LONG-TERM EFFECTS OF BROOD SIZE MANIPULATION ON ADULT METABOLIC RATE IN THE BURYING BEETLE, *NICROPHORUS PUSTULATUS*

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ABSTRACT

The environment that an organism experiences during development can shift the developmental trajectory, leading to differences in adult phenotypes. Complex life cycles that are characterized by the presence of an ontogenetic stage during which morphology, physiology, and behavior of an individual change, are thought to provide a decoupling mechanism between pre- and post-metamorphic traits in the form of metamorphosis (*i.e. adaptive decoupling hypothesis*). This would allow pre- and post-metamorphic traits to evolve independently thus maximizing an individual's lifetime fitness. Physiological changes in response to early environmental conditions can directly affect early metabolic rate, but can also have carry-over effects on adult metabolic rate. Metabolic carry-over effects have been demonstrated in vertebrates with simple as well as with complex life cycles. The purpose of my thesis was to investigate whether early developmental conditions affect adult physiology in holometabolous insects. Specifically, I addressed two questions: (1) Do environmental conditions experienced by the larvae, show a carry-over effect and influence adult metabolic rate? and (2) Will the metabolic response differ

between males and females? To answer these questions, I manipulated brood size in the burying beetle *Nicrophorus pustulatus* when the larvae were hatching, assuming that larger broods would increase larval competition. This assumption was supported by the observation that larval mass decreased with increasing brood size. Brood size manipulation had a carry-over effect on both adult mass and metabolic rate. The effect of brood size manipulation on metabolic rate was independent of body mass. Although males and females did not differ in mass, the metabolic rate of females was higher than that of males. In conclusion, my experiment showed that environmental conditions experienced by the larvae can have carry-over effects on adult metabolic rate and are not mediated through body size. My results corroborate other studies demonstrating that metamorphosis may not decouple traits as proposed by the adaptive decoupling hypothesis.

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I would also like to thank Robert Stanton for being my graduate cohort, commiserating with me, answering countless burying beetle biology questions and for joining me on this walk the last three years.

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INTRODUCTION

During development the phenotype of an individual changes not only due to genetic inputs, but also in response to environmental conditions (Schlichting & Pigliucci 1998, West-Eberhard 2003). The ability to express different ontogenetic phenotypes in response to environmental conditions (*i.e. developmental plasticity*, a sub-type of phenotypic plasticity (Piersma & van Gils 2011)) allows an organism to follow different developmental trajectories depending on the environment (Schlichting & Pigliucci 1998, Piersma & van Gils 2011). Juveniles experiencing environmental stresses, such as parasites (Kraaijeveld *et al.* 2001, Devevey .*et al.* 2008), predators (Tollrian 1995, Bernard 2004, Relyea 2007) or competition (Burness .*et al.* 2000, Verhulst .*et al.* 2006) often follow different developmental trajectories than juveniles not experiencing these environmental stresses. Such developmental trajectories can be reversible as well as irreversible, with and without long-term effects (West-Eberhard 2003).

Long-term effects of early developmental environments on adult phenotypes are well-documented for life history (Tollrian 1995; Nilsson 2002; De Block & Stoks 2008a, b; Dmitriew & Rowe 2011), morphological (*e.g.* Emlen 1994, Tollrian 1995, Crean *et al.* 2011), behavioral (*e.g.* Metcalfe *et al.* 2003, Marks *et al.* 2005, Krause *et al.* 2009), and physiological traits (*e.g.* Beck & Congdon 2000, Fischer *et al.* 2004, Verhulst *et al.* 2006, Devevey *et al.* 2008). For example, when low quantities of food are available to ladybird beetles, *Hamonia axyridis*, there is a longer lag until eggs are laid (Dmitriew & Rowe 2011). Male dung beetles, *Onthophaus taurus*, develop large horns if they experience high food availability during larval development, and small horns if they are exposed to

food shortage as larvae (Emlen 1994). Water fleas, *Daphnia pulex,* develop self-defensive "neckteeth" only in the presence of predators (Tollrian 1995). Adult zebra finches, *Taeniopygia guttata,* behave more aggressively when securing food resources if raised in nutritionally poor environments, compared to individuals raised in nutritionally rich conditions (Krause *et al.* 2009). Physiologically, adult damselflies, *Lestes virdis,* have been found to have reduced phenoloxidase activity when food availability is limited during development (De Block & Stoks 2008b).

Physiological changes in response to environmental conditions may affect overall energy use. Processes such as mounting immune responses, digestion, thermoregulation, and homeostatic maintenance, have all been demonstrated to have immediate metabolic consequences (McNab 2002, Karasov & Martínez del Rio 2007). However, developmental conditions can also have long-term metabolic consequences. Delayed photoperiod (Stoks *et al.* 2006), increased egg testosterone (Nilsson *et al.* 2011), parasites (Devevey *et al.* 2008), accelerated development (Beck & Congdon 2000, Fischer *et al.* 2004), and competition (Verhulst *et al.* 2006, Criscuolo *et al.* 2008) during early development have been shown to increase adult metabolic rate, a measurement of whole-organism physiological performance (Wikelski 2009).

