Effect of Thermal Acclimation on Organ Mass, Tissue Respiration, and Allometry in Leichhardtian River Prawns *Macrobrachium tolmerum* (Riek, 1951)

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ABSTRACT

Changes to an animal's abiotic environment—and consequent changes in the allometry of metabolic rate in the whole animal and its constituent parts—has considerable potential to reveal important patterns in both intraspecific and interindividual variation of metabolic rates. This study demonstrates that, after 6 wk of thermal acclimation at replicate treatments of 16°, 21°, and 25°C, standard metabolic rate (SMR) scales allometrically in Leichhardtian river prawns *Macrobrachium tolmerum* (mean scaling exponent = 0.61) and that the scaling exponent and normalization constant of the relationship between SMR and body mass is not significantly different among acclimation treatments when measured at 21°C. There is, however, significant variation among individuals in whole-animal metabolic rate. We hypothesized that these observations may arise because of changes in the metabolic rate and allometry of metabolic rate or mass of organ tissues within the animal. To investigate this hypothesis, rates of oxygen consumption in a range of tissues (gills, gonads, hepatopancreas, chelae muscle, tail muscle) were measured at 21°C and related to the body mass (M) and whole-animal SMR of individual prawns. We demonstrate that thermal acclimation had no effect on organ and tissue mass, that most organ and tissue (gills, gonads, hepatopancreas) respiration rates do not change with acclimation temperature, and that residual variation in the allometry of *M. tolmerum* SMR is not explained by differences in organ and tissue mass and respiration rates. These results suggest that body size and ambient temperature may independently affect metabolic rate in this species. Both chelae and tail muscle, however, exhibited a reduction in respiration rate in animals acclimated to 25° relative to those acclimated to 16° and 21°C. This reduction in respiration rates of muscle at higher temperatures is evidence of a tissue-specific acclimation response that was not detectable at the whole-animal level.

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Introduction

Metabolic rate is the rate at which an organism processes and converts materials to usable energy and as such represents the sum of all chemical reactions in an organism. The metabolic rate of an organism impacts the allocation of resources to growth and reproduction and as a consequence relates to fitness, survival, and reproductive output (Jackson et al. 2001; White and Seymour 2004; Blackmer et al. 2005; Artacho and Nespolo 2009; Biro and Stamps 2010; Burton et al. 2011; White and Kearney 2013).

Up to 95% of the variation in metabolic rate observed among species is accounted for by its relationship with body mass alone (e.g., basal metabolic rate of mammals; White and Seymour 2005). The relationship between metabolic rate (Y) and body mass (M) is generally well described by the power function $Y = aM^b$, where b is the scaling exponent and a is a normalization constant. An exponent of 1 represents isometric scaling, where metabolic rate increases in direct proportion to body mass, while an exponent different from 1 indicates that metabolic rate scales allometrically and does not increase in direct proportion to body mass (Kleiber 1947; Hayssen and Lacy 1985). Generally, interspecific scaling exponents fall between 2/3 and 1 (Niven and Scharlemann 2005; Chown et al. 2007; White et al. 2007), but intraspecific relationships show a much wider range of values (Bokma 2004; Glazier 2005; Chown et al. 2007; Killen et al. 2007, 2010; Moran and Wells 2007; Czarnoleski et al. 2008; Moses et al. 2008; White et al. 2011).

While body mass does account for much of the variation in metabolic rate, there persists a common finding of considerable residual variation in both interspecific and intraspecific scaling of metabolic rate, such that the metabolic rates of similarly sized animals can differ by several fold (Glazier 2005, 2010). The scaling relationship is also used to describe standard metabolic rates (SMRs; in inactive, nonreproductive, postabsorptive ectotherms), but it applies to a particular temperature, as the level of the scaling relationship is variable depending on the ambient temperature and the body temperature of the animal (Glazier 2005, 2010). Consequently, both body size and body temperature are recognized as central facets governing

metabolic rate and scaling thereof (Robinson et al. 1983; Gillooly et al. 2001; White et al. 2006, 2012*b*; Killen et al. 2010).

Body size and ambient temperature are thought to independently affect metabolic rate (Gillooly et al. 2001), although this assumption may be incorrect, as many studies have shown that ambient temperature may significantly alter the scaling exponent of whole-animal metabolic rate in terrestrial arthropods (Nespolo et al. 2003; Lardies et al. 2004), aquatic arthropods (Mladenova 1993), molluscs (Newell 1973), fish (Xiaojun and Ruyung 1990; Hölker 2003; Ohlberger et al. 2012), and reptiles (Al-Sadoon and Abdo 1991). The temperature dependence of metabolic rate, if present in a species, may be explained by the metabolic-level boundaries hypothesis (Glazier 2010), which considers two boundary constraints of the metabolic scaling relationship. The first physical boundary constraint concerns limits on fluxes of metabolic resources and heat across surfaces in an organism (which scale allometrically as $M^{0.67}$), and the second constraint idealizes limits on energy demands of tissues (which scale isometrically as $M^{1.00}$). In resting organisms, the scaling of metabolic rate is expected to be limited by the flux of metabolic resources and heat across surfaces when maintenance costs are high, whereas it is limited by energy demands required to sustain tissues when maintenance costs are low. For example, if cold temperatures are associated with lower maintenance costs than high temperatures, it would be predicted that there would be a decrease in the scaling exponent with temperature in that organism (Nespolo et al. 2003; Lardies et al. 2004; Glazier 2005, 2010; Ohlberger et al. 2012). The changes in scaling of metabolic rate with body mass at different ambient temperatures show no consistent trend in ectotherms; the relationship may be significantly negative, as for some fishes (Ohlberger et al. 2012); significantly positive (Newell 1973); or humped, as is the case for an aquatic arthropod (Mladenova 1993).

Ectotherms do not regulate body temperature using metabolically produced heat (Stevenson 1985), and because temperature affects nearly all biochemical and physiological processes, from an evolutionary perspective temperature has important consequences for ectotherms in that it may determine patterns of movement (Gibert et al. 2001), adult size (Atkinson 1995), and rates of reproduction and population growth (Wermelinger and Seifert 1999). As such, when measured at the same temperature, organisms from cold environments tend to have higher metabolic rates than those from warm environments (Addo-Bediako et al. 2002). This observation has been dubbed metabolic cold adaptation (MCA); it has been evidenced as an evolutionary response in insects (Addo-Bediako et al. 2002), and although there is some empirical evidence for its existence in aquatic arthropods (e.g., Rastrick and Whiteley 2011; Whiteley et al. 2011), its existence in aquatic animals in general is heavily debated (Holeton 1974; Clarke 1993; Steffensen 2002; Lurman et al. 2010; White et al. 2012*a*).

Temperature acclimation involves alteration of rates of chemical and enzymatic reactions, rates of diffusion, and changes in protein structure (e.g., Kleckner and Sidell 1985; Lucassen et al. 2003; Lurman et al. 2010; Whiteley et al. 2011). When exposed to the cold, ectothermic animals have to compensate for the slowing effect of temperature on these processes and uphold functional equilibrium between adenosine triphosphate (ATP) demand and ATP formation. As mitochondria are the sites of ATP production, acclimation to cold temperatures is well known to lead to an increase in mitochondria production and mitochondria capacity (Guderley 1990; Battersby and Moyes 1998; Lucassen et al. 2003, 2006). Mitochondria use free energy from oxidative phosphorylation to produce an electrochemical proton gradient across their inner membrane, which is passively permeable to protons. As a consequence, protons are leaked across this gradient, and a significant proportion of the SMR of an animal is expended to maintain the proton gradient during proton leak in both ectotherms and endotherms (Brand 1990; Pörtner 2002). The increased synthesis of mitochondria and the increased need for maintenance of proton gradients thus causes mitochondrial maintenance to incur a high energetic cost that is detectable as an increased SMR (Pörtner 2002).

