

# Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming

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## Abstract

Predicting species vulnerability to global warming requires a comprehensive, mechanistic understanding of sublethal and lethal thermal tolerances. To date, however, most studies investigating species physiological responses to increasing temperature have focused on the underlying physiological traits of either acute or chronic tolerance in isolation. Here we propose an integrative, synthetic approach including the investigation of multiple physiological traits (metabolic performance and thermal tolerance), and their plasticity, to provide more accurate and balanced predictions on species and assemblage vulnerability to both acute and chronic effects of global warming. We applied this approach to more accurately elucidate relative species vulnerability to warming within an assemblage of six caridean prawns occurring in the same geographic, hence macroclimatic, region, but living in different thermal habitats. Prawns were exposed to four incubation temperatures (10, 15, 20 and 25 °C) for 7 days, their metabolic rates and upper thermal limits were measured, and plasticity was calculated according to the concept of Reaction Norms, as well as  $Q_{10}$  for metabolism. Compared to species occupying narrower/more stable thermal niches, species inhabiting broader/more variable thermal environments (including the invasive *Palaemon macrodactylus*) are likely to be less vulnerable to extreme acute thermal events as a result of their higher upper thermal limits. Nevertheless, they may be at greater risk from chronic exposure to warming due to the greater metabolic costs they incur. Indeed, a trade-off between acute and chronic tolerance was apparent in the assemblage investigated. However, the invasive species *P. macrodactylus* represents an exception to this pattern, showing elevated thermal limits and plasticity of these limits, as well as a high metabolic control. In general, integrating multiple proxies for species physiological acute and chronic responses to increasing temperature helps providing more accurate predictions on species vulnerability to warming.

**Keywords:** climate change, invasive species, phenotypic buffering, phenotypic plasticity, physiological abilities, species distribution

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## Introduction

Changes in latitudinal, altitudinal and bathymetric distribution caused by global warming have been increasingly documented across terrestrial and aquatic taxa (e.g., Southward *et al.*, 1995; Menendez & Gutierrez, 1996; Parmesan & Yohe, 2003; Root *et al.*, 2003; Perry *et al.*, 2005). In general, understanding the mechanistic basis of sublethal and lethal thermal tolerance (e.g., Pörtner, 2001; Helmuth *et al.*, 2005) allows the prediction of species responses under future warming scenarios, and scaling up predictions to assemblages and ecosystems (Bernardo *et al.*, 2007; Somero, 2010, 2011).

To date, however, most studies investigating the causal mechanisms underpinning species vulnerability to warming have focused on physiological traits in isolation (e.g., Stillman & Somero, 2000; Pörtner & Knust, 2007; Calosi *et al.*, 2008; Bartolini *et al.*, 2013).

The frequency and intensity of extreme acute thermal events are predicted to increase with the global change (IPCC *et al.*, 2012). Species short-term resilience to these acute thermal events will depend on their upper thermal limits, as well as on their ability to adjust these limits, when exposed to higher temperatures, through phenotypic plasticity (e.g., acclimatization). Indeed, phenotypic plasticity may allow organisms to express broader thermal tolerance windows (Ghalambor *et al.*, 2007; Charmantier *et al.*, 2008; Bozinovic *et al.*, 2011). In the longer term, plasticity may define species scope for

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resilience to change *via* phenotypic buffering (Waddington, 1942; Bradshaw, 1965), and, in part, species scope for adaptation *via* genetic assimilation (Pigliucci *et al.*, 2006). Conventionally, plasticity of thermal limits has been studied by characterizing the magnitude of Reaction Norms (Schlichting & Pigliucci, 1998 and ref. therein). However, no comparative study has, so far, focused on both the magnitude and shape of plasticity (*sensu* Schlichting & Pigliucci, 1998; Pigliucci, 2001; c.f. Murren *et al.*, 2014).

While tolerance to heat is generally conserved across lineages (Araújo *et al.*, 2013), some species appear to have evolved extreme upper thermal limits at the expense of plasticity of these limits, reflecting an evolutionary trade-off between these traits (Stillman, 2002, 2003; see also Angilletta *et al.*, 2003). The most heat-tolerant taxa may, therefore, be at greater risk from warming (Stillman, 2003; Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; see also Araújo *et al.*, 2013; Overgaard *et al.*, 2014; Peck *et al.*, 2014), not only because they possess reduced safety margins (*sensu* Stillman, 2002; Deutsch *et al.*, 2008; see also Araújo *et al.*, 2013; Diederich & Pechenik, 2013; Overgaard *et al.*, 2014), but also because their scope for plasticity is more limited (Stillman, 2003; c.f. Calosi *et al.*, 2008; Bozinovic *et al.*, 2011).

While upper thermal limits define species ability to persist under extreme acute thermal events, physiological performances (*sensu* Bozinovic *et al.*, 2011), such as metabolic rates, mediate species resilience to chronic exposure to warming. Metabolic rate is suggested to reflect the energetic cost of adaptation to a particular thermal environment (Clarke, 2004; Clarke & Fraser, 2004; see also Watson *et al.*, 2013), rather than a purely mechanistic response to temperature. In this sense, temperature imposes a high selective pressure on maximum physiological performances: i.e., the evolution of high metabolic rates may allow organisms to exploit a broader range of environmental temperatures, but also implies higher maintenance costs. As a consequence, species living in different thermal habitats may have evolved different levels of metabolic control, suggesting different levels of vulnerability to warming (Sokolova & Pörtner, 2003; Morley *et al.*, 2009; Dillon *et al.*, 2010; Rastrick & Whiteley, 2011; Watson *et al.*, 2013). Again, while most studies to date have investigated physiological traits in isolation (e.g., Compton *et al.*, 2007; Calosi *et al.*, 2010; Rastrick & Whiteley, 2011), a more holistic approach integrating the investigation of multiple physiological traits (thermal limits and metabolic rate), and their plasticity (magnitude and shape) (Bozinovic *et al.*, 2011; Murren *et al.*, 2014) needs to be developed to more accurately elucidate species vulnerability to global warming.

Here we integrate the investigation of metabolic performance, thermal tolerance, and their plasticity to provide more accurate and balanced predictions on relative species vulnerability to both chronic and acute effects of warming. To provide an empirical test for this new paradigm, we applied our integrative, synthetic approach to an assemblage of six caridean prawns occurring in the same geographic, hence macroclimatic, region, but living in different thermal habitats. Three species inhabit broader/more variable thermal environments (including the invasive species *Palaemon macrodactylus*), and three species live in narrower/more stable thermal conditions. On the basis of the current literature (e.g., Stillman, 2002, 2003; Deutsch *et al.*, 2008; Dillon *et al.*, 2010; see also Folguera *et al.*, 2009; Bozinovic *et al.*, 2013; Peck *et al.*, 2014; Rezende *et al.*, 2014), we hypothesize that, compared to species occupying narrower/more stable thermal niches, species inhabiting broader/more variable thermal environments possess: (i) higher upper thermal limits, (ii) lower plasticity of these limits, (iii) higher metabolic rates, (iv) higher metabolic plasticity, and (v) higher metabolic costs at higher temperatures. As a consequence, species living in broader/more variable thermal environments may be less vulnerable to extreme acute thermal events, but at greater risk from chronic exposure to warming (Stillman, 2002, 2003; Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; see also Folguera *et al.*, 2009; Overgaard *et al.*, 2014), according to the idea that evolutionary trade-offs may exist between these two forms of heat tolerance (Rezende *et al.*, 2014).

Caridean prawns are both ecologically and economically important. They represent a large fraction of biomass of coastal shallow water assemblages of invertebrates (e.g., Bechmann *et al.*, 2011), and exert a great ecological impact upon benthic trophic webs as either carnivores or detritivores (Pihl & Rosenberg, 1984; Henderson, 1987; Oh *et al.*, 2001). Also, some species are targets of commercial and artisanal fisheries (e.g., *Crangon crangon*, Attrill & Thomas, 1996; Henderson *et al.*, 2006).

## Materials and methods

### *Specimen description, collection, incubation, and maintenance*

We investigated six caridean shallow water prawn species: *Palaemon elegans* (Rathke 1837), *Palaemon macrodactylus* (Rathbun 1902), *Palaemon serratus* (Pennant 1777) and *Palaemonetes varians* (Leach 1814) (Palaemonidae), *Crangon crangon* (Linnaeus 1758) (Crangonidae), and *Pandalus montagui* (Leach 1814) (Pandalidae). These species all occur in the same geographic, hence macroclimatic, region, but live in different thermal

habitats. *Palaemon elegans* and *P. varians* occupy broader/more variable thermal niches, inhabiting intertidal rock pools and salt marshes respectively. *Palaemon serratus*, *C. crangon* and *P. montagui* occupy narrower/more stable thermal niches, living in subtidal habitats. The invasive species *P. macrodactylus* also lives in subtidal habitats, but withstands a broader range of thermal conditions, which possibly explains its recent geographical expansion (Spivak *et al.*, 2006; Lavesque *et al.*, 2010; Soors *et al.*, 2010).

Adult individuals of each species were collected at four locations along the English Channel on the South coast of England (for specific details see Table S1). After collection, individuals were transported to the laboratory in plastic containers with water from the collection site within 24–48 h. The water was continuously aerated, and the temperature was measured approx. every 30 min (max. fluctuations ~0.5 °C).

