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Title:

Long-term and acute effects of temperature and oxygen on metabolism, food intake, growth and heat tolerance in a freshwater gastropod

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Abstract

Temperature affects the physiology and life-history of ectothermic animals, often increasing metabolic rate and decreasing body size. Oxygen limitation has been put forward as a mechanism to explain thermal responses of body size and the ability to survive stress. However the time-scales involved in growth performance and heat tolerance differ radically. In order to increase our understanding of oxygen and temperature effects on body size and heat tolerance and the time scale involved, we reared *Lymnaea stagnalis* under six combinations of temperature and oxygen tension from hatching up to an age of 300 days and recorded shell length during this whole period. At the end of this period, we determined scope for growth by measuring food intake rate, assimilation efficiency, respiration rate and ammonium excretion rate at two different temperatures. We also measured the snails' ability to survive heat stress (CTmax), both at normoxia and hypoxia. We found that scope for growth and long term growth performance were much more affected by interactions of chronic oxygen and temperature conditions during rearing than by acute conditions during testing. Furthermore, our study shows that individual variation in growth performance can be traced back to individual differences in rates of food and oxygen consumption. Developmental acclimation also gave rise to differences in CTmax, but these were relatively small and were only expressed when CTmax was tested under hypoxia. The large effects of rearing oxygen conditions on growth and other physiological rates compared to modest effects of test oxygen conditions on CTmax suggest that small effects of hypoxia on the short term (e.g. heat tolerance) may nevertheless have large repercussions on the long term (e.g. growth and reproduction), even in a pulmonate snail that can compensate for hypoxia to some extent by aerial respiration.

Keywords: *Lymnaea stagnalis*; Oxygen limitation; Temperature acclimation; Scope for growth; CTmax; Individual variation

1. Introduction

Temperature is an important environmental factor, especially for ectotherm animals. It affects physiological rates such as metabolism, growth and development (Gillooly et al., 2001; Van der Have and De Jong, 1996; Yamanaka et al., 2013) and life history parameters like body size and longevity (Anzueto-Sanchez et al., 2014; Goss and Bunting, 1983). Studies of thermal effects on ectotherm physiology have focussed on thermal tolerance and body size (Atkinson, 1994; Klok et al., 2004; Stillman, 2003; Sunday et al., 2012). For both heat tolerance and body size, oxygen limitation has been implicated as a key mechanism to explain thermal responses (Atkinson et al., 2006; Pörtner, 2006; Verberk and Atkinson, 2013; Verberk et al., 2016b), such that acute hypoxia may decrease survival under heat stress and chronic hypoxia may decrease body size. Warmer conditions elicit a greater oxygen demand through increased metabolism, which has been hypothesized to cause a mismatch between oxygen demand and supply (Pörtner, 2006; Winterstein, 1905), especially in an aquatic setting (Verberk and Atkinson, 2013; Verberk et al., 2011; Woods, 1999). This mismatch has been argued to arise from whole-organism capacity limitations preventing animals from increasing rates of oxygen delivery to meet tissue oxygen demand (Pörtner, 2006). Such mismatches could explain body size responses to chronic high temperatures and limit survival under acute heat (Verberk et al., 2011).

Final body size is often inversely related to rearing temperature, resulting in animals attaining smaller sizes when reared under warmer conditions. This phenomenon, termed the temperature-size rule (Atkinson, 1994), is widely documented, but the underlying mechanisms remain poorly understood (Angilletta and Dunham, 2003; Forster et al., 2012). Since final size is the result of growth, a key to understand thermal effects on size is to understand its effects on growth. Growth (increase in body mass) can be described using the net difference between resource intake and metabolism (Von Bertalanffy, 1960). Recently, Hoefnagel and Verberk (2015) have shown that oxygen availability can enhance or diminish the effects of temperature on growth in the aquatic isopod *Asellus aquaticus*. Interactive effects of temperature and oxygen were also shown in

bryozoans by Atkinson et al. (2006) and rotifera by Walczyńska et al. (2015). Finally, the temperature-size rule appears to be stronger in aquatic ectotherms (Horne et al., 2015), where oxygen limitation is perhaps more likely because of the difficulties of aquatic respiration (Verberk and Atkinson, 2013).

Given that thermal responses could potentially be better understood from an oxygen perspective, disentangling the effects of temperature and oxygen on the different components of growth (e.g. intake, absorption efficiency, respiration and excretion) may thus improve our understanding of the effect of temperature on growth. A complication here is that the thermal sensitivity of physiological rates is plastic, being affected by thermal history. For instance, Seebacher et al. (2015) showed that acclimation usually decreases the thermal sensitivity of an animal, reflecting the action of physiological processes involved in maintaining homeostasis.

Temperatures that delineate survival under heat stress (CTmax) are often determined in experiments with gradually increasing temperature. Whether oxygen universally becomes limiting at temperatures delineating the thermal performance window is currently debated (e.g. Clark et al., 2013; Ern et al., 2015; Pörtner and Giomi, 2013; Verberk et al., 2016b). A consideration of the mode of respiration of the animal may help in resolving this debate (Verberk & Bilton, 2015; Verberk et al. 2016). For instance, aquatic insects that employ aquatic gas exchange via gills or tegument are more prone to oxygen limitation than those that can rapidly increase oxygen uptake by aerial respiration (Verberk and Bilton, 2013; 2015). Similarly, an increased heat tolerance afforded by aerial gas exchange has been argued to explain why air-breathing crabs mainly occur in the tropics (Giomi et al., 2014). In gastropod molluscs, gill-breathing caenogastropod species appeared to be more prone to oxygen limitation at high temperatures than pulmonate snails (Koopman et al., 2016). Pulmonate snails can largely overcome oxygen limitations posed by a warm, aquatic environment by breathing air, thus increasing their survival at high temperatures.

Thermal history also matters for CTmax. For instance, CTmax will be higher when rapid rates of warming are employed, reflecting the fact that survival of heat stress depends on both the duration and intensity of heat stress (Rezende et al., 2014; Terblanche et al., 2007). While the time

scale employed thus matters for survival under heat stress, there is evidence that hypoxia reduces CTmax when measured in the lab on the short term and also shifts thermal niches towards colder temperatures during long term field exposures (Verberk et al., 2016a). However, there may be other reasons why an animal succumbs to heat stress that are not directly related to oxygen availability, such as loss of neuronal function (Ern et al., 2015). Studies have also shown that acclimation under higher temperatures can increase CTmax although not proportionally (Gunderson and Stillman, 2015), providing further evidence that thermal history can modulate survival under heat stress, possibly by modulating the sensitivity of an animal for oxygen limitation. Animals may similarly modulate their sensitivity for oxygen limitation in response to the oxygen conditions experienced during rearing.

Here we test the effect of rearing conditions that act on the long term and test conditions that act on the short term on metabolism, food intake, growth and survival under heat stress. Both oxygen and thermal conditions are manipulated to allow testing of the main hypothesis that an oxygen perspective can improve our understanding of thermal responses. Our study species, *Lymnaea stagnalis* (L.) is a large freshwater gastropod that employs both air breathing via a lung and tegument breathing (Jones, 1961). We hypothesized that rearing temperature and oxygen levels interactively affect growth and its components, such that growth will be maximised under warm, hyperoxic rearing conditions. We also measured the surface of the tentacles because *L. stagnalis* may be able to increase this surface in order to improve tegument breathing when reared under hypoxia. With regard to heat tolerance we hypothesized that a high rearing temperature will increase CTmax, while acute hypoxia will lower it. Moreover, hyperoxic rearing is expected to reduce CTmax under normoxia or exacerbate reductions in CTmax when tested under hypoxia, while hypoxic rearing is expected to have opposite effects.

2. Methods

2.1. Maintenance in the lab and length measurements

200 individual *Lymnaea stagnalis* were obtained from a lab at Wageningen University in the summer of 2013. These snails were kept in 10 L glass aquaria at room temperature (18-20 °C). The water (Dutch standard water: demineralized water of 0.2 µS cm^{-1} with 0.20 g l^{-1} $CaCl_2.2H_2O$, 0.18 g l^{-1} $MgSO_4.7H_2O$, 0.10 g l^{-1} $NaHCO_3$ and 0.02 g l^{-1} $KHCO_3$) was replaced weekly and food (fresh leaves of iceberg lettuce) was supplied *ad libitum* two to three times each week. Aquariums were continuously aerated with compressed air. Snails in the current experiment originated from weekly collected egg masses (February and March 2014) from F2 aquaria and assigning each weekly batch (800-5000 hatchlings) to one of the six treatments upon hatching, which took one week. Treatments consisted of a factorial design of two temperatures and 3 oxygen levels. Four large trays, containing water that was temperature controlled at either 17°C (2 trays) or 22°C (2 trays) were used and in these trays fifteen 6 L aquaria (3-4 per tray) were placed, creating 2-3 replicates per treatment. Mass stream gas flow controllers (Bronkhorst Nederland) were used to create three mixtures of oxygen and nitrogen and these were bubbled into each 6 L aquarium to keep oxygen levels in both the water and the overhead air at 10, 20 or 40 kPa. Water and food were replaced as described above. Total shell length was determined of a random sample of 20 snails per aquarium every second week or all snails in an aquarium when they numbered fewer than 20.

2.2. Scope for growth

The components of growth can be written in an equation using the concept of scope for growth (Warren and Davis, 1967).

$$\text{Scope for growth (J)} = \text{Intake} * AE - \text{Respiration} - \text{Excretion} \quad (1)$$

Where rates are given as their respective energy equivalents. Intake is all consumed energy (620 J g^{-1} wet iceberg lettuce), AE is assimilation efficiency, the fraction of intake that becomes available for processing, Respiration is an approximation of energy loss for metabolism (14.4 J mg^{-1}

O_2, Elliott and Davison (1975)) and Excretion is energy lost after metabolic processing (19.4 J mg^{-1} NH_4^+, Elliott and Davison (1975)). Snails were selected from the 18 rearing aquaria (October 2014 – February 2015) to determine scope for growth (Equation 1). Food ingestion was determined by keeping individual snails separate for 4 days and adding approximately 2 grams of pre-weighed fresh iceberg lettuce on the second day. Faeces and remaining lettuce were collected after these 4 days and weighed. The amount of faeces produced in this time frame was not sufficient to reliably determine assimilation efficiency for each treatment separately. Therefore, an assimilation efficiency of 65% was used in our calculations, which was the average assimilation efficiency across all treatments.

Oxygen consumption was determined by measuring oxygen tension in the water, using a micro optode connected to a Microx TX3 fibre-optic oxygen meter (Presens instruments, Regensburg, Germany) before and after a 4 hour run in which individual snails were kept in closed, opaque 100 mL pots without access to air. Thus, only aquatic respiration was quantified. Snails were allowed to acclimate to test conditions for one hour prior to closing the pots and the first oxygen measurement.

Ammonium production was measured in another 4 hour run in which individual snails were kept in 100 mL pots with access to air. The DSW in these pots was created with milliQ (0.060 µS cm^{-1}) rather than demineralized water (0.2 µS cm^{-1}) to avoid any ammonium contamination. Ammonium concentration was determined by an autoanalyzer (Bran&Luebbe, Norderstedt, Germany) using the Berthelot reaction.

To allow for comparisons at the level of individuals, food ingestion, oxygen consumption and ammonium excretion were measured in the same individual snail where possible. Individuals were tested at one of two temperatures (17°C or 22°C). To further explore covariates of differences in growth rate across individuals, snails in the treatment with low temperature and hypoxia were divided into four quartiles based on their shell length which is a proxy for their growth rate (Figure A.1).

2.3. CTmax trials

Temperatures delineating survival under heat stress (CTmax) was determined using thermal ramping trails. CTmax was measured for snails originating from each of the six rearing treatments (3 oxygen levels crossed with two temperatures). An electronic water bath was used to create a continuously increasing water temperature at a rate of 0.25 °C min^{-1}, starting at 20°C. Individual snails were placed in an air-sealed flow-through chamber (0.04 L s^{-1}) and each flow-through chamber also included a small head space holding a layer of air, allowing the snails to breathe air during heat trials. The air in this compartment could also be exchanged via a needle with different gas mixtures of oxygen and nitrogen, in such a way that the air and water compartments held the same gas mixture (see Verberk & Calosi, 2012 and Koopman et al., 2016 for further details). The endpoint that could be most consistently scored and therefore used as a measure of CTmax was loss of movement when snails did no longer move and often also lost grip of the sides of the box, causing them to float at the water surface. At this stage snails could still recover from heat injury, but this was not quantified as snails were transported to anoxic water after the heating trials, to prepare them for measurements of tentacle area and body mass. While many different measures are used in the literature to approximate critical temperatures during heating trials (e.g. onset of spasms, loss of equilibrium, loss of movement, inability to recover), our measure of CTmax does fit with the definition of being an empirical endpoint at which the animal becomes moribund and can no longer escape the adverse temperatures (Lutterschmidt and Hutchison, 1997). Note also that these different measures often are exhibited sequentially and in a study on damselflies their responses to oxygen conditions were largely similar (Verberk & Calosi, 2012). CTmax was determined for snails under normoxia (20 kPa) and under hypoxia (5 kPa), but not hyperoxic conditions as previous work showed very minor effects of hyperoxia in pulmonates (Koopman et al., 2016).

2.4. Body mass

After measuring components of scope for growth, snails were killed in anoxic water at 22 °C to avoid retraction of the tentacles. Tentacle surface was determined with dissection microscope (40x magnification and a microscale) and calculated by multiplying the length (in mm) of the tentacle (from the eye to the tip) with the width (in mm) of the tentacle base, which are close to perpendicular, approximating a triangle. Considering that a snail has two tentacles, the total tentacle surface (in mm^2) can be approximated as a rectangle. Both tentacles were measured and the surface of the larger of the two was doubled to correct for partial retraction. Soft body was removed from the shell and weighed after gently blotting on tissue paper. Shell free wet mass (SFWM) was measured to the nearest 0.001 gram on a micro balance (Mettler-Toledo XA105, Switzerland). A value of 4860 J g^{-1} shell free wet mass (Rumohr et al., 1987) was used to convert growth in grams to its energy equivalent. Given that body mass exerts a large influence on the components of scope for growth we used a mass correction. Mass exponents (b) were determined by regressing log transformed mass data for rates (consumption, respiration, excretion) and tentacle surface area against log-transformed shell free wet mass (M). Subsequently, data was mass-corrected by dividing the measured value by M^b (Table 3).

2.5. Statistical analysis and growth curves

Analyses were done in R version 3.2.3 using linear models from the *stats* package and mixed linear models from the *nlme* package (Pinheiro et al., 2013). The best linear model was selected based on AIC values by reducing the full model (including rearing temperature, rearing oxygen, test temperature and interactions) and we tested whether residual variation could be explained by aquarium ID. If this was the case, we included a random factor of aquarium ID nested within block. A block consisted of one cold tray and one warm tray.

Length (mm) to body mass (g) relationships (equations 2a and 2b, one for each rearing temperature) were estimated empirically on snails that were used for scope for growth

measurements. These relationships were used to calculate wet mass for the few snails that we did not have mass data for.

$$SFWM_{17} = 4.759 \cdot 10^{-5} * L^3 \quad (2a)$$

$$SFWM_{22} = 1.012 \cdot 10^{-4} * L^3 \quad (2b)$$

Modified Von Bertalanffy growth curves (equation 3) were fitted through shell free wet mass (g) values by non-linear least square (*nls*) regression for each rearing treatment separately. This served the purpose of statistically comparing its parameters between treatments.

$$SFWM = M_0 + (M_a - M_0) * (1 - e^{-K*D*t})^{3/D} \quad (3)$$

Where average mass at day zero M_0 was $2.7 \cdot 10^{-4}$ g, M_a is asymptotic body mass, K is a relative Bertalanffy growth rate, D corrects for allometric growth of respiratory organs and t is time in days which equals the age of snails. K was not estimated but fixed at 0.05 based on preliminary analysis, since M_a and K interacted too strongly.

