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Heat tolerance and its evolutionary potential along a latitudinal gradient in *Daphnia magna*

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ABSTRACT

Background: Extreme weather events follow latitudinal patterns and are increasing due to climate change. To persist, natural populations must adapt to the increased occurrence of heat waves.

Hypothesis: Heat tolerance is higher at lower latitudes and in smaller animals. Animals show evolutionary potential for heat tolerance.

Organism: The water flea, *Daphnia magna*, a common species in bodies of fresh water.

Methods: Set up a common-garden experiment using six populations spread across three latitudes in Western Europe, thereby creating a latitudinal gradient in local temperatures. Measure $CT_{\max}$, the temperature above which animals lose motor function.

Results: Thermal tolerance increased with increasing local temperatures. Body size, which was smaller at the southern latitude, negatively affected heat tolerance. Heat tolerance showed genetic variation within latitudes.

Conclusions: *Daphnia magna* possesses the evolutionary potential to increase its heat tolerance if temperatures warm. Its heat tolerance shows latitudinal adaptation to local temperatures. A parallel pattern in body size magnifies the latitudinal pattern in heat tolerance.

Keywords: $CT_{\max}$, *Daphnia magna*, evolutionary potential, latitude, thermal adaptation.

INTRODUCTION

The frequency of extreme climate events, such as heat waves, has increased significantly in the past decades due to climate change (IPCC, 2013). Extreme temperatures have a marked impact on organisms, driving them to their physiological boundaries, thereby influencing survival and other fitness components (Huey *et al.*, 2009). Given that many ectotherms have their optimum fitness at temperatures that are quite close to their upper thermal limit, the ability to respond and adapt to heat waves is essential for the local persistence of populations (Kearney *et al.*, 2009; Sinervo *et al.*, 2010; Moritz *et al.*, 2012). This has generated a surge in studies focusing on upper thermal tolerances (Hoffmann *et al.*, 2003; Huey *et al.*, 2012). Yet, our understanding of the evolutionary potential for heat tolerance is still limited and most studies are confined to terrestrial ectotherms (Hoffmann *et al.*, 2013).

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Given that mean temperatures as well as temperature extremes show latitudinal patterns (Dell *et al.*, 2011; IPCC, 2013), latitudinal gradients can provide ideal natural settings to detect past signatures of thermal evolution, including adaptation to temperature extremes (Angilletta, 2009). Despite the general pattern that upper thermal limits show little geographic variation (Addo-Bediako *et al.*, 2000), intraspecific latitudinal differences in upper thermal tolerance have been documented in many terrestrial ectotherms (Hoffmann *et al.*, 2002; Deutsch *et al.*, 2008; Overgaard *et al.*, 2011; Moritz *et al.*, 2012). In contrast, latitudinal studies on this topic in aquatic ectotherms are rare and evidence for genetic clines in thermal tolerance is limited (Stoks *et al.*, 2014; but see Yampolsky *et al.*, 2014). Despite the buffering of thermal extremes in aquatic systems, marked increases in temperature do occur (Adrian *et al.*, 2009) and may strongly impact aquatic ectotherms (Huber *et al.*, 2010; Rüdiger *et al.*, 2012). Animals that face less fluctuating temperatures are expected to show lower upper thermal tolerance limits (Peck *et al.*, 2014), which may make aquatic ectotherms particularly vulnerable to heat waves and their predicted increase in frequency under climate change (Forster *et al.*, 2012).

In the context of latitudinal clines in heat tolerance, body size is an important trait as it shows latitudinal patterns (Blanckenhorn and Demont, 2004), shapes heat tolerance, and is expected to respond to global warming (Daufresne *et al.*, 2009; Gardner *et al.*, 2011). Many ectotherms display so-called Bergmann clines with smaller-bodied individuals at warmer southern latitudes (Atkinson, 1994; Blanckenhorn and Demont, 2004). This may be adaptive with respect to the higher frequency of heat waves at southern latitudes. Indeed, smaller ectotherms are generally better able to cope with the increase in metabolic rate associated with increases in temperature because their smaller body size enables a more efficient transport of oxygen to the tissues (Gardner *et al.*, 2011; Forster *et al.*, 2012). Despite this relation, studies on latitudinal patterns in heat tolerance often ignore body size (but see Angilletta *et al.*, 2004; Sheridan and Bickford, 2011).

