

## RESEARCH ARTICLE

# Metabolic rate and climate change across latitudes: evidence of mass-dependent responses in aquatic amphipods

Milad Shokri<sup>1,\*</sup>, Francesco Cozzoli<sup>1,2,\*</sup>, Fabio Vignes<sup>1</sup>, Marco Bertoli<sup>3</sup>, Elisabetta Pizzul<sup>3</sup> and Alberto Basset<sup>1,4</sup>

**ABSTRACT**


Predictions of individual responses to climate change are often based on the assumption that temperature affects the metabolism of individuals independently of their body mass. However, empirical evidence indicates that interactive effects exist. Here, we investigated the response of individual standard metabolic rate (SMR) to annual temperature range and forecasted temperature rises of 0.6–1.2°C above the current maxima, under the conservative climate change scenario IPCC RCP2.6. As a model organism, we used the amphipod *Gammarus insensibilis*, collected across latitudes along the western coast of the Adriatic Sea down to the southernmost limit of the species' distributional range, with individuals varying in body mass (0.4–13.57 mg). Overall, we found that the effect of temperature on SMR is mass dependent. Within the annual temperature range, the mass-specific SMR of small/young individuals increased with temperature at a greater rate (activation energy:  $E=0.48$  eV) than large/old individuals ( $E=0.29$  eV), with a higher metabolic level for high-latitude than low-latitude populations. However, under the forecasted climate conditions, the mass-specific SMR of large individuals responded differently across latitudes. Unlike the higher-latitude population, whose mass-specific SMR increased in response to the forecasted climate change across all size classes, in the lower-latitude populations, this increase was not seen in large individuals. The larger/older conspecifics at lower latitudes could therefore be the first to experience the negative impacts of warming on metabolism-related processes. Although the ecological collapse of such a basic trophic level (aquatic amphipods) owing to climate change would have profound consequences for population ecology, the risk is significantly mitigated by phenotypic and genotypic adaptation.

**KEY WORDS:** Metabolic scaling, Body size, Temperature, Global warming, Species distribution, Thermal tolerance

<sup>1</sup>Laboratory of Ecology, Department of Biological and Environmental Sciences and Technologies, University of Salento, S.P. Lecce-Monteroni, 73100 Lecce, Italy.

<sup>2</sup>Research Institute on Terrestrial Ecosystems (IRET-URT Lecce), National Research Council of Italy (CNR), Campus Ecotekne, S.P. Lecce-Monteroni, 73100 Lecce, Italy. <sup>3</sup>Department of Life Science, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy. <sup>4</sup>National Biodiversity Future Center, Palermo 90133, Italy.

\*Authors for correspondence (milad.shokri@unisalento.it; francesco.cozzoli@cnr.it)

 M.S., 0000-0003-0826-6228; F.C., 0000-0002-6413-0977; F.V., 0000-0003-0579-6629; M.B., 0000-0001-8345-6107; E.P., 0000-0001-9916-5005; A.B., 0000-0002-3603-9316

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**INTRODUCTION**

Climate change has altered ecological rates by shifting patterns of energy flux in ecosystems (Kraemer et al., 2017; O'Connor et al., 2009; Santini et al., 2016) and energy allocation in species (Brown et al., 2004; Yvon-Durocher et al., 2010). Warming in aquatic ecosystems is already having measurable impacts on animal populations, manifested as changes in phenology (Poloczanska et al., 2013), body mass (Audzijonyte et al., 2020; Gardner et al., 2011) and distributional range (Angilletta, 2009; Parmesan and Yohe, 2003). The underlying mechanisms by which climate affects individuals, populations and ecosystems are thus essential for ecosystem functioning. The metabolic rate, i.e. rate of energy use, of organisms is considered a key parameter in ecology, linking individual organisms to populations and ecosystems through a unified currency of energy (Brandl et al., 2022; Cozzoli et al., 2021; Glazier, 2015). Individual ectotherm metabolic rates tend to increase with increasing temperature, owing to its effect on the kinetic energy of cellular components (Gillooly et al., 2001; but see Clarke and Fraser, 2004), although this trend has an upper thermal limit (Schulte, 2015; Sinclair et al., 2016). Thus, metabolic rate is often one of the first individual traits to respond to climate change (Bruno et al., 2015; Verberk et al., 2016b).

The metabolic theory of ecology (MTE) proposes a mechanistic approach to individual energetics, identifying body mass and temperature as the primary determinants of metabolic rate (Brown et al., 2004; Gillooly et al., 2001). This framework has been applied in ecological studies based on simple physical and biological principles. Accordingly, metabolic rate is expected to scale with body mass with an exponent of 0.75 (Kleiber, 1932; West et al., 1997) and with temperature from 0°C to 40°C in accordance with the Boltzmann–Arrhenius factor with an activation energy of ~0.65 eV (Arrhenius, 1889; Gillooly et al., 2001). The MTE thus implicitly treats the effects of temperature and body mass on individual metabolism independently (Brown et al., 2004; West et al., 1997). However, several studies have found that the effect of temperature on metabolic rate might not follow a universal mass-independent thermodynamic law (e.g. Carey and Sigwart, 2014; Clarke and Fraser, 2004; Glazier, 2020; Killen et al., 2010; Ohlberger et al., 2012). Both empirical and theoretical studies suggest that the allometric exponent of the relationship between metabolic rate and body mass varies with temperature, meaning that the effects of temperature are body-mass dependent (Bullock, 1955; Glazier, 2005, 2020; Killen et al., 2010; Naya et al., 2018; Precht et al., 1973). Several alternative hypotheses to MTE, e.g. the metabolic-level boundaries hypothesis (Glazier, 2005, 2014), the viscosity hypothesis (Verberk and Atkinson, 2013) and the acclimation hypothesis (Fossen et al., 2019), have been developed to explain the body-mass dependency of temperature effects on individual metabolic rates. The effect of temperature on metabolic rate often depends on an organism's thermal physiology and plasticity, which are affected by body mass, life stage, activity level,

predation regime/cues, latitude and local climate (Burton et al., 2011; Forster et al., 2012; Glazier, 2020; Glazier et al., 2020; Leiva et al., 2018; Schulte et al., 2011; Terblanche and Chown, 2006). The optimal physiological responses of ectotherms are seen within their local temperature range (Pörtner and Knust, 2007), outside which organism performance and fitness fall (Angilletta, 2009; Anttila et al., 2013; Schulte, 2015; Vasseur et al., 2014), potentially affecting the long-term fate of the species in terms of body mass (Audzijonyte et al., 2020), distributional range (Hickling et al., 2006; Parmesan and Yohe, 2003) and even survival (Hochachka and Somero, 2002; Verberk et al., 2020).

Thermal tolerance, i.e. the temperature zone in which growth, reproduction and survival can be maintained, varies with body mass (Peralta-Maraver and Rezende, 2021; Pörtner and Gutt, 2016). Larger aquatic ectotherms, which possess higher absolute oxygen demands, might be more susceptible to oxygen limitation because their lower surface-area-to-volume ratio constrains their capacity to extract oxygen from their environment and deliver it to their metabolizing tissues (Leiva et al., 2019; Rubalcaba et al., 2020; see also Jutfelt et al., 2018). Thus, a narrowing of thermal tolerance in larger ectothermic individuals might result from a stronger mismatch between oxygen demand and supply (Pörtner and Knust, 2007; Rubalcaba et al., 2020; Verberk et al., 2016a).

