes.
143 From the initial generation (F0), parasites were collected from the first one-third of mosquitoes to die
144 (forming the *Early* treatment) and from the final one-third to die (forming the *Late* treatment). From F1
145 onwards, these parasite lines were maintained under the same selection protocol - *Early* and *Late* - but
146 evolving independently. This selection process was repeated across seven generations for each of five
147 replicate lines per parasite treatment, allowing only the parasite to evolve, not the host [47]. After seven
148 generations, the selected parasite lines and an unselected reference line (*Stock*), maintained in parallel
149 to the artificial selection experiment, were used in the described experiments. Further details on the
150 selection experiment and its maintenance are described in Silva and Koella (2024). Experiments and
151 rearing were conducted at standard laboratory conditions, 26 ± 1ºC, 70 ± 5% relative humidity, and 12
152 h light/dark.

153 *(b) Experimental setup*

154 All experiments were performed similarly **(Table 1)**. In each experiment, freshly hatched *A. gambiae*
155 larvae were grown in groups of 50 per Petri dish (120 mm diameter x 17 mm) containing 80 ml of
156 distilled water. Five petri dishes were prepared for each parasite treatment and replicate. Larvae in these
157 dishes were fed daily with Tetramin Baby fish food according to their age: 0.04, 0.06, 0.08, 0.16, 0.32
158 and 0.6 mg/larva for 0, 1, 2, 3, 4 and 5 day-old or older larvae, respectively. Two-day-old larvae were
159 exposed to 10,000 spores per larva of their respective *V. culicis* treatment. Pupae were then transferred
160 to cages (21 x 21 x 21 cm) and left to emerge as adults. Each cage included individuals from all petri
161 dishes within a given parasite treatment and replicate. In each experiment, alive mosquitoes were
162 retrieved for the different assays. The collection was random within the same parasite treatment and
163 replicate for all traits measured. For the entirety of their adult life, the mosquitoes were provided with
164 a 6% sucrose solution up until trait measurement. Males were removed as soon as they emerged to
165 avoid mating-associated costs.

166 *(c) Proteins, carbohydrates and lipids quantification*

167 Mosquitoes were collected 5, 10, or 15 days after emergence and frozen at -80 ºC. We quantified their
168 proteins, carbohydrates, and lipid content through colourimetry, which we adapted from [49]. Protein,
169 carbohydrates, and lipid concentrations were estimated from the same sample for each of the parasite
170 treatments and respective replicates. Each individual body was weighed and then submerged in a 100
171 µL PBS pH 7.4 solution before homogenisation. To each sample, we added two stainless beads (Ø 2
172 mm) and homogenized the sample using a Qiagen TissueLyser LT at a frequency of 30 Hz for three
173 minutes. Then, the protein, carbohydrate, and lipid content were assessed for each mosquito as
174 described below. It is noteworthy that although we aimed to extract as much of the resource content
175 from the mosquito, some still hold onto the fibrous tissue. Hence, our measurements concern content
176 that is rather available and circulating, rather than structural.

177 Proteins were quantified by centrifuging the samples at 1100 RPM at 4º C for four minutes, adding 200
178 µL Bradford reagent to each solution, incubating at room temperature with soft agitation for 20 min,
179 and reading the absorbance at 595 nm with a SpectraMax i3x plate reader. To quantify protein content,
180 a standard curve of seven concentrations ranging from 400 µg/ml to 10 µg/ml of albumin in extraction
181 buffer (100 mM monopotassium phosphate, 1 mM DTT, and 1 mM EDTA in Milli-Q water) was also
182 prepared.

183 Carbohydrates were measured by first adding 20 µL of sodium sulphate at 20% to the homogenate,
184 adding 5 µL of the extraction buffer and 1500 µL Chloroform-Methanol (1:2, v:v), vortexing, and
185 centrifuging at 1100 RPM, 4ºC for 15 minutes. The supernatant was then removed, of which 300 µL
186 was transferred to a 96-well borosilicate plate and left to evaporate under the hood for approximately
187 30 minutes at room temperature. 240 µL of anthrone reagent was added to each sample and incubated

188 at room temperature for 15 minutes. The plate was sealed and incubated in a water bath at 90ºC for 15
189 minutes before measuring absorbance at 625 nm. A standard curve of seven concentrations ranging
190 from 40 µg/ml to 1 µg/ml of glucose in Chloroform-Methanol (1:2, v:v) was also prepared to quantify
191 carbohydrate content.

192 Lipids were measured by adding 200 µL of the supernatant to a 96-well borosilicate plate, then heated
193 to 90 ºC for 30 minutes. 10 µL of 98% sulfuric acid was added, and the sample was sealed and incubated
194 for two minutes in a water bath at 90 ºC. The samples were transferred to ice for three minutes before
195 adding 190 µL of vanillin reagent. Samples were sealed and incubated at room temperature for 15
196 minutes with gentle agitation. Their absorbance was measured at 525 nm. A standard curve of seven
197 concentrations ranging from 200 µg/ml to 5 µg/ml of triolein in Chloroform-Methanol (1:2, v:v) was
198 also prepared.

199 A blank sample was also included in all the standard curves to control for contamination. To acquire
200 individual resource content, the amount of resources measured was divided by the weight of the
201 mosquito. Details regarding the experiment scale and replication can be found in **Table 1**.

202 *(d) Metals quantification*

203 Five, 10 and 15 days after emergence, 30 alive females per parasite line and replicate were snap-frozen
204 in a 2 ml microcentrifuge tube at -80ºC. The samples were freeze-dried and submitted to a
205 mineralisation procedure using a Titan MPS microwave (Perkin Elmer, Waltham, MA). Each sample
206 was placed into a PTFE digestion vessel, and 2.5 mL HNO3 (Rotipuran Supra 69%, ROTH, Germany)
207 and 2.5 mL H2O2 (analytical reagent grade, Fisher Scientific, UK) were added. The vessels were heated
208 following a two-step digestion program consisting of a first step of 25 min until reaching 175°C and a
209 second step of 10 min at 175°C. After cooling, the digested sample solutions were transferred to 15 mL
210 Falcon PP tubes and stored in the fridge. A blank solution was prepared using the same procedure
211 without the sample. The elements iron, zinc, copper, calcium and manganese were simultaneously
212 analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) [24]. An Avio
213 550 max equipped with a dual view and an S23 autosampler (PerkinElmer, Waltham, MA) was used.
214 The instrument was operated in axial view with the following conditions: RF power 1450 W, cyclonic
215 spray chamber with nebuliser nitrogen gas flow rate 0.7 mL/min, plasma argon gas flow rate 8 mL/min
216 and auxiliary compressed air gas flow rate 0.2 mL/min. The sample was injected into the plasma at 1.30
217 mL/min. All the calibration curves were prepared in 2% HNO3 MilliQ water (w/w) in the concentration
218 range of 0.1-10 mg/L and using SCP Science multi-element standards (SCP Science, France). Yttrium
219 (Atomic spectroscopy standard, PerkinElmer Pure, 2% HNO3, 1000 ppm) was used as the internal
220 standard and was added at a concentration of 1 mg/L in the blank standards and samples. The elemental
221 wavelengths were 238.204 nm for Fe, 206.200 nm for Zn, 327.393 nm for Cu, 317.933 nm for Ca,

222 257.610 nm for Mn and 371.029 nm for Y. Measurements were performed in triplicates. Details
223 regarding the experiment scale and replication can be found in **Table 1**.

224 *(e) Iron metabolism assay*

225 In a separate experiment, after rearing larvae and pupae infected with their respective parasite treatment,
226 all the pupae of a given combinatorial treatment (parasite treatment x replicate) were allowed to emerge
227 within a cage. The emerging adults were then randomly split into three diet treatments, starting the
228 experiment. Mosquitoes were fed with daily prepared cotton pads with either: i) iron (1 mM $FeCl_3$ in
229 6% sucrose solution), ii) chelator (200 µM Bathophenanthroline disulfonic acid disodium salt (BPS) in
230 6% sucrose solution) or iii) or sugar (6% sucrose solution) for five days after emergence. From day six
231 onwards, the mosquitoes were provided with cotton pads with only 6% sucrose solution. Alive
232 individual mosquitoes were collected for spore load quantification 5, 10, or 15 days after emergence.
233 They were placed individually into 2 ml microcentrifuge tubes with 1 ml of distilled water and a
234 stainless-steel bead (Ø 5 mm, and homogenised with a Qiagen TissueLyser LT at a frequency of 30 Hz
235 for one minute. The number of spores was counted in a haemocytometer under a phase-contrast
236 microscope (400x magnification). Details regarding the experiment scale and replication can be found
237 in **Table 1**.

238 *(f) Statistical analysis*

239 All the analyses were performed with R version 4.3.1. Statistical analysis and their diagnosis were
240 conducted using: "DHARMa" [50], "car" [51], "lme4" [52], "muhaz" [53], "emmeans" [54] and
241 "multcomp" [55] .

242 First, we tested if the parasite treatment or the day of collection affected the mosquito's body weight.
243 For this, we used a linear mixed model with log-transformed weight as a dependent variable and parasite
244 treatment, time of collection and their interaction as explanatory variables and replicate as a random
245 factor. Body weight was not affected by any of the factors (Parasite treatment: $\chi^2 = 1.38$, $df = 2$, $p =$
246 501; Time: $\chi^2 = 0.80$, $df = 1$, $p = 0.373$; Parasite treatment * Time: $\chi^2 = 1.04$, $df = 2$, $p = 0.60$).

247 Differences in resource content were assessed using a linear mixed model for each of the three resources
248 (proteins, carbohydrates, and lipids), with parasite treatment and time of collection as explanatory
249 variables and their interaction. Replicate was considered a random factor. Individual resource content
250 was quantified by measuring the sample's quantity and adjusting it according to the respective
251 individual weight. The square root of each resource content was calculated to fit the model best.

252 We ran a linear mixed model for each metal element to test for differences in the metallomes of
253 differently infected or uninfected mosquitoes across time. The dependent variable was the log-

transformed concentration of the element obtained by ICP-OES, while the parasite treatment and assay day were explanatory variables and their interaction. Replicate was included as a random factor.

We assessed the effect of the parasite treatment and diet on spore rate and growth for each time point assayed. For each spore rate comparison, we ran a generalised linear mixed model with a binomial error structure, where parasite treatment and diet treatment were set as explanatory variables, their interaction, and the presence or absence of spores as a response variable. Replicate was included as a random factor. Concerning spore growth analyses, we only considered individuals with at least one spore. We then ran a linear mixed model with the same explanatory and random factors as the previous one.

3. Results

(a) Resource and energy use in mosquitoes infected with lines of V. culicis

The protein content was affected by parasite treatment ($\chi^2 = 9.75$, $df = 2$, $p = 0.008$), time ($\chi^2 = 45.28$, $df = 1$, $p < 0.001$) and their interaction ($\chi^2 = 24.75$, $df = 2$, $p < 0.001$; Fig. 2a). In particular, protein content remained stable until day 10 of adulthood when it significantly increased for mosquitoes infected with late-selected spores (i.e., the most virulent ones) compared to the other parasite treatments ($\chi^2 = 9.75$, $df = 2$, $p = 0.008$).

The carbohydrate content increased with infection time ($\chi^2 = 139.65$, $df = 1$, $p < 0.001$; Fig. 2b). It was higher in mosquitoes infected by the most virulent parasites (i.e., late-selected ones) than in those infected by the unselected or the early-selected ones ($\chi^2 = 16.56$, $df = 2$, $p < 0.001$).

