NTNU – Trondheim
Norwegian University of
Science and Technology

Change in Thermal Metabolic Rate Reaction Norms of *Daphnia* in Response to Rearing Temperature

Tijana Gajic

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Supervisor: Sigurd Einum, IBI
Co-supervisor: Anders Finstad, NINA

Norwegian University of Science and Technology
Department of Biology

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Abstract

Temperature is an important environmental factor that affects the distribution of organisms. It affects the biochemical and physiological processes which are the basis of life. The metabolic cold adaptation (MCA) hypothesis predicts an increase in the metabolic rate of ectotherms from cold environments compared with their more temperate counterparts. This hypothesis is one of the most controversial in ecophysiology. MCA is an example of counter gradient variation, a geographical pattern of genotypes where genetic influences on a trait oppose environmental influences. Environmental gradients are common in nature and are considered to have major effects on intraspecific variation patterns. In this study, I tested the MCA hypothesis at an intraspecific level. As a study model I utilized the water flea, *Daphnia magna*. These are planktonic crustaceans, common in lakes and ponds, and have a wide geographical and thermal distribution. I tested the MCA hypothesis in a laboratory experiment in which oxygen consumption of water fleas reared at three different temperatures for one year (12 – 35 generations), were measured at three different experimental temperatures. My results show that the animals from the coldest rearing temperature (10 °C) had the highest metabolism at all three experimental temperatures (10, 17 and 25 °C), compared with animals reared at higher temperatures (17 and 25 °C). Elevated metabolism in animals from cold environment is consistent with the metabolic cold adaptation hypothesis. The present study does however not provide conclusive evidence that clone-specific variation in thermal performance has a genetic basis. Organisms may adjust their thermal reaction norms as a response to the thermal regime in which they live through three mechanisms: acclimation, epigenetic effects and evolution, and these could not be distinguished in experimental design used here.

Sammendrag

Temperatur er en viktig miljøfaktor som påvirker distribusjon av organismer. Den påvirker biokjemiske og fysiologiske prosesser som er grunnleggende for alt liv. Hypotesen om metabolsk tilpasning til kulde (Metabolic Cold Adaptation, MCA) predikerer at ektoterme organismer fra kalde omgivelser skal ha en høyere metabolsk rate, sammenlignet med tilsvarende organismer fra tempererte strøk. Denne hypotesen en av de mest kontroversielle i økofysiologi. MCA er et spesialtilfelle av variasjon i sammensetning av genotyper langs geografiske breddegrader, hvor genetiske endringer kan motvirke miljøendringer. Miljøgradienter er vanlige i naturen og forventes å ha stor innvirkning på variasjon innen en art. I dette studiet har jeg testet MCA hypotesen på intraspesifikt nivå. Som modellorganisme i min studie har jeg brukt vannloppe, *Daphnia magna*. Det er et planktonisk krepsdyr som er vanlig i innsjøer og dammer, og har stor geografisk og termal spredning. Jeg testet MCA hypotesen i et laboratorieeksperiment. Oksygenforbruk av vannlopper dyrket på tre forskjellige temperaturer i løpet av ett år (12 – 35 generasjoner) ble målt på tre forskjellige eksperiment-temperaturer. Mine resultater viser at dyr fra den kaldeste dyrkningstemperatur (10 °C) hadde høyest metabolisme på alle tre eksperiment-temperaturer (10, 17 og 25 °C), sammenlignet med dyr oppvokst på høyere temperaturer (17 og 25 °C). Det er en indikasjon på metabolsk kompensasjon for den globale temperaturgradienten. Dette studiet kan likevel ikke bevise at denne klonspesifikke variasjonen i termal suksess har genetisk årsak. Organismer kan justere sine termale reaksjonsnormer som svar på temperaturregimet de lever under gjennom tre mekanismer: akklimatisering, epigenetiske effekter og evolusjon. Jeg kunne ikke skille mellom disse mekanismene i forsøksdesignet som ble brukt her.

The thesis is formatted according to the journal Population Ecology.

Key words: metabolic cold adaptation, thermal reaction norms, metabolic rate, oxygen consumption, phenotypic plasticity, evolutionary changes, *Daphnia magna*

Introduction

Metabolism is the universal feature of life that links organisms with their environment and with each other. Metabolism allows organisms to grow and reproduce, maintain their structures, and respond to their environments. This trait is under strong environmental control, being influenced by temperature, body size, food abundance, reproductive condition, stress, and time of day (Hill et al. 2012). Metabolic rate is a measure of the energy utilization of an organism. Changes in metabolic rate might also arise from alterations in mitochondrial density and/or capacity (Seidl et al. 2005). Thus within a species, the respiration rate might vary due to acclimation, epigenetic effects or evolution (Simčič and Brancelj 2004).

