

Resource depletion in *Aedes aegypti* mosquitoes infected by the microsporidia *Vavraia culicis*

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SUMMARY

Parasitic infection is often associated with changes in host life-history traits, such as host development. Many of these life-history changes are ultimately thought to be the result of a depletion or reallocation of the host's resources driven either by the host (to minimize the effects of infection) or by the parasite (to maximize its growth rate). In this paper we investigate the energetic budget of *Aedes aegypti* mosquito larvae infected by *Vavraia culicis*, a microsporidian parasite that transmits horizontally between larvae, and which has been previously shown to reduce the probability of pupation of its host. Our results show that infected larvae have significantly less lipids, sugars and glycogen than uninfected larvae. These differences in resources were not due to differences in larval energy intake (feeding rate) or expenditure (metabolic rate). We conclude that the lower energetic resources of infected mosquitoes are the result of the high metabolic demands that microsporidian parasites impose on their hosts. Given the fitness advantages for the parasite of maintaining the host in a larval stage, we discuss whether resource depletion may also be a parasite mechanism to prevent the pupation of the larvae and thus maximize its own transmission.

Key words: energy budget, pupation, metabolic rate, feeding rate, immunity.

INTRODUCTION

Parasites often alter the fecundity, longevity, growth, and development of their hosts. These life-history changes, which are ultimately thought to be the result of a depletion or reallocation of the host's resources, have been interpreted as being driven either by the host (to escape or minimize parasite-induced fitness losses) or by the parasite (to maximize its growth, development and ultimately its transmission). Host-driven changes can take different forms such as fecundity compensation (whereby the host increases egg production or brings forward reproduction to compensate for future parasite-induced fecundity losses, e.g. Agnew *et al.* 1999; Chadwick and Little, 2005), gigantism (whereby the host invests heavily in growth to prolong survival and increase the chances of surviving the infection, see Minchella, 1985, although this hypothesis remains controversial, e.g. Ballabeni, 1995; Keas and Esch, 1997; Ebert *et al.* 2004) and boosted immunity (whereby the host invests heavily in its immune

defence mechanisms at the cost of its fecundity or longevity, see Moret and Schmid-Hempel, 2000; Armitage *et al.* 2003; Schwartz and Koella, 2004). Parasite-driven changes, on the other hand, can take the form of castration (whereby the parasite mobilizes resources from reproductive organs to provide a richer environment for its own development, e.g. Jokela *et al.* 1993; Hurd, 1998; Sorensen and Minchella, 2001) or, more commonly, arrested development (whereby the parasite freezes the development of the host to maintain or prolong the conditions at which the parasite's growth is optimal, see Beckage, 1997).

Parasites can arrest or alter host development in 2 different ways; by acting on the endocrine or neural system of their hosts, or by changing the physiological conditions that trigger metamorphosis in the first place (Beckage, 1997). Baculoviruses, for example, arrest the development of their lepidopteran hosts by producing an enzyme that effectively inactivates ecdysone, the hormone ultimately responsible for metamorphosis (O'Reilly, 1995). Such evidence of a direct action of the parasite on the host's endocrine system provides the ultimate proof of a parasite-driven change. However, in many cases the effects of parasites on host development may be much more subtle and difficult to interpret. Parasites often deplete their hosts from key energetic resources in order to meet their own metabolic

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requirements (Jones *et al.* 1981; Chambers and Klowden, 1990, Schultz *et al.* 2006). Resource depletion may, in turn, inhibit metamorphosis or prevent larvae from attaining the critical weight or biomass required for pupation (Nijhout, 1999; Davidowitz *et al.* 2003; MacWhinnie *et al.* 2005). In these cases, arrested development may thus simply be a side-effect of nutrient theft and not a parasite tactic.