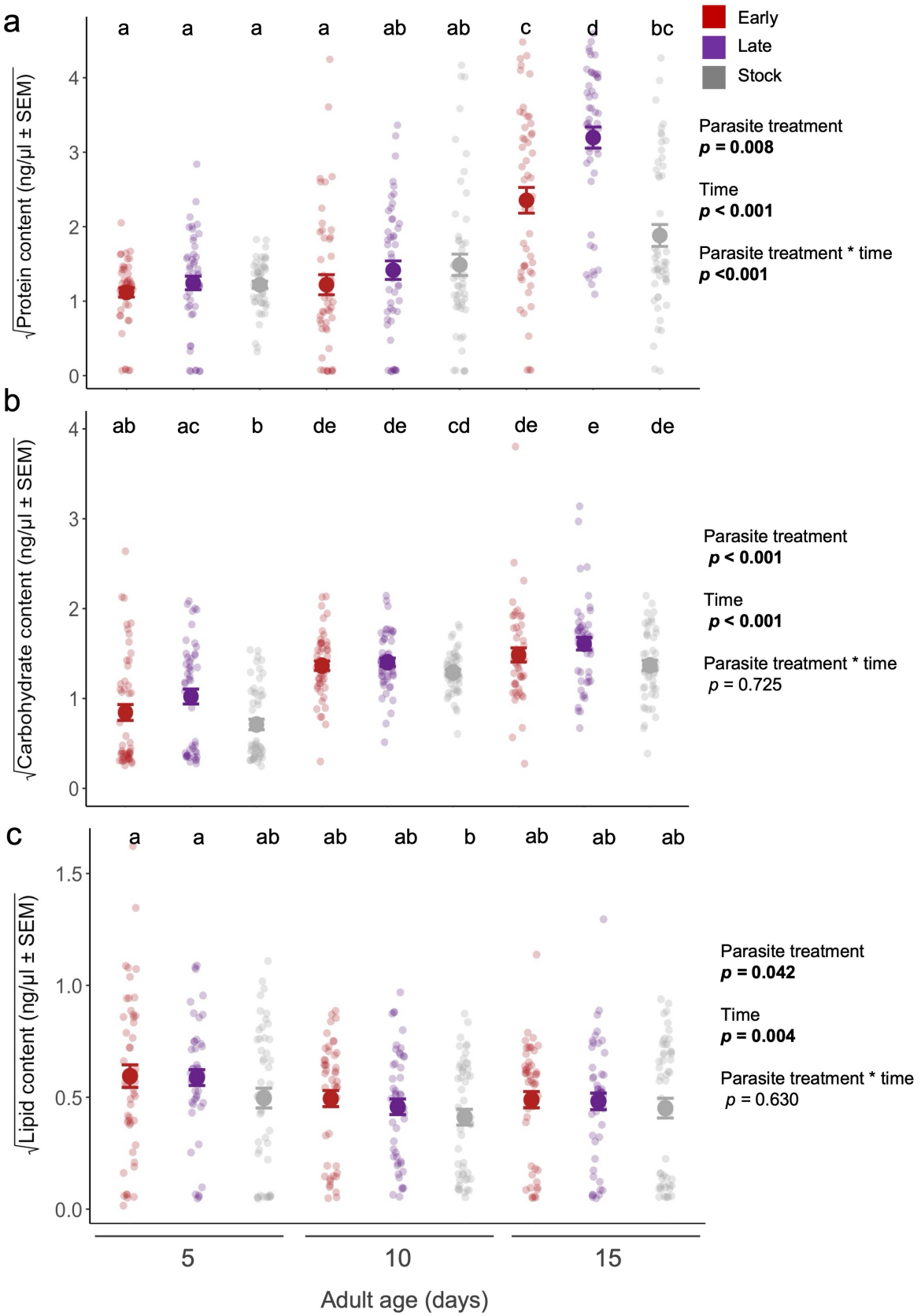
The lipid content decreased with infection time ($\chi^2 = 8.25$, $df = 1$, $p = 0.004$) irrespective of treatment, and the mosquitoes infected with selected spores (i.e., early or late) had higher lipid content than the ones infected with the unselected parasites ($\chi^2 = 6.33$, $df = 2$, $p = 0.042$; Fig. 2c).

(b) Metallomics of mosquitoes infected with lines of V. culicis

All metals (iron, copper, zinc, manganese) and calcium concentrations in the mosquito increased with time (Fe: $\chi^2 = 18.88$, $df = 2$, $p < 0.001$; Cu: $\chi^2 = 11.93$, $df = 2$, $p = 0.003$; Zn: $\chi^2 = 18.62$, $df = 2$, $p = <$ 0.001; Mn: ($\chi^2 = 17.91$, $df = 2$, $p < 0.001$; Ca: $\chi^2 = 34.08$, $df = 2$, $p < 0.001$). While iron, copper, and calcium concentrations were similar for parasite-exposed and unexposed mosquitoes and for all parasite lines (Fe: $\chi^2 = 2.26$, $df = 3$, $p = 0.521$; Cu: $\chi^2 = 0.66$, $df = 3$, $p = 0.88$; Ca: $\chi^2 = 3.19$, $df = 3$, $p = 0.36$), the zinc concentrations were lower in infected mosquitoes, irrespective of parasite line (Zn: $\chi^2 = 13.97$, $df = 3$, $p = 0.003$) and manganese concentration was higher ($\chi^2 = 32.30$, $df = 3$, $p < 0.001$) (**Fig. 3b, e**).

(c) Iron effect on parasite growth

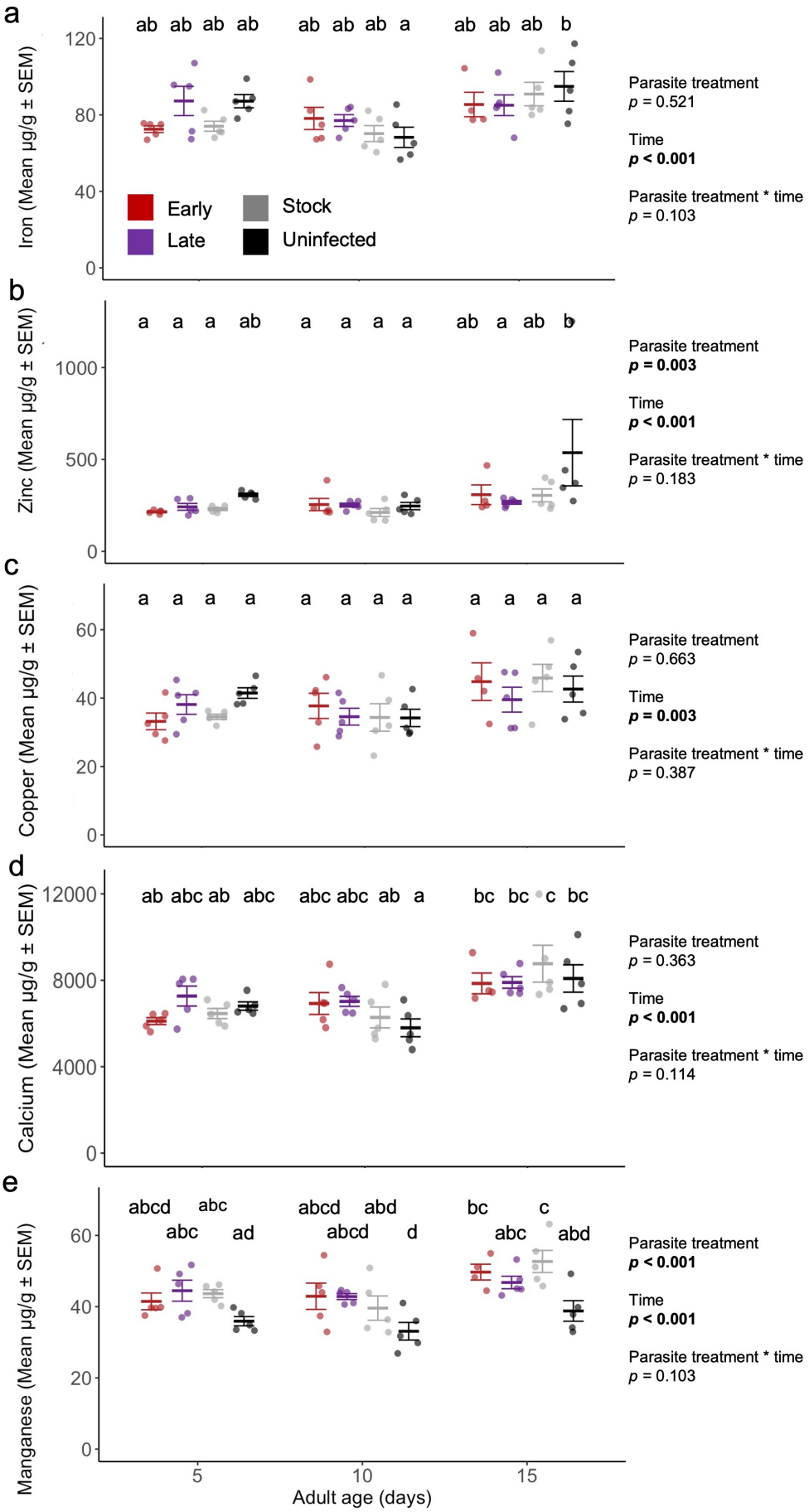
285 In the iron supplementation or chelation experiment, all mosquitoes harboured spores by day 15. At
286 days 5 and 10, the proportion of mosquitoes with spores was higher if the parasite had been late-selected
287 than if it had been early-selected or not selected (day 5: $\chi^2 = 15.43$, $df = 2$, $p < 0.001$; day 10: $\chi^2 = 8.46$,
288 $df = 2$, $p = 0.015$). On both days, iron supplementation increased while chelation decreased the spore
289 rate (day 5: $\chi^2 = 15.43$, $df = 2$, $p < 0.001$; day 10: $\chi^2 = 8.46$, $df = 2$, $p = 0.015$). A similar pattern was
290 observed for the spore load in individuals that harboured spores, with higher spore load after an iron-
291 supplemented diet and lower spore load after a chelator-supplemented diet (day 5: $\chi^2 = 12.45$, $df = 2$, p
292 $= 0.002$; day 10: $\chi^2 = 37.60$, $df = 2$, $p < 0.001$; day 15: $\chi^2 = 79.65$, $df = 2$, $p < 0.001$; **Fig. 4b**). Individuals
293 infected with late-transmitted spores also showed the highest number of spores regardless of the time
294 point or diet (day 5: $\chi^2 = 37.75$, $df = 2$, $p < 0.001$; day 10: $\chi^2 = 37.60$, $df = 2$, $p < 0.001$; day 15: $\chi^2 =$
295 26.73, $df = 2$, $p < 0.001$; **Fig. 4b**), except for time-point 15 days where infection by the unselected
296 parasite reached the same load as the late-transmitted spores for the iron-supplemented diet (interaction
297 between parasite and diet treatments: $\chi^2 = 18.60$, $df = 4$, $p < 0.001$; **Fig. 4b**).