For ectotherms, the environmental temperature is the most important environmental factor influencing metabolic rate (Angilletta 2002; Gillooly et al. 2001). Temperature controls physiological rates, tissue constituents and the rate of biochemical reactions. Element acquisition and growth are highly dependent on temperature. Temperature has a strong effect on enzyme activity. Generally, enzyme activity increases exponentially with increasing temperature. Thus, in the absence of limiting factors, a rise in temperature will increase the metabolic rate, creating what is known as thermal reaction norms (Pörtner et al. 2006, Angilletta 2009). However, there is no theoretical reason to believe that the optimal metabolism (i.e. that maximizing fitness) increases with temperature. Rather, the optimum may depend on other characteristics of the environment, such as food availability (Burton 2011). If the optimum is indeed independent of temperature, we may therefore expect to see adjustments in the thermal reaction norms, as a response to the thermal environments organisms live in (Fig. 1). This hypothesis, known as the Metabolic Cold Adaptation (MCA) hypothesis (Krogh 1916), suggests that organisms which have adapted over evolutionary time to live at low temperatures, would have a metabolic rate higher than expected from the metabolic rate/ temperature relationship established for organisms living at warmer temperatures. Specifically, ectotherms that must function at low temperatures should develop compensatory mechanisms that mitigate temperature effects (Guderley 2004). Evolution, epigenetic effects and acclimation are all such compensatory mechanisms.

MCA hypothesis can be tested by comparing the intercepts of thermal reaction norms for individuals that originate from different thermal regimes in a common garden experiment. If organisms attempt to obtain similar common optimal metabolism (RMR*) in the environment in which they live, these intercepts should be higher for organisms originating from cold environments (Fig. 1).

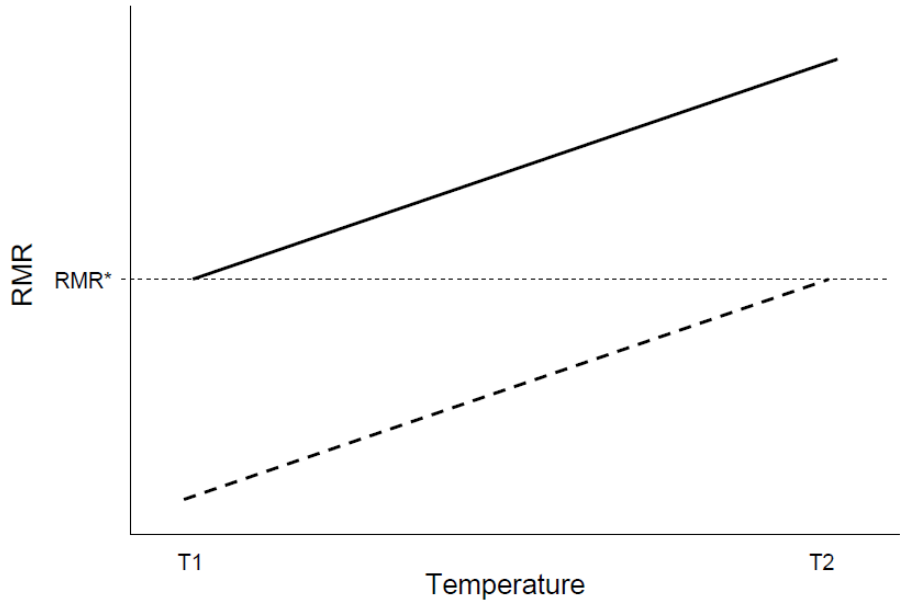

Fig. 1: Hypothesised effect of temperature on resting metabolic rate, RMR (on log scale) in organisms originating from low (T1, solid line) and high (T2, dashed line) temperature according to the Metabolic Cold Adaptation hypothesis. Due to evolutionary adaptations in the elevation of their respective reaction norms, both types obtain an optimal resting metabolic rate (RMR*) at the temperature in which they live.

The possibility of elevated metabolic rates of cold-water ectotherms has been a topic of debate over many years. Some of the early inter-species comparisons between the metabolic rates of Arctic and tropical aquatic ectotherms, concluded that there was considerable Metabolic Cold Adaptation in Arctic species (Scholander et al. 1953). These early studies have been questioned (Holeton 1974). But, insects and endotherms from cold environments have also later been shown to have relatively high metabolic rate (Addo-Bediako et al. 2002; Speakman et al. 2010). Addo-Bediako et al. (2002) used a global-scale analysis of the standard metabolic rates of insect 346 species and found that environmental temperature significantly influences interspecific variation in metabolic rate. The knowledge of adaptation mechanisms has increased a lot over the last decades. Marshall et al. (2012) found, for example, that when normalized to a common temperature, fish species with ranges that extend to high latitude had high aerobic enzyme activity, high rates of mitochondrial respiration and high standard metabolic rates. Some inter-species studies of MCA have also been performed. In the lugworm, *Arenicola marina*, individuals from colder environments in the White Sea have a higher mitochondrial activity, along with higher oxygen consumption rates, compared with their counterparts from warmer environments in the North Sea (Sommer and Pörtner 2002). Atlantic killifish, *Fundulus heteroclitus*, have been studied extensively as a model for thermal adaptation (Schulte, 2007; Fangue et al. 2009; Grim et al. 2010). Studies of killifish living along the coast of North America have shown higher metabolic rates in the northern subspecies, compared with the southern subspecies (Wells, 1935). However, the MCA hypothesis has been criticized (Holeton 1974, Steffenson 2002). Comparison of metabolic rates of different species of perciform fish (Clarke and Johnson 1999) and bivalve mollusc (Peck and Conway 2000) provided, for example, no support for MCA. It follows that the

MCA hypothesis is controversial, and the importance of MCA is vigorously debated from both evolutionary and ecological perspectives (Terblanche et al. 2009).