Modified Von Bertalanffy growth curves (equation 3) were also used to estimate average growth in the 30 days prior to scope for growth measurements on an individual basis. This was used to test for a relationship between average growth and scope for growth across individuals. To this end, the growth curve for a given rearing treatment was used as a template, and rescaled (i.e. adjusting M_a) to match the size of the individual at the time of scope for growth measurements. This curve was then used to calculate the body mass that the individual snail would have had 30 days earlier. Thus, we assumed that individuals within each rearing treatment only differed in the vertical scaling of the Von Bertalanffy growth curve.

3. Results

3.1. *Observed growth*

Snails reared under warm conditions grew faster and consequently reached a larger size than snails reared under cold conditions. The modified Von Bertalanffy growth curves fitted to biweekly size measurements captured 41-66% of the variation in the data (Table 1). Snail growth was found to differ between replicate aquaria and therefore we included aquarium ID as a random effect in the statistical models, nested within Block. Log-transforming body mass did not improve AIC of the model with respect to untransformed data, so no transformation was used. The mixed effects model on body mass with random slopes of mass over age for each aquarium confirmed that growth rate (i.e. the slope of body mass over age) was significantly increased by rearing temperature ($t_{1,3691}$ = 2.530; P = 0.011, Table A.1). Rearing oxygen did not affect growth rate ($t_{1,3691}$ = -0.072; P = 0.943, Table A.1.). Warm rearing conditions also increased final size, estimated by the asymptotic body mass of the Von Bertalanffy growth curves, while hypoxia decreased final size in both rearing temperatures (Figure 1, Table 1). Interactive effects between rearing temperature and rearing oxygen conditions on growth rate and final size appear to be absent.

3.2. *Scope for growth*

Scope for growth was determined for a total of 120 snails. Conover's (1966) method resulted in an assimilation efficiency of 65%. Energy lost in respiration consisted of roughly 10-20% of the total energy consumed while energy lost in excretion through ammonia comprised only between 0.1% and 1.6% of total consumed energy (Figure 2b). Snails from hyperoxic warm rearing conditions consumed less energy than other snails from the warm treatments, while the lowest consumption in the cold treatments was found under hypoxic conditions (Figure 2a).

The calculated scope for growth in J/d/snail was significantly explained by rearing conditions (temperature $t_{1,113}$ = 5.264; P <0.001, rearing oxygen availability $t_{1,113}$ = 1.980; P = 0.050, Table 2). Rearing temperature increased scope for growth, but less so under high rearing oxygen conditions and in large individuals, as was evident from significant interactions between rearing temperature

and both rearing oxygen conditions ($t_{1,113}$ = -3.410; P < 0.001, Table 2) and body mass ($t_{1,113}$ = 3.179; P = 0.002, Table 2). Test temperature did not affect scope for growth ($t_{1,113}$ = 0.245; P = 0.807, Table 2).

A linear model on log-log transformed data revealed a positive relation between energy potentially available for growth (i.e. scope for growth) and the energy equivalent of actual growth (i.e. the biomass they accumulated in the last 30 days; P < 0.001, R^2 = 0.28). Thus, individual snails that had grown more during the last 30 days also demonstrated a higher scope for growth. However, actual growth was lower than that predicted based on scope for growth for most snails (Figure 3).

Because of the large size differences of the snails in the different treatments, a mass correction was applied, facilitating comparisons among treatments. Mass exponents based on linear regression on log-transformed data ranged from 0.40 for scope for growth to 0.98 for food consumption (Table 3).

Mass corrected food consumption rates could not be explained by rearing conditions or test temperature when aquarium ID was included as random factor although it seemed higher in the smaller snails reared under cold conditions (Figure 4). Oxygen consumption rate, however, was related to the interaction between rearing temperature and rearing oxygen conditions ($t_{1,4}$ = -3.145; P = 0.035, Table 4). Oxygen consumption declined with increasing rearing oxygen tension, but only in the warm conditions. Test temperature was never a significant predictor except for ammonium excretion ($t_{1,110}$ = 2.360; P = 0.020), which also related to rearing oxygen ($t_{1,5}$ = 3.059; P = 0.028, Table 4 and Figure 4). Tentacle surface area was smaller under hyperoxic rearing conditions and larger under hypoxic rearing conditions ($t_{1,85}$ = -2.562; P = 0.012) but did not vary with rearing temperature.

Mass corrected food consumption was related to mass corrected oxygen consumption, indicating that snails with relatively high metabolism also consume relatively more food (Figure 5). This suggests that individual variation in their physiological activity level is important, meriting a further investigation.

3.3. *Variation across individuals from the cold hypoxic treatment*

For snails from the rearing treatment with low temperature and low oxygen we had sufficient individuals (120) for an experiment to explore differences between individuals with high physiological activity (exhibiting fast growth) and those with low physiological activity (exhibiting slow growth). These analyses showed that for snails reared under cold, hypoxic conditions, differences in average growth rate during their lifetime were positively related to differences in scope for growth ($t_{1,123}$ = 2.220; P = 0.028, Table 5 and Figure 6). This relationship was also dependent on body size, such that the fast growing snails exhibited a high scope for growth only at small size ($t_{1,123}$ = -2.614; P = 0.010, Table 5).

The correlation between growth rate and scope for growth was also reflected in food consumption being higher in fast growing snails (Figure 7), whereas oxygen consumption was mainly affected by body mass ($t_{122,128}$ = 4.711; P < 0.001, Table 5). Similar to the pattern across all treatments, no clear pattern with test temperature was observed (Figure 7), except for its effect on ammonium excretion ($t_{1,123}$ = 2.815; P = 0.006, Table 5), but since ammonium excretion represents only a minor loss of energy (0.1 – 1.6%), this effect of test temperature was not reflected in the calculated scope for growth ($t_{1,123}$ = 0.391; P = 0.697). Interactive effects between body size and growth rate for food consumption ($t_{1,123}$ = -4.029; P < 0.001, Table 5) indicate that the increase in food consumption with growth rate is weakened in large snails, suggesting that in these snails, high food consumption has a smaller contribution to achieving fast growth. Similar interactive effects were found for oxygen consumption ($t_{122,128}$ = 5.542; P < 0.001), suggesting that large snails grow better when their oxygen consumption is low. Snails with fast growth also had larger tentacles ($t_{1,96}$ = 3.573; P < 0.001) although this effect diminished in larger snails ($t_{1,96}$ = -2.916; P = 0.004, Table 5).

3.4. *CTmax*

Lymnaea stagnalis could survive temperatures up to 40.5 °C in our ramping trials. Hypoxia during tests did not affect CTmax in snails from warm rearing conditions, but it decreased CTmax of

snails from cold conditions relative to those found under normoxia ($t_{1,85}$ = 5.336; P < 0.001, Figure 8, Table 6). The oxygen conditions during rearing had a small effect on CTmax, which also differed between warm and cold rearing conditions ($t_{1,85}$ = -2.347; P = 0.021, Table 6): In warm-reared snails, snails reared under increasing levels of oxygen had reduced CTmax, while in cold-reared snails, CTmax increased with increasing oxygen conditions during rearing. Wet mass had no significant effect on CTmax of *Lymnaea stagnalis*.

4. Discussion

How animals deal with changing environmental conditions depends on the intensity of the changes and how long animals are exposed to these changes (Rezende et al., 2014). Here we show that long term differences in rearing temperature have pronounced effects on growth rate and the resulting body size (Figure 1). Scope for growth and its components likewise respond to rearing temperature, rearing oxygen conditions or their interaction (Table 2 and Table 4).

In evaluating whether thermal responses are dependent on oxygen conditions and vice versa (i.e. interactive effects of oxygen and temperature), it is clearly important to distinguish between short-term test conditions and long term rearing conditions (Seebacher et al., 2015). For our results the effects of rearing conditions on the long term appear to be more pervasive than those of the test conditions, with the exception of the test oxygen conditions during the CTmax trials. The idea underlying an oxygen perspective to understand thermal responses in survival and performance is that oxygen is more likely to become limiting under warm conditions.

The study species, *Lymnaea stagnalis* is a bimodal breather, capable of extracting oxygen from the water through the skin as well as from air by using its lung. Jones (1961) showed that at the test temperature of 15 °C, the aerial respiration and aquatic gas exchange contributed equally to total respiration of *Lymnaea stagnalis* at normoxia, but respiration increasingly relied on aerial gas exchange at progressively lower test dissolved oxygen. Previous work has shown that relative

reliance on aerial gas exchange increases with increasing temperature in bimodal breathers (e.g. Verberk and Bilton, 2015), including *Lymnaea stagnalis* (Sidorov, 2005) and other pulmonate snails (Koopman et al., 2016). This mode of gas exchange is expected to make the snails less prone to oxygen limitation when heat-stressed (Boardman and Terblanche, 2015; Davenport and Davenport, 2007; Giomi et al., 2014; Koopman et al., 2016; Verberk and Bilton, 2015; Verberk et al., 2016b). Indeed, reductions in CTmax when comparing trials under normoxia and hypoxia (Figure 8) were small compared to the 6.5 °C reduction found in aquatic ectotherms that relied on aquatic gas exchange by Verberk and Bilton (2015) and (Verberk et al., 2016a).

The observation that rearing temperature did not alter CTmax under test normoxia in *Lymnaea stagnalis* confirms that long-term acclimation has limited capacity to improve heat tolerance (Sandblom et al., 2016; Van Maaren et al., 2000), suggesting that the ability to shift thermal niches under climate warming is small (Araujo et al., 2013). Interestingly, while there was no difference in the CTmax of animals reared at 17 °C and 22 °C under test normoxia, reductions in CTmax induced by test hypoxia were greater in cold reared snails (Fig. 8). Differences in CTmax associated with rearing oxygen conditions were smaller than those associated with test oxygen conditions. Still, CTmax was lower in snails reared under hyperoxic conditions, but only in warm-reared snails (Table 6). Thus, effects of oxygen on the CTmax of snails whether acute during the CTmax trials or chronic during rearing are linked to rearing temperature. If a mismatch between oxygen supply and demand sets CTmax, then the reductions in CTmax under hypoxia in cold reared snails arises because of cold-reared snails having a higher oxygen demand than warm-reared snails in cold reared snails, which then becomes problematic under hot, hypoxic conditions. This could indeed be the case, as warm-acclimation reduces thermal sensitivity (lower Q_{10}; Seebacher et al., 2015), and the slower increase in oxygen demand with warming will result in higher CTmax (e.g. Verberk et al., 2011). Oxygen conditions during rearing have likely not consistently changed the oxygen demand (see also fig 4), but animals may have modulated their capacity to extract oxygen in response to rearing oxygen, with hypoxia reared animals likely having higher capacity for oxygen extraction, such

as enlarged tentacle surface areas (cold rearing) or increased capacity for areal gas exchange (warm rearing). For warm-reared snails, a lower capacity for aerial breathing with increasing oxygen conditions during rearing may have contributed to reductions in their CTmax. For cold-reared snails, the capacity for aerial gas likely did not change with the oxygen conditions during rearing as these snails relied more on aquatic gas exchange. This line of reasoning is supported by the observation that cold-reared snails modulated the tentacle surface area in response to rearing oxygen conditions.

While acute oxygen conditions had only modest effects on CTmax in *L. stagnalis*, concordant with its ability for aerial gas exchange, long term growth performance was clearly negatively affected in snails reared under chronic hypoxia, both when reared at 17 and 22 °C (Figure 1). Thus, the ability to breathe air in itself does not safeguard a species from effects of chronic hypoxia. Indeed, the size of the tentacles in the snails, which are an important site for gas exchange, increased with decreasing chronic oxygen availability. This indicates that snails attempt to compensate for chronic poor water oxygenation by increasing respiratory surfaces to minimise time spent surfacing. Thus the bimodal breathing strategy of *L. stagnalis* allows it to partially deal with both acute and chronic oxygen stresses related to heat stress.

To better understand thermal responses of growth, we quantified the scope for growth of individual *L. stagnalis*. The positive relationship between average growth and scope for growth (Figure 3) supports the underlying premise that thermal responses in growth, which ultimately lead to changes in body size, can be better understood by studying how temperature affects scope for growth and its components. Test temperature had much smaller influences on physiological rates than rearing temperature, indicating that thermal history can override thermal conditions (see also Giomi et al., 2016). We found interactive effects of rearing temperature and rearing oxygen on scope for growth. Scope for growth appeared to improve with chronic oxygen tension in the cold treatments, yet surprisingly it decreased with oxygen tension in the warm treatments, i.e. opposite to the expectation that oxygen is more likely to become limiting under warm conditions (Pörtner, 2006) and that hyperoxia may relieve this limitation. This is difficult to explain. Scope for growth was

calculated using a constant assimilation efficiency (see Methods), but even if assimilation efficiency was higher under warm, hyperoxic conditions, this would not compensate for the greatly reduced food consumption in that treatment. Oxygen toxicity may have played a role, since survival was similarly low in our warm, hyperoxic rearing treatment, but another issue here is the large size difference between snails reared at 17 °C and 22 °C, as size affects both oxygen demand as well as the capacity to extract oxygen from the water in non-linear ways (Gillooly et al., 2001; Verberk and Atkinson, 2013). Since warm-reared snails greatly differed from cold-reared snails in their body mass, effects could not be unambiguously attributed to either temperature or body size. As our study aimed to unravel interactive effects of temperature and oxygen, we chose to correct for differences in body size using mass scaling exponents. We derived mass scaling exponents empirically, as a single theoretical scaling exponent is unlikely to exist (e.g. White et al. 2012). While scaling exponents may differ with temperature (e.g. Killen et al, 2010; Kelly et al. 2014), the relatively small range in biomass of either cold-reared snails or warm-reared snails forced us to calculate an average scaling exponent for snails from all treatments. This procedure however, may have obscured any main effects of rearing temperature, explaining why temperature did not affect any of the components making up scope for growth. Food intake and metabolic rate provided the largest contributions to scope for growth and their relative contribution did not really change across rearing treatments (Figure 2b). Ammonium excretion was very low compared to total energy consumed, but values in our study are similar to those found by Friedl (1974). Cold hypoxic and warm hyperoxic snails both consumed relatively little food. For the cold hypoxic reared snails this may be explained by a reduced appetite in hypoxia (Bernier et al., 2012) something which also matches with presumably lower metabolic costs in colder water. However, the low respiration in snails from the warm, hyperoxic rearing treatment require a different explanation. These snails were reared under 40 kPa, but they were tested under 20 kPa in absence of an air compartment and the lower gradient in partial pressure of the dissolved oxygen may have impeded gas exchange. Thus, while the observed differences can be explained on an *ad hoc* basis, they do not fit nicely into the general framework of interactive effects of

temperature and oxygen. Alternatively, the snails may have been at a different stage of development (i.e. the warm reared snails had progressed much further into their life-cycle, perhaps approaching senescence) and this may have had repercussions for their feeding and respiration activity.

Within each treatment, we found large variations in food intake and respiration across individuals and this variation was found to be linked, also after correcting for differences in body size: individuals that consumed more food also consumed more oxygen and vice versa (Fig. 5). This consistent co-variation in individual respiration and food intake strengthens the notion that this variation is caused by individual differences in physiology and activity, which could reflect either phenotypic plasticity (Brown et al., 1985) or genetic variation (Bayne, 1999). Focussing on individual differences can help improve our understanding of the physiological responses to climatic changes (Calosi et al., 2013). Snails reared under hypoxic conditions at 17 °C displayed a large range of growth rates and because snails were sampled at different time points, we could disentangle body size and growth rate. Intriguingly, differences between fast and slow growing individual snails related most to the supply side of scope for growth (uptake of resources as indicated by food ingestion and tentacle surface area). In contrasts, the costs of growing to a certain size (reflected in metabolic rates and excretion rates) were correlated most strongly with body mass (Figure 7; Table 5). This fits with an important aspect of Von Bertalanffy's theory that the accruing costs of growth (i.e. maintenance costs to counterbalance catabolism) are strongly related to body mass. Note that growing to the point where all consumed resources are used for maintenance is not optimal, since this would make reproduction impossible (Czarnoleski and Kozlowski, 1998). Fast growing snails were found to have higher rates of food intake and larger tentacles, irrespective of their body size (Table 5). Thus, the observed increased in surface area of the tentacles under hypoxic rearing and the associated increase in capacity for aquatic gas exchange appears to be beneficial, possibly by reducing time spent surfacing for aerial gas exchange.