298

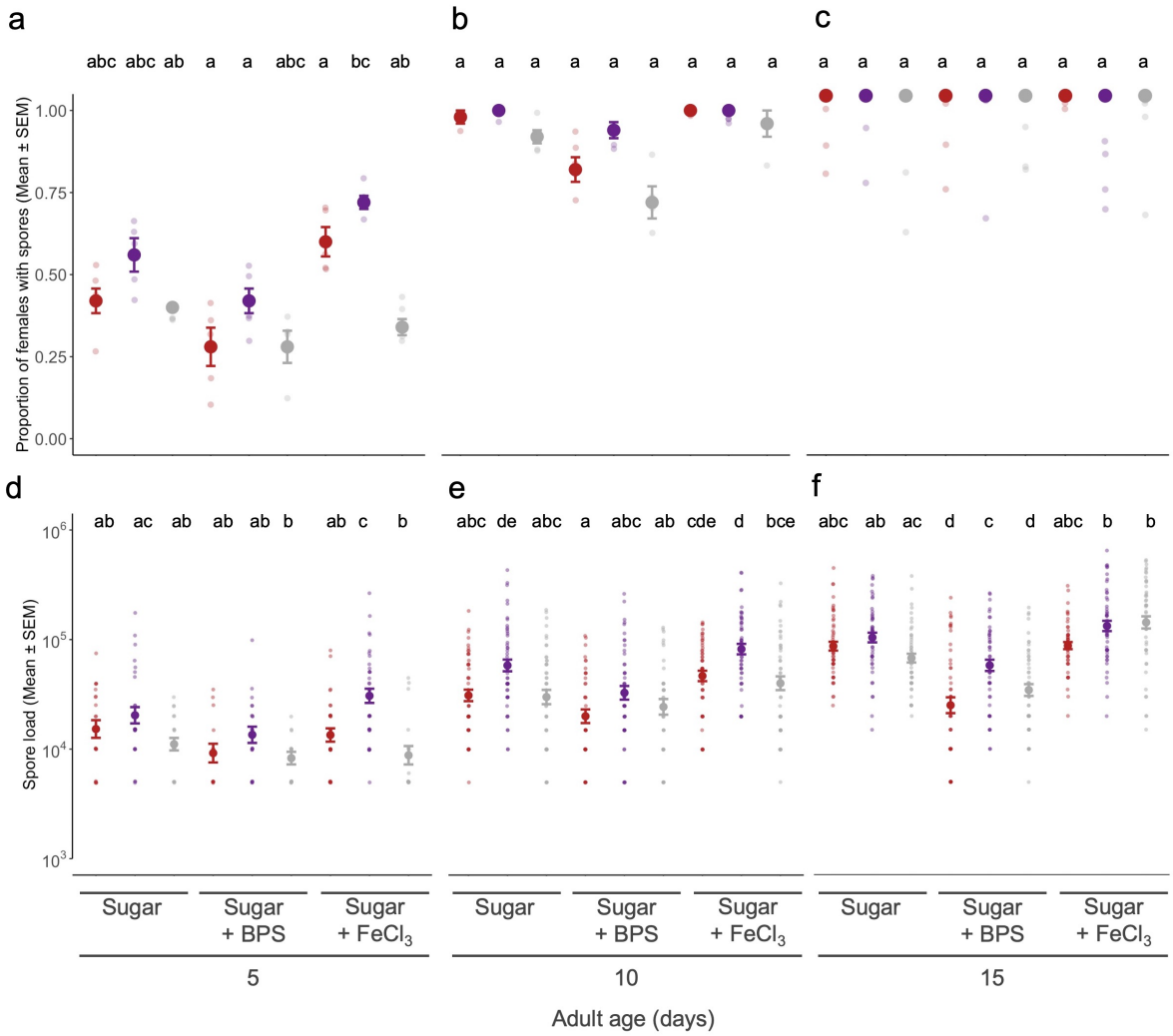
299 **Figure 2. Mean protein, carbohydrate and lipid content through infection.** Mean square root of
300 the **(a)** protein, **(b)** carbohydrate and **(c)** lipid content at day 5, 10 and 15 of adulthood upon infection

with early-, late-selected, or unselected stock parasite. Each time-point consists of individual resource content from 50 live females per parasite treatment. Statistical factors and respective *p*-values are shown on the right of each plot. Letters denote differences from multiple comparisons tests for an interaction between "parasite treatment" and "time". For statistical details, see SI Appendix Table S1.


a
Iron (Mean µg/g ± SEM)
ab ab ab ab ab ab ab a ab ab ab b
Early
Late
Stock
Uninfected
Parasite treatment
p = 0.521
Time
p < 0.001
Parasite treatment * time
p = 0.103
b
Zinc (Mean µg/g ± SEM)
a a a ab a a a a ab a ab b
Parasite treatment
p = 0.003
Time
p < 0.001
Parasite treatment * time
p = 0.183
c
Copper (Mean µg/g ± SEM)
a a a a a a a a a a a a
Parasite treatment
p = 0.663
Time
p = 0.003
Parasite treatment * time
p = 0.387
d
Calcium (Mean µg/g ± SEM)
ab abc ab abc abc abc ab a bc bc c bc
Parasite treatment
p = 0.363
Time
p < 0.001
Parasite treatment * time
p = 0.114
e
Manganese (Mean µg/g ± SEM)
abcd abc abc ad abcd abd abcd d bc c abc abd
Parasite treatment
p < 0.001
Time
p < 0.001
Parasite treatment * time
p = 0.103
Adult age (days)

305

306 **Figure 3. Metallomic profiles at adulthood.** Metal concentrations determined by ICP-OES in
307 uninfected *An. gambiae* and infected with one of three *V. culicis* parasite lines: early, late or unselected
308 stock. Iron **(a)**, zinc **(b)**, copper **(c)**, calcium **(d),** and manganese **(e)** concentrations were quantified
309 across adult age at days 5, 10 and 15 post emergences. For each time point, a pool of 30 females was
310 used to assess metal concentration for each selection treatment x replicate combination (i.e., $n = 5$ data
311 points per selection treatment). Statistical factors and respective *p*-values are shown on the right of each
312 plot. Letters denote differences from multiple comparisons tests for an interaction between "parasite
313 treatment" and "time". The mean and standard error of the mean were plotted. For further statistical
314 details, see SI Appendix Table S2.



315

316 **Figure 4. The effect of iron-supplementation and -chelation on parasite growth.** The impact of a
317 standard sugar diet (6% Sucrose), supplemented with an iron-chelator (6% Sucrose + 200 µl BPS) or
318 iron itself (6% Sucrose + $FeCl_3$ 1mM) on the presence of detectable spores (i.e., the proportion of
319 females with spores secreted) **(a-c)** and spore load **(d-f)** of differently selected, or unselected, *V. culicis*
320 treatments. Each selection treatment has a sample size of 50 females split by five experimental replicates

321 for each time point. Letters denote differences from multiple comparisons tests for an interaction
322 between "parasite treatment" and "diet" for each independent time point. For further statistical details,
323 see SI Appendix Table S3.

324

325 4. Discussion

326 Parasites are masters at host exploitation, sequestering, and manipulating host resources to their benefit.
327 In this study, we show how infection by the microsporidian parasite *V. culicis* alters host resource
328 concentrations, particularly metals and energy sources stored in the mosquito host *A. gambiae*. We
329 could also link virulence better to host exploitation by using parasite lines that differ in virulence - that
330 is, the degree of harm inflicted on the host. The findings discussed below reveal microsporidia-specific
331 mechanisms of host exploitation that may be inherently present or selectively enhanced under particular
332 conditions. In addition to characterizing these mechanisms, we consider and discuss how their
333 differential selection influences the potential role of microsporidia as biological control agents within
334 vector biology.

335 *(a) Parasite virulence drives host energy use during infection*

336 First, we observed that protein content in mosquitoes increased over time, particularly between days 10
337 and 15 of adulthood **(Fig. 2a)**. As most immune effectors and signalling pathways are protein-based,
338 this increase may indicate a host shift to parasite recognition and consequent immune response. If so,
339 our findings support the threshold hypothesis [56], which proposes that an immune response is triggered
340 only once parasite load surpasses a certain threshold. This would explain the delayed rise in proteins
341 and potential immune effectors, such as antimicrobial peptides.

342 Moreover, the magnitude of the increase varied with parasite virulence: infection with the most virulent
343 parasite line (i.e., *Late*) led to higher host protein levels by day 15 **(Fig. 2a)**. A similar pattern has been
344 observed in several other studies [7,57,58], and is likely linked to the much higher parasite burdens
345 reached by *Late* parasites, as shown in our previous work [47]. These results also highlight the mosquito
346 host's ability to detect and adjust life history investments in immunity or other fitness traits, such as
347 fecundity, based on the virulence of the infecting parasite. For instance, *Late* spores have been shown
348 to induce earlier reproduction in *A. gambiae* mosquitoes [47].

349 We also measured two fundamental energetic reserves in mosquitoes: carbohydrates and lipids.
350 Carbohydrates are typically more accessible and readily used, whereas lipids are a last resort or
351 emergency energy source. Over time, we found that lipid reserves declined slightly **(Fig. 2c)**, while
352 carbohydrate reserves increased for most parasite lines **(Fig. 2b)**. This pattern likely reflects our

353 experimental conditions, where adult mosquitoes had ad libitum access to sucrose (a carbohydrate) but
354 no lipid-based diet. Consequently, although both energy reserves are utilised during infection, as shown
355 previously for this microsporidian species [46], carbohydrate stores are replenished, whereas lipid
356 reserves are steadily depleted or sequestered. Interestingly, we do find a difference in energetic use
357 from a previous study that only focused on infection by the unselected line of *V. culicis* [46]. While in
358 this latter study, Zeferino and colleagues (2024) observed a shift in energy use later in mosquito life
359 from carbohydrates to lipids, here we observed an almost immediate use of lipids and a gradual increase
360 in carbohydrates, particularly in the selected parasite lines, *Late* and *Early*. Although perhaps
361 speculative, we can hypothesize that this difference might be driven by the selection process, which
362 selects for better-adapted parasites to *A. gambiae* and therefore, better host exploitation. To aggravate
363 this, parasite lines that better exploit the host, such as *Early* and particularly *Late*, and produce higher
364 numbers of infective spores, might have higher energy demands. Previous studies on this model system
365 have shown the ability of this parasite to change the host behaviour [59,60]. Hence, it might be
366 conceivable that either the host or the parasite is driving the mosquito to feed more and have its
367 carbohydrates replenished for the duration of the infection.

368 Therefore, parasite treatment influenced the dynamics of these energy sources **(Fig. 2bc)**.
369 Carbohydrates increased proportionally to parasite virulence, which can reflect the host's increased
370 energetic demand for immunity [61–63] and damage control [64], or the parasite's needs, as mentioned
371 above. Additionally, *V. culicis* draws upon the host's carbohydrate reserves for its own consumption
372 early in adulthood [46]. Thus, more virulent parasites, which elicit stronger immune responses and
373 produce more infective spores, impose greater energy demands on the host. The proportional increase
374 in carbohydrates with parasite virulence may therefore represent a behavioural adaptation by the host
375 to fuel investment in immunity.

376 Finally, parasite treatment also influenced baseline lipid reserves **(Fig. 2c)**, with mosquitoes infected
377 by selected parasites exhibiting higher lipid levels. The cause of this is unclear, but it may reflect greater
378 parasite growth in these treatments compared to the unselected *Stock* parasite, leading to more lipid
379 incorporation into spore production. It is also worth noting that our previous work showed that lipids
380 are heavily exploited by the parasite later in infection [46], after day 22 of adulthood - beyond the time
381 points measured here. Hence, lipid usage by the parasite may not yet have been strong enough to be
382 detectable during the sampled stages, and due to the considerable mortality in some of the treatments,
383 we could not measure later time points.

384 *(b) V. culicis infection alters host zinc and manganese levels independently of parasite selection*

385 We observed notable changes in host metal content during infection **(Fig. 3)** and its usage by the parasite
386 **(Fig. 4)**. Among the metals measured, only zinc **(Fig. 3b)** and manganese **(Fig. 3e)** were significantly

387 affected by infection. Infections with any of the parasite treatments led to a decrease in host zinc content.
388 This could reflect heavy utilization of zinc by the microsporidian parasite or represent a mechanism of
389 immune evasion [44]. Zinc is essential for parasite growth and its proper metabolism [10,33], and is
390 often linked to the production of virulence factors [65–67]. Furthermore, some fungal parasites can
391 manipulate host immunity by inducing localised zinc deficits that impair immune responses [11,67–
392 69]. However, our selection protocol had no detectable impact on zinc-related processes, as no
393 differences were observed between parasite treatments.