Once in the laboratory specimens were transferred to tanks (approx. 4.6 l, max. 10 ind. *per* tank) supplied with fully aerated sea water (salinity 33), and kept at their collection temperature for 24 h to adjust to laboratory conditions. Subsequently, individuals were haphazardly divided into four equal-size groups: 20 for *P. elegans*, seven for *P. macrodactylus*, eight for *P. serratus*, 25 for *P. varians*, 28 for *C. crangon*, and eight for *P. montagui*. Each group was exposed to one of four incubation temperatures (10, 15, 20 and 25 °C) for 7 days. This exposure period is considered to be sufficiently long to acclimate temperate species at 15–20 °C, but short enough to prevent the onset of longer-term negative effects due to too much time spent in the laboratory (e.g., Terblanche *et al.*, 2006; Calosi *et al.*, 2008, 2010). Incubation temperatures were selected within the temperature range experienced by the species across their geographical ranges, as well as potential future warming scenarios (IPCC, 2014). Specimens were incubated stepwise in constant-temperature (CT) rooms (12 : 12 h L/D regime), starting from their collection temperature. At each step aquaria were ramped to the next temperature level, and kept at these conditions for 24 h before being further ramped to the next level, with temperature increasing/decreasing with a rate of approx.  $0.015 \pm 0.005$  °C min<sup>-1</sup> (mean  $\pm$  SD). Once they had reached the desired incubation temperature, aquaria were kept at these conditions for 1 week, with a maximum water temperature fluctuation of approx. 0.6 °C.

Over the exposure period, specimens were fed daily *ad libitum* with marine flakes (New Era Aquaculture Ltd, Thorne, UK). Water changes were performed every 2 days to prevent excreta accumulation, and undesired fermentation and decomposition of leftover food. Once the exposure period was completed, metabolic rate and upper thermal limits were determined for each individual.

#### Determination of metabolic rate

Oxygen consumption rate ( $\dot{M}O_2$ ) under resting conditions was used as a proxy for Standard Metabolic Rate (SMR), as in Spicer & Eriksson (2003) and Small *et al.* (2010).  $\dot{M}O_2$  was measured in glass closed cell experimental chambers. Each chamber was supplied with fully aerated sea water at the selected incubation temperature and sealed underwater to

prevent air bubbles trapping in the chamber. Furthermore, each chamber was equipped with a magnetic flea, which was shielded on the bottom by a perforated Petri dish, and placed over a multi-channel magnetic stirrer (MS-53M, Jeio Tech, Chalgrove, UK) to ensure moderate and continuous water mixing, preventing the formation of a hypoxic layer around the prawn. Also, to provide a substrate to reduce prawn activity levels, each chamber was supplied with one or two marbles (42 mm  $\times$  42 mm  $\times$  16 mm), depending on specimen dimensions, resulting in a volume of water of either 190.3 or 218.4 ml. Additionally, to monitor prawn behaviour in the chambers, subsamples of six ind. for each species at each incubation temperature were filmed with cameras. All the specimens were quiescent during the experimental trials, and no major changes in behaviour were observed at elevated incubation temperatures (data are not presented here);  $\dot{M}O_2$  could, therefore, be effectively used as proxy for SMR. Each prawn was introduced into an experimental chamber and allowed to recover from handling and to settle into experimental conditions for 30 min (Small *et al.*, 2010; Magozzi and Calosi *pers. obs.*). The chambers were then closed and maintained sealed for experimental trials of 1.5–2 h. Preliminary tests defined this period to be sufficiently long to undertake  $\dot{M}O_2$  measurements, while preventing prawn exposure to hypoxic conditions: i.e., oxygen saturation was never allowed to fall under 70% (Vandonk & Dewilde, 1981; Small *et al.*, 2010; Oliphant *et al.*, 2011), with oxygen concentration remaining always  $>190$   $\mu\text{mol O}_2 \text{ l}^{-1}$ . To avoid sharp thermal variations, respiration chambers were kept in CT rooms inside temperature-controlled open water baths at the selected incubation temperature during the experimental trial. Oxygen concentration was measured both immediately before closing the chamber and immediately after reopening it at the end of the experimental trial by using an O<sub>2</sub> electrode (1302, Strathkelvin Instruments, Glasgow, Scotland) connected to a calibrated oxygen meter (929, Strathkelvin Instruments), and expressed as nmol O<sub>2</sub> g wet weight<sup>-1</sup> min<sup>-1</sup> STP. Before the experiments started, calibration was carried out at each incubation temperature by using 0 and 100% O<sub>2</sub> saturation as calibration points.

After  $\dot{M}O_2$  measurements were completed, wet weight (g) and body volume (mL) of each prawn were measured and then used for correction of the  $\dot{M}O_2$  values. Weight was determined by using an electronic high precision scale (PF-203, Fischer Scientific UK Ltd, Loughborough, UK), and specimen volume was determined by immersing each prawn in a Pyrex graduated cylinder (100 ml, accuracy 1 ml, Fischer Scientific UK Ltd) supplied with sea water, and measuring the water displaced by the introduction of the prawn. All individuals were returned to their aquaria, at their incubation temperature, each inside a numbered perforated screw top transparent container (either 82.0 or 149.0 ml, depending on specimen dimensions), and left to recover for 24 h before upper thermal limits were determined.

#### Determination of upper thermal limits

To measure Upper Thermal Limits (UTL) a number of observable responses were identified during preliminary tests and

used as end-points, as in Calosi *et al.* (2008) and Massamba-N'siala *et al.* (2012). A temporal sequence of responses to increasing temperature was identified as follows: (i) Mouth Gaping (MG) (wide and continuous opening of the mouth parts); (ii) Tail Flipping (TF) (fast upside down flip of the tail bringing abdomen and cephalothorax towards one another) (Arnott *et al.*, 1998); (iii) Loss of Orientation (LO) (inability of a prawn to right itself after having turned onto a side or its dorsal surface) (Brattstrom, 1968; Hopkin *et al.*, 2006; Oliphant *et al.*, 2011); (iv) Onset of Spasms (OS) (first uncontrolled, convulsive and spasmodic movements) (Zweifel, 1957; Lutterschmidt & Hutchison, 1997; Hopkin *et al.*, 2006); (v) Death (D) or total paralysis (prawn laying on the bottom of the experimental well for more than 15 s with no movement/pleopod beating).

However, not all individuals showed this complete sequence of end-points: 94.5, 90.7, and 100% of the experimental prawns showed LO, OS and D respectively, while only 79.3 and 64.8% of them showed MG and TF. Also, compared to MG and TF, LO, OS and D showed lower variance. Therefore, only LO, OS and D were assumed to represent temperature-induced mechanisms underpinning the failure of fundamental physiological functions (Lutterschmidt & Hutchison, 1997). However, to avoid redundant utilization of these end-points, correlation analyses among them were performed to include only functionally independent traits. As there were significant positive relationships between LO and OS, OS and D, and LO and D (minimum  $r_{263} = 0.840$ ,  $P < 0.0001$ ), here we mainly focused on OS, which more closely fulfils the original definition of critical thermal maximum (CT<sub>max</sub>): 'the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death' (Cowles & Bogert, 1944; see also Lutterschmidt & Hutchison, 1997).

Experiments to determine UTL were started at the temperature at which individuals of a given group had been incubated during the exposure period, and carried out by employing a ramping program, with temperature increasing at a rate of  $0.75\text{ }^{\circ}\text{C min}^{-1}$  (realised  $+ 0.668 \pm 0.016\text{ }^{\circ}\text{C min}^{-1}$ ; mean  $\pm$  SE) (Lutterschmidt & Hutchison, 1997; Rezende *et al.*, 2011; c.f. Overgaard *et al.*, 2012) performed with a computer-controlled water bath (R5, Grant Instruments Cambridge Ltd, Shepreth, UK). Each prawn was removed from its individual aquarium using a small net and rapidly, but carefully, introduced into one well (diam. 51 mm, depth 65 mm) of a generic six-well plate, whose bottom surface was painted white with Tipp-Ex<sup>®</sup> to allow an easier and more accurate visualization of the end-points. A maximum of five individuals were tested at any time and, to avoid observer biases (Terblanche *et al.*, 2007), measurements were all undertaken by one single observer (S.M.). To avoid prawn escaping, wells were covered with a lid between additions of individuals; once the experiment started, the lid was removed to allow full aeration. The actual temperature was measured every 60 s with a digital thermometer (HH802U, Omega<sup>®</sup> Engineering Inc., Stamford, USA) placed in an empty well adjacent to the prawn wells to avoid disturbance.

### Definition and calculation of plasticity

Here plasticity is intended as the ability of specimens to adjust their metabolic performance (measured as  $\dot{M}O_2$ ) and thermal tolerance (measured as UTL) following incubation to increasing temperature. Plasticity was determined according to the concept of Reaction Norms (Schlichting & Pigliucci, 1998 and ref. therein; Pigliucci, 2001) (Figure S1), as well as  $Q_{10}$  for metabolism. To determine both the magnitude and shape of the plastic response (*sensu* Pigliucci, 2001; Murren *et al.*, 2014), plasticity was calculated both within the whole temperature range examined ( $P_{\text{tot}}$ ) and within smaller temperature intervals (10–15, 15–20 and 20–25  $^{\circ}\text{C}$  –  $P_{10-15}$ ,  $P_{15-20}$ ,  $P_{20-25}$ , respectively) (Figure S1).  $P_{\text{tot}}$  was calculated as the difference between mean values of either  $\dot{M}O_2$  or UTL measured at the two extreme temperature treatments (10–25  $^{\circ}\text{C}$  –  $P_{10-25}$ ). To include *P. montagu* in the computation,  $P_{\text{tot}}$  was also calculated between 10 and 20  $^{\circ}\text{C}$  ( $P_{10-20}$ ), as this species had no replicates at 25  $^{\circ}\text{C}$  due to 100% mortality at this temperature (Table S1; see also Results).  $P_{10-15}$ ,  $P_{15-20}$  and  $P_{20-25}$  were calculated as the difference between mean  $\dot{M}O_2$  and mean UTL measured at two consecutive temperature treatments. This complementary calculation allowed not only the quantification of the magnitude of plasticity within smaller temperature intervals, but also the description of the shape of Standard Metabolic Rate–Temperature (SMR – T) and Upper Thermal Limits–Temperature (UTL – T) Reaction Norms, providing a more comprehensive understanding of plastic responses and highlighting between-species differences (Schlichting & Pigliucci, 1998; Pigliucci, 2001; Murren *et al.*, 2014). In addition, we calculated the temperature coefficient for the change in  $\dot{M}O_2$  with temperature ( $Q_{10}$ ) both within the whole temperature range (considering as extreme temperatures 10 and either 20 or 25  $^{\circ}\text{C}$ ) and within smaller temperature intervals. While the calculation of  $Q_{10}$  values enables the distinction between acclimation-induced changes in metabolism and just an expected physiological response to temperature, it does not allow an appropriate interpretation of the increase in energy expenditure associated with metabolic plastic responses to increasing temperature. Because we wish to consider the energetic implications of the metabolic plastic response, as well as to compare metabolic plasticity with that of thermal limits, here we mainly focus on Reaction Norms.