The purpose of my study is to test metabolic cold adaptation (MCA) in the model organism *Daphnia magna*. *Daphnia* are small, filter-feeding, planktonic crustaceans which are common in small lakes and ponds. They play an important ecological role as grassers. As *Daphnia* are preferred prey for both invertebrate and vertebrate predators, they act as an important link between trophic levels of the aquatic food web (Lampert, 1987). *Daphnia* have characteristics that make them suitable for this study: they are ectotherms, have wide geographical and thermal distribution. They are constantly exposed to divergent selection pressures that shape their response to temperature, because daily and seasonal temperature fluctuations in small freshwater habitats can be large. Optimum temperature range for *D. magna* has been shown to be in the range 15–25 °C, but they can tolerate temperatures between 2 – 30 °C (Lampert 1977; Goss and Bunting 1983). Different genotypes may prefer different thermal regimes. A natural population of *Daphnia* can respond to temperature change with daily vertical migrations, phenotypic plasticity of individuals, or through a change in the clonal composition of the population. Hence, *D. magna* appears to be an excellent model system to investigate the physiological basis of the thermal sensitivity of metabolism. The capacity of *Daphnia* populations to undergo rapid evolutionary change in natural systems in response to environmental challenges has received considerable attention. For nearly every trait that has been investigated, genetic variation has been reported (Ebert, 2005). *Daphnia's* life cycle involves both asexual and sexual reproduction. Parthenogenetic life cycle allows the study of epigenetic effects in the absence of confounding genetic differences. There exists also intra-clonal variation in *Daphnia*, and progeny of a single female can undergo divergent selection (Gorkhova et al 2002). The study is conducted in a laboratory, in order to examine the oxygen consumption of *D. magna* clones, reared at three different temperatures for one year. I have also quantified activity levels across temperatures, due to some unexpected observed temperature effects in the oxygen consumption experiment.

Material and methods

The study consists of two laboratory experiments: an oxygen consumption experiment and an activity experiment. Replicated clones were reared at three different temperatures over multiple generations, prior to quantification of thermal metabolic rate reaction norms in an oxygen consumption experiment. A single clone of *D. magna* was obtained from the University of Oslo in October 2011, and 13 separate lines were initiated and subsequently kept in separate 2 l plastic aquaria at three different climate rooms at the Norwegian University of Science and Technology (NTNU), Trondheim: five at temperature 10.75 ± 0.28 °C (Clone 10), five at 16.78 ± 0.85 °C (Clone 17) and three at 24.49 ± 1.28 °C (Clone 25) (the mean and standard deviation of monthly mean temperatures). Synthetically prepared water, COMBO was used as the freshwater medium during rearing (Kilham et. al. 1998) in order to keep ionic composition of water under control and to make favourable conditions for both *Daphnia* and green plankton algae (*Selenastrum sp)* that was used as food. The green algae were grown at approximately 20 °C in chemostats (see Hessen et al. 2002 for details), and added *ad libitum* 3 times a week. Tubular lamps provided light, and the photoperiod was maintained at a 14 light: 10 dark cycles. At the onset of reproduction for a given line the first 20 individuals that were born were transferred to a new aquarium to initiate the next generation. This enabled recording of the number of generations elapsed for each line, and at

the same time selected for rapid maturation. By November 2012 the number of generations elapsed varied between clones: from 12–14 generations in Clone 10, 21–24 in Clone 17 to 32–35 in Clone 25.

Oxygen consumption experiment

Table 1: Experimental design of oxygen consumption measurements in *D. magna*, giving the number of lines and the number of individuals (n) from each line, for each combination of clone rearing temperature and experimental temperature

Clone rearing temperature	*Experimental temperature*		
	10 °C	**17 °C**	**25 °C**
Clone 10	5 lines, n = 1 - 4	5 lines, n = 1 - 3	5 lines, n = 1 - 3
Clone 17	5 lines, n = 1 - 2	5 lines, n = 1 - 3	5 lines, n = 2
Clone 25	3 lines, n = 2 - 3	3 lines, n = 3	3 lines, n = 1 - 2