The microsporidian parasite *Vavraia culicis* reduces the probability of pupation of its host the mosquito *Aedes aegypti* (Bedhomme *et al.* 2004). *V. culicis* is a natural parasite of *A. aegypti* in the wild and has been reported to parasitize a number of Culicidae mosquitoes (Kelly *et al.* 1981; Fukuda *et al.* 1997). Mosquito larvae become infected by ingesting *V. culicis* spores suspended in water along with their food. Within larval cells, the parasite proliferates while passing through a series of developmental stages before producing new spores. Transmission of *V. culicis* spores is horizontal and takes place when the infected larvae die and the spores are liberated into the water. In the laboratory, the parasite cycle, from spore ingestion to the formation of new spores, requires approximately 10 days at 25 °C, while at this same temperature uninfected larvae take only about 6 days to pupate. As the emergence of the adult drastically reduces the probability of transmission of the parasite (spores do not resist desiccation), it is clearly advantageous for the parasite to prevent pupation. Previous work has shown that the decreased probability of pupation of infected larvae takes place only when mosquitoes are reared under limiting food conditions (Bedhomme *et al.* 2004), which suggests that pupation is resource dependent and that the parasite competes with the host for the available nutrients (Agnew *et al.* 2002). Microsporidia are indeed very energy-demanding parasites (Weidner *et al.* 1999), and our own results have shown that *V. culicis* spore production is limited by the nutrients available to the host larvae (Bedhomme *et al.* 2004).

In this paper we investigate the energetic budget of *A. aegypti* larvae infected by *V. culicis*. For this purpose we quantify the glycogen, sugars (glucose), lipids and protein reserves of infected and uninfected 5-day-old larvae (i.e. just before pupation) kept under a low, intermediate or high food diet. In insects, glycogen is the main energy source fuelling metamorphosis (Tolmasky *et al.* 2001). Sugars (and in particular trehalose, a glucose dimer) have been directly implicated in triggering the hormonal cascade leading to metamorphosis (Jones *et al.* 1981), but are also essential for the microsporidia (Undeen and Van der Meer, 1999; Weidner *et al.* 1999). Lipids are the key energy currency in *Aedes* larvae; their large lipid stores (which have earned them the nickname "obese mosquitoes" van Handel, 1965) are essential for survival, allowing them to live

up to 15 days in the absence of food. Proteins, on the other hand, though rarely used as energetic fuel, can be burned for energy when conditions are harsh (Clements, 1992). We predict that infected larvae will be smaller and have less energetic reserves than uninfected ones. In order to determine whether eventual differences in these energetic resources are due to differences in energy intake or expenditure we also compare the feeding rate and metabolic rates (CO₂ production, a standard measure of energy expenditure) of infected and uninfected larvae.

MATERIALS AND METHODS

Probability of pupation

The experiment took place in a room maintained at 25 °C (± 1 °C), 75% (± 5 %) humidity and 12 L:12 D photo-period. On the first day of the experiment (Day 0) a cohort of recently hatched (<5 h) *A. aegypti* larvae were placed in Petri dishes (55 mm diameter) in groups of 60 with 10 ml of mineral water (Eau de Source, Carrefour, France). Half of the Petri dishes were infected by adding 20 000 *V. culicis* spores per larva (suspended in 1 ml of mineral water). Under these experimental conditions, >95% of the larvae become infected. All Petri dishes were provided with 3.6 mg Tetramin fish food. The following day (Day 1) larvae were rinsed and transferred individually to a plastic tube (25 mm diameter, 95 mm length) containing 4 ml of mineral water (as above). Infected and uninfected larvae were then allocated to either one of 3 food treatments: 0.8 mg, 1.4 mg, or 2.2 mg Tetramin (as above). Food was provided to the larvae suspended in 1 ml of mineral water (as above).

On Day 5, 240 larvae were sampled for the quantification of energetic reserves (see below). The remaining larvae ($n=400$) were checked on a daily basis and the day of pupation and adult emergence was recorded.

Energetic resources

On Day 5 of the experiment (see above), 40 infected and 40 uninfected larvae were randomly sampled from the low (0.8 mg), intermediate (1.4 mg) and high (2.2 mg) food treatments. Randomization was carried out using JMP software (version 5.1, SAS Institute, Inc.). Larvae were measured to obtain an estimate of their body size (cubed maximum thorax width, Timmermann and Briegel, 1999) and then individually ground in 230 μ l of methanol using an electric grinder (IKA Labortechnik RW 16). From this homogenate, we extracted 70 μ l for lipid, glucose and glycogen analyses and 70 μ l for the protein analysis (see below).

Quantification of lipids, glycogen and sugars (glucose) from the same specimen was carried out using a colorimetric technique developed for analysing adult mosquitoes (van Handel, 1988; Rivero and Ferguson, 2003). The protocol was modified for the analysis of mosquito larvae as follows. To each tube we added 680 μl of a chloroform-methanol mixture (1:2) and 100 μl of sodium sulphate. Sample tubes were centrifuged (300 g for 15 min) and two 350 μl fractions of the chloroform:methanol supernatant were extracted into separate Pyrex glass tubes, one for lipid analysis and the other one for sugar analysis. The solvent in all tubes was evaporated completely in a heating block at 95 °C. The precipitate was kept for glycogen determination (see below).

