Relationship between early body condition, energetic reserves and fitness in an iteroparous insect

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Abstract

1. Body condition can be defined as the amount of energetic reserves present within an individual after structural size had been accounted for (i.e. relative amounts of energetic reserves), and estimated by Body Condition indices (BCIs)
2. Several methods have been proposed to calculate BCIs. However, they have traditionally been validated in vertebrate studies and evidence of their power to predict fitness in invertebrates is scarce. Ideally, the use of a particular BCI in an animal population should be validated based on its ability to accurately reflect the relative amount of reserves available to the animal as well as its relationship to fitness.
3. We aimed at increasing the variance in female body condition of *Tenebrio molitor* beetles by subjecting them to restricted or optimal food conditions at both the larval and/or adult stage. We then explored the predictive power of several BCIs on both the absolute and relative amount of lipids and sugars present in the insect's body, and their link with adult fitness. Using an iteroparous income breeder allowed us to assess the relative effects of larval vs. adult access to nutritional resources on fecundity along several reproductive events.
4. Simple measurements of phenotypically plastic traits (i.e. mass and volume) correlated well with absolute, but poorly with relative, measures of body reserves. Conversely, we found that BCIs that corrected for the interdependence between phenotypically plastic traits and structural size strongly correlated with relative amounts of body components.
5. We found that even though the adult feeding treatment had a stronger effect, body condition at emergence, but not larval feeding treatment, also affected fecundity. Moreover, while the effect of the adult feeding treatment varied along time (i.e. egg laying rank), the effect of body condition at emergence remained constant.

6. These results show that by carefully using simple morphometric measures and BCIs, it is possible to distinguish between the effects of structural size and body condition on fitness traits in invertebrates, and to show that an iteroparous income breeder can partially rely on its early energetic state for its later fecundity.

Introduction

Body condition is classically defined as the amount reserves present in the body of an animal after maintenance costs have been accounted for (Rowe & Houle, 1996 ; Barnett et al., 2015). Its definition can also take various shapes ranging from the “general health state” to “physiological state” of an animal, and is also sometimes referred to as its “quality” (Jakob, Marshall & Uetz, 1996 ; Wilson & Nussey, 2010). The relationship between the latter and the “quality” of an individual is likely to be more complicated than stated in most studies (Hill, 2011 ; Wilder, Raubenheime & Simpson, 2016 ; Barnett et al., 2015), since the resilience or resistance to different stressors is likely to be dependent on a different set of biomarkers (Milot et al., 2014). In an attempt to capture the concept of resilience underlying these definitions, Hill (2011) defined body condition as “the relative capacity to maintain optimal functionality of essential cellular processes”. In some cases, this is assumed to rely strongly on the amount of energetic reserves an individual has at its disposal (Schulte-Hostedde et al. 2005 ; Nandy et al., 2012), which has indeed been found to predict several fitness traits in both vertebrates and invertebrates, such as dispersal (Bargielowski et al., 2012, Evenden, Whitehouse & Sykes, 2014), resistance to diseases or chemicals (Townsend et al., 2010 ; Reid & Purcell, 2011), overwintering success (Sinclair, 2015) and fecundity (Arrese & Soulages, 2010). This amount of reserves can be estimated by the direct dosage of several body components from the organism, such as fat, glycogen and protein content, but invasive dosing is problematic when an experiment requires the animals to remain alive along several sampling events. In such cases, a non-invasive approximation of energetic reserves is needed.

Some studies use body mass or body size, because the larger the individual the more body reserves it is able to store. However, a simple measurement of mass includes the mass of body structures that are used as an energy source only in cases of extreme starvation (such as bones in vertebrates or cuticular structures in invertebrates: van Niekerk, Mitchell & Engelbrecht, 2018 ; Locke, 1991). This is the rationale behind the use of body condition indices (BCIs), which are indices based on several morphological measurements, typically the measurement of a phenotypically plastic morphological trait which varies according to the intake of resources from the environment (e.g. mass or volume), corrected for a measurement of the fixed structural body size (e.g. body length). The interdependence between these two parameters can be mathematically taken into account in different ways which are still a matter of much debate in the literature (Peig & Green 2009, 2010, Moya-Laraño et al., 2008, Labocha, Schutz & Hayes, 2014).

BCIs have been originally developed for use with vertebrates, in which ethical concerns require non-destructive ways of assessing the quantity of reserves available to animals. Simple measurements of size or mass are more frequently used by invertebrate ecologists since they have been shown to be associated with fitness traits in some studies (Bista & Omkar, 2013 ; Reid & Baruch, 2010 ; Zanchi et al., 2012). Insects have a discontinuous growth marked by the occurrence of moults in between which the structural size is fixed by a rigid exoskeleton, whereas the quantity of resources accumulated increases with the duration of the intermoult, and their adult body size is also fixed. Thus, one could argue that a measurement of structural size would reflect the resources accumulated during the larval development, and not present energy reserves of the insects, and that the use of a BCI at a given growth stage is relevant (Anderson, 1974 ; Reim, Kaufmann & Blamckenhorn, 2009 ; Knapp & Knappova, 2013).

Still, whether BCIs or simple measures of size/mass are more appropriate in predicting fitness in invertebrates remains poorly understood, since to our knowledge no studies comprehensively

validate several BCIs based on both energetic reserves and fitness. Moreover, BCIs have been criticized for their lack of predictive power on the absolute quantity of reserves present in individuals (Wilder et al., 2016). However, BCIs have been introduced to take into account the Absolute Energy Demand Hypothesis (Calder, 1984), which pertains that since large animals require more energy to sustain their body function (Blanckenhorn, 2000 ; Reim, Teuschl & Blanckenhorn, 2006), energy reserves relative to size is a better predictor of fitness than absolute measures of reserves. BCIs should therefore not be expected to correlate with absolute values of reserves, but rather with their quantity corrected for structural size (DeBano, 2008 ; Peig & Green, 2009 ; 2010).

The purpose of the present study is to compare the predictive power of several BCIs and simple morphological measurements taken from females of the mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae), on the actual quantities of energetic reserves stored in their bodies both in absolute quantity and after correction for structural size. We then compared their predictive power on the fitness of the beetles. *T. molitor* is a relatively long-lived insect and is iteroparous, meaning that it feeds at the adult stage and lays eggs in several reproductive bouts along its lifespan (Hughes, 2017). As such, it is an income breeder (Stearns, 1992, Jönsson, 1997). This feature is predicted to complicate the identification of resource-based changes in life histories which could be due to past as well as present food availability (Kasumovic, Brooks & Andrade, 2009). We use this as an opportunity to explore the effects of early body condition on the long-term fitness of adult *T. molitor* females, which we estimated by their fecundity along reproductive events.

Simple morphometric measurements were taken after metamorphosis, such as the structural size and the mass of the beetles. From these measurements, we calculated several BCIs chosen from the literature, and explore their adequacy in controlling for the interdependence between a phenotypically plastic morphometric measurement and structural size.

- The traditional and more frequently used BCI in vertebrates (Blem 1990, Brown 1996, Schulte-Hostedde et al. 2005) and invertebrates (Jakob et al., 1996 ; Reinhart, 2001 ; DeBano, 2008, Kasumovic et al., 2009) consists in the **residuals from ordinary least squares (OLS) regressions with log-transformed mass as a response variable and log-transformed structural size as an explanatory variable** (hereafter referred to as **OLS_{resid}**) (Jakob et al., 1996; Hayes and Shonkwiler, 2001). Their use has been criticized by some authors because despite controlling for the lack of independence between mass and structural size, it fails to control for the lack of independence between structural size and mass (see Green 2001, Peig and Green 2009, 2010).
- To address the latter, it has been suggested that **residuals from standardised major axis (SMA) regressions** are better suited to calculate BCIs (Green 2001, Peig and Green 2009, 2010). Indeed, the authors found that in several small vertebrate species, **Scaled Mass Index (SMI)** performed better than OLS_{resid} in predicting the amount of reserves stored in the body relative to body size. However Peig and Green (2009, 2010), did not assess which BCI predicted fitness best, which would be more pertinent in most ecological studies.
- Moreover, Moya Laraño et al. (2008) proposed that the **abdomen volume** might reflect the amount of reserves stored in this compartment more faithfully than mass. We therefore additionally calculated a **Scaled Volume Index**.