Here, we study upper thermal tolerance in a set of 36 *Daphnia magna* clones from replicated populations from three latitudes in Western Europe. Using a common garden experiment we assess current thermal adaptation to the different latitudes, reflecting past thermal evolution, as well as evolutionary potential for future thermal evolution in response to global warming. To relate thermal tolerance to body size, we also measure size at maturity in all studied clones. As a measure of heat tolerance we use $CT_{\max}$, the temperature above which animals lose motor function, in a ramping assay where temperatures are increased experimentally (Overgaard *et al.*, 2012). $CT_{\max}$ has been shown to be informative of climate change responses in several species (Chown *et al.*, 2010; Huey *et al.*, 2012). Because latitude may be a poor proxy of local thermal regimes, we explicitly tested for an effect of local temperatures on $CT_{\max}$ (Yampolsky *et al.*, 2014).

MATERIALS AND METHODS

The large-bodied cladoceran *Daphnia magna* plays a central role in lake and pond ecosystems as a main grazer of phytoplankton and preferred prey of fish and large invertebrates (Miner *et al.*, 2012). Key assets of *D. magna* include its life cycle features, such as its short generation time, the formation of clonal lineages, and production of dormant eggs that accumulate in the sediment of lakes. This set of characteristics allows for a strong test of genetic responses to changing selection pressures imposed by temperature along a latitudinal gradient.

We studied two populations at each of three latitudes: southern, central, and northern Europe. At the southern latitude we investigated two French populations in the Camargue

(Cerisière des faïsses, C9 and Picadelly, C18), at the central latitude we studied two populations in Central Belgium (Oude Meren 2, OM2 and Langerode vijver, LRV), and at the northern latitude we studied one population in Denmark (Lake Ring, LR) and one population in Sweden (Lake Bysjon, BSW; Table 1). The distance between the southern and central populations is ~820 km, while the distance between the central and northern populations is ~960 km. Sediment from each of these locations was collected and kept in the dark at 4°C. To hatch the resting eggs, we exposed them to the following hatching conditions: 20°C, fresh medium (dechlorinated tap water), and light with a photoperiod of 14/10 h light/dark (De Meester and De Jager, 1993). Six randomly selected clones per population (12 clones per latitude) were kept under standardized laboratory conditions until the start of the experiment.

Four cultures (sets of 6–10 adults in 500-mL jars) were started for each clone. This resulted in 144 experimental units (4 independent replicates per clone × 6 clones per population × 2 populations × 3 latitudes). To minimize interference from maternal effects, the experimental animals were grown for two generations under standardized conditions: in a temperature-controlled water bath system (20°C), at a long day photoperiod (14/10 h light/dark), at a density of 6–10 individuals in 500 mL, and fed *Scenedesmus obliquus* daily at a concentration of 1×10^5 cells per millilitre. Each generation was started from second-brood offspring. Twenty-four hours after individuals reached the first adult stage (i.e. giving them enough time to recover from moulting), we measured adult body size for three random individuals from each jar under a stereomicroscope at a magnitude of 20×. This was done because thermal tolerance can be size-dependent (Angilletta *et al.*, 2004; A. Geerts, personal observation). In addition, we analysed thermal tolerance, measured as $CT_{\max}$ (see below). The $CT_{\max}$-values of all adults in one jar were measured in the same run, and were averaged for statistical analysis. Thus, each clonal replicate in our statistical analysis consists of the averaged $CT_{\max}$-values over all individuals that were cultured together in one jar.

Quantification of $CT_{\max}$

As our measure of heat tolerance we used $CT_{\max}$, the upper thermal limit at which motor functions is compromised and the organism faints (Huey *et al.*, 1992; Lutterschmidt and Hutchison, 1997). In *D. magna*, this is an easily quantifiable trait because the animal stops swimming and sinks to the bottom. Measurement of $CT_{\max}$ was performed in a 4×6 thermal heater (Peqlab Digital Block Heater HX-2). The experimental animals, in their first adult instar, were individually placed in a 0.5-mL Eppendorf tube where their activity could be observed continuously. The tubes were randomly placed in the thermal heater. The starting temperature was 20°C, and the temperature was gradually increased from 20°C to 45°C in 1°C steps every 20 ± 5 seconds. Once the water fleas stopped moving, they were transferred to ambient conditions (20°C) to recover. We chose a fast ramping method to avoid confounding factors such as hardening, and to test for baseline differences in thermal tolerance between these populations (Ribeiro *et al.*, 2012). $CT_{\max}$ was measured in separate runs for each of the four replicates of each clone over a period of two months. A total of 1030 individuals were scored for $CT_{\max}$ in this study.