Most of the metabolic activity in SMR is associated with fundamental processes, such as maintenance of mitochondria (for ATP production) and protein turnover within the internal organs and tissues that together comprise a large to moderate proportion of body mass in invertebrates. The contributions of these organs and tissues to SMR is a product of both their mass and metabolic intensity. We therefore hypothesize that at least part of the reason why SMR scales allometrically with body mass is due to differences in the masses and metabolic intensity of organs and tissues among small and large individuals. The contribution of different proportions of different tissues in arthropods to SMR is poorly understood, and few studies have explored the residual effects of different organs and tissues on metabolism in invertebrates, although some studies exist for insects (Crnokrak and Roff 2002; Nespolo et al 2005, 2008).

Tail and chelae muscle respiration in prawns (*Macrobrachium*; Decapoda: Palaemonidae) may be associated with fitness benefits, as the tail is associated with escape (burst swimming) performance (Webb 1979), and chelae are associated with dominance displays (Karplus 2005). If this is the case, thermal acclimation, assuming it is beneficial to the animal, should occur in muscle tissue in prawns such that the respiration rate of cold-acclimated muscle will be higher than that of warm-acclimated muscle when measured at the same temperature. As muscle comprises >80% of body mass in prawns, it follows that they must incur a significant maintenance cost to the animal; as such, the SMR in the whole animal may likewise exhibit higher rates in cold-acclimated animals compared with warm-acclimated animals when measured at the same temperature. If this is the case, it is predicted that whole-animal SMR will be positively correlated with tail and chelae muscle metabolic rate. Alternatively, natural selection may favor low and intermediate whole-animal SMR, as has been shown for snails *Helix aspersa* (Artacho and Nespolo 2009), where, as a consequence of the high cost of muscle tissue in response to thermal acclimation, tissue and organ metabolic rate is accordingly reduced

to balance out the high maintenance cost of muscle tissue. In this instance, there would be no correlation between whole-animal SMR and muscle metabolic rate.

In this study, we test for thermal acclimation of metabolic rate at the whole-animal and organ-tissue level in Leichhardtian river ("long-arm") prawns *Macrobrachium tolmerum*. We also examine the effect of thermal acclimation on the scaling of whole-animal and organ-tissue metabolism, and we test whether residual variation in the relationship between SMR and body mass is explained by variation in tissue mass or metabolic intensity. To address these issues, animals were acclimated to temperatures within a range of 16°–25°C, and after a minimum 6-wk exposure their SMR and rates of oxygen consumption in a range of tissues (gill filaments, gonads, hepatopancreas, chelae muscle, tail muscle) were measured at 21°C.

Material and Methods

Study Organism

Macrobrachium tolmerum is a common species of freshwater prawn endemic to the east coast of Australia (Short 2004; Sharma and Hughes 2009). The distribution and abundance of *M. tolmerum* can be partially attributed to their ability to tolerate a variety of salinities and temperatures (Short 2004; Sharma and Hughes 2009). Adult *M. tolmerum* were collected at Myora Springs, North Stradbroke Island, Australia (27°30′S, 153°30′E) using baited traps and dip nets and transported to the University of Queensland, St. Lucia. Although Short (2004) reports adult tolerance of temperatures from 17° to 34°C, the sampled population encountered temperatures ranging from 15° to 22°C during the collection period. Prawns were housed in mixed-sex groups in 35-L aquariums ($N \approx 15$–20 per aquarium), with each aquarium containing a gravel base and environmental enrichment of pipes and mesh providing shelters for animals. All aquariums were housed in a controlled temperature room maintained at 16° ± 1°C. To prevent aggressive or competitive bouts and mating behavior while in captivity, claw action on the chelae of larger animals was hindered by use of cyanoacrylate glue (UHU, Bühl, Germany). It is acknowledged that this necessary action may have affected the respiration rates of chelae muscle of larger individuals, but the extent of any effect is unknown and restraining the chelae was considered preferable to housing animals in isolation or risking the animals injuring one another with unrestrained claws. Animals were fed to satiation once daily with Orca Hi-Protein Sinking Pellets (Nijimi Pet Supplies, Riverwood, Australia), with uneaten feed removed after a 5-h period. Animals were kept in tanks under controlled conditions (12L : 12D photoperiod, oxygen saturation >75%), and partial water changes were conducted once weekly to maintain water quality.

Thermal Acclimation

Acclimation temperatures were selected on the basis of pilot trials conducted to ascertain the range of temperatures that can be tolerated by *M. tolmerum* without physical impairment or mortality. These trials comprised 1 wk of laboratory observations, with animals exposed to gradual temperature changes.

Each treatment included individuals spanning a wide range of body masses (0.77–13.62 g). Animals were randomly assigned to one of three thermal exposures at 16°, 21°, or 25°C, with each thermal treatment comprising two replicate aquariums ($N \approx 15$–20 per 35-L aquarium). Acclimation temperatures for aquariums were controlled to ±0.8°C using automatic aquarium heaters (AquarWorld HT-2300; Complete Pet and Vet Supplies, Yatala, Australia). Thermal acclimation for treatment groups lasted a minimum of 6 wk before experimentation.

Measurements of Whole-Animal SMR

Animals were randomly selected from each treatment, and their molt cycle stage was determined to control for inconsistencies in animal condition. The molt cycle stage of animals was determined by physical examination of the flexibility of the rostrum, testing each animal's capability to raise its chelae and by observing any changes in pigmentation of the exoskeleton (Peebles 1977). If the animal was not early or late stage in their molt cycle and was fit and intact, then that individual met the criteria for SMR measurements in this study. Animals that met these criteria were then fasted for a minimum of 48 h, allowing them to enter a postabsorptive state before measurements of SMR. If the animal did not meet these criteria, they were either excluded from the study (i.e., individuals that were unfit or had missing appendages) or returned to their respective aquariums until such time that they reached the appropriate stage in their molt cycle (i.e., individuals that were fit and intact but at an inappropriate stage in their molt cycle). Wet body mass (0.001 g) of individuals was measured using an electronic microbalance (Sartorius, Göttingen, Germany) and converted to body volume (to 0.01 mL) assuming a density of 1 g mL^{-1}.

Rates of oxygen consumption of individuals were measured for 24 h to allow animals to recover from handling stress (approximately 30 min) and thermal stress (16°C-acclimated individuals, approximately 1 h; 25°C-acclimated individuals, approximately 1.5 h) and to become accustomed to the respirometry chamber. Values obtained during the recovery periods were excluded from $\dot{V}_{O_2}$ calculations to reduce the risk of masking any acclimation effects. Rates of oxygen consumption were measured using a two-channel intermittent flow respirometry setup similar to that used by Steffensen et al. (1984) and Daoud et al. (2007).

Resealable cylindrical chambers made of PVC-DWV (Vinidex) of three different volumes (124, 225, and 554 mL) were used as respirometry chambers. All connections were made using watertight luer integral lock rings and connectors (Cole-Parmer, Chatswood, Australia) and low-permeability tubing.

Two respirometry chambers containing one animal in each were submerged in a 60-L aquarium filled with freshwater. Water in the aquarium was continuously aerated and circulated while regulated at a constant temperature of 21° ± 0.4°C using a Frigomix FX-F cooler (B. Braun, Melsungen, Germany) and

Table 1: Summary of constituents used in freshwater crustacean Ringer's solution on the basis of van Harreveld (1936)

Compound	Concentration (mM)	g/L
Sodium chloride	205.3	12
Calcium chloride (93.0%, anhydrous)	13.55	1.5
Potassium chloride (99.0%)	5.37	.4
Magnesium chloride (−325 mesh)	2.61	.25
Sodium bicarbonate	2.38	.2

Note. Deionized water was used to mix salts to final volume (1 L), after which sodium bicarbonate was added to avoid precipitation of calcium carbonate. Final pH was equal to 7.55. All compounds were from Sigma-Aldrich (St. Louis, MO).

a Julabo-ED heater (Labortechnik, Seelbach, Germany). Good mixing within the chambers was ensured by circulating water through the chambers in a closed loop at a constant flow rate of 25 mL min^{-1} using Tygon tubing (5 mm) and a Masterflex peristaltic pump (model 77202-50; Cole-Parmer).