In addition to mass dependency, the metabolic rate response of organisms to temperature might be adaptively adjusted in specific environments through phenotypic plasticity or genotype evolution (Benavente et al., 2022; Kefford et al., 2022; Terblanche et al., 2009). A species' thermal tolerance and metabolism also varies with latitude and between climate zones (Hoffmann et al., 2005; Nati et al., 2021; Terblanche and Chown, 2006). For example, the metabolic cold adaptation (MCA) hypothesis predicts that ectotherm organisms that live at higher latitudes with a mean colder climate may show either a higher metabolic level or a higher sensitivity of the metabolic rate to temperature than organisms at lower latitudes with a warmer climate (Chown and Gaston, 1999; Clarke, 1991, 1993; Terblanche et al., 2009). In addition to mean temperature, local thermal variability represents a strong determinant of an organism's thermal niche, which reflects its metabolic rate (Barria et al., 2018; Gaston et al., 1998; Sunday et al., 2019). The climate variability hypothesis (CVH) predicts that high-latitude species often have broader thermal tolerance and possess higher thermal plasticity owing to their local adaptation to a highly variable climate than species at lower latitudes where climate variation is minimal (Bennett et al., 2019; Peralta-Maraver and Rezende, 2021; Sunday et al., 2011, 2019; but see Seebacher et al., 2015).

It is thus important to assess deviations in the mass dependency of the thermal responses of metabolic rate and how this varies biogeographically in order to predict population responses and potential vulnerability to climate change. Empirical research must seek to understand how the standard metabolic rate (SMR) of individuals across a range of body-mass classes varies with temperature, not only within the local temperature range but also with respect to the more extreme temperatures predicted by future warming scenarios. Although recent studies provide significant insight into the ecological responses of ectotherm populations to temperature (Angilletta, 2009; Clarke and Fraser, 2004; Killen et al., 2010; Schulte, 2015), few studies have considered the effects of realistic IPCC climate change scenarios on conspecific populations across latitudinal gradients down to the lower boundary of a species' distribution range (but see Bestion et al., 2015). To help bridge this knowledge gap, this study aimed to (1) assess the response of the SMR of *Gammarus insensibilis* individuals across a range of

body-mass classes (herein corresponding to successive life stages) to current annual temperature variation and to the rise in temperature above the local maximum temperature under a conservative climate change scenario, i.e. RCP2.6 (IPCC, 2014), and (2) evaluate the SMR responses to both current annual climate variation and forecasted temperature scenarios of individuals selected from three populations distributed across a range of latitudes along the Western Adriatic coast, approaching the lowest latitudinal edge of the species' distribution.

## MATERIALS AND METHODS

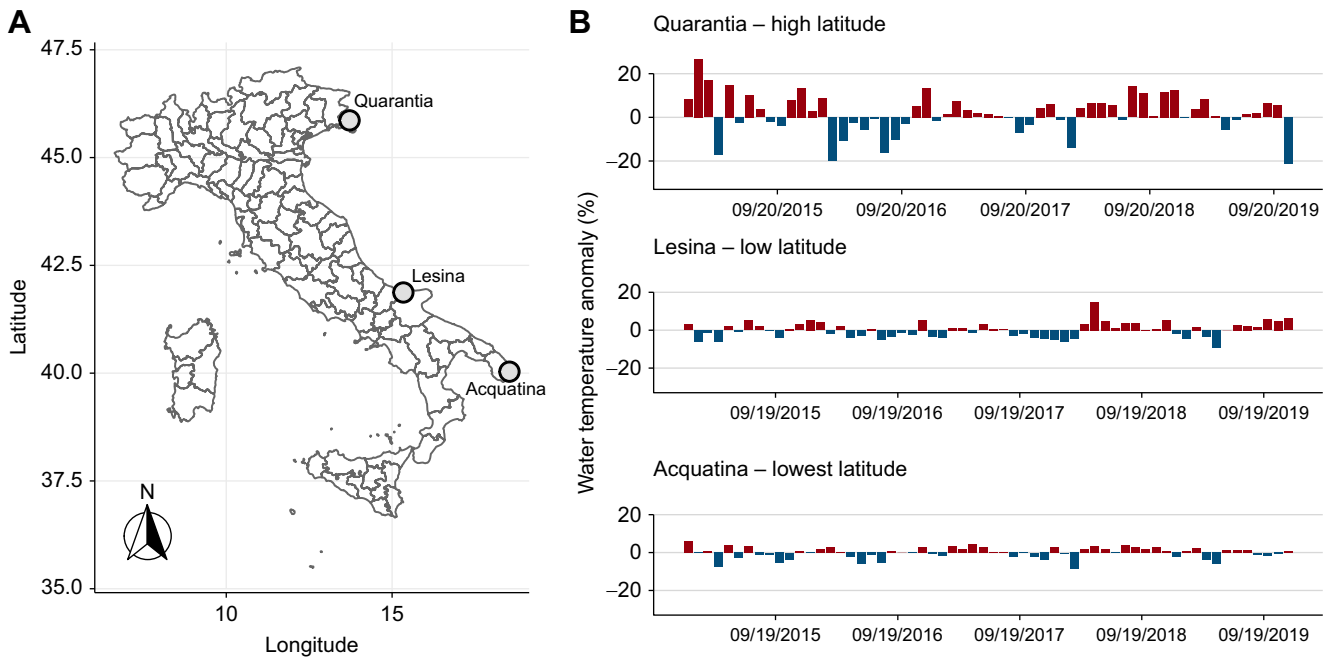
### The model organism and its distributional range

*Gammarus insensibilis* (Stock, 1966) is an ectotherm Atlantic–Mediterranean species of amphipod living in transitional and coastal waters (Costello and Emblow, 2001). They are important components of aquatic ecosystem trophic webs, feeding mainly on detritus and providing nourishment for secondary consumers (Cozzoli et al., 2022; Nelson, 2011; Shadrin et al., 2022), with a lifespan of 1 year (Gerhardt et al., 2011; Węślawski et al., 2020). The geographical distribution of *G. insensibilis* is mostly centred on Europe, Southern Greece, i.e. the Ntvari lagoon at 37.47°N, being their lower latitudinal limit (see <https://www.marlin.ac.uk/species/detail/1142>) (Tillin and White, 2017).

### Experimental design

The experiment was designed to assess the SMR response to both current annual climate variation and forecasted temperature rises of specimens of *G. insensibilis* belonging to a series of body-mass classes/life stages from a range of geographical areas. *Gammarus insensibilis* specimens were collected simultaneously in autumn from three transitional water bodies along the Western Adriatic coast (Fig. 1A): Quarantia (45.763°N, 13.498°E), Lesina (41.871°N, 15.340°E) and Acquatina (40.444°N, 18.238°E). The three locations were selected to represent a climate gradient approaching the lower latitudinal boundary of its distributional range (Fig. 1, Table 1). The high-latitude collection site, i.e. Quarantia, had relatively lower mean annual water temperature (Table 1) and higher climate variability than the lower-latitude sites, i.e. Lesina and Acquatina (Fig. 1B). The geographical distances between the collection sites were sufficient to assume that the collected specimens belong to distinct populations (Baltazar-Soares et al., 2017; Bayne, 2017).

