



The metabolic scaling exponent and respiratory quotient of the tobacco hornworm, *Manduca sexta*



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Abstract

The speed at which an organism performs the necessary chemical reactions to maintain itself is called the metabolic rate. It may be that the metabolic rate (MR) and body weight (BW) of all organisms have a direct relationship that can be expressed as $MR = aBW^b$. The correlation is represented by b , the metabolic scaling exponent. In order to take a step towards identifying whether a universal metabolic scaling exponent exists in a model species that has great size variation, and whether this exponent is isometric or allometric, we studied respiration rates in relation to body weight in the *Manduca sexta* tobacco hornworm. Larval *Manduca* were weighed daily throughout their larval stage and respirometry measurements were taken using CO₂ and differential O₂ gas analyzers. Our results showed that the respiration rates of the larvae increased with body weight. O₂ uptake over all five instars yielded metabolic scaling exponents of 0.97 and 0.96 for our first and second cohorts respectively. CO₂ released by the *Manduca* revealed a mean metabolic scaling exponent of $0.98 \pm 4.0 \times 10^{-3}$ (error=SEM). The discrepancy between b values from O₂ consumption and CO₂ production rates may be attributed to a respiratory quotient greater than 1.0 as larvae could have been beginning to store carbohydrates for their dormant pupal phase. Despite these slight differences these findings are close to those previously obtained at Kenyon College where $b=0.90$ (Vreede, Stearns, & Melbarde, unpublished work). These values for the metabolic scaling exponent support the allometric scaling theory. Future studies should use a more refined approach to analyzing O₂ consumption in order to obtain more reliable data for early instars of *Manduca sexta* larvae. Additionally, using the same methods in analyzing a variety of species may indicate whether allometric scaling is specific to tobacco hornworms or is more universal.

Introduction

All species perform a series of chemical reactions that are necessary to sustain life. This collection of reactions is known as an organism's metabolism. The ratio of these processes over time is called the metabolic rate (MR).

It has long been observed that MR has a direct correlation to body weight (BW) (Chauvi-Berlinck, 2006). This relationship is expressed by the equation:

$$MR = a(BW)^b$$

Where a is a constant and b is the metabolic scaling exponent (Banavar *et al.*, 2002).

It has been speculated that a single value for the metabolic scaling exponent may exist that is applicable to all living things. Thus, by identifying the BW of any organism it would be possible to easily determine MR (and visa versa). However, there are two highly debated hypotheses on what the value of the metabolic scaling exponent might be.

In 1883, Max Rubner proposed that MR would remain proportional to body size despite developmental stage (White and Seymour, 2005). This isometric scaling hypothesis predicts a value for b of 0.67.

Later, Max Kleiber found evidence that metabolic scaling may be allometric (non-isometric) with a b value of 0.75 (Kleiber, 1932 cited in White and Seymour, 2005).

There has been a range of metabolic scaling exponents reported from 0.4 to 1.4, however most studies (including those previously performed at Kenyon College) support the allometric scaling hypothesis (Glazier, 2005).

This investigation was performed on *Manduca sexta* larvae, more commonly known as the tobacco hornworm. *M. sexta* is an ideal subject in comparing BW to MR as larval physiology is well understood, the species has been grown in a controlled setting for decades, and the larvae increase in size by 10,000 fold rapidly without large changes in morphology (Goodman *et al.*, 1985 cited in Greenlee and Harrison, 2005 and D'Amico *et al.*, 2001).

In analyzing both O₂ consumption and CO₂ production by larvae we hoped to better identify the metabolic scaling exponent for this species as well as assessing the ratio of O₂ to CO₂ exchanged, known as the respiratory quotient (RQ). Identifying RQ values allows for insight into the amount of fats and proteins the larvae metabolize along with carbohydrates.

Materials & Methods

Manduca sexta eggs were obtained from Carolina Biologic Supply. All larvae were allowed to hatch and grow in an incubator at a constant 27 °C and with a 16L:8D photoperiod. *Manduca* were fed a wheat germ diet. Organisms used for the respirometry study were chosen at random. These larvae were kept in separate, labeled containers that allowed for easy identification as well as the ability to clearly determine molt dates. All *Manduca* were weighed daily and allowed to pupate at the end of the fifth instar in artificial wooden burrows. After eclosion, moths were frozen and weighed again. These methods were repeated in the rearing of a second cohort of experimental larvae.