We did so on groups of female beetles having experienced food shortage or not at the larval and/or the adult stage, in an attempt to increase the variance in their body condition. This experimental approach sets a context of food imbalance and limitation, in which it makes sense to restrain the definition of body condition to the amount of energetic reserves present in the bodies of *T. molitor* females, and enabling them to maintain a reproductive output in harsh conditions.

Several physiological and morphometric measurements were taken after metamorphosis and before measuring the fecundity of the beetle females. Thus, we were able to estimate whether different BCIs were performing better at identifying potential differences between the treatment groups, as well as how differences in early body condition shapes the life history traits of this iteroparous beetle species.

Materials and methods.

Insect rearing and maintenance

Young larvae (~ 1cm) were collected from a mass rearing outbred stock kept in the laboratory in wheat bran (as a substrate, is also used as a source of food) with *ad libitum* access to water, and regularly supplemented with piglet flour (as a source of proteins) and a piece of apple (for carbohydrates and vitamins). Our insects are kept at 25°C and in the dark. After collection, we reared *T. molitor* larvae either in a rich or a poor larval feeding treatment, and subsequently maintained adults in a matching or mismatching environment, i.e. similar or dissimilar to the larval feeding treatment respectively.

We later measured the effects of these treatments on female body condition and fecundity. To do so, a subset of the females generated in these treatments was killed in order to assess the quantity of energetic reserves present in their abdomen, whereas we assessed the body condition of the remaining females in a non-invasive way before allowing them to reproduce.

Generation of larval feeding treatments (L.F.T.)

After retrieving the larvae from the stock, we maintained them at a density of 250 larvae in a plastic box (L xH x l = 30 x 22 x 25 cm) in 5 L of wheat bran with *ad libitum* access to water. We kept them in 2 separate containers according to the larval feeding treatment we subjected them to while controlling for density:

- In "**poor larval feeding**" conditions (hereafter **P.L.**), larvae were simply kept in wheat bran

with unlimited access to water and no further addition of piglet flour or apple.

- In "**rich larval feeding**" conditions (hereafter **R.L.**), larvae were kept similarly to our stock, that is to say in wheat bran + piglet flour + apple.

We checked daily for the presence of pupae in these boxes.

The dynamics of pupation in our rearing conditions is such that a few larvae pupated early, preceding a "pupation peak" over 3 days, after which some larvae kept pupating late. Moreover, the growth of *T. molitor* is such that while getting closer to pupation, the food uptake of larvae stops as the insects are undergoing the physiological changes preparing metamorphosis (Connat et al., 1991). We retrieved pupae as soon as they were spotted, but we used in this experiment only the pupae formed during this "pupation peak". This way, we assumed that we minimized as much as possible the effect of the removal of conspecifics on the accumulation of energetic reserves by each individual larva.

We counted the pupae produced until the boxes were empty, in order to check that there was no difference in the number of pupae produced between the 2 boxes. There was less than 10 % difference between both. The resulting pupae were kept separately in a plastic box and checked daily for the presence of emerged adults in the teneral stage.

After emergence, adults were kept individually in Petri dishes supplied with wheat bran and a piece of apple for 5 days, time during which they reach sexual maturity. Five days after emergence the beetles were sclerotized enough to allow the determination of their sex by observation of their genitalia. As our study focused on variation in females body condition only, P.L. males were discarded, while R.L. males were transferred from their Petri dish into new plastic boxes where they were kept together according to their emergence date in wheat bran + *ad libitum* water + piglet flour + apple, waiting to be used in the later copulation experiments.

Generation of adult female treatments (A.F.T.)

Females on the other hand were still maintained individually in Petri dishes and kept differently according to the adult feeding treatment we subjected them to:

- In "**poor adult feeding**" conditions (hereafter **P.A.**), females were kept in whole grain thin wheat flour, with *ad libitum* access to water.
- In "**rich adult feeding**" conditions (hereafter **R.A.**), females were kept similarly but with addition of piglet flour and apple every second day.

In this part of the experiment, wheat bran was replaced by thin flour in order to facilitate the recovery of the eggs by sieving (600 µm).

Females of the four treatment combinations will be referred to as **P.L.P.A. for poor larval rich adult, P.LR.A. for poor larval rich adult, R.L.P.A. for rich larval poor adult, and R.L.R.A. for rich larval rich adult feeding treatments**.

Body Condition Indexes

Measure of morphological parameters

The mass of each female was measured on a Sartorius balance to a precision of 10^{-5} g before the first reproduction. The females used for assessment of the energetic reserves were weighed and measured before being frozen.

Three different measures were taken with a digital calliper (Mitutoyo, Absolute) to the nearest 0.01 mm: the length of the elytron, the width of the abdomen at the level of the second abdominal segment, and the thickness of the abdomen being the smallest thickness we can measure after pressing the elytra against the abdomen (which has been shown to correlate with fecundity in a mantid species in Maxwell, Galego & Barry, 2010). Repeatability of these measurements was assessed by taking them twice in a non-consecutive way. From these measurements we calculated the volume of the abdomen of the beetles (4/3*3.14*(length/2)*(width/2)*(thickness/2)), as advised

by Moya-Laraño et al. (2008). Being the site of storage of most energetic reserves in the fat body, we can indeed expect the abdomen volume to be condition dependent.

Dosage of the energetic reserves

We used a dosage by colorimetry to assess the lipid content as well as the glycogen and the circulating carbohydrates present in the abdomen of the beetles, by using a protocol detailed in Rivero and Ferguson (2003). In brief, frozen beetles were dissected to remove their prothorax, head, elytras and legs. The remaining abdomens were homogenized in microcentrifuge tubes containing a 2% sodium sulfate solution and incubated in a 2:1 mix of chloroform:methanol solution for 24 hours. They were then centrifuged, to separate the pellet (containing glycogen) from the supernatant (containing fat and circulating sugars). The supernatant was divided into 2 equal parts. One part we used to quantify lipids with vanillin-phosphoric acid reagent. After a brief incubation, the optical density of the resulting solution was read in a spectrophotometer (SpectraMax®, Molecular Devices) at 525 nm and compared to a standard curve made of a serial dilution of sunflower seed oil. The other part was used to quantify mono and disaccharides from the supernatant and the pellet (after breaking down of the glycogen of the pellet), by incubating each of these fractions with anthrone reagent. The optical density of the resulting solutions was read at 625 nm and compared to a standard curve made of a serial dilution of glucose. As there was a strong correlation between circulating sugars and glycogen in the beetles (Pearson's correlation of log-transformed sugars and glycogen [95% CI], r = 0.811 [0.725, 0.872], **supplementary material S1**), we considered both compounds together in the results.

Mono- and disaccharides from the supernatant represent circulating sugars mostly in the form of trehalose, as it is the main sugar present in the hemolymph of insects and serves as a storage for the glucose used in locomotory activity (Candy, 1989, Thompson, 2003), whereas glycogen stored in the fat body helps replenishing consumed trehalose (Candy, 1989). Diacylglycerol is another fuel used in locomotory activity, which is stored in the fat body

(Canavoso, 2001 ; Williams & Robertson, 2008).

Copulations and fecundity assessment

Dnervich et al. (2001) showed that the mating of T. molitor females every second day prevented their sperm depletion. Our preliminary observations showed that females could lay eggs for a week following a single copulation event, and that when mated every second day they barely laid any eggs past 2 weeks after the first copulation. We thus designed our experiment in order to prevent any sperm depletion and capture most of the fitness of the females.

Ten days after emergence females were exposed to one male for 4 hours. During copulation, a male from the R.L feeding treatment and a focal female were placed in an empty Petri dish containing only a piece of filter paper. We observed the pairs until we could record at least one copulation for each one of them, and we noticed that several events of copulation can take place in 4 hours. At the end of these 4 hours, the males were removed, put back together in a box and fed *ad libitum*, waiting to be reused 2 days later. Note that as males were not kept individually, they were randomly chosen from their stock every second day, which controls for any effect of males on female fitness. Females on the other hand were individually placed in a new Petri dish filled with flour supplemented or not with apple and piglet flour, according to their adult feeding treatment, and allowed to oviposit for 2 days until the next copulation event. The eggs of the previous Petri dish resulting from the previous reproduction event were sieved off the flour and counted, as a proxy for female fitness. There were in total 8 copulation sessions per female. Males that reached 17 days post emergence were removed from the experiment.