394 In contrast, manganese levels increased following infection with any of the parasite lines **(Fig. 3e)**. This
395 may indicate a parasite response to host immune defences, as many microsporidian antioxidant
396 enzymes, such as manganese superoxide dismutases, depend on manganese for their activity and are
397 particularly relevant for this taxon [70–72]. As with zinc, this increase in manganese was independent
398 of the parasite's selection history.

399 Across all treatments, we also noted a slight temporal increase in metal concentrations. Although the
400 underlying mechanisms remain unclear, metals may become more bioavailable as infection progresses,
401 potentially benefiting the host, the parasite, or both **(Fig. 3)**. From the parasite's perspective, enhanced
402 spore production over time would increase demand for metals sequestered from the host, making their
403 accumulation more detectable at later stages. Future studies will be essential to better understand metal
404 mobilization and dynamics during microsporidian infection and within the microsporidian cell.

405 *(c) Iron availability enhances parasite growth and spore production*

406 Given the crucial role of metals in infection dynamics, and particularly the known importance of iron
407 for parasite development, we next focused specifically on how iron availability affects *V. culicis* growth
408 within the *A. gambiae* host.

409 Iron plays a vital role as a nutrient for growth in many parasite species [16,17,21,24,39,73], and it is
410 particularly abundant in mosquitoes due to their hematophagous ecology [23,74]. In fungal parasites,
411 iron acquisition is a critical determinant of virulence [75,76]. Many fungi have evolved sophisticated
412 mechanisms to overcome host-imposed iron limitation, including the secretion of siderophores - small,
413 high-affinity iron-transporter compounds - or the expression of more specialized iron transporter
414 systems [18,77,78]. Although microsporidia lack many canonical metabolic pathways due to their
415 highly reduced genomes [31], recent genomic studies suggest they have retained or adapted specific
416 mechanisms for iron acquisition, including transporters likely involved in scavenging iron from the host
417 cytoplasm [79], which was not the focus of the previously conducted metallomics.

418 Hence, in our study, we experimentally manipulated host iron levels by supplementing or chelating
419 circulating iron in the mosquito haemolymph, consequently increasing and reducing its absorption by

420 host cells and the replicating parasitic cells inside them. We then assessed the growth of different *V.*
421 *culicis* treatments **(Fig. 4)**. Five-day iron supplementation and chelation led to an increase and decrease,
422 respectively, in the production of infective spores at days 5 and 10 **(Fig. 4ab)**, but not at day 15, by
423 which all mosquitoes harboured spores **(Fig. 4c)**. These effects were mirrored in parasite burden under
424 the two dietary treatments **(Fig. 4d-f)**.

425 Overall, selected parasites exhibited a greater capacity to utilise circulating iron within the mosquito
426 compared to the unselected *Stock*, resulting in accelerated production of infective spores. This result
427 was not evident in the results from the metallomics, likely due to the small fraction of iron diverted to
428 the parasite compared to the whole-body iron content that is not only circulating but also incorporated
429 into many host cell structures. Differences in iron usage likely reflect adaptations arising during the
430 selection experiment, favouring parasites that more efficiently sequester and use host iron. Enhanced
431 iron acquisition is a known evolutionary strategy among intracellular parasites to sustain rapid
432 proliferation under resource-limited conditions. However, the extent of these differences appeared
433 inversely related to parasite growth: *Stock* parasites, initially with lower burdens, showed a larger
434 relative increase in response to iron supplementation than selected parasites, which already maintained
435 high burdens without supplementation. This trend was evident at day 15 **(Fig. 4f)**, when the parasite
436 usually reaches its chronic persistent load, and suggests the lower increase in selected lines might be
437 due to either limiting costs in the assimilation of iron by the parasite or limited evolvability at this time
438 for the mechanism that is allowing a better iron sequestration by the parasite.

439 Interestingly, iron manipulation by fungal parasites is not only associated with growth but also with
440 evasion of host immune responses [16,75,76]. In particular, the ability to modulate host iron
441 homeostasis can impair the oxidative burst or other immune functions that are heavily dependent on
442 iron-regulated pathways [80]. Whether microsporidia, including *V. culicis*, directly manipulate
443 mosquito iron homeostasis or simply compete with the host for this resource remains an open question.
444 Nevertheless, our findings provide evidence that iron availability is a critical driver of microsporidian
445 growth within the host, and that virulence evolution in *V. culicis* may be partly linked to improved iron
446 sequestration strategies.

447 *(d) Conclusion and perspectives*

448 In conclusion, this study provides important insights into how *V. culicis* manipulates its host, *A.*
449 *gambiae*, to exploit host resources for its growth and reproduction. We have shown that the parasite
450 alters the dynamics of proteins, carbohydrates, lipids, and metals in a manner that is strongly influenced
451 by the virulence of the parasite. More virulent strains of *V. culicis* seem to induce stronger immune
452 responses in the host, resulting in higher protein levels and altered energy metabolism. Additionally,
453 the study highlights the critical role of metal homeostasis, particularly zinc, manganese, and iron, in

this host-parasite interaction, with iron availability being a key factor in parasite growth and spore production. These findings underscore the complex interplay between parasite, virulence, host resource allocation, and immune response.

Furthermore, the results suggest that the evolutionary adaptation of *V. culicis* to more efficiently exploit host resources, including iron, may play a significant role in driving within-host virulence and consequent host exploitation strategies. As the role of microsporidia as potential biological control agents in vector biology continues to be explored, these insights have implications for understanding the dynamics of parasitic disease and the potential for manipulating these interactions for disease control. Particularly concerning malaria, our findings suggest the inhibitory effect of microsporidian parasites on *Plasmodium spp.* might be due to competition for a common resource, rather than collateral immunity.

Author contributions

LMS conceived the overall idea, JCK and LMS designed the experiments, LMS collected and analysed the data, AV performed the ICP-OES elemental analysis, and LMS wrote the first draft of the manuscript. All authors contributed critically to the drafts.

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690 # Supplementary Information

691 **Table S1.** Protein, carbohydrate, and lipid content through infection.

692 **Table S2.** Metallomic profiles at adulthood.

693 **Table S3.** Iron-supplementation and -chelation on parasite growth.

694 **Supplementary Information**

695 "Mechanisms of host exploitation by a microsporidian parasite"

696 by Luis M. Silva, Armelle Vallat and Jacob C. Koella

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698 **Table S1.** Protein, carbohydrate and lipid content through infection.

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721 **Table S1. Protein, carbohydrate and lipid content through infection.** Each resource was analysed
722 independently. Differences in resource content were assessed using a linear mixed model for each of
723 the three resources (proteins, carbohydrates, and lipids), with parasite treatment and time of collection
724 as explanatory variables and their interaction. Replicate was considered a random factor. Individual
725 resource content was quantified by measuring the sample's quantity and adjusting it according to the
726 respective individual weight. The square root of each resource content was calculated to fit the model
727 best. Multiple comparisons tests were conducted to examine the interaction between "parasite
728 treatment" and "time" and show pairwise differences between all combinatorial treatments.

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Tested effect			
Model 1a - Protein content	**χ2**	***df***	***p***
Parasite treatment	9.752	2	**0.008**
Time	45.283	1	**< 0.001**
Parasite treatment: Time	24.748	2	**< 0.001**
Multiple comparisons for Model 1a (PT: T)	***df***	***t-ratio***	***p***
Early, day 5 - Late, day 5	437	-0.719	0.999
Early, day 5 - Stock, day 5	437	-0.584	0.999
Early, day 5 - Early, day 10	437	-0.583	0.999
Early, day 5 - Late, day 10	437	-1.692	0.752
Early, day 5 - Stock, day 10	437	-2.108	0.469
Early, day 5 - Early, day 15	437	-6.996	**< 0.001**
Early, day 5 - Late, day 15	437	-11.761	**< 0.001**
Early, day 5 - Stock, day 15	437	-4.326	**< 0.001**
Late, day 5 - Stock, day 5	437	0.135	1.000
Late, day 5 - Early, day 10	437	0.132	1.000
Late, day 5 - Late, day 10	437	-0.973	0.988
Late, day 5 - Stock, day 10	437	-1.386	0.903
Late, day 5 - Early, day 15	437	-6.277	**< 0.001**
Late, day 5 - Late, day 15	437	-11.042	**< 0.001**
Late, day 5 - Stock, day 15	437	-3.607	**0.010**
Stock, day 5 - Early, day 10	437	-0.002	1.000
Stock, day 5 - Late, day 10	437	-1.108	0.973
Stock, day 5 - Stock, day 10	437	-1.521	0.845
Stock, day 5 - Early, day 15	437	-6.412	**< 0.001**
Stock, day 5 - Late, day 15	437	-11.177	**< 0.001**
Stock, day 5 - Stock, day 15	437	-3.742	**0.006**
Early, day 10 - Late, day 10	437	-1.100	0.974
Early, day 10 - Stock, day 10	437	-1.512	0.850
Early, day 10 - Early, day 15	437	-6.377	**< 0.001**
Early, day 10 - Late, day 15	437	-11.118	**< 0.001**
Early, day 10 - Stock, day 15	437	-3.721	**0.007**
Late, day 10 - Stock, day 10	437	-0.408	1.000
Late, day 10 - Early, day 15	437	-5.304	**< 0.001**
Late, day 10 - Late, day 15	437	-10.069	**< 0.001**
Late, day 10 - Stock, day 15	437	-2.635	0.175
Stock, day 10 - Early, day 15	437	-4.022	**< 0.001**
Stock, day 10 - Late, day 15	437	-9.710	**< 0.001**
Stock, day 10 - Stock, day 15	437	-2.239	0.382
Early, day 15 - Late, day 15	437	-4.765	**< 0.001**
Early, day 15 - Stock, day 15	437	2.670	0.162
Late, day 15 - Stock, day 15	437	7.435	**< 0.001**
Model 1b - Carbohydrate content	**χ2**	***df***	***p***
Parasite treatment	16.558	2	**< 0.001**
Time	139.651	1	**< 0.001**
Parasite treatment: Time	0.644	2	0.725
Multiple comparisons for Model 1b (PT: T)	***df***	***t-ratio***	***p***