Dissolved oxygen was measured using fiber-optic oxygen sensors (Ocean Optics FOXY-OR125-G; Lastek, Adelaide, Australia) connected to an oxygen meter (TauTheta MFPF100-2; Lastek). Oxygen sensors were calibrated using nitrogen and air-saturated water before experimentation. Oxygen levels and temperature in both chambers were simultaneously measured and recorded at a sampling frequency of 0.5 Hz. The chamber was alternately sealed for 45 min and flushed for 15 min throughout the 24-h measurement period.

Measurements of Tissue Respiration

Individuals were identified such that further investigations of relationships between the metabolic rate of whole animals and that of their tissues could be assessed taking into account individual variations. After recording the oxygen consumption of individuals, animals were relocated to a small container of freshwater and desensitized by rapid chilling on ice before pithing. The gonads, hepatopancreas, tail muscle, chelae muscle, and gills were excised from pithed individuals, sliced thinly by hand using a stainless steel razor blade (to approximate tissue slice dimensions of 4–3 mm × 3–2 mm × 0.5 mm), washed to remove microbiota in freshwater crustacean Ringer's solution (van Harreveld 1936; table 1), and placed in individual 5-mL glass vials containing air-saturated (100%) freshwater crustacean Ringer's solution. The slice thickness of 0.5 mm allows for good diffusion in an average tissue (Elliott 1955), and tissues were not sliced thinner than 0.5 mm so that the proportion of damaged cells was kept to a minimum (Couture and Hulbert 1995). The remaining tissue for each organ-tissue type was finely minced and blotted dry before wet mass was measured to 0.1 mg on an electronic microbalance (Sartorius), and upon the respiration of the small tissue section being measured, the small tissue section was also minced finely, blotted dry, and weighed on a microbalance, and the mass of the small tissue section was combined with that of the remaining tissue of the same type for gill filaments (mean mass = 0.048 g, mass range = 1.54×10^{-4}–0.093 g), hepatopancreas (mean mass = 0.300 g, mass range = 0.059–1.006 g), tail muscle (mean mass = 1.340 g, mass range = 0.342–4.350 g), chelae muscle (mean mass = 0.218 g, mass range = 0.012–1.116 g), and gonad tissue (mean mass = 0.008 g, mass range = 0.0001–0.072 g). The respiration rate of the organ-tissue sections were converted to per-unit mass respiration rate, then multiplied by the mass of the whole tissue to obtain total organ metabolic rate. Pilot trials of tissue respiration in vials showed that the rate of oxygen depletion was not significantly different between air-saturated and oxygen-saturated Ringer's solution (Student's t-test, $P > 0.05$) for sliced hepatopancreas and tail muscle tissue.

Measurements of oxygen consumption of tissues were made using optical fluorescence-based oxygen respirometry at 21° ± 1°C in a refrigerated incubator (R190; S.E.M., Magill, Australia). Tissues were placed within glass respirometry vials containing oxygen sensor spots (type SP-PSt5-NAU-D5-YOP; PreSens, Regensburg, Germany), which were calibrated between 0% and 21% oxygen saturation using 2% sodium sulphite solution (0% air saturation) and air-saturated deionized water (Koster et al. 2008; Young et al. 2011; Alton et al. 2012).

To prevent the formation of a hypoxic boundary layer around the respiring tissue samples, tissues were placed within a fine nylon mesh basket within the respirometry vial to suspend them above the oxygen sensor spot. Vials were stirred continuously during measurements on an orbital mixer (OM1; Ratek, Victoria, Australia). A 24-channel SensorDish reader (SDR2, PreSens; AS1, Regensburg, Germany) with vials containing prepared tissue was placed in the incubator and used to measure the rate of oxygen depletion in vials. Measurements of oxygen concentration in each of the vials containing tissues and four blank vials without tissues were recorded at 2 min intervals for 1 h.

After measurement of metabolic rate, the total wet mass of each tissue (0.0001 g) was determined using an electronic microbalance.

Calculations and Statistical Analysis

Using LabChart (ver. 6.1.3; ADInstruments, Bella Vista, Australia), a linear regression was calculated for the relationship between oxygen saturation and time, and the rate of oxygen

Table 2: Summary of scaling exponents (b) for *Macrobrachium tolmerum*: whole-animal ($N = 65$) standard metabolic rate (SMR; mL h^{-1}) ± SEM and organ-tissue (gill filament: $N = 36$; hepatopancreas: $N = 61$; tail muscle: $N = 60$; chelae muscle: $N = 62$; gonad: $N = 35$) metabolic rate (OTMR; mL h^{-1}) ± SEM

	Scaling of SMR and OTMR with WAM	Scaling of OTM with WAM	Scaling of mass-specific OTMR with WAM
Whole animals	.61 ± .08	NA	NA
Gill filaments (16° and 25°C only)	1.09 ± .24	.77 ± .55	−.20 ± .17
Hepatopancreas	.61 ± .14	.89 ± .56	−.48 ± .14
Tail muscle	.71 ± .14	.83 ± .53	−.34 ± .11
Chelae muscle	.43 ± .09	1.34 ± .68	−.80 ± .12
Gonad ♂ ♀	.36 ± .20	.42 ± .74	−.20 ± .29

Note. Scaling exponents (b) are derived from the following models: for whole-animal SMR with whole-animal mass (WAM; g), $\log_{10}(\text{SMR}) \sim \log_{10}(\text{WAM})$ + temperature treatment; for OTMR with WAM, $\log_{10}(\text{OTMR}) \sim \log_{10}(\text{WAM})$ + temperature treatment; for organ-tissue mass (OTM; g) with WAM, $\log_{10}(\text{OTM}) \sim \log_{10}(\text{WAM})$ + temperature treatment; and for mass-specific (0.1 g^{-1}) OTMR with WAM, $\log_{10}(\text{mass-specific OTMR}) \sim \log_{10}(\text{WAM})$ + temperature treatment. All values are shown with SEM. Temperature treatment had no significant effect on the scaling exponents derived from these models ($P > 0.05$). NA = not applicable.

consumption ($\dot{V}O_2$; mL h^{-1}) was calculated according to Alton et al. (2007):

$$\dot{V}O_2 = -1 \times \left(\frac{m_p - m_c}{100}\right) \times V \times \beta_{O_2}, \quad (1)$$

where V is the volume of water in the respirometry chamber minus the volume of the animal, β_{O_2} is the oxygen capacitance of air-saturated freshwater at 21°C (6.23 mL L^{-1}; Riley and Chester 1971), m_p is the slope (the rate of decline in oxygen saturation) derived from the trial with a long-arm prawn (% air saturation h^{-1}), and m_c is the slope derived from the control without an animal (% air saturation h^{-1}). $\dot{V}O_2$ was calculated for each of the 24 closed periods, and the lowest value for each animal was used for analysis.

For tissue respiration, the slope of the linear regression of oxygen saturation was calculated for each tissue using SDR_v37 software (PreSens), and rate of oxygen consumption was calculated using equation (1) assuming β_{O_2} of air-saturated Ringer's solution (van Harreveld 1936) at 21°C = 5.70 mL L^{-1} (Riley and Chester 1971).

Body mass (M) of animals and $\dot{V}O_2$ data for both animals and their tissues were $\log_{10}$ transformed and analyzed statistically using JMP software (ver. 8.0.1; SAS Institute, Cary, NC). Differences in the scaling exponent (b) of animals and their tissues between treatments were assessed as an interaction between treatment (i.e., temperature) and $\log_{10}(M)$ in a full factorial ANOVA. Whenever the interaction term was not significant, treatment effects were assessed using ANCOVA with treatment as a fixed effect and $\log_{10}(M)$ as a continuous covariate. Scaling exponents for tissue mass and tissue mass–specific metabolic rate were calculated by linear regression of $\log_{10}$-transformed tissue mass and mass-specific metabolic rate against $\log_{10}$-transformed whole mass (as presented elsewhere; Oikawa and Itazawa 1984, 2003), respectively. Shapiro-Wilk tests were used to check that data satisfied assumptions of normality. Associations between whole-animal SMR and organ-tissue masses and organ-tissue metabolic rates were examined using linear models with $\log_{10}$-transformed SMR as the dependent variable and $\log_{10}$-transformed mass as a fixed predictor along with the organ-tissue mass or metabolic rate of interest.