Respirometry was performed on the day of hatch through the end of the fifth instar. Gas exchange was measured using a four-channel Qubit Systems S104 differential gas analyzer. All measurements were carried out in a temperature control chamber at 27 °C. The analyzer was calibrated at 9:00 AM each morning and again between each subsequent test. Three larvae at a time were placed in animal chambers without food and attached to the gas analysis system. A cycle in which each animal was analyzed three times for five minutes was used. Ambient air from outside of the temperature control chamber traveled through one animal chamber at a time. Qubit Systems software then compared reference gas concentrations to sample gas concentrations based on airflow rate to produce O₂ exchange rate, CO₂ exchange rate, and RQ values.

Materials & Methods Cont.

Data were compiled into Microsoft Excel for analysis. Measurements of larvae that died during the experimental period were removed from the data set as their respiration and body weights appeared irregular. CO₂ and O₂ data for respiration and body weight were log transformed for each instar and fit with a linear regression. R² values resulting from the regression lines indicate the metabolic scaling exponents observed. Body weight compared to RQ values were graphed for each instar. First instar O₂ data from cohort 1 and first and second instar O₂ data from cohort 2 were excluded from analysis along with other outliers as these data were noted as unreliable due to the size of the organisms, as well as errors in calibration.

Results

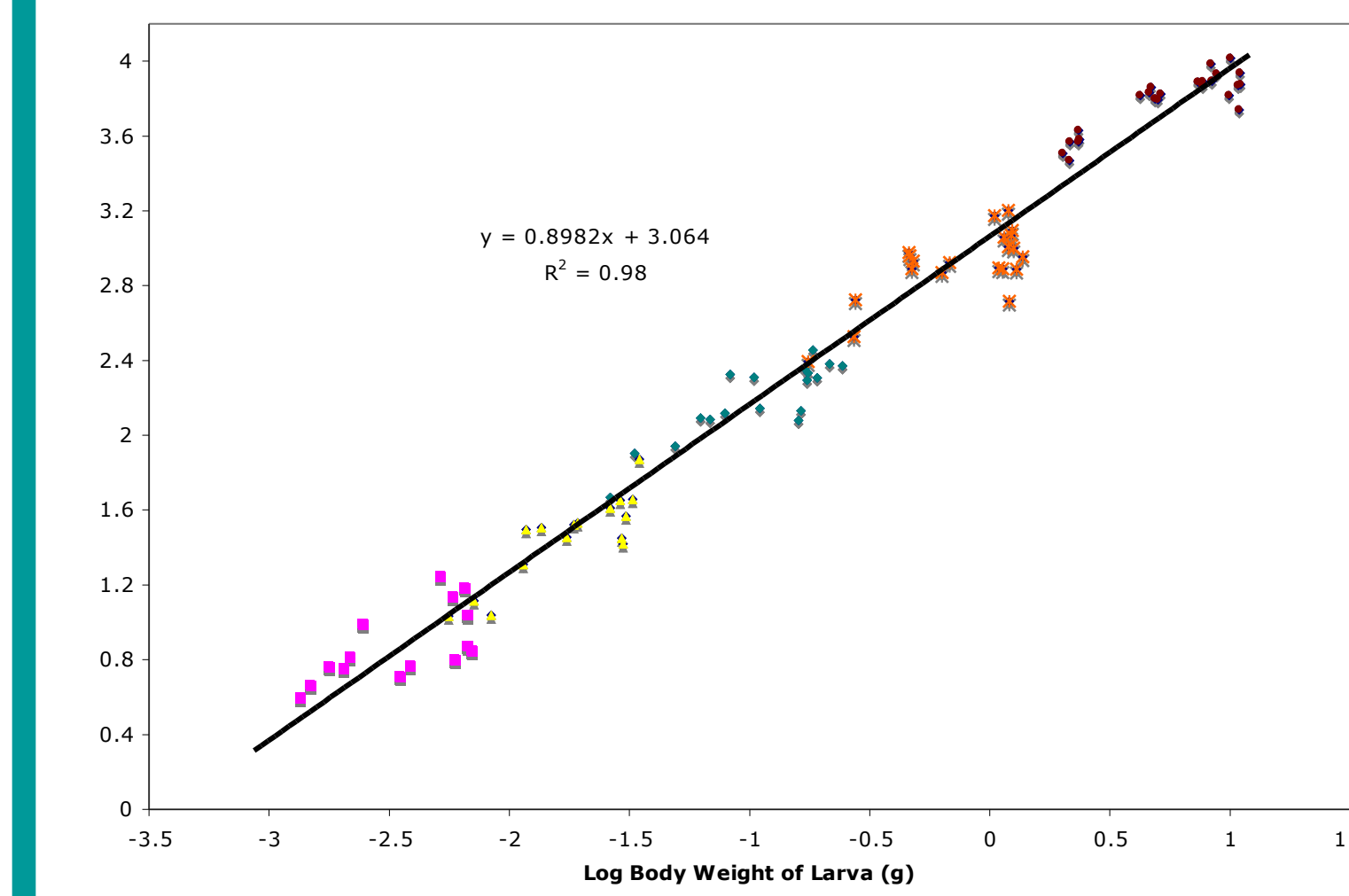


Figure 1. The relationship between body weight and rates of CO₂ released by Cohort 1 larvae at each instar. 1st, 2nd, 3rd, 4th, and 5th instar measurements are represented by pink squares, yellow triangles, aqua diamonds, orange stars, and red circles, respectively (Equation: $y = 0.8982x + 3.0641$, $R^2 = 0.98$, $n=6$).