The water temperature levels were selected to represent both the annual temperature range currently experienced by each population in accordance with their local climate and the change in temperature predicted to result from climate change (Table 1). To represent the current climate experienced by each of the studied populations, the experimental temperature levels were: (1) the local minimum winter temperature, calculated as the average coldest annual temperature recorded from 2015 to 2019, (2) the reference temperature (set at 18°C for all three sites) and (3) the local maximum summer temperature, calculated as the average warmest annual temperature recorded from 2015 to 2019 (Table 1). We assumed minimal variation in the water column temperature profile, as the waters at the collection sites were relatively shallow (less than 1 m). The daily water surface temperature data for each of the collection sites from 2015 to 2019 were collected by the Copernicus Marine Environment Monitoring Service (CMEMS, 2011; Buongiorno Nardelli et al., 2013) (Table 1). The forecasted temperature levels were chosen with reference to RCP2.6 (IPCC, 2014), i.e. the most conservative climate change scenario, which predicts a mean global water temperature rise of 0.6°C and 1.2°C by 2040 and 2100,



**Fig. 1. Specimen collection sites and the water temperature variability at each site.** (A) Map of the study areas and collection sites. (B) Time series of water temperature anomalies at the three collection sites (Quarantia, Lesina and Acquatina lagoons) from 2015 to 2019. The daily water temperature was obtained from the Copernicus Marine Environment Monitoring Service.

respectively (Genner et al., 2017; IPCC, 2014). Although, the RCP2.6 scenario predicts a temperature increase over the mean global temperature, we extended the experimental temperature gradient further by two temperature increments of (4)  $0.6^{\circ}\text{C}$  and (5)  $1.2^{\circ}\text{C}$  above the average current maxima of each collection site (Table 1), in order to estimate the individuals' metabolic responses and their vulnerability to a relatively narrow temperature increase beyond the local peak temperature that animals experienced.

### Specimen collection and acclimation

At each location, specimens with a range of body masses were collected by scraping the emergent vegetation with 2 mm mesh metal sieves, and were taxonomically identified with reference to Bellan-Santini et al. (1989, 1998). The body mass of the collected specimens was assumed to reflect different life stages of these moulting amphipods. Herein we thus use 'small' and 'large' as equivalent to 'young' and 'old', respectively. After collection, the specimens were transferred to Salento University's Biodiversity and Ecosystem Functioning Laboratory (BIO4IU) in thermo-insulated containers filled with water from the sampling sites and aerated during transport. Authorization for specimen collection was issued by the competent authorities: Friuli Venezia Giulia Regional Administration for the Quarantia site, the Gargano National Park for the Lesina site and the University of Salento for the Acquatina site. The species involved in this study are not endangered or protected. The specimens of each

population were kept in the laboratory aquaria at a salinity similar to that of the collection sites (Quarantia 20 PSU, Lesina 22 PSU and Acquatina 21 PSU). They were acclimated to the specific temperatures to be assessed (Table 1) for 2 weeks, which is a sufficient period to minimize any stress and reduce the risk of temperature shock that could severely affect individual metabolic rate (Semsar-kazerouni and Verberk, 2018). Acclimation to temperature levels above and below the collection temperature was achieved at a rate of  $\pm 1.5^{\circ}\text{C day}^{-1}$  in aquaria placed in temperature-controlled environments (KW Apparecchi Scientifici, WR UR series).

Decayed leaves of *Phragmites australis* (Cav.) Trin. ex Steud were supplied as food during the acclimation period in the aquaria and renewed depending on consumption. *Phragmites australis* is known to be one of the largest sources of organic plant detritus in transitional water ecosystems, providing food for macroinvertebrates (Able and Hagan, 2000), including *G. insensibilis* (Cozzoli et al., 2022; Shokri et al., 2021). Before starting the experiment, specimens were sorted by sex under a Nikon stereoscope (SMZ1270). Only males were selected for laboratory experiments because oocyte production in females may induce non-mass-related variability in energy requirements (Glazier et al., 2011).

### SMR setup and measurements

Following Shokri et al. (2019), Glazier and Sparks (1997) and Wrona and Davies (1984), the individual standard metabolic rate

**Table 1. Water temperature variability at three collection sites from 2015 to 2019, with corresponding acclimation and measurement temperatures**

Location	Latitude, longitude	Local temperature ( $^{\circ}\text{C}$ )			Acclimation and measurement temperature ( $^{\circ}\text{C}$ )				
		Mean $\pm$ s.d.	Min. winter	Max. summer	Min. winter	Reference	Max. summer	+0.6 (RCP <sub>2.6</sub> )	+1.2 (RCP <sub>2.6</sub> )
Quarantia	45.763°N, 13.498°E	17.1 $\pm$ 6.86	8.63	24.8	9	18	25	25.6	26.2
Lesina	41.871°N, 15.340°E	18.62 $\pm$ 5.33	10.21	28.11	10	18	28	28.6	29.2
Acquatina	40.444°N, 18.238°E	19.63 $\pm$ 3.9	12.09	29	12	18	29	29.6	30.2

(SMR,  $\text{J day}^{-1}$ ) was measured as oxygen consumption ( $V_{\text{O}_2}$ ) of *G. insensibilis* individuals. The animals were kept unfed individually in plastic beakers (200 ml) for 24 h before the SMR measurements at the specific temperatures. To assess their SMR, animals were placed individually in Strathkelvin open-flow system respirometers, also known as flow-through systems (see Fig. S1 for the diagram of the setup). The respirometer measurement system includes a glass water tank (1 litre) filled with the same water as the acclimation aquaria, which was kept magnetically stirred and oxygen-saturated throughout the experiment, using a digital ceramic magnetic stirrer (AREC.X). The stirrer speed was set to 200 rpm, and the operator had observational control over the water tank to avoid supersaturating the water. A peristaltic pump (Watson-Marlow 205 U, 12 channels) provided constant water flow ( $6 \text{ ml h}^{-1}$ ) to six respirometer chambers (6 ml volume), each containing a single individual. A 0.3 mm nylon mesh with a nominal outer diameter of 12.07 mm was placed in each respirometer chamber in order to minimize the individual's spontaneous movement. An equilibration period of 3 h was fixed as the time required to reach a steady concentration of dissolved oxygen, which also enabled specimens to adapt to the respirometer chambers and reduce their spontaneous activity. Upon exiting the chambers, the water was pumped via silicone tubes to the Clark-type microelectrodes (SI1302 Strathkelvin's oxygen electrodes), where the oxygen concentration was measured by an oximeter and recorded and stored using the Strathkelvin software (SI, 929). The operator then read the dissolved oxygen partial pressure ( $P_{\text{O}_2}$ ,  $\Delta\text{torr}$ ) for each individual for 30 min: 15 min for the oxygen concentration curve (in the presence of a specimen:  $P_{\text{O}_2,\text{in}}$ ), and 15 min for the blank (in the absence of specimens:  $P_{\text{O}_2,\text{out}}$ ) (see Fig. S2 for the oxygen traces of the three body-mass classes of model organisms). The electrodes were calibrated weekly at the base, i.e. zero calibration with a zero-oxygen solution (2% solution of sodium sulphite in distilled water), and daily to high calibration with air-saturated water (100%). After every experimental trial, we sterilized the respirometer system parts, including the respirometer chambers and silicone tubes, using an autoclave (Hiclave HV) to prevent any possible microbial growth.