The different clones were used for experiments during the period from October 2012 - January 2013. Metabolism was estimated by the measurement of oxygen consumption, using closed respirometry (Strathkelvin model 782) at 3 different experimental temperatures. Individuals from each line within each clone were measured at each of three temperatures: 10, 17 and 25 °C (Table 1). Temperatures were chosen to represent average extremes of the natural temperature range (Goss and Bunting 1983). The experiment was originally designed as a 3 x 3 x 2 replicates experiment. However, some animals died, and animals bigger than 2 mm were excluded to avoid the presence of eggs or embryos in the brood pouch (Evers and Kooijman 1989). At the occurrence of a situation in which resting metabolism cannot be measured, as is the case with Daphnia, active respiration rate is measured. It was not possible to measure standard metabolic rate in *Daphnia*, as limb movements are required for respiration (Choplet et al. 2008). The contribution of food digestion on metabolic rate, termed "specific dynamic action" (Lampert, 1986), was avoided by acclimating them to their test temperatures for 20 – 26 h in the absence of food prior to measurements.
Daphnia were placed individually into syringes containing 0.5 ml COMBO and a plastic bead, and the tip of the syringe was sealed with paraffin. Three blank syringes served as controls for each series of measurements and three start O2-measurements were taken of the COMBO. The syringes were placed horizontally within a water bath for approximately 3 hours. Prior to the measurements, a homogenous oxygen concentration was ensured by letting the plastic bead rail from one end of the syringe to the other multiple times. *Daphnia* oxygen consumption, R_D was calculated as the difference between observed consumption in syringe with *Daphnia* and oxygen consumption due to microbial consumption measured in the blank syringe:

$$R_D\ (mg\ O_2\ min^{-1}) = (Start\ O_2\ (mg) - End\ O_2\ (mg))\ x\ 0.25/1000/Time\ (min) - R_M\ (mg\ O_2\ min^{-1}) \quad (2)$$

Where R_D is Daphnia's oxygen consumption, Start O_2 is mean start value O_2, End O_2 is end value O_2 in syringe with Daphnia, Time is time in water bath and R_M is mean value of microbial consumption. I took pictures of each individual and measured their body length (top

of the eye to the base of caudal spine). Body mass, *M* was calculated from body lengths, *L* using the equation from Gorokhova et al. 2002:

$$M(g) = 0.000007 * L\ (mm)^{2.23} \quad (3)$$

Mass-specific oxygen consumption, *MR* of *D. magna* was calculated as:

$$MR\ (mg\ O_2\ h^{-1}\ g^{-1}) = R_D\ (mg\ O_2\ min^{-1}) \times 60 / M(g) \quad (4)$$

Mean mass-specific oxygen consumption, *MR* of *D. magna* was calculated as mean value of all *Daphnia* oxygen consumption (animals originating from all 3 climate rooms) at a given experiment temperature.

Activity experiment

In April 2013 I measured *D. magna* activity levels at different experimental temperatures in replicates from the same line of Clone 10. The animals in this experiment were 1.5 – 3.0 mm long and the mean lengths were similar at all experimental temperatures: 10 °C experiment: 2.28 mm, 17 °C experiment: 2.18 mm, 17 °C experiment: 2.24 mm. Prior to the measurements, the individuals were acclimated to their test temperatures for approximately 1 - 2 days (18 – 44 h) in the absence of food. I measured activity in an 11.5 x 7.5 cm plastic container filled with 2 dl COMBO. At the bottom of the container 16 equally sized squares were marked. Individual *Daphnia* were transferred to the container and upon release observations started. Activity was measured as the number of movements from one square to another during a 5 min period. At the end of the experiment I measured the length of each animal in the same way as described above.

Data processing and statistical analyses

All statistical analyses were conducted using the statistical software R, version 2.15.1. (The R Foundation for Statistical Computing, 2012). The analyses were carried out as a linear mixed model approach (*lme*), using the *nlme* package (Pinheiro et al. 2009).

Oxygen consumption experiment

I modelled *Daphnia* oxygen consumption (R_D) as a function of mass (M), experimental temperature (T) and clone rearing temperature (C) as main effects. In addition, I considered two interaction terms in the full model: interaction between experimental temperature and clone rearing temperature and interaction between mass and experimental temperature. Variation among lines was entered as a random effect (a_i). Body mass values were centered to provide more meaningful intercept estimates. Thus the full model is a random intercept mixed effects model and can be represented as:

$$R_{Di} = \alpha + \beta_1 \times T + \beta_2 \times C + \beta_3 \times M + \beta_4 \times T \times C + \beta_5 \times M \times T + a_i + \varepsilon \quad (5)$$

Residuals from this model suggested a higher variance at the experimental temperature of 17 °C. This was confirmed by comparing models with and without a function for modelling of variance structure, using likelihood ratio tests (calculated based on restricted maximum likelihood, REML and compared with ANOVA, Zuur et al. 2009), and the model with a varIdent variance structure was best ($p < 0.001$). Thus all models in the subsequent model selection procedure contained this variance structure. Assumptions of normality and homogeneity of residuals were satisfied. I then tested for random effects of line. I compared two models with and without the random effects of line, fitted using the linear mixed-effects model function *lme*, and the generalized least squares function *gls*. For comparison of these models I used likelihood ratio tests, as above. Log-likelihood comparisons of models with and without the random effect (using restricted maximum likelihood, REML) and with different fixed effects (using maximum likelihood, ML) were performed using a backward selection procedure according to Zuur et al. (2009).