For lipid determination 30 μl of sulphuric acid were added to the tubes, heated at 95 °C for 5 min, and then 800 μl of vanillin reagent were added (van Handel, 1985a). This was left for 10 min to allow the colour to develop, and then read with a spectrophotometer at OD₅₂₅ against the control. Lipid concentrations were obtained from a standard curve based on commercial vegetable oil (1 mg/ml in chloroform; van Handel, 1985a). For sugar determination 800 μl of anthrone reagent were added to the tubes, which were then heated at 95 °C for 15 min and read at OD₆₂₅ against the control (van Handel, 1985b). Sugar concentrations were obtained from a standard curve based on glucose (1 mg/ml in 25% ethanol).

For glycogen determination, the precipitate was washed with 1000 μl of 80% methanol to remove any remaining sugars. Tubes were re-centrifuged at 300 g and the supernatant was disposed of. Glycogen concentrations were determined by adding 800 μl of anthrone reagent to the washed precipitate and heating at 95 °C for 15 min. Heat breaks down the glycogen into glucose units. The sample was read at OD₆₂₅ and glycogen concentrations were obtained against a standard glucose curve.

Protein analysis was carried out using the Bradford dye-binding micro-assay procedure (Bradford, 1976). For this purpose, 70 μl of each sample homogenate (see above) were placed in a plastic tube (7.5 cm length, 1 cm diameter), to which 730 μl of physiological water and 200 μl of Bradford reagent (Bio-Rad Laboratories, Munich, Germany) were added. After 15 min, samples were read in a spectrophotometer at OD₅₉₅. Protein concentrations were obtained from a standard curve based on bovine serum albumin (Sigma-Aldrich).

Metabolic rates

Metabolic rates of infected and uninfected mosquitoes were recorded as carbon dioxide (CO₂) production. For this purpose a second cohort of larvae was set up as above and half of them were infected

with 20 000 *V. culicis* spores per larva. On Day 1, 240 infected and 240 uninfected larvae were individually transferred to *Drosophila* tubes and provided with either a low (0.8 mg), intermediate (1.4 mg) or high (2.2 mg) amount of food. The protocol followed was identical as above. On Day 5 of the experiment larvae from each infection \times food treatment were randomly sampled from the tubes and placed in glass measuring bottles in groups of 10 with 30 ml of mineral water (Eau de Source, Carrefour, France) and no food. Preliminary tests had shown 10 to be the optimum number of larvae required for CO₂ detection using our protocol. Bottles were sealed and carbon dioxide production (ppm: parts per million) was measured using a micro-gas chromatograph (Varian CP-4900). For each bottle, 2 replicate CO₂ measurements were taken 2 h after the bottle was sealed (t_{2h}), and 2 more 4 h later (t_{6h}). Measurements lasted 2 min, and the 2 replicate measurements were spaced by 1 min. The amount of CO₂ present in the bottle at each time-point (t_{2h} and t_{6h}) was calculated by averaging the 2 replicate measurements taken. The amount of CO₂ produced per h was calculated as $(\text{CO}_2 t_{6h} - \text{CO}_2 t_{2h})/4$. After the CO₂ measurements were taken, the larvae from each bottle were dried with filter paper and weighed together (Mettler Toledo MX 5 microbalance). Two blocks of replicates were set up, each with 24 replicates (12 bottles with infected, 12 bottles with uninfected larvae each).

Feeding rate

In order to test for differences in the rate of food ingestion, a third cohort of infected and uninfected larvae was set up as above and provided with one of 3 food treatments (0.8 mg, 1.4 mg or 2.2 mg). On Day 5 of the experiment, larvae were individually transferred to a new *Drosophila* tube containing green algae and left to feed *ad libitum* on the algae until their guts were filled (the dark gut contents are visible through the cuticle to the naked eye). One larva from each treatment \times infection combination was then individually transferred to a new *Drosophila* vial containing 5 ml of mineral water (as above) and 1 mg of brewer's yeast. The 6 tubes were continuously observed until the dark gut contents were entirely replaced by the white yeast. This was repeated 13 times (total $n=78$, $n=13$ for each treatment \times infection combination) on the same day. At the end of each observation, the length of the larvae (tip of the head to the base of the syphon) was measured as an estimate of the length of their digestive tube. The size of larvae (cubed maximum thorax width) was also measured. The ingestion rate was calculated as the amount of time (in min) taken for the dark gut contents to be entirely replaced, corrected by the length of the digestive tube (in millimetres).