In conclusion, our study illustrates that the thermal history and oxygen history experienced by animals can have large, interacting effects on physiological rates and modest effects on CTmax. Moreover, rearing conditions also interacted with acute temperature and oxygen conditions. Chronic rearing conditions had much larger effects on scope for growth than acute testing conditions. Similarly, rearing oxygen and temperature conditions had much larger effects on long term growth performance than on surviving acute heat stress. Our work shows that CTmax critically depends on thermal history, but only when evaluated under hypoxic conditions. This contrasts with the situation for normoxic test conditions where thermal history has only minor effects on CTmax. The large effects of rearing oxygen conditions on growth and other physiological rates compared to modest effects of test oxygen conditions on CTmax suggests that small effects of hypoxia on the short term (e.g. survival of acute heat) may nevertheless have large repercussions on the long term (e.g. growth and reproduction), even in a pulmonate snail that can compensate for hypoxia to some extent by aerial respiration. Finally, our study shows large individual variation in growth performance and this can be related to inter-individual variation in food consumption and metabolism.

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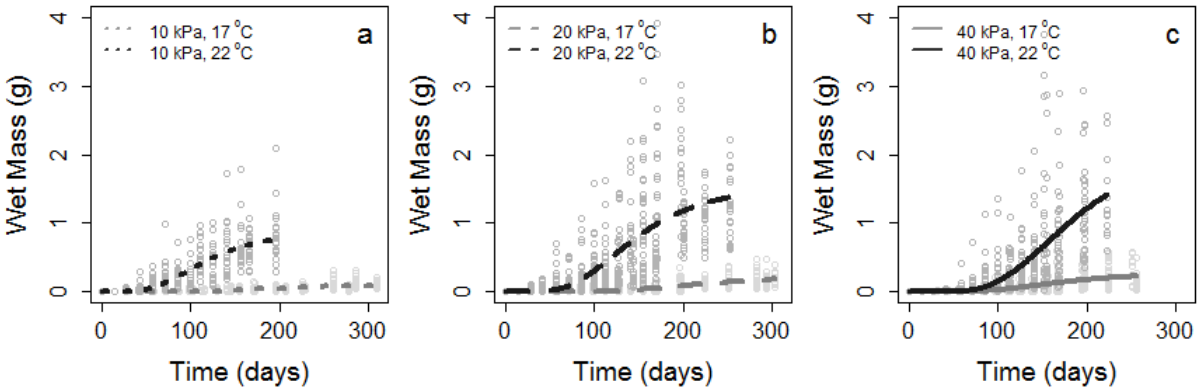


Figure 1.

640

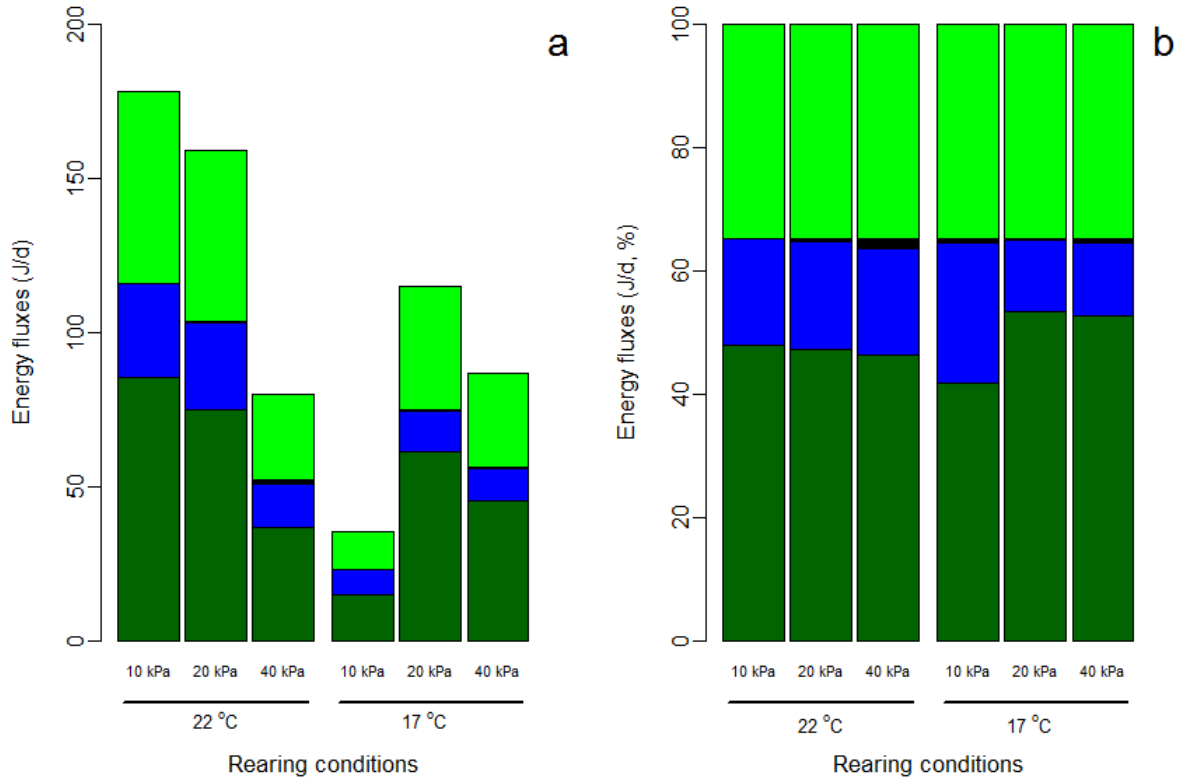
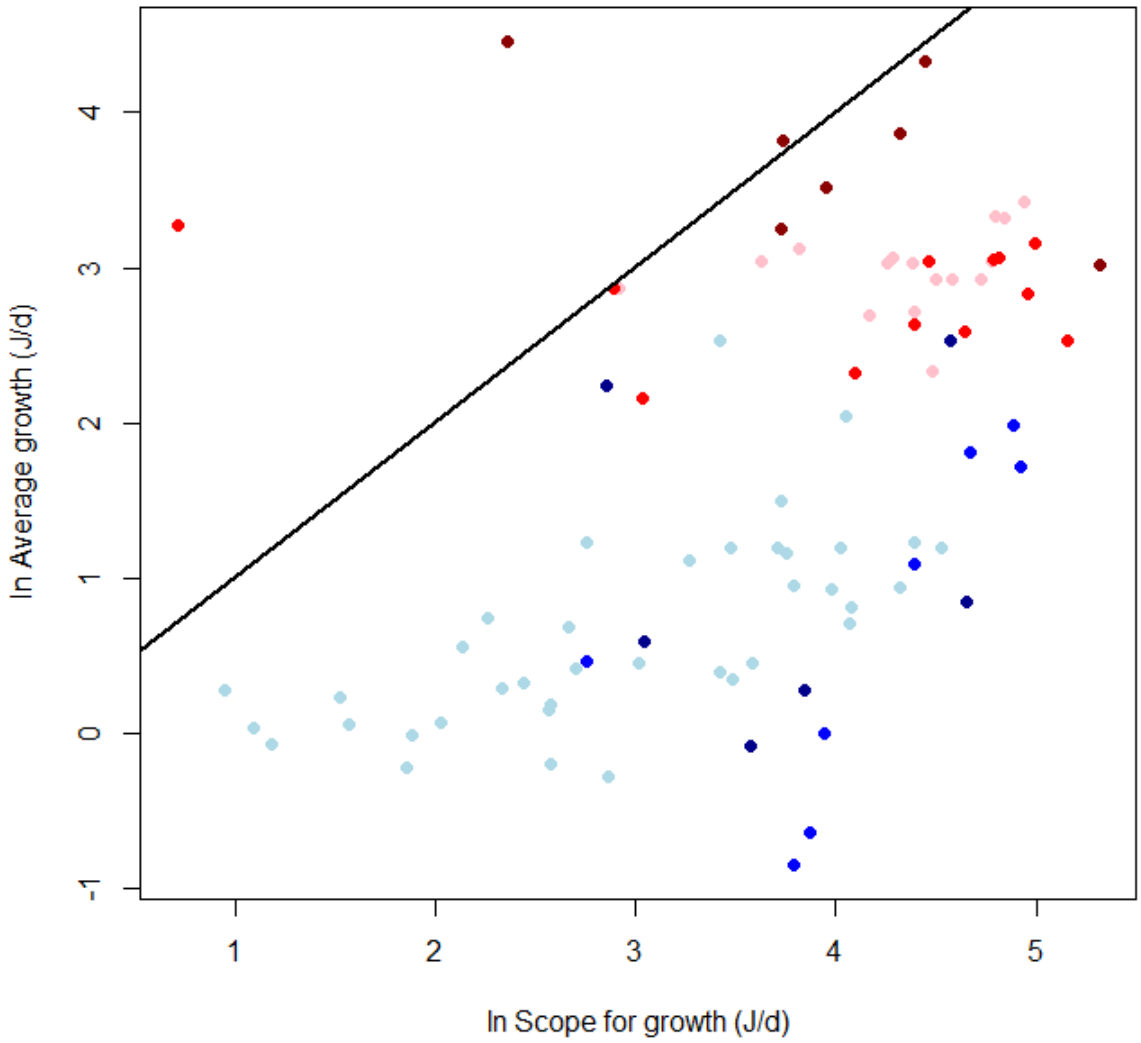


Figure 2.



645 Figure 3.

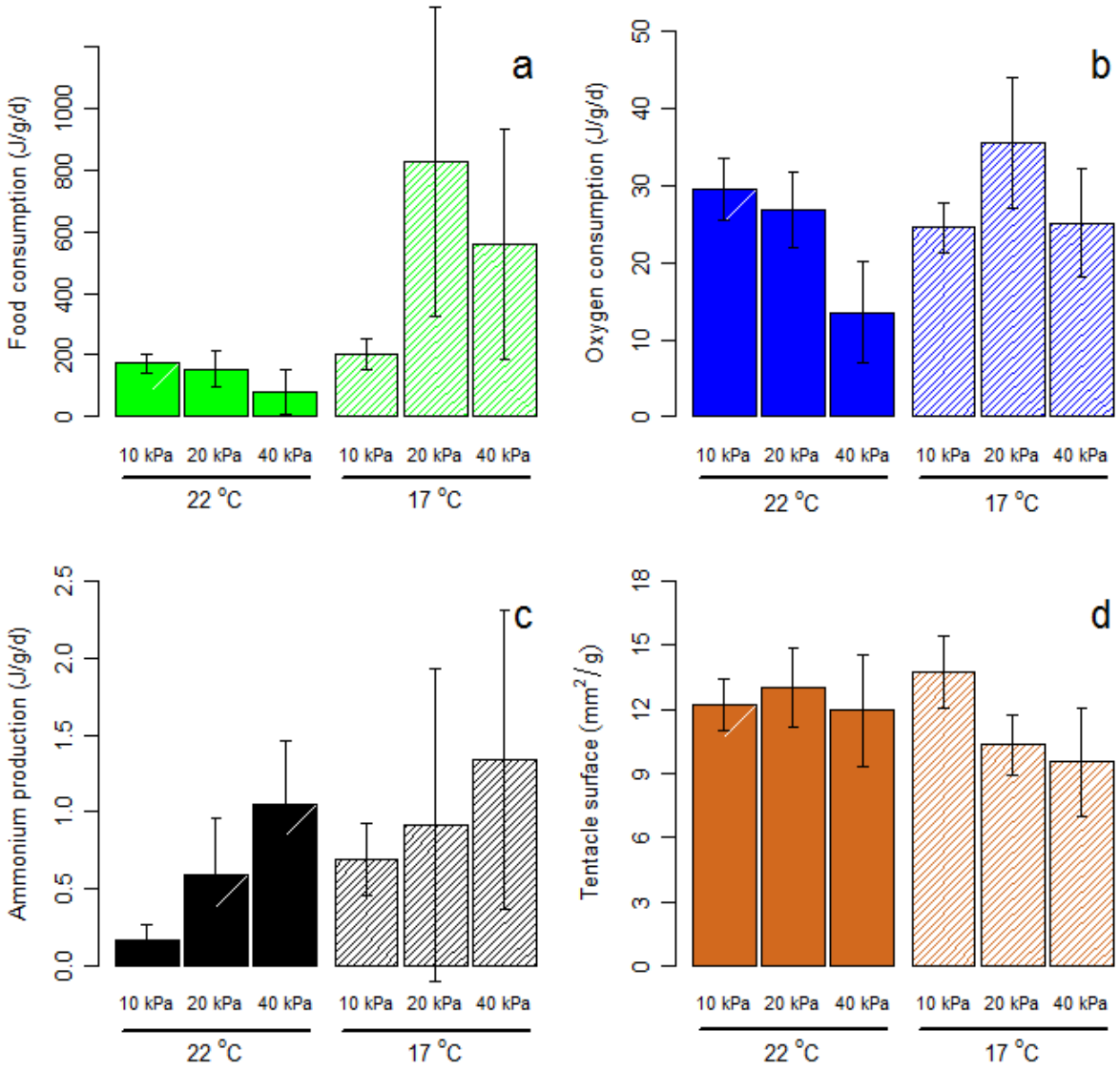
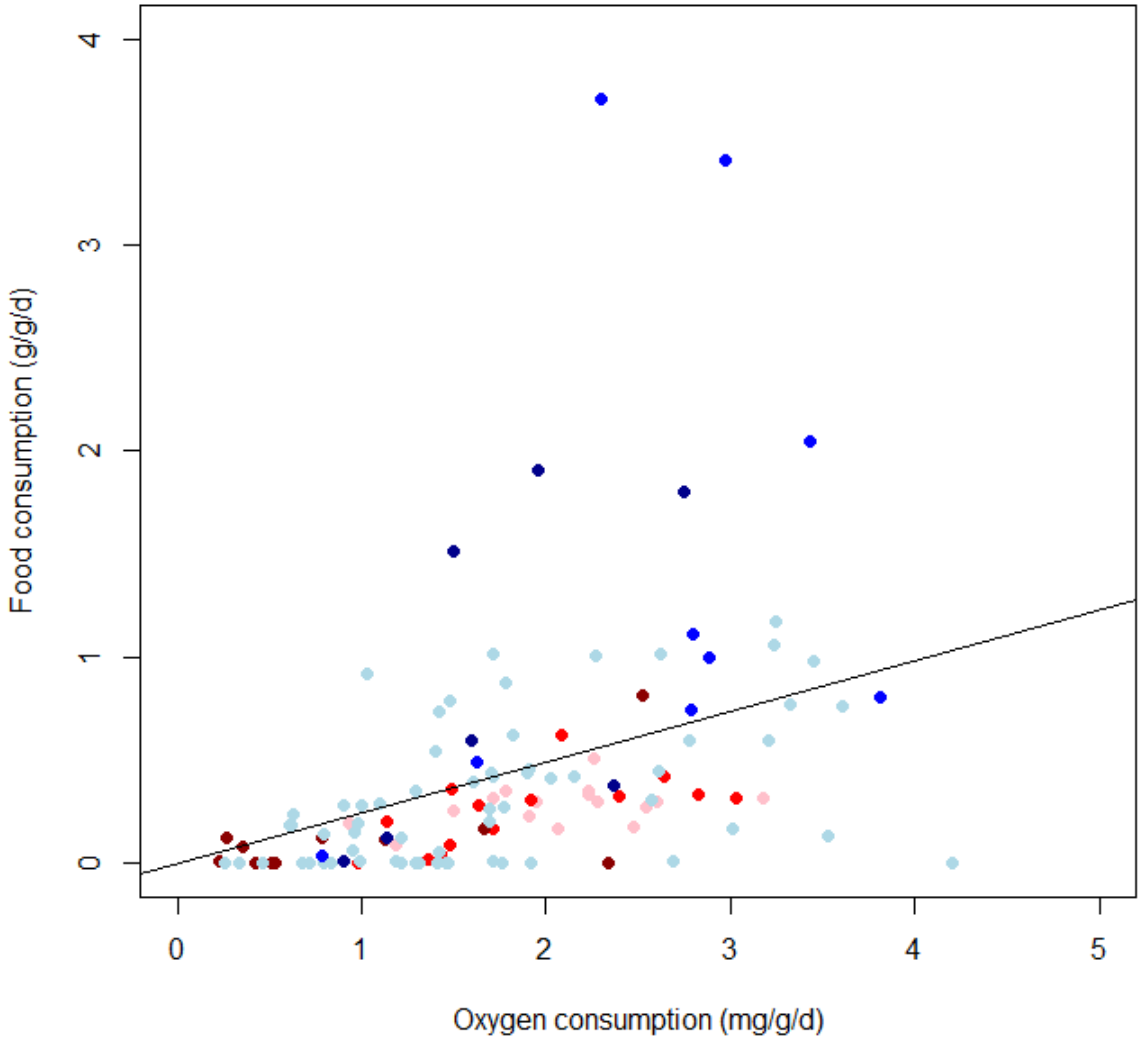

a
Food consumption (J/g/d)
0
200
400
600
800
1000
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
b
Oxygen consumption (J/g/d)
0
10
20
30
40
50
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
c
Ammonium production (J/g/d)
0.0
0.5
1.0
1.5
2.0
2.5
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
d
Tentacle surface (mm2 / g)
0
3
6
9
12
15
18
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C

Figure 4.