Activity experiment

I modelled activity as a function of body length *(L)* and experimental temperature *(T)*. In addition, I considered the interaction term between length and temperature in the full model. Thus, the full model of activity can be represented as:

$$Activity = \alpha + \beta_1 \times T + \beta_2 \times L + \beta_3 \times T \times L + \varepsilon \quad (6)$$

The models were implemented using the generalised least squares method, *gls*. Residuals from this model suggested differences between variance at different experimental temperatures and animal lengths. Variance increased with experimental temperature and animal length.

I compared a model with combination of VarIdent structure of experimental temperature and VarExp structure of animal length, with a model with only VarIdent structure of experimental temperature, using likelihood ratio tests (calculated based on restricted maximum likelihood, REML and compared with ANOVA, Zuur et al. 2009), and I did not find significant differences between the models ($p = 0.181$). The model with only varIdent variance structure is easier and best. Thus all models in the subsequent model selection procedure contained this variance structure. Assumptions of normality and homogeneity of residuals were satisfied. I compared different main effects structures. This was done using a backwards selection procedure, according to Zuur et al. (2009).

Results

Oxygen consumption experiment

Mean mass-specific oxygen consumption of *D. magna* (Table 2) was highest at the experimental temperature of 17 °C and lowest at 10 °C. Oxygen consumption was at an intermediate level at 25 °C.

Table 2: Mean mass-specific oxygen consumption (mg $h^{-1}g^{-1}$) of *D. magna* (± SD) for different combinations of experimental temperatures and clone rearing temperatures, with number of individuals (n).

Clone rearing temperature	*Experimental temperature*		
	10 °C	**17 °C**	**25 °C**
Clone 10	8.00 (±2.39) (n = 13)	23.2 (±7.30) (n = 11)	13.5 (±10.30) (n = 11)
Clone 17	8.17 (±5.37) (n = 7)	22.0 (± 8.94) (n = 7)	6.45 (±1.74) (n = 11)
Clone 25	5.26 (±3.36) (n = 7)	14.4 (±9.25) (n = 10)	8.83 (±3.11) (n = 4)
Average pr. treatment	7.34 (±3.79) (n = 27)	19.75 (± 9.09) (n = 28)	9.78 (±7.47) (n = 26)

The random effect of line was significant ($p = 0.035$). The full model gave the best fit. Experimental temperature, clone rearing temperature and body mass of the animal all had a significant effect on oxygen consumption (Tab. 3). The interaction between centred mass and experimental temperature could not be removed, without causing a significant decrease in log-likelihood ($p = 0.027$), nor could the interaction between experimental temperature and clone rearing temperature ($p < 0.001$).

Body mass had the biggest effect on oxygen consumption at the experimental temperature of 17 °C, and the lowest effect at 10 °C (Fig. 2), as shown by the interaction term between body mass and experimental temperature (Table 3). *Daphnia* reared at 10 °C (Clone 10) had the highest oxygen consumption at all experimental temperatures (Fig. 2). The interaction between experimental temperature and the clone rearing temperature was therefore primarily due to a change in relative consumption of Clone 17 and Clone 25 across experimental temperatures. Specifically, at 10 °C oxygen consumption was approximately at same level for animals originating from Clone 17 and Clone 25, at 17 °C oxygen consumption was higher for animals from Clone 17 than for animals from Clone 25 and at 25 °C oxygen consumption was higher for animals from Clone 25 than for animals from Clone 17.

