Energetic shifts reflect survival likelihood in *Anopheles gambiae*

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Abstract

Life history theory predicts that resource allocation adapts to ecological and evolutionary pressures. We investigated resource and energy in the malaria vector *Anopheles gambiae* following exposure to two stressors: blood meals and infection by the microsporidian *Vavraia culicis*. Our findings reveal the costs of blood feeding and parasitism on longevity, highlighting trade-offs in lifetime protein, carbohydrate, and lipid reserves. Notably, shifts in carbohydrate-to-lipid ratios were associated with survival likelihood, with survivors exhibiting higher resource reserves and uniquely transitioning from carbohydrate to lipid utilisation, a pattern absent in non-survivors. This study emphasises the coevolutionary dynamics between hosts and parasites, highlighting how intrinsic and extrinsic factors shape host physiology. More broadly, our results underscore the importance of integrating host metabolic responses into ecological and epidemiological frameworks to enhance understanding of parasite transmission and survival strategies.

Keywords: *Anopheles gambiae*, resource allocation, infection, energetic shift, blood meal

Highlights

- Having a blood meal did not affect mosquito longevity, resource content or *V. culicis* parasitaemia.
- Live mosquitoes harboured fewer spores than mosquitoes that just died, independently of the stage of infection, supporting the parasite load upon death hypothesis.
- Alive mosquitoes exhibited a shift in their usage of the energetic reserves (i.e., carbohydrates to lipids) late in life, which mosquitoes at death did not.
- Our findings support the hypothesis that *Plasmodium* may have coevolved with its vector's dynamics of lipid release, a nutrient essential for its development.

Introduction

According to life history theory [1,2], organisms allocate resources to traits throughout their lives to maximise their reproductive success, which leads to trade-offs among the involved traits. Traditionally, the traits considered were primarily age-specific growth rates, fecundity, and mortality rates. More recently, the approach has been expanded to study, for example, decisions about resource allocation to growth, reproduction, and immune responses theoretically [3] and experimentally [4], and experimental work has confirmed the predicted trade-offs between fecundity and immune efficacy [5].

While such work has made it clear that life-history theory can help to understand host-parasite interactions, it also shows that the approach needs more detailed data on how different resources are allocated to functions and how the allocation patterns affect the traits and underlie the trade-offs among them. One limitation in linking resource allocation and parasite-host interactions is that studies of resource sequestration by parasites often focus on specific time points rather than encompassing the host's entire adulthood, as illustrated by a review of malaria parasites in *Anopheles* mosquitoes [6].

Mosquitoes are well-suited for such studies, as they obtain their primary resources from two food sources: protein and lipids from blood [7,8] and carbohydrates from the nectar of plants [9]. Proteins, carbohydrates, and lipids play diverse roles in organisms, serving as structural components, enzymatic cofactors, and energy sources. In this study, we focus on the energetic roles of carbohydrates and lipids, as well as the reproductive role of proteins. Carbohydrates serve as an immediate energy source (e.g., glycogen), while lipids (e.g., triglycerides) provide stored energy crucial for reproduction. Proteins are essential for oogenesis, particularly in the production of vitellogenin, a yolk precursor protein that supports embryonic development [10,11]. Blood meals, however, expose mosquitoes to several risks, including parasitism and unwanted blood components [12,13], and they trigger oxidative stress responses [14,15], damage tissues because of the abdomen extension [8,16], and activate immune responses to fend off parasitic infections [17,18].

The long-term implications of blood meals on resource dynamics and their interaction with parasitism remain poorly understood. To move towards a description of the role of resource allocation on the interaction between a host's life history and parasitic infection, we investigated the impact of a blood meal and infection by the microsporidian *Vavraia culicis* [19] on the longevity and resource dynamics of the mosquito *An. gambiae*. Although *V. culicis* depletes larval resources in *Ae. aegypti* [20], it has few longevity and fecundity costs [21–23], enabling us to track long-term changes in resource allocation. In our experiment **(Fig. 1)**, we exposed some larvae to *V. culicis* and offered adults a blood meal or not, and then: a) monitored the dynamics of the total protein, total carbohydrate, and total lipid content; b) measured parasite load at several time points throughout the mosquitoes' lives; and c) evaluated a possible protective role of the concentrations of resource in mosquito survival. Through this

comprehensive approach, we aimed to shed light on the influence of mosquito ecology and physiology on infection progression and outcome. Our findings showed that although resource levels throughout the adult mosquito's life were not influenced by blood meal and infection, resources were tightly linked to adult survivorship, particularly carbohydrates and lipids, suggesting a potential correlation with their mortality likelihood. This insight is crucial for advancing our understanding of the evolution of vector- and non-vector-borne diseases. Lastly, it emphasises the need for closer integration of ecology and epidemiology in vector biology research.

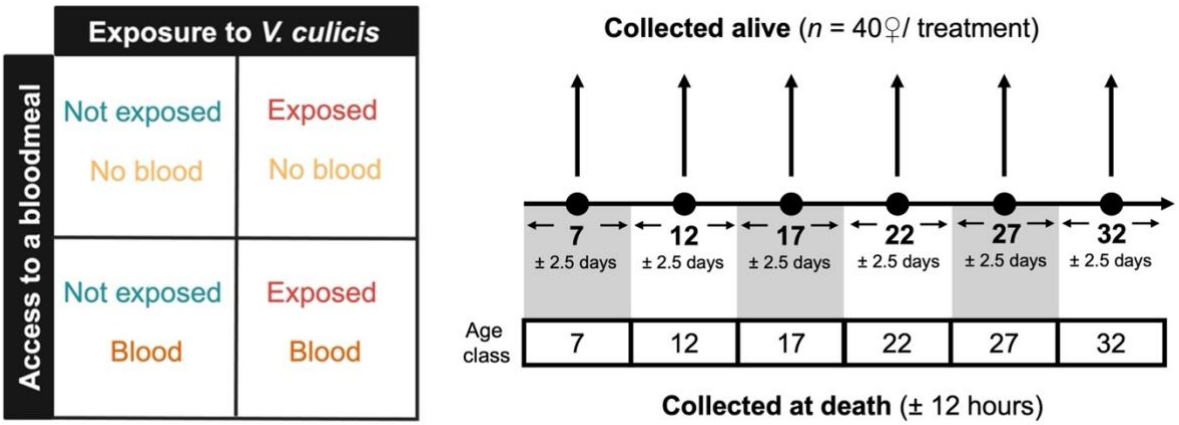


Figure 1. Experimental design. Illustration of the experiments in this study, including treatments (i.e., blood meal or not, and exposed or not to *V. culicis*), and alive and at death sample collection throughout *An. gambiae* lifetime. Individuals collected at death were allocated into subgroups (e.g., individuals that died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5 and 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals collected alive. Individuals in blood-meal treatments, had their meal on day 7 of adulthood.

Results

1. Effect of infection and blood meal on individuals that died naturally

To begin, we collected individuals that died naturally every 12 hours **(Fig. 1),** and evaluated how infection during the larval stage and blood feeding on day 7 of adulthood affected mosquito longevity and parasite development.

Mosquitoes that were not killed lived, on average, about 18.4 days. While neither exposure to *V. culicis* (χ^2 = 0.13, df = 1, p = 0.716) nor blood-feeding (χ^2 = 1.87, df = 1, p = 0.171) had a main effect on longevity, the exposure reduced the longevity of unfed mosquitoes by 4.2 days (interaction exposure * blood meal: χ^2 = 14.13, df = 1, p < 0.001, **Fig. 2a**). Exposed mosquitoes harboured, on average, about

6.6×10^5 spores, and bloodfed individuals had slightly higher parasite load ($\chi^2 = 3.91$, df = 1, p = 0.047). However, spore load increased and then decreased as age at death increased ($\chi^2 = 12.96$, df = 1, p < 0.001), particularly in unfed individuals (interaction age at death * blood meal: $\chi^2 = 5.13$, df = 1, p = 0.023, **Fig. 2b**).

Altogether, both infection with *V. culicis* or blood feeding reduced mosquito lifespan to a similar extent. However, being exposed to both stressors did not lead to greater mortality than either stressor alone, with individuals living on average 17 days.