Food consumption (g/g/d)
0
1
2
3
4
Oxygen consumption (mg/g/d)
0
1
2
3
4
5

650 Figure 5.

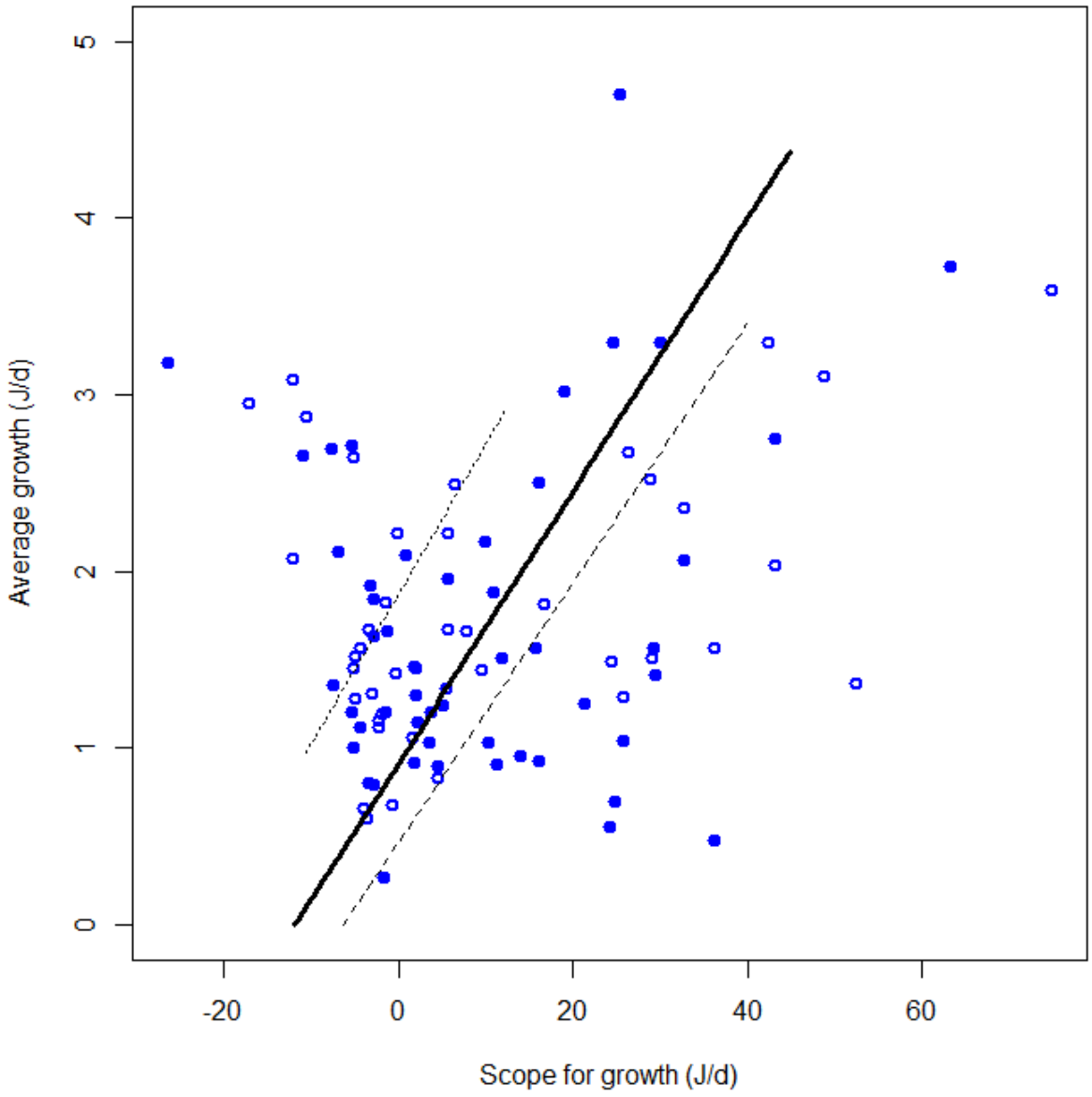


Figure 6.

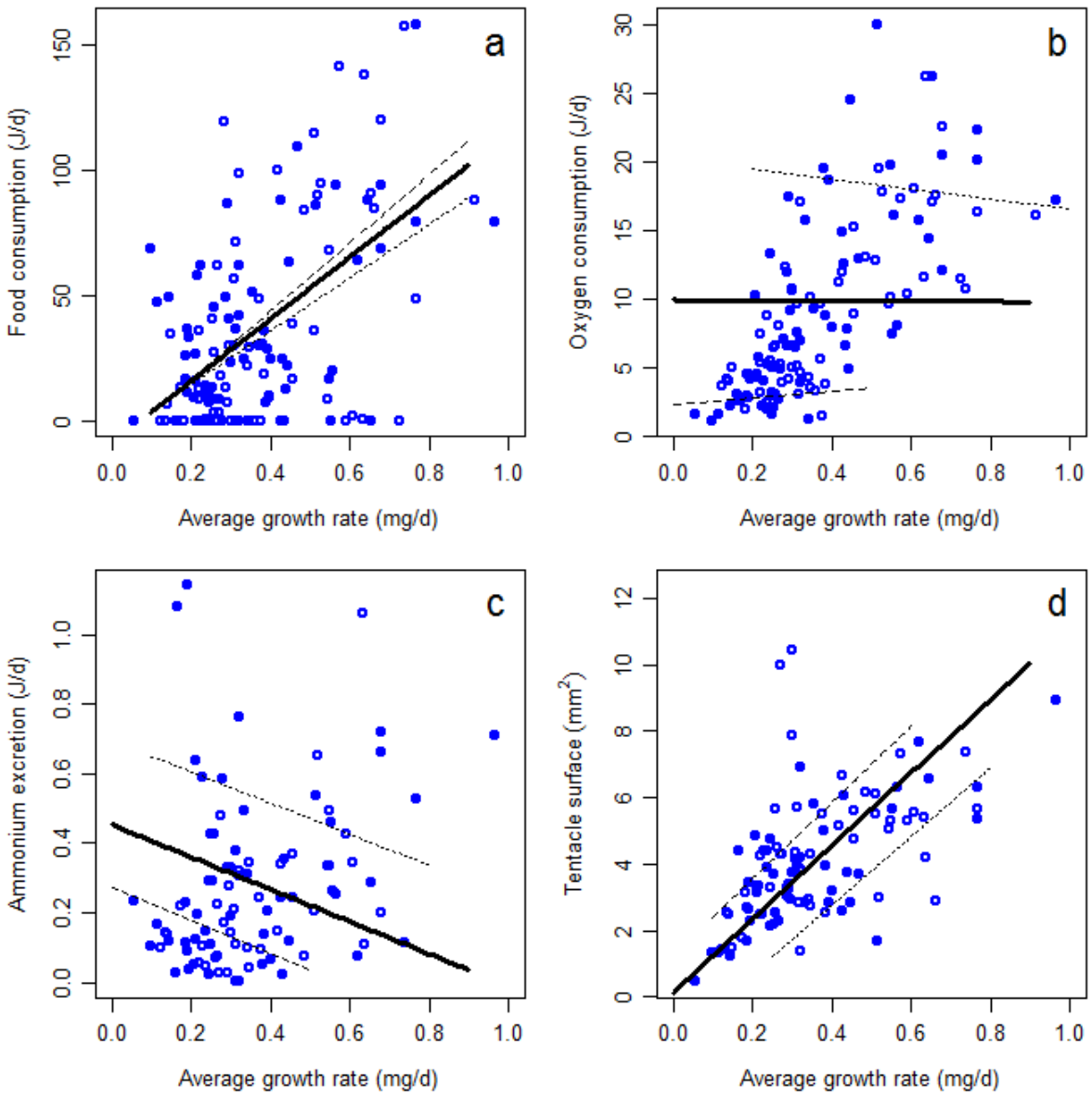
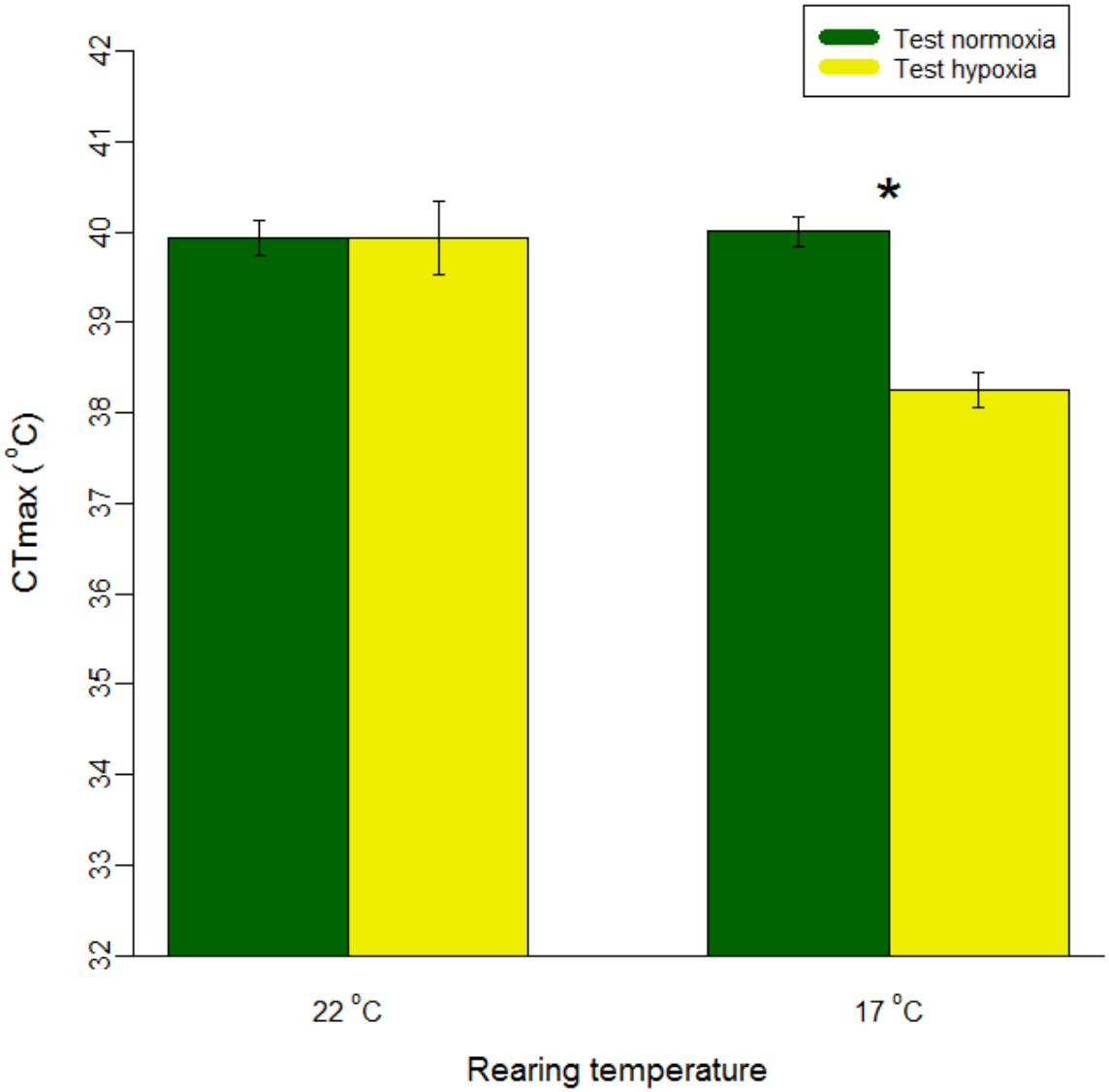

a
Food consumption (J/d)
0
50
100
150
0.0
0.2
0.4
0.6
0.8
1.0
Average growth rate (mg/d)
b
Oxygen consumption (J/d)
0
5
10
15
20
25
30
Average growth rate (mg/d)
c
Ammonium excretion (J/d)
0.0
0.2
0.4
0.6
0.8
1.0
Average growth rate (mg/d)
d
Tentacle surface (mm2)
0
2
4
6
8
10
12
Average growth rate (mg/d)

Figure 7.


Test normoxia
Test hypoxia
CTmax (°C)
42
41
40
39
38
37
36
35
34
33
32
*
22 °C
17 °C
Rearing temperature

655

Figure 8.

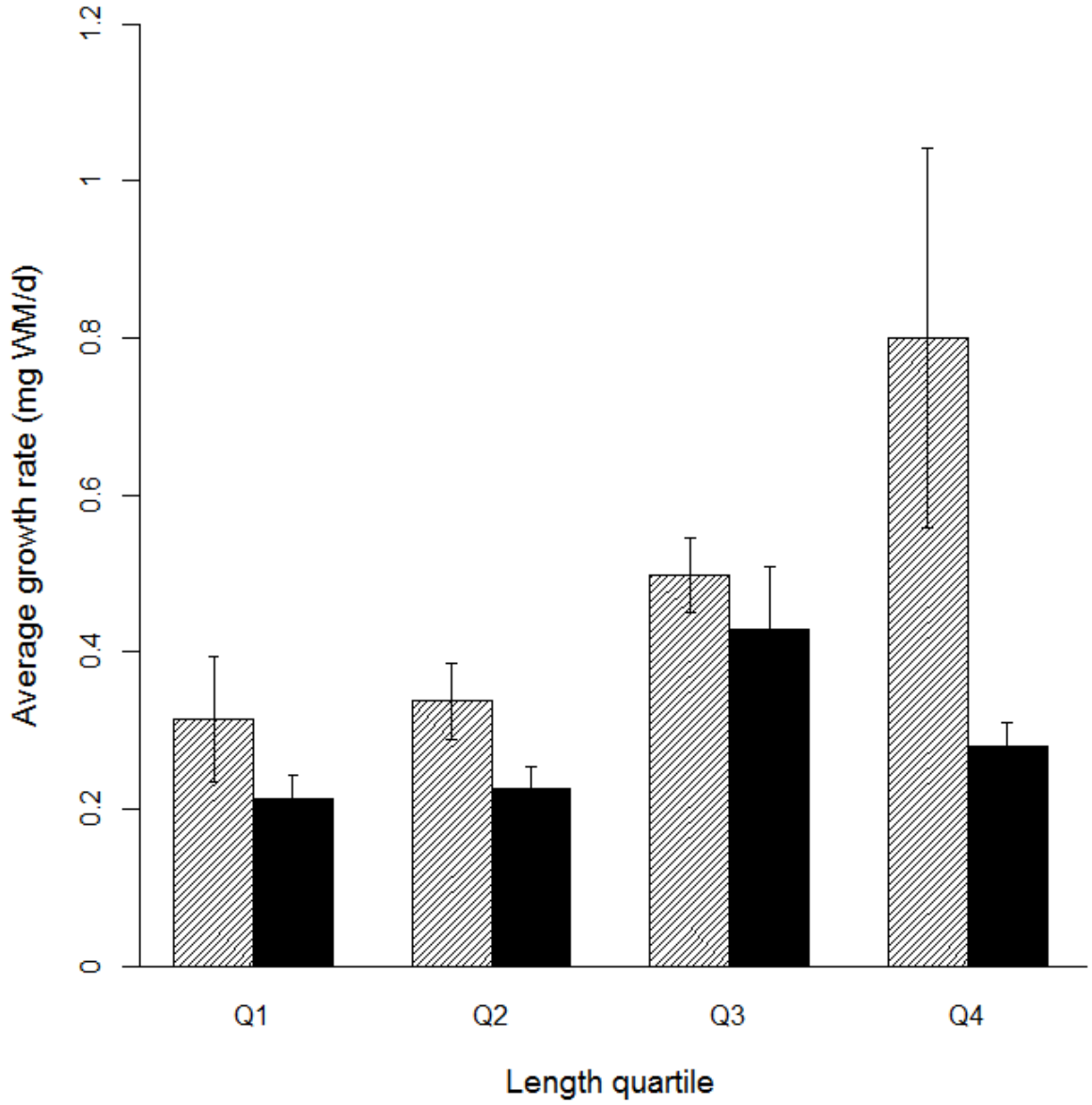


Figure A.1.