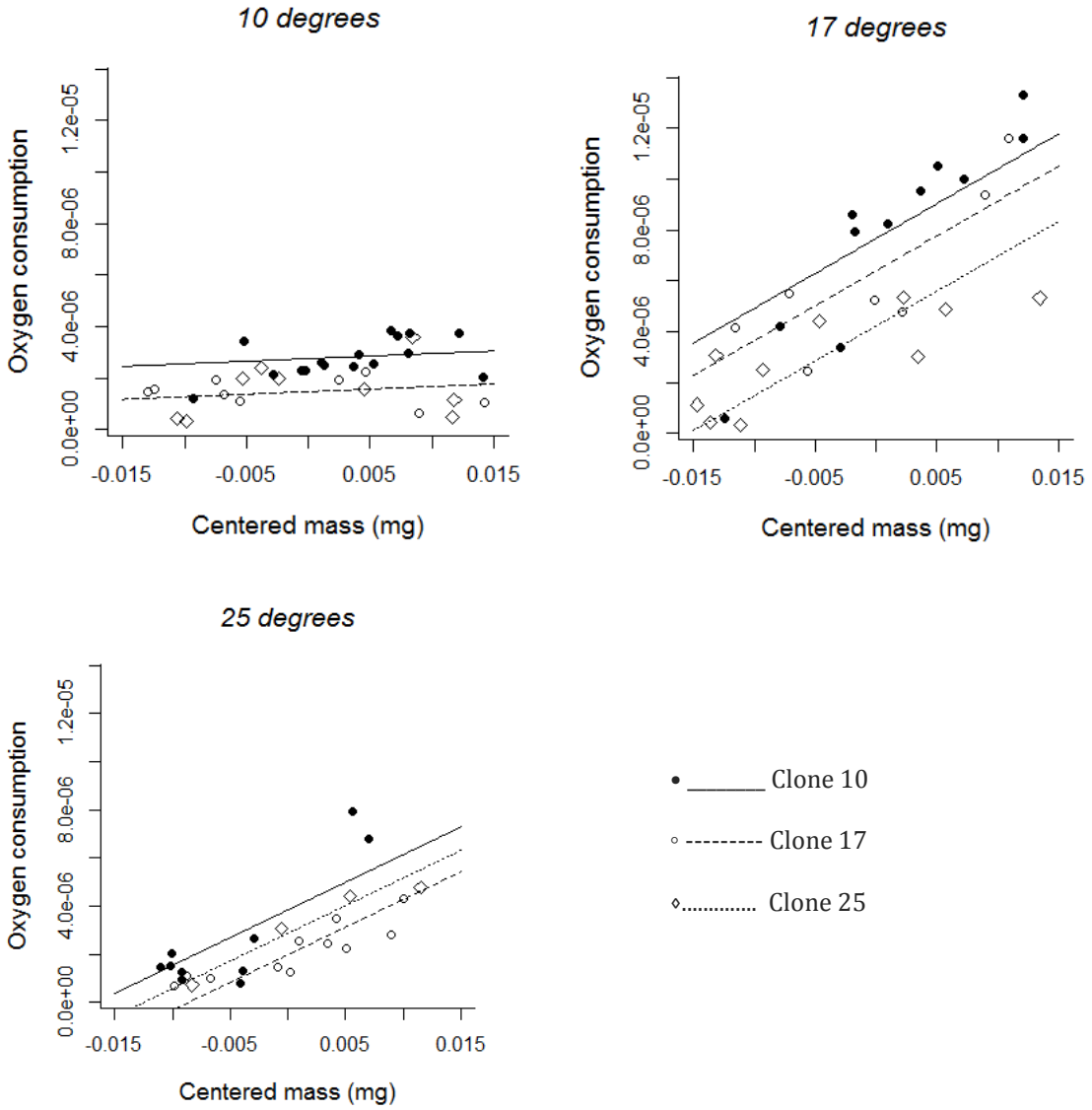

Fig. 2: Centred mass (mg) of *Daphnia magna* plotted against oxygen consumption (mg $min^{-1}mg^{-1}$) for experiment temperatures of 10, 17 and 25 °C, for animals from Clone 10 (filled circles), Clone 17 (open circles) and Clone 25 (open squares). Lines represent fitted model value from final model (Table 3), for animals from Clone 10 (solid line), Clone 17 (stippled line) and Clone 25 (dotted line).

Table 3: Parameter estimates for the final model explaining variation in oxygen consumption of *D. magna* with variation in experimental temperature, Exp. t. (°C), clone rearing temperature, Clone (°C), centered mass, Cent. mass and interactions between them.

Variable	*Parameter estimate*	*Std. error*	*T*	*P*
Intercept	2.75×10^{-6}	2.67×10^{-7}	10.29	0.0000
Exp. t. 17	4.91×10^{-6}	5.5×10^{-7}	8.92	0.0000
Exp. t. 25	1.08×10^{-6}	4.27×10^{-7}	2.53	0.0138
Clone 17	-1.28×10^{-6}	4.03×10^{-7}	-3.18	0.0098
Clone 25	-1.28×10^{-6}	4.33×10^{-7}	-2.96	0.0143
Cent. mass	1.98×10^{-5}	1.73×10^{-5}	1.14	0.2564
Exp. t. 17 x Clone 17	1.15×10^{-8}	8.91×10^{-7}	0.01	0.9897

Exp. t. 25 x Clone 17	-5.71×10^{-7}	5.94×10^{-7}	-0.96	0.3403
Exp. t. 17 x Clone 25	-2.15×10^{-6}	8.38×10^{-7}	-2.58	0.0123
Exp. t. 25 x Clone 25	3.23×10^{-7}	7.63×10^{-7}	0.42	0.6733
Exp. t. 17 x Cent. mass	2.54×10^{-4}	4.50×10^{-5}	5.67	0.0000
Exp. t. 25 x Cent. mass	2.10×10^{-4}	3.99×10^{-5}	5.28	0.0000

Activity experiment

The final model of activity shows that activity increases with temperature and animal length. The interaction between experimental temperature and body length of the animal could be removed without causing a significant decrease in log-likehoods ($p = 0.812$). In the analysis of activity, the main effects of experimental temperature and body length could not be removed ($p < 0.001$ for both). *Daphnia* were most active at 25 °C and least active at 10 °C (Table 4, Fig. 3). Big animals were more active than small ones at all three experimental temperatures (Fig. 3). Activity levels vary a lot between individuals, especially between adults (> 2 mm) at high temperatures.