Table 1. Characteristics of the six shallow lakes that hosted the studied populations

Lake	Code	Latitude, N	Longitude, E	Surface area (m^2)	Mean depth (m)	Mean maximum summer temperature (°C)	Year of sediment sampling	Year of hatching
Southern Europe								
Cerisière des faïsses	C9	43°29′31.39″	4°38′43.58″	7 954	0.33	30.01	2007	2013
Picadelly	C18	43°29′33.18″	4°40′55.21″	4 151	0.13	30.01	2007	2013
Central Europe								
Langerode vijver	LRV	50°49′42.08″	4°38′20.60″	140 000	1.20	22.77	2007	2008
Oude Meren 2	OM2	50°50′39.96″	4°43′05.16″	32 000	1.50	22.77	2007	2008
Northern Europe								
Lake Ring	LR	55°57′51.83″	9°35′46.87″	225 000	2.90	21.29	2005	2011
Lake Bysjon	BSW	57°30′37.09″	16°03′56.00″	200 000	3.60	19.78	2009	2009

Statistical analyses

All statistical analyses were done using the PROC MIXED procedure implemented in SAS v.9.3 (SAS Institute Inc., Cary, NC, USA). We chose the maximum likelihood (ML) estimation method for our models. This procedure is well suited for mixed models with nested designs. The variance component option was chosen for the covariance structure. For inference concerning the random variables we used the Wald Z-statistic. For fixed effects, significance was tested using approximate F-tests. Degrees of freedom were estimated using the Kenward-Roger method.

Differences between populations in size at maturity were tested using a general linear model with latitude and population nested in latitude as categorical fixed factors and clone nested in population as a random categorical factor.

To explicitly evaluate whether the latitudinal pattern in $CT_{\max}$ was associated with differences in local temperatures as documented by Yampolsky *et al.* (2014), we used temperature records from weather stations nearest to the six populations (National Climatic Data Centre). Based on these data, we calculated the average maximum summer air temperature (as in Yampolsky *et al.*, 2014). We only took into account the summer months (June–August) and calculated averages across the period 2000 to 2005. We tested for an effect of the average maximum summer temperature on $CT_{\max}$ by including it as a continuous variable. In the model, we also included clone as a random categorical factor, nested in population (fixed categorical factor). We additionally corrected for size by including it as a continuous fixed variable. Since thermal tolerance in *D. magna* was shown to increase with increasing local air temperatures (Yampolsky *et al.*, 2014), we tested for the temperature effect using a one-sided P-value.

We tested for evolutionary potential in $CT_{\max}$ at each latitude by evaluating clonal differences in $CT_{\max}$ within the set of 12 clones of the two populations sampled per latitude group. As we only tested six clones per population, no calculations for evolutionary potential were done at the population level. In addition, a latitudinal perspective is relevant as populations at the same latitude experience similar thermal regimes. In this model, we included latitude as a fixed categorical variable; clone nested in latitude was added as a random categorical variable. We corrected for size at maturity by including it as a fixed continuous variable.

RESULTS

Size at maturity differed significantly between latitudes ($F_{2,137} = 4.75$, $P = 0.010$) with on average larger adults in the north (Fig. 1A).

Our results showed that $CT_{\max}$ decreased with decreasing local temperatures ($t_{31.6} = 1.78$, one-sided $P = 0.043$; slope ± S.E.: -1.05 ± 0.23, Fig. 1B). $CT_{\max}$-values were higher in smaller adults ($F_{1,135} = 20.05$, $P < 0.0001$; slope ± S.E.: -1.05 ± 0.23). There was genetic variation for $CT_{\max}$ within latitude groups as indicated by the significant effect of clone nested in latitude ($Z = 2.02$, $P = 0.022$).