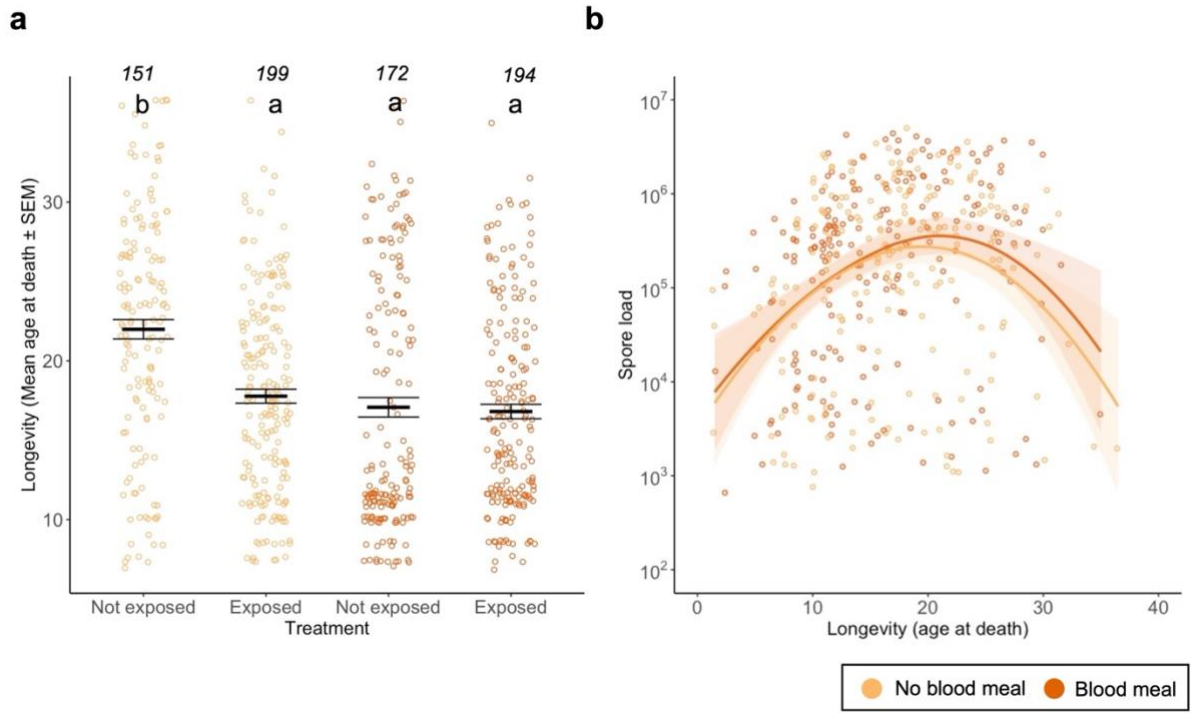


Figure 2. Effect of infection and blood meal on longevity and spore load. (a) Age at death for individuals exposed or not with *V. culicis*, that took or not a blood meal. Individuals who were collected alive were excluded from this analysis. The sample sizes (*n*) for each treatment are shown above the data points. **(b)** Individual spore load at death for individuals that had a bloodmeal (orange) or not (yellow). A smooth line was drawn for ease of visualisation with the "loess" method, and the sample sizes were 194 for females who had a blood meal and 199 if they did not. The error bars show the standard error of the mean (SEM), and the letters indicate statistically significant differences from multiple comparisons.

2. Spore load and resource dynamics in alive and dead mosquitoes across age classes

Next, we investigated spore load and energy resource dynamics in both alive and dead mosquitoes. Alive individuals were sampled every 5 days from day 7 onward, while dead individuals were collected every 12 hours and grouped into age classes to match those of live samples (**Fig. 1**).

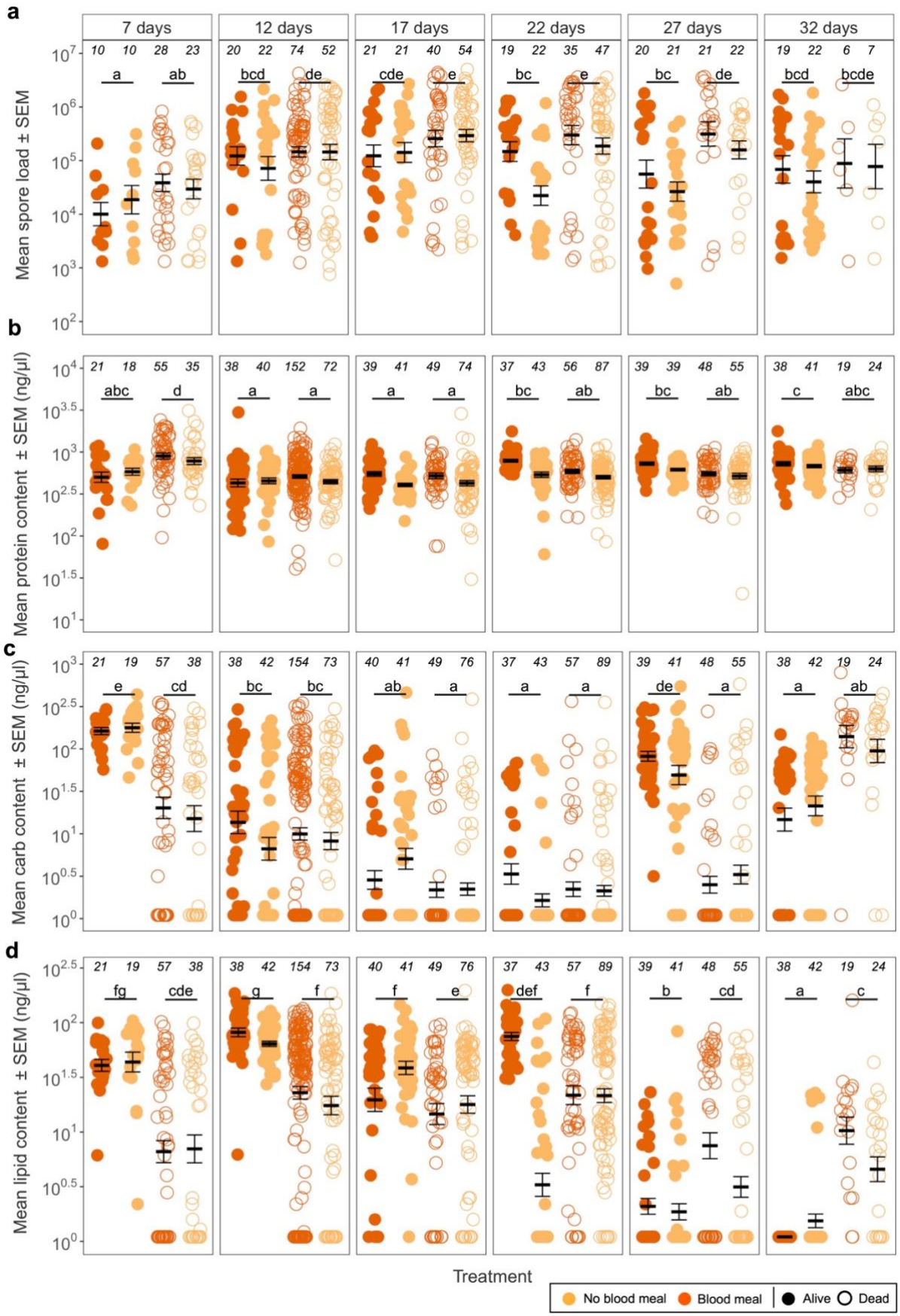
124 Spore load (**Fig. 3a**) increased with the age of mosquitoes ($\chi^2 = 27.59$, df = 5, $p < 0.001$) and was higher
125 in dead than alive mosquitoes (dead: 5.0×10^5; alive: 2.1×10^5; $\chi^2 = 8.32$, df = 1, p = 0.004, **Fig. S1**),
126 independently of their age (interaction death status * age: $\chi^2 = 3.64$, df = 5, p = 0.602). On average,
127 blood-fed mosquitoes harboured similar spore loads than unfed individuals ($\chi^2 = 1.25$, df = 1, p = 0.263)
128 with the exception of day 22 and 27 (interaction blood * age: $\chi^2 = 14.18$, df = 5, p = 0.014). These effects
129 were independent of any other combination of the treatments (for further details on the statistical
130 analysis, see **Supplementary Table S2**).