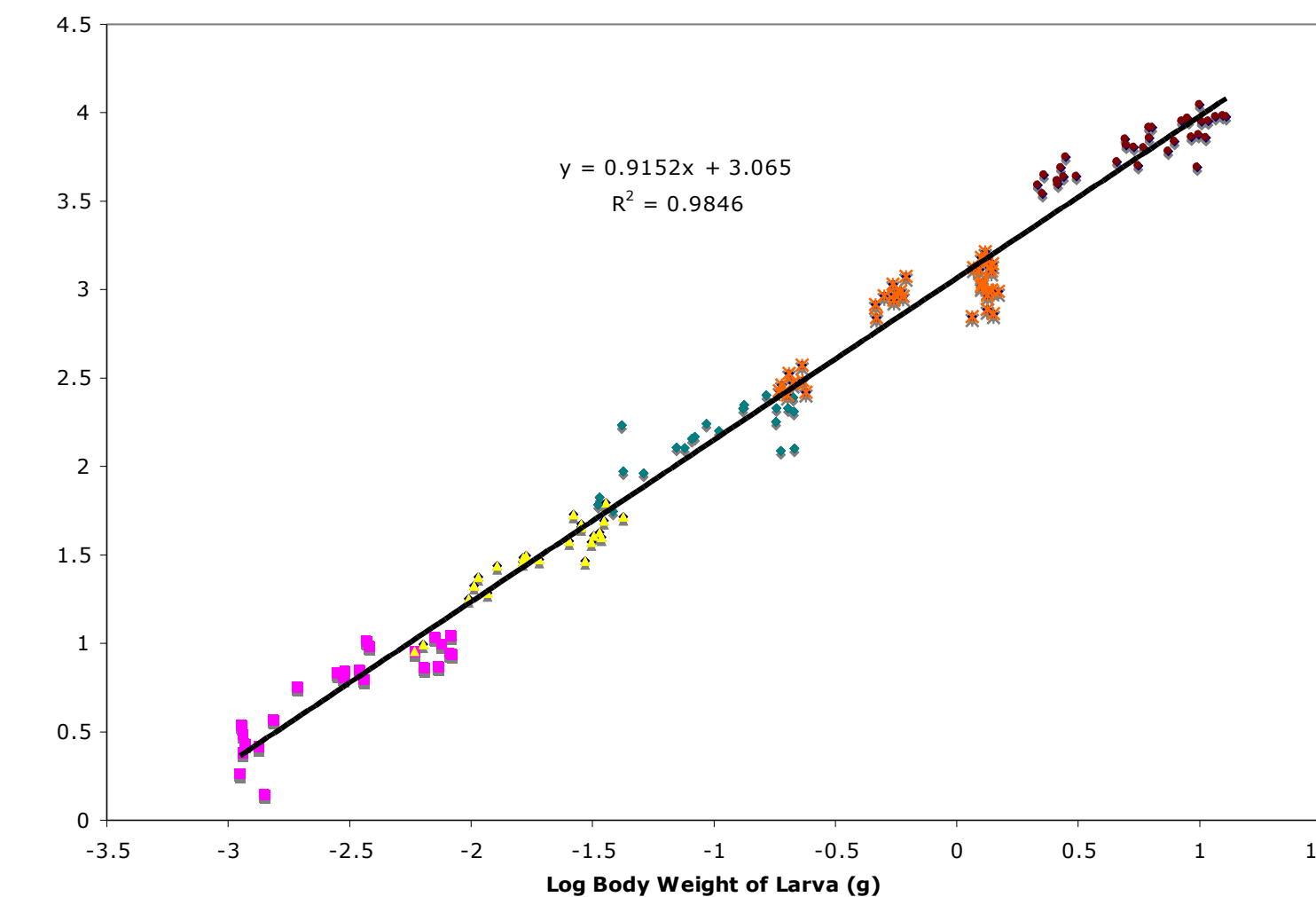


Figure 2. The relationship between body weight and rates of CO₂ released by Cohort 2 larvae at each instar. 1st, 2nd, 3rd, 4th, and 5th instar measurements are represented by pink squares, yellow triangles, aqua diamonds, orange stars, and red circles, respectively (Equation: $y = 0.9152x + 3.0653$, $R^2 = 0.9846$, $n=9$).

Ascending instars showed an increase in both body weight and rates of respiration.

As body weight increased the amount of CO₂ released by *Manduca sexta* also increased (Figure 1, $n=6$ and Figure 2, $n=9$). Measurements of CO₂ released from both cohorts of larvae yielded a mean metabolic scaling exponent of $0.98 \pm 4.0 \times 10^{-3}$ (error=SEM).