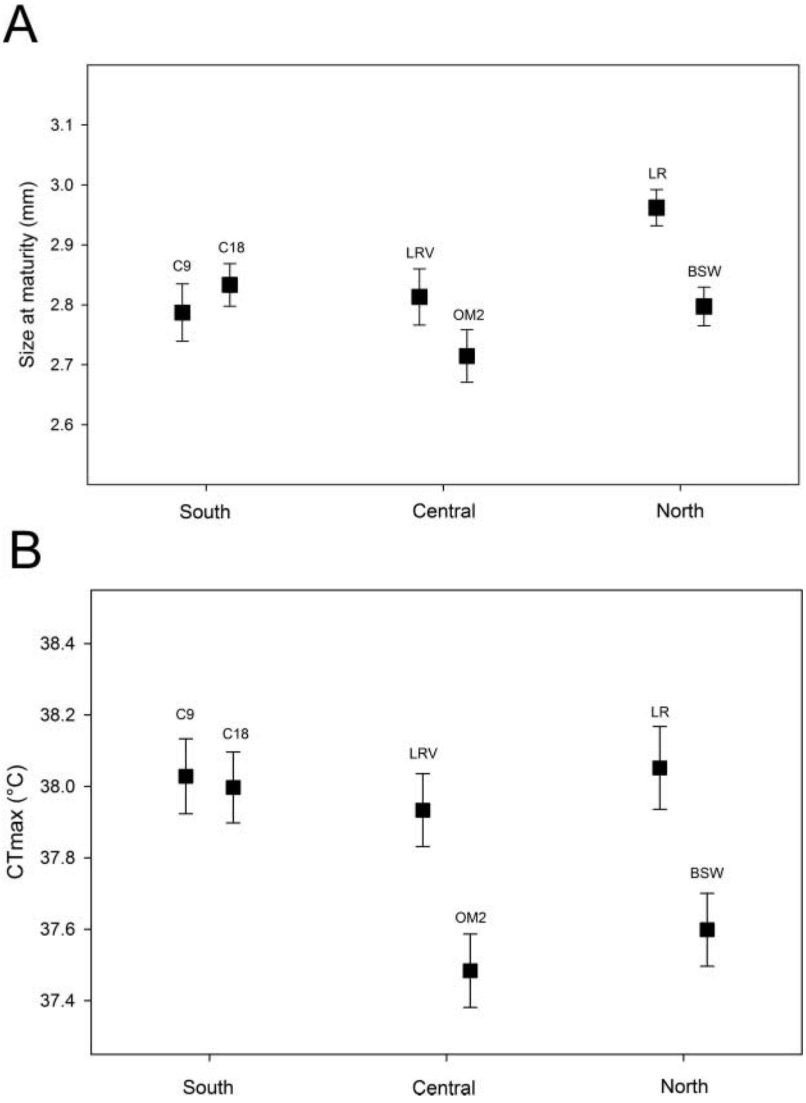

Fig. 1. Body size (A) and CT_{max}-values (B) by population for the three latitude groups. Population means ± one standard error are shown. Codes above symbols represent populations (see Table 1).

DISCUSSION

Given that populations often experience strong temperature fluctuations that may shape survival and other fitness components (Angilletta, 2009), assessing the impact of temperature extremes is very important. This is especially timely given the increased occurrence of heat waves related to climate change (IPCC, 2013). Our results indicate that populations from southern latitudes are adapted to the typically higher summer temperatures, and can be expected to withstand extreme temperatures better than populations from northern latitudes. Moreover, by studying sets of clones per latitude we were able to document genetic variation in upper thermal tolerance suggesting that natural populations of *D. magna* harbour evolutionary potential to respond genetically to increasing temperatures. It should also be noted, however, that while we did observe a latitudinal cline in $CT_{\max}$-values, the range in these values was much smaller than the range in average maximum summer temperatures that occurs along the studied latitudinal gradient.

There is ongoing debate on how to assess upper thermal tolerance, as different measures may show different patterns when comparing populations (e.g. van Heerwaarden *et al.*, 2012). Ramping assays using $CT_{\max}$ have become a popular method of estimating upper thermal tolerance (Deutsch *et al.*, 2008; Clusella-Trullas *et al.*, 2011). Compared with static assays that measure the time until knockdown using a constant stressful temperature, ramping assays are thought to be both a valid and ecologically relevant way to measure thermal resistance (Terblanche *et al.*, 2011; Overgaard *et al.*, 2012). Results obtained using ramping assays may, however, depend on the warming rates adopted (Chown *et al.*, 2009; Mitchell and Hoffmann, 2010; Sørensen *et al.*, 2013). In general, slow and presumably ecologically more relevant rates of warming negatively affect the upper thermal limit ($CT_{\max}$) (Rezende *et al.*, 2011). Yet, several researchers have advocated the use of short assays (implying fast warming rates), as these reduce interference from the starting conditions of the animals and short-term acclimatory responses (Santos *et al.*, 2011; Terblanche *et al.*, 2011). We opted for a fast ramping rate in our experiment to minimize acclimation effects and interference from the accumulation of heat stress through time. The pattern of changes in $CT_{\max}$ along the latitudinal gradient observed here is in line with that of a similar study on *D. magna* that used a static assay (Yampolsky *et al.*, 2014), confirming the latitudinal pattern in heat tolerance.