### Characterization of phylogenetic relationships among species

Sequences were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) (see Table S2 for accession numbers). The pool shrimp *Procaris ascensionis* (Chace & Manning 1972) and the banded cleaner shrimp *Stenopus hispidus* (Olivier 1811) were used as outgroups. Concatenated sequences were aligned using the ClustalW (Thompson *et al.*, 1994) algorithm within MEGA 5.05 (Tamura *et al.*, 2011). The partition homogeneity test, otherwise known as the incongruence length difference test (Farris *et al.*, 1994) was carried out in PAUP\* 4.b.10 (Swofford, 2002) to assess if the data were significantly incongruent. The test was implemented using maximum parsimony



heuristic searches (100 replicates). All other settings were left at their default values. The results of this test showed no significant incongruence between genes ( $P = 0.96$ ). Phylogenetic reconstruction was carried out using maximum likelihood (ML) as implemented in MEGA with all settings left as their default options. Support was measured with 1000 bootstrap replicates. Only clades with significant support values (defined here as  $\geq 60$  bootstrap) are shown.

Our analysis highlights that, among palaemonid species, *P. elegans* and *P. serratus* are the most phylogenetically closely related (Figure S2), with *P. varians* being more closely related to these two species than *P. macrodactylus*. *Crangon crangon* and *P. montagui* are more closely related to each other than to palaemonid species, although the reliability of their relationship is relatively low. In general, it appears that ecological competence (here defined as type of thermal habitat) is not phylogenetically confounded.

### Statistical analyses

The effects of species, incubation temperature, and their interaction on  $\dot{M}O_2$  and UTL were analysed by using a two-way ANCOVA test with 'Tank' as a random factor nested within 'Species'  $\times$  'Temperature', and 'Wet weight' as a covariate. Pairwise comparisons were based on model-estimated marginal means with Least Significant Difference test correction ( $\alpha = 0.05$ ). Data for  $\dot{M}O_2$  and UTL were non-normally distributed even following various transformations (minimum  $K-S_{274} = 1.466$ ,  $P = 0.027$ ), and variances were not homogeneous (minimum  $F_{2,252} = 2.383$ ,  $P = 0.001$ ). However, as our experimental design included 24 'Species' by 'Temperature' combinations with a minimum of seven replicates *per* treatment, the ANCOVA design was assumed to be tolerant from the assumption of normality and heteroscedasticity (Sokal & Rohlf, 1995; Underwood, 1997; see also Melatunan *et al.*, 2011). The term 'Tank' did not have a significant effect on  $\dot{M}O_2$  and UTL both among species and temperature treatments (maximum  $F_{2,177} = 2.869$ ,  $P = 0.060$ ), therefore it was removed from further analyses.

In addition, a best-fit approach was used to select regression models – considering linear, logarithmic, quadratic, cubic, power and exponential methods – to best describe the relationships between  $\dot{M}O_2$ , UTL, and their plasticity. However, when the difference in the regression coefficients ( $R^2$ ) was  $\leq 1$ , simpler relationships were favoured using a maximum parsimony approach. All analyses were conducted using IBM SPSS Statistics 19.

## Results

### Metabolic rate

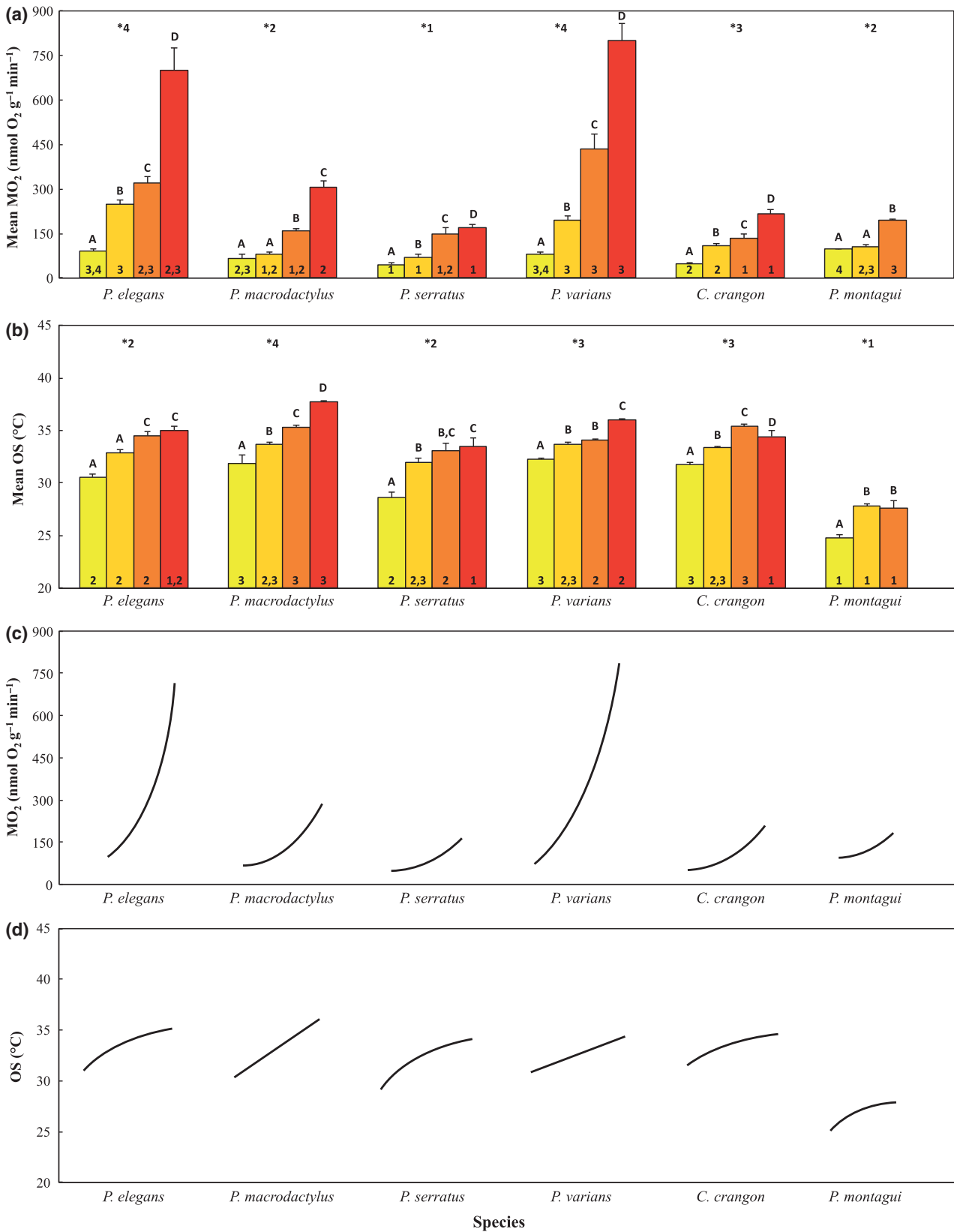
Means  $\pm$  SE for oxygen consumption rate ( $\dot{M}O_2$ ) are given in Fig. 1a and Table S3. The minimum mean  $\dot{M}O_2$  was observed in *P. serratus* incubated at 10 °C ( $46.1 \pm 7.1$  nmol  $O_2$  g wet weight $^{-1}$  min $^{-1}$ ), while the maximum was observed in *P. varians* incubated at

25 °C ( $799.9 \pm 56.7$  nmol  $O_2$  g wet weight $^{-1}$  min $^{-1}$ ). In general, greater  $\dot{M}O_2$  was observed at higher incubation temperatures in all species. Nevertheless,  $\dot{M}O_2$  response to increasing temperature was significantly different in different species (Fig. 1a), as indicated by the presence of a significant interaction between 'Species' and 'Temperature' ( $F_{14,299} = 7.1$ ,  $P < 0.0001$ ) (Table 1). At the lowest temperature tested (10 °C), *P. serratus* showed significantly lower mean  $\dot{M}O_2$  than all the other species, while *P. montagui* exhibited significantly higher mean  $\dot{M}O_2$  (being comparable to *P. elegans* and *P. varians*). *Crangon crangon* and *P. macrodactylus* showed intermediate mean  $\dot{M}O_2$  between *P. elegans*, *P. serratus* and *P. varians*, with *P. macrodactylus* being comparable to *P. elegans* and *P. varians* (Fig. 1a). At the highest temperature tested (25 °C), *P. elegans* and *P. varians*, followed by *P. macrodactylus*, showed significantly higher mean  $\dot{M}O_2$  than *P. serratus* and *C. crangon*. Since no individuals of *P. montagui* survived exposure to 25 °C, the highest temperature tested for this species was 20 °C. At this temperature, *P. montagui* showed significantly higher mean  $\dot{M}O_2$  than the other subtidal species (*C. crangon*, *P. serratus* and *P. macrodactylus*), being statistically comparable to *P. elegans* and *P. varians* (Fig. 1a). Wet weight had a positive significant effect on  $\dot{M}O_2$  ( $F_{1,299} = 166.3$ ,  $P < 0.0001$ ) (Table 1).