Early, day 5 - Late, day 5	437	-1.903	0.612
Early, day 5 - Stock, day 5	437	1.429	0.886
Early, day 5 - Early, day 10	437	-5.564	**< 0.001**
Early, day 5 - Late, day 10	437	-5.987	**< 0.001**
Early, day 5 - Stock, day 10	437	-4.771	**< 0.001**
Early, day 5 - Early, day 15	437	-7.421	**< 0.001**
Early, day 5 - Late, day 15	437	-8.193	**< 0.001**
Early, day 5 - Stock, day 15	437	-5.614	**< 0.001**
Late, day 5 - Stock, day 5	437	-3.331	**0.026**
Late, day 5 - Early, day 10	437	-3.672	**0.008**
Late, day 5 - Late, day 10	437	-4.084	**0.002**
Late, day 5 - Stock, day 10	437	-2.859	0.102
Late, day 5 - Early, day 15	437	-5.518	**< 0.001**
Late, day 5 - Late, day 15	437	-6.290	**< 0.001**
Late, day 5 - Stock, day 15	437	-3.712	**0.007**
Stock, day 5 - Early, day 10	437	-6.986	**< 0.001**
Stock, day 5 - Late, day 10	437	-7.415	**< 0.001**
Stock, day 5 - Stock, day 10	437	-6.207	**< 0.001**
Stock, day 5 - Early, day 15	437	-8.849	**< 0.001**
Stock, day 5 - Late, day 15	437	-9.621	**< 0.001**
Stock, day 5 - Stock, day 15	437	-7.043	**< 0.001**
Early, day 10 - Late, day 10	437	-0.391	1.000
Early, day 10 - Stock, day 10	437	0.845	0.996
Early, day 10 - Early, day 15	437	-1.818	0.670
Early, day 10 - Late, day 15	437	-2.586	0.200
Early, day 10 - Stock, day 15	437	-0.021	1.000
Late, day 10 - Stock, day 10	437	1.245	0.950
Late, day 10 - Early, day 15	437	-1.434	0.884
Late, day 10 - Late, day 15	437	-2.206	0.403
Late, day 10 - Stock, day 15	437	0.372	1.000
Stock, day 10 - Early, day 15	437	-2.686	0.156
Stock, day 10 - Late, day 15	437	-3.462	**0.018**
Stock, day 10 - Stock, day 15	437	-0.871	0.994
Early, day 15 - Late, day 15	437	-0.772	0.998
Early, day 15 - Stock, day 15	437	1.807	0.678
Late, day 15 - Stock, day 15	437	2.579	0.199
Model 1c - Lipid content	**χ2**	***df***	***p***
Parasite treatment	6.333	2	**0.042**
Time	8.251	1	**0.004**
Parasite treatment: Time	0.924	2	0.630
Multiple comparisons for Model 1c (PT: T)	***df***	***t-ratio***	***p***
Early, day 5 - Late, day 5	437	0.127	1.000
Early, day 5 - Stock, day 5	437	1.906	0.610
Early, day 5 - Early, day 10	437	2.003	0.542
Early, day 5 - Late, day 10	437	2.666	0.163
Early, day 5 - Stock, day 10	437	3.528	**0.014**
Early, day 5 - Early, day 15	437	2.050	0.509
Early, day 5 - Late, day 15	437	2.193	0.412
Early, day 5 - Stock, day 15	437	2.783	0.124

Late, day 5 - Stock, day 5	437	1.779	0.696
Late, day 5 - Early, day 10	437	1.877	0.639
Late, day 5 - Late, day 10	437	2.539	0.216
Late, day 5 - Stock, day 10	437	3.401	**0.021**
Late, day 5 - Early, day 15	437	1.924	0.598
Late, day 5 - Late, day 15	437	2.066	0.497
Late, day 5 - Stock, day 15	437	2.656	0.167
Stock, day 5 - Early, day 10	437	0.107	1.000
Stock, day 5 - Late, day 10	437	0.760	0.998
Stock, day 5 - Stock, day 10	437	1.613	0.798
Stock, day 5 - Early, day 15	437	0.144	1.000
Stock, day 5 - Late, day 15	437	0.287	1.000
Stock, day 5 - Stock, day 15	437	0.877	0.994
Early, day 10 - Late, day 10	437	0.649	0.999
Early, day 10 - Stock, day 10	437	1.496	0.857
Early, day 10 - Early, day 15	437	0.037	1.000
Early, day 10 - Late, day 15	437	0.178	1.000
Early, day 10 - Stock, day 15	437	0.765	0.998
Late, day 10 - Stock, day 10	437	0.849	0.997
Late, day 10 - Early, day 15	437	-0.615	1.000
Late, day 10 - Late, day 15	437	-0.473	1.000
Late, day 10 - Stock, day 15	437	0.117	1.000
Stock, day 10 - Early, day 15	437	-1.467	0.870
Stock, day 10 - Late, day 15	437	-1.324	0.924
Stock, day 10 - Stock, day 15	437	-0.732	0.998
Early, day 15 - Late, day 15	437	0.142	1.000
Early, day 15 - Stock, day 15	437	0.732	0.998
Late, day 15 - Stock, day 15	437	0.590	1.000

761 **Table S2. Metallomic profiles at adulthood.** Similarly to the previous resources, each metal was
762 analysed independently. We ran a linear mixed model for each metal element to test for differences in
763 the metallomes of differently infected or uninfected mosquitoes across time. The dependent variable
764 was the log-transformed concentration of the element obtained by ICP-OES, while the parasite
765 treatment and assay day were explanatory variables and their interaction. Replicate was included as a
766 random factor. Multiple comparisons tests were conducted to examine the interaction between "parasite
767 treatment" and "time" and show pairwise differences between all combinatorial treatments.

Tested effect			
Model 2a - Iron (Fe)	**χ2**	***df***	***p***
Parasite treatment	2.258	3	0.521
Time	18.880	2	**< 0.001**
Parasite treatment: Time	10.555	6	0.103
Multiple comparisons for Model 2a (PT: T)	***df***	***t-ratio***	***p***
Early, day 5 - Late, day 5	43	-1.942	0.727
Early, day 5 - Stock, day 5	43	-0.226	1.000
Early, day 5 - Uninfected, day 5	43	-2.073	0.643
Early, day 5 - Early, day 10	43	-0.746	1.000
Early, day 5 - Late, day 10	43	-0.666	1.000
Early, day 5 - Stock, day 10	43	0.434	1.000
Early, day 5 - Uninfected, day 10	43	0.810	1.000
Early, day 5 - Early, day 15	43	-1.680	0.868
Early, day 5 - Late, day 15	43	-1.734	0.843
Early, day 5 - Stock, day 15	43	-2.488	0.374
Early, day 5 - Uninfected, day 15	43	-2.925	0.169
Late, day 5 - Stock, day 5	43	1.716	0.851
Late, day 5 - Uninfected, day 5	43	-0.131	1.000
Late, day 5 - Early, day 10	43	1.195	0.990
Late, day 5 - Late, day 10	43	1.276	0.978
Late, day 5 - Stock, day 10	43	2.376	0.443
Late, day 5 - Uninfected, day 10	43	2.752	0.237
Late, day 5 - Early, day 15	43	0.142	1.000
Late, day 5 - Late, day 15	43	0.208	1.000
Late, day 5 - Stock, day 15	43	-0.546	1.000
Late, day 5 - Uninfected, day 15	43	0.983	0.998
Stock, day 5 - Uninfected, day 5	43	-1.847	0.783
Stock, day 5 - Early, day 10	43	-0.520	1.000
Stock, day 5 - Late, day 10	43	-0.440	1.000
Stock, day 5 - Stock, day 10	43	0.660	1.000
Stock, day 5 - Uninfected, day 10	43	1.036	0.996
Stock, day 5 - Early, day 15	43	-1.469	0.942
Stock, day 5 - Late, day 15	43	-1.508	0.930
Stock, day 5 - Stock, day 15	43	-2.262	0.517
Stock, day 5 - Uninfected, day 15	43	-2.699	0.262
Uninfected, day 5 - Early, day 10	43	1.326	0.971
Uninfected, day 5 - Late, day 10	43	1.407	0.956
Uninfected, day 5 - Stock, day 10	43	2.506	0.363
Uninfected, day 5 - Uninfected, day 10	43	2.882	0.184
Uninfected, day 5 - Early, day 15	43	0.265	1.000
Uninfected, day 5 - Late, day 15	43	0.339	1.000
Uninfected, day 5 - Stock, day 15	43	-0.415	1.000
Uninfected, day 5 - Uninfected, day 15	43	-0.852	0.999
Early, day 10 - Late, day 10	43	0.081	1.000
Early, day 10 - Stock, day 10	43	1.180	0.988
Early, day 10 - Uninfected, day 10	43	1.556	0.915
Early, day 10 - Early, day 15	43	-0.979	0.997
Early, day 10 - Late, day 15	43	-0.987	0.997

Early, day 10 - Stock, day 15	43	-1.741	0.839
Early, day 10 - Uninfected, day 15	43	-2.178	0.573
Late, day 10 - Stock, day 10	43	1.099	0.993
Late, day 10 - Uninfected, day 10	43	1.475	0.939
Late, day 10 - Early, day 15	43	-1.055	0.995
Late, day 10 - Late, day 15	43	-1.068	0.995
Late, day 10 - Stock, day 15	43	-1.822	0.797
Late, day 10 - Uninfected, day 15	43	-2.259	0.519
Stock, day 10 - Uninfected, day 10	43	0.376	1.000
Stock, day 10 - Early, day 15	43	-2.087	0.634
Stock, day 10 - Late, day 15	43	-2.167	0.580
Stock, day 10 - Stock, day 15	43	-2.921	0.170
Stock, day 10 - Uninfected, day 15	43	-3.358	0.063
Uninfected, day 10 - Early, day 15	43	-2.440	0.403
Uninfected, day 10 - Late, day 15	43	-2.543	0.342
Uninfected, day 10 - Stock, day 15	43	-3.297	0.073
Uninfected, day 10 - Uninfected, day 15	43	-3.734	**0.024**
Early, day 15 - Late, day 15	43	0.053	1.000
Early, day 15 - Stock, day 15	43	-0.654	1.000
Early, day 15 - Uninfected, day 15	43	-1.064	0.995
Late, day 15 - Stock, day 15	43	-0.754	1.000
Late, day 15 - Uninfected, day 15	43	-1.191	0.988
Stock, day 15 - Uninfected, day 15	43	-0.437	1.000
Model 2b - Zinc (Zn)	**χ2**	***df***	***p***
Parasite treatment	13.970	3	**0.003**
Time	18.622	2	**< 0.001**
Parasite treatment: Time	8.840	6	0.183
Multiple comparisons for Model 2b (PT: T)	***df***	***t-ratio***	***p***
Early, day 5 - Late, day 5	43	-0.712	1.000
Early, day 5 - Stock, day 5	43	-0.429	1.000
Early, day 5 - Uninfected, day 5	43	-2.346	0.462
Early, day 5 - Early, day 10	43	-0.934	0.998
Early, day 5 - Late, day 10	43	-1.012	0.997
Early, day 5 - Stock, day 10	43	0.229	1.000
Early, day 5 - Uninfected, day 10	43	-0.820	1.000
Early, day 5 - Early, day 15	43	-1.983	0.702
Early, day 5 - Late, day 15	43	-1.392	0.959
Early, day 5 - Stock, day 15	43	-2.119	0.613
Early, day 5 - Uninfected, day 15	43	-4.918	**< 0.001**
Late, day 5 - Stock, day 5	43	0.282	1.000
Late, day 5 - Uninfected, day 5	43	-1.635	0.886
Late, day 5 - Early, day 10	43	-0.223	1.000
Late, day 5 - Late, day 10	43	-0.300	1.000
Late, day 5 - Stock, day 10	43	0.941	0.999
Late, day 5 - Uninfected, day 10	43	-1.315	1.000
Late, day 5 - Early, day 15	43	-0.108	0.973
Late, day 5 - Late, day 15	43	-0.680	1.000
Late, day 5 - Stock, day 15	43	-1.407	0.956
Late, day 5 - Uninfected, day 15	43	-4.206	**0.006**