BCIs calculations

We decided to use three morphometric measures as indirect indices of energetic reserves: elytron length, body mass and abdomen volume.

We then computed the **residuals from ordinary least squares (OLS) regressions with log-transformed mass as a response variable and log-transformed structural size (elytron**

length in our study) as an explanatory variable (hereafter referred to as **OLSresid**) (Jakob et al., 1996; Hayes and Shonkwiler, 2001). Both mass and structural size are log-transformed because the relationships between morphological traits are known to follow a power function $Y = \alpha X\beta$ (Y being a morphological trait (e.g. mass) to be predicted, X being a second morphological trait (e.g. structural size) and α and β being constants. The constant is known as the scaling exponent (Peig and Green 2009). However OLS regressions assume that the dependent variable X is independent of the response variable Y and that X is free of errors. Both assumptions are violated since structural size will tend to be greater in individuals of higher condition and X will suffer from some error. It is also important to note that measurement is not necessarily the main source of error in structural size, a larger source of error may be variation between individuals in how well measures of structural size actually reflect true body size due to variation in body shape (Warton *et al.*, 2006, Green, 2001). The result of these violations in assumptions is that the slope of the OLS regression will be underestimated, resulting in an over-estimation of condition for large individuals and vice-versa for smaller individuals (see results).

Unlike OLS regressions, Major Axis (MA) regressions assume that X is not free of errors, that the errors in *X* and *Y* are interdependent, and that the ratio in error variance in Y and X is equal to 1 (Green, 2001; Warton *et al*., 2006). However, the error variance is expected to be greater in measurements of plastic phenotypic traits (such as mass and volume) than in measurements of structural size (Rising and Somers 1989; Green, 2001). In addition, measurements of plastic phenotypic traits and structural size are generally not measured within comparable scales. Therefore, in most cases, the error variance ratio Y/X assumption of MA regression will be violated and will lead to inflated slopes that will approach an OLS regression slope of structural size (i.e. *X*) on the plastic phenotypic trait (i.e. *Y*) (Green, 2001, see results).

To address the interdependence between mass and size, the use of **the residuals from standardised major axis (SMA) regressions** (otherwise known as reduced major axis regression) have been proposed to be a better suited BCI than OLS**resid** (Green 2001, Peig and Green 2009, 2010). SMA

regressions assume that the ratio of error variance Y/X is equal to the ratio of the true variance Y/X. Since SMA regressions standardise data, it can deal with Y and X variables measured on different scales. Its true and error variance assumptions are also more realistic than both OLS and MA regressions in most cases (Warton *et al.*, 2006, Green, 2001, see results).

Peig and Green (2009) used the Thope-Lleonar (TL) model of scaling to estimate body condition, **using the slope from an SMA regression of mass and structural size as the scaling exponent**. The benefit of Peig and Green's (2009) formula is that it retains the original mass units. We therefore corrected all of our non-fixed morphological measures (i.e. mass and volume), as well body components (i.e. lipids and sugars) for structural size (i.e. elytron length) using Peig and Green's (2009) formula. We refer to size-corrected morphological measures and body components as "**scaled**", i.e. **scaled mass index (SMI), scaled volume index (SVI), scaled lipids and scaled sugars**.

Statistical analysis

We tested the repeatability of our morphometric measures using the R package "rptR" (Stoffel and Nakagawa, 2017). We investigated Pearson correlations between morphometric measures, BCIs and measures of energetic reserves. Prior to correlations all morphometric measures and energetic reserves were log transformed. We further investigated whether log-transformed measures of energetic reserves, morphometric measures and BCIs differed between feeding treatments using linear models.

We investigated the effect of each morphological measure and BCI on total fecundity, controlling for each feeding treatment on total fecundity using a general linear model (GLM) with negative binomial distribution and a log link function, with the total number of eggs as a response variable and each morphological measure/BCI, larval feeding treatment, adult feeding treatment and the three way interaction term as explanatory variables. Because of collinearity between the

morphological measures and BCI, each measure/BCI was entered in separate models and never together in the same model. Therefore, in order to compare the relative support of each BCI, we constructed candidate models with each BCI and controlling for the effect of the adult food treatment, as well as models with each BCI but without the effect of adult food treatment, a model with only the effect of adult food treatment and the null model. We then combined all of the candidate models to assess the relative support of each morphological measure and BCI in predicting total fecundity. We calculated the sum of AICc weight of all candidate models with each morphological measure and BCI, the average R^2 across models with the measure/BCI, and the effect size, partial *r*, of the BCI on fecundity when controlling for adult feeding treatment (since the effect of adult feeding treatment was supported by model selection, see results).

T. molitor females mature eggs several times during their adult lifespan. Moreover, we noticed that some females abstained from laying eggs during some sampling periods. Since the data was zero-inflated, we considered the fecundity dynamics with a zero-inflated generalised mixed model with a negative binomial distribution and log link function. The response variable was the number of eggs laid at each egg laying event. The explanatory variables were larval feeding treatment, adult feeding treatment, egg laying rank, OLS_{resid} and the four way interaction term. The random intercept was female ID. We also present results when using mass and SMI instead of OLS_{resid}.

For all analyses, we used the information-theoretic (I–T) approach to achieve model selection (Burnham and Anderson, 2002). The relative strength of support of all possible candidate models was assessed via Akaike's Information Criterion adjusted for small sample sizes (AICc) and AICc weights (ω), the adjusted R^2 as defined by the MuMIn R package (Bartón, 2016). We also report the Cohen's D and 95% confidence intervals (Nakagawa and Cuthill, 2007) using the MBESS package (Kelley, 2018) for categorical variables. For continuous variables we report the partial *r* effect size (Nakagawa and Cuthill, 2007) and estimated the 95% confidence intervals by

bootstrap (n = 10,000) using the "boot" package (Canty and Ripley, 2019).

Cohen (1988) has proposed 'conventional' values as benchmarks for what are considered to be 'small', 'medium', and 'large' effects (r = 0.1, 0.3, 0.5 and d = 0.2, 0.5, 0.8, respectively). We will refer to such terms along the manuscript.

Results

Elytron length is a good indicator of structural size in our insect since it correlates highly with mass (Pearson's correlation [95% confidence intervals]; r = 0.776 [0.729-0.816]; **Figure 1a.**) and its size is fixed after hatching from the pupal stage, since the cuticle gets tanned and sclerotized after emergence.

The relationship between log-transformed mass and log-transformed size did not change significantly according to our larval and adult feeding treatments (SMA regressions: larval feeding treatment: LRT = 2.558, *p-value* = 0.110; adult feeding treatment: LRT = 2.080, *p-value* = 0.149). Similarly the relationship between log-transformed volume and log-transformed elytron size, did not change significantly according to our larval and adult feeding treatments (SMA regressions: larval feeding treatment: LRT = 1.824, *p-value* = 0.177; adult feeding treatment: LRT = 2.535, *p-value* = 0.111). Furthermore the relationship between all log-transformed energetic reserves (lipids and sugars) and structural size also did not significantly differ between feeding treatments (SMA regressions, all p-values > 0.05). Therefore we pooled all data together when calculating BCIs, enabling the comparison of condition between treatment groups.

Although by construction OLSresid is not correlated with structural size (**Figures 1c**.), it is positively correlated with mass (**Figures 1f**.). Therefore, as predicted, the slope of the OLS regression between the log of mass and the log of elytron size is underestimated, resulting in an over-estimation of condition for large individuals and vice-versa for smaller individuals (**Figure 1a.**; equivalent results were found when using volume instead of mass as a response variable: see **supplementary material S2**).

Once again as predicted, MA residuals did not correlate with mass (**Figure 1e.**) but strongly negatively correlated with structural size (**Figure 1h.)**. Therefore, when applying an MA regression to log-transformed mass to log-transformed elytron size, we found as stated by Green (2001) that the slope was much steeper than the slope of all other regression methods and is overestimated (**Figure 1a.**; equivalent results were also found when using volume instead of mass as an explanatory variable, see **supplementary material S2**).