The oxygen consumed by each individual  $V_{\text{O}_2}$  ( $\mu\text{mol O}_2 \text{ h}^{-1}$ ) was calculated as:

$$V_{\text{O}_2} = (P_{\text{O}_2,\text{out}} - P_{\text{O}_2,\text{in}}) \times S_{\text{O}_2} \times F, \quad (1)$$

where  $P_{\text{O}_2,\text{out}}$  is the partial pressure (torr) of dissolved oxygen in the outflow water of the blank (without specimens),  $P_{\text{O}_2,\text{in}}$  is the dissolved oxygen partial pressure (torr) of the respirometer chamber (with a specimen),  $F$  is the water flow rate ( $\text{l h}^{-1}$ ) and  $S_{\text{O}_2}$  is the solubility coefficient of dissolved oxygen in water ( $\mu\text{mol l}^{-1} \text{ torr}^{-1}$ ). For each temperature and salinity, the solubility coefficient of dissolved oxygen ( $S_{\text{O}_2}$ ) was obtained from a Loligo oxygen converter (<https://www.loligosystems.com/convert-oxygen-units>). The rate of oxygen consumption was then converted to metabolic rate ( $\text{J day}^{-1}$ ) using an oxyjoule equivalent of  $0.45 \text{ J } (\mu\text{mol O}_2)^{-1}$  (Gnaiger, 1983), and by multiplying the resulting value by 24 h. After metabolic measurement, the animals were dried individually in an oven at  $60^\circ\text{C}$  for 72 h and then weighed on a micro balance (Sartorius MC5) to the nearest  $\pm 0.001 \text{ mg}$ .

### Statistical analysis

The differences between the populations collected at each collection site in terms of dry body mass ( $M$ , mg) and SMR ( $\text{J day}^{-1}$ ) were analysed using one-way ANOVA. A linear mixed ANCOVA was

used on the complete dataset to investigate the variation of individual SMR with body mass ( $M$ , mg), temperature range ( $^\circ\text{C}$ ) and collection site. Because the relationship between SMR and body mass is commonly formulated as a power law (Glazier, 2021), they were both log-transformed. The linear mixed model was fitted with full interaction between explanatory variables, i.e. body mass, temperature and collection site, and simplified via a stepwise elimination procedure. The relative importance of the explanatory variables was then assessed by the LMG metric ( $R^2$  partitioned by averaging over orders) (Lindeman et al., 1980). Additionally, we applied an ANCOVA to the linear relationship between log-log transformed SMR and body mass to compare the mass scaling exponents and intercept variation across temperature levels at each collection site. The mass scaling exponents and intercepts of metabolic rate at the different temperatures were all compared with the scaling recorded for the lowest temperature at each collection site.

Moreover, in order to thoroughly assess the thermal response of the mass-specific SMR ( $\text{J day}^{-1} \text{ mg}^{-1}$ ) of individuals of differing body masses to current annual variation and forecasted increases, we placed individuals in three classes using body mass distribution quantiles (0.33, 0.66 and 0.99), corresponding to small (mean $\pm$ s.d.= $1.71\pm 0.51 \text{ mg}$ ), medium ( $3.76\pm 0.64 \text{ mg}$ ) and large ( $7.36\pm 1.9 \text{ mg}$ ). Following Brown et al. (2004), we regressed the logarithm of mass-specific SMR ( $\text{J day}^{-1} \text{ mg}^{-1}$ ) on inverse temperature ( $1/kT$ ), where  $k$  is Boltzmann's constant ( $8.167\times 10^{-5} \text{ eV K}^{-1}$ ) and  $T$  is the temperature in Kelvin. This enabled linear regression of the data, the scaling exponent of the regression quantifying the temperature dependency of mass-specific SMR as an activation energy ( $E$ , eV). Multiple linear regression was used to analyse the variation of mass-specific SMR with inverse temperature ( $1/kT$ ) and collection site within each body-mass class. The multivariate model was fitted with full interaction between explanatory variables and then simplified via a stepwise elimination procedure. In addition, we estimated the temperature coefficients ( $Q_{10}$ ) for each temperature with respect to the minimum temperature across body-mass classes and collection sites (see Fig. S3). The temperature anomaly was calculated as the variation from the average daily temperature for each site, relative to the last 10 years. The significance threshold level was set at  $P=0.05$ . The analyses were performed in R (<https://www.r-project.org/>), with the additional packages lme4 (Bates et al., 2015), dplyr (<https://CRAN.R-project.org/package=dplyr>), relaimpo (Groemping, 2006), Tidyverse (Wickham et al., 2019) and Lubridate (Grolemund and Wickham, 2011).

## RESULTS

### Preliminary data analysis

Overall, we measured 375 male *G. insensibilis* individuals ranging from 4.74 to 15.90 mm in body length (mean $\pm$ s.d.= $10.25\pm 2.27 \text{ mm}$ ) and from 0.4 to 13.57 mg in dry body mass ( $4.27\pm 2.65 \text{ mg}$ ). The body mass ( $M$ , mg) distribution did not differ significantly among the three studied *G. insensibilis* populations, nor did it differ across temperature levels for each population.

### Mass scaling SMRs across temperature and latitudes

Overall, 56.4% of individual SMR variance was explained by the continuous variables, i.e. body mass and temperature, and by the categorical variable, i.e. collection site (Table 2). Individual SMR increased with both temperature ( $P<0.001$ , 25.3% of explained variance in SMR) and body mass ( $P<0.001$ , 25.2% of explained variance in SMR), while 4.1% of the observed variance in SMR was

**Table 2. Summary of the linear mixed ANCOVA, with standard metabolic rate (SMR) as a response variable and body mass ( $M$ ), temperature ( $T$ ) and collection site as predictors**

Predictor	log(SMR)		$P$
	Estimate	CI	
Intercept	-1.16	-1.47–-0.86	<0.001
log( $M$ )	0.72	0.53–0.92	<0.001
$T$ (°C)	0.06	0.05–0.08	<0.001
Site (Lesina)	0.20	0.10–0.31	0.370
Site (Quarantia)	0.37	0.27–0.48	0.036
log( $M$ ) $\times$ $T$	-0.01	-0.02–-0.01	0.001
Observations	375		
$R^2/R^2$ adjusted	0.57 / 0.56		

Intercept refers to the lowest latitudinal collection site (Acquatina).

explained by the negative interaction between body mass and temperature ( $P<0.01$ ; Table 2), which implies that the SMR response to temperature decreased with increasing body mass. Across collection sites, the SMR level (intercept) was higher for the higher-latitude population than the lower-latitude populations ( $P<0.05$ ; Table 2).

In the high-latitude population of Quarantia, the estimated intercepts of SMR against body mass increased significantly with temperature from the current minimum to the additional  $0.6^\circ\text{C}$  above the current maximum, i.e.  $25.6^\circ\text{C}$ , with no significant difference in scaling exponents (slopes) compared with the minimum temperature (Fig. 2A, Table 3). However, at the highest temperature level ( $26.2^\circ\text{C}$ ), which was  $1.2^\circ\text{C}$  above the current maxima, the scaling exponent (slope) of metabolic rate against body mass was significantly lower than it was at the minimum temperature (Fig. 2A, Table 3; see Table 4 for the linear equations of the relationship between SMR and body mass across temperatures).