660

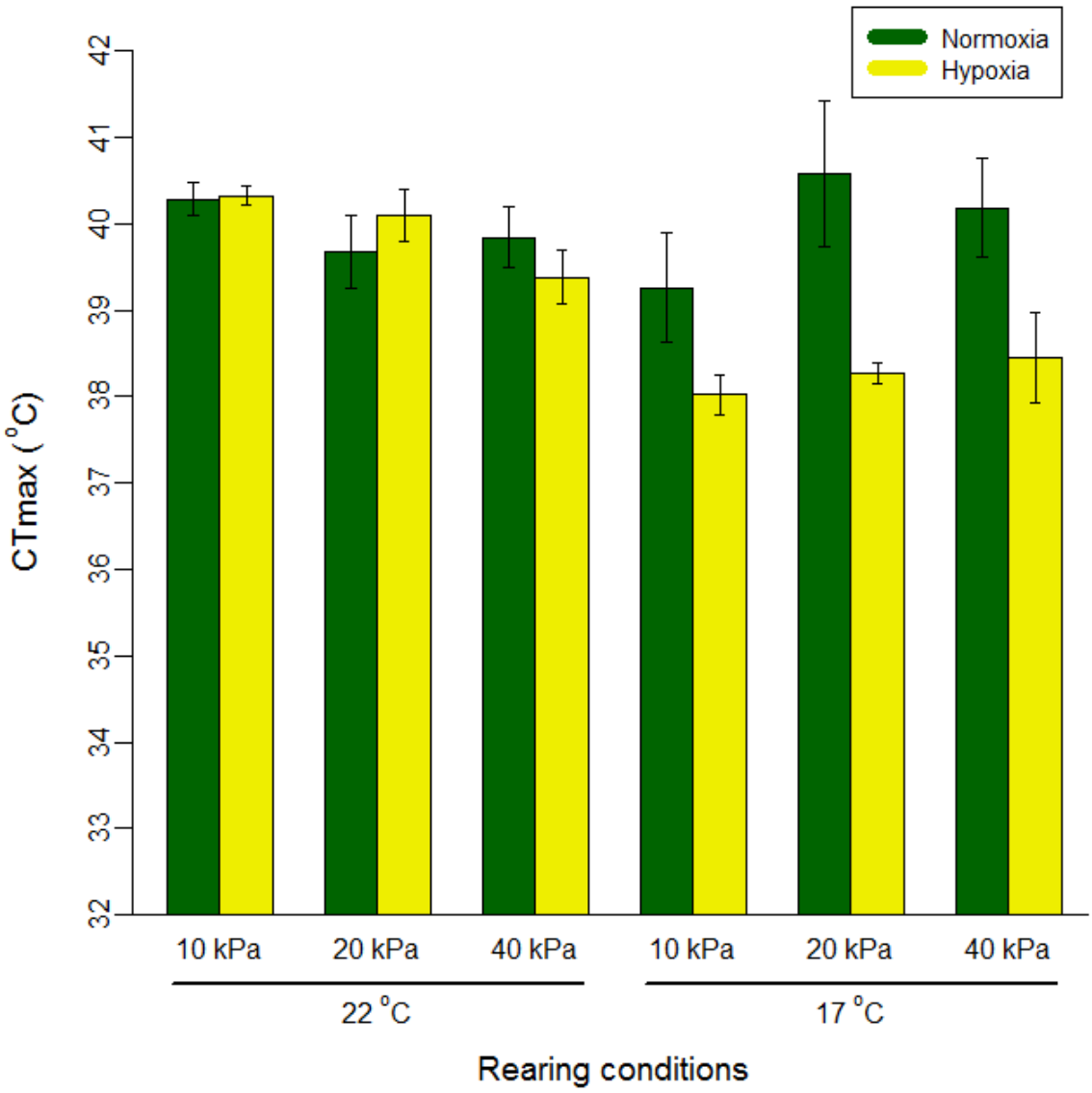

Normoxia
Hypoxia
CTmax (°C)
32
33
34
35
36
37
38
39
40
41
42
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
Rearing conditions

Figure A.2.

Table 1.

Temperature	Oxygen	D		M_0 (g)		L_a (mm)	MaxG (g^{-1})	Age M_a (d)	SSres	R^2 adj
17 °C	10 kPa	0.273	(±0.012)	0.115	(±0.011)	13.4	0.001	464	0.5	0.55
17 °C	20 kPa	0.253	(±0.011)	0.240	(±0.027)	17.1	0.001	507	1.4	0.56
17 °C	40 kPa	0.315	(±0.016)	0.269	(±0.032)	17.8	0.002	393	5.9	0.41
22 °C	10 kPa	0.415	(±0.026)	0.869	(±0.093)	20.5	0.007	285	14.6	0.66
22 °C	20 kPa	0.353	(±0.020)	1.522	(±0.164)	24.7	0.011	344	112.7	0.50
22 °C	40 kPa	0.297	(±0.018)	2.068	(±0.394)	27.3	0.012	421	86.4	0.47

Table 2.

Source	Value	Std. Error	DF	t-value	P-value	
(Intercept)	-12.903	29.584	113	-0.436	0.664	
Rearing temperature (°C)	100.544	19.101	1	5.264	6.8e-07	*
Shell free wet mass (g)	90.634	28.512	1	3.179	0.002	*
Rearing O_2 (kPa)	1.032	0.521	1	1.980	0.050	
Test temperature (°C)	0.360	1.469	1	0.245	0.807	
Rearing temperature : SFWM	-83.380	31.033	1	-2.687	0.008	*
Rearing temperature : Rearing O_2	-2.559	0.750	1	-3.410	0.001	*

Table 3.

Experiment	Rate	a	b	R^2
All rearing treatments	Food consumption (g d^{-1})	0.1264	0.9808	0.2190
	Food assimilation (g d^{-1})	0.0824	0.9808	0.2190
	Oxygen consumption (mg d^{-1})	1.5182	0.6033	0.5641
	Ammonium excretion (µmole d^{-1})	5.5332	0.5352	0.0329
	Tentacle surface (mm^2)	11.9292	0.4992	0.7987
	Scope for growth (J d^{-1})	66.6773	0.4013	0.1692

Table 4.

Response	Source	Estimate	Std. Error	DF	t-value	P-value	
Food consumption	(Intercept)	0.7629	0.5178	110	1.4733	0.1435	
	Rearing temperature	-0.0863	0.6338	4	-0.1361	0.8983	
	Rearing oxygen	0.0130	0.0156	4	0.8364	0.4500	
	Test temperature	-0.0165	0.0173	110	-0.9535	0.3424	
	Rearing temperature : Rearing oxygen	-0.0190	0.0230	4	-0.8263	0.4551	
Oxygen consumption	(Intercept)	1.8374	0.7059	110	2.6029	0.0105	*
	Rearing temperature	1.3427	0.5010	4	2.6799	0.0552	
	Rearing oxygen	0.0110	0.0131	4	0.8465	0.4450	
	Test temperature	-0.0084	0.0285	110	-0.2959	0.7679	
	Rearing temperature : Rearing oxygen	-0.0623	0.0198	4	-3.1447	0.0347	*
Ammonium production	(Intercept)	-55.4527	33.7633	110	-1.6424	0.1034	
	Rearing temperature	-21.7924	9.5897	5	-2.2725	0.0722	
	Rearing oxygen	1.2994	0.4247	5	3.0594	0.0281	*
	Test temperature	3.9603	1.6780	110	2.3602	0.0200	*
Tentacle surface	(Intercept)	15.1745	1.1364	85	13.3535	<2e-16	*
	Rearing temperature	-2.5997	1.7473	1	-1.4878	0.1405	
	Rearing oxygen	-0.1733	0.0676	1	-2.5619	0.0122	*
	Rearing temperature : Rearing oxygen	0.16280	0.0861	1	1.8910	0.0620	

675

Table 5.

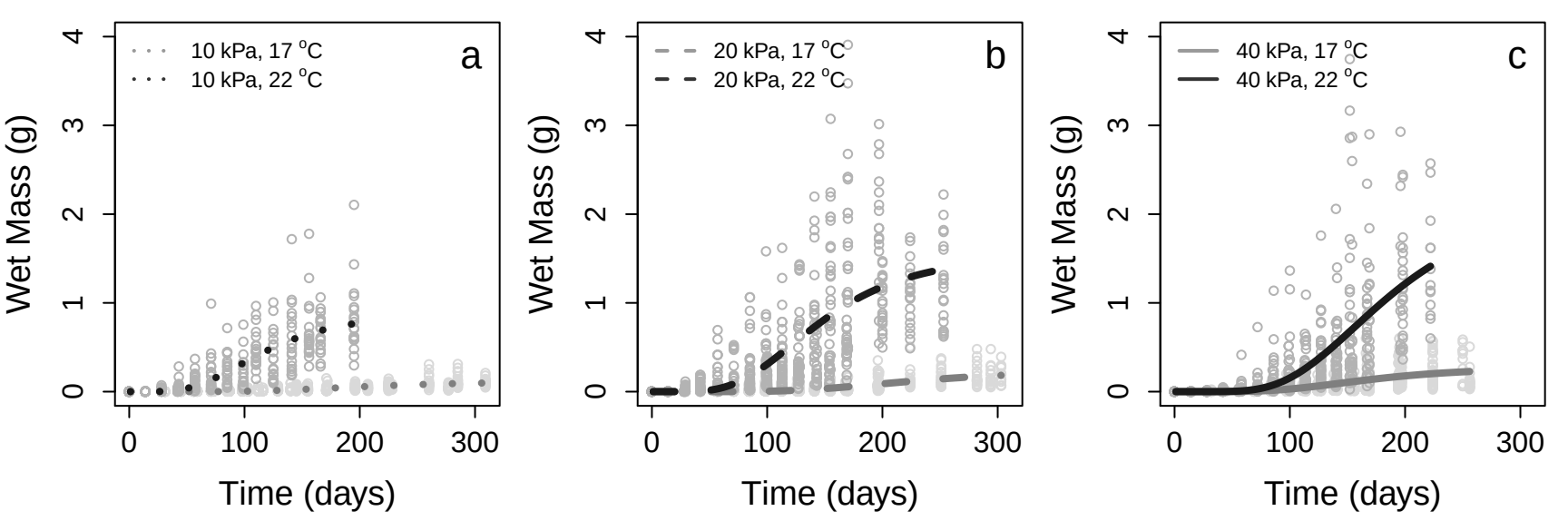
Rate	Source	Estimate	Std. Error	DF	t-value	P-value	
Food consumption	(Intercept)	-0.0466	4.023e-02	123	-1.157	0.2494	
	Growth rate	188.7	8.574e+01	1	2.201	0.0296	*
$R^2 = 0.26$	SFWM	0.1227	2.043e-01	1	0.601	0.5491	
	Test temperature	0.001575	1.924e-03	1	0.818	0.4148	
	Growth rate : SFWM	-197.1	4.891e+01	1	-4.029	< 0.0001	*
Oxygen consumption	(Intercept)	-0.3984	0.2396	128	-1.663	0.0990	
	Growth rate	5.1170	478.7973	122	0.011	0.9915	
	SFWM	5.2767	1.1202	122	4.711	< 0.0001	*
	Test temperature	0.0190	0.0105	122	1.798	0.0746	
	Growth rate : SFWM	-1565.5328	282.4945	122	-5.542	< 0.0001	*
Ammonium excretion	(Intercept)	-1.514e+01	8.876e+00	123	-1.705	0.0906	
	Growth rate	-2.564e+04	1.892e+04	1	-1.355	0.1778	
$R^2 = 0.12$	SFWM	7.432e+01	4.507e+01	1	1.649	0.1017	
	Test temperature	1.196e+00	4.246e-01	1	2.815	0.0057	*
	Growth rate : SFWM	1.436e+04	1.079e+04	1	1.330	0.1859	
Tentacle surface	(Intercept)	1.8716	0.4184	96	4.473	< 0.0001	*
	Growth rate	11761.133	3291.4975	1	3.573	0.0006	*
$R^2 = 0.42$	SFWM	-11.2951	7.2567	1	-1.557	0.1229	
	Growth rate : SFWM	-5006.451	1717.1763	1	-2.916	0.0044	*
Scope for growth	(Intercept)	-8.240	1.273e+01	123	-0.647	0.5185	
	Growth rate	6.022e+04	2.712e+04	1	2.220	0.0282	*
$R^2 = 0.11$	SFWM	-37.40	6.462e+01	1	-0.579	0.5638	
	Test temperature	2.379e-01	6.088e-01	1	0.391	0.6966	
	Growth rate : SFWM	-4.045e+04	1.547e+04	1	-2.614	0.0101	*

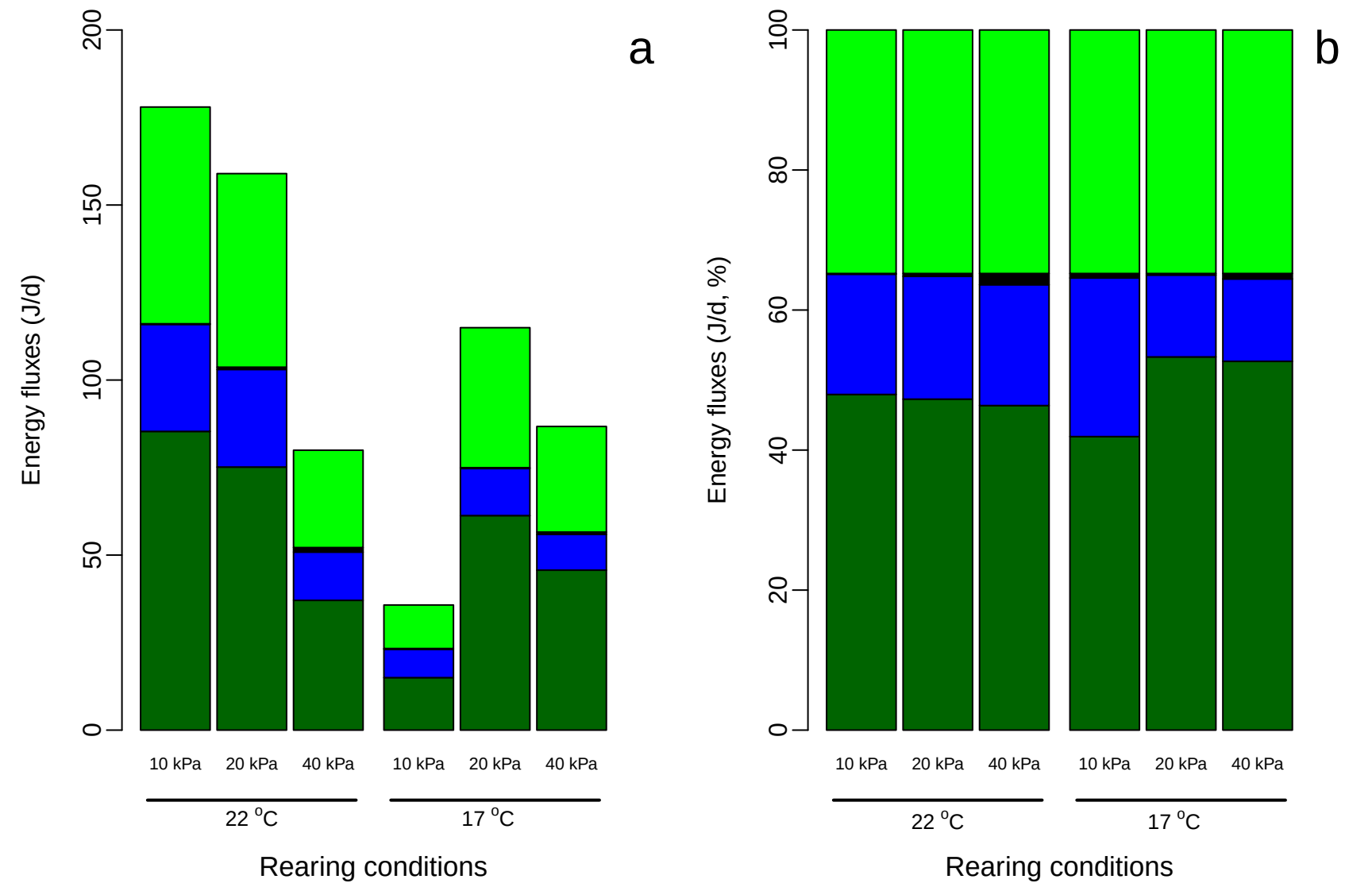
Table 6.