When applying the SMA regression to our data, the slope of the SMA regression was within the slopes of OLS and MA regression methods (**Figure 1a.**). However SMA regression does not completely correct for the dependance between mass and size since when plotting SMA residuals against mass and elytron length, they weakly correlated positively with the former (**Figure 1d.**) and negatively with the latter (**Figure 1c.**).Because the slope of the SMA regression is used as the scaling exponent, it is perfectly correlated with log-transformed SMA residuals (**Figure 1b.**).

Correlation between estimates of body condition

Morphometric measures of elytron length, abdomen width and abdomen thickness were significantly repeatable ($p < 0.05$) with an *R* [95%CI] of 0.921 [0.902, 0.936], 0.572 [0.494, 0.641] and 0.829 [0.790, 0.861] respectively. All morphometric measures (elytron length, mass and volume) were highly correlated with absolute values of body components (lipids and sugars), with correlations tending to be higher for mass and volume than elytron length. As expected, body measures correlated poorly with body components that were scaled for structural size.

BCIs (OLS_{resid}, SMI and SVI) significantly correlated with most quantities of energetic reserves which were scaled for body size. Correlation coefficients with scaled lipids or sugars tended to be significantly higher for SVI than for SMI and $OLS_{resid,}$ suggesting that SVI is the best estimator of body condition. Finally, OLS_{resid} and SVI also correlated significantly with absolute values of energetic body reserves (with the exception of SVI with lipids) but not SMI. The latter suggests that OLS_{resid} and SVI do not control adequately for the codependence between structural size and the phenotypically plastic morphological trait, whereas SMI does. The results are shown in

Table 1.

Effect of feeding treatment on body condition

1. Body components

An additive effect of larval and adult feeding treatments on lipids content was strongly supported by model selection (ΔAICc = 6.19; **Supplementary Table S3a.**; **Figure 2a.**). The rich larval feeding treatment resulted in lower lipid content than the poor larval feeding treatment (*d* = 0.640 [0.197; 1.078]) and the rich adult feeding treatment resulted in lower lipid content than the poor adult feeding treatment (*d* = 0.961 [0.512; 1.405]).

An effect of the interaction of larval and adult feeding treatments on sugar content was strongly supported by model selection (ΔAICc = 10.87; **Table S1b.**; **Figure 2b.**). Sugar content was at its lowest in the rich larval and rich adult feeding treatment (R.L/R.A vs R.L/P.A: *d* = 1.843 [1.184; 2.489]; R.L/R.A vs P.L/R.A: *d* = 3.254 [2.386; 4.107]; R.L/R.A vs P.L/P.A: *d* = 3.450 [2.567; 4.319]), followed by the rich larval and poor adult feeding treatment (R.L/P.A vs P.L/R.A: *d* = 1.412 [0.656; 2.150]; R.L/P.A vs P.L/P.A: *d* = 1.607 [0.842; 2.356]).

2. Scaled body components

There was no evidence that larval and adult feeding treatments had an effect on adult scaled lipid composition since the best supported model was the null model (AICc ω = 0.322; **Supplementary Table S4a.**; **Figure 2c.**). There was, however, strong support that individuals reared in the "poor feeding" treatment at larval stages had higher levels of scaled sugar contents than individuals reared in the "rich food" larval treatment (ΔAICc = 9.58; *d* = 0.768 [0.321; 1.211]; **Supplementary Table S4b.**; **Figure 2d.**). Model selection did not support an effect of adult feeding treatment (**Supplementary Table S4b.**).

3. Measures of absolute body condition

Elytron length could not be affected by the adult feeding treatment in our experimental

setting, since we had to keep all adults in the same conditions, regardless of their future adult feeding treatment, until the sclerotization of their cuticle. We therefore only analysed the effects of the larval feeding treatment on elytron length. Adults emerging from the rich larval feeding treatment had a smaller elytron size compared to adults emerging from the poor larval feeding treatment (ΔAICc = 7.94; **Supplementary Table S5a.**; **Figure 3a.**; RL vs. PL: *d* = 0.353 [0.133; 0.573]).

An effect of larval feeding treatment on mass was strongly supported by model selection (ΔAICc = 5.34; **Supplementary Table S5b.**; **Figure 3b.**). Adults from the rich larval feeding treatment had a lower mass than adults from the poor larval feeding treatment (*d* = 0.287 [0.067; 0.506]). An effect of adult feeding treatment on mass was also marginally supported by model selection (ΔAICc = 2.11; **Supplementary Table S5b.**; **Figure 3b.**). However, we only found a tendency (95% confidence intervals of the estimate overlap 0) for adults from the rich adult feeding treatment to have a lower mass than adults from the poor larval feeding treatment (*d* = 0.193 [-0.024; 0.411], **Figure 3b.**). An additive effect of larval food (ΔAICc = 85.40) and adult feeding treatment (ΔAICc = 13.94) on the abdomen volume of adult females was strongly supported by model selection (**Supplementary Table S5c.**; **Figure 3c.**). Adults originating from the rich larval feeding treatment had a lower volume than adults from the poor larval feeding treatment (*d* = 1.108 [0.873; 1.341]). Similarly, adults originating from the rich adult feeding treatment had a lower volume than adults from the poor adult feeding treatment (*d* = 0.443 [0.223; 0.662]).

4. Scaled body condition

We detected no effect of both larval and adult feeding treatment on OLS_{resid} and SMI, since models with feeding treatments were not better supported than the null model (**Supplementary Table S6a. and S6b.**; **Figure 3d. and e.**). However, we found strong support for an effect of the larval feeding treatment on SVI (ΔAICc = 69.66), with higher estimates for individuals reared in the “poor feeding” treatment at larval stages than in the “rich feeding” treatment (*d* = 1.239

[0.931-1.545]; **Supplementary Table S6c.**; **Figure 3f.**). The latter result further supported that SVI estimated better body component composition than OLS_{resid} and SMI since it concurred with results found with sugar components. Model selection did not support an effect of adult feeding treatment on any of the 3 BCIs.

Relationship between feeding treatments, body condition and fitness

1. **Total fecundity**

Model selection revealed a strong support for an effect of adult feeding treatment (**Figure 4a.**) and regardless of the BCI used when controlling for body condition at emergence on total fecundity (range of ΔAICc = 17.01-23.55 depending on BCI used; see **Supplementary Table S7a.; b. and c.** and **Figure 4c.; d. and e** for results using OLS_{resid}, mass and SMI at emergence respectively). Estimates were higher for individuals kept in the "rich adult feeding" treatment than the "poor adult feeding" treatment (Figure 4a.; when controlling for OLS_{resid} at emergence: d = 0.671 [0.399-0.941], when controlling for mass: d = 0.703 [0.430-0.974], when controlling for SMI: d = 0.659 [0.387-0.929]). This represents a medium effect size according to Cohen (1988). There was however a low support for an effect of larval feeding treatment and all interaction terms on total fecundity (see **Supplementary Table S7a.**).

Partial r effect sizes and 95% confidence intervals suggest support for an association between total fecundity and all BCIs (absolute and scaled) except elytron length and, surprisingly, SVI (**Figure 4b.**). OLS_{resid} and mass also had the largest effect size (partial r [95%CI] = 0.230 [0.110; 0.341] and 0.229 [0.105; 0.342] respectively), although 95%CI for all BCIs overlapped (**Figure 4b.; Table S8**). This represents a small effect size (Cohen, 1988). OLS_{resid} was by far the predictor of total fecundity with the highest AICc sum of weights with a value of 0.669, compared to 0.330 for the remaining models (**Table S8**). In addition, models with OLS_{resid} predicted a larger part of the variance (R^2 = 0.138) than models with any other BCI (next highest value was 0.125 for SMI**; Table S8**).

We previously demonstrated that mass is the best predictor absolute body reserves, SMI is the best mass based predictor of body condition, and we demonstrate here that OLS_{resid} and mass are the best predictors of fertility. Therefore, for all subsequent analyses on fecundity, we will compare the predictive power and variance explained of OLS_{resid}, mass and SMI only.