### Upper thermal limits

Means  $\pm$  SE for Upper Thermal Limits (UTL), measured as Onset of Spasms (OS), are given in Fig. 1b and Table S3. Means  $\pm$  SE for UTL, measured as Loss of Orientation (LO) and Death (D), are also given in Figure S3a, b and Table S3. The minimum mean UTL (for all end-points) was observed in *P. montagui* incubated at 10 °C (LO:  $23.6 \pm 0.2$  °C; OS:  $24.8 \pm 0.3$  °C; D:  $27.1 \pm 0.4$  °C), while the maximum was observed in *P. macrodactylus* incubated at 25 °C (LO:  $35.7 \pm 0.4$  °C; OS:  $37.8 \pm 0.5$  °C; D:  $39.9 \pm 0.2$  °C).

While in general higher UTL were observed at higher incubation temperatures in all species, UTL response to increasing temperature was significantly different in different species (minimum  $F_{14,275} = 2.2$ ,  $P = 0.010$ ) (Fig. 1b and Figure S3a, b) (Table 1). At 10 °C, *P. macrodactylus*, *P. varians* and *C. crangon* exhibited significantly higher mean OS than *P. elegans* and *P. serratus*, which in turn showed significantly higher mean OS than *P. montagui* (Fig. 1b). Between-species differences identified for OS were maintained also for LO and D with two exceptions: (i) *P. elegans* showed significantly higher mean LO than *P. serratus*, being comparable to *P. macrodactylus* and *P. varians* (Figure S3a); (ii) *P. varians* showed significantly higher mean D not only than



*P. elegans* but also than *P. macrodactylus* and *C. crangon* (Figure S3b). At 25 °C, *P. macrodactylus* showed significantly higher mean UTL (for all end-points) than all the other species, followed by *P. elegans*, *P. varians* and *C. crangon*, and finally by *P. serratus* (Fig. 1b and Figure S3a, b). UTL in *P. montagui* could not be tested at 25 °C, as no individuals survived exposure to this temperature. However, mean UTL in *P. montagui* incubated at 20 °C were significantly lower than mean UTL in all the other species incubated at both 20 and 25 °C (Fig. 1b and Figure S3a, b). Wet weight did not have a significant effect on UTL (maximum  $F_{1,275} = 0.4$ ,  $P < 0.528$  for LO), except on OS ( $F_{1,263} = 15.7$ ,  $P < 0.0001$ ) (Table 1).

#### Plasticity of metabolic rate

Data for plasticity of oxygen consumption rate ( $\Delta\dot{M}O_2$ ) are given in Table S4. Within the whole temperature range examined (10–25 °C),  $\Delta\dot{M}O_2$  ranged from 125.3 nmol O<sub>2</sub> g wet weight<sup>-1</sup> min<sup>-1</sup> in *P. serratus* to 719.8 nmol O<sub>2</sub> g wet weight<sup>-1</sup> min<sup>-1</sup> in *P. varians*. While  $\dot{M}O_2$  increased exponentially with increasing incubation temperature in all species (except in *P. montagui* – quadratic) (minimum  $R^2 = 0.672$ ,  $F_{1,89} = 182.0$ ,  $P < 0.0001$ ), there were between-species differences in  $\Delta\dot{M}O_2$ , with *P. elegans* and *P. varians*, followed by *P. macrodactylus*, showing higher  $\Delta\dot{M}O_2$  than *P. serratus* and *C. crangon* (Fig. 1c and Figure S4). While showing similar  $\Delta\dot{M}O_2$  to *P. serratus* and *C. crangon* between 10 and 20 °C, *P. macrodactylus* exhibited higher  $\Delta\dot{M}O_2$  between 20 and 25 °C, ranking after *P. elegans* and *P. varians* (Table S4). *Pandalus montagui* was the only species showing a quadratic increase in  $\dot{M}O_2$  with increasing temperature ( $R^2 = 0.896$ ,  $F_{2,17} = 73.5$ ,  $P < 0.0001$ ) (Fig. 1c and Figure S4), possibly due to the lack of measurements at 25 °C. However, while between 10 and 15 °C this species showed the lowest  $\Delta\dot{M}O_2$ , between 15 and 20 °C it showed high  $\Delta\dot{M}O_2$ , ranking after *P. varians* (Table S4). Finally,  $Q_{10}$  values are reported in Table 2.

#### Plasticity for upper thermal limits

Data for plasticity of Upper Thermal Limits ( $\Delta$ UTL) are given in Table S4. Between 10 and 25 °C, *P. serratus*, *C. crangon* and *P. varians* showed the lowest  $\Delta$ UTL measured as LO (3.4 °C), OS (2.7 °C) and D (1.2 °C) respectively. By contrast, *P. macrodactylus* showed the highest  $\Delta$ UTL measured as LO and OS (6.0 and 5.9 °C), and *P. serratus* exhibited the highest  $\Delta$ UTL measured as D (6.8 °C). Between 10 and 20 °C, *P. montagui* showed lower  $\Delta$ UTL than all the other species (except than *P. varians* for OS and D, and *C. crangon* for D). Different species increased their UTL with increasing temperature by following different patterns (Fig. 1d, Figure S3c and Figure S5a, b, c), highlighting between-species differences in  $\Delta$ UTL. In more detail, UTL increased linearly in *P. macrodactylus* and *P. varians* (minimum  $R^2 = 0.079$ ,  $F_{1,53} = 4.5$ ,  $P = 0.038$ ), logarithmically in *P. elegans* (except for LO), *P. serratus*, and *C. crangon* (minimum  $R^2 = 0.410$ ,  $F_{1,77} = 53.6$ ,  $P < 0.0001$ ), and quadratically in *P. montagui* (except for LO) (minimum  $R^2 = 0.814$ ,  $F_{2,15} = 32.9$ ,  $P < 0.0001$ ) (Fig. 1d, Figure S3c and Figure S5a, b, c). While UTL of *P. elegans*, *P. serratus*, *C. crangon* and *P. montagui* showed an asymptotic trend within the examined temperature range, those of *P. macrodactylus* and *P. varians* did not. Nevertheless, *P. macrodactylus* showed greater  $\Delta$ UTL than *P. varians*, as regression lines for LO, OS and D in this species had greater slopes (Fig. 1d and Figure S3c).

#### Relationships between O<sub>2</sub>, UTL, and their plasticity

Over the whole temperature range examined (10–25 °C), a significant positive logarithmic relationship between  $\dot{M}O_2$  and UTL (for all end-points) was found in all species (minimum  $R^2 = 0.093$ ,  $F_{1,53} = 5.4$ ,  $P = 0.023$ ) (Fig. 2 and Figure S6a, b). *Pandalus montagui* represented the only exception showing a marginally significant positive logarithmic relationship between  $\dot{M}O_2$  and UTL measured as LO ( $R^2 = 0.219$ ,

**Fig. 1** The effect of temperature on (a) mean oxygen consumption rate ( $\dot{M}O_2$ ) (here used as a proxy for Standard Metabolic Rate, SMR), (b) Upper Thermal Limits (UTL), measured as Onset of Spasms (OS), and (c, d)  $\dot{M}O_2$  and UTL plasticity in the prawn species investigated in this study: *Palaemon elegans*, *Palaemon macrodactylus*, *Palaemon serratus*, *Palaemonetes varians*, *Crangon crangon* and *Pandalus montagui*. Histograms represent mean  $\pm$  SE for (a)  $\dot{M}O_2$  or (b) UTL after 7 day exposure to one of four incubation temperatures: 10 (yellow), 15 (light orange), 20 (dark orange) and 25 °C (red). Significantly different mean values ( $P < 0.05$ ) at different incubation temperatures for the same species are indicated by different letters placed above the histograms, while significantly different mean values ( $P < 0.05$ ) at the same incubation temperature among different species are indicated by different numbers placed inside the histograms. Finally, overall significant differences in mean values ( $P < 0.05$ ) among different species are indicated by different numbers preceded by a star placed at the top of the graph above each species. Pairwise comparisons were conducted using the Estimated Marginal Means test with Least Significant Difference test correction. Lines in (c, d) represent Standard Metabolic Rate–Temperature (SMR–T) and (d) Upper Thermal Limits–Temperature Reaction Norms (UTL–T), respectively: i.e., the patterns through which species increased their  $\dot{M}O_2$  or UTL in response to increasing incubation temperature according to the best-fit regression model. Raw data, regression equation and relevant statistics for  $\dot{M}O_2$  and UTL are provided in Figures S4 and S5b respectively.