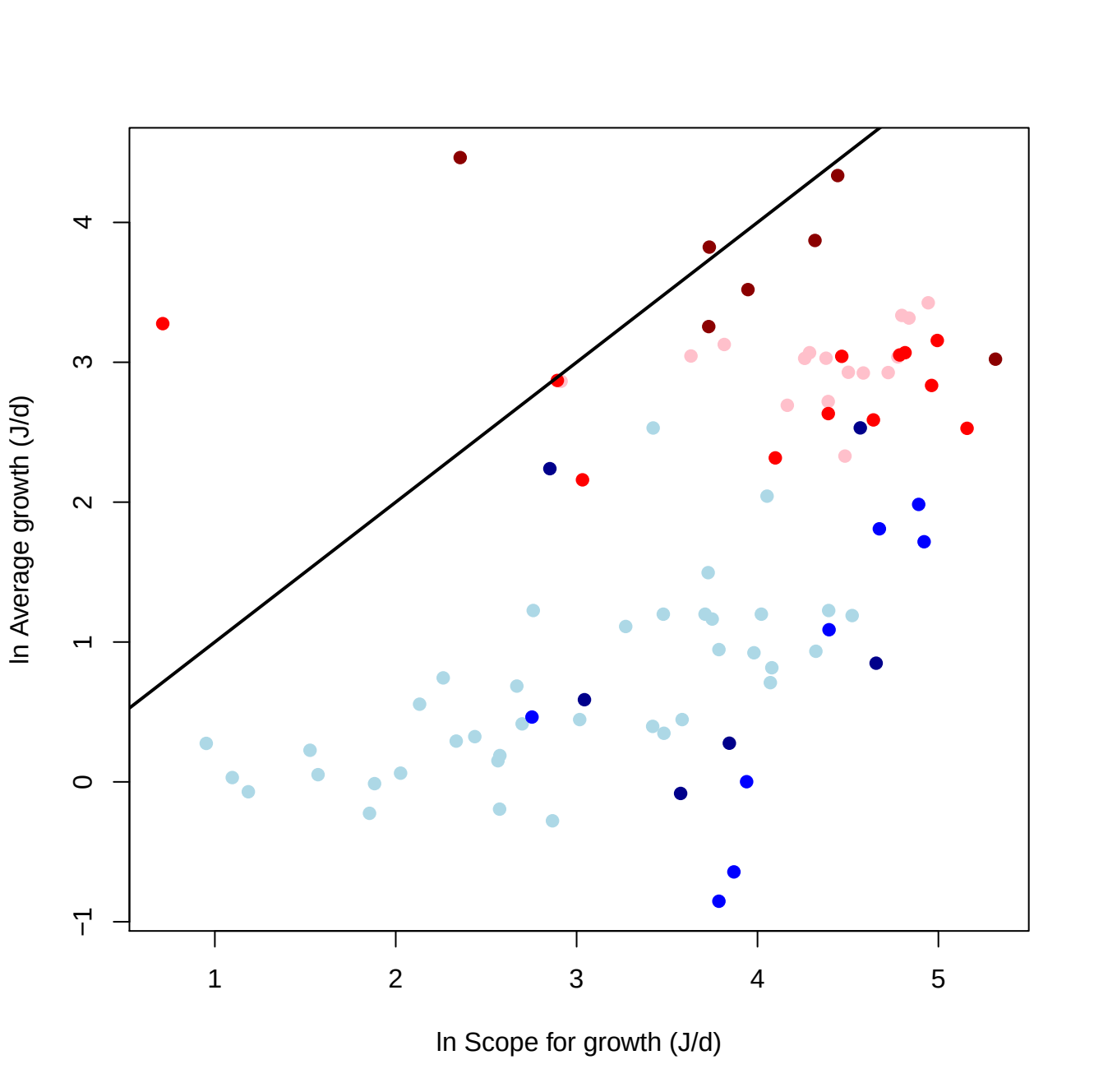
Source	Estimate	Std. Error	t-value	P-value	
(Intercept)	37.2388	0.4063	91.655	< 0.0001	***
Mass	0.5005	0.5634	0.888	0.3769	
Rearing temperature	3.0385	0.6109	4.974	< 0.0001	***
Rearing oxygen	0.0163	0.0126	1.297	0.1981	
Test oxygen	0.1172	0.0212	5.336	< 0.0001	***
Rearing temperature : Rearing oxygen	-0.0414	0.0176	-2.347	0.0213	*
Rearing temperature : Test oxygen	-0.1151	0.0311	-3.708	0.0004	***

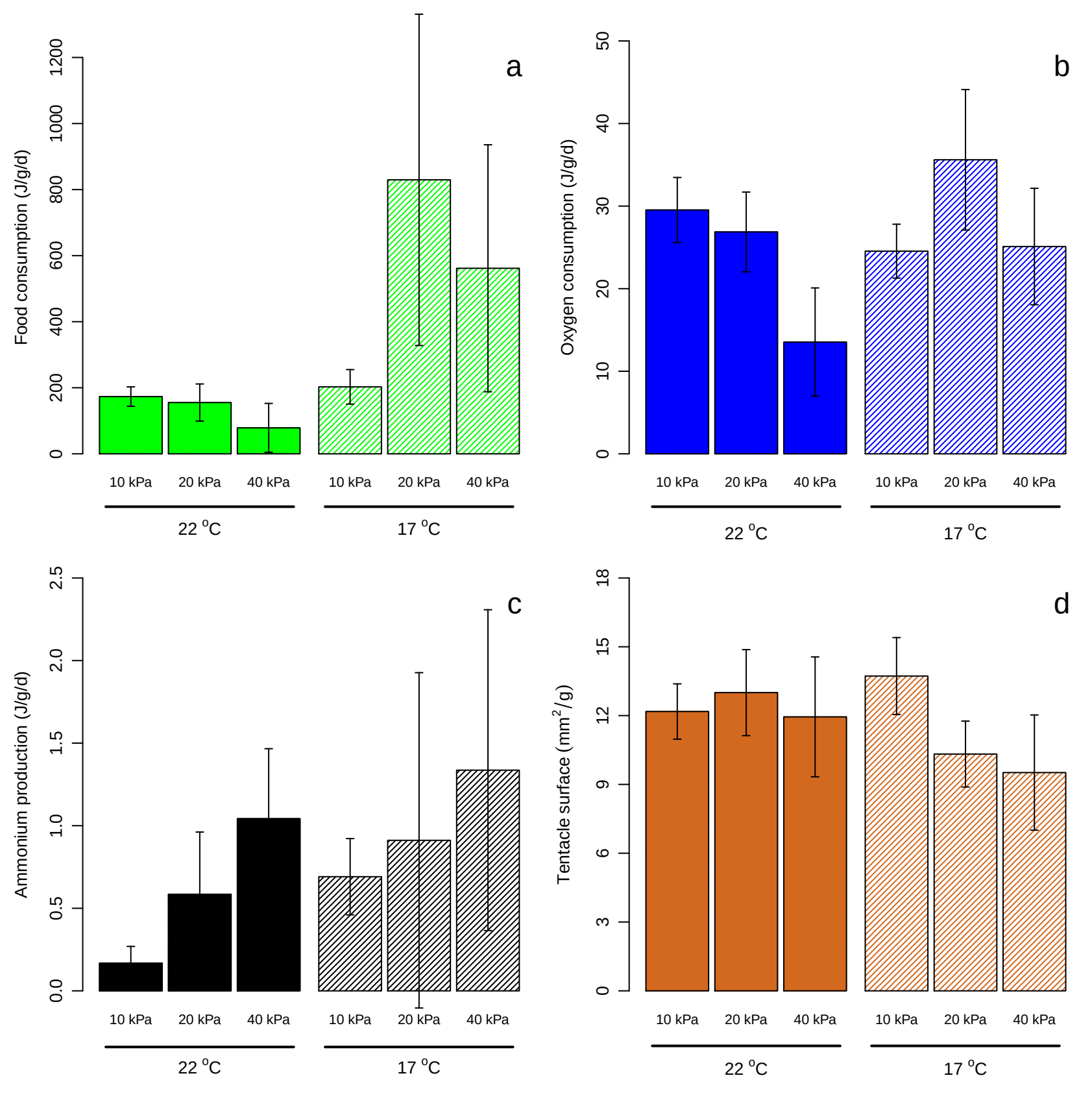
Table 7.

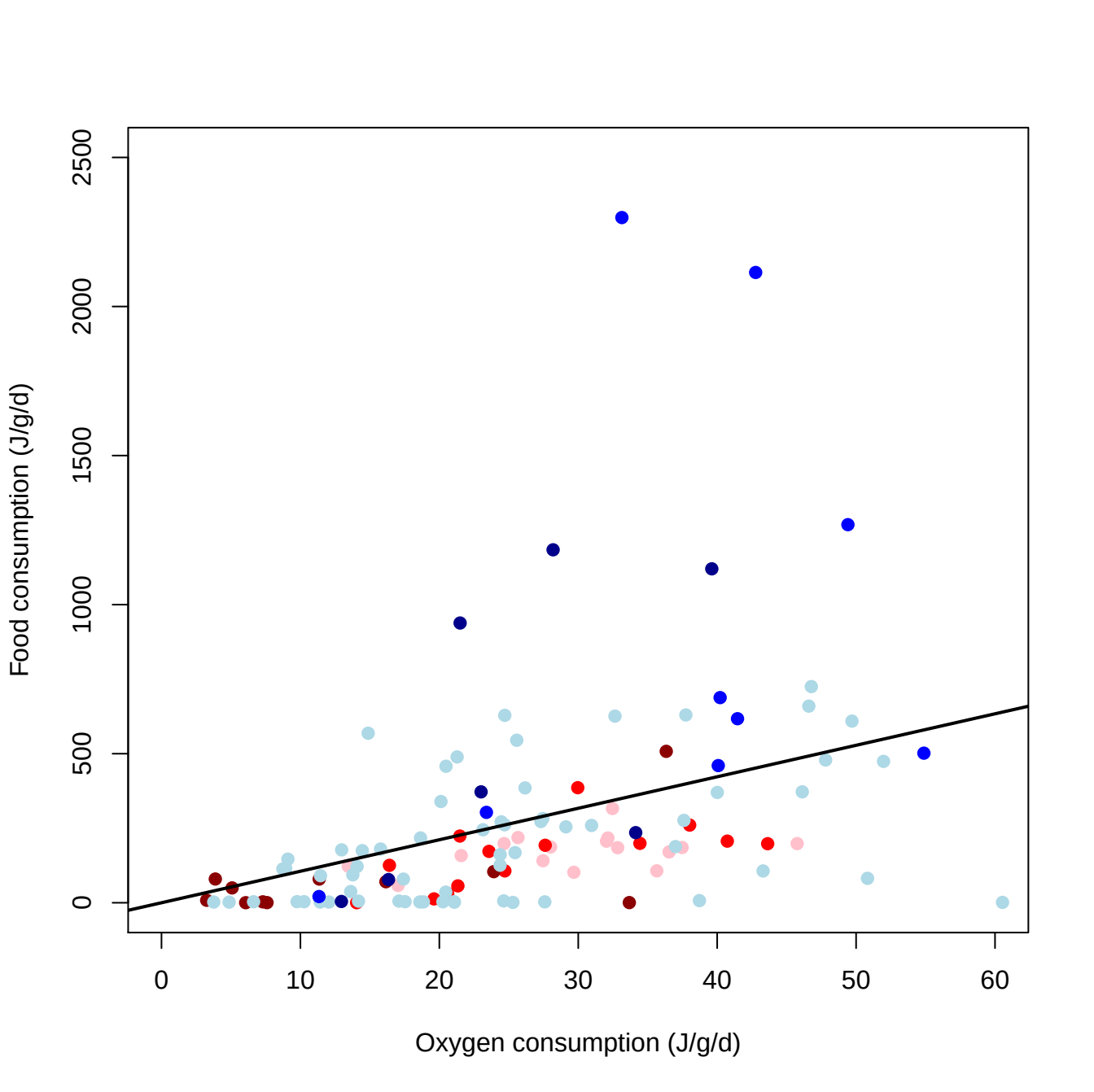
Source	Value	Std. Error	DF	t-value	p-value	
(Intercept)	-0.0873	0.0070	3691	-12.5139	0.0000	***
Age	0.0011	0.0011	3691	1.0571	0.2905	
Age : Rearing temperature	0.0039	0.0015	3691	2.5301	0.0114	*
Age : Rearing oxygen	-0.0000	0.0000	3691	-0.0721	0.9425	
Age : Rearing temperature : Rearing oxygen	0.0000	0.0000	3691	0.0687	0.9452	

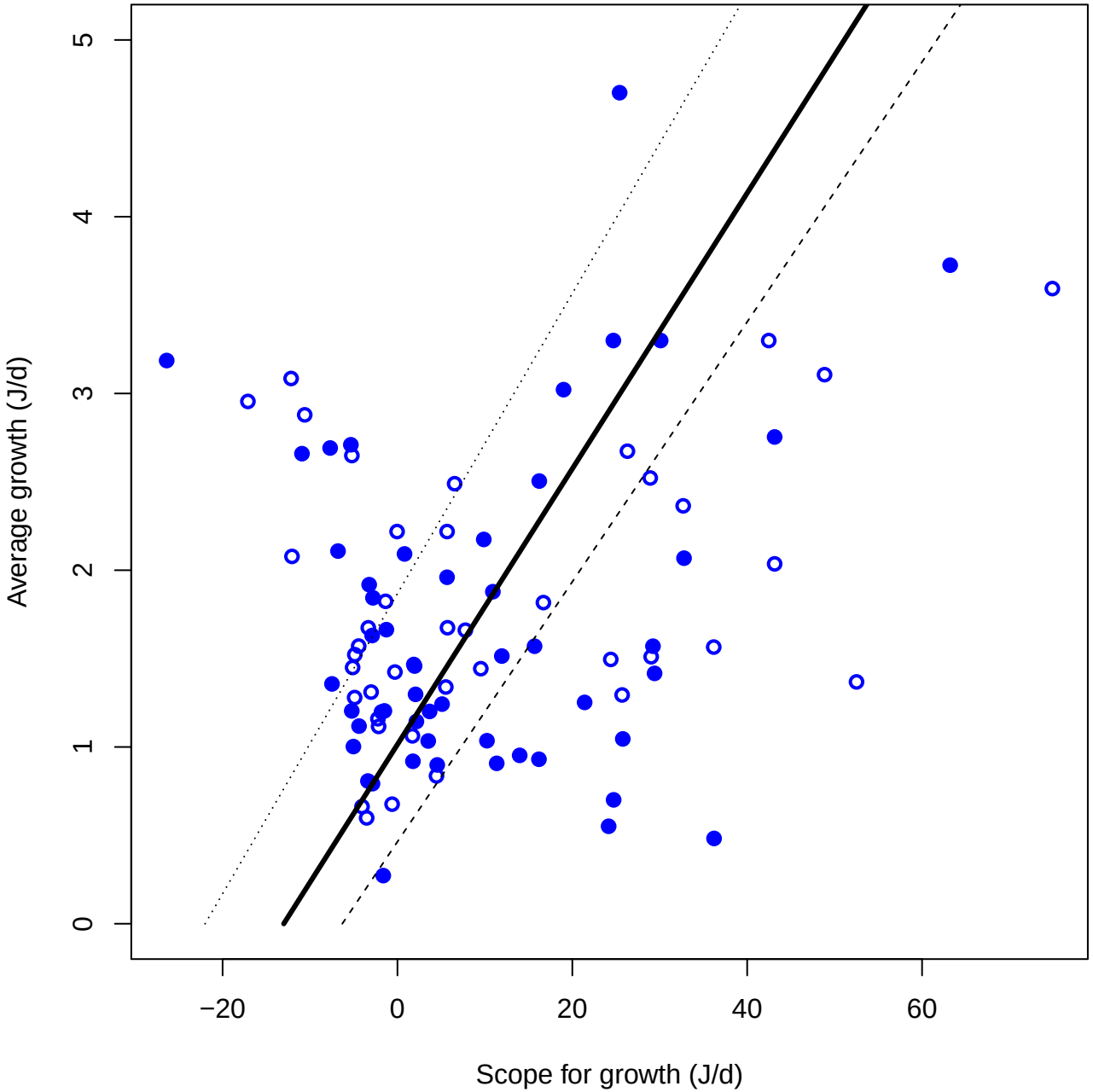

10 kPa, 17 °C
10 kPa, 22 °C
a
20 kPa, 17 °C
20 kPa, 22 °C
b
40 kPa, 17 °C
40 kPa, 22 °C
c
Wet Mass (g)
Time (days)
0
1
2
3
4
0
100
200
300