2. Egg laying dynamics

Model selection revealed a strong support for an interaction effect of adult feeding treatment and the reproductive event on the number of eggs laid, with the number of eggs laid decreasing more sharply at each successive reproductive event for individuals in the poor adult feeding treatment compared to adults in the rich adult feeding treatment (ΔAICc = 42.47 when using OLS_{resid} as a BCI; **Figure 5a.**). There was also strong support for a positive effect of body condition at emergence (estimated either as OLS_{resid}, mass or SMI; ΔAICc = 12.98; ΔAICc = 5.57 and ΔAICc = 9.94, respectively) on the number of eggs laid at each reproductive event (**Figure 5b., 5c. and 5d.** for results with OLS_{resid}, mass and SMI respectively). There was low support of larval feeding treatment and all interaction terms on the number of eggs laid at each reproductive event (**Supplementary Tables S9, S10 and S11** for results with OLS_{resid}, mass and SMI respectively).

Discussion

In our study, we performed an extensive comparison of the ability of several morphometric measurements and body condition indices to explain the variance in body components (lipids and circulating sugars) and fecundity of *T. molitor* females, as well as their effect sizes. This approach allowed us to decipher the effects of structural size, absolute and relative quantity of body reserves on this fitness parameter. We found that volume, followed by mass, had the highest correlation coefficients with lipid and sugar abdomen reserves of *T. molitor* females. Structural size, estimated through elytron length, was the worst performing morphometric measure in predicting lipid and sugar content in the abdomen. However, mass and volume did not correlate with scaled lipids and

sugars (i.e. lipids and sugars adjusted for body size). Conversely, scaled volume index (SVI; volume adjusted for body size), followed by scaled mass index (SMI; mass adjusted for body size), had the highest correlation coefficients with scaled lipid and sugar abdomen reserves, but did not or poorly correlated with their absolute measures. OLS_{resid} (i.e. residuals from an ordinary least square (OLS) regression between log mass and log structural size) was the only body condition index (BCI) that correlated equally well with both absolute and scaled measures of body reserves.

Moreover, we observed in our data that violations in assumptions that variables X (here elytron length) and Y (here mass or abdomen volume) result in an overestimation of body condition for big individuals, and an underestimation of body condition for small individuals when using the residuals of an OLS regression between X and Y as a body condition index. On the contrary, violations in the assumption that the ratio in error variance in X and Y is equal to 1 while performing an MA regression between X and Y would lead to overestimation of body condition for small individuals and an underestimation for big individuals. The use of the residuals or the slope of the SMA regression between elytron size and mass or volume as a BCI offer a compromise between OLS and MA regressions. Taken together, our results confirm results from studies in vertebrates (Peig and Green, 2009, 2010), which state that OLS_{resid} does not control adequately for the interdependence between body size and plastic morphological traits such as mass and volume. However it is important to note that using SMA regressions to calculate a BCI remains imperfect, since residuals correlate weakly with both structural size and the plastic morphological trait.

Effect of feeding treatments on morphology, body composition and body condition

We observed, though we did not quantify it, that there was approximately two months of delay between the emergence of adults from the rich compared to the poor larval feeding treatments, the larvae in the rich feeding treatment undergoing metamorphosis earlier, which desynchronized the data collection between the two feeding treatments. *T. molitor* is indeed known to be very plastic in

its larval developmental time (Cotton & St. George, 1929). It has been shown in several insect species that restricted access to food at the larval stage can sometimes lead to a delayed maturation at a smaller adult body size (Day & Rowe, 2002, Dmitriew et al., 2009). We observed the contrary, that adult females emerging from the poor larval feeding treatment were slightly bigger (2 % difference in the elytron size) than females emerging from the rich larval feeding treatment.

While looking at the body composition of adult beetles originating from both well-fed or poorly-fed larvae, the additive effects of larval and adult feeding treatment on lipid storage suggests that *T. molitor* does not adjust its propensity to store fat according to the environment experienced at the larval stage. The interaction between larval and adult feeding treatments on carbohydrates content of adult beetles however suggests that insects which have experienced adverse feeding conditions at the larval stage increase sugar storage later in life, and that even individuals which were well-fed as larvae will increase carbohydrates storage if food is lacking at the adult stage. This is the inverse pattern of what is found in a grasshopper species, where larval food restriction influenced adult lipid but not carbohydrates storage (Hahn, 2005). In a ladybird species (Dmitriew et al., 2009), food restriction at the larval stage produced adults that were lighter and storing more fat relative to their wing area, which translated into a reduced wing loading. The authors have proposed this to represent a plastic increase of the dispersal abilities of individuals having experienced adverse juvenile conditions. In our study, beetles originating from poorly fed larvae were heavier than their well-fed counterparts. Since *T. molitor* is a flightless burrowing insect, the absence of preferential storage of fat over heavier reserves such as glycogen might reflect a relaxation of the selection pressure imposed by dispersal via flight on lipid storage and mass relative to size.

Since adult beetles originating from larvae reared in rich feeding conditions were smaller, lighter and their abdomen less voluminous than beetles originating from larvae reared in poor feeding conditions, mass relative to size was similar between feeding treatments. Therefore, body condition indices OLS_{resid} and SMI were not significantly different between feeding treatments.

However, the Scaled Volume Index (SVI), which is the only scaled index that is associated with our larval feeding conditions, did differ between larval feeding treatments, suggesting that early body condition was slightly higher in individuals reared in poor feeding conditions. Interestingly, SVI is also the BCI which shows the highest correlation coefficients with our measurements of both absolute and scaled energetic reserves, mirroring the fact that abdomen volume had the highest correlation coefficients with the absolute amount of body reserves. SMI had the second highest correlation coefficient with scaled body reserves, whereas OLS_{resid} was the BCI with the lowest correlation coefficients.

Predictive power of body condition indices on female fecundity

The effect of both mass and the standardized mass index (SMI) on fecundity were supported by model selection. Interestingly, the effect size of mass is roughly equal to the effect sizes of structural size + standardized mass index on fecundity. This suggests that the effect of mass on fecundity does indeed contain the effects of both structural size and body reserves corrected for it. Following this reasoning, approximately 40 % of the effect size of mass on fecundity is represented by structural size, whereas the other 60 % are represented by the body reserves corrected by structural size. We cannot draw the same conclusions from the volume/standardized volume index (SVI), because volume was a weaker predictor of fecundity than mass, and SVI is an even poorer predictor. This might be due to the fact that the other phenotypically plastic morphological measurements of our study correlate better with components that were not measured in this study but contribute to energy storage (i.e. water, proteins, refs).

OLS_{resid} was the best supported BCI predictor of fecundity and explained the highest amount of variance. However, mass, volume and SMI all had similar effect sizes with OLS_{resid} in predicting fecundity. Similarly to mass, the effect size of OLS_{resid} on fecundity is roughly equal to the effect sizes of SMI + size. We hypothesize that OLS_{resid} was the best supported predictor of fecundity not because it is the best predictor of body condition, but rather because it incorporates both absolute and relative quantities of body reserves slightly better than mass does, both of which are associated

with fecundity. However, OLS_{resid} have a similar effect size to other BCI because it is body condition, rather than structural size, which has the strongest predictive power on fecundity, as suggested by the low predictive power of elytron length and the higher predictive power of SMI.

Effect of feeding treatments and body condition on life history

First, we can note that we found no evidence of adaptive phenotypic plasticity (Monaghan, 2008 ; Dmitriew & Rowe, 2011), in the sense that insects having experienced poor larval feeding conditions did not achieve a higher fecundity output than insects that experienced rich larval feeding conditions when placed in poor adult feeding conditions. This is likely due to the fact that *T. molitor* females, which experienced a food shortage at the larval stage, emerge as adults having a similar quantity of body reserves (in terms of OLS_{resid} and SMI) or even a little higher (absolute body reserves in terms of mass, volume and relative body reserves in terms of SVI) than beetles having been well fed at the larval stage. As a result, we found no evidence for larval feeding treatment to affect body condition at emergence. This shows that *T. molitor* females are able to maintain homeostasis in their physiology to buffer differential availability of resources. This is likely due to the high degree of developmental plasticity of this insect, allowing individuals to compensate for adverse juvenile conditions to produce an optimal phenotype at the adult stage (Beldade et al., 2011). This necessity to achieve an optimal adult phenotype might be a strong selection pressure for developmental plasticity in this insect. There was however variation in body condition at emergence.