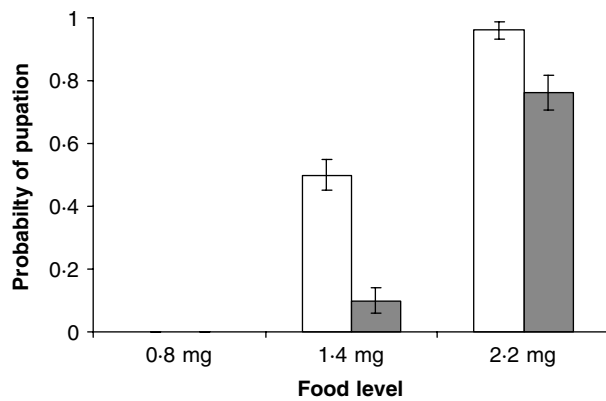


Fig. 1. Mean probability of pupation for uninfected (white columns) and infected (grey columns) larvae at each of the three different food levels. Bars represent standard errors.

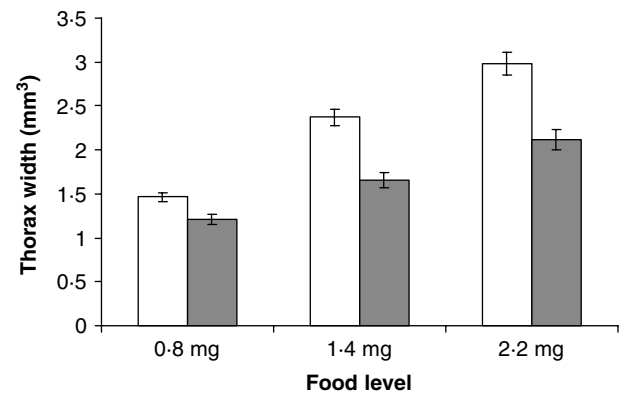


Fig. 2. Mean size (cubed thorax width) of uninfected (white columns) and infected (grey columns) larvae at each of the three different food levels. Bars represent standard errors.

Statistical analyses

The data were analysed using generalized linear modelling techniques available in the GLMStat (v.6.0, 2005, <http://www.glmstat.com>) and JMP (SAS Institute Inc, Cary, NC), statistical packages. The analyses were carried out by building up a *full model* containing all potential explanatory variables (infection treatment and food level) and their interaction. The significance of each element was assessed by removing it from the model and analysing the resulting change in deviance (Crawley, 1993). The significant values given in the text are for the *minimal model* (the model containing only significant terms and interactions) while non-significant values are those obtained prior to the deletion of the variable from the model. The energetic resources (lipids, glycogen, sugars, and protein) and the feeding rate were log transformed to correct the non-normality of errors (Sokal and Rohlf, 1995). The probability of pupation was analysed using binomial errors and the logistic link (Crawley, 1993).

RESULTS

Probability of pupation

Both infection status and food level had a strong effect on the probability of pupation of the larvae (interaction food \times infection status $\chi^2 = 10.70$, 2 D.F., $P < 0.01$). The probability of pupation increased with food level, but was significantly lower for infected larvae, whatever the food treatment (Fig. 1). These results are in accordance with those published previously (Bedhomme *et al.* 2004).

Energetic resources

Infection treatment and food levels had a significant effect on the size of larvae. Infected larvae were

significantly smaller than uninfected ones (mean \pm s.e.: 1.66 ± 0.06 mm³ and 2.27 ± 0.07 mm³ respectively, $F_{1,239} = 64.83$, $P < 0.0001$) and all larvae were significantly larger at higher food treatments (mean \pm s.e.: 1.34 ± 0.04 mm³, 2.00 ± 0.07 mm³, and 2.55 ± 0.09 mm³, for 0.8, 1.4 and 2.2 mg respectively, $F_{2,239} = 84.65$, $P < 0.0001$, see Fig. 2). The remaining analyses were carried out correcting the amount of energetic resources by the size of the individual.