131 Protein content (**Fig. 3b**) was highest at age 7, lowest at ages 12 and 17, and intermediate at ages 22, 27
132 and 32 ($\chi^2 = 17.31$, df = 5, p = 0.004), and was higher in dead than alive mosquitoes ($\chi^2 = 15.15$, df = 1,
133 $p < 0.001$) although this effect was dependent of the age of the mosquitoes (interaction death status *
134 age: $\chi^2 = 26.32$, df = 5, $p < 0.001$; **Fig. 3b**) . It was similar in blood-fed and unfed mosquitoes ($\chi^2 = 0.06$,
135 df = 1, p = 0.799), and was independent of the infection status ($\chi^2 = 0.47$, df = 1, p = 0.492) or any other
136 combination of the treatments (for further details on the statistical analysis see **Supplementary Table**
137 **S2**).

138 Carbohydrate content (**Fig. 3c**) decreased from age 7 to age 22 and then increased as mosquitoes aged
139 further ($\chi^2 = 50.57$, df = 5, $p < 0.001$). Individuals that died naturally had fewer carbohydrates than those
140 that we killed ($\chi^2 = 7.02$, df = 1, p = 0.008), and this difference was stronger in the youngest and oldest
141 mosquitoes than at other ages (interaction death status * age: $\chi^2 = 34.21$, df = 5, $p < 0.001$; **Fig. 4c**). It
142 was similar in blood-fed and unfed mosquitoes ($\chi^2 = 0.05$, df = 1, p = 0.820), and was independent of
143 the infection status ($\chi^2 = 0.01$, df = 1, p = 0.910) or any other combination of the treatments (for further
144 details on the statistical analysis see **Supplementary Table S2**).

145 Lipid content (**Fig. 3d**) increased from age 7 to 12 and then decreased to the lowest value in the oldest
146 individuals ($\chi^2 = 52.16$, df = 5, $p < 0.001$). On average, individuals that died naturally had similar levels
147 of lipids than those that we killed ($\chi^2 = 0.52$, df = 1, p = 0.472), but their content was highly dependent
148 of their age and death status, as lipid content decreased with age in alive mosquitoes while it remained
149 constant in dead mosquitoes (interaction death status * age: $\chi^2 = 24.18$, df = 5, $p < 0.001$; **Fig. 3d**). It
150 was similar in blood-fed and unfed mosquitoes ($\chi^2 = 0.01$, df = 1, p = 0.907), and was independent of
151 the infection status ($\chi^2 = 0.73$, df = 1, p = 0.391) or any other combination of the treatments (for further
152 details on the statistical analysis see **Supplementary Table S2**).

153 Altogether, spore load and energy resource levels varied with mosquito age, but dead individuals
154 consistently harboured more spores and had lower carbohydrate levels. More importantly, alive and
155 dead mosquitoes showed distinct trajectories in protein, carbohydrate, and lipid dynamics, reinforcing
156 that physiological deterioration and energy imbalance are associated with mortality risk.


a
7 days
12 days
17 days
22 days
27 days
32 days
Mean spore load ± SEM
b
Mean protein content ± SEM (ng/µl)
c
Mean carb content ± SEM (ng/µl)
d
Mean lipid content ± SEM (ng/µl)
Treatment
No blood meal
Blood meal
Alive
Dead

158 **Figure 3. Spore and resource dynamics. (a)** Individual spore load, **(b)** protein, **(c)** carbohydrate and
159 **(d)** lipid content throughout their lifetime for individuals that took a blood meal, or not, and were
160 collected alive or dead. Individuals collected at death were allocated into subgroups (e.g., individuals
161 that died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5
162 and 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals
163 collected alive (for more details see **Fig. 1)**. The error bars show the SEM, and the letters indicate
164 statistically significant differences from multiple comparisons between alive and dead individuals. The
165 sample sizes (*n*) for each treatment and comparison can be found above the respective treatment of each
166 plot. Panel assembled in Biorender.com.

167 *3. Resource ratios dynamics in alive and dead mosquitoes across age classes*

168 Finally, using the same set of individuals (**Fig. 1**), we analysed the dynamics of resource ratios (protein-
169 to-carbohydrate, protein-to-lipid, and carbohydrate-to-lipid) in alive and dead mosquitoes to further
170 characterise the metabolic shifts associated with survival likelihood observed in the previous section.

171 The ratio of proteins to carbohydrates increased until age 22 and then decreased (χ^2 = 199.24, df = 5, p
172 < 0.001). Overall, the mosquitoes that we killed had lower P:C ratio than the ones that died naturally (χ^2
173 = 96.36, df = 1, p < 0.001), but this difference was most apparent for the youngest mosquitoes and at
174 day 27 whereas at age 32 the mosquitoes that we killed had a slightly higher P:C ratio (interaction death
175 status * age: χ^2 = 123.75, df = 5, p < 0.001; **Fig. 4a**). For further details on the statistical analysis see
176 **Supplementary Table S3**.

177 The ratio of proteins to lipids was highest at ages 7, 27 and 32, and lowest at ages 17 and 22 (χ^2 = 191.11,
178 df = 5, p < 0.001). Up to age 17, mosquitoes that we killed had a lower P:L ratio those that died naturally,
179 but older mosquitoes showed the opposite pattern (interaction death status * age: χ^2 = 104.19, df = 5, p
180 < 0.001; **Fig. 4b**) leading to alive mosquitoes having overall higher P:L ratios (χ^2 = 40.96, df = 1, p <
181 0.001). For further details on the statistical analysis, see **Supplementary Table S3**.

182 The ratio of carbohydrates to lipids was highest at ages 7, 27 and 32, and lowest at ages 17 and 22 (χ^2 =
183 153.87, df = 5, p < 0.001). Overall, this ratio was lower in mosquitoes that we killed than in those that
184 died naturally (χ^2 = 10.97, df = 1, p < 0.001), particularly if they were younger than 17 days old, but
185 higher, if they were older (interaction death status * age: χ^2 = 58.72, df = 5, p < 0.001; **Fig. 4c**). For
186 further details on the statistical analysis see **Supplementary Table S3**.