As expected in an income breeder species, in which individuals rely on resources accumulated during their adult life to produce offspring, the adult feeding treatment of the females had an effect on fecundity. Even though the effect of the adult feeding treatment was stronger, we also found support for a positive effect of body condition at emergence on the total fecundity of *T. molitor*. Additionally, we could have expected to find support for an interaction between body condition and egg laying rank while analysing the fecundity dynamics. This would have indicated

that early fitness depends more on resources accumulated before metamorphosis, whereas later in life resources accumulated by the adult would play a more important role. Instead, while the decrease in fecundity over time was sharper in females from the poor adult feeding treatment compared to females from the rich adult feeding treatment, we found no evidence of such a dynamic effect of body condition at emergence, i.e. the effect was constant across egg laying ranks. Overall, our data show that it is possible to disentangle the effects of past and present food availability on life-history changes in income breeders (Kasumovic et al., 2009). In our case, *T. molitor* relies partly on resources accumulated in their early developmental stages for their lifetime fecundity as income breeders.

Conclusion

We were able to determine that even though OLS_{resid} is not the best predictor of the quantity of fat and sugars stored in the insect's body, it outperforms other BCIs in terms of variance explained of the insect's fitness, but does not outperform a simple measurement of mass in terms of effect size. SMI on the other hand is a good predictor of fecundity and adequately controls for the interdependence between mass and size. Only structural size and SVI do not predict fecundity. In our case, the use of OLS_{resid}, mass or SMI highlighted equally well that food restriction at the larval stage in *T. molitor* females results in adults having a similar early body condition. It is this early body condition as well as the adult feeding treatment, rather than the larval feeding treatment, which influence the lifetime fecundity of this iteroparous insect. The choice of a given BCI or a simple measurement of mass should therefore be dependant on the question asked:

- An investigator may not be interested in body condition or structural size when predicting fecundity, but may want to control for it effects (e.g. when investigating another unrelated variable). In such cases, OLS_{resid} should be preferred since it is the parameter which explains the highest amount of variance and has the best predictive power. Note here, however, that OLS_{resid} may not be the best predictor for other

fitness related traits (e.g. survival), thus should be compared with other BCIs when investigating other fitness related variables.

- If an investigator is interested in the effects of body condition but not structural size (i.e. relative body reserves) on any given response variable (including fecundity) then SMI or SVI should be preferred.
- If an investigator is interested in the combined effects of body condition and structural size (i.e. absolute body reserves) on any given response variable (including fecundity) then mass or volume should be preferred.

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Authors contributions: Conceived the study and designed the experiments: CZ, YM & MG. Conducted the experiments: CZ & MG. Analyzed the data: MG. Wrote the manuscript: CZ & MG with contributions from YM.

Supporting information: Supplementary Material 1, containing figures and tables S1 to S11.

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Figure 1: a. Ordinary Least Squares (OLS, in black), Major Axis (MA in grey), and Standardized Major Axis (SMA, in red) regressions between the logarithm of elytron length and the logarithm of mass of individual *Tenebrio molitor* females. The correlation between the residuals of the OLS regression, mass and elytron length are represented respectively in **c.** and **f.**. The correlation between the residuals of the SMA regression, mass and elytron length are represented in **d.** and **g.**. The correlation between the residuals of the MA regression, mass and elytron length are represented in **e.** and **h.**.
b. Correlation between the residuals of the SMA regression and the logarithm of the Standardized Mass Index (SMI) calculated using the slope of the SMA regression according to the formula of Peig & Green (2009). Each dot represents an experimental individual.

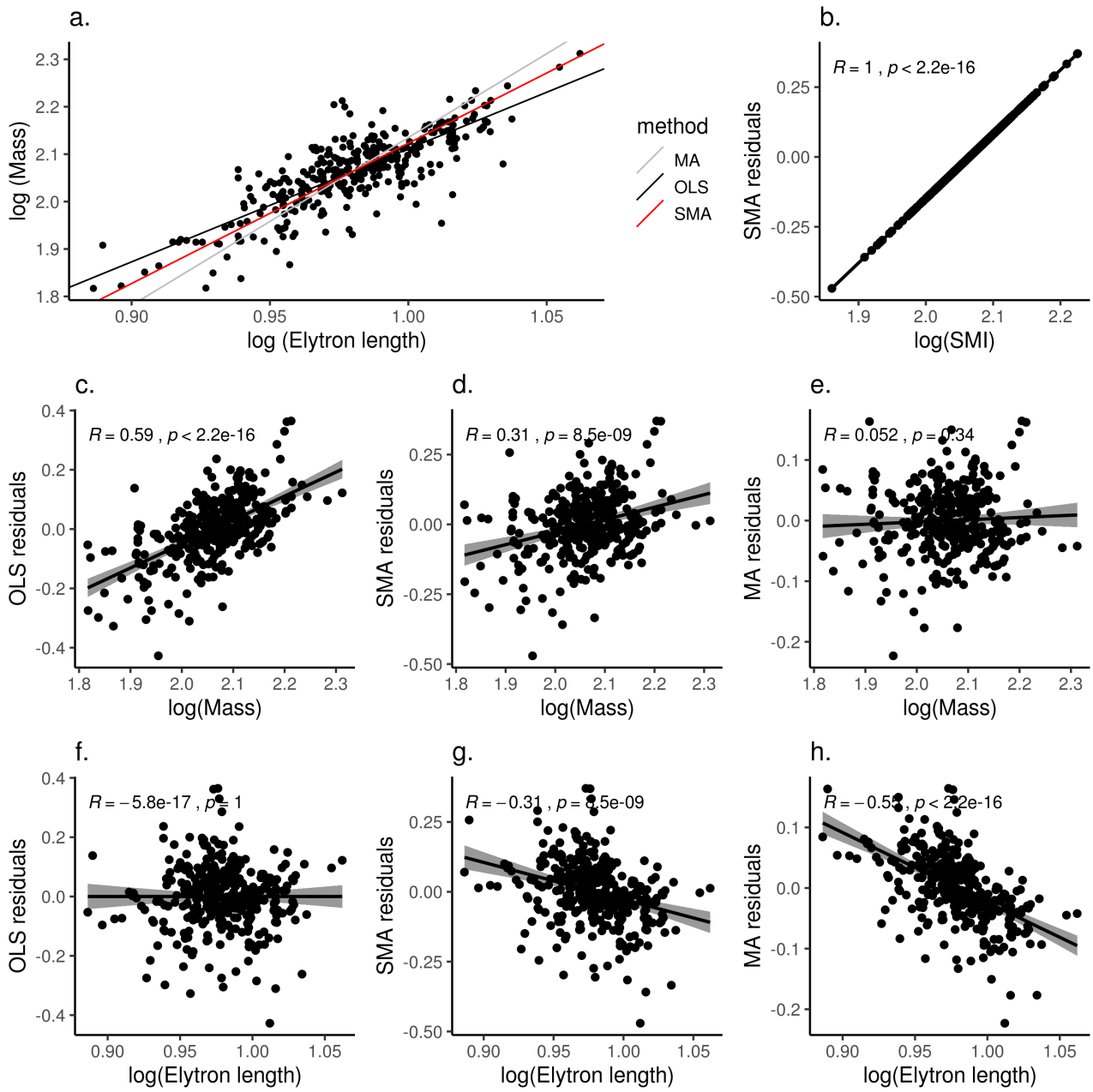

Figure 2: Relationship between the larval feeding treatment (LFT), adult feeding treatment (AFT) and **a.** the logarithm of the lipid quantities **b.** the logarithm of the quantities of sugars (glucose and glycogen) **c.** the logarithm of the lipids scaled for structural size (see methods) and **d.** the logarithm of the sugars scaled for structural size in *T. molitor* females. The boxes show the first to the third quartiles and the median. The bars indicate the 1.5 interquartile of the lower and upper quartiles. Each dot represents a data point. The open dots represent the individuals from the rich larval feeding treatment, whereas the plain dots represent individuals from the poor larval treatment. The black dots represent the rich adult feeding treatment, while the red dots represent the poor adult feeding treatment.