Despite extensive study of developmental plasticity (Schlichting & Pigliucci 1998, Pigliucci 2001, West-Eberhard 2003, ,), little is known how developmental plasticity influences adult phenotypes of organisms with complex life cycles (Pechenik 2006, Podolsky & Moran 2006, Relyea 2007, Marshall & Morgan 2011). Complex life cycles are characterized by the presence of metamorphosis, an ontogenetic life stage during which morphology, physiology, and behavior of an individual change (Wilbur

1980). During metamorphosis, holometabolic insects develop wings (Truman & Riddlford 1999), while anurans switch from dependency on gills to lungs, develop appendages, and resolve a tail (Gilbert 2000, Relyea 2001). Such changes are generally associated with changes in the individual's ecological niche (Wilbur 1980, Moran 1994, Relyea 2007). Metamorphosis is thought to have evolved in order to allow the different life stages to adapt to the environment that a particular life stage experiences (Istock 1967, Wilbur 1980, Werner 1988, Ebenman 1992). Moran (1994) proposed that metamorphosis acts as a decoupling event which allows pre- and post-metamorphic traits to evolve independently thus maximizing lifetime fitness. Empirical studies testing the adaptive decoupling hypothesis are few in numbers and the results are equivocal (Pechenik *et al.* 1998, Pechenik 2006, Podolsky & Moran 2006, Marshall & Morgan 2011). Most studies have focused on morphological and life history traits (*e.g.* Altwegg & Reyer 2007, Johansson *et al.* 2010, Ng'hadi *et al.* 2010, Crean *et al.* 2011), while only a handful investigated physiological traits such as heat-shock, resistance resource allocation, enzyme activity, or immune response (Loescke & Krebs 1996, Stevens *et al.* 1999, De Block & Stoks 2008a & b, Fellous & Lazzaro 2011). Measurements of metabolic rates before and after metamorphosis are lacking, however environmental effects on larval metabolic rate have been demonstrated. Short-term starvation causes a reduction in metabolic rate of final-instar damsel fly larvae, *Lestes virdis*, (Stoks *et al.* 2006), while increased growth rate increases metabolic rate in butterfly larvae, *Lycanea titrus*, (Fischer *et al.* 2004). To test whether environmental conditions experienced early in life have long-term effects on metabolic rate, metabolic measurements in the post-

metamorphic stage are required as have been conducted in adult birds (Verhulst *et al.* 2006, Criscuolo *et al.* 2008).

Because of the different physiological requirements of males and females for reproduction, metabolic rate often differs between males and females (McNab 2002, Stillwell *et al.* 2010). In addition, long-term effects of early developmental environments can influence males and females differently (De Block & Stocks 2005, Verhulst *et al.* 2006).

The purpose of this research is to investigate whether early developmental conditions affect adult physiology in holometabolous insects. Specifically, I will address two main questions. (1) Do environmental conditions experienced by the larvae, show a carry-over effect and influence adult metabolic rate? and (2) Will the metabolic response differ between males and females?

METHODS

Study Animal

The beetles used in this study originated from a laboratory colony that had been established in 2002 with 94 pairs of *Nicrophorus pustulatus* caught in the research forest of Berea College, KY USA. Adult beetles were maintained individually in clear plastic containers (15 cm x 10 cm x 5 cm) filled about 2 cm deep with humid peat in a light controlled room (15h light : 9h dark) at 22.0 ± 0.2 °C. Twice a week the beetles received a dime-sized portion of canned cat food (Science Diet ®, Hill's Pet Nutrition Inc., Topeka, KS, USA).

Manipulation of Developmental Conditions of Larvae

A non-sib pair of adult beetles from the colony was placed in a plastic container (15 cm x 10 cm x 5 cm) filled about 2 cm deep with moist peat, and allowed to mate. The following day, the male was removed and a mouse (descriptive statistics are in the form N, $\bar{x}$ ± SE, and range unless otherwise noted) (160, 31.01 ± 0.19 g, 19.0 – 46.9 g) was provided to each female. To simulate conditions underground, the containers were placed in a dark room at 22.0 ± 0.2 °C. The containers were checked for eggs daily in the morning and late afternoon. The day after the first eggs had been observed, the female and mouse were moved to a new container filled 2 cm deep with moist peat. The old container was searched for eggs. The eggs were placed onto a moist paper tissue in a petri dish. The eggs were allowed to develop in the same darkroom where the females were kept. The eggs were checked two times a day for newly hatched larvae.

As soon as the larvae of a female began to hatch, the female, with the mouse, was transferred to a new container to ensure that no eggs or larvae were present in the container. At at this time, the female was assigned at random to one of the three brood size manipulations: the small, medium, or large brood size. As medium brood size, I chose 15 larvae, which is the average number of larvae (mean ± SD: 15.6 ± 4.3) reared by female *N. pustulatus* on mice weighing 26.2 to 32.5 g (Rauter & Moore 2002). .Small broods, 5 larvae, corresponded to the average brood size - 2.5 SD, while large broods, 25 larvae,represented the average brood + 2.5 SD, (Rauter & Moore 2002)

All larvae that had hatched at the same time were randomly assigned to a foster mother. This distribution method minimized the number of siblings per brood and the influence of parent-offspring co-evolutionary adaptation on offspring development.