Results

Temperature treatment had no significant effect on the scaling relationship (b) between whole-animal mass and organ-tissue metabolic rates ($P > 0.05$), organ-tissue masses ($P > 0.05$), and mass-specific organ-tissue metabolic rates ($P > 0.05$). The scaling exponents (b) for these relationships are reported in table 2.

Whole-Animal SMR

There was no significant effect of sex of animals on SMR (ANOVA, $F_{1,48} = 0.78$, $P = 0.38$) and no significant interaction between $\log_{10}(M)$ and acclimation temperature (ANOVA, $F_{2,47} = 0.49$, $P = 0.61$), indicating that the scaling exponents did not differ between treatments. The relationship between body mass and SMR was significant (ANCOVA, $F_{1,49} = 58.1$, $P < 0.0001$), as was the effect of thermal acclimation (ANCOVA, $F_{2,49} = 3.6$, $P = 0.035$; fig. 1*A*).

Least squares means of mass-independent respiration rates of animals (±SEM; fig. 2) show that although thermal acclimation had a statistically significant effect on SMR, pairwise comparison showed that there was no significant difference between the 16° and 25°C acclimation groups (ANCOVA, $F_{1,37} = 0.18$, $P = 0.68$) and that 21°C was only marginally lower than these acclimation groups.

Tissue Metabolic Rate

Gill Filaments. Data for gill filaments from 21°C-acclimated animals were omitted from the analysis because of methodological inconsistencies in tissue extractions. There was no significant interaction between $\log_{10}(M)$ and acclimation treatment (ANOVA, $F_{1,32} = 0.25$, $P = 0.62$). Neither body mass nor

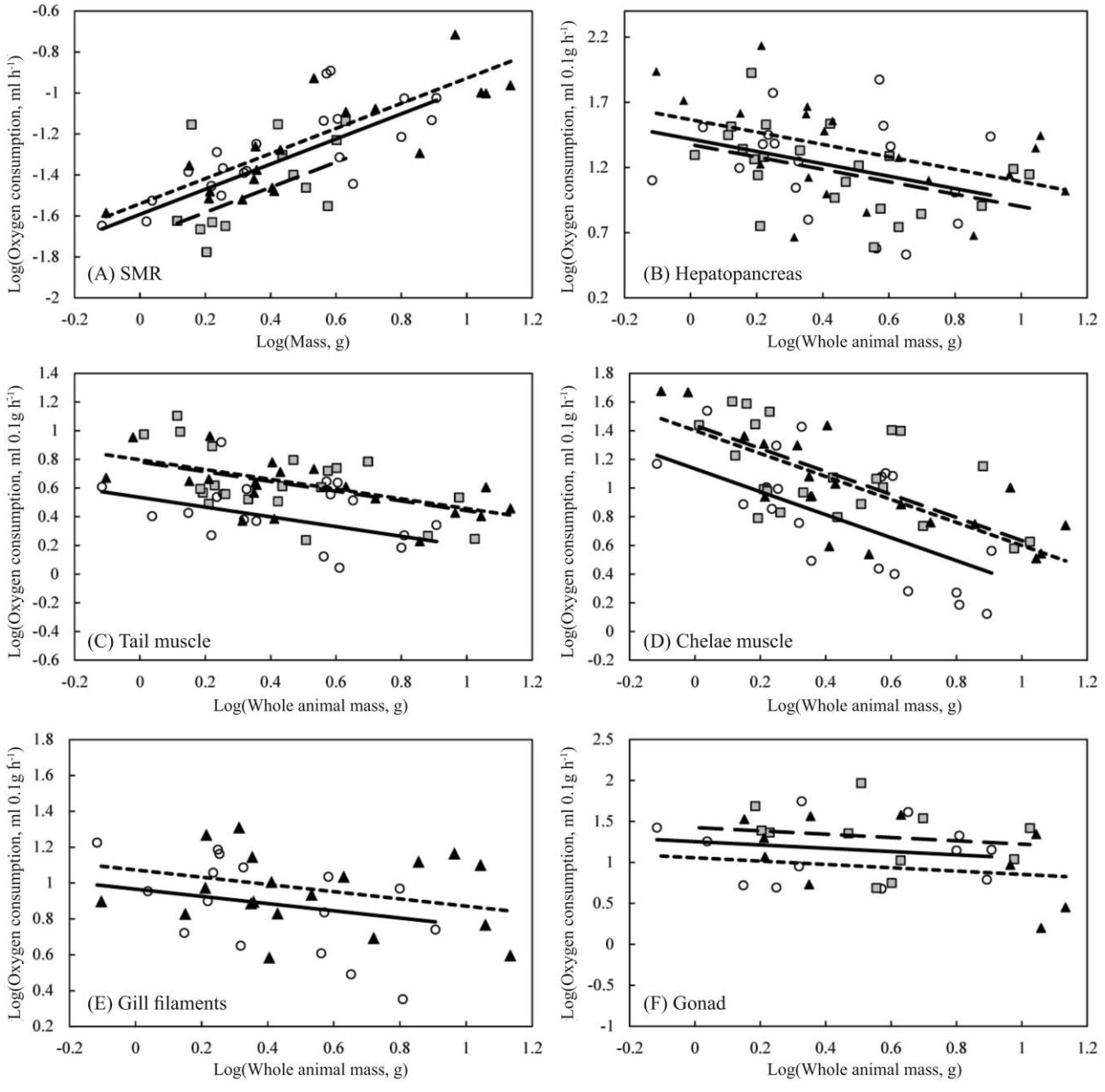

Figure 1. *A*, Effect of thermal acclimation on the allometry of respiration rate in *Macrobrachium tolmerum* measured at 21°C (25°C: $N = 20$; 21°C: $N = 14$; 16°C: $N = 21$). *B*, Effect of thermal acclimation on the allometry of respiration rate in the hepatopancreas of *M. tolmerum* measured at 21°C (25°C: $N = 18$; 21°C: $N = 23$; 16°C: $N = 20$). *C*, Effect of thermal acclimation on the allometry of respiration rate in tail muscle of *M. tolmerum* measured at 21°C (25°C: $N = 19$; 21°C: $N = 21$; 16°C: $N = 20$). *D*, Effect of thermal acclimation on the allometry of respiration rate in chelae muscle of *M. tolmerum* measured at 21°C (25°C: $N = 20$; 21°C: $N = 22$; 16°C: $N = 20$). *E*, Effect of thermal acclimation on the allometry of respiration rate in gills of *M. tolmerum* measured at 21°C (25°C: $N = 17$; 16°C: $N = 19$). *F*, Effect of thermal acclimation on the allometry of respiration rate in *M. tolmerum* gonad measured at 21°C (25°C: $N = 13$: 21°C; $N = 11$; 16°C: $N = 11$). All tissue $\dot{V}_{O_2}$ is corrected to 0.1 g of whole-tissue mass. White circles denote values obtained at 25°C, shaded squares denote values obtained at 21°C, and black triangles denote values obtained at 16°C. Solid line slopes denote linear regression of 25°C measurements, long dashed line slopes denote linear regression of 21°C measurements, and short dashed line slopes denote linear regression of 16°C measurements. SMR = standard metabolic rate.

acclimation temperature had a significant effect on gill respiration rate (body mass: ANCOVA, $F_{1,33} = 1.4$, $P = 0.24$; acclimation temperature: ANCOVA, $F_{1,33} = 0.9$, $P = 0.33$; fig. 1*E*). Acclimation temperature had no significant effect on gill mass (ANOVA, $F_{2,36} = 3.77$, $P = 0.06$).