In the lower-latitude population of Lesina, the scaling intercepts of metabolic rate against body mass increased significantly with temperature, up to the current maximum ( $28^\circ\text{C}$ ), compared with the minimum temperature, with no significant difference in scaling exponents within the current temperature range (Fig. 2B, Table 3). However, at temperatures above the current maximum ( $28.6$  and  $29.2^\circ\text{C}$ ), the mass scaling exponents of SMR significantly decreased with respect to the minimum temperature (Fig. 2B,

Table 3; see Table 4 for the linear equations of the mass scaling metabolic rate across temperatures).

At the lowest-latitude population, i.e. Acquatina, the scaling exponents of metabolic rate against body mass significantly decreased with temperature with respect to the minimum temperature ( $12^\circ\text{C}$ ) in all cases (Fig. 2C, Table 3; see Table 4 for the linear equations of the relationship between SMR and body mass across temperatures).

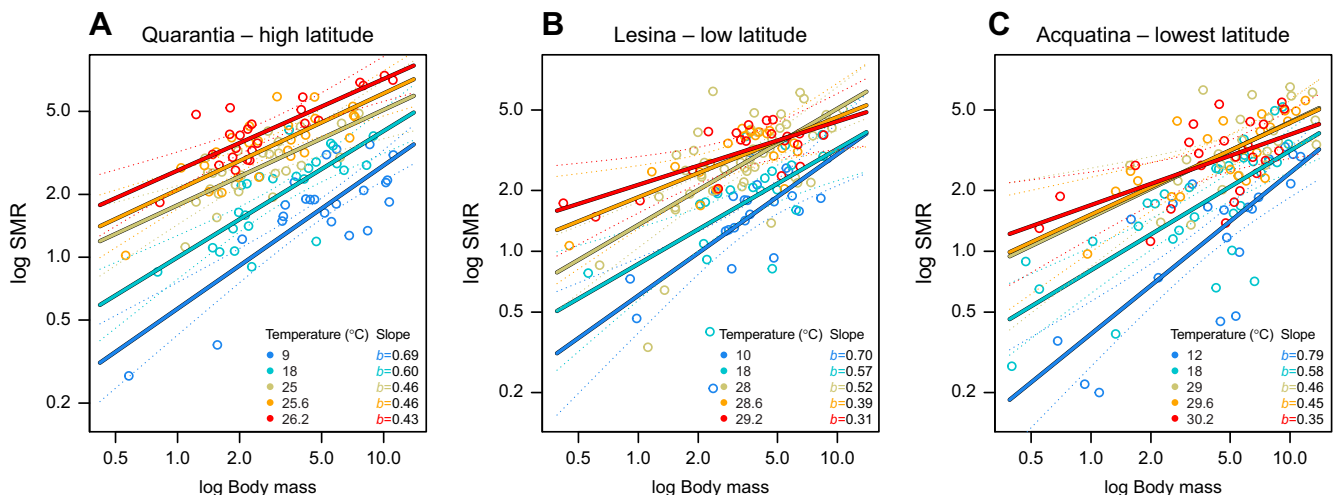
### The thermal sensitivity of mass-specific SMR across body-mass classes and latitudes

#### Current annual climate

Within the current annual temperature range (minimum to maximum temperature), the rate of change of mass-specific SMR with temperature was positive and similar across populations within each body-mass class (Fig. 3A–C, Table 5). However, within each body-mass class, the estimated intercept of the relationship of mass-specific SMR with temperature was significantly higher for the higher latitude population (Quarantia) than for the lower latitude populations (Lesina and Acquatina) (Fig. 3A–C, Table 5). Therefore, at the same temperature, high-latitude individuals had higher SMR per unit of mass than lower-latitude individuals. At all sites, the mass-specific SMR of small individuals increased with temperature, with a scaling exponent equivalent to an activation energy of  $E=0.48$  eV (Fig. 3A, Table 5). The scaling exponent of the relationship between mass-specific SMR and temperature for medium-sized ( $E=0.27$  eV) and large individuals ( $E=0.29$  eV) was lower than it was for small individuals (ANCOVA:  $F_{2,235}=39.76$ ,  $P<0.001$ ) (Fig. 3B,C, Table 5; see Fig. S3 for the  $Q_{10}$  across body-mass classes).

#### Forecasted climate change

Within the forecasted temperature rise ( $0.6$ – $1.2^\circ\text{C}$  above the current maxima), the mass-specific SMR of the small and medium individuals increased significantly in response to temperature with a similar scaling exponents across latitudes ( $E=1.15$  eV for small, and  $E=1.01$  eV for medium), and a significantly higher scaling intercept in the high-latitude population than in the lower-latitude populations (Fig. 3D,E, Table 6). However, the mass-specific SMR of large individuals responded differently across latitudes to the



**Fig. 2. Standard metabolic rate (SMR;  $\text{J day}^{-1}$ ) in relation to dry body mass ( $M$ , mg) across temperature levels at each collection site. (A) Quarantia, high-latitude population; (B) Lesina, low-latitude population; (C) Acquatina, lowest-latitude population.  $b$  represents the scaling exponent (slope) of the metabolic rate against body mass for each temperature level.**

**Table 3. Summary of ANCOVA applied to the relationship between standard metabolic rate (SMR) and body mass (*M*) (log-log transformed) across temperature levels (*T*) at each collection site**

Predictor	log(SMR)								
	Quarantia – high latitude			Lesina – low latitude			Acquatina – lowest latitude		
	Estimate	CI	<i>P</i>	Estimate	CI	<i>P</i>	Estimate	CI	<i>P</i>
Intercept	−0.57	−0.86– −0.28	<b>&lt;0.001</b>	−0.50	−0.93– −0.08	<b>0.021</b>	−0.94	−1.31– −0.57	<b>&lt;0.001</b>
log( <i>M</i> )	0.69	0.52–0.86	<b>&lt;0.001</b>	0.70	0.39–1.01	<b>&lt;0.001</b>	0.79	0.65–0.93	<b>&lt;0.001</b>
<i>T</i> (18°C)	0.56	0.19–0.94	<b>0.004</b>	0.36	−0.23–0.95	0.233	0.73	0.27–1.18	<b>0.002</b>
<i>T</i> (25°C)	1.14	0.78–1.50	<b>&lt;0.001</b>						
<i>T</i> (25.6°C)	1.31	0.95–1.67	<b>&lt;0.001</b>						
<i>T</i> (26.2°C)	1.52	1.17–1.88	<b>&lt;0.001</b>						
log( <i>M</i> )× <i>T</i> (18°C)	−0.08	−0.34–0.17	0.525	−0.13	−0.56–0.30	0.550	−0.21	−0.49–0.08	<b>0.050</b>
log( <i>M</i> )× <i>T</i> (25°C)	−0.23	−0.50–0.04	0.052						
log( <i>M</i> )× <i>T</i> (25.6°C)	−0.22	−0.47–0.02	0.055						
log( <i>M</i> )× <i>T</i> (26.2°C)	−0.25	−0.49– −0.01	<b>0.033</b>						
<i>T</i> (28°C)				0.80	0.30–1.30	<b>0.002</b>			
<i>T</i> (28.6°C)				1.12	0.56–1.68	<b>&lt;0.001</b>			
<i>T</i> (29.2°C)				1.26	0.73–1.80	<b>&lt;0.001</b>			
log( <i>M</i> )× <i>T</i> (28°C)				−0.18	−0.56–0.21	0.052			
log( <i>M</i> )× <i>T</i> (28.6°C)				−0.30	−0.72–0.12	<b>0.035</b>			
log( <i>M</i> )× <i>T</i> (29.2°C)				−0.39	−0.77– −0.00	<b>0.010</b>			
<i>T</i> (29°C)							1.33	0.66–2.00	<b>&lt;0.001</b>
<i>T</i> (29.6°C)							1.36	0.80–1.92	<b>&lt;0.001</b>
<i>T</i> (30.2°C)							1.47	0.94–2.00	<b>&lt;0.001</b>
log( <i>M</i> )× <i>T</i> (29°C)							−0.33	−0.71–0.06	<b>0.024</b>
log( <i>M</i> )× <i>T</i> (29.6°C)							−0.34	−0.69–0.00	<b>0.019</b>
log( <i>M</i> )× <i>T</i> (30.2°C)							−0.45	−0.78– −0.12	<b>0.009</b>
Observations	123			130			122		
<i>R</i> <sup>2</sup> / <i>R</i> <sup>2</sup> adjusted	0.7010.694			0.5450.511			0.5640.537		