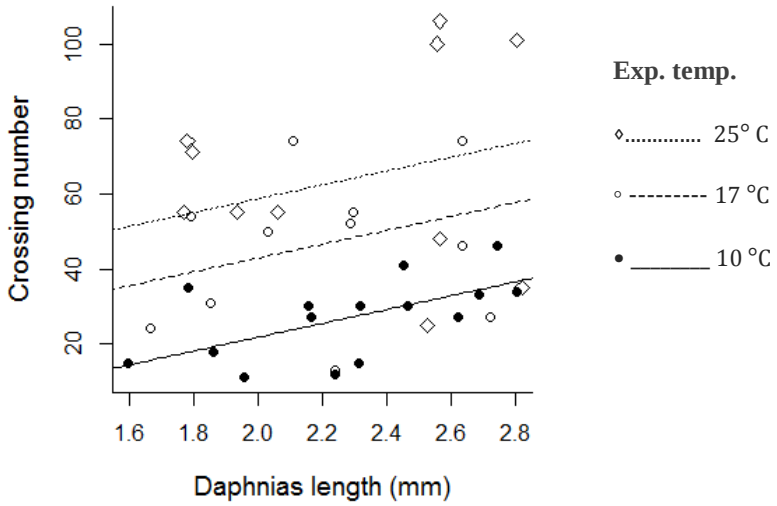

Fig. 3: Estimated activity of Daphnia with different body lengths at different experimental temperatures, measured as number of crossing from square to square in the container in a 5 min period. Circles and squares representing animals at 10°C (filled circles), 17 °C (open circles) and 25 °C (open squares). Lines represent fitted model value from final model (Table 3), for experimental temperatures of 10°C (solid line), 17 °C (stippled line) and 17 °C (dotted line). All animals were from Clone 10.

Table 4: Parameter estimates for final model explaining variation in activity of *D. magna* with variation in experimental temperature (°C) and animal body length (mm). Activity was measured as number of crossing from square to square in an 11.5 x 7.5 cm container in a 5 min period.

Variable	*Parameter estimate*	*Std. error*	*T*	*P*
Intercept	-15.08	12.43	-1.21	0.2326
Body length	18.43	5.36	3.44	0.0014
Activity at exp. t. 17 C	21.13	5.67	3.73	0.0006
Activity at exp. t. 25 C	36.96	7.87	4.70	0.0000

Discussion

The purpose of this study was to test for metabolic cold adaptation (MCA) in *Daphnia magna*, in an oxygen consumption laboratory experiment. The study provided evidence for adjustment of metabolism in *Daphnia magna* clones reared at three different thermal environments, over multiple generations. Possible adaptation to colder environment of the animals reared at a low temperature (10 °C) was illustrated by a higher unfed metabolism at all three experimental temperatures (10, 17 and 25 °C) compared with animals reared at higher temperatures (17 and 25 °C). These results are consistent with the metabolic cold adaptation hypothesis, indicating elevated metabolism in animals from cold environments. However, the present study does not provide conclusive evidence that clone-specific variation in thermal performance has a genetic basis. Organisms may adjust their thermal reaction norms as a response to the thermal regime in which they live through three mechanisms: acclimation, epigenetic effects and evolution. The present design could not separate between these mechanisms.

Thermal acclimation may enable individuals to change their thermal reaction norm throughout their life. This may be a particularly important adaptive mechanism for organisms that experience fine grained environmental heterogeneity. Acclimation during early stages of development is harder to reverse than acclimation made later in life (Angilletta 2009). Temperature acclimation has been shown to affect the thermal preference of *Daphnia* (Lagerspetz 2000; Simčič and Brancelj 2004). In my study, prior to measurements of oxygen consumption, the individuals were allowed to acclimate to their test temperatures for 20–26 hours. The purpose was to remove effects of temperature experienced earlier in their life. Because of the *Daphnia*`s short lifecycle and early egg development, I had to limit the acclimation period under the present experimental design. Thus, the different temperatures experienced earlier in life could have affected thermal performance of *Daphnia* in my study, as a pure phenotypic plasticity response.

Epigenetic effects are generalisations of more widely studied maternal effects and occur across one or a few generations. Epigenetic effects occur whenever environmental conditions experienced by parents before fertilisation result in a modification of the offspring, without modification of the gene sequence (Salinas and Munch, 2012). Additionally, as epigenetic changes can be adaptive, epigenetic information can be passed down between generations, which is called epigenetic inheritance (Vandegehuchte and Janssen, 2011). Epigenetic effects can influence thermal performance of offspring (Crill 1996). Maternal temperature conditions have earlier been shown to affect the size of eggs and offspring in cladocerans (Perrin 1988). This can in turn also affect important life history characteristics such as age and size at maturity (Ebert 1991). Epigenic effects has been demonstrated in *Daphnia* (Alekseev and Lampert 2001), and also the occurrence of DNA methylation (Vandegehuchte et al. 2009). The various epigenetic effects can interact (Alekseev and Lampert 2001). The complex set of environmental conditions of the previous generation may thus affect the performance of the next. It is therefore plausible that the different temperatures experienced by parents or grandparents could have influenced thermal performance of *Daphnia* in my study.