Hepatopancreas. There was no significant interaction between $\log_{10}(M)$ and acclimation treatment (ANOVA, $F_{2,55} = 0.21$, $P = 0.81$). Body mass had a significant effect on hepatopancreas respiration rate (ANCOVA, $F_{1,57} = 11.03$, $P = 0.002$), but acclimation temperature did not (ANCOVA, $F_{2,57} = 1.8$, $P =$

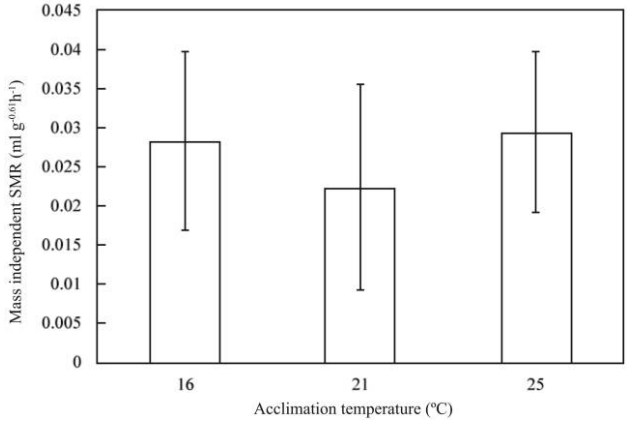

Figure 2. Least squares means of mass-independent respiration rates of *Macrobrachium tolmerum* measured at 21°C after thermal acclimation ± SEM (25°C: $N = 20$; 21°C: $N = 14$; 16°C: $N = 21$). There is no significant difference between thermal treatment groups ($P = 0.68$). SMR = standard metabolic rate.

0.17; fig. 1*B*). Acclimation temperature had no significant effect on hepatopancreas mass (ANOVA, $F_{2,55} = 0.55$, $P = 0.58$).

Tail Muscle. There was no significant interaction between $\log_{10}(M)$ and acclimation treatment (ANOVA, $F_{2,54} = 1.2$, $P = 0.33$). Both body mass and acclimation temperature had a significant effect on tail muscle respiration rate (body mass: ANCOVA, $F_{1,56} = 10.1$, $P = 0.002$; acclimation temperature: ANCOVA, $F_{2,56} = 6.7$, $P = 0.003$; fig. 1*C*). Acclimation temperature had no significant effect on tail muscle mass (ANOVA, $F_{2,55} = 1.74$, $P = 0.19$).

Post hoc analysis revealed that respiration rate in tail muscle was not significantly different between animals acclimated to 16° and 21°C but was significantly higher in animals acclimated to 16°C than those acclimated to 25°C (ANOVA, $F_{3,35} = 4.25$, $P = 0.012$; fig. 3*A*).

Chelae Muscle. There was no significant interaction between $\log_{10}(M)$ and acclimation treatment (ANOVA, $F_{2,56} = 1.5$, $P = 0.24$). The relationship between chelae muscle respiration rate and body mass was significant (ANCOVA, $F_{1,58} = 44.48$, $P < 0.0001$; fig. 1*D*), as was the effect of thermal acclimation (ANCOVA, $F_{2,58} = 6.6$, $P = 0.003$). Acclimation temperature had no significant effect on chelae muscle mass (ANOVA, $F_{2,55} = 1.12$, $P = 0.33$).

Post hoc analysis revealed that respiration rate of chelae muscle was not significantly different between animals acclimated to 16° and 21°C but was significantly higher in animals acclimated to 16°C compared with those acclimated to 25°C (ANOVA, $F_{3,36} = 14.9$, $P < 0.0001$; fig. 3*B*).

Gonad. There was no significant interaction between $\log_{10}(M)$ and acclimation treatment (ANOVA, $F_{2,28} = 1.9$, $P = 0.17$). There was no significant effect of sex on gonad respiration rate (ANOVA, $F_{1,31} = 3.3$, $P = 0.08$). Acclimation temperature had no significant effect on gonad mass (ANOVA, $F_{2,42} = 1.65$, $P = 0.20$). Neither body mass nor acclimation temperature had a significant effect on gonad respiration rate (body mass: ANCOVA, $F_{1,30} = 0.47$, $P = 0.5$; acclimation temperature: ANCOVA $F_{2,30} = 1.17$, $P = 0.32$; fig. 1*F*).

Correlation between Organ and Tissue Metabolic Rate and Mass with Whole-Animal SMR

Differences in whole-organ (hepatopancreas and gonad) and tissue (gill filaments, chelae, and tail muscle) metabolic rate were related to variations in prawn SMR. None of the correlations were significant ($P \geq 0.1$). Furthermore, differences in

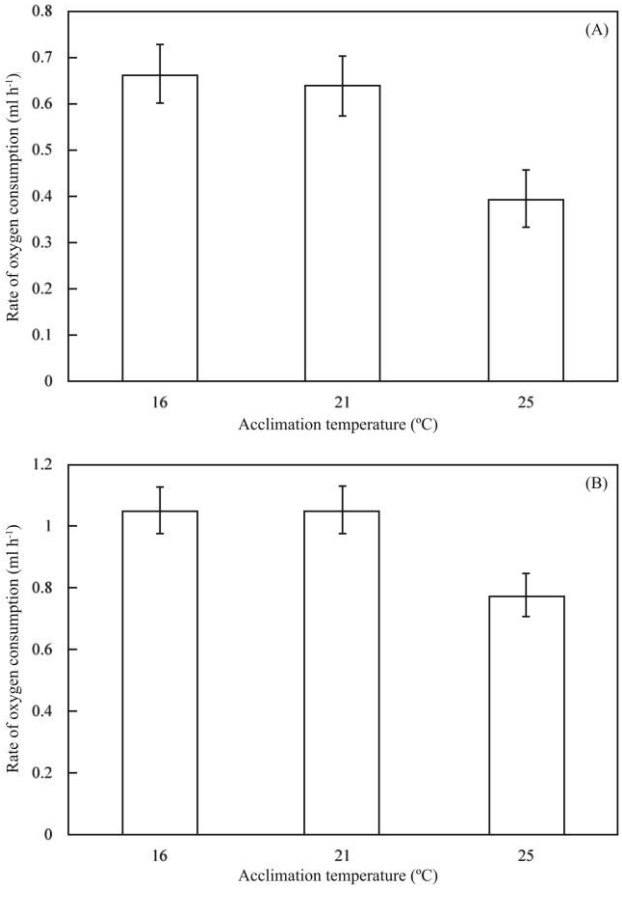

Figure 3. *A*, Least squares mean rate of oxygen consumption in tail muscle (corrected to 0.1 g of tissue) measured at 21°C after thermal acclimation ± SEM (25°C: $N = 19$; 21°C: $N = 21$; 16°C: $N = 20$). Tail muscle respiration rates are not significantly different between 16° and 21°C, but tail respiration rates are significantly higher in animals acclimated to 16°C compared with those acclimated to 25°C ($P = 0.012$). *B*, Least squares mean rate of oxygen consumption in chelae muscle (corrected to 0.1 g of tissue) measured at 21°C after thermal acclimation ± SEM (25°C: $N = 20$; 21°C: $N = 22$; 16°C: $N = 20$). Chelae muscle respiration rates are not significantly different between 16° and 21°C, but chelae respiration rates are significantly higher in animals acclimated to 16°C compared with those acclimated to 25°C ($P < 0.0001$).

whole-organ and tissue mass were not significantly correlated to variations in prawn SMR ($P \geq 0.1$).

Discussion

We aimed to determine the effect of thermal acclimation on the whole-animal SMR and organ-tissue metabolic rates of *Macrobrachium tolmerum*. We also examined the effect of thermal acclimation on the scaling of whole-animal and organ-tissue metabolism and tested whether, once variation in body mass is accounted for, residual among-individual variation in the SMR of *M. tolmerum* is attributable to among-individual differences in organ and tissue masses or respiration rates. There was a significant positive relationship between body mass and SMR, with animals from all acclimation treatments demonstrating a mean scaling exponent (b) of 0.61. There was no significant effect of thermal acclimation on the scaling exponent of whole-animal SMR or organ-tissue mass or metabolic rate in organ-tissues that comprise up to 80% of animal body mass, and there is no evidence to suggest that residual variation in SMR is attributable to differences in examined organ and tissue masses or respiration rates. There was, however, an overall effect of thermal acclimation on the metabolic rates of tail and chelae muscle (independent of mass), such that when measured at 21°C the metabolic rates of these tissues were 1.8-fold higher in animals acclimated to 16°C compared with that in animals acclimated to 25°C.