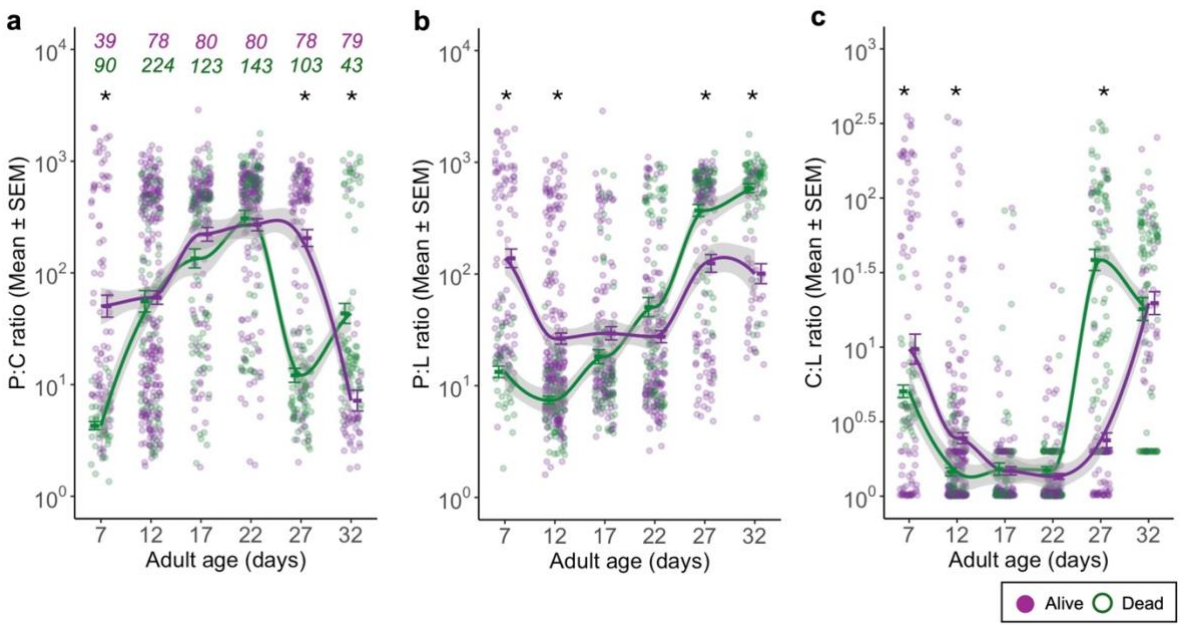


Figure 4. Resource ratios. Individual protein:carbohydrate (P:C) **(a)**, protein:lipid (P:L) **(b)** and carbohydrate:lipid (C:L) **(c)** ratios throughout their lifetime for individuals that were collected alive (pink) or dead (purple). Individuals collected at death were allocated into subgroups (e.g., individuals that died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5 and 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals collected alive (for more details see **Fig. 1)**. As carbohydrates and lipids are energetic sources, their ratio is inherently energetic. The sample sizes (*n*) are the same across the different ratios and are shown above the plot **(a)**, indicated with the corresponding treatment colour. The error bars show the SEM, and the asterisk (*) indicates statistically significant differences between dead and alive individuals for that time point.

Altogether, shifts in protein-to-carbohydrate, protein-to-lipid, and carbohydrate-to-lipid ratios consistently differed between alive and dead mosquitoes across age classes. These patterns suggest that the ability to regulate energy allocation—particularly the shift in carbohydrate-to-lipid ratio in older individuals—is closely associated with survival outcomes.

Discussion

Our findings show complex relationships between some ecological factors that the main malaria vector, *An. gambiae*, is commonly exposed to (blood meal and parasitism) and longevity, which is a fundamental life-history trait determining the mosquito's fitness and vectorial capacity. These results also suggest that shifts in resource allocation may underlie the observed patterns. In particular, although the resource levels throughout the adult mosquito's life were not influenced by blood meal or by the infection by a microsporidian, they were closely linked to survivorship.

209 Taking a blood meal shortened the mosquitoes' lives (**Fig. 2a**), most likely due to increased oxidative
210 stress and gut tissue damage in mosquitoes [12,13,24,25]. In our design, individuals were allowed to fully
211 engorge on blood during the feeding period, potentially exacerbating these costs, particularly concerning
212 tissue damage. Infection by *V. culicis* also shortened the lifespan, corroborating other studies [21,22,26]. The
213 infection cost appeared to be linked to the spore load, for mosquitoes that died naturally harboured more
214 spores than those of the same age we killed. More surprisingly, there was a cost of infection only in
215 mosquitoes that had not blood-fed, although blood-feeding increased the spore load. A possible
216 explanation is that both the production of spores and the host's survival are limited by resources
217 replenished by blood-feeding.

218 Our findings highlight that there are clear shifts in the ratios of resources during the life of the
219 mosquitoes. As protein content remained relatively stable over the lifespan (**Fig. 3b**), the shifts of P:C
220 and P:L predominantly stem from differences in carbohydrate and lipid levels, respectively (**Fig. 4ab**).
221 In particular, individuals at death consistently exhibited elevated P:C and P:L ratios over time (**Fig.**
222 **4ab**), indicative of reduced carbohydrate and lipid reserves. Conversely, alive individuals show dynamic
223 energy use, primarily relying on carbohydrates until day 22, after which lipid reserves became the
224 predominant fuel source (**Fig. 4c**). Below, we propose a few complementary explanations for these
225 findings.

226 Lipid reserves are a last resort energy source, less accessible to the host than carbohydrates, prompting
227 individuals to primarily rely on carbohydrates. Given that mosquitoes had continuous access to a 6%
228 sucrose solution, we expected them to primarily use carbohydrates for energy until their reserves were
229 depleted. Around day 20, we observed that some individuals began to struggle to reach the top of the
230 cup where the sucrose cotton balls were placed, possibly due to reduced mobility associated with aging.
231 This impaired access may have limited their ability to replenish carbohydrate stores, contributing to a
232 net decrease in carbohydrate content and triggering a metabolic shift toward lipid utilization.
233 Interestingly, this energetic shift may coincide with the timing when a new cluster of eggs is typically
234 produced. It has been proposed that in several anautogenous arthropods, including mosquitoes such as
235 *Aedes aegypti* and *Anopheles gambiae*, the initiation of vitellogenesis may rely not only on energetic
236 resources but also on iron signalling triggered by blood ingestion [8,27–29]. If such is the case for *An.*
237 *gambiae*, individuals likely begin investing in a new egg batch shortly after laying the first, around day
238 12, resulting in increased carbohydrate consumption. This is supported by a marked decrease in
239 carbohydrate content from day 12 to day 22 in alive individuals (**Fig. 4ac**). Lipids are initially used to
240 support homeostasis and later become the primary energy source. After completing the energetic
241 demands of reproduction, surviving mosquitoes appear to replenish carbohydrate reserves using the
242 available sucrose, as reflected in the increased levels observed from day 27 to day 32 (**Fig. 4bc**).

243 Moreover, when comparing the overall energetic use between individuals collected alive *versus* those
244 collected at death over the same period, distinct energetic trajectories emerge. In particular, individuals
245 that die exhibit lower energetic profiles and, as early as seven days post-emergence, tend to have fewer
246 energetic reserves compared to their surviving counterparts. This observation suggests that energetic
247 reserves measured early in adult life may hold predictive value not only for resource acquisition but also
248 for longevity. Constantly lower energetic reserves likely correspond to reduced physical condition and
249 overall fitness, increasing susceptibility to mortality. Furthermore, personal observations indicate that
250 mosquitoes nearing death exhibit reduced activity and tend to remain at the bottom of the cup. In such
251 compromised conditions, accessing sucrose at the cup's surface becomes challenging, hindering resource
252 replenishment and increasing both the usage of lipids reserves and the mortality risk. Conversely,
253 individuals with higher baseline energetic reserves may retain the energy to fly and access available
254 sucrose, as evidenced by carbohydrate replenishment observed on day 27 in surviving mosquitoes (**Fig.**
255 **4c**).

256 Thus, we propose that energetic reserves at adulthood's onset significantly influence and predict
257 longevity. Consistent with the findings of Bedhomme *et al*. (2004) in *Ae. aegypti* larvae, which showed
258 significantly reduced lipid, sugar, and glycogen levels in *V. culicis*-infected individuals compared to
259 uninfected ones [30], our results extend these insights to adults stage and to a different species, *An.*
260 *gambiae*. Although larval-stage deficits may appear modest, our data reveal the cumulative energetic
261 toll of infection over the mosquito's lifespan. By day 7, infected mosquitoes display carbohydrate levels
262 similar to uninfected individuals (189 vs. 187 ng/µL) but retain only half the lipid levels (38 vs. 61
263 ng/µL, **Fig. S3**). This underscores the long-term energetic costs of microsporidian infections on lipid
264 reserves, while also demonstrating that infected mosquitoes can recover carbohydrate levels by feeding
265 on sucrose, compensating for larval-stage deficits and achieving parity with uninfected counterparts.

266 Through a vector biology perspective, our findings reveal a noteworthy pattern with significant
267 implications for epidemiology. As mentioned above, *An. gambiae* is known as the main *Plasmodium*
268 vector. A study by Costa and colleagues (2018) demonstrated that the within-mosquito resource
269 environment affects *Plasmodium* infection success and virulence, in particular regarding lipid
270 exploitation. For instance, only 20% of the mice bitten by mosquitoes with lipid depletion were infected
271 with *Plasmodium* compared to the ones bitten by control mosquitoes, which had a 90% infection success
272 [31]. This result was attributed to the need for lipids for the correct development of *Plasmodium*
273 sporozoites and oocysts. Lipids are harder to access by the *Plasmodium* during the early stages, as they
274 are mostly stored in the ovaries. However, once the reproductive cycle is complete, lipids become much
275 more accessible. The authors proposed that the duration of *Plasmodium* sporogony, typically occurring
276 13 to 16 days after a blood meal, is an evolutionary adaptation to the temporal dynamics of mosquito
277 reproduction. Our results seem to lend support to this hypothesis as we observe that the major shifts in

lipid reserve mobility align with the expected timing of sporogony in *Plasmodium*-infected mosquitoes, suggesting an evolutionary adaptation of this parasite to timely exploit mosquito resource dynamics.