Despite the general pattern that upper thermal limits show little geographic variation (Addo-Bediako *et al.*, 2000), intraspecific latitudinal differences in upper thermal tolerance have been documented in many terrestrial species (Kellermann *et al.*, 2012; Moritz *et al.*, 2012). In contrast, few latitudinal studies on this topic in aquatic invertebrates have been performed and evidence for genetic clines in thermal tolerance is limited (Stoks *et al.*, 2014). In the mosquito *Wyeomyia smithii*, thermal tolerance measured as heat knockdown time did not show a latitudinal pattern (Ragland and Kingsolver, 2008). In the isopod *Asellus aquaticus*, heat tolerance was found to be higher in a southern than in a northern population, although maternal effects were not controlled for in this study (di Lascio *et al.*, 2011). In the water flea *D. pulex*, clones isolated from lower latitudes (Indiana, USA) showed a higher heat tolerance than clones isolated from more northerly latitudes (Ontario, USA) when tested at low and intermediate – but not at high – acclimation temperatures (Williams *et al.*, 2012). The strongest evidence for a latitudinal cline in heat tolerance in an aquatic invertebrate comes from the study of Yampolsky *et al.* (2014), who found that the time until immobilization at 37°C in *D. magna* clones spanning a range from South Africa to near arctic sites correlated positively with the local summer temperatures at the clones' sites of origin, indicating local thermal adaptation. Our study confirms these findings using *D. magna* clones spanning three latitudes within Europe, with multiple clones isolated from each of two populations at each latitude, and using a ramping assay.

The latitudinal adaptation in heat tolerance based on size-corrected $CT_{\max}$-values seems to be enhanced by the latitudinal pattern in body size. Consistent with Bergmann clines where colder climates harbour populations with larger body sizes (Atkinson, 1994; Blanckenhorn and Demont, 2004), southern clones tended to be smaller at maturity than northern clones. Moreover, as expected, smaller clones showed a higher heat tolerance (Gardner *et al.*, 2011; Forster *et al.*, 2012). Our observation that southern populations are characterized by a smaller body size is in line with other studies. For example, Van Doorslaer *et al.* (2010) showed that the body size of *D. magna* and *D. pulex* decreased significantly within one growing season when exposed to increased temperatures (ambient + 4°C) under semi-natural conditions (see also Sheridan and Bickford, 2011). This response is expected because temperature increases metabolic rate and developmental rate (Gillooly *et al.*, 2001; Bickford *et al.*, 2010), and smaller organisms are generally better able to cope with lower oxygen levels than larger organisms (Forster *et al.*, 2012). The smaller body sizes and higher size-corrected heat tolerances together determine the higher heat tolerance of populations at southern latitudes.

The thermal adaptation based on upper thermal tolerance along a latitudinal gradient in *D. magna*, as observed by us and by Yampolsky *et al.* (2014), contrasts with other studies showing no signals of thermal adaptation in life-history traits in this species. In a detailed study, Mitchell and Lampert (2000) showed that there is no adaptation to local temperatures as measured by somatic growth rate for eight European populations, despite substantial genetic variation in thermal reaction norms within populations. A similar study by Chopelet and colleagues (2008) revealed no differences in adult and juvenile growth rates between subarctic and temperate *D. magna* populations, but did find significant genetic variation within populations. This fits the general pattern that short-term exposure measurements (lethal temperatures, knockout times) typically correlate better with the local climate than long-term measurements such as thermal optima (Angilletta, 2009; Yampolsky *et al.*, 2014). This may partly be due to the fact that studies testing for patterns on long-term measurements typically have used latitude as a proxy for temperature (Stoks *et al.*, 2014) rather than more direct estimates of local thermal regimes (Yampolsky *et al.*, 2014). This is further suggested by studies directly manipulating temperature that showed rapid experimental thermal evolution in life-history traits in *D. magna* (Van Doorslaer *et al.*, 2009a, 2009b, 2010). Furthermore, short-term studies on predator–prey interactions might demonstrate thermal adaptation along a latitudinal gradient in *D. magna* (De Block *et al.*, 2013).