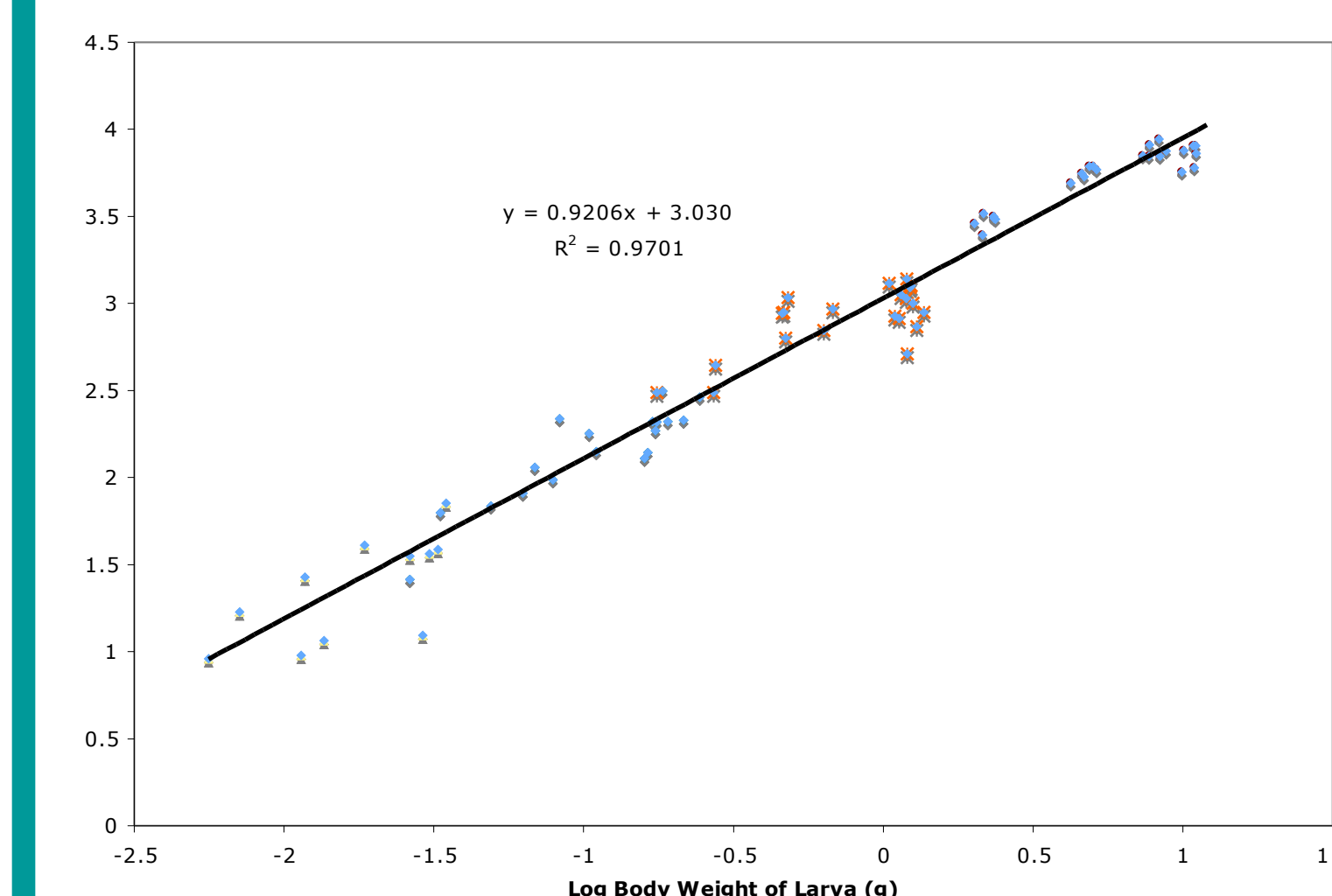


Figure 3. The relationship between body weight and rates of O₂ consumed by Cohort 1 larvae at each instar. 2nd, 3rd, 4th, and 5th instar measurements are represented by yellow triangles, aqua diamonds, orange stars, and red circles, respectively. 1st instar data were omitted as data were not reliable (Equation: $y = 0.9206x + 3.0302$, $R^2 = 0.9701$, $n=6$).

There was a direct relationship between larval body weight and O₂ consumption (Figure 3, $n=6$ and Figure 4, $n=9$). After removing outliers and unreliable data, the first cohort yielded a metabolic scaling exponent of 0.97, while the second cohort showed a metabolic scaling exponent of 0.96.