When the larvae were leaving the brood chamber (called “dispersing”), the larvae from each container were counted, weighed, and placed individually into small round containers (5.5 cm in diameter and 3.5 cm high) filled with moist peat and maintained in a darkroom at 22.0 ± 0.2 °C and allowed to pupate.

To test whether the brood size manipulation caused developmental stress, I compared the number and size of dispersing larvae among the brood size manipulations. In addition, I compared the amount of carrion consumed per larva among the brood size manipulations as a proxy for competitive stress. I estimated the amount of carrion consumed per larva as the difference in carrion mass when the mouse had been placed in the container, and the carrion mass at larval dispersal divided by the number of dispersing larvae. Considering that females also eat from the carrion, I assumed that the amount of carrion consumed by females did not differ substantially between brood size

manipulations.

As tenerals emerged, (*i.e.* immature adults not capable of reproduction), the beetles were transferred to individual containers (15 cm x 10 cm x 5 cm) filled with humid peat and placed into a light controlled room (15h light : 9h dark) at 22.0 ± 0.2 °C. Metabolic measurements were taken after adults had reached sexual maturity (at least 3 weeks old) (160, 58.99 ± 1.13 days, 21 – 95 days).

Resting Metabolic Rate Measurements

To determine resting metabolic rates, I measured CO_2 production (VCO2, ml h^{-1}) of sexually mature beetles using flow-through respirometry. Ambient air, scrubbed of CO_2 with soda lime (Fisher Scientific, USA), was pushed through the animal chamber by a Qubit Elite 800 AC gas pump (Qubit Systems Inc., Kingston ON, Canada). Air flow rate of 150 ml min^{-1} was controlled using a rotameter (Riteflow® Fisher Scientific, USA). The animal chamber, a 60 ml glass syringe with a rubber stopper fitted with airflow hoses at each end, was submerged in a water bath (Precision, Fisher Scientific, USA) set to 22.0 ± 0.2 °C., which corresponded to the average room temperature the beetles experienced during development and adulthood. Moisture in the air leaving the animal chamber was removed using a condensation column and drierite (Fisher Scientific, USA). The CO_2 concentration of the dry air was measured using an infrared CO_2 gas analyzer (S151 CO_2 analyzer; Qubit Systems Inc., Kingston ON, Canada). The CO_2 analyzer uses infrared absorbance of CO_2 to measure CO_2 concentration in a gas mixture. The amount of infrared light absorbed by the CO_2 creates a voltage potential. Between the calibration points 0 ppm and 500 ppm CO_2, the voltage potential increases

linearly with increasing CO_2 concentration. The CO_2 measurements of the infrared gas analyzer were captured by a LabProTM Interface (Vernier Software and Technology LLC, Beaverton, OR, USA), relayed to a computer and recorded using LoggerPro 2.1.1 software (Vernier Software and Technology LLC, Beaverton, OR).

Calibration

The CO_2 infrared gas analyzer was calibrated once a week. In order to calibrate the zero point, CO_2 was scrubbed from the incoming ambient air with soda lime (Fisher Scientific, USA) to provide the voltage value for 0 ppm CO_2 at 150 ml min^{-1}. A span of 500 ppm was established using a calibrated gas mixture of 500 ppm CO_2 in inert N_2 through the respiratory system at a flow rate of 150 ml/min. This provided the voltage value for 500 ppm CO_2 at 150 ml min^{-1}.

Trial

Before measurements were taken, the airflow was allowed to reach equilibrium (*i.e.* until there was no change in the CO_2 measurements), at which point all ambient CO_2 had been flushed out of the respirometry system. A baseline was allowed to run for about 15 minutes before each trial. While measuring the baseline, a beetle was selected, weighed, and placed into the animal chamber. Once the baseline was complete, the animal chamber was connected and placed into the water bath. The flow rate was monitored to ensure that a barometric pressure shift would not cause a change in flow rate. Trials were set to run for 165 minutes. However, if a continuous measurement of 30 minutes was of high quality and the beetle was continuously resting, the trial was stopped

before 165 minutes had passed. To determine the characteristics of a CO_2 measurement pattern of a beetle at rest, a preliminary study was conducted where the behavior of the beetle in the chamber was observed and compared to the CO_2 production. Good recordings had a distinct discontinuous gas exchange pattern and only recordings with this pattern were used for analysis (Fig. 1). If a beetle was active for more than 135 minutes the measurement was discarded. If a beetle was active during part of the measurement, but for less than 135 minutes, only those parts of the measurement were included in the estimation of the resting metabolic rate where the beetle was at rest (Fig. 2). A second baseline of 15 minutes was run following each trial. The average of both baselines was used to correct for the shift in the baseline during the trial and between trials.

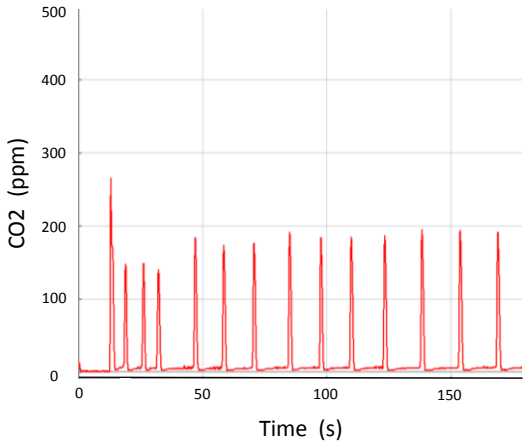

Figure 1: Example of a metabolic rate measurement of an inactive beetle.