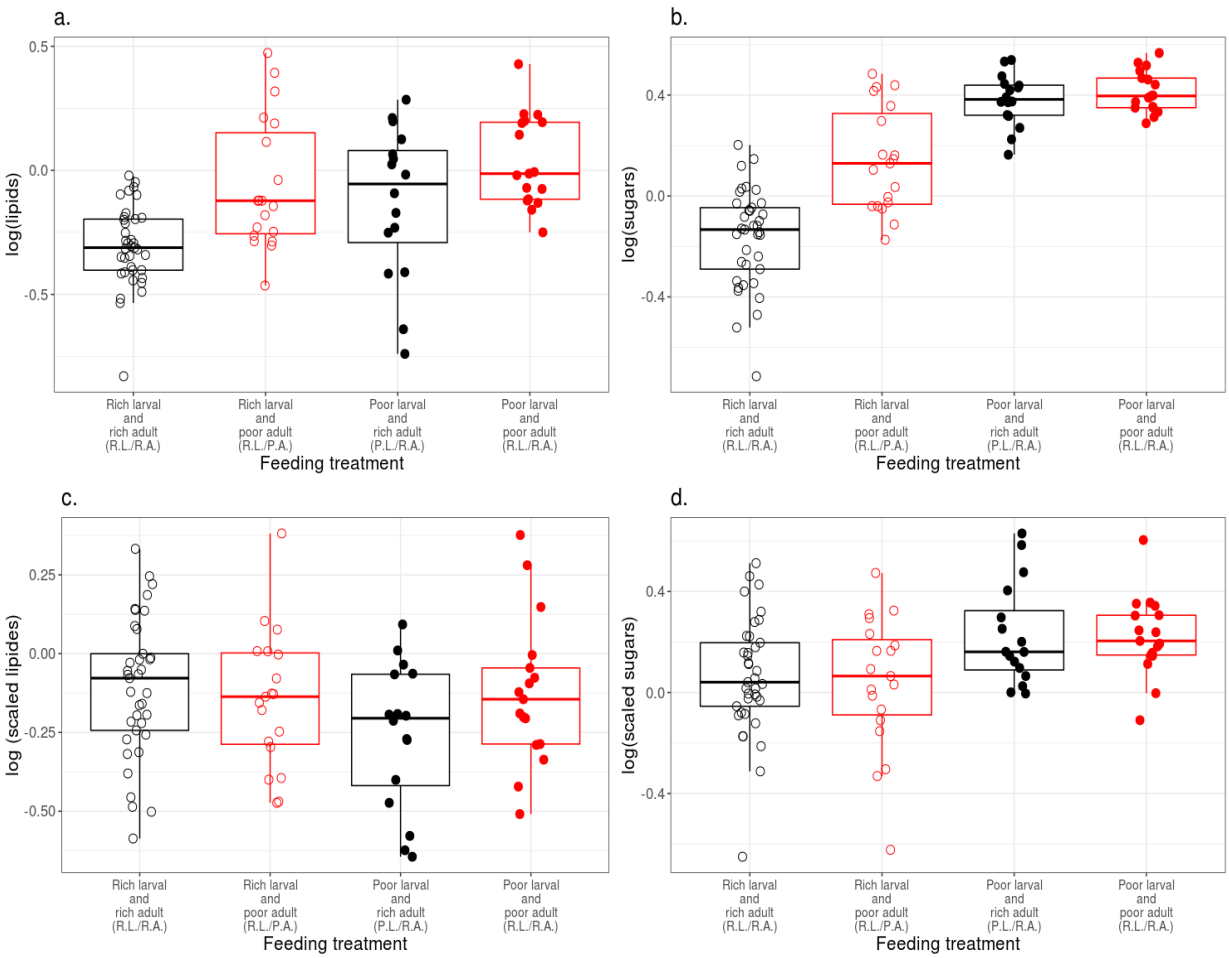

Figure 3: Effect of LFT and AFT on **a.** elytron length in centimeters (only according to LFT only), **b.** mass in milligrams, **c.** volume in cubic centimeters, **d.** OLS_{resid}, **e.** SMI in milligrams, **f.** SVI in cubic centimeters. The boxes show the first to the third quartiles and the median. The bars indicate the 1.5 interquartile of the lower and upper quartiles. Each dot represents an experimental individual. The open dots represent the individuals from the rich larval feeding treatment, whereas the plain dots represent individuals from the poor larval treatment. The black dots represent the rich AFT, while the red dots represent the poor AFT.

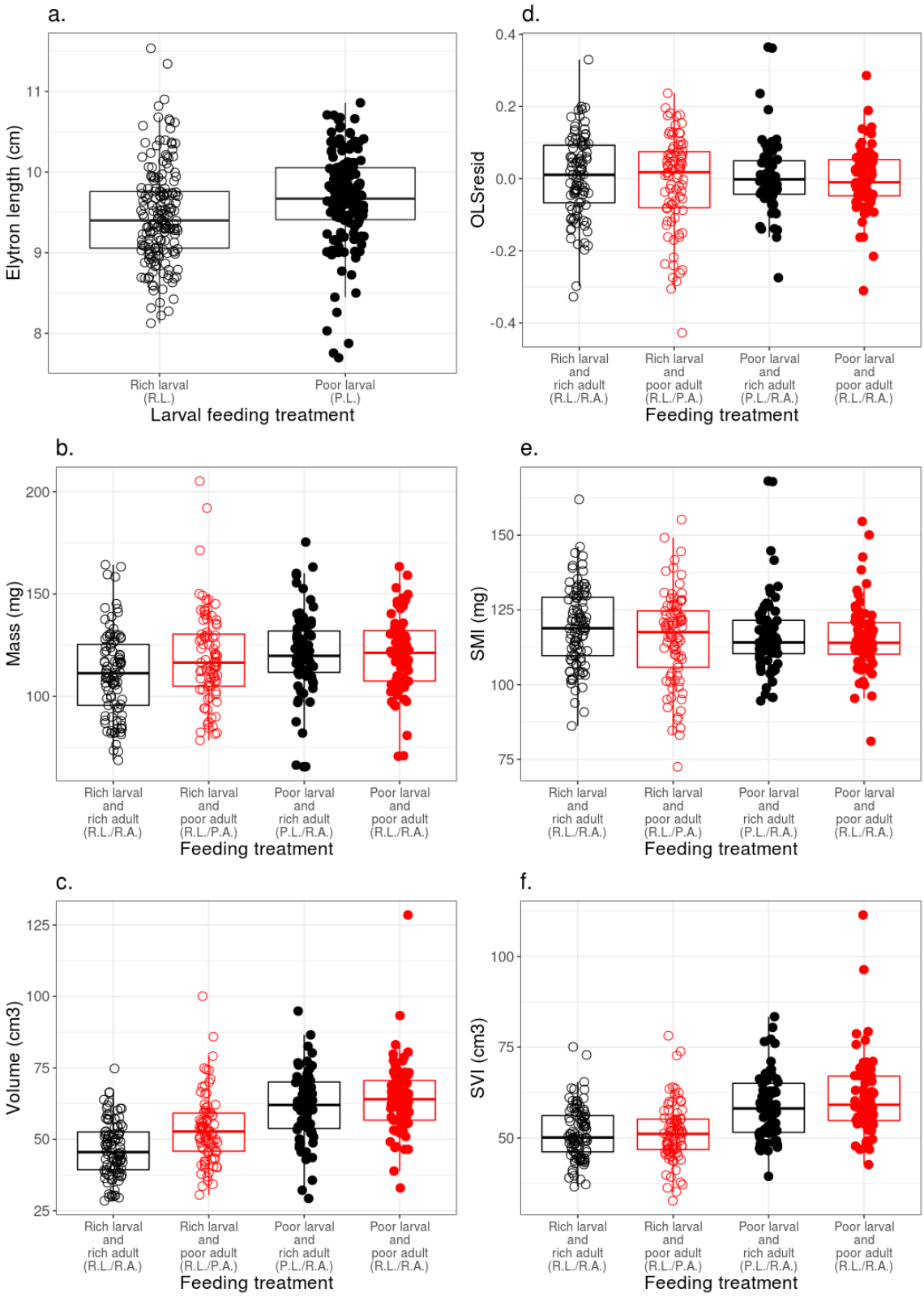

Figure 4: a. Total fecundity of *T. molitor* females according to LFT and AFT. **b.** The partial effect size of each morphological measurement and BCI on total fecundity controlling for AFT. The dots represent the estimate of each BCI, the horizontal bars represent the confidence intervals.
c., **d.** and **e.** represent the total fecundity according to respectively the residuals of OLS_{resid}, mass and SMI and the AFT. The black dots represent the rich AFT while the red dots represent the poor AFT, with the corresponding regression line and the 95% confidence intervals around it.