Intercept refers to the minimum temperature level.

forecasted temperature rises. The mass-specific SMR of large individuals from the high-latitude population increased significantly with temperature ( $E=0.89$  eV) (similar trend to what was observed for current annual temperature variation), but the thermal response of mass-specific SMR among large individuals from the two lower-latitude populations did not. Indeed, it declined, albeit not significantly (Fig. 3F, Table 6; see Fig. S3 for the  $Q_{10}$  across body-mass classes).

## DISCUSSION

We found that temperature altered the mass dependency of metabolic rate, with the mass scaling exponents of metabolic rate decreasing with temperature similarly across latitudes. We observed that the thermal response of the mass-specific SMR of *G. insensibilis* is also

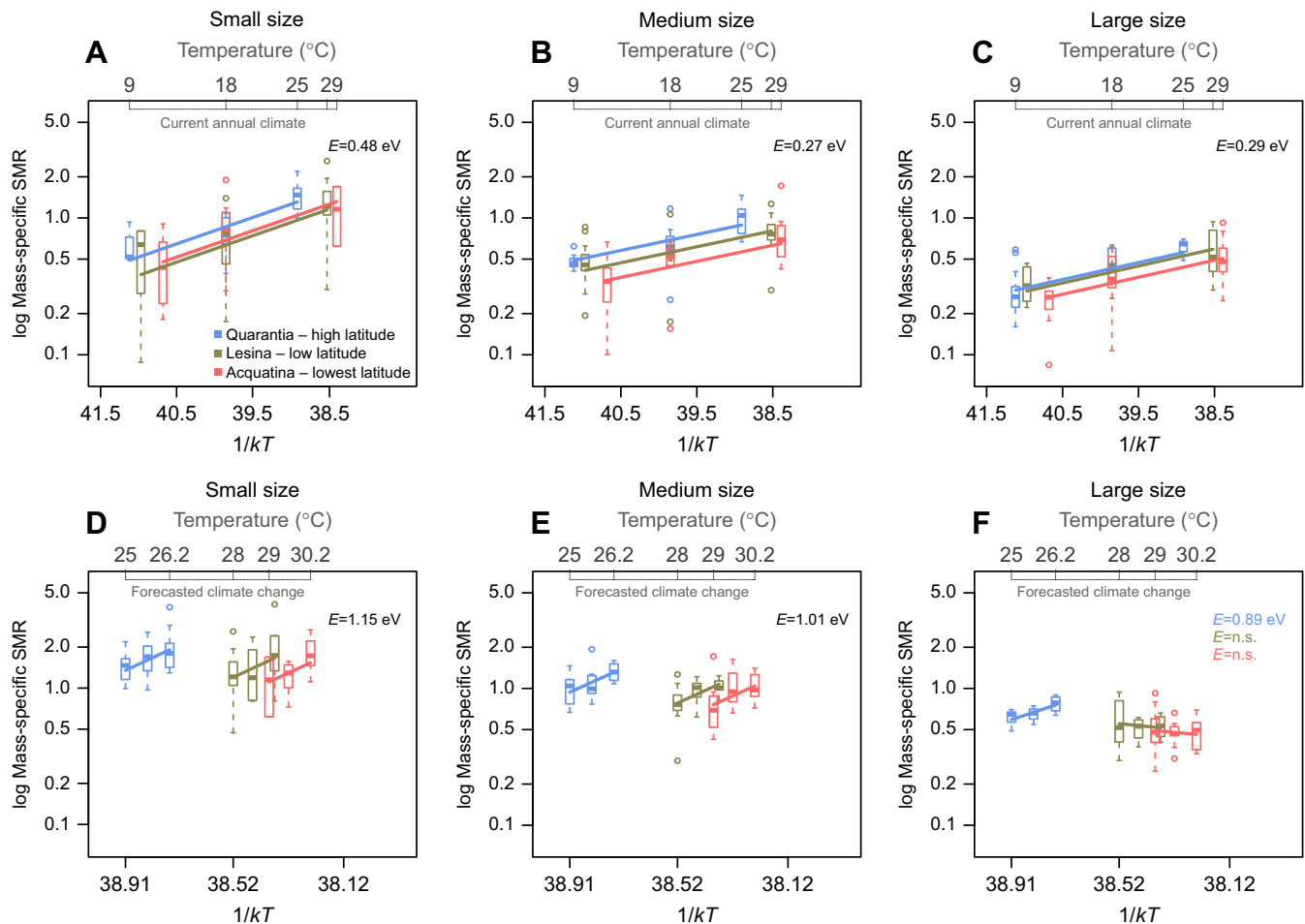
**Table 4. Linear equations for scaling relationships between standard metabolic rate (SMR) and body mass (*M*) across temperature levels at each collection site**

Site	Temperature (°C)	Linear equation
Quarantia – high latitude	9	SMR=0.69 <i>M</i> −0.57
	18	SMR=0.60 <i>M</i> −0.001
	25	SMR=0.46 <i>M</i> +0.57
	25.6	SMR=0.46 <i>M</i> +0.74
	26.2	SMR=0.43 <i>M</i> +0.95
Lesina – low latitude	10	SMR=0.70 <i>M</i> −0.50
	18	SMR=0.57 <i>M</i> −0.14
	28	SMR=0.52 <i>M</i> +0.30
	28.6	SMR=0.39 <i>M</i> +0.61
	29.2	SMR=0.31 <i>M</i> +0.75
Acquatina – lowest latitude	12	SMR=0.79 <i>M</i> −0.94
	18	SMR=0.58 <i>M</i> −0.21
	29	SMR=0.46 <i>M</i> +0.38
	29.6	SMR=0.45 <i>M</i> +0.41
	30.2	SMR=0.35 <i>M</i> +0.52

body-mass/life-stage dependent, with a stronger response (higher activation energy) in small (young) individuals than in medium and large (old) individuals. Within the current annual temperature range, the rise in mass-specific SMR with temperature was consistent across *G. insensibilis* populations within each body-mass class, with a higher mass-specific SMR level (intercept) in the high-latitude population. In contrast, at temperatures reflecting the forecasted climate scenario, although these were higher than the current maxima by only a small margin, i.e. 0.6°C and 1.2°C, we observed clear geographical variation in the mass-specific SMR response of large individuals. As was observed for the current annual climate, under the forecasted conditions, the mass-specific SMR of large individuals from the high-latitude population increased with temperature rise. However, for large individuals from the lower-latitude populations, towards the lower latitudinal edge of the geographical distribution of *G. insensibilis*, the mass-specific SMR in response to the forecasted climate change declined.