**Table 1** Results for two-way ANCOVAs testing the effect of 'Species', 'Temperature', and their interaction on the oxygen consumption rate ( $\dot{M}O_2$ ) and Upper Thermal Limits (UTL), measured as Loss of Orientation (LO), Onset of Spasms (OS) and Death (D), for the prawn species investigated in this study after 7 day exposure to one of four incubation temperatures (10, 15, 20 and 25 °C) using 'Wet weight' as a covariate. Degrees of freedom (df), mean of square (MS), *F*-ratio (*F*) and probability level (*p*) are reported

Trait	Source	df	MS	<i>F</i>	<i>P</i>
$\dot{M}O_2$	Species	5	33296.8	32.1	<0.0001
	Temperature	3	301692.2	290.7	<0.0001
	Interaction	14	7393.7	7.1	<0.0001
	Wet weight (cov)	1	172579.9	166.3	<0.0001
LO	Species	5	137.9	93.6	<0.0001
	Temperature	3	161.0	109.3	<0.0001
	Interaction	14	3.2	2.2	0.010
OS	Species	5	101.9	85.2	<0.0001
	Temperature	3	156.6	131.1	<0.0001
	Interaction	14	5.4	4.5	<0.0001
	Wet weight (cov)	1	18.8	15.7	<0.0001
D	Species	5	139.1	269.2	<0.0001
	Temperature	3	185.8	359.4	<0.0001
	Interaction	14	12.1	23.4	<0.0001

$F_{1,13} = 3.7$ ,  $P = 0.078$ ), and no significant relationships between  $\Delta\dot{M}O_2$  and UTL measured as OS and D (maximum  $R^2 = 0.211$ ,  $F_{2,17} = 2.3$ ,  $P = 0.133$ ).

In addition, a significant negative relationship between UTL and  $\Delta$ UTL was found when UTL was measured as D (Fig. 3), indicating that species showing the highest UTL also showed the lowest  $\Delta$ UTL. Particularly, as UTL increased,  $\Delta$ UTL decreased linearly between 10 and 15 °C ( $R^2 = 0.826$ ,  $F_{1,3} = 19.0$ ,  $P = 0.012$ ) (Fig. 3a), and quadratically between 15 and 20 °C and between 10 and 20 °C (minimum  $R^2 = 0.966$ ,  $F_{2,3} = 42.1$ ,  $P = 0.006$ ) (Fig. 3b, d). No significant rela-

tionship between UTL and  $\Delta$ UTL was found at 20–25 °C, while a marginally significant negative linear relationship was found at 10–25 °C (Fig. 3c). *Palaemon macrodactylus* showed both high UTL and high  $\Delta$ UTL (Fig. 3). A significant logarithmic relationship between  $\dot{M}O_2$  and  $\Delta\dot{M}O_2$  was also found, but only between 20 and 25 °C ( $R^2 = 0.890$ ,  $F_{1,3} = 24.3$ ,  $P = 0.016$ ), and, therefore, it is not represented here.

## Discussion

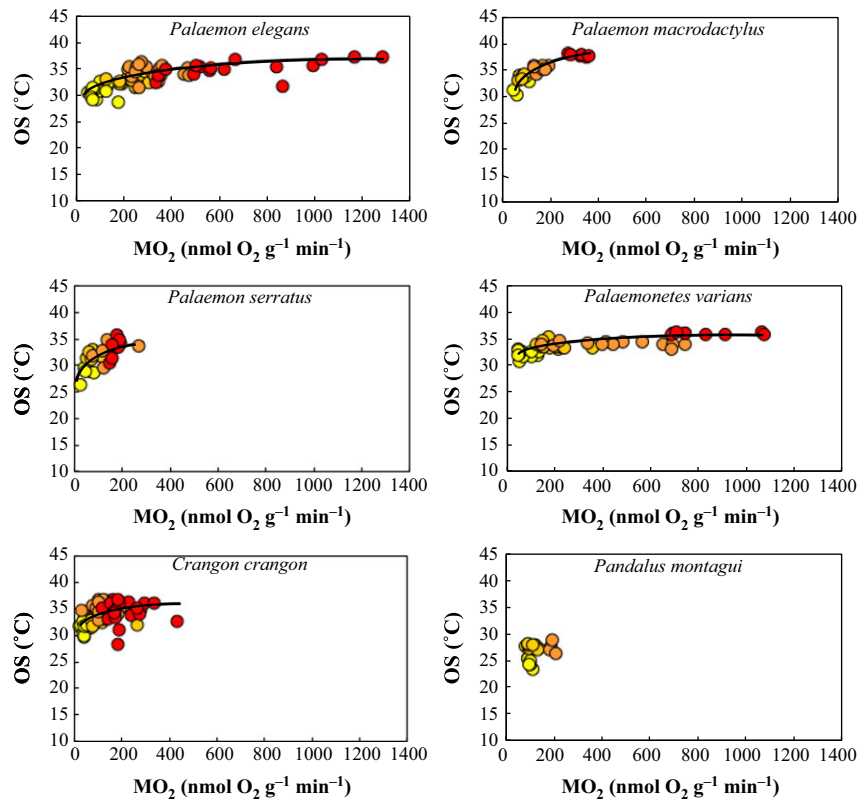
Here we demonstrate the importance of integrating the investigation of multiple physiological traits (metabolic rate and thermal limits), and their plasticity, to provide more accurate and balanced predictions on relative species vulnerability to global warming. Compared to species occupying narrower/more stable thermal niches, species inhabiting broader/more variable thermal environments appear to be more tolerant to extreme acute thermal events as a result of their higher thermal limits. Nevertheless, these species may be at greater risk from the negative effects of chronic exposure to warming due to the greater metabolic costs they incur (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; Dillon *et al.*, 2010). As a consequence, our results support the idea that evolutionary trade-offs may exist between acute and chronic heat tolerance (Rezende *et al.*, 2014). However, the invasive species *P. macrodactylus* represents an exception to this general pattern, showing elevated thermal limits and plasticity of these limits, as well as a high metabolic control. This combination of traits possibly explains the recent geographical expansion of this species (Bates *et al.*, 2013), and may make it particularly resilient to future warming scenarios (IPCC, 2014). Our findings and their likely ecological implications are discussed below, and the importance of integrating multiple physiological

**Table 2** Temperature sensitivity of  $\dot{M}O_2$ , expressed as the temperature coefficient for the change in  $\dot{M}O_2$  with temperature ( $Q_{10}$ ), in the prawn species investigated in this study. The temperature ranges used for the determination of  $Q_{10}$  are 10–20 and 10–25 °C. Mean  $Q_{10}$  for species living in broader/more variable thermal habitats and for species occupying narrower/more stable thermal niches are also reported. Types of habitat are indicated by letters placed next to species names: B stands for broader/more stable thermal environments, while N indicates species characterised by narrower/more stable thermal niches

Species	$Q_{10}$ (10–20 °C)	$Q_{10}$ (10–25 °C)	Mean $Q_{10}$ (10–20 °C)	Mean $Q_{10}$ (10–25 °C)
<i>Palaemon elegans</i> (B)	3.52	3.91	3.78	3.78
<i>Palaemon macrodactylus</i> (B)	2.41	2.79		
<i>Palaemonetes varians</i> (B)	5.42	4.64		
<i>Palaemon serratus</i> (N)	3.20	2.40	2.67	2.56
<i>Crangon crangon</i> (N)	2.80	2.72		
<i>Pandalus montagui</i> (N)	2.01	nr*		

\*nr = not recorded.





**Fig. 2** The relationship between  $MO_2$  and UTL, measured as OS, for the prawn species investigated in this study. Circles represent individual prawn  $MO_2$  and UTL measured after 7 day exposure to one of four incubation temperatures: 10 (yellow), 15 (light orange), 20 (dark orange) and 25 °C (red). Full lines represent the best-fit significant regression models, respectively; regression equation and relevant statistics are as follows: *P. elegans*:  $y = 2.082\ln(x) + 21.708$ ,  $R^2 = 0.609$ ,  $F_{1,61} = 94.9$ ,  $P < 0.0001$ ; *P. macrodactylus*:  $y = 3.290\ln(x) + 18.692$ ,  $R^2 = 0.887$ ,  $F_{1,21} = 165.2$ ,  $P < 0.0001$ ; *P. serratus*:  $y = 3.071\ln(x) + 17.766$ ,  $R^2 = 0.599$ ,  $F_{1,23} = 34.3$ ,  $P < 0.0001$ ; *P. varians*:  $y = 1.60\ln(x) + 27.491$ ,  $R^2 = 0.656$ ,  $F_{1,53} = 101.3$ ,  $P < 0.0001$ ; *C. crangon*:  $y = 1.460\ln(x) + 26.957$ ,  $R^2 = 0.244$ ,  $F_{1,77} = 24.9$ ,  $P < 0.0001$ ; *P. montagui*:  $P > 0.05$ .

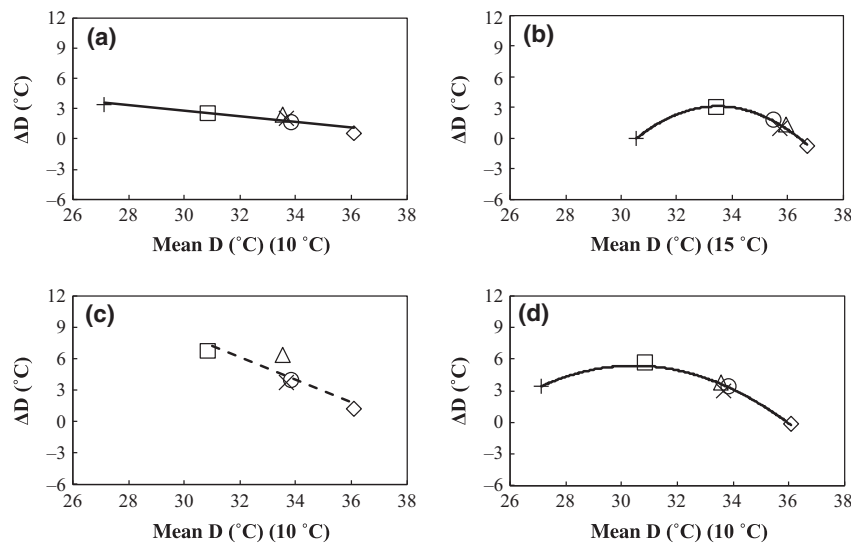
metrics to provide more accurate predictions on species and assemblage vulnerability to acute and chronic effects of global warming is highlighted.