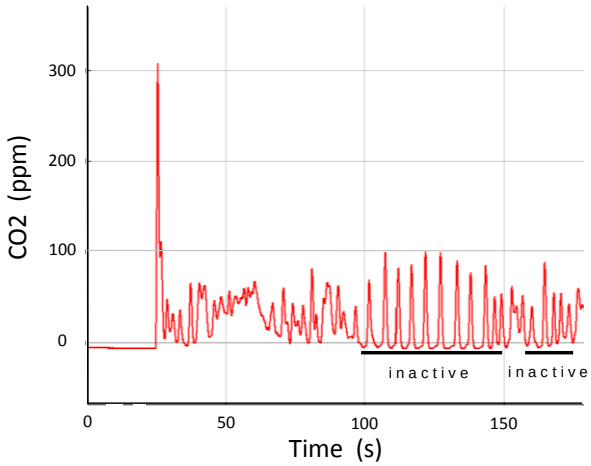

Figure 2: Example of a metabolic rate measurement of a beetle with active and inactive periods.

The beetles were weighed before and after the measurement and the average mass was used in the analysis. Measurements were taken during the day when these nocturnal beetles are inactive. However, during the measurements the beetles were kept in the dark and only photographic red light was used, because preliminary behavioral observations and measurements indicated that beetles were less active in the dark than under well-lit conditions.

Both before and after each trial, ambient temperature (160 trials, 22.27 ± 0.06 °C, 21.05 – 24.7 °C) and barometric pressure (160, 101.51 ± .06 kPa, 99.57 – 104.1 kPa) were recorded, in order to correct for standard temperature and pressure (STP):

$$STPFR = FR(BP/BPS)(TS/T) \quad \text{(Equation 1, Lighton 2008)}$$

where STPFR is the flow rate corrected for STP, FR is the flow rate, BP is recorded barometric pressure, BPS is the standard barometric pressure (101.325 kPa), TS is the standard temperature (273.15 K) and T is the recorded temperature.

Removal of water vapor before measuring CO_2 concentration, did not require

calculation of dilution errors caused by water vapor pressure (Lighton, 2008). Therefore, to calculate VCO_2 I used the following equation:

$$VCO_2 = (STPFR) * (CO_{2Excurrent} - CO_{2Incurrent}) \qquad \text{(Equation 2, Lighton 2008).}$$

Statistical Analysis

Statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary NC). To test whether developmental conditions (*i.e.* brood size manipulation) affected average larval mass at dispersal and food consumption per larva, I used a general linear model (GLM; Proc GLM in SAS) with brood size manipulation as the main effect and mouse mass as a covariate. To test for linear decrease of average larval mass with increasing brood size, a test for linear contrasts was conducted.

To test whether developmental conditions and sex affected metabolic measurements (measured as CO_2 production) in adult beetles, I used a GLM with the main effects brood size manipulation and sex and the interaction between the main effects. As covariates, I included age, body mass, and actual measurement duration. Hayes *et al.* (1992) demonstrated that the longer a metabolic trial lasts, the lower the metabolic reading becomes. Although I tried to measure at least two females and two males from each brood, not all broods had two males and females, especially the smallest broods. As a consequence, the final data set included broods with measurements for a single individual (*i.e.* only for one male or one female per brood) making it impossible to calculate variation caused by the brood environment. For this reason, I could not include rearing container (*i.e.* brood) as a random factor in the general linear model. I calculated the mean of CO_2 production, age, and body mass, respectively, for each sex within each

brood and used these averages in the statistical analysis. To test for linear increase of CO_2 production with increasing brood size, a test for linear contrasts was conducted. To test for homogeneity, the interactions between the main effects and covariates were first included in the statistical model, but dropped from the final model because the interactions were not significant.

RESULTS

Larval Measurements

For many broods, the number of larvae leaving the brood chamber and dispersing was smaller than the originally assigned brood size (Fig. 3). From small broods, on average 4.4 ± 0.2 larvae dispersed (descriptive statistics following are mean ± SE unless otherwise noted; N = 11, min = 3, max = 5), while medium broods had on average 12.2 ± 0.3 larvae dispersing (N = 10, min = 10, max = 15). Broods of the large brood size manipulation showed two modes of reduction, some broods were minimally reduced and others were greatly reduced. As this difference in magnitude of brood reduction may affect larval development, we split the originally large brood size manipulation into two groups: large-medium (LM) and large (L). Broods from the LM group had on average of 14.2 ± 0.2 larvae dispersing (N = 8, min = 10, max = 15) while from the L broods on average of 20.5 ± 0.2 larvae dispersed (N = 12, min = 19, max = 24). To keep the brood size manipulations clearly separated from each other, I excluded broods with the most extreme reduction of brood size from the medium and medium-large brood size manipulation (Fig. 3). In addition, two broods from the small brood size manipulation were excluded from the analysis, because each brood had only one larva surviving (Fig. 3). Mouse mass did not differ between brood size manipulations ($F_{3, 37} = 2.25$, $P = 0.10$).