Altogether, our study highlights the significance of accounting for resource availability and usage in mosquito life studies. Our findings indicate that resource reserves are affected by the individual life history traits and environmental stressors, such as parasites. We demonstrated that blood meals impact mosquito longevity and resource dynamics, which might contribute to changes in energetic shifts within the mosquito. Furthermore, our evidence also suggests that the quantity and dynamics of energetic reserves may serve as predictive factors for mosquito survival. We believe that these results will contribute to a better understanding and prediction of the evolution of *Plasmodium* infections and hope to inspire similar studies in other vector and non-vector infection models.

Limitations of the study

In this study, we demonstrated the predictive and deterministic role of energy reserves throughout the life history of *Anopheles gambiae*. However, one major limitation of our work is the lack of experimental testing on resource dynamics during *Plasmodium* infection and co-infection with *V. culicis*, which is crucial to evaluate our hypothesis regarding resource competition during co-infection.

Another limitation is that our initial measurements were taken on day 7. While this timing ensured that all mosquitoes harboured spores, allowing us to quantify spore load, it also gave them time to replenish their resources — particularly carbohydrates — by feeding on the sucrose source, potentially hindering the true impact of infection on some measured reserves. Measuring energy reserves immediately after adult emergence would provide a clearer baseline of resources before feeding begins. Additionally, assessing resource dynamics at critical life stages — such as immediately before pupation, during the pupal stage, and just after emergence — would offer valuable insights into the energetic costs of metamorphosis. This approach would help quantify how these transformative stages deplete resources and how infection amplifies these costs, shedding light on the trade-offs imposed by infection during key developmental transitions.

Author contributions

LMS, JCK and TGZ conceived and designed the experiments. LMS and TGZ collected, analysed and wrote the first draft of the manuscript. All authors contributed critically to the drafts.

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Competing Interests

The authors declare that they have no competing interests.

Materials and Methods

Experimental system

The host of our experiment was the Kisumu strain of *Anopheles gambiae s.s.* [32], which we had maintained at a density of about 600 individuals per cage and standard lab conditions (26 ± 1ºC, 70 ± 5% relative humidity and 12 h light/dark) for several years before the experiments. During this time, cohorts were initiated weekly and kept in separate cages throughout their lives, and eggs were obtained from several cages. Our parasite was the microsporidian *Vavraia culicis floridensis* (provided by James Becnel, USDA, Gainesville, FL), which is a generalist, obligatory, intracellular parasite that infects mosquito epithelial gut cells [22]. Before beginning the study, we maintained *V. culicis* in large numbers by alternatingly infecting *Aedes aegypti* and *An. gambiae* populations to ensure that it remains a generalist parasite.

Experimental design

To start the main experiment, we combined eggs from three cages, which held mosquitoes of different ages. We used a cross-factorial design, where one factor was exposure or not to *V. culicis,* and the other was blood-fed or not. Freshly hatched *An. gambiae* larvae were moved individually into the wells of 12-well culture plates, each containing 3 ml of distilled water. They were fed daily with Tetramin Baby ® fish food according to their age: 0.04, 0.06, 0.08, 0.16, 0.32 and 0.6 mg/larva at ages 0, 1, 2, 3, 4 and 5 days or older, respectively [33]. Two-day-old larvae were exposed to 0 or 10,000 spores of *V. culicis*. Pupae were moved to sets of three (21 x 21 x 21 cm) cages per treatment group, each containing approximately 200 individuals. Mosquitoes emerged these cages and had constant access to a 6% sucrose solution. The males were removed upon emergence, and untreated males from the colony were added to the cages at a number equal to the number of females, who were left to mate for seven days. The males were then removed, and the females were allowed, or not, to take a blood meal on human skin (TGZ) for five minutes. Mosquitoes that did not take a blood meal were excluded from the experiment and two days later, the remaining mosquitoes were moved individually to 150 ml cups (5 cm Ø x 10 cm) that were covered with a net and contained a 50 mm Ø petri dish (to keep the mosquitoes from drowning) on the surface of 50 ml deionised water, a 10 x 7 cm rectangular filter paper (to keep

339 the air humid) and a 110 mm Ø Whatman filter paper. They remained in these cups for 48 hours to allow
340 egg laying (fecundity assay). After this period, the same individuals were transferred to new 150 ml
341 cups, each covered with a net and containing a 50 mm Ø petri dish floating on 50 ml of deionised water
342 (to prevent drowning) and a 10 × 7 cm piece of filter paper to maintain humidity. These mosquitoes
343 were then monitored for the longevity assay and continued to receive 6% sucrose solution throughout
344 both assays.

345 To facilitate comparisons between live and dead individuals, we assigned an age class to mosquitoes
346 based on their collection day. Live individuals were collected at five-day intervals starting on day 7
347 (before the bloodmeal) and continuing through day 32. Dead individuals were collected every 12 hours
348 as they naturally died. The 12-hour period was based on preliminary results (further details in **Fig. S2**)
349 that showed no significant degradation of any of the resources for at least 18 hours after death. The
350 collected mosquitoes were frozen at – 80ºC until we assayed spores, proteins, carbohydrates and lipids.

351 Dead mosquitoes were assigned to the same age class as their closest live counterparts to ensure
352 meaningful comparisons between live and dead individuals. Specifically, a mosquito that died within
353 ±2.5 days of a live collection day was grouped into the corresponding age class (**Fig. 1**). For example,
354 individuals that died between days 9.5 and 14.5 were assigned to age class 12, and so on. This approach
355 ensured that dead mosquitoes were temporally proximate to the live individuals in the same age class,
356 thereby improving the comparability of the two groups. The only exception was mosquitoes that died
357 before day 7, which were excluded from the analysis because the blood-meal treatment was administered
358 on day 7. Thus, the mosquitoes dying before this point had not received their assigned treatment.

359 *Spore load*

360 Since spore load and resource content are directly related to body size, all individuals were weighed and
361 transferred to a 1.5 ml microcentrifuge tube. Then, 100 µl of extraction buffer (100 mM KH_2PO_4; 1 mM
362 dithiothreitol; 1 mM EDTA) per mg of the mosquito was added to each tube, and the mosquitoes were
363 homogenised at 30 Hz for three minutes with a stainless-steel bead (Ø 5 mm) and a Qiagen TissueLyser
364 LT. From the homogenate, 20 µl were used to measure the spore load in a haemocytometer with a phase-
365 contrast microscope (400x magnification). Spore load per individual mosquito was normalised based on
366 their body size. All of the mosquitoes exposed to the parasite were infected.

367 *Resources*

368 The remaining homogenate solution was used to quantify total protein, total carbohydrate and total lipid
369 content through colourimetry with a SpectraMax i3x plate reader [34]. For each plate (and therefore
370 resource), an eight-multipoint standard curve was prepared with 10 to 400 µg/mL of albumin (for
371 proteins), 1 to 40 µg/mL of glucose (for carbohydrates) or 5 to 200 µg/mL of triolein (for lipids). All

372 samples and points of the standard curve were measured in duplicate to account for experimental
373 variation.

374 Proteins were measured with the Bradford method [35], which consists of centrifuging the samples at
375 12,000 rpm at 4 ºC for four minutes, transferring 5 µl of the supernatant into a 96-well plate, adding 200
376 µl of Bradford reagent (Sigma-Aldrich) to the samples and standards, and incubating them at room
377 temperature with soft agitation for 15 minutes. Optical density (OD) was then read at 595 nm.