Infected larvae had significantly less lipids, glycogen, and sugars per size unit than uninfected larvae (see Table 1 for a summary of results). This effect was consistent across food treatments (no significant interactions between food and infection; lipids: $F_{1,237} = 1.81$, glycogen: $F_{1,234} = 1.42$, sugars: $F_{1,235} = 0.28$, $P > 0.05$ for all analyses, Fig. 3). The food provided to the larvae had a significant effect on the level of lipids, which increased drastically with food level ($F_{1,237} = 72.43$, $P < 0.0001$) and, to a lesser extent, of glycogen, which decreased with food level ($F_{1,234} = 3.87$, $P < 0.05$). There was no effect of food on the levels of sugars (sugars: $F_{1,238} = 1.98$, $P > 0.05$). Although infected larvae had overall less proteins ($F_{1,239} = 86.75$, $P < 0.0001$), the amount of protein per unit size did not depend on whether the larvae were infected or not (Table 1) or on the amount of food provided to the larvae ($F_{1,239} = 0.09$, $P > 0.05$). Refining the analyses to include variation in larval instar (3rd or 4th) or sex did not add much clarity to the overall trends obtained and so are not presented.

Metabolic rate

The metabolic rate (amount of CO₂ produced in 4 h) was strongly dependent on the weight of larvae, with heavier larvae producing more CO₂ ($F_{1,47} = 36.02$, $P < 0.0001$). To correct for the effect of weight, analyses were thus carried out on the amount of CO₂

Table 1. Differences in the energetic resources, respiration rate, and feeding rate between infected and uninfected larvae^a

	Uninfected	Infected	Test statistics
Energetic resources ^b	Mean (lower, upper 95% confidence limits)		
Lipids ($\mu\text{g}/\text{mm}^3$)	18.31 (17.20, 19.50)	16.39 (15.56, 17.25)	$F_{1,238} = 9.02, P < 0.01$
Glycogen ($\mu\text{g}/\text{mm}^3$)	4.74 (5.43, 4.14)	3.57 (3.98, 3.21)	$F_{1,235} = 12.22, P < 0.001$
Sugars ($\mu\text{g}/\text{mm}^3$)	5.93 (5.06, 6.95)	4.29 (3.78, 4.89)	$F_{1,238} = 11.72, P < 0.001$
Protein ($\mu\text{g}/\text{mm}^3$)	19.62 (18.75, 20.53)	19.38 (19.31, 20.11)	$F_{1,239} = 0.19, \text{ns}$
Respiration rate ^c	Mean (s.e)		
CO ₂ production (ppm/mg)	21.23 (1.050)	20.99 (1.050)	$F_{1,47} = 0.02, \text{ns}$
Feeding rate ^b	Mean (lower, upper 95% confidence limits)		
Gut emptying rate (min/mm)	5.38 (4.80, 6.02)	5.23 (4.70, 5.83)	$F_{1,70} = 0.21, \text{ns}$

^a Values are pooled across food levels (the interaction food \times infection was not significant, see text for details) and represent least square means arising from statistical models.

^b Energetic resources and feeding rate were log-transformed in order to correct the non-normality of errors. The values reported are thus the back-transformed (antilog) means and the upper and lower 95% confidence limits (Sokal and Rohlf, 2003).

^c For the respiration rate the values reported are the means and standard errors.

produced per larval mg. There was no difference between the two blocks of measurements ($F_{1,47} = 1.59, P > 0.05$) and so data from both blocks were pooled for subsequent analyses. No difference was found between the CO₂ produced per unit weight by infected and uninfected larvae (Table 1). Although larvae in the higher food treatments had a tendency towards higher CO₂ production (means \pm s.e.: $18.66 \pm 1.11, 21.87 \pm 1.28$ and 22.80 ± 1.28 ppm/mg, for 0.8 mg, 1.4 mg and 2.2 mg of food respectively), this difference was not statistically significant ($F_{1,47} = 2.87, P = 0.06$).

Feeding rate

Feeding rate (amount of time taken by larvae to empty gut contents corrected for larval length) was strongly dependent on the amount of food provided during larval growth; as food increases, larvae increase their feeding rate (mean \pm s.e.: $7.61 \pm 0.39, 5.06 \pm 0.29$ and 4.49 ± 0.18 min/mm, for 0.8 mg, 1.4 mg and 2.2 mg respectively, $F_{1,70} = 28.12, P < 0.0001$). Feeding rate was, however, independent of whether the larvae were infected or uninfected (Table 1).