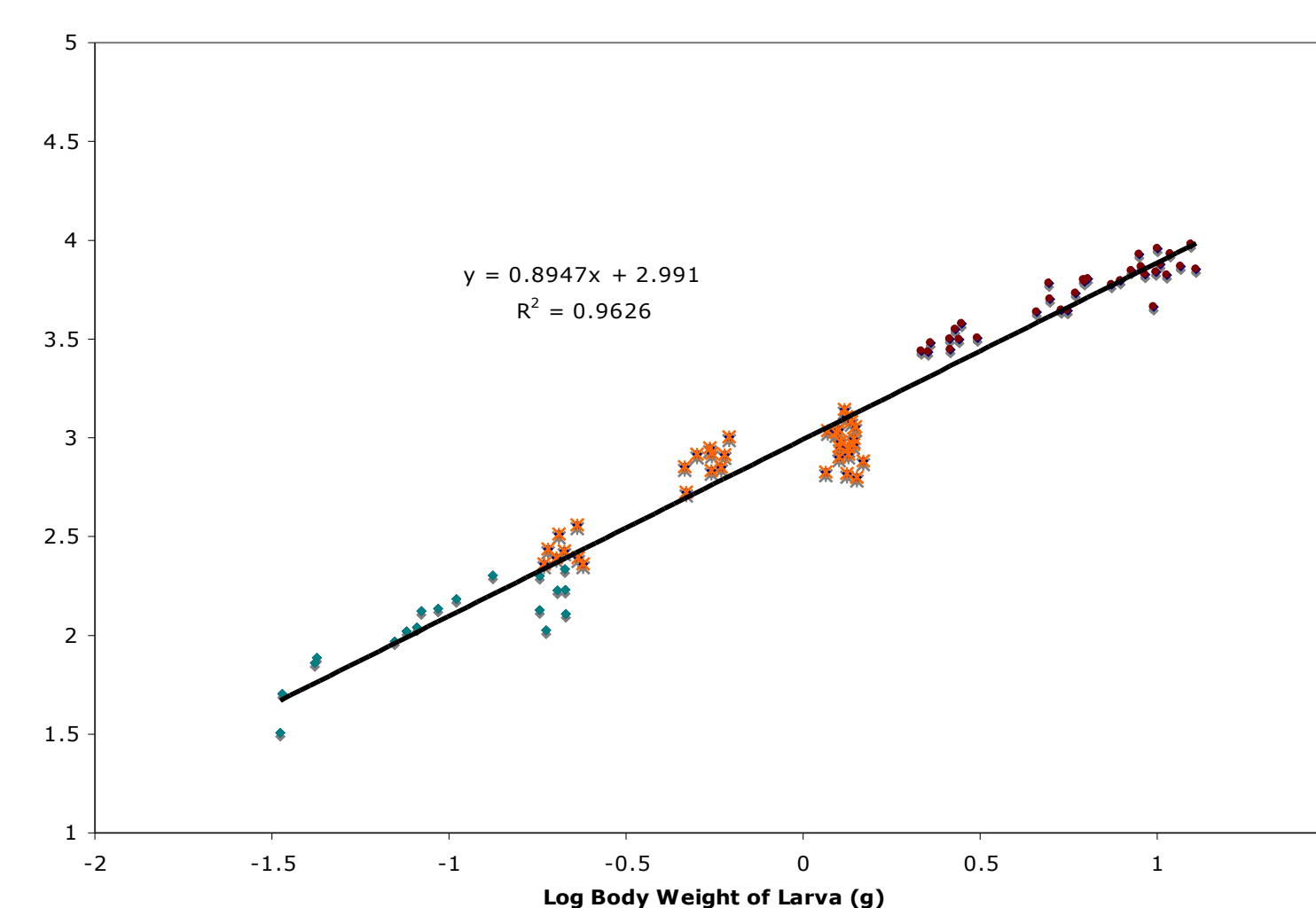


Figure 4. The relationship between body weight and rates of O₂ consumed by Cohort 2 larvae at each instar. 3rd, 4th, and 5th instar measurements are represented by aqua diamonds, orange stars, and red circles, respectively. 1st and 2nd instar data were omitted as data were not reliable (Equation: $y = 0.8947x + 2.9916$, $R^2 = 0.9626$, $n=9$).

Results cont.