## Mass scaling SMRs across temperature and latitudes

We observed that the mass scaling exponents of SMR decreased as temperature rose. This means that larger and older individuals showed a lesser response to temperature than small individuals. This finding is consistent with both observational data (Daufresne et al., 2009; Pauly, 2010; Precht et al., 1973; Rao and Bullock, 1954) and experimental research (Carey and Sigwart, 2014; Glazier, 2020; Hoefnagel and Verberk, 2015), showing that the effect of temperature on SMR is mass dependent. This is in line with the metabolic-level boundaries hypothesis (*sensu* Glazier, 2005, 2014, 2020), which predicts that the elevation (intercept) of metabolic scaling relationships should increase in response to temperature, whereas the scaling exponent (slope) should decrease owing to changes in the relative influence of surface-area- and volume-related metabolic processes (Glazier, 2005, 2014, 2020). Accordingly,



**Fig. 3.** The thermal responses of mass-specific metabolic rate across body-mass classes and latitudes under current annual climate (minimum to maximum temperature) and forecasted climate change (0.6–1.2°C above the current maxima). (A–C) Relationship between mass-specific SMR ( $\text{J day}^{-1} \text{mg}^{-1}$ ) on a logarithmic scale and inverse temperature ( $1/kT$ ), for small (A), medium (B) and large (C) body-mass classes, under current annual climate conditions. (D–F) Relationship between mass-specific SMR on a logarithmic scale and inverse temperature ( $1/kT$ ), for small (D), medium (E) and large (F) body-mass classes under the forecasted climate change scenario. Different colours represent the different populations by location, the boxes show the first, median and third quartiles, and the error bars represent the minimum and maximum values.  $E$  represents the activation energy [scaling exponent of mass-specific metabolic rate against ( $1/kT$ )]. Where the activation energies of different collection sites were not significantly different within each body-mass class, we report the pooled  $E$ . Note that the x-axis with the ( $1/kT$ ) values is inverted, reflecting actual temperature, shown at the top of each graph.

volume-related tissue demand should mainly influence SMR at low metabolic levels, but surface-related resource supply and waste removal should mostly influence SMR at high metabolic levels (Glazier, 2020). It has also been suggested that cold temperatures increase water viscosity and hence the thickness of the boundary layer enveloping the respiratory surfaces of ectotherms, which can

result in lower metabolic rates (Verberk and Atkinson, 2013). This effect is expected to be stronger in small individuals, which are more sensitive to increased viscosity and have more difficulty ventilating at low temperatures than larger individuals (Verberk and Atkinson, 2013). Furthermore, it has been hypothesized that organisms cope with the effect of temperature by upregulating or downregulating

**Table 5.** Current annual temperature range: for each size class, summary of the linear model of individual mass-specific SMR as a response to variation in inverse temperature ( $1/kT$ ) across populations

Predictor	log(mass-specific SMR)								
	Small			Medium			Large		
	Estimate	CI	$P$	Estimate	CI	$P$	Estimate	CI	$P$
Intercept	17.31	11.25–23.37	<0.001	9.91	6.32–13.51	<0.001	10.31	6.86–13.76	<0.001
$1/kT$	0.48	0.43–0.53	<0.001	0.27	0.18–0.36	<0.001	0.29	0.20–0.37	<0.001
Site (Quarantia)	0.22	0.09–0.84	0.038	0.45	0.20–0.71	0.001	0.25	0.03–0.46	0.026
Site (Lesina)	–0.08	–0.41–0.24	0.613	0.25	0.01–0.48	0.042	0.19	–0.01–0.38	0.057
Observations	73			79			86		
$R^2/R^2$ adjusted	0.374/0.347			0.365/0.340			0.370/0.347		

Intercept refers to the site at the lowest latitude (Acquatina). Note that the scaling exponent of the relationship between mass-specific SMR and inverse temperature ( $1/kT$ ), corresponding to activation energy, is sign-reversed.

**Table 6. Forecasted climate: for each size class, summary of the linear model of individual mass-specific SMR as a response to variation in inverse temperature ( $1/kT$ ) across populations**

Predictor	log(mass-specific SMR)								
	Small			Medium			Large		
	Estimate	CI	<i>P</i>	Estimate	CI	<i>P</i>	Estimate	CI	<i>P</i>
(Intercept)	73.73	19.07–128.4	<b>0.010</b>	56.48	9.95–103.01	<b>0.018</b>	–16.92	–71.48–37.63	0.098
$1/kT$	1.15	0.16–2.14	<b>0.009</b>	1.01	0.09–1.94	<b>0.018</b>	–0.42	–1.85–1.00	0.103
Site (Quarantia)	1.30	0.51–2.08	<b>0.003</b>	1.04	0.36–1.72	<b>0.003</b>	68.04	–23.31–159.39	<b>0.007</b>
Site (Lesina)	0.42	0.05–0.79	<b>0.030</b>	0.29	0.03–0.56	<b>0.027</b>	–1.54	–88.00–84.91	0.525
$1/kT \times$ Site (Quarantia)							1.31	–1.32–3.95	<b>0.017</b>
$1/kT \times$ Site (Lesina)							–0.08	–2.31–2.16	0.525
Observations	83			74			74		
$R^2/R^2$ adjusted	0.323/0.295			0.273/0.237			0.281/0.258		

Intercept refers to the site at the lowest latitude (Acquatina). Note that the scaling exponent of the relationship between mass-specific SMR and inverse temperature ( $1/kT$ ), corresponding to activation energy, is sign-reversed.

metabolic rates when growing in cold or warm environments, respectively (Fossen et al., 2019). All of these hypotheses, which are supported by several studies (Gjoni et al., 2020; Glazier, 2020; Killen et al., 2010; Kordas et al., 2022; Lindmark et al., 2018), suggest that temperature effects on the mass dependency of individual metabolism may be widespread in nature.

We observed substantial overall individual variation in SMR, as reported in many other studies of amphipods (Glazier, 2020; Glazier et al., 2011; Semsar-kazerouni and Verberk, 2018; Shokri et al., 2019). This might be due to experimental error, intrinsic factors such as cell size/number (Glazier, 2022), and the effects of ecology or specimens' lifestyles (Careau et al., 2008; Cozzoli et al., 2020; Killen et al., 2016).