378 For carbohydrates quantification, we added 20 µl of 20% sodium sulphate, 5 µl of extraction buffer and
379 1500 µL of chloroform-methanol solution (1:2) to the remaining homogenate solution, briefly mixed
380 the sample, and centrifuged it at 12,000 rpm at 4 ºC for 15 minutes. 300 µl of the supernatant were
381 placed into a quartz 96-well plate (Hellma) and left to evaporate for 40 minutes under the hood. 240 µl
382 of 1.42 g/l anthrone reagent were added to each sample and each standard. The solutions were incubated
383 for 15 minutes at room temperature and a further 15 minutes in a water bath at 90 ºC. The optical density
384 was then read at 625 nm.

385 Lipid content was assessed by pipetting 200 µl of the remaining solution into a quartz microplate and
386 incubating the plate in a preheated oven at 90 ºC for about 30 minutes. When all the solvent had
387 evaporated, 10 µl of 98% sulfuric acid was added to each well and incubated in a water bath at 90 ºC
388 for two minutes. After ice-cooling the plate for a few minutes, 190 µl of vanillin reagent was added to
389 each well, and the plate was sealed and incubated for 15 minutes with gentle agitation. Optical density
390 was read at 525 nm. All the resources were quantified according to the standard curves with the software
391 SoftMax Pro version 7.1 in the SpectraMax i3x plate reader.

392 *Statistical analysis*

393 All analyses were conducted with R version 4.4.2, using the packages "DHARMa" [36], "car" [37],
394 "glmmTMB" [38], "emmeans" [39] and "multcomp" [40]. Significance was assessed with the Anova function
395 of the "car" package [37] We used a type III ANOVA in the case of a significant interaction and a type II
396 ANOVA otherwise. When relevant, we performed post-hoc multiple comparisons with the package
397 emmeans, using the default Tukey adjustment.

398 Age at death of the mosquitoes that died naturally was analysed with a linear model with a Gaussian
399 distribution of errors, where the response variable was age at death in days and the explanatory variables
400 were infection status, blood meal and their interactions.

401 Mosquitoes with detectable spores were then used to assess the spore load. Because we counted the
402 number of spores in a haemocytometer containing 0.1 µl of the sample (i.e. 1/1000 of the total volume),
403 the detection threshold was estimated to be 1000 spores. Spore load at death (from mosquitoes that died
404 naturally) was analysed using a generalised linear model with a negative binomial distribution of errors

[41] where the response variable was spore load and the explanatory variables were age at death, blood meal and their interactions.

We then tested whether the spore load differed between blood-fed and unfed mosquitoes and between those that died naturally or that we killed. We first pooled the mosquitoes that died within 2.5 days of an age at collection (i.e., 7, 12, 17, 22, 27 and 32 days) so that the time points for the mosquitoes that died naturally or that we killed were the same. Since spores do not appear before about four days after emergence and since very few mosquitoes lived beyond 34 days, we omitted the youngest and oldest individuals. We analysed the spore load of the mosquitoes that were infected with a generalised linear model with a negative binomial distribution of errors, where the explanatory variables were blood meal, death status, age at collection and their interactions.

To analyse the mosquitoes' protein, carbohydrate and lipid content, we again pooled the mosquitoes that died naturally into groups corresponding to the ages at collection of the living mosquitoes. Because of the distribution of the resource contents, protein content was analysed with a generalised linear model with a Gamma distribution of errors whereas carbohydrates and lipids were analysed with a generalised linear model with a tweedie distribution of errors. The explanatory variables were blood meal, death status, infection by *V. culicis*, age at collection and their interactions.

Finally, we assessed differences in the ratios between resources, i.e., protein:carbohydrate (P:C), protein: lipid (P:L), and carbohydrate:lipid (C:L) ratio, as a proxy for energetic shifts in the host. Since some of the resource levels were 0, we added 1 to each value before calculating the ratio. The ratios were analysed with a generalised linear model with a Gamma distribution of errors, where the explanatory variables were blood meal, death status, infection by *V. culicis*, age at collection and their interactions.

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Figure legends

513 **Figure 1. Experimental design.** Illustration of the experiments in this study, including treatments (i.e.,
514 blood meal or not, and exposed or not to *V. culicis*), and alive and at death sample collection throughout
515 *An. gambiae* lifetime. Individuals collected at death were allocated into subgroups (e.g., individuals that
516 died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5 and
517 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals collected
518 alive. Individuals in blood-meal treatments, had their meal on day 7 of adulthood.

519 **Figure 2. Effect of infection and blood meal on longevity and spore load. (a)** Age at death for
520 individuals exposed or not with *V. culicis*, that took or not a blood meal. Individuals who were collected
521 alive were excluded from this analysis. The sample sizes for each treatment are shown above the data
522 points. **(b)** Individual spore load at death for individuals that had a bloodmeal (orange) or not (yellow).
523 A smooth line was drawn for ease of visualisation with the "loess" method, and the sample sizes were
524 209 for females who had a blood meal and 210 if they did not. The error bars show the standard error of
525 the mean (SEM), and the letters indicate statistically significant differences from multiple comparisons.

526 **Figure 3. Spore and resource dynamics. (a)** Individual spore load, **(b)** protein, **(c)** carbohydrate and
527 **(d)** lipid content throughout their lifetime for individuals that took a blood meal, or not, and were
528 collected alive or dead. Individuals collected at death were allocated into subgroups (e.g., individuals
529 that died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5
530 and 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals
531 collected alive (for more details see **Fig. 1)**. The error bars show the SEM, and the letters indicate
532 statistically significant differences from multiple comparisons between alive and dead individuals. The
533 sample sizes for each treatment and comparison can be found above the respective treatment of each
534 plot. Altogether, spore load and energy resource levels varied with mosquito age, but dead individuals
535 consistently harboured more spores and had lower carbohydrate levels. More importantly, alive and
536 dead mosquitoes showed distinct trajectories in protein, carbohydrate, and lipid dynamics, reinforcing
537 that physiological deterioration and energy imbalance are associated with mortality risk. Panel
538 assembled in Biorender.com.

539 **Figure 4. Resource ratios.** Individual protein:carbohydrate (P:C) **(a)**, protein:lipid (P:L) **(b)** and
540 carbohydrate:lipid (C:L) **(c)** ratios throughout their lifetime for individuals that were collected alive
541 (pink) or dead (purple). Individuals collected at death were allocated into subgroups (e.g., individuals
542 that died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5
543 and 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals
544 collected alive (for more details see **Fig. 1)**. As carbohydrates and lipids are energetic sources, their ratio
545 is inherently energetic. The sample sizes are the same across the different ratios and are shown above
546 the plot **(a)**, indicated with the corresponding treatment colour. The error bars show the SEM, and the

547 asterisk (*) indicates statistically significant differences between dead and alive individuals for that time
548 point.

549

550 **Supplementary information**

551 for "Energetic shifts reflect survival likelihood in *Anopheles gambiae*" by Tiago G. Zeferino, Luís M.
552 Silva, and Jacob C. Koella.

553

554 **Table S1.** Effect of infection and blood meal on individuals that died naturally.

555 **Table S2.** Spore load and resource dynamics in alive and dead mosquitoes across age classes.

556 **Table S3.** Resource ratios dynamics in alive and dead mosquitoes across age classes.

557 **Figure S1.** Average spore load in alive and dead mosquitoes.

558 **Figure S2.** Resource degradation after different times post-death.

559 **Figure S3.** Resource dynamics in alive and dead mosquitoes across age classes for infected and
560 uninfected individuals.

561 **Table S1.** Effect of infection and blood meal on individuals that died naturally.

562

Tested effect			
Model 1a – Longevity	***df***	χ^2	***p***
Infection	1	0.13	0.716
Blood meal	1	1.87	0.171
Infection : Blood meal	1	14.13	**<0.001**
Model 1b – Spore load	***df***	χ^2	***p***
Age at death	1	12.97	**<0.001**
Blood meal	1	3.91	**0.048**
Age at death : Blood meal	1	5.13	**0.023**

563

564 **Table S2.** Spore load and resource dynamics in alive and dead mosquitoes across age classes.