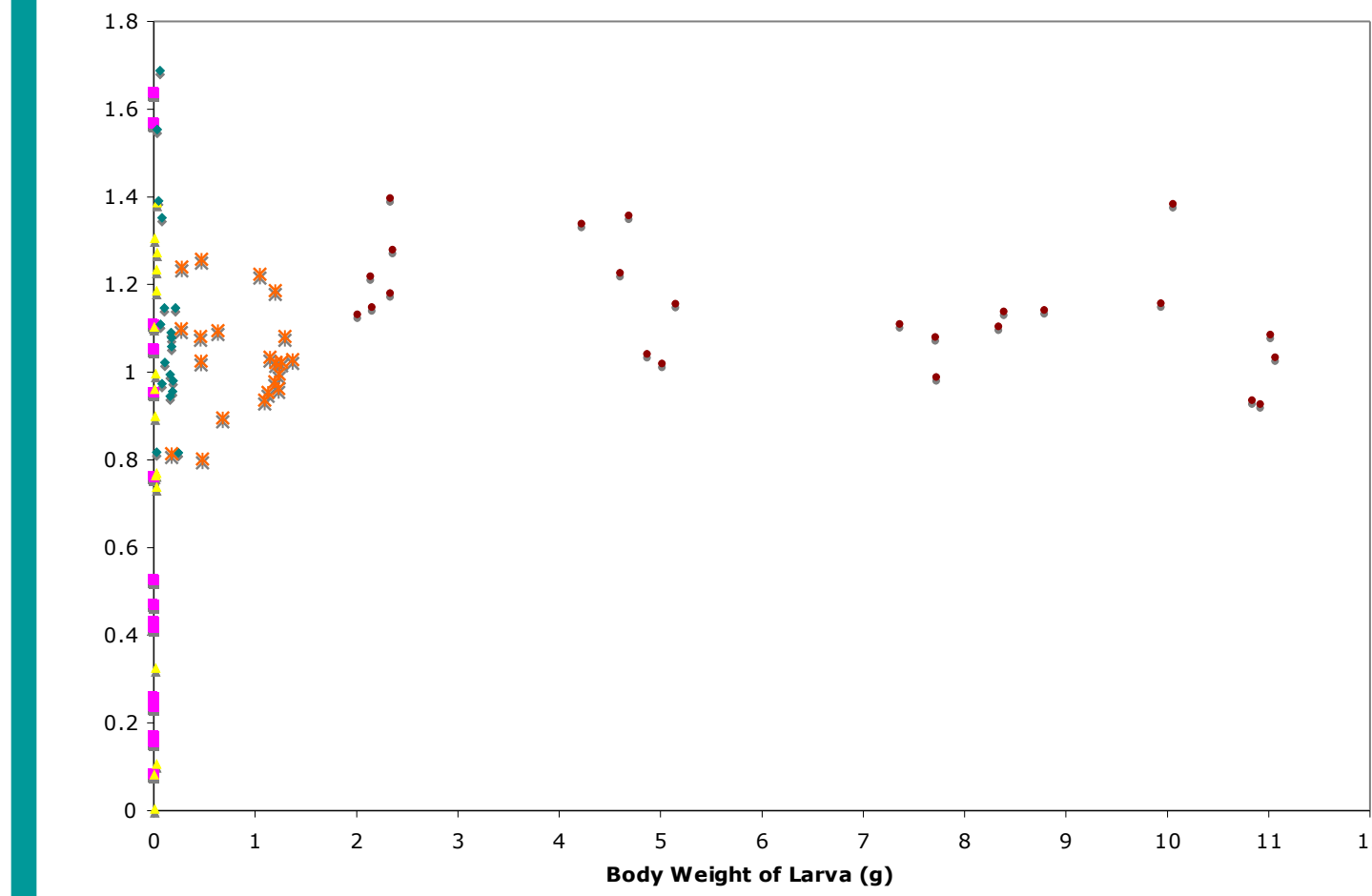


Figure 5. The relationship between body weight and respiratory quotient (RQ) values obtained from Cohort 1 larvae at each instar. 1st, 2nd, 3rd, 4th, and 5th instar measurements are represented by pink squares, yellow triangles, aqua diamonds, orange stars, and red circles, respectively ($n=6$).