The amount of carrion consumed per larva was affected by brood size manipulation and decreased with increasing brood size (Table 1; Fig. 4). Mouse size did not influence the amount of carrion consumed per larva (Table 1). Larval mass was influenced by the brood size manipulation (Table 1) and decreased as brood size increased (Table 1; Fig. 5). Larval mass was not influenced by mouse mass (Table 1).

There was, however a significant interaction between mouse mass and brood size on larval mass (Table 1).

Table 1: The results of a general linear model of analysis of average larval food consumption and larval mass.

Source	Slope	*d.f.*	*F*	*P*
Average Larval Food Consumption				
Mouse mass	0.006	1, 33	0.02	0.88
Brood size manipulation		**3, 33**	**26.59**	**<0.0001**
Test for linearity		**1, 33**	**64.12**	**<0.0001**
Larval Mass				
Mouse mass	-9.3	1, 33	0.53	0.47
Brood size manipulation		**3, 33**	**7.98**	**0.0004**
Mouse mass × Brood size manipulation		**3, 33**	**5.35**	**0.004**
Test for linearity		**1, 33**	**20.48**	**<0.0001**

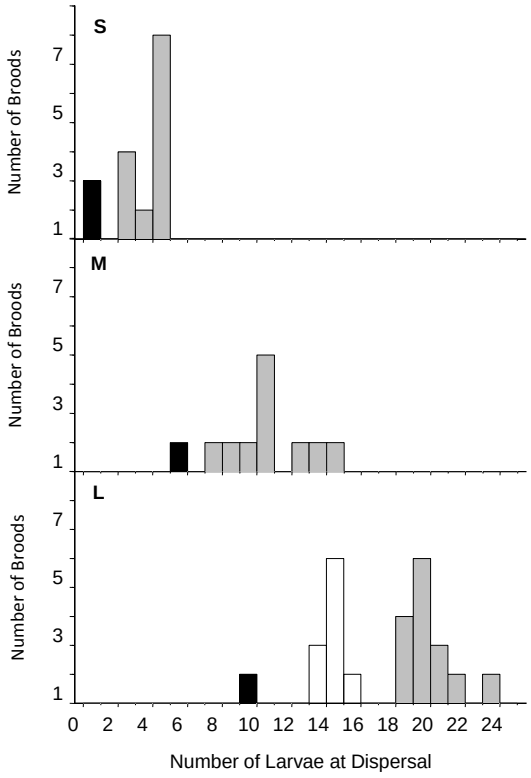

Figure 3: Distribution of brood size at dispersal for small, medium, and large broods. Black bars indicate broods with only one larva dispersing (S) or having brood size at dispersal overlapping with small-sized broods (M) or medium-sized broods (L), White bars indicate broods that were set up as large broods, but females reduced the number of larvae substantially.

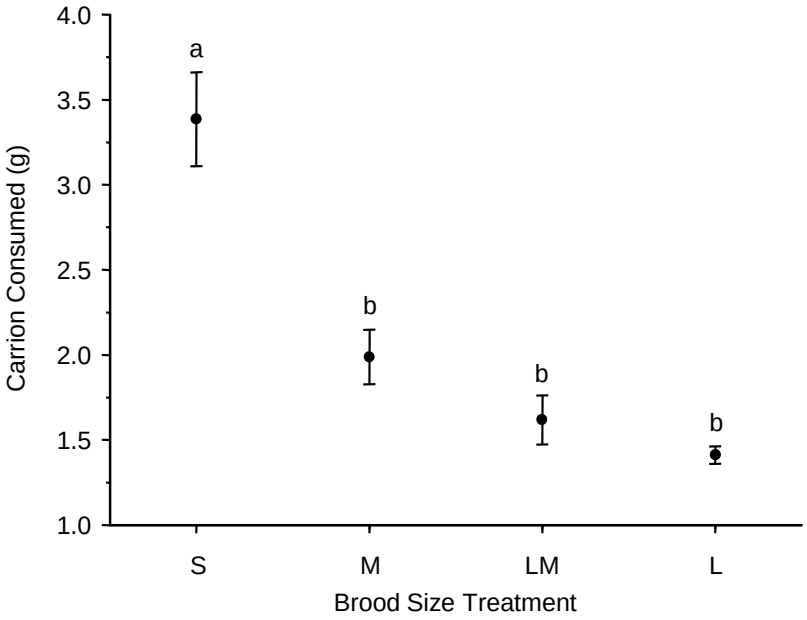

Figure 4: Effect of the brood size manipulation on the amount of carrion consumed per larva per brood. Mean and standard error are shown. Different letters indicate a significant difference at $P < 0.05$ (Post-hoc pairwise comparison using Scheffe test). S: small-sized broods; M: medium-sized broods, LM: large-sized broods reduced to medium-sized broods by females; L: large-sized broods.