#### Metabolic performance

As already demonstrated for caridean prawns (e.g., Vandonk & Dewilde, 1981; Dalla Via, 1985; Salvato *et al.*, 2001; Oliphant *et al.*, 2011), exponential Standard Metabolic Rate–Temperature (SMR–T) Reaction Norms are observed in all the species examined (except in *P. montagui* – quadratic). Indeed, metabolic rate increases exponentially with temperature due to increased kinetic energy of biochemical reactions (Gillooly *et al.*, 2001). However, between-species differences in metabolic response are also observed, possibly due to differences in mitochondrial density and aerobic capacity (e.g., Pörtner, 2001; see also Morley *et al.*, 2009) emerging from the adaptation to different thermal habitats (Clarke, 2004; Clarke & Fraser, 2004; see also Watson *et al.*, 2013).

Overall, species inhabiting broader/more variable thermal environments (particularly *P. elegans* and *P. varians*, but also, to an extent, *P. macrodactylus*) exhibit steeper SMR–T Reaction Norms than those occupying narrower/more stable thermal conditions (*P. serratus*, *C. crangon* and *P. montagui*). On average, these species also show higher mean  $Q_{10}$  values (3.78 both between 10 and 20 °C, and between 10 and 25 °C) relative to subtidal species (2.67 and 2.56 between 10 and 20 °C, and between 10 and 25 °C respectively) (see Table 2). In the assemblage investigated, the evolution of species metabolic response seems, therefore, to have been driven by species evolutionary ecology (e.g., type of thermal habitat), rather than by their phylogenetic history. If such response had been driven by species phylogeny, in fact, the response of *P. serratus* would have resembled more closely that of other palaemonid species, rather than that of *C. crangon* and *P. montagui* (see Figure S1).

While high metabolic plasticity may allow the maintenance of aerobic scope during fast and frequent



**Fig. 3** The relationship between UTL, measured as D, and plasticity for Upper Thermal Limits ( $\Delta$ UTL) at four temperature intervals: (a) 10–15 °C ( $y = -0.276x + 11.106$ ,  $R^2 = 0.826$ ,  $F_{1,3} = 19.0$ ,  $P = 0.012$ ), (b) 15–20 °C ( $y = -0.366x^2 + 25.504x - 406.748$ ,  $R^2 = 0.966$ ,  $F_{2,3} = 42.1$ ,  $P = 0.006$ ), (c) 10–25 °C ( $y = -1.077x + 40.623$ ,  $R^2 = 0.763$ ,  $F_{1,3} = 9.7$ ,  $P = 0.053$ ) and (d) 10–20 °C ( $y = -0.176x^2 + 10.739x - 157.930$ ,  $R^2 = 0.975$ ,  $F_{2,3} = 19.0$ ,  $P = 0.004$ ) °C.  $\Delta$ UTL is calculated as the difference between mean values of D either between consecutive or extreme incubation temperatures (considering either 25 or 20 °C as upper extreme temperature). Data points represent individual species UTL (measured at the incubation temperature indicated in brackets) and  $\Delta$ UTL; different symbols indicate different species: *P. elegans* (circle), *P. macrodactylus* (triangle), *P. serratus* (square), *P. varians* (diamond), *C. crangon* (cross) and *P. montagui* (plus). Full and dotted lines represent the best-fit significant ( $P < 0.05$ ) and marginally significant ( $0.05 < P < 0.08$ ) regression models, respectively.

temperature fluctuations (Via *et al.*, 1995; Bozinovic *et al.*, 2011), it also implies high energetic costs (Hulbert & Else, 2000; Rastrick & Whiteley, 2011; see also Watson *et al.*, 2013). Based on  $Q_{10}$  results (Table 2), species living in broader/more variable environments (particularly *P. elegans* and *P. varians*) seem to better compensate for temperature increases by increasing their metabolism to a greater extent, compared to species living in narrower/more stable thermal conditions. However, when evaluating SMR–T Reaction Norms, *P. elegans* and *P. varians* do show higher metabolic plasticity, but also incur considerably higher energetic costs. In general, species living in broader/more variable thermal habitats may be at great risk from the negative effects of chronic exposure to warming due to the higher metabolic costs they incur, compared to species inhabiting narrower/more stable thermal environments.

Based on SMR–T Reaction Norms, the invasive species *P. macrodactylus* maintains relatively low metabolic rates, and associated energetic costs between 10 and 20 °C, but expresses remarkably high metabolic plasticity between 20 and 25 °C. Between 10 and 25 °C, this species also shows a lower mean  $Q_{10}$  than *P. elegans* and *P. varians* (Table 2), but a higher  $Q_{10}$  in comparison to the other subtidal species (*C. crangon* and *P. serratus*) (Table 2). *Palaemon macrodactylus* seems, therefore, to

have evolved a remarkably high metabolic control, which possibly explains its recent geographical expansion (Bates *et al.*, 2013), and may make it especially resilient to future warming scenarios. By contrast, compared to the other subtidal species, *P. montagui* exhibits higher metabolic rates, showing reduced metabolic plasticity at low temperatures (10–15 °C) and elevated metabolic plasticity at relatively high temperatures (15–20 °C). This, together with the fact that *P. montagui* exhibits the lowest mean  $Q_{10}$  between 10 and 20 °C, suggests that this species may not be able to beneficially adjust its metabolic performance to temperatures within the tested range (10–20 °C), therefore being especially vulnerable to global warming.

#### Upper thermal limits

As already demonstrated for other crustaceans (e.g., Bradley, 1978; Layne *et al.*, 1985; Lagerspetz & Bowler, 1993; Cuculescu *et al.*, 1998; Stillman, 2003; Hopkin *et al.*, 2006), Upper Thermal Limits (UTL) increase with increasing temperature in all the species investigated. Nevertheless, between-species differences in UTL response to increasing temperature are also observed (e.g., Stillman, 2003; Hopkin *et al.*, 2006; Faulkner *et al.*, 2014; see also Araújo *et al.*, 2013). In addition, UTL are significantly positively  $O_2$ -dependent through a

logarithmic relationship (except in *P. montagui* – no relationship), supporting the idea that aerobic scope is maintained by increasing  $O_2$  until a *pejus* temperature is reached, then thermal tolerance becomes time-dependent (Pörtner, 2001; Verberk & Bilton, 2011). The *pejus* temperature is assumed to correspond to the point at which the relationship between  $O_2$  and UTL reaches the asymptote. In this instance, species showing higher metabolic plasticity (*P. elegans* and *P. varians*) possess: (i) a lower  $O_2$ -control for UTL, and (ii) lower *pejus* temperatures (i.e., the relationship between  $O_2$  and UTL in these species appears to tend to the asymptote at a lower temperature). Again, compared to species inhabiting narrower/more stable thermal habitats, species occupying broader/more variable thermal niches may, therefore, be at greater risk from the sublethal effects of global warming, since their scope for critical processes such as locomotion, growth and reproduction is likely to become compromised at a lower temperature.

Between-species differences in  $O_2$ -control for UTL largely reflect between-species differences in Upper Thermal Limits–Temperature (UTL–T) Reaction Norms. Indeed, species showing a greater metabolic control for UTL also possess greater plasticity of thermal limits, indicating that oxygen-limitations may be occurring at the whole-animal level (Pörtner, 2001; Verberk & Bilton, 2011; c.f. Truebano *et al.*, 2010).

A significant negative relationship between UTL and plasticity of thermal limits was also found, with species showing the highest UTL (*C. crangon*, *P. varians* and *P. elegans*) also showing the lowest plasticity of thermal limits. This suggests that the evolutionary trade-off found in porcelain crabs (Stillman, 2002, 2003; see also Bozinovic *et al.*, 2011; Araújo *et al.*, 2013) may also apply to the prawn assemblage examined here. In this instance, given that prawns with the highest UTL and plasticity of thermal limits are neither ecologically similar (i.e., type of thermal habitat) nor the most phylogenetically closely related, the evolutionary basis of such trade-off cannot be inferred, highlighting the need of exploring a larger phylogeny. However, it is relevant for conservation and commercial purposes that *P. elegans*, *P. varians* and *C. crangon* are likely to be less vulnerable to extreme acute temperatures when compared to *P. serratus* and *P. montagui*. Once again, the invasive species *P. macrodactylus* stands out showing both elevated UTL and plasticity for thermal limits. Also, as this species occurs in subtidal habitats, it is likely to possess greater thermal safety margins (*sensu* Stillman, 2002, 2003; Deutsch *et al.*, 2008; see also Diederich & Pechenik, 2013; Overgaard *et al.*, 2014), and may, therefore, be the least vulnerable species to extreme acute thermal events. By contrast, the low UTL and low plasticity of thermal limits observed in *P. montagui*, together

with their low  $O_2$ -control on UTL, further indicate that this species is likely to be the most vulnerable to warming. An alternative, not exclusive, view is that *P. montagui* shows very limited plasticity of both metabolism and UTL because it is being exposed to temperatures already above its *pejus* temperature.