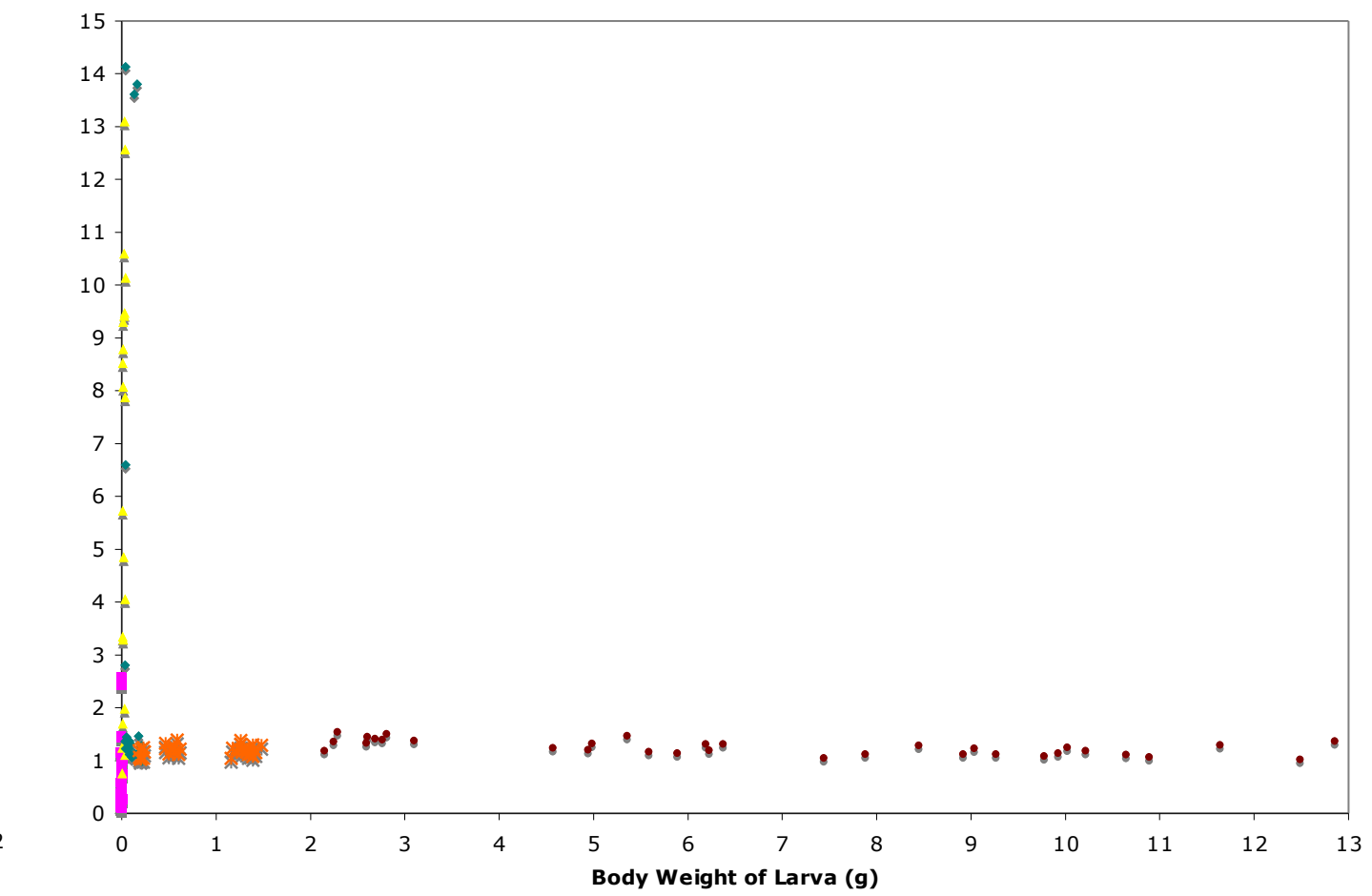


Figure 6. The relationship between body weight and respiratory quotient (RQ) values obtained from Cohort 2 larvae at each instar. 1st, 2nd, 3rd, 4th, and 5th instar measurements are represented by pink squares, yellow triangles, aqua diamonds, orange stars, and red circles, respectively ($n=9$).

Early instars showed great variation in RQ values (Figure 5, $n=6$ and Figure 6, $n=9$). Instars 2-5 in cohort 1 expressed a mean RQ value of approximately 1.05 ± 0.01 , while instars 3-5 in cohort 2 expressed a mean RQ value of approximately 1.21 ± 0.03 .

Discussion

The metabolic scaling exponents yielded by our CO₂ data from both cohorts were nearly identical after the removal of outliers. These b values were slightly higher than the metabolic scaling exponent of 0.90 found by past work at Kenyon College, but similarly supported the allometric scaling hypothesis (Vreede, Barrett, & Deimeke, unpublished work).

After the removal of outliers and unreliable data, O₂ consumption rates also revealed metabolic scaling exponents close to those previously described for *Manduca sexta* larvae. These findings further suggested that the tobacco hornworm scaled allometrically.

Metabolic scaling exponent values obtained from O₂ data were slightly smaller than those obtained from CO₂ analysis. This difference was evident in the resulting RQ values that were slightly greater than one, indicating that larvae were beginning to store carbohydrates for their dormant pupal phase (Burggren and Roberts, 1991).

In order to find more reliable b and RQ values for *Manduca sexta* larvae, it will be necessary to collect usable CO₂ and O₂ data for the earliest larval instars. Such data collection will in particular require fine-tuning calibration methods for the sensitive differential O₂ analyzer.

It may be of interest to perform similar experiments on different species in order to determine if allometric scaling is specific to the tobacco hornworm, or if it is applicable more universally.

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