Our findings indicate the presence of evolutionary potential in upper thermal tolerance in *D. magna* at the studied latitudes. Although the resolution (number of clones studied per population) of our study is not sufficient to document evolutionary potential within single populations, our results suggest that at a given latitude *D. magna* may evolve to manage increasing temperatures. Besides genetic adaptation, populations may also rely on phenotypic plasticity to cope with warming (Merilä and Hendry, 2014). In support of this, heat tolerance measured as knockdown time strongly increased at a high acclimation temperature in a set of *D. magna* clones from widely different latitudes (Yampolsky *et al.*, 2014). The few studies on this topic in terrestrial invertebrates also indicate the presence of genetic variation as well as the capacity for acclimation to heat (Bridle *et al.*, 2009; Hoffmann *et al.*, 2003, 2013; but see Overgaard *et al.*, 2011). Yet, selection and heritability experiments in *Drosophila* suggest that upper thermal limits may not increase enough to match the predicted temperature increases (Hoffmann *et al.*, 2013). Furthermore, trade-offs between upper thermal tolerance and other traits (Hoffmann *et al.*, 2003; Mori and Kimura, 2008; Roze *et al.*, 2013) may exist that slow the adaptive evolution of $CT_{\mathbf{max}}$. Moreover, the realization of evolutionary potential may strongly depend on the environmental conditions and communities in which the animals are embedded, as has been shown explicitly for thermal evolution of life-history traits in *D. magna* (Van Doorslaer, 2009; Van Doorslaer *et al.*, 2010; De Meester *et al.*, 2011). Therefore, despite accumulating evidence of genetic and plastic responses to warming in *Daphnia*, a key grazer in the pelagic zone of standing water, one should be cautious in predicting that *Daphnia* populations will be capable of genetically tracking climate warming. To address this fully, research is required that combines acclimation and thermal experimental evolution in as natural a setting as possible.

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REFERENCES

Addo-Bediako, A., Chown, S.L. and Gaston, K.J. 2000. Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. Lond. B*, **267**: 739–745.

Adrian, R., O'Reilly, C.M., Zagarese, H., Baines, S.B., Hessen, D.O., Keller, W. *et al.* 2009. Lakes as sentinels of climate change. *Limnol. Oceanogr.*, **54**: 2283–2297.

Angilletta, M.J. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. New York: Oxford University Press.

Angilletta, M.J., Steury, T.D. and Sears, M.W. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.*, **44**: 498–509.

Atkinson, D. 1994. Temperature and organism size: a biological law for ectotherms? In *Advances in Ecological Research* (M. Begon and A.H. Fitter, eds.), pp. 1–58. London: Academic Press.

Bickford, D., Howard, S., Ng, D.J. and Sheridan, J. 2010. Impacts of climate change on the amphibians and reptiles of Southeast Asia. *Biodivers. Conserv.*, **19**: 1043–1062.

Blanckenhorn, W. and Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr. Comp. Biol.*, **44**: 413–424.

Bridle, J.R., Gavaz, S. and Kennington, W.J. 2009. Testing limits to adaptation along altitudinal gradients in rainforest *Drosophila*. *Proc. R. Soc. Lond. B*, **276**: 1507–1515.

Chopelet, J., Blier, P.U. and Dufresne, F. 2008. Plasticity of growth rate and metabolism in *Daphnia magna* populations from different thermal habitats. *J. Exp. Zool A: Ecol. Genet. Physiol.*, **309**: 553–562.

Chown, S.L., Jumbam, K.R., Sorensen, J.G. and Terblanche, J.S. 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.*, **23**: 133–140.

Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta, M.J., Jr., Stenseth, N.C. and Pertoldi, C. 2010. Adapting to climate change: a perspective from evolutionary physiology. *Climate Res.*, **43**: 3–15.

Clusella-Trullas, S., Blackburn, T.M. and Chown, S.L. 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am. Nat.*, **177**: 738–751.