DISCUSSION

The probability of pupation of *A. aegypti* was strongly dependent on the nutritional resources and infection status of the larvae. Infected larvae had a significantly lower probability of pupation at all food levels except the lowest (0.8 mg), where nutrients were so limited that no pupation is possible for any larvae. Infected larvae were significantly smaller and had significantly lower energetic reserves (lipids, sugars and glycogen) per size unit than uninfected larvae.

There are at least 3 possible explanations for the fate of these missing resources. The first possibility is that the difference in larval reserves is due to differences in the acquisition of resources between infected and uninfected mosquitoes. A reduction in feeding rate is indeed a common outcome of parasitic infections, and is thought to be responsible for the decrease in developmental rate of infected hosts (Beckage, 1997). We found no significant differences in the feeding rate of infected and uninfected larvae. Feeding rate is a good indicator of energy intake unless assimilation rates differ between infected and uninfected larvae due to e.g. a damaged gut wall as a result of the infection. We are currently investigating this hypothesis using radio-isotope labelled nutrients (see, for example, Guiguère, 1981).

A second possibility is that resources may have been used by the host either to (i) repair the damage caused by the parasite or (ii) defend itself from the infection. Microsporidian infections damage the fat body, the midgut epithelium and possibly also the ovaries of insects (Becnel and Andreadis, 1999), but there is, to our knowledge, no experimental evidence that this damage is repaired. Theory indeed predicts that organisms should tolerate rather than repair damage, unless repair brings immediate fitness benefits (Kirkwood, 1977; Yearsley *et al.* 2005). In contrast, there is plenty of evidence that insects deploy efficient immune responses against parasites and that these responses are costly for the host in terms of reduced fecundity and longevity (Moret and Schmid-Hempel, 2000; Ahmed *et al.* 2002; Armitage *et al.* 2003; Schwartz and Koella, 2004). Available evidence suggests that such life-history costs arise through a reallocation of resources to fuel immunity (Koella and Sorensen, 2002; Siva-Jothy and Thompson, 2002; Freitak *et al.* 2003). It is, however, still unclear what, if any,

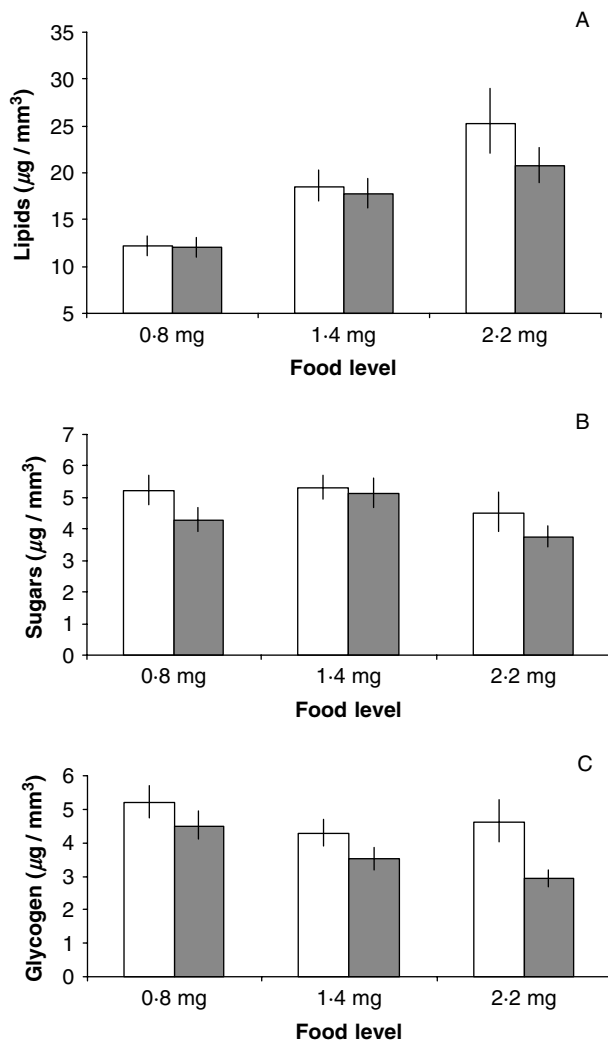


Fig. 3. Mean amount of lipids (A), sugars (B) and glycogen (C) per unit size (cubed thorax width) for uninfected (white columns) and infected (grey columns) larvae at each of the three different food levels. Figures represent the values from their respective minimal models back transformed (antilog). Bars represent the 95% confidence intervals (for details see Sokal and Rohlf, 1995).