The MCA hypothesis predicts that cold-adapted animals should have a higher metabolic rate than warm-adapted animals when measured at a common temperature, and this increase in metabolic rate is attributed to increased capacities for respiration and circulation that occur primarily in muscle and viscera (Clarke and Portner 2010). Although we do not examine MCA in our study, we do examine the effects of cold acclimation on the respiration of tissues. Temperature acclimation itself involves alteration of many physiological processes, and the increase in metabolic rate in response to cold acclimation commonly seen in ectotherms is due in part to muscle hypertrophy and mitochondrial proliferation or alteration (Johnston et al. 1998). Mitochondria generally proliferate in the cold either in response to reduced oxygen delivery (Guderley 2004) or in response to a reduced efficiency of mitochondria to produce ATP (Pörtner et al. 2000). It has been shown, however, that rather than a proliferation in mitochondria (commonly seen in ectothermic vertebrates), the likely predominant mechanism in invertebrates is to change the surface density of mitochondrial cristae in response to cold acclimation (Lurman et al. 2010). We found an overall effect of thermal acclimation on the metabolic rates of tail and chelae muscle (independent of mass), such that these tissues were 1.8-fold higher in animals acclimated to 16°C compared with animals acclimated to 25°C when measured at 21°C. Whatever the actual mechanism associated with increased muscle respiration rates in response to cold acclimation is in this study, this skeletal muscle–specific response to cold acclimation supports the predictions of the MCA hypothesis.

The effect of temperature on the scaling of metabolic rate show no consistent trends in ectotherms, and in this study we find that there is no significant effect of thermal acclimation on the scaling exponent of whole-animal SMR or organ-tissue metabolic rate. This finding has been demonstrated at the whole-animal level in other aquatic crustaceans (Paranjape 1967), and it supports the idea that body size and ambient temperature may independently affect metabolic rate (Gillooly et al. 2001). Other crustaceans, however, have demonstrated significant changes in the scaling of whole-animal metabolic rate in response to temperature (Rao and Bullock 1954; Mladenova 1993), highlighting that the species-specific response to temperature may also be due to differences in ecology and evolutionary history (Ohlberger et al. 2012).

Darveau et al. (2002) suggest that variation in metabolic scaling may be a result of differences in the metabolic contributions of different tissues, which together constitute whole-animal metabolism (see also Wang et al. 2001). The relative sizes and metabolic activities of tissues and organs should therefore change both with body mass and in response to supply-and-demand pathways and thereby alter the scaling of metabolism. For example, the studies of Crnokrak and Roff (2002) and Nespolo et al. (2008) correlated dorsolongitudinal muscle (associated with flight capacity) mass with metabolic rate in sand crickets *Gryllus firmus*, and both of these studies demonstrated that individuals with dorsolongitudinal muscle of greater mass had a higher whole-animal metabolic rate. The contribution of variations in organ mass to variations in whole-animal SMR in terrestrial vertebrates show mixed results. Garland (1984) found that approximately one-third of variation in the SMR of the iguana *Ctenosaura similis* was attributable to variation in relative liver and heart masses. Other terrestrial vertebrate studies seeking the source of variation in basal metabolic rate and SMR have indicated, however, that organ size does not account for observed variation (Song and Wang 2002; Tieleman et al. 2003; Speakman et al. 2004; Russell and Chappell 2007).

Studies examining variation in size (e.g., Crnokrak and Roff 2002; Nespolo et al. 2005, 2008) and metabolic intensity of organs and tissues in arthropods are rare, and none to date have examined contributions of both organ and tissue mass and metabolic rate to variation in whole-animal SMR. This study thus provides novel insight pertaining to the effects of thermal acclimation on tissue-specific metabolism, as it does not limit itself to just tissue masses. This study shows for the first time that variation in both the sizes of organs and organ-tissue metabolic rate are not significantly correlated with variation in SMR in an invertebrate. This may be due to variations in the metabolic rates of the isolated organ-tissues linked to difficulties in obtaining the precise mass of tissues, as mass is the denominator for metabolic rates. Furthermore, there may have been differences in the integrity of the measured tissues in this study. Although slice thickness of 0.5 mm should minimize tissue damage (Couture and Hulbert 1995), perhaps an additional assessment of tissue damage through measuring activity of glutamate dehydrogenase, a mitochondrial enzyme that

appears to be lost from damaged cells, and relating this back to glutamate dehydrogenase activity of the whole tissue (as done by Couture and Hulbert 1995) may have accounted for variations in tissue metabolic rate between individual animals.

As variation in the sizes of organs and organ-tissue metabolic rate was not significantly correlated with variation in the SMR of prawns in this study, there are likely to be a number of other factors that may account for intraspecific variation in the SMR of *M. tolmerum*. These include but are not limited to differences in behavior or personality (Biro and Stamps 2010), housing with conspecifics of different size classes (Millidine et al. 2009), and social dominance (Sloman et al. 2000). Individuals may also react differently from others when confined in a respirometer, which can lead to higher estimates of metabolic rate for those individuals (Careau et al. 2008; Killen et al. 2011). Thus, we can only conclude that it is most likely that variations in SMR within this species may be related to variations in levels of stress, social conditioning, overall body composition (e.g., Krebs 1950), and subtle differences in molecular activity (e.g., Wang et al. 2006).

Our study does not detail sex-based variation in SMR, as there was a lack of relationship between the metabolic rate of gonad tissue and body mass. Individual metabolic rates in gonad tissue may be influenced by social status and dynamics, which cannot be differentiated on the basis of respiration measurements alone (Røskaft et al. 1986; Hogstad 1987; Metcalfe et al. 1995; Piersma and Lindstrom 1997; Senar et al. 2000). In male *Macrobrachium*, it is difficult to attribute variation in gonad allometry to differences in whole-animal metabolic rate because males come in two different-sized morphotypes once sexually mature—subordinate (small sized, highly mobile) and dominant (large sized, slowgoing)—and within each morphotype there is a dominance hierarchy. Females have no different morphotypes, yet because *M. tolmerum* demonstrate no particular seasonality in breeding (Short 2004), gonad mass and gonad metabolic rate cannot be reliably correlated with body size in females. Thus, it is difficult to reliably attribute thermal acclimation effects to changes in the allometry of gonad tissue metabolic rate and mass.

Having a high or low SMR has different fitness consequences for the organism; individuals with higher SMRs may have less energy to allocate to other functions, such as reproduction and storage (Blackmer et al. 2005; Artacho and Nespolo 2009), but individuals with a higher standard metabolism may also have superior aerobic capacity or reproductive output that can also contribute to greater fitness (Nilsson 2002). Two hypotheses exist regarding the relationship between SMR and fitness. First, the "wasteful" phenotype, where some performance measure that improves fitness is associated with energy expenditure, and as a consequence SMR is also higher. In this case, fitness is expected to be positively correlated with SMR. For example, Nespolo et al. (2008) found that a higher cost of flight muscle meant a greater flight capacity; as a consequence, however, fitness correlated positively with RMR. Second, costs associated with maintenance metabolism are high enough to have fitness consequences. Assuming that reducing SMR does not interfere with any other capacity, this argument predicts that selection promotes individuals with low SMR or eliminates individuals with high SMR (Artacho and Nespolo 2009). Muscle tissue comprised >80% of body mass in prawns, and as such it was hypothesized that muscle tissue should incur a significant maintenance cost to the animal and that this cost would be reflected in the SMR of the whole animal. We hypothesized that if there was no correlation between whole-animal SMR and an increase in muscle metabolic rate, the metabolic rate of other organs and tissues would reduce to balance out the high maintenance cost of muscle tissue. This study shows that there was no correlation between whole-animal SMR and muscle metabolic rate between treatments, and although we detected no changes in the metabolic rates or masses in other organs and tissues examined in this study, this finding provides evidence that natural selection may favor low and intermediate whole-animal SMRs. Given the substantial variation in SMR observed among individuals in this study, it would be valuable to relate this variation to direct or indirect measures of fitness to determine whether this variation in SMR has consequences for the performance of individuals.

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Literature Cited

Addo-Bediako A., S.L. Chown, and K.J. Gaston. 2002. Metabolic cold adaptation in insects: a large-scale perspective. Funct Ecol 16:332–338.