Daufresne, M., Lengfellner, K. and Sommer, U. 2009. Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA*, **106**: 12788–12793.

De Block, M., Pauwels, K., Van Den Broeck, M., De Meester, L. and Stoks, R. 2013. Local genetic adaptation generates latitude-specific effects of warming on predator–prey interactions. *Global Change Biol.*, **19**: 689–696.

Dell, A.I., Pawar, S. and Savage, V.M. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl. Acad. Sci. USA*, **108**: 10591–10596.

De Meester, L. and De Jager, H. 1993. Hatching of *Daphnia* sexual eggs. I. Intraspecific differences in the hatching responses of *D. magna* eggs. *Freshw. Biol.*, **30**: 219–226.

De Meester, L., Van Doorslaer, W., Geerts, A., Orsini, L. and Stoks, R. 2011. Thermal genetic adaptation in the water flea *Daphnia* and its impact: an evolving metacommunity approach. *Integr. Comp. Biol.*, **51**: 703–718.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. *et al.* 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA*, **105**: 6668–6672.

di Lascio, A., Rossi, L. and Letizia Costantini, M. 2011. Different temperature tolerance of northern and southern European populations of a freshwater isopod crustacean species *Asellus aquaticus*. *Fund. Appl. Limnol.*, **179**: 193–201.

Forster, J., Hirst, A.G. and Atkinson, D. 2012. Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proc. Natl. Acad. Sci. USA*, **109**: 19310–19314.

Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L. and Heinsohn, R. 2011. Declining body size: a third universal response to warming? *Trends Ecol. Evol.*, **26**: 285–291.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. and Charnov, E.L. 2001. Effects of size and temperature on metabolic rate. *Science*, **293**: 2248–2251.

Hoffmann, A.A., Anderson, A. and Hallas, R. 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.*, **5**: 614–618.

Hoffmann, A.A., Sorensen, J.G. and Loeschcke, V. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.*, **28**: 175–216.

Hoffmann, A.A., Chown, S.L. and Clusella-Trullas, S. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.*, **27**: 934–949.

Huber, V., Adrian, R. and Gerten, D. 2010. A matter of timing: heat wave impact on crustacean zooplankton. *Freshw. Biol.*, **55**: 1769–1779.

Huey, R.B., Crill, W.D., Kingsolver, J.G. and Weber, K.E. 1992. A method for rapid measurement of heat or cold resistance of small insects. *Funct. Ecol.*, **6**: 489–494.

Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Álvarez Pérez, H.J. *et al.* 2009. Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. Lond. B*, **276**: 1939–1948.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M. and Williams, S.E. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. Lond. B*, **367**: 1665–1679.

Intergovernmental Panel on Climate Change (IPCC). 2013. Summary for policy makers. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.

Kearney, M., Shine, R. and Porter, W.P. 2009. The potential for behavioral thermoregulation to buffer 'cold-blooded' animals against climate warming. *Proc. Natl. Acad. Sci. USA*, **106**: 3835–3840.

Kellermann, V., Overgaard, J., Hoffmann, A.A., Flojgaard, C., Svenning, J.-C. and Loeschcke, V. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. USA*, **109**: 16228–16233.

Lutterschmidt, W.I. and Hutchison, V.H. 1997. The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Can. J. Zool.*, **75**: 1553–1560.

Merilä, J. and Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.*, **7**: 1–14.

Miner, B.E., De Meester, L., Pfrender, M.E., Lampert, W. and Hairston, N.G., Jr. 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proc. R. Soc. Lond. B*, **279**: 1873–1882.

Mitchell, K.A. and Hoffmann, A.A. 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. *Funct. Ecol.*, **24**: 694–700.

Mitchell, S.E. and Lampert, W. 2000. Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. *J. Evol. Biol.*, **13**: 371–382.

Mori, N. and Kimura, M.T. 2008. Selection for rapid and slow recovery from chill- and heat-coma in *Drosophila melanogaster*. *Biol. J. Linn. Soc.*, **95**: 72–80.

Moritz, C., Langham, G., Kearney, M., Krockenberger, A., VanDerWal, J. and Williams, S. 2012. Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. *Phil. Trans. R. Soc. Lond. B*, **367**: 1680–1687.

Overgaard, J., Kristensen, T.N., Mitchell, K.A. and Hoffmann, A.A. 2011. Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude? *Am. Nat.*, **178**: S80–S96.