Stock, day 5 - Uninfected, day 5	43	-1.917	0.743
Stock, day 5 - Early, day 10	43	-0.505	1.000
Stock, day 5 - Late, day 10	43	-0.582	1.000
Stock, day 5 - Stock, day 10	43	0.659	1.000
Stock, day 5 - Uninfected, day 10	43	-0.390	1.000
Stock, day 5 - Early, day 15	43	-1.580	0.907
Stock, day 5 - Late, day 15	43	-0.963	0.998
Stock, day 5 - Stock, day 15	43	-1.690	0.863
Stock, day 5 - Uninfected, day 15	43	-4.488	**0.003**
Uninfected, day 5 - Early, day 10	43	1.412	0.955
Uninfected, day 5 - Late, day 10	43	1.335	0.970
Uninfected, day 5 - Stock, day 10	43	2.576	0.324
Uninfected, day 5 - Uninfected, day 10	43	1.527	0.925
Uninfected, day 5 - Early, day 15	43	0.219	1.000
Uninfected, day 5 - Late, day 15	43	0.954	0.998
Uninfected, day 5 - Stock, day 15	43	0.227	1.000
Uninfected, day 5 - Uninfected, day 15	43	-2.571	0.327
Early, day 10 - Late, day 10	43	-0.077	1.000
Early, day 10 - Stock, day 10	43	1.164	0.989
Early, day 10 - Uninfected, day 10	43	0.115	1.000
Early, day 10 - Early, day 15	43	-1.106	0.993
Early, day 10 - Late, day 15	43	-0.458	1.000
Early, day 10 - Stock, day 15	43	-1.185	0.988
Early, day 10 - Uninfected, day 15	43	-3.983	**0.012**
Late, day 10 - Stock, day 10	43	1.241	0.982
Late, day 10 - Uninfected, day 10	43	0.192	1.000
Late, day 10 - Early, day 15	43	-1.033	0.996
Late, day 10 - Late, day 15	43	-0.380	1.000
Late, day 10 - Stock, day 15	43	-1.107	0.993
Late, day 10 - Uninfected, day 15	43	-3.906	**0.015**
Stock, day 10 - Uninfected, day 10	43	-1.049	0.995
Stock, day 10 - Early, day 15	43	-2.198	0.560
Stock, day 10 - Late, day 15	43	-1.621	0.892
Stock, day 10 - Stock, day 15	43	-2.349	0.460
Stock, day 10 - Uninfected, day 15	43	-5.147	**< 0.001**
Uninfected, day 10 - Early, day 15	43	-1.214	0.985
Uninfected, day 10 - Late, day 15	43	-0.572	1.000
Uninfected, day 10 - Stock, day 15	43	-1.299	0.975
Uninfected, day 10 - Uninfected, day 15	43	-4.098	**0.009**
Early, day 15 - Late, day 15	43	0.677	1.000
Early, day 15 - Stock, day 15	43	-0.006	1.000
Early, day 15 - Uninfected, day 15	43	2.631	0.294
Late, day 15 - Stock, day 15	43	-0.727	1.000
Late, day 15 - Uninfected, day 15	43	-3.526	**0.042**
Stock, day 15 - Uninfected, day 15	43	-2.799	0.217
Model 2c - Copper (Cu)	**χ2**	***df***	***p***
Parasite treatment	0.663	3	0.882
Time	11.925	2	**0.003**
Parasite treatment: Time	6.333	6	0.387

Multiple comparisons for Model 2c (PT: T)	*df*	*t-ratio*	*p*
Early, day 5 - Late, day 5	43	-1.152	0.990
Early, day 5 - Stock, day 5	43	-0.412	1.000
Early, day 5 - Uninfected, day 5	43	-1.937	0.730
Early, day 5 - Early, day 10	43	-0.983	0.997
Early, day 5 - Late, day 10	43	-0.343	1.000
Early, day 5 - Stock, day 10	43	-0.138	1.000
Early, day 5 - Uninfected, day 10	43	-0.249	1.000
Early, day 5 - Early, day 15	43	-2.271	0.511
Early, day 5 - Late, day 15	43	-1.409	0.955
Early, day 5 - Stock, day 15	43	-2.668	0.277
Early, day 5 - Uninfected, day 15	43	-2.059	0.653
Late, day 5 - Stock, day 5	43	0.740	1.000
Late, day 5 - Uninfected, day 5	43	-0.786	1.000
Late, day 5 - Early, day 10	43	0.268	1.000
Late, day 5 - Late, day 10	43	0.809	1.000
Late, day 5 - Stock, day 10	43	1.014	0.997
Late, day 5 - Uninfected, day 10	43	0.903	0.999
Late, day 5 - Early, day 15	43	-1.190	0.988
Late, day 5 - Late, day 15	43	-0.258	1.000
Late, day 5 - Stock, day 15	43	-1.516	0.928
Late, day 5 - Uninfected, day 15	43	-0.907	0.999
Stock, day 5 - Uninfected, day 5	43	-1.526	0.925
Stock, day 5 - Early, day 10	43	-0.572	1.000
Stock, day 5 - Late, day 10	43	0.069	1.000
Stock, day 5 - Stock, day 10	43	0.274	1.000
Stock, day 5 - Uninfected, day 10	43	0.163	1.000
Stock, day 5 - Early, day 15	43	-1.885	0.762
Stock, day 5 - Late, day 15	43	-0.998	0.997
Stock, day 5 - Stock, day 15	43	-2.256	0.521
Stock, day 5 - Uninfected, day 15	43	-1.647	0.881
Uninfected, day 5 - Early, day 10	43	0.954	0.998
Uninfected, day 5 - Late, day 10	43	1.594	0.902
Uninfected, day 5 - Stock, day 10	43	1.799	0.810
Uninfected, day 5 - Uninfected, day 10	43	1.688	0.864
Uninfected, day 5 - Early, day 15	43	-0.453	1.000
Uninfected, day 5 - Late, day 15	43	0.528	1.000
Uninfected, day 5 - Stock, day 15	43	-0.730	1.000
Uninfected, day 5 - Uninfected, day 15	43	-0.121	1.000
Early, day 10 - Late, day 10	43	0.640	1.000
Early, day 10 - Stock, day 10	43	0.845	0.999
Early, day 10 - Uninfected, day 10	43	0.734	1.000
Early, day 10 - Early, day 15	43	-1.348	0.967
Early, day 10 - Late, day 15	43	-0.426	1.000
Early, day 10 - Stock, day 15	43	-1.684	0.865
Early, day 10 - Uninfected, day 15	43	-1.075	0.994
Late, day 10 - Stock, day 10	43	0.205	1.000
Late, day 10 - Uninfected, day 10	43	0.094	1.000
Late, day 10 - Early, day 15	43	-1.949	0.723

Late, day 10 - Late, day 15	43	-1.066	0.995
Late, day 10 - Stock, day 15	43	-2.325	0.476
Late, day 10 - Uninfected, day 15	43	-1.716	0.851
Stock, day 10 - Uninfected, day 10	43	-0.111	1.000
Stock, day 10 - Early, day 15	43	-2.141	0.598
Stock, day 10 - Late, day 15	43	-1.271	0.979
Stock, day 10 - Stock, day 15	43	-2.530	0.350
Stock, day 10 - Uninfected, day 15	43	-1.921	0.740
Uninfected, day 10 - Early, day 15	43	-2.037	0.667
Uninfected, day 10 - Late, day 15	43	-1.160	0.989
Uninfected, day 10 - Stock, day 15	43	-2.419	0.416
Uninfected, day 10 - Uninfected, day 15	43	-1.810	0.804
Early, day 15 - Late, day 15	43	0.948	0.998
Early, day 15 - Stock, day 15	43	-0.232	1.000
Early, day 15 - Uninfected, day 15	43	0.339	1.000
Late, day 15 - Stock, day 15	43	-1.258	0.980
Late, day 15 - Uninfected, day 15	43	-0.649	1.000
Stock, day 15 - Uninfected, day 15	43	0.609	1.000
Model 2d - Calcium (Ca)	**χ2**	***df***	***p***
Parasite treatment	3.190	3	0.363
Time	34.084	2	**< 0.001**
Parasite treatment: Time	10.268	6	0.114
Multiple comparisons for Model 2d (PT: T)	***df***	***t-ratio***	***p***
Early, day 5 - Late, day 5	43	-1.999	0.691
Early, day 5 - Stock, day 5	43	-0.654	1.000
Early, day 5 - Uninfected, day 5	43	-1.295	0.975
Early, day 5 - Early, day 10	43	-1.399	0.957
Early, day 5 - Late, day 10	43	01.662	0.875
Early, day 5 - Stock, day 10	43	-0.208	1.000
Early, day 5 - Uninfected, day 10	43	0.730	1.000
Early, day 5 - Early, day 15	43	-2.786	0.222
Early, day 5 - Late, day 15	43	-3.075	0.122
Early, day 5 - Stock, day 15	43	-4.147	**0.008**
Early, day 5 - Uninfected, day 15	43	-3.234	0.085
Late, day 5 - Stock, day 5	43	1.346	0.968
Late, day 5 - Uninfected, day 5	43	0.705	1.000
Late, day 5 - Early, day 10	43	0.600	1.000
Late, day 5 - Late, day 10	43	0.337	1.000
Late, day 5 - Stock, day 10	43	1.791	0.814
Late, day 5 - Uninfected, day 10	43	2.730	0.247
Late, day 5 - Early, day 15	43	-0.910	0.999
Late, day 5 - Late, day 15	43	-1.076	0.994
Late, day 5 - Stock, day 15	43	-2.148	0.593
Late, day 5 - Uninfected, day 15	43	-1.234	0.983
Stock, day 5 - Uninfected, day 5	43	-0.641	1.000
Stock, day 5 - Early, day 10	43	-0.745	1.000
Stock, day 5 - Late, day 10	43	-1.009	0.997
Stock, day 5 - Stock, day 10	43	0.446	1.000
Stock, day 5 - Uninfected, day 10	43	1.384	0.961

Stock, day 5 - Early, day 15	43	-2.172	0.577
Stock, day 5 - Late, day 15	43	-2.421	0.414
Stock, day 5 - Stock, day 15	43	-3.494	**0.045**
Stock, day 5 - Uninfected, day 15	43	-2.580	0.322
Uninfected, day 5 - Early, day 10	43	-0.104	1.000
Uninfected, day 5 - Late, day 10	43	-0.367	1.000
Uninfected, day 5 - Stock, day 10	43	1.087	0.994
Uninfected, day 5 - Uninfected, day 10	43	2.025	0.675
Uninfected, day 5 - Early, day 15	43	-1.571	0.910
Uninfected, day 5 - Late, day 15	43	-1.780	0.819
Uninfected, day 5 - Stock, day 15	43	-2.853	0.195
Uninfected, day 5 - Uninfected, day 15	43	-1.939	0.729
Early, day 10 - Late, day 10	43	-0.263	1.000
Early, day 10 - Stock, day 10	43	1.101	0.987
Early, day 10 - Uninfected, day 10	43	2.129	0.606
Early, day 10 - Early, day 15	43	-1.473	0.940
Early, day 10 - Late, day 15	43	-1.676	0.869
Early, day 10 - Stock, day 15	43	-2.748	0.239
Early, day 10 - Uninfected, day 15	43	-1.835	0.790
Late, day 10 - Stock, day 10	43	1.454	0.945
Late, day 10 - Uninfected, day 10	43	2.392	0.432
Late, day 10 - Early, day 15	43	-1.226	0.984
Late, day 10 - Late, day 15	43	-1.413	0.955
Late, day 10 - Stock, day 15	43	-2.485	0.376
Late, day 10 - Uninfected, day 15	43	-1.571	0.910
Stock, day 10 - Uninfected, day 10	43	0.938	0.998
Stock, day 10 - Early, day 15	43	-2.590	0.316
Stock, day 10 - Late, day 15	43	-2.867	0.190
Stock, day 10 - Stock, day 15	43	-3.939	**0.014**
Stock, day 10 - Uninfected, day 15	43	-3.026	0.136
Uninfected, day 10 - Early, day 15	43	-3.471	**0.047**
Uninfected, day 10 - Late, day 15	43	-3.805	**0.020**
Uninfected, day 10 - Stock, day 15	43	-4.878	**< 0.001**
Uninfected, day 10 - Uninfected, day 15	43	3.964	**0.013**
Early, day 15 - Late, day 15	43	-0.100	1.000
Early, day 15 - Stock, day 15	43	-1.106	0.993
Early, day 15 - Uninfected, day 15	43	-0.249	1.000
Late, day 15 - Stock, day 15	43	-1.072	0.994
Late, day 15 - Uninfected, day 15	43	-0.159	1.000
Stock, day 15 - Uninfected, day 15	43	0.914	0.999
Model 2e - Manganese (Mn)	**χ2**	***df***	***p***
Parasite treatment	32.303	3	**< 0.001**
Time	17.909	2	**< 0.001**
Parasite treatment: Time	4.655	6	0.589
Multiple comparisons for Model 2e (PT: T)	***df***	***t-ratio***	***p***
Early, day 5 - Late, day 5	43	-0.803	1.000
Early, day 5 - Stock, day 5	43	-0.669	1.000
Early, day 5 - Uninfected, day 5	43	1.700	0.859
Early, day 5 - Early, day 10	43	-0.302	1.000