Alton L.A., C.R. White, and R.S. Seymour. 2007. Effect of aerial O_2 partial pressure on bimodal gas exchange and air-breathing behaviour in *Trichogaster leeri*. J Exp Biol 210: 2311–2319.

Alton L.A., C.R. White, R.S. Wilson, and C.E. Franklin. 2012. The energetic cost of exposure to UV radiation for tadpoles is greater when they live with predators. Funct Ecol 26:94–103.

Al-Sadoon M.K. and N.M. Abdo. 1991. Temperature and body mass effects on the metabolic rate of *Acanthodactylus schmidti* (Reptilia: Lacertidae). J Arid Environ 21:351–361.

Artacho P. and R.F. Nespolo. 2009. Natural selection reduces energy metabolism in the garden snail, *Helix aspersa* (*Cornu aspersum*). Evolution 63:1044–1050.

Atkinson D. 1995. Effects of temperature on the size of aquatic ectotherms: exceptions to the general rule. J Therm Biol 20: 61–74.

Battersby B.J. and C.D. Moyes. 1998. Influence of acclimation temperature on mitochondrial DNA, RNA, and enzymes in skeletal muscle. Am J Physiol 275:R905–R912.

Biro P.A. and J.A. Stamps. 2010. Do consistent individual dif-

ferences in metabolic rate promote consistent individual differences in behaviour? Trends Ecol Evol 25:653–659.
Blackmer A.L., R.A. Mauck, J.T. Ackerman, C.E. Huntington, G.A. Nevitt, and J.B. Williams. 2005. Exploring individual quality: basal metabolic rate and reproductive performance in storm-petrels. Behav Ecol 16:906–913.
Bokma F. 2004. Evidence against universal metabolic allometry. Funct Ecol 18:184–187.
Brand M.D. 1990. The contribution of the leak of protons across the mitochondrial inner membrane to standard metabolic rate. J Theor Biol 145:267–286.
Burton T., S.S. Killen, J.D. Armstrong, and N.B. Metcalfe. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proc R Soc B 278: 3465–3473.
Careau V., D. Thomas, M.M. Humphries, and D. Reale. 2008. Energy metabolism and animal personality. Oikos 117:641–653.
Chown S.L., E. Marais, J.S. Terblanche, C.J. Klok, J.R.B. Lighton, and T.M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Funct Ecol 21:282–290.
Clarke A. 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? Funct Ecol 7:139–149.
Clarke A. and H.O. Portner. 2010. Temperature, metabolic power and the evolution of endothermy. Biol Rev 85:703–727.
Couture P. and A.J. Hulbert. 1995. Relationship between body mass, tissue metabolic rate, and sodium pump activity in mammalian liver and kidney. Am J Physiol 268:R641–R650.
Crnokrak P. and D.A. Roff. 2002. Trade-offs to flight capability in *Gryllus firmus*: the influence of whole-organism respiration rate on fitness. J Evol Biol 15:388–398.
Czarnoleski M., J. Kozlowski, G. Dumiot, J.C. Bonnet, J. Mallard, and M. Dupont-Nivet. 2008. Scaling of metabolism in *Helix aspersa* snails: changes through ontogeny and response to selection for increased size. J Exp Biol 211:391–399.
Daoud D., D. Chabot, C. Audet, and Y. Lambert. 2007. Temperature induced variation in oxygen consumption of juvenile and adult stages of the northern shrimp, *Pandalus borealis*. J Exp Mar Biol Ecol 347:30–40.
Darveau C.A., R.K. Suarez, R.D. Andrews, and P.W. Hochachka. 2002. Allometric cascade as a unifying principle of body mass effects on metabolism. Nature 417:166–170.
Elliott K.A.C. 1955. Tissue slice technique. Pp. 3–11 in S.F. Colowick and N.O. Kaplan, eds. Methods in enzomology. Academic Press, New York.
Garland T., Jr. 1984. Physiological correlates of locomotory performance in a lizard: an allometric approach. Am J Physiol 247:R806–R815.
Gibert P., R.B. Huey, and G.W. Gilchrist. 2001. Locomotor performance of *Drosophila melanogaster*: interactions among developmental and adult temperatures, age, and geography. Evolution 55:205–209.
Gillooly J.F., J.H. Brown, G.B. West, V.M. Savage, and E.L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248–2251.
Glazier D.S. 2005. Beyond the "3/4-power law": variation in the intra- and interspecific scaling of metabolic rate in animals. Biol Rev 80:611–662.
———. 2010. A unifying explanation for diverse metabolic scaling in animals and plants. Biol Rev 85:111–138.
Guderley H. 1990. Functional significance of metabolic responses to thermal acclimation in fish muscle. Am J Physiol 259:R245–R252.
———. 2004. Metabolic responses to low temperature in fish muscle. Biol Rev 79:409–427.
Hayssen V. and R.C. Lacy. 1985. Basal metabolic rates in mammals: taxonomic differences in the allometry of BMR and body mass. Comp Biochem Physiol A 81:741–754.
Hogstad O. 1987. It is expensive to be dominant. Auk 104: 333–336.
Holeton G.F. 1974. Metabolic cold adaptation of polar fish: fact or artefact? Physiol Zool 47:137–152.
Hölker F. 2003. The metabolic rate of roach in relation to body size and temperature. J Fish Biol 62:565–579.
Jackson D.M., P. Trayhurn, and J.R. Speakman. 2001. Associations between energetics and over-winter survival in the short-tailed field vole *Microtus agrestis*. J Anim Ecol 70:633–640.
Johnston I.A., J. Calvo, H. Guderley, D. Fernandez, and L. Palmer. 1998. Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. J Exp Biol 201:1–12.
Karplus I. 2005. Social control of growth in *Macrobrachium rosenbergii* (De Man): a review and prospects for future research. Aquac Res 36:238–254.
Killen S.S., D. Atkinson, and D.S. Glazier. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecol Lett 13:184–193.
Killen S.S., I. Costa, J.A. Brown, and A.K. Gamperl. 2007. Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. Proc R Soc B 274:431–438.
Killen S.S., S. Marras, and D. McKenzie. 2011. Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. J Anim Ecol 80:1024–1033.
Kleckner N.W. and B.D. Sidell. 1985. Comparison of maximal activities of enzymes from tissues of thermally acclimated and naturally acclimatized chain pickerel (*Esox niger*). Physiol Zool 58:18–28.
Kleiber M. 1947. Body size and metabolic rate. Physiol Rev 27: 511–541.
Koster M., C. Krause, and G.-A. Paffenhofer. 2008. Time-series measurements of oxygen consumption of copepod nauplii. Mar Ecol Prog Ser 353:157–164.
Krebs H.A. 1950. Body size and tissue respiration. Biochim Biophys Acta 4:249–269.
Lardies M.A., T.P. Catalán, and F. Bozinovic. 2004. Metabolism and life-history correlates in a lowland and highland population of a terrestrial isopod. Can J Zool 82:677–687.

Lucassen M., N. Koschnick, L.G. Eckerle, and H.O. Pörtner. 2006. Mitochondrial mechanisms of cold adaptation in cod (*Gadus morhua* L.) populations from different climatic zones. J Exp Biol 209:2462–2471.

Lucassen M., A. Schmidt, L.G. Eckerle, and H.O. Pörtner. 2003. Mitochondrial proliferation in the permanent vs. temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. Am J Physiol 285:R1410–R1420.

Lurman G., T. Blaser, M. Lamare, K.-S. Tan, H. Poertner, L.S. Peck, and S.A. Morley. 2010. Ultrastructure of pedal muscle as a function of temperature in nacellid limpets. Mar Biol (Berl) 157:1705–1712.

Metcalfe N.B., A.C. Taylor, and J.E. Thorpe. 1995. Metabolic rate, social status and life history strategies in Atlantic salmon. Anim Behav 49:431–436.

Millidine K.J., N.B. Metcalfe, and J.D. Armstrong. 2009. Presence of a conspecific causes divergent changes in resting metabolism, depending on its relative size. Proc R Soc B 276: 3989–3993.