565

Tested effect			
Model 2a – Spore load	***df***	χ^2	***p***
Death status	1	8.32	**0.004**
Blood meal	1	1.25	0.263
Age class	5	27.59	**<0.001**
Death status : Blood meal	1	1.87	0.172
Death status : Age class	5	3.64	0.602
Blood meal : Age class	5	14.18	**0.014**
Death status : Blood meal : Age class	5	4.47	0.483
Model 2b – Proteins	***df***	χ^2	***p***
Death status	1	15.15	**<0.001**
Infection	1	0.47	0.492
Blood meal	1	0.06	0.799
Age class	5	17.31	**0.004**
Death status : Infection	1	0.53	0.466
Death status : Blood meal	1	1.84	0.175
Infection : Blood meal	1	0.51	0.476
Death status : Age class	5	26.32	**<0.001**
Infection : Age class	5	2.32	0.803
Blood meal : Age class	5	5.54	0.354
Death status : Infection : Blood meal	1	1.00	0.318
Death status : Infection : Age class	5	2.03	0.844
Death status : Blood meal : Age class	5	7.04	0.217
Infection : Blood meal : Age class	5	1.89	0.864
Death status : Infection : Blood meal : Age class	5	2.93	0.710
Model 2c – Carbohydrates	***df***	χ^2	***p***
Death status	1	7.02	**0.008**
Infection	1	0.01	0.910
Blood meal	1	0.05	0.820
Age class	5	50.57	**<0.001**
Death status : Infection	1	0.89	0.344

Death status : Blood meal	1	0.12	0.728
Infection : Blood meal	1	0.01	0.915
Death status : Age class	5	34.21	**<0.001**
Infection : Age class	5	16.54	**0.005**
Blood meal : Age class	5	9.06	0.106
Death status : Infection : Blood meal	1	3.44	0.063
Death status : Infection : Age class	5	11.86	**0.037**
Death status : Blood meal : Age class	5	7.45	0.189
Infection : Blood meal : Age class	5	21.04	**<0.001**
Death status : Infection : Blood meal : Age class	5	22.42	**<0.001**
Model 2d – Lipids	***df***	χ^2	***p***
Death status	1	0.52	0.472
Infection	1	0.73	0.391
Blood meal	1	0.01	0.907
Age class	5	52.16	**<0.001**
Death status : Infection	1	5.17	**0.023**
Death status : Blood meal	1	2.23	0.135
Infection : Blood meal	1	0.25	0.619
Death status : Age class	5	24.18	**<0.001**
Infection : Age class	5	3.69	0.594
Blood meal : Age class	5	14.36	**0.013**
Death status : Infection : Blood meal	1	3.93	**0.047**
Death status : Infection : Age class	5	6.41	0.268
Death status : Blood meal : Age class	5	17.00	**0.004**
Infection : Blood meal : Age class	5	13.08	**0.022**
Death status : Infection : Blood meal : Age class	5	14.95	**0.010**

568 **Table S3.** Resource ratios dynamics in alive and dead mosquitoes across age classes.

569

Tested effect			
Model 3a – P:C	***df***	χ^2	***p***
Death status	1	96.36	**<0.001**
Infection	1	0.10	0.751
Blood meal	1	0.21	0.644
Age class	5	199.24	**<0.001**
Death status : Infection	1	1.47	0.224
Death status : Blood meal	1	0.61	0.433
Infection : Blood meal	1	0.02	0.887
Death status : Age class	5	123.75	**<0.001**
Infection : Age class	5	81.18	**<0.001**
Blood meal : Age class	5	61.65	**<0.001**
Death status : Infection : Blood meal	1	0.95	0.330
Death status : Infection : Age class	5	7.85	0.165
Death status : Blood meal : Age class	5	6.21	0.286
Infection : Blood meal : Age class	5	128.88	**<0.001**
Death status : Infection : Blood meal : Age class	5	31.31	**<0.001**
Model 3b – P:L	***df***	χ^2	***p***
Death status	1	40.96	**<0.001**
Infection	1	0.04	0.838
Blood meal	1	0.01	0.909
Age class	5	191.11	**<0.001**
Death status : Infection	1	0.36	0.548
Death status : Blood meal	1	0.10	0.748
Infection : Blood meal	1	0.23	0.629
Death status : Age class	5	104.19	**<0.001**
Infection : Age class	5	3.75	0.586
Blood meal : Age class	5	84.52	**<0.001**
Death status : Infection : Blood meal	1	0.01	0.920
Death status : Infection : Age class	5	0.69	0.984
Death status : Blood meal : Age class	5	79.01	**<0.001**

Infection : Blood meal : Age class	5	7.82	0.166
Death status : Infection : Blood meal : Age class	5	5.42	0.366
Model 3c – C:L	***df***	χ^2	***p***
Death status	1	10.97	**<0.001**
Infection	1	0.02	0.890
Blood meal	1	0.12	0.726
Age class	5	153.87	**<0.001**
Death status : Infection	1	0.50	0.478
Death status : Blood meal	1	0.00	0.968
Infection : Blood meal	1	0.62	0.430
Death status : Age class	5	58.72	**<0.001**
Infection : Age class	5	13.46	**0.019**
Blood meal : Age class	5	24.36	**<0.001**
Death status : Infection : Blood meal	1	0.83	0.361
Death status : Infection : Age class	5	11.35	**0.045**
Death status : Blood meal : Age class	5	2.33	0.801
Infection : Blood meal : Age class	5	14.38	**0.013**
Death status : Infection : Blood meal : Age class	5	22.13	**<0.001**

570

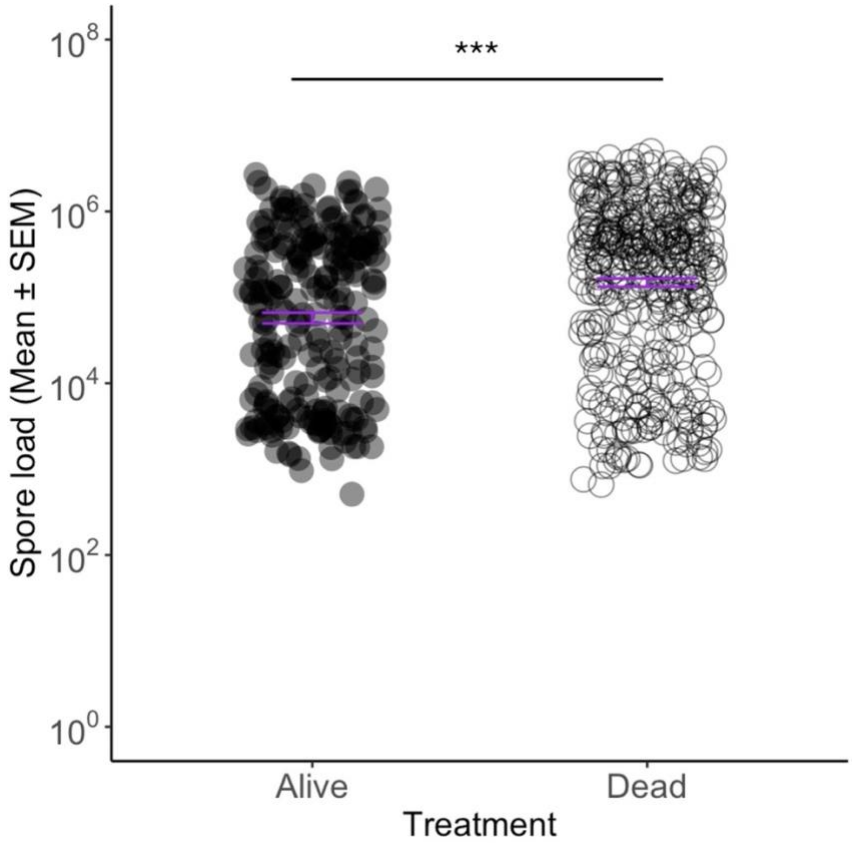


Figure S1. Average spore load in alive and dead mosquitoes. Spore load was higher in dead than alive mosquitoes (dead: 5.0×10^5; alive: 2.1×10^5; $\chi^2 = 8.32$, df = 1, p = 0.004), independently of their age (interaction death status * age: $\chi^2 = 3.64$, df = 5, p = 0.602). The error bars represent the standard error of the mean (SEM), and an asterisk indicates statistically significant differences between alive and dead individuals. The sample sizes (*n*) were 419 dead and 231 alive**.**