#### *Towards a more integrative prediction of species and assemblage vulnerability to global warming*

**Integrating the investigation of metabolic performance, thermal tolerance, and their plasticity helps to more accurately elucidate species and assemblage vulnerability to global warming** (Bozinovic *et al.*, 2011). Indeed, while thermal tolerance and metabolic performance represent useful measures of acute and chronic resilience to warming respectively (Bozinovic *et al.*, 2011), plasticity reflects the extent to which taxa are able to adjust their physiological abilities to the global change (Ghalambor *et al.*, 2007; Charmantier *et al.*, 2008; see also Murren *et al.*, 2014). In the longer term, sublethal temperatures associated with global warming are likely to compromise organismal performance in critical processes like locomotion, growth and reproduction (Pörtner & Knust, 2007; Somero, 2011), which will ultimately reduce species ability to maintain healthy populations at a specific location, possibly leading to local extinctions and/or shifts along environmental gradients (Buckley, 2008; Kearney & Porter, 2009; Cheung *et al.*, 2010). Furthermore, these sublethal temperatures are species-specific (c.f. Araújo *et al.*, 2013), leading to changes in assemblage structure and dynamics including new ecological processes such as niche competition and species invasions (Milazzo *et al.*, 2013). In the short-term, taxa may be also threatened by the lethal effects of global warming, especially due to increasing intensity and frequency of extreme acute thermal events (IPCC *et al.*, 2012).

In the assemblage investigated, compared to species inhabiting narrower/more stable thermal environments, species occupying broader/more variable thermal niches are likely to be less vulnerable to extreme acute thermal events (c.f. Diederich & Pechenik, 2013), but may be at greater risk from the negative effects of chronic exposure to warming (e.g., Folguera *et al.*, 2009). Within this study, *P. montagui* and *P. macrodactylus* highlight the types of responses seen at the ends of this acute/chronic response trade-off spectrum. The former shows both extremely low thermal limits and metabolic control, while the latter possesses high thermal limits, elevated plasticity for these limits, and a high metabolic control. On this basis, under future global change scenarios, we predict that in the English Channel area *P. montagui* may suffer a reduction in

presence and abundance, while *P. macrodactylus* may experience a further expansion (e.g., Bates *et al.*, 2013). In general, ongoing environmental changes may cause shifts in the presence and abundance of the prawn species along the European Atlantic coasts, leading to considerable changes in assemblage structure and dynamics, and ecosystem functioning, as already predicted based on results from laboratory mesocosms for other marine assemblages (e.g., Hale *et al.*, 2011; Christen *et al.*, 2013). However, it must be noted that our conclusions are solely based on responses of adult individuals for the species investigated. Future work should include various developmental stages, accounting for differences in stage-specific vulnerability, especially since early life stages often represent physiological bottlenecks (Pörtner & Farrell, 2008; Storch *et al.*, 2011; Bartolini *et al.*, 2013). In any case, the greater understanding of the mechanistic basis of acute and chronic thermal tolerance, and their evolutionary trade-offs, achieved in our study can be used to implement conservation policies aimed at protecting ecologically and economically valuable resources (Bernardo *et al.*, 2007; see also Helmuth *et al.*, 2005), such as caridean prawns.

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## References

Angilletta MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology & Evolution*, **18**, 234–240.

Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat freezes niche evolution. *Ecology Letters*, **16**, 1206–1219.

Arnott SA, Neil DM, Ansell AD (1998) Tail-flip mechanism and size-dependent kinematics of escape swimming in the brown shrimp *Crangon crangon*. *Journal of Experimental Biology*, **201**, 1771–1784.

Atrill MJ, Thomas RM (1996) Long-term distribution patterns of mobile estuarine invertebrates (Ctenophora, Cnidaria, Crustacea: Decapoda) in relation to hydrological parameters. *Marine Ecology Progress Series*, **143**, 25–36.

Bartolini F, Barausse A, Poertner H-O, Giomi F (2013) Climate change reduces offspring fitness in littoral spawners: a study integrating organismic response and long-term time-series. *Global Change Biology*, **19**, 373–386.

Bates AE, McKelvie CM, Sorte CJB *et al.* (2013) Geographical range, heat tolerance and invasion success in aquatic species. *Proceedings of the Royal Society B*, **280**, 20131958.

Bechmann RK, Taban IC, Westerlund S *et al.* (2011) Effects of ocean acidification on early life stages of shrimp (*Pandalus borealis*) and mussel (*Mytilus edulis*). *Journal of Toxicology and Environmental Health*, **74**, 424–438.

Bernardo J, Ossola RJ, Spotila J, Crandall KA (2007) Interspecies physiological variation as a tool for cross-species assessments of global warming-induced endangerment: validation of an intrinsic determinant of macroecological and phylogeographic structure. *Biology Letters*, **3**, 695–698.

Bozinovic F, Calosi P, Spicer JI (2011) Physiological correlates of geographic range in animals. *The Annual Review of Ecology, Evolution, and Systematics*, **42**, 155–179.

Bozinovic F, Catalan TP, Estay SA, Sabat P (2013) Acclimation to daily thermal variability drives the metabolic performance curve. *Evolutionary Ecology Research*, **15**, 579–587.

Bradley PB (1978) Increase in range of temperature tolerance by acclimation in the copepod *Eurytemora affinis*. *Biological Bulletin*, **154**, 177–187.

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115–155.

Brattstrom BH (1968) Thermal acclimation in anurans as a function of latitude and altitude. *Comparative Biochemistry and Physiology*, **24**, 93–111.

Buckley LB (2008) Linking Traits to Energetics and Population Dynamics to Predict Lizard Ranges in Changing Environments. *American Naturalist*, **171**, E1–E19.

Calosi P, Bilton DT, Spicer JI (2008) Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, **4**, 99–102.

Calosi P, Bilton DT, Spicer JI, Votier SC, Atfield A (2010) What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, **79**, 194–204.

Charmantier A, Mcleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC (2008) Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320**, 800–803.

Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, Zeller D, Pauly D (2010) Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. *Global Change Biology*, **16**, 24–35.

Christen N, Calosi P, McNeill CL, Widdicombe S (2013) Structural and functional vulnerability to elevated pCO<sub>2</sub> in marine benthic communities. *Marine Biology*, **160**, 2113–2128.

Clarke A (2004) Is there a universal temperature dependence of metabolism? *Functional Ecology*, **18**, 252–256.

Clarke A, Fraser KPP (2004) Why does metabolism scale with temperature? *Functional Ecology*, **18**, 243–251.

Compton TJ, Rijkenberg MJA, Drent J, Piersma T (2007) Thermal tolerance ranges and climate variability: a comparison between bivalves from differing climates. *Journal of Experimental Marine Biology and Ecology*, **352**, 200–211.

Cowles RB, Bogert CM (1944) A preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History*, **83**, 261–296.

Cuculescu M, Hyde D, Bowler K (1998) Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *Journal of Thermal Biology*, **23**, 107–110.

Dalla Via J (1985) Oxygen consumption and temperature change in the shimp *Palaeomon elegans*. *Marine Ecology Progress Series*, **26**, 199–202.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 6668–6672.

Diederich CM, Pechenik JA (2013) Thermal tolerance of *Crepidula fornicata* (Gastropoda) life history stages from intertidal and subtidal subpopulations. *Marine Ecology Progress Series*, **486**, 173–187.

Dillon ME, Wang G, Huey RB (2010) Global metabolic impacts of recent climate warming. *Nature*, **467**, 704–U788.

Farris JS, Källersjö K, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics*, **10**, 315–319.

Faulkner KT, Clusella-Trullas S, Peck LS, Chown SL (2014) Lack of coherence in the warming responses of marine crustaceans. *Functional Ecology*, **28**, 895–903.

Folguera G, Bastías DA, Bozinovic F (2009) Impact of experimental thermal amplitude on ectotherm performance: adaptation to climate change variability? *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology*, **154**, 389–393.

Ghalambor CK, Mckay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. *Science*, **293**, 2248–2251.

Hale R, Calosi P, McNeill L, Mieszowska N, Widdicombe S (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos*, **120**, 661–674.