Mladenova A. 1993. Importance of the temperature for the energetic metabolism of fresh-water isopod [*Asellus aquaticus* (L.)]. Russ J Aquat Ecol 2:55–63.

Moran D. and R.M.G. Wells. 2007. Ontogenetic scaling of fish metabolism in the mouse-to-elephant mass magnitude range. Comp Biochem Physiol A 148:611–620.

Moses M.E., C. Hou, W.H. Woodruff, G.B. West, J.C. Nekola, W. Zuo, and J.H. Brown. 2008. Revisiting a model of ontogenetic growth: estimating model parameters from theory and data. Am Nat 171:632–645.

Nespolo R.F., L.E. Castaneda, and D.A. Roff. 2005. Dissecting the variance-covariance structure in insect physiology: the multivariate association between metabolism and morphology in the nymphs of the sand cricket (*Gryllus firmus*). J Insect Physiol 51:913–921.

Nespolo R.F., M.A. Lardies, and F. Bozinovic. 2003. Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q_{10}) of oxygen consumption in a cricket. J Exp Biol 206:4309–4315.

Nespolo R.F., D.A. Roff, and D.J. Fairbairn. 2008. Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). Funct Ecol 22:624–631.

Newell R.C. 1973. Factors affecting the respiration of intertidal invertebrates. Am Zool 13:513–528.

Nilsson J.A. 2002. Metabolic consequences of hard work. Proc R Soc B 269:1735–1739.

Niven J.E. and J.P.W. Scharlemann. 2005. Do insect metabolic rates at rest and during flight scale with body mass? Biol Lett 1:346–349.

Ohlberger J., T. Mehner, G. Staaks, and F. Hölker. 2012. Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. Oikos 121:245–251.

Oikawa S. and Y. Itazawa. 1984. Allometric relationship between tissue respiration and body mass in the carp. Comp Biochem Physiol A 77:415–418.

———. 2003. Relationship between summated tissue respiration and body size in a marine teleost, the porgy *Pagrus major*. Fish Sci 69:687–694.

Paranjape M.A. 1967. Molting and respiration of euphasiids. J Fish Res Board Can 24:1229–1240.

Peebles J.B. 1977. A rapid technique for molt staging in live *Macrobrachium rosenbergii*. Aquaculture 12:173–180.

Piersma T. and A. Lindstrom. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. Trends Ecol Evol 12:134–138.

Pörtner H.O. 2002. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. J Exp Biol 205:2217–2230.

Pörtner H.O., P.L.M. van Dijk, I. Hardewig, and A. Sommer. 2000. Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. Pp. 109–122 in W. Davison and C.H. Williams, eds. Antarctic ecosystems: models for wider ecological understanding. Caxton, Christchurch.

Rao K.P. and T.H. Bullock. 1954. Q_{10} as function of size and habitat temperature in poikilotherms. Am Nat 88:33–44.

Rastrick S.P.S. and N.M. Whiteley. 2011. Congeneric amphipods show differing abilities to maintain metabolic rates with latitude. Physiol Biochem Zool 84:154–165.

Riley J.P. and R. Chester. 1971. Introduction to marine chemistry. Academic Press, London.

Robinson W.R., R.H. Peters, and J. Zimmermann. 1983. The effects of body size and temperature on metabolic rate of organisms. Can J Zool 61:281–288.

Røskaft E., T. Järvi, M. Bakken, C. Bech, and R.E. Reinertsen. 1986. The relationship between social status and resting metabolic rate in great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*). Anim Behav 34:838–842.

Russell G.A. and M.A. Chappell. 2007. Is BMR repeatable in deer mice? organ mass correlates and the effects of cold acclimation and natal altitude. J Comp Physiol B 177:75–87.

Senar J.C., V. Polo, F. Uribe, and M. Camerino. 2000. Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. Anim Behav 59:103–110.

Sharma S. and J.M. Hughes. 2009. Genetic structure and phylogeography of freshwater shrimps (*Macrobrachium australiense* and *Macrobrachium tolmerum*): the role of contemporary and historical events. Mar Freshw Res 60:541–553.

Short J.W. 2004. A revision of Australian river prawns, *Macrobrachium* (Crustacea: Decapoda: Palaemonidae). Hydrobiologica 525:1–100.

Sloman K.A., G. Motherwell, K.I. O'Connor, and A.C. Taylor. 2000. The effect of social stress on the standard metabolic rate (SMR) of brown trout, *Salmo trutta*. Fish Physiol Biochem 23:49–53.

Song Z. and D. Wang. 2002. Relationships between metabolic rates and body composition in the Mongolian gerbils (*Meriones unguiculatus*). Acta Zootaxon Sin 48:445–451.

Speakman J.R., E. Krol, and M.S. Johnson. 2004. The functional significance of individual variation in basal metabolic rate. Physiol Biochem Zool 77:900–915.

Steffensen J.F. 2002. Metabolic cold adaptation of polar fish

based on measurements of aerobic oxygen consumption: fact or artefact? artefact! Comp Biochem Physiol A 132:789–795.

Steffensen J.F., K. Johansen, and P.G. Bushnell. 1984. An automated swimming respirometer. Comp Biochem Physiol A 79:437–440.

Stevenson R.D. 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. Am Nat 125: 102–117.

Tieleman B.I., J.B. Williams, M.E. Buschur, and C.R. Brown. 2003. Phenotypic variation among and within larks along an aridity gradient: are desert birds more flexible? Ecology 84: 1800–1815.

van Harreveld A. 1936. A physiological solution for freshwater crustaceans. Proc Soc Exp Biol Med 34:428–432.

Wang W.-N., A.-L. Wang, Y. Liu, J. Xiu, Z.-B. Liu, and R.-Y. Sun. 2006. Effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimp *Macrobrachium nipponense*. Aquaculture 256:624–630.

Wang Z.M., T.P. O'Connor, S. Heshka, and S.B. Heymsfield. 2001. The reconstruction of Kleiber's law at the organ-tissue level. J Nutr 131:2967–2970.

Webb P.W. 1979. Mechanics of escape responses in crayfish (*Orconectes virilis*). J Exp Biol 79:245–263.

Wermelinger B. and M. Seifert. 1999. Temperature-dependent reproduction of the spruce bark beetle *Ips typographus*, and analysis of the potential population growth. Ecol Entomol 24:103–110.

White C.R., L.A. Alton, and P.B. Frappell. 2012*a*. Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. Proc R Soc B 279:1740–1747.

White C.R., P. Cassey, and T.M. Blackburn. 2007. Allometric exponents do not support a universal metabolic allometry. Ecology 88:315–323.

White C.R., P.B. Frappell, and S.L. Chown. 2012*b*. An information-theoretic approach to evaluating the size and temperature dependence of metabolic rate. Proc R Soc B 279: 3616–3621.

White C.R. and M.R. Kearney. 2013. Determinants of interspecific variation in basal metabolic rate. J Comp Physiol B 183:1–26.

White C.R., M.R. Kearney, P.G.D. Matthews, S.A.L.M. Kooijman, and D.J. Marshall. 2011. A manipulative test of competing theories for metabolic scaling. Am Nat 178:746–754.

White C.R., N.F. Phillips, and R.S. Seymour. 2006. The scaling and temperature dependence of vertebrate metabolism. Biol Lett 2:125–127.

White C.R. and R.S. Seymour. 2004. Does basal metabolic rate contain a useful signal? mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-history variables. Physiol Biochem Zool 77:929–941.

———. 2005. Allometric scaling of mammalian metabolism. J Exp Biol 208:1611–1619.

Whiteley N.M., S.P.S. Rastrick, D.H. Lunt, and J. Rock. 2011. Latitudinal variations in the physiology of marine gammarid amphipods. J Exp Mar Biol Ecol 400:70–77.

Xiaojun X. and S. Ruyung. 1990. The bioenergetics of the southern catfish (*Silurus meridionalis* Chen). I. Resting metabolic rate as a function of body weight and temperature. Physiol Zool 63:1181–1195.

Young K.M., R.L. Cramp, C.R. White, and C.E. Franklin. 2011. Influence of elevated temperature on metabolism during aestivation: implications for muscle disuse atrophy. J Exp Biol 214:3782–3789.