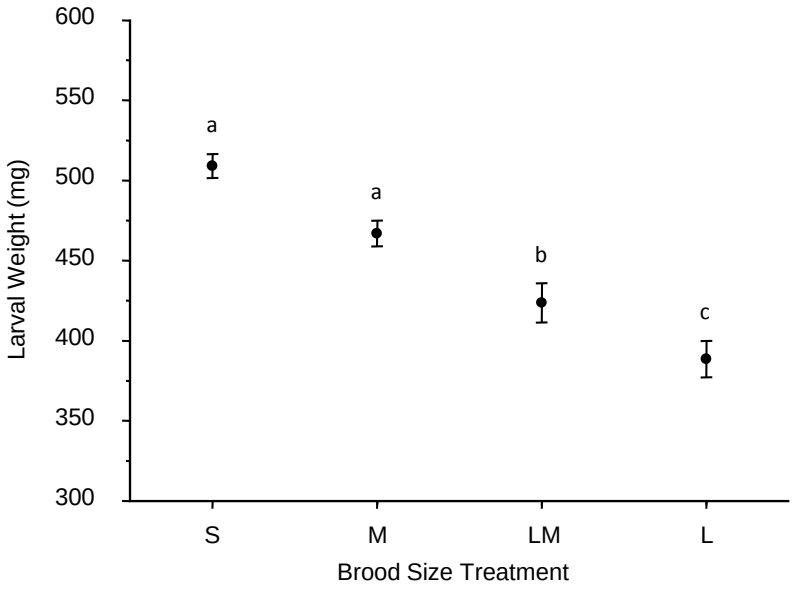

Figure 5: Effect of the brood size manipulation on larval mass at dispersal. Mean and standard error are shown. Different letters indicate a significant difference at $P < 0.05$ (Post-hoc pairwise comparison using Scheffe test). S: small-sized broods; M: medium-sized broods, LM: large-size broods reduced to medium-sized broods by females; L: large-sized broods.

Adult Measurements

The brood size manipulation had an effect on adult body mass and decreased with increasing brood size (Table 2, Fig. 6). Males and females did not differ in body mass (Table 2; Fig. 6).

VCO_2 was significantly affected by brood size (Table 2, Fig. 7). The metabolic rates of beetles reared in small broods were significantly different from beetles reared in large broods that were reduced to medium brood size by the foster female (Fig. 7). A test for linear contrasts of brood size manipulation was significant suggesting an increase in metabolic rate with increasing brood size (Table 2). Overall, females had a higher VCO_2 than males (Table 2, Fig. 7), even though at the time the metabolic measurements were taken, body mass did not differ between males and females. The interaction between sex and brood size manipulation was not significant (Table 2). VCO_2 decreased with increasing age of the beetles and longer duration of the measurements, but increased with increasing body mass (Table 2). Neither age nor the duration of the metabolic reading differed between brood size manipulations or sex (brood size manipulations: $F_{3, 55} = 2.30$; $P = 0.08$ and $F_{3, 55} = 1.45$; $P = 0.24$ respectively, and sex: $F_{1, 55} = 0.21$; $p = 0.65$ and $F_{1, 55} = 0.25$; $P = 0.62$ respectively).

Table 2: Results of a general linear model of analysis of adult mass and VCO_2.

Source	Slope	*d.f.*	*F*	*P*
Adult mass				
Sex		1, 55	1.85	0.18
Brood size manipulation		**1, 55**	**13.00**	**<0.0001**
Sex × brood size manipulation		3, 55	0.43	0.73
VCO_2				
Age	**-0.0006**	**1, 53**	**3.94**	**0.05**
Trial duration	**-0.0003**	**1, 53**	**9.25**	**0.004**
Body mass	**0.0004**	**1, 53**	**26.57**	**<0.0001**
Sex		1, 53	13.14	**0.0007**
Brood size manipulation		3, 53	4.28	**0.009**
Sex × Brood size manipulation		3, 53	0.94	0.43
Test for linearity		**1, 53**	**6.25**	**0.02**