Overgaard, J., Kristensen, T.N. and Sørensen, J.G. 2012. Validity of thermal ramping assays used to assess thermal tolerance in arthropods. *PLoS ONE*, **7**: e32758.

Peck, L.S., Morley, S.A., Richard, J. and Clark, M.S. 2014. Acclimation and thermal tolerance in Antarctic marine ectotherms. *J. Exp. Biol.*, **217**: 16–22.

Ragland, G.J. and Kingsolver, J.G. 2008. Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life-history timing in *Wyeomyia smithii*. *Evolution*, **62**: 1345–1357.

Rezende, E.L., Tejedo, M. and Santos, M. 2011. Estimating the adaptive potential of critical thermal limits: methodological problems and evolutionary implications. *Funct. Ecol.*, **25**: 111–121.

Ribeiro, P.L., Camacho, A. and Navas, C.A. 2012. Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants. *PLoS ONE*, **7**: e32083.

Roze, T., Christen, F., Amerand, A. and Claireaux, G. 2013. Trade-off between thermal sensitivity, hypoxia tolerance and growth in fish. *J. Ther. Biol.*, **38**: 98–106.

Rüdiger, J.P., Mertenskötter, A., Pinkhaus, O., Pirow, R., Gigengack, U., Buchen, I. *et al.* 2012. Seasonal and interannual changes in water temperature affect the genetic structure of a *Daphnia* assemblage (*D. longispina* complex) through genotype-specific thermal tolerances. *Limnol. Oceanogr.*, **57**: 619–633.

Santos, M., Castañeda, L.E. and Rezende, E.L. 2011. Making sense of heat tolerance estimates in ectotherms: lessons from *Drosophila*. *Funct. Ecol.*, **25**: 1169–1180.

Sheridan, J.A. and Bickford, D. 2011. Shrinking body size as an ecological response to climate change. *Nature Climate Change*, **1**: 401–406.

Sinervo, B., Mendez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Cruz, M.V.S. *et al.* 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science*, **328**: 894–899.

Sørensen, J.G., Loeschcke, V. and Kristensen, T.N. 2013. Cellular damage as induced by high temperature is dependent on rate of temperature change – investigating consequences of ramping rates on molecular and organismal phenotypes in *Drosophila melanogaster*. *J. Exp. Biol.*, **216**: 809–814.

Stoks, R., Geerts, A.N. and De Meester, L. 2014. Evolutionary and plastic responses of freshwater invertebrates to climate change: realized patterns and future potential. *Evol. Appl.*, **7**: 42–55.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., Le Roux, P.C. and Chown, S.L. 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. *J. Exp. Biol.*, **214**: 3713–3725.

Van Doorslaer, W. 2009. Micro-evolutionary responses to increased temperature in zooplankton. PhD thesis, Katholieke Universiteit Leuven.

Van Doorslaer, W., Stoks, R., Duvivier, C., Bednarska, A. and De Meester, L. 2009a. Population dynamics determine genetic adaptation to temperature in *Daphnia*. *Evolution*, **63**: 1867–1878.

Van Doorslaer, W., Vanoverbeke, J., Duvivier, C., Rousseaux, S., Jansen, M., Jansen, B. *et al.* 2009b. Local adaptation to higher temperatures reduces immigration success of genotypes from a warmer region in the water flea *Daphnia*. *Global Change Biol.*, **15**: 3046–3055.

Van Doorslaer, W., Stoks, R., Swillen, I., Feuchtmayr, H., Atkinson, D., Moss, B. *et al.* 2010. Experimental thermal microevolution in community-embedded *Daphnia* populations. *Climate Res.*, **43**: 81–89.

van Heerwaarden, B., Lee, R.F.H., Wegener, B., Weeks, A.R. and Sgró, C.M. 2012. Complex patterns of local adaptation to heat tolerance in *Drosophila simulans* from eastern Australia. *J. Evol. Biol.*, **25**: 1765–1778.

Williams, P., Dick, K. and Yampolsky, L. 2012. Heat tolerance, temperature acclimation, acute oxidative damage and canalization of haemoglobin expression in *Daphnia*. *Evol. Ecol.*, **26**: 591–609.

Yampolsky, L.Y., Schaer, T.M.M. and Ebert, D. 2014. Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. *Proc. R. Soc. Lond. B*, **281**: 20132744.