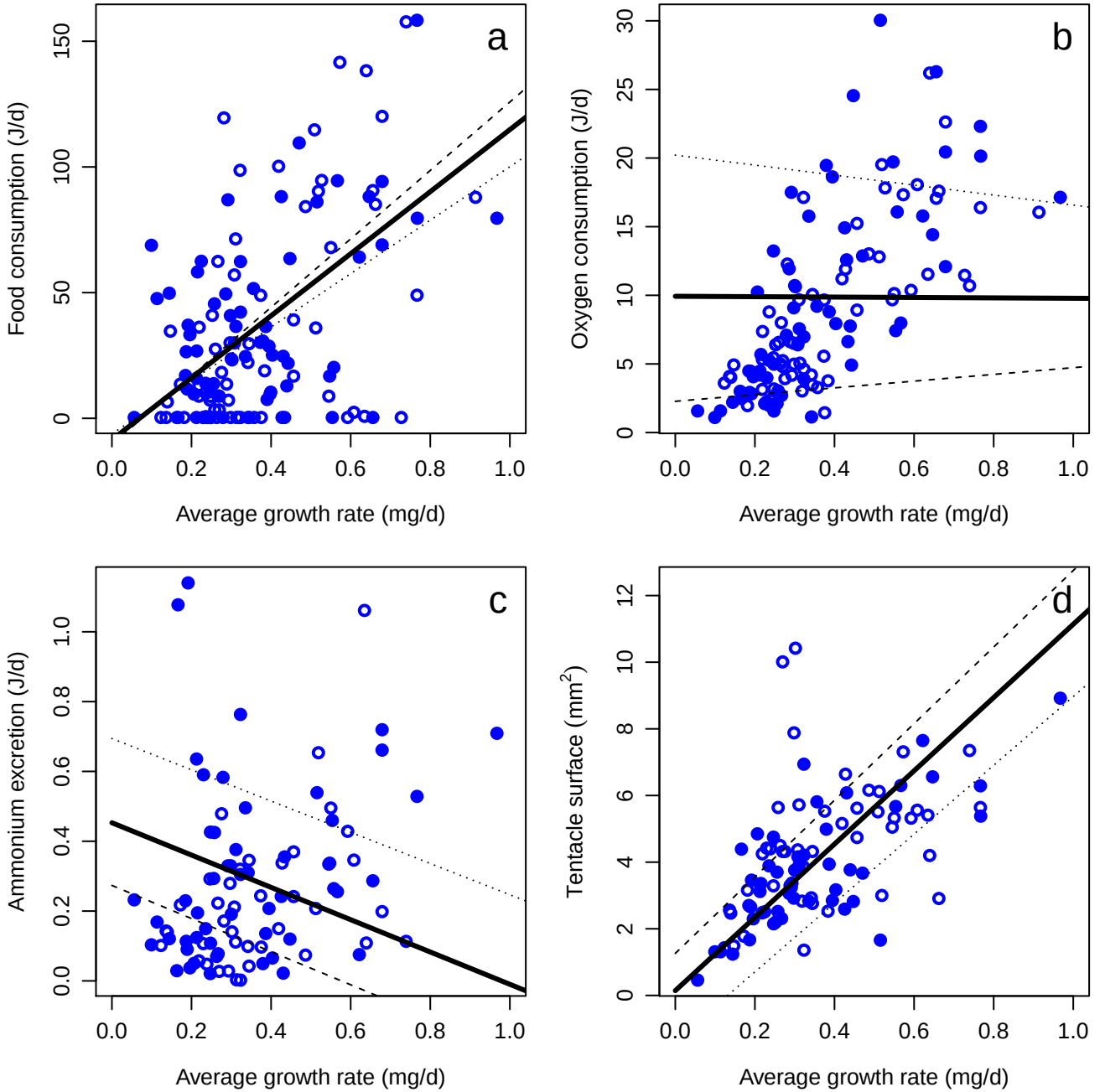

a
Energy fluxes (J/d)
0
50
100
150
200
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
Rearing conditions
b
Energy fluxes (J/d, %)
0
20
40
60
80
100
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
Rearing conditions

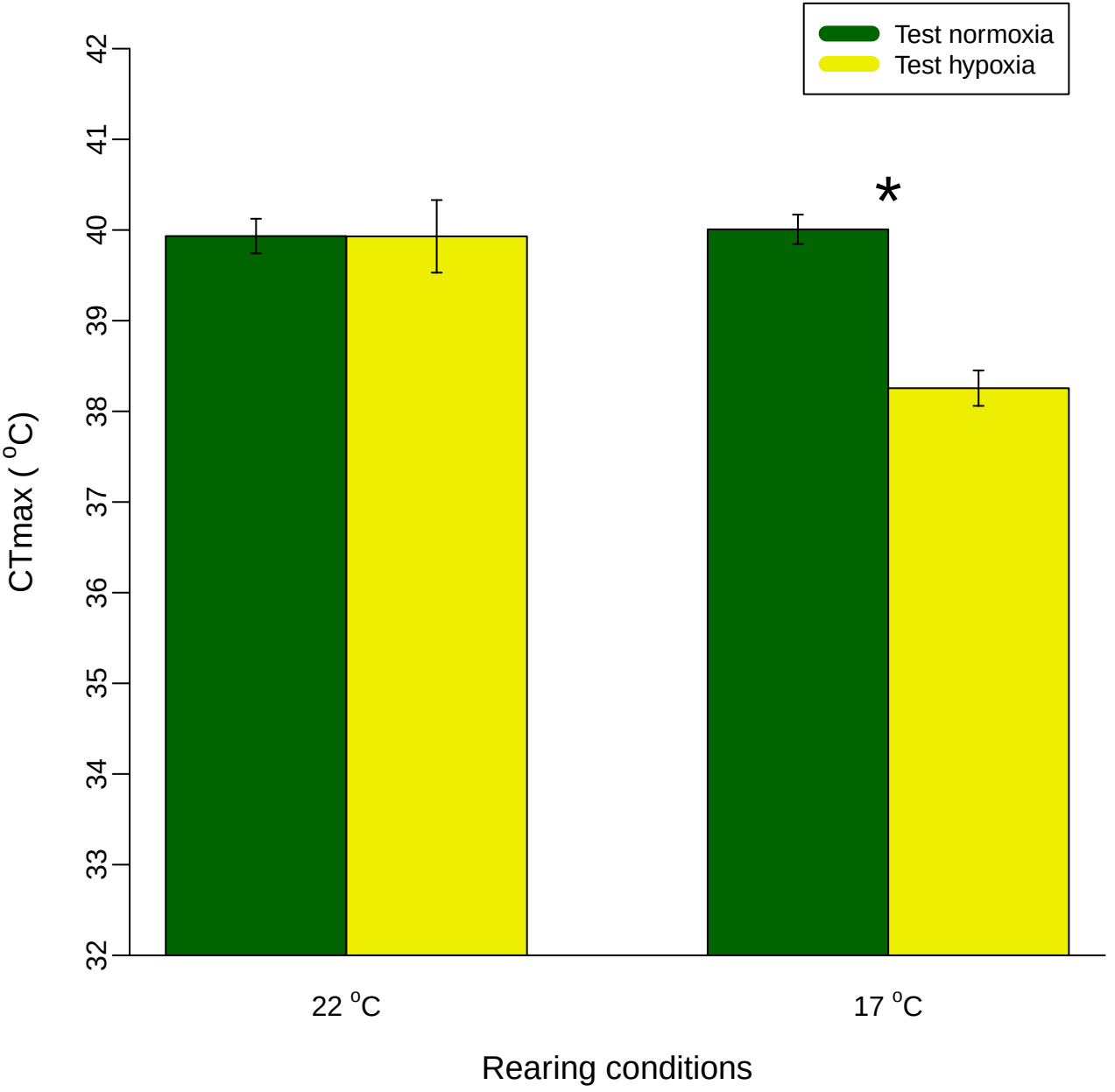

ln Average growth (J/d)
ln Scope for growth (J/d)
−1
0
1
2
3
4
1
2
3
4
5

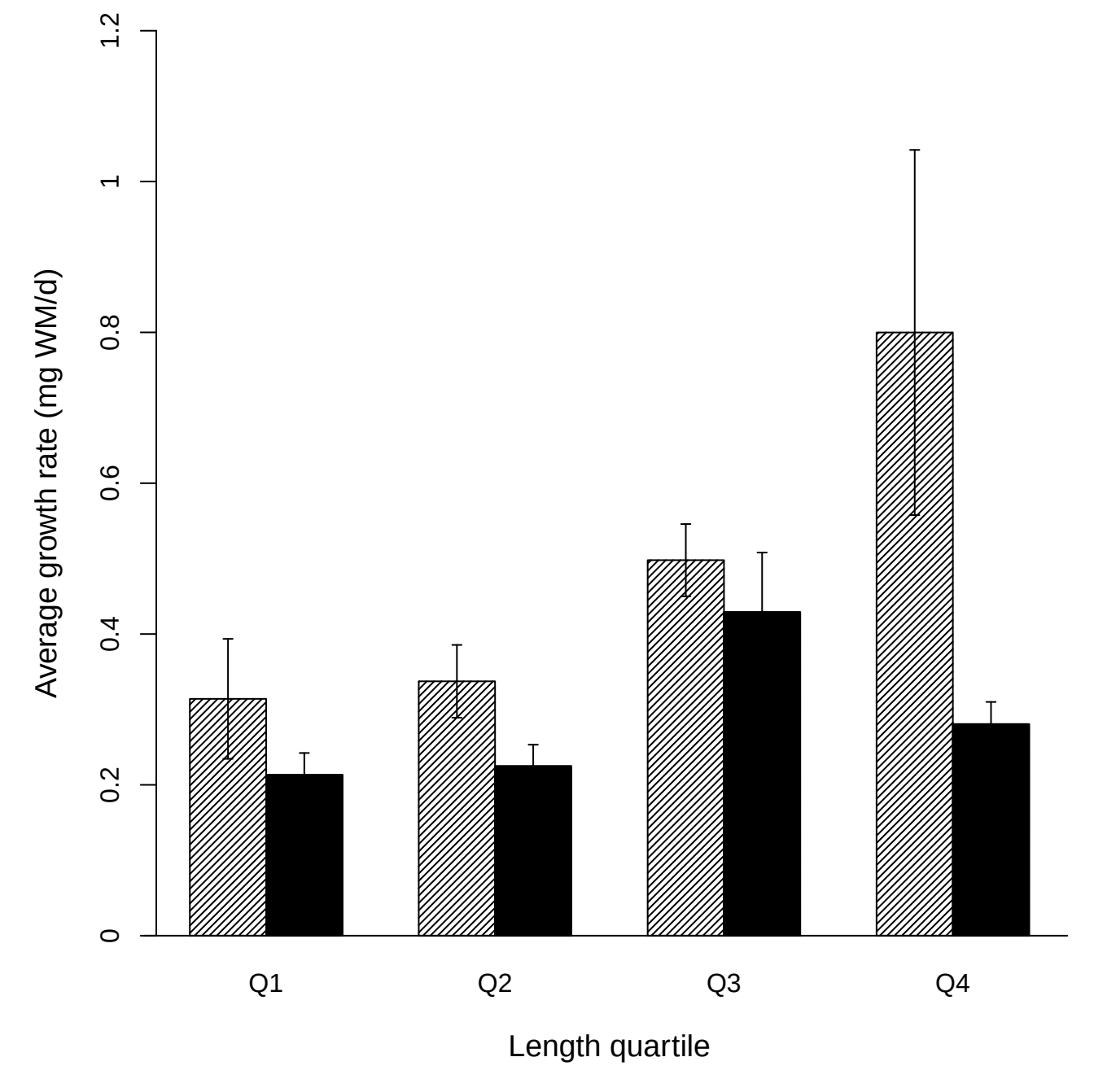

a
Food consumption (J/g/d)
0
200
400
600
800
1000
1200
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
b
Oxygen consumption (J/g/d)
0
10
20
30
40
50
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
c
Ammonium production (J/g/d)
0.0
0.5
1.0
1.5
2.0
2.5
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
d
Tentacle surface (mm²/g)
0
3
6
9
12
15
18
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C


Food consumption (J/g/d)
0
500
1000
1500
2000
2500
0
10
20
30
40
50
60
Oxygen consumption (J/g/d)


Average growth (J/d)
0
1
2
3
4
5
−20
0
20
40
60
Scope for growth (J/d)


a
Food consumption (J/d)
0
50
100
150
0.0
0.2
0.4
0.6
0.8
1.0
Average growth rate (mg/d)
b
Oxygen consumption (J/d)
0
5
10
15
20
25
30
0.0
0.2
0.4
0.6
0.8
1.0
Average growth rate (mg/d)
c
Ammonium excretion (J/d)
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0
Average growth rate (mg/d)
d
Tentacle surface (mm2)
0
2
4
6
8
10
12
0.0
0.2
0.4
0.6
0.8
1.0
Average growth rate (mg/d)


Test normoxia
Test hypoxia
CTmax (°C)
42
41
40
39
38
37
36
35
34
33
32
*
22 °C
17 °C
Rearing conditions


Average growth rate (mg WM/d)
0
0.2
0.4
0.6
0.8
1
1.2
Q1
Q2
Q3
Q4
Length quartile