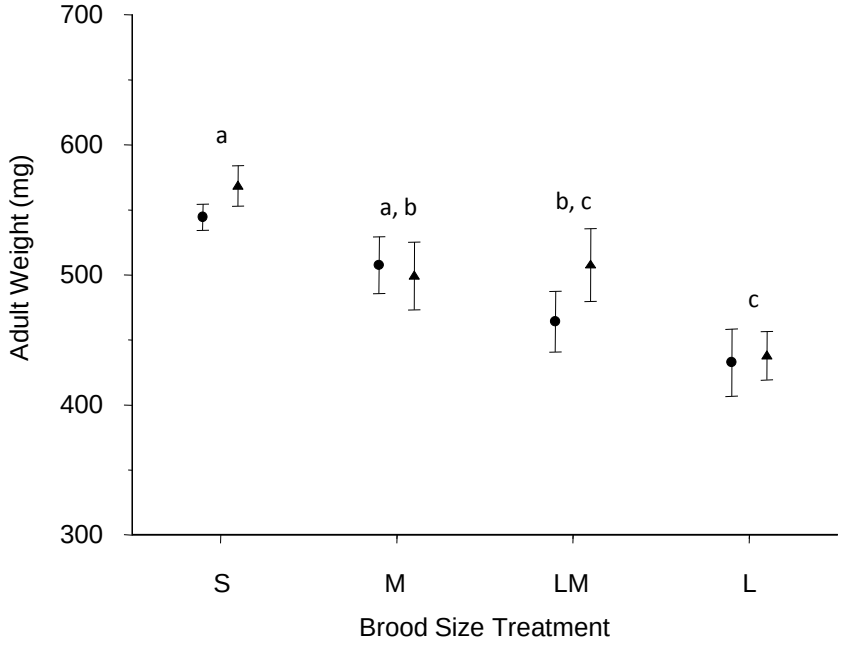

Figure 6: Effect of the brood size manipulation on adult mass of female (triangles) and male (circles) beetles. Mean and standard error are shown. Different letters indicate a significant difference at $P < 0.05$ (Post-hoc pairwise comparison using Scheffe test). S: small-sized broods; M: medium-sized broods, LM: large-sized broods reduced to medium-sized broods by females; L: large-sized broods.

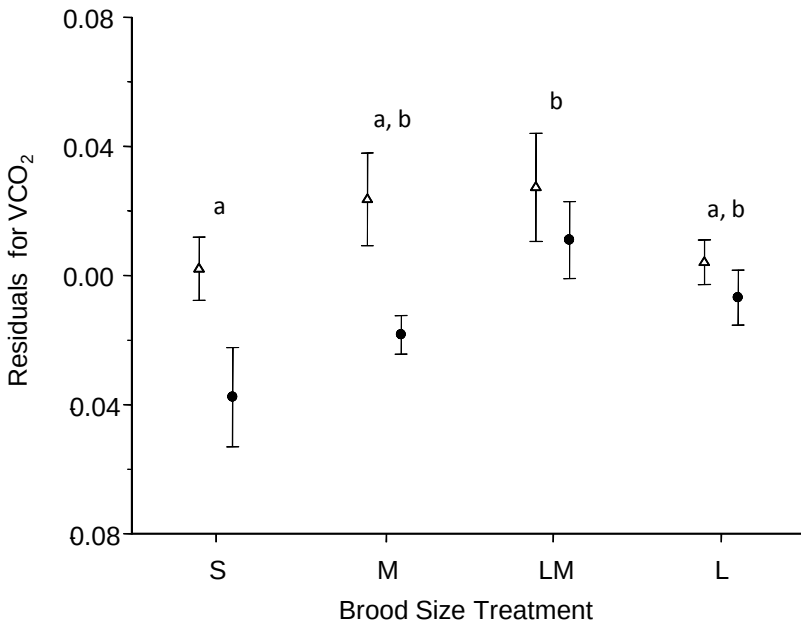

Figure 7: Effect of the brood size manipulation on VCO_2 of female (triangles) and male (circles) beetles. Residuals of VCO_2 are presented with the effects of bodymass, age, and trial duration removed. Mean and standard error are shown. Different letters indicate a significant difference at $P < 0.05$ (Post-hoc pairwise comparison using Scheffe test). S: small-sized broods; M: medium-sized broods, LM: large-sized broods reduced to medium-sized broods by females; L: large-sized broods.

DISCUSSION

Brood size manipulation had a significant effect on both larval and adult traits. The carry-over effects of the brood size manipulation on adult mass and metabolic rate did not differ between males and females. Although body mass was not different between males and females, the metabolic rate of females was higher than that of males.

Difference in Metabolic Rate Between Males and Females

Even though there was no difference in adult mass between sexes, females had a significantly higher metabolic rate than males, when body mass was controlled for. This may be due to differences in energetic investment in gamete production (Hayward & Gillooly 2011). Across a variety of vertebrate and invertebrate taxa, in general egg biomasss is 3.5 orders of magnitude higher than sperm biomasss production, and the metabolic cost of gamete production is estimated to be about three orders of magnitude higher in females (Hayward & Gillooly 2011). Although energetic measurements of gamete production in burying beetles are lacking, ovarian development during sexual maturation may explain the higher metabolic costs in females (Trumbo *et al.* 1995, Scott *et al.* 2005). Shortly after eclosion, female burying beetles begin ovarian development and both ovarian development and expression of vitellogenin genes increase gradually (Trumbo *et al.* 1995, Scott *et al.* 2005). However, after about 3 weeks ovarian development and the expression of vitellogenin genes reach a plateau (Trumbo *et al.* 1995, Scott *et al.* 2005). After encountering a suitable breeding resource, ovarian growth is resumed (Scott and Traniello 1987, Trumbo *et al.* 2001, Scott *et al.* 2005). This

developmental pattern of ovaries is assumed to reflect the requirements of a breeding resource that is scarce in time and space (Scott *et al.* 2005).

Short-term Effects of Brood Size Manipulation

Consistent with other studies that manipulated *Nicrophorus sp.* brood size (Rauter & Moore 2002, Smiseth *et al.* 2007), our results demonstrate a negative relationship between brood size and food consumed, and between brood size and larval mass. As brood size increases, so does competition for access to the carrion, and to parental feeding (Smiseth *et al.* 2007), since burying beetle larvae feed by themselves and are also fed by the parents (Smiseth *et al.* 2003).