Early, day 5 - Late, day 10	43	-0.448	1.000
Early, day 5 - Stock, day 10	43	0.663	1.000
Early, day 5 - Uninfected, day 10	43	2.786	0.222
Early, day 5 - Early, day 15	43	-2.078	0.640
Early, day 5 - Late, day 15	43	-1.490	0.935
Early, day 5 - Stock, day 15	43	-2.874	0.187
Early, day 5 - Uninfected, day 15	43	0.864	0.999
Late, day 5 - Stock, day 5	43	0.134	1.000
Late, day 5 - Uninfected, day 5	43	2.503	0.365
Late, day 5 - Early, day 10	43	0.501	1.000
Late, day 5 - Late, day 10	43	0.356	1.000
Late, day 5 - Stock, day 10	43	1.466	0.942
Late, day 5 - Uninfected, day 10	43	3.589	**0.035**
Late, day 5 - Early, day 15	43	-1.324	0.971
Late, day 5 - Late, day 15	43	-0.687	1.000
Late, day 5 - Stock, day 15	43	-2.071	0.645
Late, day 5 - Uninfected, day 15	43	1.667	0.873
Stock, day 5 - Uninfected, day 5	43	2.369	0.447
Stock, day 5 - Early, day 10	43	0.367	1.000
Stock, day 5 - Late, day 10	43	0.221	1.000
Stock, day 5 - Stock, day 10	43	1.332	0.970
Stock, day 5 - Uninfected, day 10	43	3.455	**0.050**
Stock, day 5 - Early, day 15	43	-1.450	0.946
Stock, day 5 - Late, day 15	43	-0.821	1.000
Stock, day 5 - Stock, day 15	43	-2.205	0.555
Stock, day 5 - Uninfected, day 15	43	1.533	0.923
Uninfected, day 5 - Early, day 10	43	-2.002	0.690
Uninfected, day 5 - Late, day 10	43	-2.147	0.594
Uninfected, day 5 - Stock, day 10	43	-1.037	0.996
Uninfected, day 5 - Uninfected, day 10	43	-1.087	0.994
Uninfected, day 5 - Early, day 15	43	-3.673	**0.028**
Uninfected, day 5 - Late, day 15	43	-3.190	0.094
Uninfected, day 5 - Stock, day 15	43	-4.574	**0.002**
Uninfected, day 5 - Uninfected, day 15	43	-0.836	0.999
Early, day 10 - Late, day 10	43	-0.145	1.000
Early, day 10 - Stock, day 10	43	0.965	0.998
Early, day 10 - Uninfected, day 10	43	3.088	0.119
Early, day 10 - Early, day 15	43	-1.794	0.812
Early, day 10 - Late, day 15	43	-1.188	0.987
Early, day 10 - Stock, day 15	43	-2.572	0.326
Early, day 10 - Uninfected, day 15	43	1.166	0.989
Late, day 10 - Stock, day 10	43	1.111	0.993
Late, day 10 - Uninfected, day 10	43	3.234	0.085
Late, day 10 - Early, day 15	43	-1.658	0.877
Late, day 10 - Late, day 15	43	-1.043	0.996
Late, day 10 - Stock, day 15	43	-2.426	0.411
Late, day 10 - Uninfected, day 15	43	1.312	0/.973
Stock, day 10 - Uninfected, day 10	43	2.123	0.610
Stock, day 10 - Early, day 15	43	-2.700	0.260

Stock, day 10 - Late, day 15	43	-2.153	0.590
Stock, day 10 - Stock, day 15	43	-3.537	**0.040**
Stock, day 10 - Uninfected, day 15	43	0.201	1.000
Uninfected, day 10 - Early, day 15	43	-4.692	**0.001**
Uninfected, day 10 - Late, day 15	43	4.277	**0.005**
Uninfected, day 10 - Stock, day 15	43	-5.660	**< 0.001**
Uninfected, day 10 - Uninfected, day 15	43	-1.922	0.739
Early, day 15 - Late, day 15	43	-.680	1.000
Early, day 15 - Stock, day 15	43	-0.619	1.000
Early, day 15 - Uninfected, day 15	43	2.889	0.187
Late, day 15 - Stock, day 15	43	-1.384	0.961
Late, day 15 - Uninfected, day 15	43	2.354	0.457
Stock, day 15 - Uninfected, day 15	43	3.738	**0.024**

Table S3. The effect of iron-supplementation and -chelation on parasite growth. We assessed the effect of the parasite treatment and diet on spore rate and growth for each time point assayed. For each spore rate comparison, we ran a generalised linear mixed model with a binomial error structure, where parasite treatment and diet treatment were set as explanatory variables, their interaction, and the presence or absence of spores as a response variable. Replicate was included as a random factor. Concerning spore growth analyses, we only considered individuals with at least one spore. We then ran a linear mixed model with the same explanatory and random factors as the previous one. Multiple comparisons tests were conducted to examine the interaction between "parasite treatment" and "diet" and show pairwise differences between all combinatorial treatments.

Tested effect			
Model 3a - Spore rate at day 5	**χ2**	***df***	***p***
Diet treatment	15.430	2	**< 0.001**
Parasite treatment	15.546	2	**< 0.001**
Diet treatment: Parasite treatment	4.986	4	0.289
Multiple comparisons for Model 3a (PT: D)	***df***	***t-ratio***	***p***
Early, Sugar - Late, Sugar	inf	-1.396	0.900
Early, Sugar - Stock, Sugar	inf	0.203	1.000
Early, Sugar - Early, Sugar + BPS	inf	1.460	0.874
Early, Sugar - Late, Sugar + BPS	inf	0.000	1.000
Early, Sugar - Stock, Sugar + BPS	inf	1.460	0.874
Early, Sugar - Early, Sugar + $FeCl_3$	inf	-1.790	0.689
Early, Sugar - Late, Sugar + $FeCl_3$	inf	-2.976	0.072
Early, Sugar - Stock, Sugar + $FeCl_3$	inf	0.823	0.996
Late, Sugar - Stock, Sugar	inf	1.594	0.808
Late, Sugar - Early, Sugar + BPS	inf	2.792	0.118
Late, Sugar - Late, Sugar + BPS	inf	1.396	0.900
Late, Sugar - Stock, Sugar + BPS	inf	2.792	0.118
Late, Sugar - Early, Sugar + $FeCl_3$	inf	-0.405	1.000
Late, Sugar - Late, Sugar + $FeCl_3$	inf	-1.656	0.773
Late, Sugar - Stock, Sugar + $FeCl_3$	inf	2.192	0.411
Stock, Sugar - Early, Sugar + BPS	inf	1.262	0.942
Stock, Sugar - Late, Sugar + BPS	inf	-0.203	1.000
Stock, Sugar - Stock, Sugar + BPS	inf	1.262	0.942
Stock, Sugar - Early, Sugar + $FeCl_3$	inf	-1.986	0.553
Stock, Sugar - Late, Sugar + $FeCl_3$	inf	-3.160	**0.042**
Stock, Sugar - Stock, Sugar + $FeCl_3$	inf	0.621	1.000
Early, Sugar + BPS - Late, Sugar + BPS	inf	-1.460	**0.874**
Early, Sugar + BPS - Stock, Sugar + BPS	inf	0.000	1.000
Early, Sugar + BPS - Early, Sugar + $FeCl_3$	inf	-3.160	**0.042**
Early, Sugar + BPS - Late, Sugar + $FeCl_3$	inf	-4.241	**< 0.001**
Early, Sugar + BPS - Stock, Sugar + $FeCl_3$	inf	-0.648	0.999
Late, Sugar + BPS - Stock, Sugar + BPS	inf	1.460	0.874
Late, Sugar + BPS - Early, Sugar + $FeCl_3$	inf	-1.790	0.689
Late, Sugar + BPS - Late, Sugar + $FeCl_3$	inf	-2.976	0.072
Late, Sugar + BPS - Stock, Sugar + $FeCl_3$	inf	0.823	0.996
Stock, Sugar + BPS - Early, Sugar + $FeCl_3$	inf	-3.160	**0.042**
Stock, Sugar + BPS - Late, Sugar + $FeCl_3$	inf	-4.241	**< 0.001**
Stock, Sugar + BPS - Stock, Sugar + $FeCl_3$	inf	-0.648	0.999
Early, Sugar + $FeCl_3$ - Late, Sugar + $FeCl_3$	inf	-1.262	0.942
Early, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	inf	2.574	0.200
Late, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	inf	3.705	**0.007**
Model 3b- Spore rate at day 10	**χ2**	***df***	***p***

Diet treatment	8.464	2	**0.015**
Parasite treatment	16.867	2	**< 0.001**
Diet treatment: Parasite treatment	0.504	4	0.973
Multiple comparisons for Model 3b (PT: D)	***df***	***t-ratio***	***p***
Early, Sugar - Late, Sugar	inf	-0.001	1.000
Early, Sugar - Stock, Sugar	inf	1.275	0.9387
Early, Sugar - Early, Sugar + BPS	inf	2.209	0.3993
Early, Sugar - Late, Sugar + BPS	inf	0.972	0.9883
Early, Sugar - Stock, Sugar + BPS	inf	2.785	0.1195
Early, Sugar - Early, Sugar + $FeCl_3$	inf	-0.002	1.000
Early, Sugar - Late, Sugar + $FeCl_3$	inf	-0.002	1.000
Early, Sugar - Stock, Sugar + $FeCl_3$	inf	0.575	1.000
Late, Sugar - Stock, Sugar	inf	0.001	1.000
Late, Sugar - Early, Sugar + BPS	inf	0.001	1.000
Late, Sugar - Late, Sugar + BPS	inf	0.001	1.000
Late, Sugar - Stock, Sugar + BPS	inf	0.001	1.000
Late, Sugar - Early, Sugar + $FeCl_3$	inf	0.000	1.000
Late, Sugar - Late, Sugar + $FeCl_3$	inf	-0.002	1.000
Late, Sugar - Stock, Sugar + $FeCl_3$	inf	0.001	0.878
Stock, Sugar - Early, Sugar + BPS	inf	1.451	1.000
Stock, Sugar - Late, Sugar + BPS	inf	-0.391	0.252
Stock, Sugar - Stock, Sugar + BPS	inf	2.459	1.000
Stock, Sugar - Early, Sugar + $FeCl_3$	inf	-0.002	1.000
Stock, Sugar - Late, Sugar + $FeCl_3$	inf	-0.002	0.997
Stock, Sugar - Stock, Sugar + $FeCl_3$	inf	-0.826	0.706
Early, Sugar + BPS - Late, Sugar + BPS	inf	-1.764	0.961
Early, Sugar + BPS - Stock, Sugar + BPS	inf	1.180	1.000
Early, Sugar + BPS - Early, Sugar + $FeCl_3$	inf	-0.002	1.000
Early, Sugar + BPS - Late, Sugar + $FeCl_3$	inf	-0.002	0.507
Early, Sugar + BPS - Stock, Sugar + $FeCl_3$	inf	-2.051	0.154
Late, Sugar + BPS - Stock, Sugar + BPS	inf	2.682	1.000
Late, Sugar + BPS - Early, Sugar + $FeCl_3$	inf	-0.002	1.000
Late, Sugar + BPS - Late, Sugar + $FeCl_3$	inf	-0.002	1.000
Late, Sugar + BPS - Stock, Sugar + $FeCl_3$	inf	-0.456	1.000
Stock, Sugar + BPS - Early, Sugar + $FeCl_3$	inf	-0.003	1.000
Stock, Sugar + BPS - Late, Sugar + $FeCl_3$	inf	-0.002	1.000
Stock, Sugar + BPS - Stock, Sugar + $FeCl_3$	inf	-2.837	0.105
Early, Sugar + $FeCl_3$ - Late, Sugar + $FeCl_3$	inf	-0.002	1.000
Early, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	inf	0.002	1.000
Late, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	inf	0.002	1.000
Model 3d - Spore load at day 5	$\chi 2$	***df***	***p***
Diet treatment	37.751	2	**< 0.001**
Parasite treatment	12.451	2	**0.002**
Diet treatment: Parasite treatment	6.925	4	0.140
Multiple comparisons for Model 3d (PT: D)	***df***	***t-ratio***	***p***
Early, Sugar - Late, Sugar	190	-1.257	0.942
Early, Sugar - Stock, Sugar	189	1.284	0.935
Early, Sugar - Early, Sugar + BPS	190	1.844	0.653
Early, Sugar - Late, Sugar + BPS	190	0.494	1.000