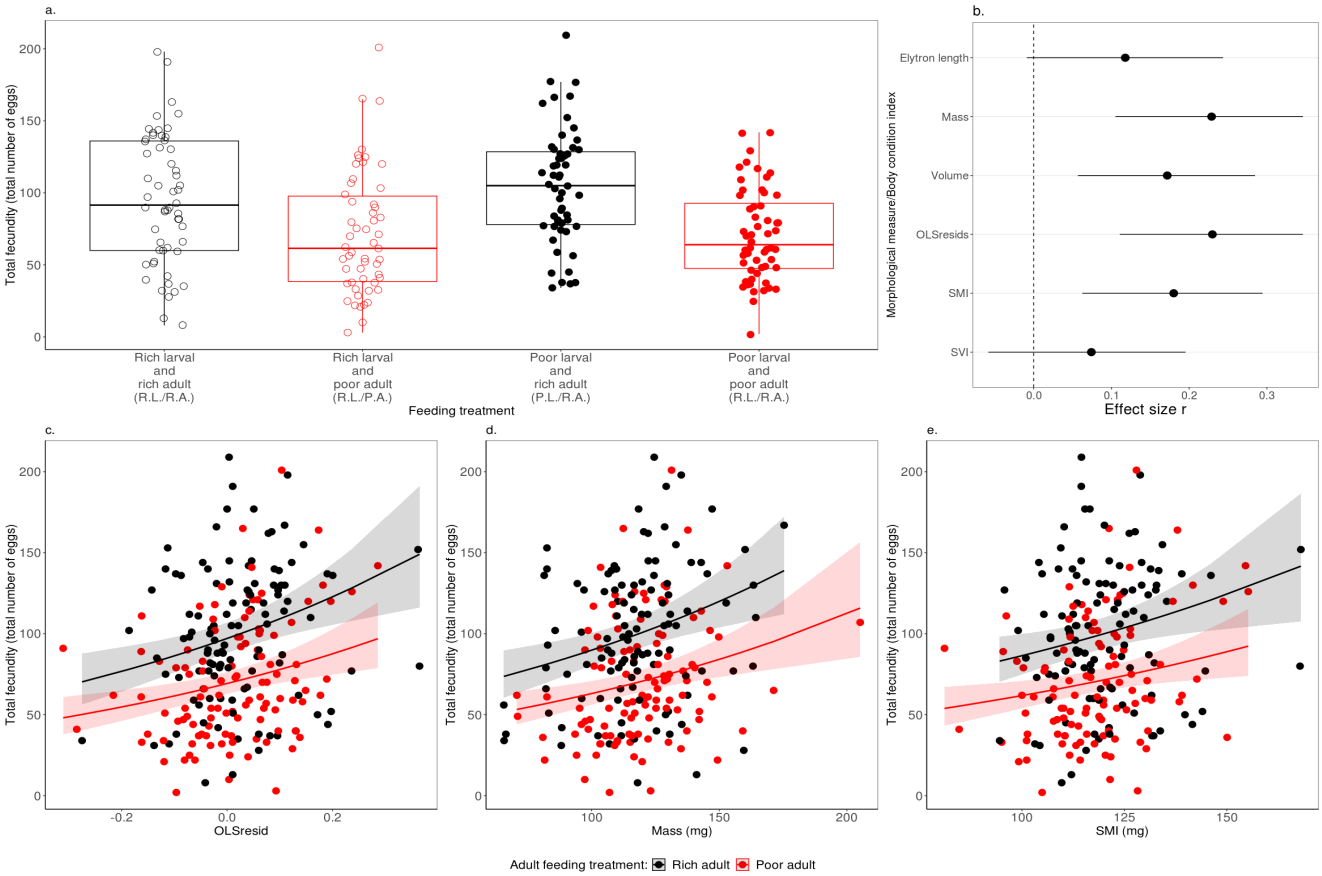
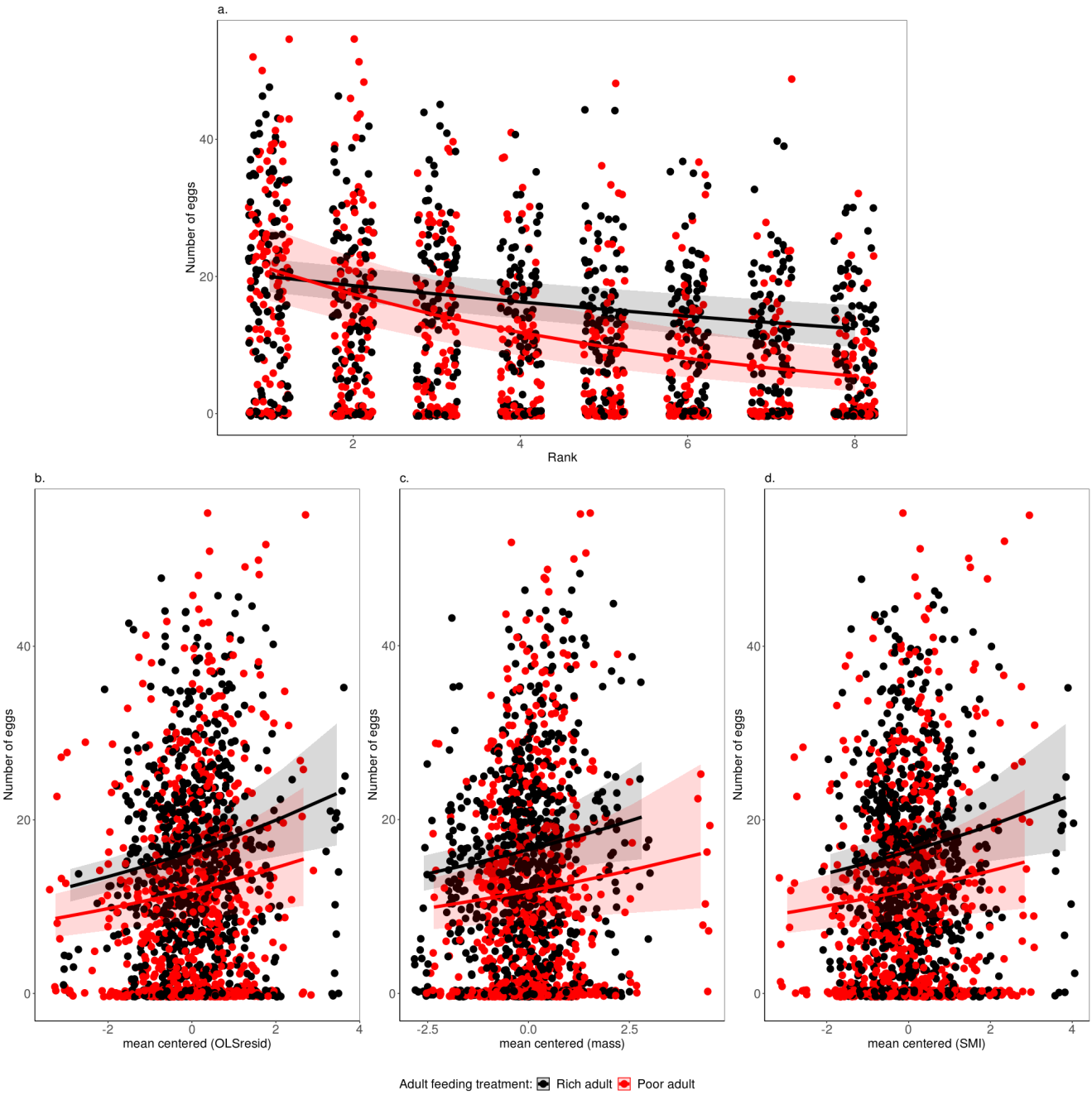
Figure 5: a. Effect of the interaction between rank (egg laying event) and AFT on the number of eggs laid, controlling for body condition at emergence as OLS_{resid}, using a zero-inflated mixed effect model with a negative binomial distribution. Effect of body condition at emergence as **b.** OLS_{resid}, **c.** mass, and **d.** SMI on fecundity controlled for the rank, using a zero-inflated mixed effect model with a negative binomial distribution. The black dots represent the rich AFT, while the red dots represent the poor AFT, with the corresponding regression lines and the 95% confidence intervals around it.