### The thermal sensitivity of mass-specific SMR across body-mass classes and latitudes

#### Current annual climate

In contrast to the MTE, which predicts a relatively constant thermal sensitivity of metabolic rate for all body masses within a range of 0–40°C, we observed that the thermal responses of mass-specific SMRs were dependent on individual body mass and life stage. Within current temperature variation, temperature-induced increases in mass-specific metabolic rates were less pronounced in large ( $E=0.29$  eV) and medium individuals ( $E=0.27$  eV) than in small individuals ( $E=0.48$  eV). This is in line with several empirical studies showing that the thermal sensitivity of metabolic rate decreases with body mass and life stage, e.g. in fish (Job, 1957; Silva-Garay and Lowe, 2021), invertebrates (Kordas et al., 2022; Schwartzkopff, 1955; Vernberg, 1959) and planktonic crustaceans (Fossen et al., 2019). Moreover, our observations highlighted geographical variation in mass-specific SMR within each body-mass class under current annual temperatures, with individuals from the high-latitude population, which experienced a more variable climate (see Fig. 1B), showing elevated mass-specific metabolic rates (higher intercepts). In line with our observations, several experimental studies have shown that organisms living in a more variable climate have higher metabolic rates (Magozzi and Calosi, 2015; Sokolova and Pörtner, 2003). Other studies have shown that populations inhabiting a higher latitude often with a colder climate also have higher metabolic rates than populations living at lower latitudes with a warmer climate (Chown et al., 1997; James, 1970; White et al., 2012; see also Terblanche et al., 2009).

#### Forecasted climate change

At temperatures reflecting global warming, our measurements showed that the thermal response of the mass-specific SMRs of

*G. insensibilis* varied in accordance with body mass/life stage and geographical distribution. As was observed under current climate conditions, the mass-specific SMR of small ( $E=1.15$  eV) and medium-sized individuals ( $E=1.01$  eV) of all populations increased in response to a 1.2°C rise above the current maximum summer temperature.

However, the response of large individuals to the forecasted temperature rise differed with latitude. Among large individuals from the lower-latitude populations, i.e. those inhabiting areas near the lower latitudinal boundary of the species' distributional range, the mass-specific SMR declined and reached the peak of their performance at temperatures above the current maxima. The inability of large individuals in the lower-latitude populations to raise their metabolic rate in line with temperature beyond the current annual maximum illustrates the functional limits of their metabolic enzymes. This indicates that future warming under more extreme scenarios could impair the performance of such enzymes, threatening the integrity of membranes, leading to a decline in metabolic rates and thereafter organism death (DeLong et al., 2017; Schulte, 2015).

Our finding also underlines that larger individuals inhabiting the lower latitudinal boundary of their distributional range were subject to metabolic homeostasis (*sensu* Precht et al., 1973), in which their metabolic rates were independent of temperature beyond the current local maxima. Several studies have shown that when facing changes in temperature, often beyond that of their usual environment (Coggins et al., 2021), metabolic homeostasis might occur in aquatic ectotherms (Bullock, 1955; Fanguie et al., 2009; Precht et al., 1973; Seibel et al., 2007; Young, 1979). This results in a degree of homeostasis at the cellular level, enabling organisms to maintain their functions in spite of temperature change (Precht et al., 1973). Homeostasis mechanisms are likely to occur where metabolically important substances would become a limiting factor following a rise in metabolic rates with temperature (Precht et al., 1973; Young, 1979). In aquatic organisms, the limiting factor of metabolic rate at the upper thermal limit is the availability of oxygen (Precht et al., 1973; Verberk et al., 2016b), because respiratory demands increase with temperature while oxygen availability and oxygen transport efficiency drop (Boardman and Terblanche, 2015; Verberk et al., 2016b). At high temperatures or under intense physical activity, in aquatic ectotherms, the oxygen supply can no longer meet the increase in oxygen demand, at which point the metabolic rate approaches a limit and its sensitivity to temperature is reduced (Rubalcaba et al., 2020). Because of their higher absolute oxygen demands, larger aquatic ectotherm individuals are likely to



experience greater oxygen limitation at high temperatures, which thus reduces aerobic scope and lowers the thermal limit (Atkinson et al., 2006; Lindmark et al., 2018; Rubalcaba et al., 2020; Verberk et al., 2011).

In contrast to the lower-latitude populations, among large individuals from the higher-latitude population with a lower mean temperature and higher thermal variability, the mass-specific SMR continued to increase ( $E=0.89$  eV) at temperatures above the current maxima. This implies that individuals across all mass classes of the higher-latitude population did not reach their thermal limits at the forecasted temperatures. In line with our observation, Rohr et al. (2018) observed that thermal tolerance increases with latitude. Physiological plasticity is often proportional to the degree of variation in local temperature that a species experiences in its original habitat: populations inhabiting more variable thermal environments are expected to have broader thermal tolerance (Rohr et al., 2018) and higher plasticity (Sun et al., 2022) than those inhabiting more temperate environments (but see Seebacher et al., 2015). This implies that populations from lower latitudes with more constant climates are more sensitive to temperature abnormalities because they also live closer to their thermal and physiological limits (Sunday et al., 2011; Thyrring et al., 2020).

In this regard, our findings show that the larger conspecifics living at lower latitudes, owing to either the limitation of the performance of metabolic enzymes or the availability of oxygen, could be the first to experience the negative impacts of future warming on performance and other metabolism-related processes. This adds to growing evidence that physiological constraints at warmer temperatures reduce the performance of large-bodied individuals to a greater degree than their small-bodied conspecifics (Lindmark et al., 2022). This in turn affects population size structure in the face of climate change, particularly in populations living at the edge of their physiological tolerance (Huss et al., 2019; Neuheimer et al., 2011).

It is important to bear in mind that although temperature manipulation experiments to test and develop the relevant theoretical frameworks and climate-based models are clearly needed, uncertainties that might affect the interpretation of the findings remain. The main limitation of our study was that the thermal acclimation period (here serving merely to minimize stress and thermal shock) was much shorter than the acclimatization of organisms in the face of climate change. In nature, animals may have a far longer period to cope with predicted climate change and may offset this effect by means of either phenotype plasticity or genotype evolution. In addition, in order to maintain functionality, ectothermic animals may seek refuge or migrate to a cooler place (behavioural buffering) as ambient water temperatures begin to exceed their upper thermal tolerance limits. Long-term experimental studies of metabolic rates involving the manipulation of climate change scenarios are an important research priority as part of efforts to develop our understanding of underlying mechanisms in the face of climate change.

In conclusion, in this study, we provide further evidence that a marginal temperature increase of 1.2°C under future climate change scenarios impacts the SMR of individuals and populations, and thus even the most conservative IPCC forecast seems likely to affect ecosystem functioning. Given the evidence for interactive temperature–mass effects on metabolic rate and their dependence on latitude, caution must be exercised, especially when predicting population responses to climate change, which have profound implications for all metabolism-related processes.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: M.S., F.C., A.B.; Methodology: M.S., F.V.; Validation: M.S., F.C., A.B.; Formal analysis: M.S.; Investigation: M.S.; Writing - original draft: M.S.; Writing - review & editing: M.S., F.C., F.V., M.B., E.P., A.B.; Supervision: A.B.; Funding acquisition: A.B.

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#### Data availability

Data are publicly accessible via the Open Science Framework at doi:10.17605/OSF.IO/56TNN.

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