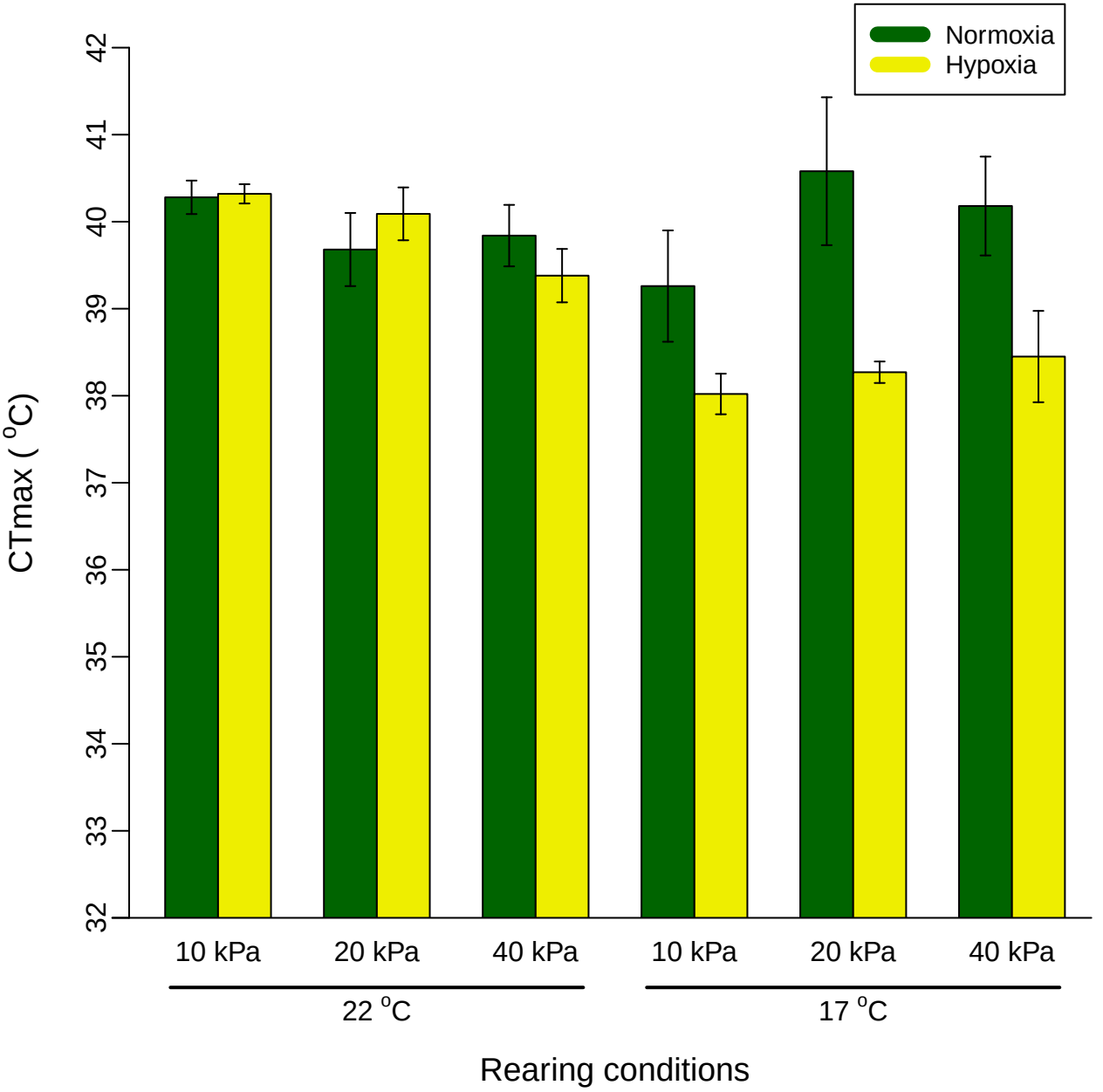

Normoxia
Hypoxia
CTmax (°C)
42
41
40
39
38
37
36
35
34
33
32
10 kPa
20 kPa
40 kPa
10 kPa
20 kPa
40 kPa
22 °C
17 °C
Rearing conditions

Figure 1. Wet mass data and Von Bertalanffy growth curves for *Lymnaea stagnalis* fitted by non-linear least square regression to biweekly length measurements in each of six rearing treatments (specified in top of each panel). Temperature treatments (17℃ and 22℃ in light grey and dark grey) are plotted separately for each rearing oxygen level (hypoxia in panel a, normoxia in panel b, hyperoxia in panel c). Curves were not extrapolated outside data range.

Figure 2. Scope for growth components in J d^{-1} $snail^{-1}$ for rearing conditions in *Lymnaea stagnalis*. Results of measurements on all rearing conditions. Top of bars marks total consumed energy, light green denotes energy not assimilated, blue and black denote energy lost in respiration and excretion respectively and dark green bars denote scope for growth. Assimilation efficiency was found to be 65%. Left panel shows measured values in Joules, right panel shows same data relative to total energy consumed.

Figure 3. Energy equivalent of observed growth (with 4860 J g^{-1} according to Rumohr et al, 1987) and calculated scope for growth in *Lymnaea stagnalis*. Colours denote rearing conditions (blue for 17 ℃ and red for 22 ℃) and darker colours denote higher rearing oxygen. Note the double logarithmic scale. The black line represents the relation y = x.

Figure 4. Mass corrected energy equivalents of physiological rates in *Lymnaea stagnalis*. Rearing oxygen (kPa) and rearing temperature (℃) are indicated below the bars. (a) Energy flux through food

consumption, (b) energy flux through oxygen consumption, (c) energy flux through ammonium excretion, (d) total surface of tentacles. Solid bars represent data from warm rearing conditions and shaded bars represent cold rearing conditions. Error bars denote 95% confidence intervals.

Figure 5. Relation between energy equivalents of mass corrected respiration rate and food ingestion rate in *Lymnaea stagnalis*. Colours denote rearing temperature (blue for 17 °C and red for 22 °C) and darker colours denote higher rearing oxygen. The line is given by mass corrected food consumption = 10.56 * oxygen consumption (R^2 = 0.46).

Figure 6. Energy equivalent of scope for growth and actual growth showing individual variation *Lymnaea stagnalis*. Data were collected from snails that were reared in hypoxic cold conditions. Open and solid symbols denote test temperature of 17 °C and 22 °C respectively. The thick line represents prediction for an average snail (0.15 g), dashed and dotted lines represent predictions for snails of 5% (0.05 g) and 95% (0.29 g) mass percentiles respectively.

Figure 7. Components of scope for growth and observed growth rate for individual variation in *Lymnaea stagnalis*. Data were collected from snails that were reared in hypoxic cold conditions. Panels show food consumption in J/d (a), oxygen consumption in J/d (b), ammonium excretion in J/d (c), and tentacle surface in mm^2 (d). Open and solid symbols denote test temperature of 17 °C and 22 °C respectively. The thick line represents prediction for an average snail (0.15 g), dashed and dotted lines represent predictions for snails of 5% (0.05 g) and 95% (0.29 g) mass percentiles respectively.

Figure 8. Maximum critical temperature (Loss of movement) of *Lymnaea stagnalis* in a ramping trial. Snails from the three rearing oxygen conditions were tested separately, but pooled in the figure, so values are averages of 15 snails. Error bars denote standard error. Green bars denote testing at normoxia (20% O_2), yellow bars denote testing at hypoxia (5% O_2). Rearing temperature is indicated at the bottom. Asterisk denotes significant difference between test conditions. See Figure A.2 for more details.

Figure A.1. Average growth rate in mg WM/d of *Lymnaea stagnalis* from the hypoxic cold rearing condition. Bars represent four length quartiles and the two aquaria in which snails were reared (shading). Error bars show 95% confidence intervals. Quartiles are based on shell length at onset of experiment.

Figure A.2. Maximum critical temperature (Loss of movement) of *Lymnaea stagnalis* in a ramping trial. CTmax values are averages of five snails, error bars denote standard error. Green bars denote testing at normoxia (20% O_2), yellow bars denote testing at hypoxia (5% O_2). Rearing conditions are indicated at the bottom.

Temperature	Oxygen	D		Wm (g)		Lm (mm)	MaxG (g^{-1})	Age Wm (d)	SSres	R^2 adj
17 °C	10 kPa	0.273	(±0.012)	0.115	(±0.011)	13.4	0.001	464	0.5	0.55
17 °C	20 kPa	0.253	(±0.011)	0.240	(±0.027)	17.1	0.001	507	1.4	0.56
17 °C	40 kPa	0.315	(±0.016)	0.269	(±0.032)	17.8	0.002	393	5.9	0.41
22 °C	10 kPa	0.415	(±0.026)	0.869	(±0.093)	20.5	0.007	285	14.6	0.66
22 °C	20 kPa	0.353	(±0.020)	1.522	(±0.164)	24.7	0.011	344	112.7	0.50
22 °C	40 kPa	0.297	(±0.018)	2.068	(±0.394)	27.3	0.012	421	86.4	0.47

Source	Value	Std. Error	DF	t-value	P-value	
(Intercept)	-12.903	29.584	113	-0.436	0.664	
Rearing temperature (°C)	100.544	19.101	1	5.264	6.8e-07	*
Shell free wet mass (g)	90.634	28.512	1	3.179	0.002	*
Rearing O_2 (kPa)	1.032	0.521	1	1.980	0.050	
Test temperature (°C)	0.360	1.469	1	0.245	0.807	
Rearing temperature : SFWM	-83.380	31.033	1	-2.687	0.008	*
Rearing temperature : Rearing O_2	-2.559	0.750	1	-3.410	0.001	*

Experiment	Rate	a	b	R^2
All rearing treatments	Food consumption (g d^{-1})	0.1264	0.9808	0.2190
	Food assimilation (g d^{-1})	0.0824	0.9808	0.2190
	Oxygen consumption (mg d^{-1})	1.5182	0.6033	0.5641
	Ammonium excretion (µmole d^{-1})	5.5332	0.5352	0.0329
	Tentacle surface (mm^2)	11.9292	0.4992	0.7987
	Scope for growth (J d^{-1})	66.6773	0.4013	0.1692

Response	Source	Estimate	Std. Error	DF	t-value	P-value	
Food consumption	(Intercept)	0.7629	0.5178	110	1.4733	0.1435	
	Rearing temperature	-0.0863	0.6338	4	-0.1361	0.8983	
	Rearing oxygen	0.0130	0.0156	4	0.8364	0.4500	
	Test temperature	-0.0165	0.0173	110	-0.9535	0.3424	
	Rearing temperature : Rearing oxygen	-0.0190	0.0230	4	-0.8263	0.4551	
Oxygen consumption	(Intercept)	1.8374	0.7059	110	2.6029	0.0105	*
	Rearing temperature	1.3427	0.5010	4	2.6799	0.0552	
	Rearing oxygen	0.0110	0.0131	4	0.8465	0.4450	
	Test temperature	-0.0084	0.0285	110	-0.2959	0.7679	
	Rearing temperature : Rearing oxygen	-0.0623	0.0198	4	-3.1447	0.0347	*
Ammonium production	(Intercept)	-55.4527	33.7633	110	-1.6424	0.1034	
	Rearing temperature	-21.7924	9.5897	5	-2.2725	0.0722	
	Rearing oxygen	1.2994	0.4247	5	3.0594	0.0281	*
	Test temperature	3.9603	1.6780	110	2.3602	0.0200	*
Tentacle surface	(Intercept)	15.1745	1.1364	85	13.3535	<2e-16	*
	Rearing temperature	-2.5997	1.7473	1	-1.4878	0.1405	
	Rearing oxygen	-0.1733	0.0676	1	-2.5619	0.0122	*
	Rearing temperature : Rearing oxygen	0.16280	0.0861	1	1.8910	0.0620	

Rate	Source	Estimate	Std. Error	DF	t-value	P-value	
Food consumption	(Intercept)	-0.0466	4.023e-02	123	-1.157	0.2494	
	Growth rate	188.7	8.574e+01	1	2.201	0.0296	*
R^2 = 0.26	SFWM	0.1227	2.043e-01	1	0.601	0.5491	
	Test temperature	0.001575	1.924e-03	1	0.818	0.4148	
	Growth rate : SFWM	-197.1	4.891e+01	1	-4.029	< 0.0001	*
Oxygen consumption	(Intercept)	-0.3984	0.2396	128	-1.663	0.0990	
	Growth rate	5.1170	478.7973	122	0.011	0.9915	
	SFWM	5.2767	1.1202	122	4.711	< 0.0001	*
	Test temperature	0.0190	0.0105	122	1.798	0.0746	
	Growth rate : SFWM	-1565.5328	282.4945	122	-5.542	< 0.0001	*
Ammonium excretion	(Intercept)	-1.514e+01	8.876e+00	123	-1.705	0.0906	
	Growth rate	-2.564e+04	1.892e+04	1	-1.355	0.1778	
R^2 = 0.12	SFWM	7.432e+01	4.507e+01	1	1.649	0.1017	
	Test temperature	1.196e+00	4.246e-01	1	2.815	0.0057	*
	Growth rate : SFWM	1.436e+04	1.079e+04	1	1.330	0.1859	
Tentacle surface	(Intercept)	1.8716	0.4184	96	4.473	< 0.0001	*
	Growth rate	11761.133	3291.4975	1	3.573	0.0006	*
R^2 = 0.42	SFWM	-11.2951	7.2567	1	-1.557	0.1229	
	Growth rate : SFWM	-5006.451	1717.1763	1	-2.916	0.0044	*
Scope for growth	(Intercept)	-8.240	1.273e+01	123	-0.647	0.5185	
	Growth rate	6.022e+04	2.712e+04	1	2.220	0.0282	*
R^2 = 0.11	SFWM	-37.40	6.462e+01	1	-0.579	0.5638	
	Test temperature	2.379e-01	6.088e-01	1	0.391	0.6966	
	Growth rate : SFWM	-4.045e+04	1.547e+04	1	-2.614	0.0101	*

Source	Estimate	Std. Error	t-value	P-value	
(Intercept)	37.2388	0.4063	91.655	< 0.0001	***
Mass	0.5005	0.5634	0.888	0.3769	
Rearing temperature	3.0385	0.6109	4.974	< 0.0001	***
Rearing oxygen	0.0163	0.0126	1.297	0.1981	
Test oxygen	0.1172	0.0212	5.336	< 0.0001	***
Rearing temperature : Rearing oxygen	-0.0414	0.0176	-2.347	0.0213	*
Rearing temperature : Test oxygen	-0.1151	0.0311	-3.708	0.0004	***

Source	Value	Std. Error	DF	t-value	p-value	
(Intercept)	-0.0873	0.0070	3691	-12.5139	0.0000	***
Age	0.0011	0.0011	3691	1.0571	0.2905	
Age : Rearing temperature	0.0039	0.0015	3691	2.5301	0.0114	*
Age : Rearing oxygen	-0.0000	0.0000	3691	-0.0721	0.9425	
Age : Rearing temperature : Rearing oxygen	0.0000	0.0000	3691	0.0687	0.9452	

Table 1. Estimated parameters for Bertalanffy growth function based on SFWM of *Lymnaea stagnalis*. M_0 and K were fixed at $2.7 \cdot 10^{-4}$ and 0.05 respectively. M_a is asymptotic mass in g, L_a is M_a converted to length in mm, MaxG is maximum growth rate in $g \cdot day^{-1}$, Age M_a is age at 98% of asymptotic weight in days, SSres is sum of squared residuals, R^2 adj is adjusted R^2. 95% confidence intervals are given in brackets.

Table 2. Linear model for effects of rearing and testing conditions on calculated scope for growth in *Lymnaea stagnalis*. Asterisks indicate significant effect. R^2 of the model is 0.32.

Table 3. Parameters of allometric equations ($Rate = a \cdot Mass^b$, with Mass in g wet mass) for scope for growth, its components and tentacle surface in *Lymnaea stagnalis*.

Table 4. Coefficients of mixed linear models with rearing and testing conditions on mass corrected physiological rates in *Lymnaea stagnalis*. Aquarium ID nested in Block was included as random factor in all models except for tentacle surface. Asterisks indicate significant effects.

Table 5. Summary table of models with individual variation on components of scope for growth in *Lymnaea stagnalis*. Data were collected from snails that were reared in hypoxic cold treatment.

Aquarium ID was included as a random factor for oxygen consumption only because it proved not significant in the other models. Colon indicates interaction, asterisks indicate significant effect. Note that R^2 are only given for linear models.

Table 6. Critical thermal maximum based on loss of movement in *Lymnaea stagnalis*. Summary table of linear model. R^2 of the model is 0.38.

Table A.1. Mixed effects model of *Lymnaea stagnalis* shell free wet mass over time. Aquarium ID nested in block was included as random effect to estimate a random slope of mass over time for each aquarium.

Highlights

- *Lymnaea stagnalis* attains a smaller body size when reared under hypoxia
- Rearing temperature and oxygen interactively affect scope for growth and its components
- Chronic rearing conditions affect growth much more than acute testing conditions
- Thermal history only affected heat tolerance when evaluated under hypoxia
- Individual variation in growth was large and related to variation in physiology and morphology