immune responses are triggered by a microsporidian infection. The traditional view is that because ingested spores rapidly penetrate cells of the digestive tube, they effectively remain hidden from the immune system of the mosquito, except for the occasional encapsulation response when new spores are released into the haemolymph (Becnel and Andreadis, 1999). Recent proteomic studies, however, seem to indicate that *V. culicis* infection stimulates the synthesis of antibacterial peptides, and possibly also triggers the nitric oxide cascade, of *A. aegypti* mosquitoes, although the functional significance of these findings has not yet been explored (Biron *et al.* 2005). We found no differences in the metabolic rate of infected and uninfected *A. aegypti* mosquito larvae, which would suggest that if

microsporidia elicit an immune response either it is not elicited at energetically costly levels or that the energy spent in this response was saved elsewhere.

The third possibility is that resources may have been used by the parasite. By virtue of the obligate intracellular nature of microsporidia, these parasites depend completely on the resources provided by the host. We have previously shown that replication of *V. culicis* within the host is limited by the amount of resources available to the mosquito (Bedhomme *et al.* 2004). Other studies have been able to prove that microsporidia have very high carbohydrate and lipid demands and that they obtain these key resources from the host (Weidner *et al.* 1999; El Alaoui *et al.* 2001). Sugars (trehalose in particular) are essential for the viability of spores; the cleavage of trehalose into 2 glucose molecules increases hydrostatic pressure within spores thus triggering their germination (Undeen and Vander Meer, 1999; Weidner *et al.* 1999). Lipids, on the other hand, are required to build the highly developed internal membranes of the microsporidian spores (El Alaoui *et al.* 2001). Interestingly, however, these lipids cannot be used to sustain the parasite's energy needs directly; the microsporidia lack mitochondria and seem to obtain ATP directly from the host (Weidner *et al.* 1999).

The consequences of the parasite's drain on host resources mean they are less likely to reach adulthood and gain reproductive success. Furthermore, infected individuals that reach adulthood, are not only smaller, but have proportionately fewer resources for their size. These two factors act in concert to increase the costs experienced by infected females. They may also affect their capacity to act as vectors of other diseases. The negative impact on vectorial capacity through the decrease of pre-adult survival is obvious. The effect through the decrease of adult body size is less trivial. Xue *et al.* (1995) have shown a positive relationship between adult body size and the number of bloodmeals, while Sumanochitrapon and colleagues (1998) showed that large females had a higher probability to become infected by dengue, and therefore vector it, than smaller females. Hence, energetically-draining parasites like *V. culicis* are potentially negatively impacting on the vectorial capacity of adult females, though this would need to be directly established. However, it is clear that parasites like *V. culicis* can adversely affect their host's potential fitness, especially in conditions of limiting food availability.

In conclusion, the lower energetic resources of infected, relative to uninfected, *A. aegypti*, mosquitoes are not the result of a decreased energy intake or increased energy expenditure and are thus probably the result of competition for resources between the larvae and the parasite, which needs high levels of lipids and carbohydrates to sustain its metabolic needs. A decrease in size and in carbohydrate and

lipid levels as a result of a microsporidian infection have been reported before (Weidner *et al.* 1999; Hoch *et al.* 2002; Overton *et al.* 2006). In our system, however, the decrease in size and energetic resources is accompanied by a decrease in the probability of pupation of the larvae. Although pupation in insects is ultimately regulated by changes in ecdysone and juvenile hormone levels that take place during the last larval instar (Lan and Grier, 2004), little is known about the causal factors that trigger changes in these hormones in the first place (Nijhout, 1999). There is, however, some indication that a decrease in carbohydrate resources prevents pupation in some insects (MacWhinnie *et al.* 2005), raising the possibility that resource depletion and pupation are linked in our system. As *V. culicis* is transmitted exclusively horizontally, there is little doubt that preventing pupation favours the transmission, and thus the fitness, of the parasite. Pending further investigations into what exactly triggers the hormonal cascades that lead to pupation in *A. aegypti*, the decrease in energetic reserves of the larvae could also suggest the existence of a double selective pressure on the parasite to obtain metabolic resources and reduce the probability of pupation of its host thereby increasing its own chances of transmission.

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