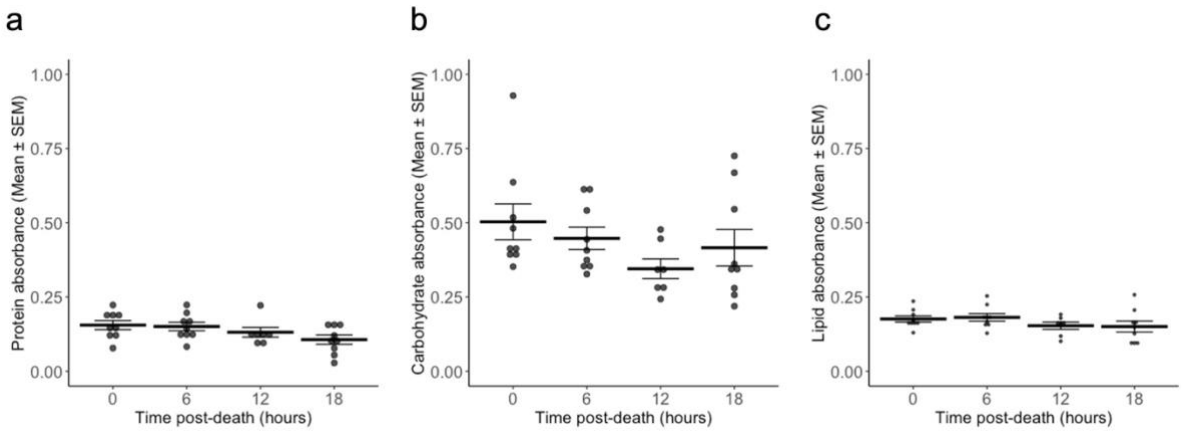
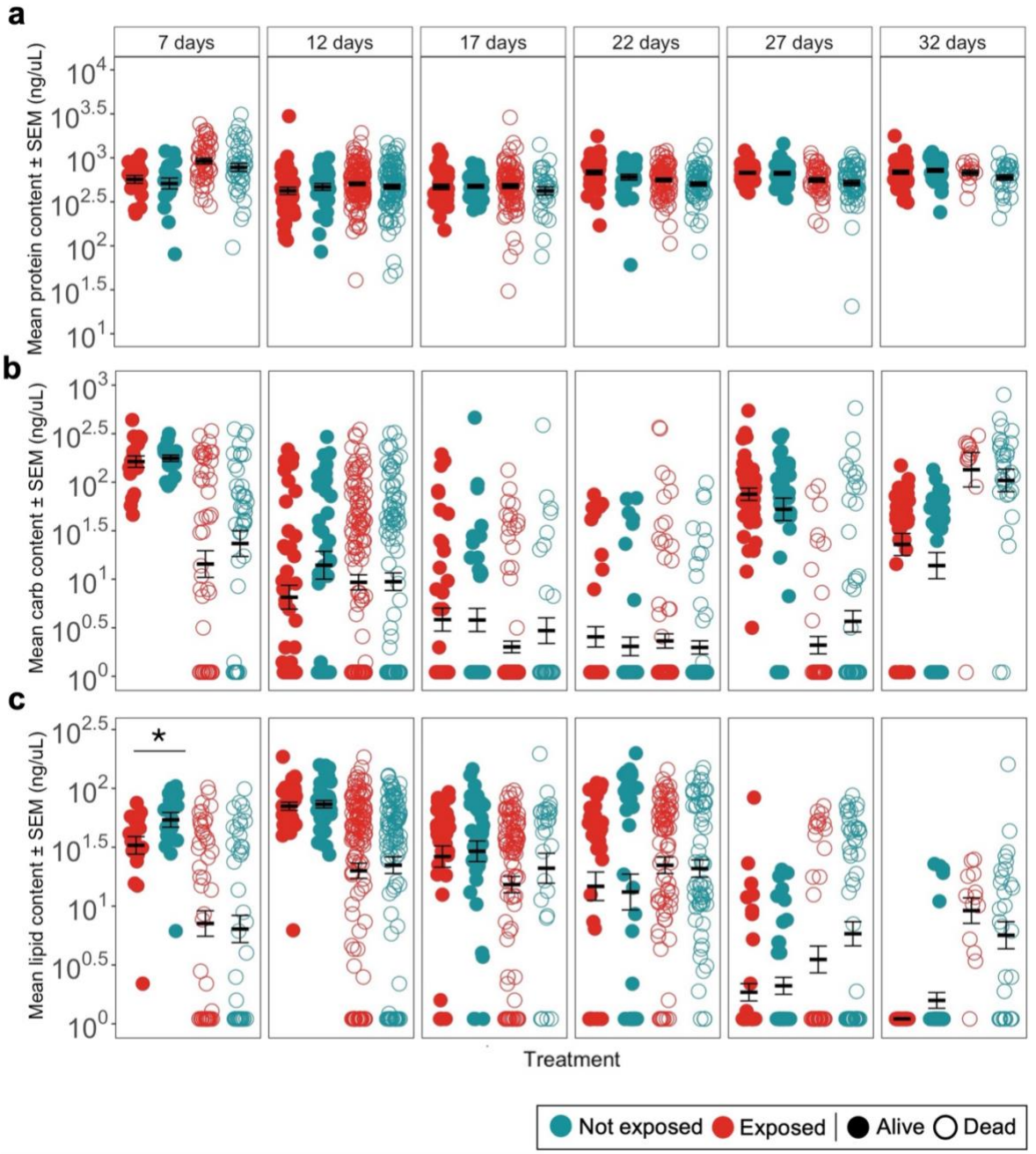


Figure S2. Resource degradation at different times after death. A preliminary experiment was carried out to estimate if there is resource degradation at different time points post mosquito death: 0, 6, 12, and 18 hours post-death. For this, eight three-day-old mosquitoes (four exposed to *V. culicis* and four unexposed) per time-point were snap-frozen at -80 ºC for 24 hours and then left at room temperature for the duration of their time treatment. At the respective time-point, each individual was frozen at -80 ºC and their resources were quantified the next morning (see the Materials and Methods). The average protein (a), carbohydrate (b) and lipid (c) content for each of the time post-death treatments is plotted above using absorbance as a proxy. The error bars show the standard error of the mean (SEM). A linear model was run for each resource category, with resource content as the response variable and time as an explanatory variable. There was no significant effect of time on the average content for any of the resource classes (Proteins: $F_{3,30} = 2.197$, $p = 0.109$; Carbohydrates: $F_{3,30} = 1.508$, $p = 0.233$; Lipids: $F_{3,30} = 1.260$, $p = 0.306$).



593

594 **Figure S3. Resource dynamics in alive and dead mosquitoes across age classes for infected and**
595 **uninfected individuals. (a)** Protein, **(b)** carbohydrate and **(c)** lipid content throughout their lifetime for
596 infected and uninfected individuals that were collected alive or dead. The error bars show the SEM, and
597 an asterisk indicates statistically significant differences from multiple comparisons between uninfected
598 and infected individuals. The sample sizes (*n*) were 19, 20, 48 and 42 for day 7; 42, 36, 123 and 101 for
599 day 12; 41, 39, 92 and 31 for day 17; 41, 39, 80 and 63 for day 22; 41, 37, 43 and 60 for day 27; and
600 lastly 41, 38, 13 and 30 for day 32 in the treatment sequence of the caption for **(a)**. The sample sizes
601 were 20, 20, 51 and 44 for day 7; 42, 38, 126 and 101 for day 12; 42, 39, 94 and 31 for day 17; 41, 39,
602 82 and 64 for day 22; 41, 39, 43 and 60 for day 27; and lastly 41, 39, 13 and 30 for day 32 in the treatment
603 sequence of the caption for **(bc)**.