- Helmuth B, Kingsolver JG, Carrington E (2005) Biophysics, physiological ecology, and climate change: does mechanism matter? *Annual Review of Physiology*, **67**, 177–201.
- Henderson PA (1987) On the population biology of the common shrimp *Crangon crangon* (L) (Crustacea, Caridea) in the Severn Estuary and Bristol Channel. *Journal of the Marine Biological Association of the United Kingdom*, **67**, 825–847.
- Henderson PA, Seaby RM, Somes JR (2006) A 25-year study of climatic and density-dependent population regulation of common shrimp *Crangon crangon* (Crustacea: Caridea) in the Bristol Channel. *Journal of the Marine Biological Association of the United Kingdom*, **86**, 287–298.
- Hopkin RS, Qari S, Bowler K, Hyde D, Cuculescu M (2006) Seasonal thermal tolerance in marine Crustacea. *Journal of Experimental Marine Biology and Ecology*, **331**, 74–81.
- Hulbert AJ, Else PL (2000) Mechanisms underlying the cost of living in animals. *Annual Review of Physiology*, **62**, 207–235.
- IPCC (2014) Working Group II (WGII) contribution to the IPCC Fifth Assessment Report Climate Change: Impacts, Adaptation and Vulnerability was accepted in Yokohama Japan, 29th March 2014.
- IPCC (2012) Managing the risks of extreme events and disasters to advance climate change adaptation. In: *A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change* (eds Field CB, Barros V, Stocker TF, Qin D, Dokken DJ, Ebi KL, Mastrandrea MD, Mach KJ, Plattner G-K, Allen SK, Tignor M, Midgley PM). Cambridge University Press, Cambridge, UK.
- Kearney M, Porter W (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters*, **12**, 334–350.
- Lagerspetz KYH, Bowler K (1993) Variation in heat tolerance in individual *Asellus aquaticus* during thermal acclimation. *Journal of Thermal Biology*, **18**, 137–143.
- Lavesque N, Bachelet G, Beguer M *et al.* (2010) Recent expansion of the oriental shrimp *Palaemon macrodactylus* (Crustacea: Decapoda) on the western coasts of France. *Aquatic Invasions*, **5**, S103–S108.
- Layne JRJ, Manis ML, Claussen DL (1985) Seasonal variation in the time course of thermal acclimation in the crayfish *Orconectes rusticus*. *Freshwater Invertebrates Biology*, **4**, 98–104.
- Lutterschmidt WI, Hutchison VH (1997) The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **75**, 1553–1560.
- Massamba-N'siala G, Calosi P, Bilton DT, Prevedelli D, Simonini R (2012) Life-history and thermal tolerance traits display different thermal plasticities and relationships with temperature in the marine polychaete *Ophryotrocha labronica* La Greca and Bacci (Dorvilleidae). *Journal of Experimental Marine Biology and Ecology*, **438**, 109–117.
- Melatunan S, Calosi P, Rundle SD, Moody AJ, Widdicombe S (2011) Exposure to elevated temperatures and pCO<sub>2</sub> reduces respiration rate and energy status in the periwinkle *Littorina littorea*. *Physiological and Biochemical Zoology*, **84**, 583–594.
- Menendez R, Gutierrez D (1996) Altitudinal effects on habitat selection of dung beetles (Scarabaeoidea: Aphodiidae) in the northern Iberian peninsula. *Ecography*, **19**, 313–317.
- Milazzo M, Mirto S, Domenici P, Gristina M (2013) Climate change exacerbates interspecific interactions in sympatric coastal fishes. *Journal of Animal Ecology*, **82**, 468–477.
- Morley SA, Lurman GJ, Skepper JN, Poertner H-O, Peck LS (2009) Thermal plasticity of mitochondria: a latitudinal comparison between Southern Ocean molluscs. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology*, **152**, 423–430.
- Murren CJ, Maclean HJ, Diamond SE *et al.* (2014) Evolutionary change in continuous Reaction Norms. *The American Naturalist*, **183**, 453–467.
- Oh CW, Hartnoll RG, Nash RDM (2001) Feeding ecology of the common shrimp *Crangon crangon* in Port Erin Bay, Isle of Man, Irish Sea. *Marine Ecology-Progress Series*, **214**, 211–223.
- Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. *Journal of Experimental Biology*, **214**, 1109–1117.
- Overgaard J, Kristensen TN, Sorensen JG (2012) Validity of thermal ramping assays used to assess thermal tolerance in arthropods. *PLoS ONE*, **7**, e32758.
- Overgaard J, Kearney MR, Hoffmann AA (2014) Sensitivity to thermal extremes in Australian *Drosophila* implies similar impacts of climate change on the distribution of widespread and tropical species. *Global Change Biology*, **20**, 1738–1750.
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.
- Peck LS, Morley SA, Richard J, Clark MS (2014) Acclimation and thermal tolerance in Antarctic marine ectotherms. *Journal of Experimental Biology*, **217**, 16–22.
- Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science*, **308**, 1912–1915.
- Pigliucci M (ed.) (2001) *Phenotypic Plasticity Beyond Nature and Nurture*. The Johns Hopkins University Press, Baltimore, MD, USA.
- Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367.
- Pihl L, Rosenberg R (1984) Food selection and consumption of the shrimp *Crangon crangon* in some shallow marine areas in Western Sweden. *Marine Ecology Progress Series*, **15**, 159–168.
- Pörtner H-O (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, **88**, 137–146.
- Pörtner H-O, Farrell AP (2008) Physiology and climate change. *Science*, **322**, 690–692.
- Pörtner H-O, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, **315**, 95–97.
- Rastrick SPS, Whiteley NM (2011) Congeneric amphipods show differing abilities to maintain metabolic rates with latitude. *Physiological and Biochemical Zoology*, **84**, 154–165.
- Rezende EL, Tejado M, Santos M (2011) Estimating the adaptive potential of critical thermal limits: methodological problems and evolutionary implications. *Functional Ecology*, **25**, 111–121.
- Rezende EL, Castañeda LE, Santos M (2014) Tolerance landscapes in thermal ecology. *Functional Ecology*, **28**, 799–809.
- Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.
- Salvato B, Cuomo V, Di Muro P, Beltrami M (2001) Effects of environmental parameters on the oxygen consumption of four marine invertebrates: a comparative factorial study. *Marine Biology*, **138**, 659–668.
- Schlichting CD, Pigliucci M (eds.) (1998) *Phenotypic evolution: a reaction norm perspective Sunderland*. Sinauer, Massachusetts, USA.
- Small D, Calosi P, White D, Spicer JJ, Widdicombe S (2010) Impact of medium-term exposure to CO<sub>2</sub> enriched seawater on the physiological functions of the velvet swimming crab *Necora puber*. *Aquatic Biology*, **10**, 11–21.
- Sokal RR, Rohlf FJ (eds) (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, New York, NY, USA.
- Sokolova IM, Pörtner HO (2003) Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes. *Journal of Experimental Biology*, **206**, 195–207.
- Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology*, **213**, 912–920.
- Somero GN (2011) Comparative physiology: a "crystal ball" for predicting consequences of global change. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, **301**, R1–R14.
- Soors J, Faasse M, Stevens M, Verbessem I, De Regge N, Van Den Bergh E (2010) New crustacean invaders in the Schelde estuary (Belgium). *Belgian Journal of Zoology*, **140**, 3–10.
- Southward AJ, Hawkins SJ, Burrows MT (1995) Seventy years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the Western English Channel in relation to rising sea temperature. *Journal of Thermal Biology*, **20**, 127–155.
- Spicer JJ, Eriksson SP (2003) Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster *Nephrops norvegicus* (L.)? *Journal of Experimental Marine Biology and Ecology*, **295**, 219–243.
- Spivak ED, Boschi EE, Martorelli SR (2006) Presence of *Palaemon macrodactylus* Rathbun 1902 (Crustacea: Decapoda: Caridea: Palaemonidae) in Mar del Plata harbor, Argentina: first record from southwestern Atlantic waters. *Biological Invasions*, **8**, 673–676.
- Stillman JH (2002) Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes*. *Integrative and Comparative Biology*, **42**, 790–796.
- Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. *Science*, **301**, 65–65.
- Stillman JH, Somero GN (2000) A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: Influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, **73**, 200–208.
- Storch D, Fernandez M, Navarette SA, Pörtner H-O (2011) Investigations on the Chilean kelp crab *Taliepus dentatus*. *Marine Ecology Progress Series*, **429**, 157–167.
- Swofford DL (2002) *PAUP\* Version 4b10*. Sinauer Associates Inc., Sunderland, Massachusetts, USA.

- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.
- Terblanche JS, Klok CJ, Krafur ES, Chown SL (2006) Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. *The American Journal of Tropical Medicine and Hygiene*, **74**, 786–794.
- Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL (2007) Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 2935–2942.
- Tewksbury JJ, Huey RB, Deutsch CA (2008) Putting the heat on tropical animals. *Science*, **320**, 1296–1297.
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.
- Truebano M, Burns G, MAS Thorne, Hillyard G, Peck LS, Skibinski DOF, Clark MS (2010) Transcriptional response to heat stress in the Antarctic bivalve *Laternula elliptica*. *Journal of Experimental Marine Biology and Ecology*, **391**, 65–72.
- Underwood AJ (ed) (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge, UK.
- Vandonk E, Dewilde P (1981) Oxygen consumption and motile activity of the brown shrimp *Crangon crangon* related to temperature and body size. *Netherlands Journal of Sea Research*, **15**, 54–64.
- Verberk W, Bilton DT (2011) Can oxygen set thermal limits in an insect and drive gigantism? *PLoS ONE*, **6**, e22610.
- Via S, Gomulkiewicz R, Dejong G, Scheiner SM, Schlichting CD, Vantienderen PH (1995) Adaptive phenotypic plasticity – Consensus and controversy. *Trends in Ecology & Evolution*, **10**, 212–217.
- Waddington CH (1942) Canalization of development and the inheritance of acquired characters. *Nature*, **150**, 563–565.
- Watson S, Morley SA, Bates AE *et al.* (2013) Low global sensitivity of metabolic rate to temperature in calcified marine invertebrates. *Oecologia*, **174**, 45–54.

Zweifel RG (1957) Studies on the critical thermal maxima of salamanders. *Ecology*, **38**, 64–69.

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Representation of plasticity.

**Figure S2.** Phylogram of the prawn species investigated in this study.

**Figure S3.** Mean  $\pm$  SE for UTL measured as Loss of Orientation (LO) and Death (D), and Upper Thermal Limits–Temperature (UTL–T) Reaction Norms for these end-points.

**Figure S4.** The relationship between  $\dot{M}O_2$  and temperature.

**Figure S5.** The relationship between UTL and temperature for all end-points.

**Figure S6.** The relationship between  $\dot{M}O_2$  and UTL measured as LO and D.

**Table S1.** Number of individuals, collection information, and mortality for the prawn species investigated in this study.

**Table S2.** GenBank accession numbers of prawn species used for phylogenetic analysis.

**Table S3.** Mean  $\pm$  SE for oxygen consumption rate ( $\dot{M}O_2$ ) and Upper Thermal Limits (UTL).

**Table S4.** Mean plasticity of oxygen consumption rate ( $\Delta\dot{M}O_2$ ) and Upper Thermal Limits ( $\Delta$ UTL).