High competition for food can lead to increased energy expenditure for food acquisition (Mueller *et al.* 2005). When competition for food is high, *Drosophila* larvae increase feeding rates. Individuals feeding at a high rate consume more food than individuals with a lower feeding rate (Joshi & Mueller 1988, Parker & Maynard Smith 1990). High feeding rates, however, are costly, because a high feeding rate leads to a less efficient use of the gained energy (Mueller 1990, Joshi & Mueller 1996).

Burying beetle larvae compete among themselves for access to carrion, and for regurgitations by the parents (Smiseth *et al.* 2003). The parents, however, can intensify the competition among the larvae (Smiseth *et al.* 2007),because parents consume some of the carrion (C. Rauter, unpublished data). As parental care effort increases asymptotically with increasing brood size (Rauter & Moore 2004)females caring for large broods would need to consume more carrion in order to maintain their body mass, thus exacerbating larval competition.

Even though total parental provisioning rates increase with increasing brood size, the feeding rates per-larva decrease as total parental provisioning rate increases (Rauter & Moore 2004, Smiseth *et al.* 2007). Consequently, larvae from larger broods spend more time begging for parental provisions and grow slower and to a smaller size than larvae from small broods (Smiseth *et al.* 2007). In burying beetles, body size has significant fitness consequences. As adults, burying beetles engage in size-dependent contests over high quality breeding resources which the larger beetles usually win(*e.g.* Pukowski 1933, Otronen 1988, Müller *et al.* 2007).

Long-Term Effects of Brood Size Manipulation

As predicted, brood size manipulation showed a carry-over effect on adult metabolic rate. The carry-over effect seemed to be non-linear with the smallest and largest brood size having the lowest adult resting metabolic rates (Fig. 7).

This non-linear pattern of adult metabolic rate may be the results of a larger sample size for large broods ($N_{♀}$ = 10, $N_{♂}$ = 11) compared to the other three brood size manipulations resulting in a much smaller variation in large broods (small broods: $N_{♀}$ = 8, $N_{♂}$ = 7; medium broods: $N_{♀}$ = 6, $N_{♂}$ = 7; large broods reduced to medium broods: $N_{♀}$ = 7, $N_{♂}$ = 7). Nonetheless, the sample sizes for all brood size manipulations were comparable to studies with similar questions, (Beck & Congdon 2000: N= 11-23; Criscuolo *et al.* 2008: N= 13 – 16; Krause *et al.* 2009: N= 10; Verhulst *et al.* 2006: N= 19 & 24).

Carry-over effects of early developmental conditions on adult metabolic rate have also been found in another holometabolous insect. Adult damselflies (*Lestes viridis*) that

have experienced time or food stress as larvae and therefore grew faster, and had higher metabolic rates than damselflies growing more slowly (Stoks *et al.* 2006). Similarly, in Southern Toads (*Buffo terrestris*), adult metabolic is increased when the tadpoles experience food stress or are exposed to colder temperatures (Beck & Congdon 2000). Also in animals without complex life cycles, carry-over effects of early developmental conditions on adult metabolic rate are known. Adult metabolic rate is elevated in Zebra finches (*Taeniopygia guttata*) that were reared in large broods (Verhulst *et al.* 2006), experienced periods of low-quality diet (Criscuolo *et al.* 2008), or were exposed to high teststosterone levels as embryo (Nilsson *et al.* 2011).

The underlying processes leading to physiological carry-over effects are not well understood. Some potential mechanisms that have been discussed (Pechenik *et al.* 1998, Eriksson *et al.* 2002, Pechenik 2006, Marshall & Morgan 2011) are genetic correlations between larval/embryonic and adult traits, damage to DNA or enzymes, as well as changes in methylation patterns of DNA, transcription and translation processes, enzymes, developmental programming, and remodeling of cells instead of de-novo development. Empirical evidence for these proposed processes is still scarce. The presence of genetic correlation between larval and adult traits has been demonstrated for *Drosophila melanogaster* where the same genetic factors control larval and adult immune traits before and after metamorphosis (Fellous & Lazzaro 2011). Substances with mutagenic effects on DNA can reduce adult survival in Pink Salmon, *Oncorhynchus gorbuscha*, when the embryos are exposed to crude oil (Roy *et al.* 1999, Heintz *et al.* 2000). Early nutrition has been linked to enzyme activities in adults as well as epigenetic gene regulation (*e.g.* Barker 1995, Desai & Hales 1997, Waterland & Jirtle 2003, Wu *et*

al. 2004). In insects, the majority of adult organs are developed de-novo from imaginal discs (Truman & Riddiford 2002). However, the cells of few organs such as the nervous system are either carried over or remodeled (Truman & Riddiford 2002). Many of the neurons (*e.g.* motor or olfactory neurons) in adult *Drosophila* exist during the larval stage as functional neurons that are remodeled during metamorphosis (Tissot & Stocker 2000, Marin *et al.* 2005)

In conclusion, my experiment showed that the environmental conditions larvae experience can have carry-over effects on adult metabolic rate that are not mediated through body size. My results are also contributing to the increasing number of studies (Pechenik *et al.* 2006, Marshall & Morgan 2011) demonstrating that metamorphosis may not function as decoupling process between pre- and post-metamorphic traits as proposed by the adaptive decoupling hypothesis (Moran 1994).

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