Evolutionary responses in thermal reaction norms may be expected under consistent differences in temperatures (Mitchell and Lampert 2000; Pinkhaus et al. 2007). *Daphnia* clones in my experiment were reared at three different temperature conditions for 12 – 35 generations. Thermal adaptation is an evolutionary change in genotype, as a consequence of thermal selection, that confers higher fitness under a certain temperature regime (Mitchell and

Lampert 2000). Carvalho and Crisp (1987) found changes in the clonal composition of a *Daphnia* population during the season. Summer clones had greater fitness at higher temperatures while winter clones performed better at lower temperatures (Carvalho and Crisp 1987; Pinkhaus et al. 2007). Thus, it seems clear that there is genetic variation in relative fitness across temperatures. In my study, individuals reproducing first within a line were used to initiate the next generation, thereby inducing strong selection for early maturation. Such divergent selection based on intraclonal variation has previously been shown to result in rapid evolution in clonal lineages of *Daphnia* (Gorokhova et al. 2002; Omilian et al. 2006). For example, Gorkhova et al. (2002) found that under divergent selection regimes, mean *Daphnia* growth rates, RNA content and P content evolved to differ significantly within only five generations. Thus, thermal selection during multiple generations could have caused evolutionary responses in thermal performance of *Daphnia* in my study.

Counter-gradient variation in metabolism, allowing partial compensation to adverse temperature conditions, has been documented in some previous studies of insects and ectotherms (Addo-Bediako et al. 2002, Sommer and Pörtner 2002). Effect of different temperature conditions on metabolism has also been documented in *Daphnia*. For example, Choplet et al. (2008) focused on effect of temperature to metabolism in populations of *Daphnia*, collected at different locations and found difference in metabolism between subarctic and temperate populations. However, one issue with such comparative studies is that they do not control for potential correlations between temperature and other environmental factors (e.g. food abundance, population density) which may also influence metabolic rates. For *Daphnia*, one previous study (Lamkemeyer et al. 2003) tested for effects of acclimation temperature by rearing individuals at different temperatures for duration of 2 weeks or more prior to testing. It is not clear whether this experiment included multiple generations of rearing (i.e. causing potential epigenetic or evolutionary effects), the sample sizes were very low (4 individuals at each temperature), and the results were mixed with respect to effects on metabolism. Thus, to my knowledge my study is the first one focusing on changes of metabolic rate within one population reared at different temperatures under controlled laboratory conditions over multiple generations. Environmental factors such as freshwater medium, photoperiod, food and density of individuals were standardized, enabling me to isolate the effect of rearing temperature.

A surprising result in my study was that mass-specific oxygen consumption was highest at the experimental temperature of 17 °C for all three clones. Standard metabolism is expected to increase monotonically with increasing temperature within their viable temperature range (Gillooly 2001). However, in the present study I did not measure standard metabolism. Mass-specific respiration rate increases with activity (Glazier 1991; Simčič and Brancelj 1997; Paul et al. 1997). One potential explanation could be highest activity at 17 °C. However, my activity measurements showed that *Daphnia* were most active at 25 °C and the least active at 10 °C, with activity being intermediate at 17 °C. Animal density and food concentration can also influence respiration rate (Lampert 1986). Another explanation for highest oxygen consumption at 17 °C in my study could be that digestion of food (specific dynamic action) during measurements was higher at 17 °C, compared with 25 °C. Since digestion is slower at cold temperatures, more food may have remained in the stomach after acclimation period at 17 °C, than at 25 °C. Some of *Daphnia* in my 10 °C experiment had visible green algae in their digestion system after the acclimation period. Choplet et al. (2008) found that oxygen consumption rates were on average twice for fed *Daphnia*, compared to starved ones. Thus, if digestion metabolism occurred at 17 °C treatment, but not at the 25 °C treatment, this may

explain the observation. Alternatively, a bias in measurements of unknown source may be responsible.

Although the exact mechanism could not be determined, the present study provides evidence for adjustment of metabolism in *Daphnia magna* clones reared at three different thermal environments for one year. Possible adaptation (genetic or non-genetic) to colder environment of animals reared at 10 °C was illustrated by a higher unfed metabolism at all three experimental temperatures, compared with animals reared at temperatures of 17 and 25 °C. Elevated metabolism in animals from a cold environment is consistent with the metabolic cold adaptation hypothesis. The present study does however not provide conclusive evidence that clone-specific variation in thermal performance has a genetic basis. Organisms may adjust their thermal reaction norms as a response to the thermal regime in which they live through three mechanisms: acclimation, epigenetic effects and evolution. Future studies should evaluate the relative importance of these three mechanisms.

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