Early, Sugar - Stock, Sugar + BPS	190	2.225	0.394
Early, Sugar - Early, Sugar + $FeCl_3$	188	0.558	1.000
Early, Sugar - Late, Sugar + $FeCl_3$	189	-3.197	**0.042**
Early, Sugar - Stock, Sugar + $FeCl_3$	189	2.128	0.458
Late, Sugar - Stock, Sugar	189	2.613	0.188
Late, Sugar - Early, Sugar + BPS	192	3.041	0.066
Late, Sugar - Late, Sugar + BPS	188	1.791	0.689
Late, Sugar - Stock, Sugar + BPS	190	3.456	**0.019**
Late, Sugar - Early, Sugar + $FeCl_3$	190	1.982	0.558
Late, Sugar - Late, Sugar + $FeCl_3$	189	-2.040	0.518
Late, Sugar - Stock, Sugar + $FeCl_3$	189	3.439	**0.020**
Stock, Sugar - Early, Sugar + BPS	190	0.676	0.999
Stock, Sugar - Late, Sugar + BPS	189	-0.795	0.997
Stock, Sugar - Stock, Sugar + BPS	190	1.057	0.980
Stock, Sugar - Early, Sugar + $FeCl_3$	188	-0.841	0.995
Stock, Sugar - Late, Sugar + $FeCl_3$	188	-4.591	**< 0.001**
Stock, Sugar - Stock, Sugar + $FeCl_3$	188	0.889	0.993
Early, Sugar + BPS - Late, Sugar + BPS	191	-1.398	0.898
Early, Sugar + BPS - Stock, Sugar + BPS	189	0.351	1.000
Early, Sugar + BPS - Early, Sugar + $FeCl_3$	190	-1.476	0.865
Early, Sugar + BPS - Late, Sugar + $FeCl_3$	190	-4.790	**< 0.001**
Early, Sugar + BPS - Stock, Sugar + $FeCl_3$	190	0.157	1.000
Late, Sugar + BPS - Stock, Sugar + BPS	189	1.786	0.691
Late, Sugar + BPS - Early, Sugar + $FeCl_3$	190	0.020	1.000
Late, Sugar + BPS - Late, Sugar + $FeCl_3$	190	-3.753	**0.007**
Late, Sugar + BPS - Stock, Sugar + $FeCl_3$	189	1.660	0.770
Stock, Sugar + BPS - Early, Sugar + $FeCl_3$	190	-1.883	0.626
Stock, Sugar + BPS - Late, Sugar + $FeCl_3$	190	-5.217	**< 0.001**
Stock, Sugar + BPS - Stock, Sugar + $FeCl_3$	190	-0.210	1.000
Early, Sugar + $FeCl_3$ - Late, Sugar + $FeCl_3$	189	-4.193	**0.001**
Early, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	189	1.764	0.706
Late, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	189	5.343	**< 0.001**
Model 3e - Spore load at day 10	**$\chi 2$**	***df***	***p***
Diet treatment	37.601	2	**< 0.001**
Parasite treatment	48.705	2	**< 0.001**
Diet treatment: Parasite treatment	3.038	4	0.552
Multiple comparisons for Model 3e (PT: D)	***df***	***t-ratio***	***p***
Early, Sugar - Late, Sugar	404	-3.455	**0.018**
Early, Sugar - Stock, Sugar	404	0.192	1.000
Early, Sugar - Early, Sugar + BPS	404	2.299	0.345
Early, Sugar - Late, Sugar + BPS	404	-0.281	1.000
Early, Sugar - Stock, Sugar + BPS	404	1.247	0.945
Early, Sugar - Early, Sugar + $FeCl_3$	404	-2.241	0.381
Early, Sugar - Late, Sugar + $FeCl_3$	404	-5.341	**< 0.001**
Early, Sugar - Stock, Sugar + $FeCl_3$	404	-1.372	0.908
Late, Sugar - Stock, Sugar	404	3.592	**0.048**
Late, Sugar - Early, Sugar + BPS	404	5.606	**< 0.001**
Late, Sugar - Late, Sugar + BPS	404	3.137	**0.048**
Late, Sugar - Stock, Sugar + BPS	404	4.429	**< 0.001**

Late, Sugar - Early, Sugar + $FeCl_3$	404	1.220	0.952
Late, Sugar - Late, Sugar + $FeCl_3$	404	-1.896	0.618
Late, Sugar - Stock, Sugar + $FeCl_3$	404	2.058	0.504
Stock, Sugar - Early, Sugar + BPS	404	2.083	0.487
Stock, Sugar - Late, Sugar + BPS	404	-0.466	1.000
Stock, Sugar - Stock, Sugar + BPS	404	1.054	0.980
Stock, Sugar - Early, Sugar + $FeCl_3$	404	-2.398	0.288
Stock, Sugar - Late, Sugar + $FeCl_3$	404	-5.448	**< 0.001**
Stock, Sugar - Stock, Sugar + $FeCl_3$	404	-1.541	0.835
Early, Sugar + BPS - Late, Sugar + BPS	404	-2.546	0.214
Early, Sugar + BPS - Stock, Sugar + BPS	404	-0.932	0.991
Early, Sugar + BPS - Early, Sugar + $FeCl_3$	404	-4.448	**< 0.001**
Early, Sugar + BPS - Late, Sugar + $FeCl_3$	404	-7.405	**< 0.001**
Early, Sugar + BPS - Stock, Sugar + $FeCl_3$	404	-3.599	**0.011**
Late, Sugar + BPS - Stock, Sugar + BPS	404	1.495	0.858
Late, Sugar + BPS - Early, Sugar + $FeCl_3$	404	-1.935	0.589
Late, Sugar + BPS - Late, Sugar + $FeCl_3$	404	-5.002	**< 0.001**
Late, Sugar + BPS - Stock, Sugar + $FeCl_3$	404	-1.078	0.977
Stock, Sugar + BPS - Early, Sugar + $FeCl_3$	404	-3.313	**0.028**
Stock, Sugar + BPS - Late, Sugar + $FeCl_3$	404	-6.163	**< 0.001**
Stock, Sugar + BPS - Stock, Sugar + $FeCl_3$	404	-2.5-6	0.232
Early, Sugar + $FeCl_3$ - Late, Sugar + $FeCl_3$	404	-3.116	0.050
Early, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	404	-0.850	0.996
Late, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	404	3.934	**0.003**
Model 3f - Spore load at day 15	**$\chi 2$**	***df***	***p***
Diet treatment	7.271	2	**0.0264**
Parasite treatment	79.910	2	**< 0.001**
Diet treatment: Parasite treatment	18.102	4	**0.001**
Multiple comparisons for Model 3f (PT: D)	***df***	***t-ratio***	***p***
Early, Sugar - Late, Sugar	437	-1.128	0.970
Early, Sugar - Stock, Sugar	437	1.553	0.830
Early, Sugar - Early, Sugar + BPS	437	7.691	**< 0.001**
Early, Sugar - Late, Sugar + BPS	437	2.497	0.236
Early, Sugar - Stock, Sugar + BPS	437	5.713	**< 0.001**
Early, Sugar - Early, Sugar + $FeCl_3$	437	-0.074	1.000
Early, Sugar - Late, Sugar + $FeCl_3$	437	-2.633	0.176
Early, Sugar - Stock, Sugar + $FeCl_3$	437	-3.095	0.053
Late, Sugar - Stock, Sugar	437	2.681	0.158
Late, Sugar - Early, Sugar + BPS	437	8.8219	**< 0.001**
Late, Sugar - Late, Sugar + BPS	437	3.625	**0.010**
Late, Sugar - Stock, Sugar + BPS	437	6.841	**< 0.001**
Late, Sugar - Early, Sugar + $FeCl_3$	437	1.053	0.980
Late, Sugar - Late, Sugar + $FeCl_3$	437	-1.505	0.853
Late, Sugar - Stock, Sugar + $FeCl_3$	437	-1.967	0.567
Stock, Sugar - Early, Sugar + BPS	437	6.139	**< 0.001**
Stock, Sugar - Late, Sugar + BPS	437	0.944	0.990
Stock, Sugar - Stock, Sugar + BPS	437	4.160	**0.001**
Stock, Sugar - Early, Sugar + $FeCl_3$	437	-1.627	0.790
Stock, Sugar - Late, Sugar + $FeCl_3$	437	-4.186	**0.001**

Stock, Sugar - Stock, Sugar + $FeCl_3$	437	-4.648	**< 0.001**
Early, Sugar + BPS - Late, Sugar + BPS	437	-5.194	**< 0.001**
Early, Sugar + BPS - Stock, Sugar + BPS	437	-1.979	0.559
Early, Sugar + BPS - Early, Sugar + $FeCl_3$	437	-7.766	**< 0.001**
Early, Sugar + BPS - Late, Sugar + $FeCl_3$	437	-10.325	**< 0.001**
Early, Sugar + BPS - Stock, Sugar + $FeCl_3$	437	-10.787	**< 0.001**
Late, Sugar + BPS - Stock, Sugar + BPS	437	3.216	**0.037**
Late, Sugar + BPS - Early, Sugar + $FeCl_3$	437	-2.572	0.202
Late, Sugar + BPS - Late, Sugar + $FeCl_3$	437	-5.130	**< 0.001**
Late, Sugar + BPS - Stock, Sugar + $FeCl_3$	437	-5.592	**< 0.001**
Stock, Sugar + BPS - Early, Sugar + $FeCl_3$	437	-5.787	**< 0.001**
Stock, Sugar + BPS - Late, Sugar + $FeCl_3$	437	-8.346	**< 0.001**
Stock, Sugar + BPS - Stock, Sugar + $FeCl_3$	437	-8.808	**< 0.001**
Early, Sugar + $FeCl_3$ - Late, Sugar + $FeCl_3$	437	-2.559	0.207
Early, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	437	-3.021	0.066
Late, Sugar + $FeCl_3$ - Stock, Sugar + $FeCl_3$	437	-0.462	1.000